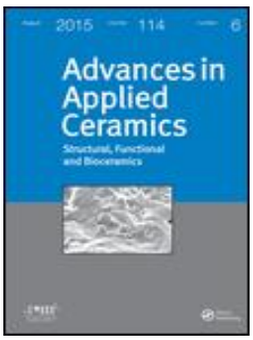

Taylor & Francis
Taylor & Francis Group

Advances in Applied Ceramics

Structural, Functional and Bioceramics

ISSN: 1743-6753 (Print) 1743-6761 (Online) Journal homepage: https://www.tandfonline.com/loi/yaac20

Preliminary evaluates of silica/β-TCP/PLGA microspheres for dentin regeneration in vivo

Kun Qian, Bingqing Li, Wenhao Zhu, Jingren Zhao & Yihong Liu

To cite this article: Kun Qian, Bingqing Li, Wenhao Zhu, Jingren Zhao & Yihong Liu (2020): Preliminary evaluates of silica/β-TCP/PLGA microspheres for dentin regeneration in vivo, Advances in Applied Ceramics, DOI: 10.1080/17436753.2020.1760460

To link to this article: https://doi.org/10.1080/17436753.2020.1760460

ADVANCES IN APPLIED CERAMICS
https://doi.org/10.1080/17436753.2020.1760460

The Institute of Materials, Minerals and Mining

Taylor & Francis
Taylor & Francis Group

Preliminary evaluates of silica/β-TCP/PLGA microspheres for dentin regeneration in vivo

Kun Qian*, Bingqing Li*, Wenhao Zhu, Jingren Zhao and Yihong Liu

Department of General Dentistry, Peking University School and Hospital of Stomatology, Beijing, People's Republic of China

ABSTRACT

Silica/β-tri-calcium phosphate/poly (lactic-co-glycolic acid) microspheres, a type of bioactive and biodegradable materials, were produced by a double emulsion and solvent evaporation methods. The biological activity of the microspheres to promote dentin regeneration was investigated in vivo. First, the ionic concentration of silicon in the microspheres was detected by inductively coupled plasma optical emission spectrometry. The results showed the release of silicon continuously and without any obvious burst. Second, the microspheres with or without human dental pulp cells adhered were transplanted into subcutaneous pockets of nude mice to evaluate the bioactivity. The implanted material specimens were obtained after 6 weeks. The specimens were evaluated after H&E staining and toluidine blue staining. The results presented the cells ingrowth of the scaffold material and collagenous tissues around the microspheres. It was demonstrated that the material had outstanding biocompatibility and bioactivity.

ARTICLE HISTORY
Received 15 September 2019
Revised 22 October 2019
Accepted 4 November 2019

KEYWORDS
Silica; microspheres scaffold; biological activity

Introduction

The health of the pulp tissue is important for the normal functioning and long-term survival of the teeth. Preservation of the pulp tissue can prevent bacterial infection, reduce the occurrence of periapical periodontitis, and maintain the normal function of the teeth. Therefore, vital pulp therapy should be an important development direction for the treatment of pulp diseases. The ideal vital pulp therapy is to regenerate rapidly dentin possessing an excellent quality using a biocompatible, bioactive agent while maintaining the health of the pulp tissue [1]. Research and development of therapeutic materials play a key role in this situation.

With the advent of modern tissue engineering and regenerative medicine, the regeneration of dental tissue has received a lot of attention. There are clinically reported cases of 'pulp regeneration', claiming that these cases use 'revascularsation' to successfully restore the vitality of some dead teeth while successfully treating apical periodontitis [2–5]. However, histological studies have shown that the tissue formed by this method is not dental pulp tissue. The root canal is filled with dental cementum, periodontal ligament, non-specific fibrous tissue and bone tissue, which is a form of wound healing [6–8].

According to the research results in the field of modern tissue regeneration, the focus of pulpal dentin regeneration should be placed on tissue engineering treatment methods. The three elements of tissue engineering include stem cells, bioactive molecules and scaffolds [9,10]. The contribution of scaffolds in tissue regeneration is indispensable as they serve as carriers to facilitate delivery of stem cells and/or growth factors at a local receptor site [11]. As a carrier material, the ideal scaffold material should be biodegradable and biocompatible, promote cellular interactions and tissue development, and possess proper mechanical properties [4,12,13].

A variety of scaffolds have been tested for dentin regeneration, including those made of collagen, polylactic acid (PLA), polyglycolic acid (PGA), a copolymer of PGA and PLA, poly (lactic-co-glycolic acid) (PLGA), and bioceramics [14–16]. These materials have been shown to promote the proliferation and differentiation of dental pulp cells, but few were able to regenerate the complete dentin tissue [17], and the newly formed hard tissue was very different from the primary dentin [18].

Silica is an essential nutrient for the natural ecosystem [19], particularly for humans and other vertebrates [20,21]. It is widely used in industrial and medical fields. At the same time, it is an important material for nanobiotechnology. Carlisle's [22] and Schwarz's study [23] first established the effect of silica on bone formation and bone repair. Previous studies confirmed that silica can be absorbed and degraded by cells through phagocytosis, and then induce bone regeneration [24]. When silica was added to human osteogenic

CONTACT Yihong Liu kqliuyh@163.com Department of General Dentistry, Peking University School and Hospital of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Laboratory for Digital and Material Technology of Stomatology & Beijing Key Laboratory of Digital Stomatology, 22 Zhongguancun South Avenue, Haidian District, Beijing 100081, People's Republic of China
*These authors contributed equally to this work and should be considered co-first authors.

sarcoma cells (SaOS-2 cells), more hydroxyapatite (HA) particles were produced, and the expression of bone morphogenetic protein 2 (BMP-2) was upregulated [24,25]. With the increase of BMP-2, the gene expression of collagen type I (COL-I) was also upregulated, providing and synthesising matrix for osteoblasts in the formed bone structure [26]. Silica upregulated the expression of COL-I and dentin sialophosphoprotein (DSPP), and promoted alkaline phosphatase (ALP) secretion and the odontogenic differentiation of human dental pulp cells (hDPCs) in the osteoinductive environment [27].

The microsphere structure provided enough riveting sites for cells and promotes adherent cell attachment better than the traditional bulk scaffold. Microspheres also effectively preserved adherent cells and their extracellular matrix, inhibited possible cell apoptosis, and improved the survival rate of transplanted cells in the transplantation environment [28].

Based on these theories, we speculated that β-tri-calcium phosphate (β-TCP)/PLGA microspheres coated with silica nanoparticles may be beneficial for the growth and differentiation of hDPCs, and is a potential scaffold material for dentin regeneration.

Accordingly, this study was intended to prepare a silica/β-TCP/PLGA microspheres, and evaluate its biocompatibility and bioactivity for dentin regeneration in vivo.

Materials and methods

Material preparation

The following materials were used. Poly (D, L-lactide-co- glycolide) (PLGA; LA:GA = 75:25, Mw 100 kDa) was obtained from Jinan Daigang Biomaterial Company Limited (Shandong province, P.R. China). Silica was provided by State Key Laboratory of New Ceramics and Fine Processing in Tsinghua University, P.R. China. Poly (vinyl alcohol) (PVA), β-tri-calcium phosphate (β-TCP), and dichloromethane were obtained from Sigma (Taufkirchen, Germany).

Microspheres were prepared by a double emulsion and solvent evaporation methods according to Wang's procedure [29]. PLGA was dissolved in dichloromethane to give a 20% (w/v) solution. Silica and β-TCP were dissolved in distilled water. Two millilitre solution was mixed with 5 mL of the PLGA/dichloromethane solution and sonicated at a voltage of 60 w by Ultrasonic Crasher Noise Isolating Chamber for 10 s and paused 10 s, repeated for 120 s. Subsequently, the viscous emulsion was injected dropwise through a syringe needle (opening 0.9 mm) into 300 mL of 1% PVA using the syringe pump at the speed of 12 mL h^{-1}. The microsphere suspension was stirred (at 650 rev min^{-1}; 4 h) on a magnetic stirrer to allow evaporation of the organic solution and to force the microspheres to harden. Then the solidified microspheres were washed in distilled water (five times) to remove the residual PVA and then dried on filter paper. After that, the microspheres were deep-frozen at −80°C overnight and then lyophilised in a freeze drier (BenchTop Pro with Omnitronics; SP Industries, RoadWarminster, PA; U.S.A.) for 24 h and stored at 4°C until further use.

The silica/β-TCP/PLGA microspheres were used as the experimental material and the β-TCP/PLGA microspheres were used as a control material. Each material was moulded into discs (diameter = 2 mm, thickness = 1.3 mm) to prepare the scaffolds used in subsequent experiments. Before use, all materials were treated with ultraviolet light for 1 h.

Determination of release amount of silicon ion in vitro

Silica/β-TCP/PLGA scaffolds were immersed in 4 mL α-MEM medium. The medium was changed every two days, and centrifuged before each measurement to obtain the supernatant, and the concentration of released silicon ions was measured by the inductively coupled plasma optical emission spectrometry (ICP-OES) method. The same amount of medium was added to continue soaking. The measurement time points were 2, 4, 6, 8, 10, 14, and 20 days.

Kolmogorov–Smirnov normality test ($\alpha = 0.05$) was performed on each group using software SPSS 20.0. Statistical analysis was performed by one-way ANOVA in combined with LSD post hoc test ($p = .05$).

Scaffolds with HDPCs subcutaneous implantation experiment in nude mice

Preparation of experimental animals

The animal study was approved by the ethical committee of Peking University Health Science Center. 6 weeks old male nude mice (BALB/c nude mice) (Beijing Weitong Lihua Experimental Animal Technology Co., Ltd.) weighing 22–24 g were used. A total of 6 mice were used for the test.

Cell culture

The isolation and culturing of human dental pulp cells (hDPCs) were performed following the previous study [27]. Normal human dental pulp tissues were isolated from two extracted third molars of a 20-year male patient at Peking University School and Hospital of Stomatology. Then, digested the pulp tissues in a solution containing 3 mg mL^{-1} collagenase type I (Worthington Biochemical, Lakewood, NJ, U.S.A.) and 4 mg mL^{-1} dispase (Roche Molecular Biochemical, Mannheim, Germany) for 1 h at 37°C. Culture was done in an alpha-minimal essential medium (α-MEM, GIBCO, NY, U.S.A.) supplemented with 10%

fetal bovine serum (GIBCO, Waltham, MA, U.S.A.), 100 U mL^{-1} penicillin G, 100 mg mL^{-1} streptomycin (Invitrogen, Carlsbad, CA, U.S.A.), and 2 mM L-glutamine (Invitrogen) in a humidified incubator containing 5% CO_2 at 37°C. The medium was changed every 3 days. Subcultured was done at 70% confluence. hDPCs between passages 4–6 were used in the following experiments.

Scaffolds groups

They were divided into 4 groups of scaffolds (6 specimens in each group):

(1) β-TCP/PLGA
(2) β-TCP/PLGA + hDPCs
(3) Silica/β-TCP/PLGA
(4) Silica/β-TCP/PLGA + hDPCs

Surgical procedure

The hDPCs (2×10^4) were seeded into each scaffold as previously described [27]. After incubation at 37°C, 5% CO_2 in the regular culture medium for 3 days, an osteogenic-inducing medium was used for another 7 days. The osteogenic-inducing medium contained fresh α-MEM with 10% fetal bovine serum, 100 U mL^{-1} penicillin G, 100 mg mL^{-1} streptomycin, 2 mM L-glutamine,100 nM dexamethasone (Sigma, Taufkirchen, Germany), 10 mM β-glycerophosphate (Sigma, Taufkirchen, Germany), and 0.2 mM L-ascorbic acid (Sigma, Taufkirchen, Germany).

Animals were anesthetised by intraperitoneal injection with 1% pentobarbital sodium (0.03 g kg^{-1}). Shaved and disinfected on the dorsal region of the mice. Subsequently, four small subcutaneous pockets were made with scissors on the backside laterally from the spine of each mice. Four groups of scaffolds were then randomly placed into the subcutaneous pockets. Finally, the incision was sutured with Prolene.

Sample acquisition and evaluation

After 6 weeks, the animals were sacrificed by carbon dioxide inhalation. The specimens were obtained and immersed in 10% buffered neutralised formalin for 4 h. Then 3 specimens in each group were subjected to H&E staining, and the other 3 specimens were stained with toluidine blue.

H&E staining group steps: 10% EDTA decalcification for 1 week, the specimens were rinsed with water, dehydrated in ascending concentration of ethanol and then embedded in paraffin. Sections were cut with a thickness of 5 μm, and then stained with H&E [30].

Toluidine blue staining group steps: The specimens were dehydrated in ascending concentration of ethanol, then embedded in light curing resin. Sections were cut with a thickness of 30 μm. Subsequently, stained with toluidine blue [31].

All sections were observed under an optical microscope to evaluate the biocompatibility of the materials and the formation of new tissue after implantation. The collagen fibers became red in H&E staining, whereas they became blue in toluidine blue staining. In view of that H&E staining could not distinguish the lesions of collagen fibers such as necrosis or hyaline degeneration, the toluidine blue staining was added to further verify the experimental results in this study.

Results and discussion

Structures of microspheres

Scanning electron microscopy images showed that the surface of microspheres was slightly coarse and demonstrated uniformly dispersed micro-pores, with diameters ranging from 2 to 10 μm (Figure 1). Based on the microsphere production methods, this superficial feature was contributed by the poly (vinyl alcohol) and the trace release of the silica and/or β-TCP on the surface, during the washing and freeze-drying process.

The superficial micro-pores increased the surface area of the microsphere, and gave rise to the

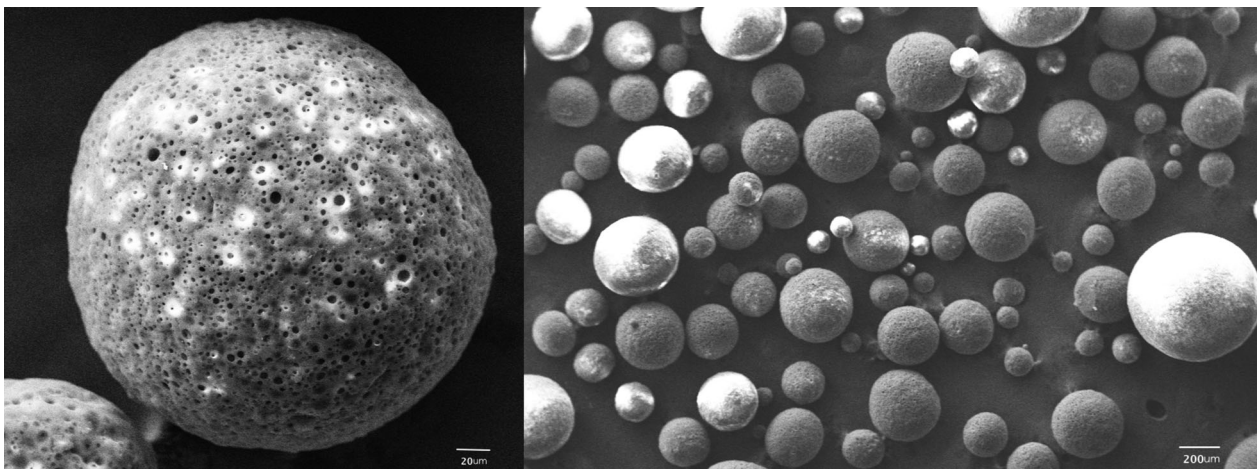

Figure 1. Scanning electron microscopy images of silica/β-TCP/PLGA microspheres.

hydrophilia of the microsphere's surface. It was regarded as favourable factors for growth factors in the interstitial fluid adhering to the microsphere and the silica releasing. Meanwhile, the rough surface of the microsphere was beneficial to the hDPCs adherence and proliferation.

Ionic concentration of silicon in the silica/β-TCP/PLGA microphere

The ionic concentrations of silicon ions in silica/β-TCP/PLGA microsphere were detected by ICP-OES in vitro. The result (seen in Figure 2) showed that the released amount of silicon ion was stable within 0–8 days (31.72 μg mL^{-1}). And then, it decreased over time. The value, 27.24 μg mL^{-1}, was the lowest at 20 days. The results had no significant difference ($p > .05$). According to these results, it was considered that the release of silicon of the microspheres was continuous within a certain range of time, without obvious burst releasing.

Review of previous studies, Shie's study [32] indicated that silicon stimulated cell adhesion via activation of MAPK/extracellular signal-regulated kinase (ERK) and p38 signalling pathways. Similarly, Sun's study [33] showed that high Si content upregulated gene expression of BMP2 and Smad1, as well as the proliferation and differentiation of cells. Based on these pathways, hDPCs were induced to odontoblast differentiation and proliferation by the silicon, which was released from silica/β-TCP/PLGA microspheres continuously.

Silica/β-TCP/PLGA microspheres hetertopic inducing hDPCs differentiation and growth to pulp-like tissue, and expected dentin-like tissues

In this study, the scaffold materials, made by silica/β-TCP/PLGA and β-TCP/PLGA microspheres, were cultured with/without hDPCs, and implanted into the back of the nude mice for 6 weeks, in order to evaluate the biocompatibility and bioactivity of the materials. The nude mice were in good health throughout the breeding period, until they were scarified 6 weeks later. The material-tissue specimens were gained and cut into slices. The slices showed the fibrous connective tissue around the scaffolds growing well under microscope in all groups (Figure 3(A1, B1, C1, D1)). At high magnification, the micrographs presented cells growth into the scaffolds and fibrous connective tissue formation among the microspheres nearby marginal area of the scaffolds in all groups (Figure 3(A2, B2, C2, D2)). These results proved the scaffold materials performed excellent biocompatibility.

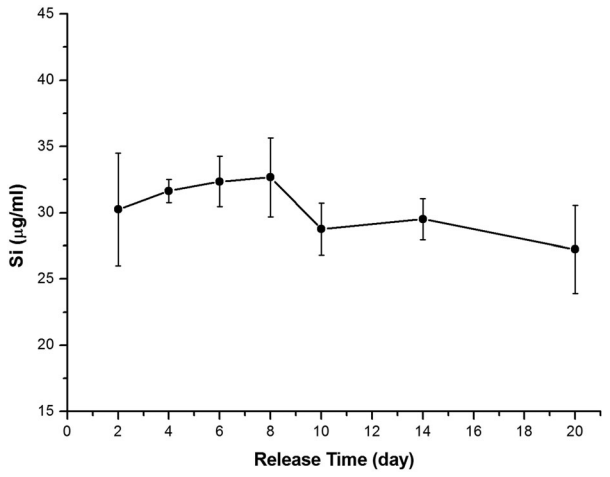

Figure 2. The variation of silicon ion concentration in solution with silica/β-TCP/PLGA. The average silicon ion concentration at 0–8 days was 31.72 μg mL^{-1}, and then the concentration decreased slightly with time, and it was 27.24 μg mL^{-1} at 20 days.

The fibrous connective tissues were observed, mainly collagen fibers both marginal and central area of the scaffolds, in each group. Further observation emerged these newly formed collagen tissues being closely attached to the microspheres surface. When the central area of the scaffolds was observed, the collagen tissues were little in group 1 (Figure 3(A2)), a little more in group 2 and 3 (Figure 3(B2,C2)). While they were much more in group 4 than other groups (Figure 3(D2)). Seeing Figure 3, the black arrow in the H&E staining pictures showed the collagen tissue, and the blue arrow showed the blood vessel. The blood vessels could be found in many specimens except group 1 (Figure 3(A1,A2)). Endothelial cells were companied with the collagen tissue formation. Therefore, blood vessels and red blood cells among the microspheres were found in the specimens of group 2 (Figure 3(B1, B2)) and group 3 (Figure 3(C1,C2)). While the blood vessels and red blood cells were observed more in group 4 (Figure 3D1, D2) than the other groups. When taking bigger magnification, star-like fibroblasts were observed (Figure 3(D3) red arrow) together with blood vessels. Based on these results, it revealed that silica/β-TCP/PLGA scaffolds induced collagen tissue and blood vessels, regardless of whether co-culture with hDPCs or not.

The toluidine blue-stained was used for staining collagen tissue, presented the density of the collagen by the depth of the colour. For example, the blue arrow showed the newly formed collagen tissue in Figure 4. Similar with H&E staining results, the amount of new collagen formation in group 1 (β-TCP/PLGA) was very small (Figure 4(A)), and a little increased in group 2 and 3 (Figure 4(B,C)). There were more new collagen formations in group 4 than the other groups, especially in the central area of the scaffolds (Figure 4(D)). Additionally, the new collagen tissue in group 4 was the most abundant and denser, appearing strong positive reaction to toluidine blue staining (Figure 4(D)). The observation after toluidine blue staining confirmed the bioactivity of the silica microspheres to induce hDPCs differentiation, collagen tissues formation.

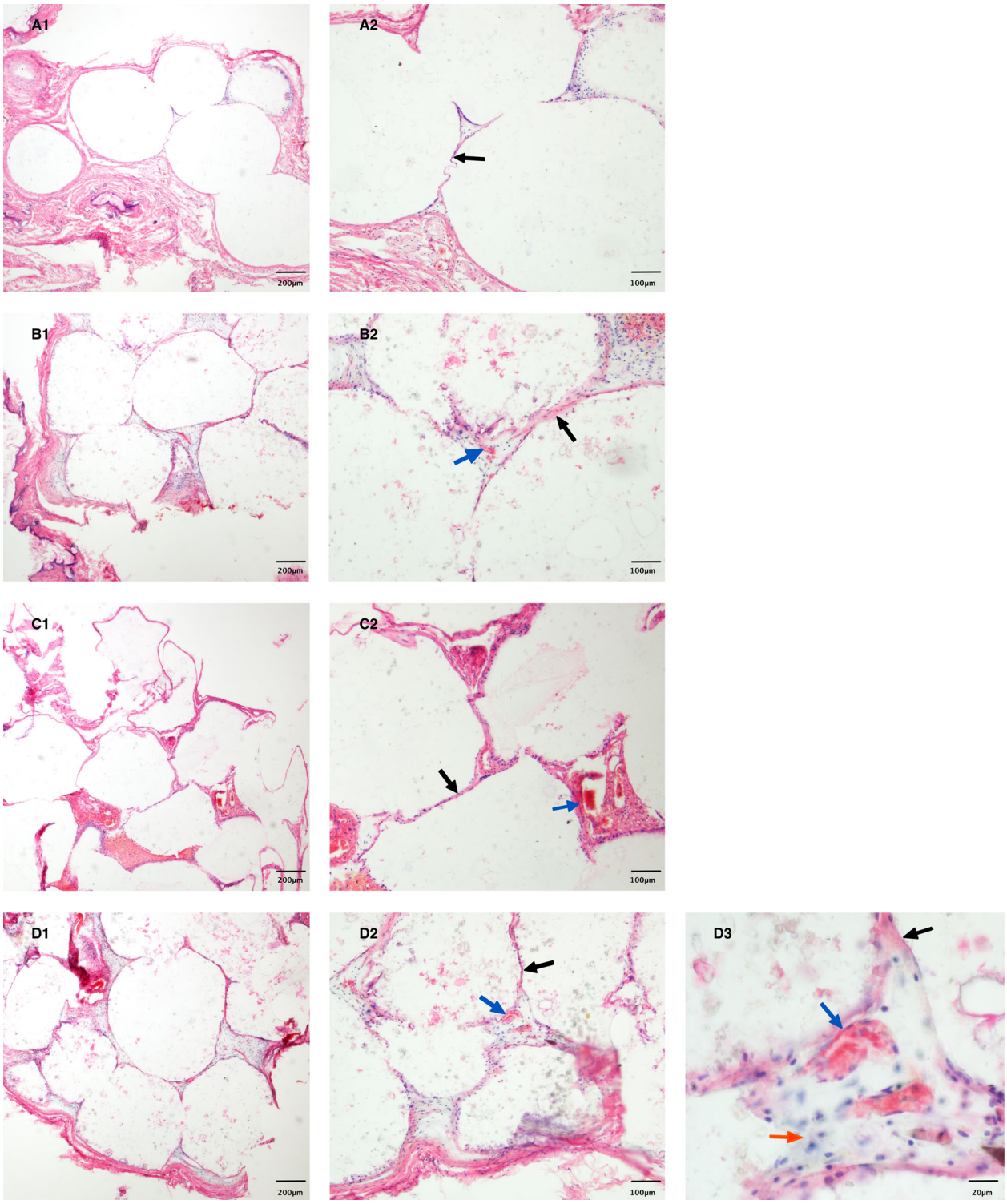

Figure 3. H&E staining images of 4 scaffolds implanted in the back of nude mice after 6 weeks . Representative examples are shown for (A1,A2) group1 (β-TCP/PLGA), (B1,B2) group 2 (β-TCP/PLGA + hDPCs), (C1,C2) group3 (silica/β-TCP/PLGA), (D1,D2,D3) group4 (silica/β-TCP/PLGA + hDPCs). The arrow shows the collagen tissue, the blood vessel, and the fibroblast.

Collagen type I is the most important constituent of the extracellular matrix of dental pulp connective tissue [34]. It has been suggested that the synthesis of collagen type I is an important step in the odontoblast differentiation process [35]. Collagen type I had been found to be associated with the production and mineralisation of dentine [36]. In addition, collagen type III is also important during odontoblast differentiation. Previous studies demonstrated that collagen type III may be a component of the predentin secreted by polarised odontoblasts [37]. In the present in vivo study, the results of 6 weeks heterotopic induction study showed that the silica/β-TCP/PLGA microspheres induced hDPCs differentiation, collagen tissues formation, furthermore indicated the bioactivity of the materials inducing dentin

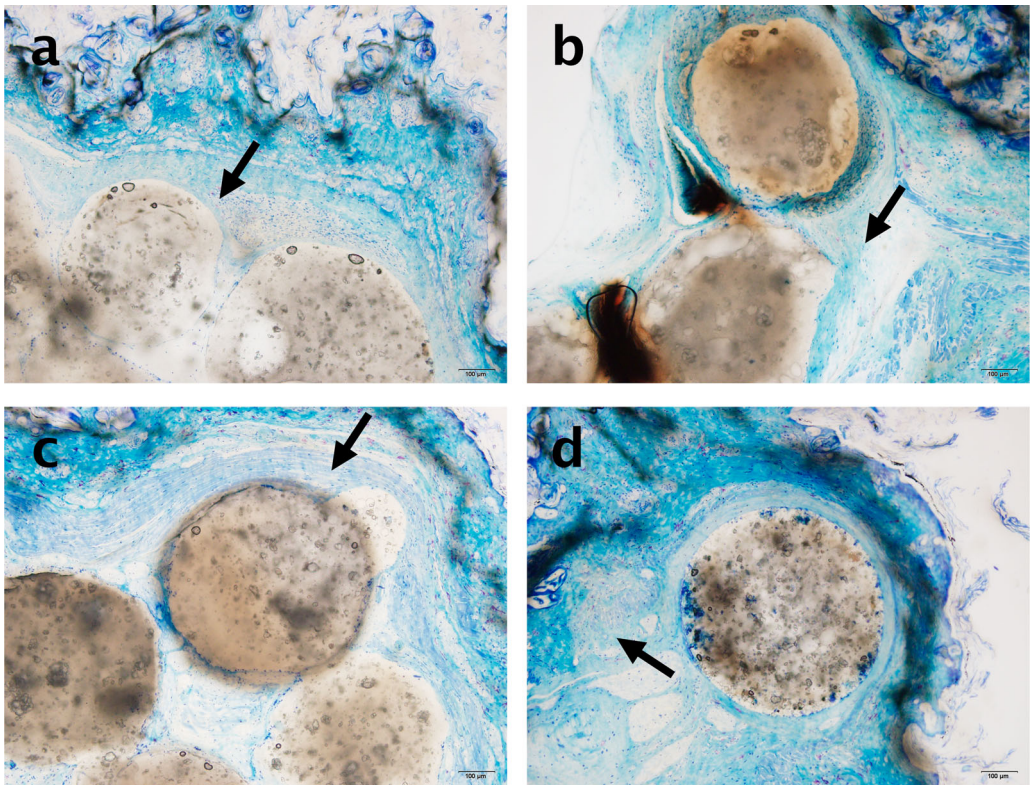

Figure 4. Toluidine blue staining images of 4 scaffolds implanted in the back of nude mice after 6 weeks. Representative examples are shown for (A) group1 (β-TCP/PLGA), (B) group 2 (β-TCP/PLGA + hDPCs), (C) group3 (silica/β-TCP/PLGA), (D) group4 (silica/β-TCP/PLGA + hDPCs). The arrow shows the collagen tissue.

generation. In addition, the present results confirmed the microspheres promoted the growth of fibroblasts, tend with cells adhesion and growth well on the surface of the microspheres, indicating its excellent biocompatibility.

Summarise the above results, that microspheres scaffold structure and silica worked together to promote the migration and differentiation of dental pulp cells, blood vessels formation, and collagen tissues formation. In this situation, silica played the core effects comparatively, meanwhile the scaffold structure provided an advantageous environment for cell growth and adhesion.

Conclusion

Based on the limited results in this study, it was confirmed that the silica microspheres had good biocompatibility and good bioactivity inducing migration and growth of hDPCs. It could to be used as a growth factor-loading scaffold material for dentin regeneration in the future research.

Acknowledgements

This study was supported by grants from the Natural Science Foundation of China (Project nos. 51772007).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by grants from the Natural Science Foundation of China [Project No. 51772007].

References

[1] Koike T, Polan MAA, Izumikawa M, et al. Induction of reparative dentin formation on exposed dental pulp by dentin phosphophoryn/collagen composite. Biomed Res Int. 2014;1:745139.
[2] Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol. 2001;17:185–187.
[3] Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod. 2004;30:196–200.
[4] Jung IY, Lee SJ, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: a case series. J Endod. 2008;34:876–887.
[5] Ding RY, Cheung GS, Chen J, et al. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J Endod. 2009;35:745–749.
[6] Wang XJ, Thibodeau B, Trope M, et al. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. J Endod. 2010;36:56–63.
[7] Martin G, Ricucci D, Gibbs JL, et al. Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. J Endod. 2013;39:138–144.
[8] Zhu W, Zhu X, Huang GT, et al. Regeneration of dental pulp tissue in immature teeth with apical

periodontitis using platelet-rich plasma and dental pulp cells. Int Endod J. 2013;46:962–970.
[9] Nakashima M, Reddi AH. The application of bone morphogenetic proteins to dental tissue engineering. Nat Biotechnol. 2003;21:1025–1032.
[10] Nakashima M, Akamine A. The application of tissue engineering to regeneration of pulp and dentin in endodontics. J Endod. 2005;31:711–718.
[11] Sharma S, Srivastava D, Grover S, et al. Biomaterials in tooth tissue engineering: a review. J Clin Diagn Res. 2014;8:309–315.
[12] Qu T, Liu X. Nano-structured gelatin/bioactive glass hybrid scaffolds for the enhancement of odontogenic differentiation of human dental pulp stem cells. J Mater Chem B. 2013;1:4764–4772.
[13] Nakashima M, Iohara K, Murakami M. Dental pulp stem cells and regeneration. Endod Topic. 2013;28:38–50.
[14] Gong T, Heng BC, Lo EC, et al. Current advance and future prospects of tissue engineering approach to dentin/pulp regenerative therapy. Stem Cells Int. 2016: 1–13.
[15] Colombo JS, Moore AN, Hartgerink JD, et al. Scaffolds to control inflammation and facilitate dental pulp regeneration. J Endod. 2014;40:S6–S12.
[16] Huang GT. Pulp and dentin tissue engineering and regeneration: current progress. Regen Med. 2009;4:697–707.
[17] Tran HB, Doan VN. Human dental pulp stem cells cultured onto dentin derived scaffold can regenerate dentin-like tissue in vivo. Cell Tissue Bank. 2015;16:559–568.
[18] Huang GT, Garcia-Godoy F. Missing concepts in de novo pulp regeneration. J Dent Res. 2014;93:717–724.
[19] Struyf E, Conley DJ. Silica: An essential nutrient in wetland biogeochemistry. Front Ecol Environ View. 2009;7:88–94.
[20] Carlisle EM. Silicon as an essential trace element in animal nutrition. In: Evered D, O'Connor M, editors. Silicon biochemistry, CIBA foundation symposium 121. Chichester: Wiley; 1986. p. 123–136.
[21] Van Dyck K, Robberecht H, van Cauwenbergh R, et al. Indication of silicon essentiality in humans. Biol Trace Elem Res. 2000;77:25–32.
[22] Carlisle EM. Silicon: a possible factor in bone calcification. Science. 1970;167:279–280.
[23] Schwarz K, Milne DB. Growth-promoting effects of silicon in rats. Nature. 1972;239(5371):333–334.
[24] Wiens M, Wang XH, Schlossmacher U, et al. Osteogenic potential of biosilica on human osteoblast-like (SaOS-2) cells. Calcif Tissue Int. 2010;87:513–524.
[25] Wang X, Schröder HC, Müller WE. Enzyme-based biosilica and biocalcite: biomaterials for the future in regenerative medicine. Trends Biotechnol. 2014;32:441–447.
[26] Wang X, Schröder HC, Wiens M, et al. Bio-silica and bio-polyphosphate: applications in biomedicine (bone formation). Curr Opin Biotechnol. 2012;23:570–578.
[27] Zhu W, Gao X, Zou X, et al. Biosilica porous microspheres promote the osteogenic/odontogenic differentiation of human dental pulp cells. J Biomater Tiss Eng. 2018;8:258–266.
[28] Malda J, Frondoza CG. Microcarriers in the engineering of cartilage and bone. Trends Biotechnol. 2006;24:299–304.
[29] Wang S, Wang X, Draenert FG, et al. Bioactive and biodegradable silica biomaterial for bone regeneration. Bone. 2014;67:292–304.
[30] Haixia L, Ruyuan Z, Chenyue L, et al. Evaluation of decalcification techniques for rat femurs using HE and immunohistochemical staining. Biomed Res Int. 2017;4:1–6.
[31] Yoko H, Yoshihito N, Ryo J, et al. Bone ingrowth to Ti fibre knit block with high deformability. J Oral Maxillofac Res. 2016;7(4):e2.
[32] Shie MY, Ding SJ. Integrin binding and MAPK signal pathways in primary cell responses to surface chemistry of calcium silicate cements. Biomaterials. 2013;34(28):6589–6606.
[33] Sun J, Wei L, Liu X, et al. Influences of ionic dissolution products of dicalcium silicate coating on osteoblastic proliferation, differentiation and gene expression. Acta Biomater. 2009;5(4):1284–1293.
[34] Karjalainen S, Söderling E, Pelliniemi L, et al. Immunohistochemical localization of types I and III collagen and fibronectin in the dentine of carious human teeth. Arch Oral Biol. 1986;31:801–806.
[35] Andujar MB, Couble P, Couble ML, et al. Differential expression of type I and type III collagen genes during tooth development. Development. 1991;111:691–698.
[36] Garcia JM, Martins MD, Jaeger RG, et al. Immunolocalization of bone extracellular matrix proteins (type I collagen, osteonectin and bone sialoprotein) in human dental pulp and cultured pulp cells. Int Endod J. 2003;36:404–410.
[37] Mao YQ, Ohsaki Y, Kurisu K. Immunohistochemical study of the relationship between extracellular matrix and root bifurcation in the mouse molar. Arch Oral Biol. 1990;35:583–591.