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Osteogenic potential of human dental pulp stem cells cultured onto poly-ε-caprolactone/poly (rotaxane) scaffolds

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ABSTRACT

Background. Bioengineering aims to develop innovative scaffolds to improve cellular activities for tissue regeneration.

Objectives. To evaluate the biological behavior of human dental pulp stem cells (hDPSCs) seeded onto an experimental polymeric-based scaffold comprising poly-ε-caprolactone/poly (rotaxane).

Material and methods. Adhesion, viability, and proliferation as well as alkaline phosphatase (ALP) activity, mineralized nodule formation (alizarin red assay), and expression of genes related to osteogenic differentiation, including ALP, type 1 collagen alpha 1 (COL1A1), Runt-related transcription factor (Runx-2), and osteocalcin (BGLAP/OCN), were evaluated in hDPSCs seeded onto polymeric scaffolds.

Results. hDPSCs expressed typical levels of mesenchymal stem cell surface markers. Cell growth increased upon cultivation on polymeric blend scaffold and the cells gained osteoblast-like appearance. Fourteen days after seeding hDPSCs on the scaffolds, irrespective to the culture medium used (clonogenic or mineralization medium), the cells presented ALP activity higher than that of control cells grown in clonogenic medium. The cells cultivated in mineralization medium on the scaffold showed significantly higher expression of all genes than the control cells, except for *BGLAP* gene expression. At 21 days, the group cultivated on the scaffold and mineralization medium showed maximum level of mineralization.

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Significance. Poly-ε-caprolactone/poly (rotaxane) blend is noncytotoxic to hDPSCs and improved genomic and functional osteogenic differentiation. Thus, poly-ε-caprolactone/poly (rotaxane) blend may serve as a promising bioactive biomaterial for bone tissue bioengineering.

1. Introduction

Bioengineering aims to develop innovative scaffolds to improve cellular activities for tissue regeneration. Bioengineering is based on three factors, namely, the cells, in particular, human mesenchymal stem cells (hMSCs) or cell substitutes, that are manipulated to gain specific functions; growth factors or bioactive molecules that induce or stimulate cell differentiation; and the scaffold as an organic or alloplastic support, which provides sustenance and allows transport of cells into regenerative sites [1].

Stem cells have the most appropriate biological characteristics for tissue regeneration. These are self-renewal undifferentiated cells, with the potential of undergoing differentiation in several tissue types. The differentiation of stem cells is dependent on the applied stimulus and favorable conditions in culture microenvironment [2,3]. There are several sources of stem cells, including hMSCs isolated from the bone marrow and human tooth tissue and the supporting tissues. HMSCs with high differentiation potential are easily isolated from dental tissues [4]. These cells may be used for functionalization of biomaterials with different structures and properties.

For bone tissue regeneration, synthetic polymers are the well-studied classes of biomaterials and have been used for their biological properties [5]. Although synthetic biomaterials provide satisfactory and controllable mechanical properties and adequate reproducibility [6], researchers are in search for new techniques to obtain or modify available scaffolds. Recent studies have shown that polymer-based membranes formed from nanofibers have large surface areas and may be obtained by different processes. Among these processes, electrospinning is a promising technique known for its versatility and cost effectiveness in the manufacturing of nanofibrous scaffolds [7].

Electrospun polymeric blends of poly-ε-caprolactone (PCL)/poly (rotaxane) have recently emerged as biomaterials for the manufacturing of scaffolds. Poly (rotaxane) is a cyclodextrin-based polymer that may be added to PCL to enhance hydrophilicity and, thus, the biodegradation of PCL [8]. These are the characteristics that would enable functionalization of these materials with stem cells for tissue engineering purposes. Therefore, we hypothesize that PCL/poly (rotaxane) may positively influence viability and osteogenic differentiation of stem cells derived from human dental pulp stem cells (hDPSCs) that may be important for bone regeneration.

2. Material and methods

2.1. Scaffold preparation and characterization

Polymeric blends of PCL/poly (rotaxane) were prepared by electrospinning and characterized as previously described [8]. Briefly, PCL (Sigma-Aldrich Corporation, St. Louis, MO, US) and poly (rotaxane) (UBE Industries Ltd., No A1000, Minato, Tokyo, Japan) were mixed at a 1:1 ratio in chloroform (Merck, Darmstadt, Germany) and acetone (Synth, Diadema, SP, Brazil) and subjected to electrospinning at a flow rate of 1 mL/h and a positive voltage of 15 kV (Test-tech, Porto Alegre, RS, Brazil). An aluminum foil was used as a collector at 23 °C, positioned 20-cm perpendicular to the needle tip. The morphology of the polymeric blend was evaluated by scanning electron microscopy (SEM) (ZEISS Model EVO-15, Oberkochen, Germany).

SEM analysis revealed porous structures with randomly oriented fibers with regular and smooth surfaces. The fibers had a mean diameter of $2.3 \pm 0.82\,\mu m$. The pores within the fibers were irregular in shape with an average diameter of $13.5\,\mu m$ and an average opening area of $87.14\,\mu m^2$. Contact angle assay was used to evaluate the hydrophilic properties of the electrospun membranes and revealed a contact angle of $108.45 \pm 3.72°$ [8]. Scaffolds were customized in the form of discs with 13-mm diameter and approximately 1-mm width and sterilized by γ-radiation in individual packages. The discs were kept in a vacuum-dehydrated chamber until experiments.

Swelling was assessed by the method described by Rodriguez-Lorenzo et al. [9]. Three samples of the scaffolds were weighed before immersion into phosphate buffer (pH 7.4) at 37 °C. Swelling was analyzed considering the percentage ratio between the liquid within the polymeric scaffold and the weight of the hydrated scaffold.

2.2. Cell culture

A Local Ethics Committee in Human Research approved this research under the protocol number 1.402.217. hDPSCs was previously isolated from the dental pulp of third molars (n = 7) extracted from both genders' healthy donors between 18 and 45 years old and characterized as described by Diniz et al. [10] and Pedroni et al. [11]. The thawed cells were cultured in clonogenic medium (CM) comprising alpha-modified minimum Eagle's medium (α-MEM; Gibco Life Technologies, Grand Island, NY, US) supplemented with 15%

Table 1 – Experimental groups.

Control CM	Cells cultured on glass coverslips in clonogenic medium
Control MM	Cells cultured on glass coverslips in mineralization medium
Poly CM	Cells cultured on scaffold in clonogenic medium
Poly MM	Cells cultured on scaffold in mineralization medium

MSC-qualified fetal bovine serum (MSC-FBS, Invitrogen/Gibco, Grand Island. NY; US), 100 μg/mL of streptomycin (Invitrogen/Gibco), 100 U/mL of penicillin (Invitrogen/Gibco), 0.1 mM ascorbic acid (Sigma-Aldrich Corporation, St. Louis, MO, US) and 2 mM L-glutamine (Gibco) under CO_2 at 37 °C. The culture medium was exchanged every 2 or 3 days. To confirm the stem cell nature of the thawed cells, the immunoprofile of surface molecules was analyzed using flow cytometry. For flow cytometry, aliquots of cells were resuspended in phosphate-buffered saline (PBS, Invitrogen/Gibco) containing saturating concentrations of conjugated primary monoclonal antibodies (1:200) specific to MSC-associated (CD44, CD146, Nanog, and STRO-1) and MSC-unrelated (CD45 and CD14) factors. All antibodies were obtained from BD Biosciences (Franklin Lakes, New Jersey, US). Cells were sorted in a flow cytometer (FACSCalibur, BD Biosciences), and a total of 50,000 events were analyzed using FlowJo Software Version 9.6.2 (Tree Star, Ashland, OR, US). Only cells until the fourth passage were used in the experiments.

2.3. Experimental protocol

The scaffolds were placed at the bottom of wells of a 12-well plate (one scaffold disc per well). Plain round sterile glass coverslips (Knittel®, Braunschweig, Germany) 13 mm in diameter were also placed at the bottom of the wells as controls. The cells (1×10^3 cells/well) were seeded on the top of the discs (scaffolds or controls) as per the experimental groups (Table 1) and cultured in a CO_2 incubator at 37 °C. Six replicates (n = 6) were performed for each experimental group (Table 1). Cells were cultured in all groups for 24 h for up to 21 days depending on the experimental assays. Cell cultures were monitored under an inverted light microscope every 48 h during the changing of culture medium.

2.4. Viability and cell growth

The viability of the cells cultured on the top of the discs (scaffolds and controls) was assessed with the MTT assay (Life Technologies, São Paulo, Brazil) at 24 h and after 7 and 14 days from seeding in six replicates (n = 6). To exclude the viability of cells that adhered to the bottom of the culture wells, the discs (scaffolds and controls) were transferred to new empty wells, followed by the addition of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) solution to each well and incubation for 4 h at 37 °C with 5% CO_2. The MTT solution was removed and the formazan crystals formed during this process were dissolved by adding 0.1-mL dimethyl sulfoxide (DMSO; Sigma-Aldrich). After 15 min, the absorbance was measured with a filter at 562 nm using a spectrophotometer (Biotek II Biochrom Ltd., Eugendorf, Austria).

2.5. Cell differentiation

For osteogenic differentiation experiments, cells were grown in mineralization medium (MM) comprising clonogenic medium supplemented with 50 μg/mL of ascorbic acid, 10 mM β-glycerophosphate, and 10 μM dexamethasone (all from Sigma-Aldrich). Osteogenic differentiation of cells was morphologically assessed by SEM, alkaline phosphatase (ALP) activity assay, alizarin red assay, and quantitative reverse-transcription polymerase chain reaction (RT-qPCR) for bone-specific gene expression (ALP, osteocalcin, Runt-related transcription factor-2 [Runx-2], and type I collagen alpha 1 [COL1A1]. All assays were applied to the experimental groups described in Table 1.

2.5.1. Scanning electron microscopy

The morphology of cells adhered to the scaffolds was analyzed with SEM. Constructs of 14 and 21 days were fixed with a 2.5% glutaraldehyde solution and subjected to routine SEM protocol [12]. Briefly, the constructs were washed with cacodylate buffer, fixed with 1% osmium solution, washed again with cacodylate buffer, and dehydrated with ethanol solution of ascending concentrations. The samples were treated with hexamethyldisilazane reagent until complete drying. After gold sputtering, the samples were observed under a scanning electron microscope.

2.5.2. Quantitative reverse-transcription polymerase chain reaction (RT-qPCR)

The expression of bone-specific genes (e.g., ALP, osteocalcin, Runx-2, and COL1A1) was evaluated using RT-qPCR in the cells from experimental groups at 14 and 21 days after seeding in four replicates (n = 4). Total RNA was extracted from the cells using the guanidine isothiocyanate technique (Life Technologies, São Paulo, Brazil) and incubated with 1-mL TRIzol reagent for cell lysis (Invitrogen/Gibco). Each sample was treated with 0.3 mL of chloroform (Invitrogen/Gibco) and centrifuged. About 300 μL of the aqueous phase containing the RNA was transferred to new tubes and treated with double the volume of isopropyl alcohol. The tubes were centrifuged, the supernatant solution was discarded, and the pellet was treated with 1 mL of 75% ethanol in water with 0.1% diethylpyrocarbonate (DEPC). After centrifugation, alcohol was discarded, and the RNA was resuspended in DEPC-free DNAse and RNAse water.

RNA concentration was determined by spectrophotometer (Nanodrop 2000). The cDNA used in RT-qPCR reactions was synthesized in a reverse-transcription (RT) reaction using the High Capacity cDNA Archive kit (Applied Biosystems Carlsbad, CA, US) at a final volume of 20 μL, starting with 600 ng of total DNA previously treated with DNAse I (Sigma Aldrich) according to manufacturer's instructions. For cDNA synthesis, samples were incubated in a thermocycler (Applied Biosystems 7500 Real-Time PCR). TaqMan was used to perform quantitative analyses of gene expression with Mastermix II on an ABI7500 equipment (Life Technologies) for the following genes: *COL1A1* (Hs00164004_m1), *RUNX2* (Hs00231692_m1), *ALP* (PDLIM3) (Hs01062534_m1), and *OCN* (BGLAP) (Hs01587814_g1).

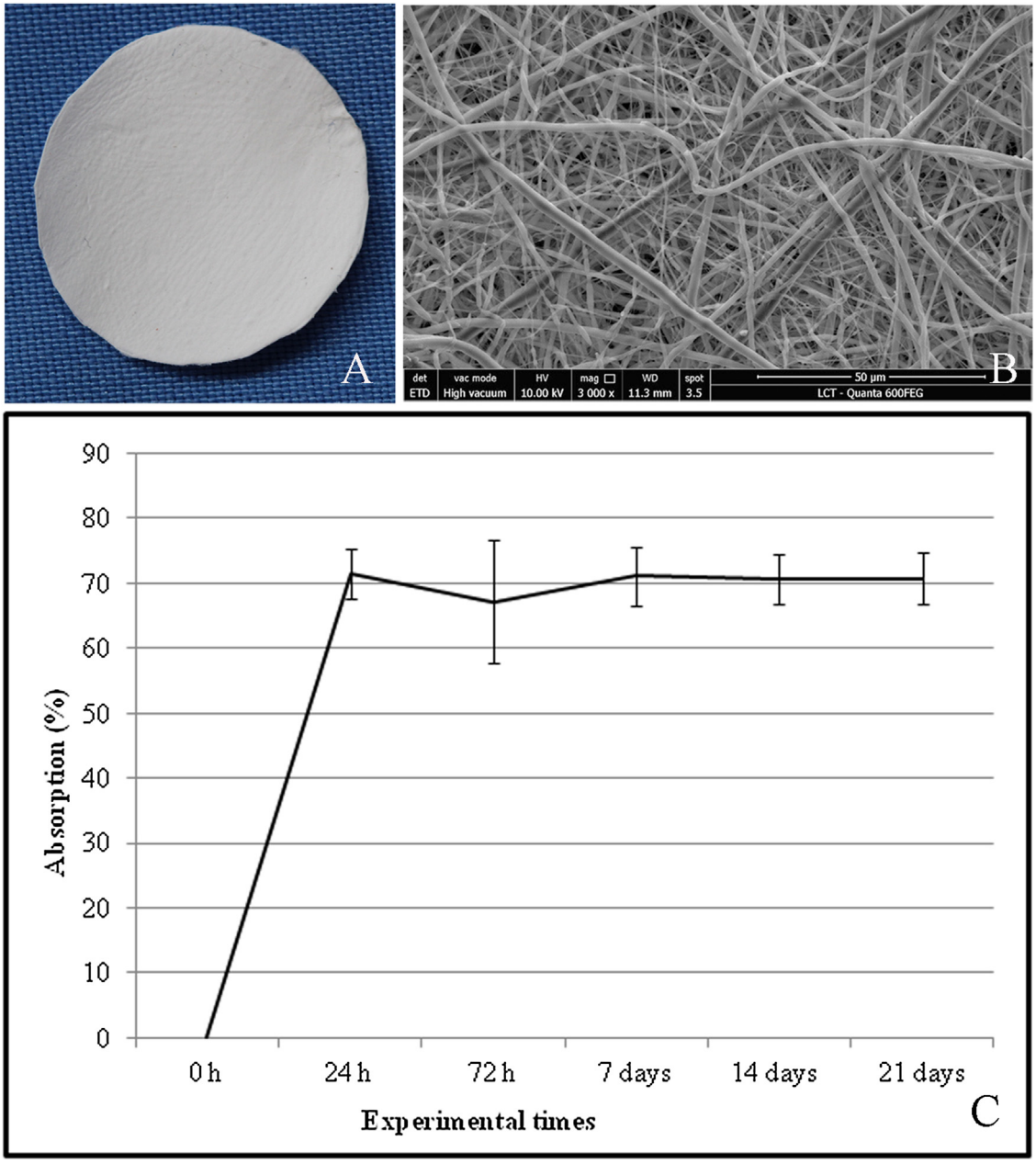

Fig. 1 – (A) PCL/poly (rotaxane) scaffolds morphology. (B) Electron microscopy images of PCL/poly (rotaxane) blend (original magnification 3000×). (C) Absorption of liquid up to 21 days (%).

The expression of the target genes was normalized based on the expression of the endogenous control glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (Hs02758991_g1). Thermo cycling comprised 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The results were analyzed based on the cycle threshold (CT). The CT_{mean} values of replicates were used to calculate the expression of the target gene after normalization with GAPDH expression level for relative quantification using the formula $2-\Delta\Delta CT$.

2.5.3. *Alkaline phosphatase activity assay*

ALP activity was assessed using Alkaline Phosphatase Yellow (pNPP) Liquid Substrate System for enzyme-linked immunosorbent assay (ELISA; Sigma-Aldrich kit) at 7 and 14 days after seeding in Control CM, Poly CM e Poly MM groups in four replicates each (n = 4). The reaction was read with a spectrophotometer (Biotek II Biochrom Ltd. Cambourne, Cambridge, UK) using a 405-nm filter.

2.5.4. *Alizarin red assay*

Briefly, 21-day-old constructs were subjected to alizarin red staining for the detection of possible mineralized nodules or calcium precipitation. Four replicates (n = 4) of Control MM e Poly MM groups and Control CM were washed twice with ice-cold PBS (pH 7.2) and fixed in 10% formaldehyde solution for 30 min at room temperature. The samples were washed twice

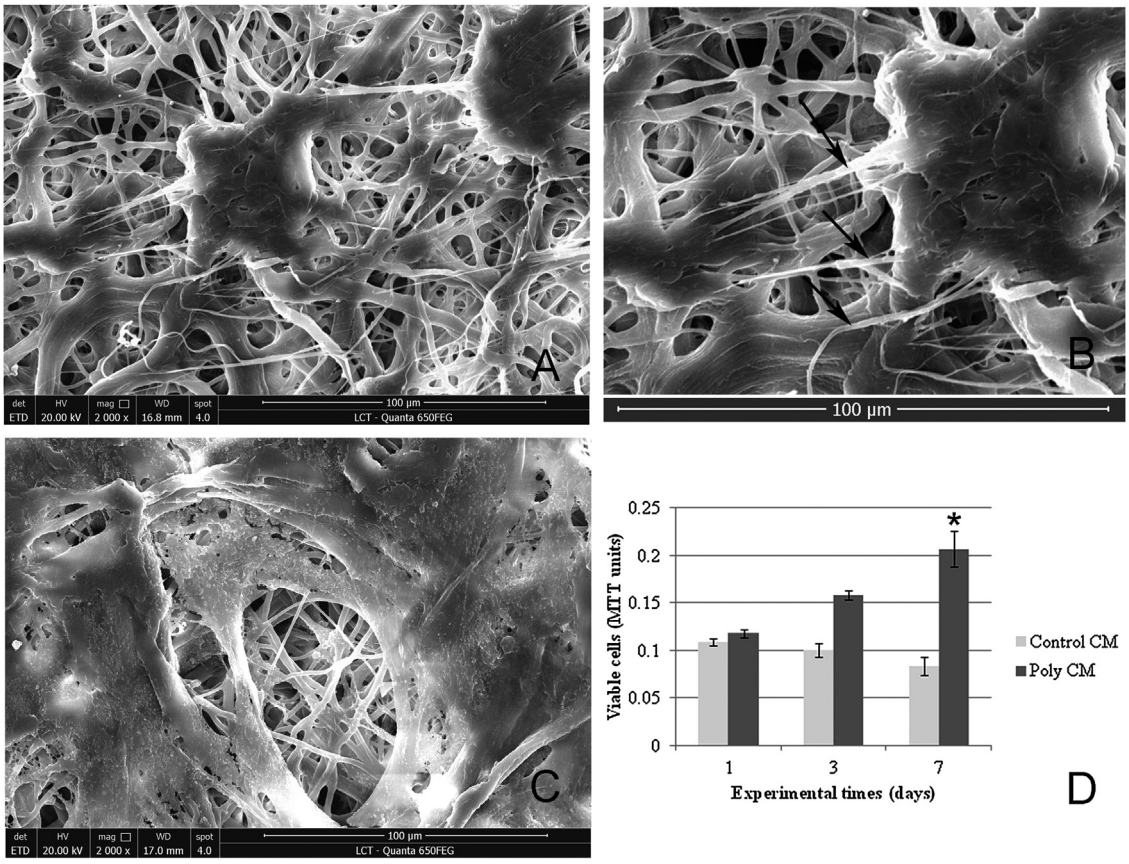

Fig. 2 – (A) SEM images of the cells cultured on scaffolds in clonogenic medium after 14 days (original magnification 2000×). (B) cell layers exhibiting bulky cytoplasm and delicate filipodia (detail from the previous image). (C) Monolayer of cells covering the entire surface of the scaffolds in 21-day cultures (D) Growth curves of hDPSCs grown on the top of scaffold and control (glass coverslips) × two-way ANOVA – significant when $p < 0.05$.

with distilled water and stained with 200 µL of 1% alizarin red solution (Alizarin red S-ARS; Sigma Aldrich) (pH 4.2) for 30 min at room temperature. The nodules were dissolved in a 10% ammonia solution and absorbance was measured at a wavelength of 550 nm.

2.6. *Statistical analyses*

Data from cell viability, proliferation (n = 6), and differentiation assays and relative gene expression (n = 4) were compared with two-way analysis of variance (ANOVA) followed by the Tukey's test (SigmaPlot 11.0, Systat Software Inc.). The level of significance was 5%. Quantitative data were expressed as the mean ± standard error of the mean (SEM).

3. Results

3.1. *Scaffold characterization*

Fig. 1A illustrates the morphology of the scaffolds of PCL/poly (rotaxane) blend, which presented a white smooth, flexible appearance. Electron microscopy images revealed porous structures with randomly oriented fibers with regular and smooth surfaces (Fig. 1B). Scaffold samples showed an average swelling of 70% of its weight after immersion into culture medium for 24 h. This percentage remained stable with no statistical difference up to 21 days (Fig. 1C).

3.2. *Cell characterization*

HDPSCs expressed typical levels of MSC surface markers. Cultures showed positive expression of CD44, CD146, STRO-1, and Nanog. Hematopoietic (CD45), endothelial (CD14), and CD34 markers showed minimal expression or were absent (Supplemental material online).

3.3. *Cell morphology, viability, and proliferation*

SEM images revealed the rounded appearance of the cells cultured on scaffolds in clonogenic medium after 14 days and presented intersections between blend fibers and cell layers, with bulky cytoplasm and delicate filipodia (Fig. 2A and B). Cells were observed in the form of a monolayer, almost covering the entire surface of the scaffolds in 21-day cultures (Fig. 2C). The growth curves of hDPSCs grown on the top of scaffold and control (glass coverslips) revealed similar growth rate up to 7 days. Poly CM group showed progressive cellular growth with significant differences between 7 and 14 days (Fig. 2D).

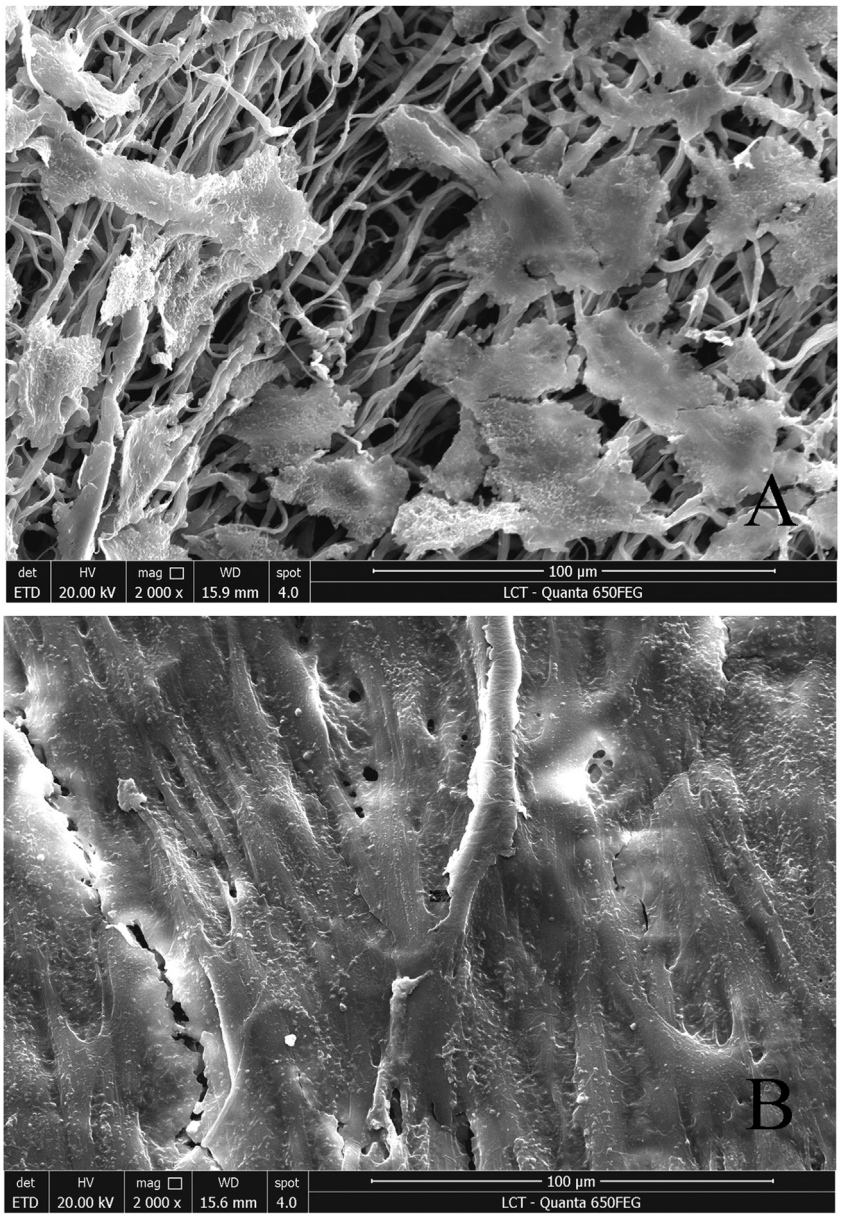

Fig. 3 – (A) Cells present thin cytoplasm and a stellar-shaped morphology in 14 days (B) At 21 days cells shows flat appearance in the form of a dense rough monolayer.

3.4. *Cell differentiation*

3.4.1. *Cell morphology*

Fig. 3 illustrates the morphological characteristics of hDPSCs grown on the scaffolds in mineralization medium. SEM images revealed small clusters of cells spread out on the scaffolds. At 14 days, cells presented thin cytoplasm and a stellar-shaped morphology (Fig. 3A). After 21 days, the cells had flat appearance in the form of a dense rough monolayer (Fig. 3B).

3.4.2. *Quantification of ALP activity*

Fig. 4A presents the graphical representation of ALP activity in all groups at both experimental time points (7 and 14 days). ALP activity significantly increased in all experimental groups from day 7 to 14. Differences among groups were only observed at day 14. hDPSCs cultured on scaffolds presented higher ALP activity in the mineralization medium than in clonogenic medium.

3.4.3. *Formation of mineralization nodules*

Mineralization nodules were formed in all experimental groups at different levels. Lowest level was observed for hDPSCs from control groups, while the highest level of mineralization was observed for the hDPSCs cultured on the scaffold in mineralization medium (Fig. 4B). Qualitative analysis revealed more intense reddish spots of mineralized nodules in Poly CM and Poly MM groups than in control (glass coverslip) group (Fig. 4C– F).

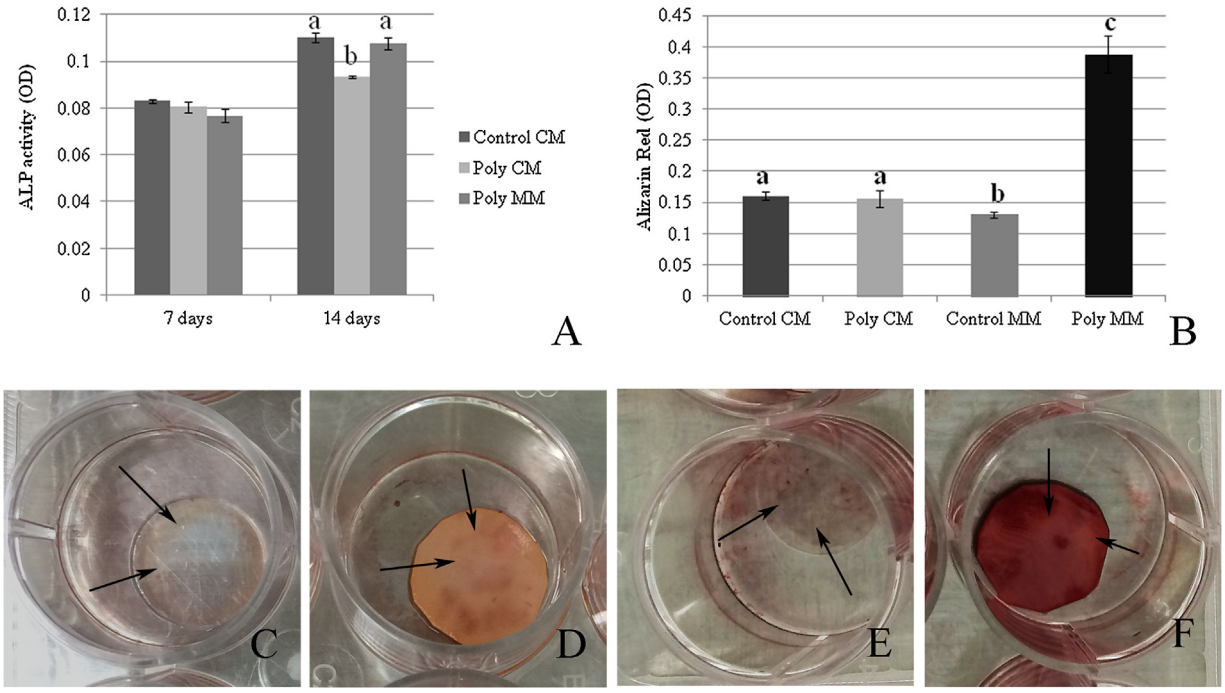

Fig. 4 – (A) Graphical representation of optical density (OP) of ALP activity in all groups (7 and 14 days). Two-way ANOVA. Different small letters significant when $p < 0.05$; (B) Graphical representation of Alizarin Red assay in all groups in 21 days. Two-way ANOVA. Different small letters significant when $p < 0.05$. Alizarin Red assay; (C) Control CM absence of mineralizing nodules (arrows points glass coverslip); (D) poly CM exhibiting slight reddish spots of mineralization; Control MM discrete reddish spots (arrows points glass coverslip); (F) poly MM shows diffuse reddish pigmentation and intense focal reddish mineralization spots.

3.4.4. *Gene expression of osteogenic markers*

Difference in the expression of *COL1A1* gene was observed between the two time periods. At 14 days, the expression of *COL1A1* was higher in Poly MM group than in Poly CM group. The expression of *ALP* gene in the control CM group was similar between two time periods. *ALP* expression differed between control MM and Poly MM groups, with significantly higher expression reported at day 14. Significant *ALP* expression was observed in control CM and Poly CM groups during the 14-day period. No difference in *ALP* expression was observed between Poly CM and Poly MM groups. At day 21, control CM group showed significantly increased *ALP* expression. The expression of *Runx-2* at day 14 was significantly higher in control MM and Poly MM groups. A decrease in *Runx-2* expression occurred at day 21. The expression of *BLGAP* was higher in control MM group at day 14 (Fig. 5).

4. Discussion

The development of innovative scaffolds is carried out to improve bioengineering for tissue regeneration processes. Biomaterials must allow functionalization with cells and growth factors to expand cell differentiation in the receptor tissue. Biomaterials should exhibit physicochemical features that allow competent cells to properly regenerate the lost or diseased tissue.

Here we studied a scaffold comprising nanofibers of two polymers, PCL and poly (rotaxane). Macroscopic observation revealed that the scaffold was thin, smooth, and flexible and may be useful for clinical applications. The presence of poly (rotaxane) enhances the hydrophilicity of the scaffold, owing to the hydroxyl functional group (OH) [13]. PCL alone is known to impart high hydrophobicity, which may act as a limitation for applications in tissue engineering [14,15]. Porosity is an important characteristic necessary for the absorption of culture medium to enhance nutrient distribution necessary for the adhesion, proliferation, and differentiation of the seeded cells [16,17]. The porosity of this scaffold exhibited values similar to those described in the literature [18,19]. This feature facilitated cellular interaction, especially in the presence of interconnectivity with these pores.

Immunophenotypic characterization of cells is an important step to evaluate stemness and differentiation potential of cells after storage and re-freezing and defrosting processes. Classic markers for stem cells were used as described by other authors [20,21]. The result revealed the positive expression of mesenchymal and undifferentiated stem cell markers [22]. These cells were originated from human third molar dental pulp. The extraction of these teeth is a routine procedure for maxillofacial surgeons; hence, dental pulp is a relatively simple source to obtain stem cells. hDPSCs are rich multipotential stem cells with low immunogenicity.

Results of the mitochondrial activity assay revealed progressive cell growth in the presence of the scaffold, which

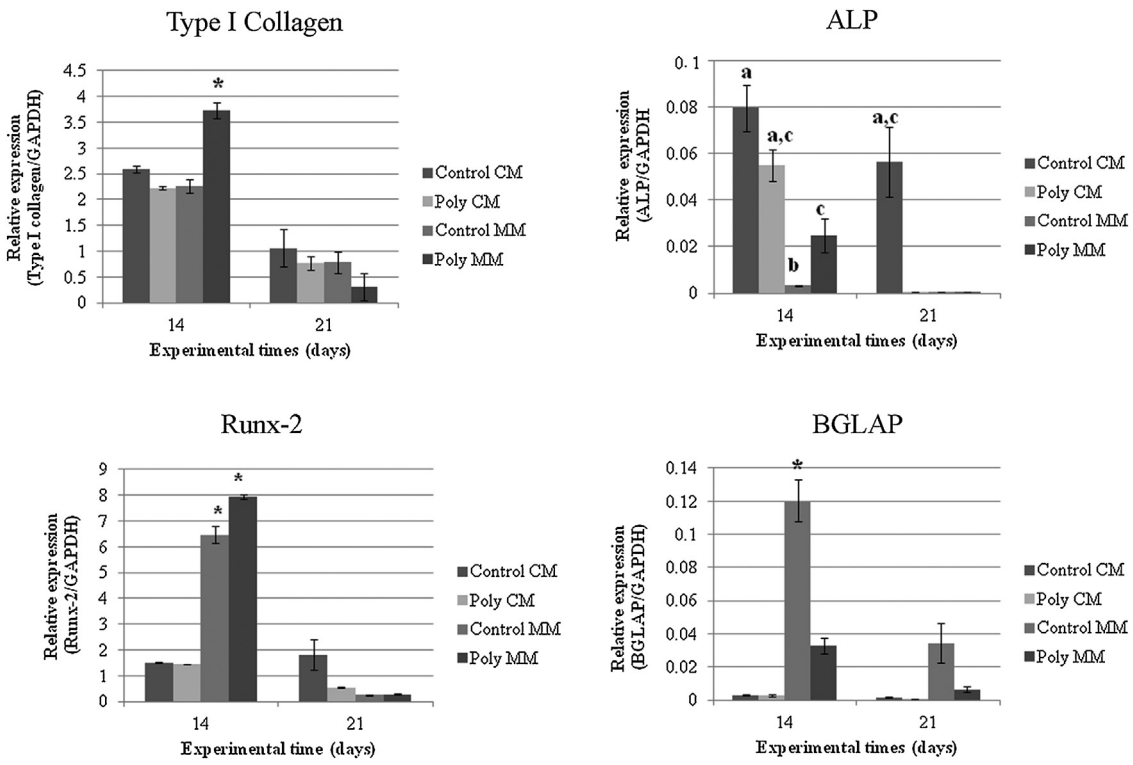

Fig. 5 – Gene expression of osteogenic markers. Type I Collagen, Alkaline phosphatase (ALP), Runt-related transcription factor 2 (Runx-2), bone gamma-carboxyglutamate protein (BGLAP) – *significant differences between groups and experimental time; different small letters mean significant differences between groups and experimental time two-way ANOVA significant when $p < 0.05$.

may be partly explained by the larger contact surface area and porosity of the scaffold. However, no differences were observed between the groups considering the time in culture. Therefore, this blend was thought to be noncytotoxic, as reported in murine fibroblasts [8]. Moreover, the cellular morphology of hDPSCs was similar in both culture media, as the cells maintained interface compatibility and confluency at the bottom of wells. During culture period, no inhibition zone at scaffold/cell interface was observed. This observation confirms the non-cytotoxic characteristics of this polymeric blend to hDPSCs. Other studies showing similar functionalization characteristics of alloplastic scaffolds by stem cells have not described this endpoint [23,24].

Here, we employed a method to selectively count cells that adhered and proliferated onto scaffold surface. The control group of cells was cultured on a glass coverslip of same seeding diameter to guarantee accuracy in cell counting. During the observation periods of 14 and 21 days, cells onto scaffolds showed better differentiation than those on glass cover slip. Cell differentiation process in mineralization medium was performed to analyze the potential of functionalization. In future, it may be important to use cells that could carry the potential to differentiate onto scaffolds within the reception tissue to enhance bone neoformation. An organic site may carry particular grown factors that could induce implanted cells to differentiate. On the contrary, implantation of differentiated cells may reduce the capacity of cell proliferation [25].

The high activity of ALP in 14 days cultures and the increase in mineralization in Poly CM cultures are consistent with the findings that hDPSCs possibly differentiate into bone cells more easily in the presence of scaffolds, as shown by others using polymeric scaffolds [9,26].

We observed reddish marks on the scaffold even in clonogenic medium, probably characterizing the presence of calcification nodules. Evaluation of the growth curve revealed similar cell growth results in the presence of the membrane, particularly on day 7. It appears that the scaffold material possibly favors the growth and differentiation of hDPSCs, probably owing to the anabolic bone effect of poly (rotaxane) [25,26]. This observation may partially explain our results. To clarify this hypothesis, replication of this assay using pure PCL is warranted. Further, our study results may encourage in vivo studies to verify the real osteogenic potential of this polymeric blend.

SEM images revealed the morphological characteristics of cells throughout the culture period. Their round shape in the initial culture period showed few branches. At the end of 7 days, a more filamentous and flattened shape with great ramifications and interconnectivity was observed. These characteristics led us conclude that these cells adhered to the material. Furthermore, we observed cell/scaffold and cell/cell interactions, as reported by other studies using different porous polymers [17,26]. During the period between 14 and 21 days of cultures, the cells in mineralization medium

showed interconnected nanofiber network. This characteristic is probably related to the differentiated cells that are ready to generate their products.

COL1A1 gene was found to be expressed on day 14 and encoded collagen, one of the main proteins in bone matrix. At day 21, collagen production was already observed; hence, the expression of this marker diminished. Collagen is an early marker of matrix formation. This matrix would be subsequently mineralized during the process of osteogenesis. *ALP* gene expression corroborates with the result of ALP activity. Runx-2 regulates the expression of other genes related to osteoblasts. Therefore, the maximum expression of *Runx-2* was observed during the intermediate period of maturation. At 21 days of cultures, the matrix matured, and *Runx-2* gene expression decreased.

The expression of *BGLAP* gene was high at day 14 in the cells cultured in clonogenic medium without the presence of the scaffold, owing to the presence of several cells with differentiation potential. Upon cell differentiation and production of mature matrix, this gene may lose its expression as observed in Poly MM group. We found that *BGLAP* gene expression was low on day 21, as evident from the strong alizarin staining intensity. In cells cultured in mineralization medium (differentiation) in the presence of the scaffold, hDPSCs differentiated and produced more mineralized matrix.

In vitro studies, such as this, are relevant to develop technology and ground-breaking biomaterials for using in regenerative therapies of tissues or organs and is one of the major challenges of translational research. The present study has shown promising data towards the potential of this material for bone regeneration. However, phase 1 stem cells clinical trials are incipient [27] and still requires several laboratory steps to demonstrate safety and efficiency. In addition, there are legal, cultural and ethical limitations that must be discussed within the scientific community to reach a consensus of precise indications for safe protocols.

We believe that the presence of the scaffold causes early differentiation of hDPSCs in mineralization medium. The benefit of this finding may be investigated in future in ex-vivo and in vivo studies complementing the findings of our previous study. The use of this polymer as a transport scaffold for stem cells for the treatment of critical bone defect might facilitate tissue repair with higher tendencies to regenerate bone than to induce fibrous tissue formation.

5. Conclusion

The scaffolds composed of PCL/poly (rotaxane) presented potential applications as a bioactive biomaterial for bone tissue engineering. The PCL/poly (rotaxane) blend had no cytotoxic effects against hDPSCs. The cells seeded on the biomaterial were capable of undergoing osteogenic differentiation and expressing markers of osteogenic differentiation. Thus, this biomaterial may be in vitro seeded with stem cells derived from human dental pulp.

Data availability

The raw data will be send on request.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.dental.2019.08.109.

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