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Research Article

BMP4-Regulated Human Dental Pulp Stromal Cells Promote Pulp-Like Tissue Regeneration in a Decellularized Dental Pulp Matrix Scaffold

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ABSTRACT

Pulp regeneration with stem cells is a promising alternative for treating periapical and pulp diseases of young permanent teeth. The aim of this study was to characterize Decellularized Dental Pulp Extracellular Matrix (dECM) and investigate whether bone morphogenetic protein 4 (BMP4) regulates Dental Pulp Stromal Cells (DPSC)-mediated pulp regeneration combined with dECM. Dental pulp isolated from healthy third molars was decellularized with 10% sodium dodecyl sulfate (SDS) and Triton X-100. H&E staining, DAPI staining and electron microscopy were used to observe the dECM structure. Cell Counting Kit-8 was used to analyse cell proliferation. Recombinant adenovirus was used to overexpress BMP4 in DPSCs. The cells were cultured in dECM and dECM+three-dimensional (3D) Vitrogel systems, and the expression of bone/dentin/angiogenesis markers was evaluated by real-time polymerase chain reaction (RT-PCR) and ALP staining. DPSCs mixed with dECM and BMP4 were transplanted into nude mice to evaluate pulp-like tissue formation. The expression of osteogenic and angioblastic genes was increased, and pulp-like tissue formed in vivo. Thus, dECM promotes DPSC proliferation, and BMP4+dECM accelerates pulp-like tissue formation by DPSCs in vitro.

Keywords: Dental pulp stromal cells; Decellularized dental pulp extracellular matrix; Bone morphogenetic proteins; BMP4; Dental pulp regeneration

INTRODUCTION

The dental pulp is the soft tissue located in the pulp cavity. Healthy dental pulp plays a significant role in the formation, sensation and nutritional function of the dental pulp-dentin complex. Young permanent teeth often suffer dental trauma and pulp and periapical lesions. Small root canals and narrow pulp cavities often result in an insufficient blood supply to the pulp. Therefore, once the pulp is inflamed, unrepairable pulp necrosis usually occurs [1]. The existing clinical treatment methods involve apexification with drugs or surgery to promote root development, which has many shortcomings. The main problems are the lack of coordination of the crown-root ratio, a thick root canal, a thin root wall, the high probability of root fracture, and poor repair of tissue defects around the apex. Pulp regeneration based on stem cells has become a promising alternative approach for the treatment of periapical and pulp diseases to overcome the inherent shortcomings of conventional root canal treatment, such as loss of vitality and brittleness of teeth [1,2].

Stem cells, growth factors and biological scaffolds are critical components of dental pulp regeneration. Recently, an increasing number of studies have shown that Mesenchymal Stem Cells (MSCs) are located at the injury site and exert their therapeutic effects by secreting soluble mediators, including angiogenic and neurotrophic factors [3]. MSCs also promote the migration of bone marrow and vascular endothelial progenitor cells and stem/progenitor cells, enhance angiogenesis and neurogenesis, improve cell survival (anti-apoptosis) and cell recovery and remodel tissue function [3,4]. Scaffolds maintain the regenerative environment and stem cell functions by providing a temporary three-dimensional structure and extracellular matrix (ECM) components and play an essential role in tissue engineering. Many scaffolds are used for pulp regeneration. In dental pulp regeneration studies, the commonly used scaffold materials include natural polymers, hyaluronic acid, chitosan, and polylactic acid (PLA). Platelet-rich fibrin and blood clots also function as scaffolds in pulp regeneration. The main issue is the lack of ECM in the pulp, which limits the ability to form dentin [5,6]. Therefore, a dental pulp regeneration scaffold

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that includes dental pulp ECM must be constructed to improve odontoblast differentiation and the dentin formation ability. Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β superfamily and play important roles in tooth generation, formation, differentiation, and matrix secretion. Currently, more than 20 different BMPs have been isolated and identified, among which BMP4 plays a central role in tooth development [7]. Bmp4 signalling inhibits tooth development inhibitors in the tooth mesenchyme, including Dkk2 and OSR2, activates mesenchymal odontogenesis and promotes tooth morphogenesis [8]. However, few reports have examined the ability of DPSCs to regenerate pulp, and the process induced by BMP4 is unknown; thus, further studies on BMP4 and DPSCs are needed.

In this study, we investigated the structure and function of decellularized human dental pulp extracellular matrix (dECM) and used BMP4 to induce the regeneration of dental pulp-like tissue by the combination of human natural dECM and human DPSCs.

MATERIALS AND METHODS

Cell culture and adenoviral infection

We used clinically healthy third molars from 18- to 22-year-olds that required extraction due to orthodontics or impaction at Stomatological Hospital Affiliated with Chongqing Medical University (China). All patients provided informed consent, and all treatments were approved by the Ethical Review Committee of Chongqing Medical University (2021 Ethics Review No. 008). Dental pulp tissue was digested with collagenase type I (3 mg/ml, Sigma-Aldrich) for 30 minutes at 37°C. Cell suspensions were cultured in T25 culture flasks with 15% foetal bovine serum (FBS, BI), 100 units of penicillin and 100 mg of streptomycin (Beyotime, Shanghai, China) at 37°C in an atmosphere containing 5% CO2. Before using DPSCs for experiments, the freshly isolated cells were assessed for their expression of cell surface markers (CD31, CD73, CD90, CD105, and NESTIN) (Zenbio, China) using flow cytometry [9]. DPSCs were cultured in a 24-well plate at a cell density of approximately 3×105. Recombinant adenoviruses were generated using AdEasy technology as previously described [10]. The Ad-Easy technology was supported by the Molecular Oncology Laboratory of the University of Chicago Medical Center (USA). The coding regions of BMP4 and Green Fluorescent Protein (GFP) were PCR amplified, cloned into an adenoviral shuttle vector and subsequently used to generate recombinant adenoviruses in HEK293 cells. The recombinant DNA experiments were approved by the Safety Committee of Chongqing Medical University (2021 No. 008). After the cells adhered to the plate, a recombinant adenovirus expressing human BMP4 (Ad-BMP4) or green fluorescent protein (Ad-GFP) was pipetted into each well. Polybrene (10 mg/mL) was used to enhance the transduction efficiency of adenoviral infection. Ad-BMP4 was constructed to express GFP as a marker for monitoring infection efficiency [10]. The GFP signals in the infected DPSCs were observed at 48 h after infection. When 30-50% of the cells expressed GFP, the corresponding virus amount was regarded as the optimal infection dose and the relative titre of the amplified virus was determined.

Preparation of the decellularized pulp ECM and cell proliferation assay

Pulp used for decellularization was isolated from the healthy third molars of 18- to 25-year-olds that required extraction due to orthodontics or impaction. The dental pulp tissue was initially treated with heparin in PBS for 15 minutes and then placed in PBS containing 10% sodium dodecyl sulfate (SDS; Sigma-Aldrich) and shaken continuously on a shaker at 25°C for 24 h, with the PBS changed every 8 h. Then, the tissue was soaked in ultrapure water containing 1% Triton X-100 (Sigma-Aldrich) for 24 h. Finally, the decellularized dental pulp tissue was thoroughly washed with PBS for 2 h and then placed in PBS containing 10 mg/ml streptomycin and 10 kU/ml penicillin in PBS. The tissue was incubated under sterile conditions for 12 h. Sections (5-μm-thick) were stained with the nuclear dyes DAPI and haematoxylin and eosin (H&E) (Beyotime, Shanghai, China). When complete nuclei were not visible after H&E and DAPI staining, decellularization was considered successful. After drying, an ultrasonic grinder was used to mix and grind the acellular matrix with PBS, and then DSPCs were inoculated into it. DPSCs were incubated in a 96-well plate. After 1-7 days, 10 μl of the Cell Counting Kit-8 solution (Beyotime, Shanghai, China) were added to every well and incubated for 4 h. The absorbance was measured at 450 nm with a microplate reader (BioTEK, USA).

Scanning electron microscopy analysis

The microstructure of the decellularized dental pulp was studied with a scanning electron microscope (SEM, Hitachi, Japan) at Chongqing Medical University (China). The samples were fixed with 2.5% glutaraldehyde for 2 h at 4°C. After 3 washes with sodium dimethyl arsenide buffer, the sample was incubated with 0.1% PBS for 1 h, dehydrated with a gradient of ethanol solutions, and then incubated with isoamyl acetate. Electron microscopy images were then captured.

Alkaline phosphatase (ALP) Alizarin Red S staining of DPSCs

Five experimental groups were established: PBS+DPSCs, a GFP+DPSCs, dECM+DPSCs, BMP4+DPSCs and BMP4+dECM+DPSCs. Cells were cultured in 24-well plates at a density of approximately 3 × 105. ALP staining of the DPSCs revealed that BMP4 induces the osteogenic/odontogenic differentiation of DPSCs on the 3rd and 5th days. The cells were cultured in the presence of 50 mg/mL ascorbic acid (Sigma-Aldrich) and 10 mM β-glycerophosphate (Sigma-Aldrich). On the 14th day of culture, the cells were collected and stained with Alizarin Red S (Sigma-Aldrich) to determine osteogenic/odontogenic differentiation. The cells were washed twice with PBS and fixed with 0.05% (v/v) glutaraldehyde for 10 minutes at room temperature. After washing with double distilled water, the cells were stained with 2% Alizarin Red S at 37°C for 20 minutes, and the results were recorded under a bright field microscope.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Five experimental groups were established: PBS+DPSCs, GFP+DPSCs, dECM+DPSCs, BMP4+DPSCs and BMP4+dECM+DPSCs. We inoculated BMP4-infected DPSCs and acellular matrix into a 6 cm culture dish and extracted RNA from the target cells on the 3rd, 5th, and 7th days using TRIzol reagent (Takara, Dalian, China) to further evaluate the effect of dECM on the endodontic pulp of DPSCs. For three-dimensional (3D) culture, the 6-well plate was coated with diluted VitroGel solution (Well Bioscience, USA), and a blank dish was used as a control. The mixture (DPSCs with dECM and BMP4) was transferred to a 6-well plate and incubated for 10-15 minutes (for gelation). Medium was added and cells were incubated with 5% CO2 at 37°C [11]. RNA was

extracted from the target cells, and the sample concentration and purity were tested prior to the analysis of osteogenic/dentinogenic gene expression. qRT-PCR was conducted using the Bio-Rad CFX Connect PCR system with SYBR Premix ExTaqTM II kits (Takara, Japan) according to the manufacturer's instructions. The markers ALP, dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP), collagen-I (COL-I), osteopontin (OPN) and the angiogenic differentiation makers vascular endothelial growth factor (VEGF) and oncostatin M (OSM) were analyzed. Target gene expression was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The RT-PCR primers (Table 1) were designed using the Primer3 system.

Table 1: Primer sequences for specific genes associated with osteogenic differentiation.

Gene	Primer sequence (5'-3')
GAPDH	5'AAGCTGTGGACGCTTTGG3'
	5'ATCCAGGGAGCGAGGAAT3'
ALP	5'-CCACGTCTTCACATTTGGTG-3'
	5'-AGACTGCGCCTGGTAGTTGT-3'
DMP1	5'-TGGGGATTATCCTGTGCTCT-3'
	5'-GCTGTCACTGGGGTCTTCAT-3'
OPN	5'-ACACAT ATGATGGCCGAGGTG-3'
	5'-GTGTGAGGTGATGTCCTCGTCTGTA-3'
DSPP	5'- TGGAGACAAGACCTCCAAGAGTGA-3'
	5'-TGCTGGGACCCTTGATTTCTATTC-3'
COL-I	5'- CGGACAGAGCCTTACGTGCC-3'
	5'-CTGCCCGGGTCCGTGG-3'
VEGF	5'- ACGTACTTGCAGATGTGACAAG-3'
	5'-GTGGCGGCCGCTCTA-3'
OSM	5'- AGCAAGCCTCACTTCCTGAG-3'
	5'-GTGGGCTCAGGTATCTCCAG -3'

Ectopic pulp tissue formation in vivo

The use and care of all animals in this study were performed in accordance with the guidelines approved by the ethics committee of Chongqing Medicine University (China). The experimental animals were divided into 5 groups: PBS+DPSCs, GFP+DPSCs, dECM+DPSCs, BMP4+DPSCs and BMP4+dECM+DPSCs. Cells were infected with Ad-BMP4 or Ad-GFP for 36 h and then collected, resuspended in PBS, and mixed with dECM. Then, the cell mixture was injected subcutaneously (1 × 106 cells/injection, 5 sites) into the backs of athymic nude mice (4 animals per group, 4–6 weeks old). Four weeks after implantation, the animals were sacrificed, and the transplantation site was excised, fixed in a neutral paraformaldehyde solution, demineralized with 10% Ethylene Diamine Tetra Acetic acid (EDTA), and embedded in paraffin. The tissue sections of the implanted specimens were stained with H&E for morphogenesis studies.

Statistical analysis

Standard deviations (SDs) were calculated with Microsoft Excel, and statistical significance was analysed with GraphPad Prism 8 software. T-test or one-way ANOVA was used to determine statistical significance. For all quantitative assays, each condition was analyzed 3 times, and the results were repeated in at least 3 independent experiments. $P<0.05$ was considered statistically significant.

RESULTS

Isolation and characterization of human DPSCs

On the 7th day after primary culture, the cells migrated from the edge of the tissue block under an inverted phase contrast microscope; the cells were mainly fibroblasts, most of which were spindle-shaped and stellate. After 14 days of culture, the cells converged and arranged into a typical spiral shape (Figure 1). Cells from passages 3-6 were employed in the study. Successful infection of DPSCs by the adenoviruses was validated by examining GFP (Figure 1). The flow cytometry analysis of the DPSCs showed that the cells were positive for expression of the surface markers CD73 and CD90, and the ratios of positive cells were 98.16% and 99.98%, respectively. In contrast, the cells were negative for the expression of CD31, CD105 and NESTIN.

Morphological observation of decellularized dental pulp

The dental pulp gradually changed from its original light red colour to a translucent milky white after decellularization, and it also became swollen (Figure 2A). H&E and DAPI staining showed that the dECM did not show visible intact cell nuclei (Figure 2A and 2B). Scanning electron microscopy images confirmed the presence of the dECM structure containing porous fibrous collagen in the decellularized tissue (Figure 2C). The cell growth curve showed that dECM had no obvious effect on the proliferation of DPSCs in the short-term culture stage. With the extension of the culture time, cell proliferation accelerated. The quantitative results were also statistically significant. Based on the results of these experiments, dECM supports the improved growth and proliferation of DPSCs without obvious cytotoxicity (Figure 2D).

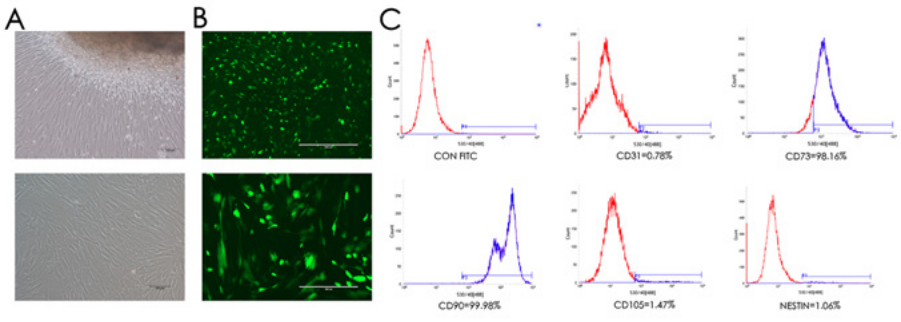

Figure 1: Equation to calculate percent by mass.

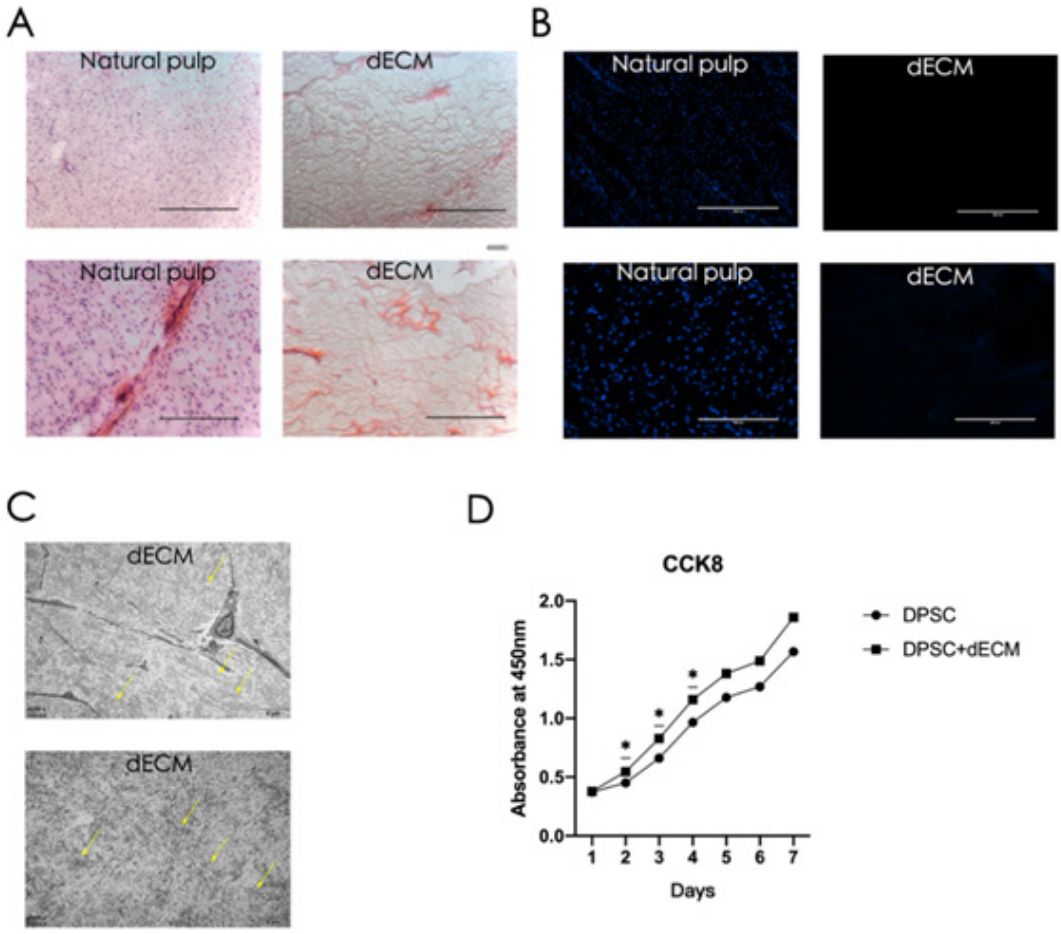

Figure 2: (A) Morphological observation of decellularized dental pulp and cell proliferation assay. H&E staining showed that the native structures of pulp were well preserved in the natural pulp. The dental pulp gradually exhibited a translucent milky white appearance after decellularization. (A-B) H&E and DAPI staining showed that the cell nuclei were well preserved in the natural pulp. After 32 h of decellularization, the pulp did not show visible intact cell nuclei. (C) Scanning electron microscopy images confirmed that the cellular components of the dental pulp were completely removed. (D) Proliferation status of cells in the DPSC and DPSC+dECM groups. From the second day to the fourth day, the cells in the DPSC+dECM group displayed a significantly higher proliferation rate than the cells in the other group; *P < 0 05.

BMP4 induced pulp regeneration in vitro

ALP staining showed more intense staining over time at the 3rd and 5th days after the Ad-BMP4 or Ad-GFP infection of DPSCs. More intense staining was observed in the BMP4 group than in GFP group, and the most intense ALP staining was observed in the BMP4+dECM+DPSC group (Figure 3A). Ad-BMP4- and Ad-GFP-infected DPSCs were seeded in 24-well cell culture plates and maintained in mineralization culture medium for 14 days before being stained with Alizarin Red S. The density and size of the mineralized nodules in each group increased with the addition of BMP4 and dECM (Figure 3B).

Quantitative RT-PCR was performed to analyses gene expression in DPSCs grown on the acellular matrix scaffold. dECM alone did not induce a significant increase in the differentiation of the DPSCs. The ALP, COL, DMP1, DSPP and OPN mRNAs were expressed at significantly higher levels in the BMP4+dECM+DPSC group than in the BMP4+DPSC groups at most time points. Specifically, the expression of ALP, DMP1, and OPN exhibited an increasing trend over time. In contrast, the expression of COL and DSPP showed a decreasing trend over time. Although VEGF was expressed at higher levels in the BMP4+dECM+DPSC group than in the other three groups, the difference was obvious only on day 7 (Figure 4A). When the cells were cultivated in the 3D VitroGel system, the expression of the COL, OPN, VEGF and OSM mRNAs in the BMP4+dECM+DPSC group was higher than that in the other groups at most time points (Figure 4B), consistent with the results obtained when the cells were not incubated in the VitroGel 3D system.

Vascularized pulp-like tissue was formed in vivo

Four weeks after stem cell transplantation, the nude mice were sacrificed by cervical dislocation, and the subcutaneous mass was removed from the injection site. No pulp-like tissue formed in the PBS+DPSC, dECM+DPSC and GFP+DPSC groups, but pulp-like tissue was observed in the dECM+BMP4+DPSC group. H&E staining showed that fibrous connective tissue was formed, fibroblast-like cells were evenly distributed in the tissue, and a small amount of adipose tissue and small blood vessels formed, similar to dental pulp tissue, but no dentin-like tissue was formed (Figure 5). Based on this result, the acellular dental pulp matrix scaffold combined with DPSCs regenerates' vascularized pulp-like tissue in vivo.

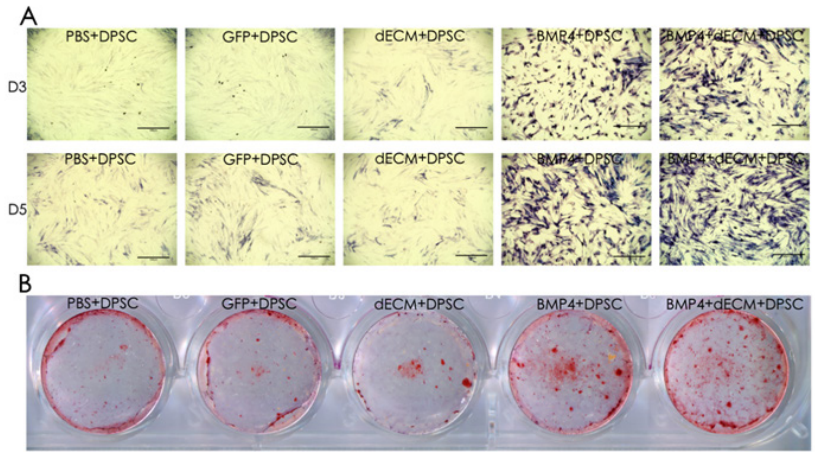

Figure 3: (A) ALP and Alizarin Red S staining of DPSCs. The most intense ALP staining was observed in the BMP4+dECM+DPSC group on the 5th day. (B) Ad-BMP4- and Ad-GFP-infected DPSCs were seeded in 24-well cell culture plates and maintained in mineralization culture medium for 14 days before being stained with Alizarin Red S. The density and size of the mineralized nodules in each group increased with the addition of BMP4 and dECM. Each assay was performed in triplicate.

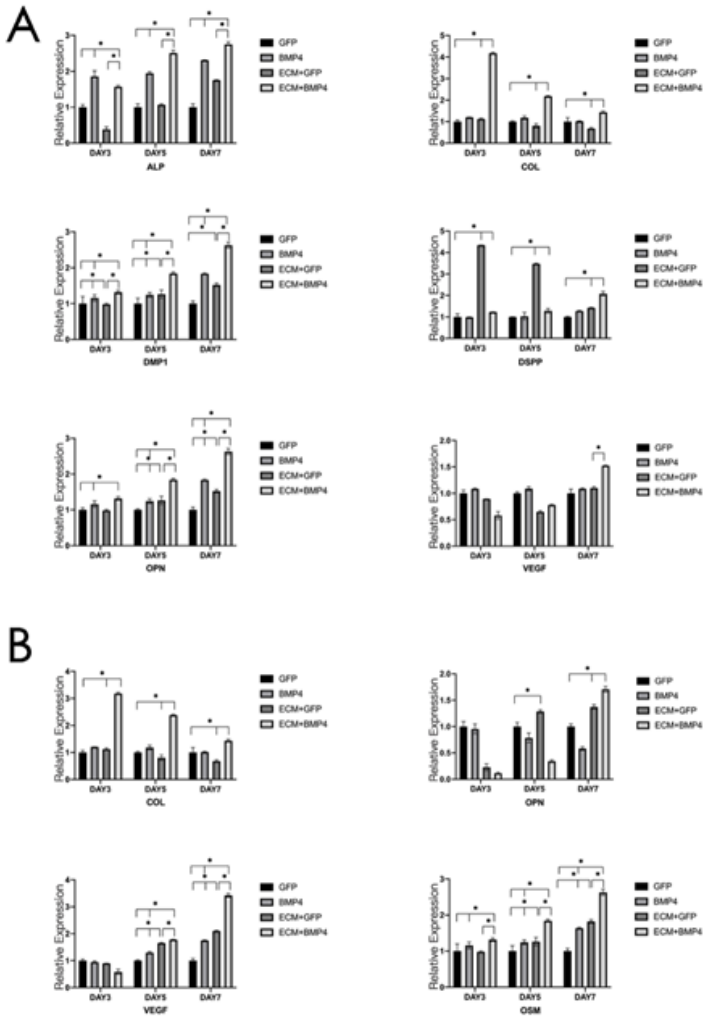

Figure 4: (A) BMP4 induces the osteo-/odonto-/angiogenic differentiation of DPSCs. Quantitative RT-PCR analysis of ALP, COL, DMP1, DSPP, OPN and VEGF expression. (B) The cells were cultivated in the 3D Vitrogel system. Samples were collected at the indicated time points. Quantitative RT-PCR was performed to determine the expression of COL, OPN, VEGF and OSM; *P<0 05.

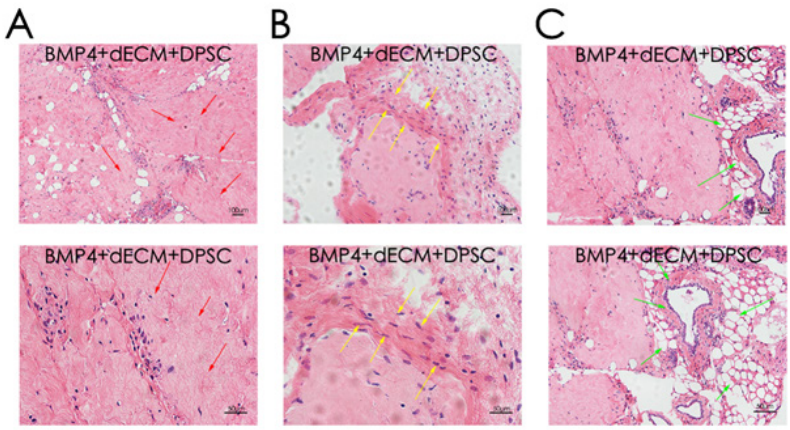

Figure 5: Vascularized pulp-like tissue formed in the BMP4+dECM+DPSC group. H&E staining showed that fibrous connective tissue formed (A, red arrow), fibroblast-like cells were evenly distributed in the tissue (B, yellow arrow), and a small amount of adipose tissue and small blood vessels formed (C, green arrow), similar to dental pulp tissue, but no dentin-like tissue was formed.

DISCUSSION

In this study, the cells isolated from the healthy pulp tissue of young permanent teeth showed the typical phenotype of DPSCs, as verified by their CD73(+), CD90(+), CD31(-), CD105(-) and NESTIN(-) status. We verified the ability of dECM and BMP4 to promote the proliferation and differentiation of DPSCs. After adding dECM to DPSCs, cell proliferation accelerated as the culture time increased. Osteogenesis- and angiogenesis-related genes were expressed in vitro, and when the mixture was implanted in the backs of nude mice, vascularized pulp-like tissue formed.

Human DPSCs derived from the pulp tissue of young and healthy third molars exhibit a strong proliferation activity and high multipotency. DPSCs have the ability to differentiate in the direction of nerves and blood vessels, and even before osteogenic induction, they express certain osteogenic proteins [12]. DPSCs combined with scaffolds regenerate dentin/pulp tissue [13-16]. In the past decade, dECM derived from humans or animals has received extensive attention as an ideal scaffold material for tissues such as the heart, liver, and nerves [2]. Common decellularization methods include physical methods, chemical methods, enzymatic methods, and the combined use of the aforementioned methods [17,18]. SDS is still the most commonly used decellularization reagent. In the present study, the cellular components of dental pulp were completely removed after decellularization, and the acellular pulp presented a porous structure. ECM scaffold constructs generally promote cellular migration, proliferation and revascularization from the surrounding host tissues [19]. Our data showed that dECM improves the growth and proliferation of DPSCs. When dECM combined with human DPSCs and the cytokine BMP4 was transplanted in vivo, the acellular dental pulp scaffold promoted the growth of pulp-like tissue.

ALP, COL and OPN are considered markers of osteoblast and odontoblast differentiation [20,21]. DMP-1 is expressed in odontoblasts, bone and the cementum, and plays an essential role in dentin mineralization [22]. Based on our results, the expression of ALP, DMP1, and OPN exhibited an increasing trend over time. DSPP is expressed at high levels during the process of odontoblastic differentiation and is considered a functional marker of odontoblasts [23]. Previous studies have reported that DSPP is a downstream effector molecule of DMP1 in dentinogenesis [22]. The expression of COL and DSPP was significantly increased in the BMP4+dECM+DPSC group on the 3rd day, indicating that BMP4+dECM stimulated the early differentiation of DPSCs into osteoblasts and odontoblasts. Researchers have confirmed that VEGF plays a role in chemotactic cells, mitosis promotion and angiogenesis, and it is the most powerful angiogenic factor identified to date. The formation of blood vessels is the basis of pulp regeneration. The formation of blood vessels provides a pathway for nutrient metabolism during the regeneration process, which is expected to support the regeneration of pulp tissue. OSM is a member of the IL-6 family that is expressed in monocytes and macrophages. OSM is a pleiotropic growth factor that plays important roles in regulating cell proliferation, differentiation, reconstruction, regeneration and other processes [24]. Compared with the classic angiogenic factors VEGF and FGF2, OSM exhibits stronger cell proliferation and luminal formation capabilities and promotes the chemotaxis of vascular endothelial cells [25-27]. BMP4 alone and BMP4+dECM upregulate the expression of VEGF and OSM in DPSCs. Furthermore, the expression of VEGF and OSM was significantly increased in DPSCs after applying the 3D hydrogel system. In this study, we showed that the use of readily available decellularized dental pulp ECM in the VitroGel 3D hydrogel system provides the structure and function of a natural scaffold and can be used to reconstruct the original microenvironment of dental pulp regeneration. Compared with traditional 2D monolayer cell culture, incubation in the 3D hydrogel system can more precisely simulate the microenvironment in the host organism. Therefore, BMP4+dECM effectively induce angiogenic processes in DPSCs cultured in a 3D scaffold environment. In the external environment, dECM may be more inclined to form soft tissue.

BMPs belong to the TGF-β superfamily and have been shown to induce the formation of bone, dentin, cartilage and connective tissue [8]. Many studies have implicated BMP2, BMP4 and BMP7

in tooth development, and BMP4 is suggested to play a central role in tooth morphogenesis. BMP4 is expressed in the enamel knot, which functions as a signaling center that controls cell proliferation and apoptosis and determines the cusp number and position and subsequent tooth patterning [28,29]. Tooth development depends on epithelial-mesenchymal interactions. BMP4 is expressed at high levels in the epithelium of the early developing tooth germ and then in the mesenchyme, and the transfer of expression is accompanied by the transfer of odontogenic potential. A low concentration of BMP4 was recently shown to stimulate the differentiation of human embryonic stem cells into oral ectoderm, and then, a high concentration of BMP4 caused the oral ectoderm to develop into dental epithelium [29]. BMP4 induces Islet1 gene expression in the incisor mesenchyme and regulates subsequent tooth development [30]. Our results indicate a role for BMP4 in dental pulp formation and regeneration [31].

CONCLUSION

In conclusion, the present study shows the upregulation of the expression of osteo-/odonto-/angiogenic markers in DPSCs upon BMP4 stimulation. In addition, dECM promotes DPSC proliferation, and BMP4+dECM accelerates the differentiation of DPSCs and formation of pulp-like tissue. Based on these results, dECM can be used as a potential scaffold for BMP4-stimulated DPSCs in dental pulp regeneration.

AUTHOR'S CONTRIBUTION

Qin Tan and Yuying Cao performed the experiments. Xiaorong Zheng and Mengtian Peng analyzed the data. Qin Tan wrote the original manuscript. Enyi Huang and Jinhua Wang designed the study and edited the original manuscript. Jinhua Wang supervised the entire project. All authors read and approved the final manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interests.

ETHICAL APPROVAL

Ethical approval was provided by the Ethics Committee of Chongqing Medical University.

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REFERENCES

1. Li J, Rao Z, Zhao Y, Xu Y, Chen L, Shen Z, et al. A Decellularized matrix hydrogel derived from human dental pulp promotes dental pulp stem cell proliferation, migration, and induced multidirectional differentiation in vitro. J Endod. 2020;46:1438-1447.
2. Choung HW, Lee JH, Lee DS, Choung PH, Park JC. The role of preameloblast-conditioned medium in dental pulp regeneration. J Mol Histol.2013;44:715-721.
3. Mathot F, Rbia N, Bishop A, Hovius S, Shin AJM. Adipose derived mesenchymal stem cells seeded onto a decellularized nerve allograft enhances angiogenesis in a rat sciatic nerve defect model. Microsurgery.2020;40:585-592.
4. Li, Q, Wang Z. Influence of mesenchymal stem cells with endothelial progenitor cells in co-culture on osteogenesis and angiogenesis: an in vitro study. Arch Med Res.2013;44:504-513.
5. Hu L, Gao Z, Xu J, Zhu Z, Fan Z, Zhang C, et al. Decellularized swine dental pulp as a bioscaffold for pulp regeneration. Bio Med Research Inter.2017:1-9.
6. Qiao W, Lu L, Wu G, An X, Li D, Guo J. DPSCs seeded in acellular nerve grafts processed by Myroilysin improve nerve regeneration. J Biomater Appl.2018;33:819-833.
7. Reddi AH, Reddi A. Bone morphogenetic proteins (BMPs): From morphogens to metabologens. Cytokine Growth Factor Rev.2009;20:341-342.
8. Jia S, Zhou J, Gao Y, Baek JA, Martin JF, Lan Y, et al. Roles of Bmp4 during tooth morphogenesis and sequential tooth formation. Develop.2013;140:423-432.
9. Ferreira LS, Diniz IMA, Maranduba CMS, Miyagi SPH, Rodrigues MFSD, Moura-Netto C, et al. Short-term evaluation of photobiomodulation therapy on the proliferation and undifferentiated status of dental pulp stem cells. Lasers Med Sci.2019;34:659-666.
10. Huang E, Zhu G, Jiang W, Yang K, Gao Y, Luo Q, et al. Growth hormone synergizes with BMP9 in osteogenic differentiation by activating the JAK/STAT/IGF1 pathway in murine multilineage cells. J Bone Miner Res.2012;27:1566-1575.
11. Xiao M, Qiu J, Kuang R, Zhang B, Wang W, Yu Q, et al. Synergistic effects of stromal cell-derived factor-1α and bone morphogenetic protein-2 treatment on odontogenic differentiation of human stem cells from apical papilla cultured in the VitroGel 3D system. Cell Tissue Res.2019;378:207-220.
12. Pisciotta A, Carnevale G, Meloni S, Riccio M, De Biasi S, Gibellini L, et al. Human dental pulp stem cells (hDPSCs): isolation, enrichment and comparative differentiation of two sub-populations. BMC Dev Biol.2015;15:14.
13. Kaneko T, Gu B, Sone PP, Zaw SYM, Murano H, Zaw ZCT, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod.2008;34:962-969.
14. Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, et al. Stem/progenitor cell–mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A.2009;16:605-615.
15. Huang GT, Al-Habib M, Gauthier P. Challenges of stem cell-based pulp and dentin regeneration: a clinical perspective. Endod Topics.2013;28:51-60.
16. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci.2000;97:13625-13630.
17. Isidan A, Liu S, Li P, Lashmet M, Smith LJ, Hara H, et al. Decellularization methods for developing porcine corneal xenografts and future perspectives. Xenotransplantation.2019;26:53-60.
18. Sesli M, Akbay E, Onur MA. Decellularization of rat adipose tissue, diaphragm, and heart: a comparison of two decellularization methods. Turk J Biol.2018;42:537-547.
19. Tavelli L, McGuire MK, Zucchelli G, Rasperini G, Feinberg SE, Wang HL, et al. Extracellular matrix-based scaffolding technologies for periodontal and peri-implant soft tissue regeneration. J Periodontol.2020;91:17-25.
20. Harris, H. The human alkaline phosphatases: what we know and what we do not know. Clin Chim Acta.1990;186:133-150.
21. Bruderer M, Richards RG, Alini M, Stoddart MJ. Role and regulation of RUNX2 in osteogenesis. Eur Cell Mater.2014;28:269-286.

22. Gibson MP, Zhu Q, Wang S, Liu Q, Liu Y, Wang X, et al. The rescue of dentin matrix protein 1 (DMP1)-deficient tooth defects by the transgenic expression of dentin sialophosphoprotein (DSPP) indicates that DSPP is a downstream effector molecule of DMP1 in dentinogenesis. J Biol Chem.2013;288:7204-7214.

23. Gu S, Liang J, Wang J, Liu B. Histone acetylation regulates osteodifferentiation of hDPSCs via DSPP. Front Biosci (Landmark Ed).2013;18:1072-1079.

24. Chen SH, Benveniste EN. Oncostatin M: a pleiotropic cytokine in the central nervous system. Cytokine Growth Factor Rev.2004;15:379-391.

25. Chakka LRJ, Vislisel J, Vidal CMP, Biz MT, KSalem A, Cavalcanti BN. Application of BMP-2/FGF-2 gene-activated scaffolds for dental pulp capping. Clin Oral Investig.2020;28:704-714.

26. Sun X, Meng L, Qiao W, Yang R, Gao Q, Peng Y. Vascular endothelial growth factor A/Vascular endothelial growth factor receptor 2 axis promotes human dental pulp stem cell migration via the FAK/PI3K/Akt and p38 MAPK signalling pathways. Int Endod J.2019;52:1691-1703.

27. Aksel H, Öztürk Ş, Serper A, Ulubayram K. VEGF/BMP-2 loaded three-dimensional model for enhanced angiogenic and odontogenic potential of dental pulp stem cells. Int Endod J.2018;51:420-430.

28. Zhang YD, Chen Z, Song YQ, Liu C, Chen YP. Making a tooth: growth factors, transcription factors, and stem cells. Cell Res.2005;15:301-316.

29. Cao Y, Tan Q, Li J, Wang J. Bone morphogenetic proteins 2, 6, and 9 differentially regulate the osteogenic differentiation of immortalized preodontoblasts. Braz J Med Biol Res.2020;3:53.

30. Li Q, Zhang S, Sui Y, Fu X, Li Y, Wei S. Sequential stimulation with different concentrations of BMP4 promotes the differentiation of human embryonic stem cells into dental epithelium with potential for tooth formation. Stem Cell Res Ther.2019;10:276.

31. Itsiadis TA, Angeli I, Ames C, Lendahl U, Sharpe PT. Role of Islet1 in the patterning of murine dentition. Development.2003;130:4451-4460.