# The Osteogenic Differentiation of Mesenchymal Stem Cells Derived from Dental Pulp in Modular Alginate-Gelatin/Nano-Hydroxyapatite Microcapsules

**Mahdieh Alipour**
Tabriz University of Medical Sciences https://orcid.org/0000-0002-9717-8213
**Nima Firouzi**
Sahand University of Technology
**Marziyeh Aghazadeh**
Tabriz University of Medical Sciences Faculty of Dentistry
**Mohammad Samiei**
Tabriz University of Medical Sciences Faculty of Dentistry
**Soheila Montazersaheb**
Tabriz University of Medical Sciences
**Ali Baradar Khoshfetrat** ( khoshfetrat@sut.ac.ir )
Sahand University of Technology
**Zahra Aghazadeh** ( Aghazadehz@tbzmed.ac.ir )
Tabriz University of Medical Sciences

**Research**

**Keywords:** Alginate-gelatin microcapsules, Bone Tissue Engineering (BTE), Human Dental Pulp Stem Cells (hDPSCs), Nano-hydroxyapatite

**Posted Date:** May 29th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-31275/v1

# Abstract

**Background** Microcapsule is considered as a promising 3D microenvironment for Bone Tissue Engineering (BTE) applications. Microencapsulation of cells in an appropriate scaffold not only protects cells against excess stress but also promotes cell proliferation and differentiation.

**Methods** In the current study, human Dental Pulp Stem Cells (hDPSCs) were cultivated in Alginate/Gelatin (Alg/Gel) and Alginate/Gelatin/nano-Hydroxyapatite (Alg/Gel/nHA) microcapsules. The proliferation and osteogenic differentiation of these cells were evaluated by MTT assay, qRT-PCR, Alkaline phosphatase, and Alizarine Red S.

**Results** The results revealed that microencapsulation by Alg/Gel/nHA could improve cell proliferation and induce osteogenic differentiation. The cells cultured in the Alg/Gel and Alg/Gel/nHA microcapsules after 28 days showed 2.5-fold and 4-fold more activity of BMP-2 gene expression in comparison with the cells cultured on the polystyrene surface as a control group. The nHA addition to hDPSCs-laden Alg/Gel microcapsule could also up-regulate the bone-related gene expressions of osteocalcin, osteonectin, and RUNX-2 during a 28-day culture period. Calcium deposition and ALP activities of the cells were obsereved in accordance with the proliferation results as well as the gene expression analysis.

**Conclusion** The study demonstrated that microencapsulation of hDPSCs inside Alg/Gel/nHA hydrogel can be a potential approach for regenerative dentistry in the near future.


# 1. Introduction

Congenital and acquired bone defects and diseases result in significant morbidity and reduction of quality of life for wide range of patients. Bone defects in critical sizes remain challenging for dentists and orthopedic surgeons (1). Allogenic and xenogenic bone grafts are available but infection transmission and limited efficacy are the main disadvantages (2). The autologous bone grafts are commonly used for these defects and considered as a gold standard method (3). However, the limitations of these grafts such as donor site morbidity as well as increased operative time have restricted the method application (4). Designing natural and synthetic scaffolds with the ability of delivering growth factors for stem cell growth and differentiation is a novel strategy desires to overcome these drawbacks in the Bone Tissue Engineering (BTE) (5, 6). Although cell-based therapies play an important role in regenerative medicine, there is a major concern for efficient delivery and resistance of transferred cells after injection or implantation. One of the novel advanced methods as a solution can be tissue modification with the fabrication of modular block(7).

The appropriate scaffold as a key part of tissue engineering should provide cell survival, induce cell bioactivity, and enhance cell retention in implanted sites. Encapsulation of cells with various polymers protects them from injection forces and immune system reactions while allowing the bidirectional diffusion of nutrients and wastes (8–10). Microcapsules as spherical microcarriers have been recently used for providing a three-dimensional hydrophilic microenvironment for supporting and transferring of

stem cells to the site of defects (11–13). This technique has shown successfully *in vitro* and *in vivo* results in the treatment of diabetes, liver dysfunction, and neurological disease (14–18).

Bone has a highly complex hierarchical structure containing a porous composite of hydroxyapatite and collagen (19). Bone Marrow Mesenchymal Stem Cells (BMSCs) are the major sources for bone tissue engineering. However, the morbidity of the donor site and the painful harvesting methods of these cells have limited the application of the cells (20, 21). Therefore, Human Dental Pulp Stem Cells (hDPSCs) were introduced as multipotent stem cells with self-renewal ability by Gronthos et al. These cells could be harvested from both primary and permanent teeth (22). hDPSCs offer the regenerative potential of different damaged or lost tissues and organs including dentin, pulp, periodontium, bone, neuronal tissue, blood vessels, muscle, cartilage, hair follicle, and cornea (23).

In bone tissue engineering, DPSCs showed more proliferation ability and number of colony clusters compared to BMSCs (24). Also, their harvesting method is less invasive and they are more accessible compared with BMSCs (25).

Alginate polysaccharide is a member of the linear polymeric acid groups, which were isolated from brown sea algae (3, 26). Alginate is a biopolymer contains G monomers (G block) which have a high affinity to $Ca^{2+}$ (27). Alginate properties such as degradability in the physiologic situation, low toxicity, and ability to support deposition of a calcified matrix, turn it to important material in bone tissue engineering (28). Also, the alginate matrix is porous and hydrophil structure, which allows oxygen, nutrients, and wastes transportation (27). The ability of alginate to make hydrogels in the presence of cations such as calcium and barium makes it one of the choice polymers in cell and protein delivery, tissue engineering, and wound dressing material (29). This material has been widely used as the main component for the capsulation of different cells (30–32).

Gelatin is a natural polymer with a similar composition to collagen, which could provide the proper structure for BTE. The development of suitable conditions in deposition and nucleation mineral phase in addition to availability and low costs compared with collagen makes gelatin a choice component in bone tissue engineering (33–35).

Bioactive materials used in BTE should enhance the osteoinductivity of the scaffold (36, 37). Nano-hydroxyapatite (nHA) is the main inorganic component of bone matrix embedded in the organic component of natural bone (collagen type I) (38, 39). Due to outstanding features such as biocompatibility, osteoconductivity, non-toxicity, and the ability to be resorbed into bone tissue, nano-hydroxyapatite could improve bone regeneration when incorporated with scaffolds. Nano-hydroxyapatite crystals increase the strength of collagen fibers, facilitate and promote bone formation with increasing cell adhesion. Also, hydroxyapatite improves the deposition of mineral compositions contain calcium ions (40–42). Studies showed that nano-hydroxyapatite particles increased surface roughness, which improved the absorption of chemical species from the surrounding environment (43, 44).

In our previous studies, osteogenic potential of Alginate/Gelatin/nano-hydroxyapatite (Alg/Gel/nHA) microcapsule for modular bone formation was evaluated by using the osteoblastic cell line (6, 44). In the current study, Alginate/Gelatin (Alg/Gel) and Alginate/Gelatin/nano-hydroxyapatite (Alg/Gel/nHA) microcapsules containing hDPSCs were used to investigate the influence of 3D spherical scaffold and nano-hydroxyapatite (nHA) on cell proliferation and osteogenic differentiation of the stem cells for bone regeneration. This study would illuminate the hDPSCs phenotype in 3D microbeads and its potential toward the formation of modular bone tissues for future *in vivo* studies.

## 2. Materials And Methods

# 2.1. Materials

Gelatin (type A, from porcine skin, 300 bloom), nHA (average size 100 nm), alginic acid sodium salt (from brown algae, medium viscosity), Alizarin red S, propidium iodide (PI), BCIP/NBT (5-Bromo–4-chloro–3-indolyl phosphate/Nitroblue tetrazolium) and barium chloride were purchased from Sigma-Aldrich. Trypsin, fetal bovine serum (FBS), high glucose Dulbecco's modified Eagle's medium (DMEM/HG), and penicillin/streptomycin were obtained from Gibco (Singapore). 3-(4,5-Dimethylthiazol–2-yl–2, 5-diphenyltetrazolium bromide) (MTT) and Trizol reagent were supplied from Invitrogen (Carlsbad, CA, USA). Complementary DNA (cDNA) synthesis kit and SYBR Green PCR Master Mix were purchased from Yekta Tajhiz Company (Tehran, Iran).

# 2.2. Isolation and adhesion of human dental pulp stem cells (hDPSCs)

This study was approved by the stem cell research center of Tabriz University of Medical Sciences under the code of TBZMED.REC.1396.654. All experimental protocols were in compliance with the Helsinki declaration.

Human dental pulp stem cells were isolated from extracted permanent teeth according to orthodontic treatment in the Oral and Maxillofacial Surgery Department of Dental Faculty of Tabriz University of Medical Sciences. All participants signed the written consent after being informed about the objective of the study.

Isolation and characterization of the hDPSCs were performed as our previous study (25). Briefly, after teeth extraction under local anesthesia, teeth were split with a chisel and the extracted pulp tissue was divided into small pieces. Pulp pieces were then digested in 3 mg/mL type I collagenase and 4 mg/ml dispase for 40 min at 37°C. After centrifuging, the harvested cells were cultured to reach 80% confluence. At the third passage, the cell suspension was transferred to round end bottom tubes and stained with immunoglobulin G–fluorescein isothiocyanate-conjugated or phycoerythrin-conjugated anti- CD105, CD90, CD166, CD73, CD11b, CD34, CD133, CD64, CD106, CD31 and CD45 (Beckman Coulter, Villepinte,

France, 20 mL each). After that, the cells were washed by fluorescence-activated cell sorting (FACS) wash solution and centrifuged for 5 min.

Dental pulp stem cells were transferred to 75 cc flasks containing DMEM high glucose amplified with 10% fetal bovine serum (FBS), 100 U/mL penicillin/ streptomycin, and 1% amphotericin B for proliferation.

# 2.3. Microcapsule fabrication and analysis

Powder of gelatin and nano-hydroxyapatite were sterilized by autoclaving and alginic acid sodium salt was suspended in ethanol 70% and kept under the laminar hood for 24h.

Cell microcapsulation was carried out according to our previous study (7). Concisely, 2% w/v sodium alginate with and without 2% w/v nHA and sterilized gelatin were dissolved in calcium-free Krebs Ringer HEPES-buffered saline (CF-KRH, pH = 7.4). Microcapsules preparation was carried out by the voltage power supply and syringe pump (Vita Teb, Iran). $2\times10^6$ cells/ml were mixed with alginate (1%) solutions containing gelatin (1.25%) with or without nHA (1%) and loaded into syringes equipped to 30-gage needles. Extruded microcapsules were dropped in CF-KRH containing 100 mM $BaCl_2$ as a crosslinking solution. The voltage and extrusion flow rate were considered 8kV and 0.08 ml/min, respectively. Continuously, the formed microcapsules were washed by CF-KRH buffer twice to remove unbounded barium ions. Then, both prepared Alginate-Gelatin (Alg/Gel) and Alginate-Gelatin/Nano-Hydroxyapatite (Alg/Gel/nHA) microcapsules were transferred to distinct flasks containing the culture medium. The flasks were then incubated under the atmosphere of 5% $CO_2$ at 37 °C and monitored for 28 days for more cell experiments and morphology evaluation by microscope (Olympus IX71). The culture medium was refreshed every 3 days. The surface morphology of the microcapsule samples was also observed by scanning electron microscopy (SEM, Tescan MW2300).

# 2.4. Proliferation and differentiation of hDPSCs

## 2.4.1. Metabolic activity and live/dead assays

Human dental pulp stem microbeads were transferred to 96-well plates to determine metabolic activity in 7, 14, and 21 days. 5 mg/ml MTT solution was added to each well. The plates were incubated under the atmosphere of 5% $CO_2$ at 37 °C for 4 h. Then, DMSO was added to wells and the absorbance was measured by the UV–160 spectrophotometer (BioQuest) at 570 nm. Dental pulp stem cells cultured on polystyrene surface (PS) were considered as control. The absorbance values were normalized with considering the number of microcapsules per sample and the experiments were carried out in triplicates and the data was reported as the mean ± SD.

To live/dead assay, after rinsing microcapsulated cells by PBS, these microcapsules were incubated in saline containing calcein-AM and propidium iodide (PI) at 37°C, as described elsewhere (45). The microcapsules were then washed with PBS for three times again after incubating in 5% carbon dioxide for 45 minutes. Finally, the microcapsules containing hDPSCs were observed under the fluorescence microscope (Olympus, IX71) at wavelengths of 488 nm (green, living cells) and 543 nm (red, dead cells).

## 2.4.2. qRT- PCR

The osteogenic gene expression was evaluated after 21 and 28 days. After washing microcapsules by PBS, samples were fractured gently. Then, Trizol reagent was used for the extraction of total RNA according to the manufacture instruction. The gel electrophoresis and Nanodrop (Thermo Scientific, Waltham, MA, USA) were applied for the determination of the yield and value of extracted RNA. 1µg of total RNA was used for cDNA synthesis by CDNA synthesis kit (YektaTajhizAzma, Iran). Synthesized cDNA, syber green master mix, and designed primers were mixed based on the manufacture instruction. Primer sequences, melting temperature, and amplicon size of designed primers were offered in Table 1. Dental pulp stem cells without microcapsules were used as the control group and all the experiments were repeated three times.

| Target | Sense and antisense sequences 5’ to 3’ | tA (°C) | bp |
|---|---|---|---|
| BMP-2 | F: GAGAAGGAGAGGCAAAGAAAG | 61/59 | 183 |
| | R:GAAGCAGCAACGCTAGAGAC | | |
| Osteocalcin | FATTGTGGCTCACCCTCCATCA : | 60 | 119 |
| | R: AGGGCTATTTGGGCGTCATC | | |
| Osteonectin | F:GCAGAGGAAACCCAAGAGGAG | 60 | 208 |
| | R: TGGCAAAGAAGTGGCAGCAG | | |
| RUNX-2 | F:ACCTTGACCATAACCGTCTTC | 67/57 | 145 |
| | R: GGCGGTCAGAGAACAAACTA | | |
| DSPP | F:CTGGTGCATGAAGGTCATAGAG | 57 | 90 |
| | R: CAATTTGCGGATCTCAGAGG | | |
| GAPDH | F: GAAGGTGAAGGTCGGAGTC | 65 | 100 |
| | R: GAAGATGGTGATGGGATTTC | | |

Table 1
Sequences, melting temperature and amplicon size of primers used for RT-PCR

### 2.4.3. Alkaline phosphatase activity assay

Alkaline phosphatase (46) tests were carried out for hDPScs microcapsulation after 21 days. For alkaline phosphatase staining, BCIP/NBT solution was prepared and added over non-medium microcapsules before incubating in a dark situation. Then microcapsules were kept in formalin solution (10%) for one min. Finally, the microcapsules were washed by PBS buffer and photographed. To quantify ALP activity, according to the method described elsewhere (44), 21 days after microcapsulation of hDPSCs the microcapsules were rinsed with PBS and centrifuged at 1200 rpm for 20 min. Then, the supernatant was collected and the ALP activity was detected using ALP assay Kit (DIALAB, Austria) according to the manufacturer protocol. ALP activity was measured by evaluating the absorbance at 405 nm by the spectrophotometer and normalized with total protein content. The experiments were carried out in triplicate and hDPSCs cultured on the conventional polystyrene culture surface was used as control.

### 2.4.4. Alizarin Red S Staining

Calcium deposition of hDPSCsin the modular structure was assessed by alizarin red S staining after 21 days. Shortly, microcapsules were removed from the flask and washed three times by HEPES buffer and fixed by 3.6% (v/v) formaldehyde solution for 15 min at 25°C. 2% alizarin red liquid (pH: 4.1–4.3) was then poured over the samples in a dark place and maintained for 15 min. Finally, the stained microcapsules were washed by deionized water several times and examined under the microscope.

## 2.5. Statistical Analysis

The data were determined as the mean ± standard deviation and comprised by using One-way ANOVA and Tukey Test analysis with Prism software (version 8.0, GraphPad, San Diego, CA, USA). P-value<0.05 was considered statistically significant.

# 3. Results

## 3.1. Influence of hydrogel composition on microcapsule stability and microcapsulated hDPSCs proliferation

Figure 1 reveals the surface morphology of both hydrogel microcapsules of Alg/Gel (Fig. 1A) and Alg/Gel/nHA (Fig. 1B) as well as the appearance of the proliferated hDPSCs in the microcapsules (Fig. 1C). Interestingly, the Alg/Gel microcapsules containing nHA showed a rough and more compact surface morphology in comparison with the Alg/Gel microcapsules (Fig. 1 A-B) revealing microcapsule stability can be improved when nHA is added to the alginate-based hydrogels.

Live and dead assay (Fig. 1C) cell proliferation in majority of the fabricated microcapsules. However, some of the cells died inside the bigger aggregates of the microcapsules. The cell viability in the micro-

carrier structures can be affected by the sizes of more than 400 μm (47). The highly hydrated 3-D alginate-based microbeads, as shown in Fig. 1C, could provide an immobilized matrix for the cells with a permeable membrane for waste, nutrients, and oxygen transmissions. Therefore, the average diameter of microcapsules was examined by BEL view software (ver.6.2) (data are not shown). More than 65% of the microcapsules at both cases had 300±40μm diameter leading to sufficient nutrient and waste transmissions to the inner core cells. The microscopy images of microcapsules were shown in Figure 2.

Mitochondrial activity as a vital cue demonstrates the viability of cells. Human dental pulp stem cells were cultured in both nHA modified and unmodified Alg/Gel microcapsules (fig3). Unmicrocapsuled stem cells cultured on the conventional PS were considered as a control group. Figure 3 shows the proliferation of hDPSCs in the microcapsules and without microcapsules for a 3-week culture period. The proliferation of stem cells increased in the microcapsules and on the PS during this period. Dental pulp stem cells in the microcapsules revealed statistically more mitochondrial activities as compared to the control group during the culture period ($P < 0.05$). Interestingly, the microcapsules containing nano-hydroxyapatite showed significant increases in all experiment times. Using nano-hydroxyapatite in microbeads increased the mitochondrial activity of the stem cells 1.26 times ($P < 0.05$) per microcapsules after 3 weeks. These outcomes can be accomplished that nano-hydroxyapatite plays an important role in the proliferation of dental pulp stem cells.

## 3.2. Influence of microcapsule composition on bone-related gene expressions of microencapsulated hDPSCs

The qRT-PCR analysis was performed to evaluate the effect of Alg/Gel and Alg/Gel/nHA microbeads on the expression levels of the key genes in bone and dentin formation including osteocalcin, BMP2, RunX2, osteonectin, and DSPP after 21 and 28 days.

According to the results shown in Figures 4 and 5, the expression levels of all the genes increased in the hDPSCs, which were microcapsulated in the Alg/Gel and Alg/Gel/nHA as compared with the control.

BMP–2 levels on day 21 significantly increased only in the Alg/Gel/nHA group. However, on day 28, the expression levels were significantly higher in both groups comparing the control group. By comparing BMP–2 expression in two distinct days, it could figure out that the expressions of this osteogenic gene in microbeads, especially in the presence of nano-hydroxyapatite were up-regulated.

21 days after microcapsulation, osteocalcin expression in both microcapsule groups was significantly higher than the control group. Although the expression levels in the Alg/Gel/nHA hydrogel microcapsule were higher than the Alg/Gel groups after 21 and 28 days, this difference was statistically significant only on the $28^{th}$ day ($P < 0.05$).

After 21 days, osteonectin expression in the Alg/Gel microcapsules was in the range of the control sample, while a significant increase of the gene expression was observed in the Alg/Gel/nHA group.

However, after 28 days, both microcapsule groups showed significant increases in the osteonectin expression levels in comparison with the stem cells without microcapsules.

In a like manner, it can be imagined that the concentrations of both osteocalcin and osteonectin can be intensified by the passing of time. These results provided great confidence in Alg/Gel/nHA microcapsule sufficiency to create bone volume in *in vivo* experiments.

RUNX–2 expression of the hDPSCs in the Alg/Gel/nHA microcapsules showed a 3.5-fold increase as compared to the control sample on the 21stday, the value of which was about 2.6-fold for the Alg-Gel microcapsule. However, after 28 days, the significant RUNX–2 up-regulation was observed in the Alg/Gel/nHA group which was 5.1-fold more than the control group ($P < 0.01$).

The results revealed that a three-dimensional microenvironment that was created by alginate-based microcapsules containing nHA could up-regulate RUNX–2 expression considerably in comparison with the conventional two-dimensional control group.

As shown in Figure 4, DSPP expression in both the Alg/Gel/nHA and Alg/Gel microcapsule groups showed no significant differences between the groups after 21 days. The expression of the DSPP gene on the 28th day for both the microcapsule groups, however, was significantly increased, the value of which was 2.5-fold and 3-fold higher than the control group,. The low up-regulation of this gene as a classic marker of odontogenic differentiation compared to other osteogenic genes demonstrated the useful application of microcapsulation of hDPSCs with Alg/Gel/nHA in bone tissue engineering.

## 3.3. Influence of microcapsule composition on hDPSCs osteogenic marker production and mineralization

To validate the presence of osteocalcin and bone sialoprotein, two distinct stainings were carried out. The enzymatic activity can be asserted directly for the dyed cells. As shown in Figure 6.A, blue-color spots have been shown in Figure 6.A indicated the positive ALP activity in the fixed days. Intensified hued microbeads with nano-hydroxyapatite presence aggravate significantly hydrolase enzyme expression, motivating ALP expression at the surface of the hDPSCs, as compared to the Alg/Gel microbeads.

Quantification of alkaline phosphatase measured in the microcapsules revealed further corroborating evidence for the hDPSCs differentiation. According to the ALP activity shown in Figure 6.B, differentiation of the hDPSCs to osteoblast-like cells increased significantly 2.7-fold and 3-fold in the Alg/Gel and Alg/Gel/nHA microcapsules, respectively. The increases in the ALP levels as early bone formation indicator in the Alg/Gel/nHA were statistically higher as compared to the Alg/Gel microbeads ($P < 0.05$). In fact, microspheres create three-dimensional space for cells to be in the exposure of factors that accelerate differentiation as well as retain cells phenotype. The quantitative analysis confirmed that ALP enzyme activity increased remarkably in the alginate-based microcapsules in comparison to non–3D scaffolds.

As also illustrated in Figure 6.A for calcium deposition, arrows show red-colored surroundings nodules, which demonstrate mineralized cells in the microcapsules. Hydroxyapatite particles in the presence of Alg/Gel composition increased calcium deposition on days 21.

The results also confirmed the formation of mineralized matrix and osteoblastic differentiation in both the microcapsules.

Consequently, it can be deduced that the 3-D microsphere network provided a network for cells to precipitate calcium and relevant enzymes content overtime to speed up modular formation.

# 4. Discussion

Microcapsules are widely used in tissue engineering including regeneration of different tissues and organs such as liver, cartilage, skin, neural tissue, and bone(9, 48–52). These microstructures with different characteristicsincluding immunoisolation, micrometer size, and providing 3D microenvironment considered a promising approach in regenerative medicine(9, 53). These modular microcarriers could directly be injected and transplanted to the defect side(54). This property is important for the reconstruction of hard tissues. The injectable microcarriers could adjust to bone defects with irregular shape and geometry, occupying the available spaces, precluding the invagination of the adjacent tissues and promoting tissue repairing(55, 56). As shown in graphical abstract, the current study aimed to use the available source of stem cells in the oral cavity and microcapsulating of theses cells for modular tissue engineering approach of bone defects in the oral and maxillofacial region.

The main findings of this study is the high osteogenic differentiation capacities of hDPSCs in Alg/Gel/nHA microcapsules. Moreover, both ALP and Alizarin red staining of micro carriers showed a greater extent of mineral deposition in nano- hydroxyl apatite modified microcapsules.

In the current study we investigated the osteogenic potential of hDPSCs as an available source of mesenchymal stem cells in Alg/Gel/nHA microcapsules for the first time.

Alginate is a natural biopolymer that has been widely used for drug delivery, dental impression materials, and tissue engineering (4, 57).

Gelatin is derived from collagen, the major organic component of the extracellular matrix of bone tissue, with adequate properties for bone tissue regeneration and revascularization (58, 59).

In the structure of Alg/Gel microcapsules, a rapid reaction between carboxyl groups of alginate and barium cations makes it possible to form an ion-crosslinking hydrogel. Microcapsules were cross-linked by the ionotropic gelation in the presence of $BaCl_2$ that has shown high stability and low degradability in comparison to $CaCl_2$ (60). Indeed, adding nHA to the system increases the crosslinking of the polymeric network, leading to an enhanced homogeneity and strength of the microcapsules (55).

Nano-hydroxyapatite (nHA) as the major inorganic mineral part of bone could increase the homogeneity and strength of microcapsules. Moreover, nHA increased the crosslinking of the polymeric network (55). These features besides, the facilitation of bone formation in the presence of nHA turn it to the important component of BTE (27).

The interaction between calcium ions of nHA and G-blocks of alginate makes a strong matrix with the rougher surface which improves cell adhesion, proliferation, and differentiation compared to smooth alginate surface. In the current study, the proliferation of hDPSCs in the Alg/Gel/nHA microcapsules was higher than Alg/Gel and control groups. Moreover, the cell viability in the micro-carrier structures was influenced by the size and mass too, which can diffuse inner layers (47). The viability and proliferation of hDPSCs in Alg/Gel and Alg/Gel/nHA microcapsules increased during the study. This viability is a result of an appropriate bead size, which could provide an immobilized matrix with a permeable membrane that facilitates waste, nutrients, and oxygen transmission for cells. This could avoid the crowd of toxic wast and lead to appropriate activity and proliferation of cells. These results were in accordance with other previous studies that used microcapsulation methods for cell transferring (7, 27).

The aim of BTE is not only to provide 3D structutres and cell proliferation without cell cytotoxicity or foreign body reaction but also the regeneration of new bone by provoking osteogenic differentiation of stem cells (4, 61).

In the oral and maxillofacial regions for the regeneration of bone defects, the non-invasive injectable methods for stem cell delivery are more useful (62). The osteo/odontogenic differentiation of hDPSCs on various scaffolds was evaluated previously (41, 43, 63, 64). However, none of these scaffolds evaluated non-invasive injectable carriers for these cells. In the current study, we transferred these accessible stem cells in the oral region to spherical injectable microcapsules, which could be useful for bone regeneration of this area.

The osteogenic differentiation of hDPSCs in Alg/Gel and Alg/Gel/nHA group was assessed by the relative expression of BMP–2, Osteocalcin, Osteonectin, and RUNX–2. Various extracellular ligands such as BMPs, WNTs, and FGFs controlled osteogenic differentiation of different multipotent mesenchymal stem cells (65). These ligands direct the three main stages of osteogenic differentiation, which associate with the expression of some genes like BMP–2, RunX–2, Osteocalcin, and Osteonectin (46).

The expression of BMP–2 as the most important growth factor in bone formation is known as an early indicator of calcified tissue generation and osteoblastic differentiation (61, 66). The expression of this factor was significantly higher in Alg/Gel/nHA group after 21 and 28 days.

Osteocalcin and osteonectin are noncollagenous proteins in the extracellular matrix of bone. Osteocalcin has an important role in the maturation of mineralized tissues and the regulation of osteogenic differentiation of mesenchymal stem cells (67). The microcapsulation of hDPSCs has positive effects on the expression levels of these genes. However, these effects were greater in Alg/Gel/nHA group.

RUNX–2 is known as a critical transcription factor associated with bone formation and plays an important role in the differentiation of pre-osteoblastic cells to mature osteoblasts. Also, this factor upregulates the VEGF factor which is important in the angiogenesis of bone tissue (29, 68). The upregulation of this gene as a late indicator of osteogenesis was higher than other genes after 21 and 28 days.

Moreover, DSPP, a classic odontogenic differentiation marker, gene expression was assessed in order to investigate any odontogenic differentiation of hDPSCs in microcapsules. As demonstrated in results, the up-regulation of this gene was not increased as well as osteogenic genes in microcapsules, which indicates the osteogenic potential of Alg/Gel/nHA microcapsules rather than odontogenic potential.

In general, hDPSCs in Alg/Gel/nHA microcapsules showed higher expression of these genes after 21 and 28 days. These results were in compliant with other previous studies, which evaluated the different alginate- nano hydroxyapatite-based scaffolds on osteogenic differentiation of stem cells (55, 61, 69).

To evaluate the mineralized deposition of hDPSCs in Alg/Gel/nHA microcapsules Alkaline phosphatase and Alizarin red staining were performed in the current study. The formation of mineralized nodules as shown in our results indicate the late stage of osteogenic differentiation. In summary, our results indicated the notable enhancement in mineralization of hDPSCs in Alg/Gel/nHA microcapsules after 21 days.

The combination of calcium with alizarin red makes orange nodules, which showed mineralization sites (41). These nodules were clearly observed in Alg/Gel/nHA group.

ALP enzyme activity as a major factor in the beginning phase of bone matrix mineralization was quantified by the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol (70).This enzyme is a universal marker for the indication of mineralization. ALP deposition occurs during osteoblast maturation and bone matrix mineralization (71). Therefore, the ALP activity could demonstrate the biological activity of osteoblasts (41). Moreover, The formation of the bone-like mineralized tissue due to calcium deposition is essential to bonding of newly regenerated bone with the former bone tissue (55, 72).

The results observed for physical and osteoconductive properties of nHA particles in the hDPSCs-laden alginate hydrogels were in agreement with the reported results of other previous studies (6, 26, 41, 44), indicating the importance of nHA bioactivity in the composite hydrogel.

In general, our results showed that the proliferation of hDPSCs increased and the expression of all osteogenesis genes upregulated in nano-hydroxyapatite containing microcapsules. Moreover the calcium deposition was increased in the presence of nHA. These results are in compliance with other studies, which used these microcapsules on different stem cells (45, 48, 73). However, this study evaluated these modular microcapsules in osteogenic differentiation of hDPSCs for the first time, based on authors' information.

## Conclusion

The cell viability and proliferation of hDPSCS in both Alg/Gel and Alg/Gel/nHA microcapsules increased. Beside, differentiation of hDPSCs was immoderately intensified in the presence of nano-hydroxyapatite. In the engineering of hard mineralized tissues, the structure of the carriers must be hardened in order to simulate the real extracellular matrix. The presence of nano-hydroxyapatite particles in microcapsules simulated the real architecture of bone tissue. In addition, these engineered scaffolds allowed the cells to maintain their original phenotype in the absence of inducers, which is important for future in vivo application.

## Abbreviations

**Alg/Gel:** Alginate/Gelatin

**Alg/Gel/nHA:** Alginate/Gelatin/nano-Hydroxyapatite

**ALP:** Alkaline phosphatase

**BMSCs:** Bone Marrow Mesenchymal Stem Cells

**BTE:** Bone Tissue Engineering

**hDPSCs:** human Dental Pulp Stem Cells

**nHA:** Nano-hydroxyapatite

**SEM:** scanning electron microscopy

## Declaration

# Availability of data and materials

All data generated and/or analyzed during this study are included in this published article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

# Acknowledgments

This article was written based on a dataset from the DDS thesis entitled "Evaluation of Osteo/odontogenic Differentiation of Human Dental Pulp Stem Cells in Alginate-Gelatin/nano Hydroxyapatite microcapsules" registered at Tabriz University of Medical Sciences, Faculty of Dentistry (reference number 58620).

# Funding

Stem Cell Research Center of Tabriz University of Medical Sciences supported this study.

# Contributions

MAgh, MS, ZAgh, and ABk contributed to the designing of the experiments, revising the manuscript, and supervising all experiments. MA and NF performed all of the experiments and analyzed the data. SMs helpt in cell culture procedure. MA wrote the manuscript and draw graphical abstracts and the diagrams. The authors read and approved the final manuscript.

# Corresponding authors

Correspondence to Ali Baradar Khoshfetrat or Zahra Aghazadeh

# Ethics declaration

## Ethics approval and consent to participate

all experimental protocols were approved by the Ethics committee of Tabriz University of Medical Sciences (TUMS) which was in compliance with the Helsinki declaration, and all participants signed the informed consent (Approval No. IR. TBZMED.REC.1396.654). There are no animal experiments carried out for this article.

# Consent for publication

Not applicable.

# Competing interests

The authors declare that they have no competing interests.

## References

1. Ge S, Zhao N, Wang L, Yu M, Liu H, Song A, et al. Bone repair by periodontal ligament stem cellseeded nanohydroxyapatite-chitosan scaffold. International Journal of Nanomedicine. 2012;7:5405.
2. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. Injury. 2005;36(3):S20-S7.

3. Moshaverinia A, Ansari S, Chen C, Xu X, Akiyama K, Snead ML, et al. Co-encapsulation of anti-BMP2 monoclonal antibody and mesenchymal stem cells in alginate microspheres for bone tissue engineering. Biomaterials. 2013;34(28):6572-9.
4. Bharadwaz A, Jayasuriya AC. Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. Materials Science and Engineering: C. 2020:110698.
5. Henkel J, Woodruff MA, Epari DR, Steck R, Glatt V, Dickinson IC, et al. Bone regeneration based on tissue engineering conceptions—a 21st century perspective. Bone research. 2013;1:216.
6. Dizaj SM, Maleki A, Lotfipour F, Sharifi S, Rezaie F, Samiei M. Porous hydroxyapatite-gelatin nanoscaffolds for bone and teeth applications. Biointerface Research in Applied Chemistry. 2018;8(6):3670-3.
7. Firouzi N, Baradar Khoshfetrat A, Kazemi D. Enzymatically gellable gelatin improves nano-hydroxyapatite-alginate microcapsule characteristics for modular bone tissue formation. J Biomed Mater Res A. 2020;108(2):340-50.
8. Zhou H, Xu HH. The fast release of stem cells from alginate-fibrin microbeads in injectable scaffolds for bone tissue engineering. Biomaterials. 2011;32(30):7503-13.
9. Huang X, Zhang X, Wang X, Wang C, Tang B. Microenvironment of alginate-based microcapsules for cell culture and tissue engineering. Journal of bioscience and bioengineering. 2012;114(1):1-8.
10. Sousa MG, Maximiano MR, Costa RA, Rezende TM, Franco OL. Nanofibers as drug-delivery systems for infection control in dentistry. Expert Opinion on Drug Delivery. 2020:1-12.
11. Drury JL, Dennis RG, Mooney DJ. The tensile properties of alginate hydrogels. Biomaterials. 2004;25(16):3187-99.
12. Alsberg E, Anderson K, Albeiruti A, Franceschi R, Mooney D. Cell-interactive alginate hydrogels for bone tissue engineering. Journal of dental research. 2001;80(11):2025-9.
13. García-Astrain C, Avérous L. Synthesis and behavior of click cross-linked alginate hydrogels: Effect of cross-linker length and functionality. International journal of biological macromolecules. 2019;137:612-9.
14. Iansante V, Dhawan A, Masmoudi F, Lee CA, Fernandez-Dacosta R, Walker S, et al. A new high throughput screening platform for cell encapsulation in alginate hydrogel shows improved hepatocyte functions by mesenchymal stromal cells co-encapsulation. Frontiers in Medicine. 2018;5:216.
15. Aebischer P, Buchser E, Joseph J, Favre J, De Tribolet N, Lysaght M, et al. Transplantation in humans of encapsulated xenogeneic cells without immunosuppression: a preliminary report. Transplantation. 1994;58(11):1275-7.
16. Zurn A, Henry H, Schluep M, Aubert V, Winkel L, Eilers B, et al. Evaluation of an intrathecal immune response in amyotrophic lateral sclerosis patients implanted with encapsulated genetically engineered xenogeneic cells. Cell transplantation. 2000;9(4):471-84.

17. Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, et al. Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. Diabetes care. 2011;34(11):2406-9.
18. Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, et al. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. Diabetes care. 2006;29(1):137-8.
19. Vidal ON, Sjögren K, Eriksson BI, Ljunggren Ö, Ohlsson C. Osteoprotegerin mRNA is increased by interleukin-1α in the human osteosarcoma cell line MG-63 and in human osteoblast-like cells. Biochemical and biophysical research communications. 1998;248(3):696-700.
20. Chung IH, Yamaza T, Zhao H, Choung PH, Shi S, Chai Y. Stem cell property of postmigratory cranial neural crest cells and their utility in alveolar bone regeneration and tooth development. Stem cells. 2009;27(4):866-77.
21. Bueno EM, Glowacki J. Cell-free and cell-based approaches for bone regeneration. Nature Reviews Rheumatology. 2009;5(12):685.
22. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences. 2000;97(25):13625-30.
23. Nakashima M, Iohara K, Murakami M. Dental pulp stem cells and regeneration. Endodontic Topics. 2013;28(1):38-50.
24. Kanafi MM, Ramesh A, Gupta PK, Bhonde RR. Influence of hypoxia, high glucose, and low serum on the growth kinetics of mesenchymal stem cells from deciduous and permanent teeth. Cells Tissues Organs. 2013;198(3):198-208.
25. Samiei M, Aghazadeh M, Movassaghpour AA, Fallah A, Aminabadi NA, Pakdel SMV, et al. Isolation and characterization of dental pulp stem cells from primary and permanent teeth. J Am Sci. 2013;9(12):153-7.
26. Zamani D, Moztarzadeh F, Bizari D. Alginate-bioactive glass containing Zn and Mg composite scaffolds for bone tissue engineering. International journal of biological macromolecules. 2019;137:1256-67.
27. Westhrin M, Xie M, Olderøy MØ, Sikorski P, Strand BL, Standal T. Osteogenic differentiation of human mesenchymal stem cells in mineralized alginate matrices. PLoS One. 2015;10(3):e0120374.
28. Polini A, Wang J, Bai H, Zhu Y, Tomsia AP, Mao C. Stable biofunctionalization of hydroxyapatite (HA) surfaces by HA-binding/osteogenic modular peptides for inducing osteogenic differentiation of mesenchymal stem cells. Biomaterials science. 2014;2(12):1779-86.
29. Kanczler J, Oreffo R. Osteogenesis and angiogenesis: the potential for engineering bone. Eur Cell Mater. 2008;15(2):100-14.
30. Liu ZC, Chang TMS. Increased viability of transplanted hepatocytes when hepatocytes are co-encapsulated with bone marrow stem cells using a novel method. Artificial Cells, Blood Substitutes, and Biotechnology. 2002;30(2):99-112.

31. Kim YM, Jeon YH, Jin GC, Lim JO, Baek WY. Immunoisolated chromaffin cells implanted into the subarachnoid space of rats reduce cold allodynia in a model of neuropathic pain: a novel application of microencapsulation technology. Artificial organs. 2004;28(12):1059-66.
32. Haque T, Chen H, Ouyang W, Martoni C, Lawuyi B, Urbanska AM, et al. In vitro study of alginate–chitosan microcapsules: an alternative to liver cell transplants for the treatment of liver failure. Biotechnology letters. 2005;27(5):317-22.
33. Bigi A, Boanini E, Panzavolta S, Roveri N, Rubini K. Bonelike apatite growth on hydroxyapatite–gelatin sponges from simulated body fluid. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2002;59(4):709-15.
34. Kavya K, Jayakumar R, Nair S, Chennazhi KP. Fabrication and characterization of chitosan/gelatin/nSiO2 composite scaffold for bone tissue engineering. International journal of biological macromolecules. 2013;59:255-63.
35. Dizaj SM, Mokhtarpour M, Shekaari H, Sharifi S. Hydroxyapatite-gelatin nanocomposite films; production and evaluation of the physicochemical properties. Journal of advanced chemical and pharmaceutical materials (JACPM). 2019;2(2):111-5.
36. Cornell CN. Osteoconductive materials and their role as substitutes for autogenous bone grafts. Orthopedic Clinics. 1999;30(4):591-8.
37. LeGeros RZ. Properties of osteoconductive biomaterials: calcium phosphates. Clinical Orthopaedics and Related Research®. 2002;395:81-98.
38. Dorozhkin SV. Calcium orthophosphate-based biocomposites and hybrid biomaterials. Journal of Materials Science. 2009;44(9):2343-87.
39. Verrier S, Blaker JJ, Maquet V, Hench LL, Boccaccini AR. PDLLA/Bioglass® composites for soft-tissue and hard-tissue engineering: an in vitro cell biology assessment. Biomaterials. 2004;25(15):3013-21.
40. Ogata K, Imazato S, Ehara A, Ebisu S, Kinomoto Y, Nakano T, et al. Comparison of osteoblast responses to hydroxyapatite and hydroxyapatite/soluble calcium phosphate composites. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials. 2005;72(2):127-35.
41. Gu Y, Bai Y, Zhang D. Osteogenic stimulation of human dental pulp stem cells with a novel gelatin-hydroxyapatite-tricalcium phosphate scaffold. Journal of Biomedical Materials Research Part A. 2018;106(7):1851-61.
42. Yazdani J, Ahmadian E, Sharifi S, Shahi S, Dizaj SM. A short view on nanohydroxyapatite as coating of dental implants. Biomedicine & Pharmacotherapy. 2018;105:553-7.
43. Hokmabad VR, Davaran S, Aghazadeh M, Alizadeh E, Salehi R, Ramazani A. A Comparison of the Effects of Silica and Hydroxyapatite Nanoparticles on Poly (ε-caprolactone)-Poly (ethylene glycol)-

Poly (ε-caprolactone)/Chitosan Nanofibrous Scaffolds for Bone Tissue Engineering. Tissue engineering and regenerative medicine. 2018;15(6):735-50.

44. Yan J, Miao Y, Tan H, Zhou T, Ling Z, Chen Y, et al. Injectable alginate/hydroxyapatite gel scaffold combined with gelatin microspheres for drug delivery and bone tissue engineering. Materials Science and Engineering: C. 2016;63:274-84.
45. Nabavinia M, Khoshfetrat AB, Naderi-Meshkin H. Nano-hydroxyapatite-alginate-gelatin microcapsule as a potential osteogenic building block for modular bone tissue engineering. Materials Science and Engineering: C. 2019;97:67-77.
46. Ghosh M, Halperin-Sternfeld M, Grinberg I, Adler-Abramovich L. Injectable alginate-peptide composite hydrogel as a scaffold for bone tissue regeneration. Nanomaterials. 2019;9(4):497.
47. Al-Rammah TY. Alginate microencapsulation of stem cells as alternative source to the limited supply of donor tissue. 2014.
48. Lou R, Xie H, Zheng H, Ren Y, Gao M, Guo X, et al. Alginate-based microcapsules with galactosylated chitosan internal for primary hepatocyte applications. International journal of biological macromolecules. 2016;93:1133-40.
49. Shi T, Yang X, Zhou H, Xi J, Sun J, Ke Y, et al. Activated carbon N-acetylcysteine microcapsule protects against nonalcoholic fatty liver disease in young rats via activating telomerase and inhibiting apoptosis. PloS one. 2018;13(1).
50. Kupikowska-Stobba B, Lewińska D. Polymer microcapsules and microbeads as cell carriers for in vivo biomedical applications. Biomaterials Science. 2020;8(6):1536-74.
51. Calafiore R, Basta G, Montanucci P. Microencapsulation of islets for the treatment of type 1 diabetes mellitus (T1D). Cell Microencapsulation: Springer; 2017. p. 283-304.
52. Saghebasl S, Davaran S, Rahbarghazi R, Montaseri A, Salehi R, Ramazani A. Synthesis and in vitro evaluation of thermosensitive hydrogel scaffolds based on (PNIPAAm-PCL-PEG-PCL-PNIPAAm)/Gelatin and (PCL-PEG-PCL)/Gelatin for use in cartilage tissue engineering. Journal of Biomaterials science, Polymer edition. 2018;29(10):1185-206.
53. Galvez-Martin P, M Martin J, M Ruiz A, Clares B. Encapsulation in cell therapy: methodologies, materials, and clinical applications. Current pharmaceutical biotechnology. 2017;18(5):365-77.
54. Declercq HA, De Caluwé T, Krysko O, Bachert C, Cornelissen MJ. Bone grafts engineered from human adipose-derived stem cells in dynamic 3D-environments. Biomaterials. 2013;34(4):1004-17.
55. Barros J, Ferraz MP, Azeredo J, Fernandes M, Gomes P, Monteiro F. Alginate-nanohydroxyapatite hydrogel system: Optimizing the formulation for enhanced bone regeneration. Materials Science and Engineering: C. 2019;105:109985.
56. Tozzi G, De Mori A, Oliveira A, Roldo M. Composite hydrogels for bone regeneration. Materials. 2016;9(4):267.
57. Sun J, Tan H. Alginate-based biomaterials for regenerative medicine applications. Materials. 2013;6(4):1285-309.

58. Zein N, Harmouch E, Lutz J-C, Fernandez De Grado G, Kuchler-Bopp S, Clauss F, et al. Polymer-based instructive scaffolds for endodontic regeneration. Materials. 2019;12(15):2347.
59. Valcourt U, Merle B, Gineyts E, Viguet-Carrin S, Delmas PD, Garnero P. Non-enzymatic glycation of bone collagen modifies osteoclastic activity and differentiation. Journal of Biological Chemistry. 2007;282(8):5691-703.
60. Bajpai S, Sharma S. Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca2+ and Ba2+ ions. Reactive and Functional Polymers. 2004;59(2):129-40.
61. Sancilio S, Gallorini M, Di Nisio C, Marsich E, Di Pietro R, Schweikl H, et al. Alginate/hydroxyapatite-based nanocomposite scaffolds for bone tissue engineering improve dental pulp biomineralization and differentiation. Stem Cells International. 2018;2018.
62. Bendtsen ST, Wei M. Synthesis and characterization of a novel injectable alginate–collagen–hydroxyapatite hydrogel for bone tissue regeneration. Journal of Materials Chemistry B. 2015;3(15):3081-90.
63. Hokmabad VR, Davaran S, Aghazadeh M, Rahbarghazi R, Salehi R, Ramazani A. Fabrication and characterization of novel ethyl cellulose-grafted-poly (□-caprolactone)/alginate nanofibrous/macroporous scaffolds incorporated with nano-hydroxyapatite for bone tissue engineering. Journal of biomaterials applications. 2019;33(8):1128-44.
64. Samiei M, Aghazadeh M, Alizadeh E, Aslaminabadi N, Davaran S, Shirazi S, et al. Osteogenic/odontogenic bioengineering with co-administration of simvastatin and hydroxyapatite on poly caprolactone based nanofibrous scaffold. Advanced pharmaceutical bulletin. 2016;6(3):353.
65. Zhang Y, Xie R-l, Croce CM, Stein JL, Lian JB, Van Wijnen AJ, et al. A program of microRNAs controls osteogenic lineage progression by targeting transcription factor Runx2. Proceedings of the National Academy of Sciences. 2011;108(24):9863-8.
66. Bessa PC, Casal M, Reis R. Bone morphogenetic proteins in tissue engineering: the road from laboratory to clinic, part II (BMP delivery). Journal of tissue engineering and regenerative medicine. 2008;2(2-3):81-96.
67. Tsao Y-T, Huang Y-J, Wu H-H, Liu Y-A, Liu Y-S, Lee O. Osteocalcin mediates biomineralization during osteogenic maturation in human mesenchymal stromal cells. International journal of molecular sciences. 2017;18(1):159.
68. Jensen ED, Gopalakrishnan R, Westendorf JJ. Regulation of gene expression in osteoblasts. Biofactors. 2010;36(1):25-32.
69. Wang X-F, Lu P-J, Song Y, Sun Y-C, Wang Y-G, Wang Y. Nano hydroxyapatite particles promote osteogenesis in a three-dimensional bio-printing construct consisting of alginate/gelatin/hASCs. RSC advances. 2016;6(8):6832-42.
70. Khatami N, Khoshfetrat AB, Khaksar M, Zamani ARN, Rahbarghazi R. Collagen-alginate-nano-silica microspheres improved the osteogenic potential of human osteoblast-like MG-63 cells. Journal of cellular biochemistry. 2019.

71. Tomlinson MJ, Dennis C, Yang XB, Kirkham J. Tissue non-specific alkaline phosphatase production by human dental pulp stromal cells is enhanced by high density cell culture. Cell and tissue research. 2015;361(2):529-40.
72. Bertazzo S, Zambuzzi WF, Campos DD, Ogeda TL, Ferreira CV, Bertran CA. Hydroxyapatite surface solubility and effect on cell adhesion. Colloids and Surfaces B: Biointerfaces. 2010;78(2):177-84.
73. Xia Y, Mei F, Duan Y, Gao Y, Xiong Z, Zhang T, et al. Bone tissue engineering using bone marrow stromal cells and an injectable sodium alginate/gelatin scaffold. Journal of Biomedical Materials Research Part A. 2012;100(4):1044-50.

# Figures

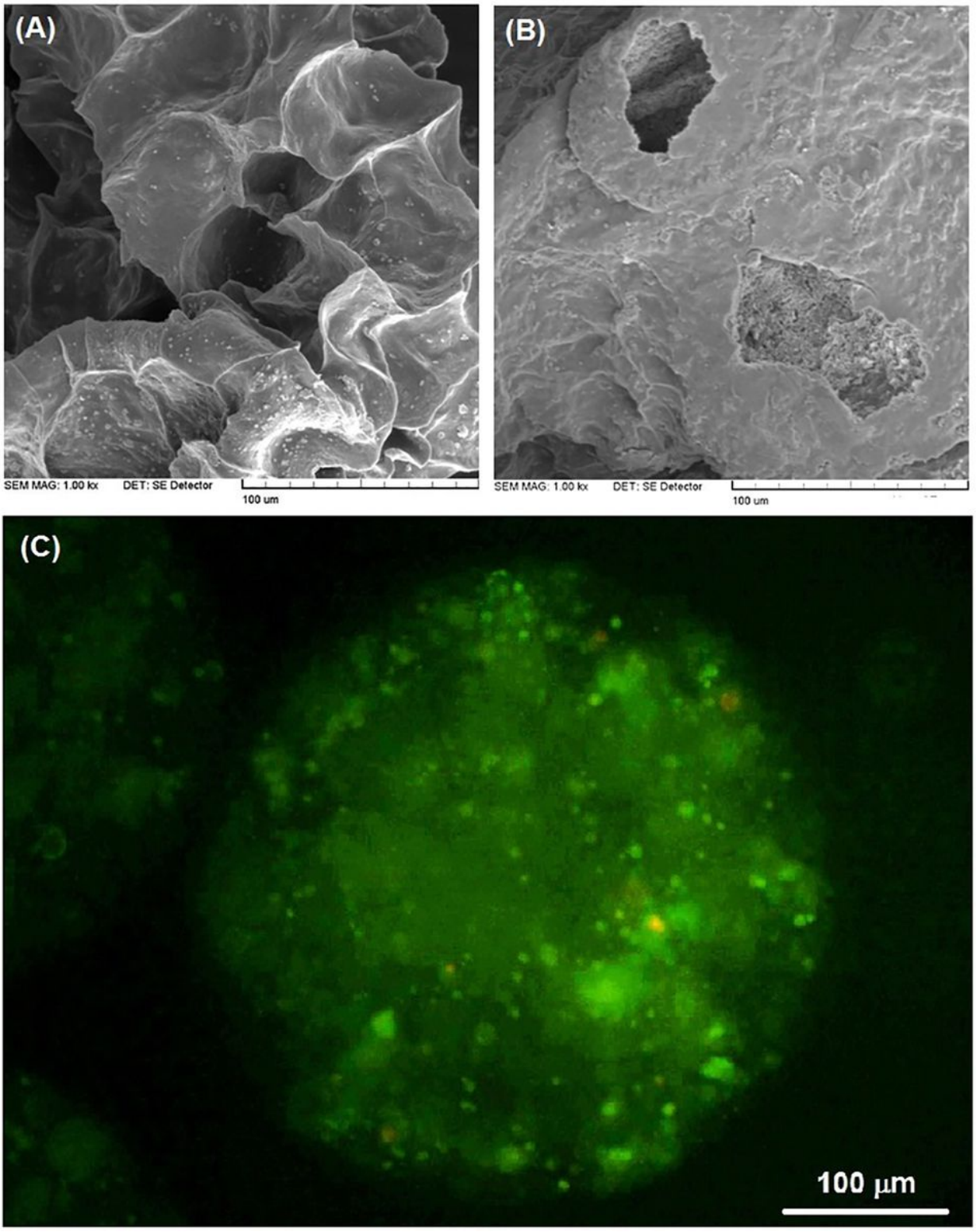
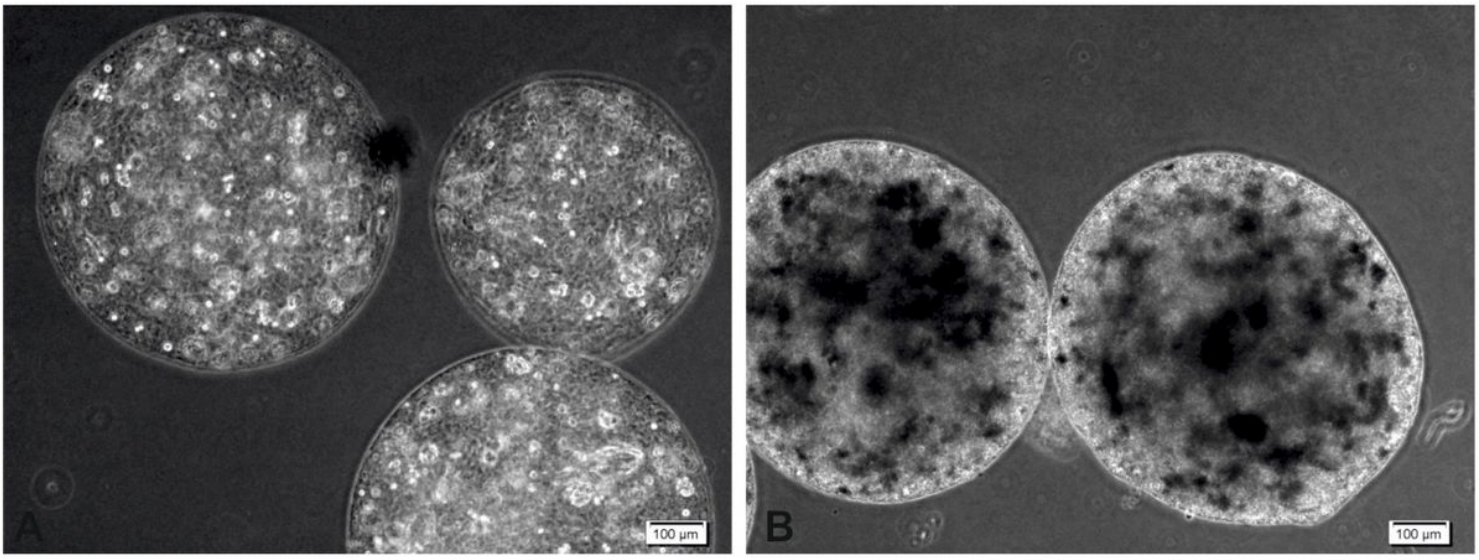


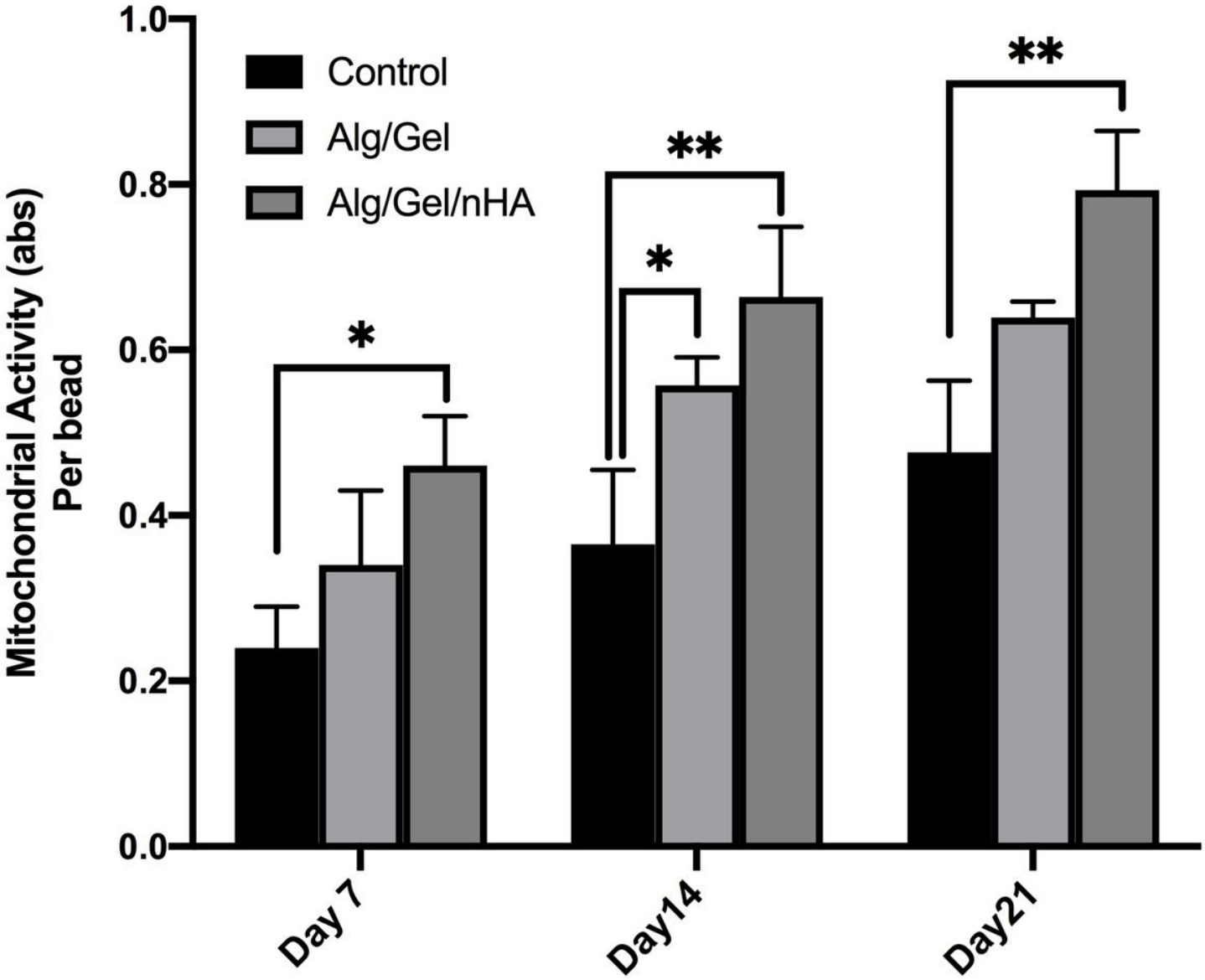

**Figure 1**

SEM images of microcapsule surface after gelation for hydrogel compositions of Alg/Gel (A), Alg/Gel/nHA (B) and live/dead staining for the cells microencapsulated at Alg/Gel/nHA after 21 days (C) (Scale bar: 100 µm).



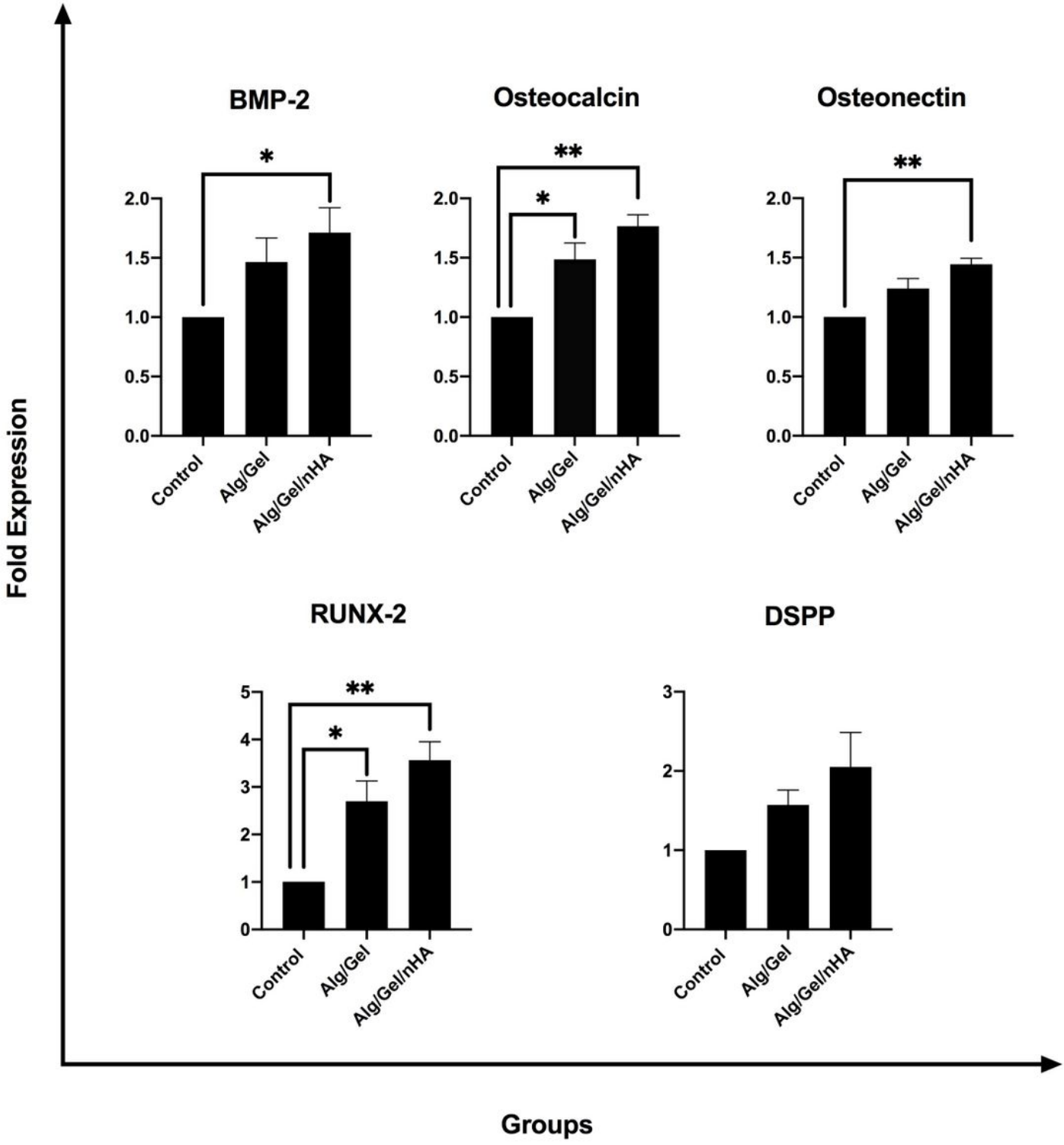

## Figure 2

Microscopy images of microcapsules containing hDPSCs after 21 days. (A) Alg/Gel microcapsules containing hDPSCs (B) Alg/Gel/nHA microcapsules containing hDPSCs



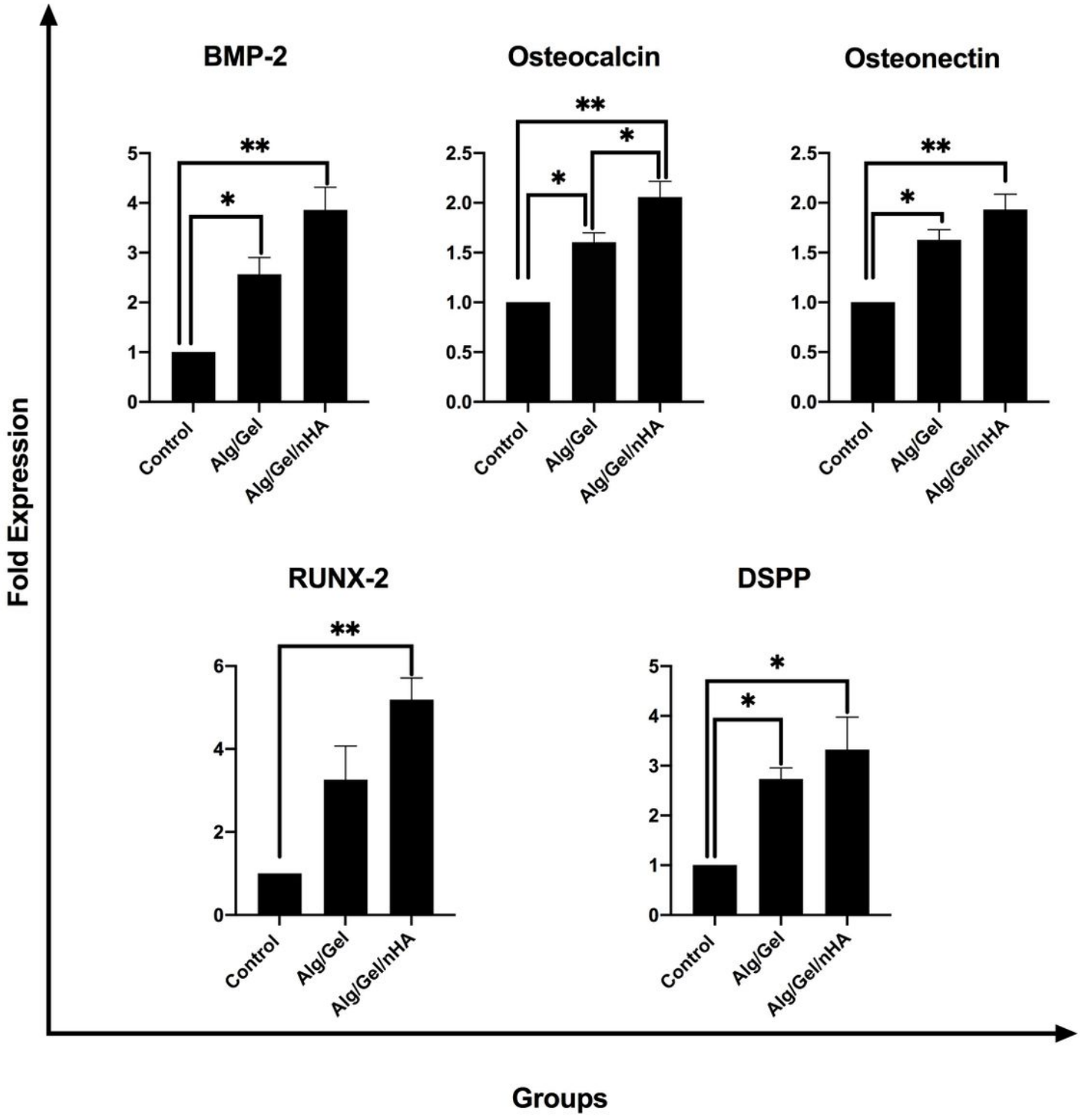

## Figure 3

MTT assay results for Alg/Gel and Alg/Gel/nHA microcapsules after 7, 14, and 21 days (*P< 0.05) and (**P<0.01).




## Figure 4

The expression levels of osteogenic differentiation gens of hDPSCs grown on PS, Alg-Gel, and Alg-Gel-nHA microcapsules after 21 days (*P< 0.05) and (**P<0.01).




## Figure 5

The expression levels of osteogenic differentiation gens of hDPSCs grown on PS, Alg-Gel, and Alg-Gel-nHA microcapsules after 28 days (*P< 0.05) and (**P<0.01).

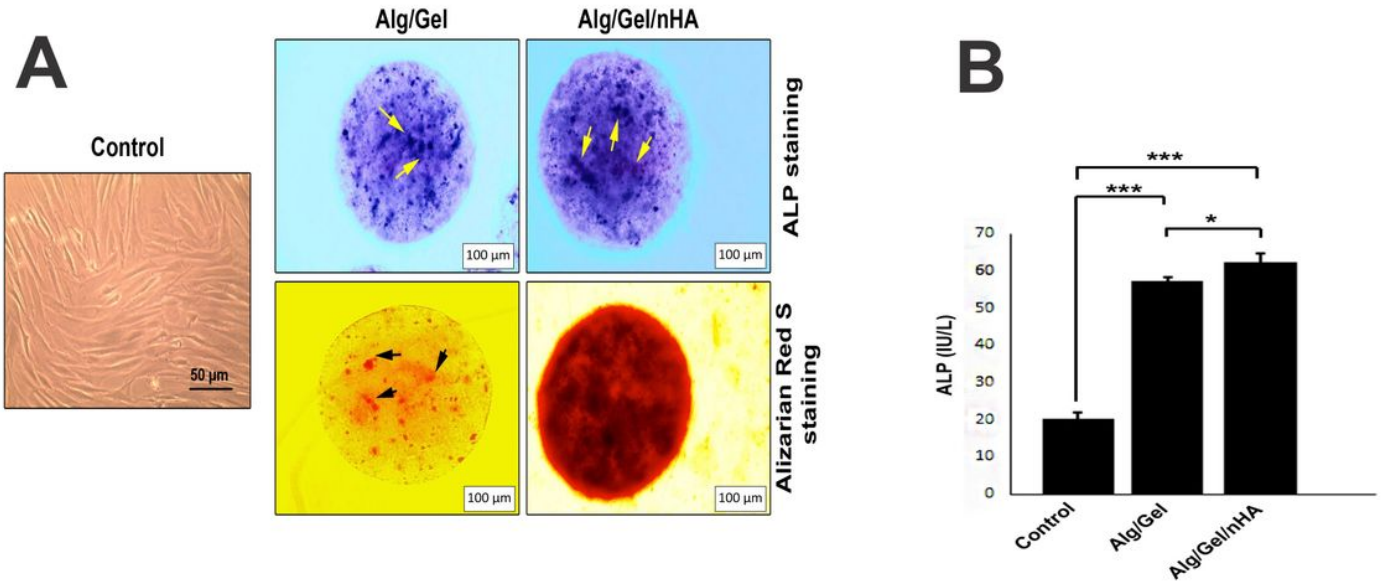



**Figure 6**

(A) Calcium deposition and ALP excretion of hDPSCs cells in Alg/Gel microcapsules and Alg/Gel/nHA microcapsules. (B) ALP Enzyme Activity of hDPSCs grown on culture medium (control), Alg/Gel microcapsules, and Alg/Gel/nHA microcapsules after 21 days. *P< 0.05, **P<0.01, *** P<0.001 and **** P< 0.0001

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- graphicalabstract.jpg
- Titlepage.docx