Submitted: 24 January, 2024 Accepted: 09 April, 2024 Published: 03 March, 2025 DOI:10.22514/jocpd.2025.034

ORIGINAL RESEARCH

Journal of Clinical Pediatric Dentistry

The effect of Matrigel in deciduous tooth pulp transplantation: an *in vitro* study

Jiangjingjun Xu[1], Yanni Jing[1], Yan Huang[1,*]

[1]The Affiliated Stomatological Hospital, Jiangxi Medical College, Nanchang University, Jiangxi Province Key Laboratory of Oral Biomedicine, Jiangxi Province Clinical Research Center for Oral Diseases, 330000 Nanchang, Jiangxi, China

***Correspondence**
ndfskqyy152@ncu.edu.cn
(Yan Huang)

Abstract

Background: Pulp regeneration in immature permanent teeth depends on whether deciduous teeth's pulp can establish early blood supply connections with surrounding tissues after transplantation. This study aimed to explore the effect of Matrigel on early blood supply establishment after transplantation. **Methods**: Empty root canals of permanent teeth were prepared and divided into 3 groups (n = 18 each). Group A (Deciduous Pulp group): The pulp of deciduous teeth was extracted, transplanted into an empty root canal, and then implanted subcutaneously into nude mice. Deciduous pulp/Matrigel group as Group B, empty root group as Group C. **Results**: Histochemical and immunohistochemical examinations were performed 8 weeks after implantation. Both Groups A and B had pulp tissue and fibrous connective tissue interwoven within the root canals. Immunohistochemical staining revealed that human platelet endothelial cell adhesion molecule-1 (CD31) positive cells were scattered over pulp tissue area, while mice CD31 positive cells were scattered over connective tissue area. Meanwhile, human Nestin immunohistochemistry was positive, and positive cells were distributed in pulp tissue. The deciduous tooth pulp/Matrigel group presented significantly higher microvascular count and optical density of nerve fibers ($p < 0.05$). **Conclusions**: This study indicated that that Matrigel can promote primary teeth pulp viability after transplantation.

Keywords

Matrigel; Dental pulp transplantation; Pulp regeneration

1. Introduction

Pulp and periapical tissue regeneration in immature permanent teeth can be promoted mainly by apexification and pulp revascularization [1, 2]. However, the former is primarily aimed at enhancing hard tissue development in immature permanent teeth. The prognosis of affected teeth varies depending on the developmental state of the root and the degree of lesions [3, 4]. Consequently, apexification falls short of restoring the physiological structure and long-term function of roots [5]. During pulp revascularization, strict disinfection is used to control inflammation, apical blood extraction is stimulated, and a tight crown seal is performed. Animal studies show pulp revascularization can cause calcification in the root and complicate treatment after reinfection with newly formed organisms similar to periodontal tissue [6–8]. The use of tissue bioengineering techniques to implant dental stem cells into root canals of immature permanent teeth has increased in recent years [9–13]. Despite some success in restoring pulp tissue, this approach has mostly resulted in fragmented regeneration. To regenerate the real pulp of immature permanent teeth, we proposed transplanting deciduous teeth pulp directly into root canals. We observed odontoblast-like cells generated after autotransplantation of deciduous tooth pulp into a Beagle model of immature permanent tooth periapical inflammation in our preliminary study [14]. However, the establishment of early blood supply is a prerequisite for deciduous teeth pulp survival and function. During the early stage of transplantation, the pulp tissue of primary teeth lacks connections to surrounding tissues, and the transient ischemic and hypoxic environment will influence stem cell activity in the pulp tissue. So, we aim to find a material that promotes the establishment of blood supply to pulp tissue in deciduous teeth after transplantation. Using Matrigel, a soluble proteoglycan substrate of the basement membrane, endothelial cells can be cultured to form interconnected, capillary-like structures that mimic basement membrane characteristics [15–17]. Therefore, in this study, we tested the effect of Matrigel on pulp blood supply in deciduous teeth after transplantation by using an ectopic transplantation model of nude mice.

2. Materials and methods

2.1 Experimental animals

Six-week-old female nude mice were provided by Nanchang Leyou Biotechnology Co., LTD. (Animal qualification certifi-

cate No: SYXK (Gan) 2017-0002). Each nude mouse weighed 20–25 g (22.16 ± 1.39).

2.2 Acquisition of *in vitro* teeth

Eighteen mandibular premolars for orthodontic extraction at the Affiliated Stomatological Hospital of Nanchang University from May to August 2021 were collected. Consent forms were signed by parent or legal guardian of patients. Patients are required to be 12–15 years old and free of odontogenic or periodontal diseases. Based on the Cone Beam Computed Tomography (CBCT) analysis, the extracted premolars had single canals and roots at least 10 mm long.

2.3 Acquisition of deciduous teeth

We selected children from May to August 2021 who had retained deciduous teeth that needed to be extracted. 12 qualified deciduous anterior teeth were collected. Extracted primary teeth should have healthy dental and periodontal tissues, root resorption less than 1/2, and tooth mobility less than II°.

2.4 Preparation of dental cavities

In vitro teeth were scraped with a surgical blade to remove residual periodontal tissue on the root surface. Crowns were removed from the enamel cementum boundary along the dental long axis with a fissure bur (MANI, Japan). We cut off a small amount of the root tip in order to establish an apical foramen, which was about 2 mm wide, and we prepared the root to be about 8 mm long. With a pull-out needle, the dental pulp was completely removed and prepared to 35# with a nickel titanium file (M3, United Dental Group, Jiangsu, China). For irrigation, 17% Ethylene Diamine Tetraacetic Acid (EDTA, 03690, Sigm, St. Louis, Missouri, USA) solution and 5.25% sodium hypochlorite (NaOCl, TMD, Beijing, China) solution were alternated. Once the dental cavity had been prepared, it was rinsed 2–3 times with phosphate buffered saline (PBS, Biorebo, Beijing, China), followed by ultrasonic oscillation with 5.25% NaOCl solution for 10 min, ultrasonic oscillation with PBS for 10 min, and immersion in 17% EDTA for 12 h. Shock the deionized water for 30 min. Finally, the prepared dental cavity tube was soaked in sterile triple antibiotic solution (Solebault Technologies, China), and stored at a sterile low temperature of 4 °C (Fig. 1).

2.5 Pulp extraction from deciduous teeth

To prepare for the procedure, participants were gargled with 0.2% chlorhexidine solution (Bond, Jiangsu, China) for 60 s, disinfected the operative area with 0.1% iodophor (20172140782, HYNAUT, Qingdao, Shandong, China), and underwent a complete extraction of residual deciduous teeth under local anesthesia. A dental diamond bur is used to longitudinal grind and incise the proximal and distal wall of the tooth root after deciduous teeth are extracted. Pulp tissue was excavated quickly using a small, sharp excavator to minimize damage. Pulp sample was quickly placed in the sterile triple antibiotic solution, sealed, and transported to the laboratory at 4 °C. The time it takes to extract the retained deciduous tooth and transport the pulp to the laboratory should not exceed 30 min (Fig. 2).

2.6 Experimental method

The experiment was divided into three groups, control group, treatment group and negative control group.

Group A represents the control group that only consists of deciduous pulp. Deciduous teeth pulp was gently placed in the dental cavity, ensuring that it exceeded the apical foramina. Crown root canals were sealed with mineral trioxide aggregate (MTA, Dentsply TulsaDental, Tulsa, OK, USA) rebase and glass ionomer cement.

Group B represents the treatment group comprises deciduous pulp supplemented with Matrigel (356234, Corning Co., Corning, NY, USA). Matrigel was thawed at 4 °C. Matrigel liquid was injected into the dental cavity. Deciduous teeth pulp was fully covered by Matrigel liquid, and gently placed in the dental cavity. After ensuring pulp exceeds apical foramina, MTA rebase and glass ionomer cement are used to close the crown root canal opening. The pulp was left to gel at room temperature.

Group C serves as a negative control, contains an empty root canal without deciduous pulp. Dental cavity treatment was not performed. Crown root canals were sealed with MTA rebase and glass ionomer cement.

Continuous inhalation anesthesia was administered to nude mice and they were fixed to an operating plate after muscle tension was weakened. During the operation, the condition of nude mice was always noted. Nude mice were incised longitudinally about 1 cm long on the shoulder blade of one side of their backs using sterile iodophor cotton balls disinfection on a super-clean bench (Suzhou Antai Air Technology Co., LTD., China). The subcutaneous tissue was bluntly separated, a dental cavity was implanted, the suture was closely sutured, and the suture was disinfected again with iodophor cotton balls. All treated nude mice had their ears tagged. Nude mice were still kept at Nanchang Leyou Biotechnology Co., LTD. after the operation. They were not given any special treatment before or after the experiment, nor was the feeding environment modified. To ensure a constant sample size for each group, samples were supplemented whenever possible during experiment. This was to prevent accidental mortality of nude mice during surgery or feeding. Make sure each sample lives under the skin of a nude mice for 8 weeks. This study was approved by the Ethics Committee of Nanchang Royo Biotech Co,. Ltd. (IACUC Issue No: RYE2021032601).

2.7 Postoperative operation, histological and immunohistochemical analysis

8 weeks after the operation, nude mice were killed and the tooth root was detached. The sample was then placed in 10% neutral-buffered formalin for 48 h, decalcified with 17% EDTA, and the demineralized solution was changed every 24 h. After 3 months of demineralization, sections were washed off with buffered saline, dehydrated, and embedded in paraffin wax. Hematoxylin and eosin (HE) staining was performed on successive slices of paraffin embedded blocks at 5 mm thick using the Rotary Microtome (RM2255, Leica, Solms, WZ, Germany). Each sample was examined histopathologi-

FIGURE 1. Preparation of dental cavities. (A) Extracted premolars; (B) Residual periodontal tissue on the root surface of *in vitro* teeth were scraped with a surgical blade; (C,D) Process for preparing dental cavities; (E) Ultrasonic scouring; (F) Sterile triple antibiotic solution containing dental cavities.

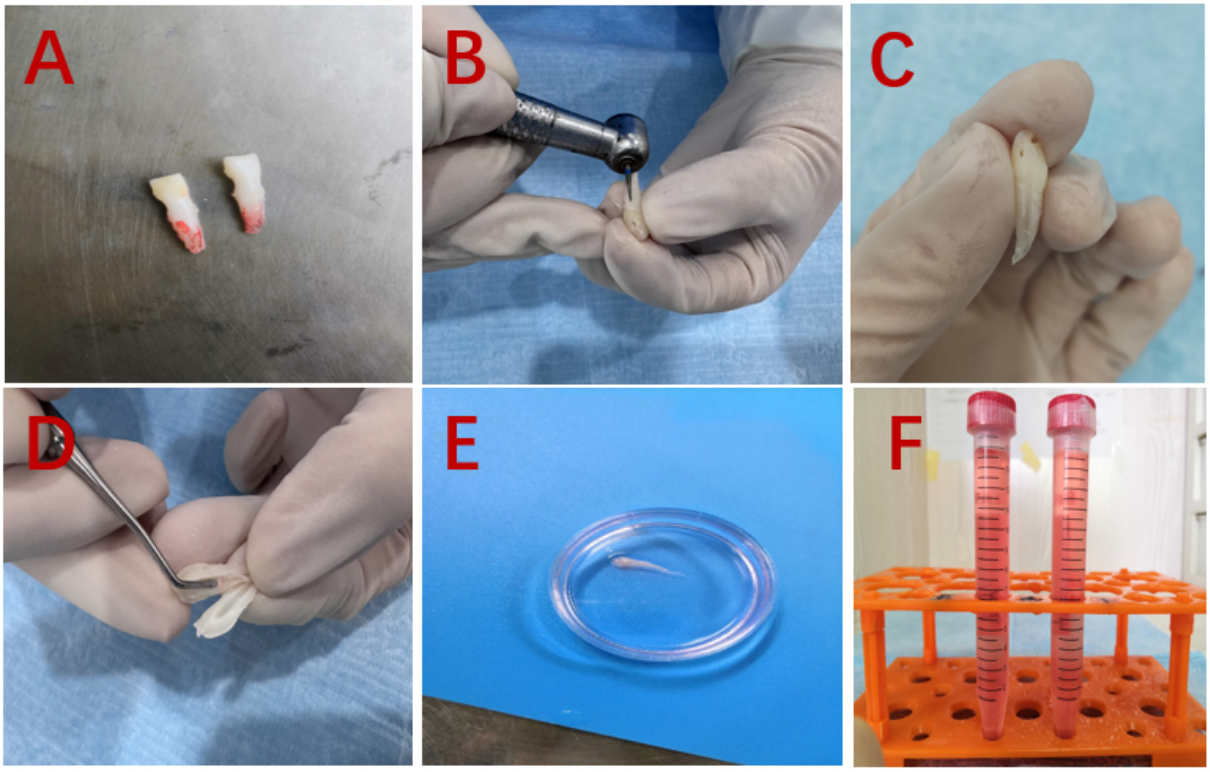

FIGURE 2. Pulp extraction from deciduous teeth. (A) Extracted deciduous teeth; (B,C) Cavities were prepared along the long axis of the deciduous teeth, and the hard tissue was cleaved to expose the pulp; (D) Pulp tissue was extracted with a small, sharp excavator; (E) Pulp tissue of deciduous teeth; (F) Sterile triple antibiotic solution containing pulp tissue of deciduous teeth.

cally under a light microscope (DM2500, Leica, Solms, WZ, Germany) by the same oral pathologist who was blind to treatments. Histopathological parameters assessed included soft tissue composition and blood vessel distribution within root canals.

Immunohistochemical analyses were also performed to evaluate angiogenesis and neurogenesis following pulp transplantation. Firstly, serial sections were prepared according to the method described above. Proteinase K (30 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) was subjected to antigen retrieval and incubated with 0.3% hydrogen peroxide in methanol for 30 minutes. We then incubated the sections with antibodies-rabbit anti-CD31 (EPR3094, Boster Bio, Wuhan, China), an immunomarker for human vascularization, CD31 Polyclonal Antibody (28083-1-AP, Sanying, Co., Ltd., Wuhan, China), an immunomarker for mice vascularization, and rabbit anti-Nestin (EPR1301(2), Omin MAbs, Alhambra, CA, USA), a biomarker for human nerve fibers—all antibodies were diluted in 1× phosphate-buffered saline. In the negative group, regular rabbit serum was used to replace the indicated antibodies. After washing with PBS, sections were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG, SV0002, Boster Bio), diluted in 1× phosphate-buffered saline for 30 minutes. Additional washes in PBS were followed by incubation with incubated with 3,3′-diaminobenzidine substrates (DAB) (CWBIO, Beijing, China). The stained slides were inserted into the slide-reading driver of a digital microscopic imaging system for image information analysis. For quantitative analysis of differences between groups, Human CD31 Microvascular density (MVD) and Human Nestin Optical density were calculated. CD31 and Nestin were expressed in vascular endothelial cells and nerve fibers, respectively, and stained yellow or brown-yellow in the cytoplasm. A 200-fold field of view was used to photograph the three areas with the highest density of positive cells in each specimen. Following staining, Image-Pro Plus 6.0 (MEDIA CYBERNETICS, MD, USA) was used to review each staining result individually. The average microvascular densitometer value and light density value were used as detection indexes.

2.8 Statistical analysis

Based on the resource equation, the sample size is calculated. Kruskal-Wallis single factor analysis of variance was performed using SPSS 20.0 (IBM Information Management, Armonk, NY, USA) and GraphPad Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). $p < 0.05$ indicates statistical significance.

3. Results

3.1 Histological result

HE staining showed interwoven growth of pulp tissue and fibrous connective tissue in the lumen of Group A (Fig. 3A). And in Group B, HE staining also revealed contact growth of dental pulp tissue in root canal with mice connective tissue (Fig. 3B). While, only a small amount of fibrous connective tissue containing blood vessels of different sizes was found in root canal in Group C (Fig. 3C).

Immunohistochemical staining of Group A (Fig. 4) showed that human CD31 positive cells were scattered in pulp tissue region, while mice CD31 positive cells were scattered in connective tissue. Meanwhile, human Nestin immunohistochemistry was positive. Similar to human CD31, the positive cells were distributed in the dental pulp tissue. It was evident from the above results that the dental pulp tissue was still functioning after transplantation, since the nude mice were capable of supplying blood to it. And in Group B, immunohistochemistry (Fig. 5) revealed that dental pulp tissue contained a wide distribution of human CD31 and human Nestin positive cells. Human Nestin positive cells have a similar distribution area to human CD31 positive cells. However, only mice CD31 positive cells were found in mouse connective tissue. It was demonstrated that nude mice provided a rich blood supply for the transplanted pulp. But immunohistochemical staining showed that only mice CD31 staining was positive in Group C (Fig. 6).

3.2 Statistical analysis

MVD was used to quantitatively compare the amount of angiogenesis between the three groups. Table 1 show that there were significant differences among the three groups ($p < 0.001$). Similarly, Table 2 shows the difference in average optical density values of Nestin between the groups. The statistical results show that, Nestin's average optical density was significantly different among the deciduous pulp group, the deciduous pulp/Matrigel group and the negative control group ($p < 0.001$).

TABLE 1. Comparison of microvascular counts M (P25, P75).

	n	M (P25, P75)	H	*p* value
Deciduous Pulp Group	18	8.00 (4.00, 12.50)	41.41	<0.001
Deciduous Pulp/ Matrigel Group	18	25.00 (17.75, 31.50)		
Negative Control Group	18	0.00 (0.00, 0.00)		

Microvascular counts differ significantly among the deciduous pulp group, the deciduous pulp/Matrigel group and the negative control group.

4. Discussion

An autotransplantation model in Beagle confirmed the feasibility of pulp transplantation of deciduous teeth. Additionally, we observed that the transplanted pulp tissue from deciduous teeth was able to establish a blood supply connection with the surrounding tissue in an ectopic regeneration model of nude mice. This is significant since the pulp is enclosed within a pulp cavity with only one blood supply. Therefore, the early establishment of blood supply can reduce ischemia-induced damage and aid pulp regeneration [18]. In this study, we used Matrigel to optimize the pulp tissue treatment strategy

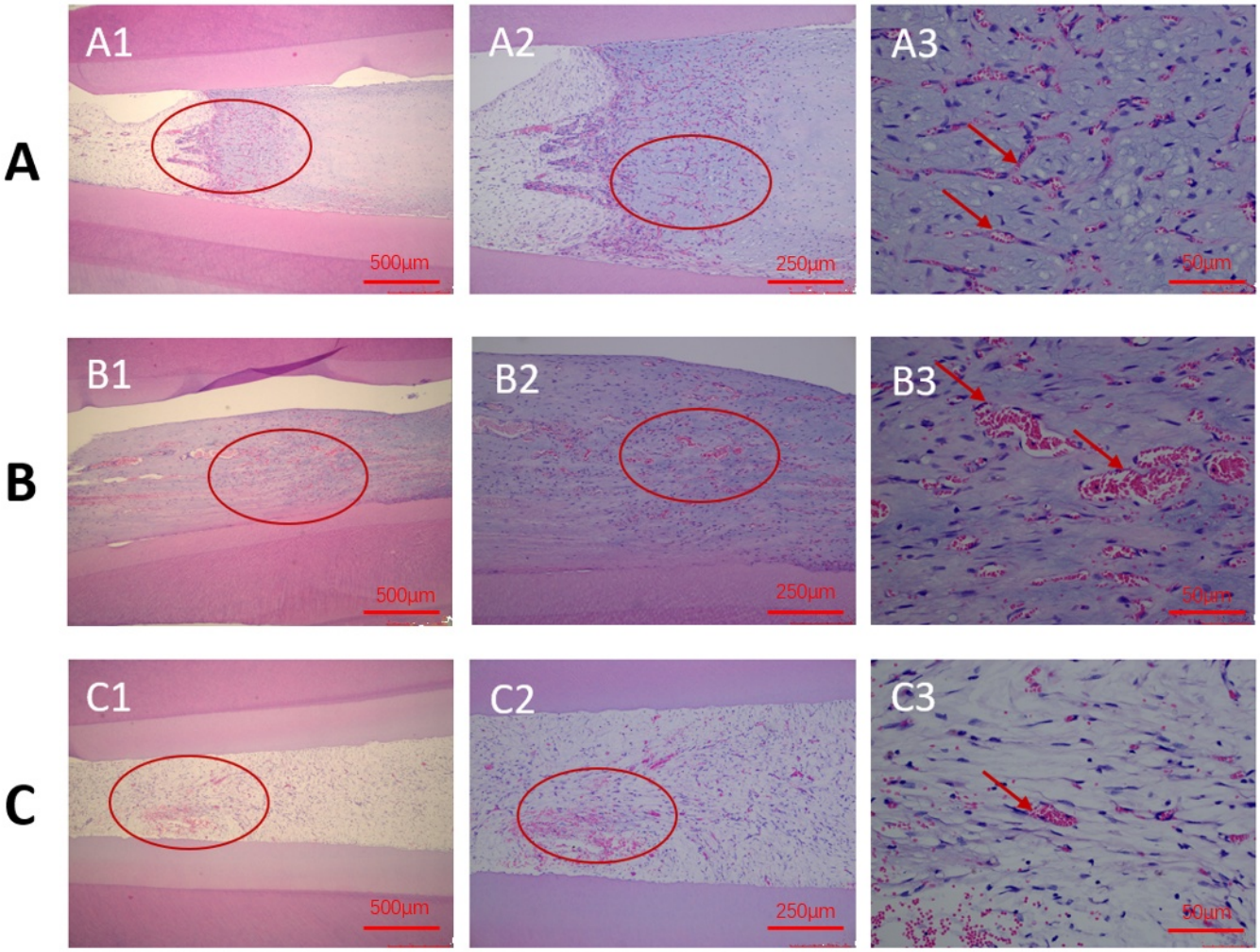

FIGURE 3. Histological analysis. Fig. 3A shows the deciduous pulp group (Group A). (A1) Detection of fibrous connective tissue and pulp tissue in the root canal (×20); (A2) Enlargement of the circled area in Fig. 3A1 (×40); (A3) Enlargement of the circled area in Fig. 3A2 displays vascular endothelial cells form obvious vascular cavities, most of which are small vascular cavities, with red arrows indicating neovascularization lumens (×200). Fig. 3B shows the deciduous pulp/Matrigel group (Group B). (B1) Root canal contains many fibrous connective tissue and dental pulp tissue (×20); (B2) Enlargement of the circled area in Fig. 3B1 (×40); (B3) Enlargement of the circled area in Fig. 3B2 displays vascular endothelial cells form an obvious vascular cavity, most of which are small vascular cavities, with red arrows indicating neovascular cavity (×200). Fig. 3C shows the negative control group (Group C). (C1) A small amount of fibrous connective tissue is present in the root canal and blood vessels are visible (×20); (C2) Enlargement of the circled area in Fig. 3C1 (×40); (C3) Enlargement of the circled area in Fig. 3C2, with the red arrow indicating vascular cavity (×200).

TABLE 2. Comparison of average optical density of Nestin M (P25, P75).

	n	M (P25, P75)	H	*p* value
Deciduous Pulp Group	18	0.0062 (0.0005, 0.0125)	34.783	<0.001
Deciduous Pulp/ Matrigel Group	18	0.0166 (0.0099, 0.0195)		
Negative Control Group	18	0.0004 (0.0001, 0.0011)		

Nestin's average optical density was significantly different among the deciduous pulp group, the deciduous pulp/Matrigel group and the negative control group.

to help immature permanent teeth develop. We compared the early vascular and nerve formation between the deciduous pulp/Matrigel group and the deciduous pulp group after transplantation. As compared to direct transplantation of deciduous pulp tissue, Matrigel promotes early blood supply after transplantation. As a soluble substrate for basement membrane proteoglycan, Matrigel is widely found on the surface of cell membranes and in the cell matrix. Biologically active substrates resemble the main components of mammalian basement membrane, which can better simulate basement membrane characteristics in all aspects [19]. Matrigel has been used in previous studies for various experimental or research applications, such as angiogenesis, tumor invasion, *in vivo* disease model construction and regenerative medicine. According to studies, Matrigel is histocompatible, and its three-dimensional

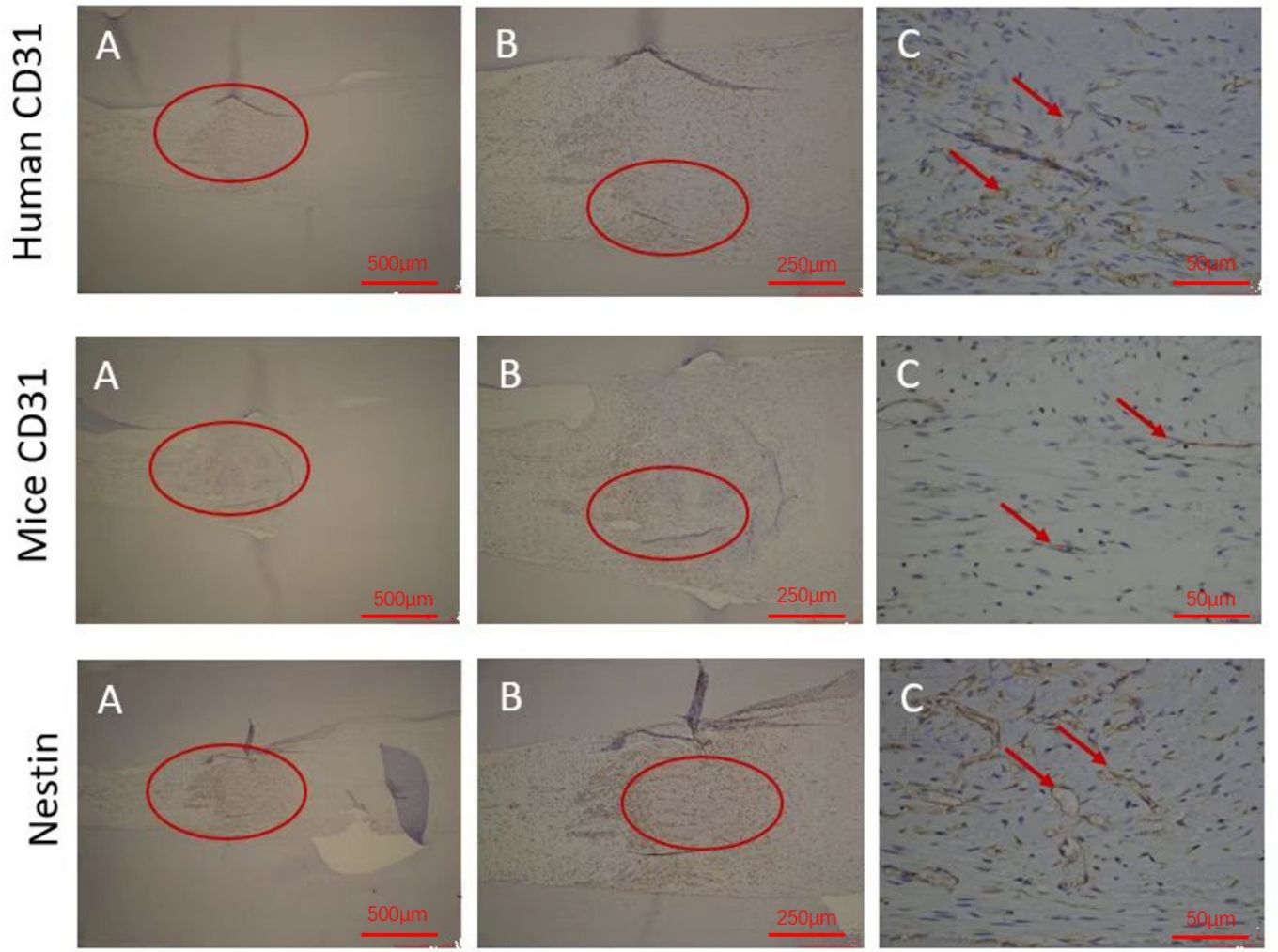

FIGURE 4. Immunohistochemical staining of the deciduous pulp group (Group A). The first row of images shows Human CD31 localization. (A) Canal tissue rich in blood vessels (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrows indicate blood vessels. The second row of images shows Mice CD31 localization. (A) Canal tissue is rich in blood vessels (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrows indicate blood vessels. The third row of images shows localization of anti-Nestin antibody binding to nerve fibers. (A) Canal tissue is rich in nerve fibers (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrows indicate nerve fibers.

support environment can reduce stem cell apoptosis after pulp stem cell transplantation and promote pro-angiogenic factor expression [15, 20]. In addition, Matrigel contains rich nutrients that can nourish deciduous pulp prior to re-establishing blood supply, and can improve survival rates after transplantation of deciduous pulp [21–23]. Co-culturing spinal ganglia with Matrigel has also been shown to increase neuronal cell differentiation [24, 25]. This study demonstrated that HE staining and CD31 immunohistochemistry improved the angiogenesis of the deciduous pulp/Matrigel group compared to the deciduous pulp group. Vascular endothelial cells formed a large number of vascular cavities, most of which were newly formed small vascular cavities. Human CD31 positive cells were used for microvascular counting, and statistical analysis that angiogenesis differed between the deciduous pulp/Matrigel group and the deciduous pulp group. Furthermore, the deciduous pulp/Matrigel group showed superior angiogenesis to the deciduous pulp group, and Matrigel allowed the deciduous pulp to survive after transplantation more effectively. In addition, the results confirmed Matrigel's role in promoting pulp blood supply reconstruction. A similar distribution of human CD31 and Nestin positive expression areas was observed in dental pulp tissue in this study. Also, Nestin's average optical density was further statistically analyzed. Deciduous pulp/Matrigel had a significantly different average optical density than deciduous pulp. This result is also consistent with statistical analysis of angiogenesis values. After transplantation, the nude mice provide blood supply to the dental pulp tissue, consistent with previous studies.

Dental tissue engineering involves ontogenic stem cells, scaffold structure and growth factors [26]. Deciduous teeth pulp is a natural three-dimensional scaffold containing pulp stem cells and many growth factors. In the replacement period, deciduous teeth have fibrosis in the pulp tissue, which will affect the effect after transplantation. Matrigel contains a variety of growth factors including transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF),

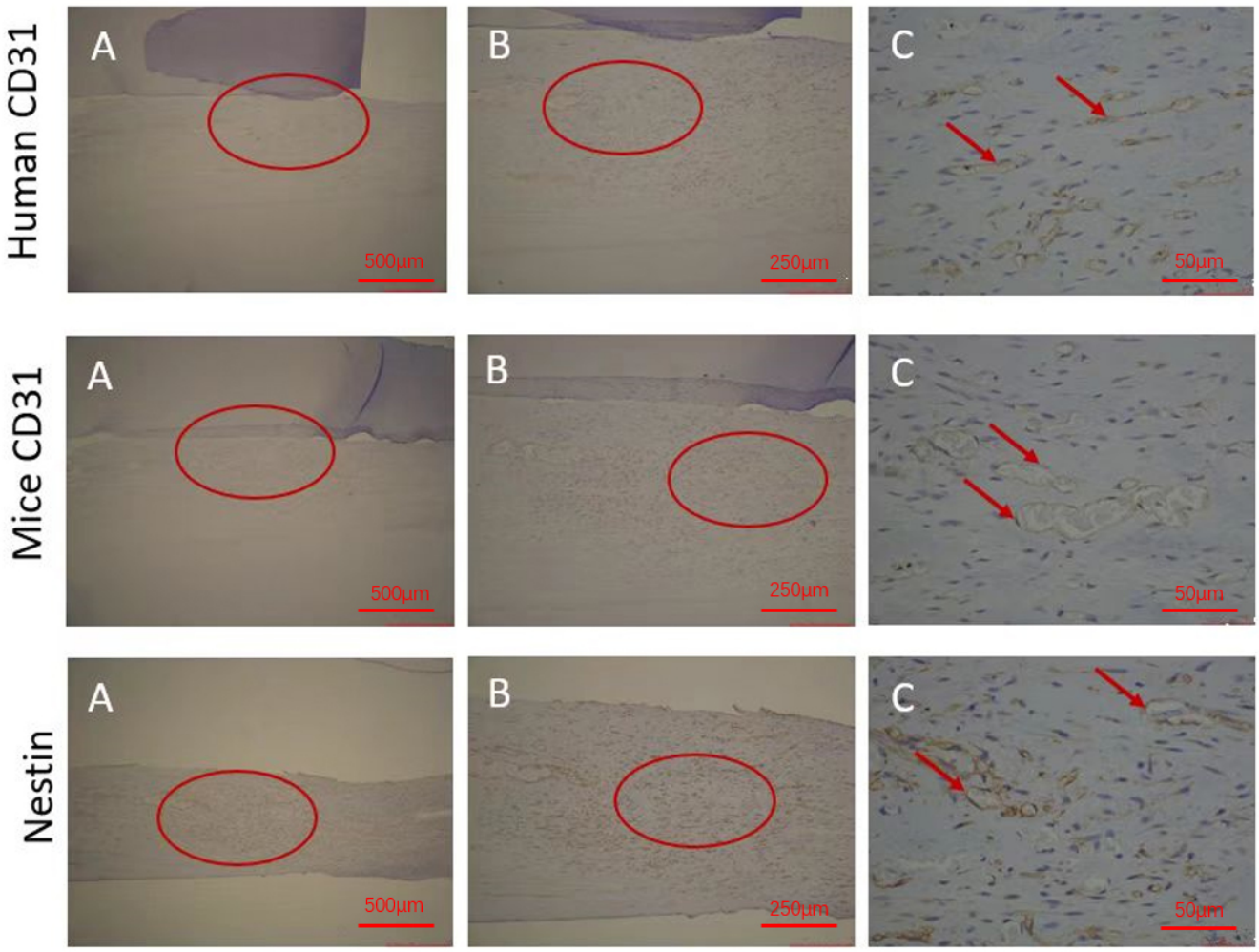

FIGURE 5. Immunohistochemical staining of the deciduous pulp/Matrigel group (Group B). The first row of images shows Human CD31 localization. (A) Canal tissue rich in blood vessels (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrows indicate blood vessels. The second row of images shows Mice CD31 localization. (A) Canal tissue is rich in blood vessels (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrows indicate blood vessels. The third row of images shows localization of anti-Nestin antibody binding to nerve fibers. (A) Canal tissue is rich in nerve fibers (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrows indicate nerve fibers.

platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF), which have been shown to improve blood vessel formation [27]. Both immunohistochemical results and microvascular counts in the experiment showed that the deciduous pulp/Matrigel group vascular regeneration was better than the deciduous pulp group. After transplantation, Matrigel can promote pulp blood supply, further confirming its potential.

Besides being an important part of dental tissue engineering, pulp-dentin regeneration is also an ideal target for treating dental pulp diseases on a clinical level [28–30]. Pulp-dentin complex regeneration aims to restore pulp sensibility, repair capability by mineralization, immunity and other physiological functions [31]. Dental tissue engineering has often used Matrigel as scaffolds. The study showed that after co-culturing rat dental pulp cells with Matrigel and implanting them into pulpotomized maxillary first molars of rats, neovascularization occurred [20]. Furthermore, implanting Human Dental Pulp Stem Cell Sheet/Treated Dentin Matrix/Matrigel sandwich structures subcutaneously into nude mice for 3 months showed the formation of periodontium, dentin, and pulp-like tissues [32]. Despite the fact that the deciduous pulp/Matrigel group had stronger vascular regeneration ability than the deciduous pulp group, neither the experimental group nor the control group could be able to observe the endodontic complex structure. The possible reasons are as follows: (1) Under hypoxia conditions following pulp extraction, transplanted pulp stem cells first differentiated into vascular endothelial cells to form blood vessels under hypoxia environment, lacking the regulation of odontoblast differentiation. (2) Embedded under the skin of nude mice were isolated teeth, which lack the support of the periodontal tissue physiological environment, and stem cells have limited differentiation ability. (3) The experimental observation time was too short, and the transplanted deciduous teeth's pup did not differentiate into odontoblast cells. Observing pulp-dentin regeneration is crucial to real pulp regeneration, so we must observe this further in future

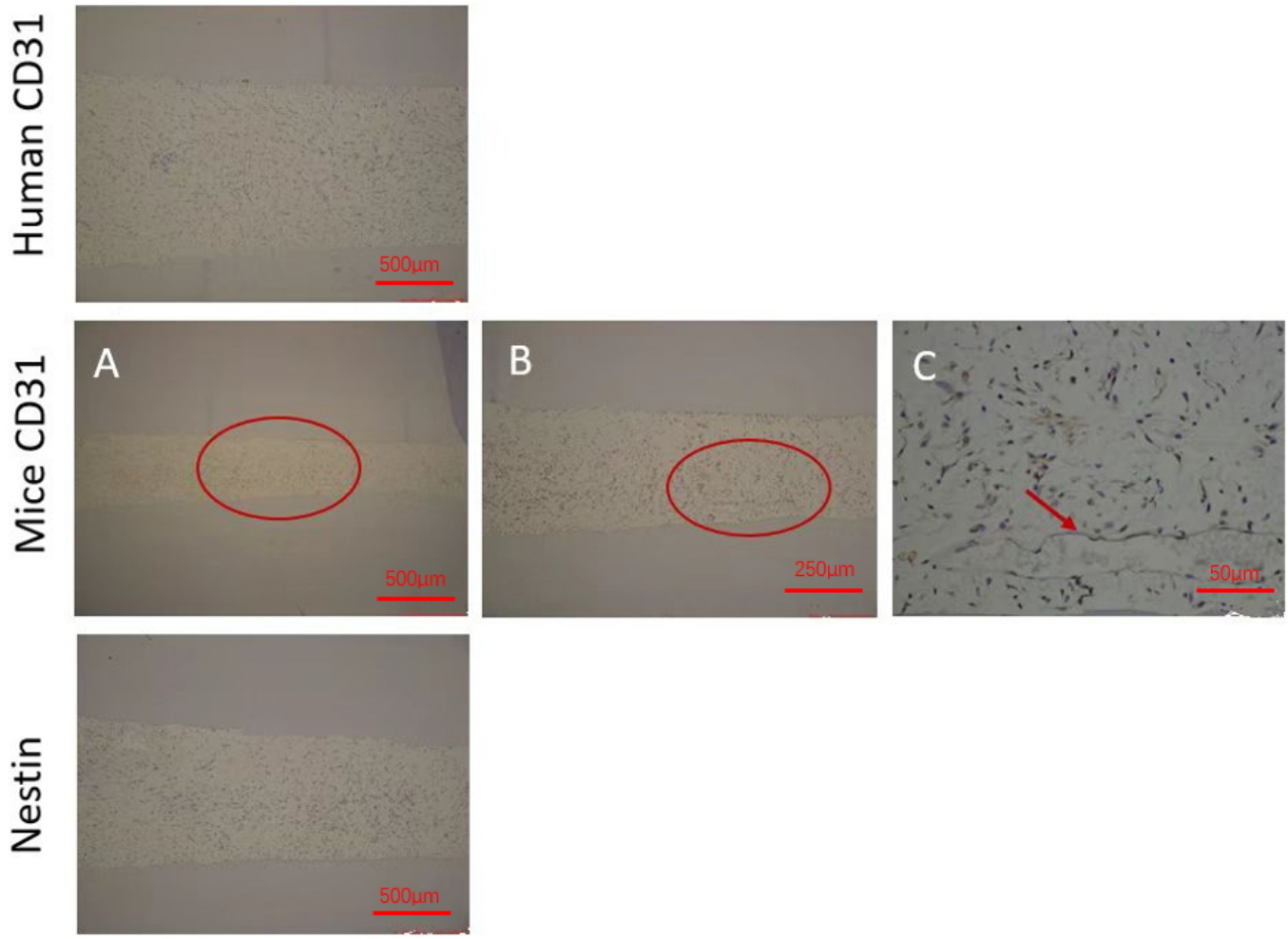

FIGURE 6. Immunohistochemical staining of the negative control group (Group C). Human Nestin and CD31 immunohistochemical were negative (×20). The second row of images localization of Mice CD31 localization. (A) Canal tissue is rich in blood vessels (×20); (B) enlargement of the circled area in panel A (×40); (C) red arrow indicates blood vessel.

experiments. Despite the fact that this study shows that Matrigel promotes the development of immature permanent teeth by stimulating the pulp tissue of transplanted deciduous teeth, some limitations remain. It is unclear which specific cells Matrigel is affecting in deciduous teeth due to the complex composition of pulp tissue. As a result of the short time frame of the experiment, further observation is required to determine how the transplanted pulp tissue will be incorporated back into the primary teeth.

5. Conclusions

This study demonstrated that Matrigel can lead to the establishment of a blood supply after pulp transplantation in deciduous teeth. However, there is still a need for further research on how Matrigel promotes revascularization of deciduous tooth pulp after transplantation.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

YH—designed the research study. YNJ and JJJX—performed the research. YNJ—analyzed the data. JJJX—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This retrospective study was conducted according to the guidelines of the Declaration of Helsinki and all procedures performed were in accordance with the Ethics Committee of the Affiliated Stomatological Hospital of Nanchang University Medical Ethics Committee (Ethics Code: 2024#052). Consent forms were signed by parent or legal guardian of patients.

ACKNOWLEDGMENT

We acknowledge the Affiliated Stomatological Hospital of Nanchang University for providing their experimental platform.

FUNDING

This work was supported by the Science and Technology Department of Jiangxi, PR China (20171BBG70042).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Murray P E. Review of guidance for the selection of regenerative endodontics, apexogenesis, apexification, pulpotomy, and other endodontic treatments for immature permanent teeth. International Endodontic Journal. 2023; 56: 188–199.

[2] Li J, Cheng J, Yang F, Yu J, Song G. Treatment outcomes of immature permanent necrotic evaginated teeth: a retrospective study comparing regenerative endodontic procedures with apexification. International Journal of Paediatric Dentistry. 2023; 33: 595–606.

[3] Kandemir DG, Kaval ME, Guneri P, Çalışkan MK. Treatment of immature teeth with nonvital pulps in adults: a prospective comparative clinical study comparing MTA with $Ca(OH)_2$. International Endodontic Journal. 2020; 53: 5–18.

[4] Pereira AC, Oliveira ML, Cerqueira-Neto ACCL, Vargas-Neto J, Nagata JY, Gomes BPFA, *et al*. Outcomes of traumatised immature teeth treated with apexification or regenerative endodontic procedure: a retrospective study. Australian Endodontic Journal. 2021; 47: 178–187.

[5] Panda P, Mishra L, Govind S, Panda S, Lapinska B. Clinical outcome and comparison of regenerative and apexification intervention in young immature necrotic teeth—a systematic review and meta-analysis. Journal of Clinical Medicine. 2022; 11: 234–246.

[6] Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? Journal of Endodontics. 2004; 30: 196–200.

[7] Iwaya S, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dental Traumatology. 2001; 17: 185–187.

[8] Swaikat M, Faus-Matoses I, Zubizarreta-Macho Á, Ashkar I, Faus-Matoses V, Bellot-Arcís C, *et al*. Is revascularization the treatment of choice for traumatized necrotic immature teeth? A systematic review and meta-analysis. Journal of Clinical Medicine. 2023; 12: 2656.

[9] Wang Y, Mao J, Wang Y, Jiang N, Shi X. Multifunctional exosomes derived from M2 macrophages with enhanced odontogenesis, neurogenesis and angiogenesis for regenerative endodontic therapy: an *in vitro* and *in vivo* investigation. Biomedicines. 2024; 12: 441.

[10] Li K, O'Dwyer R, Yang F, Cymerman J, Li J, Feldman JD, *et al*. Enhancement of acellular biomineralization, dental pulp stem cell migration, and differentiation by hybrid fibrin gelatin scaffolds. Dental Materials. 2023; 39: 305–319.

[11] Piglionico SS, Varga B, Pall O, Romieu O, Gergely C, Cuisinier F, *et al*. Biomechanical characterization of a fibrinogen–blood hydrogel for human dental pulp regeneration. Biomaterials Science. 2023; 11: 6919–6930.

[12] Brizuela C, Huang GT, Diogenes A, Botero T, Khoury M. The four pillars for successful regenerative therapy in endodontics: stem cells, biomaterials, growth factors, and their synergistic interactions. Stem Cells International. 2022; 2022: 1580842.

[13] Xuan K, Li B, Guo H, Sun W, Kou X, He X, *et al*. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. Science Translational Medicine. 2018; 10: eaaf3227.

[14] Huang Y, Tang X, Cehreli ZC, Dai X, Xu J, Zhu H. Autologous transplantation of deciduous tooth pulp into necrotic young permanent teeth for pulp regeneration in a dog model. Journal of International Medical Research. 2019; 47: 5094–5105.

[15] Kastana P, Zahra FT, Ntenekou D, Katraki-Pavlou S, Beis D, Lionakis MS, *et al*. Matrigel plug assay for *in vivo* evaluation of angiogenesis. Methods in Molecular Biology. 2019; 1952: 219–232.

[16] Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. Seminars in Cancer Biology. 2005; 15: 378–386.

[17] Zoeller JJ, Whitelock JM, Iozzo RV. Perlecan regulates developmental angiogenesis by modulating the VEGF-VEGFR2 axis. Matrix Biology. 2009; 28: 284–291.

[18] Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocrine Reviews. 2004; 25: 581–611.

[19] Gan Z, Qin X, Liu H, Liu J, Qin J. Recent advances in defined hydrogels in organoid research. Bioactive Materials. 2023; 28: 386–401.

[20] Zaw ZCT, Kawashima N, Kaneko T, Okiji T. 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*. Journal of Dental Sciences. 2022; 17: 1160–1168.

[21] Engbring JA, Kleinman HK. The basement membrane matrix in malignancy. The Journal of Pathology. 2003; 200: 465–470.

[22] Luzuriaga J, Irurzun J, Irastorza I, Unda F, Ibarretxe G, Pineda JR. Vasculogenesis from human dental pulp stem cells grown in matrigel with fully defined serum-free culture media. Biomedicines. 2020; 8: 483.

[23] Abbass Marwa M S, El-Rashidy Aiah A, Sadek Khadiga M, Moshy SE, Radwan IA, Rady D, *et al*. Hydrogels and dentin-pulp complex regeneration: from the benchtop to clinical translation. Polymers. 2020; 12: 2935.

[24] Braun H, Bühnemann C, Neumann J, Reymann KG. Preparation of a tissue-like cortical primary culture from embryonic rats using Matrigel and serum free Start V Medium. Journal of Neuroscience Methods. 2006; 157: 32–38.

[25] Gonzalez H, Narasipura SD, Shull T, Shetty A, Teppen TL, Naqib A, *et al*. An efficient and cost-effective approach to generate functional human inducible pluripotent stem cell-derived astrocytes. Cells. 2023; 12: 2357.

[26] Guo X, Li J, Wu Y, Xu L. Recent advancements in hydrogels as novel tissue engineering scaffolds for dental pulp regeneration. International Journal of Biological Macromolecules. 2024; 264: 130708.

[27] Vukicevic S, Kleinman H K, Luyten F P, Roberts AB, Roche NS, Reddi AH. Identification of multiple active growth factors in basement membrane Matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Experimental Cell Research. 1992; 202: 1–8.

[28] Moussa DG, Aparicio C. Present and future of tissue engineering scaffolds for dentin-pulp complex regeneration. Journal of Tissue Engineering and Regenerative Medicine. 2019; 13: 58–75.

[29] Zou J, Mao J, Shi X. Influencing factors of pulp-dentin complex regeneration and related biological strategies. Journal of Zhejiang University. 2022; 51: 350–361.

[30] Wei X, Yang M, Yue L, Huang D, Zhou X, Wang X, *et al*. Expert consensus on regenerative endodontic procedures. International Journal of Oral Science. 2022; 14: 55.

[31] Sismanoglu S, Ercal P. Dentin-pulp tissue regeneration approaches in dentistry: an overview and current trends. Advances in Experimental Medicine and Biology. 2020; 43: 79–103.

[32] Meng H, Hu L, Zhou Y, Ge Z, Wang H, Wu C, *et al*. A sandwich structure of human dental pulp stem cell sheet, treated dentin matrix, and Matrigel for tooth root regeneration. Stem Cells and Development. 2020; 29: 521–532.

How to cite this article: Jiangjingjun Xu, Yanni Jing, Yan Huang. The effect of Matrigel in deciduous tooth pulp transplantation: an *in vitro* study. Journal of Clinical Pediatric Dentistry. 2025; 49(2): 145-153. doi: 10.22514/jocpd.2025.034.