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Review article

Electrospinning For Drug Delivery Applications: A Review

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Abstract

Drug delivery devices are promising tools in the pharmaceutical field, as they are able to maximize the therapeutic effects of the delivered drug while minimizing the undesired side effects. In the past years, electrospun nanofibers attracted rising attention due to their unique features, like bio-compatibility and broad flexibility. Incorporation of active principles in nanofibrous meshes proved to be an efficient method for *in-situ* delivery of a wide range of drugs, expanding the possibility and applicability of those devices. In this review, the principle of electrospinning and different fields of applications are treated to give an overview of the recent literature, underlining the easy tuning and endless combination of this technique, that in the future could be the new frontier of personalized medicine.

Keywords: electrospinning, drug delivery, nanofibers, tissue engineering, controlled release

1. Introduction

Pharmaceutical research of new drug candidates is one of the most challenging tasks for academics and industries.[1] It is estimated that in 2018 pharmaceutical industries spent 179 billion dollars globally for research and development of new pharmaceuticals.[2] However, approximately only 11% of new candidates have the probability of reaching the market.[3] The most common failure appears during phase II clinical trials, were most drug candidates show previously unknown toxic side effects or insufficient efficacy to treat the medical condition being tested.[4] Still, drugs reaching the market are not free of possible side effects: for example, due to their intrinsic toxicity anticancer chemotherapeutics remain a concern in both therapists and patients. Besides their potency and target selectivity has been improved over the years, severe side effects like infections, vomiting, fatigue, loss of taste, anemia and destruction of the immune system are still present.[5] Another rising threat associated with the use of antibiotics is the selection of multi-drug resistant (MDR) bacteria strain. Given the decline of discovery of new antibiotics, recent estimations predict that in 2050 antimicrobial resistance could cause up to 50 million deaths per year all over the world.[6,7]

During the past few decades, it became clear that the method of delivery influences the therapeutic benefit of a drug, affecting numerous factors, including pharmacokinetics, distribution, pharmacodynamics, and metabolism as well as toxicity.[8] Together with the discovery of nanotechnologies such as nanoparticles, nanofiber, nanogels, micelles, and microspheres, the development of new approaches to drug delivery systems became a new promising tool in the pharmaceutical field.[9] Nanocarriers can be used to wrap and deliver pharmaceuticals that are too toxic, insoluble, rapidly cleared, or unstable as free molecules by passive or active targeting strategies based on the final formulation.[10,11] A recently developed approach, for instance, consists in the use of a delivery system based on cells or their derivative products such as erythrocytes, platelets, stem cells and extracellular vesicles as nanocarriers for drug delivery, which have been recently applied to many fields.[12]

Among all these alternatives, nanofibers produced with bio-degradable and bio-compatible polymers gained increasing interest due to their broad flexibility, effectiveness and the unique physiochemical properties such as a large surface area, small diameter, and high aspect ratio.[13,14] Also,

targeted in-situ application of nanofibrous scaffolds could minimize the disadvantages of systemic perfusion with the free drug or other drug delivery system, and on the other hand, maximize the action of the active pharmaceutical by a controlled and sustained release directly at the site of action.[15] For instance, a nanofibrous scaffold can reduce the threat of antibiotic-resistant bacteria and multi-drug resistance in cancer therapy by site-specific, dose-specific and timed release of different types of drugs.[16–18]

Another great advantage is given by the similarity of the fibers with the natural fibrillary extracellular matrix (ECM), which facilitates cell attachment and proliferation for biomedical applications.[9,14] During the years, electrospinning proved to be one of the most cost-effective, simple and flexible fabrication technique in the choice of polymer techniques for the production of nanofibers.[19] Electrospinning is performed applying a high voltage electrostatic field to a suitable polymer solution flowing through a needle. A specific feature of the final electrospun fiber is that structural design parameters such as porosity, morphology and surface area could be tuned easily by modification of the environmental and processing conditions, according to the specific requirements for the delivery conditions.[13]

Drugs can be incorporated in the fiber by different approaches. Starting from a direct blending between the drug and the polymer solution, a surface immobilization after the spinning process, or by using an emulsion, each of them provides a different profile of drug release. A large variety of drugs has been successfully incorporated into electrospun fibers from small molecules to proteins and nucleic acids. More sophisticated devices are also able to deliver multiple drugs with synergistic effects or to selectively tune the release of the incorporated drug in response of specific stimuli.[20] The purpose of this review is to give an overview of the possible approaches of electrospinning for drug delivery purposes by giving an insight into the different techniques and field applications.

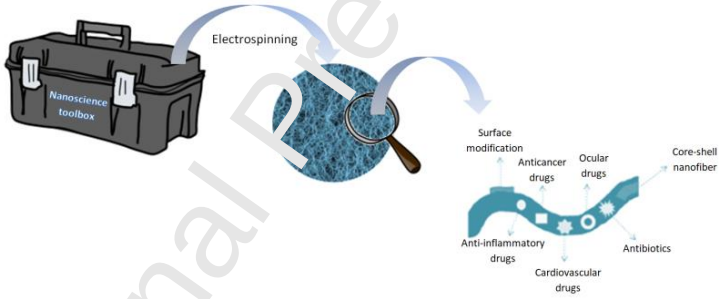


Fig. 1. Nanofibers for drug delivery

2. Electrospinning

Electrospinning uses an electrostatic potential characterized by high voltage and very low current for creating ultrafine fibers. Historically, the first observation of an electrospinning process for such purpose was in 1902 by J. F. Cooley who patented the technique with the name of “Apparatus for electrically dispersing fibers”.[21] The popularity of electrospinning raised during the end of the 20th century when many publications started to appear and continue today, where many applications for electrospun fibers, such as drug delivery[22–24], wound healing[25,26], tissue engineering[27,28], textiles[29] as well as sensors[30], cosmetics[31] and food packaging[32] are studied.

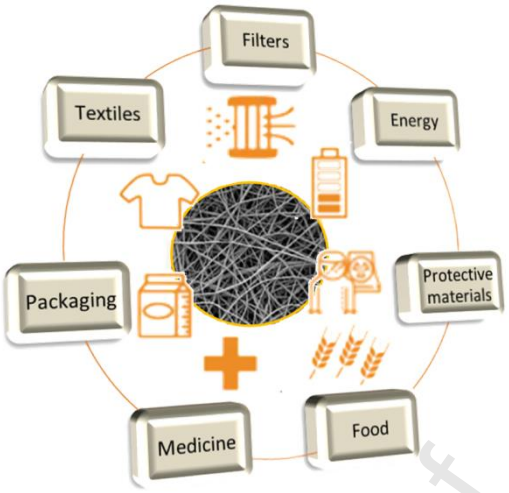


Fig. 2. Fields of application for electrospun nanofibers

The overall process is carried out by using a polymer solution or directly a melted polymer. The polymer must be pumped through a spinneret (usually a syringe needle), to which a high voltage is applied. The applied voltage induces a charge movement in the polymer liquid, able to stretch the shape of the pendant drop, normally a sphere formed by the surface tension. Once the electrostatic repulsion of the charged polymer liquid becomes higher than the surface tension, a conical shape known as a Taylor's cone is formed and the jet initiation starts from the cone tip. Remarkably, the two forces that induces the formation of the Taylor's cone are controlled indirectly by flow rate and applied voltage. Therefore a good balance benefits the formation of a stable jet. If enough cohesive force exists in the polymer liquid, a stable jet is ejected from the Taylor's cone, allowing the polymer chains to stretch each other and forming a uniform filament. The process is accompanied by the evaporation of the solvent causing a vigorous whipping of the formed filament.[25,33] Fibers deposition occurs over a grounded metallic collector, usually formed by a simple aluminum foil placed at optimized distance.[21] Normally, fibers deposition occurs randomly over the collector. However, some fields of application require structured scaffolds with aligned fibers. For this reason, several approaches were developed for the creation of ordered structures. The most straightforward strategy consists in the use of a rotating mandrel or a wheel-like bobbling collector. In some cases, the bending instability of the jet disrupts the collection of the fiber along the rotational direction.[34,35] Auxiliary electrodes, able to manipulate the electric field in the space between the needle and the collector, could efficiently reduce the bending instabilities and improve fibers alignment.[36–38]

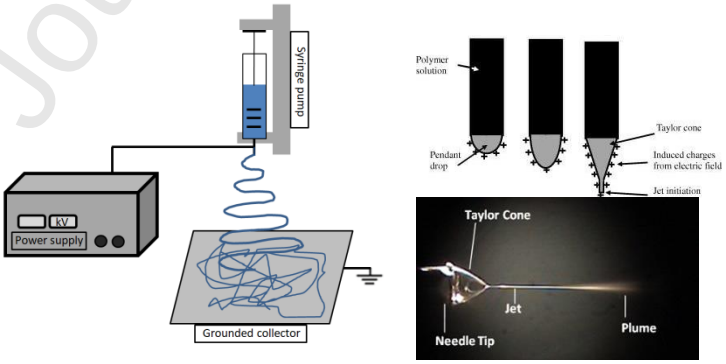


Fig. 3. (a) Schematic representation of an electrospinning apparatus. (b) the formation of the Taylor cone.

Eslamian *et.al.* (2019) compared the release profile of the drug dexamethasone from randomly aligned poly(lactic-co-glycolic acid) (PLGA) nanofibers and highly aligned ones. [39] The authors optimized all the working parameters, including the angular velocity of the wheel collector, to obtain fibers with more than 99% of the spatial orientation index, the degree of alignment along one axis. *In vitro* release showed less burst and a longer sustained release wherein the aligned fibers, presumably due to the higher porosity

of random fibers and anisotropic degradation of aligned fibers respectively. Exploiting the same methodology, Han *et.al.* (2019) developed astatic acid embedded poly(lactic acid) (PLA) nanofibers for diabetic wound healing. [40] Astatic acid is a molecule extracted from *C.asiatica* that proved to promote the gene expression of TGF-β (Transforming growth factor-β), VEGF (Vascular endothelial growth factor), and FGFs (Fibroblast Growth Factors) expression in fibroblasts as well as antiflammatory and anti-bacterial effects. The high fibers alignment, obtained with a rotating drum, granted to accelerate the re-epithelization, angiogenesis, and extracellular matrix formation of the wound in *in vivo* models. Also, the combination with the drug and its slow release of the drug over 7 days decreased the oxidative stress, inflammation, and infection at the wound site.

Other collectors types are useful for the creation of 3D coiled scaffolds. One example is a coagulation bath made of a non-solvent.[41] In this system, fibers collection occurs in a coagulation bath of deionized water, ethanol, or methanol. Employing this strategy, Sonseca *et.al.* were able to successfully create 3D helically coiled scaffolds from segmented co-polyester of poly (butylene succinate-co-dilinoleic succinate) for the future development of architectures able to mimic the behavior of human soft tissues, such as the heart muscle perimysium. This approach granted high specific surface area, high porosity, and good elasticity of the final product. This architecture could be exploited for the development of drug delivery devices, for instance as patches for cardiac drugs delivery in the heart failure treatment.[42]

2.1 Kinetic of release

The treatment of a specific disease requires a proper curve of drug release from the polymeric scaffold. The knowledge of the release kinetics allows a simple tuning of the desired behavior by choosing over the different methodologies for fiber production.[9]

Different methodologies, morphology, and drug loading strongly influence the release profiles. For example, Li *et.al.* (2020) created a tri-layered structure for the treatment of breast cancer by combining different drugs in different polymers. [43] By exploiting different drug-polymer combination, the authors were able to achieve a time-programmed release of different chemotherapeutics agents with a synergistic effect. Blended fibers represent the simplest nanofibers producible by electrospinning. In this case, the release strongly depends on the degree of drug encapsulation inside the polymeric matrix and the drug-polymer affinity.

Two main points are mandatory for the achievement of a sustained-release: the first is a good affinity between the polymer and the drug polarity, the second is a complete solubility of the drug in the polymer solution. If those requirements are not met, usually, the drug is burst released from the polymeric matrix in a small time window.[44]

Wu *et.al.* (2020) proposed a possible mechanism by analyzing the behavior of poly(D, L- lactide-co-glycolide) (PLGA) blends embedded with ciprofloxacin. [45] Release kinetics drove to the identification of three different stages: during the first few hours release occurs through stage one, described by a first-order equation in which the diffusion of the embedded molecules is controlled by the fibers swelling. The second stage, whose duration is around a few days, has a more flattered and sustained release, described by the zero-order equation of the Higuchi model. In this stage, the rate-determining step is the movement of the drug to the surface of the fiber. The release became proportional to time but independent from the concentration of the molecule. The third and final stage is mainly characterized by the hydrolysis of small oligomers from the scaffold, their diffusion together with the entrapped molecules controls the rate of release. This stage has a square-root time dependence and continues until the fiber is completely discharged.

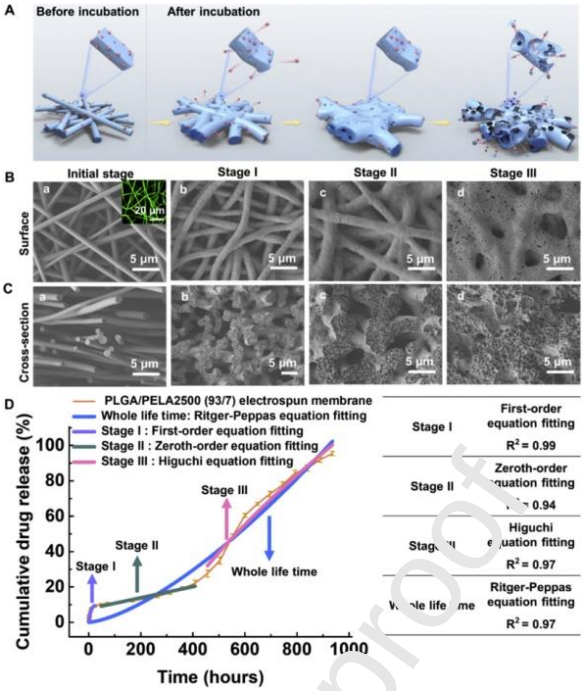


Fig. 4. Typical release process of drug delivery system developed by Wu *et.al.* (A) Schematic illustration of the drug release mechanism divided into three stages. (B) Surfaces of PLGA/PELA2500 (93/7) electrospun membrane imaged by SEM. The insets (a) show the distribution of ciprofloxacin (green fluorescence) imaged by CLSM. (C) Cross-sections of PLGA/PELA2500 (93/7) electrospun membrane imaged by SEM. (D) In vitro drug release profile of PLGA/PELA2500/ciprofloxacin together with fitting by the four kinetic equations relative to the three different stages and the overall time equation. Figure reprinted from Wu *et al.* [45].with permission from Elsevier.

Core-shell nanofibers produced by coaxial electrospinning could mitigate the drawbacks relative to the burst release. Typically, the polymeric core is drug embedded, while the shell acts as a physical barrier between the core and the solution. The presence of the barrier in coaxial fibers allows sustained release for a longer time and more protected drug from environmental degradation. [46]

Still, coaxial electrospinning is not as simple as monoaxial electrospinning and requires a specific apparatus or at least a coaxial needle and two syringe pumps. Once optimized, the process can be not so challenging. However, the selection of appropriate polymers and process parameters could require longer time compared to other techniques. An alternative approach for the creation of core-shell nanofibers is emulsion electrospinning. In this case, polymer and micelles contribute to achieving a slower and more prolonged release.[47] Besides changing the emulsion parameters, such as concentration and surfactant type, can easily control the obtaining of the desired release profile.[48] Qi *et.al.* (2006) fabricated bovine serum albumin (BSA) loaded Ca-alginate microspheres before electrospinning in poly (L-lactic acid) (PLLA) W/O emulsion. [49] The authors demonstrated not only efficient incorporation of BSA, but also a sustained release 12 times longer than the naked microspheres.

2.2 Types of Polymers

For each different application, nanofibers need to fulfill specific requirements in terms of mechanical proprieties, hydrophilicity, morphology and biocompatibility. The chemical composition of the fiber, namely the polymer structure, governs these features. Polymer structure influences the release rate of the loaded drug and the duration of the treatment, and the main factors playing a role are: 1) polymer swelling in water, 2) polymer affinity with the drug, and 3) polymer degradation rate. Also, polymer molecular weight influences some of the physical features of the fiber, as for instance its thickness and physical stability. Molecular weight is involved as well in controlling the polymer concentration at which electrospinning can be performed, as a consequence of the direct dependence between polymer molecular weight and solution viscosity at fixed concentration. [23,50]

An exceedingly high number of polymers, both natural and synthetic, are employed in fibers production in the biomedical field. [36]–[51] Natural polymers, as gelatin, chitosan, and silk fibroin possess unique features like high cellular affinity and exceptional biocompatibility, together with the presence of many functional groups allowing easy chemical modification.[52,53] Ansari *et.al.* (2019) produced levodopa-loaded Zein protein nanofibers for the treatment of Parkinson's disease. [54] Zein, a protein derived from corn endosperm, proved a good efficiency in encapsulating the drug and giving an excellent sustained release.

By taking advantage of silk fibroin functional groups Mehraz *et.al.* (2020) developed β-cyclodextrin-grafted silk fibroin nanofibers. [55] Silk fibroin was covalently attached to cyclodextrin before the electrospinning process. Citric acid was used as an ester linker between primary hydroxyl of CD and hydroxyl of silk fibroin using a double esterification process. This approach allowed a post-spinning ciprofloxacin loading by the formation of a host-guest complex between the covalently attached cyclodextrin and the drug. In this way, authors were able to reach a steady release of drug after more than 50 hours with a general slower delivery of drug compared to the pure silk fibroin scaffold, having the possibility to control the total amount of drug released by modification of the polymer-cyclodextrin ratio. Exploiting a similar approach Murali *et.al.* developed chitosan modified nanofibers for the delivery of simvastatin. [56] Since chitosan is extremely hydrophilic inducing a loss of its nanofibrous structure, acylation of amino groups of its chemical structure with fatty acid grants protection and enhanced hydrophobicity. In this case the hydrophobicity depending from acyl chains length controls the release: since simvastatin is a hydrophobic drug, shorter is the chain faster is the release. Longer chains were able to release the hydrophobic drugs up to 90 days. Preliminary *in vitro* studies confirmed the efficacy of the delivery. Furthermore, easy modification of this polymer could be useful for the delivery of other classes of hydrophobic drugs.

On the other hand, the majority of natural polymers lack stability in physiological condition and have generally poor mechanical proprieties. Conversely, synthetic biopolymers like poly(ethylene glycol) (PEG), poly(lactic acid) (PLA), Poly(ε-caprolactone) (PCL), and poly(lactic-co-glycolic acid) (PLGA) satisfy the stability requirement in physiological conditions mutually with easy tunability for the specific physicochemical and mechanical requirements by chemical modification depending on the targeted application.[57] García-Salinas *et.al.* (2020) addressed the production of wound healing patches loaded with essential oils as anti-inflammatory agents by using PCL as polymer. [58] The authors were able to produce nanofibers with the ability to reduce significantly the production of pro-inflammatory cytokines using *in vitro* models. In another work, Ide *et.al.* (2020) were able to develop an innovative delivery platform for local anesthetic by the use of PLGA electrospun scaffolds. [59] The produced nanofibers showed an improved *in vitro* release compared to other local anesthetic and an *in vivo* prolonged analgesia compared to lidocaine infiltration in rat model of postoperative pain. In particular, authors were able to achieve a sustained release of vivobupivacaine for about 35 days and prolonged analgesic effects in a rat model of postoperative pain. Furthermore, the biocompatible profile of PLGA and the slow release achieved granted *in vivo* safety profile confirmed by symptomatic, histological, and pharmacokinetic analysis. In the same way, Budai-Szűcs *et.al.* (2020) obtained interesting preliminary results for the treatment of periodontitis by creating PLA nanofibrous scaffolds embedding the antibiotic metronidazole. [60] Authors were able to enhance the scaffold roughness and surface porosity by physical compression of the neat fiber, boosting at the same time the drug release compared to the non-compressed scaffolds. The hydrophobic polymer matrix demonstrated the ability to slowly reach a release plateau in 24 hours with the capability of *in vitro* inhibition of bacterial growth up to 13 days. Another minor advantage of this system is the masking of the undesirable bitter taste of the metronidazole, thus hiding its local application as a drug for periodontal treatment.

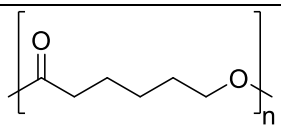
A major advantage of nanofibrous delivery systems is the possibility of implantation directly at the site of action, thus reducing the systemic toxicity of the embedded drug.[15] To further increase the specificity of drug action several stimuli-responsive nanofibrous devices were developed. This special kind of scaffolds is able to release the drug only when certain environmental conditions were realized.[61] For example, it is known that the tumor microenvironment is characterized by more acidic pH than healthy tissue, this peculiar feature can be used for the creation of delivery systems able to release a chemotherapeutic only in such pH conditions.[62] Jassal *et.al.* (2015) exploited this possibility by creating a partially hydrolyzed PCL scaffold, exposing carboxylic groups on the surface. [63] The exposed carboxyls were used for doxorubicin loading as well as a sensor for the surrounding environment. In this way, the obtained scaffolds were able to release a higher quantity of the drug in acidic media with respect

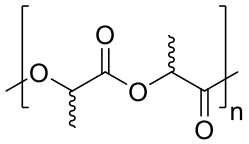
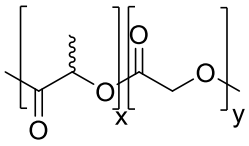
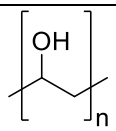
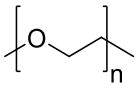
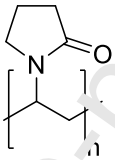
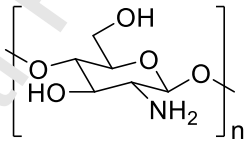
to neutral pH with expected safer profile for future development. To overcome a big challenge in small-diameter vascular grafts creation, which is achieving rapid endothelialization and long-term anticoagulation, Wang *et.al.* (2020) developed small-diameter PCL nanocoated vascular grafts with a metallopeptidase 2 regulative mechanism. [64] In this way, bioactive molecules such as gelatin, poly-lysine, and heparin were delivered with a long-term release for more than 35 days without burst, exhibiting *in vitro* greatly improved biocompatibility and endothelial cell affinity and proliferation, setting the basis for more advanced studies.

Fazio *et.al.* (2018) created coaxially spun nanofibrous composites to obtain a dual-sensitive drug delivery systems for antitumor applications. [65] PEG-PLGA emulsion with Au or Ag and the therapeutic agent silibinin was used as the core, while Fe_2O_3 magnetic nanoparticles in PVA were used as a shell. The presence of nanoparticles allows these systems to control both the time and amount of the active agent to be released at a specific target site by fine-tuning with a light source and a magnetic field, achieving sustained release for more than 60 hours without burst.

A very simple but effective strategy for combining the advantage of different types of polymers is blending. The dissolution of two or more polymers in the same media could facilitate the achievement of desired proprieties combining the different advantages of the materials [65]. For example, blending a synthetic polymer with a natural polymer can result in the production of a scaffold with both high mechanical properties and cell-affinity features. Remarkably, the only limitation to this technique is the thermodynamic compatibility between the two polymers: if the two materials possess adverse thermodynamics, they can phase separate when mixed together. [66,67] To overcome phase separation issues between keratin and polyamide-6, a synthetic polymer with molecular structure similar to proteins, Aluigi *et.al.* studied the rheological proprieties to identify a suitable system for electrospinning. [66] The miscibility degree and the super-molecular organization of the cast films obtained from blend solutions were compared to electrospun nanofibers produced from the same blends. Results highlighted that, in this case, the production of smooth and beadles nanofibers was possible due to the rapid solvent evaporation during electrospinning, which prevented polymer separation of the fresh prepared solution. Ramalingam *et.al.* (2019) used this technique for the creation of nanofibers loaded with *Gymnema sylvestre* extract for antimicrobial and wound dressing applications, using a blend between PCL and gelatin for the scaffold. [67] The mixture of the two polymers succeeded in the achievement of the desired materials proprieties, where the use of the polymers alone could not. For the creation of biomimetic tissue-engineered vascular graft, Wan *et.al.* (2020) used a PCL/keratin blend. [68] In this work, the formulation allowed not only to efficiently obtain smooth and suitable nanofibers capable to promote adhesion and growth of human umbilical vein endothelial cells (HUVECs) but, due to the keratin functional groups, to covalently bind VEGF growth factors and heparin. The dual loading allowed heparin to stabilize VEGF toward proteolytic degradation and subsequently prolong the sustained release, with prolonged blood clotting time and decrease platelet adhesion without erythrolysis. Nur Akşit *et.al.* (2020) used a blend of PLGA and gelatin for the development of membranes containing different amounts of *Hypericum capitatum var. capitatum* extract. [69] The blend combines the biocompatible and biodegradable properties of PLGA with gelatin able to increase cell proliferation and attachment due to the presence of the RGD sequence enabling the recognition of the integrin protein on the cell surface. Also, the hydrophilic nature of the extract required an adequate modulation of the hydrophobicity features of the polymer composition to obtain a proper drug release. Biological results showed low cytotoxicity of the scaffolds together with enhanced proliferation and bacteria growth inhibition, suggesting potential interest of this formulation in the wound dressing field.

Table 1 Examples of synthetic and natural polymers used for the creation of nanofibers for drug delivery purposes, their molecular structure, and the experimental conditions used for the spinning solution.

	Polymer-abbreviation	Molecular structure	Solvent	Reference
Synthetic	Polycaprolactone or poly(ε-caprolactone) PCL		10% DCM 10% DCM:DMF 9:1	[140][106]

	poly (D,L-lactic acid) or polylactic acid PLA poly (L-lactic acid) or polylactic acid PLLA		4% DCM 30% dimethyl carboxylate (DMC)	[113][130]
	poly(D,L-lactic-co- glycolic acid) PLGA		25% ACN	[163]
	Polyvinylalcohol PVA		8% water	[125]
	Poly(ethylene glycol) polyethylene oxide PEG/PEO		1 to 3% water:ethanol 7:3	[122]
	Polyvinylpyrrolidone PVP		10% ethanol 10% ethanol:CAN 1:1	[158][163]
Natural	Gelatin (protein)	-	50% DCM:DMF 75:25	[139]
	Silk fibroin (protein)	-	10 to 16% formic acid	[46][168]
	Chitosan (polysaccharide)		5.5% TFA:DCM 7:3 2% acetic acid	[47][148]

2.3 Electrospinning methods

Fibers production by electrospinning takes place by application of high voltage to a liquid polymer flowing through a spinneret. A classification of different electrospinning methods is possible by analyzing the liquid source and the type of spinneret.[21]

Two different techniques are employed for the generation of liquid polymers: The first involves the dissolution of polymer in a suitable solvent; the second make uses of the melting point of the polymer itself. In solution electrospinning, the liquid is a solution of the polymer itself in a suitable solvent or mixture of different solvents. Melt electrospinning, on the other hand, uses the heat to liquefy the polymer without using any solvent.[70,71]

Both setup have their advantage and disadvantage: melt electrospinning eliminates the need of a large amount of solvent, is cheaper and highly reproducible. However, for drug delivery purposes the use of high temperatures required for reaching the melting point of the polymer could degrade the drug.[72] Moreover, the presence of the solvent in solution electrospinning helps the whole spinning process by the easy modification of viscosity and conductivity parameters of the liquid. In addition, the whole process occurs at room temperature.[23,73]

Lian *et.al.* (2017) compared the release of curcumin-loaded PCL nanofibers produced with melt and solution electrospinning. [74] PCL has the advantage of displaying the lowest melting point among hydrophobic polymers making it suitable for the purpose. The morphology obtained by melt electrospinning presented higher crystallinity and lower porosity compared to the same fiber produced by the solution technique. Those characteristics allowed a slower and sustained drug release without heavy burst in the first phase, in the melt fibers. In another work, Lian *et.al.* (2017) employed melt electrospinning for the

development of daunorubicin hydrochloride-loaded PCL fibrous scaffolds. [75] Again, the high crystallinity of the spun material allowed an approximately linear drug release profile, with slow-release rates for 3-4 days and long-term release periods for more than 16 days, without any burst. At the same time, scaffolds possessed excellent antitumor properties, significantly inhibiting tumor cell growth, with the possibility of efficiently tuning the amount of drug embedded and consequently increasing biological activity by increasing drug content.

Spinneret or nozzle geometry governs the production of fibers with different morphologies. In practice, the nozzle is the component in which the polymer flows through and the voltage is applied.

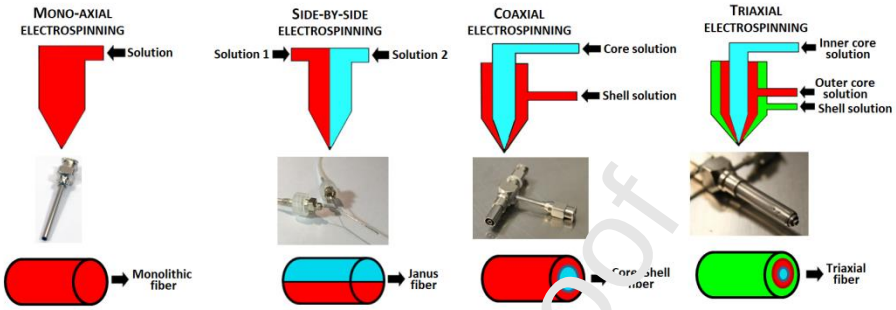


Fig. 5. Schematic examples of electrospinning setup and their fibers output, (a) Mono-axial electrospinning. (b) Side-by-side electrospinning. (c) Coaxial electrospinning and (d) Triaxial electrospinning

Single-axial represents the easiest setup for electrospinning. This technique uses a single capillary or a syringe needle as the nozzle.[76] A more sophisticated setup involves multi-channel or multi-axial technologies. In particular, side-by-side and coaxial spinneret are composed of two or more capillaries, placed one adjacent to the other and one inside the other, respectively.[9,77] Both techniques allow the use of several polymers. However, conversely from blending, by using those techniques the polymers will coexist in the final fiber without physical mixing. For example, coaxial electrospinning allows the production of core-shell nanofibers.[9] Ye *et.al.* (2020) in order to provide long-term effect against the resistant bacteria strains of *S. aureus* developed by coaxial electrospin nanofibers encapsulating the antibiotic emodin in the core of hydrophilic PVP, with a hygroscopic cellulose acetate sheath. [78] By employing this technique, the authors were able to obtain a release profile with significant inhibition of bacteria growth from 24 h up to 9 days. This work is in contrast with other mono-axial emodin nanofibers having a high burst release with complete clearance of the drug up to 90 minutes. Sruthi *et.al.* (2020) created veratric acid sustained-release device with osteogenic potential by coaxial electrospinning of PCL as shell and PVP as the core. [79] Veratric acid (3,4 di-methoxy benzoic acid) is a benzoic acid derivative extracted from different natural sources like plants and mushrooms; the molecule exhibited antibacterial, anti-inflammatory, antioxidant, anti-hypertensive, and UV protective properties. The osteogenic potential was investigated in this study. The sustained release was facilitated with the creation of veratric acid-loaded chitosan nanoparticle embedded in the polymeric core instead of simple free drug blending. Results suggested physiochemical and material properties biocompatible with mouse mesenchymal stem cells. The sustained release over 25 days highlighted the capability of the scaffold to promote osteoblast differentiation through monitoring calcium deposit and other biological markers.

By proper modification of the mechanical setup, coaxial electrospinning can also be used for the preparation of more complex architectures for example the tri-axial fibers. Nagiah *et.al.* (2020) developed triaxial fibers formed by PCL as the core layer, a 50:50 PLGA as the sheath layer, and gelatin as the intermediate layer. [80] Two model compounds were embedded within the fiber: Rhodamine B and Fluorescein isothiocyanate-Bovine Serum Albumin conjugate, respectively inside the sheath and middle layers. The fibers produced with this technique, not only demonstrated a dual simultaneous release up to 600 hours, but also higher tensile proprieties compared to the uniaxial PLGA (50:50) and coaxial PLGA (50:50) (sheath)-gelatin (core) fibers used as control. In particular, the authors were able to strongly reduce the known drawback of fiber shrinking in cell culture media, typical of PLGA fibers. The result was achieved thanks to the excellent affinity between different layers of the triaxial fibers. Also, the biocompatibility of the scaffold remained unaltered.

With a similar approach, Liu *et.al.* (2019) were able to create scaffolds with a precise tuned zero-order release of ferulic acid. [81] The process was carried out using two un-electrospinnable liquids as the outer and middle working fluids, with only the core solution being individually electrospinnable into fibers. In particular, the outer liquid was a mixture of acetone and acetic acid, while the middle fluid was a dilute solution of cellulose acetate, and the core fluid was an electrospinnable co-dissolving solution of ferulic acid and gliadin. The solvents in the outer layers were used mainly for two reasons: on one hand, to prevent the clinging of cellulose acetate solution on the nozzle of the spinneret; on the other hand, to eliminate the negative influences from the environment. The produced scaffolds possess a fibrous core, coated with an even layer of cellulose acetate controlled in terms of thickness by variation of the flow rate. The main improvement achieved with this approach was the elimination of the initial profound burst release observed with the uncoated ferulic acid-gliadin fibers, and also the easily tunable zero-order release profiles which could be incrementally adjusted by variation of the coating thickness. Both these two preliminary studies could be an inspiration for further modification and application in specific fields, where the scaffolds could exploit their real potential as drug delivery devices.

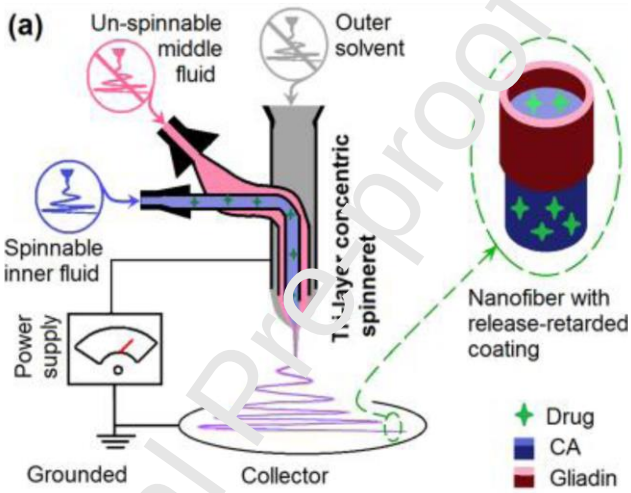


Fig. 6. The schematic rapresentation of the experimental setup used by Liu et.al., inner fiber coating is produced from a un-spinnable solution of cellulose acetate, stabilized thanks to the unspinnable solvents in the shell. Figure reprinted from Liu *et al.* [81] with permission from Elsevier.

An example of side-by-side electrospinning process is the work by Yu *et.al.* (2016), in which the dual delivery of ketoprofen was developed through the production of Janus nanofibers with one side of the fiber composed of PVP, while the other from ethyl cellulose. [82] The different behavior of the two polymers in aqueous media granted two different rates of release. While the PVP side dissolves very rapidly with complete discharges of the active ingredient, the ethyl-cellulose side allows a sustained release of the remaining ketoprofen. This dual strategy grants a powerful, controllable system capable of maximizing the therapeutic effect by tuning of the two sides. Similarly, Wang *et.al.* (2018) developed structural Janus nanocomposites for the oral delivery of helicid, an herbal medicine with poor water solubility, through rapid dissolution and transmembrane permeation. [83] Janus fibers were composed of PVP K10-sodium dodecyl sulfate and PVP K90–helicid, in which the first component is a non-spinnable solution that the authors were able to simultaneously spin with the second solution through the use of an eccentric spinneret. The Janus nanofibers exhibited improved dissolution and transmembrane permeation of helicid for potentially faster onset of therapeutic action with respect to the classical monoaxial blended nanofibers used as a control, providing a promising platform for the oral delivery of insoluble drugs.

2.4 Polymer morphology

A large number of different elements play a crucial role in controlling the outcome of the electrospinning process. Three different categories group the parameters able to influence the formation and the morphology of the final fibers. In most cases, the desired outcome of the electrospinning process is smooth, uniform, and bead-less nanofibers. In the majority of the studies present in literature, a fine-tuning of all the parameters can produce this outcome. However, besides the general definition in which

beaded fibers are considered as low-quality fibers, in some studies beads are produced intentionally as a drug deposit.[84] For instance, Xi *et.al.* (2019) compared the different release behavior of three pH-responsive silk fibroin scaffold: smooth, bead-on-string, and coaxial bead-on-string fiber materials. [85] The authors chose the cytotoxic doxorubicin embedding for anticancer treatment applications, in which a selective delivery is critical for limiting side effects. I*n vitro* test highlighted a similar release curve in an acidic environment, as a mimic of the tumoral tissue. yet, the bead-on-string scaffolds, especially the coaxially fabricated ones, showed substantially lower rates of release in a neutral environment, as a mimic of the healthy tissue, boosting the efficiency and the safety of the treatment. With a similar approach, Ma *et.al.* (2018) developed camptothecin loaded silk fibroin beaded meshes, in which beads act as a drug deposit. [86] Fibers showed excellent mechanical proprieties and a sustained release for more than 50 days with the absence of any burst phase, with moderate antiproliferative activity *in vitro*.

Indeed, ambient, solution and processing parameters are independent variables capable of cooperating to affect every single feature of the final fibers. Processing parameters are relative to the mechanical setup of the electrospinning machine, including voltage applied, flow rate, needle-to-collector distance and geometry of the collector. Voltage controls the jet initiation. Usually, at least 6 kV are required to initiate the process. A rise in the voltage causes greater electrostatic interaction in the charged solution leading to thinner fibers.[87] The flow rate of the polymer solution has a great effect on the uniformity of the fibers. Generally thinner fibers production takes place at lower flow rates. An increase in flow can increase the fiber diameter; however, too high flow rates lead to beaded fibers.[84]

Another parameter of great importance is the distance between the needle and the collector. Even though a minimum distance is mandatory for the initiation of the process, an excessive distance gives rise to beads and non-uniform fibers.[87] Depending on the final application of the fiber, different types of collectors could satisfy different needs. Flat surfaces are the most common and simple collectors, usually, an aluminum foil is used. Varying the geometry in favor of more sophisticated ones enables the production of aligned fibers or 3D structured fibers. Some examples are rotating mandrel, dual collection ring, water bath, moving platform and helical spring.[88]

Solution parameters include concentration, viscosity, volatility and dielectric constant of the solvent. Concentration and viscosity are proportional: higher the concentration, higher the viscosity of the solution.[89] Optimizing concentration and viscosity allows the polymer to flow through the nozzle and being spinnable. Moreover, higher concentration forms greater diameter. [73] Meanwhile, a high viscosity enables the formation of beadless fibers, yet, increasing too much the solution viscosity results in beads formation.[90]

Solvent parameters play a key role in the formation of better fibers; a wise optimization could extremely facilitate the whole process. The dielectric constant and volatility, in particular, strongly contribute to the formation of beadless fibers. Solvents with higher dielectric constant such as acetic acid, acetone or hexafluoroisopropanol (HFIP) reduce the fiber diameter and increase the deposition area, due to rising in bending instability of the electrospinning jet.[73] Meanwhile, higher volatility is associated with higher fiber porosity.[73]

Ambient parameters are the most complex to control due to their nature. This category includes temperature and humidity. Both parameters contribute to governing of the solvent evaporation rate. At the same time, the temperature affects the viscosity and the dimension of the fibers: higher temperature lowers solution viscosity, causing higher stretching in the process and thinner fibers.[91] Humidity affects the porosity of the final scaffold: in the presence of high humidity rates. The evaporative cooling caused by the solvent leads to the condensation of water over the surface of the fiber contributing to rising the porosity.[92] In Table 1, the effect of the different working parameters on the fiber morphology are summarized.

Table 2 List of parameters affecting the output of the electrospinning process.

Parameter	Effect
Processing parameters	

Voltage	↓	No fibers formation
	↑	Fiber diameter decrease
Flow rate	↓	Fiber diameter decrease
	↑	Beaded fibers are formed
Distance needle-collector	↓	No fiber formation
	↑	Non uniform beaded fibers are formed
Collector		Type of the collector controls fiber alignment and 3D structure
Solution parameters		
Concentration	↓	if too low sputtering can happen
	↑	Fibers formation with higher diameter and less beads. If too high nozzle clogging can be observed
Viscosity	↓	Finer and shorter nanofibers
	↑	Thicker and continuous nanofibers. If too high, beads and nozzle clogging are observed
Solvent parameters		
Volatility	↓	Difficult removal of the solvent
	↑	High porosity and surface area
Dielectric constant	↓	Beaded fiber are formed
		Fiber diameter decrease
Ambient parameters		
Temperature		Temperature affects viscosity and solvent evaporation rate. Higher temperature means lower viscosity and more efficient is evaporation of solvent.
Humidity		Humidity affects solvent evaporation rate. In addition, at higher humidity porosity increases

2.5 Drug loading

The incorporation of drugs in the electrospun fibers is carried out by different techniques. Drug loading heavily affects the drug release profile, making the correct choice over the best loading method for the desired application essential.[93] The simplest approach is the direct blending between the polymer and the drug by the dissolution of the two components in a suitable solvent. Blending has the highest loading rate compared to other techniques. The strength of the polymer-drug interaction will govern the release profile together with the drug solubility properties. Balancing hydrophobicity of drug and polymer is a crucial task for constant release over a defined time window.[94] The drawbacks of this technique are associated mainly with the presence of the organic solvent, often capable of denaturing bioactive molecules. Moreover, a burst release of the drug generally is observed.[95]

Emulsion electrospinning gives a possible alternative, enabling the formation of core-shell nanofibers by encapsulation of the drug inside micelles. Usually, the formation of drug-containing micelles

occurs by addition of a supernatant to a water solution of the drug itself. Vigorous mixing of the so formed micelles with an oil solution of the polymer form a stable emulsion suitable for electrospinning. The advantages are mainly two: the first is a minimized contact of the bioactive molecule and the organic solvent, allowing the use of various combinations of hydrophilic drugs and hydrophobic polymers; the second advantage is due to the easy formation of uniform core-shell structure without the use of specific coaxial apparatus. [96,97]

Coaxial electrospinning, besides being a technique for core-shell nanofibers formation, is also considered a loading methodology. As already discussed, the applicability of the coaxial technique requires a specific apparatus and optimization time. However, it does not only give an infinite combination of polymers for the core and the shell, but can be a modular platform for the loading of different drugs in different compartments of the fiber. Also, the coaxial loading of a single drug gives the great advantage of inserting the drug in the core polymer with the presence of the shell acting as a physical barrier preventing burst release. Disadvantages consist of the difficulty of optimizing parameters and the difficult scalability of the technique.[71,98]

Another approach for drug loading involves surface immobilization of the bioactive molecule after the electrospinning process. Thus, it is possible to avoid every contact between the active molecule and the organic solvent, preventing any undesired degradation. Another advantage is the preservation of the original degradation and mechanical proprieties of the polymeric matrix.[99,100] However, achieving a longer release over time requires strong non-covalent bonding between the polymer and the drug and usually a cross-linking process.[101]

Another strategy adopted by Celebioglu *et.al.* (2020) for the fabrication of fast dissolving oral involves the supramolecular complexation of drugs before the spinning process. [102] In this case, the use of cyclodextrin (CyD) allowed the formation of a complex with hydrocortisone, before the direct spinning of the solution. The so formed CyD-hydrocortisone fibers proved to be a very promising material for oral delivery application due to the increase in solubility and fast dissolution.

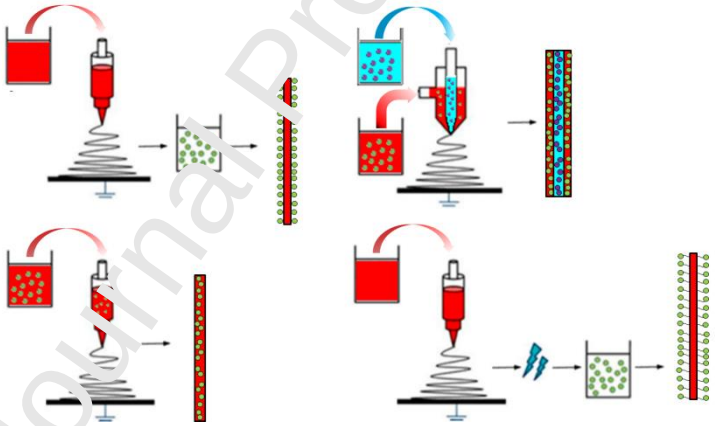


Fig. 7. Schematic representation of different approaches for the drug loading. (a) physical absorption after the electrospinning. (b) Blend solution between drug and polymer. (c) Coaxial electrospinning and (d) chemical surface modification after the electrospinning process.

3. Pharmacological applications

The unique feature and the easy tunability of nanofibrous scaffolds made them a highly flexible tool for drug delivery for the treatment of different pathologies. Since the intrinsic difference in the pathologies, every different field of application requires a specific release and mechanical trait of the nanofiber.[16]

This section will be focused on the principal and most widespread application of nanofibers for drug delivery found in literature, and the strategy adopted for their development and characterization. In particular general applications, like antibiotics and anticancer, will precede more specific uses in tissue engineering, like wound healing, cardiovascular, and ocular regeneration.

3.1 Antibiotics

Bacterial infections are one of the most current challenges for medicine. The final result of a severe infection could be sepsis, one of the leading causes of death in the world.[103] Also, bacteria have a strong inclination to develop resistance towards antibiotics. It is estimated that in 2050 antimicrobial resistance could cause 50 million deaths per year all over the world.[7] Antimicrobial resistance is the ability of a microbe to grow in an inhibitory concentration of an antibiotic. Usually, antibiotic combinations are used to improve efficacy and to prevent the emergence of antibiotic resistance.[104] Besides, some pathophysiological conditions, like for example cystic fibrosis, needs long term and repetitive antibiotics cycle to control the rising of chronic infections.[105]

For these reasons, the development of new, more tunable and efficient systems for antibiotics delivery could minimize the drawbacks due to overdosage and the rise of bacteria resistance by a maximized action directly in the site of action. Electrospun nanofibers, due to their unique features could be an attractive platform for the creation of a new drug delivery system for antibiotics therapy.[8,106]

Pisani *et.al.* (2019) produced gentamicin loaded polylactide-co-polycaprolactone electrospun nanofibers with possible application for preventing bacteria biofilm formation after surgical operation. [107] Gentamicin sulfate, an aminoglycoside antibiotic characterized by poor oral bioavailability, and the high occurrence of side effects, such as ototoxicity and toxicity in the kidney, when the drug is administered by intravenous or intramuscular routes. The development of a localized drug delivery system could overcome the side effect and maximize the antibacterial action. Scaffolds were produced by simple blend electrospinning. The release rate was characterized by diffusion through porous thin films, demonstrating that degradation of the polymeric matrix was not involved in the release. This preliminary work sets a good starting point for the release study of gentamicin sulfate, with different possible applications. However, no rational highlights were made over the polymer choice, the lack of toxicity. Also, bacterial growth inhibitions data does not give a complete overview of the possibility of this kind of scaffold.

Another possibility investigated by Li *et.al.* (2020) is the production of gastro-retentive drug delivery system, with a potential application in the everyday life. The system was based on *B.striata* polysaccharide, a natural glucomannan material. [108] However, the glucomannan was not used as a starting material for electrospinning, but as a lyophilization wafer embedded with levofloxacin hydrochloride. PCL electrospun fibers were used as a coating for the tablets. This kind of approach granted a reduced water permeation within the microchannel, regulating the drug release rate and reducing the erosion rate. The tablets exhibited high activity against *H. pylori*, one of the main pathogenic microorganisms of chronic active gastritis, with no significant cytotoxic effects. The high drug loading and good gastric retention capacity of the wafer allowed a better treatment of *H. pylori* infection compared with the free drug, both *in vitro* and *in vivo*.

Similarly, Behbood *et.al.* (2017) created blended fibers of chitosan and gelatin loaded with vancomycin, a glycopeptide antibiotic, as a mucoadhesive oral delivery system. [109] These implants have three main advantages, such as improved absorption and bioavailability, predictable release, and avoidance of hepatic first-pass metabolism. Since vancomycin suffers low absorption in the gastrointestinal tract and severe side effects, its controlled release could be a striking approach to maximize the dosage and the beneficial effects of the drug. The fibers created by blend electrospinning were crosslinked with glutaraldehyde to improve the water stability and mechanical property of the nanofibrous material. The *in vitro* release showed that crosslinked material is stable for more than three days, with a Fickian release mechanism for vancomycin. However, the lack of more in depth biological studies leaves a gap in the understanding of the behavior, the toxicity, and the actual action of the mucoadhesive electrospun scaffold and to validate its applicability for infection control.

Patients subjected to surgical intervention could develop surgical site infections, which can increase the length of postoperative hospital and the probability of patient death. Prophylaxis and post-surgical treatment could reduce the risk of rising this kind of infection.[110] For this reason, Chen *et.al.* (2017) prepared coaxially spun nanofibers with gentamicin/pluronic F127 in the core and silver/PCL in the sheath as sutures with drug delivery behavior. [111] Pluronic F127 is a biocompatible tri-block copolymer of poly(ethylene oxide), poly(propylene oxide), and poly(ethylene oxide) with remarkable surfactants properties. Since gentamicin is nephrotoxic and ototoxic while silver can accumulate in many organs exhibiting cytotoxicity, the encapsulation, and a slow and controlled release could easily limit such negative effects. boosting on the other hand the therapeutic ones. The *in vitro* release profiles exhibited an initial burst followed by a sustained release over 5 weeks, with no evidence of cytotoxicity. Interestingly, scaffolds showed higher antibacterial efficacy than silver or gentamicin alone loaded sutures, indicating a synergistic effect. These studies set the basis for more in-depth exploration where, thanks to the already

optimized coaxial spinning, other therapeutic molecules for imparting multiple functions could easily be incorporated into the fibers, for example, immune-modulating agents or growth factors, producing a final optimized tool capable of absolving different antibacterial roles, retaining the biocompatibility and non-toxicity of the scaffold as principal features.

For the same reason, Boncu *et.al.* (2020) formulated linezolid, an oxazolidinone antibiotic, loaded electrospun PLGA and PCL fibers for controlled drug release, applicable for the treatment and prophylaxis of skeletal prosthesis related infections. [112] The aim was to accelerate healing in the damaged and infected surrounding tissue and bone, and control the infection through controlled linezolid release to achieve effective treatment by optimal antibiotic dosage. The scaffolds, thanks to a fine-tuning of the composition, demonstrated a good positive effect on tissue healing in a rat model of tibia fracture. Also, the antibiotic loading granted therapeutic and prophylactic effects with more efficacy than intraperitoneal treatment with commercial linezolid two times a day. The efficiency of the electrospun meshes eliminates the need for two injections per day in favor of a one-time application, with a 37-fold reduction of antibiotic administration compared to conventional treatment. The approach could prevent rising of antibiotic resistance and allows for cost-efficient treatment.

A correlated issue appears in orthopedic surgery where infection of the orthopedic implant can damage the self-healing ability of bone tissue, leading to severe bone loss, implant failure, and even amputation. In this field, scaffolds should act synergistically, by helping bone regeneration and preventing the rising of infections.[113] Shi *et.al.* (2019) developed infection responsive electrospun nanofibers for targeted and efficient release of anti-infection drugs. [114] Fibers coating with polydopamine, after the electrospinning process, allowed further functionalization with silexane to introduce amino groups. The nitroimidazole antibiotic metronidazole was successively attached by esterification to the functionalized surface of the fibers. Drug release is possible by cleavage of the ester bond by the action of cholesterol esterase, enzyme regularly secreted in the inflammation site with a concentration directly correlated to the gravity of the inflammation itself. Fibers showed no cytotoxicity and a very interesting response to bacterial infection. The *in situ* smart drug release could potentially reduce drug resistance of the bacteria by a controlled dosage of the drug. A more detailed study *in vivo* could validate this model, and possibly making a new tool for treating the long term infection that can develop after surgery.

Bakhsheshi-Rad *et.al.* (2019) took advantage of poly-L-lactic acid nanofibers for the creation of a coating for åkermanite, a magnesium alloy. [115] Magnesium has attractive features for orthopedics application. Yet, its low corrosion resistance leads to the generation of large amounts of hydrogen gas and increasing pH in the surrounding tissue. In this study, nanofibers formed a physical shield for the redox protection of magnesium alloy. Therefore, incorporation of antibiotics into polymer nanofibers enhanced the implant performance by lower viability, adhesion, and growth of microbes on the surface. Corrosion characterization showed an almost halved degradation rate of the coated magnesium with respect to the uncoated ones. The presence of the coating did not affect mechanical proprieties of magnesium alloy. Doxycycline release is characterized by an initial burst and a sustained release boosting the antibacterial performance of the scaffold against *S.aureus* and *E.coli*. The presence of åkermanite improves the biocompatibility towards bone cells thanks to the generation of essential ions such as Ca, Si, and Mg. Further biological characterization of this kind of composite fibers produced by simple blending electrospinning could provide an effective tool for bone infection treatment.

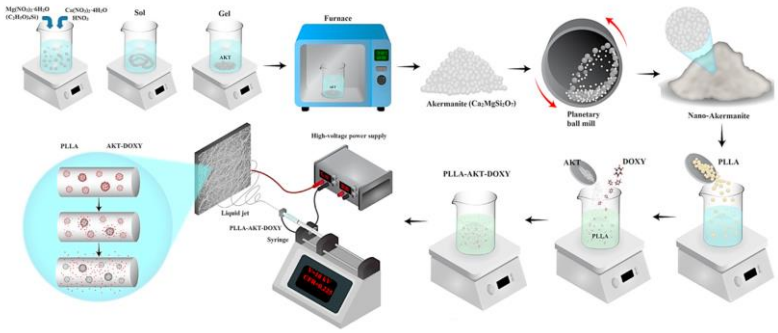


Fig. 8. A schematic representation of the synthesis of åkermanite nanopowders and electrospun PLLA-AKT-DOXY nanofiber-coated with Mg alloys by Bakhsheshi-Rad *et.al.*. Figure reprinted from Bakhsheshi-Rad *et al.* [115] with permission from Elsevier.

Another strategy explored by Wei *et.al.* (2018) for orthopedic surgery applications, concerned the creation of PCL fibers for vancomycin delivery in infected critical bone defects. [116] The scaffolds have the function to assists bone regeneration and, at the same time, control the bacteria growth to prevent infections. Scaffolds showed excellent biocompatibility, and allowed a sustained release of vancomycin for more than 14 days, without noticeable burst release. However, such slow-release needs a deep debridement of the wound from the necrotic tissue. The scaffolds are not able to control the infection. The membrane was able to block only partially the migration of fibroblast, probably because fibroblasts involved in chemotactic migration may have adaptively changed their shape in order to pass through the scaffold small pores. This study is a good starting point, supported with interesting *in vivo* data. However, a more in-depth screening of materials and antibiotics combinations, to achieve on one hand an initial burst release followed by a sustained one, and on the other hand a reduction of pore size of the fibers could allow the production of more efficient scaffolds for this kind of application.

3.2 Antitumoral drugs

Despite significant advances in therapy, diagnosis, and prevention, cancer remains one of the most dreaded diseases to haunt mankind in the world today, being still one of the leading causes of death worldwide.[117] Cancer is a complex and heterogeneous pathology in which clusters of cells display unlimited growth and could spread around the body. Usually, an early-stage diagnosis is associated with improved patient survival. Indeed, identification of the malignant tumor site before the spreading and appearance of metastasis could open the route to chemotherapy or surgical removal of the tumor solid mass.[118]

Cancer treatment by chemotherapy aims to control the growth of the tumor by the administration of cytotoxic drugs, for example, doxorubicin, able to interrupt the cell cycle and induce apoptosis.[119] The high growth of the tumor requires more nutrients delivery with respect to the healthy tissue, resulting in a high vascularization. For this reason, the biodistribution of the drug is mainly within the tumor, where its action is required.[120] However, cancer chemotherapy is very well known for its severe side effects.[5] Hence, the development of localized delivery of chemotherapeutic drugs could focus on the cytotoxic action of the drug, reducing the systemic toxicity in the patient. The high biocompatibility of electrospun scaffolds together with their high tunability toward the drug release makes them suitable for the application as chemotherapeutics delivery systems.[121]

Kuang *et.al.* (2018) developed scaffolds with a controlled release kinetic of doxorubicin. [122] The authors employed the principle of blend electrospinning between a hydrophilic polymer and a hydrophobic one. In this case, the choice fell respectively on PEO and PLLA. To optimize the therapeutic effect the release was tuned in two stages. The first stage allows a fast release of a fraction of the drug to suppress the tumor in the early stage. The second stage, on the contrary, shows a sustained release to prolong the time of the therapy. The desired release profile was found in fibers composed of 10% of poly(ethylene oxide) and 90% of poly(L-lactide). It is noticeable that fibers composed by only poly(ethylene oxide) completely dissolved within one hour with a complete release of the drug, the possible change towards less soluble polymer could probably result in a more interesting release profile. *In vivo* experiments showed a localized biodistribution within the tumor site and no significant toxicity. However, the initial doxorubicin burst may not be sufficient for effective tumor suppression, and the antitumor effect was barely satisfactory. This approach of biphasic release could be valid in tumor control, and reduction of adverse effects of chemotherapy. Yet, other combinations of polymers could be more effective in the treatment.

In another kind of approach, Akpan et.al. (2020) designed a scaffold composed of poly(D,L-lactic-co- glycolic acid), gelatin, and pluronic F127 for breast cancer treatment with prodigiosin. [123] Prodigiosin is a red pigment produced by many strains of the proeobacteria with several biological activities, including activities as antimalarial, immunosuppressant, and antibiotic. Blend electrospinning was used for the creation of the fibers, assuring a simple process. Pluronic F127 plays a double role in ensuring a high loading and a more controlled release of the drug. The kinetic of the release is divided into three stages: the first stage involves a drug burst release, the second stage is diffusion controlled, while the third is a bit slower and regulated by the scaffold degradation. Fibers showed interesting mechanical proprieties with the ability to induce apoptosis in tumoral cell lines. However, scaffolds without the loaded drug exhibited high affinity with the tumoral line, boosting its proliferation. Such behavior could be considered as a two-faced coin: on one hand, it could help the regeneration of the tissue after chemotherapy; on the other

hand, if the tumor is not eradicated, the scaffold could behave as a seeding point. Also, detailed toxicity study over healthy tissue remains a point with the need for clarification.

With the same purpose, Aytac *et.al.* (2020) developed a series of core-shell nanofibers based on Eudragit S100, a copolymer of methacrylic acid and methyl methacrylate, as shell and polyethylene oxide as the core. [124] Fibers core embedded 5-fluorouracil or ferulic acid or their cyclodextrin inclusion complex as antitumoral agents. Also, the core contains a gadolinium complex as a contrast agent for magnetic resonance imaging. The approach could combine the possibility of treating the tumor with the possibility of monitoring the action of the fibers. Besides, the presence of cyclodextrin could be striking in enhancing the solubility of the drugs, boosting their biological activity. However, the shell polymer revealed a non-compatible behavior toward the desired application because of its rapid degradation in the acidic environment. *In vitro* release, showed a complete release of every embedded drug in 2 hours or less, due to the formation of big pores in the shell. Also, the effect of gadolinium was not investigated. Modification of the shell with different techniques, for example, by polymer blending, could slow the degradation rate of the shell, assuring a more sustained and stable release. Also, the role of gadolinium and the behavior of the scaffold need a deeper clarification.

Tumor chemotherapy involves a large array of possible therapies alternative to the classic pharmacological treatment. Among different strategies hyperthermia is an alternative cancer treatment to chemotherapy involving a localized heat of the tumor of its destruction instead of drug administration. To create an highly localized hyperthermia device, Hu *et.al.* developed PCL-Fe3O4 scaffolds by melt electrospinning. [125] The simple principle of melt electrospinning permitted the authors to develop a portable device with a rechargeable battery to provide a high voltage and heating power, which can work without extra electricity supply. Fibers met the conditions necessary for magnetic hyperthermia, with the possibility of alternating cycles of high and low temperatures. The presence of the fibers could enhance the targeting of the heat reducing damage to other tissues. However, more comparative data between the efficiency of the loaded nanofibers with respect to the free iron nanoparticles are needed to validate the further investigation.

Tumors exhibit some peculiar features due to their high requirement of nutrients and the high inflammatory state, which causes respectively high vascularization and an acidic pH. In particular, pH could efficiently be utilized for the creation of responsive material, able to release the drug only within a specific environment, reducing even more the toxicity of the chemotherapeutics drugs.[62]

Zhang *et.al.* (2020) created pH-responsive scaffolds for the delivery of 5-fluorouracil. [126] The drug, in a preliminary stage, was covalently attached to keratine by a nucleophilic substitution involving the terminal cysteine of keratine. The obtained polymer was mixed with poly (L-lactide) and used for electrospinning to fabricate a nanofibrous scaffold, for local tumor chemotherapy. The presence of keratine assures a pH-dependent release with a controlled release in acidic environments. When triggered, the fibers show a rapid release of about 83% of the drug during the first 120h correlated to a potent antitumor effect. However, the limited amount of drug remaining in the fibers after the burst release, may not be sufficient to control the proliferation of the remaining tumoral tissue after the first treatment. To avoid a second implantation, it could be necessary to achieve a prolonged sustained release after the burst stage. Also, such rapid burst release could result *in vivo* toxicity toward healthy tissue.

Similarly, Yan *et.al.* (2020) developed pH-sensitive core-shell nanofibers by coaxial electrospinning in which polyvinyl alcohol and PCL formed, respectively, the core and shell layers. [127] Doxorubicin embedded in the core layer showed sustained and pH-responsive release. Different fibers were created by adjusting the flow rate of the shell and the core solution. However, little difference in the surface morphology of the fibers was observed. TEM analysis indicated that the shell flow rate determined the thickness of the shell itself. Acidic condition release experiments revealed a small burst release, attributed to a little leakage of the shell during the electrospinning process, followed by a more sustained release. Increasing the thickness of the hydrophobic shell helps to reduce the first burst stage and reduces the cumulative release. Therefore, the fibers with the biggest shell possessed the lowest amount of doxorubicin released. In a neutral environment, the release is even slower, revealing a pH-dependent behavior. Fibers were tested over a cervical tumor cell line, where they exhibited their action only after three days. The slow degradation of the shell is the cause of such moderate action. However, after 7 days the morphology of the cells could not be identified, implying that cells were killed by the drug. Besides the interesting behavior and the low toxicity, a more rapid release in the first stage could be helpful to control the tumor in a small time-window.

Most cancer deaths are due because their disseminated tumors do not respond to available chemotherapies. Tumors usually respond to chemotherapy developing a variety of mechanisms that result in the loss of their initial hypersensitivity to anticancer drugs.[128] Much was learned about drug action, and efforts to elucidate the molecular basis for resistance have revealed a large variety of mechanisms that either prevent a drug from reaching its target, deploy compensatory mechanisms promoting survival, or lull cancer cells into a dormant state. These phenomena are known as multi-drug resistance.[129] A combination of drugs with multiple targets might prevent treatment failure due to drug resistance, but at the cost of increased side effects caused by long-term multiple-drug treatments.[130] Despite the high expectations, no compound became available for therapy, because of either intrinsic toxicity or changes in the pharmacokinetic properties of the chemotherapeutics resulting in strong toxic side effects.[131] However, localized co-delivery of compounds able to block the multidrug resistance mechanism and chemotherapeutics could enhance their action with a lower requirement of dosage. In this context, the usage of electrospun scaffold could be striking in the development of new and more effective therapies with reduced toxicity.

He *et.al.* (2019) developed an implantable hierarchical-structured ultrafine fiber device by microfluidic electrospinning for localized co-delivery of doxorubicin and apatinib. [132] Fibers were formed in two-step: first, the actively targeted polymer micelles were formed by self-assembly of 3-aminophenylboronic acid-poly(ethylene glycol)-PCL copolymers and doxorubicin. Then, an aqueous solution containing the above micelles, glycerin, and free doxorubicin and an oil solution of poly(D,L lactic acid) and apatinib were monodispersed to obtain a water-in-oil solution through glass capillary microfluidic device. The obtained emulsion was further used for electrospinning. The copolymer used in the first stage, thanks to the presence of 3-aminophenylboronic acid exposed in the surface, giving active tumor targeting by binding to the sialic acid receptor, overexpressed on the surface of various solid tumors. Also, the degree of encapsulation inside the fibers proved to be very high, with maximized usage of the drugs. Release experiments revealed a dual pattern: while doxorubicin micelles were rapidly released with the fracture of the cavities, apatinib was released slowly with a ratio dependent on the degradation of the fiber matrix. *In vivo* experiments confirmed the interesting behavior of the scaffolds revealing a highly controlled biodistribution of the drugs within the tumor site and excellent antitumor effect with single implantation. Tumor mass in the treated mice was four-time smaller than the untreated ones after 21 days and higher survival rates. The synergistic effect of doxorubicin and apatinib has great potential for the creation of devices able to achieve a great therapeutic effect with low systemic toxicity.

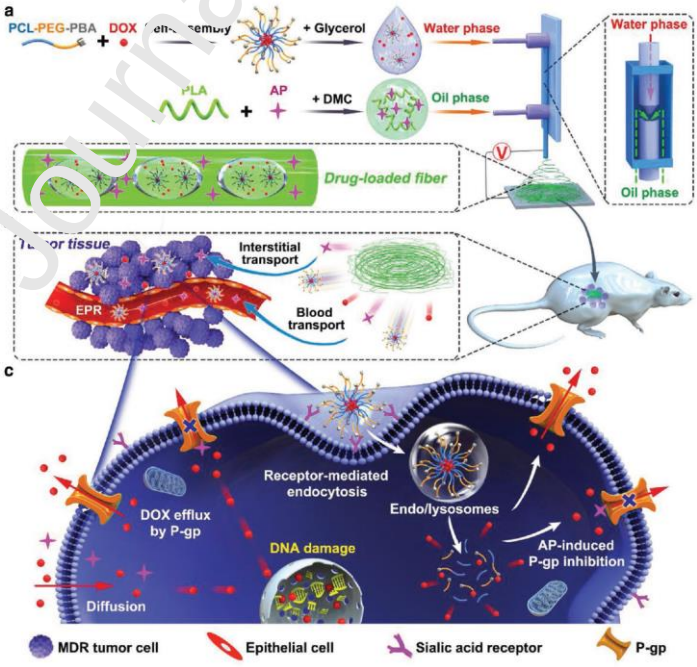


Fig. 9. Schematic illustrations of the fabrication and application of the implantable hierarchical-structured micelle-/drug loaded fibrous device. Developed by He *et.al.* (a) The fabrication of the hierarchical-structured micelle-/drug-loaded fibers through a microfluidic assisted electrospinning. (b) Local delivery of the Doxorubicin-loaded micelles, free doxorubicin, and apatinib from the fibrous device to tumor

tissues after implantation. (c) apatinib continuously inhibiting the P-gp drug pump of MDR tumor cells, thereby enhancing the doxorubicin antitumor action. Figure reprinted from He *et al.* [132] with permission of Wiley Online Library.

In a similar approach by Li *et.al.* (2020) combined multiple chemotherapeutics with time-programmed administration from a single tri-layered carrier for the treatment of breast cancer. [43] The tri-layer structure was fabricated through a modified -triaxial electrospinning technique. The layers were composed of glycerol and doxorubicin in the inner core, and poly(l-lactic acid) and PCL containing the multidrug resistance inhibitor apatinib form the double walls of the fiber. The morphology of the scaffold was intentionally not completely smooth but presented discrete ellipsoidal bulges along the fiber axis composed of glycerol-doxorubicin. The cavity rapture assures a rapid and burst release of doxorubicin to reduce the tumoral mass, while the slow degradation of the fiber-matrix assures a sustained release of apatinib for the final elimination of the tumor. The synergistic effect was evaluated *in vivo* where the time-programmed release revealed excellent therapeutic effect without significant toxicity. Also, the biodistribution of the drug is significantly higher in the tumor site than other organs, helping to limit side-effects. This study furnishes an interesting platform for the control of tumor growth and the development of new combined synergistic treatment of cancer.

Another great treat of cancer is the possibility of local recurrence after surgical resection. Besides decreasing the survival of the patients, fear of cancer recurrence has a great impact on the psychology of people cured by cancer. Therefore, the prevention and treatment of these malignancies represent a great point of interest in the oncological field.[133,134]

Electrospun scaffolds could help in preventing cancer recurrence. For example, Rasouli *et.al.* (2020) evaluated the efficacy of simple blend electrospun nanofibers co-loaded with Curcumin and Chrysin against breast cancer recurrence. [135] Fibers were composed of a copolymer of Poly (lactic-co-glycolic acid) and PEG, which showed high encapsulation efficiency. Also, co-loading improved mechanical properties with respect to single drug-loaded fibers. Drugs exhibited an almost identical and prolonged release profile with synergistic effects *in vitro*, without burst release, but at the same time, with an anti-proliferative and pro-apoptotic effect on breast cancer cells. The purpose of the applicability of fibers for after surgical treatment could be compatible with the release profile. These preliminary results need to be confirmed within *in vivo* data to exploit the real potential of these fibers and clarify the biodistribution of the fiber to exclude systemic toxic effects.

Sedghi *et.al.* (2020) prepared chitosan derivative nanofibers for the prevention of local breast cancer recurrence. [136] Chitosan was first chemically modified by a five-step synthesis for the introduction of a tetramethyl urea thiosemicarbazone group, resulting in enhanced hydrophilicity. Curcumin was blended with obtained chitosan and polyvinylalcohol before electrospinning, to obtain a loaded scaffold. The thiocarbonyl groups in the chemical structure the derivative provide good anticancer activity *in vitro* and no cytotoxic effects on the healthy cells. Also, the blending with Curcumin inside the fibers, showing a slow and sustained release, is claimed to enhance the anti-proliferative and antibacterial features of the fibers itself. However, lack in comparative data between non-loaded fibers and curcumin loaded fibers gives rise to question about the real mechanism of action of the scaffold, sometimes attributed to curcumin and sometimes to the chitosan derivative. Also, biodistribution and more consistent *in vivo* data could clarify the real potential of these scaffolds.

3.3 Wound healing

Skin is the largest organ and the most outer layer of the body with three main functions: protection, regulation, and sensation. Since skin acts as a barrier in bodily defense, protecting from microbes and damage between the internal and external environment, such function makes skin highly prone to injuries.[137,138]

Fast regeneration of the wound could prevent complications or chronic infections, usually resulting in the amputation of the body part. Wound healing is a complicated physiological process involving tissue regeneration and repair, affected by both intrinsic and extrinsic factors. Despite the numerous progress in the past decade, the development of more efficient wound coverage patches and skin substitutes is still a challenging task.[139]

Scaffolds for wound healing application need specific features like mimicking of the extracellular matrix, the ability to absorb the wound exudates, and impermeability to bacteria. Electrospinning could be a valuable technique for the fulfillment of such features. Moreover, the possibility of incorporation with

active substances and drugs can be exploited to boost regeneration or for the delivery of antimicrobial agents to significantly reduce wound infection.[140]

Varshosaz *et.al.* (2020) exploited the fabrication of a wound dressing membrane based on modified polybutylene adipate-co-terephthalate and gelatin nanofibrous structures loaded with doxycycline using the double electrospinning technique. [141] Polybutylene adipate-co-terephthalate is a biodegradable insoluble polyester with interesting mechanical proprieties without toxicity to the cells. The combination with gelatin allowed the introduction of a water-soluble part inside the scaffold to modulate the release and achieve a better ECM mimicry. To further enhance cell adhesion, a post-spinning modification with RGD peptide, the most common peptide motif responsible for cell adhesion to the extracellular matrix, was adopted. The presence of the metalloproteinase inhibitor doxycycline allowed to achieve significant antibacterial proprieties on strains of *S.aureus* and *P.aeruginosa*. The fibers exhibited notable wound healing *in vivo* within three days after initiation of the treatment, without any noticeable cytotoxicity. Despite the presence of RGD peptide enhanced cell attachment and wound healing, the polymer choice for the composition of the fibers did not introduce any significant improvement in the mechanical proprieties of the scaffold.

Another example by Guo *et.al.* (2020) projected pH-responsive coaxial nanofibers for co-load and sequential co-delivery of two drugs. [142] Herein, fibers were composed of a chitosan–polyethylene oxide blend embedded with a shell of lidocaine hydrochloride, used for pain relief, and PCL embedded curcumin, an anti-inflammatory agent within the core. The pH-responsive behavior was achieved by the combined presence of chitosan and sodium bicarbonate in the core. Protonation of chitosan in an acidic environment triggers the reaction with the sodium bicarbonate with the generation of CO_2, which creates holes in the surface of the fibers boosting the release of the two drugs. The design contributes on one hand to rapidly release lidocaine in the early stage of wound healing and reduce pain immediately; on the other hand, when the inflammatory stage begins and the pH becomes more acidic than physiological conditions, the release of curcumin accelerated. Besides, the presence of curcumin grants an antibacterial activity against *E.coli* and *S.aureus*, especially in the first 24h. However, the lack of *in vivo* experiments does not confirm if the time scale of release is compatible with the time window of the drug release.

Yang *et.al.* (2020) created PVP and ethylcellulose with side-by-side technique producing nanofiber with a synergistic release of respectively ciprofloxacin and silver nanoparticle, both with antibacterial features. [143] This Janus strategy allows the fibers to burst release ciprofloxacin within 30 minutes. Then, the sustained release of silver nanoparticles maintains the antibacterial effect up to 72h, resulting in potent inhibition of bacterial growth. Such behavior makes this scaffold a promising tool in preventing infections during the wound healing process. Yet, toxicity data and cell attachment studies are needed to reveal their potential.

Natural polymers are broadly used to efficiently mimic the native tissue matrix. These natural polymers have shown great potential for skin regeneration as wound healing patches or dressings in the treatment of various types of wounds.[144] Faccendini *et.al.* (2020) compared different types of polysaccharide-blend based scaffolds as dermal substitutes. [145] Norfloxacin, a fluoroquinolone antibiotic, loading allowed the use of such scaffolds for the treatment of infected wounds. Fibers were manufactured employing a simple one-step electrospinning, and norfloxacin was loaded as a free drug or as montmorillonite nanocomposite. Scaffold degradation and drug delivery occurred through lysosomes, thus eliciting drug release during the inflammatory process. Despite montmorillonite loading resulted in higher deformability, lower elasticity, and decreased mechanical resistance of the nanofibrillar meshes, these nanocomposites demonstrated to possess adequate stiffness to support fibroblast proliferation and the capability to sustain antimicrobial properties through norfloxacin release. Also, a cell viability decreased with respect to the cells growth in standard condition used as control, if norfloxacin was loaded as a free drug. This study furnishes a great tool for the treatment of infected wounds. However, *in vivo* validation of the scaffold behavior are still needed. Also, other types of fiber blends of chitosan with synthetic polymers like PCL or the use of other loading techniques, for example emulsion loading, could help to overcome the mechanical limit of the fibers.

Asadi *et.al.* (2020) tried to overcome the limited applicability of zein in wound dressing applications by the creation of composite nanofibers with graphene oxide. [146] Tetracycline hydrochloride was prior encapsulated inside graphene oxide nanosheets. Then, dispersion and blending with the polymeric matrix allowed the emulsion electrospinning and the creation of composite core-shell scaffolds. Graphene oxide granted increased mechanical properties and prolonged release profile compared to zein nanofibers alone.

Furthermore, the material exhibited excellent bactericidal properties and very low cytotoxicity. Despite their promising activity, no evidence of anti-inflammatory activity was shown.

With the same purpose, Bakhsheshi-Rad *et.al.* (2020) exploited the production of gentamicin loaded chitosan-alginate blended fibers. [147] Even though scaffolds exhibited good antibacterial performance, good cell attachment, and proliferation *in vitro* hand in hand with enhanced skin regeneration in mice, metabolic activity assessment showed dose-related cytotoxicity with increasing gentamicin concentration. Also, gentamicin itself plays a key role in the modulation of mechanical and cell attachment proprieties of the scaffold. Despite an obvious optimization in the gentamicin concentration, the modulation of the intrinsic proprieties of the fibers alone could modulate the mechanical and biological proprieties, while allowing a reduction in the gentamicin concentration reducing the cytotoxicity of the scaffold.

Another kind of skin damages is represented by burns, a major life-threatening event that significantly affects the quality of life. The compromised integrity of the skin results in a dangerous avenue for infections leading to delayed wound healing process.[148] Hadisi *et.al.* (2020) combined the production with core-shell nanofibers composed by hyaluronic acid and silk fibroin. [149] Hyaluronic acid was chosen due to its great ability to modulate the three main phases of the wound healing process, including the inflammatory response, migration of cells, and angiogenesis. However, its poor mechanical properties, high swelling, non-controlled drug delivery, and fast degradation rate forced the combination with another polymer. The choice was silk fibroin, which could overcome the drawback of hyaluronic keeping good biocompatibility. The fibers were embedded with zinc oxide, which possesses incredible antibacterial proprieties. The fibers embedded with 3% of zinc oxide revealed good cell attachment and interesting wound healing behavior in the scratch assay, together with antibacterial activity against both *E. coli* and *S. aureus*. *In vivo*, scaffolds were able to enhance the stimulation of epidermis, hair follicles, sebaceous glands formation, and promote collagen deposition. Also, a decreased inflammatory response was observed. Despite these promising activity, zinc oxide was found cytotoxic over a 3% concentration. The use of such borderline concentration needs a really careful optimization of the production process to not exceed such limit. In alternative, other antibacterials like more common and efficient antibiotics such as norfloxacin or gentamicin, or mix should be considered.

Bayat *et.al.* (2019) studied the applicability of bromelain-loaded chitosan nanofibers for burn wounds repair. Bromelain, a mixture of proteolytic enzymes present in all tissues of pineapple (*Ananas comosus*), is already known for its efficient debriding action in burn treatment. [150] Fibers were produced by simple blend electrospinning and showed good mechanical proprieties. However, at 4% of bromelain concentration fibers showed noticeable cytotoxicity. Scaffolds were tested *in vivo* and confronted with non-loaded chitosan scaffolds. Interesting proprieties of wound healing in mice, with more regular collagen fibers, reduced inflammation and tissue necrosis appeared after 14 days of treatment, with an overall acceleration of the wound healing process. Further improvement of the fibers could involve a chemical modification of chitosan, in order to improve the lipophilic profile and thus the release profile, or by exploiting other techniques, like polymer blending with, for example, hyaluronic acid could result in an even better tool for the treatment of burn injuries.

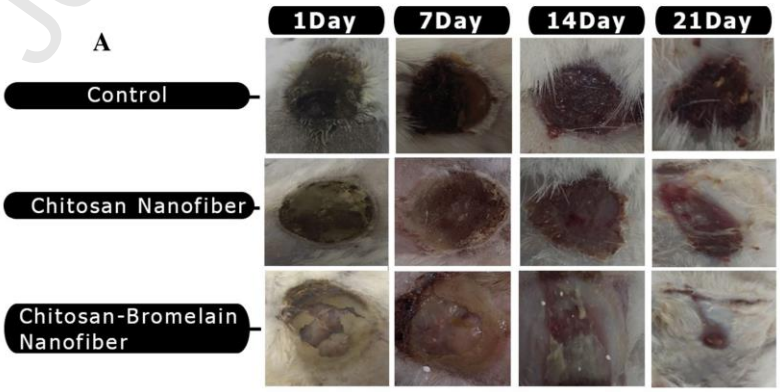


Fig. 10. The work of Bayat et.al. on the effect of the bromelain loaded nanofibers on damaged tissues (2nd burn degree mice model) respect the non-loaded fibers and negative control. After 21 days is visible the complete regeneration of the skin. Figure reprinted from Bayat *et al.* [150] with permission from Elsevier.

3.4 Cardiovascular diseases

The cardiovascular system permits blood to circulate and transport fundamental nutrients, its essential components are blood vessels, blood, and the heart. Diseases involving the cardiovascular system could affect every component, resulting in a heterogeneous array of diseases. The most common include coronary artery diseases, stroke, heart failure, hypertensive heart disease and represent the leading cause of death in the world.[151,152]

Despite the strong correlation of cardiovascular disease and environmental and lifestyle factors, during the years different therapies were developed for the treatment of various pathologies. For instance, the treatment of coronary heart disease was revolutionized by the use of arterial stents acting as support to keep the artery open and maintain the blood flow without obstruction.[153] To enhance the therapeutic effects, Bakola *et.al.* (2018) developed polylactic acid nanofibers as stents coating. [154] Fibers were embedded with Dipyridamole, an inhibitor of blood clot formation, to cope with artery thrombosis that often occurs after stent implantation. The authors were able to achieve a uniform distribution of the drug within the fibers, allowing a good and controlled sustained release dependent on the fiber degradation. *In vitro* preliminary studies confirm excellent biocompatibility over the tested cell line with enhanced cell viability. For the same purpose Kersani *et.al.* (2020) used electrospun nanofibers of chitosan and β-cyclodextrin polymer for covering self-expandable NiTiNOL stent, with nanofibers sheath for the elution of simvastatin, a drug commonly used for restenosis prevention. [155] Cyclodextrin forms a host-guest complex with simvastatin enhancing drug solubility in aqueous media, prolonging the release with respect to stent coverage with chitosan only. Also, mechanical tests showed promising resistance of nanofibrous covering toward stent insertion in the catheter. This approach could easily overcome the limited possibilities of classical coated active stents in which drug loading is limited by the reduced area of the stent structure.

In both cases, further *in vivo* studies will investigate the actual efficiency and biocompatibility of those implants. Yet, the easy and efficient approach could effectively set the basis for further development of similar tools with improved features.

A similar approach by Rychter *et.al.* (2018) studied the potential application of tubular structured PCL electrospun nanofibers. [156] Scaffolds were loaded with different amounts of cilostazol, a drug used stroke prevention, by simple blend electrospinning. Morphology studies located the drug near the fiber surface, causing a rapid *in vitro* release of the drug after only 48 hours, with similar trends for all the concentration tested. This release profile is compatible with the time-frame of the subacute phase following device implantation and vascular injury, which could facilitate the re-endothelialization process. Also, mechanical properties matched those of collagen fibrils found in blood vessels. The promising aspects of the electrospun nanofibers are not supported with biological data of biocompatibility and cytotoxicity, leaving a question mark in the potential applicability of this kind of scaffold. Furthermore, the implementation of a more sustained drug release phase, by changing polymer composition, could prolong the therapeutic window of the implanted device.

Another work by Rychter *et.al.* (2019) exploited the behavior of different blends of PCL and pluronic P123 for the improvement of cilostazol loaded tubular nanofibers. [157] To achieve a sustained release of the chosen hydrophobic drug, and facilitate tissue regeneration, is crucial to obtain hydrophobic fibers with high wettability. To evaluate the impact of P123 on those parameters of the electrospun materials, water contact angle was measured, showing that pluronic P123 is mainly distributed on the surface of the fibers gaining improved wettability even in very small quantities. The evaluation of mechanical proprieties, also, showed improved tensile proprieties of the blended fibers compared to pure PCL fibers. *In vitro* release study showed an increase of burst release with decreasing pluronic P123 concentration, but at the same time a more controlled sustained release in the same fibers. This kind of behavior reflects the complex release mechanism caused by polymer matrix relaxation and the spatial location of pluronic P123. *In vitro* evaluation of cell viability showed a negative impact of pluronic P123 in the fiber formulation compared to pure PCL fibers. The same trend was observed in primary cells line were no benefit was observed for both fibers formulation. It is crucial to underline potential cytotoxicity toward endothelial and smooth muscle cells applying pluronic for combination product development for cardiovascular applications. This preliminary data suggest the need to move toward different formulation of polymer blends avoiding the use of pluronic. For this purpose modified natural polymer like cellulose acetate could be examined in the future, a creation of a library of different nanofiber formulation could help the identification of a composition with superior features compared to pure PCL.

Disease like heart failure, involving directly the heart muscle, didn't find many applications in drug delivery devices probably due to the difficulties of implanting this kind of devices. However, the combination with electronic devices could give rise to smart and long-time implants for monitoring and delivery of drugs.[158] Feiner *et.al.* (2019) developed a hybrid microelectronic tissue construct capable of withstanding the dynamic environment of the beating heart without compromising electronic or mechanical functionalities. [159] The device is made by a freestanding electronic chip selectively coated in defined zones by a positively charged polypyrrole layer. The presence of an electroactive polymer on the electronics enables it to release multiple negatively charged drugs, attached to the polypyrrole, in parallel under stimuli response control. Gelatin and PCL composite nanofibers assure a straightforward strategy for successfully promote cell attachment, together with a protective function over the chip coating. Preliminary release studies were afforded with anti-inflammatory drugs, such as aspirin and indomethacin, showing a release only dependent on current stimuli. The device successfully supported cardiomyocytes growth, with the possibility of monitoring the parameters of cardiac cells function. The produced construct has indeed several advantages with respect to the use of nanofibers alone. However, in terms of drug release, its applicability is limited only to charged molecules. Further works could explore more specific drugs for the treatment of cardiac disease, evaluating the effective impact of this kind of device in patients.

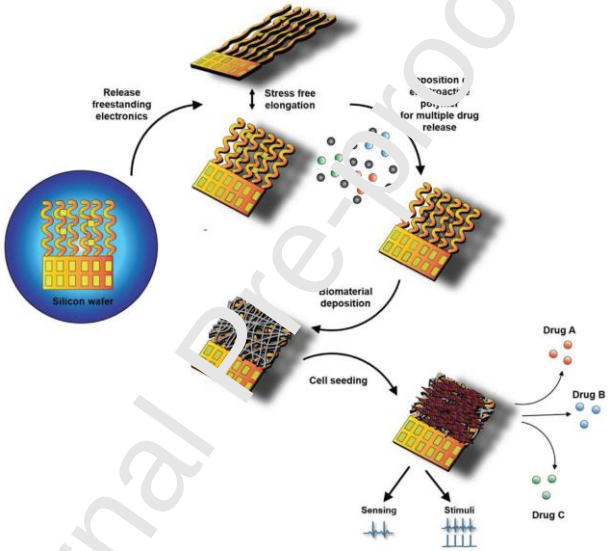


Fig. 11. Schematic illustration of the hybrid microelectronic tissue concept. Drug are loaded in the electro-active polymer layers, deposited on the central electrodes. PCL-gelatin electrospun nanofibers enables the cell seeding and attachment. The resulting hybrid tissue can then be used to monitor tissue function, intervene through electrical stimulation and controlled release of drugs. Figure reprinted from Feiner *et al.* [159] with permission from Wiley Online Library.

As already seen for other pathologies, another approach could involve the oral delivery through sublingual implants, Li et.al. (2020) prepared carvedilol, a non-cardioselective beta-receptor inhibitor used for the treatment of hypertension, loaded electrospun nanofibers for this purpose. [160] The fibers were mainly composed of PVP, while polyethylene glycol was used as a plasticizer to promote the interaction of the fibers and improve flexibility. Drug loading occurred by blending with the polymers and allowed to achieve a homogenous distribution. Nanofiber films exhibited excellent fast dissolutions and enhanced *in vitro* permeation behavior with respect to the simple drug solution. Sublingual delivery could be an attractive platform especially for patients with swallowing difficulties. Yet, more toxicity data are needed to validate this kind of platform.

3.5 Ocular diseases

The eye is a complex organ debited to the visual system able to capture light from the surrounding environment, regulates its intensity through a diaphragm, and focus through an adjustable assembly of lenses to form an image. Eyes are regularly lubricated by tears, suited to remove irritants and aid the immune system.[161,162] Treatment of eye disease usually occurs through the usage of eye drops, saline

solution of the active pharmaceutical applied directly over the eye. However, this system suffers from poor bioavailability due to the small volume, the fast turnover of the tear film, and the presence of several physiological barriers in which drug molecules have to pass by. Solid delivery systems for eye disease are attracting rising attention for their potential higher bioavailability due to decreased clearance of the system compared to liquid ones.[163,164] Tawfik *et.al* (2020) developed coaxial electrospun nanofibers incorporated with two different drugs in different compartments to treat corneal abrasion and prevent the rise of bacterial infection. [165] The shell composition was poly(lactic-co-glycolic acid), a hydrophobic polymer, loaded with pirfenidone, an anti-fibrotic drug used in clinics to treat idiopathic pulmonary fibrosis. On the other hand, the hydrophilic PVP was used as the core, loaded with the antibiotic moxifloxacin. Besides the success in coaxial fiber production, and excellent drug loading rate, a high burst release was observed with a complete discharge of pirfenidone after 4 hours, and about 70% of the antibiotic released in 30 minutes. Such behavior could be explained with a non-optimal combination of drug and polymer, leaving space to further optimization and expansion of the study. Nevertheless, no information about the transparency of the scaffold, crucial for ocular application, was given by the authors. This preliminary work introduced an original approach in terms of drugs used, but still needs proper optimization.

Another approach from Göttel *et.al.* (2020) exploited a solid *in situ* gelling system for the treatment of topical ocular diseases based on gellan gum/pullulan electrospun nanofibers. [166] Since the eye curvature makes the application of solid dosage forms more complex than the instillation of eye drops, authors developed a system able to bend the scaffold in a defined shape. This approach also furnishes a higher contact between the fiber and the eye, improving the drug delivery feature. As preliminary study, scaffolds were embedded with fluorescein and their residence time in the eye compared with conventional eye drops in an *ex vitro* porcine model. Results underlined a more homogeneous distribution over the whole cornea surface where fibers were applied with an extremely higher residence time of the model compound. Besides the exciting results achieved and the successful creation of an efficient delivery system, only a model compound was used in this study leaving open the possibility that, in case of other kinds of drugs, a too-long exposure could result in toxic side effects. Also, the lack of time-release study does not give information about the dosage needed to achieve a therapeutic effect.

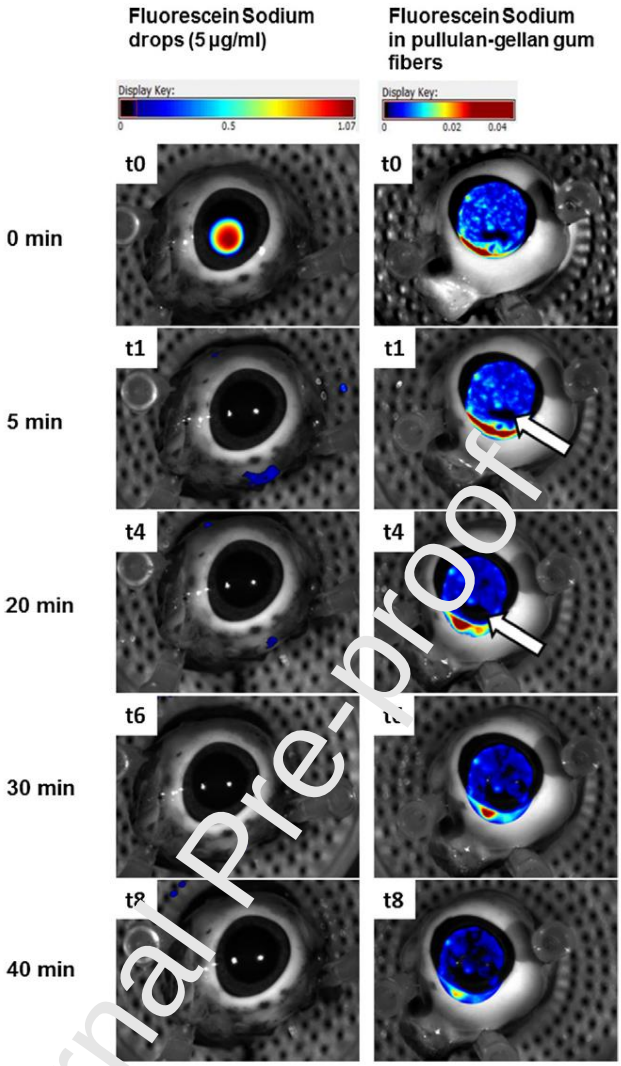


Fig. 12. Overlay of regular (photo) and fluorescence images of porcine eyes eyes treated with fluorescein sodium eye drops (5 µg/ml) (left), and with pullulan-gellan gum nanofiber lens developed by Göttel *et.al.*. The presence of the fluorescence in the nanofiber treated eye, indicates a slightly higher permanence of the drug respect the normal eye drops. Figure reprinted from Göttel *et al.* [166] with permission from Elsevier.

Grimaudo *et.al.* (2020) designed a nanofibrous ophthalmic mesh composed of a blend of hyaluronan and PVP as a coupled delivery platform of ferulic acid, an antioxidant, and ε-polylysine, an antimicrobial peptide, for the treatment of different ocular surface diseases. [167] The two drugs were loaded with two different techniques: while ferulic acid was blended with polymers, ε-polylysine was cross-linked after the electrospinning process. Preliminary *in vitro* assays showed that scaffolds caused no hemorrhage, vessel lysis or coagulation, and performed the same as the saline solution control indicating that the designed inserts can be recognized as non-irritant. Additional cytocompatibility studies were carried out with fibroblasts with promising results. Yet, antibacterial assays resulted in effective inhibition of *P.aeruginosa* and *S.aureus* growth. The drug release study revealed a remarkably fast erosion of the scaffolds with consequent release of the two drugs in 20 minutes, limiting the applicability of this kind of nanofibrous platform only for short time treatment, without any efficacy in the long term medication.

Meanwhile, Di Prima *et.al.* (2019) developed triamcinolone acetonide loaded poly(1,4-butylene succinate) scaffolds for ocular delivery purposes. [168] The bulk electrospun material resulted in soft, flexible, and characterized by a pearl-necklace highly porous structure. After the spinning process, nanofibers were treated with plasma-assisted chemical surface functionalization to confer biomimetic properties like wettability, mucoadhesion, and cytocompatibility on human corneal epithelial cells. The modified nanofibers revealed promising features, including excellent stability in simulated fluid, high

loading, non-bioerodible surface, and low swelling. Moreover, all the produced scaffolds present a complete drug release after 30 days without short term burst, with adequate mucoadhesive and cytocompatibility features granting high penetration and permeation through *ex vivo* corneal tissue compared to the pure drug solution. This promising approach could furnish an efficient topical ocular drug delivery system. Still, elucidation over the interference with the sight in in vivo models has to be clarified since the long term treatment could be invasive for the patients.

Corneal transplantation is still the best clinical treatment for corneal disease, the leading cause of blindness in the world. However, qualified donors are not always equal to the demand, leaving the route for alternative treatments. Also, the very fragile nature of the eye requires extreme care before, during, and after a surgical procedure to minimize or prevent further damage. For this reason, the creation of engineered tissues could open new horizons for alternative therapies as well as post-operative care for the prevention of infection or adverse effects. In this field, nanofibrous scaffolds could be a promising platform for delivery purposes of drugs and tissue regeneration.[169]

Forouzideh *et.al.* (2020) produced loaded with epigallocatechin gallate silk fibroin-based scaffolds with anti-angiogenesis properties for corneal tissue engineering. [170] To induce crystallinity of silk fibroin and enhance the mechanical proprieties and lipophilicity, non-loaded fibers were treated with methanol, a standard protocol to convert random coils or silk I conformation into β-sheets. Loaded-scaffolds were not treated with any organic solvent to avoid the waste of the active compound, but at the same time leaving a random coiled structure in this fibers. *In vitro* release studies revealed a controlled and sustained drug release profile over more than 5 days, and showed the *in vitro* ability of dose-dependent inhibition of cell proliferation. The scaffolds were able to sustain the growth of limbal cells, although this was observed only with the non-loaded structures, presenting more crystalline and hydrophobic features, thus leaving doubts over the behavior of loaded fibers, organized as random coils, on cell growth.

In another work, Da Silva *et.al.* (2019) exploited the applicability of Dexamethasone acetate loaded PCL nanofibers for targeted delivery in the vitreous cavity in the treatment of retinal diseases. [171] Fibers production occurred by blend electrospinning. The release study revealed the complete release of the drug in 12 days, without burst release. The fibers degradation follows the same trend of release, with an almost complete degradation at the same time window. Since fiber production occurred through the usage of acidic solvents, the possibility of the inclusion of acidic residues into the mats, which could trigger damage to the delicate ocular tissues and consequently disrupt the neurosensorial retina and other ocular structures, could not be discarded. However, preliminary *in vitro* results showed excellent biocompatibility with cellular growth and morphology similar to control tissue culture polystyrene (TCPS), as well as the absence of cytotoxicity. *In vivo* tests revealed the lack of eye enucleation and other signals of toxicity. Scaffolds exhibited no interferences with the sight of the animals since remained light-sensitive and able to detect moving objects. The work set a promising approach for the post-operative treatment of vitreoretinal surgery, giving a platform able to prevent inflammation and adverse effects that could arise after the surgery.

3.6 Clinical development of nanofibers

The promising potential of nanofibers delivery technology, at the time being, is not so well explored in clinical trials. Currently, most of the studies limits the biological characterization of the scaffolds to the pre-clinical phase. Despite many works observe significant results in *in vitro* or *in vivo* models, the development of translational studies to patients is still a limitation to this technique. [172]

Clinical development of new devices requires time and economical efforts higher than the pre-clinical phase that could hamper the initial investment. The production of the device should also meet specific requirements to fulfill ISO 10993 standards and should be produced in GMP conditions.[173] Still, the existence of already FDA and European Medicines Agency (EMA) approved nano-formulation of different drugs, like the liposomal formulations of daunorubicin commercialized as DaunoXome®, should persuade more researcher and medics to investigate deeper the nanofibers technology in the clinics.[174] Nowadays, only a few literature examples of clinical trials are reported. Chaturvedi *et.al.* (2013) developed a PCL based nanofibrous delivery system for the treatment of periodontal infections.[175] Nanofibers were prepared by solution electrospinning, blended with doxycycline, which provided *in vitro* release for 11 days. Scaffolds were studied in 7 patients affected by Chronic Periodontitis divided into two groups: A) scaffold treated plus surgical scaling and root planning; B) surgical only scaling and root planning control. The patients were evaluated with three indexes: probing depth (PD), plaque index (PI), and gingival index (GI).

The three indexes were significantly better in those patients treated with the scaffolds, confirming a synergism between the surgical treatment and the drug delivery device. Although positive results were obtained, this treatment revealed similar effects to similar investigations made with doxycycline gels applied topically. However, scaffolds were easy to place in the periodontal pocket, less time-consuming, and more cost-effective than gels, also providing the capacity to embed anti-inflammatory drugs in addition to doxycycline, which could further improve the impact of the medication on the disease.

Additional examples involving nanofibers clinical trials regard scaffolds that lack drug embedding but exploit the intrinsic capability of scaffolds to promote cellular growth and tissue regeneration, like the work of Kossovich *et. al.* in which chitosan/PEO nanofibers were used to induce regeneration of burning injuries in patients.[176] The efficacy in terms of pain reduction, wound healing and protection from infections indicates the potential of these tools that could be further improved with the addition of drugs.

One of the key advantages of electrospun nanofibers is their capability of limiting the peripheral side effect of the drugs by direct application at the specific site of action.[177] The latter, in many cases, requires surgical intervention that limits the self-usage and the large-scale market of the technology. Still, in cases of severe conditions or chronic diseases, the one-time surgical application could limit the admission in the hospital over time.

4. Conclusion and future perspective

The endless possibilities of electrospinning are an exceptional platform for the development of innovative drug delivery systems, able to maximize the therapeutic benefits of drugs, minimizing at the same time their undesired side effects. Drug and polymer choice could be tuned simply for the specific field of application or the precise requirement. By changing the mechanical properties or the kinetic of release, electrospun scaffolds could become a new horizon for personalized medicine.

Besides the great advantage given by this technique, only a few clinical trials were reported in literature during the years, and still regulation agencies like FDA did not approve any devices.[178] In many cases, this outcome is the result of toxic residue of the solvent used in the spinning process, remaining trapped in the fiber and being released with the drug.[53,179] Since electrospinning is still a facile and easy technique for the development of smart and controlled drug delivery devices, it is remarkable the development of new approaches exploiting the use of greener and biocompatible solvents, for instance water, deploying the usage of more aggressive and toxic ones like chloroform and HFIP. Alternatively, one of the most promising techniques could be melt electrospinning, in which the production of nanofibers occurs without the use of any solvent. Yet, it will be necessary to protect the drug from the heat bypassing its degradation.

Additional issues are always linked to the tuning of the drug release. In many works, a proper correction in the drug release profile, sometimes linked to a bad polymer-drug combination, could easily result in an improvement of the whole work. Thus, the creation of a database resuming information like the scaffolds features, composition, and the final output in terms of drug release could give a simple overview of what can be the successive step for the optimization of that kind of system. Also, the development of new materials, and blends could help the creation of even more platforms, which could enlarge the available toolbox when planning new works. Also, the biological testing of electrospun nanofibers in many cases are limited to the *in vitro* testing on cell lines. However, for the complete understanding of the real performance and toxicity, moving *in vivo* could is a necessary step; a shortcoming, where is possible, is to use primary cells or patient-derived tissue, to have a more realistic view of the behavior of the scaffold.

Besides, the continuous advance of the technology and the creation of more sophisticated combined systems could also help to develop innovative smart devices able to precisely modulate the quantity of drug released from the scaffold in the response of body stimuli. Such kind of improvement could also open the gates to applications that nowadays finds lesser application for electrospun nanofibers, for instance, in fields like diabetes, hormones therapies, or even autoimmune diseases that nowadays are described only by few, or none, works.[180]

Overcoming such limitation could result in an even more powerful tool towards an efficient and noninvasive tissue engineering and for moving towards personalized medicine. Remarkably, the cooperation of different disciplines with competence in fields like engineering, chemistry, and biology could offer an appealing approach for the creation of this kind of system.

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