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Novel Double-Layered Conduit Containing Highly Bioactive Glass Fibers for Potential Nerve Guide Application

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Peripheral nerve injuries are frequent conditions that currently have very few treatment alternatives. This study aimed to incorporate aligned bioactive glass microfibers (that belong to the SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5 system) in nanofibrous poly ε-caprolactone (PCL) membranes to develop a new biocomposite that is potentially able to facilitate nerve growth and increase the polymer matrix's biological and mechanical properties. For the manufacture of this novel tubular nerve guide, electrospinning of PCL was performed on the surface of bioactive glass fibers, resulting in a two-layer microcomposite. The mechanical strength, bioactivity, wettability, degradation, and permeability of this new material were characterized. The preliminary results indicate that the incorporation of the bioactive glass fibers into PCL led to the development of a highly bioactive biocomposite with significantly improved mechanical properties and wettability compared with the PCL matrix alone.

Keywords: *bioactive glass; glass fibers; biocomposite; nerve guide; poly ε-caprolactone*

Introduction

Peripheral nerve injuries are quite frequent and represent a very complicated condition that commonly results from severe trauma, for example, invasive surgical procedures or traffic, domestic, or industrial accidents. These injuries can impact profoundly and permanently impact the lives of patients by affecting their ability to perform daily activities.[1] In Europe, more than 300,000 cases are reported each year, and in the United States, approximately 200,000 patients per year undergo surgical repairing procedures due to this type of injury.[2]

In current clinical practice, autografts remain the gold standard in nerve repair; however, they present some disadvantages, such as requiring the sacrifice of a

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functional nerve that could lead to morbidity, such as scarring and loss of sensation at the donor site. In addition, the autograft technique requires two surgical procedures, making the treatment time-consuming, painful, cost intensive, and challenging. Another disadvantage of autografts is the small quantity of collected material, which is normally limited, and the deficient functional recovery of the tissue.[1]

Nerve allografts are also possible; however, the need for a systemic immunosuppressant and the high rejection risk limit this option. Synthetic grafts thus appear as a suitable alternative; however, the number of commercially available nerve guides is still limited.[3] Recently, synthetic biomaterials have drawn attention for potential application in peripheral nerve regeneration. Several studies have reported positive results using collagen tubes and biodegradable polymers, such as PGA, PLGA, PLLA, PLCL, and PHB, among others.

One polymer that is continuously investigated and has led to satisfying outcomes is poly ε-caprolactone (PCL). This material is a strong candidate for nerve conduit applications.[4] PCL has several advantages, such as an inexpensive manufacturing process, good reproducibility, easy handling and sterilization capability, and being biodegradable, nontoxic, and nonimmunogenic.[4]

In short-term studies, PCL scaffolds have demonstrated satisfying results in nerve regeneration approaches, for example, bridging a rat sciatic 1-cm nerve gap in only 2 weeks postinjury and presenting compatibility with both nerve cells and Schwann cells.[5]

However, PCL's nerve growth stimulation is poor, limiting its effectiveness in supporting nerve regeneration for clinical applications. One solution to increase the biointeraction of PCL is to incorporate a bioactive phase, such as bioactive glass. Given the increasing interest in utilizing bioactive glasses in soft tissue repair due to its interesting biological properties, this biomaterial can also be considered for nerve guide applications.[6]

The concept of using bioactive glasses (BGs) in the regeneration of nerve tissue injuries is fairly new and has scarcely been explored so far. Some studies have suggested a potential use for bioactive glasses doped with ZnO and CeO_2,[7] dense and rigid phosphate glass tubes[8,9] and 150 to 200 μm diameter hand-drawn fibers of 45S5 bioactive glass inserted in silicone tubes.[10] However, little is known about the effects linked to the use of BGs in this type of injury. It is anticipated, for example, that the presence of ions such as Ca^{2+}, which can be easily leached from bioactive glasses, have an extremely important role in the regeneration of nerve tissue because these ions can promote the stability of the cytoskeleton and regulation of nerve cells, as well as stimulate axonal growth.[10]

In most approaches, bioactive glass particles or fibers have been combined with biodegradable polymers. For example, Zhang *et al.*[11] developed a composite made of PGA/F127 and SiO_2–Na_2O–CaO–ZnO–CeO_2 bioactive glass. The results indicated that the achieved mechanical properties were similar to those of commercial nerve guide conduits and the material presented good cytocompatibilty *in vitro*. Regarding tests with bioactive phosphate glass tubes, Gilchrist *et al.*[9] reported that there were no significant differences between the treated group and the control group for the velocity of conduction or nerve morphometry after 10 months of implantation of such tubes in sheep. In an early effort, Bunting *et al.*[10] showed that hand-drawn fibers of Bioglass 45S5 with 150–200 μm in diameter were suitable for *in vitro* growth of Schwann cells and fibroblasts. Additionally, they provided qualitative and quantitative evidence of axonal regeneration through a Silastic conduit filled with Bioglass® fibers *in vivo* for a 0.5-cm interstump gap in the sciatic nerves of adult rats. For this study, the axonal regrowth after 4 weeks was indistinguishable from that which occurs across an autograft. Jeans *et al.*[12] developed a biodegradable phosphate glass fiber wrap using a polymer solution and fibrin glue or polyglactin. It was shown that, after 7 months, nerve regeneration had occurred at a similar rate in all the studied groups for the treatment of a divided median nerve in the upper forelimb of sheep. Further studies on the application of bioactive glasses in peripheral nerve and spinal cord repair have been reviewed recently.[13] Thus, the evidence in the literature indicates the possibility of incorporating bioactive glasses into PCL for developing biocomposites that are able to induce or accelerate peripheral nerve regeneration.

In targeting a more effective regeneration process, the presence of an aligned substrate is both important and more appealing. Studies have shown that substrate alignment is greatly important for nerve tissue regeneration. Ebental[14] showed that substrate orientation affects cell migration and that axons can be oriented by contact guidance along an aligned substrate. Chew *et al.*[15] reported that the presence of aligned PCL electrospun

fibers could emulate the formation of bands of Büngner, which are structures composed of longitudinally aligned Schwann cell strands that selectively guide axons regeneration.[16] These bands result in elongation and alignment of cells along the axes of the material fibers and promote Schwann cells maturation. When Schwann cells are aligned, *in vitro* studies have shown that they can increase the rate of regeneration and facilitate the extent of neurite elongation from dorsal root ganglia.[17,18] Therefore, an aligned substrate creates gaps and elongated spaces that can provide suitable channels for directed axonal growth, which is an extremely relevant feature for a nerve guide biomaterial. Taking this important feature into account, the incorporation of aligned bioactive glass fibers in a PCL matrix could greatly benefit nerve regeneration. So, this study aimed to develop a new type of "scaffold" for nerve repair by incorporating aligned bioactive silicate glass microfibers into a nanofibrous PCL membrane, which resulted in a novel composite with enhanced biological and mechanical properties. This biocomposite is potentially capable of facilitating nerve growth.

Materials and Methods

Glass Fiber Fabrication

For manufacturing the bioactive glass fibers, a brand new highly bioactive glass formulation was used, designated F18. This composition was developed by researchers of the Vitreous Materials Laboratory (LaMaV—Department of Materials Engineering, Federal University of São Carlos, São Carlos, São Paulo, Brazil) presenting itself as a reabsorbable bioactive glass in *in vitro* and *in vivo* studies.[19,20] The glass fibers fabrication process is described in detail elsewhere.[19,20] Briefly, this new composition belongs to the silicate system SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5 and the glass is prepared by melting the analytical grade raw materials at 1350°C in a platinum crucible, followed by crushing and remelting at 1350°C to provide homogenization. This new glass composition allowed the fabrication of continuous fibers with a precise diameter control by the downdrawing process. The downdrawing process is simple and widely known. For our laboratory scale production, 300 g of glass was placed into the apparatus's furnace and heated to approximately 1350°C, which is above the *liquidus* temperature. Then, the temperature was lowered, and the viscosity was adjusted to be approximately 10^3 to 10^4 dPa.s. Finally, the glass slowly drained from the crucible's nozzles forming continuous fibers. The obtained glass fibers had a mean diameter of approximately 20 μm.

Fabrication of the Nerve Conduit

For the development and characterization of the new nerve guide, two types of biocomposite samples were manufactured. The first sample type was a double-layered membrane form (one layer of electrospun PCL nanofibers upon a layer of aligned bioactive glass fibers—DL), whereas the second sample type was a tubular form with the aligned glass fibers in its interior.

For the manufacture of the double-layered biocomposite (DL), a PCL solution with concentration of 10 wt.% was prepared by dissolving PCL of 70,000 to 90,000 Mn (Sigma-Aldrich, St. Louis, MO) in acetone (Fisher Scientific, Leicestershire, UK). This solution was stirred for 2 h at 40°C for complete homogenization. Then, the polymer solution was electrospun (at room temperature) on the glass fibers on an aluminum collector, using a 10-mL syringe with a needle of 0.4 mm in diameter and a mass flow rate of 1 mL/h. A high voltage of 17 kV was applied to the tip of the needle attached to the syringe. After 4 h of electrospinning, the double-layered fiber mats (DL biocomposite) were cut in the desired shape for characterization.

For the fabrication of the tubular nerve guides, a rotating device with an aluminum wire with a 1 mm in diameter was used. Before the electrospinning process, the bioactive glass fibers were attached to the aluminum wire with a PCL solution (0.1 g PCL in 3 mL of acetone). The fibers were rapidly attached to the rod, wrapping its entire circumference. After the solvent evaporation, the set "wire + fibers" was placed in a rotating apparatus at approximately 70 rpm. The electrospinning process was carried out by the same processing parameters, namely a high voltage of 17 kV and a flow rate of 1 mL/h. To obtain approximately the same thickness of the PCL nanofiber layer in the DL membrane, the electrospinning process was carried out for approximately 1 h. After that period, the tube was removed from the aluminum rod and cut into the desired length.

The final double-layered membrane (DL) possessed approximately 50 fibers/mm^2 with a thickness of approximately 0.5 mm, being 0.3 mm of the PCL layer and 0.2 mm from the fiber layer. Figure 1 shows

a schematic diagram of the manufacturing process of the biocomposite conduits.

Characterization of the Biocomposites

For sample characterization, the double-layered membranes (DL biocomposite) were used because it is easier and more reliable to analyze the reactions that occur on a flat surface. A pure PCL electrospun nanofiber membrane (PCL membrane) and/or a mesh made with slightly woven glass fibers (BGF Mesh) were used as control groups.

Optical Microscopy and SEM Analysis: The morphology of the electrospun PCL nanofibers as well as of the whole biocomposite structure was evaluated using optical microscopy (Nikon Eclipse LV100N POL, Tokyo, Japan) and scanning electron microscopy (SEM) (FEG XL30; Philips, Eindhoven, the Netherlands). The diameter of the fibers was measured from SEM images using image analysis software (Image J; National Institutes of Health, Bethesda, MD).

Dissolution Tests: To analyze the rate of formation of the HCA layer on the new biocomposite, DL biocomposite membranes with an area of 10 × 10 mm were soaked in 25 mL of SBF-K9 solution, which was prepared according to the procedure proposed by Kokubo *et al.*,[21] and incubated *in vitro* at 37°C for different periods of time (i.e., 16 h, and 2 and 14 days). After each period, the samples were dried at room temperature for 24 h and subjected to X-ray diffraction (XRD) analysis (Ultima IV, Rigaku, Tokyo, Japan), data were recorded in the range of 10–60°. SEM images were used to analyze the changes in the glass fibers and in the PCL nanofibers morphology during these periods of incubation. These tests were conducted to determine whether the presence of PCL would affect the bioactivity of the glass fibers. The formation of HCA itself was not the aim of the study; however, the rate of its formation was of interest, because this would indicate whether a nonreactive phase (PCL) would cause a significant alteration in the bioactive glass fibers' response *in vitro*.

To analyze mass and pH changes over time, degradation tests were carried out according to a modified

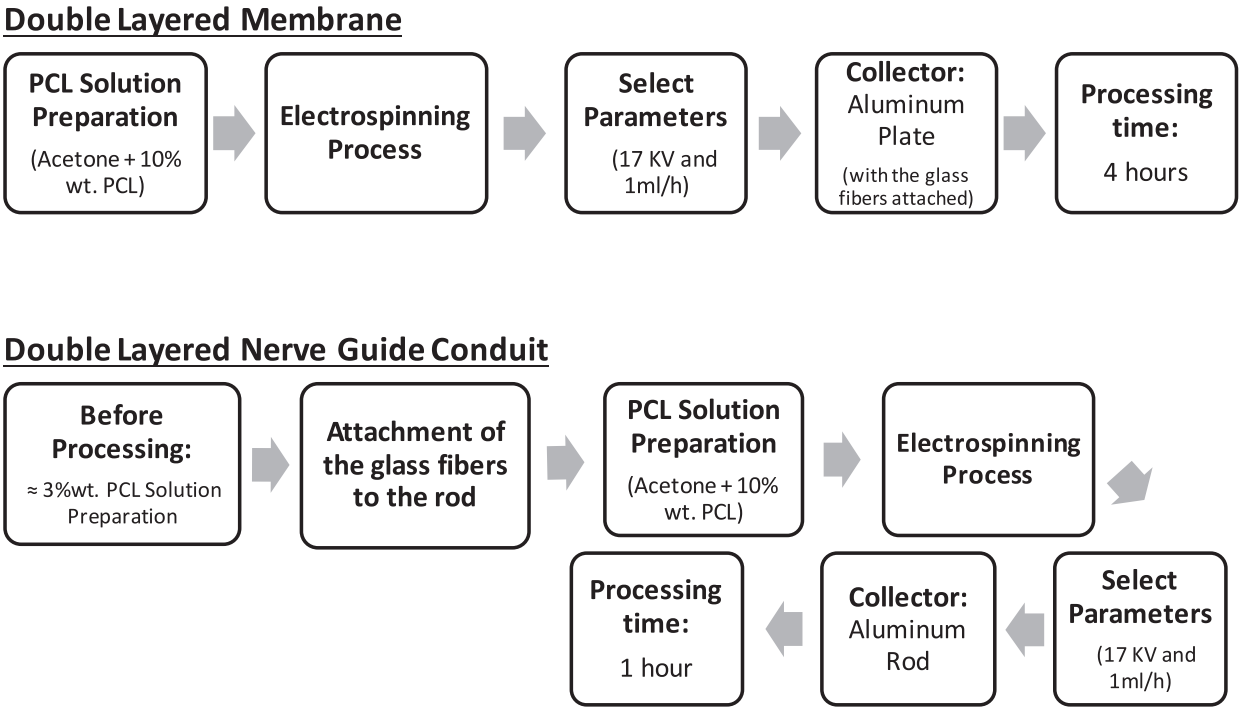

Fig. 1. Schematic diagram for the manufacture of the 2 types of composites: DL membranes and DL nerve guide conduits.

ISO standard 10993-14.[22] A Tris–HCl solution (ISO degradation solution) was used, and samples were incubated for 16 h and 2, 7, 14, and 21 days. After the soaking times, the samples were dried at room temperature for 24 h, and then, they were weighed. All these measurements were conducted in duplicate. After these analyses, ion-selective tests were performed to quantify ion release (Ca^{2+}, Na^{+}, PO_4^{3-}, and K^{+}) over time.

Mechanical Properties: The strength of the DL biocomposites was determined by a universal testing machine (Zwick, Ulm, Germany, DE) using a 50 N load cell at a crosshead speed of 5 mm/min at ambient conditions. All samples were prepared in a prismatic shape with dimensions of 40 × 5 × 0.5 mm. At least six samples were tested for each group (DL biocomposite, PCL membrane, and BGF Mesh), and the average value of the uniaxial tensile strength was reported with the standard deviation (±SD).[23]

Wettability: For the determination of wettability (or hydrophilicity) of the biocomposites, the contact angle was measured by a video contact angle system (DSA, Krünn, Aachen, Germany). The droplet size was set at 3 μL. Ten measurements were used for each group and the average value was reported with a standard deviation (±SD). The wettability of the biocomposite was also monitored after soaking the pure PCL and DL membranes in SBF solution for 16, 24, 48, and 96 h to track wettability changes over time.

Permeability Test: Water vapor permeability (WVP) tests were performed according to a modified ASTM standard test E96:95.[24] The membranes were fixed at the circular opening of a glass bottle (10 mm in diameter and 4 cm in height). Then, they were stored in a climatic chamber at 50 ± 1% relative humidity at 30 ± 1°C. The weight of the bottle with water was measured at different time intervals (24, 48, and 72 h) with a five-decimal precision scale (Shimadzu, Tokyo, Japan, AUW220D). The WVP of the DL biocomposite, the PCL membrane, and the BGF Meshes was calculated by the following equation:

$$\mathrm{WVP} = \frac{\Delta W}{A \times \Delta t} \quad (1)$$

where ΔW represents the change in the amount of the water weight, A represents the exposure area of the membrane, and Δt represents the exposure time.[24,25] For this test, a glass bottle without a membrane cover was used as control.

Results

Biocomposite Structure

The approach used in this study to produce a double-layered nerve guide that possessed aligned bioactive glass fibers in its interior is presented in the schematic diagrams of the 2 developed concepts (Fig. 1). Figures 2(a and b) show the obtained PCL electrospun nanofiber + bioactive glass fiber tube at different magnifications and Fig. 2(c) depicts the obtained alignment of the fibers in the double-layered (DL biocomposite) membrane.

SEM Analysis

Figure 3 shows an SEM image of a typical DL membrane (as prepared). The bioactive glass fibers were approximately 20 μm in diameter (SD ± 2.3 μm), and electrospun PCL fibers can be observed to be attached to them, forming a double-layered biocomposite. The PCL nanofibers had a mean diameter of 750 nm (SD ± 540 nm).

Dissolution Tests: SBF tests were conducted to determine the rate of HCA formation for the DL and PCL membrane samples. Specimens with and area of 10 × 10 mm were soaked in a SBF-K9 solution for 16 h, 2 and 14 days and were then analyzed by XRD and SEM analyses. As previously mentioned these tests were conducted to assess whether the presence of PCL would affect the rate of HCA formation of the bioactive glass fibers.

Figure 4 presents the XRD spectra for the PCL membrane before the SBF tests (as prepared) and for PCL membranes and DL biocomposites after 16 h soaked in SBF solution. The spectra confirmed the formation HCA phase only for the DL biocomposites. As reported in literature, the typical HCA peaks appear at ≈26°, ≈31° (covered peak) and at ≈46°[26–28]; while the two main peaks for PCL appear at ≈21° and 23°.[29]

SEM images obtained from the samples after soaking in SBF solution are shown in Fig. 5. As detected by XRD analysis, a pronounced precipitated HCA layer

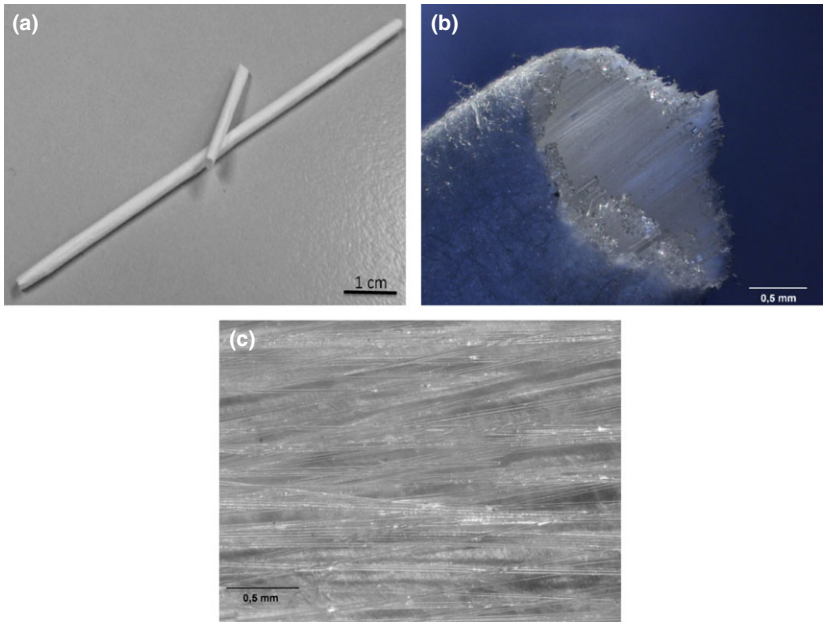

Fig. 2. (a) Double-layered nerve guides (DL) as prepared; (b) DL nerve guide with aligned fibers in its interior; and (c) aligned bioactive glass fibers in the interior of the nerve guide conduits.

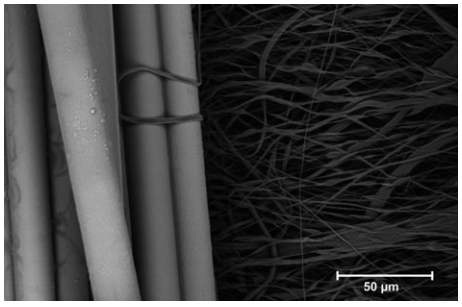

Fig. 3. SEM image of the interface between PCL nanofibers and bioactive glass microfibers in the double-layered biocomposites.

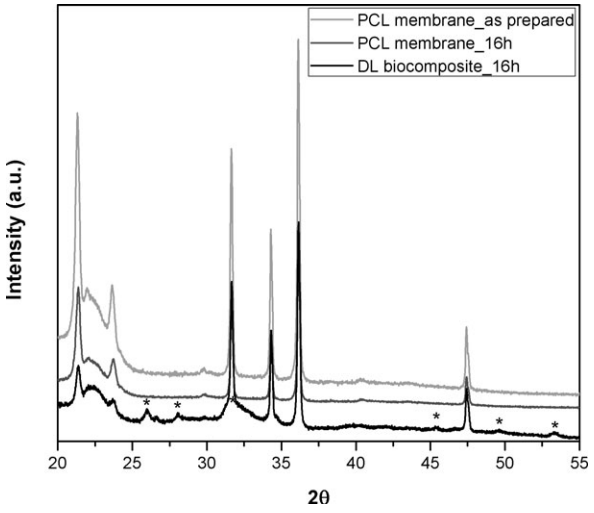

Fig. 4. XRD spectra of the PCL membrane (as prepared) and PCL membrane and DL biocomposite after in vitro studies in SBF solution for 16 h. HA peaks are market by "".*

on the surface of the bioactive fibers was observed after only 16 h in SBF solution, as shown in Fig. 5(a). The characteristic globular structure associated with this bioactive phase was present not only on the fiber surface but also connecting two or more bioactive glass fibers, especially after longer exposure times in SBF solution (Fig. 5b).

Degradation and weight loss over time in the ISO degradation solution are shown in Fig. 6. For the pure PCL membranes, a swelling caused by water absorption was observed as the samples had a weight gain of approximately 10% during the initial period and presented no significant weight change over longer incubation periods. For the DL biocomposites, the degradation of the glass fibers was more significant than the swelling process, which reflected in the decrease of the sample's weight with time. After 16 h, the DL

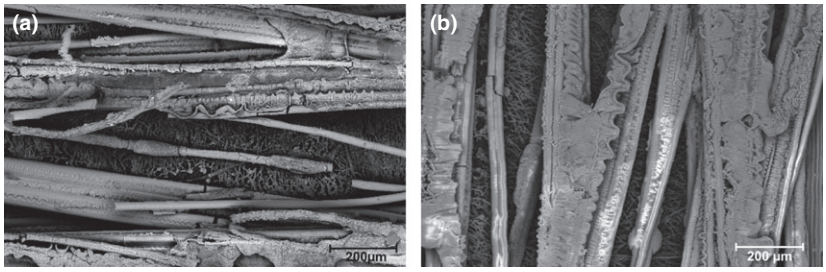

Fig. 5. SEM images of the DL biocomposite soaked in SBF solution for (a) 16 h; (b) 14 days.

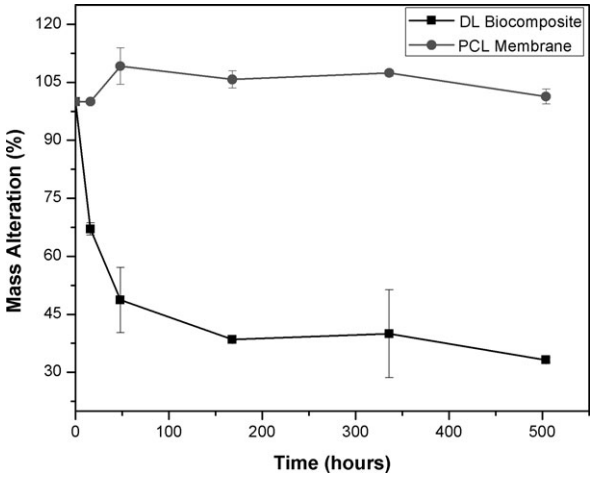

Fig. 6. Weight loss (%) of the pure PCL membrane and DL biocomposite samples in ISO degradation solution, showing a significant mass loss from the DL samples over time.

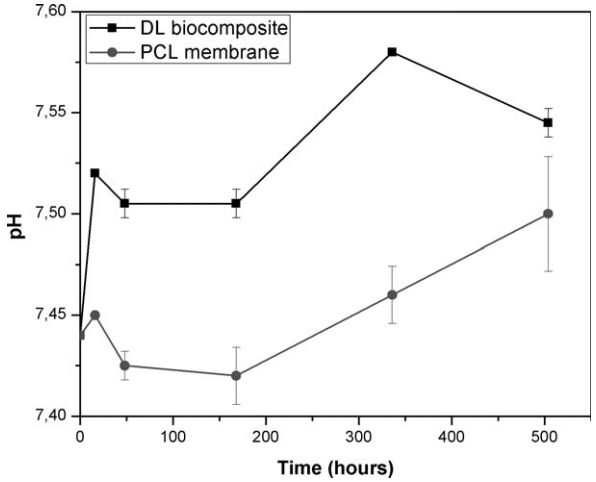

Fig. 7. pH variation over time in a ISO degradation solution, indicating a general increasing trend for both samples with different rates.

biocomposite sample lost more than 30% of its weight and after 21 days approximately 77% of the sample weight had been lost.

The variation of pH over time can be observed in Fig. 7. For the DL membranes, an increase in pH was observed, reaching a maximum value of 7.6 at day 14. For the pure PCL samples, a slight drop in pH was observed at first, and this was followed by an increase in pH during the final incubation period.

Ion-selective tests (Fig. 8) showed that the amount of Ca^{2+}, PO_4^{3-}, K^+, and Na^+ released into the ISO degradation solution was stable for the pure PCL samples in all experimental periods. However, for the DL samples, an increase in the amount of Ca^{2+}, PO_4^{3-}, and K^+ was observed. The quantity of ions reached a maximum value at 48 h and then decreased up to day 14; this ion release burst is well known and reported in literature.[30] The amount of Na^+ released remained constant at 115 mg/L for both samples and all experimental times, mainly to limitations of the chosen technique. For the DL biocomposites, the ionic concentrations of Ca^2, P, and K^+ showed a slight decrease for longer experimental times.

Mechanical Properties: Typical tensile stress–elongation curves for the pure electrospun PCL membrane, bioactive glass fiber mesh, and DL biocomposite are shown in Fig. 9.

The mean values for the maximum tensile strength and the maximum deformation, as well as their standard deviations, are presented in Table 1.

The final tensile strength achieved for the DL biocomposite membrane was 60 MPa with a maximal elongation of 10%, while the PCL electrospun fibrous membrane exhibited a tensile strength of 17 MPa and

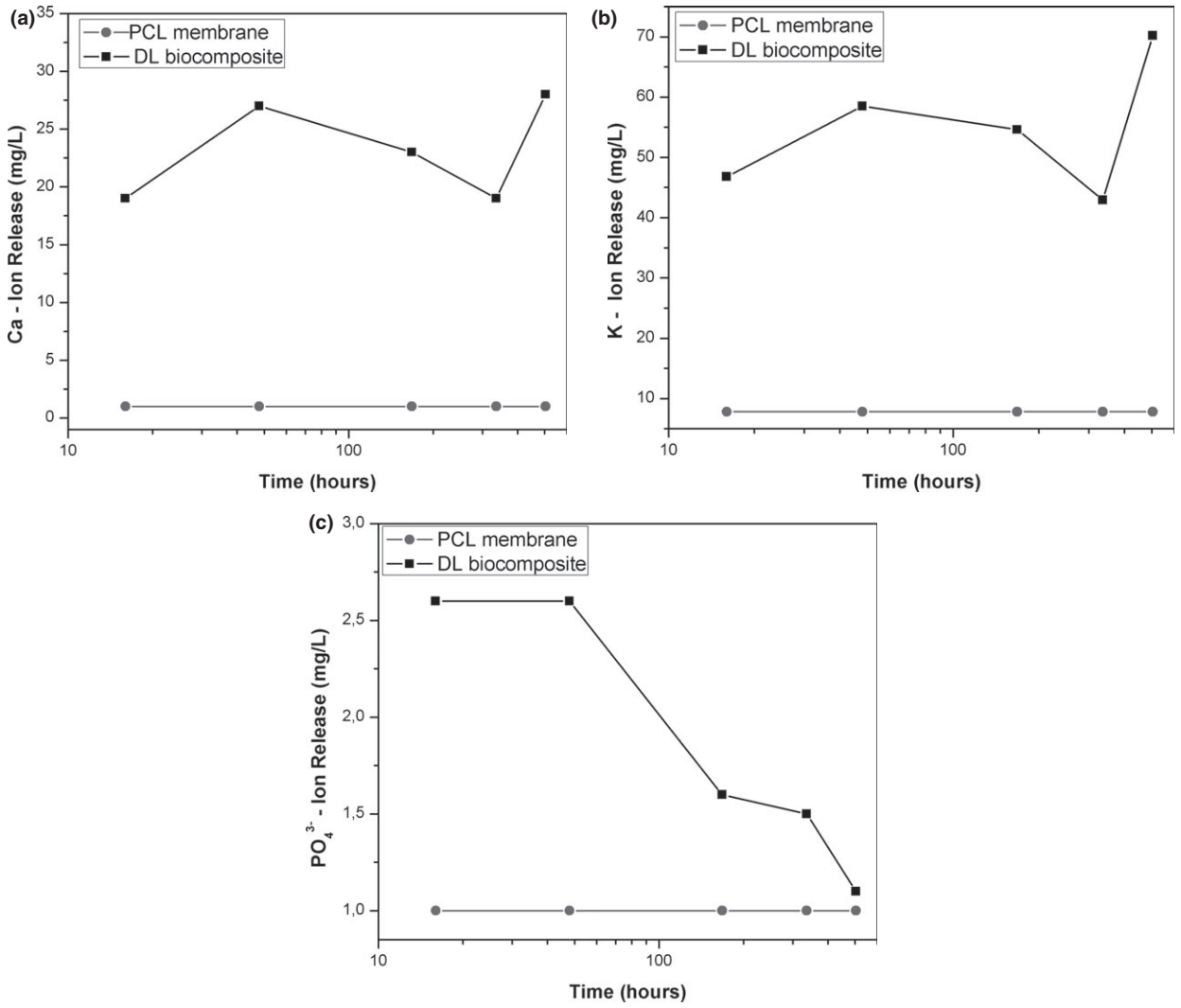

Fig. 8. Ion release vs time in ISO degradation solution for PCL membranes and DL biocomposites.

a maximal elongation of 50%. A *t*-test showed that the *P* value was <0.05 when these two groups were compared, but there was no significant difference when the DL biocomposite was compared to the bioactive glass fiber mesh sample (BGF Mesh).

Wettability: The hydrophobicity of the biocomposites was characterized by measuring the contact angle using water as the test liquid. The results are shown in Table 2.

The PCL membranes exhibited a decrease in the contact angle value with increasing soaking time in SBF solution. The DL biocomposite was tested for both layers, on the bioactive glass side no droplet was formed, as the water drop disappeared in less than a few seconds; therefore, the obtained value was considered zero. However, the PCL nanofiber side exhibited a large decrease in contact angle after 16 h ($\theta = 128°$ to 11°) and reached zero after 24 h.

Permeability: Water vapor tests showed that the control group (uncovered glass bottle) presented more intense water evaporation, as expected. However, the

trend in the permeability rate was similar for all samples (Fig. 10). A *t*-test and ANOVA showed that even though the control group presented a higher value of permeability, there was no significant differences between the groups for all experimental times investigated ($P > 0.05$).

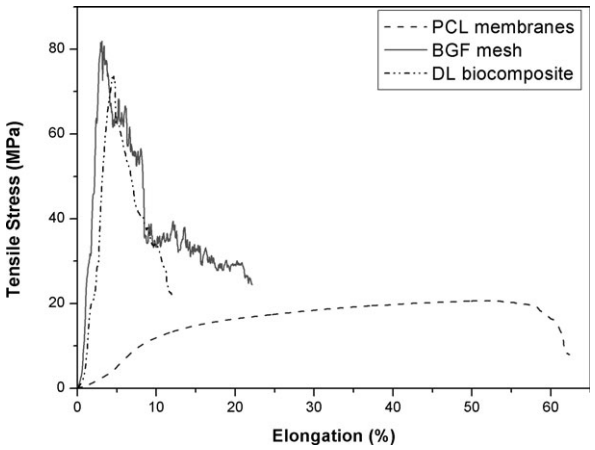

Fig. 9. Typical tensile stress–elongation curves for pure electrospun PCL membrane, BGF mesh, and DL biocomposite.

Table 1. Elongation at Break (%) and Tensile Stress for Pure PCL Membranes, BGF Mesh, and DL Biocomposite

Material	Mean elongation (%)	Mean tensile strength (MPa)
Pure PCL nanofibers	50 ± 22	17 ± 3
Bioactive glass fiber mesh	18 ± 4	59 ± 15
DL biocomposite	10 ± 2	60 ± 16

Discussion

Electrospinning is indeed a versatile technique to produce micro- and nanofibers.[31] The manufacture of fibers at nanoscale allows the production of structures that can mimic the extracellular matrix (ECM) of human tissues,[32] and the possibility of combining this technique with bioactive glass fibers to develop novel nerve guides exhibiting aligned inner structures, as proposed in this study, could have a huge impact on the further development of nerve regeneration strategies based on oriented fibrous scaffolds. As could be observed in Fig. 2(c), the glass fibers could be displaced in an aligned form and, as indicated by Ebental and Chew *et al.*,[14,15] this feature is relevant for a rapid nerve growth.

Our bioactivity tests with SBF-K9 solution demonstrated the rapid formation of an HCA layer on the surface of the constructs after only 16 h in SBF solution, as confirmed by SEM images and XRD analyses. The formation of an HCA phase is not necessarily an intended component of nerve regeneration, but it was a viable test to analyze whether PCL incorporation would affect the surface reactivity of the glass fibers by insulating them from the biological environment. The results indicated that after 16 h in SBF, the DL biocomposites could form the HCA phase on the bioactive glass fibers surface which was confirmed by the XRD (Fig. 4), indicating that the high bioactivity and solubility of the glass, as shown previously by Gabbai-Armelin *et al.*,[19,20] were not significantly affected by the incorporation of PCL.

Degradation properties are of crucial importance in biomaterial selection and design of nerve regeneration scaffolds. The rate of degradation may affect a range of processes, such as cell growth, tissue regeneration, and host response. Our results, using an ISO degradation solution, indicated that pure PCL membrane samples gained weight during the experimental period. This phenomenon is linked to water

Table 2. Contact Angle Results for the Biocomposites as a Function of Soak Time in SBF Solution

Material	As prepared	16 h	24 h	48 h	96 h
Pure PCL nanofibers	134.0 ± 0.1	134.5 ± 0.2	133.0 ± 0.2	51 ± 4	36 ± 4
DL biocomposite—glass fiber side	0	0	0	0	0
DL biocomposite—PCL nanofiber side	129.0 ± 0.2	11.0 ± 0.5	0	0	0

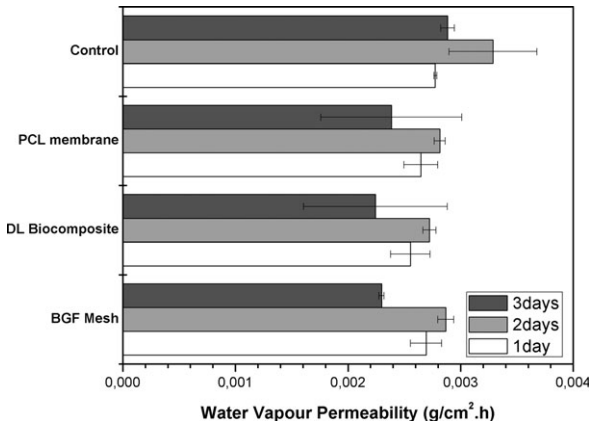

Fig. 10. Permeability rate for pure PCL membranes, DL biocomposite, BGF mesh, and the control group at 1, 2, and 3 days.

reabsorption, which is a mechanism that occurs during PCL hydrolytic degradation.[33]

It is generally accepted that the degradation of PCL in aqueous media occurs in two stages. In the first stage, water diffusion into the amorphous regions occurs, resulting in the hydrolytic scission of ester bonds. Several studies have indicated that the molar mass decreases continuously during this first stage of degradation, but there is no weight loss.[33–36] The second phase is characterized by the onset of weight loss due to the formation of low molar mass fragments that are small enough to diffuse out of the bulk.[34–37]

For the DL biocomposite samples, a significant weight loss was observed. This result was mainly linked to the glass fiber degradation, as approximately 75% of the initial sample weight was from the glass fiber layer. The mass loss and the mechanisms of the formation of the HCA in bioactive glasses have been widely discussed in the literature.[38–41] An important phenomenon that occurred in the DL biocomposite samples was that the release of alkaline ions from the glass fibers into the medium created a catalytic effect on the degradation of the PCL matrix. The effect of OH^- anions and an alkaline pH on the degradation of aliphatic polyesters has been widely described in the literature[42–44] and may have contributed to the greater weight loss observed for these samples (DL biocomposites). According to Loh,[44] OH^- can cleave the ester bonds in the PCL chains resulting in faster hydrolysis and accelerating the polymer degradation.

However, the greater mass loss for DL biocomposites did not cause any alteration to the sample form, and as the glass fiber is highly reabsorbable, the material is designed to stimulate the early stages of tissue regeneration and then be gradually replaced by the newly formed host tissue.

The alteration of pH over time for the DL samples was due to the degradation of the bioactive glass fibers, because they leach ions, such as Na^+ and Ca^{2+} into the medium. The value decreased after 48 h probably due to the precipitation of calcium phosphates and the formation of HCA, thereby consuming the alkaline ions and decreasing the pH. For the pure PCL membrane samples, a slight drop in pH value was observed because the polymer degradation led to acidic byproducts; then, this acid gradient was buffered by the surrounding medium, slightly increasing the pH again.[36]

The ion release results indicate that Ca^{2+} and PO_4^{3-} ions were promptly released from the glass to the solution and that they were rapidly consumed for the formation of the HCA layer. This trend has also been observed in other studies and is a well-known phenomenon related to the HCA precipitation.[30,45,46] Although changes in sodium release have not been observed, perhaps because of the chosen analysis method, the potassium release rate can be taken as a parameter of the overall ion release from the glass as well, as its concentration is also not considerably affected by apatite precipitation, as described by Groh *et al.*[45]

Regarding mechanical strength, the present results indicate that the addition of the bioactive glass fibers restricted the flexibility and elongation of the PCL matrix, limiting its plastic deformation. This result would be expected from any composite in which a rigid ceramic phase had been added to a polymer matrix.[47] At the same time, the DL biocomposite tensile strength could be tripled ($P < 0.05$ according to a *t*-test for both properties). This feature is important for a nerve guide, as the tube has to maintain a satisfactory flexibility and, at the same time, it should be strong enough to support the surgical and suturing procedures.

Another interesting feature of the present constructs is that the addition of the glass fibers could help to control the swelling effect of the polymer by restricting its enlargement. Regarding wettability, it is known that PCL is highly hydrophobic, and the addition of an alkaline source or incubation in alkaline medium can increase hydrophilicity.[48–50] The addition of bioactive glass particles to a PCL matrix has also been

reported to increase the hydrophilicity of PCL films by affecting its hydrolysis rate.[44–46] This improvement of wetting behavior affects PCL biodegradation and can lead to a superior biointeraction with the biological environment *in vivo*. Our results showed that the incorporation of bioactive glass fibers could dramatically reduce contact angle, even in the samples that were not immersed in SBF solution. These results could be explained by the macro roughness created by the aligned fibers. The fibers created water flow channels that dispersed the water drop almost instantly. Furthermore, the addition of the fibers could reduce the hydrophobicity of the PCL layer in the DL biocomposite after some hours of soaking in SBF solution. The high pH achieved locally by the degradation of the glass fibers created a catalytic environment for PCL matrix degradation, and in only 16 h, the hydrophobicity of the PCL decreased by more than 90%. This effect can improve the interaction between the polymer and the host cells, facilitating the tissue regeneration process.[51,52]

Permeability tests revealed that the developed DL biocomposite is permeable to water vapor. This feature is important because an ideal nerve guide should allow the exchange of cell growth factors and excretions between the inner portion of the nerve guide and the medium. Additionally, nerve guides should ideally have a semi-permeable structure that can prevent access of different cells to the regenerating nerve region. Blocking the infiltration of inflammatory cells into the lumen of the nerve guide is a critical step for the regeneration of this type of tissue.[53]

Conclusion

The incorporation of bioactive glass fibers into a PCL fibrous matrix permitted the manufacture of a more reactive biocomposite with better mechanical properties when compared with those of the polymer alone. The fabrication of the nerve guide using electrospinning led to a double-layered tube, with bioactive glass fibers aligned in its interior and PCL electrospun nanofibers on its exterior. Initial characterization indicated that the presence of the glass fibers improved PCL wettability, increased construct bioactivity (as the fibers are highly reactive), and tripled the tensile strength without affecting the nerve guides flexibility, which is expected to facilitate handling during any surgical procedure. These preliminary tests have demonstrated promising results for application of the novel bioactive composite containing glass fibers as nerve conduits. Therefore, plans for future work include *in vitro* and *in vivo* studies.

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