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Original Article

De novo regeneration of dentin pulp complex mediated by Adipose derived stem cells in an immunodeficient albino rat model (Histological, histochemical and scanning electron microscopic Study)

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ABSTRACT

Background: Dental tissue engineering is an alternative procedure for restoring damaged dental tissues. Adipose-derived stem cells are a new source of cells for regenerative endodontics in combination with scaffold materials. The descriptive data about this regenerative process is still insufficient.
Objective: To evaluate the regenerative potential of Adipose-derived stem cells using a self-assembling polypeptide scaffold for the dentin-pulp complex in an emptied root canal space.
Material and Methods: 40 root segments of human single-rooted teeth were transplanted into the albino rats' dorsal subcutaneous tissue. Root segments were divided into two groups: group I contained only a self-assembling polypeptide scaffold, and group II contained fluorescent-labeled Adipose-derived stem cells embedded in a self-assembling polypeptide scaffold. The newly formed tissues were assessed on the 60th and 90th days post-transplantation using routine histological examination, Masson trichrome staining, and scanning electron microscopy.
Results: Group I showed granulation tissue without any signs of predentin formation or odontoblast-like cells. Group II revealed the presence of predentin tissue along the dentin margin, with arranged odontoblast-like cells. An organized connective tissue with abundant vasculature and calcific masses was observed in the pulp space.
Conclusion: Adipose-derived stem cells can be considered as alternative stem cells for regenerating the dentin-pulp complex. Dentin pulp complex regeneration utilizing a self-assembling polypeptide scaffold alone would not yield successful results.

1. Introduction

The restoration of dental tissues by traditional procedures has many limitations caused by the complex organization and different biological properties of dental tissues. Tissue engineering (TE) targets to overcome these limitations by stimulating the dental tissues' growth and regeneration and by developing biomimetic functional substitutes that can integrate with the original tissues (Kim et al., 2023; Matichescu et al., 2020).

TE is defined as a multidisciplinary field that implements the principles of engineering and life sciences to develop biological substitutions to restore, maintain, and enhance tissue function. TE is based on three main components: cells (e.g., stem cells) for matrix formation, scaffolds for transplantation and support, and signaling molecules for induction and modulatory functions. These three constituents may be used separately or combined to regenerate the tissues (de Isla et al., 2010).

Scaffolds are three-dimensional constructions that give support and manage the growth and organization of cultured cells (Fahimipour et al., 2019). Hydrogel scaffolds have received noticeable interest because they are similar to the extracellular matrix in composition and structure. Additionally, they have an appropriate framework, allowing cell proliferation (El-Sherbiny et al., 2013). Hydrogel scaffolds act as transporters for vital cells and growth factors because of their biocompatibility, increased permeability, and their great capacity to hold water. Cell-loaded hydrogels are able to restore damaged tissues and give opportunities for cell-based treatments (Li et al., 2023).

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Stem cell-based treatment has recently been considered a key factor in tissue engineering (Hoang et al., 2022). Adipose tissue stem cells (ADSCs) belong to the mesenchymal stem cells that were first isolated from the stromal vascular fraction attained via processing adipose tissue (Zuk et al., 2001). ADSCs have self-renewal and multipotency, giving several cell lineages, including adipocytes, osteoblasts, and nerve cells (Miana et al., 2018). Over the past decade, ADSCs have gained increased consideration in tissue engineering. ADSCs are considered the most valuable cells for regenerative medicine due to their minimal invasive accessibility, multipotency, and their active paracrine activity (Qin et al., 2023).

ADSCs have differentiation potential that can be maintained with aging. They could also be induced into odontogenic lineage (Jing et al., 2008). Previous studies reported the development of tissues resembling dentin-pulp complex with blood vessels and nerves, using ADSCs with a collagen scaffold (Iohara et al., 2011; Ishizaka et al., 2012). Furthermore, Khazaei et al., (2021) isolated ADSCs and reported their capability of differentiating into odontoblast-like cells. However, other studies reported relatively weak ADSCs' odontogenic and angiogenic differentiation potential (Davies et al., 2014; Murakami et al., 2015). More descriptive studies are needed to evaluate the odontogenic and angiogenic capacity of ADSCs and the hydrogel scaffolds. This work aimed to evaluate the dentin-pulp complex regeneration potential of the ADSCs accompanied with a self-assembling polypeptide scaffold.

2. Materials and methods

2.1. Experimental design

The present work is a cross-sectional animal study performed on twenty male albino rats weighing 200–250 g at the Medical Research Center, Ain Shams University after ethical approval from the Bioethical Committee in the Faculty of Dentistry - Ain Shams University (Approval ID: FDASU Rec-D140521).

Forty roots of recently extracted human single-rooted teeth were horizontally sectioned in the mid-root region to obtain 6–7 mm segments. The root canals were widened to a diameter of 1–2.5 mm and sealed on one side with 1 mm mineral trioxide aggregate (MTA). Samples were immersed in 17 % ethylenediaminetetraacetic acid (EDTA) (10 min) at room temperature and 19 % citric acid (1 min) to eliminate the smear layer. The samples were sterilized with Betadine (30 min) and 5.25 % sodium hypochlorite (10–15 min), rinsed, and immersed in phosphate-buffered saline (PBS), then incubated at 37–38 °C for 3–7 days to eliminate the residual sterilizing agents and microbial contamination (Huang et al., 2010).

Root segments were implanted into the dorsal subcutaneous region of rats, with 2 segments in each rat, and divided into two groups (n = 20 root segments in each group):

Group I (control) received a self-assembling polypeptide scaffold.

Group II (experimental) received fluorescent-labeled adipose-derived stem cells (ADSCs) embedded in a self-assembling polypeptide scaffold. Each group was equally subdivided into two subgroups (A and B) according to the time of segment collection (60 and 90 days after transplantation, respectively). The rats' sample size was verified to be sufficient based on the equation (E = Number of animals – number of groups), where any sample size with an E value between 10 and 20 is considered adequate. (In our work, E = 20 – 4 = 16) (Ilyas et al., 2017).

2.2. Scaffold preparation and seeding of stem cells

HydroMatrix™ Peptide Cell Culture Scaffold (Catalog Number A6982) was prepared according to the manufacturer's instructions (Sigma-Aldrich®). In group II, ADSCs (106/ml), immunolabeled by PKH26 dye, were seeded onto the scaffold with a seeding density (1x106 cells/ml) for 24 h in CO2 incubator. The cell-seeded scaffolds were loaded inside the root segments and left for 5 min before insertion in the rats (Yuan et al., 2022). The 24-hour incubation was essential for cell-scaffold interaction and cell nesting. However, this incubation hindered the proper observation of the co-cultures under a phase contrast microscope.

2.3. Surgical procedure

Rats were immunosuppressed by receiving cyclosporine (10 mg/kg/day) (Tamburrino et al., 2015) one day before surgery and continued daily thereafter. The rats were anesthetized, and their dorsal surface was shaved and disinfected with Betadine. Two linear incisions of 1.5–2 cm were made on the dorsal skin, 1 cm from the midline on each side. Blunt dissection by artery forceps was done to expose the subcutaneous space. Two root segments were implanted into the subcutaneous space (one segment was assigned for each group), then the wounds were sutured. The rats received post-operative intramuscular injections of 15–20 mg/kg of Cephotaxime and 75 mg/ml of Diclofenac sodium once daily for 3 days.

2.4. Sample preparation and examination procedures

Rats were clinically examined, and those with healing complications were excluded. Rats were sacrificed, and root segments were retrieved and freed from soft tissues. Specimens were fixed in 10 % formaldehyde (buffered in pH 7.2 PBS) for five days. Samples were decalcified by 12 % EDTA solution, changed every 2 days, for two weeks. Samples were prepared for Hematoxylin and Eosin (H&E) and Masson trichrome (MTC) staining (Bancroft and Gamble, 2002). Specimens for the scanning electron microscope were fixed in 10 % buffered glutaraldehyde for seven days. Samples were cut longitudinally to examine the dentin-pulp interface and root canal space by SEM (JEOL-JSM-5500LV). Phase-contrast fluorescent microscopic examination was used to detect the immuno-labeled stem cells in the 60-day duration subgroups due to the limited half-life of the dye.

3. Results

3.1. Histological results (H&E)

Subgroup IA showed root canal space with rare evidence of predentin deposition or cells at the pulpal margin of dentin. Granulation tissue was observed inside the root canal space, enclosing connective tissue cells, and red blood corpuscles, and rare distinct blood vessels. Subgroup IB showed similar findings, but there were some zones of fat cells and hyaline masses entrapping a few cells (Fig. 1). Subgroup IIA possessed a predentin layer along the dentin-pulp margin with globular calcification sites. Occasional odontoblast-like cells were detected at the dentin margin. The pulp core showed organized connective tissue with collagen fibers, cellular elements, calcific masses, and relatively distinct blood vessels. Subgroup IIB showed apparent thickening in the predentin layer, with dentinal tubules occasionally observed. The core of the root canal exhibited fibrocellular connective tissue with distinct vessels and calcific masses, entrapping cells inside (Fig. 2).

3.2. Histochemical results (Masson Trichrome)

Subgroups IA and IB revealed limited vasculature and new collagen formation, particularly in subgroup IB. Specimens of subgroup IIA showed collagen formation attached to the dentin. Subgroup IIB revealed extended tissue formation with cellular elements, mature collagen fibers, and a well-developed vascular supply (Fig. 3).

3.3. Immunofluorescence results (Phase contrast fluorescent Microscope)

Group I revealed no positive cells for PKH26 in the pulp core. Meanwhile, Group II revealed positive cells to PKH26 with different

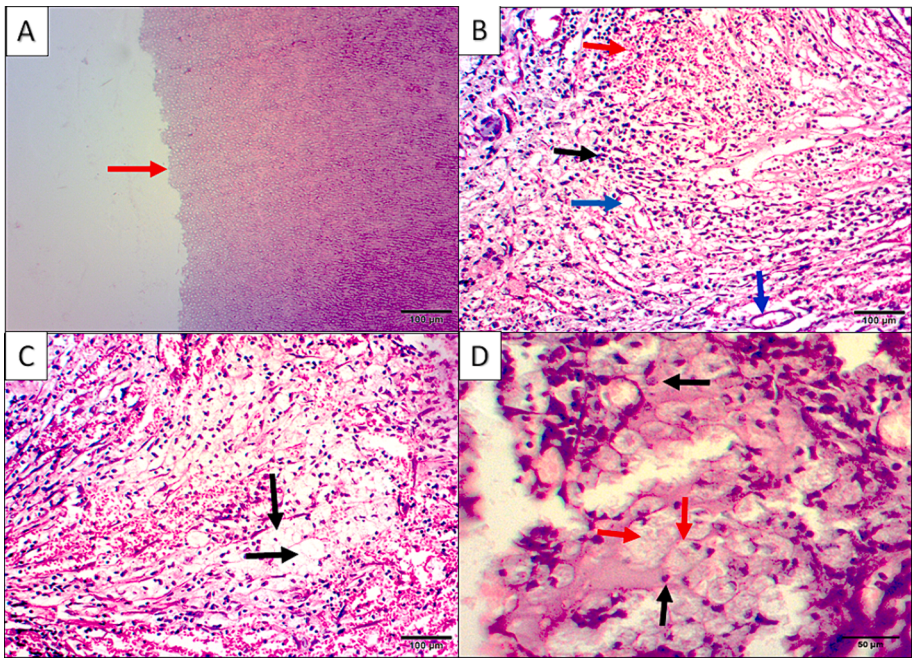

Fig. 1. Photomicrographs of Group I: (A, B: subgroup IA. C, D: subgroup IB). (A): Dentin border (arrow) with no predentin or lining cells. (B) Pulp core with granulation tissue, dark rounded cells (black arrows), RBCs (red arrows), and small vessels (blue arrows). (C): Pulp core with granulation tissue with regions of fatty degeneration (arrows). (D) Clusters of adipocytes (red arrows) and hyaline masses which entrap few cells (black arrows) [H&E x 400]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

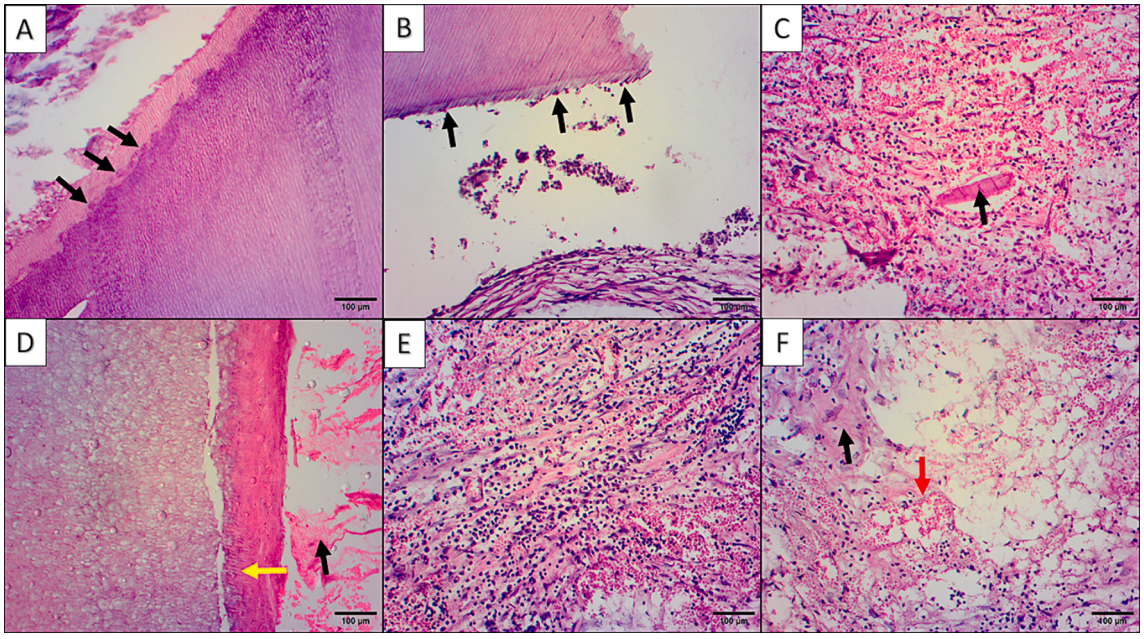

Fig. 2. Photomicrographs of Group II: (A-C: Subgroup IIA. D-F: Subgroup IIB). (A): Dentin border with predentin and calcification globules (arrows). (B) Odontoblast-like cells (arrows) lining the dentin. (C): Organized connective tissue with linear calcification (arrow). (D): Tubular predentin (yellow arrows) and developing fibrous connective tissue (black arrow). (E): Fibrocellular connective tissue (F): Calcific mass (black arrow) and distinct vessel (red arrow) [H&E x200]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

morphologies (Fig. 3).

3.4. Scanning electron microscopic results

Subgroup IA showed bare dentin in some regions with open dentinal tubules. In other areas, dentin was covered by granulation tissue formed of fibers and cells with variable sizes. Subgroup IB showed mass of cellular connective tissue. The dentin-scaffold interface revealed intimate contact between the scaffold and dentin with no predentin formation. Scaffold fibrils interdigitated with dentinal tubules (Fig. 4). Subgroup IIA showed a predentin layer in some regions. The pulp cavity had a well-organized fibrous tissue matrix with connective tissue cells. In subgroup IIB, the predentin layer was evident, and the pulp space showed connective tissue containing cells and an intercellular matrix. The dentin integrated with neighboring developed tissue, and the fibers were inserted into the dentinal tubules (Fig. 5).

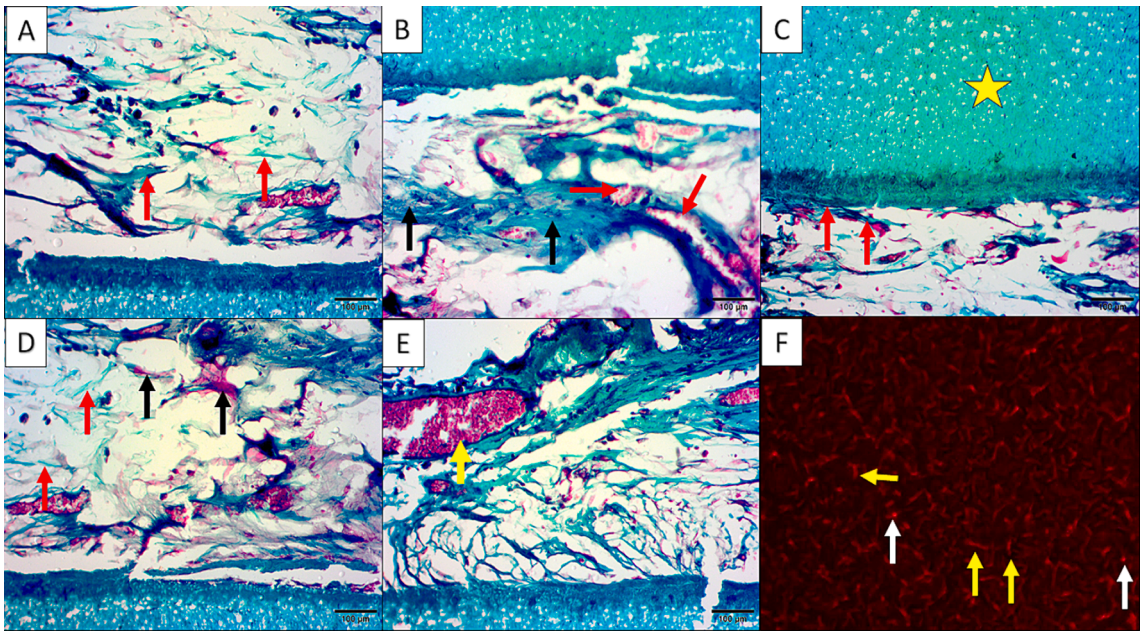

Fig. 3. Photomicrographs (A): Subgroup IA showing new collagen (arrows). (B): Subgroup IB is showing new collagen (red arrow), and small blood vessels (black arrow). (C): Subgroup IIA shows organized collagen (arrows), which is attached to dentin walls (star). (D): Subgroup IIB shows new collagen fibers (red arrows) and mature fibers (black arrows). (E): Subgroup IIB shows a blood vessel (arrow). (F): Subgroup IIA shows rounded positive cells (white arrows) and spindle cells (yellow arrows) (A-E: Masson trichrome. X200. F: Phase-contrast fluorescent microscope PKH26 x 400]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

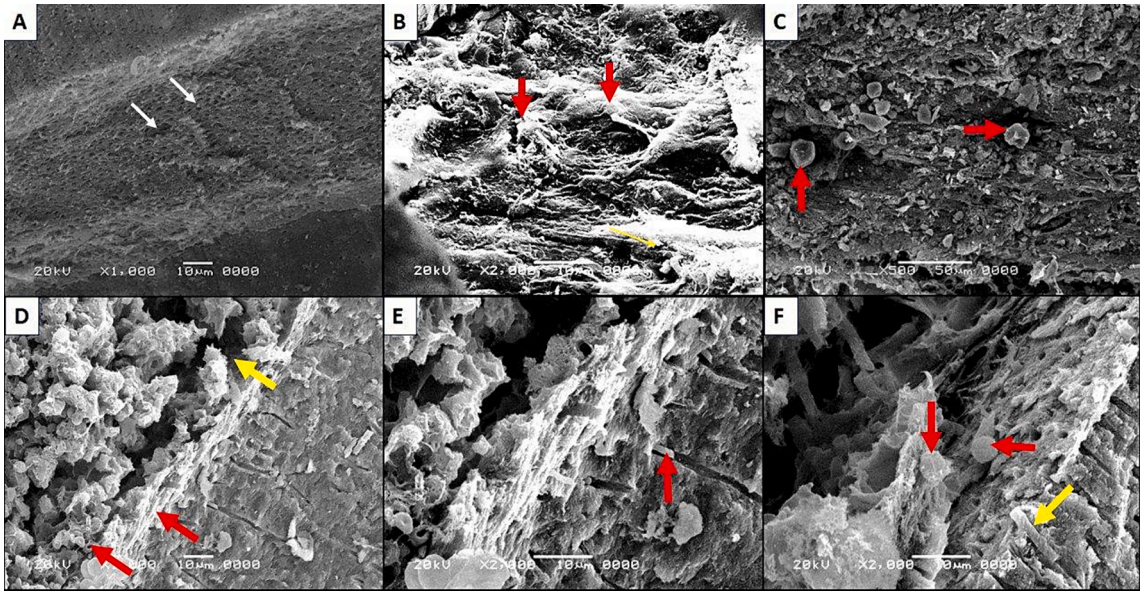

Fig. 4. SEM micrographs of Group I: (A-C: Subgroup IA. D-F: Subgroup IB). (A): Bare dentin with opened dentinal tubules (Arrows). (B): Granulation tissue on the scaffold (arrows). (C): Granulation tissue on the scaffold with large cells (arrows). (D): Dentin-scaffold integration (red arrow) and narrow gaps in other areas (yellow arrow). (E): Scaffold-dentin interface with interdigitation (arrow). (F): Connective tissue cells (red arrows) and a fibril from scaffold inserted in dentinal tubule (yellow arrow) [SEM. B, E, F: x2000; A, D: x1000; C: x500]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The current study investigated the formed tissues in root segments embedded in rat subcutaneous tissue using ADSCs and a self-assembled scaffold. ADSCs were selected due to their multipotency (Qin et al., 2023; Zuk et al., 2001). Adipose tissue gives different populations of stem cells. This tissue is easily accessible compared to dental-pulp stem cells. Several studies reported the application of ADSCs in regenerative medicine and bio-root regeneration. Their transplantation into periodontal tissue defects in rats can promote regeneration. ADSCs can regenerate dentin and alveolar bone in rabbits. (Khazaei et al., 2021; Palumbo et al., 2015; Qin et al., 2023; Raposio et al., 2016; Raposio et al., 2017; Si et al., 2019). We used a self-assembling polypeptide scaffold, which allows development of extracellular matrix-like materials, provides precise control at the molecular level, and produces injectable materials appropriate for small defects (Galler et al., 2011).

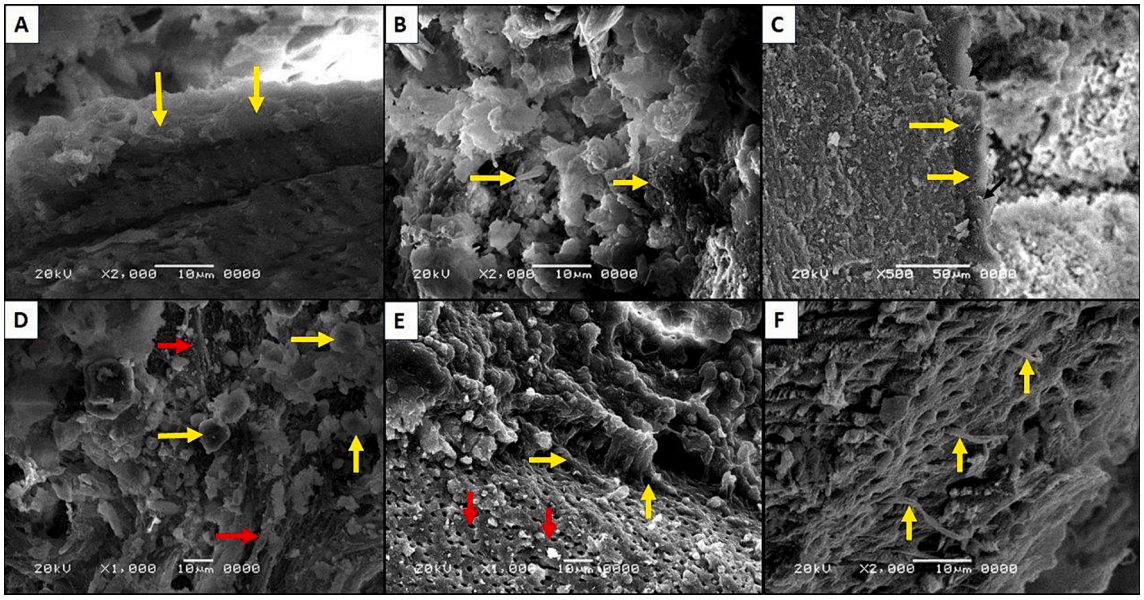

Fig. 5. SEM micrographs of Group II: (A,B: Subgroup IIA. C-F: Subgroup IIB). (A): Homogenous predentin (arrows) (B): New tissue with areas of matrix formation (arrows). (C): Predentin (arrows) (D): Connective tissue cells (yellow arrows) and matrix formation (red arrow) on the scaffold. (E): Bare dentin (red arrows) in some regions, and others integrated with neighboring tissue (yellow arrows) (F): Fibers inserted inside dentinal tubule (arrows) [SEM. A, B, F: x2000; D, E: x1000; C: x500]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the current study, rats were immunosuppressed to avoid host-foreign body reactions, which caused root segments dissolution in our pilot study. This can be explained by Anderson et al. (2008), who detected macrophages and giant cells following biomaterial implantation. In our study, the root canal was widened to a diameter of 1–2.5 mm because the apex narrower than 1.0 mm did not show revascularization of the pulp (Kling et al., 1986). Furthermore, using PKH26 for cell labeling ensured that the endothelial cell function remained unchanged, as reported by (Ford et al., 1996).

In the present study, subgroup IA (scaffold only) showed few unorganized granulation tissues and rare predentin. The formation of granulation tissue coincides with **El-Sherbiny et al. (2013),** who reported the effectiveness of self-assembled polypeptide scaffolds in forming tissue-like hydrogels. The authors reported poor mechanical features of these scaffolds; therefore, they are unsuitable for tissue engineering requiring high mechanical integrity. The rare predentin and blood vessels could be due to the absence of forming cells in this group.

Group II (ADSCs and scaffold) showed organized connective tissue and predentin after 60 days, which was thickened after 90 days, indicating continuous cellular activity. This conforms to the outcomes of Huang et al. (2010), who concluded that stem cells, when combined with scaffold, developed a dentin-like layer on the dentin walls. This could be due to the release of growth factors (Sloan et al., 2000), which induce odontoblastic differentiation of the stem cells (Huang et al.,2006). The differentiation of ADSCs into odontoblast-like cells was reported by Khazaei et al. (2021).

We detected collagenous connective tissue in group II, which agreed with a previous study that reported a high collagenous matrix containing odontoblast-like cells in the ADSCs group (Murakami et al., 2015). The calcified masses detected in our work could be due to the excellent osteoinductivity of the hydrogel scaffolds (Hao et al., 2023). However, this study could not achieve distinct zones of pulp tissue, which might be due to the lack of external growth factors.

In our work, Masson trichrome stain presented more vascularity in group II. This coincides with Huang et al. (2010) and King et al. (2014), who supported the role of stem cells in angiogenesis. ADSCs play a crucial role in new blood vessel formation, which might be due to the high vascular endothelial growth factor expression (Fujiwara et al., 2020). Furthermore, the ADSCs' secretomes could enhance angiogenesis through stimulation of differentiation and migration of endothelial cells (Mazini et al., 2020). Additionally, a hydrogel scaffold combined with stem cells might enable forming a capillary-like network, as Fuenteslópez et al. (2023) reported.

Our study's SEM examination revealed an intimate relationship between scaffold and root dentin in both groups. This coincides with Gotlieb et al. (2008), who observed cell adherence in pulp constructs with different scaffolds. Neunzehn et al. (2014) investigated stem cell-based pulp regeneration in root constructs and found that the developed dentin was attached to a fibrillar constitution.

5. Conclusions

Adipose-derived mesenchymal stem cells are an accessible alternative for dentin-pulp complex regeneration. Using a self-assembling polypeptide scaffold alone would not yield successful results.

CRediT authorship contribution statement

Khaled El-Haddad: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Investigation, Validation, Formal analysis, Methodology, Supervision. **Medhat A. El-Zainy:** Conceptualization, Writing – review & editing, Validation, Formal analysis, Methodology, Supervision. **Mohamed Nagy:** Conceptualization, Validation, Methodology, Supervision, Resources. **Iman Fathy:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization, Investigation, Validation, Formal analysis, Methodology, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Anderson, J.M., Rodriguez, A., Chang, D., 2008. Foreign body reaction to biomaterials. Semin Immunol. 20 (2), 86–100. https://doi.org/10.1016/j.smim.2007.11.004.

Bancroft, J.D., Gamble, M., 2002. Theory and practice of histological techniques, fifth ed. Churchill Livingstone publishers, Philadelphia.
Davies, O.G., Cooper, P., Shelton, R.M., Smith, A.J., Scheven, B.A., 2014. A comparison of the in vitro mineralisation and dentinogenic potential of mesenchymal stem cells derived from adipose tissue, bone marrow and dental pulp. J. Bone Miner. Metab. 33, 371–382. https://doi.org/10.1007/s00774-014-0601-y.
De Isla, N., Huseltein, C., Jessel, N., Pinzano, A., Decot, V., Magdalou, J., Bensoussan, D., Stoltz, J.F., 2010. Introduction to tissue engineering and application for cartilage engineering. Biomed. Mater. Eng. 20 (3), 127–133. https://doi.org/10.3233/BME-2010-0624.
El-Sherbiny, I.M., Yacoub, M.H., 2013. Hydrogel scaffolds for tissue engineering: Progress and challenges. Glob. Cardiol. Sci. Pract. 2013 (3), 316–342. https://doi.org/10.5339/gcsp.2013.38.
Fahimipour, F., Dashtimoghadam, E., Hasani-Sadrabadi, M.M., Vargas, J., Vashaee, D., Lobner, D.C., Kashi, T.S.J., Ghasemzadeh, B., Tayebi, L., 2019. Enhancing cell seeding and osteogenesis of MSCs on 3D printed scaffolds through injectable BMP2 immobilized ECM-mimetic gel. Dent. Mater. 35, 990–1006. https://doi.org/10.1016/j.dental.2019.04.004.
Ford, J.W., Welling, T.H., Stanley, J.C., Messina, L.M., 1996. PKH26 and 125I-PKH95: characterization and efficacy as labels for in vitro and in vivo endothelial cell localization and tracking. J. Surg. Res. 62 (1), 23–28. https://doi.org/10.1006/jsre.1996.0167.
Fuenteslópez, C.V., Thompson, M.S., Ye, H., 2023. Development and optimisation of hydrogel scaffolds for Microvascular network formation. Bioeng. 10 (8), 964. https://doi.org/10.3390/bioengineering10080964.
Fujiwara, O., Prasai, A., Perez-Bello, D., El Ayadi, A., Petrov, I.Y., Esenaliev, R.O., Petrov, Y., Herndon, D.N., Finnerty, C.C., Prough, D.S., Enkhbaatar, P., 2020. Adipose-derived stem cells improve grafted burn wound healing by promoting wound bed blood flow. Burns. Trauma. 8, tkaa009. https://doi.org/10.1093/burnst/tkaa009.
Galler, K.M., D'Souza, R.N., Federlin, M., Cavender, A.C., Hartgerink, J.D., Hecker, S., 2011. Dentin conditioning codetermines cell fate in regenerative endodontics. J. Endod. 37, 1536–1541. https://doi.org/10.1016/j.joen.2011.08.027.
Hao, L., Wang, A., Fu, J., Liang, S., Han, Q., Jing, Y., Li, J., Li, Q., Bai, S., Seeberger, P.H., Yin, J., 2023. Biomineralized dipeptide self-assembled hydrogel with ultrahigh mechanical strength and osteoinductivity for bone regeneration. Colloids Surf. a: Physicochem. Eng. Asp. 657 (A), 130622 https://doi.org/10.1016/j.colsurfa.2022.130622.
Hoang, D.M., Pham, P.T., Bach, T.Q., Ngo, A.T.L., Nguyen, Q.T., Phan, T.T.K., Nguyen, G. H., Le, P.T.T., Hoang, V.T., Forsyth, N.R., Heke, M., Nguyen, L.T., 2022. Stem cell-based therapy for human diseases. Signal. Transduct. Target. Ther. 7 (1), 272. https://doi.org/10.1038/s41392-022-01134-4.
Huang, G.T., Sonoyama, W., Chen, J., Park, S.H., 2006. In vitro characterization of human dental pulp cells: various isolation methods and culturing environments. Cell. Tissue. Res. 324 (2), 225–236. https://doi.org/10.1007/s00441-005-0117-9.
Huang, G.T., Yamaza, T., Shea, L.D., Djouad, F., Kuhn, N.Z., Tuan, R.S., Shi, S., 2010. 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. 16 (2), 605–615. https://doi.org/10.1089/ten.TEA.2009.0518.
Ilyas, M.N., Adzim, M.K.R., Simbak, N.B., Atif, A.B., 2017. Sample size calculation for animal studies using degree of freedom (E); an easy and statistically defined approach for metabolomic and genetic research. Curr. Trends. Biomed. Eng. & Biosci. 10 (2), 555785. https://doi.org/10.19080/CTBEB.2017.10.555785.
Iohara, K., Imabayashi, K., Ishizaka, R., Watanabe, A., Nabekura, J., Ito, M., Matsushita, K., Nakamura, H., Nakashima, M., 2011. Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue, Eng. Part A 17, 1911–1920. https://doi.org/10.1089/ten.tea.2010.0615.
Ishizaka, R., Iohara, K., Murakami, M., Fukuta, O., Nakashima, M., 2012. Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials. 33, 2109–2118. https://doi.org/10.1016/j.biomaterials.2011.11.056.
Jing, W., Wu, L., Lin, Y., Liu, L., Tang, W., Tian, W., 2008. Odontogenic differentiation of adipose-derived stem cells for tooth regeneration: necessity, possibility, and strategy. Med. Hypotheses. 70 (3), 540–542. https://doi.org/10.1016/j.mehy.2007.07.010.
Khazaei, S., Khademi, A., Nasr Esfahani, M.H., Khazaei, M., Nekoofar, M.H., Dummer, P. M.H., 2021. Isolation and Differentiation of Adipose-Derived Stem Cells into Odontoblast-Like Cells: A Preliminary In Vitro Study. Cell J. 23(3), 288-293. 10.22074/cellj.2021.7325.
Kim, S., Hwangbo, H., Chae, S., Lee, H., 2023. Biopolymers and their application in bioprinting processes for dental tissue engineering. Pharmaceutics. 15 (8), 2118. https://doi.org/10.3390/pharmaceutics15082118.
King, A., Balaji, S., Keswani, S.G., Crombleholme, T.M., 2014. The role of stem cells in wound angiogenesis. Adv. Wound. Care. (new Rochelle) 3 (10), 614–625. https://doi.org/10.1089/wound.2013.0497.
Kling, M., Cvek, M., Mejare, I., 1986. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors".Endod. Dent. Traumatol. 2 (3), 83–89. https://doi.org/10.1111/j.1600-9657.1986.tb00132.x.
Li, W., Hu, J., Chen, C., Li, X., Zhang, H., Xin, Y., Tian, Q., Wang, S., 2023. Emerging advances in hydrogel-based therapeutic strategies for tissue regeneration. Regen. Ther. 20 (24), 459–471. https://doi.org/10.1016/j.reth.2023.09.007.
Matichescu, A., Ardelean, L.C., Rusu, L.C., Craciun, D., Bratu, E.A., Babucea, M., Leretter, M., 2020. Advanced biomaterials and techniques for oral tissue engineering and regeneration—A review. Materials (basel). 13 (22), 5303. https://doi.org/10.3390/ma13225303.
Mazini, L., Rochette, L., Admou, B., Amal, S., Malka, G., 2020. Hopes and limits of adipose-derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) in wound healing. Int. J. Mol. Sci. 21 (4), 1306. https://doi.org/10.3390/ijms21041306.
Miana, V.V., González, E.A.P., 2018. Adipose tissue stem cells in regenerative medicine. Ecancermedicalscience. 12, 822. https://doi.org/10.3332/ecancer.2018.822.
Murakami, M., Hayashi, Y., Iohara, K., Osako, Y., Hirose, Y., Nakashima, M., 2015. Trophic effects and regenerative potential of mobilized mesenchymal stem cells from bone Marrow and adipose tissue as alternative cell sources for pulp/dentin regeneration. Cell Transplant. 24, 1753–1765. https://doi.org/10.3727/096368914X683502.
Neunzehn, J., Weber, M.T., Wittenburg, G., Lauer, G., Hannig, C., Wiesmann, H.P., 2014. Dentin-like tissue formation and biomineralization by multicellular human pulp cell spheres in vitro. Head. Face. Med. 10, 25. https://doi.org/10.1186/1746-160X-10-25.
Palumbo, P., Miconi, G., Cinque, B., La Torre, C., Lombardi, F., Zoccali, G., Orsini, G., Leocata, P., Giuliani, M., Cifone, M.G., 2015. In vitro evaluation of different methods of handling human liposuction aspirate and their effect on adipocytes and adipose derived stem cells. J. Cell. Physiol. 230 (8), 1974–1981. https://doi.org/10.1002/jcp.24965.
Qin, Y., Ge, G., Yang, P., Wang, L., Qiao, Y., Pan, G., Yang, H., Bai, J., Cui, W., Geng, D., 2023. An update on adipose-derived stem cells for regenerative medicine: where challenge meets opportunity. Adv. Sci. (weinh) 10 (20), e2207334.
Raposio, E., Caruana, G., Petrella, M., Bonomini, S., Grieco, M.P., 2016. A Standardized method of isolating adipose-derived stem cells for clinical applications. Ann. Plast. Surg. 76 (1), 124–126. https://doi.org/10.1097/SAP.0000000000000609.
Raposio, E., Simonacci, F., Perrotta, R.E., 2017. Adipose-derived stem cells: Comparison between two methods of isolation for clinical applications. Ann. Med. Surg. (lond) 20, 87–91. https://doi.org/10.1016/j.amsu.2017.07.018.
Si, Z., Wang, X., Sun, C., Kang, Y., Xu, J., Wang, X., Hui, Y., 2019. Adipose-derived stem cells: sources, potency, and implications for regenerative therapies. Biomed. Pharmacother. 114, 108765 https://doi.org/10.1016/j.biopha.2019.108765.
Sloan, A.J., Perry, H., Matthews, J.B., Smith, A.J., 2000. Transforming growth factor-beta isoform expression in mature human healthy and carious molar teeth. Histochem. J. 32 (4), 247–250. https://doi.org/10.1023/a:1004007202404. PMID: 10872890.
Tamburrino, A., Churchill, M.J., Wan, O.W., Colino-Sanguino, Y., Ippolito, R., Bergstrand, S., Wolf, D.A., Herz, N.J., Sconce, M.D., Björklund, A., Meshul, C.K., Decressac, M., 2015. Cyclosporin promotes neurorestoration and cell replacement therapy in pre-clinical models of Parkinson's disease. Acta Neuropathol. Commun. 3, 84. https://doi.org/10.1186/s40478-015-0263-6.
Yuan, Y., Zhang, X., Zhan, Y., Tang, S., Deng, P., Wang, Z., Li, J., 2022. Adipose-derived stromal/stem cells are verified to be potential seed candidates for bio-root regeneration in three-dimensional culture. Stem. Cell. Res. Ther. 13, 234. https://doi.org/10.1186/s13287-022-02907-y.
Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J., Benhaim, P., Lorenz, H.P., Hedrick, M.H., 2001. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue. Eng. 7 (2), 211–228. https://doi.org/10.1089/107632701300062859.