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GEORG-MARTEN LANNO

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Contribution of Georg-Marten Lanno to original publications (I–III)

Publication I: Study design, experiments (preparation of different structured PCL scaffolds, viscosity measurements, ATR-FTIR, *in vitro* drug release experiments). Sample preparation and analysis (BET, SEM, XRPD, HPLC). Data analysis and writing.

Publication II: Study design, experiments (mechanical analysis, contact angle measurements, swelling and degradation studies, biological relevance: fibroblast attachment and biocompatibility, antibacterial and antibiofilm properties). Data analysis and writing.

Publication III: Experiments, data analysis (Agar diffusion assay and fibroblast attachment and biocompatibility). Writing the publication.

ABBREVIATIONS

Abbreviation *List in alphabetic order*	Explanation
API	Active pharmaceutical ingredient
AA	Acidic acid
ACE	Acetone
CAM	Chloramphenicol
CF	Chloroform
CoNS	Coagulase-negative *Staphylococci*
DCM	Dichloromethane
DDS	Drug Delivery System
DETCs	Dendritic epidermal T cells
DMSO	Dimethyl sulfoxide
DMF	Dimethylformamide
ECM	Extracellular matrix
EPS	Extracellular polymeric matrix
ES	Electrospinning
FA	Formic acid
MIC	Minimum inhibitory concentration
PCL	Polycaprolactone
PEO	Polyethylene oxide
PHMB	Poly hexamethylene biguanide
PS	Polystyrene
SA	Stearic acid
SC	Stem cells
TET	Tetracycline
THF	Tetrahydrofuran
TIPS	Thermally induced phase separation
VIPS	Vapor induced phase separation
ROS	Reactive oxygen species

1 INTRODUCTION

Wound treatment is a worldwide problem with annually increasing costs and insufficient treatment options. (Mirhaj et al., 2022) There is always contamination related to wounds which increases the risk for the development of infection. Therefore, the main treatment strategy has been to restore the homeostasis in the wound site as well as control the bacterial load. (Mathes, 2008) The problem arises when the patient's medical condition (for example diabetes) inhibits the native immune response causing failed and long-lasting treatment. (Q. Zhang et al., 2017)

The administration of antibacterial drugs is a vital part of wound treatment strategy. In order to prevent the development of antimicrobial resistance the antibiotic treatment plan should be chosen wisely regarding the choice of drug and the route of administration (systemic or local). (Mathes, 2008) The systemic administration of antibiotics to treat wounds has its risks related with suitable dose (high risk of toxicity) and low drug concentrations at the wound site. (D. Leaper et al., 2015b) Topical treatment with antibiotics (conventional drug formulations such as gels, creams) is frequently used but its efficiency in most cases is poorly determined. (Smith et al., 2020) Therefore novel drug delivery research is focused on finding the site-specific drug delivery systems (DDS) with enhanced antibacterial properties (Luraghi et al., 2021)

Electrospinning is a straightforward method for the production of polymeric fibers ranging from nano- to microscale. (Doshi & Reneker, 1995) The fiber scaffolds have specific features: controlled surface morphology, large specific surface area, tunable porosity and relatively simple incorporation of drugs giving them potential to be used as DDS. (Qu et al., 2013) The drug release from fiber scaffolds is mostly based on diffusion however it is tunable by manipulating the surface porosity on the fibers or porosity of the fiber scaffold. (Zupančič et al., 2018) Controlling the surface or scaffolds porosity is therefore critical for achieving desired antibacterial properties of the scaffold. (Preem et al., 2017; Preem, Bock, et al., 2019; Zupančič et al., 2018)

Electrospun scaffolds have potential in wound healing because of their overall structure which is similar to the extracellular matrix (ECM). (Haider et al., 2018) Furthermore, the high specific surface area in combination with porous structure enables absorption of wound exudate as well as enhanced gas exchange. (Preem et al., 2017) The mechanical properties of the scaffold are also modifiable by ES material, process and environmental parameters. (Rashid et al., 2021) Ideal scaffold for wound healing should be highly elastic and flexible to ease application and removal as well as offering suitable substrate for cells in charge of native wound healing. (Gao et al., 2021)

An antibacterial scaffold needs to be non-toxic and biocompatible towards cells responsible for native wound healing process. Therefore, the cell/scaffold interactions need to be studied in order to estimate the scaffold performance. (Ninan et al., 2015).

When developing antibacterial scaffold for wound healing the bacteria/ scaffold interactions should be studied. Ideally the scaffold should inhibit bacterial growth at the wound site and protect the wound from contamination. It has been shown that some electrospun fiber scaffolds can promote the bacterial growth on the scaffold surface. (Pompa-Monroy et al., 2020) Even more, fiber scaffolds have shown to promote bacterial biofilm formation. (Tamayo-Ramos et al., 2018) However, this can be avoided by suitable composition, morphology, mechanical and drug release properties of the scaffold. (Kargar et al., 2012; Mitik-Dineva et al., 2006, 2009).

Electrospun scaffolds are still considered novel DDS as only a few commercial products have been released. (Omer et al., 2021) Regarding scaffolds for wound healing there is still a lack of knowledge about the material properties and material/structure relationship that supports wound healing as well as offers enhanced protection from bacteria.

The aim of this study was to design and develop electrospun antibacterial drug loaded scaffolds using different polymers and antibacterial drugs for wound healing purposes. Differently structured scaffolds were produced in order to study the structure/activity relationships as well as interactions with bacterial and eukaryotic cells. The antibacterial properties, biocompatibility and safety studies enabled to examine the scaffold's suitability to be used for wound healing.

2 LITERATURE REVIEW

2.1 Skin wounds, wound healing and treatment

Human skin is the primary barrier defending the organism from external environment. It offers protection against dehydration, pathogens, regulates the thermic dispersion as well as signalling via cutaneous receptors.(Azzimonti et al., 2016) Furthermore, skin has several self-renewal mechanisms in order to maintain the homeostasis being exposed to many physical, chemical and biological irradiations. (Azzimonti et al., 2016; Blanpain & Fuchs, 2009) Skin consists of epidermis, dermis and hypodermis where the resident cells are hematopoietic and mesenchymal stem cells (SC) originating from bone marrow SC. (Azzimonti et al., 2016) Hematopoietic SC, as precursors to endothelial progenitor cells, are responsible for neovascularization. Mesenchymal SC, as precursors to fibroblasts, adipocytes and osteoblasts, are all responsible for the physiological integrity and communication. (Azzimonti et al., 2016; Cha & Falanga, 2007) Epidermis as basal layer consists of epidermal SC, long-living quiescent SC that are progenitors for tissue renewal. (Azzimonti et al., 2016; De Rosa & De Luca, 2012) Furthermore, epidermal SC are also found in sebaceous glands and hair follicles and are known to promote epithelialization during wound healing. (Blanpain & Fuchs, 2009; Ito et al., 2005)

Wounds are damages of biological tissue, including skin, mucous membranes, and organ tissues (Herman & Bordoni, 2021) which are broadly categorized as acute (caused by trauma, burns, surgery) or chronic wounds. Acute wounds usually heal within acceptable timeframe, however chronic wounds tend to persist over longer period. (Whitney, 2005) Furthermore, in chronic wounds, the functionality of cells is altered which makes the native wound healing insufficient so that keratinocytes are unable to close the wound making it vulnerable against pathogens. (Azzimonti et al., 2016; Brem et al., 2007)

The native wound healing process has the goal to restore the structure of the skin and homeostasis and it has 4 steps: hemostasis, inflammation, proliferation and remodelling (maturation) (**Figure 1**). (Azzimonti et al., 2016)

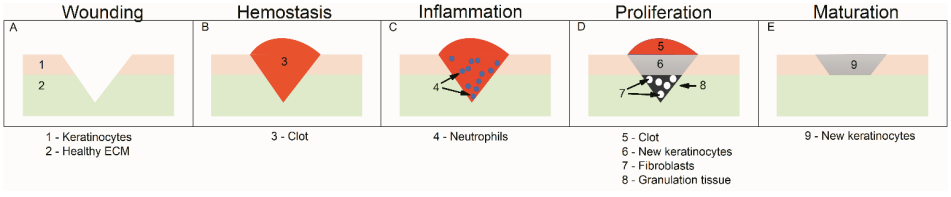


Figure 1. The main factors and stages of wound healing: **A** (Wounding)– The keratinocytes and extracellular matrix (ECM) are damaged; **B** (Hemostasis) – clot formation as the preliminary ECM; **C** (Inflammation) – Infiltration of neutrophils; **D** (Proliferation) – the formation of granulation tissue by keratinocytes and fibroblasts; E (Maturation) – the remodelling of ECM and wound maturation. (Modified from Rieger, Birch, and Schiffman 2013)

Wound healing process starts immediately after wounding (e.g., tissue damage) (**Figure 1A)**. (Rieger et al., 2013) The injured site is filled with blood and lymphatic fluid which is followed by hemostasis stage during the first 15 minutes (**Figure 1B**). At this stage, the main goal is to stop the bleeding by vasoconstriction in blood and lymphatic vessels. Localized clot is formed by migration of trombocytes to the wound site which forms as a provisional ECM for cell migration. (Baum & Arpey, 2005; Rieger et al., 2013)

The next step is inflammation (**Figure 1C**) where vasodilation and increased capillary permeability occurs by chemical signalling from injured tissue and mast cells. (Avi & Howard, 2005) The site is also colonized with polymorphonuclear cells which activate the production of growth factors and are in charge of removing foreign substances (bacteria, debris etc). (Baum & Arpey, 2005; Wilgus, 2008) The migration of monocytes, which transform into macrophages at the wound site, is also stimulating collagen production and reepithelialization of the wound. (Baum & Arpey, 2005)

The proliferation phase is mostly focused on reepithelialization occuring 24 – 48 h after wounding (**Figure 1D**). (Rieger et al., 2013) The degradation of the provisional ECM starts by enzymes released from keratinocytes which migrate to the wound boundary. (Baum & Arpey, 2005) The migration of keratinocytes is dependent on the number of platelets, but slowed down by the presence of neutrophiles and macrophages. (Rieger et al., 2013) After 2 days of healing, endothelial cells migration occurs and angiogenesis starts which is controlled by cytokines, the presence of ECM and the absence of neighbouring endothelial cells. Furthermore, matrix metalloproteinases are responsible for degrading the basement membrane and ECM and laminin production starts from endothelial cells producing new basement membrane. (Baum & Arpey, 2005; Rieger et al., 2013) After 4 days collagen-based granulation tissue replaces the provisional ECM formed in hemostasis.(Wilgus, 2008) The closure is aided by fibroblasts converted to myofibroblasts that produce actin filaments. (Baum & Arpey, 2005)

In the maturation phase the granulation tissue is replaced by scar tissue and this can take several months. The collagen fibrils increase in diameter together with interfibril binding and rearrangement. The tissue gains strength as the binding increases, level of inflammation is decreased and the scarring matures. (Baum & Arpey, 2005)

Chronic wounds have the tendency not to follow the normal wound healing phases and their order, usually the problems occur in the inflammation phase. (Frykberg & Banks, 2015) The excessive number of cytokines, proteases, reactive oxygen species (ROS), senescent cells, existing persistent infection and dysfunctional stem cells are usually the related features with chronic wounds. In acute wounds the proteases are regulated by the inhibitors, however in chronic wounds they are exceeding the level of inhibitors resulting in the destruction of ECM and degradation of growth factors. This amplifies the inflammation cycle and inhibits the normal cycle of wound healing (Frykberg & Banks, 2015) Furthermore, increased ROS causes ECM and cellular damage and stimulation of proteases and inflammatory cytokines. (Schreml et al., 2010) Another problem

with chronic wounds is the increased amount of senescent cells which is correlated to the failure of wound healing. (Lobmann et al., 2002; Stanley & Osler, 2001; Tsourdi et al., 2013) Mesenchymal SC are known to have an important role as precursors to many important cells in wound healing and the mesenchymal SC deficiency has been seen in diabetic patients with chronic wounds. (Cianfarani et al., 2013; Ennis et al., 2013; Rodriguez-Menocal et al., 2012) Problems with reepithelialization are seen with chronic wounds. (Lazarus et al., 1994) Hence, to achieve normal wound healing, the balance needs to be restored for cytokines, growth factors, proteases and normally proliferating cells at the wound site. (Frykberg & Banks, 2015; Schultz et al., 2003)

2.1.1 Wound infections

There are always bacteria in the wound. The major factors that determine the development of wound infection from contamination and colonization are the number of bacteria, their virulence and the immune system response. (Frank et al., 2005) Contamination is usually caused by bacteria from the surrounding skin, local environment or the bacteria are of endogenous origin. (Siddiqui & Bernstein, 2010b) Diagnosis of the wound infection nowadays consists of clinical observations, laboratory tests, bacterial culturing and wound biopsy. (Yi-Fan et al., 2022) A high risk of wound infection is considered when bacterial load exceeds 10^5 microorganisms/g of tissue. (Daeschlein, 2013) That being said the threshold depends on the host immune system, and the origin and virulence of the microorganisms. (Kingsley, 2003) For instance, in some cases the local inflammation may lead to increased wound bed perfusion promoting the healing process. (Laato et al., 1988) The shift to infection is seen when the host immune response is overruled by the bacterial proliferation. (Salcido, 2007) The immune response is affected by various aspects – diabetes, malnutrition, comedication, obesity, and age of the host as well as site specific factors – poor perfusion, necrosis, foreign bodies. (Bowler et al., 2001; Siddiqui & Bernstein, 2010b) The microenvironment changes in the wound – pH, prolonged presence of macrophages and neutrophils that increases the degradation of growth factors and leads to incomplete ECM assembly. (Percival et al., 2016) In serious cases amputations need to be performed and sepsis can occur which increases the mortality. (Daeschlein, 2013). Therefore, as the ECM assembly is vital for native wound healing, scaffolds/ substrates (that promote assembly) could be used.

Infection can be caused by both aerobic and anaerobic bacteria. Usually, the first bacteria above threshold in wounds are Gram+ bacteria with the pH optimum of 7 for instance coagulase-negative staphylococci (CoNS). In later stages *Pseudomonas aeruginosa* and *Enterococcus faecalis* appear that have wider pH optimum. For normal skin the pH is slightly acidic, which has been assumed favourable for native wound healing, however it is not always the case. (Percival et al., 2014) With chronic wounds the alkaline pH increases the anaerobic bacterial population. (Daeschlein, 2013) Too low pH in the wound, caused by bacterial

produced high lactate levels, may be unsuitable for vulnerable fibroblasts and endothelial cells. (Britland et al., 2012; Percival et al., 2016) Chronic wounds are more likely to be colonized by anaerobic bacteria and fungi and have a complex bacterial microbiota for example in diabetic wound ulcers you can find elevated concentrations of *Bacteroides, Peptoniphilus, Finegoldia, Anaerococcus,* and *Pepetostreptococcus* species. (Frykberg & Banks, 2015) Importantly, it is said that the non-healing wound is not caused by individual bacterium but more of the combination of bacteria being part of the microbiota of the wound which should be specifically diagnosed before the treatment. (R. Wolcott & Dowd, 2011)

The infection can be caused either by planktonic state bacteria or bacteria that form a biofilm. It is seen that microorganisms tend to aggregate and form a complex structure in natural and clinical environments, which is known to be responsible for more than 80% of microbial infections in human bodies. (Davies, 2003) Biofilms are complex aggregates of microorganisms that protect themselves with a self-produced polymeric ECM which represents a unique microenvironment. (Thomson, 2011) In this microenvironment the bacteria alter their phenotype (Fisher et al., 2010) and develop quorum sensing in response to population density which gives them the ability to regulate biofilm formation, adaption and response to injury. (Miller & Bassler, 2001) One critical aspect about biofilm is that it increases the bacterial resistance to mechanical, chemical and antimicrobial treatments. (Fisher et al., 2010) Chronic wounds are a good environment for biofilm formation due to the presence of superficial debris, necrotic tissue and altered vascularization that prevents the natural immune system to develop an effective response. (G. Zhao et al., 2013) It is said that about 60% of chronic wounds are colonized by biofilm forming bacteria which also makes a huge impact on mortality and increased costs on healthcare. (James et al., 2008; Siddiqui & Bernstein, 2010a) Biofilms have also been related to various conditions like venous leg ulcers, diabetic foot ulcers and pressure ulcers which are the main ones to develop into non-healing chronic wounds. (Siddiqui & Bernstein, 2010a) Studies have shown that bacteria isolated from skin wounds have higher biofilm formation potential than bacteria isolated from normal skin. (Percival et al., 2012) Therefore, it is important to inhibit biofilm formation in wounds at any cost as it limits or inhibits the wound infection treatment.

In clinical practice the first signs of critically infected wounds are the decrease in progression of healing which is also relevant in diagnosis. Other clinical signs are increased pain, oedema, unusual malodorous discharge which leads to cellulitis with infection. (Mathes, 2008) After diagnosis, a proper treatment strategy needs to be set up. It is known that extensively used systemic administration of antibiotics can be insufficient in the wounds due to low local drug concentrations and permeability in the wound. Furthermore, it can cause the risk for toxicity during long-term use and development of antibacterial resistance. (Bowler et al., 2001; James et al., 2008; Siddiqui & Bernstein, 2010a)

2.1.2 Wound infection treatment

Current wound care is based on the TIME concept, where T stands for tissue observation and cleaning, I for diagnosis and treatment of the infection and inflammation, M – looking after the environment (moisturization) and E – observation of the wound edges and the surrounding tissues. TIME is used for the initial treatment of the chronic wound followed by choosing the proper dressing to protect the site and aid the wound healing. (D. J. Leaper et al., 2012) Recently it has been updated to TIMERS where the additional letter R stands for repair/regeneration and S – social factors (**Figure 2**). (Atkin et al., 2019)

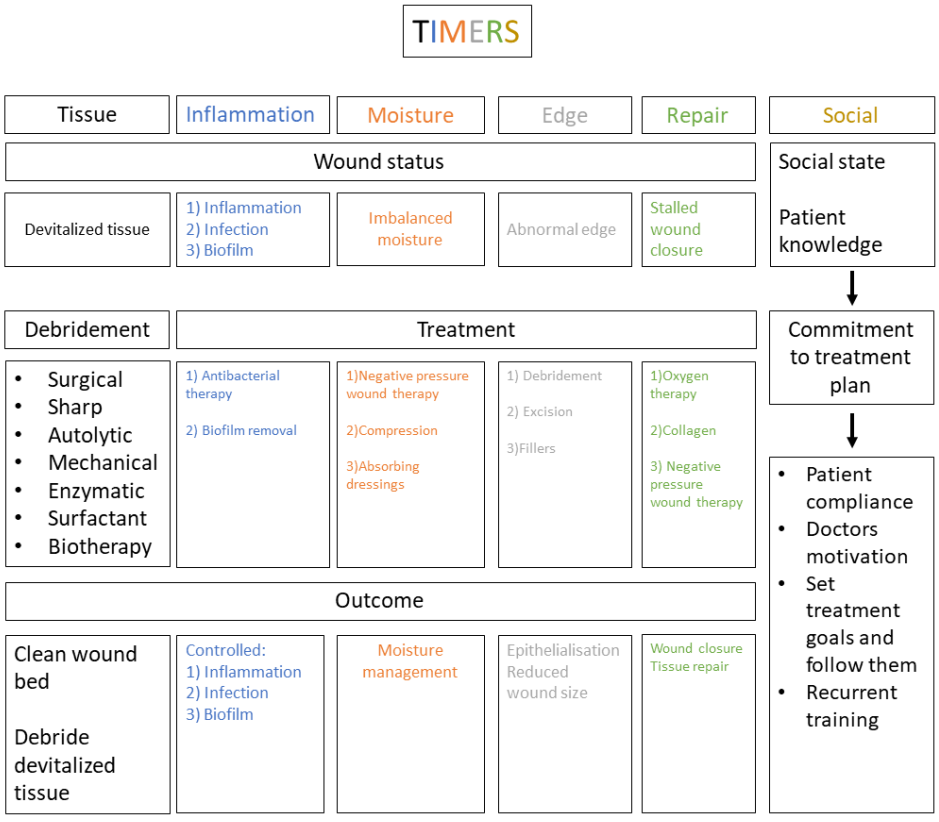


Figure 2. TIMERS approach for the treatment of chronic, non-healing wounds due to critical colonisation and/or biofilm. (modified from Alves et al. 2021)

The treatment strategies at the moment are to restore the homeostasis of the wound and control the bacterial load. Usually this means regular cleaning procedures of the wound, surgical interference by removing any necrotic tissue (debridement), reduction of oedema and use of an appropriate medication – local or systemic treatment. (Mathes, 2008) With some diseases, wounds need specific treatment since the native wound healing process in compromised. One of those

is diabetes where the patients have incompetent inflammatory response, insufficient angiogenesis, ECM imbalance and peripheral neuropathy. (Q. Zhang et al., 2017) Diabetic ulcers are limited in the wound healing process to the inflammatory/proliferation phase that increases the risk of secondary infection, prolonged inflammation which leads to delayed healing. Furthermore, with chronic wounds, the ECM imbalance, problems with cellular communication and the lack of growth factors increase the risks. (Sundaramurthi et al., 2014)

The most cost-effective method until now has been surgical interference by biofilm removal. (R. D. Wolcott et al., 2009) Regarding antimicrobial therapy it has been demonstrated that the time is a crucial factor – newly formed biofilms are easier to treat with antibiotics. Successful chronic wound treatment should be the combination of both – regular tissue removal that disrupts the biofilm combined with thorough cleaning (debridement) and local antimicrobial treatment. (R. D. Wolcott et al., 2010) However new strategies have been also suggested, one of them is called biofilm-based wound care. (Scalise et al., 2015) The strategy consists of debridement, antiseptics, antibiotics in combination with anti-biofilm agents that interfere with the bacterial adhesion, degrade the biofilm matrix and inhibit the quorum-sensing of the bacteria. (R. Wolcott & Dowd, 2011)

2.1.2.1 Systemic treatment

Prophylactic systemic use of antibiotics is shown to be ineffective for the treatment of infected wound except dog and human bites on hands. (Medeiros & Saconato, 2001) Similarly after small surgeries there is no clinical evidence that systemic antibiotic treatment is needed. (O'Mathúna, 2016) Systemic use of antibiotics has been used for chronic wound infection treatment for a long time. (D. Leaper et al., 2015b) The list of used antibiotics is long and main classes are beta-lactams, aminoglycosides, macrolides, quinolones, lincosamides, nitroimidazoles, sulfonamides and combinations. (D. Leaper et al., 2015a) The antimicrobials help to decrease the concentration of planktonic bacteria, inhibit their adhesion properties as well as inhibit their ability to form biofilm. (Simões et al., 2018) The limiting factors for the used antibiotics are toxicity as well as limited amount of cellular uptake by the host cells as well as the development of resistance. (Hoss, 2005) It has been studied that the use of systemic antibiotics to treat and prevent biofilm related infections has the efficiency of 25–31%. (Marr et al., 1997) It has been suggested that the role of extracellular polymeric substances (EPS) is conferring tolerance to aminoglycosides by diffusion-reaction inhibition phenomena resulting in enzymatic degradation of the drug. (Billings et al., 2015; Fux et al., 2005; Khan et al., 2010; Oubekka et al., 2012) Another factor is the stationary phase of biofilm bacteria which is in correlation with antibiotic tolerance that increases together with the maturation of the biofilm. (Amato et al., 2014; Maisonneuve & Gerdes, 2014) Furthermore, the biofilm is usually populated with persisters that have multidrug tolerance phenotype and are prevalent in stationary phase communities. (Ayrapetyan et al., 2015; Helaine & Kugelberg, 2014; Keren et al., 2011) The systemic use of antibiotics for the

treatment of chronic wounds such as diabetic ulcers has been proven to be insufficient. (D. Leaper et al., 2015a) It has also been studied that for example drug concentrations within chronic diabetic ulcers fail to exceed the minimum inhibitory concentration (MIC) for inhibiting and killing wound pathogens. (Seabrook et al., 1991) Venous ulcers are another condition where the systemic treatment with antibiotics should always be the second choice of action depending on the patient, costs and wound type. (Brölmann et al., 2012) There have been studies that have shown that pentoxifylline has been strongly effective for the treatment of venous ulcers with the combination of compression therapy. (Jull et al., 2007) However, as mentioned, the long term and continuous use of systemic antibiotics increases the risk for the development of antibiotic resistance which should be minimized at any cost. (Brölmann et al., 2012)

2.1.2.2 Local treatment

Topical antibiotic wound treatment is very controversial. It has historically been used due to its localized site of action with smaller side effects and problems with toxicity. (Smith et al., 2020) The list of topical antibiotic agents is substantially shorter and includes mupirocin, fusidic agents, neomycin, polymyxin and bacitracin (Bowler et al., 2001) The problem with topical antibiotic treatment of wounds is that the efficiency is still questionable since when applied too late and in a wrong concentration it helps the bacteria to develop resistance. (Smith et al., 2020)

Antiseptics is another topical treatment method frequently used. Their main mode of action is to reduce microbial burden on the skin surface prophylactically. (Smith et al., 2020) Antiseptics have 4 different classes: emulsifiers (chlorhexidine, octenidine, polyhexamethylene biguanide, benzalkonium salts), oxidizers (hydrogen peroxide, iodine compounds), acids (acidic acid, boric acid) and heavy metal compounds (silver compounds, bismuth compounds, copper and mercury). (Cambiaso-Daniel et al., 9000; D. Leaper et al., 2015a) The problem with antiseptics is that their continuous usage can be harmful for the human skin. The most commonly used antiseptics are silver compounds, polyvinylpyrrolidone (PVP) iodine, octenidine, and polyhexamethylene biguanide (PHMB).

Silver compounds (silver nitrate solutions, ointments, silver containing dressings) are very popular as topical antimicrobials, especially for the treatment burn wounds. (Cambiaso-Daniel et al., 2018) Silver ions have an affinity to bind to microorganism's DNA, proteins and enzymes causing cellular death. (Durán et al., 2016a) Silver nitrate solutions are used in various concentrations 0.5–50% and it has shown bacteriostatic effect with most relevant pathogens found in wounds. (Durán et al., 2016b) Silver sulfadiazine (used in ointments) has not shown to be that effective against *Pseudomonas spp* and enteric bacteria, however, it provides coverage against fungi. (Greenhalgh, 2009) The problem with using silver ions is their limited penetration within the wound as the ions tend to bind on the surface of proteins. (Aziz et al., 2012) Furthermore, recent studies have raised the problems with cytotoxicity regarding these silver formulations

which could limit their use as a topical antimicrobial in the future. (Atiyeh et al., 2007) The treatment of chronic wounds with silver compounds has also shown to be ineffective. For example, silver nitrate 100 mg/L is incapable to reduce the number of microorganisms present in the biofilm. (Silvestry-Rodriguez et al., 2007) However, there have been studies with silver nanoparticles with the concentration of 100–150 g/mL that have been effective against biofilms caused by *P. aeruginosa* and *S. proteamaculans.* (X. Chen & Schluesener, 2008) Additionally, they found out that biofilm bacteria were about 25 times more tolerant to silver than planktonic bacteria.

Another popular antiseptic used to treat acute wounds is PVP iodine (liquid and ointment formulations), which has been used for over 150 years. (Cambiaso-Daniel et al., 2018) It acts as a broad-spectrum antimicrobial against pathogens, fungi and yeast. (Georgiade, 1973) It has been shown that PVP iodine is cytotoxic against fibroblasts and keratinocytes which could alter the wound healing. (Thomas et al., 2009) In contrast for the treatment of chronic wounds PVP iodine has shown to be effective in *in vitro* model with *Pseudomonas* and *Staphylococcus* biofilms. (Hill et al., 2010) PVP iodine has also shown higher antibiofilm activity than PHMB and silver acetate. (Oates et al., 2018)

PHMB is a widely used antiseptic having the highest spectrum of activity from biguanides as well as an effect against *P. aeruginosa*. (Chindera et al., 2016a) The mode of action is thought to be electrostatic bonding to the bacterial membrane which causes it to break. (Chindera et al., 2016b) PHMB based products have shown to be less toxic in comparison with other antiseptics together with the positive effect on the wound closure. (Mulder et al., 2007) PHMB based formulations have also shown to be effective for critically colonised chronic wounds and shown antibiofilm activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) in wound models. (Davis et al., 2017; Dissemond et al., 2010) However, recent studies have shown the insufficiency of PHMB in eliminating biofilm from wound tissues. (Borges et al., 2018) and its cytotoxicity towards cells involved in wound healing process. (Alves et al., 2021)

One more approach is the use of antibiofilm agents like xylitol, salicylic acid, erythritol, farnesol, and Sanguitec gel. (Dowd et al., 2009). There have been studies that have evaluated the activity of antibiofilm agents and found that 20% xylitol, 10% erythritol, 1 g/mL farnesol, 20 mM salicylic acid and 0.1% Sanguitec gels were able to inhibit biofilm formation – some had selective and non-selective inhibition against *S. aureus* and *P. aeruginosa* in the model used. (Dowd et al., 2009) The effect could be enhanced using combined therapy of antiseptics, antibiotics in combination with antibiofilm agents which could have synergistic effect against biofilm related chronic wounds. (Percival et al., 2015) The complexity of the treatment of chronic wounds needs appropriate measures, where the native wound healing process needs to be aided in every way possible. Wound dressings could play a significant role in the wound healing process offering sufficient moisture management, contributing to the prevention of additional contamination of the wound bed. During recent years novel wound dressings have been developed using nanotechnology, where polymer based fiber wound

dressings have shown to be capable to offer enhanced properties in moisture control of the wound bed, localised antimicrobial DDS in clinically relevant concentrations and improved properties for cellular activity in the wound bed. (Preem & Kogermann, 2018)

2.2 Wound dressings

Wound dressings are an important part of the treatment of non-healing chronic wounds. Historically, the dressings have been used as a physical barrier to prevent further contamination of the wound. It was also thought to be important that the dressing should keep the wound dry until 1962 when the moisturisation in wound healing was found to be important. (Winter, 1962) After that the development of dressings changed. One of the most important roles of wound dressings is to preserve and provide optimal moisture in the wound in order to support sufficient wound repair. (Andreu et al. 2015) The dressings are classified in three groups depending on their mode of action as seen in **Table 1**.

Passive dressings are known to be used for wound concealment and inhibiting bacterial colonisation. (Andreu et al., 2015) The negative effect for passive wound dressing is that its continuous use can lead to skin maceration due to the entrapment of water and wound exudate. (Weller & Sussman, 2006) Interactive dressings have the ability to promote debridement, enhance granulation and re-epithelisation, control the exudate levels and bacterial colonisation. Some examples of these materials are hydrocolloids, alginates, collagen, hyaluronic acid products, foams, hydrogels and semipermeable films. (Andreu et al., 2015) The last group of dressings is bioactive dressings that have incorporated APIs like antimicrobials, growth factors, nanoparticles and natural products that change the microenvironment and promote wound healing – such as fibroblast attachment and endothelial cell migration. (Andreu et al., 2015; Mihai et al., 2019; S. Sharma, A. Dua, 2014; Zahedi et al., 2010) Bioactive dressing is usually prepared from various bio- or synthetic polymers in foam, sponge, film, hydrogel or nanofiber membrane structure. (Ambekar & Kandasubramanian, 2019; Fahimirad & Ajalloueian, 2019) In an ideal world a wound dressing should have the following properties: maintain a moist environment, debridement of wound site, control the amount of wound exudate, avoid contamination, non-toxic and non-allergenic, provide sufficient gas exchange, protection against additional trauma, easily removable, inhibition of bacterial invasion and colonisation, thermal insulation, minimize the frequency of dressings change, enhanced shelf-life, user friendly and cost effective. (Boateng et al., 2008; Mayet et al., 2014; Seaman, 2002) Until today there is no single dressing that has all the properties discussed above. The most promising dressings that have been shown to have a lot of the properties are polymeric fiber scaffolds that could potentially be used as novel bioactive wound dressings. (Miguel et al., 2018)

Table 1. Wound dressings, description and mechanism of action (Dhivya et al., 2015; Preem & Kogermann, 2018; Rheinecker, 1995)

	Passive (inert) dressing	**Interactive dressing**	**Bioactive dressing**
Material	Gauze (polyester or cotton), tulle (petroleum jelly)	Semi-permeable polymeric films and foams, hydrogels, hydrocolloids, hydrofibers.	Dressings including bioactive polymers such as alginate, collagen, gelatin, chitosan, or dressings consisting of antibiotics, antiseptics, growth factors, enzymes, steam cells, plant extracts, phages etc. Skin grafts, skin substitutes.
Description	Non-occlusive. Cover the wound to restore its function underneath.	Semi-occlusive or occlusive, highly elastic and flexible, nonabsorptive and /or moderately to highly absorptive. Barrier against penetration of bacteria to the wound environment, permeable to water vapor and oxygen.	Semi-occlusive or occlusive dressings, barrier against contamination, highly elastic and flexible, tunable absorptiveness. Specific mechanism of action depends on the properties of active substance and the carrier polymer, the incorporation method of the active substance and its release.
Mechanism of action	No regulatory function, some dressings may absorb exudate.	Regulate wound healing by simple physicochemical means, control moisture level (e.g. gel formation).	Delivering bioactive substances that assist in wound healing or dressing is constructed from material having endogenous activity. Regulate wound healing by means of physiologically active substances. Control the moisture balance in the wound.
Application	Superficial acute wounds.	Epithelializing wound, superficial wound and shallow wound with low ammount of exudates (dry wound). Burn wounds. Moderately to highly exudating wounds. Chronic wounds.	Infected wound, burns, chronic wounds (e.g. pressure ulcers, venous ulcers and diabetic foot ulcers).

2.3 Electrospinning technique and electrospun drug delivery systems (DDS)

There are various methods producing nanofibers like drawing techniques, spinneret-based tunable engineered parameter method, phase separation, self-assembly, template synthesis, freeze-drying, and interfacial polymerization of nanofibers, amongst all others electrospinning is thought to be the most promising method for novel bioactive wound scaffold production. (Alghoraibi & Alomari, 2019) (Andreu et al., 2015) Electrospinning is a polymeric fiber production method using high voltage and obtaining fiber sizes ranging from nano to macro-scale. Electrospinning is derived from electrospraying method first mentioned and patented in 1902 that was used to disperse fluids using electrostatic forces. Electrospinning method for fiber production was first patented in 1934 (Alghoraibi & Alomari, 2019), however the concept was discussed already in 1600s. (Formhals A, 1934; Gilbert, 1958) Typical solution-based electrospinning process has 3 important components: 1) polymeric solution that is pushed towards the needle using syringe pump; 2) high voltage supply; 3) grounded collector as seen in **Figure 3**.

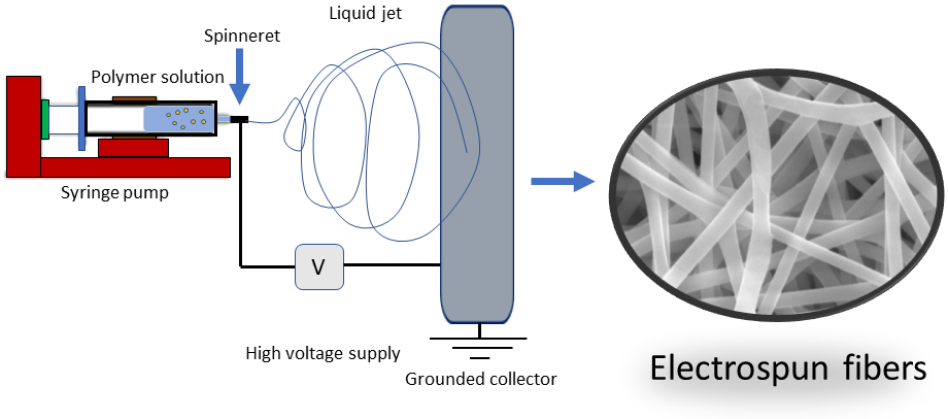


Figure 3. Schematics of electrospinning process

Basically, a droplet of the polymeric solution in the tip of the needle is affected by two forces – electrostatic forces that try to deform the shape of the droplet and surface tension that tries to retain the spherical shape of the droplet. The charge of the electrical field in the system is manipulated by the high-voltage power supply. When increasing the electrical field to the critical point, where the electrostatic forces overcome surface tension forces – the droplet of the liquid is deformed into smaller droplet or droplets. This process is known as electrospraying. Using high molecular-weight polymers, the polymeric chains can be entangled forming a steady stream of ultrafine fibers which are ejected towards the collector – this process is called electrospinning. The elongated droplet is called Taylor cone, from which the fibers eject. The electrospinning jet formation has

3 main steps: 1) the formation of the Taylor cone; 2) the ejection of the straight jet; 3) the unstable whipping jet region. (Alghoraibi & Alomari, 2019; Angammana & Jayaram, 2011a) The whipping region is where the interactions between the electrostatic force and surface tension lead to the evaporation of the solvent. Eventually thin fiber filaments solidify on the grounded collector.

Conventional electrospinning can be modified using specific needles for more sophisticated fiber production. One of those is the use of two concentric needles that generate co-axially electrified jet called co-axial electrospinning. (Qu et al., 2013) It is possible to produce core-shell structured fibers using co-axial electrospinning which can be used to enhance the electrospinning capabilities of various materials. For example, co-axial electrospinning has been used to fabricate thermally crosslinking polymers (S. Lu et al., 2013), modified DDSs (C. L. He et al., 2006; X.-Y. Li et al., 2013), and for the incorporation of biological material such as proteins (H. Jiang et al., 2005) and bacteria (Nagy et al., 2014) etc. The system can also be modified by increasing the number of concentric needles, forming multiaxial (three or more) needle system that can offer three or more layered structures for desired applications. (Y. Zhao et al., 2007) However, multichannel system has a restriction that needs to be considered. One thing is that the used fluids need to have similar electrospinning properties in order to produce fibers. The viscosities of the liquids need to be sufficient for electrospinning, the fluids need to be immiscible, should have similar dielectric properties, and the flow properties of both liquids need to be optimised. (G. Lee et al., 2010) Another approach that can be considered to produce core-shell structured fibers is emulsion electrospinning, where the system consists of lipophilic and hydrophilic immiscible phases and after electrospinning forms the desired structure. (Cai et al., 2017) One drawback with emulsion electrospinning is the lack of precise control on the location of incorporated component, such as API, within the core or the shell of fibers. (Y. Zhao et al., 2007)

In order to increase the amount of material produced people have been improving the electrospinning set-up. One is the capillary-based system from which needleless electrospinning systems have been developed. (Yarin & Zussman, 2004) They used a magnetic fluid together with magnetic field in order to generate nanofibers from PEO solution, however this also made the system complex as the polymeric solution could not be mixed with the magnetic solution. (Yarin & Zussman, 2004) Another needleless system nowadays frequently used is called NanoSpider^{TM} developed by scientists from the Technical University of Liberci. (Jirsak et al., 2003) In this system a rotating spinneret is dipped to the reservoir of polymeric solution and the collection occurs on moving substrate material. (Molnár & Vas, 2012) Bubble electrospinning invented by several scientist from China, South-Africa and United States is also a newer needleless electrospinning system that can utilize a static or rotating spinneret and gas in order to prepare the fibers. (Jakapon Sunthornvarabhas, Darrell H. Reneker, George G. Chase, 2012; Y. Liu & He, 2007; Molnár & Vas, 2012; Smit et al., 2005; R. Yang et al., 2009) This process uses bubbles generated from air/nitrogen which play a role for achieving stable Taylor cones. (Molnár & Vas, 2012) Another novel electro-

spinning method is high-intensity focused ultrasound electrospinning (USES), where alternate current is transformed to ultrasound. (Laidmäe et al., 2016) USES method enables rapid manipulation of gradient structures during electrospinning as ultrasound parameters can be modified during process. Overall, there are numerous other methods not mentioned that have been developed for electrospinning and the research keeps evolving as the interest in nanotechnology increases.

Electrospinning is largely dependent on the process, material and environmental parameters. The process parameters are applied voltage, distance between spinneret and collector, flow rate of the solution and the size of the capillary used. The material parameters are mainly the polymer, solvent, polymer concentration, and the viscosity and conductivity of the solution.

Crucial factor affecting electrospinning is the solvent used for polymer solution preparation as the volatility of the vapor pressure of the solvent determines the evaporation rate which is in correlation with the solidification rate of the jet. The volatility needs to be optimal for the polymer solution in order to avoid premature solidification of fibers or the deposition of wet fibers on the collector. The volatility rate of solvent is largely dependent on the dielectric constant, so the stability of the jet can be optimized by the modification of the dielectric constant. (Luo et al., 2012) Some of the most commonly used solvents are dichloromethane, chloroform, dimethylsulfoxide (DMF), tetrahydrofuran (THF), acetone, dimethylsulfoxide (DMSO), hexafluoro isopropanol (HFIP) and trifluoroethanol. Water is not so favourable due to a high dielectric constant value that affect the solvent evaporation rate. However in some cases, solvents are combined in the formulation phase in order to achieve the desired results, for example for the preparation of nanoporous fibers from PCL. (Katsogiannis et al., 2015a)

Today more than 200 polymers have been electrospun, both synthetic and natural polymers. (W.-J. Li & Tuan, 2009) Some synthetic polymers like polystyrene (PS) and polyvinylchloride (PVC) are commercially used to produce nanofibers. Solution-based electrospinning for biomedical applications is mostly performed using biocompatible and biodegradable synthetic polymers like polycaprolactone (PCL), polylactic acid (PLA) and poly (lactic-co-glycolic acid) (PLGA) in order to produce scaffolds. Natural polymers for instance fibrinogens, dextran, chitin, chitosan, alginate, collagen and gelatin have also been successfully electrospun. (J. Xue et al., 2019) The advantage of natural polymers is biodegradability, biocompatibility, low antigenicity, sometimes having antimicrobial, anti-inflammatory properties and contributing to tissue repair. (Juncos Bombin et al., 2020) The disadvantages for natural polymers are the accelerated degradation and poor mechanical properties compared to the synthetic polymers.

The resemblance between natural ECM and electrospun fiber scaffolds has focused the research of nanofibers towards tissue engineering, wound dressings, drug delivery systems (DDS) and enzyme immobilization in order to achieve faster biological response. (Metreveli et al., 2014) The material selection is largely dependent on the final application. In biomedical applications for instance, the materials used for preparing the wound healing scaffolds need to be non-toxic,

biodegradable, biocompatible, have suitable mechanical properties and approved by the international legal agencies like United States Food and Drug Administration (FDA) or European Medical Agency (EMA). (Preem & Kogermann, 2018)

Environmental parameters are relative humidity and temperature. (Haider et al., 2018) All these process, material and environmental parameters play a significant role in the fiber formation and enhance the design space of the desired product, however in some cases it might be difficult to control the parameters, for example the environmental parameters. The effects of different parameters are summarized in **Table 2.** (Bhattarai et al., 2018; J. Xue et al., 2019)

Table 2. Parameters affecting the electrospinning process and fiber morphology (Bhattarai et al., 2018; J. Xue et al., 2019)

Parameters			**Effect**	**Reference**
Material properties	Polymer	Polymer	Hydrophilicity, mechanical properties	(Abbasi et al., 2014; J. Xue et al., 2019)
		Molecular weight	Rheology and electrical properties	
	Solvent	Solvent	Solubility of the polymer (homogeneity)	(Luo et al., 2010; Zheng et al., 2014)
		Boiling point	Related to the volatility (evaporation of the solvent)	(Haider et al., 2015, 2018)
	Solution	Polymer concentration	Fiber morphology. Higher viscosity = homogenous fibers (to a critical value)	
		Conductivity	Increased conductivity = decreased fiber diameter (to critical value)	(Angammana & Jayaram, 2010, 2011b)
		Viscosity/surface tension	Related to the stability of the jet and bead formation	(Haider et al., 2014; J. Xue et al., 2019)
Process parameters		Voltage	Fiber diameter and bead formation, higher voltage = smaller fibers	(Haider et al., 2018; Sill & von Recum, 2008)
		Flow rate	Fiber diameter, pore size and bead formation	(Henriques et al., 2009; Zeng et al., 2003)
		Distance	Fiber diameter, smaller distance = bigger fibers	(Geng et al., 2005; Ingavle & Leach, 2014)
Environmental parameters		Humidity	Controls the solidification process of the charged jet and porosity	(De Vrieze et al., 2009; Haider et al., 2018; Katsogiannis et al., 2015b)
		Temperature	Related to the evaporation of solvent and decrease in viscosity	

2.3.1 Fiber morphology

The electrospinning process as mentioned before can be used to produce fiber scaffolds from nano to microscale with modifiable surface morphology. (Z. Wang et al., 2017) A list of parameters affecting fiber morphology is concluded in **Table 2**. The surface morphology of the electrospun fiber scaffolds can be modified with various parameters: solution parameters (Ge et al., 2016; Huan et al., 2015; Zaarour, Zhang, et al., 2018; Zaarour, Zhu, & Jin, 2019), environmental parameters (Ramos et al., 2020; G.-Z. Yang et al., 2017; Zaarour, Zhu, et al., 2018) and process parameters (Acik et al., 2019; Bakar et al., 2018; Jian et al., 2018; Z. Li & Wang, 2013; Meng et al., 2019; Ramakrishna et al., 2005). Furthermore, one of the modifiable process parameter is the collector type like rotating collector (CHAO et al., 2019; Zaarour, Zhu, Huang, et al., 2019), parallel electrodes (L. Yu et al., 2019), disk collector (Ng & Supaphol, 2018), patterned electrodes (G. H. Kim et al., 2018), parallel rings (W. Song et al., 2019), microfiber collector modification (Thorvaldsson et al., 2008) and liquid bath collector. (Wu & Hong, 2016). Furthermore, the electrospinning set-up can be modified to result in desired morphology. *In-situ* microfluidics electrospinning has been developed for tunable microporous structure production (W. Liu et al., 2016) and water bath collector in order to develop nanoporous fibers. (Zhu et al., 2020) Core-shell type systems have been developed using multi-channel needles. (Pan et al., 2015; Y. Zhao et al., 2007) The main fiber morphology types developed are hollow, (L.-Y. Wang et al., 2018), porous, (Dersch et al., 2005) grooved, (Dou et al., 2022; W. Liu et al., 2015) wrinkled, (Zaarour, Zhu, & Jin, 2019) rough (C. Huang & Thomas, 2018) that is going to be discussed below.(Zaarour et al., 2020) (**Figure 4**)

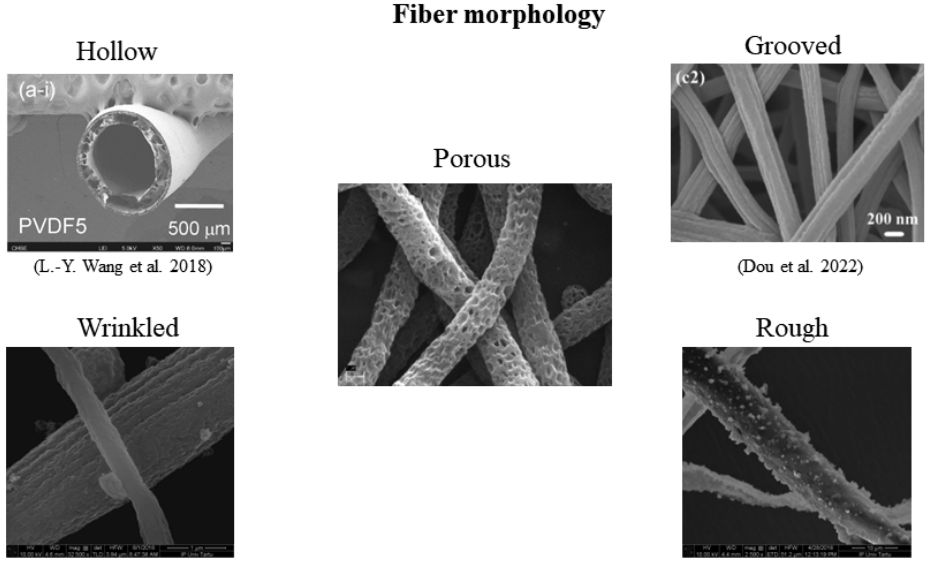


Figure 4. Examples of different electrospun fiber's morphology. (L.-Y. Wang et al., 2018) © 2018 Elsevier B.V. (Licence no: 5398711142833). (Dou et al. 2022) © 2022 Elsevier B.V. (Licence no: 5398720191603)

Increased porosity and specific surface area of the fiber scaffold improves the performance of absorption that could be beneficial for wound healing to keep the moisturization under control (L. Liu et al., 2019; Z. Liu et al., 2019). There are numerous methods to achieve porous fibers, most of them are based on the phase separation or using sacrificial templating. (P. Lu & Xia, 2013; Y. Wang et al., 2017) Using sacrificial templating for pore formation is not ideal as the method involves harsh post-treatment conditions which may damage the material. (Zaarour et al., 2020) Therefore, phase separation method is preferred which can be thermally (TIPS) or vapor induced phase separation (VIPS). (P. Lu & Xia, 2013) Different polymers and solvents used for porous fiber production can be seen in **Table 3**.

Table 3. Polymers and solvents used for the production of porous electrospun fibers

Polymer	**Solvent/solvent systems**	**Reference**
PCL	THF:DMSO GAA	(Katsogiannis et al., 2015a; W. Li et al., 2019)
PVDF	ACE	(Zhu et al., 2019)
PVC	DMF THF:DMF	(Ding et al., 2019)
PLLA	DMF:CF	(Asli et al., 2012)
PS	DMF	(Tang et al., 2019)
EC	THF:DMAc	(JY et al., 2007)

Key: polycaprolactone (PCL), polyvinylidene fluoride (PVDF), polyvinyl chloride (PVC), poly-(L-lactic acid) (PLLA), polystyrene (PS), ethylcellulose (EC), tetrahydrofuran (THF), dimethyl-sulfoxide (DMSO), glacial acetic acid (GAA), acetone (ACE), dimethylformamide (DMF), chloroform (CF), dimethylacetamide (DMAc)

TIPS is more commonly used for pore formation on electrospun fiber surfaces by condensation of the water vapor in the vicinity of the jet-air interface caused by the evaporation of high volatile solvents. (Zaarour et al., 2020) The positive charges dispersed on the surface of water attract condensed water droplets that form numerous water pockets that generate pores after drying. (P. Lu & Xia, 2013) Internal porosity is formed using VIPS. The penetrating non-solvent from vapor phase into the polymer solution causes phase separation for the polymer. (Zaarour et al., 2020) The polymer is precipitated by the non-solvent out of solution that generates the solid matrix where solvent-rich phase evolves into porous regions. (P. Lu & Xia, 2013). Several polymers have been used in order to generate porous fibers using VIPS, for example: PMMA (Bae et al., 2013), polyoxymethylene (Kongkhlang, Kotaki, et al., 2008), and PCL (Katsogiannis et al., 2015b) using low volatile solvents or a combination of high and low volatile solvents. In some cases, interior porous fibers were fabricated using ternary system of polymer/ solvent/non-solvent. (Zaarour et al., 2020)

Grooved fibers have shown potential in tissue engineering, superhydrophobic surfaces and oil clean up. (Liang et al., 2017) There are 3 main mechanisms in the production of grooved fibers: void-based elongation, wrinkle-based elongation, and collapsed jet-based elongation. (W. Liu et al., 2015) Void-based elongation is based on the fast evaporation of high volatile solvents with phase separation, where glassy skin and voids on the jet surface are formed, which continue with the elongation and solidification of tiny voids into grooved fibers. (W. Liu et al., 2015) Wrinkle-based elongation is similar at the start of forming glassy skin, however the wrinkled structures are formed due to buckling of a cylindrical polymer shell under compressive radial stresses. (Zaarour et al., 2020) Collapsed jet-based elongation is again similar at first, forming glassy skin, however the jet is collapsed due to slow evaporation rate and increased viscosity of HBPS, resulting in smooth grooved structures. (W. Liu, Huang, and Jin 2015, Bilal Zaarour, Zhu, and Jin 2020)) One study successfully produced grooved electrospun fibers from cellulose acetate butyrate (CAB) using void-based elongation that has found application in neural science. (C. Huang et al., 2011) The effect on hydrophilicity has also been studied with randomly oriented and aligned electrospun PLLA grooved fibers. (Liang et al., 2017) The results indicated that randomly oriented grooved fiber scaffolds made of PLLA had the highest hydrophilicity.

Wrinkled structures have shown increased specific surface area, enhanced mechanical properties and increased surface roughness making them ideal candidates for several applications like self-cleaning surfaces, energy harvesting and biomedical field. (C. Huang & Thomas, 2018; L. Wang et al., 2009; Zaarour, Zhu, Huang, et al., 2019)

2.3.2 Mechanical properties

Mechanical properties of a material play a huge role for the selection of suitable material for a particular application. Electrospun fiber scaffolds are known to have unique characteristics as mentioned before, however their superior mechanical properties are gaining interest, giving them even more potentials in various applications(Rashid et al., 2021), specifically in DDS (Khodadadi et al., 2020; Ramos et al., 2020; Rychter et al., 2018), tissue engineering (M. M. Islam et al., 2020; McCullen et al., 2009; Rahmati et al., 2021; Vasita & Katti, 2006) and wound dressings (F. Wang et al., 2020). In all these applications the electrospun fiber scaffold is affected by stresses from the surrounding environment that it needs to withstand in order to remain functional. The mechanical properties of the scaffold are related with the structure of the fibers, e.g. morphology, surface properties, diameter and fiber diameter which are modifiable as discussed before (*2.3 Morphology*). (Rashid et al., 2021)

Nanofibers (fiber with smaller diameter, below 1 μm) are known to have higher Young's modulus and tensile strength. (Papkov et al., 2013). In contrast, increased mechanical properties have also been seen with microfiber scaffolds. (Nair & Parameswaran, 2016; Xu et al., 2017) Controversial results may be

related with the combination of different properties (environmental vs processing vs material) which all affect the electrospinning and the obtained electrospun fiber's behaviour. Solvents or solvent systems are affecting scaffold's properties and consequently affecting the mechanical properties. (Demir et al., 2002; C. Liu et al., 2018; Z. Song et al., 2018) Polymer concentration is also known to affect the morphology of the fibers which also will affect the mechanical properties. As with all parameters there is a limit – for polystyrene solutions up to 40% solutions can be used in order to produce homogenous fibers. (Doshi & Reneker, 1995; Huan et al., 2015; Lasprilla-Botero et al., 2018; Zong et al., 2002) Polymer concentration is linked with the viscosity of the electrospinning solution. Increased concentrations together with higher viscosity causes molecular entanglement and increase in surface tension resulting in increased mechanical strength. (Rashid et al., 2021)

Fiber orientation is also known to be important parameter which affects the mechanical properties. Collector type is usually affecting the isotropic or anisotropic alignment of the fibers in scaffolds. (Kongkhlang, Tashiro, et al., 2008) The shear and elongation forces, when using rotational collector, influence the alignment of polymeric chains and improve crystalline orientation when fibers are formed. (Rashid et al., 2021) Another crucial factor influencing the mechanical properties of the fiber scaffold is the adhesion forces between the scaffold and the collector which might limit the practical application of the scaffold. Distance between the needle and collector (electrode gap) is influencing the fiber diameter. The electrode gap affects the behaviour of the electrospun jet, shorter gap increases electrical field strength and accelerates the speed of the electrospun jet resulting reduced solvent evaporation. The residual solvent results in merged fibers on the collector. (Angammana & Jayaram, 2016) Depending on the materials and conditions increasing the electrospinning distance may increase, decrease or have no impact on the fiber diameter. (X. Yuan et al., 2004) However, in most cases increasing the distance decreases the fiber diameter and consequently induce changes in mechanical properties. (Doustgani, 2016)

Two very important parameters affecting the mechanical properties are the thickness and porosity of the scaffold. (Rashid et al., 2021) The thickness of the samples is relevant when analysing the sample – uneven porosity and void spaces manipulate the obtained data. (Rashid et al., 2021) Therefore, ideally samples of similar thicknesses should be analyzed. One study has focused on the effect of PCL fiber scaffolds thickness and fiber orientation on the mechanical properties. (Mubyana et al., 2016) It was seen that peak stress decreased with thicker samples and tensile strength was higher with aligned fiber scaffolds.

In addition to the morphology and structure of fibers and fiber matrix, also process-related factors indirectly affect the mechanical properties of electrospun fiber scaffolds. Changes in process parameters during the production (electrical field, environmental parameters) of the scaffold can dramatically affect the mechanical properties. (Croisier et al., 2012a; Gu et al., 2005; Richard-Lacroix & Pellerin, 2013) Applied voltage can indirectly affect the mechanical properties of fibers. (Doshi & Reneker, 1995; Nair & Parameswaran, 2016; Zong et al., 2002)

The voltage affects the shape of the droplet that change the shape of formed fibers. (Rashid et al., 2021) One study concluded that the increased voltage causes the rapid evaporation of solution on the tip of needle that results in the oscillated shape of the Taylor cone and formation of beaded fibers – which decrease the mechanical properties of the fibers. (Zong et al., 2002) Environmental parameters are also indirectly affecting scaffolds mechanical properties. Humidity is known to decrease the vapor pressure that increases surface tension and inhibits sufficient solvent evaporation. Changes in temperature usually affect the viscosity of the solution, fiber to fiber adhesion within the scaffold, and chain orientation of fibers. All these factors are directly affecting the morphology of the scaffold and indirectly mechanical properties. (Rashid et al., 2021)

Until today, quite a lot of electrospun scaffolds made from different polymers and used for wound healing applications have been developed. **Table 4** summarises some of these scaffolds together with the actual formulation composition and their mechanical properties.

Clinically relevant wound scaffolds have the potential to be developed with desired mechanical properties similar to the native skin. Ideal scaffold for wounds should be highly elastic and flexible for the application and removal. (Gao et al., 2021) The mechanical properties are mainly presented by tensile strength, elongation at break and Young's modulus (describes the elasticity of the scaffold). (Memic et al., 2019) The tensile strength of native skin is about 20 MPa (Kennedy et al., 2017), Young's modulus from 0.008 MPa to 70 MPa (Pailler-Mattei et al., 2008; Shevchenko et al., 2010) and elongation at break from 35% to 115%. (S. Chen, Liu, et al., 2017) There have been several studies that have measured the mechanical properties of native skin however the values (in some cases) vary quite a lot. It is desired to obtain fibers with the mechanical properties mimicking native ECM for signalling, transportation and anchoring of the cells which affects the wound healing process. (Gao et al., 2021)

The mechanical properties of the fiber scaffolds are usually analysed using scaffolds (entire fiber matrix), however single fiber mechanical properties can be also studied, but not using conventional methods. (Croisier et al., 2012a; Gu et al., 2005) The production of single fiber for mechanical analyses (e.g. modelling and predictions for the entire matrix behaviour) as well as the analyses itself are challenging. (Rashid et al. 2021; Tan et al. 2005)

Even for scaffold the choice of the mechanical properties measurement technique is crucial as it affects the results almost as much as the process parameters for the fiber production. (Jeong et al., 2007)

Table 4. List of mechanical properties of electrospun polymeric scaffolds developed for wound healing applications (Rashid et al., 2021)

Polymer (s)	Solvent (s)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	Reference
Cellulose acetate	DMAc ACE	12.1	1170	1.31	(C. Sun et al., 2015)
Collagen	HFIP TFA	4.5	205	12	(Z. Chen et al., 2009)
Chitosan		0.5	10	8	
Chitosan/Collagen (20:80)		1.7	30	78	
Chitosan/PEO	AA H_2O	8.94	1.5	15	(Kohsari et al., 2016)
Gelatin	HFIP	1.6	0.490	17	(J. Lee et al., 2008)
PCL		4.7	0.0084	960	
PCL/Gelatin (50:50)		5.1	0.340	86	
Nylon 6	FA	10.45	19.4	250	(Bazbouz & Stylios, 2010)
PAN	DMF	45.7	1800	10.7	(Hou et al., 2005)
PAN/ 5% multiwall carbon nanotubes		80	3100	2.5	
PVA	H_2O	5.8	175	102	(Jeong et al., 2007)
PVA/ 1% multiwall carbon nanotubes		9.3	178	133	
PVC	THF DMF	2.2	12.3	90	(Carrizales et al., 2008)
PVDF	ACE DMF	3.7	168.9	NA	(C. Liu et al., 2018)
		5.8	184.3	NA	
PVDF/HFP	DMAc ACE	10	10	NA	(Kimura et al., 2014)
PET	TFA DCM	3.7	60	NA	(Veleirinho et al., 2008)
PCL	THF DMF	4.5	≈3.8	≈170	(Croisier et al., 2012a)
PLA (random)	DMF CF	3.9	43.8	87.6	(Huan et al., 2018)
PLA (aligned)		4.5	62.7	27.4	
PMMA	THF DMF	0.3	12.9	40	(Carrizales et al., 2008)
PVP	DMF	2.3	NA	9.1	(S. Huang et al., 2016)
PVP / 4% CNC		3.1	NA	3.25	
PVP / 4% CNC		2.81		2.5	

Key: AA – acetic acid; CF – chloroform; DMAc – dimethylacetamide, DMF – dimethylformamide; DCM – dichloromethane ACE – acetone; HFIP – 1,1,1,3,3,3 – hexafluoroisopropanol; TFA – trifluoroacetic acid; THF – tetrahydrofuran; FA – formic acid.

2.3.3 Solid state properties, drug release and stability of DDS

When developing electrospun scaffolds as DDS the solid-state properties of the carrier polymer(s) and API(s) are crucial since these may affect their performance. Polymer crystallinity determines the degradability, water and drug release properties of the scaffold. (Natu et al., 2010) The physicochemical properties (stability, solubility, dissolution rate, and bioavailability) are key factors affecting the morphology (crystalline, semi-crystalline and amorphous) of the API. It is known that amorphous forms of the API exhibit higher apparent aqueous solubility and dissolution rate compared to crystalline forms. (Martínez-Pérez, 2020) Furthermore, this is known to affect the drug release properties – crystalline forms of APIs mainly deposit outside the fibers causing the burst release of the drug and amorphous form of API is deposited within the fibers offering sustained release. (Natu et al., 2010; J. Xie & Wang, 2006) It is quite often seen that during electrospinning the rapid solvent evaporation results in solid state phase transformation of the API from crystalline to amorphous form. (Natu et al., 2010; Preem et al., 2017; Zamani et al., 2010) These phenomena could be beneficial to poorly water-soluble drugs however the physical stability of the APIs needs to be thoroughly studied due to possible recrystallization during storage. (D.-G. Yu et al., 2009) The drug release from the electrospun DDS is affected by the scaffolds thickness and morphology (Okuda et al., 2010), fiber diameter and porosity (Cui et al., 2006), scaffold composition (carrier polymer) (Buschle-Diller et al., 2007), polymer crystallinity (Kenawy et al., 2002), scaffolds hydrophilicity, drug loading (Cui et al., 2006; Z. Xie & Buschle-Diller, 2010), drug morphology (crystallinity) (J. Xie & Wang, 2006; Zamani et al., 2010), drug molecular weight (Buschle-Diller et al., 2007; Taepaiboon et al., 2006), drug solubility in dissolution media (Z. Xie & Buschle-Diller, 2010), interactions between carrier polymer, polymer solution (solvents) and drug (Chew et al., 2005; Zeng et al., 2005).

Drug loading influences the release rate of the drug – usually higher drug loadings show faster and higher burst release (Cui et al., 2006; Z. Xie & Buschle-Diller, 2010; Zamani et al., 2010), however rapidly dissolving drug increases cavitation and porosity which could alter the biorelevant activity of the scaffold. (Cui et al., 2006) For example, antibacterial DDS should have sufficient antibacterial activity (burst release) to inhibit the proliferation of the bacteria at the site followed by sustained release which is above MIC. (Kaiser et al., 2021) However, when only burst release occurs then the scaffold could become a substrate for bacterial growth and proliferation as electrospun scaffolds are known to be good substrates for bacteria. (Abrigo et al., 2015b) Another especially crucial factor affecting drug release are the interactions between the polymeric solution and the drug. Materials with similar polarity form a homogenous mixture which improves the drug incorporation within electrospun DDS. Therefore, lipophilic polymers should be used for lipophilic drugs to inhibit drug deposition on the fibers which is in correlation with burst release. (Zeng et al., 2005) Polymer-drug interactions are also relevant as the recrystallization of the drug within the scaffold is altered. (D.-G. Yu et al., 2009) Furthermore, the release rate from the scaffolds can be

controlled even with drugs in crystalline forms in correlation with the polymeric-drug interactions. (Taepaiboon et al., 2006)

The stability of drug substances in electrospun DDS is often recognized as one of their key advantages for drug delivery and storage. (Haider et al., 2018) As already mentioned above the stability of the DDS is influenced by several properties related to its composition. When talking about the shelf-life of electrospun DDS then the carrier polymer(s) degradation is usually the limiting factor. (Natu et al., 2010) Scaffolds made from synthetic polymers (or combinations) are mostly stable and degradation properties well documented. (Cui et al., 2008; M. Liu et al., 2019; Woodruff & Hutmacher, 2010) However, in some cases, the scaffold needs to be pretreated (sterilized) prior application, which can cause premature degradation of the API as well as the scaffold itself. Preem et al. studied the effect of sterilisation (using different methods) on model drug (CAM) and the scaffold. (Preem, Vaarmets, et al., 2019) The study concluded that depending on the hydrophilicity and morphology of the scaffold the sterilisation methods had an impact on the stability of the drug and carrier polymer – causing premature degradation. Therefore, regarding the application (and possible pretreatment needed prior use) of electrospun DDS the stability issues also need to be addressed in the development stage.

2.4 Electrospun scaffolds as wound dressings

There are unique characteristics of nanofibers, such as high surface area to volume ratio, sufficient mechanical stability and highly porous structure. (Andreu et al., 2015) In wound dressing perspective, high porosity offers enhanced gas exchange, moisture control and nutrient exchange at the wound site, high surface area contributes to the cellular attachment, proliferation and differentiation in tissue regeneration and the mechanical properties offer similarities to the natural ECM. (Andreu et al., 2015)

One of the key factors in wound healing is the cellular migration to the wound site. (Qin et al., 2015) Major parameters of electrospun scaffolds that are known to help to improve the wound healing by promoting the cell migration and growth are the 1) morphology of the fibers and fiber scaffolds, 2) 3D structure and orientation/alignment of the fibers, 3) and the material of fibers (chemical properties of carrier polymers and added active substances). The morphology of the scaffolds could be designed with similar structures as the collagen fibrils of the native skin. (L. Sun et al., 2018) Crossed nanofibers have shown improved cellular migration of fibroblasts and keratinocytes *in vivo* compared to randomly or uniaxially aligned fibers. (L. Sun et al., 2018) Cotton wool like fluffy structured scaffold prepared from chitosan/PCL using emulsion electrospinning have shown positive impact on infiltration and growth of fibroblasts and keratinocytes *in vitro* (Pal, Srivas, et al., 2017) The regeneration of dermal ECM is largely dependent on the fibroblast infiltration and specific sandwich type scaffolds have been designed in order to improve or aid the infiltration rate. (B. Ma et al., 2014) A

combination of aligned nanofibers at the bottom, a micro skin tissue in between and square arrayed nanofiber microwells at the top has been designed as a 3D scaffold in order to be used as a wound dressing. (B. Ma et al., 2014; J. Xue et al., 2019) 3D scaffolds from silk fibroin nanoparticle loaded PCL nanofibers have also been developed that have shown improved native cellular infiltration using rat *in vivo* model and have shown comparable efficacy to the commercial Matriderm® artificial dermis. (J. M. Lee et al., 2016) Another approach is the immobilization of biological cues to scaffolds that have shown improved healing and tissue regeneration in the wound. (J. Xue et al., 2019) Various compounds like proteins, growth factors, genes, have also been incorporated in nanofibers. (Norouzi et al., 2018; Z. Xie et al., 2013) For example, previously mentioned porous wool like scaffolds (Pal, Srivas, et al., 2017) with collagen coating have shown enhanced healing properties with burn wounds in a rat model compared to a commercially used Tegaderm® dressing. (Pal, Dadhich, et al., 2017) The release rate of growth factors can also be manipulated from nanofiber-based scaffolds. (J. Xue et al., 2019) Collagen and hyaluronic acid fiber scaffolds have been designed in order to understand the chronic wound healing in diabetic rats. (Lai et al., 2014) It was seen that initial delivery of fibroblast and epidermal growth factors stimulated the early stages of wound healing and slow release of vascular endothelial and platelet-derived growth factors aided the late stages of skin reconstruction. (J. Xue et al., 2019)

Every wound has a source of contamination, so specific antibacterial wound dressings could inhibit the infection development or treat the cause of infection. Antimicrobial drug delivery is a really important research topic due to the fast development of antibiotic resistance that is estimated to cause over 50 million deaths by 2050. (Neill, 2014) Novel antibiotic delivery systems are needed in order to enhance the efficiency of antibiotic treatment and minimize the risks of toxicity and the development of bacterial resistance. There has been an increased interest for the last 10 years on studying electrospun fiber scaffolds as potential antimicrobial DDS due to their suitable properties. For example, one group developed gentamycin loaded electrospun PCL scaffold to be used as antibiofilm matrices after surgery, but these were not as successful as hoped regarding for their actual activity. (Pisani et al., 2019) Electrospun fibers have also been studied as mucoadhesive oral DDS. Vancomycin loaded chitosan/gelatin fibers were developed potentially offering better absorption and bioavailability, predictable release and avoidance of hepatic first-pass mechanism. (Behbood et al., 2017) However, the study failed to give sufficient biological data in order to evaluate the possible action and effect of the developed mucoadhesive DDS. Electrospun fibers have also been studied as potential antimicrobial medical devices that can limit the possibilities of post-operative infection. One group prepared fibers with co-axial electrospinning which consisted of gentamycin/pluronic F127 in the core and silver/PCL in the sheath as a suture for possible DDS. (S. Chen, Ge, et al., 2017) The study concluded that the scaffolds showed synergistic effects compared to sutures loaded with silver or gentamycin alone. Electrospun fiber scaffolds as DDS have also been studied in orthopaedics (implantation). One group developed

PCL fibers with polydopamine coating which allowed further functionalization of fibers with metronidazole. (Shi et al., 2019) The drug release was controlled by cleavage of the ester bond by cholesterol esterase correlated to the severity of the infection. In another study commonly used antibiotics (amoxicillin, tetracycline, ciprofloxacin, levofloxacin, moxifloxacin, cefazolin, fusidic acid etc) were incorporated into electrospun nanofibers. (E. J. Lee et al., 2017; Y. Lu et al., 2016) Furthermore, electrospun scaffolds incorporated with a combination of antimicrobial and antifungal agents have shown positive effects. (Dhand et al., 2017) In addition, silver nanoparticle loaded (Mofidfar et al., 2019) and surface modified silver nanoparticle loaded fiber scaffolds have shown long lasting antibacterial activity against multidrug-resistant bacteria. (E. J. Lee et al., 2017; Mofidfar et al., 2019; X. Yang et al., 2017)

Electrospun fiber scaffolds have also been used in order to control the scar formation which is usually caused by abnormal fibroblast proliferation and collagen deposition. (E. J. Lee et al., 2017) With hypertrophic scarring several factors can be modulated, like inhibition of transforming growth factor-β1 signalling, that can prevent scar formation. (L. Wang et al., 2017) Additionally, ginsenoside-Rg3 loaded fibers with surface immobilized fibroblast growth factors have shown to promote early stage wound healing and inhibit late-stage hypertrophic scarring *in vivo*. (L. Cheng et al., 2016)

There are studies that have investigated nanoparticle loaded nanofiber scaffolds to treat chronic diabetic wounds. (L. Yu et al., 2019) For example, SiO_2 nanoparticle loaded PCL/gelatin fiber scaffolds have shown to be effective improving angiogenesis, collagen deposition, re-epithelialization and inhibiting inflammation at the wound site in a diabetic mouse model. (Lv et al., 2017) SiO_2 nanoparticle loaded DDS have also been successfully incorporated into nanofiber scaffolds that have shown improved neo-vascularization, re-epithelialization, collagen formation and inhibited inflammatory response with diabetic wounds. (Ren et al., 2018)

In recent years, electrospun scaffolds have also been studied in the antitumor field. Scaffolds for localized skin tumor therapy in combination with skin tissue regeneration have shown promising results avoiding recurrence as well as treatment of tumor induced wounds. (J. Xue et al., 2019) In one study, Cu_2S nanoparticles were incorporated in PLA/PCL nanofiber scaffolds and near-infrared irradiation caused effective mortality of skin tumor cells as well as inhibition of tumor growth, whereas the scaffold promoted wound healing of skin defects by improving proliferation, migration of skin cells and angiogenesis during wound healing. (X. Wang et al., 2017)

2.4.1 Cytotoxicity, cell attachment and biocompatibility

In order to develop electrospun scaffolds as DDS their clinical safety needs to be addressed. The scaffold, its components (polymers, solvents, APIs) must be safe, biocompatible and non-cytotoxic. There is a lack of standardized assays to evaluate the safety of electrospun DDS products. (Repanas et al., 2016a) Several

methods have been used *in vitro* such as cell viability testing (Live/dead cell kit) (R. Xue et al., 2017), cellular metabolism activity testing using MTS or MTT assay, WST-1 assay (Molnar et al., 2020; Pezeshki-Modaress et al., 2018; Sadeghi et al., 2016a), visual inspection of cellular morphology (Shahhosseininia et al., 2018). However, the problem is that these experiments are very dependent on the cell lines used (Sadeghi et al., 2016a; R. Xue et al., 2017) and subjective in some cases (interpretation of the cellular morphological studies depends largely on the operator). Therefore, the safety needs to be thoroughly addressed by combining different methods and using several cell lines before any conclusions can be made. (Repanas et al., 2016b)

Mammalian cells, usually 10–100 μm in diameter, are known to be constantly sensitive to their surroundings. Typical cell outer membrane consists of various receptor systems that are activated by interacting with surrounded cells, by the ligands in the ECM and the excreted signalling molecules. (S. M. M. & H., 2005) Numerous proteins have a role in the stimulation of the cellular receptors that determines the response type and rate that is important for adequate tissue development and functionalization. (Aumailley & Gayraud, 1998; Gullberg & Ekblom, 1995; Wallner et al., 1998; Zagris, 2001) Furthermore, the ECM plays an important role in the regulation of growth factors signalling by releasing and activating them as needed. (Loh & Choong, 2013) Specific amino acid sequences are characterized within receptors as motifs that target and bind to specific cellular receptors. The most characterised receptors are transmembrane integrin receptors, recognized motifs as Arg-Gly-Asp (RGD), that were found in ECM proteins – fibronectin and vitronectin. (Bökel & Brown, 2002) These receptors bind cell cytoskeleton to the fibers in ECM and form local focal adhesions. (S. M. M. & H., 2005) When activated, a cascade of intracellular signalling pathways occur that affect gene expression and most aspects of cellular behaviour – differentiation, proliferation, expression of ECM proteins, activation of growth factors, and the prevention of apoptosis. (Giancotti & Ruoslahti, 1999) Cellular membrane receptors usually form a multicomponent system (several receptors are involved) that expand the signalling pathways. (Plopper et al., 1995; Tran et al., 2004)

Designing new scaffolds to control or promote cellular behaviour in the wound needs to be thoroughly investigated. In order to regenerate tissue, the host cells need to be originated from the native tissue. (S. M. M. & H., 2005) One parameter of electrospun fiber scaffolds, which is known to affect the cell behaviour, is the mechanical properties of the designed scaffold. For example scaffold engineered to be used in bone tissue needs to have higher mechanical strength (L. & M., 2002) and elasticity is needed in scaffolds used for vascular tissue regeneration. (Y. Wang et al., 2002) Scaffolds with optimal fiber organization, diameter and porosity is needed to aid wound healing. Studies have shown enhanced wound healing with crossed nanofiber scaffolds compared to aligned and random nanofibers. (L. Sun et al., 2018) The cellular activity can also be controlled by modifying the scaffold's porosity and fiber diameter that is illustrated in **Figure 5.** (S. M. M. & H., 2005)

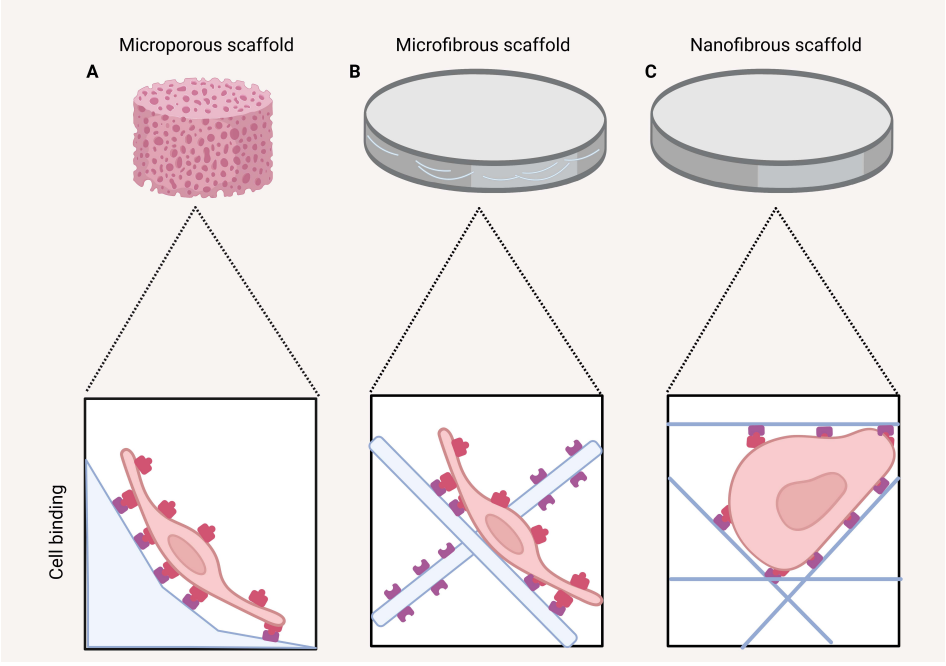


Figure 5. Scaffold architecture affects cell binding and spreading. (**A** and **B**) Cells binding to scaffolds with microscale architectures that changes cellular morphology while spreading on flat surfaces (**C**) Scaffolds with nanoscale architectures have larger surface areas to adsorb proteins, presenting many more binding sites to cell membrane receptors. The adsorbed proteins may also change conformation, exposing additional cryptic binding sites (S. M. M. & H., 2005) Created with BioRender.com

Ideal scaffold regarding cellular response has nanoscale morphology and consists of biopolymers derived from nature for example collagen and elastin due to their structure and suitable binding sites with ECM. (S. M. M. & H., 2005) However as mentioned before the limiting factor for the electrospinning of natural polymers is often their poor electrospinnability, and their accelerated degradation in aqueous environment and poor mechanical properties. For that reason, synthetic or hybrid (synthetic/natural) polymers have been used, for example PCL/chitosan hybrid for nanofiber scaffold production via electrospinning. (H. Chen, Fan, et al., 2011; Hong & Kim, 2011; Jhala et al., 2019) One study evaluated the nanofiber PCL/chitosan scaffolds performance on C3H10T1/2 ECM production and proliferation. (Jhala et al., 2019) They found out that nanofiber scaffolds exhibited lower initial cell number compared to tissue culture polystyrene (TCPS), followed by steady increase up to 15 days which was higher than on TCPS. The cell cycle analysis suggested that after 7 days more cells were in proliferation phase compared to TCPS. Furthermore, the ECM production (fibronectin and collagen IV) was higher on nanofiber scaffold at 7 and 14 days compared than TCPS. These phenomena were related to the enhanced cellular adherence on the scaffold which boosts ECM production. Moreover, the nanofiber scaffold's performance on

cellular activity was confirmed with nanoparticle internalization, as it was 25 times higher on nanofiber scaffold in comparison to TCPS, indicating enhanced ECM production and proliferation phases in cell cycle.

Particularly important property of the electrospun scaffold in the biomedical field is its biocompatibility. Biocompatibility by definition is the materials ability to function as desired, inducing adequate host response without the rish of injury, toxicity and immunorejection.(Ghasemi-Mobarakeh et al., 2019) Regarding wound healing applications low biocompatibility means restricted wound healing properties and problems with cellular toxicity caused by the scaffold. (Gaharwar et al., 2014; Ninan et al., 2015; Parani et al., 2016; X. Wang et al., 2018) There have been a lot of studies that have shown the biocompatibility of electrospun fiber scaffolds made from varied materials. For example, nanofiber scaffold that consisted of type I collagen showed enhanced proliferation, attachment and growth of human epidermal keratinocytes and *in vivo* studies revealed improved wound healing properties at an early stage of wound healing. (Rho et al., 2006) Another study focused on the biocompatibility of silk fibroin fiber scaffold and showed positive results on the proliferation of primary human fibroblasts and improved healing properties in *ex-vivo* human skin wound model. (Hodgkinson et al., 2014) Another study focused on collagen nanofiber scaffold and found enhanced human dermal fibroblast elongation with focal adhesion clustering which resulted in improved cell migration and differentiation rate to myoblasts. (C. Huang et al., 2012) Fibrinogen nanofiber scaffolds have also shown higher fibroblast migration and expression of differentiated phenotype α-smooth muscle actin. (Shalaby et al., 2022) Synthetic polymers have also been used in the production of scaffolds for wound healing applications, amongst all different polymers the most popular is PCL. PCL based scaffolds are known to have superior mechanical properties, tunable hydrophobicity, and sufficient degradation properties. (T. K. Dash & Konkimalla, 2012) For example, PCL/collagen hybrid scaffold loaded with growth factor (e.g., TGFβ1) has shown to promote the differentiation of myofibroblasts which is very important in native wound healing process. (W. Cheng et al., 2016) PCL scaffolds have also shown to aid differentiation of human hair-follicle derived and normal human epidermal melanocytes and rat hair follicle stem-cells. (Savkovic et al., 2016; Yari et al., 2016) Natural polymer based electrospun nanofiber scaffolds have also shown many positive aspects regarding wound healing and are concluded in **Table 5**.

Table 5. Natural polymer based electrospun scaffolds in wound healing (Shalaby et al., 2022)

Polymer (s)	Properties	Applications	Reference
Polymers of protein origin	**Collagen** – Nontoxic – Not mitogenic – Better cellular response – Malleable – Bioresorbable – Highly compatible – Combinations with other copolymers ***Disadvantages*** – ↑ purification cost – Difficult work involved – Risk of disease transmission	Suitable for nanofiber scaffold	(Coelho, 2020; Ghasemi-Mobarakeh et al., 2008)
	Silk Fibroin – Biocompatible and biodegradable – Low inflammatory reactions – Adequate oxygen WVTR	Tissue engineering Drug delivery	(Shen et al., 2010; Wenk et al., 2011)
Algae poly-saccharides	– Biodegradable and affordable – Versatile and abundant – Tissue-compatible – Immunomodulatory – Antioxidant – Anticoagulant and antitumour – ↑ wound closure ***Disadvantages*** – Limited electrospinnability	Wound dressings Skin regeneration	(Hao et al., 2020; Rashtchian et al., 2020; Shalumon et al., 2011)
Plant poly-saccharides	**Starch** – Abundant – Physically or chemically amended to be utilised in wound dressings – **Electrospun starch-hyaluronic acid/polyurethane-based electrospun nanofiber patch**: ✔ biocompatible ✔ biodegradable ✔ confers surface hydrophilicity ✔ improves the mechanical durability	Wound healing Drug delivery	(Adeli et al., 2019; Movahedi et al., 2020)
	α-cellulose nanofibers – Antibiotic-loaded α-cellulose nanofibers diminish the wound size	Wound healing	(Yazdanbakhsh et al., 2018)
	Pectins – Rapid exudate absorption – Moderate hydrophilicity – Antibacterial	Wound healing Drug development Tissue engineering	(Augustine et al., 2016; S. Chen, Cui, et al., 2017)
	Zein – Biodegradable – Amphiphilic and able to self-assemble – Antioxidant and antimicrobial properties	Drug delivery Tissue engineering Coatings in food industry	(Paliwal & Palakurthi, 2014; Yao et al., 2007)

Polymer (s)	Properties	Applications	Reference
	Disadvantages – poor electrospinnability – low water stability – poor mechanical properties for the electrospun scaffolds		
Animal poly-saccharides	**Chitosan** – Antioxidant and anti-inflammatory – Antibacterial – Biocompatible – **Electrospun nanofiber scaffold with chitosan-graft-PCL:** ✔ excellent cell attachment and proliferation ***Disadvantages*** – Pristine chitosan fibers have limited biomedical applications because of its poor mechanical properties	Wound healing Drug development Tissue engineering	(Alavarse et al., 2017; H. Chen, Huang, et al., 2011; Lopes-da-Silva et al., 2009; Veleirinho et al., 2013; M. Wang et al., 2016)
	Hyaluronic acid – Nonimmunogenic – Biodegradable – Biocompatible – High wettability – High ability to be chemically modified	Wound healing Drug delivery Tissue engineering	(El-Aassar et al., 2020)
Fungal poly-saccharides	**Pullulan** – Nontoxic – Nonmutagenic – Tasteless – Odourless ***Disadvantages*** – The high hydrophilicity of pullulan hinders its tissue engineering applications, limiting cellular attachment and pro-liferation and preventing protein adsorption	Drug delivery Tissue engineering Wound healing	(Aguilar-Vázquez et al., 2018; Dalgic et al., 2022; M. S. Islam & Yeum, 2013; R. Li et al., 2017)
	Sonifilan (SPG) – Embrace molecular components, functional polymers, and nanoparticles to form water-soluble one-dimensional nanocomposites	Drug delivery	(Y. Zhang et al., 2013)
Bacterial poly-saccharides	**Dextran Gum** – Dextran crosslinking tailors its biodegradation stability and retains its mechanical features in moist conditions – **Electrospun dextran-boric acid:** – prepare a steady network of dextran-boric acid electrospun nanofibers with controlled degradation time	Wound healing	(Innocenti Malini et al., 2019)
	Xanthan Gum – Thermostable – Generates self-assembled micro or nanoscale structure	Regenerative medicine Tissue engineering Controlled drug delivery	(Faralli et al., 2019)

2.4.2 Bacterial attachment and biofilm formation

Polymeric fiber scaffolds are promising candidates for wound healing applications due to their functionality which can be combined (e.g., antibacterial, wound healing etc) (H. Li et al., 2021) It is important to understand the antibacterial efficacy of the electrospun fiber scaffolds using relevant pathogenic wound bacteria. (Adeli et al., 2019) According to the literature the most common clinically relevant wound pathogens are *S. aureus* (Alavarse et al., 2017) and *E. coli* (mostly related to burn wound infections). (Abdelgawad et al., 2014) However, the bacterial-scaffold interaction is regularly overlooked, but known to be a key factor to affect the behaviour of electrospun fiber wound scaffolds. As with eukaryotic cells, bacteria are also known to attach on substrates which is influenced by morphology, mechanical properties and the composition. (Kargar et al., 2012; Mitik-Dineva et al., 2006, 2009) As for wound healing, the designed scaffold should prevent bacterial colonisation or biofilm formation as it limits the wounds to heal. Fiber diameter and morphology is known to influence bacterial viability. For example, with polystyrene(PS) fiber scaffold it has been shown that highest bacterial proliferation was observed with an average fiber diameter close to the bacterial size which offered the best support for bacterial adhesion and spreading. (Abrigo et al., 2015a) Rod shaped elongated cells (*E. coli* and *P. aeruginosa*) tended to wrap onto fibers with diameters smaller than bacteria which limited the colony formation, however round *S. aureus* showed high proliferation throughout the nanofiber scaffold. PCL and PLA based scaffolds have also shown to be a good substrate for biofilm forming bacteria like *E. coli*, *P. putida*, *B. diminuta* and *S. fulinginis.* (Tamayo-Ramos et al., 2018) There are reports about polyamide (PA) based scaffolds as substrate for bacterial growth and biofilm formation. (Lencova et al., 2021) They confirmed the previous statement that biofilm tends to grow preferably on thicker (microscale) and non-homogenous regions of the fibers. This was thought to be caused by decreased porosity and sufficient air permeability of the scaffold which aided biofilm formation with *E. coli*, *S. aureus* and *S. epidermidis*. Another study was focused on *P. aeruginosa* adhesion and biofilm formation on PS based scaffolds. (Kargar et al., 2012) It was seen that with small diameter of fibers (91 ± 17 nm) the bacteria adhered in the crossed spacing mode of the substrate. With larger diameters adhesion density increased in the space between two fibers. They also concluded that bacterial adhesion is affected by the topology deformation which decreases the available binding sites for adhesion. Cellulose acetate scaffold has also been studied as a substrate for *L. plantarum* biofilm formation. (Hu et al., 2019) It was observed that biofilm formation was observed after 12 h of incubation, but after 24 h the nanofibers in the scaffolds were no longer visible in SEM micrographs. This was also confirmed with CFU/g measurements where rapid increase of bacterial density was seen after 16 h of culturing followed by decreased bacterial growth due to nutrient deficiency. It was also seen that stacking 3 membranes on top of each other increased biofilm formation and higher number of stacked membranes had a negative impact by limited diffusion

of nutrients through the membranes. It was also interesting that the *L. plantarum* biofilm was interlocked within the nanofiber scaffold so in order to remove the biofilm from scaffolds, combined detachment protocol was used (ultrasonication and enzymatic degradation). So to conclude – when designing scaffolds for wound healing application and wound infection treatment, the scaffold should have sufficient antibacterial and antibiofilm properties as well as offering suitable substrate for aided native wound healing process rather than increase the risk of further contamination.

Summary of literature

In summary, non-healing wounds that need long term treatment are a major problem for the healthcare system. The most problematic are patients diagnosed with chronic diseases that has a negative impact on the native wound healing process. An important part in wound treatment strategy is the administration of antibacterial drugs. The main problems with antibacterial drugs for treating wounds (especially with long term treatment) are the toxicity and low drug concentrations at the wound site. For this reason, there is a demand for novel and site-specific drug delivery systems (DDS) which could have multiple functionalities (antibacterial properties, aided wound healing).

Electrospinning is a versatile method that is used to produce polymeric matrices with unique characteristics (high surface area, mechanical stability, porosity) and incorporated APIs – all in favour for novel DDS. The unique characteristics as well as drug release properties are tunable which could give the scaffold multiple functionalities. Regarding aided wound healing – the scaffold should have a suitable morphology and structure that would resemble the native ECM. The drug release properties from antibacterial DDS should reach the MIC as fast as possible followed by prolonged release above MIC. However, there are a lot of factors affecting the performance of the scaffold which need to be addressed in the development stage.

Electrospinning is largely dependent on the material, process and environmental parameters which all affect the performance of the developed scaffold. In biomedical field synthetic and hybrid (natural/synthetic) are preferred over natural polymers due to problems with stability and poor mechanical properties of the electrospun scaffold. The chosen materials should be non-toxic, biodegradable and biocompatible. Process and environmental parameters need to be optimized in order to achive scaffolds with desired morphology and structure.

When developing electrospun scaffold as DDS the polymer/solvent/drug interactions have to be characterized. The scaffold needs to have suitable morphology, stability and structure to have the desired drug release properties needed for wound healing. The chosen API has to be stable in the solution (polymeric solution) as well as the scaffold in order to have sufficient activity. In order to have multifunctionality the cellular/scaffold interactions have to be tested. The mechanical properties of the antibacterial scaffold are important 1) in order to ease application

and removal and 2) favour cellular adhesion and proliferation supporting wound healing. Furthermore, the scaffold should have sufficient antibacterial properties to inhibit bacterial growth at the wound site.

When developing antibacterial scaffolds as DDS the clinical safety also needs to be addressed. The problem is that due to the novelty of electrospun scaffolds there are not many standardized tests to evaluate their safety. There are various *in vitro* assays already in use however they seem to be very cell line specific. Therefore, the safety needs to be thoroughly investigated before any conclusions are made about the safety.

3 AIMS OF THE STUDY

The aim of the study was to develop novel antibacterial drug delivery systems for wound healing applications using electrospinning technology. Our focus was to study the aspects that are required in order to achieve surface porosity on the fibers as well as improving the electrospinnability of materials that is known to be challenging. Specific objectives of the study were to:

- design and develop antibacterial drug chloramphenicol loaded porous fibers from biodegradable polymer PCL with different solvent systems (I,II) and tetracycline loaded zein core-shell fibers (III)
- understand the effects of electrospinning (ES) process and environmental conditions (e.g., humidity) and the presence of CAM on the pore formation within the fibers (I)
- characterize morphological, physicochemical and mechanical properties the fiber wound scaffolds. (I,II)
- understand the structure/activity relationships and reveal how the mechanical properties and porosity of the scaffolds and surface porosity on fibers affects their interactions with the living bacterial and eukaryotic fibroblast cells (II)
- evaluate the antibacterial and antibiofilm activity of the fiber scaffold (II, III)
- assess the biocompatibility and safety of fiber scaffolds (II, III)

4 MATERIALS AND METHODS

A detailed explanation of the materials, preparation, methods can be found in the Materials and Methods sections of Publications I–III.

4.1 Materials

Polycaprolactone (PCL; Mn 80,000) was used for monoaxial electrospinning (I, II) and PEO (Mv ~900,000), stearic acid, SA (synthesis grade), and zein (19~22 kDa) were used for co-axial electrospinning (III) as carriers and active substance (biopolymer zein) for zein fiber formation. Antibacterial agent chloramphenicol (CAM) (PubChem CID: 5959) (I,II) and tetracycline hydrochloride (TET, ≥95% purity) (III) was used as a model drug. All solvents (acetone, chloroform, dichloromethane, dimethyl sulfoxide, ethanol, tetrahydrofuran, acetic acid, and formic acid) were obtained from Sigma-Aldrich Inc. (Darmstadt, Germany). Growth media and buffers used in this study were of reagent grade and were used as received without any further purification.

4.2 Methods

4.2.1 Preparation and characterisation of electrospinning solutions (I)

Different PCL solutions for electrospinning were prepared by varying the polymer concentration as well as the solvents/solvent systems (**Table 6**). Solutions were allowed to mix overnight by the aid of a magnetic stirrer. Drug (CAM) was added to the electrospinning solution after polymer (PCL) dissolution in solvent mixtures and was further stirred for 1 h before electrospinning. The amount of CAM was 4% w/w based on the dry weight of the solid material. The viscosity of the polymer solutions was measured with Anton Paar Physica MCR 101 (Anton Paar GmbH, Ostfildern, Germany) rotary rheometer. The viscosity of the solutions was measured in rotational shear test at controlled shear rates between 100 s^{-1} to 0 s^{-1}. Viscosity was measured at 25.0 ± 0.2 °C using triplicate measurements to confirm the reproducibility. In addition, the viscosity of two weeks aged PCL 15% w/V (THF:DMSO; 90:10 V/V%) and PCL 15% w/V (AA:FA; 75:25 V/V%) solutions (room temperature, RT (18–24 °C) under constant mixing) was measured in order to understand the possible viscosity changes during storage.

4.2.2 Electrospinning of the fiber scaffolds (I, II, III)

Electrospinning was performed using ESR200RD robotized electrospinning system (NanoNC, Seoul, Republic of Korea). Relative humidity (RH) within electrospinning chamber was varied using humidifier AEG LBF 7138 (EHT Haustechnik GmbH, Nürnberg, Germany) in order to achieve single fiber porosity. For co-axial electrospinning different concentrations of PEO/SA/Zein were used with electrospinning system Fluidnatek LE-50 (Bionicia, Valencia, Spain) at fixed 25 °C and 45% RH.

4.3 Morphology and structure of electrospun fibers (I, II, III)

4.3.1 Scanning electron microscopy (SEM) (I, II, III)

The morphology, diameter and surface topography of pristine PCL and CAM-loaded fibers were investigated using SEM (Zeiss EVO 15 MA, Germany). Randomly selected areas of the fiber scaffolds were mounted on aluminum stubs and magnetron-sputter coated with a 3-nm platinum layer in an argon atmosphere prior to microscopy. Mean fiber diameter was calculated (N=100) and presented together with standard deviation (SD). For co-axial samples they were coated and imaged with SEM TM3030 (Hitachi, Tokyo, Japan). Porosity was calculated using Eq.1:

$$Porosity = \left(1 - \frac{\rho f}{\rho m}\right) \cdot 100 \ (Equation\ 1)$$

where ρf is the apparent density of fiber scaffold and ρm is the bulk density of corresponding materials, hence PCL 1.145 g/cm3 and CAM 1.547 g/cm3 were used.

Surface porosity and specific surface area (S_{BET}) were determined using Accelerated Surface Area and Porosimetry System (ASAP) 2020 (Micromeritics, Norcross, GA, USA). The image analysis of SEM images was also performed to estimate single fiber pore diameters using ImageJ1.52e (National Institutes of Health, Bethesda, MD, USA). In addition, the fiber scaffolds covered with eukaryotic cells, were also visualised under SEM (cells were fixed as described in manuscript II).

4.3.2 Confocal fluorescent microscopy (CFM) (II)

CFM using LSM710 (Carl Zeiss, Munich, Germany) and Zen software (Zeiss) was used for the visualisation of fibroblast cells after the cell attachment and growth studies (as described in manuscript II). To evaluate the cell penetration through the fiber scaffolds orhto view and 3D microraphs were constructed by the z-stack

images from CFM using Zen software. The z-stack image consisted of 101 slices with the depth of 134.95 μm and the cell infiltration was measured using 3D measuring.

4.4 Solid state analysis (I)

4.4.1 X-ray diffraction (XRD)

The XRD patterns of the pure starting materials and electrospun fiber scaffold were obtained with the X-ray diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany). The XRD experiments were carried out in a symmetrical reflection mode (Bragg–Brentano geometry) with CuKa radiation (1.54 Å). The scattered intensities were measured with the LynxEye one-dimensional detector including 165 channels. The angular range was from 5° to 40° 2θ and the step size of 0.0198° 2θ.

4.4.2 Attenuated total reflection fourier transformed infrared (ATR-FTIR) spectroscopy

ATR-FTIR spectroscopy was performed on pure substances and electrospun fiber scaffold using an IR Prestige-21 spectrophotometer (Shimadzu Corp., Kyoto, Japan) and Specac Golden Gate Single Reflection ATR crystal (Specac Ltd., Orpington, UK). The spectra were collected between 600 and 4,000 cm^{-1}, each spectrum was the average of 60 scans. Spectra were collected using Shimadzu IRsolutions 1.5 software (Shimadzu Corp., Kyoto, Japan) and all spectra were normalized with OriginLab 6.1 software (OriginLab Corporation, Northampton, USA).

4.5 Mechanical analysis (II)

Mechanical behaviour of the dry and wet fiber scaffolds was studied by Brookfield CT3 Texture Analyzer (Middleboro, MA, USA) equipped with a 10 kg load cell. Tensile test method was used for analysis in-line with the ASTM D-638 and ISO10350:1993 mechanical testing guidelines. TexturePro CT software (AMTEK Brookfield, Middleboro, MA, USA) was used for data collection and analysis. Roller Cam accessory grips (TA-RCA; width of 25 mm) were used to fix the sample. All measurements were performed at ambient conditions (temperature of 22 ± 1 °C and RH of 20 ± 2). Each sample group comprised at least 3–5 specimens. The mean thickness of the fiber scaffolds varied from 0.09 up to 0.11 mm measured using Precision-Micrometer 533.501 (Scalamesszeuge, Dettingen, Germany) with a resolution of 0.01 mm. Young's modulus (MPa, linear region) and tensile strength (zero slope) were calculated from each corresponding stress–strain curve. The same TexturePro CT software was used to obtain the elongation at break (%) values.

4.6 Drug loading and release (I, III)

To determine the drug loading and its distribution within PCL electrospun fiber scaffold, high performance liquid chromatography (HPLC) analyses were performed using Shimadzu Prominence LC20 with PDA detector (wavelength at 275 nm) (Shimadzu Europa GmbH, Duisburg, Germany) and according to the official European Pharmacopeia method for a related substance CAM sodium succinate. The *in vitro* drug release testing of CAM from drug-loaded electrospun PCL fiber scaffold was carried out using 1 cm^2 samples ($N = 3$) cut from the scaffold. These were weighed and placed into 10 mL of 1 × PBS (pH 7.4) at 37 °C in 50 mL plastic tubes. The tubes were put into dissolution apparatus vessel (Dissolution system 2100, Distek Inc., NJ, USA) containing water and maintained at 37 °C. The tubes were rotated by the paddles at the speed of 100 rpm. Aliquots of 2 mL were removed and replaced with the same amount of buffer solution at set time points. The aliquots were analyzed using UV-spectrophotometer (Shimadzu UV-1800). Wavelength of maximum absorption ($\lambda = 278$ nm) was chosen for the drug release analysis.

4.7 Contact angle, swelling and early in vitro degradation (II)

In order to understand the hydrophilic/hydrophobic nature of the fiber scaffolds and their wettability behaviour, the contact angle between the fiber scaffolds (m = 11.5 ± 0.01 mg; size 2 × 2 cm) and biorelevant 1 × PBS (pH 7.4) was measured with the sessile drop method (OCA 15EC, DataPhysics Instruments GmbH, Filderstadt, Germany). A drop of 1 x PBS buffer solution (5 µL) was applied onto the fiber scaffolds. The contact angle measurements were taken at time-point 0 and 30 s after the liquid drop touched the surface of the fiber scaffold. This test was conducted at RT (22 ºC ± 0.3). The contact angle was analysed using the SCA20 software (DataPhysics Instruments GmbH, Filderstadt, Germany). Each sample was measured at least in triplicate. Co-axial scaffolds were also analyzed similarly with Drop Shape Analyzer (DSA100, Krüss, Hamburg, Germany).

For swelling index and weight loss measurements (early *in vitro* degradation) a set of 4 cm^2 square-shape samples (N = 3) were cut from the fiber scaffolds and weighed, then immersed into 10 mL of 1 × PBS solution at 37 °C for 24 h. After that, the samples were placed on a plastic Falcon Cell stainer sieves (mesh size 40 µm) (Fisher scientific, Thermo Fischer, USA) placed on a 50 mL Falcon tube to remove free surface solution and weighed. Swelling index and weight loss were calculated as reported previously. (Nazemi et al., 2014)

4.8 Biological evaluation of differently designed electrospun fiber scaffolds (II, III)

4.8.1 Bacterial strains and preparation of stocks (II,III)

Clinically relevant for wound infection facultative anaerobic and Gram-negative bacterial strains (*Escherichia coli* DSM No. 1103 (clinical isolate); *Pseudomonas aeruginosa* DSM No.: 1117 (, blood isolate) used in this study were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. Overnight culture was diluted 100 times in fresh LB medium (100 µL on bacteria in 10 mL medium) and grown at 37 °C aerobically to an exponential phase. At optical density of 0.8 (OD600), dimethylsulfoxide (DMSO) was added to a final concentration of 8%. 120 µL of suspension was added to PCR tubes and frozen in liquid nitrogen. The tubes were stored in –80 °C and used within 6 months.

4.8.2 Eukaryotic cells and culturing media (II, III)

Baby hamster kidney cells (BHK-21) were used for cell studies. Fibroblasts were grown in Glasgow Modified Essential Medium (GMEM) (500 mL) supplemented with 7.5% FBS, 11 mL 2% TPB, 10 mL 1 M HEPES, 100 µg/mL penicillin, and 100 µg/mL streptomycin. Cells were maintained at 37 °C in 5% CO_2 incubator.

4.8.3 Eukaryotic cell attachment and growth (II, III)

Fiber scaffolds were placed into 24-wellplates using cell crown inserts (Cell-Crown®, Scaffdex Oy, Finland), 500 µL of BHK-21 cell suspension (consisting approximately 10 000 cells) were seeded on the scaffolds and 700 µL of DMEM (Dulbecco's Modified Eagle Medium) with phenol red and serum was added. The number of cells was determined using trypan blue exclusion (Invitrogen, Thermo Fischer, USA). After 24 h incubation, 500 µL of medium was added. After 48 h the scaffolds were removed from the inserts, placed carefully into 1 × PBS, and transferred to 400 µL of DMEM (phenol red and serum free medium). Another 100 µL of DMEM was added and also 80 µL of MTS reagent (K300-500, Biovision, USA). The cells were incubated at 37 °C and 5% CO_2. After 45 min, the scaffolds were removed and obtained colored media were pipetted onto 96-wellplate and absorbances were measured using a plate-reader (Invitrogen, Thermo Fischer, USA) at 490 nm. The graphs show the MTS activity of cells. From the same experiment, cell attachment and proliferation was visualised using SEM and CFM as explained under the paragraph: *4.3 Morphology and structure*. At minimum three technical replicates were performed.

4.8.4 Agar diffusion assay (III)

Samples were cut and sterilized and bacterial susceptibility measured using an agar diffusion method described previously. (Zupančič et al., 2018) *S. aureus* and *E. coli* were selected as model Gram-positive and Gram-negative bacteria, respectively. Briefly, overnight bacterial cultures in LB (grown from DMSO stocks) were seeded at approximately 10^6 colony forming units (CFU per plate after which samples of 6 mm diameter circles were placed on top together with controls and allowed to incubate in aerobic conditions for 24 h at 37 °C. Inhibition zones were measured using ImageJ software (1.52a version). (Schneider et al., 2012) The test was conducted in triplicate.

4.8.5 Bacterial attachment, growth and bacterial biofilm formation (II)

Biofilm formation protocol was established based on the work of Brackman et al (Brackman et al., 2011) and already proved suitable in our previous study. (Preem et al., 2017) Overnight liquid culture of bacteria in LB was grown from DMSO stocks. The culture was diluted to about $5x10^7$ colony-forming units (CFU)/mL with DMEM supplemented with 10% heat-inactivated FBS. 1 mL of the bacterial dispersion was added to 1 cm^2 square-shaped samples in 24 well-plates. The well-plates were incubated at 37 °C for 24, 48 or 72 h. After that, the samples were rinsed twice with 1 × PBS and put into 1 mL of fresh 1 × PBS in 1.5 mL eppendorf tube. For disrupting the biofilm alternating vortexing (Vortex-Genie 2, Scientific Industries) and sonication (Bandelin Sonorex digital 10 P, operating at 20% of maximum power) was performed in 30 s cycles. Each cycle was repeated 6 times, as this was seen to provide the best compromise between biofilm disruption and bacterial viability. The CFUs were determined by making 10-times dilutions of the dispersion, plating these as 5 µL drops on LB agar plates and counting the CFUs at optimal dilutions after 18 h of incubation. Planktonic bacteria in each time point were always plated as controls in order to verify the growth of bacteria in each condition. A minimum of three technical replicates were performed.

4.9 Statistical analyses (I, II, III)

The results are expressed as an arithmetic mean ± SD. Statistical analysis was performed by applying one-way ANOVA and post hoc pairwise *t*-tests with MS Excel 365 software ($p < 0.05$). In case of multiple comparisons, Holm's method was used for adjusting *p*-values.

5 RESULTS AND DISCUSSION

5.1 Development of electrospun antibacterial fiber scaffolds as DDS

5.1.1 Discussion about general development flow with schematics

In the present work, the development of antibacterial electrospun fiber scaffolds for wound healing and infection treatment was performed using the development flow presented in **Figure 6**.

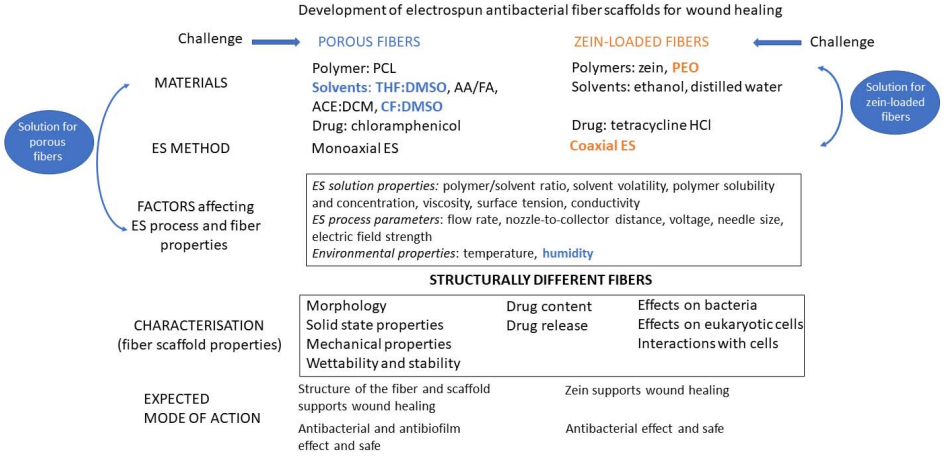


Figure 6. Schematics of the development of antibacterial electrospun fiber scaffolds as DDS for wound healing purposes.

In order to develop electrospun scaffolds as DDS several factors need to be addressed already in a preformulation phase. Firstly, the material selection is crucial for the desired application. The core polymer used for fiber scaffold preparation needs to be fully dissolved and stable in the solvent or solvent system. Furthermore, when designing antibacterial DDS for wound healing the chosen antibacterial drug has to fulfil the same criteria in addition to being clinically relevant. As mentioned before there are several parameters affecting ES process and fiber properties. With ES solutions the main parameters are: polymer/solvent composition (Abbasi et al., 2014; Luo et al., 2010), solvent volatility (Haider et al., 2018), polymer solubility and concentration (Luo et al., 2010), viscosity (J. Xue et al., 2019), surface tension (Haider et al., 2014), and conductivity (Angammana & Jayaram, 2011b). ES process parameters such as flow rate (Henriques et al., 2009), nozzle-to-collector distance (Ingavle & Leach, 2014), voltage (Sill & von Recum, 2008), needle size (Abunahel et al., 2018), electric field strength (Haider et al., 2018) also have an impact on the fiber properties. Furthermore, the environmental properties (temperature and relative humidity)

can affect ES process and scaffold properties. (De Vrieze et al., 2009; Katsogiannis et al., 2015b) The scaffolds need to be thoroughly characterised in order to understand the effect of ES process parameters on the scaffold's performance. The morphology, mechanical properties, wettability and stability have to be suitable for wound healing application. With antibacterial drug loaded scaffolds the antibacterial drug concentration in the scaffold as well as its release behaviour needs to be sufficient and suitable for desired antibacterial activity. In addition to the mentioned properties, the scaffold should be non-toxic and biocompatible.

5.2 Design and characterisation of electrospinning solutions (I, III)

The selection of polymer and solvent is crucial for electrospinning therefore, careful design of electrospinning (electrospinning solutions and electrospinning experiment parameters) needs to be conducted. In our studies we used hydrophobic polymers and hydrophilic/hydrophobic polymer combination as well as various solvents and solvent systems in order to prepare electrospun antibacterial DDS. To obtain antibacterial electrospun scaffolds, antibiotics were included into the formulation. In this case chloramphenicol (CAM) and tetracycline hydrochloride (TET) were selected which are antibiotics known to have severe side-effects and toxicity when used during systemic delivery but hold immense potential to be used for the local delivery. Both drugs are broad spectrum antibiotics belonging to protein synthesis inhibitors group. TET inhibits aminoacyl tRNA attachment to ribosomal site (Ian & Marilyn, 2001) and CAM bonds to bacterial ribosomal subunit (50S) directly inhibiting bacterial protein synthesis. (Ehrlich et al., 1947)

In order to produce porous fibers we used hydrophobic PCL as a carrier polymer which is widely used in various fields as it is biocompatible, biodegradable and nontoxic (T. K. Dash & Konkimalla, 2012; L. He et al., 2011). Indeed, the effect of solvent systems on PCL fiber morphology has been previously studied and these reports were carefully considered while designing the compositions for the formation of PCL fibers with high surface porosity.(Kanani & Bahrami, 2011; Katsogiannis et al., 2015a) Furthermore, we used CAM as an antibacterial drug to develop electrospun antibacterial DDS. CAM, with 4% (w/w) concentration based on the dry weight of the polymer, was chosen as model drug that we have also successfully used in previous studies. (Preem et al., 2017)

The electrospinning of amphiphilic zein is known to be challenging.(Selling et al., 2007; Yao et al., 2007) Zein is a non-toxic biodegradable hydrophobic biopolymer, a prolamine protein found in corn, which is classified amongst the safest biomaterial excipients by FDA. (Q. Li et al., 2011; Torres-Giner et al., 2008). Zein was selected as it is known to be biodegradable, biocompatible and enables to provide controlled release of drugs (Hawkins et al., 2008; Q. Jiang et al., 2010; H.-J. Wang et al., 2005; Zhong & Jin, 2009) Co-axial electrospinning was used in order to improve zein electrospinnability and avoid premature solvent evaporation

which are known problems for traditional electrospinning of zein. (Kanjanapongkul et al., 2010) As zein based electrospun scaffolds demonstrate weak mechanical properties, plasticizers were added polyethylene oxide (PEO) and stearic acid (SA) to the system. Furthermore, TET (5% w/w) was used as an antibacterial drug to produce zein based antibacterial electrospun DDS.

The viscosity of the polymer solution is one of the main factors in order to conduct successful electrospinning and prepare bead-free fibers. (Deitzel et al., 2001; Haider et al., 2014) The viscosity is related to the molecular chain entanglements of polymer molecules which needs to be optimal for electrospinning. When viscosity is too high, the stretching of the fibers (from the polymeric solution) by electrical charges is limited leading to unsuccessful electrospinning. When viscosity is too low the chain entanglements are insufficient causing breakups in electrically driven jets into droplets.(Zong et al., 2002) The viscosity of the solution depends on the polymer and solvent properties. In order to produce bead-free fibers the suitable polymer and solvent need to be chosen and optimized. These factors highly influence the fiber formation which affect the morphology, mechanical properties, wettability and biorelevance of the scaffold. When increasing shear rate the viscosity decreases. (Chhabra et al., 2008) Pseudoplastic flow lacks yield value so viscosity can be compared with rheogram profiles. All solvent systems selected (**Table 6**) were known to be suitable for PCL electrospinning based on their solubility, boiling point and dielectric constants. (Woodruff & Hutmacher, 2010)

Table 6. Polymer and solvent systems used for electrospinning.

Polymer (PCL) %, w/V	Solvent system (ratio % V/V)	Viscosity at near zero shear rate, Pa*s	Viscosity at $20 s^{-1}$ shear rate, Pa*s
15	CF:DMSO (90:10)	2.53	2.01
15	ACE:DCM (50:50)	2.12	1.65
15	THF:DMSO (90:10)	3.55	2.14
15	AA:FA (75:25)	2.57	1.18

Key: AA – acetic acid; ACE – acetone; CF – chloroform; DCM – dichloromethane; DMSO – dimethyl sulfoxide; FA – formic acid; PCL – polycaprolactone; THF– tetrahydrofurane.

Although all these solution parameters give information about system properties, further characterisation was performed using viscosity measurements and finding viscosity – electrospinnability correlations. The shear rates of chosen systems (PCL and solvent systems) were measured from $100\ s^{-1}$ to $0\ s^{-1}$ at 25.0 ± 0.2 °C. Similar polymer concentration in different solvent systems results in different viscosity (**Publication I, Figure 1**). Even though we were able to electrospin PCL in all solvents, AA:FA solvent system exhibited lower polymer solution viscosity compared to other solvent systems (more information in **Publication I**). CF:DMSO, ACE:DCM, and THF:DMSO polymer solutions had similar viscosities. Such large differences in viscosity values are known and nicely supported

by the literature hence was not unexpected.(Dobrzański et al., 2014) Increasing PCL concentration (12.5 vs 15% PCL in AA/FA) resulted in elevated viscosity. The addition of CAM increased the viscosity of the polymer solutions.

Aqueous ethanol (80% v/v) was used to prepare zein solutions (core) where 20% (w/V) of zein was added 2 h prior electrospinning to avoid degradation. PEO and SA containing zein solutions (core) were prepared by adding 1% (w/w compared to zein) and dissolved overnight. 5% TET (w/w compared to zein) was added 10 min prior electrospinning to the core solution. The shell solution consisted of either 80% ethanol or 1% (w/w compared to zein) PEO in 80% ethanol. The viscosity of zein formulations were not measured in the present study, but it is reasonable to assume that the addition of drug may change the viscosity of the solution and affect the electrospinnability and morphology of electrospun scaffolds.

The stability of PCL electrospinning solutions was studied with the chosen solvent systems (AA:FA, THF:DMSO) in order to design the experiment for large-scale manufacturing. It was seen that PCL 15% w/V with AA:FA solvent system exhibited decreased viscosity after few weeks of storage (2 weeks). PCL is known to undergo acidic hydrolysis with acetic acid which could increase its hydrolytic degradation.(Ekram et al., 2017) Interestingly PCL solution with THF:DMSO solvent system showed increased viscosity after storage, which was thought to be caused by the evaporation of the high volatile THF.

5.3 Electrospinning and characterisation of antibacterial fiber scaffolds (I, III)

Two different syringe-based electrospinning methods were used in the present study namely monoaxial and co-axial electrospinning. Monoaxial electrospinning is the most common needle-based electrospinning set-up (**Figure 7A**). This was successfully used in order to produce fibers with different morphology (porous vs non-porous fibers) from PCL polymer solutions (**Figure 7A**). The effect of different parameters (e.g. solvents, RH) on monoaxial ES were tested in order to obtain porous antibacterial drug-loaded fibers. However, for the electrospinning of zein-based wound matrices, modified ES set-up- co-axial electrospinning method, was applied. This method was used as the monoaxial electrospinning was unsuccessful to produce zein fibers. Co-axial method is often chosen when materials used for electrospinning might be unstable during monoaxial ES. Co-axial electrospinning set-up consists of concentric needle that can be used to produce core-shell structured fibers (**Figure 7B**). For successful co-axial ES of zein the key elements were the selected materials for ES in addition to zein (polymers and solvents). The flow rates and voltage depended on the optimized conditions of ES.

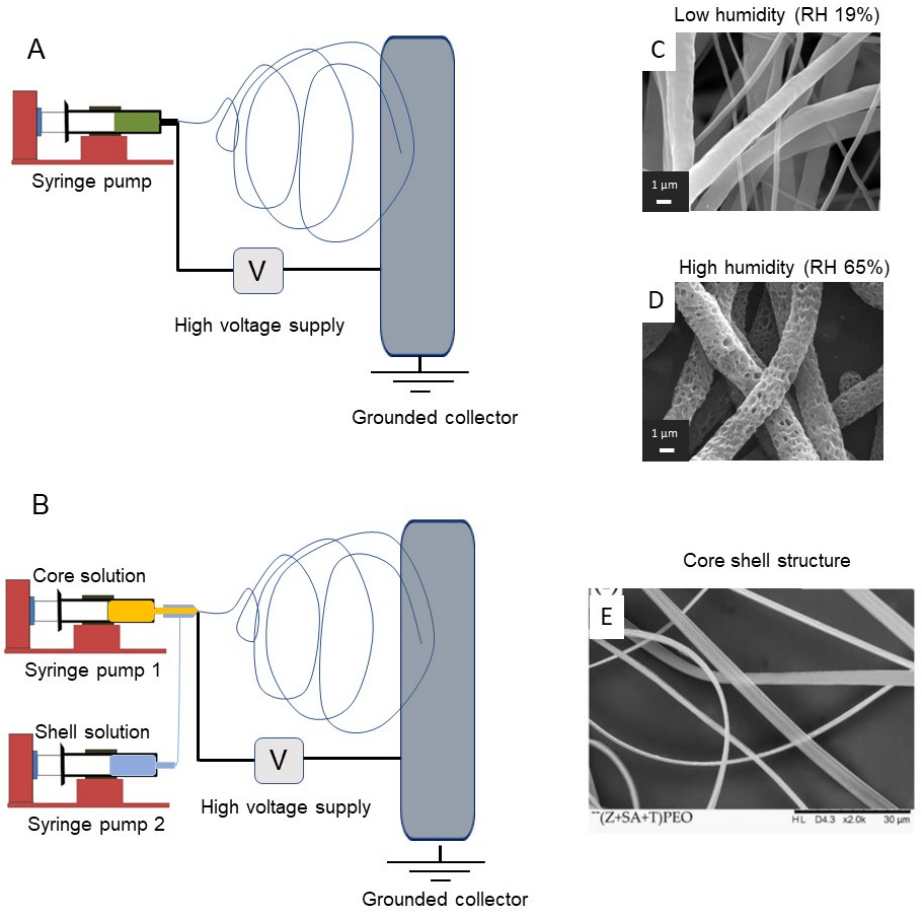


Figure 7A. Schematics of monoaxial electrospinning; **B:** co-axial electrospinning. Scanning electron microscopy (SEM) micrographs of; **C:** 15% PCL fibers with THF:DMSO solvent system without surface pores (low RH); **D:** 15% PCL fibers with THF:DMSO solvent system with surface pores (high RH). (magnification 10,000×). Mean fiber diameter and standard deviation values provided ($N = 70 – 100$). **E:** SEM image of core-shell zein microfibers consisting of core: 20% zein (Z) + 1% SA + 5% tetracycline (TET) and shell: 1% PEO in aqueous ethanol (1/4 v/v). (magnification 2,000×)

Key: DMSO – dimethyl sulfoxide; PCL – polycaprolactone; PEO – polyethylene oxide; SA – stearic acid; THF – tetrahydrofuran

5.4 Morphology of fibers (I, III)

The morphology of the fiber scaffold is one of the key properties regarding its performance and application. There are various parameters affecting the morphology of fibers and fiber scaffolds, one of them is the solvent used for producing electrospinning solutions. In our study we saw that the solvent system affected the fiber diameters as well as pore formation (**Figure 8**). Various polymer

concentrations and solvent systems were tested keeping the polymer the same (PCL) and in addition the effect of drug on pore formation was studied.

Surface porosity was achieved with specific solvent systems (THF:DMSO; CF:DMSO) only at elevated RH (high RH)(**Publication I, Figure 2**). Therefore, the effect of solvents on fiber morphology was tested at different RH-s and it was seen that the combination of the solvent/RH determines the morphology (e.g. presence of surface pores). The importance of humidity regarding surface porosity was confirmed with CF:DMSO and THF:DMSO solvent systems **(Publication I**, **Figure 2**). Evaporation rate of the solvents used was different which affected the fiber formation. Desired surface porosity was achieved with 15% PCL in THF: DMSO solvent system at high RH. Signs of surface porosity with the same composition was also seen with 30% RH, however it was not that evident and at low RH (19%) there were no signs of surface porosity (**Publication I, Figure 3**). Surface porosity was not achieved with 15% PCL in AA:FA solvent system regardless the humidity. Katsogiannis *et al.* have stated that surface porosity is achieved by using solvent systems where polymer has different solubility rates. (Katsogiannis et al., 2015b; Nasouri et al., 2015) The importance of humidity on surface porosity has also been discussed before.(Lubasova & Martinova, 2011) From the results obtained we decided to prepare three scaffolds with different morphology for further characterisation. For nanofiber scaffold preparation we choose AA:FA solvent system (non-porous nanofibers), microfiber scaffold without surface porosity THF:DMSO at low RH (non-porous microfibers) and microfiber scaffold with surface porosity at high RH (porous microfibers).

Surface porosity can be measured with different methods. One method we used was the measurement of pore area on SEM 2D images. In order to evaluate the overall porosity and pore size distribution of electrospun scaffolds mercury porosimetry and capillary flow porometry can be used. (Guo et al., 2018; Moradi et al., 2018; Pham et al., 2006) With electrospun scaffolds these methods are not ideal due to the inhomogeneity of pores. In our study we focused on the pore area measurements – specifically pore diameter measurements. More sophisticated pore size and distribution analysis would need the combination of various methods – CFM, segmentation analysis of the micrographs etc. (Choong et al., 2015; Guo et al., 2018; Hotaling et al., 2015) The mean pore diameter for porous microfiber's pore diameter was 0.28 μm (**Publication I, Table 2**). Furthermore, the fiber scaffold porosity was measured (**Publication I, Table 2**). There were signs of correlation with applied voltage and surface porosity, however it was not that evident. Electrospun fibers prepared with THF:DMSO solvent system had a mean diameter of approximately 1.22 ± 0.21 μm (**Figure 8**). AA:FA solvent system with PCL resulted in nanofibers with a mean diameter of 0.25 ± 0.11 μm regardless of the RH (high or low) (**Figure 6**). As discussed in the literature increased viscosity causes enlarged fiber diameters. Vapor pressure affects fiber formation and morphology of the fiber scaffold.(Lubasova & Martinova, 2011) Solvent evaporation rate is affected by the differences in vapor pressure. Increased humidity decreases the pressure difference between vapor pressure and saturated vapor pressure which limits the solvent evaporation rate. (Y. Yang et al., 2006)

Addition of drug changed the fibers morphology. With PCL fibers we observed that surface porosity changed when adding 4% (w/w) CAM. The drug content was confirmed with HPLC with some changes in nominal values (**Publication I; Table S2**). The fiber mean diameters increased with CAM loaded samples (**Figure 8**). This has previously been stated in the literature that the incorporation of APIs increases the PCL fiber diameters. (Arbade et al., 2018; Z. M. Huang et al., 2006) Although changes in morphology was seen with PCL fibers, surface porosity still remained with THF:DMSO solvent system. It was evident when analysing SEM micrographs that the surface porosity had decreased compared to porous microfibers without CAM. The mean pore diameter for porous fibers with CAM was approximately 0.22 µm, however no statistical differences were seen with pristine PCL fibers (**Publication I, Table 2**).

Co-axially prepared zein fibers were in microscale and either ribbon or tubular shaped **(Figure 5B**, **Publication III, Table 2**). The shape was largely dependent on the PEO content. When PEO was present in shell, the fibers exhibited both shapes ribbon and tubular, accordingly. PEO in core and shell resulted in tubular shaped fibers (**Publication III, Figure 4E**). Beads formation was seen when the core consisted of zein or zein + stearic acid (SA) and shell – absolute ethanol. The addition of PEO in the core was able to overcome the bead formation and ribbon shaped fibers were successfully electrospun. TET loaded fibers were mostly ribbon shaped which increased the fiber diameter distribution.

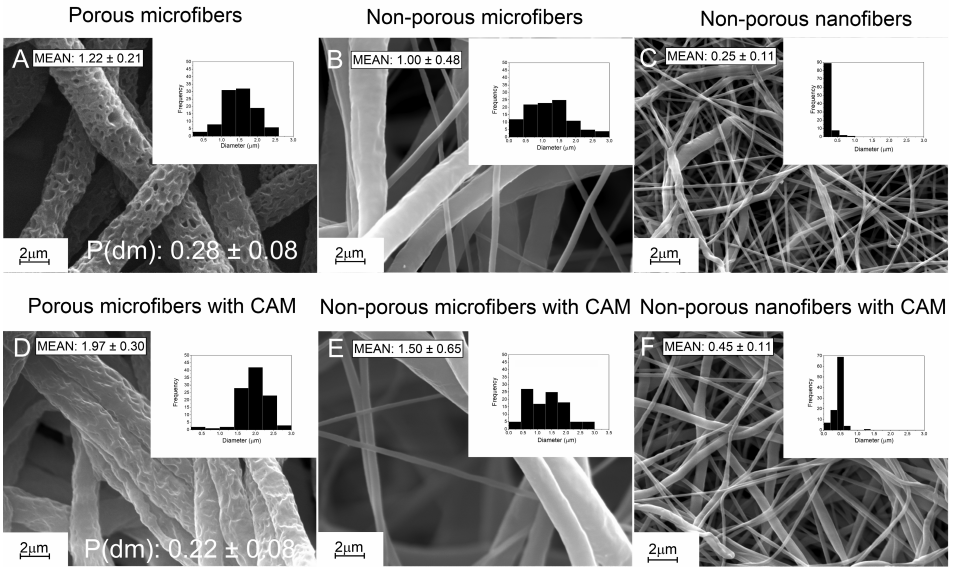


Figure 8. SEM micrographs of porous and non-porous electrospun PCL micro and nanofiber scaffolds without and with CAM (magnification 10,000×). Porous microfibers without (**A**) and with CAM (**D**). Non-porous microfibers without (**B**) and with CAM (**E**). Non-porous nanofibers without (**C**) and with CAM (**D**). Key: MEAN – mean fiber diameters ± SD (N=100); P(dm) – mean surface porosity (pore diameter on fibers) ± SD (N=30) n.± SD

5.5 Porosity of fiber scaffolds (I)

It is known that the porosity on the scaffold has an impact on the drug release, mechanical properties, and stability in biorelevant solutions (such as a buffer solution). In order to evaluate the PCL scaffold's porosity, we used calculations (described in **Publication I)**. The calculated porosity for PCL scaffolds was 71–78% with no statistical differences (**Publication I, Table 2**). These values are in correlation with the literature. (Cortez Tornello et al., 2014) Differences were seen with surface porosity diameter analysis. Microfiber (porous and non-porous) PCL fiber scaffolds had a pore diameter ranging from 13.24 to 21.87 µm whereas nanofiber PCL fiber scaffold's pore diameter ranged from 2.88 to 3.65 µm (**Publication I, Table 2**). This is in correlation with the literature that electrospun fibers with larger diameter exhibit increased scaffold porosity. (Pham et al., 2006)

Brunauer–Emmett–Teller (BET) analysis was used to evaluate the surface area of the electrospun scaffolds. Our samples had differences in surface area according to BET analysis (**Publication I, Table 2**). PCL porous microfiber matrix with and without CAM had significantly smaller specific surface area values compared to the non-porous microfibers and non-porous nanofibers with and without CAM. The largest surface area was seen with non-porous nanofiber scaffolds. With these samples N^2 adsorption isotherms were measured which characterize the sample in a larger relative pressure (P/Po) range in comparison with the isotherms measured using krypton. At the beginning of isotherm ($p/p0 < 0.2$) some indication about the microporosity was observed (resembles the I type isotherm) followed by non-porous II isotherm. We were unsuccessful differentiating surface porosity with this analysis from porous microfibers, as it is known that nanofibers have larger specific surface area which outweights the effect of pores on the microfiber surface. (Z. M. Huang et al., 2003)

5.6 Solid state characterisation of fiber scaffolds (I)

Materials (polymers and drugs) may undergo different changes in solid state during electrospinning process. In order to understand the quality and estimate the efficiency of the developed fiber scaffold solid state characterisation is especially important. Fourier transform infrared (FTIR) is a vibrational spectroscopy technique that can be used to evaluate possible intramolecular interactions between the materials used for electrospinning. (M. S. Islam et al., 2019) Furthermore, this method gives an additional information about the polymorphism and amorphous forms. (Bunaciu et al., 2015) Attenuated total reflectance (ATR) enhances the evanescent infrared signal which improves spectral acquisition and quality and therefore, ATR-FTIR setup is mostly used for infrared spectroscopy. X-ray diffraction (XRD) is a gold standard method to identify polymorphs and the amorphous form. In addition, thermal methods, such as differential scanning calorimetry (DSC), are frequently used to evaluate the changes in the crystallinity of substances by analysing the thermal properties of the materials. The solid-state

analysis gives us valuable information that is in correlation with the mechanical properties, stability of the scaffold in biorelevant media and its interactions with cells together with its antibacterial properties.

ATR-FTIR spectroscopy analysis of PCL based electrospun fiber scaffolds showed distinctive PCL peaks with all samples (**Publication I, Figure 6**). Characteristic PCL peaks were seen at 1,161 cm^{-1}, 1,238 cm^{-1} and 1,720 cm^{-1} in correlation with the literature. (G. K. Kim et al., 2013) It was interesting to see that despite using different solvent systems for electrospinning, PCL spectra remained unchanged. Characteristic CAM peaks were seen with drug loaded PCL scaffolds at 650 cm^{-1}, 813 cm^{-1}, 1,514 cm^{-1}, and 1,681 cm^{-1} (**Publication I, Figure 6**). These peak positions suggest that CAM was in an amorphous form. CAM is known to transform from crystalline to amorphous form during electrospinning. (Preem et al., 2017) The CAM concentration used in this study was previously confirmed to be detectable. (Preem et al., 2017) Interestingly, the IR spectra of CAM-loaded scaffolds showed differences in the characteristic CAM peak appearances, positions and intensities. The CAM peak at 1,685 cm^{-1} could only be detected with non-porous nanofiber scaffolds (**Publication I, Figure 6**). Furthermore, CAM loaded non-porous nanofiber scaffold had the highest number of characteristic CAM peaks. In contrast porous and non-porous CAM loaded scaffolds had CAM distinctive peak only at 1,514 cm^{-1} (**Publication I, Figure 6**). This might be caused by intramolecular interactions, however additional experiments need to be done to prove the statement. Additionally, CAM placement within the fibers rather than on the surface could diminish the detection of the distinctive peaks. (Cui et al., 2006)

XRD analysis confirmed the presence of amorphous CAM as no distinctive CAM reflections were observed with drug loaded PCL fiber scaffolds (**Publication I, Figure 6**). Furthermore, PCL was able to retain its semicrystalline structure in the fiber scaffolds. Some PCL reflection changes in XRD diffractograms were seen between samples however this is probably caused by differences in structure and surface properties according to the solvent system used. Some changes were seen with the intensity ratio of two main peaks of PCL (I_{110}/I_{200}) related to decreased crystallinity. (K. H. Lee et al., 2003) The crystallinity calculations were not possible due to the differences in structure and surface properties between the samples.

With co-axially prepared zein based fiber scaffolds the solid-state characterisation was done with DSC and XRD (**Publication III, Figure 4**). DSC analysis showed that the zein glass transition temperature (Tg) for raw material (zein) was 158.0 ± 0.9 °C and within fiber scaffolds 161.9 ± 1.3 °C in correlation with the literature. (Torres-Giner et al., 2008, 2009) TET loaded zein fiber scaffolds showed decreased Tg values, however it was not statistically proven. XRD analysis of zein based fiber scaffolds showed that all components were in amorphous forms (zein, SA, PEO, TET). Furthermore, in order to characterise the samples and see the changes after storage, XRD analysis was performed after 4 months which confirmed that the amorphous form was retained.

5.7 Effect of fiber scaffold structure on the mechanical properties (II, III)

The mechanical properties of the fiber scaffold are one of the key parameters for its application. For wound healing application the dressings need to be easily applicable, comfortable, and non-adherent for the patient. However, if fiber scaffolds are produced, it is desired that cells preferentially adhere on their surface. Electrospun scaffolds should be designed with suitable mechanical properties (tensile strength, Young's modulus, elongation) similar to native skin and favouring cell adhesion and proliferation supporting the wound healing. (Gao et al., 2021)

In our study we saw differences in the mechanical properties among the scaffolds with different morphology: porous microfiber, non-porous microfiber and non-porous nanofiber scaffolds (pristine and CAM loaded scaffolds) (**Table 7** and **Publication II, Figure 2**). Highest elongation at break was seen with the porous microfiber scaffold. This means that mentioned scaffold has highest degree of deformation before breaking which could also be beneficial in cell-scaffold interactions.(Wolf et al., 2013) Non-porous nanofiber scaffold had significantly lower elongation at break making them stiffest of the samples. Regarding the tensile strength and Young's moduli – these were significantly higher with non-porous microfiber scaffolds. It was seen that scaffolds prepared with the same composition, but different morphology (porous vs non-porous microfibers) exhibited differences in plastic deformation (**Table 7** and **Publication II, Figure 2**). There was also an indication of higher tensile strength and Young`s modulus with non-porous nanofiber scaffold (compared to porous microfiber scaffold), however it was not statistically relevant. There have been discussions in the literature that decreased fiber diameter of scaffolds results in increased tensile strength. (Chew et al., 2006; Tan et al., 2005) Generally, drug-loaded fibers exhibited increased tensile strength and Young's modulus with the exception of non-porous microfiber scaffold **(Table 7**). There exists a correlation between the macrostructural and microstructural porosities of fiber scaffolds, which can be tracked by the free volume changes due to the stability of the drug within the scaffold (Sebe et al., 2013, 2017) The size of the free volume of pores (e.g., holes) determines the mechanical properties of the fiber scaffolds and also the drug release from the samples. (J. Zhang et al., 2017; Scheler, 2014) The decreased free volume is known to increase the tensile strength of fiber scaffolds. (D'Amato et al., 2018)

Surface porosity increased the elongation at break with means highest level on deformability of the scaffold (**Table 7**). There are studies that have shown the importance of PCL fiber diameter on mechanical properties. Fibers with diameters below 500 nm had increased tensile strength. (Baji et al., 2010) In our study, we saw that fiber diameter is not the only factor affecting mechanical properties, as this effect comes in combination with the morphology of the scaffolds. According to the literature we were able to confirm that elongation at break increased together with larger diameter of PCL fibers (**Table 7**). (Tan et al., 2005)

When designing scaffolds for wound healing, these need to withstand the forces that are needed for the application as well as removal without harming premature tissue and its growth. The cell-scaffold interactions are dictated by the stiffness of the fibers or fiber scaffold. (S. Jiang et al., 2018) Focal adhesions give cells push and pull mechanism which the scaffold needs to withstand without breaking. (Kennedy et al., 2017) When electrospun scaffolds are designed to mimic the skin, these need to withstand the tensile strength and Young's modulus of native skin which is approximately 20 MPa in tensile strength (Shevchenko et al., 2010) and from 0.008 MPa (Pailler-Mattei et al., 2008) up to 70 MPa for Young's modulus. (Shevchenko et al., 2010) Elongation at break values for the skin range between 35 and 115%.(S. Chen, Liu, et al., 2017) Our developed PCL scaffolds fulfil the criteria having similar mechanical properties to native skin.

Table 7. Mechanical properties (Young's modulus, tensile strength, and elongation at break) of dry and wet (24 h wet) electrospun fiber scaffolds without and with CAM. MF = microfiber; NF = nanofiber. (N=3)

Formulations		**Tensile strength MPa)**	**Elongation at break (%)**	**Young's modulus (MPa)**	**Thickness of the fiber scaffold (mm)**
PCL THF: DMSO (porous microfibers)	Dry	12.72 ± 3.20	526.73 ± 124.03	18.35 ± 7.11	0.11 ± 0.02
	Wet (24h in PBS)	14.35 ± 2.78	477.73 ± 66.60	19.08 ± 6.07	
PCL THF: DMSO (non-porous microfibers)	Dry	86.91 ± 16.16	461.63 ± 206.38	150.60 ± 9.63	0.05 ± 0.02
	Wet (24h in PBS)	118.45 ± 22.95	477.66 ± 66.60	152.59 ± 40.21	
PCL AA:FA (non-porous nanofibers)	Dry	32.37 ± 0.86	180.23 ± 7.20	38.50 ± 6.62	0.09 ± 0.01
	Wet (24h in PBS)	22.01 ± 11.10	187.20 ± 13.00	23.97 ± 16.25	
PCL THF: DMSO + CAM (porous microfibers)	Dry	15.44 ± 1.13	341.86 ± 75.10	25.63 ± 5.06	0.09 ± 0.01
	Wet (24h in PBS)	15.58 ± 1.07	380.53 ± 83.37	21.31 ± 7.94	
PCL THF: DMSO + CAM (non-porous microfibers)	Dry	15.84 ± 3.82	285.26 ± 35.26	30.27 ± 10.17	0.09 ± 0.01
	Wet (24h in PBS)	16.96 ± 3.48	231.60 ± 49.00	35.74 ± 6.78	
PCL AA:FA + CAM (non-porous nanofibers)	Dry	39.86 ± 5.84	161.00 ± 21.58	57.24 ± 3.54	0.11 ± 0.01
	Wet (24h in PBS)	23.78 ± 5.40	163.30 ± 23.14	36.64 ± 14.22	

Besides fiber and fiber scaffold structure, the impact of solvent systems on PCL crystallinity is also relevant, which is known to affect the mechanical properties of the scaffold. (Pok et al., 2010) In our study, we saw that the solvents used for electrospinning had an effect on the morphology of the scaffold that affected the mechanical properties. The fiber and scaffold mechanical properties have been thoroughly discussed in the literature with varied results depending on several parameters: morphology, solvent composition, fiber diameters and thickness of the scaffold. (Croisier et al., 2012b) There are suggestions that the evaporation rate of the solvents used for electrospinning have a big impact on the mechanical properties of the scaffolds due to their impact on the solidification of the carrier polymer. (Arinstein & Zussman, 2011) PCL fiber scaffold from acetic acid solutions is known to be stiffer in mechanical properties.(Bahrami & Gholipour Kanani, 2011) These phenomena were also seen in our study with non-porous nanofiber scaffolds.

The mechanical properties of zein based scaffolds were also tested and these showed low Young's modulus and tensile strengths with all samples. PEO in the core showed trends of increased tensile strength and Young's modulus, however it was not statistically relevant. However, the composition of the scaffolds had an impact on the profiles of the stress-strain curves and elongation profiles. As the zein based scaffolds were less strong than PCL based scaffolds and native skin, it could be problematic to use them as scaffolds for wound healing alone.

Drug incorporation into the scaffolds can have a positive or negative effect on the mechanical properties. (Chew et al., 2006) Linezolid incorporation in PCL scaffolds has been shown to have a positive effect on its mechanical properties. (Tammaro et al., 2015) It has been shown that drug-polymer interactions have an impact on the mechanical properties of polyester-based fiber scaffolds. (Chou & Woodrow, 2017) We have shown that the solvent systems used in polymer solutions have different drug-polymer interactions which leads to changes in the mechanical properties of the scaffolds (**Publication II, Figure 2**). CAM incorporation in the scaffolds showed some increase in tensile strength with porous microfiber scaffolds and non-porous nanofiber scaffolds. However, CAM incorporation made the scaffolds stiffer as the elongation at break decreased with all samples (**Table 7**). The mechanical properties of TET loaded zein based scaffolds were also tested and these showed decreased Young's modulus and tensile strength values except the sample with PEO in the shell. It was interesting to see that (Z+PEO+TET) PEO samples had increased elongation at break.

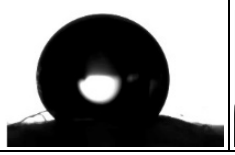
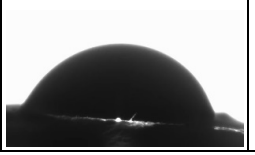
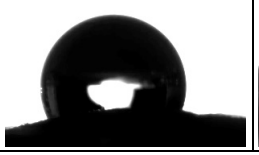
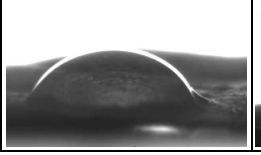
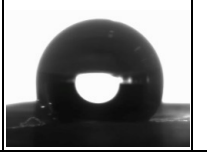
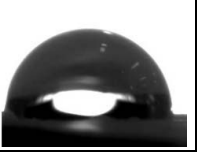
When designing scaffold for wound healing its properties also need to be studied in wet environment. In our study, we wanted to see if and how the mechanical properties change when the scaffolds are placed in buffer (1 × PBS) at 37 °C for 24 h. It was interesting to see that no significant changes in the mechanical properties of PCL scaffolds were seen after 24 h in wet conditions compared to dry samples. Furthermore, it was interesting to see that even CAM loaded samples did not show significant changes when comparing the mechanical properties of dry and wet scaffolds. This indicates that PCL based scaffolds (in short term) are stable enough in biorelevant media which is important for wound

healing purposes. One thing to mention is that we saw decreased tensile strength with CAM loaded non-porous nanofiber scaffold after wetting. This is most probably due to the higher drug release (*more information in 5.9 drug release paragraph*) (**Table 7**). The mechanical properties of zein based scaffolds were also tested in liquid media (2 min in PBS). The elongation at break drastically increased after wetting with all the samples. The highest change in elongation at break (dry vs wet) was seen with (Z+SA) PEO scaffolds where it increased from 19.3% to 411.2%. The drug loaded zein scaffolds showed lower Young's modulus, tensile strength, and elongation at break values. The most drastic change was seen with (Z+SA) PEO scaffolds where the elongation at break (compared to drug free scaffold) in wet conditions decreased to 24.0%. This indicates that zein based scaffolds alone would not have sufficient mechanical properties in order to be used as wound dressings.

5.8 Wettability, swelling, and early *in vitro* degradation behaviour (II, III)

The wettability, swelling and *in vitro* degradation largely affect the scaffolds performance as wound dressings. Furthermore, buffer penetration into the scaffold can increase the degradation rate as well as affect the drug release properties. (Cui et al., 2008; Zupančič et al., 2018) There are various methods which allow testing the scaffold performance in biorelevant media. (Uhljar et al., 2021; Walther et al., 2022; J. Wang et al., 2018) One method is contact angle measurement which gives valuable information about the wettability and hydrophobicity/hydrophilicity of the scaffold. (Z. Xie & Buschle-Diller, 2010) Determining the swelling index of the scaffold is also relevant because it provides information about the absorption capability of the scaffold. (Dwivedi et al., 2019) In our study higher swelling indexes were seen with non-porous nanofiber and non-porous microfiber PCL scaffolds compared to porous microfiber scaffold (**Table 8**). This phenomenon most likely is due to the morphological differences of the scaffolds. During experiment there was still some residual liquid within the scaffolds that could sufficiently be removed only from porous microfiber scaffold. Drug incorporated scaffolds showed increased swelling index with all samples which can be related to the increased hydrophobicity of the scaffolds. The contact angle measurements confirmed that pristine PCL scaffolds were hydrophobic in nature. However, with CAM loaded scaffolds the scaffolds hydrophilicity was increased. The most hydrophilic scaffold in our study according to the contact angle measurements was porous CAM loaded microfiber scaffold.

Table 8. Contact angle, swelling index and weight loss of electrospun fiber scaffolds with and without chloramphenicol (CAM). Data presented as mean ± SD (N=3).

Formu-lations	**PCL_ THF:DMSO (porous microfibers)**	**PCL_ THF:DMSO + CAM (porous microfibers)**	**PCL_ THF:DMSO (non-porous microfibers)**	**PCL_ THF:DMSO + CAM (non-porous microfibers)**	**PCL_AA:FA (non-porous nanofibers)**	**PCL_AA:FA + CAM (non-porous nanofibers)**
Swelling index (%)	248.40 ± 29.49	462.98 ± 28.25	389.75 ± 124.99	619.36 ± 124.92	348.34 ± 35.87	589.04 ± 58.35
Weight loss (%)	0.54 ± 0.31	2.39 ± 2.10	0 ± 0	1.63 ± 0.92	1.68 ± 0.97	1.15 ± 0.43
Contact angle (0 s)	125 ± 22	107 ± 31	121 ± 8	134± 5	100 ± 38	114 ± 16
Contact angle and images after 30 s	124 ± 22 	29 ± 51 	120 ± 9 	56 ± 22 	113 ± 12 	54 ± 47 

Studies have shown that nanoscale fiber scaffolds exhibit increased surface roughness which causes air entrapment between the contacting liquid droplet and the scaffold. (M. Ma et al., 2005) In our study pristine PCL scaffolds did not have significant changes in contact angle values at 0 s and 30 s. However, CAM loaded samples showed decreased contact angle values at 30 s when in contact with the buffer (**Table 8**). This indicates that CAM loading increased the surface hydrophilicity of the scaffolds as drug started to dissolve when in contact with the liquid. Furthermore, the drug release aided buffer penetration into the scaffold as dissolved drug vacated cavities and increased the surface porosity of the fibers. Unfortunately, we were not able to see any significant differences in the contact angle values with porous microfiber scaffolds (compared to controls). It has been stated that hydrophobic materials with increased surface area have high contact angles, whilst with hydrophilic materials the contact angles decrease. (Chau et al., 2009) So, modifying the surface roughness of the material could be used to improve the hydrophilicity of the material.

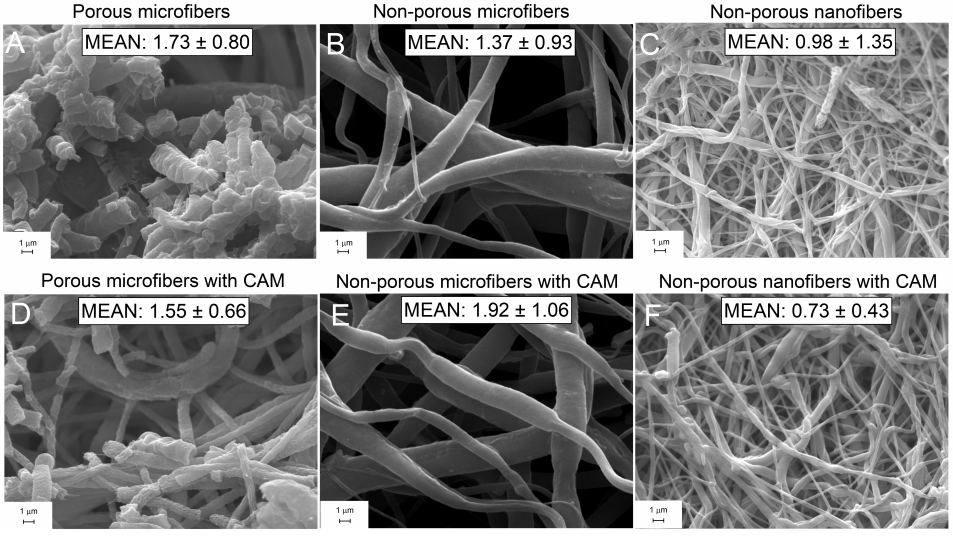


Figure 9. Early *in vitro* degradation of porous and non-porous electrospun PCL micro and nanofiber scaffolds without and with CAM after 24 h in 1 × PBS (magnification 10,000×). Porous microfibers without (**A**) and with CAM (**D**). Non-porous microfibers without (**B**) and with CAM (**E**). Non-porous nanofibers without (**C**) and with CAM (**F**).

Key: MEAN – mean fiber diameters ± SD (N=100)

Early *in vitro* degradation studies showed changes in fiber morphology (**Figure 9**). Porous microfiber scaffolds with and without CAM showed the highest degradation where the fibers had lost their integrity. Non-porous microfiber scaffold with and without CAM showed trends of increased fiber diameters, however these differences were not found to be significant due to large variances (compared to diameters discussed in paragraph *5.4 Morphology of fibers* and **Figure 8**). The same phenomena were seen with non-porous nanofiber scaffolds.

As zein based scaffolds are known to be hydrophilic in nature it was no surprise that the contact angle measurements showed no droplet after 1–3 s. (**Publication III, Table 2**). Nevertheless, further stability analysis was performed in order to understand the water stability of the zein based scaffolds. The scaffolds were incubated for 48 h pH 7.4 at 37 °C and visualized under SEM in order to see the changes in morphology (**Publication III, Figure 3**). Fiber scaffolds without PEO in the shell were completely dissolved after 48 h of incubation and therefore, removed for further analysis. Other scaffolds exhibited excess of swelling, but their structure was retained. TET loaded scaffolds exhibited degradation and drug dissolution as surface porosity was observed after 48 h of degradation testing in SEM (**Publication III, Figure 3**). The most evident surface porosity was seen with (Z+SA+TET) PEO scaffolds (**Publication III, Figure 3i**).

5.9 Drug release from electrospun scaffolds (I, III)

The polymer properties (affecting drug release from DDS) such as molecular weight, viscosity of the polymer solution, surface tension of the polymer solution can be modified with solvents used for electrospinning. (D'Amato et al., 2017) Furthermore, the volatility of the solvent has a huge impact on the fiber and scaffold structure which is also in correlation with the differences seen in the drug release properties. (D'Amato et al., 2017) Furthermore, there have been studies that have changed the polymers as well as manipulating the humidity which have a huge effect on the drug release properties. (Y. Yuan et al., 2018) Our results showed that the solvents used for electrospinning have a significant impact on the drug release properties. Our group has previously developed a DDS with prolonged CAM release when CF:MET solvent system was used for preparing the PCL-CAM scaffolds. (Preem et al., 2017) Present study results however showed fast release from the PCL-CAM fiber scaffolds (**Publication I, Figure 7**). The effect of porosity (more specifically pore size of the fiber scaffold) on drug release from electrospun scaffolds has thoroughly been investigated (Naseri et al., 2015; Soliman et al., 2011). However, the effect of surface porosity of the single fibers has only been discussed in few previous studies.(Ren et al., 2018; Yaru et al., 2018) Co-axially electrospun fibers with surface porosity have shown increased drug release in comparison to the fibers with no pores.(Nguyen et al., 2012) Furthermore, surface porosity increases the amount of drug released from the scaffold, since with non-porous fiber scaffolds some of the drug may be entrapped within the fibers.(Yaru et al., 2018) From this it was of interest to study the effect of differently structured fibers (however similar thickness) on the drug release properties from the scaffold. We hypothesised whether the pores on fibers (without any modification on the formulation composition) could be used as an approach to modify and control the drug release from fibers.

We observed initial burst release properties of CAM with all scaffolds despite the differences in their structure (pores *vs* no pores on single fibers) (**Publication I, Figure 7**). This indeed made the comparison and understanding the

effect of pores more challenging. Nevertheless, we saw interesting differences regarding the burst release and total amount of drug released. The highest burst release of CAM was seen with non-porous nanofiber scaffold which was 66.9 ± 4.0% after 3 min. Porous microfiber scaffold burst release was 53.5 ± 7.0%, respectively. The burst release was followed by slower release properties for porous microfiber and non-porous nanofiber scaffold. At 96 h timepoint the highest CAM cumulative release was seen with non-porous nanofiber scaffold where 72.6 ± 7.7% of CAM was released. Interestingly porous microfiber scaffolds had 59.2 ± 3.2% of CAM released at the same timepoint which suggests that some of the drug was still entrapped within the scaffold/fibers. To confirm that the phenomenon was not only caused by higher surface area provided with nanofiber structure we also tested CAM release properties from non-porous microfiber scaffolds. The results confirmed our hypothesis as non-porous microfiber scaffold showed only 20% of CAM release at 96 h. Most probably most of CAM was located within the non-porous microfibers and only the drug located on the surface of the fibers was able to be released. As porous microfiber scaffold and non-porous microfiber scaffold were prepared using the same polymer/solvent composition it was interesting to observe these major differences. The surface porosity, which was achieved with the suitable polymer/ solvent composition combined with high humidity during electrospinning, changed the drug release behaviour from the scaffold. For that the dissolution media have to penetrate into the scaffold in order to provoke the diffusion. Regarding the results seen with non-porous microfiber scaffold, where only 20% of the drug was able to diffuse, the hydrophobic nature of PCL might have caused insufficient buffer penetration.

With zein loaded scaffolds the TET release behaviour was also studied from different fiber scaffolds. As the zein based systems were prepared co-axially potentially having the core-shell structure, the release properties were also thought to be different. However, the burst release effect was seen with all compositions where about 70% of TET was released at 5 min timepoint. This could be related to the high instability of the scaffolds in biorelevant media confirmed by contact angle, drug release and degradation/stability measurements. Contact angle measurements showed that zein based scaffolds were very hydrophilic in nature (paragraph 5.8 Wettability, swelling, and early *in vitro* degradation behaviour). Furthermore, TET loaded samples showed degradation and in some cases ((Z+SA+TET) PEO) pore formation through the fibers (**Publication III, Figure 2**). All this most probably ended up dissolving the drug in the dissolution medium very rapidly which corresponds to the high burst release values.

There are various drug release mechanisms described for the electrospun fiber scaffolds (S. Dash et al., 2010) and studying these mechanisms enables to understand better the structure/behaviour relationships relevant for the design and development of such novel DDSs. It has previously been discussed that the drug release from PCL scaffolds is mainly based on diffusion.(McInnes et al., 2018) The ATR-FTIR confirmed the differences in interactions between PCL and solvent systems used for electrospinning (**Publication I, Figure 6**). Even though PCL and used model drug CAM are hydrophobic the solvent systems used are

different in nature. AA:FA compared to THF:DMSO and CF:MET solvent systems, is thought to be more hydrophilic. Due to this the polymer/drug interactions with AA:FA are different. There might have been interactions between hydrophobic polymer and drug and hydrophilic solvent system that could have caused the drug to be deposited rather on the surface of the fibers than the inner structure of the fibers.(Širc et al., 2012) Acidic environment might increase the surface charge of PCL during dissolution which could increase an affinity to water as well as drug release from the scaffolds. The surface morphology of the fiber scaffolds is also affected by the dissolution of the polymer in the chosen solvent system. Higher soluble solvents for PCL like CF and THF increase PCL aggregation and less soluble solvents such as ACE result in increased filament structure. (Pok et al., 2010) With hydrophobic system (polymer, drugs, solvents) the interactions and affinity increases. To sum up, the structural differences of the fibers within the scaffold, interactions between the components that have been used for electrospinning, and surface changes (surface porosity) are amongst some of the parameters that affect the drug release properties from the scaffold.

5.10 Biological relevance of differently designed fiber scaffolds (II, III)

5.10.1 Fibroblast attachment, biocompatibility and cytotoxicity (II, III)

The investigations involving the scaffold – eukaryotic cell interactions are especially important when designing electrospun scaffolds for wound healing. The scaffold needs to be suitable substrate for cells to attach and grow on in order to aid the native wound healing process. Furthermore, all used materials need to be biocompatible and non-toxic. There are many reports showing the cell interactions with electrospun scaffolds, however the results are very different and mainly depend on the cell line used. (Sadeghi et al., 2016b; R. Xue et al., 2017) We used BHK-21 cell line that has been successfully used in many studies. (Hasbiyani et al., 2020; S. H. Huang et al., 2010) MTS assay was chosen for the fibroblast attachment testing on differently structured electrospun scaffolds and understanding their biocompatibility. MTS test contains tetrazolium salts (specifically phenazine methosulfate) which interact with metabolically active cells that start to produce formazan dye. The metabolism is carried out by dehydrogenase enzymes and the dye can be quantified by measuring absorption at 490–500 nm. (V. Kuete et al., 2017) CAM loaded porous microfiber scaffold had the highest MTS activity (**Publication II, Figure 6**). This indicates that microfibers with surface porosity offered best substrate for fibroblast attachment and proliferation. Furthermore, as drug loaded scaffolds had no negative effect on BHK-21 cells it can be concluded that CAM concentration was optimal and non-toxic in line with the literature.

No significant differences in MTS activity were seen when comparing different microfiber CAM loaded scaffolds (porous vs non-porous). The trends showed that cells preferred microfibers with surface pores and the significance was confirmed with non-drug loaded scaffolds, where porous microfiber scaffold had significantly higher MTS activity than non-porous microfiber scaffold. ($p < 0.05$).

Zein based microfiber scaffolds were also tested with BHK-21 cell line and MTS test to evaluate the metabolic activity of the cells. (**Publication III, Figure A4**) The MTS activity was somewhat lower than porous PCL scaffolds (0.85–0.95), at about 0.75–0.80. TET did not affect the viability of fibroblasts which means that the drug concentration used in scaffolds was not toxic. It was seen that the zein based scaffolds were not stable enough in biorelevant media and formed a gel (**Publication III, Figure 3).** Most probably this resulted in changed fiber and scaffold morphology which inhibited the penetration of cells into the fiber scaffold. It was seen that due to the dense structure of the fiber scaffolds and small pore size between the fibers in the scaffold, cell migration was observed only into the upper surface layer of the scaffold.

In order to get a better understanding of the cell-scaffold interactions visual analysis with confocal fluorescence microscopy (CFM) was performed. (**Figure 10A–E**).

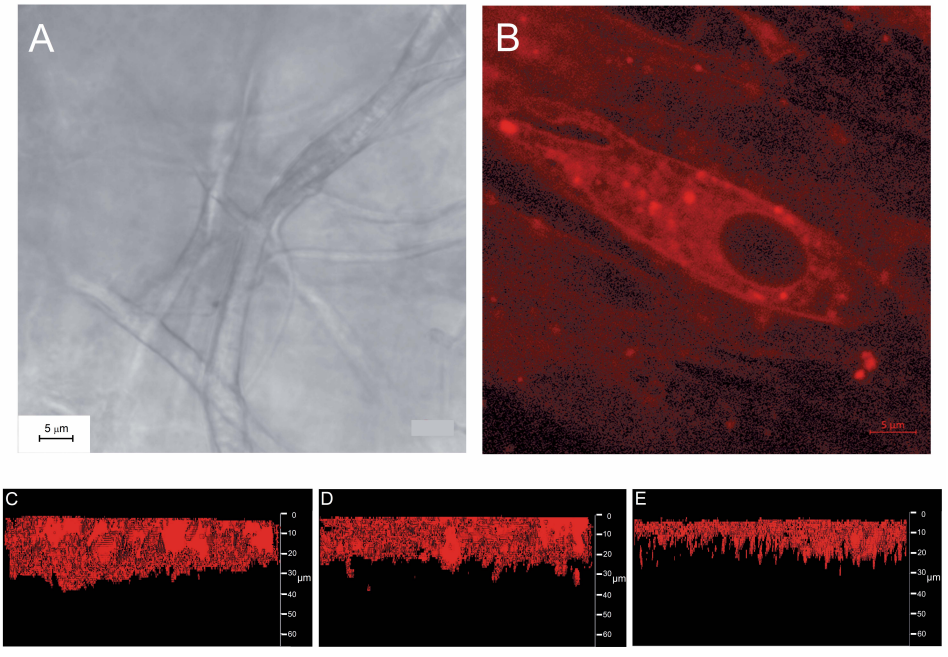


Figure 10. Confocal fluorescence microscopy (CFM) 2D transmitted light micrograph of porous microfiber scaffold (**A**) and the same image showing a fluorescent signal that comes from the FM 4–64 dyed fibroblast cells (**B**). CFM cross-section micrographs of fibroblast cells attached onto the electrospun porous microfiber scaffold consisting of (**C**) CAM; pristine nonporous microfiber scaffold (**D**); pristine nonporous nanofiber scaffold (**E**).

The BHK cells were deformed in shape and differentiating of the fibers and cells was exceedingly difficult. Therefore, we decided to use confocal microscopy to investigate the cell-scaffold interactions. The z-stack images of the samples were used to construct the 3D micrographs. (**Figure 10C–E**). Specific membrane dye (FM4-64) helped to get a better understanding of the shape, size and distribution of the cells. This analysis also confirmed the results from MTS assay, changes in morphology, more even distribution and infiltration were observed with CAM loaded porous microfiber scaffolds (**Figure 10C**). The distribution and morphology of cells on non-porous microfibers were similar to the porous ones, however infiltration seemed to be less evident as lower parts of the scaffold had weaker intensity of FM4-64 dye (**Figure 10D**) In contrast non-porous nanofiber scaffold covered with eukaryotic cells showed a strong fluorescent signal only on the upper layer of the scaffold indicating that the structure was not ideal for cell infiltration (**Figure 10E**).

Fibroblast attachment and growth studies indicated correlations between used BHK-21 cells and the structural/morphological properties of the fiber scaffold. Our study showed the tendency for the cells to prefer elastic microfiber scaffold for attachment and growth rather than stiffer nanofiber scaffold. Furthermore, the surface porosity on fibers (which further increased due to drug release) had an impact on the scaffolds' hydrophilicity and morphology which aided fibroblast attachment and proliferation. In our study we saw less cell-scaffold interactions with nanofiber scaffolds which could be related with the fact that the pore size affects the cellular infiltration properties. (Farooque et al., 2014) Previously it has been stated that cell-scaffold interactions can be provoked by the substrate. (Sharifi et al., 2016) One of the parameters affecting cell growth and spreading is the pore size of the fiber scaffold. (Farooque et al., 2014) There have been studies that have shown that the pore size above 20 μm in the scaffold boosts the fibroblast spreading along the walls and within the scaffold. (Wolf et al., 2013) Pores smaller than cells have induced changes in attached cell morphology however the limiting factor for entry of cells into the scaffold pores is the size of nucleus (Wolf et al., 2013) We also saw in our study that nanoporous scaffolds had too small pores between the fibers for the fibroblasts to infiltrate into the scaffold. However, surface porosity together with microfiber structure seemed to create ideal substrate for the BHK-21 cells.

5.10.2 Bacterial attachment, biofilm formation and antibacterial activity (II, III)

When developing antibacterial scaffolds for wound healing the material needs to be either antibacterial or at least it would not promote bacterial growth in the wound. Ideally the scaffold can also have antibiofilm properties. Pristine PCL based fiber scaffolds have shown enhanced bacterial growth on the fiber scaffold surfaces with *E. coli* and *S. aureus.* (Pompa-Monroy et al., 2020) Furthermore, the colonies were not only on the surface of the material but were also located in

the scaffolds. PCL based scaffolds have also shown to be substrates for bacterial biofilm formation. (Høiby, 2017; Nickel et al., 1985; Oates et al., 2014; Preem et al., 2017; Tamayo-Ramos et al., 2018) For the present study, we evaluated the biofilm formation and antibiofilm activity of the PCL scaffolds with previously developed biofilm assay. (Preem et al., 2017) We used *E. coli* DSM 1103 clinical isolate in this experiment as it is known to be clinically relevant pathogen in wound infections. (Smith et al., 2020) For zein based microfiber scaffolds we tested their antibacterial efficiency against *E. coli* DSM 1103 and *S. aureus* DSM 2569 wound isolates using agar diffusion assay.

The developed PCL based scaffolds showed no differences in planktonic bacteria/scaffolds interactions regardless of their structure and composition (**Publication II, Figure 6**). However, differences were seen with biofilm bacteria, where *E. coli* biofilm formation was evident with scaffolds without the drug. We confirmed that PCL based fiber scaffolds offer a good substrate for *E. coli* biofilm formation.

Higher biofilm formation was seen on non-porous nanofiber scaffold at 24 h and 72 h timepoints compared to microfiber scaffolds (porous and non-porous) (**Publication II, Figure 6**). Microfiber scaffolds (porous and non-porous) showed similar biofilm formation at all timepoints, only slightly favouring porous fiber structure.

All drug loaded samples showed reduced number of biofilm bacteria in every measured and following timepoints (24 h, 72 h). The latter means that these scaffolds have sufficient antibiofilm properties (**Figure 11**).

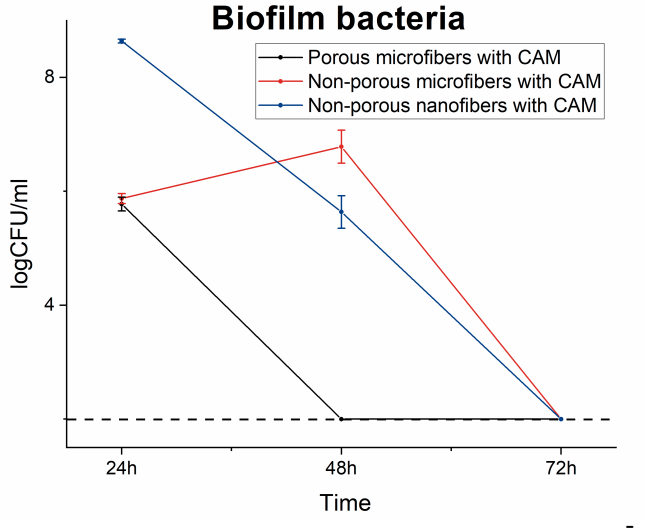


Figure 11. Biofilm formation and antibiofilm activity of porous and non porous electro spun PCL microfiber and nanofiber scaffolds with CAM on *E. coli*.

Key: biofilm bacteria–biofilm bacteria washed off from the fiber scaffold; CAM – chloramphenicol; detection limit refers to the CFU that can be counted by the plate method (200 CFU/mL).

However, some differences in biofilm structure and antibiofilm activity were seen. The highest antibiofilm activity was observed with CAM loaded porous microfiber and non-porous nanofiber scaffolds. In 48 h timepoint the scaffolds (porous microfibers and non-porous nanofibers) had inhibited the bacterial growth. The killing of biofilm bacteria was more evident with porous microfiber scaffolds. The CAM loaded non-porous microfiber scaffold was not so efficient in inhibiting the bacterial growth at 48 h timepoint. This could be related to the limited drug release where only 20% of CAM was released (more thoroughly discussed in paragraph *5.9 Drug release from electrospun fibers*). At 72 h timepoint the bacterial growth was inhibited with all tested CAM loaded scaffolds.

The bacterial growth and proliferation within the electrospun scaffold are affected by the size and shape of bacteria. One factor within the scaffold that affects the growth and proliferation rate is a fiber diameter. Higher proliferation rate of bacteria has been seen when the fiber diameter is similar to the dimensions of bacteria. (Abrigo et al., 2015a) With biofilm bacteria on PCL based fiber scaffolds it has been seen that it is largely dependent on the bacterial attachment abilities. (De Cesare et al., 2019) De Cesare *et. al* showed conditioning film production by *B. terracola* when the cells were incubated in growth medium on electrospun scaffolds improving bacterial adhesion. In our study we wanted to see the effect on biofilm formation on differently structured fiber scaffolds as well as their antibiofilm properties. It was seen that biofilm formation was higher with non-porous nanofiber scaffolds compared to microfiber scaffolds. It has been shown that substrates with submicron and nanometric surfaces reduce the contact area between bacterial cells and substrate surfaces that have a negative effect on bacterial attachment.(Y. Cheng et al., 2019; Hsu et al., 2013) The surface nanoporosity on fibers has also shown to decrease the bacterial attachment and biofilm formation as it increases the repulsive surface-bacteria interaction forces. (Anselme et al., 2010) In our study the surface porosity had a little to no effect on the bacterial growth and biofilm formation.

With CAM loaded scaffolds it was observed that the differences in drug release rate as well as drug amount entrapped within the scaffold also affected *E. coli* biofilm formation. Non-porous nanofiber scaffold had the highest drug release rate, so the small amount of drug still left within the scaffold was unable to inhibit *E. coli* biofilm formation. Porous microfiber scaffolds exhibited lower drug release rate which provided enhanced antibiofilm effect compared to the non-porous microfiber scaffold. Furthermore, there is a good correlation with the antibacterial properties on planktonic bacteria and the drug release rates from drug loaded PCL scaffolds. The scaffolds were also tested with *P. aeruginosa* and as expected the scaffold acted as a good surface for bacterial growth and biofilm formation without any antibacterial nor antibiofilm effect with drug loaded samples. This is not surprising as *P. aeruginosa* is known to be resistant against CAM. (X. Z. Li et al., 1994; Morita et al., 2013)

We also evaluated the antibacterial activity of TET – loaded zein fibers with agar diffusion assay against clinically relevant wound pathogens *S. aureus* and *E. coli*. (Smith et al., 2020) TET is a suitable antibacterial agent against these

pathogens. (Ahmad et al., 2015) With our experiment the inhibition zones were about 23 mm at 24 h timepoint regardless of the scaffold composition (**Publication III, Figure A2**). TET is stated to have lower antibacterial activity against *E. coli* than *S. aureus.* (H.-A. S. M., 1976) However, in our study we were unable to see any relevant differences. (Giske, 2020) Hence all TET loaded samples were able to inhibit the bacterial growth.

6 CONCLUSIONS

In the present work antibacterial CAM-loaded porous biocompatible PCL and TET-loaded core-shell zein based fiber scaffolds were successfully developed. It can be concluded that both developments were challenging and required modifications of different parameters. More specific conclusions were:

- High humidity (RH of 65%) enabled to produce porous (pores on fibers) PCL microfiber scaffolds from binary solvent mixtures (THF:DMSO and CF: DMSO). Incorporation of hydrophobic antibacterial drug CAM reduced the pore formation on fibers. It was seen that high humidity was one of the key parameters for pore formation as low RH electrospinning with same parameters resulted in non-porous PCL fiber scaffold.
- Changing of the solvent systems changed the morphology, structure and drug release properties of the scaffold. Non-porous PCL nanofibers exhibited the highest drug release, followed by porous PCL microfiber scaffold and non-porous PCL microfiber scaffold. CAM was entrapped in non-porous PCL microfibers and only 20% of drug was released into the buffer. Surface porosity was able to increase the drug release from PCL microfiber scaffolds by at least two times.
- The mechanical properties of scaffolds are especially important for wound healing and tissue regeneration applications. In our study the tensile strength was highest with non-porous microfiber PCL scaffolds. Surface porosity increased the elasticity of the microfiber scaffold. Drug encapsulation within the fibers decreased the tensile strength, and Young' s modulus of non-porous microfiber scaffolds and elasticity of all the samples.
- The mechanical properties in biorelevant conditions (mimicking wounds) had a negligible impact on the scaffold's performance. The tensile strength of non-porous CAM loaded nanofiber scaffolds decreased under wet conditions – this was most likely due to the drug release.
- Using different solvent systems also affected the scaffold's interactions with living cells (bacterial and eukaryotic cells). Porous CAM loaded microfiber scaffolds support better fibroblast attachment and growth compared to the non-porous microfiber and nanofiber scaffolds. Furthermore, the porous CAM loaded microfiber scaffolds offered the best antibiofilm activity against *E. coli*. Surface porosity, in addition to the nano- and microscale diameter of the fibers, changes the living cell-fiber interactions affecting the antibiofilm efficacy and biocompatibility of the scaffolds for the local treatment of wounds.

- The core-shell zein based fiber scaffolds (without TET and with TET) were successfully developed with the addition of plasticizers (PEO and SA) and use of co-axial electrospinning method. All zein based scaffolds were highly hydrophilic, furthermore drug release studies showed high burst release (most of the drug released after 5 min). Although the stability studies in biorelevant media (degradation study) indicated that the structure was retained, no differences in antibacterial properties were seen against *E. coli* or *S. aureus* regardless of the composition. This might be related to the high burst release of TET from the fiber scaffolds. Fibroblasts on zein based core-shell structured scaffolds showed lower MTS activity than on PCL based scaffolds indicating that the zein fiber scaffold is less efficient in supporting cell adhesion and growth. This can be related to the insufficient mechanical properties (tensile strength, elongation) and problems with the stability of fiber scaffolds.

7 REFERENCES

Abbasi, A., Nasef, M. M., Takeshi, M., & Faridi-Majidi, R. (2014). Electrospinning of nylon-6,6 solutions into nanofibers: Rheology and morphology relationships. *Chinese Journal of Polymer Science (English Edition)*, *32*(6), 793–804. https://doi.org/10.1007/s10118-014-1451-8

Abdelgawad, A. M., Hudson, S. M., & Rojas, O. J. (2014). Antimicrobial wound dressing nanofiber mats from multicomponent (chitosan/silver-NPs/polyvinyl alcohol) systems. *Carbohydrate Polymers*, *100*, 166–178. https://doi.org/https://doi.org/10.1016/j.carbpol.2012.12.043

Abrigo, M., Kingshott, P., & McArthur, S. L. (2015a). Electrospun polystyrene fiber diameter influencing bacterial attachment, proliferation, and growth. *ACS Applied Materials and Interfaces*, *7*(14), 7644–7652. https://doi.org/10.1021/acsami.5b00453

Abrigo, M., Kingshott, P., & McArthur, S. L. (2015b). Bacterial response to different surface chemistries fabricated by plasma polymerization on electrospun nanofibers. *Biointerphases*, *10*(4), 04A301. https://doi.org/10.1116/1.4927218

Abunahel, B. M., Azman, N. Z. N., & Jamil, M. (2018). Effect of Needle Diameter on the Morphological Structure of Electrospun n-Bi2O3/Epoxy-PVA Nanofiber Mats. *International Journal of Chemical and Materials Engineering*, *12*(6), 296–299.

Acik, G., Cansoy, C. E., & Kamaci, M. (2019). Effect of flow rate on wetting and optical properties of electrospun poly(vinyl acetate) micro-fibers. *Colloid and Polymer Science*, *297*(1), 77–83. https://doi.org/10.1007/s00396-018-4443-3

Adeli, H., Khorasani, M. T., & Parvazinia, M. (2019). Wound dressing based on electrospun PVA/chitosan/starch nanofibrous mats: Fabrication, antibacterial and cytocompatibility evaluation and in vitro healing assay. *International Journal of Biological Macromolecules*, *122*, 238–254. https://doi.org/10.1016/j.ijbiomac.2018.10.115

Aguilar-Vázquez, G., Loarca-Piña, G., Figueroa-Cárdenas, J. D., & Mendoza, S. (2018). Electrospun fibers from blends of pea (Pisum sativum) protein and pullulan. *Food Hydrocolloids*, *83*, 173–181. https://doi.org/https://doi.org/10.1016/j.foodhyd.2018.04.051

Ahmad, A., Zachariasen, C., Christiansen, L. E., Græsbøll, K., Toft, N., Matthews, L., Damborg, P., Agersø, Y., Olsen, J. E., & Nielsen, S. S. (2015). Pharmacodynamic modelling of in vitro activity of tetracycline against a representative, naturally occurring population of porcine Escherichia coli. *Acta Veterinaria Scandinavica*, *57*(1), 79. https://doi.org/10.1186/s13028-015-0169-0

Alavarse, A. C., de Oliveira Silva, F. W., Colque, J. T., da Silva, V. M., Prieto, T., Venancio, E. C., & Bonvent, J.-J. (2017). Tetracycline hydrochloride-loaded electrospun nanofibers mats based on PVA and chitosan for wound dressing. *Materials Science and Engineering: C*, *77*, 271–281. https://doi.org/https://doi.org/10.1016/j.msec.2017.03.199

Alghoraibi, I., & Alomari, S. (2019). Different Methods for Nanofiber Design and Fabrication. In *Handbook of Nanofibers*. https://doi.org/10.1007/978-3-319-53655-2_11

Alves, P. J., Barreto, R. T., Barrois, B. M., Gryson, L. G., Meaume, S., & Monstrey, S. J. (2021). Update on the role of antiseptics in the management of chronic wounds with critical colonisation and/or biofilm. *International Wound Journal*, *18*(3), 342–358. https://doi.org/https://doi.org/10.1111/iwj.13537

Amato, S. M., Fazen, C. H., Henry, T. C., Mok, W. W. K., Orman, M. A., Sandvik, E. L., Volzing, K. G., & Brynildsen, M. P. (2014). The role of metabolism in bacterial persistence. *Frontiers in Microbiology*, *5*, 70. https://doi.org/10.3389/fmicb.2014.00070

Ambekar, R. S., & Kandasubramanian, B. (2019). Advancements in nanofibers for wound dressing: A review. *European Polymer Journal*, *117*, 304–336. https://doi.org/10.1016/j.eurpolymj.2019.05.020

Andreu, V., Mendoza, G., Arruebo, M., & Irusta, S. (2015). Smart dressings based on nanostructured fibers containing natural origin antimicrobial, anti-inflammatory, and regenerative compounds. *Materials*, *8*(8), 5154–5193. https://doi.org/10.3390/ma8085154

Angammana, C. J., & Jayaram, S. H. (2010). A Modified Electrospinning Method for Conductive and Insulating Materials. *Proc. ESA Annual Meeting on Electrostatics*, *3*, 1–7. http://www.electrostatics.org/images/ESA2010_L3_Angammana.pdf

Angammana, C. J., & Jayaram, S. H. (2011a). A Theoretical Understanding of the Physical Mechanisms of Electrospinning. *ESA Annual Meeting on Electrostatics*, 1–9.

Angammana, C. J., & Jayaram, S. H. (2011b). Analysis of the Effects of Solution Conductivity on Electrospinning Process and Fiber Morphology. *IEEE Transactions on Industry Applications*, *47*(3), 1109–1117. https://doi.org/10.1109/TIA.2011.2127431

Angammana, C. J., & Jayaram, S. H. (2016). Fundamentals of electrospinning and processing technologies. *Particulate Science and Technology*, *34*(1), 72–82. https://doi.org/10.1080/02726351.2015.1043678

Anselme, K., Davidson, P., Popa, A. M., Giazzon, M., Liley, M., & Ploux, L. (2010). The interaction of cells and bacteria with surfaces structured at the nanometre scale. *Acta Biomaterialia*, *6*(10), 3824–3846. https://doi.org/10.1016/j.actbio.2010.04.001

Arbade, G. K., Jathar, S., Tripathi, V., & Patro, T. U. (2018). Antibacterial, sustained drug release and biocompatibility studies of electrospun poly(ε-caprolactone)/ chloramphenicol blend nanofiber scaffolds. *Biomedical Physics & Engineering Express*, *4*(4), 045011. https://doi.org/10.1088/2057-1976/aac1a4

Arinstein, A., & Zussman, E. (2011). Electrospun Polymer Nanofibers : Mechanical and Thermodynamic Perspectives. *Journal of Polymer Science Part B*, 691–707. https://doi.org/10.1002/polb.22247

Asli, M. M., Pourdeyhimi, B., & Loboa, E. G. (2012). Release Profiles of Tricalcium Phosphate Nanoparticles from Poly(L-lactic acid) Electrospun Scaffolds with Single Component, Core–Sheath, or Porous Fiber Morphologies: Effects on hASC Viability and Osteogenic Differentiation. *Macromolecular Bioscience*, *12*(7), 893–900. https://doi.org/10.1002/mabi.201100470

Atiyeh, B. S., Costagliola, M., Hayek, S. N., & Dibo, S. A. (2007). Effect of silver on burn wound infection control and healing: Review of the literature. *Burns*, *33*(2), 139–148. https://doi.org/10.1016/j.burns.2006.06.010

Atkin, L., Bućko, Z., Conde Montero, E., Cutting, K., Moffatt, C., Probst, A., Romanelli, M., Schultz, G. S., & Tettelbach, W. (2019). Implementing TIMERS: the race against hard-to-heal wounds. *Journal of Wound Care*, *23*(Sup3a), S1–S50. https://doi.org/10.12968/jowc.2019.28.Sup3a.S1

Augustine, R., Augustine, A., Kalarikkal, N., & Thomas, S. (2016). Fabrication and characterization of biosilver nanoparticles loaded calcium pectinate nano-micro dual-porous antibacterial wound dressings. *Progress in Biomaterials*, *5*(3), 223–235. https://doi.org/10.1007/s40204-016-0060-8

Aumailley, M., & Gayraud, B. (1998). Structure and biological activity of the extracellular matrix. *Journal of Molecular Medicine (Berlin, Germany)*, *76*(3–4), 253–265. https://doi.org/10.1007/s001090050215

Avi, S., & Howard, I. M. (2005). *Wound Healing and Ulcers of the Skin Diagnosis and Therapy — The Practical Approach* (1st ed.). Springer, Berlin, Heidelberg. https://doi.org/10.1007/b138035

Ayrapetyan, M., Williams, T. C., & Oliver, J. D. (2015). Bridging the gap between viable but non-culturable and antibiotic persistent bacteria. *Trends in Microbiology*, *23*(1), 7–13. https://doi.org/10.1016/j.tim.2014.09.004

Aziz, Z., Abu, S. F., & Chong, N. J. (2012). A systematic review of silver-containing dressings and topical silver agents (used with dressings) for burn wounds. *Burns*, *38*(3), 307–318. https://doi.org/10.1016/j.burns.2011.09.020

Azzimonti, B., Sabbatini, M., Rimondini, L., & Cannas, M. (2016). Manipulating the healing response. *Wound Healing Biomaterials*, *1*, 101–116. https://doi.org/10.1016/B978-1-78242-455-0.00004-5

Bae, H.-S., Haider, A., Selim, K. M. K., Kang, D.-Y., Kim, E.-J., & Kang, I.-K. (2013). Fabrication of highly porous PMMA electrospun fibers and their application in the removal of phenol and iodine. *Journal of Polymer Research*, *20*(7), 158. https://doi.org/10.1007/s10965-013-0158-9

Bahrami, S. H., & Gholipour Kanani, A. (2011). Effect of changing solvents on poly(ε-Caprolactone) nanofibrous webs morphology. *Journal of Nanomaterials*, *2011*. https://doi.org/10.1155/2011/724153

Baji, A., Mai, Y.-W., Wong, S.-C., Abtahi, M., & Chen, P. (2010). Electrospinning of polymer nanofibers: Effects on oriented morphology, structures and tensile properties. *Composites Science and Technology*, *70*(5), 703–718. https://doi.org/10.1016/J.COMPSCITECH.2010.01.010

Bakar, S. S. S., Fong, K. C., Eleyas, A., & Nazeri, M. F. M. (2018). Effect of Voltage and Flow Rate Electrospinning Parameters on Polyacrylonitrile Electrospun Fibers. *IOP Conference Series: Materials Science and Engineering*, *318*, 12076. https://doi.org/10.1088/1757-899x/318/1/012076

Baum, C. L., & Arpey, C. J. (2005). Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatologic Surgery : Official Publication for American Society for Dermatologic Surgery [et Al.]*, *31*(6), 674–686; discussion 686. https://doi.org/10.1111/j.1524-4725.2005.31612

Bazbouz, M. B., & Stylios, G. K. (2010). The tensile properties of electrospun nylon 6 single nanofibers. *Journal of Polymer Science Part B: Polymer Physics*, *48*(15), 1719–1731. https://doi.org/10.1002/polb.21993

Behbood, L., Moradipour, P., Moradi, F., & Arkan, E. (2017). Mucoadhesive electrospun nanofibers of chitosan/gelatin containing vancomycin as a delivery system. *Journal of Reports in Pharmaceutical Sciences*, *6*(2), 150–160. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85047728269&partnerID=40&md5=0b903e819ce775a28380bad77e611086

Bhattarai, D. P., Aguilar, L. E., Park, C. H., & Kim, C. S. (2018). A Review on Properties of Natural and Synthetic Based Electrospun Fibrous Materials for Bone Tissue Engineering. In *Membranes* (Vol. 8, Issue 3). https://doi.org/10.3390/membranes8030062

Billings, N., Birjiniuk, A., Samad, T. S., Doyle, P. S., & Ribbeck, K. (2015). Material properties of biofilms-a review of methods for understanding permeability and mechanics. *Reports on Progress in Physics. Physical Society (Great Britain)*, *78*(3), 36601. https://doi.org/10.1088/0034-4885/78/3/036601

Blanpain, C., & Fuchs, E. (2009). Epidermal homeostasis: A balancing act of stem cells in the skin. *Nature Reviews Molecular Cell Biology*, *10*(3), 207–217. https://doi.org/10.1038/nrm2636

Boateng, J. S., Matthews, K. H., Stevens, H. N. E., & Eccleston, G. M. (2008). Wound healing dressings and drug delivery systems: a review. *Journal of Pharmaceutical Sciences*, *97*(8), 2892–2923. https://doi.org/10.1002/jps.21210

Bökel, C., & Brown, N. H. (2002). Integrins in development: moving on, responding to, and sticking to the extracellular matrix. *Developmental Cell*, *3*(3), 311–321. https://doi.org/10.1016/s1534-5807(02)00265-4

Borges, E. L., Frison, S. S., Honorato-Sampaio, K., Guedes, A. C. M., Lima, V. L. de A. N., Oliveira, O. M. M. de, Ferraz, A. F., & Tyrone, A. C. (2018). Effect of Polyhexamethylene Biguanide Solution on Bacterial Load and Biofilm in Venous Leg Ulcers: A Randomized Controlled Trial. *Journal of Wound, Ostomy, and Continence Nursing : Official Publication of The Wound, Ostomy and Continence Nurses Society*, *45*(5), 425–431. https://doi.org/10.1097/WON.0000000000000455

Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, *14*(2), 244–269. https://doi.org/10.1128/CMR.14.2.244-269.2001

Brackman, G., Cos, P., Maes, L., Nelis, H. J., & Coenye, T. (2011). Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. *Antimicrobial Agents and Chemotherapy*, *55*(6), 2655–2661. https://doi.org/10.1128/AAC.00045-11

Brem, H., Stojadinovic, O., Diegelmann, R. F., Entero, H., Lee, B., Pastar, I., Golinko, M., Rosenberg, H., & Tomic-Canic, M. (2007). Cholinergic Anti-Inflammatory Pathway Activity and High High Mobility Group Box-1 (HMGB1) Serum Levels in Patients with Rheumatoid Arthritis. *Molecular Medicine*, *13*(9), 30–39. https://doi.org/10.2119/2006

Britland, S., Ross-Smith, O., Jamil, H., Smith, A. G., Vowden, K., & Vowden, P. (2012). The lactate conundrum in wound healing: clinical and experimental findings indicate the requirement for a rapid point-of-care diagnostic. *Biotechnology Progress*, *28*(4), 917–924. https://doi.org/10.1002/btpr.1561

Brölmann, F. E., Ubbink, D. T., Nelson, E. A., Munte, K., van der Horst, C. M. A. M., & Vermeulen, H. (2012). Evidence-based decisions for local and systemic wound care. *The British Journal of Surgery*, *99*(9), 1172–1183. https://doi.org/10.1002/bjs.8810

Bunaciu, A. A., Aboul-Enein, H. Y., & Hoang, V. D. (2015). Vibrational spectroscopy used in polymorphic analysis. *TrAC Trends in Analytical Chemistry*, *69*, 14–22. https://doi.org/10.1016/j.trac.2015.02.006

Buschle-Diller, G., Cooper, J., Xie, Z., Wu, Y., Waldrup, J., & Ren, X. (2007). Release of antibiotics from electrospun bicomponent fibers. *Cellulose*, *14*(6), 553–562. https://doi.org/10.1007/s10570-007-9183-3

Cai, N., Han, C., Luo, X., Chen, G., Dai, Q., & Yu, F. (2017). Fabrication of Core/Shell Nanofibers with Desirable Mechanical and Antibacterial Properties by Pickering Emulsion Electrospinning. *Macromolecular Materials and Engineering*, *302*(3), 1600364. https://doi.org/10.1002/mame.201600364

Cambiaso-Daniel, J., Boukovalas, S., Bitz, G. H., Branski, L. K., Herndon, D. N., & Culnan, D. M. (2018). Topical Antimicrobials in Burn Care: Part 1-Topical Antiseptics. *Annals of Plastic Surgery*. https://doi.org/10.1097/SAP.0000000000001297

Cambiaso-Daniel, J., Boukovalas, S., Bitz, G. H., Branski, L. K., Herndon, D. N., & Culnan, D. M. (9000). Topical Antimicrobials in Burn Care: Part 1 – Topical Antiseptics. *Annals of Plastic Surgery*. https://journals.lww.com/annalsplasticsurgery/Fulltext/9000/Topical_Antimicrobials_in_Burn_Care__Part.97554.aspx

Carrizales, C., Pelfrey, S., Rincon, R., Eubanks, T. M., Kuang, A., McClure, M. J., Bowlin, G. L., & Macossay, J. (2008). Thermal and mechanical properties of electrospun PMMA, PVC, Nylon 6, and Nylon 6,6. *Polymers for Advanced Technologies*, *19*(2), 124–130. https://doi.org/10.1002/pat.981

Cha, J., & Falanga, V. (2007). Stem cells in cutaneous wound healing. *Clinics in Dermatology*, *25*(1), 73–78. https://doi.org/10.1016/j.clindermatol.2006.10.002

CHAO, T.-C., SAKTHINATHAN, S., CHIU, T.-W., & FU, Y. (2019). Anisotropic delafossite-type $CuFeO_2$ thin films deposited by electrospinning with rotating collector. *Journal of the Ceramic Society of Japan*, *127*(7), 498–503. https://doi.org/10.2109/jcersj2.19040

Chau, T. T., Bruckard, W. J., Koh, P. T. L., & Nguyen, A. V. (2009). A review of factors that affect contact angle and implications for flotation practice. *Advances in Colloid and Interface Science*, *150*(2), 106—115. https://doi.org/10.1016/j.cis.2009.07.003

Chen, H., Fan, X., Xia, J., Chen, P., Zhou, X., Huang, J., Yu, J., & Gu, P. (2011). Electrospun chitosan-graft-poly (ε-caprolactone)/poly (ε-caprolactone) nanofibrous scaffolds for retinal tissue engineering. *International Journal of Nanomedicine*, *6*, 453–461. https://doi.org/10.2147/IJN.S17057

Chen, H., Huang, J., Yu, J., Liu, S., & Gu, P. (2011). Electrospun chitosan-graft-poly (ε-caprolactone)/poly (ε-caprolactone) cationic nanofibrous mats as potential scaffolds for skin tissue engineering. *International Journal of Biological Macromolecules*, *48*(1), 13–19. https://doi.org/10.1016/j.ijbiomac.2010.09.019

Chen, S., Cui, S., Hu, J., Zhou, Y., & Liu, Y. (2017). Pectinate nanofiber mat with high absorbency and antibacterial activity: A potential superior wound dressing to alginate and chitosan nanofiber mats. *Carbohydrate Polymers*, *174*, 591–600. https://doi.org/10.1016/j.carbpol.2017.06.096

Chen, S., Ge, L., Mueller, A., Carlson, M. A., Teusink, M. J., Shuler, F. D., & Xie, J. (2017). Twisting electrospun nanofiber fine strips into functional sutures for sustained co-delivery of gentamicin and silver. *Nanomedicine: Nanotechnology, Biology and Medicine*, *13*(4), 1435–1445. https://doi.org/10.1016/j.nano.2017.01.016

Chen, S., Liu, B., Carlson, M. A., Gombart, A. F., Reilly, D. A., & Xie, J. (2017). Recent advances in electrospun nanofibers for wound healing. *Nanomedicine*, *12*(11), 1335–1352. https://doi.org/10.2217/nnm-2017-0017

Chen, X., & Schluesener, H. J. (2008). Nanosilver: a nanoproduct in medical application. *Toxicology Letters*, *176*(1), 1–12. https://doi.org/10.1016/j.toxlet.2007.10.004

Chen, Z., Wei, B., Mo, X., Lim, C. T., Ramakrishna, S., & Cui, F. (2009). Mechanical properties of electrospun collagen–chitosan complex single fibers and membrane. *Materials Science and Engineering: C*, *29*(8), 2428–2435. https://doi.org/10.1016/j.msec.2009.07.006

Cheng, L., Sun, X., Zhao, X., Wang, L., Yu, J., Pan, G., Li, B., Yang, H., Zhang, Y., & Cui, W. (2016). Surface biofunctional drug-loaded electrospun fibrous scaffolds for comprehensive repairing hypertrophic scars. *Biomaterials*, *83*, 169–181. https://doi.org/10.1016/j.biomaterials.2016.01.002

Cheng, W., Xu, R., Li, D., Bortolini, C., He, J., Dong, M., Besenbacher, F., Huang, Y.,

& Chen, M. (2016). Artificial extracellular matrix delivers TGFb1 regulating myofibroblast differentiation. *RSC Adv.*, *6*(26), 21922–21928. https://doi.org/10.1039/C5RA26164C

Cheng, Y., Feng, G., & Moraru, C. I. (2019). Micro-and nanotopography sensitive bacterial attachment mechanisms: A review. *Frontiers in Microbiology*, *10*(FEB), 1–17. https://doi.org/10.3389/fmicb.2019.00191

Chew, S. Y., Hufnagel, T. C., Lim, C. T., & Leong, K. W. (2006). Mechanical properties of single electrospun drug-encapsulated nanofibres. *Nanotechnology*, *17*(15), 3880–3891. https://doi.org/10.1088/0957-4484/17/15/045

Chew, S. Y., Wen, J., Yim, E. K. F., & Leong, K. W. (2005). Sustained release of proteins from electrospun biodegradable fibers. *Biomacromolecules*, *6*(4), 2017–2024. https://doi.org/10.1021/bm0501149

Chhabra, R. P., Richardson, J. F. (John F., & Chhabra, R. P. (2008). *Non-Newtonian flow and applied rheology : engineering applications*. Butterworth-Heinemann/Elsevier. https://www.sciencedirect.com/science/book/9780750685320

Chindera, K., Mahato, M., Kumar Sharma, A., Horsley, H., Kloc-Muniak, K., Kamaruzzaman, N. F., Kumar, S., McFarlane, A., Stach, J., Bentin, T., & Good, L. (2016a). The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. *Scientific Reports*, *6*(1), 23121. https://doi.org/10.1038/srep23121

Chindera, K., Mahato, M., Kumar Sharma, A., Horsley, H., Kloc-Muniak, K., Kamaruzzaman, N. F., Kumar, S., McFarlane, A., Stach, J., Bentin, T., & Good, L. (2016b). The antimicrobial polymer PHMB enters cells and selectively condenses bacterial chromosomes. *Scientific Reports*, *6*(1), 23121. https://doi.org/10.1038/srep23121

Choong, L. T., Yi, P., & Rutledge, G. C. (2015). Three-dimensional imaging of electrospun fiber mats using confocal laser scanning microscopy and digital image analysis. *Journal of Materials Science*, *50*(8), 3014–3030. https://doi.org/10.1007/s10853-015-8834-2

Chou, S.-F., & Woodrow, K. A. (2017). Relationships between mechanical properties and drug release from electrospun fibers of PCL and PLGA blends. *Journal of the Mechanical Behavior of Biomedical Materials*, *65*, 724–733. https://doi.org/10.1016/J.JMBBM.2016.09.004

Cianfarani, F., Toietta, G., Di Rocco, G., Cesareo, E., Zambruno, G., & Odorisio, T. (2013). Diabetes impairs adipose tissue-derived stem cell function and efficiency in promoting wound healing. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *21*(4), 545–553. https://doi.org/10.1111/wrr.12051

Coelho, D. S. (2020). *Electrospinning Technology: Designing Nanofibers toward Wound Healing Application* (B. Veleirinho (ed.); p. Ch. 9). IntechOpen. https://doi.org/10.5772/intechopen.81530

Cortez Tornello, P. R., Caracciolo, P. C., Cuadrado, T. R., & Abraham, G. A. (2014). Structural characterization of electrospun micro/nanofibrous scaffolds by liquid extrusion porosimetry: A comparison with other techniques. *Materials Science and Engineering C*, *41*, 335–342. https://doi.org/10.1016/j.polymer.2016.02.046

Croisier, F., Duwez, A. S., Jérôme, C., Léonard, A. F., Van Der Werf, K. O., Dijkstra, P. J., & Bennink, M. L. (2012a). Mechanical testing of electrospun PCL fibers. *Acta Biomaterialia*, *8*(1), 218–224. https://doi.org/10.1016/j.actbio.2011.08.015

Croisier, F., Duwez, A. S., Jérôme, C., Léonard, A. F., Van Der Werf, K. O., Dijkstra, P. J., & Bennink, M. L. (2012b). Mechanical testing of electrospun PCL fibers. *Acta Biomaterialia*, *8*(1), 218–224. https://doi.org/10.1016/j.actbio.2011.08.015

Cui, W., Li, X., Zhou, S., & Weng, J. (2008). Degradation patterns and surface wettability of electrospun fibrous mats. *Polymer Degradation and Stability*, *93*(3), 731–738. https://doi.org/10.1016/j.polymdegradstab.2007.12.002

Cui, W., Li, X., Zhu, X., Yu, G., Zhou, S., & Weng, J. (2006). *Investigation of Drug Release and Matrix Degradation of Electrospun Poly (DL-lactide) Fibers with Paracetanol Inoculation*. 1623–1629. https://doi.org/10.1021/bm060057z

D'Amato, A. R., Bramson, M. T. K., Corr, D. T., Puhl, D. L., Gilbert, R. J., & Johnson, J. (2018). Solvent Retention in Electrospun Fibers Affects Scaffold Mechanical Properties. *Electrospinning*, *2*(1), 15–28. https://doi.org/10.1515/esp-2018-0002

D'Amato, A. R., Schaub, N. J., Cardenas, J. M., Fiumara, A. S., Troiano, P. M., Fischetti, A., & Gilbert, R. J. (2017). Removal of Retained Electrospinning Solvent Prolongs Drug Release from Electrospun PLLA Fibers. *Polymer*, *123*, 121–127. https://doi.org/10.1016/j.polymer.2017.07.008

Daeschlein, G. (2013). Antimicrobial and antiseptic strategies in wound management. *International Wound Journal*, *10 Suppl 1*(Suppl 1), 9–14. https://doi.org/10.1111/iwj.12175

Dalgic, A. D., Koman, E., Karatas, A., Tezcaner, A., & Keskin, D. (2022). Natural origin bilayer pullulan-PHBV scaffold for wound healing applications. *Biomaterials Advances*, *134*, 112554. https://doi.org/10.1016/j.msec.2021.112554

Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica*, *67*(3), 217–223.

Dash, T. K., & Konkimalla, V. B. (2012). Poly-ϵ-caprolactone based formulations for drug delivery and tissue engineering: A review. *Journal of Controlled Release*, *158*(1), 15–33. https://doi.org/10.1016/j.jconrel.2011.09.064

Davies, D. (2003). Understanding biofilm resistance to antibacterial agents. *Nature Reviews. Drug Discovery*, *2*(2), 114–122. https://doi.org/10.1038/nrd1008

Davis, S. C., Harding, A., Gil, J., Parajon, F., Valdes, J., Solis, M., & Higa, A. (2017). Effectiveness of a polyhexanide irrigation solution on methicillin-resistant Staphylococcus aureus biofilms in a porcine wound model. *International Wound Journal*, *14*(6), 937–944. https://doi.org/10.1111/iwj.12734

De Cesare, F., Di Mattia, E., Zussman, E., & Macagnano, A. (2019). A study on the dependence of bacteria adhesion on the polymer nanofibre diameter. *Environmental Science: Nano*, *6*(3), 778–797. https://doi.org/10.1039/c8en01237g

De Rosa, L., & De Luca, M. (2012). Cell biology: Dormant and restless skin stem cells. In *Nature* (Vol. 489, Issue 7415, pp. 215–217). https://doi.org/10.1038/489215a

De Vrieze, S., Van Camp, T., Nelvig, A., Hagström, B., Westbroek, P., & De Clerck, K. (2009). The effect of temperature and humidity on electrospinning. *Journal of Materials Science*, *44*(5), 1357–1362. https://doi.org/10.1007/s10853-008-3010-6

Deitzel, J., Kleinmeyer, J., Harris, D., & Beck Tan, N. . (2001). The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, *42*(1), 261–272. https://doi.org/10.1016/S0032-3861(00)00250-0

Demir, M. M., Yilgor, I., Yilgor, E., & Erman, B. (2002). Electrospinning of polyurethane fibers. *Polymer*, *43*(11), 3303–3309. https://doi.org/10.1016/S0032-3861(02)00136-2

Dersch, R., Steinhart, M., Boudriot, U., Greiner, A., & Wendorff, J. H. (2005). Nanoprocessing of polymers: Applications in medicine, sensors, catalysis, photonics. *Polymers for Advanced Technologies*, *16*(2–3), 276–282. https://doi.org/10.1002/pat.568

Dhand, C., Venkatesh, M., Barathi, V. A., Harini, S., Bairagi, S., Goh Tze Leng, E., Muruganandham, N., Low, K. Z. W., Fazil, M. H. U. T., Loh, X. J., Srinivasan, D. K., Liu, S. P., Beuerman, R. W., Verma, N. K., Ramakrishna, S., & Lakshminarayanan, R. (2017). Bio-inspired crosslinking and matrix-drug interactions for advanced wound dressings with long-term antimicrobial activity. *Biomaterials*, *138*, 153–168. https://doi.org/10.1016/j.biomaterials.2017.05.043

Dhivya, S., Padma, V. V., & Santhini, E. (2015). Wound dressings – a review. *BioMedicine*, *5*(4), 22. https://doi.org/10.7603/s40681-015-0022-9

Ding, J., Zhang, J., Li, J., Li, D., Xiao, C., Xiao, H., Yang, H., Zhuang, X., & Chen, X. (2019). Electrospun polymer biomaterials. *Progress in Polymer Science*, *90*, 1–34. https://doi.org/https://doi.org/10.1016/j.progpolymsci.2019.01.002

Dissemond, J., Gerber, V., Kramer, A., Riepe, G., Strohal, R., Vasel-Biergans, A., & Eberlein, T. (2010). A practice-oriented recommendation for treatment of critically colonised and locally infected wounds using polihexanide. *Journal of Tissue Viability*, *19*(3), 106–115. https://doi.org/10.1016/j.jtv.2010.06.002

Dobrzański, L. A., Hudecki, A., Chladek, G., Król, W., & Mertas, A. (2014). Surface properties and antimicrobial activity of composite nanofibers of polycaprolactone with silver precipitations. *Archives of Materials Science and Engineering*, *70*(2), 53–60.

Doshi, J., & Reneker, D. H. (1995). Electrospinning process and applications of electrospun fibers. *Journal of Electrostatics*, *35*(2), 151–160. https://doi.org/10.1016/0304-3886(95)00041-8

Dou, S., Ke, X.-X., Zhong, L.-B., Fan, J.-J., Paul Chen, J., & Zheng, Y.-M. (2022). Novel ultraporous polyimide-based hollow carbon nanofiber mat: Its polymer-blend electrospinning preparation strategy and efficient dynamic adsorption for ciprofloxacin removal. *Separation and Purification Technology*, *295*, 121341. https://doi.org/10.1016/j.seppur.2022.121341

Doustgani, A. (2016). Optimization of mechanical and structural properties of PVA nanofibers. *Journal of Industrial Textiles*, *46*(3), 901–913. https://doi.org/10.1177/1528083715601511

Dowd, S. E., Sun, Y., Smith, E., Kennedy, J. P., Jones, C. E., & Wolcott, R. (2009). Effects of biofilm treatments on the multi-species Lubbock chronic wound biofilm model. *Journal of Wound Care*, *18*(12), 508, 510–512. https://doi.org/10.12968/jowc.2009.18.12.45608

Durán, N., Durán, M., de Jesus, M. B., Seabra, A. B., Fávaro, W. J., & Nakazato, G. (2016a). Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine*, *12*(3), 789–799. https://doi.org/10.1016/j.nano.2015.11.016

Durán, N., Durán, M., de Jesus, M. B., Seabra, A. B., Fávaro, W. J., & Nakazato, G. (2016b). Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine*, *12*(3), 789–799. https://doi.org/10.1016/j.nano.2015.11.016

Dwivedi, C., Pandey, H., Pandey, A. C., Patil, S., Ramteke, P. W., Laux, P., Luch, A., & Singh, A. V. (2019). In Vivo Biocompatibility of Electrospun Biodegradable Dual Carrier (Antibiotic + Growth Factor) in a Mouse Model-Implications for Rapid Wound Healing. *Pharmaceutics*, *11*(4). https://doi.org/10.3390/pharmaceutics11040180

Ehrlich, J., Bartz, Q. R., Smith, R. M., Joslyn, D. A., & Burkholder, P. R. (1947). Chloromycetin, a New Antibiotic From a Soil Actinomycete. *Science*, *106*(2757), 417. https://doi.org/10.1126/science.106.2757.417

Ekram, B., Abdel-Hady, B. M., El-kady, A. M., Amr, S. M., Waley, A. I., & Guirguis, O. W. (2017). Optimum parameters for the production of nano-scale electrospun poly-caprolactone to be used as a biomedical material. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *8*(4), 045018. https://doi.org/10.1088/2043-6254/aa92b4

El-Aassar, M. R., Ibrahim, O. M., Fouda, M. M. G., El-Beheri, N. G., & Agwa, M. M. (2020). Wound healing of nanofiber comprising Polygalacturonic/Hyaluronic acid embedded silver nanoparticles: In-vitro and in-vivo studies. *Carbohydrate Polymers*, *238*, 116175. https://doi.org/10.1016/j.carbpol.2020.116175

Ennis, W. J., Sui, A., & Bartholomew, A. (2013). Stem Cells and Healing: Impact on Inflammation. *Advances in Wound Care*, *2*(7), 369–378. https://doi.org/10.1089/wound.2013.0449

Fahimirad, S., & Ajalloueian, F. (2019). Naturally-derived electrospun wound dressings for target delivery of bio-active agents. *International Journal of Pharmaceutics*, *566*, 307–328. https://doi.org/10.1016/j.ijpharm.2019.05.053

Faralli, A., Shekarforoush, E., Ajalloueian, F., Mendes, A. C., & Chronakis, I. S. (2019). In vitro permeability enhancement of curcumin across Caco-2 cells monolayers using electrospun xanthan-chitosan nanofibers. *Carbohydrate Polymers*, *206*, 38–47. https://doi.org/10.1016/j.carbpol.2018.10.073

Farooque, T. M., Camp, C. H., Tison, C. K., Kumar, G., Parekh, S. H., & Simon, C. G. (2014). Measuring stem cell dimensionality in tissue scaffolds. *Biomaterials*, *35*(9), 2558–2567. https://doi.org/10.1016/j.biomaterials.2013.12.092

Fisher, T. K., Wolcott, R., Wolk, D. M., Bharara, M., Kimbriel, H. R., & Armstrong, D. G. (2010). Diabetic foot infections: A need for innovative assessments. *The International Journal of Lower Extremity Wounds*, *9*(1), 31–36. https://doi.org/10.1177/1534734610363459

Formhals A. (1934). *Process and apparatus for preparing artificial threads* (Patent No. U.S. patent 1975 504). U.S. patent 1975 504.

Frank, C., Bayoumi, I., & Westendorp, C. (2005). *Approach to infected skin ulcers*. *5*, 1352–1359.

Frykberg, R. G., & Banks, J. (2015). Challenges in the Treatment of Chronic Wounds. *Advances in Wound Care*, *4*(9), 560–582. https://doi.org/10.1089/wound.2015.0635

Fux, C. A., Costerton, J. W., Stewart, P. S., & Stoodley, P. (2005). Survival strategies of infectious biofilms. *Trends in Microbiology*, *13*(1), 34–40. https://doi.org/10.1016/j.tim.2004.11.010

Gaharwar, A. K., Peppas, N. A., & Khademhosseini, A. (2014). Nanocomposite hydrogels for biomedical applications. *Biotechnology and Bioengineering*, *111*(3), 441–453. https://doi.org/10.1002/bit.25160

Gao, C., Zhang, L., Wang, J., Jin, M., Tang, Q., Chen, Z., Cheng, Y., Yang, R., & Zhao, G. (2021). Electrospun nanofibers promote wound healing: theories, techniques, and perspectives. *Journal of Materials Chemistry B*, *9*(14), 3106–3130. https://doi.org/10.1039/D1TB00067E

Ge, J., Zhao, H.-Y., Zhu, H.-W., Huang, J., Shi, L.-A., & Yu, S.-H. (2016). Advanced Sorbents for Oil-Spill Cleanup: Recent Advances and Future Perspectives. *Advanced Materials*, *28*(47), 10459–10490. https://doi.org/10.1002/adma.201601812

Geng, X., Kwon, O.-H., & Jang, J. (2005). Electrospinning of chitosan dissolved in concentrated acetic acid solution. *Biomaterials*, *26*(27), 5427–5432. https://doi.org/10.1016/j.biomaterials.2005.01.066

Georgiade, N. G. H. (1973). Open and closed tratment of burns with povidone iodine. *Plastic and Reconstructive Surgery*, *52*(6). https://journals.lww.com/plasreconsurg/Fulltext/1973/12000/OPEN_AND_CLOSED_TREATMENT_OF_BURNS_WITH.6.aspx

Ghasemi-Mobarakeh, L., Kolahreez, D., Ramakrishna, S., & Williams, D. (2019). Key terminology in biomaterials and biocompatibility. *Current Opinion in Biomedical Engineering*, *10*, 45–50. https://doi.org/10.1016/j.cobme.2019.02.004

Ghasemi-Mobarakeh, L., Prabhakaran, M. P., Morshed, M., Nasr-Esfahani, M.-H., & Ramakrishna, S. (2008). Electrospun poly(epsilon-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials*, *29*(34), 4532–4539. https://doi.org/10.1016/j.biomaterials.2008.08.007

Giancotti, F. G., & Ruoslahti, E. (1999). Integrin signaling. *Science (New York, N.Y.)*, *285*(5430), 1028–1032. https://doi.org/10.1126/science.285.5430.1028

Gilbert, W. (1958). *De Magnete* (Dover Publications Inc (ed.)).

Giske, C. (2020). *T.E.C.o.A.S. Breakpoint Tables for Interpretation of MICs and Zone Diameters*. https://www.eucast.org/clinical_breakpoints/

Greenhalgh, D. G. (2009). Topical Antimicrobial Agents for Burn Wounds. *Clinics in Plastic Surgery*, *36*(4), 597–606. https://doi.org/10.1016/j.cps.2009.05.011

Gu, S.-Y., Wu, Q.-L., Ren, J., & Vancso, G. J. (2005). Mechanical Properties of a Single Electrospun Fiber and Its Structures. *Macromolecular Rapid Communications*, *26*(9), 716–720. https://doi.org/https://doi.org/10.1002/marc.200400667

Gullberg, D., & Ekblom, P. (1995). Extracellular matrix and its receptors during development. *The International Journal of Developmental Biology*, *39*(5), 845–854.

Guo, J., Lin, Z., Chang, C., Lu, C., & Chen, J. (2018). Applied Surface Science Protein valves prepared by click reaction grafting of poly (N-isopropylacrylamide) to electrospun poly (vinyl chloride) fibrous membranes. *Applied Surface Science*, *439*, 313–322. https://doi.org/10.1016/j.apsusc.2017.12.215

Haider, A., Gupta, K. C., & Kang, I.-K. (2014). Morphological Effects of HA on the Cell Compatibility of Electrospun HA/PLGA Composite Nanofiber Scaffolds. *BioMed Research International*, *2014*, 308306. https://doi.org/10.1155/2014/308306

Haider, A., Haider, S., & Kang, I. K. (2018). A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology. *Arabian Journal of Chemistry*, *11*(8), 1165–1188. https://doi.org/10.1016/j.arabjc.2015.11.015

Haider, A., Kim, S., Huh, M.-W., & Kang, I.-K. (2015). BMP-2 Grafted nHA/PLGA Hybrid Nanofiber Scaffold Stimulates Osteoblastic Cells Growth. *BioMed Research International*, *2015*, 281909. https://doi.org/10.1155/2015/281909

Hao, Y., Zhao, W., Zhang, L., Zeng, X., Sun, Z., Zhang, D., Shen, P., Li, Z., Han, Y., Li, P., & Zhou, Q. (2020). Bio-multifunctional alginate/chitosan/fucoidan sponges with enhanced angiogenesis and hair follicle regeneration for promoting full-thickness wound healing. *Materials & Design*, *193*, 108863. https://doi.org/10.1016/j.matdes.2020.108863

Hasbiyani, N. A. F., Hikmawati, D., & Siswanto. (2020). Electrospun Collagen-based Scaffold as Therapeutic Agent for Ocular Chemical Injury. *Journal of Physics: Conference Series*, *1445*(1), 7–15. https://doi.org/10.1088/1742-6596/1445/1/012022

Hawkins, M. J., Soon-Shiong, P., & Desai, N. (2008). Protein nanoparticles as drug carriers in clinical medicine. *Advanced Drug Delivery Reviews*, *60*(8), 876–885. https://doi.org/10.1016/j.addr.2007.08.044

He, C. L., Huang, Z. M., Han, X. J., Liu, L., Zhang, H. S., & Chen, L. S. (2006). Coaxial electrospun poly(L-lactic acid) ultrafine fibers for sustained drug delivery. *Journal of Macromolecular Science, Part B: Physics*, *45 B*(4), 515–524. https://doi.org/10.1080/00222340600769832

He, L., Chen, J., Farson, D. F., Lannutti, J. J., & Rokhlin, S. I. (2011). Wettability modification of electrospun poly(ε-caprolactone) fibers by femtosecond laser irradiation in different gas atmospheres. *Applied Surface Science*, *257*(8), 3547–3553. https://doi.org/10.1016/j.apsusc.2010.11.072

Helaine, S., & Kugelberg, E. (2014). Bacterial persisters: formation, eradication, and experimental systems. *Trends in Microbiology*, *22*(7), 417–424. https://doi.org/10.1016/j.tim.2014.03.008

Henriques, C., Vidinha, R., Botequim, D., Borges, J. P., & Silva, J. A. M. C. (2009). A systematic study of solution and processing parameters on nanofiber morphology using a new electrospinning apparatus. *Journal of Nanoscience and Nanotechnology*, *9*(6), 3535–3545. https://doi.org/10.1166/jnn.2009.ns27

Herman, T. F., & Bordoni, B. (2021). *Wound Classification*. StatPearls Publishing, Treasure Island (FL). http://europepmc.org/abstract/MED/32119343

Hill, K. E., Malic, S., McKee, R., Rennison, T., Harding, K. G., Williams, D. W., & Thomas, D. W. (2010). An in vitro model of chronic wound biofilms to test wound dressings and assess antimicrobial susceptibilities. *The Journal of Antimicrobial Chemotherapy*, *65*(6), 1195–1206. https://doi.org/10.1093/jac/dkq105

Hodgkinson, T., Yuan, X.-F., & Bayat, A. (2014). Electrospun silk fibroin fiber diameter influences in vitro dermal fibroblast behavior and promotes healing of ex vivo wound models. *Journal of Tissue Engineering*, *5*, 2041731414551661. https://doi.org/10.1177/2041731414551661

Høiby, N. (2017). A short history of microbial biofilms and biofilm infections. *APMIS : Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *125*(4), 272–275. https://doi.org/10.1111/apm.12686

Hong, S., & Kim, G. (2011). Fabrication of electrospun polycaprolactone biocomposites reinforced with chitosan for the proliferation of mesenchymal stem cells. *Carbohydrate Polymers*, *83*(2), 940–946. https://doi.org/10.1016/j.carbpol.2010.09.002

Hoss, S. (2005). Essential microbiology. In E. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ (Ed.), *British Library Cataloguing* (1st ed., Vol. 328, Issue Suppl S4). John Wiley & Sons Ltd.

Hotaling, N. A., Bharti, K., Kriel, H., & Simon, C. G. (2015). DiameterJ: A validated open source nanofiber diameter measurement tool. *Biomaterials*, *61*, 327–338. https://doi.org/10.1016/J.BIOMATERIALS.2015.05.015

Hou, H., Ge, J. J., Zeng, J., Li, Q., Reneker, D. H., Greiner, A., & Cheng, S. Z. D. (2005). Electrospun Polyacrylonitrile Nanofibers Containing a High Concentration of Well-Aligned Multiwall Carbon Nanotubes. *Chemistry of Materials*, *17*(5), 967–973. https://doi.org/10.1021/cm0484955

Hsu, L. C., Fang, J., Borca-Tasciuc, D. A., Worobo, R. W., & Moraru, C. I. (2013). Effect of micro- and nanoscale topography on the adhesion of bacterial cells to solid surfaces. *Applied and Environmental Microbiology*, *79*(8), 2703–2712. https://doi.org/10.1128/AEM.03436-12

Hu, M. X., Li, J. N., Guo, Q., Zhu, Y. Q., & Niu, H. M. (2019). Probiotics Biofilm-Integrated Electrospun Nanofiber Membranes: A New Starter Culture for Fermented Milk Production [Research-article]. *Journal of Agricultural and Food Chemistry*, *67*(11), 3198–3208. https://doi.org/10.1021/acs.jafc.8b05024

Huan, S., Liu, G., Cheng, W., Han, G., & Bai, L. (2018). Electrospun Poly(lactic acid)-Based Fibrous Nanocomposite Reinforced by Cellulose Nanocrystals: Impact of Fiber Uniaxial Alignment on Microstructure and Mechanical Properties. *Biomacromolecules*, *19*(3), 1037–1046. https://doi.org/10.1021/acs.biomac.8b00023

Huan, S., Liu, G., Han, G., Cheng, W., Fu, Z., Wu, Q., & Wang, Q. (2015). Effect of Experimental Parameters on Morphological, Mechanical and Hydrophobic Properties of Electrospun Polystyrene Fibers. In *Materials* (Vol. 8, Issue 5). https://doi.org/10.3390/ma8052718

Huang, C., Fu, X., Liu, J., Qi, Y., Li, S., & Wang, H. (2012). The involvement of integrin β1 signaling in the migration and myofibroblastic differentiation of skin fibroblasts on anisotropic collagen-containing nanofibers. *Biomaterials*, *33*(6), 1791–1800. https://doi.org/10.1016/j.biomaterials.2011.11.025

Huang, C., Tang, Y., Liu, X., Sutti, A., Ke, Q., Mo, X., Wang, X., Morsi, Y., & Lin, T. (2011). Electrospinning of nanofibres with parallel line surface texture for improvement of nerve cell growth. *Soft Matter*, *7*(22), 10812–10817. https://doi.org/10.1039/C1SM06430D

Huang, C., & Thomas, N. L. (2018). Fabricating porous poly(lactic acid) fibres via electrospinning. *European Polymer Journal*, *99*, 464–476. https://doi.org/10.1016/j.eurpolymj.2017.12.025

Huang, S. H., Chien, T. C., Hung, K. Y., & Chung, Y. C. (2010). Selective deposition of electrospun alginate-based nano-fibers on cell-repelling hydrogel surfaces for cell-based microarray. *14th International Conference on Miniaturized Systems for Chemistry and Life Sciences 2010, MicroTAS 2010*, *3*, 1892–1894. https://doi.org/10.2174/157341311794653703

Huang, S., Zhou, L., Li, M.-C., Wu, Q., Kojima, Y., & Zhou, D. (2016). Preparation and Properties of Electrospun Poly (Vinyl Pyrrolidone)/Cellulose Nanocrystal/Silver Nanoparticle Composite Fibers. In *Materials* (Vol. 9, Issue 7). https://doi.org/10.3390/ma9070523

Huang, Z. M., He, C. L., Yang, A., Zhang, Y., Han, X. J., Yin, J., & Wu, Q. (2006). Encapsulating drugs in biodegradable ultrafine fibers through co-axial electrospinning. *Journal of Biomedical Materials Research – Part A*, *77*(1), 169–179. https://doi.org/10.1002/jbm.a.30564

Huang, Z. M., Zhang, Y. Z., Kotaki, M., & Ramakrishna, S. (2003). A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites Science and Technology*, *63*(15), 2223–2253. https://doi.org/10.1016/S0266-3538(03)00178-7

Ian, C., & Marilyn, R. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews*, *65*(2), 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Ingavle, G. C., & Leach, J. K. (2014). Advancements in electrospinning of polymeric nanofibrous scaffolds for tissue engineering. *Tissue Engineering. Part B, Reviews*, *20*(4), 277–293. https://doi.org/10.1089/ten.TEB.2013.0276

Innocenti Malini, R., Lesage, J., Toncelli, C., Fortunato, G., Rossi, R. M., & Spano, F. (2019). Crosslinking dextran electrospun nanofibers via borate chemistry: Proof of concept for wound patches. *European Polymer Journal*, *110*, 276–282. https://doi.org/10.1016/j.eurpolymj.2018.11.017

Islam, M. M., Shahruzzaman, M., Biswas, S., Nurus Sakib, M., & Rashid, T. U. (2020). Chitosan based bioactive materials in tissue engineering applications-A review. *Bioactive Materials*, *5*(1), 164–183. https://doi.org/10.1016/j.bioactmat.2020.01.012

Islam, M. S., Ang, B. C., Andriyana, A., & Afifi, A. M. (2019). A review on fabrication of nanofibers via electrospinning and their applications. *SN Applied Sciences*, *1*(10), 1248. https://doi.org/10.1007/s42452-019-1288-4

Islam, M. S., & Yeum, J. H. (2013). Electrospun pullulan/poly(vinyl alcohol)/silver hybrid nanofibers: Preparation and property characterization for antibacterial activity. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *436*, 279–286. https://doi.org/10.1016/j.colsurfa.2013.07.001

Ito, M., Liu, Y., Yang, Z., Nguyen, J., Liang, F., Morris, R. J., & Cotsarelis, G. (2005). Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nature Medicine*, *11*(12), 1351–1354. https://doi.org/10.1038/nm1328

J. Zhang, H., Sellaiyan, S., Kakizaki, T., Uedono, A., Taniguchi, Y., & Hayashi, K. (2017). Effect of Free-Volume Holes on Dynamic Mechanical Properties of Epoxy Resins for Carbon-Fiber-Reinforced Polymers. *Macromolecules*, *50*(10), 3933–3942. https://doi.org/10.1021/acs.macromol.7b00472

Jakapon Sunthornvarabhas, Darrell H. Reneker, George G. Chase, O. (2012). *Bubble Launched Electrospinning Jets*.

James, G. A., Swogger, E., Wolcott, R., Pulcini, E. deLancey, Secor, P., Sestrich, J., Costerton, J. W., & Stewart, P. S. (2008). Biofilms in chronic wounds. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *16*(1), 37–44. https://doi.org/10.1111/j.1524-475X.2007.00321.x

Jeong, J. S., Moon, J. S., Jeon, S. Y., Park, J. H., Alegaonkar, P. S., & Yoo, J. B. (2007). Mechanical properties of electrospun PVA/MWNTs composite nanofibers. *Thin Solid Films*, *515*(12), 5136–5141. https://doi.org/10.1016/j.tsf.2006.10.058

Jhala, D., Rather, H., Kedaria, D., Shah, J., Singh, S., & Vasita, R. (2019). Biomimetic polycaprolactone-chitosan nanofibrous substrate influenced cell cycle and ECM secretion affect cellular uptake of nanoclusters. *Bioactive Materials*, *4*, 79–86. https://doi.org/10.1016/j.bioactmat.2018.12.004

Jian, S., Zhu, J., Jiang, S., Chen, S., Fang, H., Song, Y., Duan, G., Zhang, Y., & Hou, H. (2018). Nanofibers with diameter below one nanometer from electrospinning. *RSC Advances*, *8*(9), 4794–4802. https://doi.org/10.1039/c7ra13444d

Jiang, H., Hu, Y., Li, Y., Zhao, P., Zhu, K., & Chen, W. (2005). A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents. *Journal of Controlled Release*, *108*(2), 237–243. https://doi.org/10.1016/j.jconrel.2005.08.006

Jiang, Q., Reddy, N., & Yang, Y. (2010). Cytocompatible cross-linking of electrospun zein fibers for the development of water-stable tissue engineering scaffolds. *Acta Biomaterialia*, *6*(10), 4042–4051. https://doi.org/10.1016/j.actbio.2010.04.024

Jiang, S., Li, S. C., Huang, C., Chan, B. P., & Du, Y. (2018). Physical Properties of Implanted Porous Bioscaffolds Regulate Skin Repair: Focusing on Mechanical and Structural Features. *Advanced Healthcare Materials*, *7*(6), 1–17. https://doi.org/10.1002/adhm.201700894

Jirsak, O., Sanetrnik, F., Lukas, D., Kotek, V., Martinova, L., & Chaloupek, J. (2003). A method of nanofibres production from a polymer solution using electrostatic spinning and a device for carrying out the method. *1*(19), 1–6.

Jull, A. B., Arroll, B., Parag, V., & Waters, J. (2007). Pentoxifylline for treating venous leg ulcers. *Cochrane Database of Systematic Reviews*, *3*. https://doi.org/10.1002/14651858.CD001733.pub2

Juncos Bombin, A. D., Dunne, N. J., & McCarthy, H. O. (2020). Electrospinning of natural polymers for the production of nanofibres for wound healing applications. *Materials Science and Engineering: C*, *114*, 110994. https://doi.org/10.1016/j.msec.2020.110994

JY, P., SW, H., & IH, L. (2007). Preparation of Electrospun Porous Ethyl Cellulose Fiber by THF/DMAc Binary Solvent System. *Journal of Industrial and Engineering Chemistry*, *13*(6), 1002–1008. https://www.cheric.org/research/tech/periodicals/view.php?seq=692385

Kaiser, P., Wächter, J., & Windbergs, M. (2021). Therapy of infected wounds: overcoming clinical challenges by advanced drug delivery systems. *Drug Delivery and Translational Research*, *11*(4), 1545–1567. https://doi.org/10.1007/s13346-021-00932-7

Kanani, A. G., & Bahrami, S. H. (2011). Effect of Changing Solvents on Poly(ε-Caprolactone) Nanofibrous Webs Morphology. *J. Nanomaterials*, *2011*. https://doi.org/10.1155/2011/724153

Kanjanapongkul, K., Wongsasulak, S., & Yoovidhya, T. (2010). Investigation and prevention of clogging during electrospinning of zein solution. *Journal of Applied Polymer Science*, *118*(3), 1821–1829. https://doi.org/10.1002/app.32499

Kargar, M., Wang, J., Nain, A. S., & Behkam, B. (2012). Controlling bacterial adhesion to surfaces using topographical cues: a study of the interaction of Pseudomonas aeruginosa with nanofiber-textured surfaces. *Soft Matter*, *8*(40), 10254–10259. https://doi.org/10.1039/C2SM26368H

Katsogiannis, K. A. G., Vladisavljević, G. T., & Georgiadou, S. (2015a). Porous electrospun polycaprolactone (PCL) fibres by phase separation. *European Polymer Journal*, *69*, 284–295. https://doi.org/10.1016/j.eurpolymj.2015.01.028

Katsogiannis, K. A. G., Vladisavljević, G. T., & Georgiadou, S. (2015b). Porous electrospun polycaprolactone (PCL) fibres by phase separation. *European Polymer Journal*, *69*, 284–295. https://doi.org/10.1016/j.eurpolymj.2015.01.028

Kenawy, E.-R., Bowlin, G. L., Mansfield, K., Layman, J., Simpson, D. G., Sanders, E. H., & Wnek, G. E. (2002). Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. *Journal of Controlled Release*, *81*(1), 57–64. https://doi.org/10.1016/S0168-3659(02)00041-X

Kennedy, K. M., Bhaw-luximon, A., & Jhurry, D. (2017). Acta Biomaterialia Cell-matrix mechanical interaction in electrospun polymeric scaffolds for tissue engineering : Implications for scaffold design and performance. *Acta Biomaterialia*, *50*, 41–55. https://doi.org/10.1016/j.actbio.2016.12.034

Keren, I., Minami, S., Rubin, E., & Lewis, K. (2011). Characterization and transcriptome analysis of Mycobacterium tuberculosis persisters. *MBio*, *2*(3), e00100-11. https://doi.org/10.1128/mBio.00100-11

Khan, W., Bernier, S. P., Kuchma, S. L., Hammond, J. H., Hasan, F., & O'Toole, G. A. (2010). Aminoglycoside resistance of Pseudomonas aeruginosa biofilms modulated by extracellular polysaccharide. *International Microbiology : The Official Journal of the Spanish Society for Microbiology*, *13*(4), 207–212. https://doi.org/10.2436/20.1501.01.127

Khodadadi, M., Alijani, S., Montazeri, M., Esmaeilizadeh, N., Sadeghi-Soureh, S., & Pilehvar-Soltanahmadi, Y. (2020). Recent advances in electrospun nanofiber-mediated drug delivery strategies for localized cancer chemotherapy. *Journal of Biomedical Materials Research Part A*, *108*(7), 1444–1458. https://doi.org/10.1002/jbm.a.36912

Kim, G. H., Nam, H., Choi, W., An, T., & Lim, G. (2018). Electrospinning Nanofiber on an Insulating Surface with a Patterned Functional Electrolyte Electrode. *Advanced Materials Interfaces*, *5*(5), 1701204. https://doi.org/10.1002/admi.201701204

Kim, G. K., Trang, H., Rainer, A., & Trombetta, M. (2013). *Electrospinning of PCL / PVP blends for tissue engineering scaffolds*. 1425–1442. https://doi.org/10.1007/s10856-013-4893-6

Kimura, N., Sakumoto, T., Mori, Y., Wei, K., Kim, B.-S., Song, K.-H., & Kim, I.-S. (2014). Fabrication and characterization of reinforced electrospun poly(vinylidene fluoride-co-hexafluoropropylene) nanofiber membranes. *Composites Science and Technology*, *92*, 120–125. https://doi.org/10.1016/j.compscitech.2013.12.002

Kingsley, A. (2003). The wound infection continuum and its application to clinical practice. *Ostomy/Wound Management*, *49*(7A Suppl), 1–7.

Kohsari, I., Shariatinia, Z., & Pourmortazavi, S. M. (2016). Antibacterial electrospun chitosan–polyethylene oxide nanocomposite mats containing bioactive silver nanoparticles. *Carbohydrate Polymers*, *140*, 287–298. https://doi.org/10.1016/j.carbpol.2015.12.075

Kongkhlang, T., Kotaki, M., Kousaka, Y., Umemura, T., Nakaya, D., & Chirachanchai, S. (2008). Electrospun Polyoxymethylene: Spinning Conditions and Its Consequent Nanoporous Nanofiber. *Macromolecules*, *41*(13), 4746–4752. https://doi.org/10.1021/ma800731r

Kongkhlang, T., Tashiro, K., Kotaki, M., & Chirachanchai, S. (2008). Electrospinning as a New Technique To Control the Crystal Morphology and Molecular Orientation of Polyoxymethylene Nanofibers. *Journal of the American Chemical Society*, *130*(46), 15460–15466. https://doi.org/10.1021/ja804185s

Kuete, V., Karaosmanoğlu, O., & Sivas, H. (2017). *Chapter 10 – Anticancer Activities of African Medicinal Spices and Vegetables* (V. B. T.-M. S. and V. from A. Kuete (ed.); pp. 271–297). Academic Press. https://doi.org/10.1016/B978-0-12-809286-6.00010-8

L., H. L., & M., P. J. (2002). Third-Generation Biomedical Materials. *Science*, *295*(5557), 1014–1017. https://doi.org/10.1126/science.1067404

Laato, M., Niinikoski, J., Lundberg, C., & Gerdin, B. (1988). Inflammatory reaction and blood flow in experimental wounds inoculated with Staphylococcus aureus. *European Surgical Research. Europaische Chirurgische Forschung. Recherches Chirurgicales Europeennes*, *20*(1), 33–38. https://doi.org/10.1159/000128738

Lai, H.-J., Kuan, C.-H., Wu, H.-C., Tsai, J.-C., Chen, T.-M., Hsieh, D.-J., & Wang, T.-W. (2014). Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. *Acta Biomaterialia*, *10*(10), 4156–4166. https://doi.org/10.1016/j.actbio.2014.05.001

Laidmäe, I., Nieminen, H., Salmi, A., Paulin, T., Rauhala, T., Falck, K., Yliruusi, J., Heinämäki, J., & Haeggström, E. (2016). *Device and method to produce nanofibers (EP3274491B1)*.

Lasprilla-Botero, J., Álvarez-Láinez, M., & Lagaron, J. M. (2018). The influence of electrospinning parameters and solvent selection on the morphology and diameter of polyimide nanofibers. *Materials Today Communications*, *14*, 1–9. https://doi.org/10.1016/j.mtcomm.2017.12.003

Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Pecoraro, R. E., Rodeheaver, G., & Robson, M. C. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Archives of Dermatology*, *130*(4), 489–493.

Leaper, D., Assadian, O., & Edmiston, C. E. (2015a). Approach to chronic wound infections. *British Journal of Dermatology*, *173*(2), 351–358. https://doi.org/10.1111/bjd.13677

Leaper, D., Assadian, O., & Edmiston, C. E. (2015b). Approach to chronic wound infections. *British Journal of Dermatology*, *173*(2), 351–358. https://doi.org/10.1111/bjd.13677

Leaper, D. J., Schultz, G., Carville, K., Fletcher, J., Swanson, T., & Drake, R. (2012). Extending the TIME concept: what have we learned in the past 10 years?(*). *International Wound Journal*, *9 Suppl 2*(Suppl 2), 1–19. https://doi.org/10.1111/ j.1742-481X.2012.01097.x

Lee, E. J., Huh, B. K., Kim, S. N., Lee, J. Y., Park, C. G., Mikos, A. G., & Choy, Y. Bin. (2017). Application of Materials as Medical Devices with Localized Drug Delivery Capabilities for Enhanced Wound Repair. *Progress in Materials Science*, *89*, 392–410. https://doi.org/10.1016/j.pmatsci.2017.06.003

Lee, G., Song, J.-C., & Yoon, K.-B. (2010). Controlled Wall Thickness and Porosity of Polymeric Hollow Nanofibers by Coaxial Electrospinning. *Macromolecular Research*, *18*, 571–576. https://doi.org/10.1007/s13233-010-0607-9

Lee, J. M., Chae, T., Sheikh, F. A., Ju, H. W., Moon, B. M., Park, H. J., Park, Y. R., & Park, C. H. (2016). Three dimensional poly(ε-caprolactone) and silk fibroin nanocomposite fibrous matrix for artificial dermis. *Materials Science & Engineering. C, Materials for Biological Applications*, *68*, 758–767. https://doi.org/10.1016/j.msec.2016.06.019

Lee, J., Tae, G., Kim, Y. H., Park, I. S., Kim, S.-H., & Kim, S. H. (2008). The effect of gelatin incorporation into electrospun poly(l-lactide-co-ε-caprolactone) fibers on mechanical properties and cytocompatibility. *Biomaterials*, *29*(12), 1872–1879. https://doi.org/10.1016/j.biomaterials.2007.12.029

Lee, K. H., Kim, H. Y., Khil, M. S., Ra, Y. M., & Lee, D. R. (2003). Characterization of nano-structured poly(ε-caprolactone) nonwoven mats via electrospinning. *Polymer*, *44*(4), 1287–1294. https://doi.org/10.1016/S0032-3861(02)00820-0

Lencova, S., Svarcova, V., Stiborova, H., Demnerova, K., Jencova, V., Hozdova, K., & Zdenkova, K. (2021). Bacterial Biofilms on Polyamide Nanofibers: Factors Influencing Biofilm Formation and Evaluation. *ACS Applied Materials & Interfaces*, *13*(2), 2277–2288. https://doi.org/10.1021/acsami.0c19016

Li, H., Chen, X., Lu, W., Wang, J., Xu, Y., & Guo, Y. (2021). Application of Electrospinning in Antibacterial Field. *Nanomaterials (Basel, Switzerland)*, *11*(7). https://doi.org/10.3390/nano11071822

Li, Q., Liu, C., Zhao, X., Zu, Y., Wang, Y., Zhang, B., Zhao, D., Zhao, Q., Su, L., Gao, Y., & Sun, B. (2011). Preparation, characterization and targeting of micronized 10-hydroxycamptothecin-loaded folate-conjugated human serum albumin nanoparticles to cancer cells. *International Journal of Nanomedicine*, *6*, 397–405. https://doi.org/10.2147/IJN.S16144

Li, R., Tomasula, P., De Sousa, A. M., Liu, S.-C., Tunick, M., Liu, K., & Liu, L. (2017). Electrospinning Pullulan Fibers from Salt Solutions. In *Polymers* (Vol. 9, Issue 1). https://doi.org/10.3390/polym9010032

Li, W.-J., & Tuan, R. S. (2009). Fabrication and application of nanofibrous scaffolds in tissue engineering. *Current Protocols in Cell Biology*, *Chapter 25*, Unit-25.2. https://doi.org/10.1002/0471143030.cb2502s42

Li, W., Shi, L., Zhou, K., Zhang, X., Ullah, I., Ou, H., Zhang, W., & Wu, T. (2019). Facile fabrication of porous polymer fibers via cryogenic electrospinning system. *Journal of Materials Processing Technology*, *266*(June 2018), 551–557. https://doi.org/10.1016/j.jmatprotec.2018.11.035

Li, X.-Y., Li, Y.-C., Yu, D.-G., Liao, Y.-Z., & Wang, X. (2013). Fast Disintegrating Quercetin-Loaded Drug Delivery Systems Fabricated Using Coaxial Electrospinning. *International Journal of Molecular Sciences*, *14*(11), 21647–21659. https://doi.org/10.3390/ijms141121647

Li, X. Z., Livermore, D. M., & Nikaido, H. (1994). Role of efflux pump(s) in intrinsic resistance of Pseudomonas aeruginosa: resistance to tetracycline, chloramphenicol, and norfloxacin. *Antimicrobial Agents and Chemotherapy*, *38*(8), 1732–1741.

Li, Z., & Wang, C. (2013). *Effects of Working Parameters on Electrospinning BT – One-Dimensional nanostructures: Electrospinning Technique and Unique Nanofibers* (Z. Li & C. Wang (eds.); pp. 15–28). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-36427-3_2

Liang, M., Chen, X., Xu, Y., Zhu, L., Jin, X., & Huang, C. (2017). Double-grooved nanofibre surfaces with enhanced anisotropic hydrophobicity. *Nanoscale*, *9*(42), 16214–16222. https://doi.org/10.1039/C7NR05188C

Liu, C., Shen, J., Liao, C. Z., Yeung, K. W. K., & Tjong, S. C. (2018). Novel electrospun polyvinylidene fluoride-graphene oxide-silver nanocomposite membranes with protein and bacterial antifouling characteristics. *Express Polymer Letters*, *12*(4), 365–382. https://doi.org/10.3144/expresspolymlett.2018.31

Liu, L., Lin, Z., Niu, J., Tian, D., & He, J. (2019). Electrospun polysulfone/poly(lactic acid) nanoporous fibrous mats for oil removal from water. *Adsorption Science & Technology*, *37*(5–6), 438–450. https://doi.org/10.1177/0263617419828059

Liu, M., Zhang, Y., Sun, S., Khan, A. R., Ji, J., Yang, M., & Zhai, G. (2019). Recent advances in electrospun for drug delivery purpose. *Journal of Drug Targeting*, *27*(3), 270–282. https://doi.org/10.1080/1061186X.2018.1481413

Liu, W., Huang, C., & Jin, X. (2015). Electrospinning of Grooved Polystyrene Fibers: Effect of Solvent Systems. *Nanoscale Research Letters*, *10*(1), 237. https://doi.org/10.1186/s11671-015-0949-5

Liu, W., Zhu, L., Huang, C., & Jin, X. (2016). Direct Electrospinning of Ultrafine Fibers with Interconnected Macropores Enabled by in Situ Mixing Microfluidics. *ACS Applied Materials & Interfaces*, *8*(50), 34870–34878. https://doi.org/10.1021/acsami.6b11362

Liu, Y., & He, J.-H. (2007). Bubble Electrospinning for Mass Production of Nanofibers. *International Journal of Nonlinear Sciences and Numerical Simulation*, *8*, 393–396.

Liu, Z., Tang, Y., Zhao, K., & Zhang, Q. (2019). Superhydrophobic SiO2 micro/nanofibrous membranes with porous surface prepared by freeze electrospinning for oil adsorption. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *568*, 356–361. https://doi.org/10.1016/j.colsurfa.2019.02.038

Lobmann, R., Ambrosch, A., Schultz, G., Waldmann, K., Schiweck, S., & Lehnert, H. (2002). Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. *Diabetologia*, *45*(7), 1011–1016. https://doi.org/10.1007/s00125-002-0868-8

Loh, Q. L., & Choong, C. (2013). Three-Dimensional Scaffolds for Tissue Engineering Applications: Role of Porosity and Pore Size. *Tissue Engineering Part B: Reviews*, *19*(6), 485–502. https://doi.org/10.1089/ten.teb.2012.0437

Lopes-da-Silva, J. A., Veleirinho, B., & Delgadillo, I. (2009). Preparation and characterization of electrospun mats made of PET/chitosan hybrid nanofibers. *Journal of Nanoscience and Nanotechnology*, *9*(6), 3798–3804. https://doi.org/10.1166/jnn.2009.NS70

Lu, P., & Xia, Y. (2013). Maneuvering the Internal Porosity and Surface Morphology of Electrospun Polystyrene Yarns by Controlling the Solvent and Relative Humidity. *Langmuir*, *29*(23), 7070–7078. https://doi.org/10.1021/la400747y

Lu, S., Duan, X., Han, Y., & Huang, H. (2013). Silicone rubber/polyvinylpyrrolidone microfibers produced by coaxial electrospinning. *Journal of Applied Polymer Science*, *128*(4), 2273–2276. https://doi.org/10.1002/app.37848

Lu, Y., Huang, J., Yu, G., Cardenas, R., Wei, S., Wujcik, E. K., & Guo, Z. (2016). Coaxial electrospun fibers: applications in drug delivery and tissue engineering. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology*, *8*(5), 654–677. https://doi.org/10.1002/wnan.1391

Lubasova, D., & Martinova, L. (2011). Controlled Morphology of Porous Polyvinyl Butyral Nanofibers. *Journal of Nanomaterials*, *2011*, 292516. https://doi.org/10.1155/2011/292516

Luo, C. J., Nangrejo, M., & Edirisinghe, M. (2010). A novel method of selecting solvents for polymer electrospinning. *Polymer*, *51*(7), 1654–1662. https://doi.org/10.1016/j.polymer.2010.01.031

Luo, C. J., Stride, E., & Edirisinghe, M. (2012). Mapping the Influence of Solubility and Dielectric Constant on Electrospinning Polycaprolactone Solutions. *Macromolecules*, *45*(11), 4669–4680. https://doi.org/10.1021/ma300656u

Luraghi, A., Peri, F., & Moroni, L. (2021). Electrospinning for drug delivery applications: A review. *Journal of Controlled Release*, *334*, 463–484. https://doi.org/10.1016/j.jconrel.2021.03.033

Lv, F., Wang, J., Xu, P., Han, Y., Ma, H., Xu, H., Chen, S., Chang, J., Ke, Q., Liu, M., Yi, Z., & Wu, C. (2017). A conducive bioceramic/polymer composite biomaterial for diabetic wound healing. *Acta Biomaterialia*, *60*, 128–143. https://doi.org/10.1016/j.actbio.2017.07.020

M., H.-A. S. (1976). Comparison of Tetracycline Action on Staphylococcus aureus and Escherichia coli by Microbial Kinetics. *Antimicrobial Agents and Chemotherapy*, *10*(2), 223–228. https://doi.org/10.1128/AAC.10.2.223

M., S. M., & H., G. J. (2005). Exploring and Engineering the Cell Surface Interface. *Science*, *310*(5751), 1135–1138. https://doi.org/10.1126/science.1106587

Ma, B., Xie, J., Jiang, J., & Wu, J. (2014). Sandwich-type fiber scaffolds with square arrayed microwells and nanostructured cues as microskin grafts for skin regeneration. *Biomaterials*, *35*(2), 630–641. https://doi.org/10.1016/j.biomaterials.2013.09.111

Ma, M., Mao, Y., Gupta, M., K. Gleason, K., & C. Rutledge, G. (2005). Superhydrophobic Fabrics Produced by Electrospinning and Chemical Vapor Deposition. *Macromolecules*, *38*(23), 9742–9748. https://doi.org/10.1021/ma0511189

Maisonneuve, E., & Gerdes, K. (2014). Molecular mechanisms underlying bacterial persisters. *Cell*, *157*(3), 539–548. https://doi.org/10.1016/j.cell.2014.02.050

Marr, K. A., Sexton, D. J., Conlon, P. J., Corey, G. R., Schwab, S. J., & Kirkland, K. B. (1997). Catheter-related bacteremia and outcome of attempted catheter salvage in patients undergoing hemodialysis. *Annals of Internal Medicine*, *127*(4), 275–280. https://doi.org/10.7326/0003-4819-127-4-199708150-00003

Martínez-Pérez, C. A. (2020). *Electrospinning: A promising technique for drug delivery systems*. *59*(1), 441–454. https://doi.org/doi:10.1515/rams-2020-0041

Mathes, D. (2008). Grabb and Smith's Plastic Surgery. *JAMA*, *299*(20), 2450. https://doi.org/10.1001/jama.299.20.2450-a

Mayet, N., Choonara, Y. E., Kumar, P., Tomar, L. K., Tyagi, C., Du Toit, L. C., & Pillay, V. (2014). A comprehensive review of advanced biopolymeric wound healing systems. *Journal of Pharmaceutical Sciences*, *103*(8), 2211–2230. https://doi.org/10.1002/jps.24068

McCullen, S. D., Ramaswamy, S., Clarke, L. I., & Gorga, R. E. (2009). Nanofibrous composites for tissue engineering applications. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *1*(4), 369–390. https://doi.org/10.1002/wnan.39

McInnes, S., Macdonald, T., Parkin, I., Nann, T., & Voelcker, N. (2018). Electrospun Composites of Polycaprolactone and Porous Silicon Nanoparticles for the Tunable Delivery of Small Therapeutic Molecules. *Nanomaterials*, *8*(4), 205. https://doi.org/10.3390/nano8040205

Medeiros, I. M., & Saconato, H. (2001). Antibiotic prophylaxis for mammalian bites. *Cochrane Database of Systematic Reviews*, *2*. https://doi.org/10.1002/14651858.CD001738

Memic, A., Abdullah, T., Mohammed, H. S., Joshi Navare, K., Colombani, T., & Bencherif, S. A. (2019). Latest Progress in Electrospun Nanofibers for Wound Healing Applications. *ACS Applied Bio Materials*, *2*(3), 952–969. https://doi.org/10.1021/acsabm.8b00637

Meng, N., Zheng, Y., & Xin, B. (2019). Surface morphologies of electrospun polystyrene fibers induced by an electric field. *Textile Research Journal*, *89*(18), 3850–3859. https://doi.org/10.1177/0040517518824843

Metreveli, G., Wågberg, L., Emmoth, E., Belák, S., Strømme, M., & Mihranyan, A. (2014). A Size-Exclusion Nanocellulose Filter Paper for Virus Removal. *Advanced Healthcare Materials*, *3*(10), 1546–1550. https://doi.org/10.1002/adhm.201300641

Miguel, S. P., Figueira, D. R., Simões, D., Ribeiro, M. P., Coutinho, P., Ferreira, P., & Correia, I. J. (2018). Electrospun polymeric nanofibres as wound dressings: A review. *Colloids and Surfaces B: Biointerfaces*, *169*, 60–71. https://doi.org/10.1016/j.colsurfb.2018.05.011

Mihai, M. M., Dima, M. B., Dima, B., & Holban, A. M. (2019). Nanomaterials for Wound Healing and Infection Control. *Materials*, *12*(13). https://doi.org/10.3390/ma12132176

Miller, M. B., & Bassler, B. L. (2001). Quorum Sensing in Bacteria. *Annual Review of Microbiology*, *55*(1), 165–199. https://doi.org/10.1146/annurev.micro.55.1.165

Mirhaj, M., Labbaf, S., Tavakoli, M., & Seifalian, A. M. (2022). Emerging treatment strategies in wound care. *International Wound Journal*, *n/a*(n/a). https://doi.org/10.1111/iwj.13786

Mitik-Dineva, N., Stoddart, P. R., Crawford, R., & Ivanova, E. P. (2006). Adhesion of Bacteria. In *Wiley Encyclopedia of Biomedical Engineering*. https://doi.org/10.1002/9780471740360.ebs0027

Mitik-Dineva, N., Wang, J., Truong, V. K., Stoddart, P., Malherbe, F., Crawford, R. J., & Ivanova, E. P. (2009). Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus Attachment Patterns on Glass Surfaces with Nanoscale Roughness. *Current Microbiology*, *58*(3), 268–273. https://doi.org/10.1007/s00284-008-9320-8

Mofidfar, M., Kim, E. S., Larkin, E. L., Long, L., Jennings, W. D., Ahadian, S., Ghannoum, M. A., & Wnek, G. E. (2019). Antimicrobial Activity of Silver Containing Crosslinked Poly(Acrylic Acid) Fibers. *Micromachines*, *10*(12), 829. https://doi.org/10.3390/mi10120829

Molnar, K., Varga, R., Jozsa, B., Barczikai, D., Krisch, E., Nagy, K. S., Varga, G., Jedlovszky-Hajdu, A., & Puskas, J. E. (2020). Investigation of the Cytotoxicity of Electrospun Polysuccinimide-Based Fiber Mats. *Polymers*, *12*(10). https://doi.org/10.3390/polym12102324

Molnár, K., & Vas, L. M. (2012). Electrospun Composite Nanofibers and Polymer Composites. In *Synthetic Polymer-Polymer Composites* (pp. 301–349). Carl Hanser Verlag GmbH & Co. KG. https://doi.org/10.3139/9781569905258.010

Moradi, G., Rajabi, L., Dabirian, F., & Zinadini, S. (2018). *Biofouling alleviation and flux enhancement of electrospun PAN microfiltration membranes by embedding of para- aminobenzoate alumoxane nanoparticles*. *1*, 1–13. https://doi.org/10.1002/app.45738

Morita, Y., Tomida, J., & Kawamura, Y. (2013). Responses of Pseudomonas aeruginosa to antimicrobials. *Frontiers in Microbiology*, *4*(JAN), 1–8. https://doi.org/10.3389/fmicb.2013.00422

Movahedi, M., Asefnejad, A., Rafienia, M., & Khorasani, M. T. (2020). Potential of novel electrospun core-shell structured polyurethane/starch (hyaluronic acid) nanofibers for skin tissue engineering: In vitro and in vivo evaluation. *International Journal of Biological Macromolecules*, *146*, 627–637. https://doi.org/10.1016/j.ijbiomac.2019.11.233

Mubyana, K., Koppes, R. A., Lee, K. L., Cooper, J. A., & Corr, D. T. (2016). The influence of specimen thickness and alignment on the material and failure properties of electrospun polycaprolactone nanofiber mats. *Journal of Biomedical Materials Research Part A*, *104*(11), 2794–2800. https://doi.org/10.1002/jbm.a.35821

Mulder, G. D., Cavorsi, J. P., & Lee, D. K. (2007). Polyhexamethylene Biguanide (PHMB): An Addendum to Current Topical Antimicrobials. *Wounds: A Compendium of Clinical Research and Practice*, *19*(7), 173–182.

Nagy, Z. K., Wagner, I., Suhajda, Á., Tobak, T., Harasztos, A. H., Vigh, T., Sóti, P. L., Pataki, H., Molnár, K., & Marosi, G. (2014). Nanofibrous solid dosage form of living bacteria prepared by electrospinning. *Express Polymer Letters*, *8*(5), 352–361. https://doi.org/10.3144/expresspolymlett.2014.39

Nair, P. A., & Parameswaran, R. (2016). Synthesis, Characterization, and Electrospinning of Calcium-Containing Polyurethane Urea. *Advances in Polymer Technology*, *35*(3), 326–334. https://doi.org/10.1002/adv.21559

Naseri, N., Mathew, A. P., Girandon, L., Fröhlich, M., & Oksman, K. (2015). Porous electrospun nanocomposite mats based on chitosan–cellulose nanocrystals for wound dressing: effect of surface characteristics of nanocrystals. *Cellulose*, *22*(1), 521–534. https://doi.org/10.1007/s10570-014-0493-y

Nasouri, K., Shoushtari, A. M., & Mojtahedi, M. R. M. (2015). Effects of polymer/solvent systems on electrospun polyvinylpyrrolidone nanofiber morphology and diameter. *Polymer Science Series A*, *57*(6), 747–755. https://doi.org/10.1134/S0965545X15060164

Natu, V., Sousa, H. C. De, & Gil, M. H. (2010). *Effects of drug solubility , state and loading on controlled release in bicomponent electrospun fibers*. *397*, 50–58. https://doi.org/10.1016/j.ijpharm.2010.06.045

Nazemi, K., Moztarzadeh, F., Jalali, N., Asgari, S., & Mozafari, M. (2014). Synthesis and characterization of poly(lactic-co-glycolic) acid nanoparticles-loaded chitosan/ bioactive glass scaffolds as a localized delivery system in the bone defects. *BioMed Research International*, *2014*, 898930. https://doi.org/10.1155/2014/898930

Neill, U. S. (2014). A conversation with Marc Feldmann. *The Journal of Clinical Investigation*, *124*(1), 1–2. https://doi.org/10.1172/JCI71860

Ng, J.-J., & Supaphol, P. (2018). Rotating-disk electrospinning: needleless electrospinning of poly(caprolactone), poly(lactic acid) and poly(vinyl alcohol) nanofiber mats with controlled morphology. *Journal of Polymer Research*, *25*(7), 155. https:// doi.org/10.1007/s10965-018-1540-4

Nguyen, T. T. T., Ghosh, C., Hwang, S.-G., Chanunpanich, N., & Park, J. S. (2012). Porous core/sheath composite nanofibers fabricated by coaxial electrospinning as a potential mat for drug release system. *International Journal of Pharmaceutics*, *439* (1–2), 296–306. https://doi.org/10.1016/j.ijpharm.2012.09.019

Nickel, J. C., Ruseska, I., Wright, J. B., & Costerton, J. W. (1985). Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. *Antimicrobial Agents and Chemotherapy*, *27*(4), 619–624. https://doi.org/10.1128/ AAC.27.4.619

Ninan, N., Muthiah, M., Park, I.-K., Wong, T. W., Thomas, S., & Grohens, Y. (2015). Natural Polymer/Inorganic Material Based Hybrid Scaffolds for Skin Wound Healing. *Polymer Reviews*, *55*(3), 453–490. https://doi.org/10.1080/15583724.2015.1019135

Norouzi, M., Abdali, Z., Liu, S., & Miller, D. W. (2018). Salinomycin-loaded Nanofibers for Glioblastoma Therapy. *Scientific Reports*, *8*(1), 9377. https://doi.org/10.1038/ s41598-018-27733-2

O'Mathúna, D. P. (2016). Therapeutic touch for healing acute wounds. *The Cochrane Database of Systematic Reviews*, *9*(9), CD002766. https://doi.org/10.1002/14651858. CD002766.pub6

Oates, A., Bowling, F. L., Boulton, A. J. M., Bowler, P. G., Metcalf, D. G., & McBain, A. J. (2014). The visualization of biofilms in chronic diabetic foot wounds using routine diagnostic microscopy methods. *Journal of Diabetes Research*, *2014*, 153586. https://doi.org/10.1155/2014/153586

Oates, A., Lindsay, S., Mistry, H., Ortega, F., & McBain, A. J. (2018). Modelling antisepsis using defined populations of facultative and anaerobic wound pathogens grown in a basally perfused biofilm model. *Biofouling*, *34*(5), 507–518. https://doi.org/ 10.1080/08927014.2018.1466115

Okuda, T., Tominaga, K., & Kidoaki, S. (2010). Time-programmed dual release formulation by multilayered drug-loaded nanofiber meshes. *Journal of Controlled Release*, *143*(2), 258–264. https://doi.org/10.1016/j.jconrel.2009.12.029

Omer, S., Forgách, L., Zelkó, R., & Sebe, I. (2021). Scale-up of Electrospinning: Market Overview of Products and Devices for Pharmaceutical and Biomedical Purposes. *Pharmaceutics*, *13*(2). https://doi.org/10.3390/pharmaceutics13020286

Oubekka, S. D., Briandet, R., Fontaine-Aupart, M.-P., & Steenkeste, K. (2012). Correlative Time-Resolved Fluorescence Microscopy To Assess Antibiotic Diffusion-Reaction in Biofilms. *Antimicrobial Agents and Chemotherapy*, *56*(6), 3349–3358. https://doi.org/10.1128/AAC.00216-12

Pailler-Mattei, C., Bec, S., & Zahouani, H. (2008). In vivo measurements of the elastic mechanical properties of human skin by indentation tests. *Medical Engineering and Physics*, *30*(5), 599–606. https://doi.org/10.1016/j.medengphy.2007.06.011

Pal, P., Dadhich, P., Srivas, P. K., Das, B., Maulik, D., & Dhara, S. (2017). Bilayered nanofibrous 3D hierarchy as skin rudiment by emulsion electrospinning for burn wound management. *Biomater. Sci.*, *5*(9), 1786–1799. https://doi.org/10.1039/C7BM00174F

Pal, P., Srivas, P. K., Dadhich, P., Das, B., Maulik, D., & Dhara, S. (2017). Nano-/Microfibrous Cotton-Wool-Like 3D Scaffold with Core–Shell Architecture by Emulsion Electrospinning for Skin Tissue Regeneration. *ACS Biomaterials Science & Engineering*, *3*(12), 3563–3575. https://doi.org/10.1021/acsbiomaterials.7b00681

Paliwal, R., & Palakurthi, S. (2014). Zein in controlled drug delivery and tissue engineering. *Journal of Controlled Release*, *189*, 108–122. https://doi.org/10.1016/j.jconrel.2014.06.036

Pan, C. T., Yen, C. K., Wang, S. Y., Lai, Y. C., Lin, L., Huang, J. C., & Kuo, S. W. (2015). Near-field electrospinning enhances the energy harvesting of hollow PVDF piezoelectric fibers. *RSC Advances*, *5*(103), 85073–85081. https://doi.org/10.1039/c5ra16604g

Papkov, D., Zou, Y., Andalib, M. N., Goponenko, A., Cheng, S. Z. D., & Dzenis, Y. A. (2013). Simultaneously Strong and Tough Ultrafine Continuous Nanofibers. *ACS Nano*, *7*(4), 3324–3331. https://doi.org/10.1021/nn400028p

Parani, M., Lokhande, G., Singh, A., & Gaharwar, A. K. (2016). Engineered Nanomaterials for Infection Control and Healing Acute and Chronic Wounds. *ACS Applied Materials & Interfaces*, *8*(16), 10049–10069. https://doi.org/10.1021/acsami.6b00291

Percival, S. L., Finnegan, S., Donelli, G., Vuotto, C., Rimmer, S., & Lipsky, B. A. (2016). Antiseptics for treating infected wounds: Efficacy on biofilms and effect of pH. *Critical Reviews in Microbiology*, *42*(2), 293–309. https://doi.org/10.3109/1040841X.2014.940495

Percival, S. L., Hill, K. E., Williams, D. W., Hooper, S. J., Thomas, D. W., & Costerton, J. W. (2012). A review of the scientific evidence for biofilms in wounds. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *20*(5), 647–657. https://doi.org/10.1111/j.1524-475X.2012.00836.x

Percival, S. L., McCarty, S., Hunt, J. A., & Woods, E. J. (2014). The effects of pH on wound healing, biofilms, and antimicrobial efficacy. *Wound Repair and Regeneration*, *22*(2), 174–186. https://doi.org/10.1111/wrr.12125

Percival, S. L., McCarty, S. M., & Lipsky, B. (2015). Biofilms and Wounds: An Overview of the Evidence. *Advances in Wound Care*, *4*(7), 373–381. https://doi.org/10.1089/wound.2014.0557

Pezeshki-Modaress, M., Zandi, M., & Rajabi, S. (2018). Tailoring the gelatin/chitosan electrospun scaffold for application in skin tissue engineering: an in vitro study. *Progress in Biomaterials*, *7*(3), 207–218. https://doi.org/10.1007/s40204-018-0094-1

Pham, Q. P., Sharma, U., & Mikos, A. G. (2006). Electrospun poly (ε-caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: Characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules*, *7*(10), 2796–2805. https://doi.org/10.1021/bm060680j

Pisani, S., Dorati, R., Chiesa, E., Genta, I., Modena, T., Bruni, G., Grisoli, P., & Conti, B. (2019). Release Profile of Gentamicin Sulfate from Polylactide-co-Polycaprolactone Electrospun Nanofiber Matrices. *Pharmaceutics*, *11*(4). https://doi.org/10.3390/pharmaceutics11040161

Plopper, G. E., McNamee, H. P., Dike, L. E., Bojanowski, K., & Ingber, D. E. (1995). Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. *Molecular Biology of the Cell*, *6*(10), 1349–1365. https://doi.org/10.1091/mbc.6.10.1349

Pok, S. W., Wallace, K. N., & Madihally, S. V. (2010). In vitro characterization of polycaprolactone matrices generated in aqueous media. *Acta Biomaterialia*, *6*(3), 1061–1068. https://doi.org/10.1016/J.ACTBIO.2009.08.002

Pompa-Monroy, D. A., Figueroa-Marchant, P. G., Dastager, S. G., Thorat, M. N., Iglesias, A. L., Miranda-Soto, V., Pérez-González, G. L., & Villarreal-Gómez, L. J. (2020). Bacterial Biofilm Formation Using PCL/Curcumin Electrospun Fibers and Its Potential Use for Biotechnological Applications. *Materials (Basel, Switzerland)*, *13*(23). https://doi.org/10.3390/ma13235556

Preem, L., Bock, F., Hinnu, M., Putrinš, M., Sagor, K., Tenson, T., Meos, A., Østergaard, J., & Kogermann, K. (2019). Monitoring of antimicrobial drug chloramphenicol release from electrospun nano-and microfiber mats using UV imaging and bacterial bioreporters. *Pharmaceutics*, *11*(9), 1–19. https://doi.org/10.3390/pharmaceutics11090487

Preem, L., & Kogermann, K. (2018). *Electrospun Antimicrobial Wound Dressings: Novel Strategies to Fight Against Wound Infections*. 213–253. https://doi.org/10.1007/15695_2018_133

Preem, L., Mahmoudzadeh, M., Putrinš, M., Meos, A., Laidmäe, I., Romann, T., Aruväli, J., Härmas, R., Koivuniemi, A., Bunker, A., Tenson, T., & Kogermann, K. (2017). Interactions between Chloramphenicol, Carrier Polymers, and Bacteria-Implications for Designing Electrospun Drug Delivery Systems Countering Wound Infection. *Molecular Pharmaceutics*, *14*(12), 4417–4430. https://doi.org/10.1021/acs.molpharmaceut.7b00524

Preem, L., Vaarmets, E., Meos, A., Jõgi, I., Putrinš, M., Tenson, T., & Kogermann, K. (2019). Effects and efficacy of different sterilization and disinfection methods on electrospun drug delivery systems. *International Journal of Pharmaceutics*, *567*, 118450. https://doi.org/10.1016/j.ijpharm.2019.118450

Qin, S., Clark, R. A. F., & Rafailovich, M. H. (2015). Establishing correlations in the en-mass migration of dermal fibroblasts on oriented fibrillar scaffolds. *Acta Biomaterialia*, *25*, 230–239. https://doi.org/10.1016/j.actbio.2015.06.030

Qu, H., Wei, S., & Guo, Z. (2013). Coaxial electrospun nanostructures and their applications. *Journal of Materials Chemistry A*, *1*(38), 11513–11528. https://doi.org/10.1039/C3TA12390A

Rahmati, M., Mills, D. K., Urbanska, A. M., Saeb, M. R., Venugopal, J. R., Ramakrishna, S., & Mozafari, M. (2021). Electrospinning for tissue engineering applications. *Progress in Materials Science*, *117*, 100721. https://doi.org/10.1016/j.pmatsci.2020.100721

Ramakrishna, S., Fujihara, K., Teo, W.-E., Lim, T.-C., & Ma, Z. (2005). *An Introduction to Electrospinning and Nanofibers*. WORLD SCIENTIFIC. https://doi.org/10.1142/5894

Ramos, C., Lanno, G.-M., Laidmäe, I., Meos, A., Härmas, R., & Kogermann, K. (2020). High humidity electrospinning of porous fibers for tuning the release of drug delivery systems. *The International Journal of Poymeric Materials and Polymeric Biomaterials*. https://doi.org/10.1080/00914037.2020.1765361

Rashid, T. U., Gorga, R. E., & Krause, W. E. (2021). Mechanical Properties of Electrospun Fibers—A Critical Review. *Advanced Engineering Materials*, *23*(9), 2100153. https://doi.org/10.1002/adem.202100153

Rashtchian, M., Hivechi, A., Bahrami, S. H., Milan, P. B., & Simorgh, S. (2020). Fabricating alginate/poly(caprolactone) nanofibers with enhanced bio-mechanical properties via cellulose nanocrystal incorporation. *Carbohydrate Polymers*, *233*, 115873. https://doi.org/10.1016/j.carbpol.2020.115873

Ren, X., Han, Y., Wang, J., Jiang, Y., Yi, Z., Xu, H., & Ke, Q. (2018). An aligned porous electrospun fibrous membrane with controlled drug delivery – An efficient strategy to accelerate diabetic wound healing with improved angiogenesis. *Acta Biomaterialia*, *70*, 140–153. https://doi.org/10.1016/j.actbio.2018.02.010

Repanas, A., Andriopoulou, S., & Glasmacher, B. (2016a). The significance of electrospinning as a method to create fibrous scaffolds for biomedical engineering and drug delivery applications. *Journal of Drug Delivery Science and Technology*, *31*, 137–146. https://doi.org/10.1016/j.jddst.2015.12.007

Repanas, A., Andriopoulou, S., & Glasmacher, B. (2016b). The significance of electrospinning as a method to create fibrous scaffolds for biomedical engineering and drug delivery applications. *Journal of Drug Delivery Science and Technology*, *31*, 137–146. https://doi.org/10.1016/j.jddst.2015.12.007

Rheinecker, S. B. (1995). Wound management: the occlusive dressing. *Journal of Athletic Training*, *30*(2), 143–146.

Rho, K. S., Jeong, L., Lee, G., Seo, B.-M., Park, Y. J., Hong, S.-D., Roh, S., Cho, J. J., Park, W. H., & Min, B.-M. (2006). Electrospinning of collagen nanofibers: Effects on the behavior of normal human keratinocytes and early-stage wound healing. *Biomaterials*, *27*(8), 1452–1461. https://doi.org/10.1016/j.biomaterials.2005.08.004

Richard-Lacroix, M., & Pellerin, C. (2013). Molecular Orientation in Electrospun Fibers: From Mats to Single Fibers. *Macromolecules*, *46*(24), 9473–9493. https://doi.org/10.1021/ma401681m

Rieger, K. A., Birch, N. P., & Schiffman, J. D. (2013). Designing electrospun nanofiber mats to promote wound healing – a review. *Journal of Materials Chemistry B*, *1*(36), 4531–4541. https://doi.org/10.1039/c3tb20795a

Rodriguez-Menocal, L., Salgado, M., Ford, D., & Van Badiavas, E. (2012). Stimulation of skin and wound fibroblast migration by mesenchymal stem cells derived from normal donors and chronic wound patients. *Stem Cells Translational Medicine*, *1*(3), 221–229. https://doi.org/10.5966/sctm.2011-0029

Rychter, M., Baranowska-Korczyc, A., Milanowski, B., Jarek, M., Maciejewska, B. M., Coy, E. L., & Lulek, J. (2018). Cilostazol-Loaded Poly(ε-Caprolactone) Electrospun Drug Delivery System for Cardiovascular Applications. *Pharmaceutical Research*, *35*(2), 32. https://doi.org/10.1007/s11095-017-2314-0

S. Sharma, A. Dua, A. M. (2014). Third Generation Materials for Wound Dressings. *International Journal of Pharmaceutical Sciences and Research*, *5*(6), 2113–2124. http://dx.doi.org/10.13040/IJPSR.0975-8232.5(6).2113-24

Sadeghi, A. R., Nokhasteh, S., Molavi, A. M., Khorsand-Ghayeni, M., Naderi-Meshkin, H., & Mahdizadeh, A. (2016a). Surface modification of electrospun PLGA scaffold with collagen for bioengineered skin substitutes. *Materials Science and Engineering: C*, *66*, 130–137. https://doi.org/10.1016/j.msec.2016.04.073

Sadeghi, A. R., Nokhasteh, S., Molavi, A. M., Khorsand-Ghayeni, M., Naderi-Meshkin, H., & Mahdizadeh, A. (2016b). Surface modification of electrospun PLGA scaffold with collagen for bioengineered skin substitutes. *Materials Science and Engineering: C*, *66*, 130–137. https://doi.org/10.1016/j.msec.2016.04.073

Salcido, R. (2007). What is bioburden? The link to chronic wounds. *Advances in Skin & Wound Care*, *20*(7), 368. https://doi.org/10.1097/01.ASW.0000280205.88754.57

Savkovic, V., Flämig, F., Schneider, M., Sülflow, K., Loth, T., Lohrenz, A., Hacker, M. C., Schulz-Siegmund, M., & Simon, J.-C. (2016). Polycaprolactone fiber meshes provide a 3D environment suitable for cultivation and differentiation of melanocytes from the outer root sheath of hair follicle. *Journal of Biomedical Materials Research Part A*, *104*(1), 26–36. https://doi.org/10.1002/jbm.a.35536

Scalise, A., Bianchi, A., Tartaglione, C., Bolletta, E., Pierangeli, M., Torresetti, M., Marazzi, M., & Di Benedetto, G. (2015). Microenvironment and microbiology of skin wounds: the role of bacterial biofilms and related factors. *Seminars in Vascular Surgery*, *28*(3–4), 151–159. https://doi.org/10.1053/j.semvascsurg.2016.01.003

Scheler, S. (2014). The Polymer Free Volume as a Controlling Factor for Drug Release from Poly(lactide-co-glycolide) Microspheres. *Journal of Applied Polymer Science*, *131*. https://doi.org/10.1002/app.39740

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schreml, S., Szeimies, R. M., Prantl, L., Karrer, S., Landthaler, M., & Babilas, P. (2010). Oxygen in acute and chronic wound healing. *British Journal of Dermatology*, *163*(2), 257–268. https://doi.org/10.1111/j.1365-2133.2010.09804.x

Schultz, G. S., Sibbald, R. G., Falanga, V., Ayello, E. A., Dowsett, C., Harding, K., Romanelli, M., Stacey, M. C., Teot, L., & Vanscheidt, W. (2003). Wound bed preparation: a systematic approach to wound management. *Wound Repair and Regeneration: Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *11 Suppl 1*, S1-28. https://doi.org/10.1046/j.1524-475x.11.s2.1.x

Seabrook, G. R., Edmiston, C. E., Schmitt, D. D., Krepel, C., Bandyk, D. F., & Towne, J. B. (1991). Comparison of serum and tissue antibiotic levels in diabetes-related foot infections. *Surgery*, *110*(4), 671–677. http://europepmc.org/abstract/MED/1925956

Seaman, S. (2002). Dressing selection in chronic wound management. *Journal of the American Podiatric Medical Association*, *92*(1), 24–33. https://doi.org/10.7547/87507315-92-1-24

Sebe, I., Ostorházi, E., Bodai, Z., Eke, Z., Szakács, J., Kovács, N. K., & Zelkó, R. (2017). In vitro and in silico characterization of fibrous scaffolds comprising alternate colistin sulfate-loaded and heat-treated polyvinyl alcohol nanofibrous sheets. *International Journal of Pharmaceutics*, *523*(1), 151–158. https://doi.org/10.1016/j.ijpharm.2017.03.044

Sebe, I., Szabó, B., Nagy, Z. K., Szabó, D., Zsidai, L., Kocsis, B., & Zelkó, R. (2013). Polymer structure and antimicrobial activity of polyvinylpyrrolidone-based iodine nanofibers prepared with high-speed rotary spinning technique. *International Journal of Pharmaceutics*, *458*(1), 99–103. https://doi.org/10.1016/j.ijpharm.2013.10.011

Selling, G. W., Biswas, A., Patel, A., Walls, D. J., Dunlap, C., & Wei, Y. (2007). Impact of Solvent on Electrospinning of Zein and Analysis of Resulting Fibers. *Macromolecular Chemistry and Physics*, *208*(9), 1002–1010. https://doi.org/10.1002/macp.200700056

Shahhosseininia, M., Bazgir, S., & Joupari, M. D. (2018). Fabrication and investigation of silica nanofibers via electrospinning. *Materials Science and Engineering: C*, *91*, 502–511. https://doi.org/10.1016/j.msec.2018.05.068

Shalaby, M. A., Anwar, M. M., & Saeed, H. (2022). Nanomaterials for application in wound Healing: current state-of-the-art and future perspectives. *Journal of Polymer Research*, *29*(3), 91. https://doi.org/10.1007/s10965-021-02870-x

Shalumon, K. T., Anulekha, K. H., Nair, S. V, Nair, S. V, Chennazhi, K. P., & Jayakumar, R. (2011). Sodium alginate/poly(vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. *International Journal of Biological Macromolecules*, *49*(3), 247–254. https://doi.org/10.1016/j.ijbiomac.2011.04.005

Sharifi, F., Irani, S., Zandi, M., Soleimani, M., & Atyabi, S. M. (2016). Comparative of fibroblast and osteoblast cells adhesion on surface modified nanofibrous substrates based on polycaprolactone. *Progress in Biomaterials*, *5*(3–4), 213–222. https://doi.org/10.1007/s40204-016-0059-1

Shen, Y., Qian, Y., Zhang, H., Zuo, B., Lu, Z., Fan, Z., Zhang, P., Zhang, F., & Zhou, C. (2010). Guidance of Olfactory Ensheathing Cell Growth and Migration on Electrospun Silk Fibroin Scaffolds. *Cell Transplantation*, *19*(2), 147–158. https://doi.org/10.3727/096368910X492616

Shevchenko, R. V., James, S. L., & James, S. E. (2010). A review of tissue-engineered skin bioconstructs available for skin reconstruction. *Journal of the Royal Society Interface*, *7*(43), 229–258. https://doi.org/10.1098/rsif.2009.0403

Shi, R., Ye, J., Li, W., Zhang, J., Li, J., Wu, C., Xue, J., & Zhang, L. (2019). Infection-responsive electrospun nanofiber mat for antibacterial guided tissue regeneration membrane. *Materials Science and Engineering: C*, *100*, 523–534. https://doi.org/10.1016/j.msec.2019.03.039

Siddiqui, A. R., & Bernstein, J. M. (2010a). Chronic wound infection: Facts and controversies. *Clinics in Dermatology*, *28*(5), 519–526. https://doi.org/10.1016/j.clindermatol.2010.03.009

Siddiqui, A. R., & Bernstein, J. M. (2010b). Chronic wound infection: Facts and controversies. *Clinics in Dermatology*, *28*(5), 519–526. https://doi.org/10.1016/j.clindermatol.2010.03.009

Sill, T. J., & von Recum, H. A. (2008). Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials*, *29*(13), 1989–2006. https://doi.org/10.1016/j.biomaterials.2008.01.011

Silvestry-Rodriguez, N., Sicairos-Ruelas, E. E., Gerba, C. P., & Bright, K. R. (2007). Silver as a disinfectant. *Reviews of Environmental Contamination and Toxicology*, *191*, 23–45. https://doi.org/10.1007/978-0-387-69163-3_2

Simões, D., Miguel, S. P., Ribeiro, M. P., Coutinho, P., Mendonça, A. G., & Correia, I. J. (2018). Recent advances on antimicrobial wound dressing: A review. *European Journal of Pharmaceutics and Biopharmaceutics*, *127*(December 2017), 130–141. https://doi.org/10.1016/j.ejpb.2018.02.022

Širc, J., Hobzová, R., Kostina, N., Munzarová, M., Juklíčková, M., Lhotka, M., Kubinová, Š., Zajícová, A., & Michálek, J. (2012). Morphological Characterization of Nanofibers: Methods and Application in Practice. *Journal of Nanomaterials*, *2012*, 327369. https://doi.org/10.1155/2012/327369

Smit, E., Bűttner, U., & Sanderson, R. D. (2005). Continuous yarns from electrospun fibers. *Polymer*, *46*(8), 2419–2423. https://doi.org/10.1016/j.polymer.2005.02.002

Smith, R., Russo, J., Fiegel, J., & Brogden, N. (2020). Antibiotic Delivery Strategies to Treat Skin Infections When Innate Antimicrobial Defense Fails. *Antibiotics*, *9*(2). https://doi.org/10.3390/antibiotics9020056

Soliman, S., Sant, S., Nichol, J. W., Khabiry, M., Traversa, E., & Khademhosseini, A. (2011). Controlling the porosity of fibrous scaffolds by modulating the fiber diameter and packing density. *Journal of Biomedical Materials Research – Part A*, *96 A*(3), 566–574. https://doi.org/10.1002/jbm.a.33010

Song, W., Zhao, B., Wang, C., & Lu, X. (2019). Electrospun nanofibrous materials: A versatile platform for enzyme mimicking and their sensing applications. *Composites Communications*, *12*, 1–13. https://doi.org/10.1016/j.coco.2018.12.005

Song, Z., Chiang, S. W., Chu, X., Du, H., Li, J., Gan, L., Xu, C., Yao, Y., He, Y., Li, B., & Kang, F. (2018). Effects of solvent on structures and properties of electrospun poly(ethylene oxide) nanofibers. *Journal of Applied Polymer Science*, *135*(5), 45787. https://doi.org/https://doi.org/10.1002/app.45787

Stanley, A., & Osler, T. (2001). Senescence and the healing rates of venous ulcers. *Journal of Vascular Surgery*, *33*(6), 1206–1211. https://doi.org/10.1067/mva.2001. 115379

Sun, C., Boluk, Y., & Ayranci, C. (2015). Investigation of nanofiber nonwoven meshes produced by electrospinning of cellulose nanocrystal suspensions in cellulose acetate solutions. *Cellulose*, *22*(4), 2457–2470. https://doi.org/10.1007/s10570-015-0665-4

Sun, L., Gao, W., Fu, X., Shi, M., Xie, W., Zhang, W., Zhao, F., & Chen, X. (2018). Enhanced wound healing in diabetic rats by nanofibrous scaffolds mimicking the basketweave pattern of collagen fibrils in native skin. *Biomater. Sci.*, *6*(2), 340–349. https://doi.org/10.1039/C7BM00545H

Sundaramurthi, D., Krishnan, U. M., & Sethuraman, S. (2014). Electrospun Nanofibers as Scaffolds for Skin Tissue Engineering. *Polymer Reviews*, *54*(2), 348–376. https://doi.org/10.1080/15583724.2014.881374

Taepaiboon, P., Rungsardthong, U., & Supaphol, P. (2006). Drug-loaded electrospun mats of poly(vinyl alcohol) fibres and their release characteristics of four model drugs. *Nanotechnology*, *17*(9), 2317–2329. https://doi.org/10.1088/0957-4484/17/9/041

Tamayo-Ramos, J. A., Rumbo, C., Caso, F., Rinaldi, A., Garroni, S., Notargiacomo, A., Romero-Santacreu, L., & Cuesta-López, S. (2018). Analysis of Polycaprolactone Microfibers as Biofilm Carriers for Biotechnologically Relevant Bacteria. *ACS Applied Materials & Interfaces*, *10*(38), 32773–32781. https://doi.org/10.1021/acsami.8b07245

Tammaro, L., Saturnino, C., D'Aniello, S., Vigliotta, G., & Vittoria, V. (2015). Polymorphic solidification of Linezolid confined in electrospun PCL fibers for controlled release in topical applications. *International Journal of Pharmaceutics*, *490*(1–2), 32–38. https://doi.org/10.1016/J.IJPHARM.2015.04.070

Tan, E. P. S., Ng, S. Y., & Lim, C. T. (2005). Tensile testing of a single ultrafine polymeric fiber. *Biomaterials*, *26*(13), 1453–1456. https://doi.org/10.1016/j.biomaterials.2004.05.021

Tang, Y., Liu, Z., & Zhao, K. (2019). Fabrication of hollow and porous polystyrene fibrous membranes by electrospinning combined with freeze-drying for oil removal from water. *Journal of Applied Polymer Science*, *136*(13), 47262. https://doi.org/10.1002/app.47262

Thomas, G. W., Rael, L. T., Bar-Or, R., Shimonkevitz, R., Mains, C. W., Slone, D. S., Craun, M. L., & Bar-Or, D. (2009). Mechanisms of Delayed Wound Healing by Commonly Used Antiseptics. *Journal of Trauma and Acute Care Surgery*, *66*(1). https://journals.lww.com/jtrauma/Fulltext/2009/01000/Mechanisms_of_Delayed_Wound_Healing_by_Commonly.10.aspx

Thomson, C. H. (2011). Biofilms: do they affect wound healing? *International Wound Journal*, *8*(1), 63–67. https://doi.org/10.1111/j.1742-481X.2010.00749.x

Thorvaldsson, A., Stenhamre, H., Gatenholm, P., & Walkenström, P. (2008). Electrospinning of Highly Porous Scaffolds for Cartilage Regeneration. *Biomacromolecules*, *9*(3), 1044–1049. https://doi.org/10.1021/bm701225a

Torres-Giner, S., Gimenez, E., & Lagaron, J. M. (2008). Characterization of the morphology and thermal properties of Zein Prolamine nanostructures obtained by electrospinning. *Food Hydrocolloids*, *22*(4), 601–614. https://doi.org/10.1016/j.foodhyd.2007.02.005

Torres-Giner, S., Ocio, M. J., & Lagaron, J. M. (2009). Novel antimicrobial ultrathin structures of zein/chitosan blends obtained by electrospinning. *Carbohydrate Polymers*, *77*(2), 261–266. https://doi.org/10.1016/j.carbpol.2008.12.035

Tran, K. T., Griffith, L., & Wells, A. (2004). Extracellular matrix signaling through growth factor receptors during wound healing. *Wound Repair and Regeneration : Official Publication of the Wound Healing Society [and] the European Tissue Repair Society*, *12*(3), 262–268. https://doi.org/10.1111/j.1067-1927.2004.012302.x

Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A., & Bornstein, S. R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *BioMed Research International*, *2013*, 385641. https://doi.org/10.1155/2013/385641

Uhljar, L. É., Kan, S. Y., Radacsi, N., Koutsos, V., Szabó-Révész, P., & Ambrus, R. (2021). In Vitro Drug Release, Permeability, and Structural Test of Ciprofloxacin-Loaded Nanofibers. In *Pharmaceutics* (Vol. 13, Issue 4). https://doi.org/10.3390/pharmaceutics13040556

Vasita, R., & Katti, D. S. (2006). Nanofibers and their applications in tissue engineering. *International Journal of Nanomedicine*, *1*(1), 15–30. https://doi.org/10.2147/nano.2006.1.1.15

Veleirinho, B., Berti, F. V, Dias, P. F., Maraschin, M., Ribeiro-do-Valle, R. M., & Lopes-da-Silva, J. A. (2013). Manipulation of chemical composition and architecture of non-biodegradable poly(ethylene terephthalate)/chitosan fibrous scaffolds and their effects on L929 cell behavior. *Materials Science and Engineering: C*, *33*(1), 37–46. https://doi.org/10.1016/j.msec.2012.07.047

Veleirinho, B., Rei, M. F., & Lopes-DA-Silva, J. A. (2008). Solvent and concentration effects on the properties of electrospun poly(ethylene terephthalate) nanofiber mats. *Journal of Polymer Science Part B: Polymer Physics*, *46*(5), 460–471. https://doi.org/10.1002/polb.21380

Wallner, E. I., Yang, Q., Peterson, D. R., Wada, J., & Kanwar, Y. S. (1998). Relevance of extracellular matrix, its receptors, and cell adhesion molecules in mammalian nephrogenesis. *The American Journal of Physiology*, *275*(4), F467–77. https://doi.org/10.1152/ajprenal.1998.275.4.F467

Walther, M., Rohde, F., Kielholz, T., & Windbergs, M. (2022). Physico-chemical analysis of electrospun fibers – A systematic approach. *European Journal of Pharmaceutics and Biopharmaceutics*, *171*, 60–71. https://doi.org/10.1016/j.ejpb.2022.01.001

Wang, F., Hu, S., Jia, Q., & Zhang, L. (2020). Advances in Electrospinning of Natural Biomaterials for Wound Dressing. *Journal of Nanomaterials*, *2020*, 8719859. https://doi.org/10.1155/2020/8719859

Wang, H.-J., Lin, Z.-X., Liu, X.-M., Sheng, S.-Y., & Wang, J.-Y. (2005). Heparin-loaded zein microsphere film and hemocompatibility. *Journal of Controlled Release : Official Journal of the Controlled Release Society*, *105*(1–2), 120–131. https://doi.org/10.1016/j.jconrel.2005.03.014

Wang, J., Planz, V., Vukosavljevic, B., & Windbergs, M. (2018). Multifunctional electrospun nanofibers for wound application – Novel insights into the control of drug release and antimicrobial activity. *European Journal of Pharmaceutics and Biopharmaceutics*, *129*, 175–183. https://doi.org/10.1016/j.ejpb.2018.05.035

Wang, L.-Y., Yu, L. E., Lai, J.-Y., & Chung, T.-S. (2018). Developing ultra-high gas permeance PVDF hollow fibers for air filtration applications. *Separation and Purification Technology*, *205*, 184–195. https://doi.org/10.1016/j.seppur.2018.05.036

Wang, L., Pai, C.-L., Boyce, M. C., & Rutledge, G. C. (2009). Wrinkled surface topographies of electrospun polymer fibers. *Applied Physics Letters*, *94*(15), 151916. https://doi.org/10.1063/1.3118526

Wang, L., Yang, J., Ran, B., Yang, X., Zheng, W., Long, Y., & Jiang, X. (2017). Small Molecular TGF-β1-Inhibitor-Loaded Electrospun Fibrous Scaffolds for Preventing Hypertrophic Scars. *ACS Applied Materials & Interfaces*, *9*(38), 32545–32553. https://doi.org/10.1021/acsami.7b09796

Wang, M., Roy, A. K., & Webster, T. J. (2016). Development of Chitosan/Poly(Vinyl Alcohol) Electrospun Nanofibers for Infection Related Wound Healing. *Frontiers in Physiology*, *7*, 683. https://doi.org/10.3389/fphys.2016.00683

Wang, X., Chang, J., & Wu, C. (2018). Bioactive inorganic/organic nanocomposites for wound healing. *Applied Materials Today*, *11*, 308–319. https://doi.org/10.1016/j.apmt. 2018.03.001

Wang, X., Lv, F., Li, T., Han, Y., Yi, Z., Liu, M., Chang, J., & Wu, C. (2017). Electrospun Micropatterned Nanocomposites Incorporated with Cu(2)S Nanoflowers for Skin Tumor Therapy and Wound Healing. *ACS Nano*, *11*(11), 11337–11349. https://doi.org/10.1021/acsnano.7b05858

Wang, Y., Ameer, G. A., Sheppard, B. J., & Langer, R. (2002). A tough biodegradable elastomer. *Nature Biotechnology*, *20*(6), 602–606. https://doi.org/10.1038/nbt0602-602

Wang, Y., Huang, H., Li, G., Zhao, X., Yu, L., Zou, C., & Xu, Y. (2017). Electrospun TiO2–SiO2 fibres with hierarchical pores from phase separation. *CrystEngComm*, *19*(19), 2673–2680. https://doi.org/10.1039/C7CE00471K

Wang, Z., Crandall, C., Sahadevan, R., Menkhaus, T. J., & Fong, H. (2017). Microfiltration performance of electrospun nanofiber membranes with varied fiber diameters and different membrane porosities and thicknesses. *Polymer*, *114*, 64–72. https://doi.org/ 10.1016/j.polymer.2017.02.084

Weller, C., & Sussman, G. (2006). Wound dressings update. *Journal of Pharmacy Practice and Research*, *36*(4), 318–324. https://doi.org/10.1002/j.2055-2335.2006.tb00640.x

Wenk, E., Merkle, H. P., & Meinel, L. (2011). Silk fibroin as a vehicle for drug delivery applications. *Journal of Controlled Release*, *150*(2), 128–141. https://doi.org/10.1016/j.jconrel.2010.11.007

Whitney, J. D. (2005). Overview: Acute and Chronic Wounds. *Nursing Clinics of North America*, *40*(2), 191–205. https://doi.org/10.1016/j.cnur.2004.09.002

Wilgus, T. A. (2008). Immune cells in the healing skin wound: influential players at each stage of repair. *Pharmacological Research*, *58*(2), 112–116. https://doi.org/10.1016/j.phrs.2008.07.009

Winter, G. (1962). Formation of the Scab and the Rate of Epithelization of Superficial Wounds in the Skin of the Young Domestic Pig. *Nature*, *193*(4812), 293–294. https://doi.org/10.1038/193293a0

Wolcott, R. D., Kennedy, J. P., & Dowd, S. E. (2009). Regular debridement is the main tool for maintaining a healthy wound bed in most chronic wounds. *Journal of Wound Care*, *18*(2), 54–56. https://doi.org/10.12968/jowc.2009.18.2.38743

Wolcott, R. D., Rumbaugh, K. P., James, G., Schultz, G., Phillips, P., Yang, Q., Watters, C., Stewart, P. S., & Dowd, S. E. (2010). Biofilm maturity studies indicate sharp debridement opens a time – dependent therapeutic window. *Journal of Wound Care*, *19*(8), 320–328. https://doi.org/10.12968/jowc.2010.19.8.77709

Wolcott, R., & Dowd, S. (2011). The role of biofilms: are we hitting the right target? *Plastic and Reconstructive Surgery*, *127 Suppl*, 28S-35S. https://doi.org/10.1097/PRS.0b013e3181fca244

Wolf, K., te Lindert, M., Krause, M., Alexander, S., te Riet, J., Willis, A. L., Hoffman, R. M., Figdor, C. G., Weiss, S. J., & Friedl, P. (2013). Physical limits of cell migration: Control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *Journal of Cell Biology*, *201*(7), 1069–1084. https://doi.org/10.1083/jcb.201210152

Woodruff, M. A., & Hutmacher, D. W. (2010). The return of a forgotten polymer – Polycaprolactone in the 21st century. *Progress in Polymer Science (Oxford)*, *35*(10), 1217–1256. https://doi.org/10.1016/j.progpolymsci.2010.04.002

Wu, J., & Hong, Y. (2016). Enhancing cell infiltration of electrospun fibrous scaffolds in tissue regeneration. *Bioactive Materials*, *1*(1), 56–64. https://doi.org/10.1016/j.bioactmat.2016.07.001

Xie, J., & Wang, C.-H. (2006). Electrospun Micro- and Nanofibers for Sustained Delivery of Paclitaxel to Treat C6 Glioma in Vitro. *Pharmaceutical Research*, *23*(8), 1817–1826. https://doi.org/10.1007/s11095-006-9036-z

Xie, Z., & Buschle-Diller, G. (2010). Electrospun poly(D,L-lactide) fibers for drug delivery: The influence of cosolvent and the mechanism of drug release. *Journal of Applied Polymer Science*, *115*(1), 1–8. https://doi.org/10.1002/app.31026

Xie, Z., Paras, C. B., Weng, H., Punnakitikashem, P., Su, L.-C., Vu, K., Tang, L., Yang, J., & Nguyen, K. T. (2013). Dual growth factor releasing multi-functional nanofibers for wound healing. *Acta Biomaterialia*, *9*(12), 9351–9359. https://doi.org/10.1016/j.actbio.2013.07.030

Xu, H., Jiang, S., Ding, C., Zhu, Y., Li, J., & Hou, H. (2017). High strength and high breaking load of single electrospun polyimide microfiber from water soluble precursor. *Materials Letters*, *201*, 82–84. https://doi.org/10.1016/j.matlet.2017.05.019

Xue, J., Wu, T., Dai, Y., & Xia, Y. (2019). Electrospinning and electrospun nanofibers: Methods, materials, and applications. *Chemical Reviews*, *119*(8), 5298–5415. https://doi.org/10.1021/acs.chemrev.8b00593

Xue, R., Qian, Y., Li, L., Yao, G., Yang, L., & Sun, Y. (2017). Polycaprolactone nanofiber scaffold enhances the osteogenic differentiation potency of various human tissue-derived mesenchymal stem cells. *Stem Cell Research & Therapy*, *8*(1), 148. https://doi.org/10.1186/s13287-017-0588-0

Yang, G.-Z., Li, H.-P., Yang, J.-H., Wan, J., & Yu, D.-G. (2017). Influence of Working Temperature on The Formation of Electrospun Polymer Nanofibers. *Nanoscale Research Letters*, *12*(1), 55. https://doi.org/10.1186/s11671-016-1824-8

Yang, R., He, J., Xu, L., & Yu, J. (2009). Bubble-electrospinning for fabricating nanofibers. *Polymer*, *50*(24), 5846–5850. https://doi.org/10.1016/j.polymer.2009.10.021

Yang, X., Yang, J., Wang, L., Ran, B., Jia, Y., Zhang, L., Yang, G., Shao, H., & Jiang, X. (2017). Pharmaceutical Intermediate-Modified Gold Nanoparticles: Against Multidrug-Resistant Bacteria and Wound-Healing Application via an Electrospun Scaffold. *ACS Nano*, *11*(6), 5737–5745. https://doi.org/10.1021/acsnano.7b01240

Yang, Y., Jia, Z., Li, Q., & Guan, Z. (2006). Experimental investigation of the governing parameters in the electrospinning of polyethylene oxide solution. *IEEE Transactions on Dielectrics and Electrical Insulation*, *13*(3), 580–584. https://doi.org/10.1109/TDEI. 2006.1657971

Yao, C., Li, X., & Song, T. (2007). Electrospinning and crosslinking of zein nanofiber mats. *Journal of Applied Polymer Science*, *103*(1), 380–385. https://doi.org/10.1002/app.24619

Yari, A., Teimourian, S., Amidi, F., Bakhtiyari, M., Heidari, F., Sajedi, N., Veijouye, S. J., Dodel, M., & Nobakht, M. (2016). The role of biodegradable engineered random polycaprolactone nanofiber scaffolds seeded with nestin-positive hair follicle stem cells for tissue engineering. *Advanced Biomedical Research*, *5*, 22. https://doi.org/10.4103/2277-9175.175911

Yarin, A. L., & Zussman, E. (2004). Upward needleless electrospinning of multiple nanofibers. *Polymer*, *45*(9), 2977–2980. https://doi.org/10.1016/j.polymer.2004.02.066

Yaru, W., Lan*, X., & Chenxu, S. J. and F. (2018). Preparation, Characterization and Drug Release of Salicylic Acid Loaded Porous Electrospun Nanofibers. In *Recent Patents on Nanotechnology* (Vol. 12, Issue 3, pp. 208–217). http://dx.doi.org/10.2174/ 18722105126661810291549 08

Yazdanbakhsh, M. F., Rashidi, A., Rahimi, M. K., Khajavi, R., & Shafaroodi, H. (2018). The Effect of Impregnated Alpha-Cellulose Nanofibers with Ciprofloxacin Hydrochloride on Staphylococcus aureus In Vitro and Healing Process of Wound in Rat. *Regenerative Engineering and Translational Medicine*, *4*(4), 247–256. https://doi.org/10.1007/s40883-018-0066-y

Yi-Fan, L., Peng-Wen, N., Yao, H., & Ting, X. (2022). Therapeutic strategies for chronic wound infection. *Chinese Journal of Traumatology*, *25*(01), 11–16. https://doi.org/10.1016/j.cjtee.2021.07.004

Yu, D.-G., Shen, X.-X., Branford-White, C., White, K., Zhu, L.-M., & Annie Bligh, S. W. (2009). Oral fast-dissolving drug delivery membranes prepared from electrospun polyvinylpyrrolidone ultrafine fibers. *Nanotechnology*, *20*(5), 055104. https://doi.org/10.1088/0957-4484/20/5/055104

Yu, L., Zhou, P., Wu, D., Wang, L., Lin, L., & Sun, D. (2019). Shoepad nanogenerator based on electrospun PVDF nanofibers. *Microsystem Technologies*, *25*(8), 3151–3156. https://doi.org/10.1007/s00542-018-4217-3

Yuan, X., Zhang, Y., Dong, C., & Sheng, J. (2004). Morphology of ultrafine polysulfone fibers prepared by electrospinning. *Polymer International*, *53*(11), 1704–1710. https://doi.org/https://doi.org/10.1002/pi.1538

Yuan, Y., Choi, K., Choi, S. O., & Kim, J. (2018). Early stage release control of an anticancer drug by drug-polymer miscibility in a hydrophobic fiber-based drug delivery system. *RSC Advances*, *8*(35), 19791–19803. https://doi.org/10.1039/c8ra01467a

Zaarour, B., Zhang, W., Zhu, L., Jin, X. Y., & Huang, C. (2018). Maneuvering surface structures of polyvinylidene fluoride nanofibers by controlling solvent systems and polymer concentration. *Textile Research Journal*, *89*(12), 2406–2422. https://doi.org/10.1177/0040517518792748

Zaarour, B., Zhu, L., Huang, C., & Jin, X. (2018). Controlling the Secondary Surface Morphology of Electrospun PVDF Nanofibers by Regulating the Solvent and Relative Humidity. *Nanoscale Research Letters*, *13*(1), 285. https://doi.org/10.1186/s11671-018-2705-0

Zaarour, B., Zhu, L., Huang, C., & Jin, X. (2019). Enhanced piezoelectric properties of randomly oriented and aligned electrospun PVDF fibers by regulating the surface morphology. *Journal of Applied Polymer Science*, *136*(6), 47049. https://doi.org/10.1002/app.47049

Zaarour, B., Zhu, L., & Jin, X. (2019). Controlling the surface structure, mechanical properties, crystallinity, and piezoelectric properties of electrospun PVDF nanofibers by maneuvering molecular weight. *Soft Materials*, *17*(2), 181–189. https://doi.org/10.1080/1539445X.2019.1582542

Zaarour, B., Zhu, L., & Jin, X. (2020). A Review on the Secondary Surface Morphology of Electrospun Nanofibers: Formation Mechanisms, Characterizations, and Applications. *ChemistrySelect*, *5*(4), 1335–1348. https://doi.org/10.1002/slct.201903981

Zagris, N. (2001). Extracellular matrix in development of the early embryo. *Micron*, *32*(4), 427–438. https://doi.org/10.1016/S0968-4328(00)00011-1

Zahedi, P., Rezaeian, I., Ranaei-Siadat, S.-O., Jafari, S.-H., & Supaphol, P. (2010). A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polymers for Advanced Technologies*, *21*(2), 77–95. https://doi.org/10.1002/pat.1625

Zamani, M., Morshed, M., Varshosaz, J., & Jannesari, M. (2010). Controlled release of metronidazole benzoate from poly ε-caprolactone electrospun nanofibers for periodontal diseases. *European Journal of Pharmaceutics and Biopharmaceutics*, *75*(2), 179–185. https://doi.org/10.1016/j.ejpb.2010.02.002

Zeng, J., Haoqing, H., Schaper, A., Wendorff, J. H., & Greiner, A. (2003). Poly-L-lactide nanofibers by electrospinning – Influence of solution viscosity and electrical conductivity on fiber diameter and fiber morphology. *E-Polymers*, *3*(1). https://doi.org/10.1515/epoly.2003.3.1.102

Zeng, J., Yang, L., Liang, Q., Zhang, X., Guan, H., Xu, X., Chen, X., & Jing, X. (2005). Influence of the drug compatibility with polymer solution on the release kinetics of electrospun fiber formulation. *Journal of Controlled Release*, *105*(1), 43–51. https://doi.org/10.1016/j.jconrel.2005.02.024

Zhang, Q., Oh, J.-H., Park, C. H., Baek, J.-H., Ryoo, H.-M., & Woo, K. M. (2017). Effects of Dimethyloxalylglycine-Embedded Poly(ε-caprolactone) Fiber Meshes on Wound Healing in Diabetic Rats. *ACS Applied Materials & Interfaces*, *9*(9), 7950–7963. https://doi.org/10.1021/acsami.6b15815

Zhang, Y., Kong, H., Fang, Y., Nishinari, K., & Phillips, G. O. (2013). Schizophyllan: A review on its structure, properties, bioactivities and recent developments. *Bioactive Carbohydrates and Dietary Fibre*, *1*(1), 53–71. https://doi.org/10.1016/j.bcdf.2013.01.002

Zhao, G., Usui, M. L., Lippman, S. I., James, G. A., Stewart, P. S., Fleckman, P., & Olerud, J. E. (2013). Biofilms and Inflammation in Chronic Wounds. In *Advances in wound care* (Vol. 2, Issue 7, pp. 389–399). https://doi.org/10.1089/wound.2012.0381

Zhao, Y., Cao, X., & Jiang, L. (2007). Bio-mimic Multichannel Microtubes by a Facile Method. *Journal of the American Chemical Society*, *129*(4), 764–765. https://doi.org/10.1021/ja068165g

Zheng, J.-Y., Liu, H.-Y., Wang, X., Zhao, Y., Huang, W.-W., Zheng, G.-F., & Sun, D.-H. (2014). Electrohydrodynamic Direct-Write Orderly Micro/Nanofibrous Structure on Flexible Insulating Substrate. *Journal of Nanomaterials*, *2014*, 708186. https://doi.org/10.1155/2014/708186

Zhong, Q., & Jin, M. (2009). Nanoscalar Structures of Spray-Dried Zein Microcapsules and in Vitro Release Kinetics of the Encapsulated Lysozyme As Affected by Formulations. *Journal of Agricultural and Food Chemistry*, *57*, 3886–3894. https://doi.org/10.1021/jf803951a

Zhu, L., Zaarour, B., & Jin, X. (2019). Unexpectedly high oil cleanup capacity of electrospun poly (vinylidene fluoride) fiber webs induced by spindle porous bowl like beads. *Soft Materials*, *17*(4), 410–417. https://doi.org/10.1080/1539445X.2019.1614060

Zhu, L., Zaarour, B., & Jin, X. (2020). Direct generation of electrospun interconnected macroporous nanofibers using a water bath as a collector. *Materials Research Express*, *7*(1), 15082. https://doi.org/10.1088/2053-1591/ab6c1e

Zong, X., Kim, K., Fang, D., Ran, S., Hsiao, B. S., & Chu, B. (2002). Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *Polymer*, *43*(16), 4403–4412. https://doi.org/10.1016/S0032-3861(02)00275-6

Zupančič, Š., Preem, L., Kristl, J., Putrinš, M., Tenson, T., Kocbek, P., & Kogermann, K. (2018). Impact of PCL nanofiber mat structural properties on hydrophilic drug release and antibacterial activity on periodontal pathogens. *European Journal of Pharmaceutical Sciences*, *122*, 347–358. https://doi.org/10.1016/J.EJPS.2018.07.024

8 SUMMARY IN ESTONIAN

Uudsete antibakteriaalsete haavakatete valmistamine kasutades elektrospinnimise tehnoloogiat

Sissejuhatus

Tõusvate kulude ning aina keerulisemate ravijuhtude tõttu on halvasti paranevad kroonilised haavandid muutunud kaasaegses ühiskonnas väga suureks probleemiks. (Mirhaj et al., 2022) Kuna haavadega kaasneb alati infektsioonioht, näeb kaasaegne haavaravi ette, et taastatakse haava homeöstaas ning hoitakse kontrolli all bakterite arvukus. (Mathes, 2008) Kõige probleemsemaks osutuvad kaasuvate haigustega patsientidid (näiteks diabeedihaiged), kelle häirunud immuunvastus võib takistada ravi efektiivsust ning pikendada ravi kestvust. (Q. Zhang et al., 2017)

Haavaravi skeemis on antibiootikumide manustamine väga olulisel kohal. Kahjuks on tänapäeval üha enam levinud antibiootikumresistentsus. Selle vältimiseks tuleks antibiootikume ordineerides jälgida raviskeemi ning valida sobiv raviviis (süsteemne või lokaalne). (Mathes, 2008) Süsteemse ravi määramisega peab alati arvestama võimaliku toksilisuse ning madala raviaine kontsentratsiooniga sihtkohas. (D. Leaper et al., 2015b) Lokaalne antibakteriaalne haavaravi (kasutades enamlevinud traditsioonilisi ravimvorme) on väga levinud, kuid selle efektiivsus on siiani küsitav nende lühikese toimeaja ja pideva manustamise tõttu. (Smith et al., 2020) Seetõttu on kaasaegne ravimvormide teadustöö keskendunud just kohaspetsiifiliste lokaalsete antibakteriaalsete ravimkandursüsteemide väljatöötamisele, mis omaksid veel täiendavat funktsionaalsust (suurem antibakteriaalne toime). (Luraghi et al., 2021)

Elektrospinnimine (eesti keelne sünonüümnimetus ka elektroketrus) on lihtne ja laialtlevinud meetod polümeersete fiibermaatriksite tootmiseks. Antud meetodiga on võimalik toota erineva läbimõõduga fiibreid nanomeetritest mikromeetriteni. (Doshi & Reneker, 1995) Fiibrimaatriksitel on mitmeid häid omadusi: kontrollitav morfoloogia, suur eripind ning poorsus. Lisaks on maatriksitesse võimalik lihtsa vaevaga inkorporeerida erinevaid raviaineid, mis võimaldab neid kasutada ravimkandursüsteemidena. (Qu et al., 2013) Raviaine vabanemine maatriksitest baseerub enamasti difusioonil, kuid seda on võimalik modifitseerida, muutes fiibrite pinnaomadusi (poorid fiibritel) või maatriksi poorsust. (Zupančič et al., 2018) Manipuleerides raviaine vabanemiskiirusega on võimalik muuta maatriksi antibakteriaalseid omadusi. (Preem et al., 2017; Preem, Bock, et al., 2019; Zupančič et al., 2018)

Elektrospinnitud fiibermaatriksid sobivad ideaalselt haavakateteks, kuna omavad nahale omase rakuvälise maatriksiga sarnast struktuuri. (Haider et al., 2018) Lisaks on elektrospinnitud maatriksid suure poorsuse ning eripinnaga, andes neile hea haavaeksudaadi absorbeerimise võime ning võimaluse gaaside transpordiks haavas. (Preem et al., 2017) Maatriksite mehaanilised omadused on muudetavad vastavalt vajadusele. (Rashid et al., 2021) Haavakatte seisukohalt peaks maatriks olema piisavalt elastne ning veniv, et seda oleks lihtne paigaldada

ning eemaldada, samas peab maatriks pakkuma soodsat pinda rakkudele, mis vastutavad haavade paranemise eest. (Gao et al., 2021) Oluline on, et maatriks ning selle komponendid oleksid ohutud ning ei esineks toksilisust eukarüootsetele rakkudele. Seetõttu on väga oluline uurida maatriksite mõju rakkudele ja nende omavahelisi interaktsioone. (Ninan et al., 2015). Palju on uuritud maatriksite mõju rakkudele *in vitro,* kuid enamasti sõltub see katseks valitud rakuliini eripäradest. (Sadeghi et al., 2016b; R. Xue et al., 2017)

Antibakteriaalsete omadustega haavakatete väljatöötamisel on oluline uurida ka nende mõju bakterirakkudele. Maatriks peaks inhibeerima bakterite arvukuse tõusu haavas ning kaitsma haava kontaminatsiooni eest. Artiklites on kinnitust leidnud fakt, et elektrospinnitud maatriksid loovad bakteritele soodsa kasvukeskkonna. (Pompa-Monroy et al., 2020) Samuti on leitud, et bakterid on maatriksile moodustanud biokile. (Tamayo-Ramos et al., 2018) Seda saab vältida valides sobivad lähteained, modifitseerides maatriksi morfoloogiat, mehhaanilisi omadusi ning raviaine vabanemist. (Kargar et al., 2012; Mitik-Dineva et al., 2006, 2009).

Elektrospinnitud maatriksid on siiani uudsete ravimkandursüsteemide hulgas ning ainult üksikud on neist turule jõudnud. (Omer et al., 2021) Haavakatete puhul on jätkuvalt vähe teadmisi materjalide ja nende sobivate omaduste kohta, mis toetaks haavaravi ning pakuks head antibakteriaalset efektiivsust. Samuti puuduvad standardiseeritud meetodid testimaks elektrospinnitud ravimkandursüsteemide omadusi ja ohutust. (Repanas et al., 2016a)

Käesoleva doktoritöö eesmärgiks oli disainida ning uurida erinevatest polümeeridest ja raviainetest valmistatud antibakteriaalsete omadustega elektrospinnitud fiibermaatrikseid, mida saaks kasutada lokaalseks haavaraviks. Maatriksite struktuuri varieeriti, et uurida selle mõju maatriksite efektiivsusele. Samuti sooviti välja selgitada struktuuri mõju bakteritele ning eukarüootsetele rakkudele. Antibakteriaalsete omaduste, bioloogilise sobivuse ning ohutuse uurimine aitas välja selgitada maatriksite võimaliku sobivuse kasutamaks neid lokaalses haavaravis.

Eesmärgid

Doktoritöö eesmärgiks oli välja töötada ning uurida erineva struktuuriga antibakteriaalsete omadustega elektrospinnitud fiibermaatrikseid, mida saaks kasutada lokaalselt haavade raviks ning infektsioonide ennetamiseks. Töö täpsemad eesmärgid olid:

- Disainida ja valmistada antibakteriaalsete omadustega mudelravimit (klooramfenikooli) sisaldavad poorsed (poorid fiibritel) fiibermaatriksid (I, II) ning (tetratsükliini) sisaldavad tuum-kest struktuuriga fiibermaatriksid (III)
- Uurida protsessi ning keskkonnaparameetrite mõju elektrospinnimisele ning raviaine (klooramfenikooli) mõju fiibrite poorsusele (I)
- Iseloomustada maatriksite morfoloogilisi, füsikokeemisi, ning mehhaanilisi omadusi (I,II)

- Uurida maatriksite struktuuri mõju nende antibakteriaalsele aktiivsusele ning mõista mehhaaniliste omaduste, poorsuse ning fiibrite pinnaomaduste mõju bakteri- ning eukarüoodi rakkudele. (II)
- Hinnata maatriksite antibakteriaalseid omadusi ning biokile vastast aktiivsust (II, III)
- Hinnata maatriksite biosobivust ning ohutust (II, III).

Materjalid ja meetodid

Fiibermaatriksid valmistati elektrospinnimise meetodil. Kandjapolümeerina kasutati hüdrofoobset polükaprolaktooni (PCL) ning hüdrofiilset polüetüleenglükooli (PEO) ja gluteenijahust toodetud maisivalku (zein). Abainetena kasutati formulatsioonis zeiniga ka stearhapet (SA). Mudelraviainetena kasutati hüdrofoobseid antibakteriaalseid raviaineid klooramfenikooli (CAM) ning tetratsükliini (TET).

Fiibermaatriksite morfoloogiat analüüsiti skanneeriva elektronmikroskoopia (SEM) abil. Maatriksite poorsust uuriti gaasi absorptsiooni (BET) ning SEMi piltide analüüsil (fiibrite pooride läbimõõtude mõõtmine). Tahke faasi muutusi analüüsiti kasutades röntgendifraktomeetriat (XRD) ja täieliku sisepeegeldumise kristalliga (ATR) FT-infrapunaspektroskoopiat. Maatriksite mehhaanilisi omadusi määrati tõmbetestidega kasutades tekstuurianalüsaatorit. Raviaine sisalduse ning vabanemise määramiseks maatriksitest kasutati kõrgsurvevedelikkromatograafiat (HPLC) ning modifitseeritud dissolutioonikatset puhverlahusesse (pH 7.4, 37 °C). Maatriksite hüdrofiilsust hinnati kontaktnurga analüüsil, samuti hinnati maatriksite *in vitro* degradatsiooni. Maatriksite antibakteriaalset efektiivsust määrati agar difusiooni meetodil. Lisaks uuriti bakterite kinnitumist maatriksitele ning biokile vastast toimet. Maatriksite toksilisust ning eukarüootsete rakkude kinnitumist ja infiltreerumist määrati BHK-21 rakuliinil nii MTS testi abil kui kasutades konfokaalmikroskoopiat (CFM).

Tulemused ja arutelu

Elektrospinnimine õnnestus kahe eri meetodiga – monoaksiaalne ning koaksiaalne elektrospinnimine. PCL-st õnnestus monoaksiaalse elektrospinnimise meetodiga valmistada erineva morfoloogiaga maatriksid (fiibrid pooridega ning ilma). Fiibrite poorsuse saavutamiseks oli vajalik sobiv lahutite valik (THF: DMSO) ning keskkond (kõrge suhteline õhuniiskus, 65%). Raviaine lisamine muutis fiibrite morfoloogia ning pooride kuju. Fiibrite diameetrid olid enne raviaine lisamist keskmiselt 1,2 µm ning pärast 2,0 µm. Fiibri diameetrite muutust raviaine lisamisel on kirjanduses täheldatud ka varem. (Arbade et al., 2018; Z. M. Huang et al., 2006) Maisivalgu (zein) elektrospinnimine monoaksiaalse meetodiga ei õnnestunud, mistõttu kasutati koaksiaalset meetodit. Maisivalgu elektrospinnimisel kasutati PEO ja SA abiaineid, mis aitasid stabiliseerida kogu elektrospinnimise protsessi.

Maatriksite poorsuse analüüsil leiti, et saadud PCLi mikrofiibrid olid suurema poori diameetriga (13–22 μm) kui PCLi nanofiibermaatriksid, mille poori diameeter oli 2–4 μm. Maatriksite poorsuse muutust fiibri diameetri suurenedes on ka varem kirjanduses täheldatud. (Pham et al., 2006) Tahke faasi muutuse analüüsil leiti, et PCL oli elektrospinnimise järgselt säilitanud oma poolkristallilise struktuuri. XRD analüüsil leiti, et mudelraviaine (CAM) oli elektrospinnimise järgselt muutunud kristallilisest amorfseks. ATR-FTIR analüüsi tulemused kinnitasid saadud tulemusi. Maisivalgust valmistatud maatriksite tahke faasi analüüs näitas, et kõik lähteained olid elektrospinnimise järgselt amorfses vormis.

Mehhaaniliste omaduste analüüs näitas, et PCList valmistatud mattide mehhaanilised omadused sõltusid oluliselt maatriksi ja fiibri struktuurist. Poorsetel mikrofiibermaatriksitel oli kõige suurem venivus, mis näitab materjali deformatsioonivõimet enne purunemist. Võrdluseks – kõige madalam deformatsioonivõime oli mittepoorsel nanofiibermaatriksil. Kirjanduses on näiteid mehhaaniliste omaduste sõltuvuse kohta maatriksi struktuurist, väiksemate fiibritega maatriksitel on suurem tõmbetugevus ning erimoodul (*Young's modulus*). (Chew et al., 2006; Tan et al., 2005) Raviainet sisaldavate maatriksite mehhaaniliste omaduste uurimisel selgus, et raviaine lisamine suurendas tõmbetugevust ning erimoodulit. Maisivalgust valmistatud maatriksite mehhaaniliste omaduste määramisel selgus, et kõik proovid olid madala erimooduli ning tõmbetugevusega.

Fiibermaatriksite märgumisomaduste ja hüdrofiilsuse/hüdrofoobsuse uurimiseks kasutati kontaktnurga määramise meetodi. PCList valmistatud maatriksitest oli kõige hüdrofiilsem raviainet sisaldav poorne mikrofiibermaatriks. Samuti uuriti PCList valmistatud maatriksite *in vitro* degradatsiooni. Katse tulemusest järeldus, et kõige enam muutus poorsete fiibermaatriksite struktuur (peale 24 h puhverlahuses, 37 °C). Poorsete fiibrite diameetrid olid suurenenud, samuti esines fiibrite katkemist. Maisivalgust valmistatud fiibrite kontaktnurga määramisel selgus, et tegemist oli väga hüdrofiilsete maatriksitega. Kontaktnurga analüüsil absorbeerisid kõik maisivalgu maatriksid tilga 1–3 sekundi jooksul (PCLi maatrikseid analüüsiti kuni 60 sekundini). *In vitro* degradatsiooni määramisel selgus, et abiaineteta (PEOta) maatriksid lagunesid täielikult 48 h jooskul. Teiste maisivalgu maatriksite degradatsiooni analüüsil oli näha, et esines paisumist, kuid fiiberstruktuur säilis (fiibrid olid SEM piltidel alles).

Raviaine vabanemiskatsetest oli näha, et PCList toodetud maatriksitel esines esmalt kiire vabanemise faas (*burst release)*, millele järgnes aeglane raviaine vabanemise faas. Kõige rohkem raviainet ja kõige kiiremini vabanes raviaine mittepoorsel nanofiibermaatriksil – 3 min jooksul vabanes ligi 67% raviainest. Poorsest mikrofiibermaatriksist vabanes 3 min jooksul 53% raviainest ning mittepoorsest mikrofiibermaatriksist 20%. Katse viimases ajapunktis (96 h) vabanes raviainet vastavalt 73%, 60% ja 21%. Katse tulemusest järeldus, et fiibermaatriksi struktuur mõjutas raviaine vabanemise kiirust. Tõenäoliselt toimus kõige suurem raviaine vabanemine mittepoorsetest nanofiibermaatriksist seetõttu, et raviaine asus enam fiibrite pinnal. Puutudes kokku dissolutsioonikeskkonnaga toimus kiire raviaine vabanemine. Mittepoorses mikrofiibermaatriksis paiknes raviaine enamasti fiibrite sees ning seetõttu jäi suurem osa (ligi 80%)

raviainest vabanemata. Samast lahusest (kuid kõrge suhtelise õhuniiskuse juures) valmistatud poorsest mikrofiibermaatriksist vabanes sama aja jooksul ligi 3× rohkem raviainet. Fiibrite pinnal asuvate pooride tõttu oli puhverlahusel lihtsam fiibritesse ning maatriksisse absorbeeruda, suurendades difusiooni. Maisivalgust valmistatud maatriksite vabanemiskatse näitas, et kiire vabanemise faas esines kõikidel proovidel, 5 minuti ajapunktis oli maatriksitest vabanenud ligi 70% tetratsükliini.

Maatriksite bioloogilist olulisust ning võimalikku toksilisust uuriti eukarüootsete rakkudega kasutades BHK-21 rakuliini. Selleks, et maatriksid omaks haavade parandamist soodustavat mõju, peaksid rakud olema võimelised substraadile kinnituda ning seal paljuneda. Lisaks peaksid kõik materjalid olema ohutud (mitte toksilised). Eukarüootsete rakkude kinnitumise määramiseks kasutati MTS testi. MTS reaktiivi aktiivseks komponendiks on fenasiin metosulfaat, mis reageerides metaboolselt aktiivsete rakkudega metaboliseerub formasaaniks (testis määratakse absorptsiooni 490–500 nm juures) (Kuete, Karaosmanoğlu, and Sivas 2017). Kõrgeim MTSi aktiivsus oli rakkudel, mis kasvasid poorsetel raviainet sisaldavatel PCLi maatriksitel. See näitab, et fiibrite poorsus soodustas BHK rakkude kinnitumist ning paljunemist. Kõikide raviainet sisaldavatel mattidel ei täheldatud negatiivset efekti BHK rakkudele. Sellest võib järeldadada, et kloramfenikooli kontsentratsioon oli optimaalne ning ei olnud rakkudele toksiline. Maisivalkul baseeruvate fiibermaatriksite MTS aktiivsus (0.75–0.85) oli madalam kui PCLi maatriksitel (0.85–0.95). Tetratsükliini sisaldavatel maatriksitel ei täheldatud negatiivset mõju BHK rakkudele, mis kinnitab, et tetratsükliini konsentratsioon oli samuti optimaalne. PCL-il baseeruvatel fiibermaatriksitel uuriti ka BHK rakkude infiltreerumist maatriksisse kasutades FM 4–64 membraanivärvi ning analüüsides maatrikseid konfokaalmikroskoobiga. Analüüsist järeldus, et enim rakke ning suurimat infiltreerumist oli märgata raviainet sisaldavatel poorsetel mikrofiibermaatriksitel. Mittepoorsetel raviainet sisaldavatel mikrofiibermaatriksitel oli BHK rakke maatriksi pinnal samal määral, kuid maatriksi sisse infiltreerumist täheldati vähem. Võrdluseks mittepoorsetel nanofiibermaatriksitel (nii raviainega kui ilma) paiknesid rakud põhiliselt maatriksi pealmises kihis ning infiltreerumist esines kõige vähem. Sellest võib järeldada, et antud struktuur ei olnud ideaalne substraat BHK rakkudele infiltreerumiseks.

Järeldused

Kasutades kandurpolümeere (PCL, PEO ja zein) ning antibakteriaalseid raviaineid (kloorafenikool ja tetratsükliin) õnnestus monoaksiaalse ja koaksiaalse elektrospinnimise meetoditega valmistada erineva koostise ja struktuuriga nanoning mikrofiibermaatrikseid. Fiibrite poorsust oli võimalik modifitseerida sobivate solventidega (THF:DMSO) ning muutes keskkonnaparameetreid (kõrgendatud suhteline õhuniiskus). Solventide valik mõjutas saadud maatriksi morfoloogiat, struktuuri ning raviaine vabanemist.

Raviaine vabanemine sõltus maatriksi struktuurist. Kõikidel maatriksitel esines raviaine kiire vabanemise faas (*burst release*). PCLil baseeruvatest fiiibermaatriksitest oli suurim raviaine vabanemine mittepoorsel nanofiibermaatriksil, järgnesid poorne mikrofiibermaatriks ning mittepoorne mikrofiibermaatriks. Kuna fiibermaatriksitest raviaine vabanemine sõltub peamiselt difusioonist, määras erinev struktuur maatriksi märgumisvõime ning puhverlahuse tungimise kiiruse maatriksisse. Kõige hüdrofoobsemast, mittepoorsest mikrofiibermaatriksist vabanes ainult 20% raviainet ehk suurem osa raviainest jäi fiibrite koostisse.

Maatriksite mehhaanilised omadused on haavaravi seisukohast väga olulised. Maatriksile olulised omadused on elastsus ja venivus ning soodne keskkond haavaravis osalevatele rakkudele. Kõrgeimat deformatsioonivõimet täheldati poorsetel mikrofiibermaatriksitel. Mehhaanilisi omadusi testiti ka biorelevantses keskkonnas (puhverlahuses, imiteerimaks haava), kuid suuri muutusi ei täheldatud.

Erineva struktuuriga maatriksid mõjutasid rakkude/maatriksite omavahelisi interaktsioone. Maisivalgul (zein) baseeruvatel maatriksitel oli madalam eukarüootsete rakkude kinnitumine ning paljunemine (võrreldes PCLi maatriksitega). PCLil baseeruvatest maatriksitest oli parim fibroblastide kinnitumine ning kasv poorsetel raviainet sisaldavatel mikrofiibermaatriksitel. Sama täheldati *E. coli* vastase antibakteriaalse ning biokile vastase toime uurimisel. Sellest järeldati, et sobiva suuruse (mikrofiibrid) ning struktuuri olemasoluga (poorsed fiibrid) on võimalik mõjutada maatriksi/rakkude omavahelisi interaksioone. Maatrikseid on võimalik disainida selliselt, et suurendada nende häid antibakteriaalseid ja biofilmi vastaseid omadusi ning nende biosobivust, ja muutes nad seeläbi sobivaimaks lokaalses haavaravi jaoks.

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PUBLICATIONS

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List of publications:

Ramos, Celia*; **Lanno, Georg-Marten***; Laidmäe, Ivo; Meos, Andres; Härmas, Riinu; Kogermann, Karin (2020). High humidity electrospinning of porous fibers for tuning the release of drug delivery systems. *International Journal of Polymeric Materials and Polymeric Biomaterials* 70(12), 880–892. https://doi.org/10.1080/00914037.2020.1765361
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