Odontogenic differentiation of inflamed dental pulp stem cells (IDPSCs) on polycaprolactone (PCL) nanofiber blended with hydroxyapatite

Vellore Kannan GOPINATH¹, Sheela SOUMYA², V. YOGESHWAR CHAKRAPANI³ and T.S. SAMPATH KUMAR³

¹ College of Dental Medicine, University of Sharjah, University City, Sharjah 27272, UAE
² Sharjah Institute for Medical Research, University of Sharjah, University City, Sharjah 27272, UAE
³ Medical Material Laboratory, Indian Institute of Technology Madras, Chennai 600036, India
Corresponding author, Vellore Kannan GOPINATH; E-mail: gopinathvk@yahoo.com

INTRODUCTION

Dental pulp tissue located in the center of the tooth is innervated by nerves and blood vessels, which provide good vascular supply to the tissue. Injury to the dental pulp tissues could result from dental caries, trauma or accidentally during cavity preparation resulting in dental pulp irritation, thereby altering the dynamic equilibrium of the pulp cavity. Hence, there is a need for the injured dental pulp tissues to repair and heal by reparative dentin formation and restore normal tissue homeostasis. The robust cellular component of the pulp such as fibroblasts, odontoblasts, and dental pulp stem cells help in pulp regeneration when the dental pulp tissue is subjected to injury¹⁻². Therapeutic interventions are usually required to facilitate this healing and repair process, which include the placement of suitable biocompatible material on the damaged and exposed dental pulp tissue³.

Materials such as calcium hydroxide and MTA (Mineral trioxide aggregate) have been utilized to facilitate repair by reparative dentin formation with varying levels of success and limitation⁴⁻⁶. Therefore, an innovative approach could be, the placement of a scaffold impregnated with calcium-based cement to facilitate the repair and regeneration of the pulp. It is interesting to know that in regenerative medicine application, numerous biomimetic scaffolds mimicking the natural extracellular matrix (ECM) have been developed employing different fabrication processes. Electrospun membranes made from synthetic and natural polymers exhibit excellent similarity with the ECM⁸. Production of an electrospun membrane with synthetic polymer blends and the ceramic compound is relatively easy and cost-effective. Polycaprolactone (PCL) is an FDA approved biodegradable polymer which can be incorporated with calcium-based cement and can be effortlessly electrospinnable¹⁰.

The potential of using PCL electrospun membranes as a pulp capping material has been investigated in vivo. In this pre-clinical trial, direct pulp capping procedures were performed on premolars with PCL fiber meshes being positioned on the exposed pulp and MTA was placed over it. This approach resulted in the production of a thicker dentin bridge when compared to exposed pulp tissues directly capped with MTA¹¹. Similar results were seen when direct pulp capping was done on human teeth utilizing PCL fiber meshes when placed on the exposed dental pulp followed by MTA¹². However, one of the limitations of the PCL electrospun membrane is its bioinert nature lacking the ability to signal biological reactions essential for tissue repair and regeneration¹³⁻¹⁴. Therefore, PCL electrospun scaffolds when incorporated with materials such as hydroxyapatite, fluorapatite, and bioactive glass could enhance their capabilities to be utilized in tissue regeneration and reconstruction. Fluorapatite coating on the electrospun PCL nanofiber surface facilitated adhesion, proliferation, and differentiation of DPSCs to odontoblasts¹⁰. An in-depth study on the same material indicated that osteogenic differentiation of DPSCs in in vitro cultures was modulated by Hedgehog, insulin, and Wnt signal pathways¹⁶. These studies indicated the potential clinical
application of using this biomimetic material to promote odontogenic differentiation of DPSCs. Incorporating calcium-based materials within the PCL nanofiber matrix could modulate bioactivity of the material. This was investigated in cultures by incorporating bioactive glasses (BGs) into the PCL matrix which resulted in the release of calcium and silicon ions from the BGs-PCL matrix facilitating the differentiation of DPSCs to odontoblastic and osteoblastic lineages.

Hydroxyapatite being the major mineral present in bone and teeth is a candidate material to be used in tissue engineering to facilitate regeneration of tissues owing to its outstanding osteoinductive potential. Biodegradable electrospun PCL scaffolds with hydroxyapatite (HA) incorporation facilitated attachment and proliferation of osteoblast-like cells (MG-63), human periodontal ligament cells (hPDL) and human dental pulp stem cells (hDPSCs). It can be postulated that the presence of HA in the nanofiber can act as a molecular signal to attract cells to enter, attach and proliferate on the matrix. It should also be noted that there is lacking information on the ability of IDPSCs to attach, proliferate and differentiate when cultured on PCLHA electrospun scaffolds. Therefore, there is a need to explore these scaffolds with biomimetic properties in dental pulp regeneration applications.

This study aimed to develop biodegradable electrospun PCL composite membranes blended with hydroxyapatite (HA) and evaluate its biocompatibility and differentiation potential of IDPSCs into odontoblasts.

**MATERIALS AND METHODS**

**Electrospinning**

A 20 wt% PCL (Mₐ ~ 80,000, Sigma-Aldrich, St. Louis, MO, USA) solution was prepared in chloroform: methanol (3:1) solvent system. Pure nano-hydroxyapatite (HA) was prepared using a previously described accelerated microwave irradiation method. Composite solutions of PCL with HA were formed by adding 10 and 15 wt% of HA to the PCL solution. Electrospinning of the various solutions was carried out using custom-made electrospinning set up, which comprises of a high voltage (HV) unit (Model 2-A, Zeonics Systech, Bengaluru, Karnataka, India), a syringe pump (NE1000, New Era Pump Systems, Farmingdale, NY, USA) and a stationary metallic collector which is grounded. The solutions were loaded into a 5 mL syringe fitted with a 22 gauge (G) blunted needle. The anode portion of the high voltage unit was connected to the needle tip. The electrospun samples were collected onto the metallic collector covered with an aluminium foil.

The parameters used for spinning the polymer and composite solutions, namely flow rate, the voltage applied and tip to collector distance (TTCD) are given in Table 1. The electrospun PCL, PCL/HA (10 wt%) and PCL/HA (15 wt%) mats were coded as PCL, PCL10% HA and PCL15% HA respectively.

**Scanning electron microscopy (SEM) analysis**
The electrospun mats were sputter-coated with gold using a precision coating system (Gatan, Pleasanton, CA, USA) and the morphology of PCL, PCL10% HA, and PCL15% HA were observed using a scanning electron microscope (FEI Quanta 400, Hillsboro, OR, USA) under an accelerating voltage of 10 kV. The images were analyzed using image analysis software, Digimizer.

**X-ray diffraction analysis (XRD)**
The crystalline nature of the synthesized HA, PCL, PCL 10% HA and PCL 15% HA were analyzed using a X-ray diffractometer (D8 DISCOVER, Bruker, Billerica, MA, USA) with Cu Kα radiation (λ=1.54 Å) at a scanning speed of 0.1°/step and at a scanning rate of 1 step per second.

**Fourier transform infrared (FTIR) spectroscopy**
HA, PCL, PCL10% HA, and PCL15% HA were analyzed using a Fourier-transform infrared spectrometer (Spectrum One FTIR spectrometer, Perkin-Elmer, Waltham, MA, USA) for identifying the functional groups present and to study the molecular interactions in the blend and composite electrospun mats.

**Contact angle analysis**
The contact angle made by water on PCL, PCL10% HA and PCL15% HA were measured using a contact angle analyzer (Easy Drop, Krüss, Hamburg, Germany) by the sessile drop method at room temperature. The measurements were made on well-dried sample surfaces. Averages of 5 measurements were taken from triplicates of each sample.

**Isolation of inflamed dental pulp stem/progenitor cells**
The dental pulp stem/progenitor cells were isolated from patients diagnosed with irreversible pulpitis according to the protocol previously described by our research team.

| Parameter | Flow rate (mL/h) | Applied voltage (kV) | TTCD (cm) |
|-----------|-----------------|---------------------|-----------|
| Samples   |                 |                     |           |
| PCL       | 0.9             | 17                  | 13        |
| PCL10% HA | 0.75            | 23                  | 13        |
| PCL15% HA | 0.7             | 24                  | 13        |
The viability of the IDPSCs on the membranes, HA alone and glass coverslips (control) was assessed by Live/Dead assay. IDPSCs were seeded at a density of 1×10^4 cells/cm^2 were seeded on top of the membranes and incubated for 48 h. The cells were washed three times with phosphate-buffered saline (PBS) and were fixed in 4% paraformaldehyde (Sigma-Aldrich) for 20 min at room temperature. The fixed cells were permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 5 min. Subsequently, the cells were stained with DAPI and were visualized using a fluorescence microscope (IX53 Inverted Microscope, Olympus, Tokyo, Japan).

SEM analysis was performed after fixing the samples with 2.5% (v/v) glutaraldehyde in phosphate buffer for 1 h at room temperature. After rinsing with PBS, the samples were dehydrated in ascending alcohol series and were gold-palladium sputter coated for SEM analysis (VEGA3 XM, TESCAN, Brno, Czech Republic).

**Odontogenic differentiation potential**

1. **Gene expression studies**

Real-time PCR analysis was done to evaluate the odontogenic differentiation potential of the electrospun membranes and HA on IDPSCs when cultured in normal DMEM medium with 10% FBS and 1% Pen-Strep (NM) and in DMEM medium supplemented with odontogenic differentiation factors (OM). For relative gene expression of odontoblast specific genes such as ALP and DSPP, IDPSCs at a density of 5×10^5 cells were seeded onto the membranes and HA placed in a 6-well plate for 21 days in NM and in OM. For the membranes placed in OM, initially till confluency, the cells are grown in NM only. Upon confluency, the media was changed to odotogenic media (OM), which contains DMEM with 10% FBS and 1% Pen-Strep with 10 nM dexamethasone (Sigma-Aldrich), 10 mM β-glycerophosphate (Sigma-Aldrich) and 50 μg/mL ascorbic acid (L-Ascorbic acid 2-phosphate, Sigma-Aldrich). Total RNA was extracted from cells using the Qiagen RNA isolation kit (Qiagen, Hilden, Germany). The quantity of the isolated nucleic acid was measured in a nanodrop (ND-1000, NanoDrop Technologies, Wilmington, DE, USA). The total RNA isolated was first transcribed to cDNA using RT² HT First Strand Kit (Qiagen, Hilden, Germany) in a PCR system. The real-time PCR analysis was carried out using GoTaq® qPCR Master Mix (Promega, Madison, WI, USA) in a Rotor-Gene Q PCR machine. The PCR cycling conditions included an initial hot Start Polymerase activation at 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. GAPDH was used as an internal control. The expression levels of the target genes were calculated by normalizing the Ct values from the reference gene and the fold change was calculated using the formula 2^ΔΔCt. The genes and the respective primer sequences are represented in Table 2.

**SEM-EDS analysis for the calcium-phosphate deposition**

The calcium phosphate deposition ability of the test
materials when seeded with IDPSCs was analyzed after 21 days. The lysed electrospun membranes after gene expression study were fixed in 2.5%(v/v) glutaraldehyde for 1 h at room temperature. The membranes were then thoroughly washed with PBS and dehydrated in ascending alcohol series. The fixed samples were dried at 37°C and mounted on aluminum stubs, sputter-coated with gold-palladium and were visualized under SEM. The calcium phosphate deposition on the membranes was quantified in the EDS attachment of SEM (SEM-EDS, TESCAN).

**Statistical analysis**

All results were obtained from three separate experiments with each experiment consists of six replicates and the statistical significance was calculated by One Way Anova and Tukey’s post-hoc test. Data are expressed as mean±SD and p≤0.05 was considered as statistically significant.

**RESULTS**

**SEM analysis**

The SEM analyses of electrospun mats were done for evaluating the morphology of the fibers (Fig. 1(i)). PCL shows fibers with relatively uniform diameters compared to the composite fibers. Both PCL10% HA and PCL15% HA show thick and thin fibers. PCL, PCL10%HA, and PCL15%HA reveal an average fiber diameter of 140, 220 and 250 nm respectively.

**FTIR spectroscopic analysis**

The FTIR spectra of PCL, PCL10% HA, PCL15% HA and HA alone are represented in (Fig. 1(ii)). All the electrospun PCL and PCLHA membranes displayed asymmetric CH2 stretching at 2,923 cm\(^{-1}\), symmetric CH2 stretching at 2,857 cm\(^{-1}\), carbonyl stretching at 1,720 cm\(^{-1}\), C–O and C–C stretching at 1,293 cm\(^{-1}\) and asymmetric COC stretching at 1,240 cm\(^{-1}\) corresponding to the polymer PCL. In addition, the CH2 bending vibrations were observed at 1,464, 1,418, 1,397 and 1,366 cm\(^{-1}\). In the PCLHA composites, the infrared vibration bands corresponding to (PO\(_4\))\(^{3-}\) of HA were also observed at 1,023 and 560 cm\(^{-1}\) respectively.

**XRD analysis**

The X-ray diffractograms of the electrospun mats and HA are shown in (Fig. 1(iii)). PCL shows two sharp peaks at 2θ=21.7° and 23.7° along with a shoulder at 2θ=22°. The diffraction pattern of HA has been indexed according to JCPDS file no. 9-432.

---

| Genes | Primer sequence | Gene bank accession numbers |
|-------|-----------------|-----------------------------|
| DSPP  | ATATTGAGGGCTGGAGATGGGGA TTTGTGGCTCCAGCATTGCA | NM_014208.3 |
| ALP   | ACCTGGCTAAGAGATGTCATC CTGGTAGGCGATGTCCTTA | NM_000478.5 |

Table 2: The list of genes and primer sequences used in the qPCR study
**Contact angle analysis**
The contact angle made by a water droplet on all the electrospun mats showed a hydrophobic nature as shown in (Fig. 1(iv)). PCL exhibits a highly hydrophobic surface with a contact angle value of 150±3° which on mixing with HA marginally lowers the contact angle for PCL10%HA (145±2°) and PCL15%HA (142±3°).

**IDPSC characterization**
The isolated cells expressed positive expression for CD markers with CD-90, CD-105 (>90%) and CD-73 (50%). The cells did not express the negative markers CD-14 and CD-45 (Fig. 2).

**Cytocompatibility analysis by MTT assay**
To check the in vitro cytocompatibility of the test membranes such as PCL, PCL10%HA and PCL15%HA, MTT assay was carried out for 3 days. To check the cytotoxicity of the HA particles on the cells, HA extract with an extraction ratio of 0.2g/mL following the ISO standards was taken as one of the test groups along with the control (glass coverslips). The MTT analysis after day 1 and day 3 demonstrated the nontoxic behavior of the test membranes. All the samples exhibited ~100% cell viability after 24 h of culture conditions. After day 3 also the cell viability was not significantly compromised in any of the test samples with respect to control indicating the nontoxic behavior of the test materials (Fig. 3).

**Live/Dead analysis by flow cytometry**
To further evaluate the cytotoxicity of the test samples, a one-step live/dead assay protocol was carried out by flow cytometry. The live-cell dye in the kit labels the viable cell in green and the dead cells dye stains the cells with not so intact cell membranes in red color. The flow cytometric data also demonstrated the nontoxic behavior of the test samples. All the materials showed ~90% cell viability when compared to the control samples. These data also supported the MTT assay findings, indicating the cytofriendly nature of the test membranes.

**Adhesion of IDPSCs on test samples- SEM and fluorescent analysis**
Adhesion behavior of IDPSCs on the membranes checked...
by SEM displayed a well-spread morphology on all the membranes (Fig. 4). The cells on control glass coverslips and HA exhibited the elongated morphology as that of MSCs. The IDPSCs adhesion on the membranes was further determined by DAPI staining. The nuclear staining showed a higher number of adhered cells on control glass coverslips and HA than the PCL membranes. Between the membranes analyzed, more cells were found to attach to PCL10%HA compared to PCL and PCL15%HA. The quantitative analysis by ImageJ also showed a significantly higher cell number on PCL10%HA compared to PCL and PCL15%HA. However, the cell adhesion was greater on PCL15%HA when compared to PCL (Fig. 4(f)).

**mRNA level expression of DSPP and ALP**

The relative gene expression studies revealed a significant enhancement in the odontogenic specific gene DSPP in HA alone treated cells after 21 days of induction with odontogenic factors. The difference was highly significant when compared to all the PCL membranes. Between the membranes, PCL10% and 15% showed a similar level of DSPP expression than PCL. However, the expression of ALP was found to be higher on PCL10%HA compared to PCL and PCL15%HA (Fig. 5(b)). The membranes when incubated with cells for 21 days in noninducing medium i.e., NM also showed some amount of DSPP and ALP expression, particularly in PCL10%HA and PCL15%HA than PCL alone samples.


Fig. 6  Representative SEM-EDS analysis of calcium phosphate depositions on test membranes after 21 days of treatment with IDPSCs.
PCL, PCL10%HA and PCL15%HA were analyzed for calcium phosphate deposition by IDPSC both in inducing (OM) and non-inducing conditions (NM). EDS analysis of the respective membranes in NM is represented in the upper panel with a) PCL, b) PCL10%HA and c) PCL15%HA. The corresponding membranes in OM is represented in the lower panel with a(i) PCL, b(i) PCL10%HA and c(i) PCL15%HA. The SEM images of the mineral deposition of each membranes is represented along with the EDS data. The Calcium and phosphorous deposition was higher on the membranes cultured with IDPSCs in OM.

Fig. 7  Graphical representation demonstrating the Ca and P levels on PCL, PCL10%HA and PCL15%HA after 21 days of treatment with IDPSCs in NM and OM.
Statistical significance between PCL10%HANM and OM and PCL15%HANM and OM and was denoted by * and **, which represents the \( p \leq 0.05 \) and 0.01 respectively. The difference in the Ca and P deposition on PCL10%HANM and PCL15%HANM membranes was found to be statistically significant and denoted as # and & with \( p \leq 0.05 \). PCL10%HANM had higher mineral deposition than PCL15%HANM.

(Fig. 5a). Higher expression of genes was noticed in all the samples when induced with odontogenic factors than culture conditions without inducing factors.

**Calcium phosphate deposition on membranes and quantification by EDS**
The biomineralization ability of the IDPSCs on the membranes was analyzed after a period of 21 days either in inducing (OM) and non-inducing conditions (NM). SEM images taken after 21 days demonstrated a higher deposition of minerals on all the membranes treated with odontogenic factors. The respective elemental analysis by EDS also demonstrated significantly higher calcium and phosphorous deposit on the membranes cultured with IDPSCs in OM than those in NM (Fig. 6). The quantitative analysis of the Ca and P were done after selecting three respective areas from each SEM image. The quantitative elemental analysis data also depicted a higher Ca and P wt% for all the membranes when induced with odontogenic factors (Fig. 7). However, statistical significance was found between, PCL10%HANM and OM, and PCL15%HANM and OM, in terms of Ca and P deposition. PCL10%HANM also had significantly higher mineral deposition than PCL15%HANM.

**DISCUSSION**
The concentration and parameters used for the electrospinning of the polymer blend composite were based on literature and finalized through trial runs. Since HA ceramic particles were added to the polymer blend, the voltage used is also higher for the composite than that is used for the polymer blend. The variation in fiber morphology is more evident in PCL 15% HA, and can be attributed to the presence of HA which is an insulator, tends to decrease the
orthorhombic crystal structure of crystalline PCL28). The shoulder corresponding to the (111) planes of the peaks corresponding to the (110) and (200) planes while the surface of the membrane29). A worthy observation is the necessary cues for the cells to attach and spread on distribution of HA in PCL10% HA could have provided by the respective elemental analysis EDS. The even cells. This could be attributed to high baseline levels of HA in the interconnected structure of the material facilitated (PCL), 220 (PCL10%HA) and 250nm (PCL15% HA) with staining images. The average fiber diameter of 140 glass when cultured with DPSCs were also found to be hydrophobic, it did not intently affect the cell adhesion as these membranes had significantly higher percentages of attached cells while compared to PCL. This could be attributed to the incorporation of HA in the membrane which could have triggered the downstream signaling events in the cells29).

Favorable metabolic activity and a high percentage of live cells were seen when IDPSCs were incubated with the tested membranes, indicating the cytofriendly nature of the material. This showed that the inflamed dental pulp stem/progenitor cells were able to thrive in the presence of the tested material, which is in favor of utilizing these membranes in dental pulp tissue regeneration studies. Related studies also indicated that PCL electrospun membranes incorporated with materials such as hydroxyapatite, fluorapatite, and bioactive glass when cultured with DPSCs were also found to be biocompatible15–17). When the test materials were seeded with IDPSCs, the cells were able to attach on the surface of the membrane as evident from our SEM and DAPI staining images. The average fiber diameter of 140 (PCL), 220 (PCL10%HA) and 250nm (PCL15% HA) with the interconnected structure of the material facilitated a favorable attachment and spread of the cells on the surface of the membrane. However, it should be noted that while assessing the number of cells attached per square millimeter of the tested material, it was observed that PCL10%HA showed a higher number of attached cells. This could be attributed to high baseline levels of calcium and phosphate in PCL10%HA as assessed by the respective elemental analysis EDS. The even distribution of HA in PCL10% HA could have provided the necessary cues for the cells to attach and spread on the surface of the membrane29). A worthy observation is that in the absence of HA particle incorporation in the PCL membrane, the cell attachment was significantly low.

The differentiation potential of IDPSCs seeded on the membrane to odontoblasts was evaluated by the expression of the DSPP gene which is expressed mainly in odontoblasts and known to play a vital role during primary dentinogenesis30) along with ALP gene which is indicative of early odontoblast development31). In our membranes, the expression of DSPP and ALP was high in PCL10%HA and PCL15%HA when compared to PCL alone in the normal and odontogenic medium. This indicates that the incorporation of HA particles into the membranes has facilitated the upregulated expression of odontogenic specific genes. In confirmation to this fact HA alone treated cells showed significantly higher levels of expression of DSPP and ALP when compared to all PCL membranes, which further reinforces the role of HA in odontogenic differentiation of IDPSCs. In support of the present finding the role of calcium in odontogenic differentiation and mineralization of DPSCs have already been reported in many studies32). Since the ALP gene plays a vital role in the initiation of matrix mineralization, its relatively lower expression is acceptable when compared to DSPP in the present experimental setting as samples were harvested after 21 days of culture. In support of the present findings, in a previous study ALP gene expression was upregulated by day 7 but its expression was down-regulated after 15 days in human dental pulp cells cultures with calcium silicate-based materials30). It is important to note that the levels of expression of odontogenic genes were lower in all test membranes cultured in a non-induction medium when compared to the odontogenic medium. Although the gene expression levels were lower, yet it indicated the potential of the membrane in inducing odontogenesis even in the absence of any induction factors available in the cultures. Between the membranes PCL, PCL10%HA and PCL15%HA when cultured with IDPSC in non-induction medium, PCL10%HA showed upregulated expression of DSPP gene when compared to PCL. It could be hypothesized that the release of calcium from the HA incorporated PCL membranes could have resulted in an upregulated expression of odontogenic specific genes30).

Nodules of mineral deposits which appeared as decorations on the PCL mesh network are apparent on all test membranes when examined after 21 days of its incubation with IDPSC. In support of the expression of odontogenic specific genes in the present study, the biomineralization ability of the tested material also followed a similar trend in calcium and phosphate deposition as assessed by SEM-EDS. The present findings are in correlation with a previous study reporting mineral deposition on fluorapatite modified scaffold when cultured with DPSCs for 21 and 28 days15). The test membranes (PCL10%HA & PCL15%HA) cultured in the non-induction medium had a lower Ca/P ratio when compared to the induction medium. This indicates the mineralization potential of the material even in the absence of the inducing factor in the culture. The reason for the high Ca/P ratio in the control (PCL10%HA and PCL15%HA) when compared to the levels estimated after 21 days in the non-induction medium could be attributed to biodegradation of the membrane resulting in the release of calcium and phosphates. Concurrently odontogenic differentiation of IDPSCs could have
contributed to the deposition of calcium and phosphate on the same membrane, which was seen as nodular deposits in our SEM images. The higher level of mineral deposition seen on the PCL10%HA in non-induction media when compared to PCL15%HA after 21 days could be attributed to the fact that the membranes itself possess odontogenic differentiation potential.

In our study, although we used 15 wt% HA with PCL for electrospinning, the baseline estimation of the Ca and P by SEM-EDS indicated lower levels in PCL15%HA than in PCL10%HA. The lower levels of HA present in PCL15%HA were reflected as lower cell attachments, gene expression and Ca/P ratio. Hence it can be postulated that 10% incorporation of HA in PCL for electrospinning resulted in uniform distribution of HA particles in the membrane which favored positive cell attachments, upregulated expression of DSPP and ALP gene and higher Ca/P ratio.

CONCLUSION

Given the favorable biocompatible behavior of all the membranes tested and robust odontogenic differentiation properties of IDPSC seen in PCL10%HA, this material has a promising potential to be used as a pulp capping material on vital pulp exposure with inflamed pulp cells. However, further exploration of the in-depth signaling events and pre-clinical evaluation should be carried out for the successful implementation of the test material in clinical settings.

ACKNOWLEDGMENTS

This work was funded by the University of Sharjah, Competitive Research Grant (Project No. 1901100237).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1) Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009; 88: 792-806.
2) Nuti N, Corallo C, Chan BM, Ferrari M, Gerami-Naini B. Multipotent differentiation of human dental pulp stem cells: A literature review. Stem Cell Rev 2016; 12: 511-523.
3) Goldberg M, Smith AD. Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. Crit Rev Oral Biol Med 2004; 15: 13-27.
4) Gandolfi MG, Siboni F, Botero T, Bossu M, Riccielli F, Prati C. Calcium silicate and calcium hydroxide materials for pulp capping: biointeractivity, porosity, solubility and bioactivity of current formulations. J Appl Biomater Funct Mater 2015; 13: 43-60.
5) Kang JY, Lee BN, Son HJ, Koh JT, Kang SS, Son HH, et al. Biocompatibility of mineral trioxide aggregate mixed with hydration accelerators. J Endod 2013; 39: 497-500.
6) Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, et al. Response of human dental pulp capped with biodentine and mineral trioxide aggregate. J Endod 2013; 39: 743-747.
7) Okiji T, Yoshioka K. Reparative dentinogenesis induced by mineral trioxide aggregate: a review from the biological and physicochemical points of view. Int J Dent 2009; 2009: 464280.
8) See MS, Hwang KG, Lee J, Kim H, Baek SH. The effect of mineral trioxide aggregates on odontogenic differentiation in dental pulp stem cells. J Endod 2013; 39: 242-248.
9) Ma PX. Biomimetic materials for tissue engineering. Adv Drug Deliv Rev 2008; 60: 184-198.
10) Ruckl TT, Carroll DA, Weaver JR, Popat KC. Mineralization content alters osteogenic responses of bone marrow stromal cells on hydroxyapatite/polycaprolactone composite nanofiber scaffolds. J Funct Biomater 2012; 3: 776-798.
11) Lee W, Oh JH, Park JC, Shin HI, Baek JH, Ryoo HM, et al. Performance of electrospray poly(epilon-caprolactone) fiber meshes used with mineral trioxide aggregates in a pulp capping procedure. Acta Biomater 2012; 8: 2986-2995.
12) Lee LW, Hsiao SH, Hung WC, Lin YH, Chen PY, Chiang CP. Clinical outcomes for teeth treated with electrospray poly(epilon-caprolactone) fiber meshes/mineral trioxide aggregate direct pulp capping. J Endod 2015; 41: 628-636.
13) Lin HM, Lin YH, Hsu FY. Preparation and characterization of mesoporous bioactive glass/polycaprolactone nanofibrous matrix for bone tissues engineering. J Mater Sci Mater Med 2012; 23: 2619-2630.
14) Vatankhah E, Semnani D, Prabhakaran MP, Tadayon M, Razavi S, Ramakrishna S. Artificial neural network for modeling the elastic modulus of electrospray polycaprolactone/gelatin scaffolds. Acta Biomater 2014; 10: 708-721.
15) Guo T, Li Y, Cao G, Zhang Z, Chang S, Czajka-Jakubowska A, et al. Fluorapatite-modified scaffold on dental pulp stem cell mineralization. J Dent Res 2014; 93: 1290-1295.
16) Guo T, Cao G, Li Y, Zhang Z, Nor JE, Clarkson BH, et al. Signals in stem cell differentiation on fluorapatite-modified scaffolds. J Dent Res 2018; 97: 1331-1338.
17) Kim GH, Park YD, Lee SY, El-Fiqi A, Kim JJ, Lee EJ, et al. Odontogenic stimulation of human dental pulp cells with bioactive nanocomposite fiber. J Biomater Appl 2015; 29: 864-866.
18) Jeong J, Kim JH, Shim JH, Hwang NS, Heo CY. Bioactive calcium phosphate materials and applications in bone regeneration. Biomater Res 2019; 23: 4.
19) Hokmabad VR, Davaran S, Aghazadeh M, Rahbarghazi R, Salehi R, Ramazani A. Fabrication and characterization of novel ethyl cellulose-grafted-poly (ε-caprolactone)/alginate nanofibrous/macroporous scaffolds incorporated with nano-hydroxyapatite for bone tissue engineering. J Biomater Appl 2019; 33: 1128-1144.
20) Kim W, Jung CH, Kim G. Optimally designed collagen/polycaprolactone biocomposites supplemented with controlled release of HA/TCP/rhBMP-2 and HA/TCP/PRP for hard tissue regeneration. Mater Sci Eng C Mater Biol Appl 2017; 78: 763-772.
21) Nowwarote N, Chanjanavakul P, Kondecha P, Clayhan P, Chumprasert S, Manokawinchoke J, et al. Characterization of a bioactive Jagged1-coated polycaprolactone-based membrane for guided tissue regeneration. Arch Oral Biol 2018; 88: 24-33.
22) Venugopal J, Prabhakaran MP, Zhang Y, Low S, Choon AT, Ramakrishna S. Biomimetic hydroxyapatite-containing composite nanofibrous substrates for bone tissue engineering. Philos Trans A Math Phys Eng Sci 2010; 368: 2065-2081.
23) Rameshbabu N, Prasad Rao K, Sampath Kumar TS. Accelerated microwave processing of nanocrystalline hydroxyapatite. J Mater Sci 2005; 40: 6019-6023.
24) Gopinath VK, Soumya S, Jayakumar MN. Osteogenic and odontogenic differentiation potential of dental pulp stem cells isolated from inflamed dental pulp tissues (I-DPSCs) by two
different methods. Acta Odontol Scand 2020; 78: 281-289.
25) Fattahi P, Dover JT, Brown JL. 3D near-field electrospinning of biomaterial microfibers with potential for blended microfiber-cell-loaded gel composite structures. Adv Healthc Mater 2017; 6: 10.
26) Sachot N, Castano O, Planell JA, Engel E. Optimization of blend parameters for the fabrication of polycaprolactone-silicon based ormoglass nanofibers by electrospinning. J Biomed Mater Res B Appl Biomater 2015; 103: 1287-1293.
27) Siva Rama Krishna D, Siddharthan A, Seshadri SK, Sampath Kumar TS. A novel route for synthesis of nanocrystalline hydroxyapatite from eggshell waste. J Mater Sci Mater Med 2007; 18: 1735-1743.
28) Liu JY RL, Wei Q, Wu JL, Liu S, Wang YJ, et al. Fabrication and characterization of polycaprolactone/calcium sulfate whisker composites. Express Polym Lett 2011; 5: 742-752.
29) Venugopal JR, Low S, Choon AT, Kumar AB, Ramakrishna S. Nanohioengineered electrospun composite nanofibers and osteoblasts for bone regeneration. Artif Organs 2008; 32: 388-397.
30) Simon S, Smith AJ, Lumley PJ, Berdal A, Smith G, Finney S, et al. Molecular characterization of young and mature odontoblasts. Bone 2009; 45: 693-703.
31) Larmas M. Pre-odontoblasts, odontoblasts, or “odontocytes”. J Dent Res 2008; 87: 198.
32) Sohn S, Park Y, Srikanth S, Arai A, Song M, Yu B, et al. The role of ORAI1 in the odontogenic differentiation of human dental pulp stem cells. J Dent Res 2015; 94: 1560-1567.
33) Chen CC, Shie MY, Ding SJ. Human dental pulp cell responses to new calcium silicate-based endodontic materials. Int Endod J 2011; 44: 836-842.