Research Article

Biocompatibility of Developing 3D-Printed Tubular Scaffold Coated with Nanofibers for Bone Applications

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3D printing with controlled microarchitectures has gained traction in a wide variety of fields, including bone tissue engineering, because it represents an exciting alternative for the synthesis of new scaffolds due to its rapid manufacturing process, high precision, cost-effectiveness, and ease of use. Thus, this study is aimed at evaluating the biocompatibility response of a 3D-printed tubular scaffold coated by a layer of 7% PLA nanofibers. The morphology, structure, and chemical composition of the 3D-printed tubular scaffold were characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier Transform Infrared (FTIR), thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), and surface property analysis by profilometry. The biocompatibility response of the scaffold was assessed by cell adhesion, proliferation, and cell-material interactions of human fetal osteoblasts. Our results showed that 3D printing allowed obtaining similar and reproducible structures and the biocompatibility assays showed that nanofiber coating of the surface of the 3D tubular scaffold promoted an improvement on cell attachment, proliferation, and the morphology of osteoblast cells when compared with a noncoated scaffold. In conclusion, the surface of the 3D-printed tubular scaffold could be improved by the deposition of a nanofiber layer to render a more mimetic and active topography with excellent cellular biocompatibility for bone tissue applications.

1. Introduction

Bone defects have a high impact on a patient’s quality of life, leading to a high demand for bone substitutes in the orthopedic and maxillofacial fields. Moreover, current surgical procedures for bone regeneration utilize transplantation (auto- and allografts) that must deal with the repair, renewal, and replacement of the bone tissue defect. However, these treatments have severe drawbacks such as donor site morbidity, severe pain, unavailability of large tissue volumes, risk of infections, immunogenicity, and the risk of communicable diseases [1–4].

Bone tissue engineering has emerged as promising strategies to replace or regenerate critical bone size defects with the ambition of circumventing the complications associated with traditional techniques [5–7]. Current procedures involve the construction of a scaffold with a combination of materials and cells [8]. The ideal scaffold should be tailored to mimic the native tissue characteristics to facilitate localization and distribution of a cell to specific sites in the body,
maintain a three-dimensional architecture (3D), have a highly porous structure with an interconnected network for cell growth and flow transport of nutrients and metabolic waste, and be biocompatible and nontoxic, with optimal physical and mechanical properties that allow and guide the formation of new tissues with an appropriate function [9]. However, one of the biggest challenges for bone tissue engineering is to design a 3D scaffold structure that could mimic the in vivo extracellular matrix and cell environment because cells lack the ability to grow in favored 3D orientations by themselves. Moreover, one of the critical factors to bridge this gap is the possibility of modulating scaffold characteristics so that specific biological, clinical, manufacturing, economic, and regulatory prerequisites can be met [10].

In recent years, 3D printing or additive manufacturing techniques have emerged as a useful tool for the generation of 3D scaffolds with controlled microarchitectures and have gained traction in bone tissue engineering because they represent an attractive alternative technique for the synthesis of new scaffolds allowing the modulation and control of the geometry with high precision over the pore size, porosity, and outer shape of the scaffold. They could also be used to reproduce specific bone defect shapes because of their rapid manufacturing process, cost-effectiveness, and ease of use [11–13]. Thus, 3D printing in the synthesis of bone scaffolds has been used due to its high flexibility in biocompatible, biodegradable, and biocomposite polymeric material handling [13–18]. In particular, in the 3D printing technique, polylactic acid (PLA) has been utilized to develop synthetic scaffolds. PLA is a linear aliphatic polyester which is widely used in bone tissue engineering because its mechanical integrity is preserved in vitro or in vivo, it is approved by the FDA, and it has extensively been used as a biomaterial in the human body for its adsorbability and nontoxicity after degradation, its thermoplastic properties, and its low viscosity [19]. 3D printing technology holds great promise for tissue engineering because 3D printing can easily control roughness at the micron scale; however, controlling roughness at the nanoscale level has proved more difficult. That is why it is necessary to improve the 3D-printed surface so that they promote cell interactions as well as mimic the nanotopography of natural bone. One strategy is to coat the 3D structure with a nanofiber layer to achieve a suitable surface for cell attachment, proliferation, and differentiation and provide an open, porous network for uniform cell distribution during cell seeding [20]. In this work, we propose the use of the air jet spinning technique (AJS) as a simple method for surface modification based on a specialized spinning system nozzle (such as a commercial airbrush), a surface for collecting polymer fibers, and compressed gas; through this method, the polymer solution and pressurized gas are simultaneously ejected to form the fiber morphology [21]. Thus, this work is aimed at synthesizing and fabricating 3D tubular scaffolds by using 3D printing to coat the surface of a printed scaffold with a layer of PLA fibers by AJS. The improved 3D-printed tubular scaffold was characterized by SEM, XRD, FTIR, TGA, and DSC, and the influence of the submicrometric fiber surface coating in the biological response of human fetal osteoblast cells (hFOB) on cell adhesion, cell-material interaction, and proliferation assays was evaluated.

2. Materials and Methods

2.1. Manufacture of 3D Tubular Scaffold. Cylindrical scaffolds were proposed using a dual technique that employs both an inner core of PLA built by 3D additive manufacturing and an outer layer functionalization of PLA nanofibers. The inner core geometries of the scaffold were digitally designed with Cura 3D printing software 3.0, and 3D tube scaffolds were fabricated by additive manufacturing (Maker-Mex Printers, León, México) of polylactic acid (PLA). The outer functionalization of the tubular scaffold was done by a cover with fiber spun mats fabricated with the air jet spinning technique (AJS). Briefly, PLA pellets (C3H6O3; MW 192,000 from NatureWorks) were dissolved in chloroform/acetone (volume ratio of 3:1) to obtain a 7% (w/v) polymer solution. Then, the polymer solution was placed in commercially available ADIR model 699 airbrushes with a 0.3 mm nozzle diameter and with a 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, México), and a pressure of 35 psi and a distance of 10 cm from the nozzle to the 3D tubular scaffold were constantly maintained for fiber deposition.

2.2. Characterization of 3D Tubular Scaffold. 3D-printed tubular scaffolds were characterized by SEM, XRD, FTIR, topographical analysis, DSC, and TGA. The morphology and structure of the 3D tubular scaffold were observed by field emission scanning electron microscopy (JEOL JSM-7600F FE-SEM). The samples were sputter coated with 5 nm of a thin gold layer (EMS 150R, Quorum, USA) to analyze outer and inner surfaces. Specific observations were done to analyze the interphase between nanofibers and the core material. Scaffold structures were determined by XRD employing a D8 FOCUS Bruker AXS diffractometer with Cu-Kα radiation in the 2θ range between 5° and 50°. The chemical structure of 3D tubular scaffolds was analyzed by FTIR employing the IRAffinity-1S (Shimadzu, Kyoto, Japan) within the 400-4,000 cm⁻¹ range. The topographic images were taken with a contact profilometer (Bruker Dektak XT, USA). A 2 μm radius needle tip was used. The sweep area was taken with an applied force of 4 mg, and the resolution was adjusted to 0.033 μm/point (corresponding to a needle tip speed of 10 μm/s covering a total area of 500 x 500 μm). The image was constructed by joining 500 scans with a separation of 1 μm. The roughness values were evaluated using the entire measured area to determine the average roughness (Ra) and the average maximum depressions (Rq). TGA and DSC evaluated thermal response. TGA (TGA Q500, TA instruments, Delaware, USA) was done with 4-6 mg of each sample, running a ramp from 25°C to 1000°C, with a heating rate of 20°C/min. Onset point (To), inflection points (Tp), and maximum mass loss point (Tmax) were calculated. DSC (DSC Q200, TA instruments, Delaware, USA) was performed with 2.5-3 mg of each sample, running a ramp
from 25°C to 250°C, with a heating rate of 10°C/min. Glass transition temperature (Tg) and melting point (Tm) were calculated. Tg was confirmed by calculating the tan δ with a dynamic mechanical analysis (DMA) of the samples. DMA analysis began at 20°C, and a ramp of 3°C/min with a strain level of 0.1% and an angular frequency of 6.28 rads/s was used.

2.3. Processing of 3D Tubular Scaffold for Cell Seeding. Before the biological assays, 3D tubular scaffolds were placed in 24-cell culture plates and sterilized by immersion in 70% ethanol (v/v) with an antibiotic solution (streptomycin 100 μg/mL and penicillin 100 U/mL) for 30 min. After sterilization, nanofiber scaffolds were rinsed with PBS, washed with distilled water three times, and air-dried.

2.4. Cell Culture. Biological assays to evaluate the cell biocompatibility response of 3D tubular scaffolds were performed using human fetal osteoblasts (hFOB, 1.19 ATCC) cultured in a 75 cm² cell culture flask in Dulbecco’s Modified Eagle Medium (DMEM, Corning) supplemented with 10% Fetal Bovine Serum (FBS, Corning), antibiotic solution (streptomycin 100 μg/mL, penicillin 100 U/mL, and fungizone 0.3 μg/mL, Sigma-Aldrich, USA), and 2 mM L-glutamine (Sigma-Aldrich, USA). For all the experimental procedures, hFOB cells at 2-6 passages were used and incubated at 37°C in a 100% humidified atmosphere with 5% CO₂ and 95% air.

2.5. Cellular Adhesion Assay. hFOB cells were seeded at 1 × 10⁴ cells/mL onto 3D tubular scaffolds with and without a fiber layer coating placed in 24-well culture plates and allowed to adhere in a standard cell culture for 4 and 24 h. After the prescribed time, the 3D tubular scaffolds were rinsed three times employing PBS to remove nonadherent cells. The evaluation of cell attachment was performed according to a crystal violet assay. Briefly, adherent cells were fixed with 4% paraformaldehyde and incubated with 0.1% crystal violet solution for 15 min. Then, the dye was extracted with 0.1% of sodium dodecyl sulfate (SDS), and optical absorption was quantified by spectrophotometry at 545 nm with a plate reader (ChroMate, Awareness Technology).

2.6. Cell Proliferation Assay. The cell viability of hFOB cells plated at a concentration of 1 × 10⁴ cells/mL in triplicate onto 3D tubular scaffolds with and without a fiber layer coating was checked by the WST-1 assay for 1, 3, 7, 14, and 21 days of culture. This assay is based on the ability of the mitochondrial succinate-tetrazolium reductase of living cells to reduce a WST-1 salt (4-(3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolo)-1,3-benzene disulfonate) to produce a water-soluble formazan dye product. The concentration of the formazan product is directly proportional to the number of metabolically active cells. hFOB cells seeded onto a 3D tubular scaffold at the prescribed times were washed with PBS and incubated with 400 μL fresh culture medium containing 40 μL of the cell proliferation reagent WST-1 for 4 h at 37°C. Then, 200 μL of the supernatant was removed, and the absorbance was quantified by spectrophotometry at 450 nm with a plate reader (ChroMate, Awareness Technology). During the experimental time, the culture medium was exchanged every other day with fresh media.

2.7. Cell Morphology. The cell morphology, spreading pattern, and cell-material interaction of hFOB cells seeded at 1 × 10⁴ cells/mL onto 3D tubular scaffolds with and without a fiber layer coating were examined using SEM and fluorescence microscopy (AMSCOPE) after 24 h of cell culture. For SEM analysis, at the end of the incubation culture time, 3D tubular scaffolds were washed three times with PBS, fixed with 2% glutaraldehyde for 1 h, and then dehydrated with a graded series of ethanol (25-100%); finally, samples were subjected to critical point drying. The samples were sputter coated with a thin layer of gold-palladium and examined by SEM.

For fluorescence microscopy, before seeding onto 3D tubular scaffolds, the hFOB culture cells were incubated with CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate) in phenol red-free medium at 37°C for 30 min. Subsequently, the cell culture was washed with PBS and incubated for 1 h in complete medium. After recovery, hFOB cells were trypsinized and counted to the desired cell concentration (1 × 10⁴ cells/mL) and incubated for 24 h onto 3D tubular scaffolds and evaluated for spreading and cell-material interaction.

2.8. Statistical Analysis. All quantitative data were expressed as the average ± standard error of the mean. Numerical data were analyzed via Student’s t-test to determine the differences among the groups. Statistical significance was considered at p < 0.05.

3. Results and Discussion

3.1. Morphological Characterization of 3D Tubular Scaffold. This study is aimed at creating a polymeric scaffold by combining two different techniques: 3D printing and air jet spinning (AJS). The morphological, physicochemical, and biological properties of this innovative option were also evaluated. The approach to fabricate a bicomponent scaffold that could mimic the extracellular matrix and morphology of long bone structures was followed first by the creation of a cylindrical core built by additive printing of PLA to acquire the mechanical properties for long bones and then by adding a coating of PLA fibers obtained by air jet spinning to mimic Sharpey’s fibers of compact bones in the periosteum. 3D-printed tubular scaffolds were observed using SEM both at high and low magnification micrographs. Both 3D tubular scaffolds (Figures 1(a), 1(b), 1(d), and 1(e)) and 3D tubular scaffolds coated with fibers (Figures 2(a), 2(b), 2(d), and 2(e)) analyzed at low magnification showed a similar macrostructure with similar geometry and homogeneous nonporous microporosity with evident wrinkles which exhibit small extruded triangle-shaped gaps. Moreover, 3D tubular scaffolds at high magnification micrographs (Figures 1(c) and 1(f)) showed a smooth homogeneous surface morphology of the polymer after the layer-by-layer deposition of the polymer, whereas the 3D tubular scaffolds coated with fibers...
showed the interphase between the printed material and fiber layer, displaying a roughness morphology (Figures 2(c), 2(f), and 2(i)), which confirms the significant difference in the surface topography of the two scaffolds. Additionally, high magnification micrographs of the coating distribution showed the morphology of the random fibers with an average diameter of $0.430 \pm 0.205 \mu m$ demonstrating that the coating was well distributed in the printed matrix (Figures 2(g)–2(i)) that covers all of the surface giving a marked roughness in comparison with the scaffold alone. This finding further proved the suitability of the combination of the dual approach (3D printing and coating with AJS) to obtain a fiber layer dispersion well adapted to the 3D surface scaffold with a homogeneous thickness distribution of submicrometric fibers in all the areas, a thickness which could be dependent on the deposition time of coating. The deeper nanofibers showed good adaptation and merged with a printed scaffold. This is in agreement with studies that use additive manufacturing and rapid prototyping for producing composite constructs for tissue engineering applications [22–25].

3.2. Structural Characterization

3.2.1. XRD Analysis. The comparison of the crystalline structure of the PLA filament with that of the 3D tubular scaffold was further characterized by XRD (Figure 3). The diffractograms showed that both 3D tubular scaffolds have a signal with the same value as a broadband characteristic of an amorphous peak that increased significantly in comparison with the PLA filament with a lower intensity. These results are in concordance with different studies that reported that PLA is amorphous and depends on the crosslinking of dopants or printed parameters [26–29].

3.2.2. FTIR Analysis. The chemical identity for interpreting and determining the functional groups were analyzed using FTIR during the printing of the 3D tubular scaffolds. For a comparison of the 3D-printed scaffold spectra, the pure PLA filament and PLA fibers were also examined. The peak at 2922 cm$^{-1}$ is the stretching vibration absorption of C-H. Peaks were also observed at 758 and 873 cm$^{-1}$ (-CH bending vibration), 1043 and 1182 cm$^{-1}$ (C-O stretching vibration), 1450 cm$^{-1}$ (-CH$_3$ bending vibration), and 1751 cm$^{-1}$ (C=O stretching vibration on ester group) as shown the Figure 4. From the infrared spectra absorption, a slight difference could be seen between all samples. Moreover, these results are supported and in agreement with several studies that used FTIR to investigate the structural features of printing samples before and after extrusion and to analyze if the PLA filament does not contain any significant contaminants [23, 30–33].

3.2.3. Profilometry. The surface topographies of the 3D tubular scaffolds with and without coating with fibers were analyzed by profilometry (Figure 5). The results showed the smoothest topography of the 3D tubular scaffold with an average roughness (Ra) of 0.049 μm and average maximum depression (Rq) of 0.064 μm (Figure 5(a)), in comparison with the 3D tubular scaffold coated with fibers that showed the influence of the nanofibers on the average roughness (Ra) of 0.586 μm and the average maximum depression (Rq) of 0.727 μm (Figure 5(b)). These results are in concordance with SEM images and studies of 3D composite
blends that reported the effect on the structural, surface, and morphological properties onto the scaffold with increased roughness values with clear peaks and valleys related to composites [25, 34–36]. Moreover, the coating of nanofibers is in agreement with a study where the adhesion properties of the 3D printing of polymers and nanocomposites onto textiles was analyzed; this study reported that the adhesion force of the deposited PLA and PLA nanocomposites onto the PLA fabrics was very strong, which was determined in all samples according to the strength of the deposited layer and structure [37].

3.3. Thermal Characterization. The thermal stability of the different 3D tubular scaffolds was analyzed by thermal gravimetric analysis (TGA) for measuring the weight change of the scaffolds as a function of the temperature in a controlled atmosphere. Derivative thermogravimetric analysis (DTG) was used to determine the decomposition temperature of the 3D scaffolds. From the spectra (Figure 6), the determination of the onset point (To), the inflection point (Tp), and the mass loss temperature (Tmax) of the 3D tubular scaffolds could be seen in comparison with the pure PLA filament as a single-stage degradation mechanism for all three samples. For the 3D tubular scaffold coated with fibers, the highest To = 331°C could be seen in comparison with the 3D tubular scaffold and pure PLA filament which both started their mass loss at 327°C. Moreover, the 3D tubular scaffold coated with fibers was completely degraded at around 369°C in comparison with the 3D tubular scaffold that degraded at 394°C and the pure PLA filament that degraded at 355°C. Finally, for DTG curve related to the inflection points, the 3D tubular scaffold showed an increase of the TGA curve of 21°C compared with the 3D tubular scaffold coated with fibers that led to an increase of 17°C; however, both 3D tubular scaffolds showed to be increased when compared with the pure PLA filament (Figure 6). Based on the TGA and DTG curves,
the incorporation of the fiber layer coating by air jet spinning onto the tubular 3D-printed scaffold influenced the thermal degradation temperature which enhances the thermal stability of the scaffold. These results are in agreement with several studies that use TGA for analyzing the scaffold fabrication process considering that the polymer matrix is subjected to high temperatures. These results also show that (1) the polymer does not undergo changes in its properties and thus avoids any degradation phenomena due to the process, (2) the composites show an increase in the thermal stability properties of the scaffolds, and (3) the extrusion process does not have any influence on the chemical properties of the 3D sample scaffolds [30, 38–40].

Figure 7 shows the change of the thermal properties using the DSC curves for the set of studies of the following materials: pure PLA filament, 3D tubular scaffold, and 3D tubular scaffold coated with fibers. DSC analyses showed a similar behavior for the three samples, with a clear glass transition
point (Tg) between 56 and 62°C, followed by an exothermic signal and a marked melting point (Tm) around 169°C. When comparing the analyses of the three samples, pure PLA showed a lower Tg and a higher Tm, indicating that pure bulk PLA requires an increased heat flow to achieve a different physical state. It is possible that printed material with smaller polymeric structures, especially those functionalized with fibers, may easily distribute the heat flow achieving a faster melting point. DMA analyses supported these results (Figure 8), where the same behavior was observed. Pure PLA showed a tan (δ) of 70.4°C, while 3D tubular scaffolds (functionalized with and without fibers) showed an increased tan (δ) of 76°C. The thermal behavior of the analyzed materials is consistent with findings from the literature where it is reported that the processing parameters used to elaborate the scaffolds did not induce significant changes in terms of temperature and the variation is not substantial enough for affirming decomposition of the material. Moreover, the crystallization of PLA could be the result of the lamellar rearrangement of the polymer that depends on the molecular characteristics and composition, as well as the reorganization of less crystalline

Figure 5: Optical profiler data showing the topography of a 3D-printed tubular scaffold. (a) Images show the uncoated smooth surface and (b) the coated surface where roughness is strongly enhanced by the presence of nanofibers.
regions, resulting in the expansion of the amorphous areas [28, 35, 41-43]. This was also supported by the XRD analysis of the 3D tubular scaffolds.

3.4. Biological Evaluation of the 3D Tubular Scaffolds. The evaluation of the biocompatibility of osteoblast cells on the surface of biomaterials is a key step for bone tissue engineering and regeneration applications. For this reason, the response of cell adhesion of hFOB cells seeded onto 3D tubular scaffolds coated with and without fibers was analyzed by crystal violet according to the defined time intervals, as shown in Figure 9(a). As can be seen clearly, more cells adhered on the 3D tubular scaffold coated with nanofibers than on the 3D tubular scaffold; however, both 3D tubular scaffolds showed higher cell adhesion when compared with control PLA filament groups after 4 and 24 h of cell culture (p < 0.05). For the cell attachment study, the percent of cells in the 3D tubular scaffold coated with a fiber layer increased by 13% after 4 h and 15% after 24 h, when compared with the 3D tubular scaffold (p < 0.05). This indicates that the combination of the fiber membrane and rough surface morphologies played a decisive role in the attachment of human osteoblast cells supported by SEM and fluorescence analysis (Figures 10–12).

The cell viability of hFOB cells cultured onto 3D tubular scaffolds coated with and without fibers after seeding for 1, 3, 7, 14, and 21 days was characterized by the WST-1 assay (Figure 9(b)). The results showed that after 1 day of culture, the cell viability of hFOB cells cultured onto 3D tubular scaffolds is higher than that on the PLA filament, which is consistent with the results of cell attachment after 4 and 24 h of culture (Figure 9(a)). Moreover, the response of the cell that grew well over time and as incubation time increased could be seen; the cell viability increases onto the 3D tubular scaffolds during the experimental periods, and it could be seen that there was no significant difference in the cell response between scaffolds after culturing for 7-21 days (p < 0.05). These results showed that printed scaffolds did not induce any cytotoxicity effects on the behavior of human osteoblast cells and the coating improved the viability of the cells.

To determine whether cells could grow, attach, and interact with the 3D tubular scaffolds, SEM and fluorescence analysis were performed to observe the morphology of cultured hFOB cells. For fluorescence analysis, a representative SEM image of the surface of 3D tubular scaffolds with and without fiber coating is shown (Figures 12(a) and 12(e)). From the SEM and fluorescence analysis, the images show that osteoblasts cells adhered to and grew on the surface of the scaffold (Figures 10–12). The cells appeared to attach well on the 3D PLA tubular scaffold coated with fibers where osteoblasts cover all of the surface of the scaffold and on the reinforced spun fibrous morphology where the lamellipodia projection of their cytoplasm could be seen after interacting with neighboring cells that could contribute to the
Figure 7: DSC thermograms of the PLA filament (red), 3D tubular scaffold (purple), and 3D tubular scaffold coated with nanofibers (black).

Figure 8: DMA results of the PLA filament (red), 3D tubular scaffold (purple), and 3D tubular scaffold coated with nanofibers (black).
formation of deposited tissue as shown by SEM images (Figure 10). After the osteoblast cells attached to the surface of these scaffolds, a widened distribution with cellular extensions or lamellipodia was revealed to have contact with other cells and with the surface, maintaining a polygonal characteristic morphology as shown by the fluorescence images (Figures 12(f)–12(h)). Moreover, the hFOB cells on the 3D tubular scaffold also showed a cell affinity with isolated cells or with cells in small disperse groups over the surface, presenting a rounded cytoplasm with few spreading cells that exhibited a flat shape as shown by SEM (Figure 11) and fluorescence micrography (Figures 12(b)–12(d)). From the cell-material interaction analysis of the spreading and morphology of hFOB cells, the 3D tubular scaffold did not induce visible changes in cellular morphology and is not cytotoxic, indicating that the nanotopology surface is more favorable for the spread of osteoblast cells supporting the cell adhesion assay where cells adhered to a greater degree on the 3D tubular scaffold coated with fibers and indicating that the scaffolds were more favorable for cell adhesion and proliferation than the 3D PLA tubular scaffold without coating. This coating gives to the 3D tubular scaffold a property that is important in bone tissue engineering because the success of the scaffold for bone induction depends on the scaffold design for controlled geometries, i.e., a specific microenvironment by surface topography that

Figure 9: Biocompatibility response of human fetal osteoblasts seeded onto the 3D-printed tubular scaffold. (a) Cell adhesion at 4 and 24 h of cell culture. (b) WST assay showing the viability of cells evaluated at 1 to 21 days of cell culture. Statistical significance is indicated by *p < 0.05.

Figure 10: SEM micrographs of the 3D tubular scaffold surface seeded with hFOB cells showing some cells with an oval to spindle-shaped morphology typical of osteoblasts cells.
improves cell biological response to the material allowing the promotion of osteoblast activity and bone deposit formation [44, 45]. The 3D tubular scaffold produced in this study allows the detection of the differentiation of hFOB cells into mature osteoblasts because the coating of the fiber layer should mimic the structural and biochemical functions of the natural extracellular matrix of bone. This imitation is important for cell attachment and proliferation as we reported in the study; however, it could also stimulate the cell differentiation and mineralization of the osteoblasts. This expected differentiation response by surface characteristics could start with the induction of a common marker of osteoblasts (such as alkaline phosphatase) as an early marker of bone formation, and then the scaffolds may provide osteoconductive communication with the surrounding cells for the expression of noncollagenous proteins that regulated the 3D nodules and mineral deposit as a later marker of bone differentiation. Moreover, considering our preliminary results, we propose that the 3D tubular scaffold presented in this study is a promising material that could facilitate...
mineralization for future guided bone regeneration applications. However, more studies on the differentiation properties of the 3D tubular scaffold need to be performed.

4. Conclusion

The additive manufacturing technique employing an ordinary commercial 3D printer has been successfully used for the fabrication of a 3D scaffold with a defined tubular shape and an inner structure that provided regular and sufficient porosity. Moreover, the successfully fabricated 3D tubular scaffold was homogeneously coated by a layer of submicrometric fiber membrane by the AJS technique. The 3D surface of the printed tubular scaffold exhibited distinctive morphologies and structures analyzed by SEM, and the surface roughness of the tubular scaffolds increased with the incorporation of the coating functionalization by the fiber membrane. Moreover, scaffolds coated with submicrometric fibers allow hFOB cells to adhere and proliferate better than uncoated 3D tubular scaffolds showing that the fibers work as a platform to improve cell biocompatibility (being not toxic to cells) and provide support to colonization and cell growth by the osteoblast cells. Moreover, the 3D tubular scaffold coated with fibers needs more studies as a biomineralization process in order for it to have a potential future use in bone tissue engineering or for it to have an application in the vascularization process.

Data Availability

All data generated or analyzed during this study are included in this manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest and no financial interest related to this study.

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References


