Influence of diameter of fiber membrane scaffolds on the biocompatibility of hPDL mesenchymal stromal cells

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This study evaluated the influence in the biocompatibility of human periodontal ligament (hPDL) mesenchymal stromal cell onto poly lactic-acid (PLA) films and PLA fiber membrane. Fiber scaffold was prepared via air jet spinning (AJS) from PLA solutions (6, 7, and 10%) and analyzed using SEM, AFM and FTIR. Biocompatibility was evaluated by adhesion, proliferation and cell-material interaction. PLA film exhibited a smooth and homogeneously surface topography in comparison with random orientation of PLA fiber with roughness structure where diameter size depends on PLA solution. Moreover, cell adhesion; proliferation and cell-material interaction has the best respond on random orientation nanofiber of 10, followed by 7, and 6% of PLA in comparison with PLA films. It could be concluded that AJS is an attractive alternative technique for manufacture fiber scaffolds with a tunable random orientation geometry of fibers that allow to produce interconnected porous formed by nanometric fiber diameter structures that could be a potential scaffold for periodontal tissue engineering applications.

Keywords: Biocompatibility, Nanofibers, Periodontal ligament, Cell viability, Biomaterials

INTRODUCTION

Tissue engineering is a novel approach that is emerging as an alternative to regenerating tissues lost or damage by trauma, congenital anomalies, or chronic diseases1-5. In dentistry, the main concern is to attempt to regenerate lost tissue caused by periodontal disease (PD)6-9. PD is considered one of the most destructive dental pathologies and as a chronic inflammatory disorder. It is characterized by the collection of dental biofilm on the tooth surface, which affects the integrity of the periodontal system that maintain the tooth in the alveolar socket, leading to damage and destruction of periodontal tissues and it may cause tooth loss and several associated health problems6. A variety of tech-scaling approaches have been employed for periodontal therapy, e.g., scaling and root planning, subgingival scaling, gingivectomy and full or split thickness flap procedures with or without osseous recontouring7,8. Furthermore, several strategies for periodontal tissue regeneration have been developed in the past few decades and include soft tissue grafts, bone replacement grafts, root surface biomodifications, and guided tissue/bone regeneration9-11. However, it was not until the recently that experimental approaches for tooth regeneration have been made possible by advances in cell biology and engineering, in which many different biomaterials are currently available for periodontal tissue regeneration, including bioactive ceramics, composites and allo- or autografts that are utilized for designing biomatrices12-15. These biomatrices or biological scaffolds are currently acquiring relevance in periodontal regeneration because they provide the anatomic dimensions of temporary matrices in which cells synthesize tissues, thus playing important, if not indispensable, roles in tooth regeneration16. Furthermore, the most important role of this biomatrices scaffold in tissue regeneration comprises mimicking the native geometry of the extracellular matrix (ECM) of tissues. For example, the natural ECM of PDL tissue has a specific geometrical random architecture with nanofibrous structures that function as natural supportive platform that provides biophysical and mechanical support for cell recruitment, adhesion, proliferation, differentiation, and/or metabolism for the fibroblasts or mesenchymal stromal stem cell of PDL tissue11,15,16. Thus, new geometry is frequently taken into consideration during the scaffold design process, because there is an information arising from the inherent structure geometry of ECM that often receives less attention. This geometrical feature that are on the length scale at which a cell interacts with its substrate that is, on the order of sub-microns or nanometers is referred to as matrix microarchtecture17. Thus, many methods have been developed to synthesize scaffolds for tissue engineering applications; however, only a limited number of methods can generate the nanoscale...
features necessary to mimic the ECM microarchitecture. Electrospinning is one nanotechnological approach that could reproduce the physical properties of the ECM; including; porosity, nanofiber diameter, geometry (random or aligned) and composition, however this technique is expensive and represents certain risks, due to the use of high voltage\(^{19,20}\). One emerging technique that could provide good results in tissue engineering is the novel air jet spinning (AJS), which consists of a commercial available airbrush, commonly used for paint, connected to a gas supply and any surface that could be painted. This new technique is easy to use, less expensive, and saving that it does not need applied high voltage to produce fiber spun membranes. The AJS technique has been utilized for synthesized micro- or nano- fiber scaffolds with a broad range of polymers, such as polystyrene, polymethyl methacrylate, polycaprolactone or poly lactic-acid (PLA)\(^{19,20}\). In order to know whether the synthesized fibrous membrane of PLA via AJS technique are good candidates as biomatrices for periodontal therapy that could mimic the natural geometry and sizes of ECM microarchitecture of PDL tissue, the adhesion, proliferation and cell material interaction respond of human periodontal ligament (hPDL) mesenchymal stromal cells were evaluated.

**MATERIALS AND METHODS**

**Materials**

PLA pellets (C\(_{3}\)H\(_{6}\)O\(_{3}\); MW 192,000) were purchased from Nature Works, Minnetonka, MN, USA. Chloroform (CHCl\(_{3}\)) and anhydrous absolute ethyl alcohol (CH\(_{3}\)CH\(_{2}\)OH) were supplied by J. T. Baker, Ecatepec, Mexico. All products were utilized as received without further purifications. Three organic solutions with 6, 7, and 10 %wt of PLA, respectively, were prepared as follows: dissolved in CHCl\(_{3}\) and stirred for 20 h and subsequently CH\(_{3}\)CH\(_{2}\)OH was added and the solution was submitted to stirring for up to 30 min to obtain homogeneous solution (polymeric solution). The volume ratio of chloroform/ethanol was 3:1.

**Preparation of films and spun membranes**

Fibrous spun scaffolds were produced via AJS process from PLA polymeric solutions (6, 7, and 10%). In all cases, the polymeric solution was placed in a commercially available ADIR model 699 airbrush with a 0.3 mm nozzle diameter and with gravitational feed of the solution to synthesize the fiber membrane scaffold. The airbrush was connected to a pressurized argon tank (CAS number 7740-37, concentration >99%, PRAXAIR México, CDMX, Mexico) and for fiber deposition, a pressure of 35 psi with a 15 cm distance from the nozzle to the target was maintained constant. Optimized parameters of an 8 mL volume of a polymer solution rate with respect to 30 min of time were performed according to previously described protocols\(^{22}\).

For PLA films, the polymeric solution was cast on a polytetrafluoroethylene (PTFE) disc and the solvent used was dried at room temperature in a dark place and under hood during 24 h in static condition.

**Airbrushed fiber scaffold characterization**

Chemical structure of fibers was analyzed by Fourier transform infrared spectrophotometer (FTIR) employing an IRAffinity-1S (Shimadzu, Kyoto, Japan) within the 400–4,000 cm\(^{-1}\) range. To determine the surface topography of PLA of film and membrane spun mat atomic force microscopy was used with an auto-Probe with ultrasharp silicon cantilever (NSC15, µMasch, CA, USA) in tapping mode (JSP-4210 JEOL, Tokyo, Japan). The morphology and structure of the fibers were observed with a scanning electron microscope (SEM) (Cambridge-Leica Model Stereoscan 440, Leica, Cambridge, UK). Fiber diameter was measured from SEM micrographs employing image Analysis software (Image J. National Institutes of Health, Rockville, MD, USA).

**Isolation and culture of hPDL mesenchymal stromal cells**

hPDL mesenchymal stromal cells were obtained from premolars extracted for orthodontics reasons. hPDL mesenchymal stromal cells biopsy specimens were obtained from healthy subjects (aged 18–25 years) without systemic and oral diseases who signed an informed consent form prior to specimen collection. hPDL mesenchymal stromal cells were removed along the surface using a sterilized surgical blade and digested utilizing trypsin 0.25% for 20 min at 37°C. Cell suspensions were treated with fresh medium to stop trypsin action, were then centrifuged at 5,000 rpm for 5 min; the supernatant was discarded and fresh medium was added, this was resuspended and placed onto 24 well culture plates in order to attach the tissue, and was then cultured in alpha Minimal Essential Eagle Medium (α-MEM) supplemented with 10% Fetal Bovine Serum (FBS, Sigma-Aldrich, St Louis, MO, USA) and 100 µg/mL Penicillin/Streptomycin at 37°C in a humidified atmosphere with 5% CO\(_2\). After 48 h, the cell culture medium was changed to remove non-adherent cells. Afterward, the remaining cells grew as a small cluster after approximately 15–20 days in culture. After this time, the cells were trypsinized and passed into a T25 flask. After the culture had reached confluence at between 2 and 4 weeks, the cells were characterized as described later.

**Morphological analysis**

To identify the morphology of mesenchymal stromal cells obtained from periodontal ligament, second-passage cells were grown in a Petri dish (Corning, Monterrey, Mexico) at a density of 4,000 cells/cm\(^{2}\). After 6 days of culture, cells were stained with Toluidin Blue (Sigma-Aldrich) and examined under a phase-contrast microscope.

**Characterization of hPDL mesenchymal stromal cells**

Immunophenotypic characterization and differentiation capacities of hPDL mesenchymal stromal cells were performed according to previously described protocols\(^{22}\). FITC, PE, or APC-conjugated monoclonal antibodies against CD73, CD90, and CD45 (BD Biosciences,
San Diego, CA, USA), CD105, CD13, CD14, (Caltag, Buckingham, UK), and HLA-ABC, HLA-DR, CD31, and CD34 (Invitrogen, Carlsbad, CA, USA) were employed for flow cytometric analysis of cultured hPDLSC. A total of 1–2×10^5 cells, previously blocked with Fc receptor blocker (Human Blocking Reagent; Miltenyi Biotec, San Diego, CA, USA), was resuspended in 100 mL of Phosphate-Buffered Saline (PBS) with 3% FBS and 1mM EDTA, and these were incubated with the appropriate antibodies for 20–30 min. The cells were subsequently washed with 1 mL of PBS (with 3% FBS and 1 mM EDTA) and fixed with FACS Lysing Solution (BD Biosciences). The labeled cells were analyzed in a Coulter Epics Altra Flow Cytometer (Beckman Coulter, Brea, CA, USA), and at least 10,000 events were collected per sample. The data were analyzed with FlowJo ver. 2.6 software (FlowJo LLC, Ashland, OR, USA).

Adipogenic and osteogenic differentiation was induced with Stem Cells KitsTM (Stemcell Technologies, Vancouver, BC, Canada). Adipogenic differentiation was determined by visualizing the presence of Oil Red O-stained (Sigma-Aldrich) lipid vacuoles. Osteogenic differentiation was assessed by alkaline phosphatase staining. Chondrogenic differentiation was induced with a commercial induction medium (Cambrex Bio Science Walkersville, MD, USA) that was supplemented with 10 ng/mL of transforming growth factor beta (TGF-β, Cambrex). The resulting micromasses were fixed, embedded and sliced. Cross-sections were stained with Alcian blue dye (Sigma-Aldrich).

Processing scaffold for cell seeding
Prior to the biological assays PLA spun fiber scaffolds and PLA films were cut into a round shape (8 mm of diameter), placed in 24 cell culture plates and sterilized by immersion in 70% of ethanol (v/v) with antibiotic solution (streptomycin 100 µg/mL and penicillin 100 U/mL) for 30 min. After sterilization, nanofiber scaffolds were rinsed with PBS, distilled water three times, and air-dried.

Adhesion assay
hPDL mesenchymal stromal cells were seeded at 1×10^4 cells/mL onto films and fiber spun scaffolds, placed in 24 well culture plates and allowed to adhere in standard cell culture for 4 and 24 h. After the prescribed time period, substrates were rinsed three times employing PBS to remove non-adherent cells. Evaluation of cell attachment was performed according to crystal violet assay. Briefly, adherent cells were fixed with 4% paraformaldehyde and incubated with 0.1% crystal violet solution for 15 min. The dye was extracted with 0.1% of sodium dodecyl sulfate (SDS) and optical absorption was quantified by spectrophotometry at 550 nm with a plate reader (ChroMate, Awareness Technology, Palm City, FL, USA). Conventional polystyrene 24-well culture plates were used as control.

MTT assay
The cell viability of hPDL mesenchymal stromal cells plated at a concentration of 1×10^4 cells/mL in triplicate onto PLA films and fibrous spun scaffolds were checked by MTT assay for 3, 5, and 7 days of culture. This assay is based on the ability of the mitochondrial dehydrogenases of living cells to oxidize a tetrazolium salt (3-[4, 5-dimethylthiazolyl-2-y]-2, 5-diphenyltetrazolium bromide), into an insoluble blue formazan product. The concentration of the blue formazan product is directly proportional to the number of metabolically active cells. hPDL mesenchymal stromal cells seeded onto films, fiber mat scaffolds of PLA and control cultures at the prescribed times were washed with PBS and incubated with fresh cultured medium containing 0.5 mg/mL of MTT for 4 h at 37°C in the dark. Then, the supernatant was removed and dimethyl sulfoxide (DMSO) was added to each well. After 60 min of slow shaking, absorbance was quantified by spectrophotometry at 570 nm with a plate reader. Conventional polystyrene 24-well culture plates were used as control. During the experimental time, the culture medium was exchanged every other day with fresh media.

Cell morphology
The cell morphology and the cell spreading pattern interaction of hPDL mesenchymal stromal cells seeded at 1×10^4 cells/mL onto films and PLA nanofiber scaffolds were examined by using field emission scanning electron microscopy (FE-SEM 7600F, JEOL) after 24 h of cell culture. At the end of the incubation time, scaffolds were washed three times with PBS, fixed with 4% formaldehyde for 1 h and then dehydrated with a graded series of ethanol (25–100%) and air dried. The samples were sputter-coated with a thin layer of gold-palladium and examined by FE-SEM.

Statistical analysis
All data are presented as means with standard deviation (SD). For the purpose of this work, an SD is the combined standard uncertainty of the mean. To evaluate for statistically significant differences, one-way analysis of variance (ANOVA), with Tukey’s test were employed for comparison of 3 or more conditions (p<0.05).

RESULTS
Characterization of PLA spun membrane scaffold
Representative SEM micrographs of spun PLA fibers showed that the morphologies and diameters of fibers in each sample of PLA membrane were nearly uniform, with good interconnected fibers that formed interconnected porous and without the presence of beads, using 35 psi with a 15 cm distance from nozzle. Under these conditions of synthesis, the average fiber diameters of PLA membrane obtained depended on the composition of the solution utilized for spinning. At a 6% PLA concentration, the average fiber diameter was 0.880±0.53 µm. For the 7% PLA concentration, this was 0.675±0.26 µm, and for the higher PLA polymer concentration (10%), the average diameter was 0.704±0.29 µm (Fig. 1). Moreover the interconnected fiber spun mat were 3.4±0.7 µm for 6% PLA; 2.6±0.8 µm for PLA 7% and 3.4±1.2 µm
Fig. 1 Scanning electron microscopy micrograph of the AJS spun membrane mats and the histogram of fiber diameter estimation via the imaging analysis of (A) PLA 6%, (B) PLA 7% and (C) PLA (10%). Scale bar=10 µm.

for PLA 10%, that is a estimated interconnected porous diameter of the synthesized fiber membranes by AJS.

Figure 2 depicts the typical PLA absorption peaks at 1,740, 1,183, 1,148, and 1,074 cm\(^{-1}\) of the functional groups, which represent the backbone ester group of PLA identified by FTIR spectroscopy. After spinning the polymeric solution at different concentrations (6, 7, and 10%), these peaks are clearly observed in the spectra.

Figure 3 showed the representative surface topography of the PLA films and fiber scaffold analyzed by AFM where could be seen differences on the surface of both scaffolds. In PLA films AFM image showed a smooth and homogeneously surface topography with a roughness of 0.042±0.019 µm in comparison with fiber interconnected network in random orientation and nanotextured surface of PLA fiber scaffold with a roughness of 0.273±0.122 µm.

Characterization of hPDL mesenchymal stromal cells

hPDL mesenchymal stromal cells isolated from periodontal ligament tissue demonstrated that cells were adherent in serum-containing growth medium exhibited
the typical characteristic morphology of multipotent mesenchymal stromal cells, such as fibroblast-like morphology, colony-forming efficiency and were successfully expanded ex vivo (Fig. 4A). Additionally, hPDL mesenchymal stromal cells showed positive staining and expressed specific mesenchymal stromal cells (MSC) surface markers such as CD73, CD90, CD105, and CD13, and negative staining for hematopoietic surface markers CD14, CD34, CD45, CD31, and HLA-DR. Furthermore, hPDL mesenchymal stromal cells
exhibited high expression of the histocompatibility surface marker HLA-ABC, as illustrated in Table 1.

The differentiation potencies of hPDL mesenchymal stromal cells were evaluated with appropriated inductive cell culture media. Results disclosed that under differentiation culture conditions, hPDL mesenchymal stromal cells revealed Oil Red O positive lipid droplet accumulations for adipogenic commitment (Fig. 4B); positive staining for alkaline phosphatase for osteogenic commitment (Fig. 4C) and positive staining for Alcian blue as chondrogenic-like matrix commitment (Fig. 4D).

**Biocompatibility assay of hPDL mesenchymal stromal cells onto PLA nanofiber scaffolds**

To investigate the influence on the biocompatibility of smooth and homogenously surface of PLA films and nanofiber with random orientation of PLA membrane scaffolds MTT and colorimetric adhesion assay were evaluated. The adhesion assay allows observing the behavior of hPDL mesenchymal stromal cells on PLA films and PLA nanofiber membranes at 4 and 24 h after cell seeding (Fig. 5). Our results showed significantly differences in the cellular attachment ability of hPDL mesenchymal stromal cells between the PLA scaffolds. After incubation time of 4 and 24 h, the attachment of hPDL mesenchymal stromal cells was different among the concentrations (6, 7, and 10%) of PLA films and PLA nanofiber scaffolds. hPDL mesenchymal stromal cells were found to attach more readily and efficiently to 10% of PLA nanofibers scaffolds at 4 and 24 h of incubation time, followed by 7 and 6% of PLA fibrous spun mats, when compared with PLA films scaffolds ($p<0.05$). However, when these differences focused on investigate the effect of nanotopography structure onto cell adhesion, our results showed significantly more hPDL mesenchymal stromal cells adhered to the spun nanofibrous mat than to the flat surface of films, suggesting that the diameter, size and the interconnected fibers spun mat showed greater advantages in improving cell response of hPDL mesenchymal stromal cells.

While it is noteworthy that cell adhesion response obtained on PLA nanofiber scaffolds is a good indicator that the surface is not toxic; the MTT assay was carried out to evaluate hPDL mesenchymal stromal cells viability onto films and spun fiber of PLA scaffolds. The MTT assay involves a reduction reaction that reduces the MTT reagent to formazan when incubated with viable cells, providing information on cell growth and the metabolic activity of cells. Thus, the level of MTT reduction to blue formazan can reflect the level of cell metabolism. Figure 6 demonstrates the time course of blue formazan accumulation for hPDL mesenchymal

### Table 1 Immunophenotypic characterization of surface markers of hPDL mesenchymal stromal cells expressed in percent

| Surface markers | Percent |
|-----------------|---------|
| CD73            | 98.5    |
| CD90            | 99.5    |
| CD105           | 97.8    |
| CD13            | 97.6    |
| CD14            | 0.4     |
| CD34            | 0.2     |
| CD45            | 0.3     |
| CD31            | 0.2     |
| HLA-ABC         | 44.5    |
| HLA-DR          | 3.1     |

Fig. 5  Cell adhesion response of hPDL mesenchymal stromal cells seeded onto the surface of PLA films and nanofiber mats at different concentrations (6, 7, and 10%) at 4 and 24 h of culture. Tissue culture plate (polystyrene) were used as a control.

Fig. 6 MTT cell viability measurement of hPDL mesenchymal stromal cells seeded onto the surface of PLA films and nanofiber mats at different concentrations (6, 7, and 10%) at 3, 5, and 7 days of culture. Tissue culture plate (polystyrene) were used as a control.
stromal cells viability onto films and spun fiber membranes of PLA scaffolds after 3, 5, and 7 days of culture. We found when comparing between scaffolds, the MTT value indicated that the growth rate were superior onto PLA spun fiber mats, indicating a good cytocompatibility condition. Although MTT conversion of cells cultured on all PLA scaffolds also indicated an increase in cell viability throughout the culture time, it is notice that cell density increased over time onto fiber surfaces, indicating a best response behavior on PLA concentrations of 7 and 10% followed by 6% spun mat.

**Morphology observation of hPDL mesenchymal stromal cells cultured onto PLA scaffolds**

Cell morphology and cell-material interaction of hPDL mesenchymal stromal cells onto films and spun fiber mats of PLA scaffolds were examined by FE-SEM after 24 h of cell seeding (Fig. 7). hPDL mesenchymal stromal cells appear more homogeneously distributed and spread well on the nanofiber spun mats of PLA when comparing with PLA films scaffolds in agreement with the quantitative adhesion assay reported in Fig. 4. The morphology of hPDL mesenchymal stromal cells showed an extended and elongated shape, exhibiting a more flattened morphology with filopodia contacts in proximity with the fiber surface that made able to adhere to the fibers and colonize the interconnected fibers of the scaffold, which confirm highest tendency of cells to spread on the substrates nanosurface; in comparison with less spreading and less elongated morphology showed by the hPDL mesenchymal stromal cells morphology onto PLA films scaffolds.

**DISCUSSION**

For evaluating, the ability for periodontal application of PLA spun fiber mats, different samples were generated by AJS based on 6, 7, and 10% of polymeric solution. The AJS technique allows the easy synthesis of fiber scaffolds from the experimental polymer solutions employing a commercially available airbrush with a 0.3 mm nozzle diameter. From the surface images (Figs. 1 and 3), it could be observed that fibers and the interconnected fibers of the spun mat has a geometry with randomly assembled and, the large spaces formed among the fibers comprised nanotextured surface features. These data are consistent with previous reports, which found a similar average diameter and reported this condition for producing nanofiber spun mats, depending on the control of several conditions, such as solvent evaporation, polymer molecular weight, solvent concentration, gas pressure, and nozzle diameter as critical parameters and as an important key for obtaining scaffold surfaces with topographical characteristics for application in tissue engineering. Furthermore, the characterization of the chemical structure of the PLA spun fibers mats by FTIR spectroscopy demonstrated that after the spinning process, the polymer does not undergo any change in its composition.

For periodontal applications and the therapeutic approach, mesenchymal stromal cells are becoming a promising tool for regenerative strategies. In the present study, hPDL mesenchymal stromal cells from periodontal ligament tissue were isolated as tools for exploring the biocompatibility of fiber spun membranes for periodontal tissue engineering application. Data showed that we were successful in isolating a population of hPDL mesenchymal stromal cells, which
exhibited typical multipotent MSC characteristics. Moreover, all of our results demonstrated the potential to differentiate into several phenotypes. Additionally, this is combine with their ease of access for isolation from adult teeth via routine extraction; rendering hPDL mesenchymal stromal cells as a good source for potential use in periodontal tissue regeneration as autologous mesenchymal stromal cell application in biomaterial characterization\textsuperscript{30,37}.

In clinical periodontal therapy, it has been demonstrated that the treatment option through the utilization of the membrane scaffold is viable. For the therapy, the scaffolds must meet certain requirements, such as specific geometry architecture similar to that of the ECM periodontal microenvironment, which permit tissue integration, cell attachment for attracting desirable cells, and maintenance to allow new attachment formation and, more importantly, biocompatibility for ensuring material safety\textsuperscript{10,28}. In addition, nanomaterials such as nanofibers could mimic the microarchitecture, which not only provides mechanical support for cellular interactions, but also has a direct impact on cellular response\textsuperscript{29}. Moreover, nanofabrication technology has been proven to be a powerful tool to develop geometric and topographical features for mimicking the natural architecture of the ECM environment. Micro- and nanoscale topographical cues can be used to precisely control adhesion, morphology, migration, and differentiation in a wide variety of cell types ranging from fibroblasts to MSCs, as well as be applied to measure cell generated forces\textsuperscript{30}. On considering the latter, we thus conducted preliminary experiments to manufacture a topographical fiber scaffold with random orientation geometry based on the AJS technique as a potential biomatrices for \textit{in situ} periodontal therapy. In our study, we were able to synthesize a nanofiber scaffold that could mimic the fibrillar architecture geometry of the ECM of periodontal tissue, and our preliminary evaluation of biocompatibility revealed that fiber spun membranes exhibit good response of hPDL mesenchymal stromal cells as compared with smooth materials (Figs. 5 and 6). This could be attributable to the nanofiber morphology with a random geometry that allow generated an interconnected fibrillar porous, which may be an advantageous microenvironment and microarchitecture that hPDL mesenchymal stromal cell recognize as an excellent and beneficially interfacial topographical cue to maintain a 3D morphology and form stronger focal adhesions comparing with smooth materials\textsuperscript{29-31}. Moreover, our results showed that, during the experimental period of viability, PLA scaffolds did not induce any cytotoxic effects on the behavior of hPDL mesenchymal stromal cells indicating that there is no evidence of any influence from the solvent residues after synthesis of the biomatrices. From the images (Fig. 7), it could be suggested that PLA fiber morphology with random distribution geometry of the fibers demonstrates effectiveness in improving cell recognition, cell-material interaction and the biocompatibility response. This effect of geometry on the spun membrane consists of a network of long interconnected fibers, creating a bioactive meshwork that provides the microenvironment between the interacting fibers and cells depending of the concentration of polymeric solution in which cells conduct their activities\textsuperscript{12,33}. Thus, the fiber geometry in random morphology create a 3D environment like native ECM microarchitecture, because the PLA fiber spun mat are much smaller than an individual hPDL mesenchymal stromal cells\textsuperscript{34}. This is important because, within the context of mesenchymal stromal cells, nanotopography has been shown to maintain hMSC stemness, facilitating cell adhesion because anchorage to the stem cell niche is necessary for the cell self-renewal capacity. In addition, it is well documented that many cells respond to nanotopography surface features by changing their proliferation, adhesion, migration and cell orientation, and this phenomenon is reported as contact guidance that could provide information for migration out of the niche and influences stem cell differentiation\textsuperscript{34-37}.

**CONCLUSION**

In this study, concentration of polymeric solution was chosen as the main factors to investigate the effect on the resultant properties of PLA fibers in the AJS process for mimicking the natural architecture of the ECM periodontal microenvironment. The morphology, geometry and topographical features of PLA fibers was affected by the concentration of polymeric solution. Although, the PLA fiber morphology with random distribution geometry of the fibers used as a simple topographical feature provided a microenvironment for the biocompatibility response of hPDL mesenchymal stromal cells, supported by adhesion, cell-material interaction and cell viability. Moreover, this random nanofiber feature depends on the concentration because in our study the cells seeded in the scaffold had appropriate interactions with their surroundings, adhered and proliferated better on 10% followed by 7 and 6% of PLA. Together with the manufacture of fiber-spun structures, which attempts to mimic the fibrillar features of the native ECM periodontium microenvironment, could serve as future therapeutic clinic applications for repairing periodontal tissues damaged due to PD. Further studies are needed for evaluating the applications of these nanofibers scaffolds synthesized by AJS that assess the differentiation and biomineralization process for achieving this.

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