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Harnessing the Potential of Fibrous Polyester Composites Meant for Bioactive Medical Devices

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Abstract. Fibrous-based composite polyester mats have gained importance in the biomedical area due to their morphological characteristics and mechanical performance. These materials can be modified by incorporating different inorganic micro- or nanoparticles, thus leading to promising potential applications as medical devices with antimicrobial and/or bioactive properties. In this regard, this chapter reports a compilation of different studies related to the development of sub-micron fibers based on two important polyesters for biomedical use, namely poly(3-hydroxybutyrate) and poly(lactic acid). Studies focus on fibers developed through hydrodynamic techniques, namely electrospinning, electrospraying, and centrifugal spinning. The incorporation of zinc oxide or hydroxyapatite on the polyester-based fibers results in materials that inhibit bacterial growth or induce bone cell proliferation. Studies have reported on the effect of the fabrication method as well as the concentration of nanoparticles on the antimicrobial effectiveness against bacteria such as *Escherichia coli* and *Staphylococcus aureus*. Particularly, the development of polyesters and their corresponding composites have emerged using a novel and scalable technique named Forcespinning®. These recent studies report on the influence of morphology and chemical nature on cell adhesion and proliferation and more notable on the ability to mass-produce fibrous-based polyester composite systems with practical applications.

Keywords: Electrospinning · electrospraying · forcespinning · polyesters · bioactive composites

1 Fibrous scaffolds for biomedical applications

During the past decades, scientific advances in molecular and cellular biology, as well as a progressive understanding of tissue regeneration and wound healing processes have stimulated the development of a novel and multidisciplinary field of research known as *Tissue Engineering*. This research field applies engineering and life science to design *biodegradable and bioactive materials* with specific structural properties that allow for the simulation of the extracellular matrix (ECM), with the purpose of promoting its regeneration or function [1, 2]. Most often, the ultimate goal is implantation in the body to repair an injury or replace the function of a damaged organ. Critical functions can be structural (bone, cartilage), supportive, transport (skin, blood vessels), secretory, and biochemical (liver and pancreas). The concept of *scaffold* in tissue engineering is to mimic the functions of native ECM [3]. Therefore, the properties of engineered scaffolds are related to the ECM functions in native tissues and are connected with their structural, biological, and mechanical features [4].

The morphological features associated with *fibrous 3D materials* closely mimic the natural microenvironment constituted of fibrous proteins present in native ECM [5]. In combination with their porous nature and high surface area to volume ratio, these qualities have made micro-/nano-fibrous scaffolds attractive not only from the academic point of view but also in terms of applied science to be used in tissue engineering applications. In this sense, they have been used as supports for drug delivering agents, dressings for wound healing, and porous scaffolds for the regeneration of tissues, including skin, blood vessels, nerves, tendons, bone, and cartilage [6-8]. In the field of bone tissue regeneration, efforts are being made to address diseases, such as osteogenesis imperfecta, osteoarthritis, osteomyelitis, osteoporosis, traumatic injuries, and orthopedic surgeries (i.e., total joint arthroplasty, arthrodesis of the spine, fixation of implants, etc.). These efforts have produced a remarkable clinical and economic impact in the treatment of bone defects. For this reason, the demand for bone implants has been increasing exponentially, until it has become the second tissue with the highest requirement for implants [9, 10].

1.1 Polymer matrices

A polymer matrix for medical applications has to be categorized as a biomaterial, *i.e.* a material able to interact with a biological system to perform a specific action of regeneration or replacement of a tissue, organ, or body function [11]. A biomaterial has to be biocompatible, namely, it must have the ability to develop, with an appropriate host, a specific response without causing a cytotoxic effect. There are a wide variety of factors that affect the tissue response in contact with an implant and are mostly related to its biological and physicochemical properties, as well as to its shape and structure [3].

Some of the most important properties that should define a biodegradable material are listed below [12]:

- Prevent a sustained inflammatory or toxic response after implantation in the body.
- Possess an acceptable duration of action, which should coincide with the healing or regeneration process.
- Possess mechanical properties appropriate for the indicated application, where the variation of mechanical properties with degradation should be compatible with the healing or regeneration process.
- Provide non-toxic by-products and those should be capable of being metabolized and eliminated by the body.
- Present feasible processability for the intended application.

Polymeric biomaterials are currently used for biomedical applications as their mechanical and degradation properties are suitable for mimicking various tissues. Especially, scaffolds based on polyesters (poly(ϵ -caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PLG), poly(lactic-*co*-glycolic acid), and poly(hydroxyalcanoate)s (PHAs) among others) have gained increasing attention given their biodegradability, biocompatibility, and ease of processing [13].

1.2 Production techniques for fibrous scaffolds

1.2.1 Fiber production techniques

Several methods have already been applied for generating micro-/nano-fiber, such as melt-blown [14], three-dimensional (3D) printing [15, 16], phase separation [17], template synthesis [1], and self-assembly [2, 18]. However, the selection of materials that can be produced by these methods is restricted, limiting their practical application [19]. On the contrary, electrospinning [20-22] is an extensively studied and widely

applied method for nanofiber production from a remarkable range of organic and inorganic materials. Compared with traditional nanofiber preparation technologies, electrospinning can produce fibers with high specific surface areas, uniform pore sizes, and high porosity, which significantly improves the performance of nanofibers. In terms of adaptability, the versatility of the electrospinning technique has allowed for producing a vast range of materials. Moreover, it enables the fiber diameter to be adjusted from nano- to micrometers [23].

In general, spinning technologies for the production of fibers have been based on an extrusion process, which allows for the continuous production of materials with one or more filaments. The resulting product exhibits different properties depending on the used process and selected parameters [24]. Polymeric fibers can be obtained from melts, solutions, and emulsions. The polymer melt process offers unique advantages as it avoids the use of solvents and possesses economic advantages given the lack of solvent recovery steps. On the other hand, using solvent-based methods possesses the advantage of increasing the selection of materials to be used while also offering lower energy consumption and the potential to develop smaller fiber diameters. Emulsion spinning is a method that is selected to produce multiphase polymer fibers with different morphologies, active component encapsulations, etc [25, 26].

Electrospinning typically consists of a syringe and needle through which a polymer solution is passed, and the flow is controlled by a metering pump. A power source applies several tens of kilovolts on the needle, and therefore on the polymer solution. The high applied voltage causes a significant density of charges on the protruding polymer droplet (Taylor cone) at the needle tip, to a point where the repulsive forces of the charges overcome the surface tension and a jet of polymer is generated that goes to the grounded collector, the solvent is evaporated during the path from the needle tip to the collector. The final morphology of the materials depends not only on polymer molar mass, viscoelasticity, electrical conductivity, and surface tension [27], but also on the voltage applied during electrospinning, the distance from the tip to the collector [28], and the feeding speed [29]. By adjusting these parameters and determining the appropriate values, electrospun micro/nanofibers with desirable morphologies can be obtained.

On the other hand, electrospinning and electrospraying are two electrohydrodynamic processes in which a suspension or solution can be spun or sprayed upon applying a high direct current voltage to generate fibers or particles, respectively. However, when

nanostructures have to be incorporated into fibrous materials, it is difficult to uniformly disperse and distribute fillers within the electrospun fibers. In this regard, electrospraying is a flexible and effective method for fabricating nano-/micro-structures composites by spraying a solution in the form of droplets or beads. The simultaneous electrospinning and electrospraying technique is a combined strategy that has typically been used in which fibers are formed by electrospinning, while nanoparticles are deposited on the fiber surfaces through electrospraying.

Despite the versatility and effectiveness of electrospinning, there are still some challenges that need to be addressed, including the accurate and reproducible control of fiber morphology, structure, and uniformity as well as the slow collection speed, the need for a high voltage electric field, the sensitivity in the conductivity of the solution, and the difficulty in manufacturing three-dimensional (3D) structures. There are some strategies that allow for expanding the versatility of electrospinning [30, 31]; however, there is still a need for techniques allowing for easy and fast generation of sub-micron polymeric fibers [32].

A new process called Forcespinning® has been developed to obtain nanofibers from a wide variety of polymers, at a high production speed. This method uses centrifugal force, as opposed to the electrostatic force used in the electrospinning technique. Furthermore, through Forcespinning®, the fiber can be obtained from a polymer solution or from a molten polymer [33]. Conductivity and/or electrostatic charges are not relevant parameters for the properties of the precursor solution; therefore, the spectrum of materials that can be spun is broader.[34] One of the important features of the centrifugal spinning process is its high production speed. The average production rate is 50 g/h and meters per minute on industrial scale systems [34, 35], which is at least two orders of magnitude higher than typical laboratory-scale electrospinning processes. This advantage makes this centrifugal spinning system a low-cost, large-scale approach to deliver practical applications of fiber-based systems [36].

During fiber formation, the polymeric fluid is placed in the spinneret which contains multiple nozzles around the side walls. When the rotation speed reaches a critical value, the centrifugal force overcomes the surface tension of the polymer fluid (polymer solution or molten polymer), causing fine streams of polymer fluid from the tips of the spin head nozzles. The centrifugal force together with the frictional force of the air elongate the liquid thread and lead to the nanofiber formation. The material is deposited on the surface of a collector, forming a non-woven mesh of nanofibers. The polymer concentration and the associated viscosity of the precursory solution, temperature, the

rotational speed of the spinneret and the collection distance are parameters influencing the morphology of centrifugally spun fibers.

1.2.2 Design consideration of fibrous scaffolds

For a 3D scaffold construction, the mass transport is an important factor to consider. Oxygen and nutrient delivery, waste removal, cell migration and penetration are some of the processes involved in a natural ECM environment. These processes are associated with the morphological architecture of the scaffold, which is mainly characterized by its pore size, total porosity, pore interconnectivity and surface area [37]. Pore size controls the tissue in-growth and the internal surface area available for cell attachment. If pores are too small for the cell type of interest, they could be blocked by cells preventing cellular penetration, extracellular matrix production and vascularization into the internal region of the scaffold, where diffusion limitations have been one of the mayor obstacles in the production of 3D engineered tissues [38]. There is controversial information about the optimal pore size needed for cell growth and tissue formation. It has been reported optimal sizes between 5-15 μm for fibroblast, 20-125 μm for keratinocytes and, 200-400 μm for osteoblasts [39-41]. Additionally, highly porous structures fully interconnected with a large surface-to-area volume ratio are required to allow for cell ingrowth and facilitate neovascularization. An adequate diffusion and enough surface area for cell-polymer matrix interactions have been found in porosity ratios higher than 90%. However, high porosity has a negative effect on the mechanical strength of a scaffold [42, 43]. Therefore, the scaffold design should be optimized to get the maximum total porosity with appropriate mechanical needs based on the particular application.

Moreover, fiber diameter also plays an important role in cell behavior. Nanofiber scaffolds have resulted suitable for enhancing cell differentiation, adhesion, and proliferation [44]. However, the small pore sizes, associated with nanoscale fibrous scaffold, limit cell migration, thus producing a cell monolayer on the scaffold surface. On the other hand, microfiber scaffolds could be potentially advantageous due to their large pore sizes, enabling cellular infiltration and nutrient diffusion. These results suggest that a multiscale scaffold should be ideal to promote an accurate environment for tissue regeneration. Pham *et al.* [45] developed a bimodal scaffold using a sequential electrospinning technique producing multiscale layers of different morphologies. They

evaluated the fiber diameter effect on the cell morphology, observing a larger number of cells (mesenchymal cells) spread throughout the nanofiber membrane. Additionally, the authors obtained a significant reduction in infiltration distance of cells when the nanofiber layer increased compared with other systems. Shalumon *et al.* [46] produced a 3D PLA multiscale fibrous scaffold using a layer by layer method combining nano- and micro-fibers in an optimum ratio. They found cell (MG63) penetration through the macropores and a well spread by taking advantage of available nanofibers within them. Deepthi *et al.* [47] designed an electrospun multiscale fibrous scaffold combining aligned and non-aligned microfibers. They used a dual electrospinning setup producing random PCL nanofibers and aligned microfibers at the same time. Their results showed improvement in cell attachment (Rabbit ligament fibroblast) and infiltration within the random microfiber systems.

In the case of composite fibrous materials, the concentration and morphology of micro- or nanoparticles incorporated to the polymeric matrix have to be considered as main factors affecting the morphology and final performance of the obtained composites, which will be addressed in the following sections.

2 Composite Fibrous scaffolds for biomedical applications

2.1. Tailoring the antimicrobial properties of fibrous polyester composites

Materials based on polymer fibers have suitable morphological characteristics that make them attractive for applications in the area of tissue engineering since they promote cell adhesion, proliferation, and migration, which are necessary mechanisms for tissue regeneration. However, their morphological characteristics could also promote the adhesion of bacteria (cells of pathogenic microorganisms), eventually leading to the formation of a biofilm.

In this context, composites materials based on polymeric matrices and metal oxide antimicrobial nanoparticles, have been extensively investigated over the last decade.

The resulting findings have evidenced the interactions between the composite materials and microorganisms, which have to be circumvented to minimize the adhesion and proliferation of the pathogenic microorganism. As a summary, a sequence of interactions is explained below:

- i. Firstly, bacteria are transported near the fibrous material surface by physical forces, such as gravitational and Brownian forces, or by protein-based organelles called flagella;
- ii. Then, the attraction between microorganism and fiber surface takes place by physico-chemical interactions (Van der Waals and hydrogen bond) or chemical reactions, which can lead to reversible and irreversible adhesion, respectively;
- iii. After anchoring on the material surface, the microorganism synthesizes the biofilm matrix which protects and facilitates its proliferation.
- iv. Finally, biofilm maturation is achieved and subsequent detachment occurs.

Considering the aforementioned composite material-microorganism interactions in conjunction with the well-known antimicrobial mechanism of metallic and metal oxide nanoparticles, it could be hypothesized that the antimicrobial activity of fibrous polymeric composites derived from different spinning techniques could be tailored by modifying the morphological features of fibers and distribution/dispersion of antimicrobial agents. For example, Shalumon *et al.* [48] reported ternary fibrous composites consisting of sodium alginate, poly(vinyl alcohol) (PVA), and several concentrations of ZnO nanoparticles. The ability of the mats to inhibit the growth of both *S. aureus* and *E. coli* strains was proportional to ZnO concentration, and interestingly ZnO did not cause a detrimental effect on the adhesion of fibroblast cells. These materials have proven suitable for wound dressing or cell scaffold applications. Biodegradable and/or biocompatible fibrous mats have been developed utilizing other matrices, such as chitosan (which has an inherent antibacterial activity) [49], cellulose acetate, and PCL containing ZnO nanoparticles which showed excellent antimicrobial activity against *Candida albicans*, *E. coli*, *S. aureus*, and *Citrobacter freundii* [50]. Augustine *et al.* [51,52] found the existence of an optimal ZnO concentration to induce inflammation in animals and inhibit the growth of bacteria; furthermore, in the whole range of ZnO concentration, PCL/ZnO membranes showed an excellent fibroblast attachment, which is very important for the application as a wound healing materials.

The combination of electrohydrodynamic techniques represents a versatile approach for tailoring morphological features and consequently the antimicrobial properties of fibrous composites. For instance, Rodríguez-Tobías *et al.* [53, 54] investigated the

influence of the electrohydrodynamic technique employed and nanoparticles concentration on the antibacterial activity and mechanical performance of biodegradable polyesters (PLA or PHB) fibers containing nano-ZnO. In the first instance, it was evidenced that increasing the ZnO concentration (from 1% to 5%) the viscosity and conductivity of polyester precursory solution remains in the same order of magnitude, thus leading to fibers with similar fiber diameter ranging from 0.7 to 0.8 microns, porosity from 40-50%, and pore sizes from 10 to 300 microns. In spite of the homogeneity in morphological features, some aggregates into the fibers and semi-exposed on their surface were detected by SEM/TEM. Figure 1 gathers SEM and TEM images of the PLA electrospun fibers containing 5 wt.% ZnO nanoparticles, in which it could be observed a homogeneous fibrous morphology with a wide diameter distribution, and the ZnO aggregates near the fiber surface. Similar trends were also found in the case of the electrospinning of PHB/ZnO solutions, but the formed fibers were thinner due to the lower viscosity and higher conductivity compared with PLA solutions.

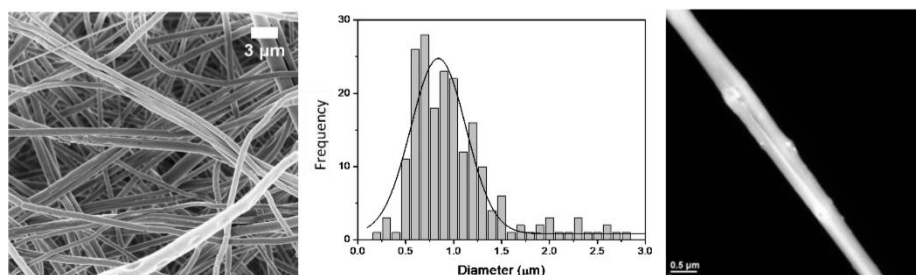


Fig. 1 SEM image of PLA/ZnO electrospun fibers (left), their fiber diameter distribution (center) and TEM image showing the inner and semi-exposed ZnO nanoparticles in one PLA fiber (right). Reprinted from Rodríguez-Tobías *et al.*, [53] Copyright (2014), with permission from Elsevier.

Concerning the combination of electrohydrodynamic techniques, it was reported [53-55] the tandem process based on electrospinning of polyester solutions and simultaneous electrospraying of ZnO dispersion with the aim to obtain ZnO-coated fibrous materials and determine its antibacterial activity as a function of nanoparticles concentration. It was found that fiber diameter remains unchanged due to the absence of ZnO nanoparticles in the precursory solutions, and the electrospraying technique leads to a deposition system of ZnO aggregates onto the fibers surface, which ranged from several nanometers to 2-5 microns.

The evaluation of mechanical performance of fibrous composites materials is of paramount importance since they could be subjected to tensile forces not only during their handling but also once exposed to tissues by the corresponding movements. Rodríguez-Tobías *et al.* [53-55] demonstrated that the mechanical performance of PLA or PHB fibrous composites is dependent on the electrohydrodynamic technique as well as the ZnO concentration. Taking into account the ZnO concentration, both fibrous materials, *i.e.* electrospun and electrospun/electrosprayed polyesters, exhibited an increase in tensile strength when increasing ZnO concentration up to an optimal concentration of 3 wt.%. Higher ZnO concentrations caused a deterioration of mechanical performance probably due to the presence of a high concentration of aggregates, which eventually acts as failure points.

Regarding the main topic of this section, antimicrobial properties of the aforementioned polyester fibrous materials were evaluated against *E. coli* and *S. aureus* strains by immersion tests during 24 h. In general, antibacterial activity was dependent on the type of bacteria, ZnO concentration, chemical nature of the polyester and the electrostatic technique used. Considering the bacterium, *E. coli* is more resistant to the fibers containing different concentrations of ZnO nanoparticles, which has been attributed to the ability of this bacterium to metabolize low concentrations of Zn^{2+} as an oligoelement in conjunction with the nature of its cell wall structure, which is more complex than that presented by *S. aureus*, and their components impart a partial negative charge. These findings have been reported in several research works thus demonstrating the importance to evaluate the fibrous composite materials against several microorganisms to ensure a suitable antimicrobial performance and increase the viability to scale up to medical devices.

On the other hand, the ZnO concentration has an optimal value (3 wt.%) above which no longer antibacterial activity is observed. This behavior is associated with the agglomeration trend at high ZnO concentration, thus lowering the surface area prone to attack the bacteria. As previously mentioned, the chemical nature of the polyester can influence the antimicrobial performance of the corresponding fibrous materials since the polyester chain polarity can promote the attraction or repulsion of the bacteria as a function of its cell wall composition. The effect of nanoparticles agglomeration on antimicrobial performance has also been detected for a wide range of polymers and inorganic nanoparticles, so that the research scope for these materials has been to determine the optimal concentration to lead a good balance in mechanical and antimicrobial performance.

Korina *et al.* [56, 57] investigated the combination of different electrohydrodynamic techniques, thus evidencing that the use of electrospraying and electrospinning leads to the metal oxide-coated fibrous material with high surface area prone to attack more efficiently to microorganisms. In our research works, when electrospinning/electrospraying was used with at least 1 wt.% ZnO nanoparticles, values higher than 94% of growth inhibition were achieved. On the contrary, fibrous composites materials derived from mere electrospinning exhibited a moderate antibacterial activity, which could be related to the low degree of ZnO dispersion/exposition and consequently minor interactions between nanoparticles and bacteria.

Recently, Padilla-Gainza *et al.* [58] have taken advantage of the Forcespinning® technique to obtain polyester/ZnO fibrous materials with potential to suppress *E. coli* and *S. aureus*. The authors evaluated the antibacterial efficiency of PHB- and PLA-based fibrous composites containing ZnO. In the case of PLA, at a concentration of 5 wt.%, an inhibition of bacterial growth for both strains exceeded 97%. On the other hand, PHB showed an excellent antibacterial performance against both microorganisms, observing a slightly higher efficiency at a concentration of 1 wt.%. It is noteworthy that the presence of ZnO did not affect the osteoblasts viability, thus increasing the potential use of this mats in bone regeneration.

2.2. Tailoring the bioactive properties of fibrous polyester composites

In many cases, for the design of polymeric fiber scaffolds, it is necessary to consider the incorporation of substances that would promote cell-scaffold interactions. Scaffold bioactivity in bone tissue regeneration can be increased by incorporating ceramic biomaterials, such as laponite nanosilicates, bioactive glasses, calcium phosphates, biphasic calcium phosphate, among others [59, 60]. Nevertheless, hydroxyapatite (Hap) $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ is the most commonly used bioactive component, since it is the main inorganic constituent of the bone matrix; specifically, around 69% of the human hard tissue bone is Hap [61]. Hap has a Ca/P molar ratio of 1.67 and mostly made up of a hexagonal crystal structure. The different crystalline structure of Hap allows it to have compositional variations in a biological system. In different reports [62], it has been

shown that Hap contributes to important biological processes for the regeneration of bone tissue, such as osteoconduction [50] (bone growth at the superficial level), osteoinduction [63] (pluripotent cells are stimulated to develop bone-forming cells, process that induces osteogenesis), and osteointegration (stable anchoring of an implant, obtained by direct contact between the bone tissue and the implant) [64]. However, the understanding of how Hap stimulates the mechanisms of cell adhesion, proliferation and differentiation is a controversial issue that is still under investigation. Currently, different fibrous hybrid systems based on biopolyesters and Hap have been studied. In this context, it has been reported that these systems offer efficient support for the adhesion, growth and osteogenic differentiation of human mesenchymal stem cells (hMSCs) [65-67], osteoblasts (MG-63) [68], rat bone marrow cells (rBMSCs) [69], and mouse pre-osteoblasts (MC3T3-E1) [70]; properties that have been attributed mainly to the presence of Hap.

As described above, PLA and PHB have received special attention for the design of biomedical materials due their versatility to produce systems with different properties. In Table 1, hybrid fibrous scaffolds based on PLA and PHB with Hap obtained by different spinning techniques are described, with special emphasis on the fiber production technique and their biological performance.

It is interesting to observe the relationship between the fiber diameter and its production technique, even considering its dependence with precursory solution properties and operational conditions (see the section 1.2.1). Among the techniques described in Table 1, the systems with the smallest diameters were obtained with the needle/needle-less electrospinning techniques, which in turn produced narrower fiber size distributions than those obtained with Forcespinning® (see standard deviations). These results have a correlation with the scaffold porosity (space between fibers) and pore sizes. High porosity and standardized pore sizes improve the 3D material performance to be used as scaffolds for tissue regeneration.

Based on the biological performance, it is difficult to make comparison between fiber production techniques, because the results are dependent on type of cells, assay, and experimental design. However, it is clear that the Hap incorporation through electrospraying improves cell adhesion. The Hap covering on the fibers provides a highly rough surface and enables direct and efficient interactions between cells and the bioceramic compound. Therefore, the electrospinning/electrospraying approach showed improved cell performance (Figure 2) for the systems displayed in Table 1.

Derivatives of the PHA family, including PHB and poly(3-hydroxybutyric acid-co-3-hydrovaleric acid) (PHBV), and the three stereochemical forms of PLA, *i.e.* PLLA, PDLA and PDLLA, have shown favorable results in their interactions with different types of cells. Hybrid systems including Hap concentration between 15 and 50 wt.% have been studied with mesenchymal stem and osteoblast cells. It has been demonstrated an inductive effect on the differentiation of h/BMSCs into osteoblasts and the osteogenesis. The differentiation has been confirmed by the increase in alkaline phosphatase (ALP) activity, indicating an early marker relevant in matrix mineralization [71]. Enhanced viability has been found at 20 wt.% which was correlated with the high porosity (91%) that could facilitate the diffusive transport of nutrients improving cell viability [65]. On the other hand, filopodia formation from osteoblast cells, specifically MG63, have been promoted at 11 wt.% of Hap combined with PHBV polymer matrix [72]. Osteoblast differentiation have been determined by the presence of osteocalcin, obtaining a concentration significantly higher in MG63 cells grown on PLA with 15 wt.% Hap than in those grown on pristine PLA. Additionally, at this Hap concentration, it was found an increased performance in cell growth and viability. A combined effect between an adequate Hap concentration content in a 3D structure with wide distribution of fiber diameters allows for an efficient cell interaction with the scaffold at 15 wt.% system [69].

Concerning the Forcespinning[®] technique, a couple of studies are described in Table 1. Padilla *et al.* [73] reported the design and characterization of PDLLA and PHB scaffolds and their performance interacting with human osteoblast-like cells (Saos-2) to evaluate their potential in bone tissue regeneration applications. The biological performance was evaluated for both polymers at 5 wt.% of Hap. The results showed improved cellular response for PDLLA with a cell viability higher than 60%, where the PDLLA-Hap5% system promoted an increase of 10% in cell viability test by day 7 of cell interaction. This behavior could be attributed to the accessibility to the Hap nanoparticles (NPs) within the fibers because most of them were embedded in the PDLLA (Figure 3-f), avoiding the NPs-cells interactions for the first and third days of study. The fiber surface erosion (hydrolytic degradation) could produce the exposition of greater number of NPs, resulting in an increased cell metabolic activity by day 7. Additionally, the surface roughness of the final PDLLA fibers (Figure 3-b) could have played an important role in the different biological performance observed in the PHB fibers.

Padilla-Gainza *et al.* [74] reported an in-depth biological study of a bioactive (Hap) and antibacterial (ZnO) PDLLA scaffolds with potential applications for bone regeneration. It is important to highlight that the antibacterial evaluation, optimization process, and

cell viability study [73] previously reported by our research team defined the appropriate ZnO and Hap concentrations used for the design of this dual (bioactive/antibacterial) hybrid scaffold. The idea to combine ZnO in the final material was to diminish the risk to promote the adhesion and growth of pathogenic microorganisms. ZnO has demonstrated a selective toxicity for bacteria but exhibits minimal effect on human cells [75]. In addition, it has been proven that it exerts a positive osteoconductive and osteoinductive effect in mesenchymal cells [76]. The results showed an increased cell viability, above 80%, over the test course of days 1, 3, 5 and 7, which could be consistent with increased cell number. From day 5, the PDLLA scaffold showed viabilities above 100% with respect to the positive control, thus suggesting that cells in the positive control were near confluence. In the case of fibrous scaffolds, due to a higher surface area and adequate pore size, cells continuously proliferated during the tested period. For day 7, mitochondria morphology and proliferation studies were further addressed. All the scaffolds promoted the growth of MC3T3-E1 cells in clusters within the nanofiber matrix. Additionally, the mitochondria morphology showed an interconnected network, representing an efficient system to deliver energy or transfer calcium between different areas of cells (Figure 4). Complementary osteoblast cells morphology was also studied by labeling actin cytoskeletal filaments (Phalloidin) and nucleus (DAPI). It is clearly observed in Figure 5, that MC3T3-E1 cells lay on the fibers and surround them, while extending in the direction of the fiber. The systems revealed normal cell morphology and no signs of necrosis/apoptosis, showing widely spread and spindle-like morphology for blank and PDLLA-ZnO 5%-Hap 5%, respectively. As it could be observed in Figure 5-b, cells were completely in contact with the fibers and some filopodia-like extensions were evidenced coming up from the cells towards the fibers (see the arrows), indicating favorable cell-fiber interactions. Regarding cell migration (Figure 6), cells were found at different depth levels of the scaffold, finding cells down to 37 μm depth from the surface. This behavior showed biocompatibility since cells were able to grow embedded within the scaffolds. These results suggest that the scaffold could promote the exchange of nutrients at different depths given the developed porosity, a feature that is desirable in biocompatible materials. This demonstrates a favorable design of the scaffold, as a result of its chemical nature, surface porosity, porosity (inter-fiber space) and fiber/pore size distribution.

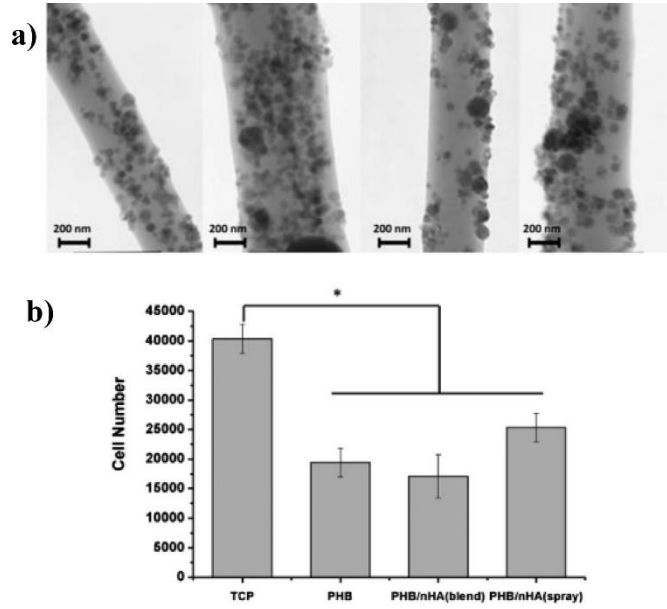


Fig. 2 PHB/Hap scaffolds at 15 wt.% of Hap (a) TEM images of single fibers from blend (left side) and spray (right side) and (b) MTS assay of hMSC after 1 h of incubation on fibrous scaffolds. Bars represent mean values \pm standard deviations ($n = 4$). Asterisks indicate significant difference obtained by t-test ($p \leq 0.05$). Reprinted from Ramier et al.,[66] Copyright (2014), with permission from Elsevier.

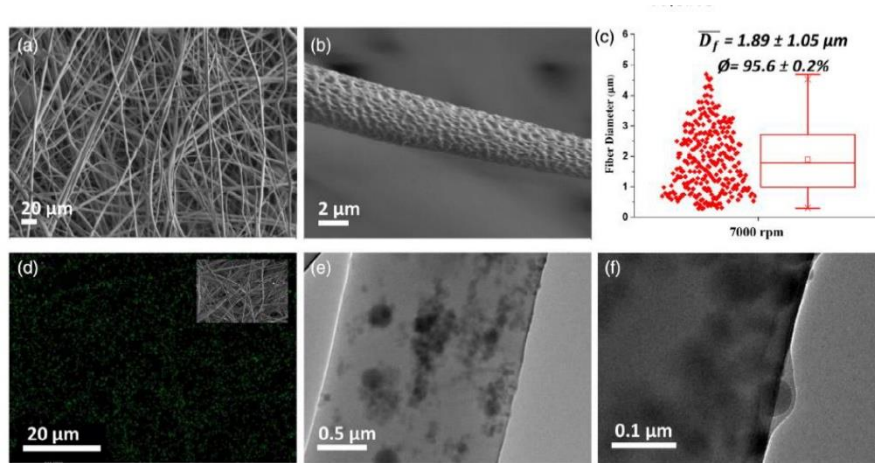


Fig. 3 SEM images (a, b, and d), TEM (f, g), box chart of fiber diameter (c) and mapping of phosphorous element obtained through EDS of PDLLA-Hap 5% system produced at ω 7000 rpm, $\overline{D_f}$ and ϕ are the average fiber diameter and porosity, respectively. Reprinted from Padilla et al.,[74] Copyright (2021), with permission from Elsevier.

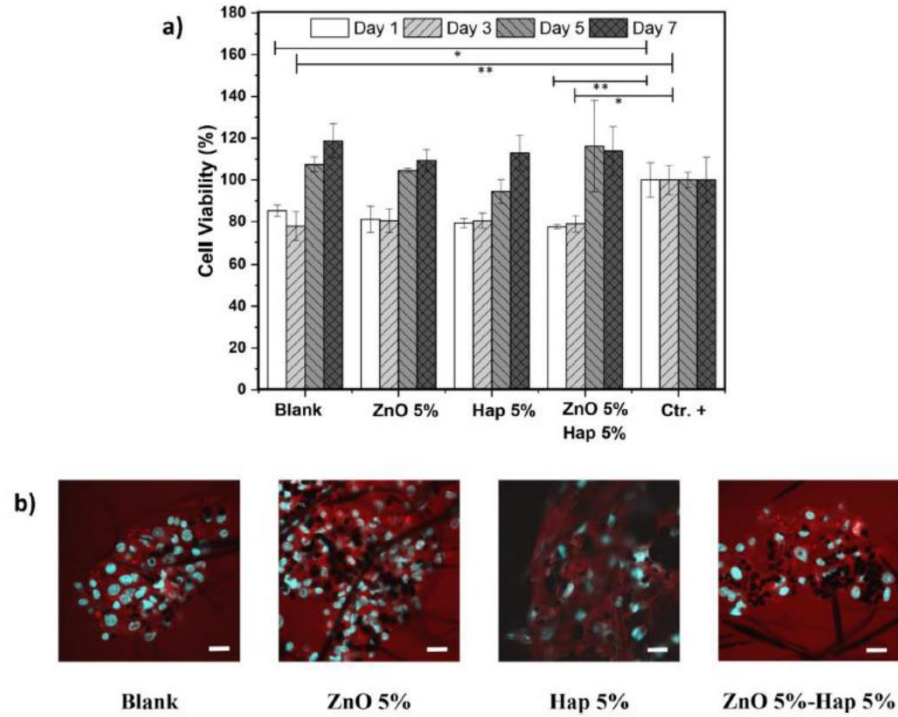


Fig. 4 (a) Resazurin-MC3T3-E1 cell viability test; Ctr.+ : positive control (cells grown on glass coverslips) was taken as reference to determine the cell viability of the systems. Blank is defined by cell interacting with a PDLLA fiber without NPs. The results are presented as the mean of three experiments \pm the standard deviation of the mean (* $p < 0.05$, ** $p < 0.01$). (b) MC3T3-E1 Confocal micrographs (mitochondria are labeled with Mitotracker (red), while DAPI labels cell nuclei (blue)) obtained at day 7 of scaffold-cell interaction, after being subject to the cell viability test (scale bar: 20 μ m). Reprinted from Padilla et al.,[74] Copyright (2021), with permission from Elsevier.

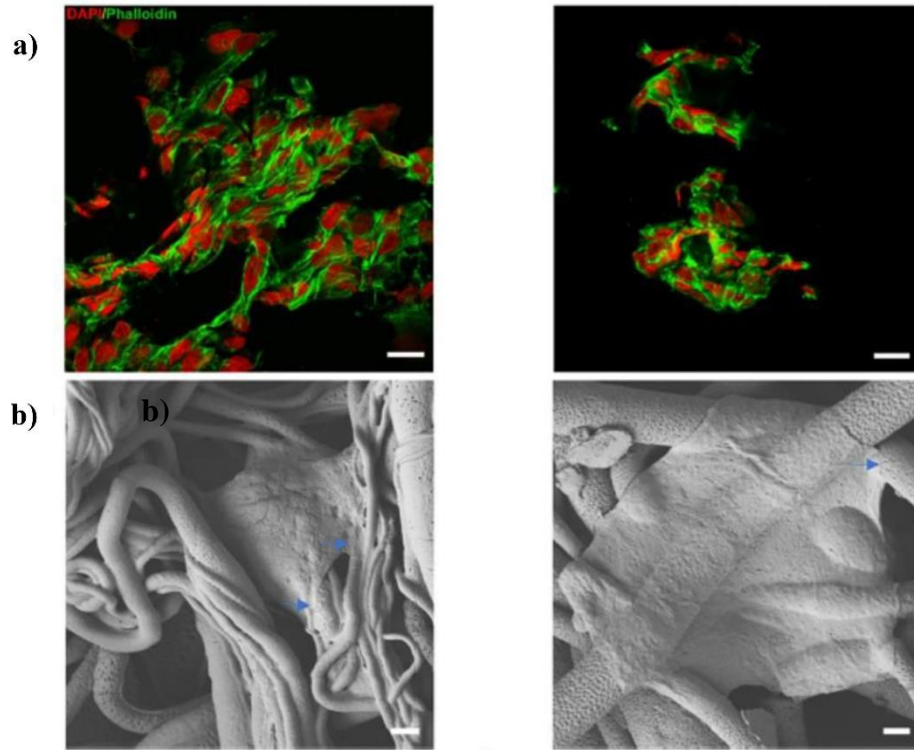


Fig. 5 Osteoblast cells (MC3T3-E1) morphology observed in PDLLA fiber scaffolds obtained through confocal microscopy, cytoskeletal filaments (green) and nucleus (blue): (a) Cell clusters found in day 5 (PDLLA blank) and 7 (PDLLA-ZnO 5%-Hap 5%) of the scaffold-cell interaction test (scale bar: 20 μm), and (b) SEM images of the osteoblast cells interacting with the fibers from PDLLA blank and PDLLA-ZnO 5%-Hap 5%, left and right side, respectively (day 7) (scale bar: 2 μm). Reprinted from Padilla et al.,[74] Copyright (2021), with permission from Elsevier.

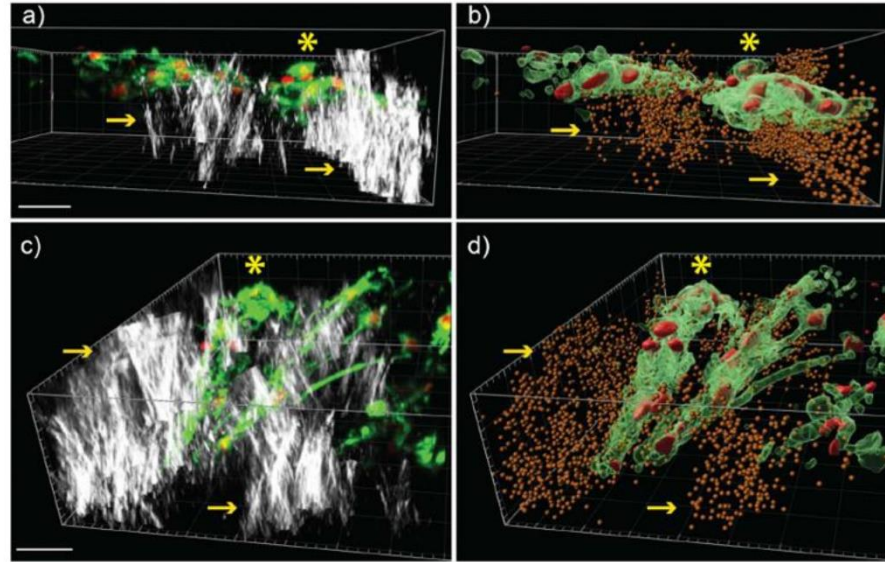


Fig. 6 MC3T3-E1 cell growth embedded in PDLLA-ZnO 5%-Hap 5% fiber scaffolds in three-dimensions. Lateral and upper view of fluorescence confocal Z-stacks (a and c, respectively) showing the cell cytoplasm (green) and nucleus (red) of MC3T3-E1 cells (yellow asterisks) into the PDLLA-ZnO 5%-Hap 5% fiber scaffolds (white), pointed by yellow arrows, for day 7 of cell-scaffold interaction. Imaris three-dimensional reconstruction of the fluorescent signal for cytoplasm, nucleus, and fiber scaffolds (represented as orange dots for better viewing) are shown in (b) and (d). Scale bar for all figures is 30 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article). Reprinted from Padilla et al.,[74] Copyright (2021), with permission from Elsevier.

Table 1 Main characteristics of hybrid fibrous scaffolds based on PLLA, PDLLA, PHB or PHBV with Hap nanoparticles: technique, Hap concentration (C_{Hap}), average fiber diameter ($\overline{D_f}$), pore size, porosity (\emptyset , inter-fiber space) and biological performance

System	Technique	C_{Hap} (wt.%)	$\overline{D_f}$ (μm)	Pore size (μm)	\emptyset (%)	Biological Performance	Ref
PHB/Hap(blend)	Electrospinning	0 15	950 \pm 160 640 \pm 80	5-15 ^a 1-7	61 ^a 61	<u>MTS assay</u> Cell: hMSC - PHB/Hap (spray) shown a higher cell adhesion after 1 h cell-scaffold interaction than PHB/Hap (blend)	[56]
PHB/Hap (spray)	Electrospinning /Electrospraying	15	950 \pm 70	2-11 ^a	77 ^a		
PLLA/Hap	Electrospinning	0 20 50	770 \pm 350 840 \pm 330 1030 \pm 390	NR	80 \pm 3 ^a 91 \pm 2 70 \pm 4	<u>MTT assay and ALP activity</u> Cell: hMSCs -Induce osteogenic and osteoclastogenic differentiation of human mesenchymal stem cells.	[55]
PHB/Hap	Gas-jet/electrospinning	10	NR	NR	NR	<u>MTS assay</u> Cell: BMSC -Good support for the attachment, proliferation and differentiation	[57]
PLLA/Hap	Needle-less electrospinning	0 5 15	160-340 287-545 242-729	NR	NR	<u>Cell viability (live/dead viability kit) and osteogenic cell differentiation</u> Cell: MG63 -No considerable cytotoxic effect in human osteoblast-like MG63. 15 wt.% of Hap presented a higher potential for tissue engineering application.	[59]
PHBV/Hap	Electrospinning	0 11	2920 \pm 280 3760 \pm 370	NR	NR	<u>Resazurin assay</u> Cell: MG63 -Hap promoted the filopodia formation increasing the specific cell activities	[62]
PDLLA/Hap	Forcespinning [®]	0 5 10 15	1030 \pm 779 1890 \pm 1050 - -	NR	NR 95.6 \pm 0.1 ^b	<u>Resazurin assay</u> Cell: Saos-2 -PDLLA systems promoted higher cellular response than those formulated with PHB.	[63]
PHB/Hap		0 5 10 15	1720 \pm 550 1920 \pm 710 1820 \pm 830 1890 \pm 680	5 to 200 ^c	NR 92.9 \pm 0.1 ^b 94.4 \pm 0.4 96.2 \pm 0.5		
PDLLA/Hap/ZnO		5	1840 \pm 1030	2 to 2561.7 ^c	92.7 \pm 0.16 ^b		
						<u>Resazurin assay, proliferation and migration study</u> Cell: MC3T3	[64]

						- PDLLA scaffold promoted a favorable cell response, with viabilities above 80%. - PDLLA-ZnO 5%-Hap 5% scaffold promoted favorable cell morphology. - Scaffold design promoted a sustained increase in cell proliferation and migration into the 3D structure.	
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MTT assay, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide): it is based on reduction of the MTS tetrazolium by viable mammalian cells to generate a colored formazan dye that is insoluble in cell culture media.

MTS assay, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium): it is based on reduction of the MTS tetrazolium by viable mammalian cells to generate a colored formazan dye that is soluble in cell culture media.

Resazurin assay, is based on the reduction of oxidized non-fluorescent blue resazurin to a red fluorescent dye (resorufin) by the mitochondrial respiratory chain in live cells.

NR: not reported

^a Mercury intrusion porosimetry

^b $\phi = \left(1 - \frac{m}{Z \cdot A \cdot H \cdot \rho}\right) \cdot 100$ where m, Z, A, H, and ρ stand for the mass, thickness, width, length of the scaffold and the density of the polymer

^c Pore area in μm^2 , as determined by the *Image J* software.

3 Conclusions

In this chapter, important aspects about the design of polymeric fibrous composite materials for biological applications were addressed. Fiber size and their distribution, porosity (inter-fiber space), surface morphology, type of polymer matrix and the incorporation of agents in accurate concentration to prevent bacterial growth and improve cell interaction are crucial factors to design promising 3D fibrous structures for tissue engineering applications. Electrospinning and centrifugal spinning have been the most extensive technologies used to produce fibrous scaffolds. Particularly, PHB and PLA based fibrous scaffolds have been widely used in medical applications, and their combination with ZnO and/or Hap have produced promising antibacterial and/or bioactive alternatives for wound healing, and bone tissue regeneration.

4 References

- [1] Dlaska, C.E., Andersson, G., Brittberg, M., Suedkamp, N.P., Raschke, M.J., Schuetz, M. A.: Clinical Translation in Tissue Engineering—The Surgeon's View. *Curr. Mol. Biol. Reports.* 1, 61–70 (2015). <https://doi.org/10.1007/s40610-015-0013-3>
- [2] Berthiaume, F., Maguire, T.J., Yarmush, M.L.: Tissue engineering and regenerative medicine: history, progress, and challenges. *Annu. Rev. Chem. Biomol. Eng.* 2, 403–430 (2011). <https://doi.org/10.1146/annurev-chembioeng-061010-114257>
- [3] Nair, L.S., Laurencin, C.T.: Biodegradable polymers as biomaterials. *Prog. Polym. Sci.* 32, 762–798 (2007). <https://doi.org/10.1016/j.progpolymsci.2007.05.017>
- [4] Chung, S.: Fibrous Scaffolds for Tissue Engineering Applications. Dissertation, NC State University (2010).
- [5] Cao, H., Liu, T., Chew, S.Y.: The application of nanofibrous scaffolds in neural tissue engineering. *Adv. Drug Deliv. Rev.* 61, 1055–1064 (2009). <https://doi.org/10.1016/j.addr.2009.07.009>
- [6] Onuwaje, I., Phillips, J.B.: Three-dimensional culture systems in central nervous system research. In: Salgado, A.J.: *Handbook of Innovations in Central Nervous System Regenerative Medicine*, 571–601, Elsevier Inc. (2020). <https://doi.org/10.1016/B978-0-12-818084-6.00016-7>
- [7] Kumbar, S.G., James, R., Nukavarapu, S.P., Laurencin, C.T.: Electrospun nanofiber scaffolds: engineering soft tissues. *Biomed. Mater.* 3, 1–15 (2008). <https://doi.org/10.1088/1748-6041/3/3/034002>
- [8] Kurimoto, R., Niiyama, E., Ebara, M.: Fibrous Materials, In: Ebara, M.: *Biomaterials and Nanoarchitectonics*, Elsevier Inc., 267–278 (2016). <https://doi.org/10.1016/B978-0-323-37127-8.00016-9>
- [9] Oryan, A., Alidadi, S., Moshiri, A., Maffulli, N.: Bone regenerative medicine: Classic options, novel strategies, and future directions. *J. Orthop. Surg. Res.* 9, 1–27 (2014). <https://doi.org/10.1186/1749-799X-9-18>
- [10] Porter, J.R., Ruckh, T.T., Popat, K.C., Bone tissue engineering: A review in bone biomimetics and drug delivery strategies. *Biotechnol. Prog.* 25, 1539–1560 (2009). <https://doi.org/10.1002/btpr.246>
- [11] Williams, D.F.: *The Williams dictionary of biomaterials*, Liverpool University Press, Liverpool (1999) <https://doi.org/10.5949/UPO9781846314438>
- [12] Lloyd, A.: Interfacial bioengineering to enhance surface biocompatibility. *Med. Device Technol.* 13, 18–21 (2002).
- [13] Bhattarai, D.P., Aguilar, L.E., Park, C.H., Kim, C.S.: A review on properties of natural and synthetic based electrospun fibrous materials for bone tissue engineering. *Membranes* 8, 62–85 (2018). <https://doi.org/10.3390/membranes8030062>
- [14] Ellison, C. J., Phatak, A., Giles, D. W., Macosko, C. W., Bates, F. S.: Melt blown nanofibers: Fiber diameter distributions and onset of fiber breakup. *Polymer* 48, 3306–3316 (2007). <https://doi.org/10.1016/j.polymer.2007.04.005>
- [15] Song, J.H., Kim, Y.T., Cho, S., Song, W.J., Moon, S., Park, C.G., Park, S., Myoung, J.M., Jeong, U.: Surface-embedded stretchable electrodes by direct printing and their uses to fabricate ultrathin vibration sensors and circuits for 3D structures. *Adv. Mater.* 29, 1702625 (2017). <https://doi.org/10.1002/adma.201702625>

- [16] Liu, S., Li, L.: Ultrastretchable and self-healing double-network hydrogel for 3D printing and strain sensor. *ACS Appl. Mater. Interfaces* 9, 26429–26437 (2017). <https://doi.org/10.1021/acsami.7b07445>
- [17] Zhang, Y., Lim, C.T., Ramakrishna, S., Huang, Z.M.: Recent development of polymer nanofibers for biomedical and biotechnological applications. *J. Mater. Sci. Mater. Med.* 16, 933–946 (2005). <https://doi.org/10.1007/s10856-005-4428-x>
- [18] Chen, C., Tang, Y., Vlahovic, B., Yan, F.: Electrospun polymer nanofibers decorated with noble metal nanoparticles for chemical sensing. *Nanoscale Res. Lett.* 12, 451 (2017). <https://doi.org/10.1186/s11671-017-2216-4>
- [19] Park, J.H., Kim, B.S., Yoo, Y.C., Khil, M.S., Kim, H.Y.: Enhanced mechanical properties of multilayer nano-coated electrospun nylon 6 fibers via a layer-by-layer self-assembly. *J. Appl. Polym. Sci.* 107, 2211–2216 (2008) <https://doi.org/10.1002/app.27322>
- [20] Tiwari, A., Terada, D., Yoshikawa, C., Kobayashi, H.: An enzyme-free highly glucose-specific assay using self-assembled aminobenzene boronic acid upon polyelectrolytes electrospun nanofibers-mat. *Talanta* 82, 1725–1732 (2010). <https://doi.org/10.1016/j.talanta.2010.07.078>
- [21] Villarreal-Gómez, L.J., Cornejo-Bravo, J.M., Vera-Graziano, R., Grande, D.: Electrospinning as a powerful technique for biomedical applications: a critically selected survey. *J. Biomater. Sci. Polym. Ed.* 27, 157–176 (2016). <https://doi.org/10.1080/09205063.2015.1116885>
- [22] McCullen, S.D., Stevens, D.R., Roberts, W.A., Ojha, S.S., Clarke, L.I., Gorga, R.E.: Morphological, electrical, and mechanical characterization of electrospun nanofiber mats containing multiwalled carbon nanotubes. *Macromolecules* 40, 997–1003 (2007). <https://doi.org/10.1021/ma061735c>
- [23] Taepaiboon, P., Rungsardthong, U., Supaphol, P.: Vitamin-loaded electrospun cellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and vitamin E. *Eur. J. Pharm. Biopharm.* 67, 387–397 (2007). <https://doi.org/10.1016/j.ejpb.2007.03.018>
- [24] Deitzel, J.M., Kleinmeyer, J., Harris, D., Beck Tan, N.C.: The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer* 42, 261–272 (2001). <https://doi.org/10.1016/j.ejpb.2007.03.018>
- [25] Wang, Z., Crandall, C., Sahadevan, R., Menkhaus, T.J., Fong, H.: Microfiltration performance of electrospun nanofiber membranes with varied fiber diameters and different membrane porosities and thicknesses. *Polymer* 114, 64–72 (2017). <https://doi.org/10.1016/j.polymer.2017.02.084>
- [26] Persano, L., Camposeo, A., Tekmen, C., Pisignano, D.: Industrial upscaling of electrospinning and applications of polymer nanofibers: A review. *Macromol. Mater. Eng.* 298, 504–520 (2013). <https://doi.org/10.1002/mame.201200290>
- [27] Liu, Z., Zhao, J.H., Liu, P., He, J.H.: Tunable surface morphology of electrospun PMMA fiber using binary solvent. *Appl. Surf. Sci.* 364, 516–521 (2016). <https://doi.org/10.1016/j.apsusc.2015.12.176>
- [28] Lee, J.H., Shin, D.W., Nam, K.B., Gim, Y.H., Ko, H.S., Seo, D.K., Lee, G.H., Kim, Y.H., Kim, S.W., Oh, T.S.: Continuous bundles of aligned electrospun PAN nano-fiber using electrostatic spiral collector and converging coil. *Polymer* 84, 52–58 (2016). <https://doi.org/10.1016/j.polymer.2015.11.046>
- [29] Tiwari, A.P., Joshi, M.K., Kim, J.I., Unnithan, A.R., Lee, J., Park, C.H., Kim, C.S.: Bimodal fibrous structures for tissue engineering: Fabrication, characterization and in vitro biocompatibility. *J. Colloid Interface Sci.* 476, 29–34 (2016). <https://doi.org/10.1016/j.jcis.2016.02.048>
- [30] Weitz, R. T., Harnau, L., Rauschenbach, S., Burghard, M., Kern, K.: Polymer Nanofibers via Nozzle-Free Centrifugal Spinning. *Am. Chem. Soc.* 8, 1187–1191 (2008). <https://doi.org/10.1021/nl080124q>
- [31] Arumuganathar, S., Jayasinghe, S. N.: Living scaffolds (specialized and unspecialized) for regenerative and therapeutic medicine. *Biomacromolecules* 9, 759–766 (2008). <https://doi.org/10.1021/bm701322k>
- [32] Badrossamay, M. R., McIlwee, H. A., Goss, J. A., Parker, K. K.: Nanofiber assembly by rotary jet-spinning. *Nano Lett.* 10, 2257–2261 (2010). <https://doi.org/10.1021/nl101355x>
- [33] Sarkar, K., Gomez, C., Zambrano, S., Ramirez, M., de Hoyos, E., Vasquez, H., Lozano, K.: Electrospinning to Forcespinning. *Mater. today* 13, 13–15 (2010). [https://doi.org/10.1016/S1369-7021\(10\)70199-1](https://doi.org/10.1016/S1369-7021(10)70199-1)
- [34] Sebe, I., Szabó, B., Nagy, Z. K., Szabó, D., Zsidai, L., Kocsis, B., Zelkó, R.: Polymer structure and antimicrobial activity of polyvinylpyrrolidone-based iodine nanofibers prepared with high-speed rotary spinning technique. *Int. J. Pharm.* 458, 99–103 (2013). <https://doi.org/10.1016/j.ijpharm.2013.10.011>
- [35] Padron, S., Patlan, R., Gutierrez, J., Santos, N., Eubanks, T., Lozano, K.: Production and characterization of hybrid BEH-PPV/PEO conjugated polymer nanofibers by Forcespinning. *J. Appl. Polym. Sci.* 125, 3610–3616 (2012). <https://doi.org/10.1002/app.36420>
- [36] Zhang, X., Lu, Y.: Centrifugal Spinning: An Alternative Approach to Fabricate Nanofibers at High Speed and Low Cost. *Polym. Rev.* 54, 677–701 (2014). <https://doi.org/10.1080/15583724.2014.935858>

- [37] T.S. Karande, J.L. Ong, C.M. Agrawal, Diffusion in musculoskeletal tissue engineering scaffolds: Design issues related to porosity, permeability, architecture, and nutrient mixing. *Ann. Biomed. Eng.* 32 (2004). 1728–1743. <https://doi.org/10.1007/s10439-004-7825-2>
- [38] Yang, S., Leong, K., Du, Z., Chua, C.: The design of scaffolds for use in tissue engineering. Part I. traditional factors. *Tissue Eng.* 7, 679–689 (2001). <https://doi.org/10.1089/107632701753337645>
- [39] Salgado, A.J., Coutinho, O.P., Reis, R.L.: Bone tissue engineering: State of the art and future trends. *Macromol. Biosci.* 4, 743–765 (2004). <https://doi.org/10.1002/mabi.200400026>
- [40] Silva, M.M.C.G., Cyster, L.A., Barry, J.J.A., Yang, X.B., Oreffo, R.O.C., Grant, D.M., Scotchford, C.A., Howdle, S.M., Shakesheff, K.M., Rose, F.R.A.J.: The effect of anisotropic architecture on cell and tissue infiltration into tissue engineering scaffolds. *Biomaterials* 27, 5909–5917 (2006). <https://doi.org/10.1016/j.biomaterials.2006.08.010>
- [41] Itälä, A.H., Ylänen, A.I., Ekholm, H.O., Karlsson, C.: Pore diameter of more than 100 microm is not requisite for bone ingrowth in rabbits. *J. Biomed. Mater. Res.* 58, 679–83 (2001). <https://doi.org/10.1002/jbm.1069>
- [42] Liu, R., Ma, L., Liu, H., Xu, B., Feng, C., He, R.: Effects of pore size on the mechanical and biological properties of stereolithographic 3D printed HAp bioceramic scaffold. *Ceram. Int.* 47, 28924–28931 (2021). <https://doi.org/10.1016/j.ceramint.2021.07.053>
- [43] Van Tienen, T.G., Heijkants, R.G.J.C., Buma, P., De Groot, J.H., Pennings, A.J., Veth, R.P.H.: Tissue ingrowth and degradation of two biodegradable porous polymers with different porosities and pore sizes. *Biomaterials* 23, 1731–1738 (2002). [https://doi.org/10.1016/S0142-9612\(01\)00280-0](https://doi.org/10.1016/S0142-9612(01)00280-0)
- [44] Rampichová, M., Chvojka, J., Buzgo, M., Prosecká, E., Mikeš, P., Vysloužilová, L., Tvrdík, D., Kochová, P., Gregor, T., Lukáš, D., Amler, E.: Elastic three-dimensional poly (ϵ -caprolactone) nanofibre scaffold enhances migration, proliferation and osteogenic differentiation of mesenchymal stem cells. *Cell Prolif.* 46, 23–37 (2013). <https://doi.org/10.1111/cpr.12001>
- [45] Pham, Q.P., Sharma, U., Mikos, A.G.: Electrospun poly (ϵ -caprolactone) microfiber and multilayer nanofiber/microfiber scaffolds: Characterization of scaffolds and measurement of cellular infiltration. *Biomacromolecules* 7, 2796–2805 (2006). <https://doi.org/10.1021/bm060680j>
- [46] Shalumon, K.T., Chennazhi, K.P., Nair, S. V., Jayakumar, R.: High thick layer-by-layer 3D multiscale fibrous scaffolds for enhanced cell infiltration and its potential in tissue engineering. *J. Biomed. Nanotechnol.* 9, 2117–2122 (2013). <https://doi.org/10.1166/jbn.2013.1702>
- [47] Deepthi, S., Jeevitha, K., Nivedhitha M., Sundaram, K.P. Chennazhi, R.: Chitosan-hyaluronic acid hydrogel coated poly(caprolactone) multiscale bilayer scaffold for ligament regeneration. *Chem. Eng. J.* 260, 478–485 (2015). <https://doi.org/10.1016/j.cej.2014.08.106>
- [48] Shalumon, K.T., Anulekha, K.H., Nair, S.V., Chennazhi, K.P., Jayakumar, R.: Sodium alginate/poly(vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. *Int. J. Biol. Macromol.* 49, 247–254 (2011). <https://doi.org/10.1016/j.ijbiomac.2011.04.005>
- [49] Son, B., Yeom, B.-Y., Song, S.H., Lee, C.-S., Hwang, T.S.: Antibacterial electrospun chitosan/poly(vinyl alcohol) nanofibers containing silver nitrate and titanium dioxide. *J. Appl. Polym. Sci.* 111, 2892–2899 (2009). <https://doi.org/10.1002/app.29233>
- [50] Anitha, S., Brabu, B., Thiruvadigal, D.J., Gopalakrishnan, C., Natarajan, T.S.: Optical, bactericidal and water repellent properties of electrospun nano-composite membranes of cellulose acetate and ZnO. *Carbohydr. Polym.* 87, 1065–1072 (2012). <https://doi.org/10.1016/j.carbpol.2011.08.030>
- [51] Augustine, R., Malik, H.N., Singhal, D.K., Mukherjee, A., Malakar, D., Kalarikkal, N., Thomas, S.: Electrospun polycaprolactone/ZnO nanocomposite membranes as biomaterials with antibacterial and cell adhesion properties. *J. Polym. Res.* 21 (2014). <https://doi.org/10.1007/s10965-013-0347-6>
- [52] Augustine, R., Dominic, E.A., Reju, I., Kaimal, B., Kalarikkal, N., Thomas, S.: Electrospun polycaprolactone membranes incorporated with ZnO nanoparticles as skin substitutes with enhanced fibroblast proliferation and wound healing. *RSC Adv.* 4, 24777 (2014). <https://doi.org/10.1039/C4RA02450H>
- [53] Rodríguez-Tobías, H., Morales, G., Ledezma, A., Romero, J., Grande, D.: Novel antibacterial electrospun mats based on poly(D,L-lactide) nanofibers and zinc oxide nanoparticles. *J. Mater. Sci.* 49, 8373–8385 (2014). <https://doi.org/10.1007/s10853-014-8547-y>
- [54] Rodríguez-Tobías, H., Morales, G., Ledezma, A., Romero, J., Saldívar, R., Langlois, V., Renard, E., Grande, D.: Electrospinning and electrospraying techniques for designing novel antibacterial poly(3-hydroxybutyrate)/zinc oxide nanofibrous composites. *J. Mater. Sci.* 51, 8593–8609 (2016). <https://doi.org/10.1007/s10853-016-0119-x>

- [55] Rodríguez-Tobías, H., Morales, G., Grande, D.: Improvement of mechanical properties and antibacterial activity of electrospun poly(d,l-lactide)-based mats by incorporation of ZnO-graft-poly(d,l-lactide) nanoparticles. *Mater. Chem. Phys.* 182, 324-331 (2016). <https://doi.org/10.1016/j.matchemphys.2016.07.039>
- [56] Korina, E., Stoilova, O., Manolova, N., Rashkov, I.: Multifunctional Hybrid Materials From Poly(3-Hydroxybutyrate), TiO₂ Nanoparticles, and Chitosan Oligomers by Combining Electrospinning/Electrospraying and Impregnation. *Macromol. Biosci.* 13, 707-716 (2013). <https://doi.org/10.1002/mabi.201200410>
- [57] Korina, E., Stoilova, O., Manolova, N., Rashkov, I.: Poly(3-hydroxybutyrate)-based hybrid materials with photocatalytic and magnetic properties prepared by electrospinning and electrospraying. *J. Mater. Sci.* 49, 2144-2153 (2013). <https://doi.org/10.1007/s10853-013-7907-3>
- [58] Padilla-Gainza, V., Rodríguez-Tobías, H., Morales, G., Ledezma-Pérez, A., Alvarado-Canché, C., Rodríguez, C., Gilkerson, R., Lozano, K.: Processing-structure-property relationships of biopolyester/zinc oxide fibrous scaffolds engineered by centrifugal spinning. *Polym. Adv. Technol.* 31, 2601-2614 (2020). <https://doi.org/10.1002/pat.4987>
- [59] D.M. Ibrahim, E.S. Sani, A.M. Soliman, N. Zandi, E. Mostafavi, A.M. Youssef, N.K. Allam, N. Annabi, Bioactive and elastic nanocomposites with antimicrobial properties for bone tissue regeneration. *ACS Appl. Bio Mater.* 3, 3313–3325 (2020). <https://doi.org/10.1021/acsabm.0c00250>
- [60] Turnbull, G., Clarke, J., Picard, F., Riches, P., Jia, L., Han, F., Li, B., Shu, W.: 3D bioactive composite scaffolds for bone tissue engineering. *Bioactive Mater.* 3, 278-314 (2018). <https://doi.org/10.1016/j.bioactmat.2017.10.001>
- [61] Armentano, I., Puglia, D., Luzi, F., Arciola, C.R., Morena, F., Martino, S., Torre, L.: Nanocomposites Based on Biodegradable Polymers. *Materials* 11, 795 (2018). <https://doi.org/10.3390/ma11050795>
- [62] Bhat, S., Uthappa, U.T., Altalhi, T., Jung, H.-Y., Kurkuri, M.D.: Functionalized Porous Hydroxyapatite Scaffolds for Tissue Engineering Applications: A Focused Review. *ACS Biomater. Sci. Eng.* In Press (2021). <https://doi.org/10.1021/acsbiomaterials.1c00438>
- [63] Ripamonti, U.: Osteoinduction in porous hydroxyapatite implanted in heterotopic sites of different animal models. *Biomater.* 147, 31-35 (1996). [https://doi.org/10.1016/0142-9612\(96\)80752-6](https://doi.org/10.1016/0142-9612(96)80752-6)
- [64] Albrektsson, T., Johansson, C.: Osteoinduction, osteoconduction and osseointegration. *Eur. Spine J.* 10, S96-S101 (2001). <https://doi.org/10.1007/s005860100282>
- [65] Morelli, S., Salerno, S., Holopainen, J., Ritala, M., De Bartolo, L.: Osteogenic and osteoclastogenic differentiation of co-cultured cells in polylactic acid-nanohydroxyapatite fiber scaffolds. *J. Biotechnol.* 38, 53-62 (2015). <https://doi.org/10.1016/j.jbiotec.2015.03.023>
- [66] Ramier, J., Boudierlique, T., Stoilova, O., Manolova, N., Rashkov, I., Langlois, V., Renard, E., Albanese, P., Grande, P.: Biocomposite scaffolds based on electrospun poly(3-hydroxybutyrate) nanofibers and electrosprayed hydroxyapatite nanoparticles for bone tissue engineering applications. *Mater. Sci. Eng. C* 38,161-169 (2014). <https://doi.org/10.1016/j.msec.2014.01.046>
- [67] Guan, D., Chen, Z., Huang, C., Lin, Y.: Attachment, proliferation and differentiation of BMSCs on gas-jet/electrospun nHAP/PHB fibrous scaffolds. *Appl. Surf. Sci.* 255, 324-327 (2008). <https://doi.org/10.1016/j.apsusc.2008.06.093>
- [68] Zhang, H., Fu, Q.W., Sun, T.W., Chen, F., Qi, C., Wu, J., Cai, Z.Y., Qian, Q.R., Zhu, Y.J.: Amorphous calcium phosphate, hydroxyapatite and poly(d,l-lactic acid) composite nanofibers: Electrospinning preparation, mineralization and in vivo bone defect repair. *Colloids Surf. B* 136, 27-36 (2015). <https://doi.org/10.1016/j.colsurfb.2015.08.015>
- [69] Novotna, K., Zajdlova, M., Suchy, T., Hadraba, D., Lopot, F., Zaloudkova, M., Douglas, T.E.L., Munzarova, M., Juklickova, M., Stranska, D., Kubies, D., Schaubroeck, D., Wille, S., Balcaen, L., Jarosova, M., Kozak, H., Kromka, A., Svindrych, Z., Lisa, V., Balik, K., Bacakova, L.: Polylactide nanofibers with hydroxyapatite as growth substrates for osteoblast-like cells. *J. Biomed. Mater. Res. A* 102, 3918-3930 (2014). <https://doi.org/10.1002/jbm.a.35061>
- [70] Sadat-Shojai, M., Khorasani, M.-T., Jamshidi, A.: A new strategy for fabrication of bone scaffolds using electrospun nano-HAp/PHB fibers and protein hydrogels. *Chem. Eng. J.* 289, 38-47 (2016). <https://doi.org/10.1016/j.cej.2015.12.079>
- [71] Hessle, L., Johnson Kristen, A., Anderson, H.C., Narisawa, S., Sali, A., Goding James, W., Terkeltaub, R., Millán J.L.: Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. *Proc. Nat. Acad. Sci.* 99, 9445-9449 (2002). <https://doi.org/10.1073/pnas.142063399>

- [72] Karbowniczek, J.E., Kaniuk, Ł., Berniak, K., Gruszczyński, A., Stachewicz, U.: Enhanced Cells Anchoring to Electrospun Hybrid Scaffolds With PHBV and HA Particles for Bone Tissue Regeneration. *Front. Bioeng. Biotechnol.* 9, 632029 (2021). <https://doi.org/10.3389/fbioe.2021.632029>
- [73] Padilla-Gainza, V.M., Rodríguez-Tobías, H., Morales, G., Saucedo-Salazar, E., Lozano, K., Montaña-Machado, V., Mantovani, D.: Centrifugally spun mats based on biopolyesters/hydroxyapatite and their potential as bone scaffolds. *J. Appl. Polym. Sci.* 138, app50139 (2021). <https://doi.org/10.1002/app.50139>
- [74] Padilla-Gainza, V.M., Rodríguez-Tobías, H., Morales, G., Ledezma-Pérez, A., Alvarado-Canché, C., Loera-Valencia, R., Rodríguez, C., Gilkerson, R., De Leo, C.T., Lozano, K.: Development of zinc oxide/hydroxyapatite/poly(D,L-lactic acid) fibrous scaffold for tissue engineering applications. *Biomater. Adv.* 133, 112594 (2022). <https://doi.org/10.1016/j.msec.2021.112594>
- [75] Xie, Y., He, Y., Irwin, P. L., Jin, T., Shi, X.: Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 77, 2325-2331 (2011). <https://doi.org/10.1128/AEM.02149-10>
- [76] Yusa, K., Yamamoto, O., Takano, H., Fukuda, M., Iino, M.: Zinc-modified titanium surface enhances osteoblast differentiation of dental pulp stem cells in vitro. *Sci. Rep.* 6, 29462-29462 (2016). <https://doi.org/10.1038/srep29462>