Osteogenic Differentiation of Human Dental Pulp Stromal Cells on 45S5 Bioglass[®] Based Scaffolds *In Vitro* and *In Vivo*

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The increasing clinical demand for bone substitutes has driven significant progress in cell-based therapies for bone tissue engineering. The underpinning goals for success are to identify the most appropriate cell source and to provide three-dimensional (3D) scaffolds that support cell growth and enhance osteogenic potential. In this study, human dental pulp stromal cells (HDPSCs) were cultured under basal or osteogenic conditions either in monolayers or on 3D Bioglass[®] scaffolds *in vitro* for 2 or 4 weeks. Cell-scaffold constructs were also implanted intraperitoneally in nude mice for 8 weeks. Osteogenic potential was assessed using quantitative real-time polymerase chain reaction and histological/immunohistochemical assays. In monolayer culture, osteoinductive conditions enhanced HDPSC expression of osteogenic gene markers (*COL1A1*, *RUNX2*, *OC*, and/or *OCN*) compared with basal conditions while culture of HDPSCs on 3D scaffolds promoted osteogenic gene expression compared with monolayer culture under both basal and osteogenic conditions. These results were confirmed using histological and immunohistochemical analyses. *In vivo* implantation of the HDPSC 3D Bioglass constructs showed evidence of sporadic woven bone-like spicules and calcified tissue. In conclusion, this study has demonstrated the potential of using a combination of HDPSCs with 3D 45S5 Bioglass scaffolds to promote bone-like tissue formation *in vitro* and *in vivo*, offering a promising approach for clinical bone repair and regeneration.

Introduction

RESTORATION OF BONE or complex tissue loss due to trauma, tumors, or degenerative disease is still a major medical challenge.^{1–4} To date, autologous bone grafts are considered to be the optimum choice for fracture repair and bone restoration. However, the major disadvantages of these include limited bone stock, the requirement for additional surgical intervention, and complications at the donor site.^{5–10} Other types of bone grafts, for example, allograft or xenograft, carry risks of immunogenicity, poor bio-incompatibility, and the potential transmission of communicable viral infections.^{5–8} Stem-cell-based bone tissue engineering may provide an alternative approach to address these problems.^{1,5–7,9,11,12}

Bone tissue engineering requires cells that are capable of differentiating down the osteogenic lineage to produce bone-

relevant extracellular matrix (ECM).^{13,14} However, a three-dimensional (3D) scaffold framework is essential to support cell growth and allow vascular invasion and ECM deposition. Scaffolds can be also osteoinductive to accelerate osteogenic differentiation.^{15–17} Human dental pulp stromal cells (HDPSCs) are known to be highly proliferative, multipotent, and capable of producing mineralized nodules in monolayer culture^{18,19} as well as producing dentine-like regenerative tissue or mineralized tissue similar to bone.^{20–22}

Silicate bioactive glasses, first investigated by Hench *et al.* (1971),²³ have been well researched as 3D bone tissue scaffolds. The application of bioactive glasses and glass-ceramics in bone tissue engineering is expanding.^{24–26} Further, bioactive glasses can also serve as carriers for the local delivery of metal ions to control cellular functions.²⁷ The dissolution products from such glasses can upregulate expression of genes that control osteogenesis.^{28,29} In addition, there is

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increasing evidence for the positive effects of bioactive glass on vascularization of tissue engineering constructs.^{29–31}

The aim of this study was to investigate the potential use of HDPSCs in combination with 45S5 Bioglass® scaffolds for bone tissue engineering.

Materials and Methods

Cell culture plastics were purchased from Corning. Alpha-modified minimum essential medium (α -MEM), phosphate-buffered saline solution, and fetal bovine serum (FBS) were obtained from Lonza. Antibiotics, growth factors, enzymes, and other reagents were purchased from Sigma unless stated otherwise.

The starting bioactive glass powder selected for this work was 45S5 Bioglass with the standard composition of 45 wt% silicon dioxide, 24.5 wt% sodium oxide, 24.5 wt% calcium oxide, and 6 wt% phosphorus pentoxide.³² Scaffolds were produced by the foam replication technique developed earlier and the foams exhibited porosity of ~90%.³³ Briefly, a polyurethane (PU) foam, which serves as a sacrificial template, is immersed in a Bioglass slurry leading to a homogeneous glass particle coating adhering on the PU foam surfaces. The slurry contains polyvinyl alcohol (PVA) (Sigma Aldrich) as binder. After air drying, the foams are sintered at 1100°C with a presintering step to burn off the binder and the PU template. During this process, the foam struts densify and the glass crystallizes, leading to a Bioglass-based scaffold of suitable structural integrity for further investigation.

Cell isolation and in vitro expansion

Three wisdom teeth were obtained from one male (age 19 years) and two female (age 20 years and 37 years) donors with full patient consent and ethical approval (LREC 07/H1306/93). HDPSCs were isolated using the collagenase digestion method previously described by Ricordi *et al.* (1992)³⁴ and Gronthos *et al.* (2000).¹⁸ The cells were maintained in basal media (α -MEM supplemented with 20% FBS, 200 mM L-glutamine, and 100 unit/mL penicillin-streptomycin) at 37°C and 5% CO₂ until 80% confluent. Passage 4 cells (P4) were used for this study.

HDPSC culture as monolayers in vitro

HDPSCs were seeded in six-well plates (5×10^5 cells per well, $n=3$) and cultured under basal or osteogenic (basal medium supplemented with 0.1 nM dexamethasone and 100 μ M of L-ascorbic-2-acid) conditions for up to 2 and 4 weeks. The samples were then collected for quantitative real-time polymerase chain reaction (qRT-PCR).

HDPSC seeding and growth on 3D scaffolds

The 3D Bioglass scaffolds were cut into standardized cubes of $5 \times 5 \times 5$ mm³ and sterilized for 45 min under UV light (253.7 nm wavelength) using a reflective surface to allow simultaneous sterilization of all scaffold surfaces.

HDPSCs (5×10^5 cells per scaffold) were seeded dynamically on 3D Bioglass scaffolds for 5 days before being statically cultured in 12-well plates ($n=3$). The cell-scaffold constructs were cultured under basal or osteogenic conditions for 2 and 4 weeks. Monolayer cultures were used as controls.

TABLE 1. DETAILS OF PRIMERS USED FOR TAQMAN® GENE EXPRESSION ASSAYS

Gene name	Gene group	Taqman gene primer
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Dehydrogenase (housekeeping gene/control)	Hs99999905-m1
Collagen type I, alpha 1 (COL1A1)	Extracellular matrix structural protein (gene of interest)	Hs00164004-m1
Alkaline phosphatase (ALP)	Phosphatase (bone marker, gene of interest)	Hs01029144-m1
Runt related transcription factor 2 (RUNX2)	Transcription factor (bone marker, gene of interest)	Hs00231692-m1
Bone gamma-carboxyglutamate (gla)/Osteocalcin (OC)	Select calcium binding protein (bone marker, gene of interest)	Hs00609452-g1

Determination of osteogenic gene expression using qRT-PCR

Expression of osteogenic marker genes (COL1A1, ALP, RUNX2, and OC) was assessed using qRT-PCR. GAPDH was used as housekeeping gene. RNA was extracted using the trizol reagent kit (Invitrogen) according to the manufacturer's instructions. One microgram of RNA from each sample was used for reverse transcription using the ABI high-capacity RNA to cDNA kit (Applied Bioscience) according to the supplier's instructions. cDNA was then amplified using ABI TaqMan primers (Table 1) in a 20 μ L reaction mix in 96-well plates (Roche). Amplification was performed using a Roche LC480 cyclor. The results were analyzed using the $2^{-\Delta\Delta ct}$ method³⁵ where ct values at each time point were normalized to the house keeping gene in the same sample and further normalized to ct values of control samples (monolayers or constructs cultured under basal conditions) at the corresponding time points. Results were then expressed as mean $\pm$ SD.

Cell viability and growth on 3D Bioglass scaffolds

At 2 and 4 weeks, the constructs were labeled with Cell Tracker™ Green (CMFDA). Cell viability and growth on the scaffolds was visualized under the confocal laser scanning microscope (CLSM; LEICA TCS SP2). Cell morphology and tissue formation were observed under the scanning electron microscope (SEM; JEOLJSM35).

In vivo implantation of HDPSCs and 3D Bioglass scaffolds

After dynamic seeding for 5 days and *in vitro* culture under basal conditions for a further 2 days, the constructs were sealed in diffusion chambers (Millipore) prior to intraperitoneal implantation in male immunocompromised nude mice (MF1-Nu/Nu, 4–5 weeks old).^{36,37} After 8 weeks, the diffusion chambers were retrieved and samples were fixed in 10% NBF or 98%

TABLE 2. DETAILS OF ANTIBODIES/COUNTER STAINS USED IN IMMUNOHISTOCHEMICAL ANALYSES

Primary antibody name/number	Antigen retrieval	Primary antibody concentration and incubation time	Counter staining
Collagen type I (mouse monoclonal, ab6308)	Pressure cooker with vector unmasking solution for 5 min	1/100 Overnight	H&E
Osteocalcin (mouse monoclonal, ab13420)	0.1% chymotrypsin (0.05 g of chymotrypsin) for 15 min	1/100 Overnight	H&E

H&E, hematoxylin and eosin.

ethanol (for ALP staining) prior to being processed for histology and immunohistochemistry.

Histological and immunohistochemical examination of cell–scaffold constructs

To detect alkaline phosphatase (ALP) activity, HDPSC–Bioglass constructs were stained directly using the ALP staining kit (Sigma)^{38,39} and viewed using a 3D light microscope.

In addition, sections were prepared of the fixed and wax-embedded constructs and stained with either standard hematoxylin and eosin (H&E) or Alizarin red [2% aqueous alizarin red (pH 4.3) for 30 s] to detect calcium deposits.

Immunohistochemical staining of the sections was carried out using primary antibodies against collagen type I (Abcam; mouse monoclonal, ab6308) and osteocalcin (Abcam; mouse monoclonal, ab13420) (Table 2). The Envision kit (Dako) was used to provide secondary antibodies and substrate in each case.

Statistical analysis

qRT-PCR data were statistically analyzed using an analysis of variance one-way test followed by Bonferroni multiple comparison tests. The statistical analyses were carried out using the GraphPad InStat software (version 3). Each experiment was repeated three times from three different donors for each cell type. Results shown are presented from one representative donor per cell type.

Results

HDPSC viability, growth, and osteoblastic differentiation on 3D Bioglass scaffolds under basal culture conditions in vitro

HDPSCs on 3D Bioglass scaffolds were cultured under basal conditions to determine any osteogenic effect of the scaffolds themselves. Live/dead fluorescent staining coupled with CLSM images showed that the majority of the attached HDPSCs had maintained viability following culture on the scaffolds. Cells appeared to have a fibroblastic morphology—indicative of cell spreading—and had covered the scaffolds after 2 weeks of culture, suggesting good proliferation (Fig. 1A). SEM images confirmed the presence of confluent cell layers and cell bridge formation after both 2 (Fig. 1B) and 4 weeks (Fig. 1C). Intense positive staining for ALP was seen within the constructs even under basal conditions, indicating osteoblastic differentiation after 4 weeks of culture *in vitro* (Fig. 1D).

Effect of osteogenic culture conditions on gene expression of HDPSCs on 3D Bioglass scaffolds

Under osteogenic culture conditions, the levels of *COL1A1* gene expression in HDPSC–Bioglass scaffold constructs were

lower at both 2 and 4 weeks compared with those of the basal culture group. In contrast, the levels of expression of *ALP*, *RUNX2*, and *OC* were significantly greater at 2 weeks ($p < 0.001$) under osteogenic compared with basal conditions. However, there was a dramatic drop in the levels of expression of these genes at 4 weeks (Fig. 2A).

Effect of 3D Bioglass scaffolds on HDPSC osteogenic marker gene expression compared with monolayer culture

When comparing the levels of gene expression in 3D constructs with monolayer controls cultured under the same conditions, the levels of *COL1A1* in the constructs under basal conditions were lower at 2 weeks but higher at 4 weeks compared with those of monolayer culture group ($p \leq 0.001$). The levels of *ALP* expression in 3D culture were lower than in monolayer culture at both time points. *RUNX2* and *OC* showed a significantly higher expression at 2 weeks ($p \leq 0.001$) with a dramatically lower expression at 4 weeks compared with monolayer cultures (Fig. 2B). Under osteogenic culture conditions, *COL1A1* levels were downregulated at 2 weeks in the 3D constructs compared with monolayer controls but were significantly upregulated at 4 weeks ($p \leq 0.001$). In contrast, *ALP*, *RUNX2*, and *OC* levels were raised significantly at 4 weeks ($p \leq 0.001$) compared with monolayer controls and then drastically decreased at 4 weeks.

Histological appearance of neotissue formation by HDPSCs on 3D Bioglass scaffold constructs in vitro

After 6 weeks of *in vitro* culture under basal and osteogenic conditions, H&E staining showed that all scaffold pores appeared to have been filled with cells and ECM. In many cases, the appearance was typical of mesenchymal tissue condensation, comprising of dense ECM. Cells in these regions sometimes acquired a more cuboidal, osteoblast-like morphology irrespective of the culture conditions used (Fig. 3A, B).

Alizarin red staining was used to indicate any calcium-rich deposits within the constructs. The Bioglass scaffolds themselves contained calcium and are known to be able to initiate hydroxyapatite deposition^{40–42} and this was reflected by the presence of high amounts of the red/orange Alizarin red staining associated with the scaffolds. However, it was still possible to discern small Alizarin-red-positive deposits within the surrounding cell layers and/or matrix for constructs cultured under both basal (Fig. 3C) and osteogenic cultures (Fig. 3D).

Immunohistochemistry revealed that the “tissue” formed within the constructs stained positively for collagen type I. Positively stained fibers showed a compact bundle arrangement and were oriented parallel to the inner or outer scaffold surface (Fig. 4A, B), which was comparable in the osteogenic culture group. Osteocalcin was also detected in the

FIG. 1. Human dental pulp stromal cell (HDPSC) viability and growth on three-dimensional (3D) 45S5 Bioglass® scaffolds. **(A)** Confocal laser scanning microscope image showing HDPSC viability (arrow—CMFDA) on 45S5 Bioglass scaffolds after 2 weeks of *in vitro* culture under basal condition. **(B, C)** scanning electron microscope (SEM) images showing HDPSC spreading and cell bridge formation (arrows) on 45S5 Bioglass scaffolds after *in vitro* culture under basal conditions for 2 weeks **(B)** and 4 weeks **(C)**. **(D)** Alkaline phosphatase (ALP) staining (arrow) indicative of osteogenic differentiation of HDPSC–45S5 Bioglass scaffolds after 4 weeks of culture in basal medium. Color images available online at www.liebertpub.com/tea

FIG. 2. Relative changes in osteogenic marker gene expression for HDPSCs cultured on 45S5 Bioglass scaffolds *in vitro*. **(A)** The effect of osteogenic culture conditions on the expression of osteogenic marker genes by HDPSCs on 3D Bioglass scaffolds after 2 and 4 weeks ($n=3$). The data for osteogenic conditions were normalized to corresponding controls cultured under basal conditions using the $\Delta\Delta\text{Ct}$ method. **(B)** The effect of 3D Bioglass scaffolds on osteogenic marker gene expression by HDPSCs cultured under basal or osteogenic condition on 3D Bioglass scaffolds for 2 and 4 weeks ($n=3$). The data for 3D culture were normalized to corresponding controls in monolayers using the $\Delta\Delta\text{Ct}$ method. The data are presented as $\log_{10}$ of the mean $2^{-\Delta\Delta\text{Ct}} \pm \text{SD}$ ($*p < 0.05$, $***p < 0.001$). Color images available online at www.liebertpub.com/tea

FIG. 3. Hematoxylin and eosin (H&E) and Alizarin red staining of HDPSCs cultured on 3D Bioglass scaffolds *in vitro*. (A, B) H&E staining following 6 weeks of culture under basal conditions (A) and osteogenic conditions (B). Arrows point to regions of extracellular matrix condensation/woven bone-like spicules. (C, D) Alizarin red staining for calcium deposits in the cell-scaffold constructs cultured *in vitro* for 6 weeks under basal conditions (C) and osteogenic conditions (D). Despite the fact that the calcium-rich scaffolds themselves ("S") are intensely stained with the Alizarin red, staining can also be seen in the "tissue" surrounding the scaffold (arrows). Scale bars = 100 μ m. Color images available online at www.liebertpub.com/tea

constructs under basal conditions (Fig. 4C) with higher staining intensity apparent under osteogenic conditions (Fig. 4D).

Osteogenic differentiation of HDPSCs in 3D Bioglass scaffolds in vivo

After 8 weeks of intraperitoneal implantation in nude mice, H&E staining revealed extensively condensed mesenchymal tissue formation within the constructs, including apparently polarized cuboidal/columnar cells with a parallel orientation adjacent to the scaffold surface, together with the presence of larger, woven bone-like spicules (Fig. 5A).

Alizarin red staining showed evidence of calcium deposition within the neotissue formed around the scaffolds. Further, the scaffolds themselves seemed to be "thickened" with Alizarin red deposits at the interface between the scaffolds and the ECM (Fig. 5B).

Immunohistochemistry confirmed that newly formed "tissue" in the *in vivo* constructs was positive for osteogenic markers, including collagen type I (Fig. 5C) and osteocalcin (Fig. 5D). In addition, the cell-scaffold constructs had evidence of condensed bundles of collagen type I-positive fibers parallel to the outer surface of the scaffold (Fig. 5C).

Discussion

The emerging field of cell-based tissue engineering currently offers great promise to meet the clinical need for bone substitutes. There has been growing interest in using HDPSCs for mineralized tissue regeneration.^{18,19} Despite the fact that we do not yet know enough in respect of the total stem cell number and type within dental pulp and how this compares with cells

from other sources (e.g., bone marrow or adipose tissue), there is evidence to suggest that the stem cells from dental pulp are more "immature" and have a higher colony-forming unit-fibroblast (CFU-F) capacity compared with human bone marrow stem/stromal cells (HBMSCs) (Gronthos *et al.*, 2000).¹⁸ In addition, deciduous teeth, permanent premolars, and wisdom teeth are readily accessible during everyone's life time. This has led to the establishment of many dental cell banks worldwide (e.g., Biobank, Tayside Tissue Bank, UK Stem Cell Bank, UK Biobank, Precious Cells, Store-a-Tooth Stem Cell Bank, BioEden Tooth Cell Bank, and Dhruv Dental Stem Cell Bank),⁴³ emphasizing the need for more research on stem cells from this tissue source.

Various criteria have been set out for selection of appropriate 3D scaffolds for tissue engineering applications.^{13,14,16,44} Yang *et al.* (2006)³⁷ showed that Bioglass-containing composite scaffolds supported HBMSC attachment, growth, and osteogenic differentiation *in vitro* and *in vivo*. Therefore, in the present study, a Bioglass-based scaffold was used due to its reported porosity, bioactivity, and osteoconductivity.^{42,45–47}

This study has confirmed that the Bioglass scaffolds are biocompatible as evidenced by good cell attachment and spreading and maintenance of cell viability within the constructs. This agrees with our previous report for HBMSCs³⁷ and extends it to HDPSCs. HDPSCs seeded on to Bioglass scaffolds were shown to form neotissue structures *in vitro* and *in vivo* with evidence of osteogenic differentiation as evidenced by the PCR data and immunohistochemistry.

Most previous publications related to HDPSC gene expression were carried out in monolayer culture.^{48–50} In this study, HDPSC expression of osteogenic marker genes was investigated in 3D culture using the Bioglass scaffolds to

FIG. 4. Immunohistochemical staining of HDPSC-scaffold constructs cultured *in vitro*. After 6 weeks of culture in basal condition *in vitro*, positive immunohistochemical staining was seen for collagen type I (A, B); arrows indicate collagen-I-positive fibers/bundles running parallel to the construct surface. The constructs were also positive for osteocalcin (C, D). All sections were counterstained with Harris hematoxylin. Scale bars = 100 μ m. Color images available online at www.liebertpub.com/tea

FIG. 5. H&E and Alizarin red staining of HDPSC-3D Bioglass scaffold constructs following intraperitoneal implantation *in vivo* in diffusion chambers. After 8 weeks *in vivo* implantation, H&E staining (A) indicated extracellular matrix condensation/woven bone-like spicule formation (arrows). Alizarin red staining (B) for calcium deposits in HDPSC-scaffold constructs showed that despite the fact that the calcium-rich scaffolds themselves ("S") were intensely stained with the Alizarin red, additional staining was also seen in the "tissue" surrounding the scaffold (arrows). (C) Immunohistochemical staining for collagen type I shows parallel collagen-I-positive fiber/bundle formation (arrows). (D) Immunohistochemical staining for osteocalcin was positive in all samples (sections counterstained with Harris hematoxylin). Scale bars = 100 μ m. Color images available online at www.liebertpub.com/tea

provide a construct. A number of studies have shown that Bioglass scaffolds stimulate osteoblastic differentiation^{37,51,52} and the ionic dissolution products of 45S5 Bioglass are known to affect the gene expression profile of human osteoblasts in monolayer cultures.^{29,53} However, these studies used relatively short culture periods of 48 h⁵² and/or 5 days compared with the longer periods used here.⁵³

This study showed that in monolayer culture, *COL1A* was downregulated at both 2 and 4 weeks under osteoinductive conditions compared with controls in basal medium. Previous studies have suggested that *COL1A1* is closely correlated with the osteoblast proliferation phase. It would therefore be expected to be expressed highly during proliferation and at the beginning of ECM production and to be downregulated in more advanced stages of the active osteoblasts, when mineralization starts.^{54,55} Alternatively or in addition, this may also be due to the inhibitory effect of Dexamethasone (present in the osteoinductive medium) on proliferation of the cells under osteogenic culture conditions.⁵⁶

ALP, *RUNX2*, and *OC* were all upregulated in the monolayer HDPSCs under osteogenic conditions at 2 weeks compared with controls in basal medium. This was followed by a dramatic downregulation at 4 weeks, commensurate with advancing osteoblast differentiation.⁵⁴ The expression patterns seen in this monolayer study were very similar to those reported by Yamada (2010).⁵⁷

In comparison with monolayers, HDPSCs cultured on 45S5 Bioglass scaffolds and in osteoinductive culture showed enhanced expression of osteogenic gene after 2 weeks. These findings confirmed that 45S5 Bioglass scaffolds are osteoinductive and able to promote mineralization as confirmed by the downregulation of *ALP*, upregulation of *OC*, and by the Alizarin red staining data. Osteogenic growth factors, such as BMPs, are known to stimulate inhibitory growth factors and antagonist binding proteins, leading to a feedback inhibitory loop⁵⁸ that can explain the overall downregulation of osteogenic marker genes by the constructs after 4 weeks of culture.

The histological and immunohistochemical results suggested that constructs cultured under basal and osteogenic conditions *in vitro* showed a shift in cell morphology toward more plump and cuboidal cells, which produce an organized ECM with evidence of calcium-rich deposits. *In vivo* results showed a greater extent of organized ECM and more evidence of calcium-rich deposits within the constructs. These data were further supported by the immunohistochemistry findings, where *in vivo* constructs showed extensive staining for both collagen type I and OCN, characteristic of more mature osteoblasts.^{59–61} Further, bone-like spicules were seen only within *in vivo*-retrieved constructs. This might be attributed to the longer period of culture (8 weeks) or due to the provision of an appropriate microenvironment *in vivo* that enhances blood supply and exchange of fluids, together with cytokines and host diffusible products.^{22,62–64}

Zhang *et al.* (2006) demonstrated the formation of thick capsules and ECM by HDPSCs under osteogenic conditions on three types of 3D scaffolds *in vivo*. However, mineralization was only observed for titanium scaffold constructs.⁴⁸ The results presented here showed that constructs using 45S5 Bioglass formed tissue condensations with osteoblast-like cells (e.g., cuboidal morphology) and Alizarin red positive that are indicative of calcium-rich deposits. Although the scaffolds themselves picked up the Alizarin red stain due to

their intrinsic calcium content, constructs seeded with HDPSCs showed more staining between and/or surrounding the scaffolds compared with scaffolds alone, an indication of the well-documented bioactivity of Bioglass.⁴⁵

Mineralized tissue formation has been observed previously when HDPSCs have been implanted *in vivo*. Otaki *et al.* (2007) implanted HDPSCs with hydroxyapatite/tricalcium phosphate (HA/TCP) powder subcutaneously in immunocompromised mice for 7 and 15 weeks and demonstrated lamellae bone formation.⁴⁹ Graziano *et al.* (2008) showed formation of bone nodules by combining human dental pulp tissue with poly(lactic-co-glycolic acid) scaffolds in a subcutaneous implantation module in immunocompromised rats.⁶⁵ Other studies demonstrated that HDPSCs formed dentine-like tissue *in vivo* while HBMSCs formed bone and these results may be attributed to the use of the subcutaneous implantation model where epithelial tissue from the host can contribute to reciprocally inducing the HDPSCs into odontoblasts rather than osteoblasts.^{18,66–68} This is in contrast to the present study, where there was no evidence of odontoblastic differentiation in the intraperitoneal diffusion chamber that does not permit any cellular contribution to the constructs from the host cells. The diffusion chamber model provided a simple *in vivo* bioreactor for this work. However, a bone defect model may provide a better microenvironment for angiogenesis and bone remodeling for future studies.^{69,70}

Conclusion

45S5 Bioglass scaffolds can enhance osteogenic differentiation of HDPSCs both *in vitro* and *in vivo*, indicating the potential use of this combination for bone tissue engineering for clinical applications.

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Disclosure Statement

No competing financial interests exist.

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