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Original Article

# Angiogenesis during coronal pulp regeneration using rat dental pulp cells: Neovascularization in rat molars *in vivo* and proangiogenic dental pulp cell-endothelial cell interactions *in vitro*

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## KEYWORDS

Angiogenesis;  
Cell transplantation;  
Coculture;  
Dental pulp  
regeneration;  
Vascular endothelial  
growth factor

**Abstract** *Background/purpose:* Angiogenesis is considered a crucial event for dental pulp regeneration. The purpose of this study was to demonstrate neovascularization during coronal pulp regeneration in rat molars using rat dental pulp cells (rDPCs) and to examine whether rDPC-endothelial cell interactions promote proangiogenic capacity *in vitro*.

*Materials and methods:* Maxillary first molars of Wistar rats ( $n = 42$ ) were pulpotomized and rDPCs isolated from incisors were implanted with a porous poly (L-lactic acid) (PLLA) scaffold and hydrogel (Matrigel). After 3, 7, and 14 days, coronal pulp tissues were examined histologically and by nestin and CD146 immunohistochemistry. rDPCs and rat dermal microvascular endothelial cells (rDMECs) were cocultured for 4 days and vascular endothelial growth factor (VEGF) synthesis and angiogenic factor gene expression were determined by enzyme-linked immunosorbent assays and real-time polymerase chain reaction, respectively. Effects of cocultured medium on tube formation by rDMECs were also evaluated.

*Results:* Implantation of rDPC/PLLA/Matrigel induced coronal pulp regeneration with dentin bridge formation and arrangement of nestin-positive odontoblast-like cells at 14 days. PLLA/Matrigel without rDPCs did not induce pulp regeneration. CD146-positive blood vessels increased in density in the remaining pulp tissues at 3 and 7 days, and in the regenerated pulp tissue at 14 days. rDPC/DMEC coculture significantly promoted VEGF secretion and mRNA

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expression of nuclear factor-kappa B, angiogenic chemokine *CXCL1*, and chemokine receptor *CXCR1*. Cocultured medium significantly promoted tube formation.

**Conclusion:** Coronal pulp regeneration with rDPC/PLLA/Matrigel was accompanied by neovascularization. rDPC-rDMEC interactions may promote angiogenic activity represented by proangiogenic factor upregulation and tube formation *in vitro*.

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## Introduction

Dental pulp is a specialized connective tissue that consists of a combination of cells, which include odontoblasts, fibroblasts, and immunocompetent cells, and rich blood and nerve supplies.<sup>1</sup> This tissue has several protective functions against exogenous stimuli such as reparative dentinogenesis<sup>2</sup> and activation of immunocompetent cells.<sup>3</sup> Pulpless teeth lack such protective functions and conservation of pulp tissue is critical for tooth longevity.<sup>4</sup> Furthermore, the risk of tooth fracture increases in non-vital teeth.<sup>5</sup> Accordingly, pulp/dentin regeneration has been attempted with promising results.<sup>6</sup> In addition to so-called pulp revascularization induced by blood-born stem cells of the apical papilla,<sup>7</sup> pulp regeneration after intracanal transplantation of dental pulp stem cells (DPSCs) has been reported by several animal studies.<sup>8,9</sup> A pilot clinical study of human DPSC transplantation into pulpotomized root canals has reported a relatively high success rate.<sup>10</sup>

Angiogenesis establishes a vascular network that carries oxygen, nutrients, and paracrine signals required for efficient tissue growth<sup>11</sup> and is considered a critical event for regeneration of the dentin-pulp complex.<sup>12</sup> For whole pulp tissue regeneration, the blood supply is provided only from the apical foramen, which can hinder successful outcomes. Thus, as an alternative procedure, coronal pulp regeneration has been investigated in pulpotomized teeth where abundant host blood supply from the remaining pulp tissue is expected.<sup>13–17</sup> Indeed, successful regeneration of coronal pulp tissue has been reported after implantation of bone marrow stem cells (BMSCs) or DPSCs with appropriate scaffolds into pulpotomized rat molars.<sup>14–17</sup> Moreover, coimplantation of rat BMSCs and endothelial cells promotes coronal pulp regeneration concomitant with increased angiogenic factor expression.<sup>14</sup>

Angiogenesis is a complex and dynamic process of sprouting new capillaries from existing blood vessels, which involves coordinated endothelial cell proliferation, invasion, migration, and tube formation.<sup>18</sup> During tumor angiogenesis, vascular endothelial growth factor (VEGF), a potent endothelial cell mitogen, activates prosurvival B-cell lymphoma 2 (Bcl-2) protein via the phosphoinositide 3-kinase/Akt signaling pathway.<sup>19,20</sup> Bcl-2 promotes expression of endothelial cell-derived angiogenic CXC chemokines, such as *CXCL1* and *CXCL8*, through nuclear factor-kappa B (NF- $\kappa$ B).<sup>20</sup> DPSCs cocultured with endothelial cells upregulate expression of proangiogenic factors, such as VEGF, Bcl-2, and *CXCL8*, and blockade of NF- $\kappa$ B activity inhibits the coculture-induced proangiogenic factor upregulation.<sup>21</sup>

The purpose of this study was to test the hypothesis that proangiogenic functions of dental pulp cells (DPCs) play a major role in coronal pulp regeneration. The specific aims were (1) to demonstrate neovascularization during coronal pulp regeneration using isolated rat dental pulp cells (rDPCs) in rat molars and (2) to examine whether rDPC-endothelial cell interactions promote the proangiogenic capacity *in vitro*.

## Materials and methods

All animal experiments were conducted under the approval of the institutional laboratory animal care and use committee of Tokyo Medical and Dental University (approval no. A2019-193A). The experimental procedures mentioned below are summarized in Fig. 1.

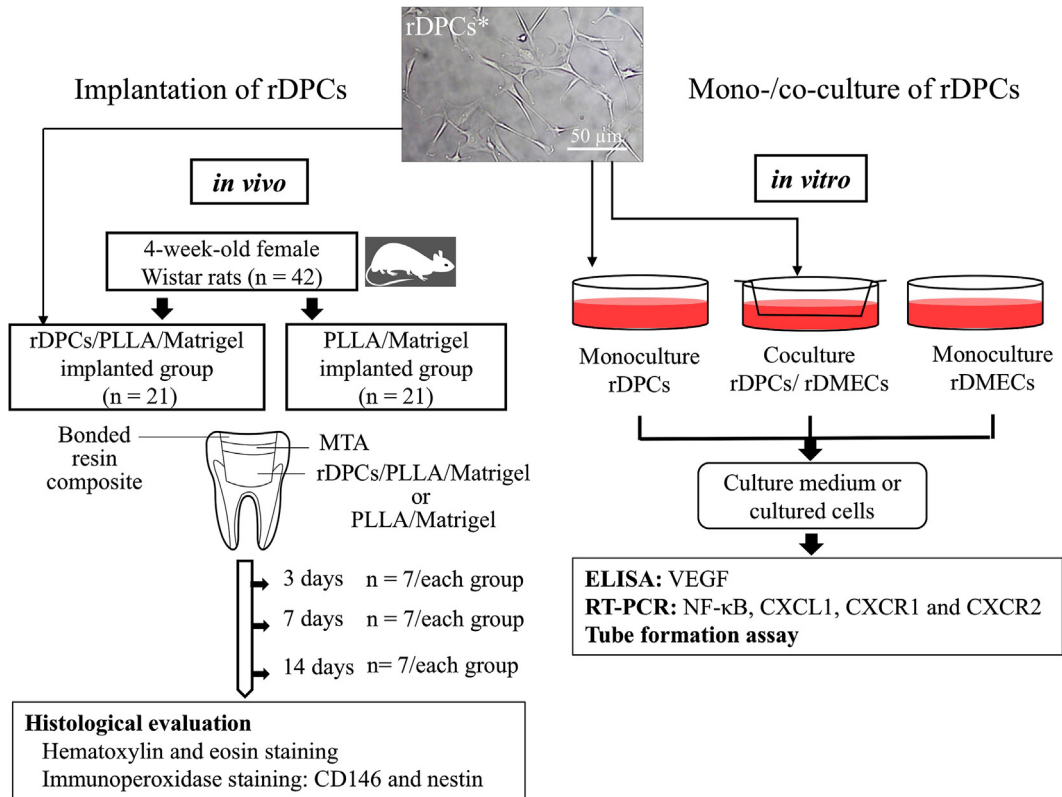
### Cell culture

Minced pulp tissues from upper incisors of 4-week-old female Wistar rats ( $n = 12$ ) were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 medium (Gibco/Thermo, Waltham, MA, USA) supplemented with 15% fetal bovine serum (Gibco/Thermo) and penicillin and streptomycin (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C with 5% CO<sub>2</sub> and 100% relative humidity. Outgrown cells were passaged at 70% confluence and cells at passage 5 were used for experiments. Rat dermal microvascular endothelial cells (rDMECs, RA-6064; Cell Biologics, Chicago, IL, USA) were cultured in endothelial basal medium-2 (Lonza, Basel, Switzerland) supplemented with SingleQuots (Lonza) under the same incubation conditions described above.

### Implantation of rDPCs with a scaffold

rDPCs ( $2 \times 10^5$  cells) in 400  $\mu$ l DMEM/F12 medium were mixed with 400  $\mu$ l Matrigel hydrogel (Corning, Bedford, MA, USA) on ice and 1  $\mu$ l of the rDPC/Matrigel/DMEM/F12 suspension was allowed to absorb into a porous poly (L-lactic acid) (PLLA) scaffold (approximately 0.5 mm<sup>3</sup>; 180  $\mu$ m average pore diameter)<sup>22</sup> in a CO<sub>2</sub> incubator for 30 min.

Four-week-old female Wistar rats ( $n = 42$ ) kept in a specific pathogen-free animal facility were injected intraperitoneally with a mixture of cyclosporine (15 mg/kg; Novartis Pharma, Basel, Switzerland), methylprednisolone (10 mg/kg; Sawai Pharmaceutical, Osaka, Japan), ciprofloxacin (15 mg/kg; Meiji Seika Pharma, Tokyo, Japan), fluconazole (10 mg/kg; Pfizer, New York, NY, USA),



**Figure 1** Flowchart of experimental procedures. rDPCs, rat dental pulp cells. rDMECs, rat dermal microvascular endothelial cells. PLLA, poly (L-lactic acid). \*rDPCs were isolated from pulp tissues of upper incisors of 4-week-old female Wistar rats (n = 12).

subactam/ampicillin (600 mg/kg; Pfizer), and vancomycin (10 mg/kg; Kobayashi Kako, Tokyo, Japan).<sup>14–17</sup> The rats were randomly divided into rDPC/PLLA/Matrigel and PLLA/Matrigel groups (n = 21, each). Right or left maxillary first molars were randomly assigned as experimental teeth and contralateral untreated molars served as normal controls. Under anesthesia induced by 8% chloral hydrate (350 mg/kg, intraperitoneal; Hisamitsu Pharmaceutical, Tokyo, Japan), experimental teeth were subjected to local anesthesia (2% lidocaine with 1:80,000 epinephrine; Astra Pharmaceutical, Worcester, MA, USA; 0.3 ml), followed by disinfection with 2.5% sodium hypochlorite. Cavity preparation and partial pulpotomy were performed with #1/2 (ISO 006) round burs (Dentsply Sirona, Ballaigues, Switzerland), followed by irrigation with 2.5% sodium hypochlorite, 15% EDTA, and phosphate buffered saline. PLLA/Matrigel with or without rDPCs was implanted into the pulpotomized area and the cavity was sealed with mineral trioxide aggregate (white ProRoot MTA; Dentsply Sirona), followed by an adhesively bonded resin composite.

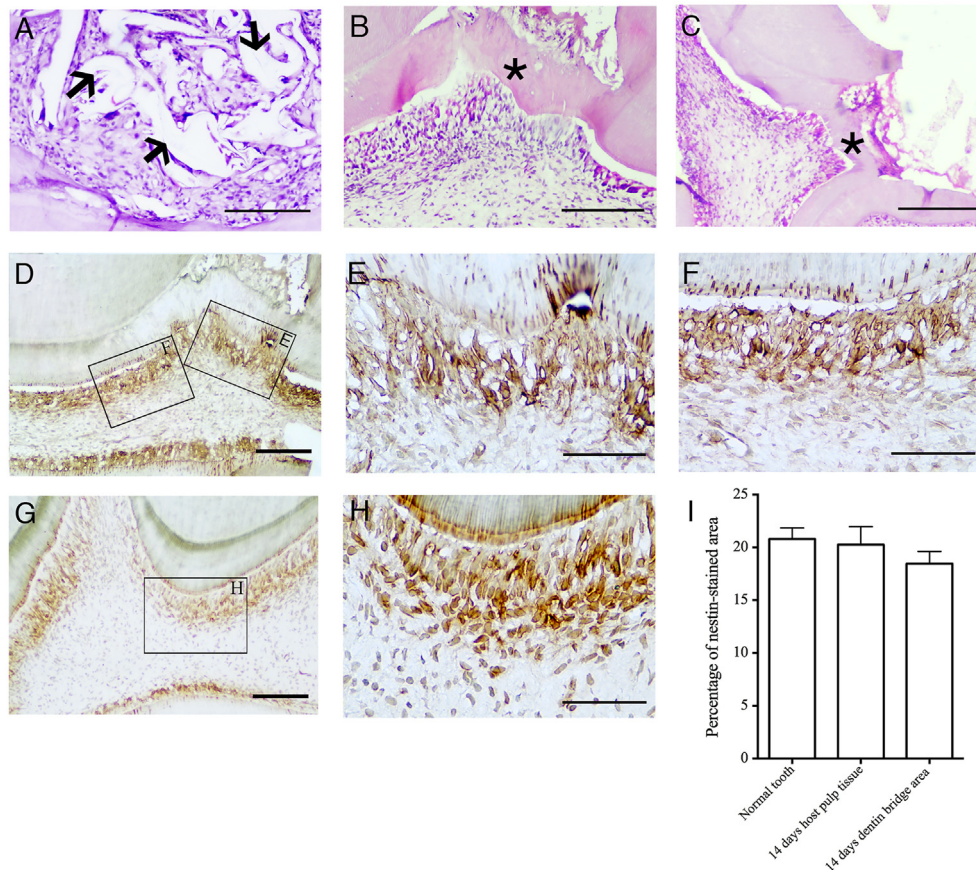
### Immunohistochemistry

The animals were sacrificed at 3, 7, and 14 days (n = 14 each) by transcardiac perfusion with 4% paraformaldehyde and 0.01% glutaraldehyde. Maxillary first molars were retrieved

and immediately immersed in 3% paraformaldehyde at 4 °C for 12–16 h. After demineralization with 10% EDTA, samples were cut into 8- $\mu$ m-thick serial frozen sections and subjected to hematoxylin–eosin staining.

Four teeth from each group were subjected to immunoperoxidase staining. After incubation with 0.3% hydrogen peroxide in methanol and then normal horse serum for 30 min each, sections were incubated with a mouse anti-nestin monoclonal antibody (1:1000, MAB353; Chemicon, Temecula, CA, USA) or mouse anti-CD146 monoclonal antibody (1:400, MAB3250; R&D Systems, Minneapolis, MN, USA) at 4 °C overnight, followed by sequential incubation with biotinylated horse anti-mouse IgG (1:100; rat adsorbed; Vector, Burlingame, CA, USA; 1 h) and avidin-biotin-peroxidase complex (Vector; 30 min) at room temperature and development with 3,3-diaminobenzidine HCl peroxidase substrate (Vector).

For quantitative analysis, three typical sections that contained the center or near center of the pulp tissue were chosen for each specimen and images of the remaining pulp tissue adjacent to the implanted area and regenerated pulp tissue (only rDPC/PLLA/Matrigel group at 14 days) were obtained under a light microscope with a 40 $\times$  objective lens (Nikon, Tokyo, Japan). Pixels of the positively stained area and the whole area in each image were measured by ImageJ software (Version 1.37v; NIH, Bethesda, MD, USA) and their ratio was expressed as a percentage.



**Figure 2** (A–C) Coronal pulp regeneration in rat molars after implantation of rDPC/PLLA/Matrigel or PLLA/Matrigel. Hematoxylin and eosin staining. (A) Three days after rDPC/PLLA/Matrigel implantation showing the PLLA scaffold (arrows) remaining in the implanted area. (B) Fourteen days after rDPC/PLLA/Matrigel implantation showing regenerated pulp-like tissue accompanied by a complete dentin bridge (\*). (C) Fourteen days after PLLA/Matrigel implantation. No tissue regeneration was observed and a dentin bridge (\*) had formed along the border of the remaining pulp tissue. Scale bars: 40  $\mu\text{m}$ . (D–H) Nestin immunoreactivity in rDPC/PLLA/Matrigel-implanted and normal rat molar pulp tissue. Immunoperoxidase staining. (D–F) Fourteen days after rDPC/PLLA/Matrigel implantation showing nestin-immunoreactive odontoblast-like cells in the dentin bridge area (D, E) and remaining host pulp tissue (F). (G, H) Nestin-immunoreactive odontoblast-like cells in the periphery of normal pulp. Scale bars: 200  $\mu\text{m}$  (D, G) and 40  $\mu\text{m}$  (E, F, H). (I) Quantitative analysis of the nestin-stained area. The percentage of nestin-stained area in the dentin bridge area at 14 days was not significantly different from that in the host pulp tissue ( $P > 0.05$ ; paired  $t$ -test) and corresponding area of the normal pulp ( $P > 0.05$ ; Student's  $t$ -test;  $n = 4$ ).

### Coculture of rDPCs and rDMECs

rDPCs ( $1 \times 10^5$  cells/well) and rDMECs ( $1 \times 10^5$  cells/well) were seeded in six-well plates and hanging cell culture inserts (0.4  $\mu\text{m}$  pore size, Millicell; EMD Millipore, Billerica, MA, USA), respectively, and cultured in DMEM/F12 medium with 15% fetal bovine serum for 4 days. For monocultures, cells ( $1 \times 10^5$ /well) were seeded in six-well plates. VEGF in the medium was measured with an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems), following the manufacturer's instructions.

Real-time reverse transcription polymerase chain reaction (RT-PCR) was carried out to measure mRNA expression of angiogenesis-related molecules, namely *NF- $\kappa$ B*, *CXCL1* (angiogenic chemokine), and chemokine receptors *CXCR1* and *CXCR2*. Total RNA was extracted with Nucleospin RNA Plus (Macherey-Nagel, Düren, Germany) and first-strand

cDNA was synthesized from the extracted RNA (10 ng) with TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA, USA). The probe and primer sets for TaqMan gene expression assays (Applied Biosystems) were *Nfkb*; Rn01399572\_m1, *Cxcl1*: Rn01413936\_g1, *Cxcr1*; Rn00570857\_s1, *Cxcr2*; Rn02130551\_s1, glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*); Rn01775763\_g1. Real-time PCR was conducted with the StepOne Sequence Detection System (Applied Biosystems). Target gene expression was normalized to the housekeeping gene *Gapdh*. Relative gene expression values were calculated by  $\Delta\Delta\text{CT}$ -based fold-change calculations.

### Tube formation assay

rDMECs ( $5 \times 10^3$  cells/well) seeded on six-well Matrigel-coated plates (BioCoat Matrigel multiwell plate; Applied



Biosystems) were cultured in conditioned media from rDMEC monocultures or rDPC/rDMEC cocultures for 4 days. Images were then obtained from 10 randomly selected areas in each well under a light microscope with a  $10\times$  objective lens (Nikon) and the total tube length, total branching points, and total number of tubes were quantified by ImageJ software (version 1.37v; National Institutes of Health, Bethesda, MD, USA).

### Statistical analysis

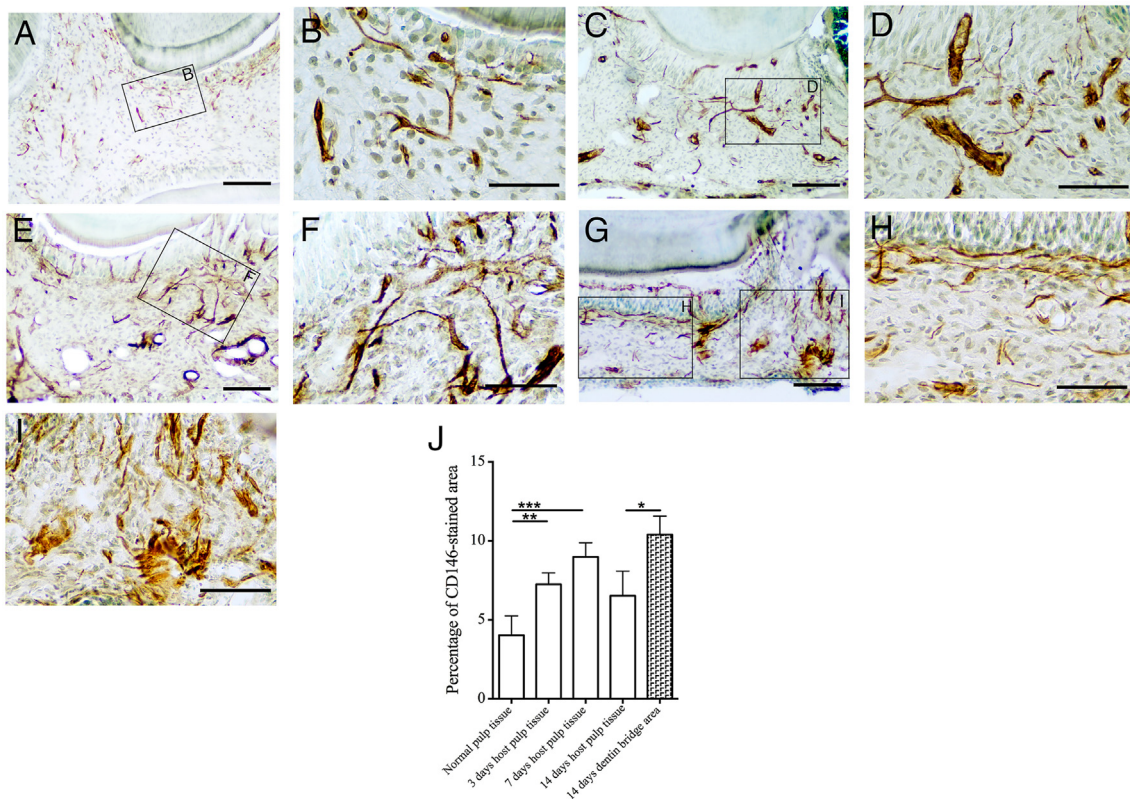
Kruskal-Wallis nonparametric analysis of variance (ANOVA) followed by the Mann-Whitney *U*-test with Bonferroni correction or one-way ANOVA followed by the Tukey-Kramer test were used for multiple group comparisons. The paired *t*-test and Student's *t*-test were used for comparisons of dependent and independent values, respectively. All calculations were performed using SPSS 19.0 software (IBM, Armonk, NY, USA). A *p*-value of  $<0.05$  was considered statistically significant.

## Results

### Coronal pulp regeneration after implantation of rDPCs

In the rDPC/PLLA/Matrigel group, the implanted area was filled with unresolved scaffolds at 3 days (Fig. 2A), whereas resorption of scaffolds had progressed over time and, at 14 days (Fig. 2B), the pulpotomized area was filled with a pulp-like tissue. The pulp exposure site had closed completely with a reparative dentin-like mineralized tissue (dentin bridge). No pulp-like tissue regeneration was observed in the implanted area of the PLLA/Matrigel group at 14 days (Fig. 2C).

Nestin-positive odontoblast-like cells were aligned beneath the dentin bridge and original dentin in the rDPC/PLLA/Matrigel group at 14 days (Fig. 2D–F). Similar alignment of these cells was also seen in normal pulp tissue (Fig. 2G and H). The Nestin-stained area beneath the dentin bridge (dentin bridge area) was not significantly different



**Figure 3** CD146-expressing blood vessels in rDPC/PLLA/Matrigel-implanted and normal rat molar pulp tissue. (A, B) Normal pulp. (C–F) Remaining host pulp at 3 days (C, D) and 7 days (E, F) after rDPC/PLLA/Matrigel implantation. (G–I) Fourteen days after rDPC/PLLA/Matrigel implantation showing the dentin bridge area (G, I) and remaining host pulp tissue (G, H). Immunoperoxidase staining. Scale bars, 200 μm (A, C, E, G) and 40 μm (B, D, F, H, I). (J) Quantitative analysis of the CD146-stained area. The percentage of the CD146-stained area at 3 and 7 days in the remaining host pulp tissue was significantly higher than that in normal tissue.  $**P < 0.01$  and  $***P < 0.001$  (one-way analysis of variance followed by the Tukey-Kramer test). At 14 days, the percentage of the CD146-stained area in the dentin bridge area was significantly higher than that in the remaining host pulp tissue.  $*P < 0.05$  (paired *t*-test;  $n = 4$ ).

from that beneath the original dentin (remaining host pulp tissue) and the corresponding area of normal teeth ( $P > 0.05$ ; Fig. 2I).

CD146-positive blood vessels were abundantly observed in normal pulp tissues, particularly under the odontoblastic layer (Fig. 3A and B). In the rDPC/PLLA/Matrigel group, the CD146-stained area was significantly increased in the remaining host pulp tissues at 3 and 7 days ( $P < 0.01$  and  $P < 0.001$ ; Fig. 3C–F, J). Pulp-like tissue in the dentin bridge area at 14 days had a significantly larger CD146-stained area than the remaining host pulp tissue at 14 days ( $P < 0.05$ ; Fig. 3G–J).

### rDPC/rDMEC coculture promotes angiogenic factor expression and tube formation

Under monoculture conditions, rDMECs synthesized a larger amount of VEGF than rDPCs ( $P < 0.05$ ; Fig. 4A). rDPC/rDMEC coculture resulted in a significantly higher level of VEGF compared with monocultured rDPCs and rDMECs ( $P < 0.01$ , Fig. 4A).

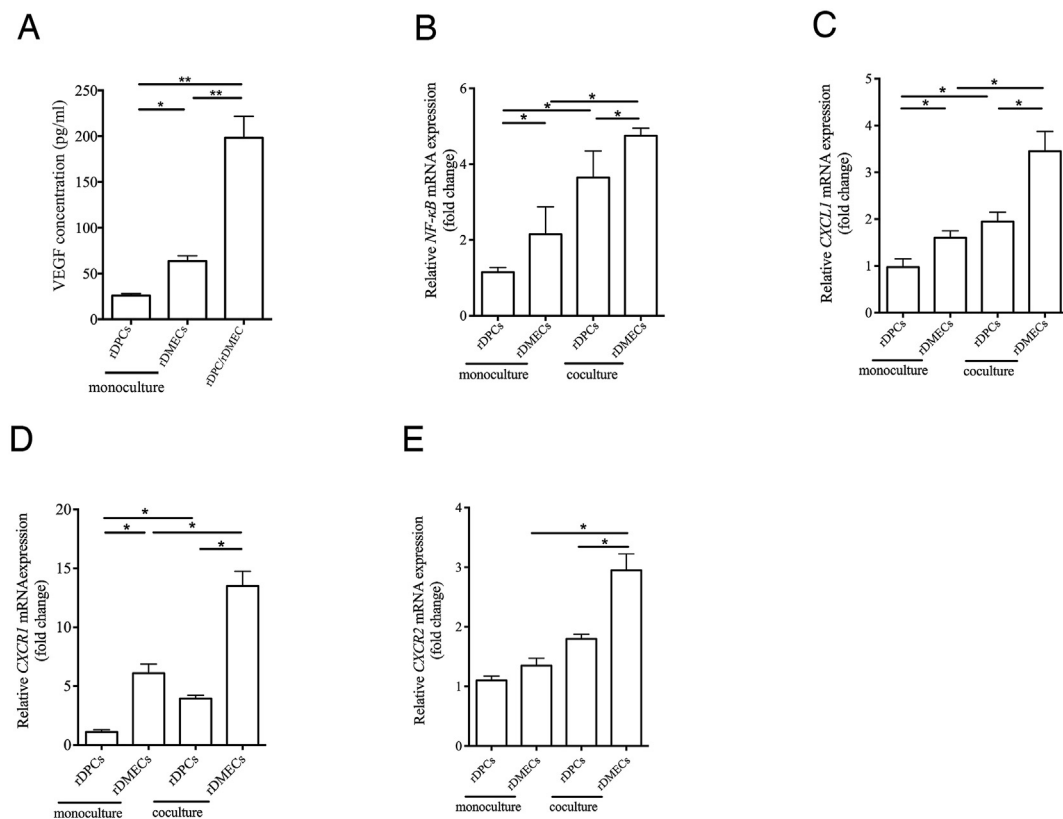
mRNA expression of *NF- $\kappa$ B*, *CXCL1* and *CXCR1* in monocultured rDMECs was significantly higher than that in monocultured rDPCs ( $P < 0.05$ , Fig. 4B–D). rDPC/rDMEC coculture induced significantly higher *NF- $\kappa$ B*, *CXCL1*, and *CXCR1* mRNA expression in both cell types compared with

the corresponding monocultures ( $P < 0.05$ , Fig. 4B–D). *CXCR2* mRNA levels were not significantly different between cocultured and monocultured rDPCs ( $P > 0.05$ , Fig. 4E).

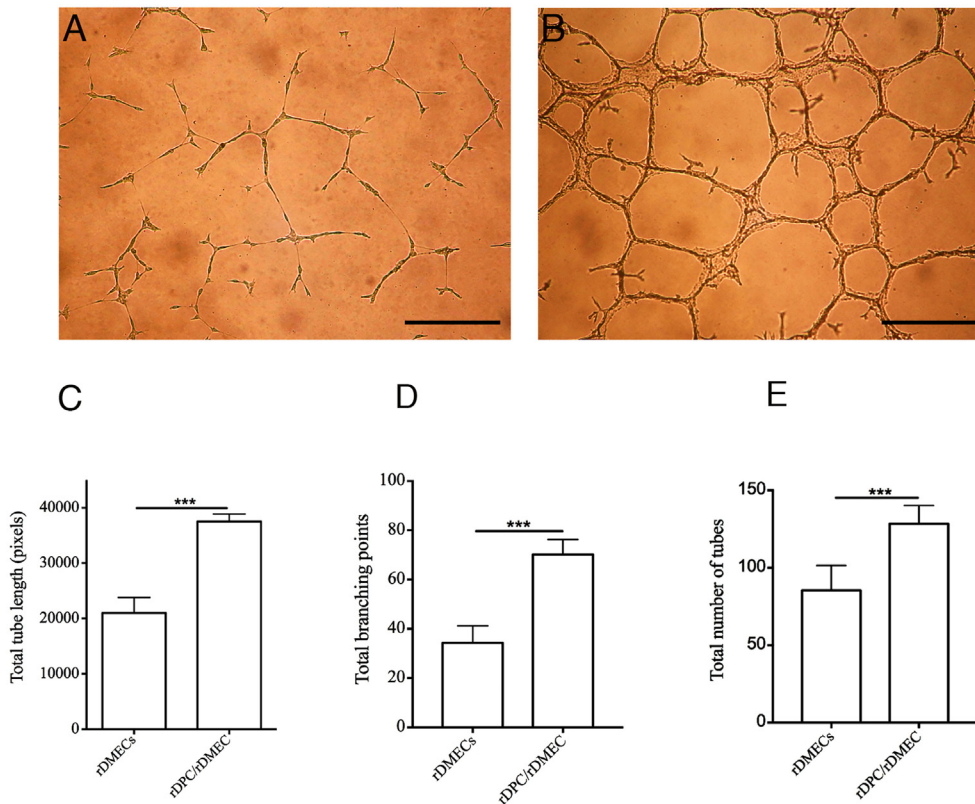
As shown in Fig. 5, rDPC/rDMEC cocultured medium induced more capillary-like tubes and yielded significantly higher values of the total tube length, total branching points, and total number of tubes ( $P < 0.001$ ) than the monocultured medium of rDMECs.

### Discussion

In this study, implantation of rDPC/PLLA/Matrigel into pulpotomized rat molars induced regeneration of pulp-like tissue accompanied by a dentin bridge and nestin-expressing odontoblast-like cells at 14 days (Fig. 2B, D, E). This is in line with studies that show successful coronal pulp regeneration after implantation of rat BMSCs (rBMSCs) with PLLA/Matrigel in rat molars.<sup>14–17,23</sup> This also indicates regeneration of functional odontoblast-like cells because nestin is a marker of odontoblasts that are mature enough to secrete dentin matrix.<sup>24</sup> Transplantation of rDPCs alone was sufficient to promote complete dentine bridge formation, which was in contrast to a previous study in which rBMSC/rDMEC co-transplantation accelerated regeneration and was thus necessary to form a complete dentin bridge.<sup>14</sup>



**Figure 4** (A) VEGF synthesis from rDPCs and rDMECs mono- or co-cultured for 4 days measured by an ELISA. \* $P < 0.05$  and \*\* $P < 0.01$  (one-way analysis of variance followed by the Tukey–Kramer test;  $n = 5$ ). (B–E) Gene expression of angiogenic factors in mono-/co-cultured rDPCs and rDMECs. RT-PCR was used to determine the mRNA expression levels of *NF- $\kappa$ B* (B), *CXCL1* (C), *CXCR1* (D), and *CXCR2* (E) in mono- or co-cultured rDPCs and rDMECs. \* $P < 0.05$  (Kruskal–Wallis nonparametric analysis of variance followed by the Mann–Whitney U-test with the Bonferroni correction;  $n = 5$ ).



**Figure 5** (A, B) Tube formation of rDMECs cultured in the medium of monocultured rDMECs (A) or cocultured rDPC/rDMEC (B) on Matrigel-coated dishes for 4 days. Scale bars, 50  $\mu$ m. (C–E) Quantitative analysis of the total tube length (C), total branching points (D), and total number of tubes (E) in 10 randomly selected areas. \*\*\* $P < 0.001$  (Student's  $t$ -test,  $n = 3$ ).

Collectively, the present results support the notion that rat incisor pulp tissue is a useful source of mesenchymal stem cells<sup>25</sup> and that the rDPCs used in this study possessed more potent functions to induce dentin bridge formation than rBMSCs.

Angiogenesis is essential for tissue regeneration because rapid establishment of oxygen diffusion and nutrient supply is necessary for survival of transplanted cells and further tissue regeneration.<sup>26</sup> Although CD146 is regarded as a mesenchymal stem cell marker,<sup>27</sup> it was originally identified as an endothelial biomarker for angiogenesis,<sup>28</sup> which is constitutively expressed on endothelial cells, smooth muscle cells, and pericytes.<sup>29</sup> CD146 plays an important role in vascular development and remodeling into functional tubes.<sup>30</sup> The present study revealed an increase of the CD146-stained area in remaining pulp tissues and regenerated tissue (Fig. 3), which indicated that rDPC/PLLA/Matrigel implantation promoted neovascularization in both of these tissues. The lack of pulp tissue regeneration in the PLLA/Matrigel group suggested that the transplanted rDPCs played a decisive role in stimulating angiogenesis and subsequent tissue formation.

DPSCs secrete paracrine factors, such as VEGF, angiopoietin-1, and platelet-derived growth factor, which alter the host environment to promote vascularization via local endothelial cells.<sup>31</sup> VEGF regulates both vasculogenesis and angiogenesis prominently<sup>32</sup> and stimulation by VEGF results in various distinct responses during neovascularization.<sup>33</sup>

In this study, rDPC/rDMEC coculture produced a significantly higher amount of VEGF than monocultures (Fig. 4A). This is consistent with previous studies showing that coculture of endothelial cells and stem cells from human exfoliated deciduous teeth (SHEDs) enhances VEGF synthesis compared with monocultures<sup>21,34</sup> and further indicates that rDPCs possess equivalent functions to SHEDs for stimulating endothelial cells to promote VEGF synthesis. Moreover, the rDPC/rDMEC coculture medium significantly promoted tube formation compared with medium from monocultured rDMECs (Fig. 5), which may be attributable to the higher concentration of VEGF in the cocultured medium. It is tempting to speculate that such interactions between transplanted rDPCs and host vascular endothelial cells have significant implications in the process of coronal pulp regeneration, although further investigation is required.

Regarding proangiogenic chemokine expression in stem cells, SHED-DMEC crosstalk has been reported to promote CXCL8 mRNA expression in an NF- $\kappa$ B-dependent manner.<sup>21</sup> In human tumor angiogenesis, VEGF secreted from tumor cells upregulates Bcl-2 in tumor-associated endothelial cells via VEGF receptor 2 (VEGFR2) and Bcl-2 in turn activates NF- $\kappa$ B through mechanisms dependent on I $\kappa$ B kinase  $\beta$  activity, which subsequently promotes expression of CXCL8 and CXCL1 in endothelial cells.<sup>20</sup> CXCL1 binds specifically to CXCR2, which in turn leads to VEGF upregulation by STAT3 phosphorylation.<sup>35</sup> Binding of CXCL8 to CXCR2 increases VEGF mRNA and protein levels in endothelial cells by



autocrine activation of VEGFR2<sup>33</sup> and binding of CXCL8 to CXCR1 modulates endothelial cell anti-apoptotic pathways and regulates angiogenesis.<sup>36</sup>

In this study, coculture promoted *NF-κB*, *CXCL1*, and *CXCR1* mRNA expression in rDPCs and rDMECs compared with the corresponding monocultured cells (Fig. 4B–E). This is not contradictory to the aforementioned previous findings, although it is important to remember that rats lack a CXCL8 peptide homologue.<sup>37</sup> Moreover, *CXCR2* mRNA expression was not significantly different between monocultured and cocultured rDPCs (Fig. 4E). This suggests that CXCR1 played a more essential role than CXCR2 in the higher VEGF secretion of cocultured rDPCs (Fig. 4A).

In conclusion, the present study showed that coronal pulp regeneration with rDPC/PLLA/Matrigel was accompanied by neovascularization and that rDPC-rDMEC interactions may promote angiogenic activity represented by proangiogenic factor upregulation and tube formation *in vitro*. These findings suggest that interactions between transplanted rDPCs and host capillary endothelial cells play some essential roles in the process of successful coronal pulp regeneration.

## Declaration of competing interest

The authors declare that they have no conflict of interest.

## Acknowledgments

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