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Development of Electrospun Nanofibers for Biomedical Applications: State of the Art in Latin America

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REVIEW

Electrospinning is a powerful processing technique with huge potential in many attractive and cutting-edge research fields. This technique allows the production of non-woven micro/nanofibrous materials, including polymers, ceramics and metals, with a wide range of morphologies and functionalities. The highly porous electrospun scaffolds are ideal for biomedical applications, in particular for tissue engineering and drug delivery of biologically active compounds. In this review, we summarize the works on electrospun micro/nanofibers for biomedical applications carried out by research groups from Latin American countries. Studies are mainly focused on nanofibrous polymeric systems for drug delivery of therepeutic and bioactive agents, tissue engineering scaffolds and sensors, as well as other biomedical applications.

Keywords: Nanofibers, Electrospinning, Tissue Engineering, Drug Delivery Systems, Biomedical Applications.

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1. INTRODUCTION

Nanostructured systems are promising for many different and novel applications. Current nanotechnological advances provide opportunities to characterize, manipulate and organize matter at the sub-micron and nanometer scale. Biomaterials with nano-scale organizations have been used as controlled drug delivery systems and artificial scaffolds for tissue engineering.[1,2] However, the development of nano-scale drug and biomacromolecule delivery systems with optimal bioavailability, effective targeting and no cytotoxicity is still a big challenge. On the other hand, the tradicional tissue engineering strategy uses bioresorbable porous scaffolds with appropriate requirements to regenerate functional tissues. Recent efforts are addressed to the control over cell behavior and tissue formation. The native extracellular matrix is mimicked by nano-scale topography.[3,4] The controlled release of bioactive agents is also a focus of attention, since it will enhance the performance of tissue engineered matrices.

The broad interest in nanomaterials is leading to the creation of a huge variety of nanostructured systems, including nanoparticles, nanocapsules, nanogels, nanofibers, nanotubes, and dendrimers, just to mention a few.[1,5–8] Electrospun nanofibers have served as a highly versatile platform for a broad range of applications in widely different areas such as catalysis, nanofluidics, sensors, medicine, energy, environmental engineering, biotechnology, defense and security, and healthcare.[9–12]

The development of biomimetic highly-porous scaffolds is essential for successful tissue engineering.[13] Polymeric nanofiber-based scaffolds provide a structural support for the cells to accommodate and nutrient transportation. Also, guide cell growth in the three-dimensional space into a specific tissue.[14] In this way, biomimetic electrospun

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matrices facilitate cell attachment, support cell growth, and regulate cell differentiation.[15–17]

Processing techniques to produce polymeric nanofibers include phase separation, self-assembly, and electrospinning.[18,19] Phase separation does not requiere specialized equipment, and constructs can be prepared in molds with specific geometry. On the other hand, this method requires several steps to produce nanofibers of a limited number of polymers, and it is difficult to scale-up to a commercial stage. Self-assembly can be used for nanofiber fabrication,

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but relies on intermolecular forces to assemble small molecules, peptides, proteins and nucleic acids into fibers. This process is limited to a few polymers and only creates short fibers of several micrometers. Electrospinning is currently the most promising technique to produce continuous nanofibers. In this process, solid fibers are produced from a polymeric fluid stream (solution or melt), forming highly porous non-woven micro/nanofibrous membranes with excellent pore interconnection. Advantages of electrospinning include a relatively inexpensive setup, and the possibility to create different fiber architectures for use in many areas of application.

In this review, we summarize the works reported on electrospun nanofibers for biomedical applications carried out by research groups from Latin American countries. These studies are mainly focused on nanofibrous polymeric systems for the delivery of therapeutic and bioactive agents, tissue engineering scaffolds and regenerative medicine, sensors and other applications to a lesser extent. First, a short historical introduction and process description are presented. Second, the advances in each one of the above mentioned areas are described and commented. Finally, in the concluding remarks section, a vision of the future research on this topic is presented.

2. THE ELECTROSPINNING TECHNIQUE

2.1. Brief Historical Introduction

Electrospinning (also known as electrostatic spinning) is an electrohydrodynamic process discovered by Lord Rayleigh as part of his investigations in electrospraying in the late 1800s. The first patents for electrospinning were granted in 1902 to Cooley and Morton, but major commercialization did not occur until the advances made by A. Formhals (patents for the fabrication of textile yarns in 1934–1944) and C. L. Norton (patents in the area of electrospinning from melts in 1936). The main theoretical basis for electrospinning was made by Sir Geoffrey Taylor (in 1964–1969) when he developed mathematical models for the shape of the cone formed by the fluid droplet at the tip of the polymer reservoir under the effect of an electric field (known as the Taylor cone).[2,20]

After Taylor's work on the jet-forming process, attention focused on the understanding of the relationships between processing parameters and structural properties. Since the 1990s, when the rising interest in nanotechnology reawakened the interest in many technological areas, electrospinning has gained more attention. The great interest on the electrospinning process was quickly reflected by the large number of articles and patents published in the literature.[21–23] In recent years, the availability of commercial nanofiber electrospinning units has prompted further research in nanoscience and nanotechnology.

2.2. The Process

Nano/microfibers are produced when a polymer solution (or polymer melt) is subjected to a high-voltage electrostatic field operated between a metallic nozzle and a grounded collector as counter electrode. The transition from a charged droplet emerging from a die to a solid fiber is controlled by a set of complex physical instabilities.[24] A number of excellent reviews that describe and analyze the process can be found in the literature.[2,10,20,23,25,26]

Electrospinning is a unique and versatile process to produce polymeric fibers in the diameter range of few nanometers to several micrometers (usually between 3 nm–5 μm).[15] Electrospun meshes form three-dimensional scaffolds with high porosity, interconnected pore structure, and high surface area-to-volume ratio.

The fibers produced from melt polymers are thicker (due to the higher viscosity and the lack of solvent evaporation), but the process has significant advantages in that no volatile solvent is required and much higher volumes of material can be produced. Advantages are clear, and progresses in the technology behind the process are being made to scale this processing technique in order to get equipments and products commercially available.

Although the major attraction of electrospinning is its simplicity, the process is governed by a number of parameters that greatly affect fiber formation and structure. The main parameters are polymer structure and molecular weight, polymer-solution properties (concentration, solvent, viscosity, conductivity and surface tension), processing parameters, such as applied electrical potential, polymer solution flow rate, distance between spinneret and collector (working distance), position, geometry and static or rotatory nature of the grounded or charged targets, and ambient parameters (temperature, humidity, and air velocity). Therefore, this process is not as simple and easy as it appears. In order to produce defect-free nanofibers with controlled fiber diameter distribution and orientation, these parameters must be precisely controlled. Detailed explanations of each parameter and its influence on the electrospinning process can be found in the literature.[2,15]

In tissue engineering applications, the small fiber size intrinsic to electrospinning process can hinder efficient cellular infiltration.[27] In recent years, the preparation of electrospun scaffolds with controllable macroscopic porosity by several techniques, such as the combination of electrospinning with salt leaching[27–30] and salt leaching/gas foaming,[31] the use of auxiliary electrodes and chemical blowing agents,[32] cryogenic electrospinning,[33] and the use of tailored collectors,[34] have been reported. Nanofibers can be functionalized through encapsulation, grafting, coating or blending of biologically active compounds such as proteins, enzymes, and growth factors. Moreover, nanofibers can be assembled into a variety of arrays or architectures by manipulating their alignment, stacking, or folding.[17,24,35]

The production of individual fibers, random non-wovens, or orientationally highly ordered non-wovens can be achieved by an appropriate selection of electrode arrays or collector configurations.[14] The orientation of nanofibers is interesting for many tissue engineering applications where a distinct growth direction for the cells is required.[36–38] The basic setup has also been extended towards coaxial electrospinning of core–shell micro- and nanofibers, increasing the possibilities and potential areas of application.[39]

Although electrospinning is predominantly applied to polymer based materials including natural polymers, synthetic polymers, and composites,[10,40] recently, its use has been extended towards the production of metal, ceramic and glass nanofibers exploiting precursor routes.[41]

3. ELECTROSPUN NANOFIBERS IN DRUG DELIVERY SYSTEMS

The controlled release of drugs and active principles is an efficient process to balance the delivery kinetics, minimizing the toxicity and side effects. To achieve a controlled and sustained release system, the active substance is included into a device that ensures a predictable release rate *in vivo*, being administered topically, by an injected or non-injected route.

Electrospun nanofibers exhibit highly attractive functional characteristics as potential drug delivery carriers. Their porous structure with interconnected pores, high specific surface area, and short diffusion passage length increase the dissolution rate of a particulate drug and help in mass transfer and efficient drug release.[2,20,42,43] Moreover, depending on nanofiber morphology, porosity and composition, the release kinetics from a pharmaceutical dosage form can be designed as rapid, immediate, delayed or modified dissolution. The preparation of electrospun drug-loaded membranes offers other advantages, such as the ease of drug loading in simultaneous with fiber formation.

The bioavailability of poorly water-soluble drugs with pharmacological activity for the treatment of several diseases is a well known problem in the pharmaceutical industry. Besides, up to 40% of the active principles discovered in the last years are poorly soluble or lipophilic compounds, which lead to limited oral bioavailability, unpredictable absorption and lack of dose proportionality. Several strategies are available to solve these limitations, such as the use of solid dispersions, emulsions, liposomes, nanoparticles, and cyclodextrins, among others. In addition, the use of polymer-based electrospun micro/nanofibers containing dispersed drugs has also been reported for potential use in oral and topical drug delivery.[44]

The development of topical devices containing poorly water-soluble drugs for the treatment of skin diseases must achieve several requirements. The devices must contain solubilized drugs in a non-ionized state to accomplish therapeutical concentrations and to be optimally bioavailable to the skin. These systems can be developed by several strategies, including solution/dispersion technologies.[45,46] Although these techniques are at an early stage respect to pharmaceutical applications, they are suggested to be of high value in topical drug administration and wound healing.[44,47] Moreover, new drugs developed for efficient treatment of infections are unable to distinguish between the membranes of the fungi and mammalian cells, resulting in undesirable side effects when administered orally for the treatment of epithelial fungal diseases.[48]

To reduce undesired side effects at the systemic level, our group developed polymer systems with dispersed antifungal agents for topical applications.[49] Microfibrous poly(lactide-co-glycolide) (PLGA 50:50) meshes containing Ketoconazole or Chalcone 1, both poorly water-soluble drugs, were prepared by electrospinning. Ketoconazole is a commercial drug with antifungal properties, whereas Chalcone 1 (2,4-dihydroxy-3-metoxichalcone) is a natural active principle with proven antifungal properties,[50] extracted from natural species *Zuccagnia punctata Cav.* (Fabaceae).[51] The fiber diameters resulted higher for drug-loaded meshes (Fig. 1). The absence of characteristic melting peaks of Ketoconazole and Chalcone 1 in the polymeric matrices suggests complete dissolution of the active principles. The hydrophilic and amorphous nature of PLGA, added to the observed plastification effects due

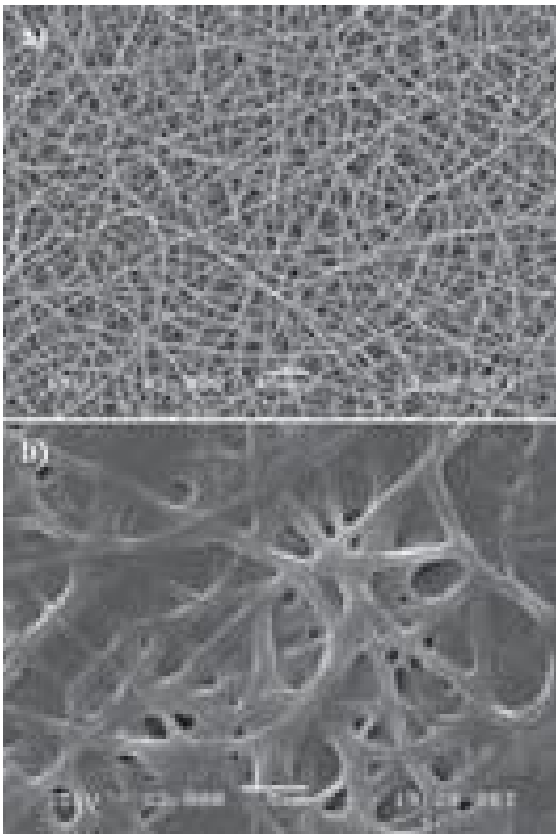

Fig. 1. SEM micrographs of electrospun meshes: (a) PLGA (×1000), (b) PLGA/Chalcone 1 (×3000). (Manuscript in preparation.)

to drug loading, should lead to drug release by hydrolytic degradation mechanism. The loaded amounts of the active principles resulted suitable for the treatment of fungal diseases by topical application, being potentially applicable for short-time exposures. Further studies to assess the release behavior of these matrices are being performed.

We have also developed microfibrous systems for antimycotic drug delivery applications. A poorly water-soluble antimycotic and bioactive agent, embelin, was dispersed in a biodegradable and biocompatible polymer matrix of poly(ε-caprolactone) (PCL).[52] Due to its hydrophobic character and semicrystalline structure, PCL exhibits a degradation time around two years under physiological conditions. PCL is degraded by hydrolysis, being the speed of this process dependent on the shape and size of the device, the dispersed bioactive agent, and the physiological characteristics of the biological environment. Embelin (2,5-dihydroxy-3-undecil-1,4-benzoquinone) is an active principle obtained from *Oxalis erythrorhiza* Gillies ex Hooker et Arnott (Oxalidaceae) collected in San Juan, Argentina.[53–55] This compound shows a diversity of relevant biological activities, such as *in vitro* cytotoxicity against B16 and XC tumor cell lines, anti-fertility effects, anticonvulsant, antidiabetic and antimicrobial activities. To our knowledge, this is the first work incorporating embelin into polymeric matrices without using diluents. The amount of drug in meshes preparation was selected to ensure the minimum inhibitory concentration of embelin (MIC index, 100 μg ml^{-1}) for both bactericidal and trypanocidal activities.[55,56] Microfibrous embelin-loaded PCL meshes exhibited a significative decrease in drug crystallinity, indicating its good solubilization in the polymeric matrices. The polymer carrier stabilized the amorphous drug dispersion/solution, suppressing or retarding the tendency for the drug to recrystallize for a period long enough to make the system pharmaceutically useful.[57] Although the *in situ* bioavailability of a drug-loaded mesh strongly depends on its size and geometrical parameters, the morphological analyses commonly found in the literature do not take into account the real morphology of the matrices, which is considered in this work. Drug-loaded fibrous membranes showed higher bioavailability of the bioactive agent due to an increase of 86% in the area-to-volume ratio, providing an effective area per unit mass 5.8-fold higher than that found for the corresponding film. Embelin showed an initial burst release, probably the drug dispersed on the surface of the membrane (Fig. 2), followed by a slow release, suggesting a mechanism involving the diffusion of the active agent molecules through the PCL matrix to the aqueous solution. The better capacity of the membranes to expose amorphous embelin make them potential drug-loaded systems with improved efficiency, being the *in vivo* antifungal activity against dermatophytes *(Trichophyton mentagrophytes)* currently under investigation.

Recently, hyperbranched polyglycerol (HPGL) gels gained attention as biomaterials due to their topology,

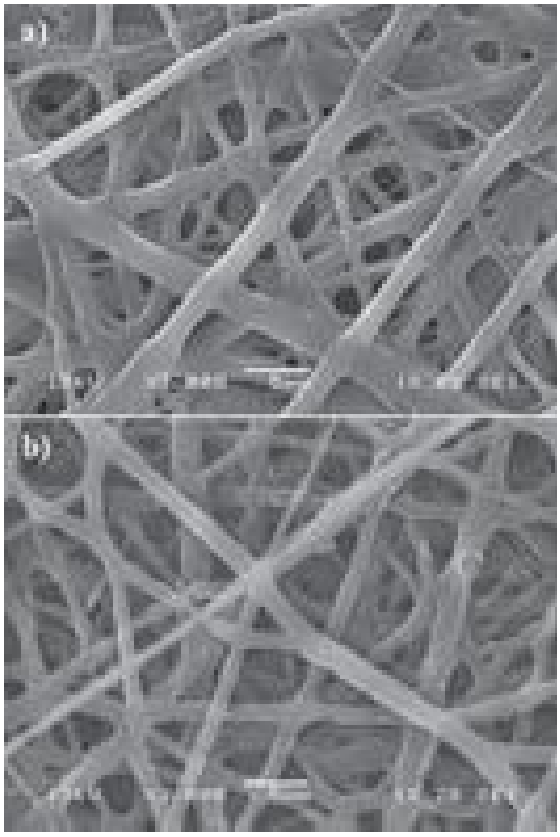

Fig. 2. SEM micrographs of electrospun meshes (×3000), (a) PCL and (b) PCL/Embelin (surface exposed to air). Adapted with permission from [52], P. R. C. Tornello et al., Dispersion and release of embelina from electrospun, biodegradable, polymeric membranes. *Polym. J.* **(2012)**, doi:10.1038/pj.2012.80. © 2012, Nature Publishing Group.

which can lead to molecular capsules and compartments for guest bioactive molecules.[58] Thus, therapeutic agents can be loaded into this polymer to be topically absorbed, providing the desirable therapeutic effects within wound sites. Moreover, HPGL hydrogels can potentially increase both cell attachment and cell growth, due to their swelling ability, non-toxicity and biocompatible properties, acting as possible wound-healing accelerators. Motivated by the development of bioactive wound dressings, Vargas et al. worked on the preparation of electrospun HPGL hydrogel nanofibers containing *Calendula officinalis* (CO), and tested the anti-inflammatory activity for wound-healing applications.[59] A rapid release of CO from the electrospun HPGL nanofibers at physiological temperature was exhibited as result of the high swelling ability of the carrier,[60] as well as the high porosity and surface area of the electrospun HPGL–CO membranes, and probably their erosion within the medium. The CO release process, dependent on the CO content in the HPGL nanofibers, is diffusion-controlled, and the release kinetic agrees with the reservoir-type drug release system. Electrospun HPGL matrix was tough and hard, whereas electrospun HPGL–CO membranes resulted more soft and flexible, presenting higher elongation at break and lower tensile strength values with

increasing concentrations of the bioactive agent, possibly due to its plasticization effects. The electrospun HPGL–CO demonstrated a significantly higher work of adhesion relative to HPGL on biological tissue. The increase in flexibility of the HPGL–CO membranes could have improved the contact between the membrane and the biological tissue, hence promoting the penetration of the hydrated nanofibers into the tissue to form a strong bonding, leading to an increase in the adhesion strength.[61] The loaded electrospun HPGL–CO nanofibers displayed a low inflammatory reaction and fast reepithelialization after implantation in rats. These results of *in vivo* experiments suggest that HPGL–CO membranes might be interesting bioactive wound dressing materials for clinical applications.

Castillo-Ortega et al. also reported the preparation of a fibrous membrane containing a poorly water-soluble drug, obtained by coaxial electrospinning. The fibers were composed of poly(vinyl pyrrolidone) (PVP) as core polymer and cellulose acetate (CA) as shell material.[62] CA nanofibers were used for controlled drug release and tissue engineering applications, while PVP was widely explored for microencapsulation, controlled drug release, and articular cartilage replacement. The CA/PVP/CA membrane was not loaded during the electrospinning process, being the drug incorporated by immersion into an amoxicillin salt solution. The release behavior of amoxicillin resulted dependent on the pH of the medium. The amount of drug released at pH 7.2 was approximately three times higher than that observed at pH 3.0 for any given time. This pH-dependent behavior suggests a higher interaction by hydrogen-bonding at pH 3.0, between the protonated carboxylic groups in amoxicillin and the carbonyl groups present in CA and PVP, resulting a lower drug release. Even more, the equilibrium release of amoxicillin from the fibrous membrane was reached after 2 weeks approximately, regardless of the pH employed. Based on these results, the fibrous CA/PVP/CA membranes would have potential applications in oral administration as well as in controlled-release transdermal patches.

Besides drugs with limited solubility in aqueous media, proteins also have particular delivery requirements. There are many proteins of nutritional and therapeutic relevance, but due to their structural instability once they are extracted from their natural source, new and effective protein delivery systems are needed. Enzymes are proteins responsible in catalysing most of the chemical reactions taking place in the body, that, when affected by several pathologies, can lose its capacity to produce certain enzymes or the enzymes produced lack of catalytic activity. Hence, there is a need for proper and effective enzyme sustained release systems. Electrospun nanofibers are interesting alternatives, due to the generation of nanofibers in one step. Thereby, the exposure of proteins to harsh organic solvents and elevated temperatures can be avoided, reducing the risk of denaturalization. Several substances of biological interest, like plasmids, nerve growth factors and bovine serum albumin,[63–65] and enzymes, such as β-galactosidase, lysozyme and luciferase,[65–68] have been encapsulated and released from nanofibers. Moreno et al. successfully encapsulated the enzyme lactate dehydrogenase (LDH) in poly(vinyl alcohol) (PVA) nanofibers, using coaxial electrospinning.[69] This setup was chosen due to the poor miscibility of the enzyme in the PVA solution. The systems employing the enzyme as core solution displayed entangled fibers with bead defects, while the shell-enzyme membranes showed the presence of numerous agglomerates (Fig. 3). In all cases, the enzyme conserved its catalytic function after processing into nanofibers. The core-enzyme membranes displayed a burst release within the first two days, followed by a sustained release for about three weeks. The hydrophilic nature of PVA produces an immediate swelling of the shell layers, probably generating that both enzyme molecules immobilized in the interior of the nanofibers and the ones present in their surface are liberated to the medium. On the other hand, the shell-enzyme systems displayed a burst release during the first five days, due to the desorption of the enzyme molecules adsorbed at the surface of the nanofibers. After a 10-day release, a strong decrease in enzyme activity was observed. Also, the core-enzyme systems were soaked in methanol to generate a physical crosslinking by hydrogen-bonding among PVA chains, increasing its crystallinity and avoiding the loosening of the fibrous morphology. In this case, there is a steady increment in enzyme release until the fourth week. The crosslinking process increases the water resistant quality of the PVA nanofibers. These crosslinked-polymer membranes work in similar way to reservoir-type release devices, acting as diffusive barriers that regulate the rate of release of the enzyme molecules. This is an interesting high molecular-weight model for protein sustained release systems, and a potential enzyme controlled delivery system in the clinical treatment for the LDH deficiency disease.

The topical administration of nitric oxide (NO) has been presented as a potential treatment for diabetic foot ulcers (DFU) as well as for ulcers generated by the cutaneous leishmaniasis (CL) disease. It is estimated that 1.5 million people suffer from CL annually and more than 350 million are at risk of infection. In Latin America 62,000 new cases are reported every year, being endemic in 18 countries.[70] Ulcers have been treated with meglumine antimoniate (Glucantime) for more than 60 years, showing a cure rate greater than 85%.[71] However, this drug has been associated to several side effects (cardiac abnormalities, increased hepatic aminotransferase, pancreatitis). Additionally, the adherence to the treatment is affected by its duration (several weeks). Thus, new safe therapeutic options are needed. In several *in vitro* and *in vivo* studies, it has been demonstrated that NO plays a key role in the elimination of Leishmania intracellular amastigotes. On the other

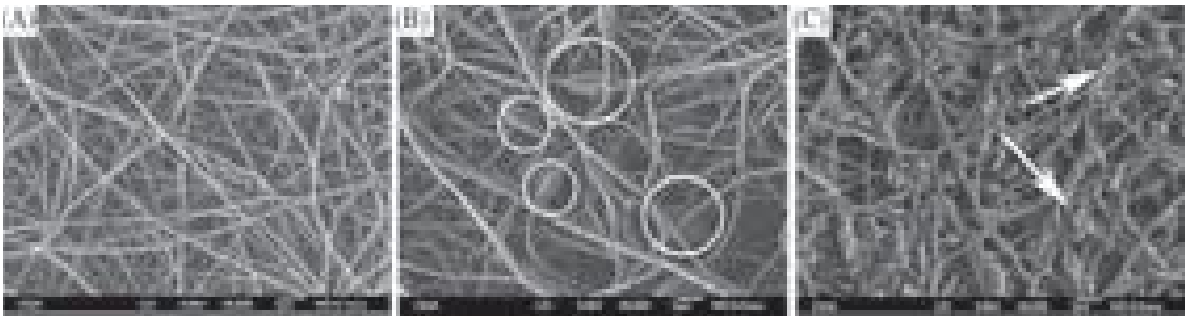

Fig. 3. SEM micrographs of PVA nanofibers (A) without enzyme, (B) employing the enzyme-solution as core-solution, and (C) employing the enzyme-solution as shell-solution. Adapted with permission from [69], I. Moreno, et al., Control release of lactate dehydrogenase encapsulated in poly(vinyl alcohol) nanofibers via electrospinning. *Eur. Polym. J.* 47, 1264 **(2011)**. © 2011, Elsevier.

hand, diabetes mellitus constitutes one of the most important public health problems due to its high prevalence and enormous social and economic consequences. It is believed that there are more than 135 million diabetics worldwide, and this number is expected to increase to 300 million in the next 25 years.[72] DFU, one of the chronic consequences of diabetes mellitus, constitute the most important cause of non-traumatic amputation of inferior limbs. It has been described that the alteration of the immune and wound healing responses in diabetic patients is characterized, among others, by a minor production of NO.[73]

The topical treatments employing NO against CL ulcers in humans showed encouraging results and minimal side effects, but the main problem was the unsustained NO release, requiring multiple applications and hindering the adherence of the patients.[74,75] To overcome this problem, Smith et al. developed a nanofiber NO releasing patch (NOP).[76] This device is a four-layer polymeric transdermal patch (Fig. 4), in which the principal components are stabilized and encapsulated in nanofibers which guarantee a constant NO release upon its hydration. The NO is generated by acidifying nitrite with ascorbic acid. Both components are encapsulated in polyurethane electrospun nanofibers to avoid an instant blast of NO, ensuring a maintained release of 3.5 μmol/cm^2 during 12 h or more depending upon the dosage.

With respect to CL disease, a clinical trial was conducted to determine the effectiveness of NOP respect to Glucantime, with less adverse events and lower cost. The cure rates after a 3-month treatment were 94.8% for the group that received Glucantime compared to 37.1% in the NOP group.[77] Despite the lower efficacy of the patch versus Glucantime, a significantly lower frequency of adverse events and a reduced variation in serum markers were observed in patients treated with the patch. These facts, added to the facility of topic administration justify the development of new systems for the treatment of CL. Thus, studies are currently in place to increase the amount of NO released to the infected area and to mimic the response of innate immune cells to pathogen infection. In addition, as topically administered NO is a potential treatment for chronic ulcers, a clinical trial has been proposed to evaluate the effectiveness and safety of this novel NO release wound dressing for the treatment of DFU.[78]

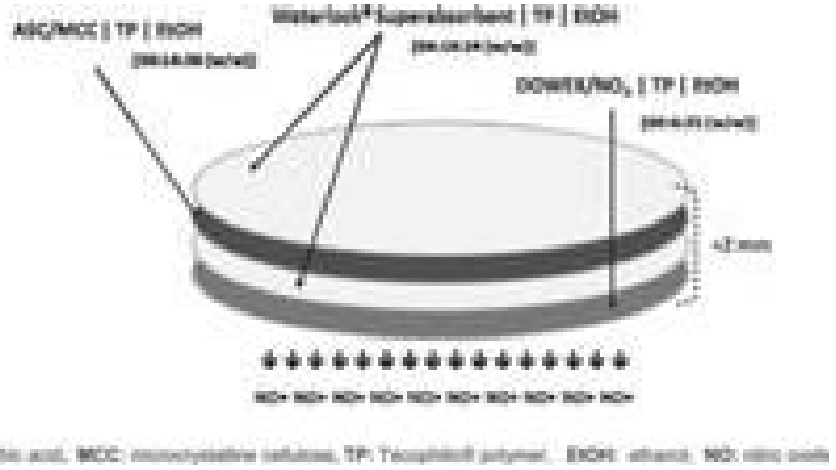

Fig. 4. Electrospun nitric oxide releasing patch (NOP). Cited with permission from [76], D. J. Smith, et al., Topical nitric oxide donor devices, [WO/2006/058318], Ref Type: Patent **(2006)**. © 2006, The American Society of Tropical Medicine and Hygiene.

4. ELECTROSPUN SCAFFOLDS FOR TISSUE ENGINEERING

Millions of surgical procedures, requiring the use of tissue or organ substitutes to repair or replace those damaged or severely affected by a disease, are performed every year. The growth in life expectancy has also become an important factor of this enlarged demand. Although being the traditional approaches, biological implants present several disadvantages. Among others, xenograft implants generate thrombosis and calcification events; allograft transplantation is limited by the number of available donors; and autograft transplantation has restricted applications, generating pain and discomfort. Therefore, efforts in terms of healing and reconstruction of human tissue are being carried in the medical, biological, chemical and engineering fields. Tissue engineering has emerged in the last decades as an alternative multidisciplinary approach to circumvent these limitations.

Tissue engineering has recently been defined as 'the creation (or formation) of new tissue for the therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells through a systematic combination of molecular and mechanical signals.'[79] Although there is no mention of a biomaterial in this definition, in the classical tissue engineering approximation[80] a scaffold biomaterial is usually employed to support cell growth and provide shape to the developing tissue engineered construct, as well as to facilitate the delivery of those molecular and mechanical signals. Current challenges lie in the election/design of natural, synthetic or composite biocompatible and bioresorbable materials to produce scaffolds mimicking the biological functions of natural extracellular matrix (ECM).[81,82] The ECM is a three-dimensional network in which collagen embedded in proteoglycans organizes in nanometer scale multifibrils.[83] The structure and morphology of the ECM also greatly contribute to the properties and functions of each organ. Hence, the development of nanostructured porous scaffolds with interconnected pores, wide pore size distributions, large surface areas and adequate mechanical properties also constitutes a dare. Together with the election of appropriate materials, these parameters affect not only cell survival, signalling, adhesion, proliferation, and reorganization, but also their gene expression and the preservation, or not, of their phenotype,[84] or their differentiation for the case of stem cells. Moreover, the ideal scaffold should also present seeded cells uniformly distributed throughout the entire matrix. Therefore, another challenge to be overcome by tissue engineering is the maintenance of cell viability after implantation. A functional vasculature is fundamental for the delivery of nutrients and removal of metabolites from the cells, otherwise cell survival is not possible. The support of this vasculature is mainly dependent on the surface and pore size characteristics of the scaffold, which control the adhesion and organization of the vascular endothelial cells into blood vessels.[85]

Up to date, a wide variety of materials and processing methods have been proposed for the design of scaffolds to regenerate human tissues. Bone, cartilage, skin, cardiovascular and neural tissues, spinal cord, blood vessels, tendons, ligaments, and organ tissue regeneration show promise for a large portion of individuals with special needs. Electrospinning recently emerged as a promising technology for the generation of non-woven nanofiber-based scaffolds, which not only mimic the nanoscale fibrous structure of natural ECM but also its spatial organization on the mesoscopic scale (control over fiber orientation and spatial placement). Matrices with appropriate fiber diameters, topology, texture, pore size, chemical compatibility and mechanical properties adequate for specific target tissues can be obtained.[86] In this way, electrospun matrices facilitate cell attachment, support cell growth, and regulate cell differentiation,[15–17,87] providing an easy passage for nutrient intake and metabolic waste exchange,[88] which make them potentially applicable as wound dressing, vascular grafts, and tissue engineering scaffolds.[23,89–91]

4.1. Soft Tissue Engineering Applications

Electrospinning has been used for the fabrication of nanofibrous scaffolds from numerous bioresorbable synthetic and natural polymers, blends, and composites.[23] Classical synthetic polyesters can provide the necessary strength for structural stability, and their electrospun scaffolds have been explored for the regeneration of bone tissue,[92,93] musculoskeletal tissue,[94] myocardial tissue grafts,[95,96] and blood vessel substitutes.[97,98] However, they are relatively stiff, non-elastic materials and not ideally suited for engineering of soft flexible tissues such as cardiovascular, urological, or gastrointestinal tissues.

The development of soft-tissue engineering needs bioresorbable materials exhibiting elastomeric properties. Elastomeric polyurethane scaffolds can withstand the action of stress and load and undergo an elastic recovery with little or no hysteresis. In recent years, biocompatible and biodegradable segmented polyurethanes (SPU) have been investigated for applications in the tissue engineering field, such as cardiovascular tissue engineering,[99–101] musculoskeletal applications (anterior cruciate ligament,[102] knee joint meniscus,[103] smooth muscle cell constructs for contractile muscle[104,105]) and nerve regeneration.[106] Their highly variable chemistry allows the preparation of biocompatible materials with controlled physico-chemical, mechanical, and biodegradation properties that can be achieved through the appropriate selection of monomers and the manipulation of hard and soft content. Biodegradation into non-toxic components can be promoted by the use of aliphatic diisocyanates. Bioresorbable polyester soft segments are commonly used to provide hydrolytically

labile soft segments,[107] whereas chain extenders containing easily hydrolyzable linkages increase the SPU degradation rate.[108]

Surprisingly, SPU and poly(urethane urea)s (SPUU) have been infrequently used for the fabrication of electrospun scaffolds. So far, only limited studies on nanofibrous polyurethanes have been reported as tissue engineering scaffolds.[109,110] Prado et al. attempted to produce polyurethane fibers in the nanoscale for potential applications in tissue engineering.[111] Different solvent mixtures and a rotating collector were employed, but beaded nanofibers were obtained at best. The presence of interchain hydrogen bonding is significant enough to affect the viscosity, increasing the cohesiveness of the solution, and hindering or impeding the electrospinning process.[112] Among polyurethanes, SPUU exhibit three-dimentional networks of hydrogen-bonding due to the inter-urea hydrogen bonding.[113]

Our group reported the preparation, characterization and properties of novel electrospun elastomeric polyurethane scaffolds, employing bioresorbable SPU and SPUU, based on PCL, hexamethylene diisocyanate, and novel chain extenders containing urea groups or an aromatic amino acid derivative with ester groups.[114] These polymers were chosen because of their unique composition and mechanical properties,[115] as well as their promising *in vitro* biological properties.[116,117] The optimization of the electrospinning parameters to obtain defect-free SPU and SPUU fibers with controlled diameters was difficult to achieve, due to the strong hydrogen-bonding interactions present in the polymeric structures. Common highly polar organic solvents, such as dimethylformamide (DMF) and dimethylacetamide (DMAc), solubilize SPU allowing the preparation of cast films, but usually fail when employed to electrospin their solutions, generating electrosprayed or bead-on-string systems. Figure 5 shows a bead-free fibrous structure for the SPU containing an aromatic chain extender, obtained from a high-enough concentrated solution to cause chain entanglements with a low-enough viscosity to allow motion induced by the electric field. However, the processing of the SPUU in the same solvent mixture was not possible, obtaining at best beaded fibers. The highly hydrogen-bonded structure inhibits the formation of fibers using such solvents.

1,1,1,3,3,3-hexafluoro-2-propanol (HFP) has been reported to be a good solvent for electrospinning highly hydrogen-bonded macromolecules, such as proteins,[118,119] polyamides,[120] SPU, and SPUU.[121] For this reason, its solutions were employed to process our SPU and SPUU. An effect of the concentration in the morphology was clearly observed. Diluted solutions of the SPUU led to electrospraying instead of electrospinning, due to the lack of sufficient polymer-chain entanglements. As the concentration increased, the solution became viscoelastic, taking a longer time to break up into drops, and obtaining a 'bead-on-string' morphology (Fig. 6(a)). With a further increase in concentration, the critical concentration was reached, and the polymer was able to form a network of entanglements, making the solution electrospinnable into uniform fibers (Fig. 6(b)).

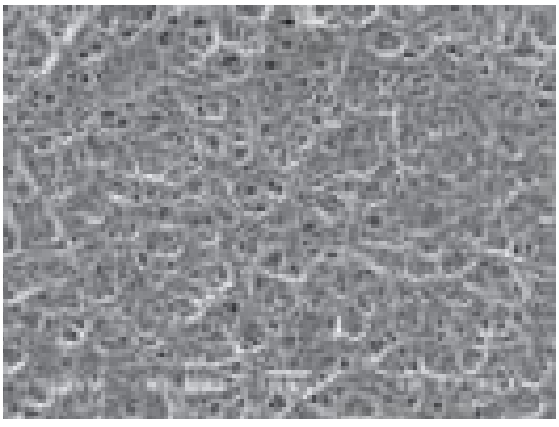

Fig. 5. SEM micrograph of electrospun SPU matrix obtained from DMF/THF (50/50) solution 30 wt%. Adapted with permission from [114], P. C. Caracciolo et al., Electrospinning of novel biodegradable poly(ester urethane)s and poly(ester urethane urea)s for soft tissue engineering applications. *J. Mater. Sci.—Mater. Med.* 20, 2129 **(2009)**. © 2009, Springer.

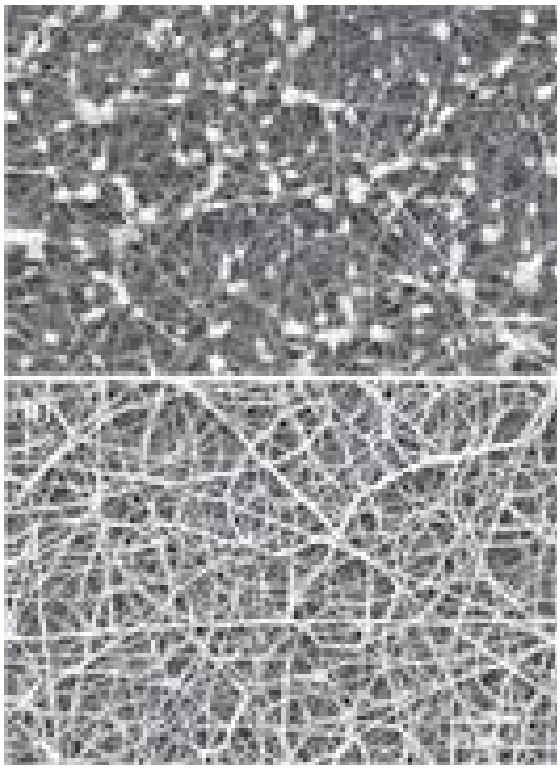

Fig. 6. SEM micrographs of electrospun SPUU matrices obtained from HFP solutions at different concentrations (a) 15 wt%; (b) 20 wt%. Adapted with permission from [114], P. C. Caracciolo et al., Electrospinning of novel biodegradable poly(ester urethane)s and poly(ester urethane urea)s for soft tissue-engineering applications. *J. Mater. Sci.—Mater. Med.* 20, 2129 **(2009)**. © 2009, Springer.

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The degradation properties of the SPU and SPUU electrospun membranes and films were also studied. The fibrous scaffolds exhibited lower mass-loss values and higher hydrolytic stability than the corresponding films after short-time assays, but they also experienced higher mass-loss under accelerated conditions.[122] These results suggest that the degradation rate is not constant, but depends on the chemical structure of the chain extenders, as well as the crystallinity and morphology of the materials.

In particular, SPUU matrices present low-enough hysteresis and elastic modulus to be a candidate for the design of cardiac patches along with the seeding of cardiomyocytes, to restore the functionality of infarcted tissue regions. To improve its performance for use in cardiac and vascular tissue regeneration, SPUU/polydioxanone (PDO) blends were processed into tubular electrospun scaffolds.[123] In order for vascular grafts to succeed, they need to match the biomechanical properties of the natural blood vessels. PDO is a semicrystalline, flexible and biodegradable polymer whose mechanical strength is capable of withstanding pulsatile blood flow. It also presents shape memory, preventing kink formation in vascular structures. The scaffolds showed randomly oriented bead-free fiber meshes with relatively narrow distribution of fiber diameters, comparable to the typical diameters of collagen fibers found in natural arteries (50 nm to 500 nm). The blend-fibers resulted rougher than pure SPUU fibers, which could be attributed to a higher crystallinity for the former. The porosity of the blend was also higher, which favors cell attachment and proliferation. The addition of PDO led to tubular scaffolds with improved mechanical properties respect to the corresponding plain SPUU scaffold, being both comparable to those of elastomeric polymers reported for cardiac tissue engineering.[124]

Our group also reported preliminary results for the development of poly(L-lactic acid) (PLLA) tubular electrospun scaffolds for potential applications as small-diameter vascular grafts in cardiovascular tissue engineering.[125] The matrices presented bead-free nanofibrous morphologies (Fig. 7), with narrow and unimodal diameter distributions independently of the rotation rate. The fiber orientation seemed to be in between a range of values (90–100°), however, the standard deviation resulted high, indicating that no preferential orientation was obtained. In spite of this, orientation of the nanofibers could be achieved for the 4 mm inner diameter graft with a non conductive mandril and electric field modifications. The thermal analysis did not show an influence in the rotation speed neither in the collector used. A decrease in the crystallinity was observed in contrast with the raw polyester. Shorter times for crystallization and spatial restrictions during the electrospinning process are expected to reduce chain mobility and thus, lower crystallization values are obtained. The more amorphous polymer should favor a faster hydrolytic degradation. Further studies regarding the incorporation of a bioresorbable polyurethane into the scaffold to mimic the properties of natural collagen/elastin ECM are being carried out.

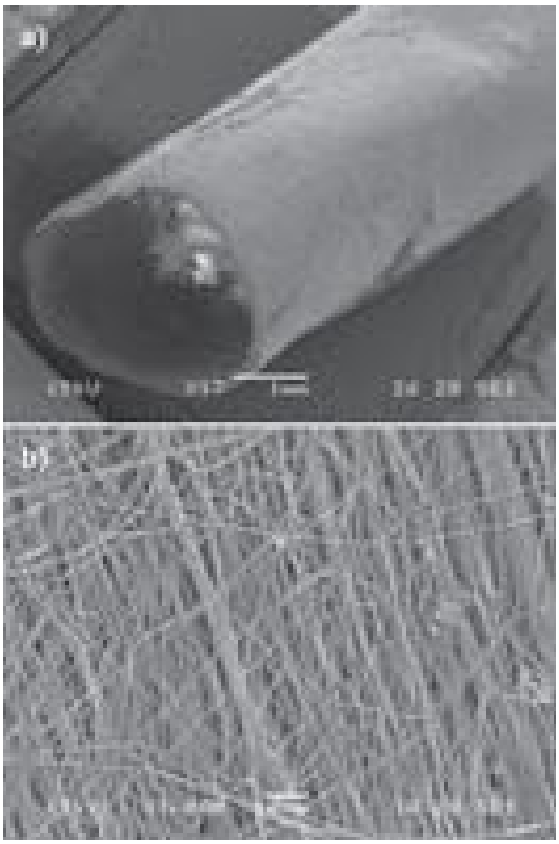

Fig. 7. SEM micrograph of a PLLA nanofibrous vascular graft: (a) tubular structure of 4 mm inner diameter, (b) fiber morphology at the inner surface (manuscript in preparation).

Electrospun PDLLA scaffolds were also extensively reported in the literature. Branciforti et al. studied the influence of the solvent type, solution concentration and processing conditions on the morphology and properties of electrospun PDLLA matrices.[126] Regardless of the solvent mixture employed, all the scaffolds presented low crystallinity values, containing very small and imperfect crystals.

Motivated by the development of cardiac-tissue engineered scaffolds to restore cardiac function after myocardial infarction, Fernandes et al. prepared electrically active electrospun nanofibers based on hyperbranched poly(L-lysine) dendrimer (HPLys) containing polyaniline (PANI).[127] Because of its similarity to biological tissues, elasticity and biocompatible properties, hydrogels have been frequently used in combination with conductive polymers to obtain nanofibers for cardiac tissue engineering by electrospinning. The use of PANI, with tunable electroactive properties, is based on the evidence that cell functions could be modulated through electrical stimulation. Moreover, the biocompatibility of PANI can be improved by the incorporation of adhesive peptides, such

as HPLys.[128] HPLys hydrogels may act as surgical adhesives, via their swelling ability, promoting cardiomyocyte infiltration through their mesh structure. The HPLys-PANI nanofibers displayed a high swelling behavior, which decreases with higher PANI content, due to the formation of a rigid network with higher density of physical crosslinks. The matrices did not induce toxic effects on Chinese hamster ovary cells. Moreover, they promoted proliferation and differentiation of cardiomyocytes cells when exposed to electrical stimulation, increasing viability with the applied voltage. Both electroactivity and biocompatibility of the electrospun HPLys-PANI nanofibers suggest their use for the culture of cardiac cells as well as biocompatible electroactive scaffolds in cardiac tissue engineering, although cardiomyocyte viability assays during scaffold degradation should be performed.

In other work, de Morais et al. obtained electrospun scaffolds incorporating Spirulina (*Arthrospira* LEB 18),[129] a microalgae that exhibits highly favorable biological functions for tissue engineering. Clinical studies suggest that Spirulina contains polysaccharides with antiinflammatory effects and fatty acids with antibacterial and antifungal properties.[130] To obtain well-defined nanofiber matrices incorporating Spirulina, poly(ethylene oxide) (PEO) was used as a well known electrospinnable polymer. Electrospinning was facilitated by the increment in biomass composition, possibly due to the presence of salts in the cultivation medium which increased the conductivity of the solutions. However, the resulting fiber diameter and morphology seemed to be insensitive to conductivity variations. PEO bead-free nanofibrous matrices containing biomass contents up to 67 wt% were produced for subsequent studies in tissue engineering applications. These matrices are suggested as candidates for extracellular scaffolds for stem cell culture and future treatment of spinal cord injury.

Several electrospun matrices have been evaluated with different cell types for potential applications in tissue engineering. This approximation has limitations such as cell mobility after seeding and small pore dimensions to allow cell infiltration. Therefore, Zanatta et al. studied the incorporation of cells into PVA porous scaffolds during the electrospinning process, employing mesenchymal stem cells (MSCs) and mononuclear cells (MNCs).[131] Cell viability after electrospinning was assessed in order to study cell damage due to fiber formation. A reduction of MNCs viability from 80% in the PVA solution control group to 8.4% in the resulting nanofibers was observed, whereas MSCs showed a reduction from 64% to 19.6%. The viability decreased significantly after electrospinning, but the MSCs displayed a higher resistance to the process. The loss of cell viability was attributed to the limited access to nutrients due to the high viscosity of the solution, as well as the electric and mechanical stress during the process.[132] Studies by confocal laser scanning microscopy suggested that cells were not encapsulated in the fibers, probably because of their small diameter. The cells were disposed among the fibers, providing a three-dimensional distribution along the scaffolds (Fig. 8). These results suggest that the incorporation of cells during the electrospinning process is viable, and it can be improved by the adjustment of the solution viscosity.

In other approach, Ramos et al. developed PCL electrospun matrices composed of random fibers with a wide range of diameter sizes (500 nm–5 μm).[133] The large spacing between the fibers resulted in a high porosity, which favors cellular penetration. In this way, the porosity generated proved to be a positive factor for the MSCs penetration, enabling the development of a cell support scaffold suitable for cartilage reconstruction.

Furlan et al. investigated the formation of oriented fibers containing PEO and pectin.[134] These morphologies were achieved by using a non-conventional electrospinning configuration, introducing two parallel electrodes instead of a plate. Pectin was incorporated into fibers as a bioactive, biodegradable and biocompatible heteropolysaccharide, having previously been employed for applications that include controlled drug release and implantable cell carriers. PEO was employed to favor the formation of fibers containing pectin, because of its biocompatibility and good electrospinnability. The incorporation of pectin led to a higher variation of fiber diameters, as well as larger sizes, probably due to cluster formation. The resulting flexible matrices presented a decrease in PEO chain alignment, as determined by FTIR. Nanofibers were also collected forming a perpendicular configuration by rotating the substrate 90°. The deposition of transversal fibers can be adopted to obtain scaffolds with defined shapes for application in tissue engineering.

4.2. Hard Tissue Engineering Applications

Around 200 million people are affected every year by musculoskeletal problems caused by accidents or diseases. The slow process of bone regeneration, added to the need for better filler materials in the reconstruction of large orthopaedic defects, as well as orthopaedic implants more suitable to their biological environment, are the main clinical reasons to develop bone tissue-engineering alternatives.[135] The use of scaffolds together with growth factors plays an important role in bone regeneration. The ideal matrix for such applications should be biodegradable, biocompatible, osteoconductive or osteoinductive, cheap, and with high surface area to volume ratio. A way to obtain a tridimensional scaffold mimicking bone-like properties is by coping its scale and composition.

Human bone is mainly a hydroxyapatite (HA) and type I collagen based composite. HA is the best bioactive calcium phosphate, being used in bone-tissue repairing applications due to its biocompatibility and osteoconductivity.

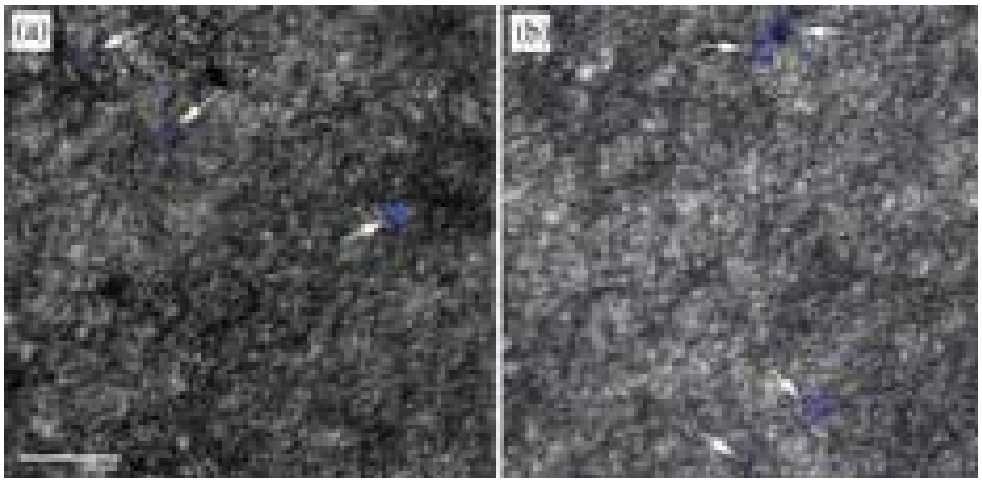

Fig. 8. Images of confocal laser scanning microscopy showing cells among the fibers: (a) Mononuclear cells, (b) mesenchymal stem cells. Arrows indicate cell nuclei. Nuclei stained with DAPI solution. Cited with permission from [131], G. Zanatta et al., Viability of mesenchymal stem cells during electrospinning. *Braz. J. Med. Biol. Res.* 45, 125 (**2012**). © 2012, Brazilian Journal of Medical and Biological Research.

The most important biomedical applications of HA are bone graft substitution in dental and orthopaedic applications and coating of biomedical implants. In recent years, many methods and techniques have been developed and applied to design advanced materials for bone replacement. The incorporation of nanoparticles can lead to scaffolds with improved *in vivo* and *in vitro* mechanical, chemical and biological properties. Thus, polymeric electrospun matrices incorporating HA nanoparticles could achieve the desired bone properties. The adhesion, proliferation and differentiation of osteoblast cells will depend on the structure and composition of the fibers, added to surface and mechanical properties, among others. Moreover, the attachment and migration of these anchorage-dependent cells inside the scaffold are expected to be favored by the high surface area and porosity of the electrospun fibers.

Recently, Rodriguez et al. prepared electrospun fibrous PLLA membranes containing dispersed HA nanoparticles (2–9 wt%).[136] Smooth bead-free fibers were obtained for all cases. The incorporation of HA was evidenced by the shift of the infrared carbonyl band of PLLA due to the interaction by hydrogen-bonding with the hydroxyl groups in HA. Furthermore, the membranes displayed a decrease in glass transition of PLLA, probably due to the enhanced mobility of the polymer chains because of the presence of HA. The mean diameter of the fibers increased considerably with the increment in HA content, generating an increment in pore diameter which could favor cell infiltration (Fig. 9). These scaffolds are promising for bone tissue engineering applications, and studies regarding cell adhesion and proliferation should be performed.

Vázquez-Hernández et al. reported the preliminary results on the preparation of three-dimensional HA/chitosan composite electrospun scaffolds.[137] Uniform fibers could not be obtained probably due to the high HA:chitosan ratios employed. Although none of the samples displayed a Ca/P ratio according to the obtained for HA or native bone, the minerals were identified by X-ray diffraction as HA and monobasic calcium phosphate, which are constituents of human bones. Further studies improving the processing conditions could lead to better results for this system.

Electrospun scaffolds composed of hyperbranched polyglycerol (HPGL) nanofibers containing HA nanoparticles were recently reported.[138] HPGL hydrogels can potentially act as orthopaedic adhesives via their swelling ability, and also encourage osteoblast infiltration and bone formation through their mesh structure. Matrices with different HA content (0.5–5 wt%) consisting of uniform fibers, resulted elastic and flexible. The increasing content of HA reduced the swelling ability of the HPGL fibers, indicating the formation of higher physical intermolecular crosslinking. The *in vitro* evaluation of the scaffolds afforded no cytotoxicity, showing high cell viability. To evaluate the potential use of the HPGL-HA matrices as scaffolds for bone regeneration, the materials were cultured with human sarcoma osteogenic cells ($SaOS_2$), monitoring the alkaline phosphatase (ALP) activity as an indicator of the cellular activity on the scaffold. The ALP activity values resulted high, appearing to promote both a better proliferation and differentiation of $SaSO_2$ than the corresponding film scaffolds. HPLG-HA matrices could be suitable for bone tissue engineering, although studies regarding their physicochemical and mechanical properties are needed to support these results.

In another work, Pantojas et al. prepared three-dimensional electrospun membranes composed of a PLLA/poly(ethylene glycol) (PEG) blend.[139] Although PLLA is one of the most widely used polymers for biomedical applications, it is a stiff and hydrophobic material. Nevertheless, the addition of a small fraction of low molecular weight PEG improved the hydrophilicity of the blend. Meshes presented slightly lower pore sizes than the ones

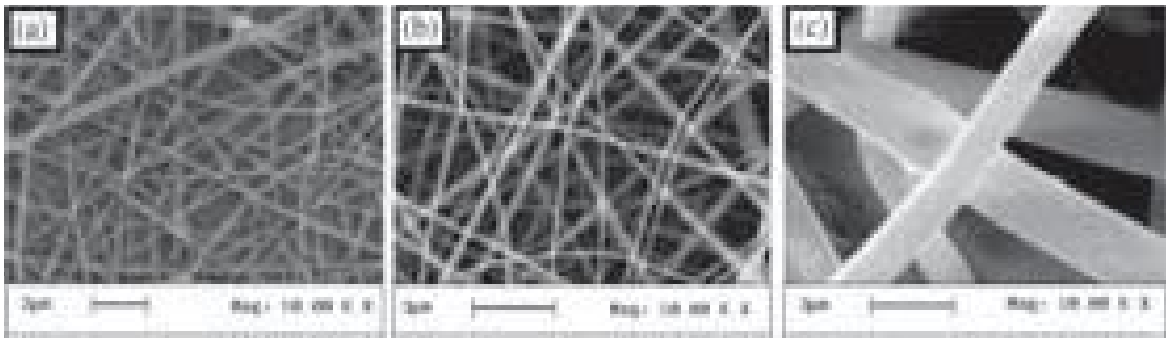

Fig. 9. Nanocomposite matrices containing HA/PLLA: (a) 1.96 wt%, (b) 4.76 wt%, and (c) 9 wt%. Cited with permission from [136], G. N. P. Rodriguez et al., Electrospun Scaffolds composed of poly(L-lactic acid) and hydroxyapatite. *Key Eng. Mater.* 493—494, 872 **(2012)**. © 2012, Trans Tech Publications.

necessary to assess cell infiltration (25 to 100 μm). Fibers with porous surfaces were obtained when electrospinning 9% wt solutions. This morphology could be attributed to a rapid phase separation during electrospinning, where the solvent-rich regions apparently generate porous surfaces after evaporation. Surface pores can modify the wetting properties, increase surface area, influence the kinetics of biodegradation of the scaffold, and improve cell adhesion, acting as anchoring points for cells. Cell adhesion and proliferation studies should be performed to assess the effect of this surface modification.

The use of polymeric matrices loaded with silica, alumina or carbon nanotubes (CNT) is another approach to obtain composite materials for bone tissue engineering.[140,141] These fillers have led to composite systems with significantly improved mechanical properties. Particularly, CNT rank among the highest modulus and strongest fibers known, and their structure allows surface functionalization for matrix-compatibility improvement or the introduction of new functional properties. The group of Volpato et al. reported the preparation of electrospun scaffolds composed of aligned fibers of polyamide 6 (PA 6) and carboxyl-functionalized multi-walled carbon nanotubes (MWCNT) for biomedical applications.[142] Since CNT biocompatibility is still under investigation with controversial results, a non-resorbable polymer was employed to avoid a potentially harmful CNT release.[143–145] The increased electrical conductivity of PA 6/MWCNT solutions respect to PA 6 solutions caused higher stretching and thus smaller fiber diameters, but also affected negatively the fiber alignment. The composite membranes contained the dispersed fillers aligned within the nanofiber axes, which allowed specific morphological and mechanical properties of the networks. The presence and alignment of the MWCNT, added to solvent evaporation and fiber stretching, led to the formation of superficial roughness and defects that could improve cell adhesion.

As expected by fiber alignment, anisotropic mechanical properties were found for the matrices. Only few works have reported a study of the mechanical behavior of CNT-reinforced electrospun membranes,[146,147] finding an enhancement in the mechanical properties by the addition of CNT. However, the stiffness, ultimate tensile stress and strain decreased for these MWCNT-based networks. This decrease was attributed to the different net architecture, which displayed lower fiber alignment, and higher void and defect content. Consequently, these matrices could potentially be use for applications where low mechani-

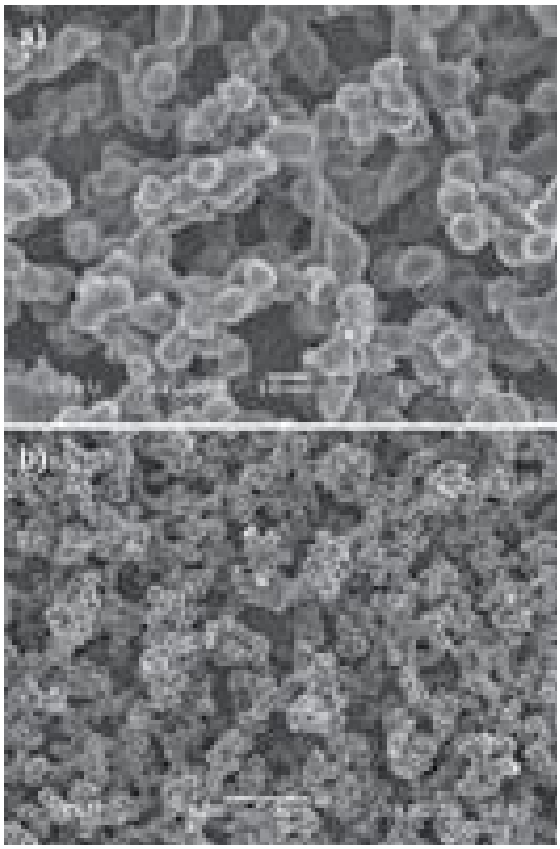

Fig. 10. SEM micrographs showing the surface morphology of PCL/PDIPF glass substrates covered with multilayers of microparticles formed by electrospraying at (a) 1000× and (b) 400× magnification. Adapted with permission from [151], J. M. Fernandez et al., Osteoblast behavior on novel porous polymeric scaffolds. *J. Biomater. Tissue Eng.* 1, 1 **(2011)**. © 2011, American Scientific Publishers.

cal support is required, such as vertebra and spongy bone scaffolds.

In order to assess the effect of CNT-filled scaffolds on cell response, membranes were cultivated with MG63 cell line of osteoblasts. Cell activation and proliferation were enhanced by the surface modification caused by the addition of MWCNT. A significant increase in the ALP activity on the composite network was also observed, which means that cells are able to differentiate and produce an extracellular matrix. It is suggested that the surface roughness of the composite fibers governed the observed cell response due to protein interaction with the nanoscale patterning.[148, 149] Although longer term biological tests need to be performed regarding the CNT biological behaviour, these networks have encouraging profiles for use in biomedical applications.

Electrospraying of polymer solutions also enables the possibility of producing porous scaffolds to meet current challenges in tissue engineering applications, drug delivery systems and surface modification of medical implants. Although the process is affected by several variables, as for electrospinning, the breaking of the jet into droplets is a consequence of a lower concentration of the polymer solution.[43, 150] Our group has made a contribution in this field, preparing highly porous three-dimensional scaffolds obtained by electrosprayed microparticle deposition (Fig. 10).[151] In a previous work, films prepared from poly(ε-caprolactone)/poly(diisopropyl fumarate) proved to support adhesion, proliferation and differentiation of UMR106 and MC3T3E1 osteoblast-like cell lines without evidence of cytotoxicity.[152] Fernandez et al. employed this blend to obtain random polymer-microparticle matrices as better osteophilic environments for the growth and differentiation of osteoblasts. *In vitro* biological studies were carried out by culturing UMR106 and MC3T3E1 on the matrices. Electrosprayed scaffolds presented higher cell adhesion and proliferation, as well as higher ALP activity and type-I collagen production[153] than flat films of the same blend. The electrosprayed morphology improved cell incorporation into the scaffold (Fig. 11), being this fact crucial because cell infiltration is difficult to achieve in other porous membranes.[154] Despite the general finding that hydrophobic materials do not support adhesion and spreading of cells,[155] the rougher and more hydrophobic surfaces of the matrices enhanced osteoblast response as has also been reported in literature less frequently,[156] being this a controversial discussion.

5. SENSORS BASED ON NANOFIBROUS STRUCTURES

Typical polymeric sensors exist in the form of thin films for sensing a wide variety of substances in gas and fluid states. They have been extensively used for environment protection, industrial-process control, safety, security, defense and medical diagnosis applications. Common requirements for a good sensor are small dimensions, low fabrication cost, fast response, easy operability, high sensitivity, selectivity and reliability.[157] To achieve a higher sensitivity and a faster response, an increase in specific surface area of the sensor is needed. Therefore, the use of electrospun nanofiber-based sensing materials is promising to obtain high-performing gas and fluid sensors. The development of sensors based on nanofibrous structures is the less explored area by Latin American research groups.

Several methods have been employed to obtain nanofibers with a sensing capacity, such as the use of a sensing polymer, the incorporation of sensing molecules during the electrospinning process, or after fiber formation via the coating/grafting technique. Humidity sensors based on polymeric layers enjoy a fast response time for

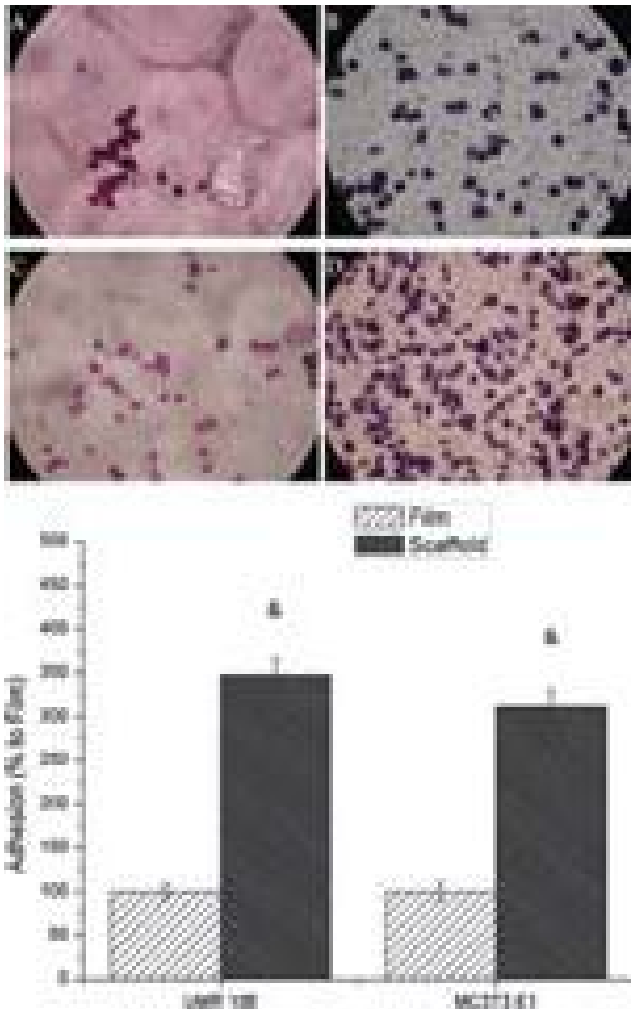

Fig. 11. Adhesion of UMR106 (A), (B) and MC3T3E1 (C), (D) osteoblasts plateled over the solvent-cast films (A), (C) and the electrosprayed scaffold (B), (D). Cells were incubated for 1 h and stained with Giemsa. The cells in ten fields per samples were counted and expressed as percentage of the cells in the cast film. Cited with permission from [151], J. M. Fernandez et al., Osteoblast behavior on novel porous polymeric scaffolds. *J. Biomater. Tissue Eng.* 1, 1 **(2011)**. © 2011, American Scientific Publishers.

increasing humidity changes. However, the recovery time when the humidity decreases is longer than the response time, reducing drastically the overall dynamic performance. Poly(vynilidene fluoride) (PVdF) presents excellent resistance to heat, aging and abrasion, non toxicity, and unique electrical properties. It is generally used in structural health, monitoring systems as pressure and volume displacement sensors. Its exceptional chemical stability and mechanical flexibility at physiological temperature ($T_g \approx -35$ °C), makes it easy to conform to complex surfaces. Moreover, its biocompatibility makes PVdF a desirable candidate for biological environments.[158] Besides its sensing capability, PVdF is also applied in actuation mechanisms, which can be thermally, optically or electrically stimulated.

The group of Corres et al. developed a new optical fiber humidity sensor composed of a Multimode–Hollowcore–Multimode (MHM) structure coated with an electrospun nanoweb.[159] The MHM structure consists of one short silica hollow-core fiber segment spliced between two standard multimode fibers, where a PVdF nanoweb is deposited. The piezoelectric nature of PVdF has been employed for the development of gas and humidity film sensors. The low humidity permeability and the high humidity sensitivity of PVdF thin films are improved when employing an electrospun membrane with high surface to volume ratio and highly porous structure, generating a sensor that combines high sensitivity and reproducibility with a faster response to relative humidity changes. When exposed to human breathing, a repetitive response in the range from 50 to 70% of relative humidity with a rise time of 100 ms was found. This response is fast enough for the sensor to be used in medical applications, which require high dynamic performances, especially in the ranges in which human breathing has to be monitored. This sensor has a reduction in the response time (from 300 ms to 100 ms) compared to others based on ESA polymeric nano-film (PDDA/Poly-R478), and a comparable performance respect to superhydrophobic devices.[160]

González-Morán et al. also electrospun PVdF in order to develop a temperature sensor for biomedical applications.[161] This sensor was obtained by positioning the membrane on a couple of plates with cooper rings to ensure an electrical contact. The heat-sensing abilily is due to detection of changes on the membrane dielectric constant, which are then converted to frequency changes. These signals are transmitted to a receiver by means of frequency modulation, and make a telemetry temperature-sensor system. This sensor has several advantages for biomedical applications, as it is non invasive due to its pyroelectrical detection, there are no chemical reactions with the body or possible current discharges because it is completely isolated, and it does not require external electrical supply due its pyroelectricity. This cheap sensor displays a linear response in the range of 25–80 °C. It can be obtained in any shape and size, being appropriate for monitoring different regions of adult human beings (37 °C), neonatals (36.5 °C to 37.5 °C) and biological environments.

6. OTHER BIOMEDICAL APPLICATIONS

6.1. Conducting Nanofibers

The development of ultrathin fibers from electroactive polymers has recently received much attention due to their useful properties with several potential applications such as electronic devices, optics and biomedical materials, protective clothing, filtration media, charge storage devices, sensors and actuators. In this context, PANI has been one of the most investigated conducting polymers due to its simple and relatively easy doping/dedoping chemistry, high environmental stability, and low cost of synthesis.[162] Multi-component polymer fibers have also been obtained by electrospinning. The blending of PLLA and its copolymers with other polymers has generated great interest and is considered one of the most innovative materials developed. Recently, PLLA/PANI blends have been used to prepare conducting nanofibers as promising materials for sensors and other electroactive applications.

Picciani et al. studied the structure and properties of electrospun conductive micro/nanofibers of PLLA/PANI blends and cast films with the same composition.[163] The electrical resistivity values for electrospun fibers were found to be about two orders of magnitude higher than those for the corresponding cast films. In addition, electrospun fibers presented a mechanical behavior more appropriate for their use in the preparation of electronic devices. The stress–strain behavior showed that PLLA/PANI electrospun matrices are stiffer than those without PANI. The group of Picciani et al. also electrospun PLLA/PANI blends doped with *p*-toluene sulfonic acid (TSA),[164] obtaining ultrafine fibers with diameters in the order of 100–200 nm and a significant reduction of bead formation. The fibers resulted homogeneous, indicating a good interaction between the components of the blend. The electrical conductivity of the electrospun matrices was lower than that for blend films produced by casting, probably because of the lower degree of crystallinity of PANI dispersions and the high porosity of the non-woven matrices. The presence of PANI in the blends significantly decreased bead formation because of changes in the dielectric constant, which was associated with an increase in the solution viscosity and charge density. The crystallinity of these fibers was lower than that for the corresponding blends prepared by film casting, and this could be attributed to the rapid evaporation of the solvent during the electrospinning process, which resulted in an amorphous structure.[165]

The influence of the electrospinning parameters on polyurethane and conductive composite nanofibers was studied by Ballarin.[166] Single wall carbon nanotubes (SWCNT) and PANI nanoparticles were used to prepare

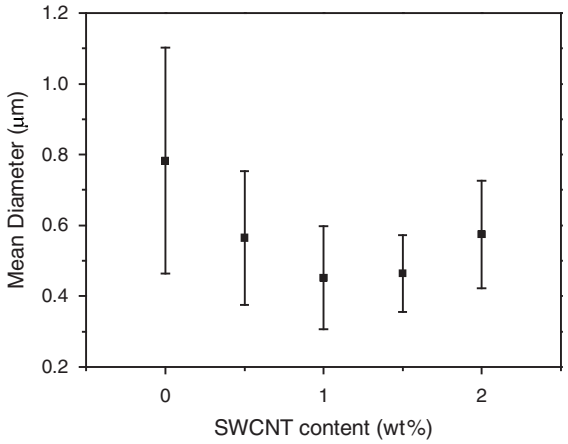

Fig. 12. Mean diameter as a funcion of SWCNT content in electrospun polyurethane nanocomposites. Cited with permission from [166], F. M. Ballarin, Engineering final project: Nanofibras Poliuretánicas y Compuestas de Interés Biomédico, Universidad Nacional de Mar del Plata, Argentina **(2009)**. © 2009, Universidad Nacional de Mar del Plata, Argentina.

polyurethane nanocomposites. A biostable poly(ether urethane) was employed in this case, since the biocompatibility of the fillers is still under discussion. The effect of the solution intrinsic properties and processing parameters in fiber diameters were studied. As in the work of Picciani et al.[164] fiber diameters were reduced by the increase in the conductive nanoparticle concentration. Figure 12 shows the variation of mean diameters as a funcion of SWCNT content in polyurethane nanocomposites. A minimun in diameter was observed at 1 wt% nanotube concentration.[166] Conductivity values were in agreement with the change in fiber diameters, being these materials interesting candidates for biomedical applications.[167]

6.2. Nanofibers with Antimicrobial Properties

Martínez-Camacho et al. reported the preparation and properties of chitosan nanofibers, as well as the theoretical basis for understanding how the antimicrobial activity of chitosan could occur in the form of polymer nanofibers.[168] In most cases, the proposed mechanism is related either to the presence of positively charged amino groups at the surface of the nanofibers,[169] or is associated to the release of small chitosan oligomers that could penetrate bacterial cells and interact with DNA. Chitosan–PVA nanofibers showed a high antimicrobial activity against *Escherichia coli* and *Staphilococcus aureus* for large diameters, possibly due to a more effective contact surface area and, consequently, to a greater presence of amino groups to carry out the inhibition of microbial growth.[170] In other approach, Hidalgo et al. prepared electrospun PLLA scaffolds coated with chitosan by impregnation of the mats with chitosan solutions.[171]

Antimicrobial materials can be chemically engineered by adding functional antimicrobial agents onto their surface or within the matrix to either eliminate or inhibit the growth of microorganisms. Silver (Ag) is a powerful antimicrobial agent that inactivates several microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. There are few works employing Ag as antimicrobial agent in PVA fibers prepared by electrospinning. In these works, either heat treatments, UV radiation or chemical reduction were employed to obtain Ag nanoparticles. However, it was demonstrated that it is possible to obtain PVA nanofibers containing Ag nanoparticles by electrospinning aqueous PVA solutions with $AgNO_3$ in a nitric acid medium (pH = 2–4), without any further processing.[172] Despite the fact that most of the produced nanoparticles should be occluded into the fibers, the composite matrices showed good values of antibacterial activities against *Staphylococcus aureus* (87.8%) and *Escherichia coli* (85.0%).

Novel hybrid fibrous membranes were developed by electrospinning blend solutions of poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) and chitosan.[173] The effect of solvent, employing different proportions of trifluoracetic acid and HFP, PHBV/chitosan ratio, and chitosan molecular weight on solution spinnability, fiber morphology and size, and *in vitro* degradation were investigated. Continuous nanofiber structures were obtained. In general, mean fiber diameter increased as chitosan content and molecular weight increased, and decreased with the decrease of HFP content in the solvent. *In vitro* dissolution tests revealed that the hybrid fibers show much higher degradation rates than the neat PHBV.

6.3. Morphology and Adhesion Measurements

The fiber diameter, orientation and membrane porosity strongly influence cellular response, drug release, mechanical and adhesion properties. For this reason it is important to evaluate and quantify the morphology of the electrospun membranes in an automatic manner.

Gonzalez et al. developed an image processing technique to characterize grey level SEM micrographs.[174] The granulometric size function (SGF) was used to develop algorithms that allow the characterization of shape, size, stocking density and orientation of the electrospun fibers present in SEM images. Different morphologies were obtained by varying the electrospinning setup, the intrinsic properties of the polymer solution and processing parameters. 164 sample images belonging to 42 electrospun scaffolds of PLLA and PCL were processed. Different structural elements were used to scan the images and study diverse aspects of the membrane morphology. The occupancy rate, orientation and diameter of the nanofibers were properly estimated by the proposed algorithm. One of the main advantages of this algorithm is that it is not necessary to binarize images. Besides, the SGF also provides features on nanofiber statistics.

Topology of electrospun scaffolds is other important feature to analyze in electrospun membranes. Nanofibers

present a biomimetic fibrillar structure. It was probed that the hierarchical fibrillar structures of natural creatures produce dry adhesion by van der Waals (vdW) forces.[175,176] For this reason, it is of interest to measure the adhesion properties of electrospun fibers. Our group studied the adhesion between PCL nanofibrous membranes by T-peel test for the first time.[177] The influence of fiber orientation on the adhesion properties was studied. Aligned fibers presented higher adhesion strength (758.7 ± 211.7 kPa) than the randomly oriented non-wovens (613.1 ± 79.9 kPa). These energy values probed that the surface asperities present in electrospun membranes contribute to enhanced adhesion. Even more, a well-aligned structure enhanced adhesion by introducing more contact points and a band-like morphology.[178] Adhesion strength changed marginally with peeling rates and conditioning pressures on the membranes. The morphology of the electrospun membranes after performing the peel test was studied. Neither plastic deformation nor interlocking was observed, demonstrating that the adhesion between the nanofibers was a result of interactions in the nanometer range. Finally, a single contact adhesion energy value (83.1 ± 32.5 mJ $\cdot$ m^2) was calculated by Johnson Kendall and Roberts (JKR) contact mechanics. This value was consistent with vdW adhesion forces, suggesting that vdW was the primary adhesion mechanism. Overall, this work demonstrated the increase in vdW adhesion of electrospun membranes by a change in fiber orientation.

7. CONCLUDING REMARKS

Nanofibrous scaffolds manufactured by electrospinning offer many advantages for the design of highly porous biomaterials useful in tissue engineering applications and drug delivery systems, among other emerging fields. In the last few years, this technique has become widely accepted in academia and industry. Despite this, research on electrospinning for biomedical applications is still at an early stage of development in Latin America, as demonstrated by the number of published works. Brazil, Argentina and Mexico are currently the leading Latin American countries in this field, followed by Colombia and Puerto Rico. Most of these research teams work in collaboration with groups having large expertise in the field. Main efforts are addressed to the development of novel electrospun biomaterials, drug-loaded systems, electrospun nanocomposites, characterization tools, biomedical textiles with functional properties and novel applications of nanofibrous non-woven mats.

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