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ORIGINAL ARTICLE

Hydroxyapatite–collagen augments osteogenic differentiation of dental pulp stem cells

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Abstract

The objectives of this study were to isolate and culture dental pulp stem cells (DPSCs) and to investigate their proliferation and osteogenic differentiation on hydroxyapatite–collagen (HA–Col) scaffold. DPSCs were characterized by fluorescence-activated cell sorting (FACS). Cultured cells were $CD73^+$, $CD90^+$, $CD105^+$ and $CD31^-$, $CD45^-$. A commercially available HA–Col scaffold was used for culture of DPSCs. Cell attachment and viability of DPSCs cultured on scaffold was studied by sulforhodamine assay. Osteoblast differentiation capacity was studied by alkaline phosphatase assay and the effects of growth factors such as PDGF, IGF1 and FGF2 were further studied. Scanning electron microscopy (SEM) of cell seeded scaffolds was also performed. We found that DPSCs cultured exhibited the characteristic mesenchymal stem cells (MSCs) morphology and differentiation properties. Scaffold was found to be non-cytotoxic and had good biocompatibility in vitro. Osteoblast differentiation ability was found to increase at higher concentration of scaffold and additive effects were observed with the use of growth factors. In SEM, cells appeared to cover the entire surface of the scaffold forming continuous cell layer and extending filopodial extensions. HA–Col scaffold is apt for MSCs attachment and proliferation in vitro. Their unique self-renewal and multilineage differential potential make them ideal for use in regenerative medicine. The limitations of currently available bone graft materials have led to the emergence of tissue engineering using mesenchymal stem cells (MSCs). Since, HA–Col scaffold potentiated the proliferation and osteogenic differentiation of DPSCs, this biomimetic material may be an ideal one for maxillofacial and alveolar bone regeneration.

Keywords Mesenchymal stem cells · Biomimetic scaffold · Growth factors · Osteoblasts · Bone regeneration

Introduction

Mesenchymal stem cells (MSCs) are described as multipotent stem cells having self-renewal potential and ability to differentiate into various specialized cell types such as osteoblasts, adipocytes and chondrocytes [1, 2]. Their unique high self-renewal property and multi-lineage differentiation potential make them ideal for use in regenerative medicine.

Mesenchymal stem cells have attracted much attention regarding their clinical use as they are easily available, expandable in vitro and do not elicit immediate immune response [3]. These cells can be isolated from different sources such as bone marrow, adipose tissue, umbilical cord and peripheral blood. MSCs from bone marrow (BMMSCs) have been widely isolated and expanded for experimental studies [4, 5]. However, the problems associated with it include pain, morbidity, limited cell number and activity. Alternative sources have therefore been sought for years.

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Dental tissues are excellent source of MSCs. Human dental pulp stem cells (DPSCs) can be obtained from the extracted tooth, which is discarded as a medical waste. The procedure is common, effective and less painful as compared to the surgical procedure of harvesting BMMSCs. Dental pulp tissue has been reported as competent enough to provide an ample number of cells for a prospective clinical application. Their differentiation ability towards osteogenic lineage makes them an ideal source for repair of skeletal defects [6, 7].

In craniofacial region, bone loss due to trauma, infection or radical tumor resection often complicates successful rehabilitation of the patient. Several grafts available include autografts, allografts and xenografts and each has its own shortcomings. An ideal bone tissue engineering construct is still lacking.

The basis of tissue engineering is three-dimensional (3-D) cell culture on a biodegradable scaffold. Ability of MSCs to self-renew and undergo differentiation towards bone-forming cells makes them specifically valuable for repair and regeneration of bone tissue. Scaffolds are considered crucial for bone regeneration, as they act as a template for cell function while maintaining volume and bearing external load. Hydroxyapatite (HA) and collagen (Col) are components of natural bone and hence considered biomimetic.

In our study, HA–Col scaffold was used for culture with MSCs with the objectives to investigate the attachment of DPSCs on the HA–Col scaffold for proliferation and osteogenic differentiation; and it was hypothesized that HA–Col scaffold would support DPSCs attachment, proliferation and osteogenic differentiation in vitro.

Materials and methods

Non-carious, non-pathological impacted third molars were collected from 10 patients of 18–30 years age from the Department of Oral & Maxillofacial Surgery, Faculty of Dental Sciences, King George's Medical University, Lucknow, India. Ethical clearance was obtained from the Institutional Stem Cell Ethical Committee. Informed consent was obtained from all individual participants after explaining the experimental procedures to be followed. Before extraction, the individuals were screened for any oral infection or systemic disease. The extracted tooth was transported to the lab in sterile chilled PBS supplemented with antibiotics (100 U/mL penicillin, 0.2 mg/mL streptomycin).

Isolation and culture of DPSCs

Tooth was washed twice with PBS and wiped with 10% w/v povidone–iodine. Pulp tissue was obtained by splitting the tooth at cemento-enamel junction (CEJ) by creating a groove using a bur in high speed head piece followed by sectioning with chisel and mallet to avoid overheating and damage to pulp tissue. The pulp tissue obtained was sectioned into small pieces, digested for 30 min at 37 °C with 2 mg/mL Type 1 collagenase (Sigma-Aldrich, USA) followed by filtration through 70 µ nylon mesh.

All cells were cultured in complete culture media (CCM) consisting of α-minimum essential media (α-MEM), non-essential amino acids (Hi-media laboratories, India) supplemented with 10% fetal bovine serum (Gibco, Life Technologies) 100 U/mL penicillin, 0.2 mg/mL streptomycin and incubated at 37 °C in 5% CO_2. After 2 days of initial plating, media was replenished to remove non-adherent cells and adherent MSCs were maintained in expansion media. Before reaching to confluence, DPSC culture was trypsinized and expanded through sequential passaging and up to 5th passage was used for experiments.

Flow cytometry

Mesenchymal stem cells were analyzed for expression of cell surface antigens by multispectral imaging flow cytometer (Fluorescence-activated cell sorting (FACS) Imagestream, Amnis Imaging Flow Cytometer (Millipore). Fluorochrome tagged CD90-BV421, CD105-FITC and CD73-PerCP Cy5.5 cell surface antigens were considered as positive markers while CD31 and CD45 biotin (secondary streptavidin PE) were considered as negative markers. 0.5×10^6 cells were resuspended in 100 µL staining buffer (PBS + 0.2% BSA) and incubated with antibodies on ice for 45 min. After staining cells were washed twice (1500 rpm for 5 min) with staining buffer at 4 °C. Samples were acquired in imaging flow cytometer and data was analyzed using the IDEAS Analysis Software.

Osteogenic, adipogenic and chondrogenic differentiation of MSCs

Osteogenic, adipogenic and chondrogenic differentiation of MSCs was performed as previously described [1]. The osteogenic media consisted of CCM supplemented with 100 nM dexamethasone, 50 µM ascorbic acid, and 10 mM β-glycerophosphate (Sigma-Aldrich, USA) while adipogenic media comprised of 100 nM dexamethasone, 10 mg/mL insulin, and 0.5 m misobutylxanthine (Sigma-Aldrich, USA). MSCs were induced in pellet culture for chondrocyte differentiation using media consisting of StemX Vivo Chondrogenic Supplement (R&D Systems) in CCM for 3–4 weeks. For staining of osteoblasts, adipocytes and chondrocytes; alkaline phosphatase (ALP), Oil Red O and alcian blue staining was carried out, respectively, using standard protocol.

HA–Col scaffold and cell seeding

HA–Col scaffold (G-Graft, Surgiwear, India) was used as scaffold in the present study and was sectioned into strips of 2 mm thickness to facilitate nutrient delivery to cells throughout the scaffold. The scaffold pieces were transferred to 24 well plate and incubated in PBS at 37 °C in 5% CO_2. After 48 h of incubation, 115 µL of cell suspension, which is equivalent to 300 cells/mm^3 was added onto each scaffold. The porous scaffold pieces were left undisturbed for 2 h to allow initial attachment of the cells before adding 500 µL of the culture or differentiation medium.

Sulforhodamine B (SRB) colorimetric assay

Sulforhodamine B assay determines cell density based on the measurement of cellular protein content. 5×10^3 DPSCs were seeded in 96 well plate in complete culture media (CCM). After 24 h, HA–Col scaffold was added at different concentrations, i.e. 5, 20, 40, 60 mg/mL with proper control (without scaffold) and incubated for 24 and 72 h. At the end of incubation, SRB assay was performed according to a previously published protocol [8]. Briefly, cells were fixed by adding 100 µL of 10% (w/v) trichloroacetic acid at 4 °C for one hour followed by washing 4 times with running tap water. After drying at room temperature (20–25 °C), cells were stained with 100 µL of 0.057% SRB in 1% acetic acid (v/v) for 30 min. Unbound excess dye was removed by washing four times with 1% acetic acid. To solubilize the protein-bound dye, 200 µL of 10 mM Tris (pH 10.5) was added to each well. OD was measured at 510 nm in microplate reader (SpectraMax M5^e, Molecular Devices, USA).

Measurement of osteoblast differentiation

HA–Col scaffold powder was added in 96 well plate at different concentration and centrifuged at 2000 rpm for 2 min. 3×10^3 DPSCs/well were seeded on the scaffold with differentiation media. To measure the effect of growth factors on osteoblast differentiation, DPSCs were seeded on 60 mg/mL HA–Col without (control) and with FGF2, IGF1 and PDGF at 1 ng/mL, 50 ng/mL and 5 ng/mL, respectively. Media was changed twice a week. Cell culture was maintained up to 2 weeks. At the end of experiment, total ALP activity was measured colorimetrically using a substrate p-nitrophenylphosphate [9, 10] at 405 nm in microplate reader.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Human dental pulp derived mesenchymal stem cells (DPMSCs) were cultured on HA–collagen scaffold at a density of 25,000 cells/well in 24 well plate in α-MEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotics solution and after 24 h cells were treated with osteoblast differentiation media as mentioned above for 10 days. RNA isolation was performed according to Qiagen RNeasy Mini Kit protocol and cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad). qRT-PCR was performed in StepOne Real-Time PCR System (Applied Biosystems) using Hot Fire-Pol EvaGreen QPCR Mix (SOLISBIODYNE) for osteoprotegerin (OPG) and a housekeeping gene GAPDH (sequence in supplementary Table 1).

ELISA assay

For the estimation of RANKL in DPMSCs cultured for 10 days on HA–collagen scaffold in differentiation media, we performed ELISA assay with Human TNFSF-11/RANKL Elisa kit (ImmunoTag). Briefly after 10 days of differentiation, cells were washed twice with PBS and treated with 1× trypsin for 5–10 min. After trypsin digestion cell were collected in fresh microfuge tube and centrifuged at 2000 rpm for 5 min at 4 °C. Supernatant was discarded and fresh 1× PBS was added to each sample and sonicated for 1–2 min on ice. After sonication cell lysate was transferred to new centrifuge tube for ELISA assay. Absorbance was measured at 450 nm.

Scanning electron microscopy (SEM)

Scanning electron microscopy of cell seeded scaffolds was performed at days 2 and 21. Scaffolds seeded with DPSCs were washed twice with PBS and fixated with 2.5% glutaraldehyde for 30 min followed by 2% osmium tetroxide treatment for 30 min. After further washing scaffolds were dehydrated with increasing concentration (10% to 100%) of ethanol. For SEM analysis, cell seeded scaffolds were sputter coated with a gold–palladium layer using Jeol JEC-3000FC Auto Fine Coater. The scaffolds were examined by a Field Emission Scanning Electron Microscope (FESEM 7610F, JEOL, Japan).

Statistical analysis

Statistical analysis was performed using the Statistical package for social services (SPSS) software. Data were presented as mean ± standard error and analyzed using the two-way student *t* test. Differences between groups were considered statistically significant when $^{***}p \leq 0.001$; $^{**}p \leq 0.01$; $^{*}p \leq 0.05$.

Results

Isolation and expansion of DPSCs in vitro

Single cell suspension of dental pulp was seeded for in vitro culture. After the first passage, cells were seeded at

plating density of 10^4 cells/cm^2. DPSCs formed a monolayer 6–7 days after first passage. The cells were spindle-shaped, elongated, homogeneous and had fibroblast-like morphology when viewed under phase contrast microscope (Fig. 1a). During in vitro culture, MSCs possessed a limited lifespan and finally exhibited a loss of proliferation and alteration in morphology which indicates replicative senescence. We used DPSCs up to 5th passage for experiments.

Multilineage differentiation potential of DPSCs in vitro

To check the stemness of DPSCs in vitro differentiation was performed. DPSCs were seeded into chondrogenic, adipogenic and osteogenic differentiation media individually. Osteogenic differentiation was assayed in osteogenic media for 21 days. Osteoblasts were morphologically distinct from DPSCs (Fig. 1b). Osteogenic differentiated cells were stained positive for ALP whereas control wells were negative for ALP (Fig. 1c). Adipogenic differentiation was confirmed by the accumulation of neutral lipid vacuoles assayed by Oil Red O stain (Fig. 1d). Chondrogenic differentiation was confirmed by the formation of a sphere in micro-mass culture and secretion of cartilage-specific proteoglycans stained with Alcian blue. The samples tested showed a cartilage-like phenotype with chondrocyte-like lacunae (Fig. 1d).

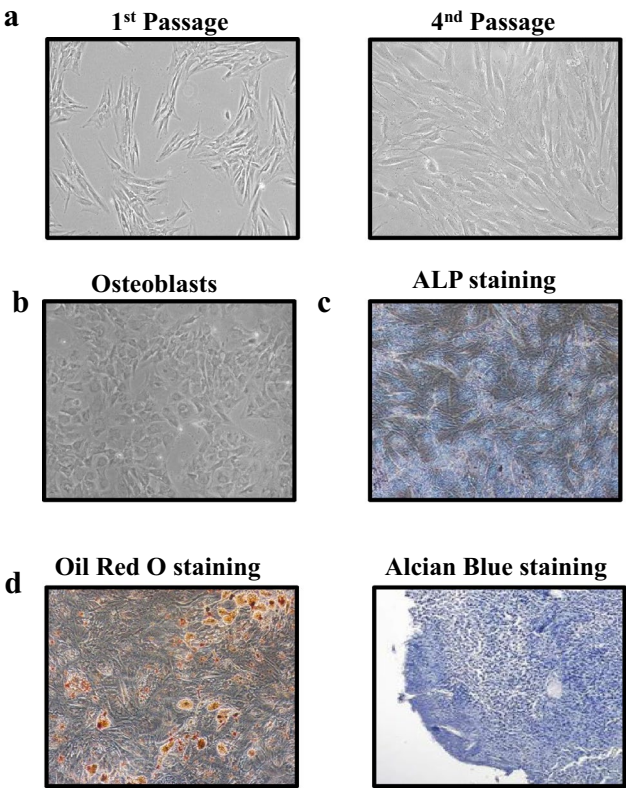

Fig. 1 Culture and differentiation of DPSCs in vitro. Cell morphology of dental pulp stem cells (DPSCs) observed under phase contrast microscope at passage 1 and passage 4 (**a**). Change in the morphology of DPSCs after osteogenic differentiation (**b**). Alkaline phosphatase (ALP) staining at 21 days after osteogenic induction for staining of differentiated osteoblasts (**c**). Adipogenic differentiation (left panel) was confirmed by the formation of neutral lipid-vacuoles stainable with Oil Red O (**d**). Chondrogenic differentiation (right panel) confirmed by alcian blue staining (**d**)

Immunophenotypic characterization

Before seeding cells on scaffold, immunophenotypic characterization of MSCs by image flow cytometry was done to assess the expression of cell surface antigens at passages 2–4. Cells were labeled with fluorochrome tagged CD90, CD73, CD105 and biotinylated CD31, CD45 antibodies. After acquisition, DPSCs were CD73$^+$, CD90$^+$and CD105$^+$and endothelial marker CD31$^-$ and pan hematopoietic marker CD45$^-$. Phase contrast image shows the shape and size of MSCs (Fig. 2a). In analysis, dot plots were plotted with normalized frequency against gradient RMS to select the focused cells. Aspect ratio versus area helped to gate single cells. These single cells were gated for CD90 and CD105. Further, CD73 expression was analyzed in double positive cells (CD90$^+$ and CD105$^+$). 97% of single cells (CD31$^-$, CD45$^-$) were positive for CD73, CD90 and CD105 (Fig. 2b). This result clearly indicates that our culture was maintaining MSCs.

Toxicity of HA–Col to cultured DPSCs

It was very important to assess the toxic effect of HA–Col on DPSCs. DPSCs were cultured on HA–Col scaffold and after exposure to different concentrations of HA–Col scaffold, cell viability was assessed by SRB assay. At 24 h, no reduction in cell viability was observed till 20 mg/mL concentration. At 40 mg/mL and 60 mg/mL slight reduction in viability was observed. At 72 h, a slight reduction in viability was seen with increasing concentration. At both the time periods and at varying concentration, more than 60% of cells remained viable with the scaffold (Fig. 3) [11].

Differentiation of DPSCs into osteoblast on HA–Col scaffold

Cells were grown on slices of HA–Col scaffold with and without differentiation media. Differentiation was monitored by SEM at different days after seeding. SEM data demonstrated that the scaffold had heteroporous morphology and a highly interconnected three-dimensional structure. SEM images of DPSCs seeded on HA–Col scaffold showed excellent biocompatibility. At day 2 of culture, it was observed that cells presenting a spindle-like

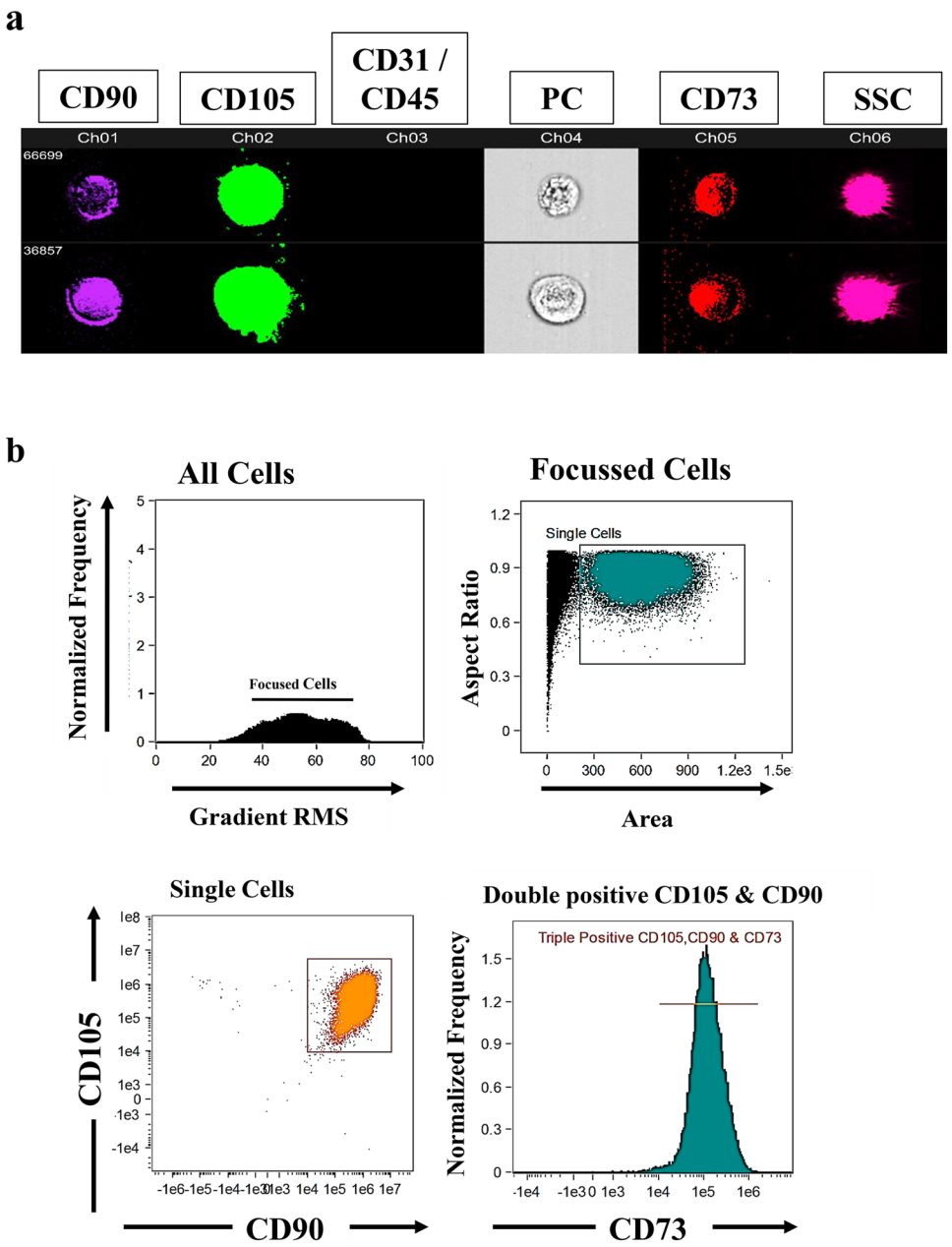

Fig. 2 Characterization of DPSCs by cell surface antigen phenotyping using FACS. Cultured DPSCs were stained with CD90 BV421, CD105 FITC, CD73 PerCP Cy5.5, and biotinylated CD31, CD45—streptavidin PE antibodies. Channel 04 (Ch04) and channel 06 (Ch06) shows phase contrast (PC) image and side scatter (SSC), respectively (**a**). Focused cells were gated and moved for single cell analysis. All the single cells were used to study CD90 and CD105 double positive. These double positive cells were looked for CD73. All single cells were double negative for CD31 and CD45 (**b**)

morphology were well attached to the HA–Col scaffold (Fig. 4a). Further, it was also noticed that the cells cultured on scaffold were well adhered to the surface and flattened, exhibited numerous filopodial-like extensions and cell-to-cell contact points. On day 21, the surface of the scaffold was almost entirely covered with cells that formed continuous layer (Fig. 4b). Few cells migrated into the pores of scaffold. In SEM images of DPSCs cultured in osteogenic media on HA–Col scaffold, leafy nodule-like structures were observed suggestive of calcifications (Fig. 4c). No cytotoxicity response was observed in any sample.

Osteogenic differentiation of DPSCs increased in the presence of HA–Col and growth factors

As up to 60 mg/mL HA–Col was not very toxic to DPSCs, we coated 6 well plate with different concentrations of HA–Col and seeded DPSCs on them, with and without differentiation media. After 14 days, ALP assay was performed, and osteogenic differentiation was significantly ($p \leq 0.001$) upregulated at higher concentrations of HA–Col (Fig. 5a).

Osteogenic differentiation also enhanced in the presence of certain growth factors like MMP-7, IGF1 [12]. DPSCs

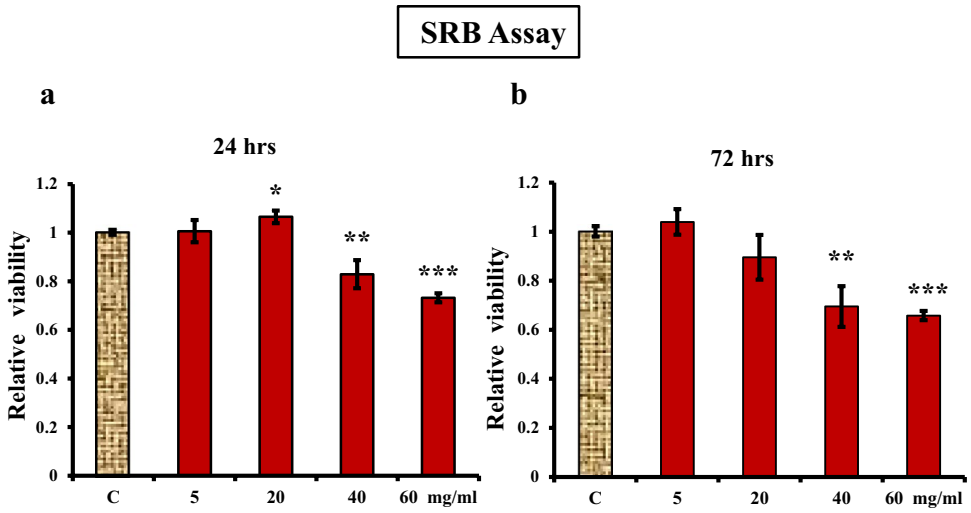

Fig. 3 Assessment of toxicity of HA–Col on DPSCs using SRB assay. 5×10^3 DPSCs were seeded in 96 well plate and 24 h after seeding different concentration of HA–Col powder was added and cell viability was assessed after 24 h (**a**) and 72 h (**b**) after treatment. The result is the average of two experiments with triplicates. Data shown as mean ± SEM; $n = 3$; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ compared with control cells (**c**)

were grown on HA–Col scaffold with 5 ng/mL PDGF, 1 ng/mL FGF2, 50 ng/mL IGF1. DPSC differentiation was induced in osteogenic lineage in vitro. Osteogenic differentiation was further enhanced with the addition of HA–Col scaffold (Fig. 5b). This result clearly suggests that combination of growth factors with HA–Col scaffold enhances the osteogenic differentiation.

we performed RT PCR analysis for expression of osteogenic gene Osteopontin to evaluate the effect of HA–collagen scaffold on DPMSCs differentiation after a week in differentiation medium (Fig. 5c). The expression of Osteopontin, (SPP-1) a major component of the extracellular matrix of the bone was significantly increased in DPMSCs grown on HA scaffold compared to control (Carlo Mangano, 2011 plos one).

To estimate RANKL concentration in cell lysate we performed ELISA assay at 10 day of differentiation of DPMSCs grown on HA–collagen scaffold. There was no significant difference in RANKL amount between DPMSCs grown with or without HA–collagen scaffold. That suggest there was no on osteoclast differentiation of DPMSCs (Supplementary Fig. 1).

Discussion

Bone tissue engineering involves the use of materials to regenerate bone in vivo. The gold standard for bone regeneration is autologous bone graft. However, restriction of size, additional injury and morbidity associated with it limits its use. The use of osteogenic cells in combination with a natural bone mimicking scaffold is a promising alternative to conventional bone grafts.

A biocompatible scaffold is a basic prerequisite for bone regeneration. Scaffold provides a framework for cell attachment and proliferation. An ideal scaffold should support cell adhesion, proliferation and differentiation must have good tissue biocompatibility and degradation profile [13].

In bone tissue engineering, HA is often used for its osteoinductive property, high mechanical strength and excellent biocompatibility. It has been suggested that HA hastens the formation, precipitation, and deposition of calcium phosphate from the body fluid, leading to fast bone regeneration and improved the strength of the bone–matrix interface [14].

However, HA is highly brittle. The ductile properties of collagen help to overcome the poor fracture toughness of HA and provide a favorable environment for cell attachment [15]. Collagen coating increases bone cell proliferation enhances cell adhesion and leads to better osteogenic cell differentiation and handling efficiency on implantation [16, 17]. Thus, a scaffold with HA and collagen has the advantages of both the materials and may offer the best condition for both osteogenesis and proliferation of MSCs. Hence, we aimed to study the cell viability, attachment, proliferation and osteogenic potential of DPSCs on HA–Col scaffold.

Cultured DPSCs had all the typical characteristics of MSCs with respect to morphology, expansion and differentiation characteristics. Differentiation studies agree with earlier studies which showed differentiation potential towards osteogenic, adipogenic and chondrogenic lineages [6, 12]. We have also demonstrated that cultured DPSCs express MSC-specific cell surface antigens, CD73, CD90, CD105 and do not express CD31 and CD45, which are associated with endothelial and hematopoietic lineages, respectively.

It is known that the scaffold contains additives like glycerol, certain components of low molecular weight and initiator fragment that may affect cell viability [18]. However, our results of SRB assay revealed that the scaffold did not affect the viability of cells at lower concentration, though inhibition (< 50%) was observed at higher concentration. Biocompatibility of MSCs on HA–Col disc has also been demonstrated by fluorescent live-dead imaging

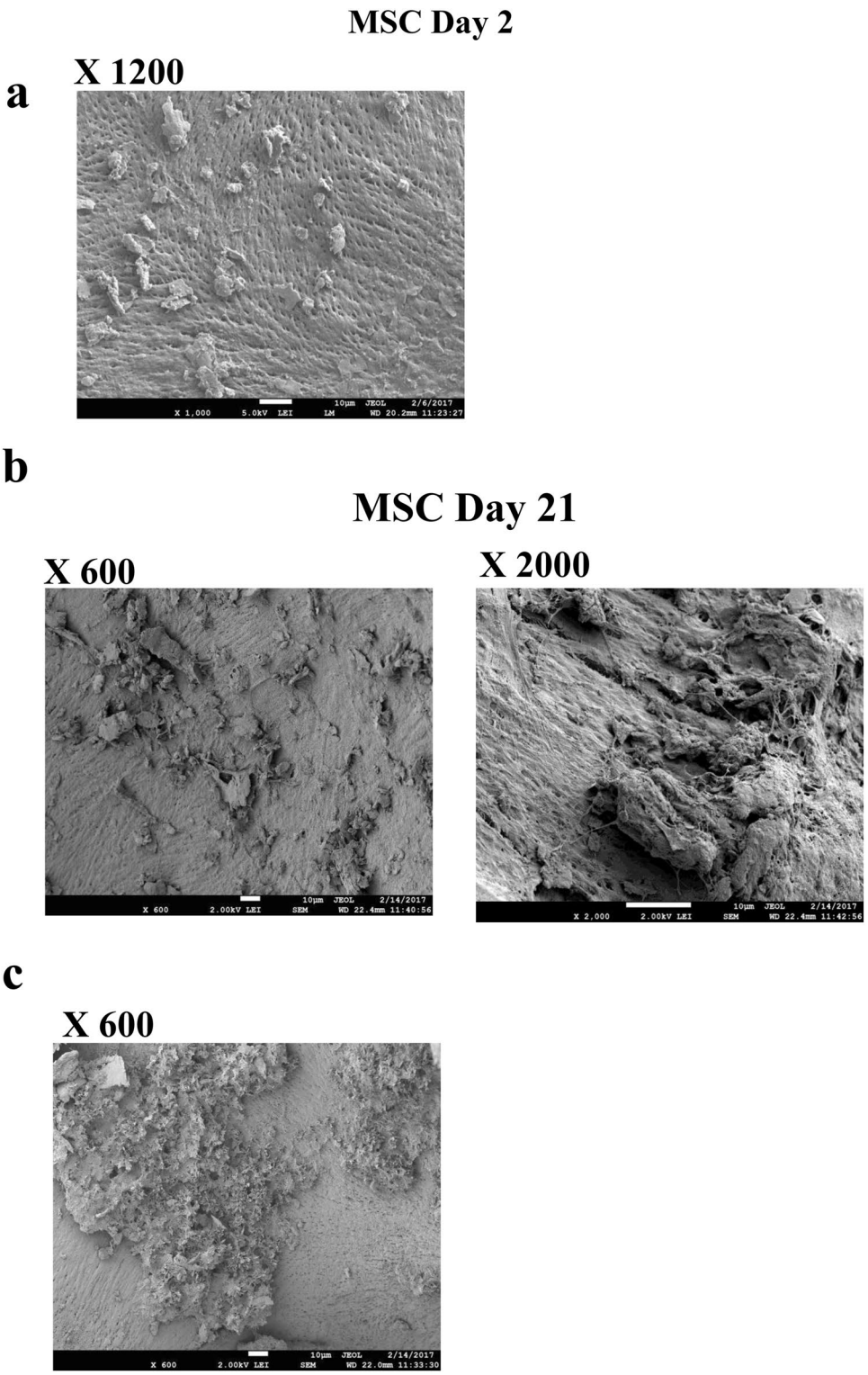

Fig. 4 Scanning electron microscopy (SEM) images of DPSCs grown on HA–collagen scaffold. SEM images taken at ×1200 magnification at day 2 in culture (**a**). At day 21, cells were spread throughout the surface of scaffold by extending filopodial extensions and undergoing change in morphology (×600 and ×2000) (**b**). Nodule-like structures at ×600, suggestive of calcification seen on cells cultured with scaffold in osteogenic media at day 21 (**c**)

[19]. Similar findings were reported by Ning et al. where mouse MSCs and human periodontal ligament stem cells were cultured on collagen–HA scaffolds with different collagen:HA ratios [20]. Cell proliferation and biocompatibility assays were performed and it was found that the porous Col-HA composites have good biocompatibility and biomimetic properties.

In SEM study, cells were found to adhere to the surface at day 2 and increased in number with time. DPMSCs have been differentiated in 3D culture where they undergo some

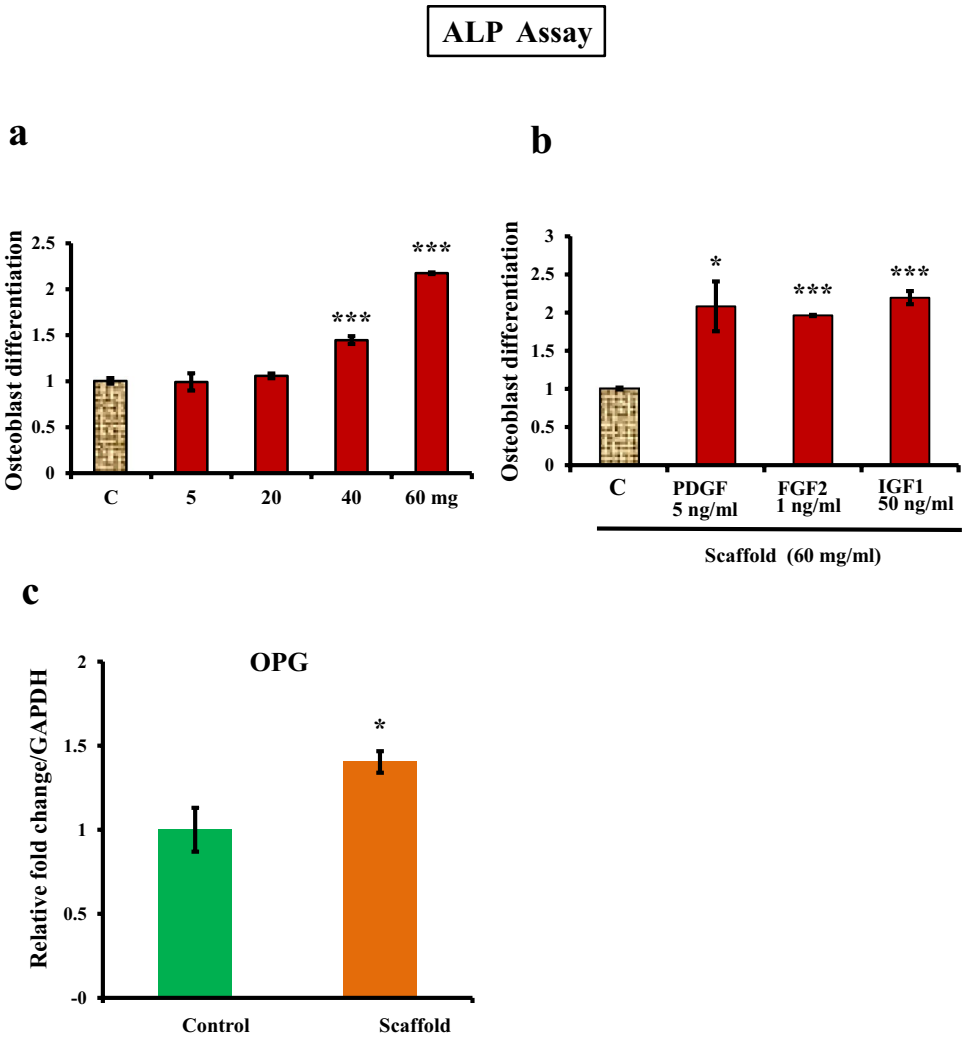

Fig. 5 Measurement of osteoblast differentiation of DPSCs in the presence of the HA–Col scaffold using alkaline phosphatase assay. HA–Col granules were added in media at different concentration (5, 20, 40, 60 mg/mL) in 96 well plate and plate was centrifuged to settle down the granules. 3×10^3 DPSCs were seeded on the scaffold with osteoblast differentiation media for 14 days. Osteogenic differentiation was measured by the ALP assay (**a**). Additive effect of growth factors was measured on DPSCs at 60 mg/mL HA–Col with FGF2 (1 ng/mL), IGF1 (50 ng/mL) and PDGF (5 ng/mL) for 2 weeks (**b**). Data shown as mean ± SEM; $n = 3$; ***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$ compared with control (**c**). HA–collagen scaffold increases mRNA level of osteoprotegerin (OPG) significantly on day 10. Data are presented as mean ± SEM ($n = 3$). Data shown as mean ± SEM; $n = 3$; ***$p \leq 0.001$; **$p \leq 01$; *$p \leq 0.05$ compared with control

morphological changes [21]. SEM image demonstrated that at day 21, cells were fully confluent on the surface of scaffold. The key events of cell attachment, i.e., cell adherence to the substrate, extension of filopodia, networks of cytoplasmic extensions and flattening of the cell mass were observed. We also found that osteogenic differentiation of DPCSs increased with increasing concentration of scaffold. This suggests that HA–Col creates a microenvironment that is conducive to the osteogenic differentiation of DPSCs.

As growth factors are an inevitable part of the tissue engineering triad, we further aimed to study the effects of growth factors on osteogenic differentiation. 60 mg/mL concentration of scaffold demonstrated highly significant osteogenic differentiation potential, so this concentration was used to study the additive effects of PDGF, FGF2 and IGF1. All the above growth factors studied, promoted the osteogenic differentiation significantly. In a recent study, platelet rich plasma (PRP) which is also a growth factor was found to promote the osteogenic differentiation potential of human adipose-derived MSCs as demonstrated by ALP activity and calcium content [22]. In another study, IGF1at a concentration of 400–800 ng/mL lead to significantly higher osteogenic differentiation in human iliac crest bone marrow MSCs [12]. In the present study, we have used a lower concentration of IGF1 and still found convincing results. In concordance Li et al. opined that PDGF regulates osteogenic differentiation and migration via BMP-Smad 1/5/8 signaling [23]. On the contrary, another study revealed that PDGF receptor signaling does not affect osteogenic differentiation of MSCs [24].

In conclusion, HA–collagen scaffold is an apt material for MSCs attachment, proliferation and osteogenic differentiation in vitro; and may serve as an ideal for maxillofacial and alveolar bone regeneration, for which further studies may be planned.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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