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Properties, Engineering and Applications of Polymeric Nanofibers: Current Research and Future Advances

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The subject of nanomedicine has seen a surge in research activity over the past decade, with nanofibers being a particularly active field. Nanofibers are solid, dry fibers with nanometer diameters, made of various polymers, whereas electrospinning is a versatile, simple, elegant, reproducible, continuous and scalable technology for their preparation. Nanofibers are a unique class of materials in the biomedical field, since they provide a biomimetic environment on the nanometer scale, a three-dimensional architecture with the desired surface properties on the micrometer scale, combined with mechanical strength and physiological acceptability on the macro scale. In particular, their ability to imitate the fibrillar elements of a natural extracellular matrix in a very realistic way is crucial. In this paper we introduce the fundamental aspects of the electrospinning process and the properties of nanofibers, as well as highlighting the enormous potential of nanofibers as drug-delivery systems and tissue scaffolds.

Key words:
Nanofibers, electrospinning, polymer, drug delivery, tissue scaffolds

Introduction

In the past decade nanomedicine has experienced an unprecedented rate of advancement, with nanofibers being a particularly active field, due to their enormous potential in biomedicine. A bibliometric analysis of scientific publications in the "Web of Science" database clearly shows the tremendous interest in polymeric nanofibers, with the number of publications rising from just 100 articles in 2000 to more than 2300 in 2011, with drug delivery being the largest research field[1]. Moreover, it is estimated that the global market for nanofiber products will be worth \$176 million in 2012. This market is forecast to grow at a compound annual rate of 34 %, and this despite the fact that there are just 50 companies in the world producing nanofibers[2].

Nanofibers are solid fibers with several remarkable nanoscale features, among them is a very large ratio of surface area to mass, a porous structure, and a theoretically unlimited length, together with a better mechanical performance and flexibility than any other form of the same material (Fig.1)[3,4]. They are a unique class of materials in the biomedical field since they provide a biomimetic environment on the nanometer scale, a three-dimensional architecture with the desired surface properties on the micrometer scale, combined with mechanical strength and physiological acceptability on the macro scale.
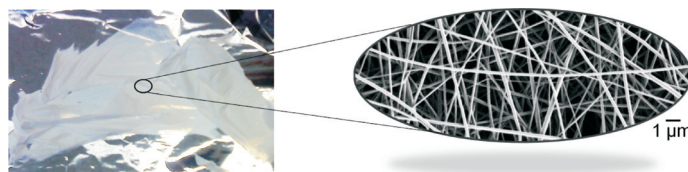

F i g . 1 − *Polymeric nanofibers on an aluminum collector as seen by the naked eye and a magnified image of the same sample under a scanning electron microscope.*

Of the various processing methods available, nanofibers are most often prepared by electrospinning. Compared with other fabrication techniques, such as drawing, template synthesis and phase separation, electrospinning is a simple, elegant, reproducible, continuous and scalable technology with the ability to produce nanofibers from a wide variety of polymers [5].

The aim of this paper is to describe the fundamental aspects of the electrospinning process and the properties of nanofibers as well as to show the tremendous potential of nanofibers for applications in the biomedical field. In particular, the use of nanofibers in drug-delivery systems and tissue scaffolds is highlighted. In this paper we discuss current research and future challenges.

Electrospinning process

Mechanism of the electrospinning process

Electrospinning is a process for preparing nanofibers from a polymer solution by the applica-

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tion of a high voltage that induces the formation of a thin liquid jet[3]. A standard electrospinning system is simple in terms of equipment; however, the physics governing the process is extremely complex. In a standard system, a polymer dissolved in the proper solvent is put into a syringe fitted with a metal needle and set on a syringe pump, which produces a constant flow rate. At a low voltage the polymer solution drips from the needle, but when the applied voltage is high enough, it causes the formation of a cone (the so-called Taylor cone) and from the tip of this cone a charged fluid jet is ejected and accelerated towards a grounded collector (Fig. 2). The jet is continuously stretched and whipped, which means its diameter is reduced from several hundred micrometers to as little as tens of nanometers. Simultaneously, the solvent evaporates and dry nanofibers are deposited on the collector. Optimum conditions cause the formation of smooth nanofibers (Fig. 2A), whereas inappropriate parameters lead to a bead-on-string morphology as a result of the incomplete stretching of the polymer chains due to an unsuitable proportion of electrostatic forces during the nanofibers' formation (Fig. 2B)[4].
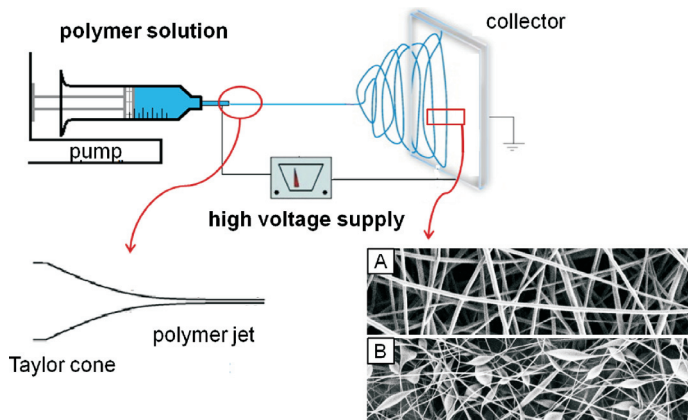

Fig. 2 – *A schematic illustration of the basic principles of the electrospinning process. Representative figures taken with a scanning electron microscope showing two distinctive morphologies of the obtained nanofibers: (A) smooth nanofibers, (B) bead-on-string morphology.*

The success of the electrospinning process and the morphology of the obtained nanofibers depend on many different, but interrelated, parameters. Clear evidence for this is seen in Fig. 3, which shows significant changing of the parameters of PVA solutions when varying the concentration of the polymer [6]. It is clear that the interdependence of the parameters complicates the nanofiber design; however, on the other side minor changes of the parameters results in the formation of an electrospun product with the desired morphology and topography. Nanofibers have already been successfully pre-
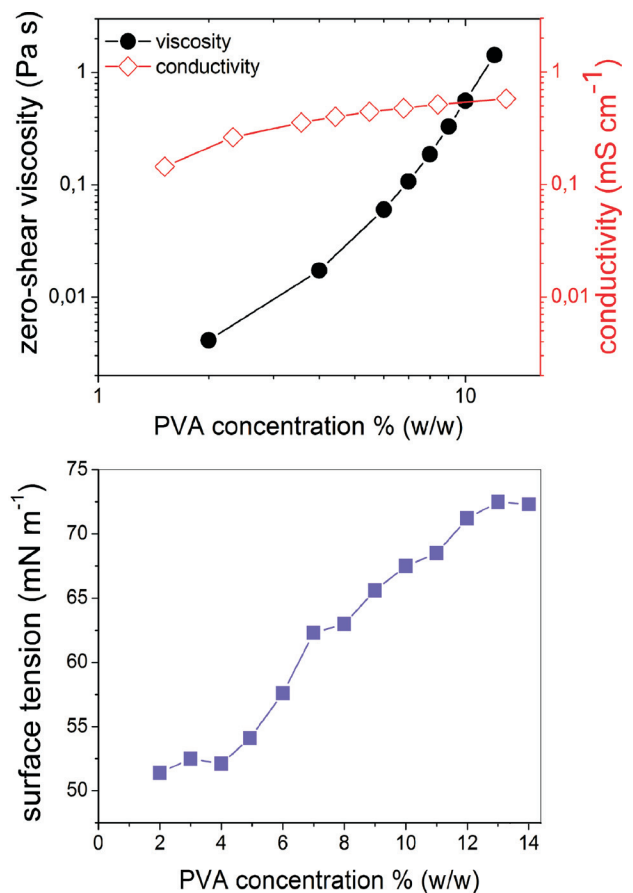

Fig. 3 – *Zero-shear viscosity, conductivity and surface tension in relation to the concentration of PVA [6]*

pared from more than 200 natural and synthetic polymers; nevertheless, the applicability of electrospinning is not easily transferable between various polymer solutions, which means that for each combination of polymer and solvent the appropriate parameters have to be determined individually[4].

Control of the nanofiber's diameter and morphology

The parameters affecting the morphology of the obtained nanofibers can be divided into the solution, process and environmental conditions. In terms of the solution parameters, the solution viscosity, surface tension and conductivity are the most determining, while among the process parameters the most decisive are the applied voltage, the feed rate and the distance to the collector[4,7,8]. Although it is well known that the previously mentioned groups of parameters have a predominant influence on the nanofibers' formation and morphology, the effect of environmental conditions (temperature and humidity) is not negligible. In the text below some selected parameters are briefly described and the effects of all the parameters on the

Table 1

Solution parameter	Effect on nanofiber morphology	Ref.
• Concentration	Increase in concentration leads to increase in fiber diameter.	4,6,7
• Viscosity	Increasing viscosity leads to thicker and beadless nanofibers. Too high viscosity causes generation of beads.	4,7,12
• Surface tension	No conclusive correlation has been established between the surface tension and the nanofiber morphology.	9,10,11
• Conductivity	Increase in conductivity leads to thinner nanofibers.	12–15
• Molecular weight of polymer	Increase in polymer molecular weight leads to formation of a nanofiber with fewer beads.	9,16,17
• Volatility of solvent	Higher volatility requires higher flow rate and leads to formation of a nanofiber with fewer beads.	4,18,19
• Dielectric constant	Sufficient dielectric constant of the solvent is needed for successful electrospinning.	4,15
Process parameter		
• Flow rate	Lower flow rate results in thinner nanofibers. Too high flow rate causes the generation of beads.	19,20
• Applied voltage	Thinner fiber with higher applied voltage.	4,5,12
• Needle tip to collector distance	Minimum distance required to obtain dry nanofibers. Generation of beads when the distance is too small or too large.	4,9
• Geometry of collector	Metal collectors are preferred. With conductive frame or rotating drum aligned nanofibers are obtained.	21
Ambient parameter		
• Humidity	Lower humidity enables higher flow rate and the generation of beads is reduced.	22,23
• Temperature	A thinner nanofibers are obtained when the temperature is higher.	23

nanofibers' morphology are schematically presented in Table 1.

The fiber diameter tends to increase with an increasing polymer concentration and viscosity, but decreases with an increasing conductivity, while no strong correlation between the surface tension and the nanofiber's morphology has been established[9–11]. However, the solution's surface tension determines the threshold voltage needed for the electrospinning process to occur, since the surface-tension forces have to be overcome by the electrostatic forces for the formation of a jet[7].

Increasing the applied voltage – as the most important process parameter – generally leads to a smaller fiber diameter due to the stronger electrostatic forces; however, it can also cause an increased mass flow, leading to a thicker fiber[12]. The distance between the needle and the collector determines the strength of the electric field and together with the applied voltage governs the stretching of the polymer jet. The distance should be sufficient to allow the evaporation of the solvent. In addition, the solution feed rate should ensure the continuous formation of the Taylor cone, even though some of the solution is carried away by the jet[24]. The nanofibers that are formed can be oriented randomly or aligned on the collector, whereas the arrangement is mainly a result of the type of collector. The fibers collected on a planar aluminum foil are usually randomly oriented, while the usage of a rotary drum or patterned electrodes enables the formation of aligned nanofibers (Fig. 4)[21,25].

Progress in electrospinning techniques resulted in the development of new methods for the preparation of nanofibers, enabling the mass production and formation of various nanofibrillar structures[21,26–28]. An example of the latter that has gained wide popularity is coaxial electrospinning, where two solutions are coaxially and simultaneously electrospun through different feeding capillary channels in one needle to generate nanofibers with a core-shell structure[3,24].

Biocompatible polymers

The selection of a material plays a pivotal role in the design of nanofibers for biomedical applications. The ideal biomaterial should be biocompatible,
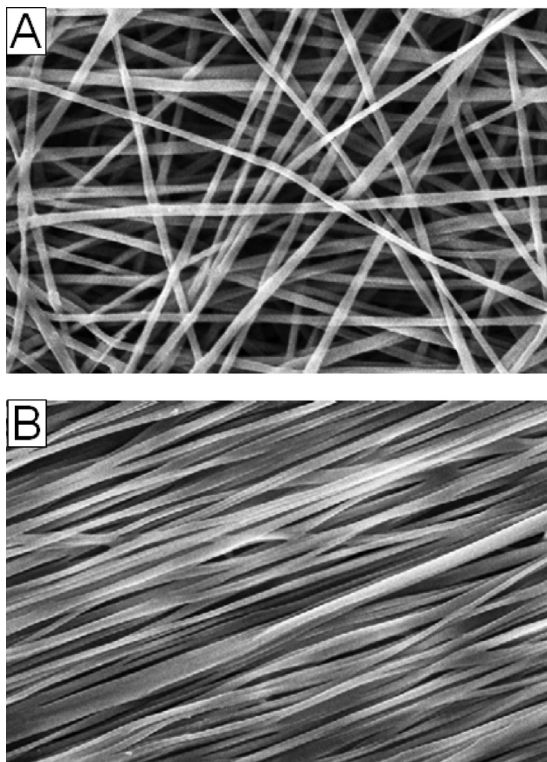
F i g . 4 – *Representative images of (A) randomly oriented and (B) aligned nanofibers under a scanning electron microscope. Adapted with permission from[25].*
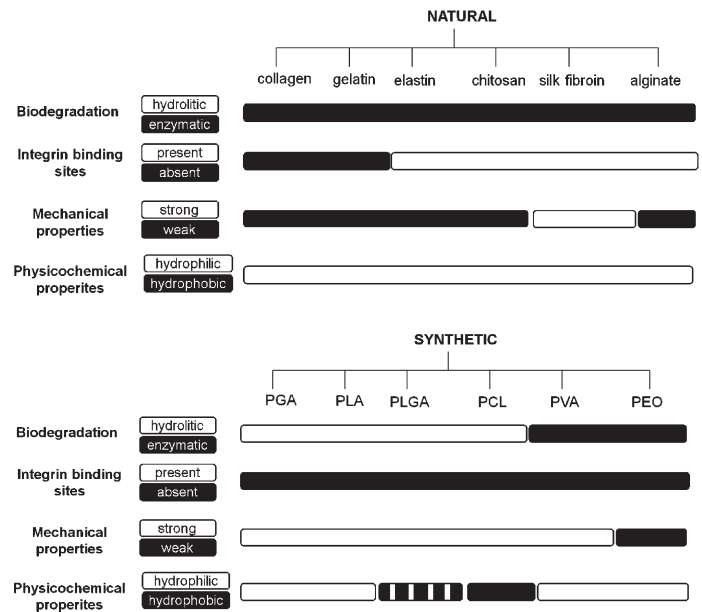

F i g . 5 – *Biological, mechanical and physicochemical properties of commonly used polymers. (PGA – poly(glycolic acid), PLA – poly(lactic acid), PLGA – poly(lactic-co-gylcolic acid), PCL – poly(caprolactone), PVA – poly (vinyl alcohol), PEO – poly (ethylene oxide).*

biodegradable, nontoxic, hydrophilic and with the proper mechanical strength[29]. In theory, the choice of the polymer for electrospinning is not limited, providing the polymer allows the preparation of a solution or a melt with the proper characteristics[4]. Up to now many different polymers have been electrospun into nanofibers and can be broadly classified as either synthetically or naturally derived[29]. Among the synthetic ones the most commonly used are poly(vinyl alcohol) (PVA), poly(ethylene oxide) (PEO) and biodegradable aliphatic polyesters, such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(caprolactone) (PCL), while chitosan, alginate, collagen, gelatin, hyaluronic acid and silk are examples of frequently used natural polymers[5,30,31]. The key biological, mechanical and physicochemical properties of the commonly used polymers are presented in Fig. 5.

Synthetic materials are strong, cheap, reliable, often easily electrospinnable and exert physico-chemical characteristics that can be controlled through the production process. However, they lack cell-recognition sites, causing poor affinity for cell attachment. On the other hand, natural polymers are preferred due to their similarity with the macro-molecular substances present in the human body[23]. Therefore, the biological environment recognizes and favorably interacts with the natural polymers. The preparation of nanofibers from natural polymers is challenging due to their polyelectrolyte nature and their high viscosity at low concentrations. Thus, natural polymers are often blended with synthetic polymers, which represent a spinnable carrier and enable the formation of nanofibers[12]. Importantly, the nanofibers prepared from polymer blends retain the biological functionability of the natural polymer as well as the mechanical strength and durability of the synthetic component[32]. Using this approach we have successfully prepared nanofibers from chitosan and alginate with the addition of PEO or PVA. However, the research was mainly focused on chitosan, due to its outstanding properties, such as biocompatibility, biodegradability, safety, hydrophilicity, the ability to suppress an inflammation response during healing and antimicrobial activity, and its enormous potential as an effective biomaterial for drug-delivery applications, wound dressings and tissue substitutes. Our results have shown that the morphology of nanofibers obtained from chitosan/PEO solutions depends strongly on the solution composition, since significant changes in the product morphology were observed, when the amount of chitosan in the polymer blend was decreased[12]. Recent results have also indicated that the role of humidity in the electrospinning process was underestimated in previous studies, since the success of the process can be significantly improved only by lowering the humidity[22].

The selection of an optimal solvent for each polymer or polymer blend is fundamental to the success of the electrospinning. Solvent selection is pivotal in determining the critical minimum solution concentration to allow the electrospinning as well as contributing to the solution surface tension and conductivity, thereby affecting the solution's spinnability and the morphology of the electrospun nanofibres[33]. Frequently used solvents for nanofiber preparation are tetrahydrofuran, dimethylformamide, chloroform, acetic acid, acetone, ethanol, 2,2,2–trifluoroethanol and distilled water[5]. Distilled water is a favorable solvent when nanofibers are intended for biomedical applications, since solvent residues in the formulated nanofibers do not lead to any safety concerns. Polymers are often not completely soluble in water; therefore, the addition of co-solvents in a relatively small proportion is necessary. For example, chitosan/PEO nanofibers can be successfully prepared from acidic aqueous solutions containing 2 % acetic acid[12]. Moreover, a mixture of solvents is not only used for the needs of solubility, but also to accelerate the solvent's evaporation in the electrospinning process. The addition of a volatile, usually organic, solvent increases the solvent evaporation rate and, therefore, decreases the necessary needle-to-collector distance in electrospinning. Solvent volatility also affects the fiber porosity, being higher when the solvent evaporates quickly[3]. Our results showed that the addition of an organic solvent is only beneficial in the case when the polymer is completely soluble in the solvent mixture[4].

The addition of surfactants and salts to the polymer solutions is another well-established practice to achieve spinnability of natural and semi-synthetic polymers, improve the reproducibility of the electrospinning process or transform the product morphology from beads to fibers[4,13]. For this purpose various salts and surfactants can be applied[9,10,12,14,15]. In one of our studies the effects of Tween 80 and NaCl on the electrospinning of a hydroxylethyl cellulose solution were examined. Both supplements were chosen due to their safety and widespread use in several pharmaceutical and cosmetic products. The addition of Tween 80 lowered the surface tension of the solution, which resulted in the elongation of the beads into fibers, an increase in the fiber diameter and an improved process efficacy. The addition of the salt significantly increased the conductivity of the polymer solution, resulting in a reduced bead formation and a larger fiber diameter. It was shown that the addition of a surfactant improved the nanofiber morphology to a much greater extent than the addition of salt[4].

Drug loading

Active substances can be incorporated inside the nanofibers, physically adsorbed or chemically bound to the surface (Fig. 6)[34]. However, knowledge of the drug's behavior during its incorporation in the nanofibers and its subsequent release from the nanofibers is much more limited, compared to the knowledge available for drug incorporation and release from, for example, solid lipid nanoparticles. The loading of many different drugs and their localization in the lipid matrix have been systematically investigated[34–36]. The results showed that the drug incorporation, localization and release depend on the physicochemical properties of the drug and the carrier matrix. Therefore, it is also expected that for nanofibers the loading mechanism will be governed by the drug solubility in the polymer solution and the drug–polymer interactions in the solid state.
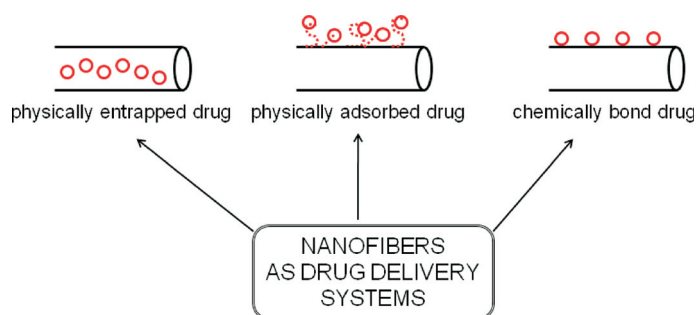

physically entrapped drug   physically adsorbed drug   chemically bond drug

NANOFIBERS AS DRUG DELIVERY SYSTEMS

F i g . 6 – *Scheme presenting the possibilities of drug loading in/on nanofibers*

Of the various loading possibilities, physical entrapment is currently the most widespread, since the drug in the nanofibers is protected against unfavorable environmental conditions and it offers good control over the drug's release. A typically observed release profile from such nanofibers exhibits an initial burst effect followed by an almost linear, sustained release[24]. Furthermore, the preparation of core-shell nanofibers provides a drug-reservoir system with a shell barrier protecting the incorporated drug and controlling the drug diffusion rate. The burst effect from such nanofibers is small and the entire release profile is more sustained[31,37].

The incorporation of a drug in nanofibers, either in the form of a matrix or as a core-shell system, is relatively easy to perform, since the drug is simply dissolved in the polymer solution prior to electrospinning. The formation of an amorphous drug, which shows a higher solubility with respect to the crystalline form[38,39], is favored, due to a very limited time being available for the drug's recrystallization during the electrospinning process[40–42]. Furthermore, a reasonable question can be

raised concerning the preservation of the chemical and biological integrity of the incorporated drug due to the application of a high voltage during the electrospinning. Various studies using H-NMR, DSC, X-ray and IR spectroscopy have proven that the electrospinning process does not affect the structural integrity of the incorporated drug[3,31,40,43].

The physical adsorption of a drug on the surface of the preformed nanofibers is achieved by dipping the nanofibers into a solution of the drug, which associates with the nanofibrillar surface via electrostatic interactions[36]. However, this technique is seldom used due to poor control over the drug's release and an undesirable competitive displacement of the drug with the components of biological fluids.

The third approach to drug loading is the covalent immobilization of the drug on the nanofibrillar surface via the formation of chemical bonds. The latter is predominately used for the modification of the surface properties of nanofibers, since the technique is technically complex. However, there are some reports dealing with this approach for the delivery of active substances. The drug is released after the enzymatic or environmental degradation of the chemical bond[37].

Use of nanofibers in biomedicine

Electrospun nanofibers as 2D and 3D scaffolds

Tissue engineering is an interdisciplinary field with the fundamental aim being to utilize the body's natural biological response to achieve successful tissue regeneration. Currently, two strategies have emerged as the most promising tissue-engineering approaches: one being the implantation of pre-cultured cells and a synthetic scaffold at the site of a tissue defect and the other being the application of an acellular scaffold at the site of the tissue defect immediately after the injury[37]. There has been increased interest in the latter approach, also due to the progress in the area of nanofibers. The underlying rationale for the use of nanofibrillar tissue scaffolds is a biomimetic approach. Electrospun nanofibers can mimic the physical structure of the major constructive elements of the native extracellular matrix (ECM) in terms of shape, size and mechanical properties. Furthermore, the fiber can also resemble the tissue biology, since almost all the tissues are constructed and hierarchically organized from fibrous structural elements[23]. All this suggests that such scaffolds could promote cell attachment, proliferation and differentiation. Tissue damaged as a result of an injury or disease is often accompanied by the loss of the ECM, which has to be regenerated in the healing process. In some cases, such as chronic wounds, the reconstruction of the natural ECM is disrupted, due to the imbalance between the regeneration and degeneration processes, leading to delayed or even prevented healing. Therefore, it is reasonable to expect that the application of ECM analogues will help with the recovery[44,45].

The preparation of nanofibers for tissue regeneration can be adapted for a specific place in the body, because of the wide range of biocompatible polymers available and the flexibility of the electrospinning process. For example, studies have shown that nanofibers used for artery-wall reconstruction should be applied in a sufficiently thick layer to withstand the pulsating blood flow. Aligned nanofibers resemble more closely the structure of the vasculature and the nerves than randomly oriented fibers[23,46]. The nanofibers for application in bone remodeling usually contain hydroxapatite, since the inorganic component improves the mechanical properties of the scaffold, makes it comparable to the natural bone and improves its osteoconductivity and osteointegration[21].

Various independent studies have proven the influence of nanofibers on the morphology of different cells[47–51]. Our results indicated that cells grown on nanofibers have an altered morphology in comparison to cells grown on a flat glass surface. Furthermore, the thickness and orientation of the nanofibers also affect the cell behavior. While cells grown on a randomly oriented, relatively thick, nanofibrillar support adopted a more rounded morphology, the others grown on aligned nanofibers had a distinctly elongated morphology and an improved mobility. The orientation of the nanofibers can govern the cell shape and the direction of the cell migration. Additionally, it was shown that the cell attachment to the nanofibers is slightly delayed compared to the attachment to a glass surface; however, it is much stronger due to the improved focal adhesion and the physical entrapment of cells in the electrospun scaffold. The cells grown on nanofibers exert an increased metabolic activity, which clearly indicates that the nanosized elements of the artificial ECM play a crucial role in the stimulation of the cell proliferation[52].

There is an increasing demand for bioactive scaffolds that can, besides physical support, provide a local release of incorporated biomolecules, which stimulate the regeneration of the surrounding tissue. The ECM is much more than just a physical support in the tissue, it represents a substrate with expressed specific ligands for cell adhesion, enables and orients the cell migration and regulates the cell growth and functions through the release of different bioactive factors. As a result, various growth factors as well as glucosaminoglycans have been incorporated into and on the surface of the nanofibers to improve

the tissue-regeneration properties[37]. However, additional investigations are required.

Drug delivery

Polymeric nanofibers have been used as carriers for the local delivery of therapeutic agents to target sites in the body[24]. The first report about nanofibers as drug-delivery systems was published by Kenawy et al., who incorporated tetracycline hydrochloride into nanofibers prepared from PLA or poly(ethylene-co-vinyl acetate) or from a blend of both polymers in a ratio 50:50 (w/w). The release profiles showed a relatively smooth release over 5 days, with a greater total percentage released than from the corresponding films, while eliminating the initial burst effect[53]. Later studies investigated the incorporation of various types of active substances, such as antibiotics[46,54], antiseptics[55], non-steroidal anti-inflammatory drugs[56,57], anti-cancer drugs[47,58,59] as well as biomolecules such as proteins[5,60,61] and nucleic acids[62].

Taepaiboon et al. reported the development of electrospun PVA nanofibers for the transdermal drug delivery of four, non-steroidal, anti-inflammatory drugs differing in aqueous solubility, i.e., sodium salicylate, diclofenac sodium, naproxen and indomethacin. The molecular weight of a drug has an important effect on both the rate and the total amount of drug released, with both decreasing for an increasing molecular weight of the drug. The drug-loaded electrospun PVA mats exhibited better controlled release characteristics of the model drugs than the drug-loaded films[56].

One of the largest areas in drug-delivery research is the targeted and controlled delivery of anti-cancer drugs. Xie and Wang developed PLGA-based nanofibers as implants for the sustained delivery of the anti-cancer drug paclitaxel to treat C6 glioma cells *in vitro*. The results showed the sustained release of paclitaxel over a period of more than 60 days, while the citotoxicity was comparable to the commercial paclitaxel formulation known as Taxol® [47]. Xu et al. incorporated two different anti-cancer drugs, i.e., hydrophilic doxorubicin hydrochloride and lipophilic paclitaxel, in PLA-PEO nanofibers[63]. The results indicated that the release behavior of both drugs correlated with their aqueous solubility and distribution in the fibers. Therefore, a faster release was observed for the more soluble hydrophilic doxorubicin hydrochloride. An *in vitro* cytotoxicity assay indicated the higher inhibition and apoptosis of the cells in the case of a dual drug combination compared to the effectiveness of a single-drug-loaded system, which suggests promise for multi-drug delivery and successful combined therapies.

Nowadays, more and more active substances belong to the class of proteins, and nanofibers can also be successfully applied for the delivery of such biomolecules. Since proteins are sensitive molecules, special attention has to be given to the preservation of their native structure in the electrospinning process. Therefore, coaxial electrospinning is frequently used. Chew et al. investigated the feasibility of encapsulating the human β-nerve growth factor in copolymer nanofibers from PCL and ethylene phosphate and proved its sustained release for at least 3 months [49]. Human epidermal growth factor (EGF) was immobilized on the PCL and PCL-PEO electrospun nanofibers for the treatment of diabetic ulcers by Choi et al[48]. The EGF nanofibers exerted superior *in vivo* wound-healing properties compared to the control groups or the group treated with the EGF solution. Furthermore, Yang et al. indicated that wounds, created in the dorsal area of diabetic rats, treated with nanofibers incorporating basic fibroblast growth factor, showed a significantly faster wound-recovery rate. The complete re-epithelialization and phenomenon of mature capillary vessels was observed 2 weeks after the onset of the treatment[50]. The above results demonstrate the unique potential of nanofibers to rapidly restore the structural and functional properties of wounded skin in patients with diabetes mellitus.

Another step forward was the incorporation of live cells into fibers with a micrometer diameter. Townsend-Nicolson and Jayasinghe successfully encapsulated cells into poly(dimethyl siloxan) fibers using coaxial electrospinning, whereas the cell viability stayed high throughout the electrospinning process and no change in the cell morphology was observed[64]. On the other hand, Canbolat et al. proved that a large proportion of cells embedded in nanofibers were incapacitated during the electrospinning when a single needle was used. The results indicated that the underlying reasons are fiber stretching and dehydration in the dry fiber[65].

To sum up, all the aforementioned results clearly indicate the suitability and uniqueness of nanofibers as a delivery system, which does not ensure only the desired release profile of the specific drug, but also provides a biomimetic environment in the place of application.

Future challanges

Despite the intensive research in the field of nanofibers a number of unanswered questions still remain to act as a driving force for further studies. The largest challenge is a complete understanding of the electrospinning mechanism. In order to control the properties, orientation and mass production

of the nanofibers, it is necessary to understand quantitatively how electrospinning transforms the fluid solution through a millimeter-sized needle into solid fibers having diameters that are four-to-five orders smaller. The next bottleneck in the electrospinning is the process efficiency and repeatability. Furthermore, the construction of a proper, three-dimensional scaffold remains a technological challenge, while from the point of view of drug delivery the drug loading has to be increased and the initial burst release has to be reduced in many cases.

Last but not the least, the future should see a move towards more *in vivo* testing, since the majority of work is currently done *in vitro,* in order to evaluate the performance of nanofibers in a biological environment. Consequently, more animal studies are needed to fully explore the potential of nanofibers for clinical applications. A close cooperation between laboratories and clinics may help to translate this promising technique from lab to bed and confirm the therapeutic benefit of nanofibers in regenerative medicine in the near future.

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