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ORIGINAL ARTICLE

A comparative study of the therapeutic effect of bone marrow mesenchymal stem cells versus insulin on mandibular dento-alveolar complex collagen formation and beta-catenin expression in experimentally induced type I diabetes

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KEYWORDS

β-catenin;
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Insulin;
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Abstract *Objective:* To assess and compare the therapeutic effect of bone marrow mesenchymal stem cells (BM-MSCs) versus insulin on mandibular dento-alveolar complex collagen formation and beta-catenin (β-catenin) expression in experimentally induced type I diabetes in albino rat.

Design: Twenty-eight male albino rats were equally divided as follows; Group I: was composed of rats which received no drug. The remaining rats were administrated a single streptozotocin (STZ) (40 mg/kg) intra-peritoneal injection. After affirmation of diabetes induction, the rats were divided into: Group II: Diabetic rats were given no treatment. Group III: Diabetic rats received a single BM-MSCs intravenous injection ($1x10^6$ cells). Group IV: Diabetic rats were given a daily insulin subcutaneous injection (5 IU/kg). After 28 days, mandibles were processed and stained by Hematoxylin & Eosin (H&E), Masson's trichrome and anti-β-catenin antibody. A statistical analysis was performed to measure positive area% of Masson's trichrome and β-catenin.

Results: Dento-alveolar complex tissues and cells of Group II showed destructive changes histologically, while Groups III and IV demonstrated improved histological features. Group II presented almost old collagen in all dento-alveolar complex tissues, and nearly negative β-catenin expression. Groups III and IV revealed a newly formed collagen intermingled with very few areas of old

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collagen, and both groups showed positive β-catenin immunoreactivity. Statistically, Groups III and IV represented the highest mean values of Masson's trichrome area% and β-catenin area%, while Group II reported the lowest mean.

Conclusions: Streptozotocin has a destructive effect on the dento-alveolar complex structure and function. BM-MSCs and insulin show regenerative capacity in STZ-affected periodontal tissues, and statistically, they increase collagen formation and β-catenin expression.

1. Introduction

Beta-catenin (β-catenin) is a constituent of Wingless/Integrated (Wnt) signal cascade and it triggers transcription of Wnt-specific genes which regulates cellular growth, development, and homeostasis (Valenta et al., 2012; Bao et al., 2021).

The Wnt/β-catenin signaling activation in dento-alveolar complex promotes cementum regeneration, periodontal ligament (PDL) formation (Lim et al., 2015), and alveolar bone osteoblast differentiation (Kobayashi et al., 2016). Conversely, inhibition of Wnt/β-catenin signaling following administration of streptozotocin (STZ) owing to hyperglycemia develops as consequence of impairment in insulin secretion by pancreatic β-cells (Chen et al., 2017; Salem et al., 2021).

Hyperglycemia associated with STZ-induced diabetes mellitus type I increases susceptibility to infections and periodontitis, that induces destruction of periodontal tissue, loss of alveolar bone, and impairs PDL regeneration (Kim et al., 2006; Preshaw et al., 2012).

Application of bone marrow-mesenchymal stem cells (BM-MSCs) is an innovative promising therapy for diabetes mellitus type I. They can differentiate into β-cells so increasing insulin secretion, protect β-cells from autoimmune attack by inhibiting T-cell proliferation, and improve the survival of β-cells by secreting cytokines that enhance antioxidant and proliferation ability of cells (Wan et al., 2022).

In contrast, daily insulin injection therapy is mandatory for diabetes mellitus type I to compensate total endogenous insulin deficiency because of autoimmune β-cells destruction (Forbes & Cooper, 2013). Both stem cells and insulin therapies can regulate Wnt/β-catenin pathway activation that elevates pancreatic β-cells proliferation and helps in tissue repair (Wang et al., 2017; Cabrae et al., 2020).

From abovementioned data, this research aimed to compare the therapeutic effect of bone marrow mesenchymal stem cells versus insulin on mandibular dento-alveolar collagen formation and β-catenin expression in experimentally induced type I diabetes in albino rat.

2. Materials and methods

Research Ethics Committee of Faculty of Dentistry, Ain Shams University, Cairo, Egypt, approved this study protocol under the approval number: FDASU-Rec IR092201.

2.1. BM-MSCs preparation

Hind limb long bones of male albino rats were cut and medulla was flushed by a syringe "18-gauge needle" containing (3 ml) low-glucose Dulbecco's modified Eagle's medium supplemented with (10%) fetal bovine serum and (1%) L-gluta mine/penicillin/streptomycin antibiotics. The collected bone marrow suspensions were cultured in polystyrene dishes, incubated in 5% humidified CO_2 at 37 °C. The culturing medium was changed each two days for two weeks until the adhering cells at the third passage reached 80–90% confluence (Mohsen et al., 2019).

2.2. Animals

Twenty-eight male Wistar albino rats (200–250 g) were housed in separate cages at the animal house of "Medical Research Center" of Ain-Shams University and kept in a controlled, clean environment with free access to food and water.

Rats were randomly assigned into four groups (Seven rats/group):

Group I (Control): received no drug.

The remaining rats were given a single intra-peritoneal injection of STZ (40 mg/kg) dissolved in 1 ml citrate buffer (pH: 4.5). For affirmation of diabetes induction, blood was taken from rats' tail vein after 72 h of STZ injection and defined diabetic if the readings of fasting blood glucose level were increased above (250 mg/dl) (Pari & Rathinam, 2016).

After diabetic confirmation, rats were randomly divided into:

Group II (Diabetic): seven diabetic rats received no treatment.

Group III (BM-MSCs treated): seven diabetic rats, each was given a single intravenous injection of BM-MSCs $1x10^6$ suspended in 1 ml phosphate buffered saline through tail vein (El Qashty et al., 2018).

Group IV (Insulin treated): seven diabetic rats received a single insulin subcutaneous injection (5 IU/kg)/day for 28 days (Pinheiro et al., 2011).

2.3. Specimen preparation

After 28 days from diabetes induction affirmation, rats were euthanized by ketamine overdose (148 mg/kg) (Rebuelto et al., 2002). Mandibles were carefully dissected, divided sagitally into two halves, fixed in 10% phosphate buffered formalin solution for five days, decalcified for 30 days in 10% ethylenediamine tetra-acetic acid solution, dehydrated, infiltrated and embedded in paraffin wax (Bancroft et al., 2013). Finally, sections of 4 μm thickness were stained by: hematoxylin and eosin (H&E), Masson's trichrome special stain and β-catenin immunohistochemical stain.

2.3.1. H&E stain

Sections were deparaffinized and rehydrated then stained by H&E (Bancroft et al., 2013).

2.3.2. Masson's trichrome special stain

After sections deparaffinization and rehydration, slides were soaked in Bouin's solution at 56 °C for 15 min, washed for 5 min by water, stained by Weigert's hematoxylin for 5 min, and then rinsed. Afterwards, stained for 5 min by Biebrich scarlet-acid fuchsin, rinsed, treated with phosphotungstic phosphomolybdic acid for 5 min, stained with aniline blue for 5 min, fixed for 2 min in 1% acetic acid, rinsed, dehydrated and mounted (Rieppo et al., 2019). New collagen areas were detected as blue color while old collagen areas were stained red.

2.3.3. β-catenin immunohistochemical staining

Sections used for immunolocalization of β-catenin were mounted on positive charged microscope slides. After deparaffinization, rehydration, and antigen retrieval completion, sections were placed in goat serum for 30 min to reduce nonspecific staining, incubated with primary antibody at dilution 1:200 (Rabbit polyclonal β-catenin antibody, Catalog # AHO0462), and incubated in secondary biotinylated antibody. Subsequently, washed in phosphate buffer and incubated with streptavidin. Diaminobenzidine was added to sections for 10 min to develop brown color. Finally, counterstaining was performed by using Mayer hematoxylin (Rabea, 2020). The brown cytoplasmic and nuclear staining is considered as positive immunoreaction (Liu et al., 2018).

Molar region of dento-alveolar complex was captured at a magnification (x200) and (x400) by using a digital video camera (TVO.5XC, Olympus, China).

2.4. Histomorphometric analysis

By using image J software (Version 1.41a, NIH, USA), the area% of newly formed collagen (blue color) in Masson's trichrome sections and positive cytoplasmic and nuclear immunoreactivity (brown color) in β-catenin immunohistochemical sections was measured. From each specimen, seven representative non-overlapping fields were captured at magnification 200x for Masson's trichrome sections and at 400x for β-catenin immunohistochemical sections. Images were then converted into 8-bit monochrome type and color thresholding was performed, so the selected areas were labeled by black, then finally area% was obtained.

2.5. Statistical analysis

Area% was analyzed using statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA) and presented as mean ± standard deviation and ranges. F - One-way analysis of test to compare between all groups, and Post Hoc test: Least Significant Difference for multiple comparisons between each two groups were used. Confidence interval was set to 95% with 5% accepted error margin. P-value was highly significant at ≤ 0.001, significant at ≤ 0.05, and non-significant at > 0.05 (Kotz et al., 2006; McBey et al., 2021).

3. Results

3.1. H&E results

3.1.1. Group I (Control)

Acellular cementum and alveolar bone exhibited regular outlines. Alveolar bone showed fibrocellular marrow cavities lined with osteoblasts. Dense, well organized and apparently normal PDL fibers were noticed (Fig. 1a). Multiple cementoblasts with basophilic cytoplasm were seen distributed on the cementoid tissue of both cementum types. Oval osteoblasts exhibited basophilic cytoplasm lining the alveolar bone surfaces were noticed. Cementocytes and osteocytes were observed. Spindle-shaped fibroblasts overlying PDL fibers were detected (Fig. 1b & c).

3.1.2. Group II (Diabetic)

Alveolar bone showed irregular outline with apparently wide marrow cavities (Fig. 1d). Some flat cementoblasts appeared on some areas and absent in others with no obvious cementoid tissue. Fewer cementocytes were apparently noticed in cellular cementum. Osteoblasts appeared flattened in some areas and lost in others. Alveolar bone showed reversal "scalloped" lines, many empty osteocytes lacunae in some areas and shrunken osteocytes with pyknotic nuclei in other areas. PDL seemed to be disorganized with some acellular areas. Flattened fibrocytes with dark nuclei were seen (Fig. 1e, f & g).

3.1.3. Group III (BM-MSCs treated)

Alveolar bone showed relatively regular outline and detectable reversal lines. PDL appeared dense with regularly arranged fibers (Fig. 2a). Some cementoblasts and osteoblasts showed regular shape, while others appeared flattened or absent in some areas. Some cementocytes and osteocytes appeared with ordinary shape, while others were shrunken and altered. Most of PDL fibrocytes exhibited spindle shape, while others appeared flattened (Fig. 2b & c).

3.1.4. Group IV (Insulin treated)

Both cementum and alveolar bone showed regular outline. PDL appeared dense and regularly arranged (Fig. 2d). Multiple cementoblasts and regular cementocytes were detected. Oval osteoblasts, many osteocytes and reversal lines were noticed in the alveolar bone. Plump fibroblasts and flattened fibrocytes overlying PDL fibers were observed (Fig. 2e, f & g).

3.2. Masson's trichrome results

3.2.1. Group I (Control)

The PDL revealed apparently wide areas of new collagen with few areas of old collagen. Cementum exhibited old collagen. Alveolar bone showed markedly large areas of old collagen intermingled with few areas of new collagen (Fig. 3a).

3.2.2. Group II (Diabetic)

Both alveolar bone and PDL revealed areas of old collagen intermingled with new one, while only old collagen area was noticed in cementum (Fig. 3b).

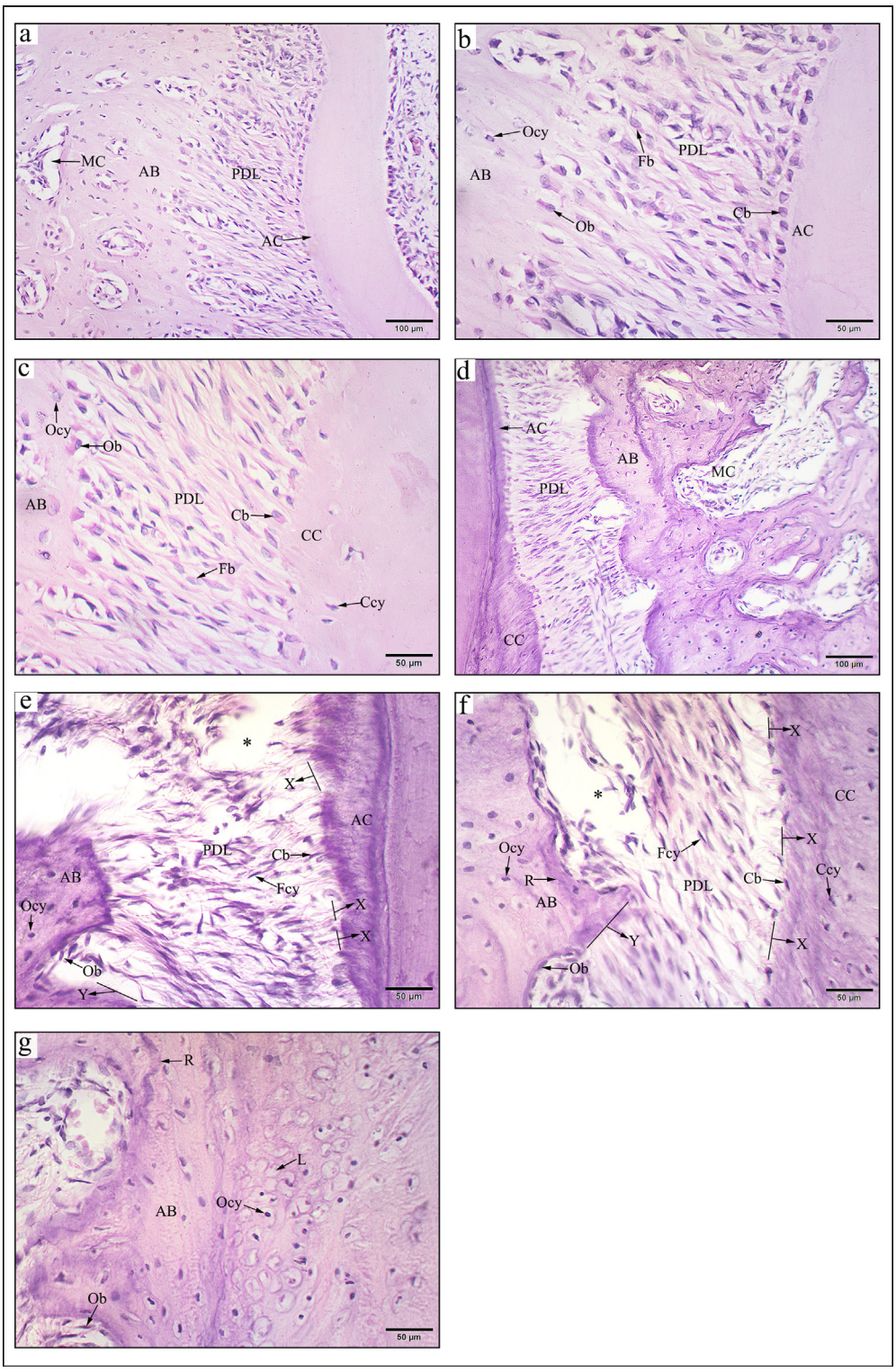

Fig. 1 Photomicrographs of dento-alveolar complex showing; Group I: **(a)-** Acellular cementum and alveolar bone with regular outlines. Alveolar bone with marrow cavities. Dense and apparently normal PDL. **(b)-** Acellular cementum, **(c)-** Cellular cementum showing; Multiple cementoblasts on the cementoid tissue. Oval osteoblasts. Cementocytes and osteocytes. Spindle-shaped fibroblasts overlying PDL fibers. Group II: **(d)-** Alveolar bone with irregular outline and wide marrow cavities. **(e)-** Acellular cementum, **(f)-** Cellular cementum showing; Some flat cementoblasts. Flattened osteoblasts with loss of lining in some areas. Fewer cementocytes and osteocytes. Disorganized PDL with some acellular areas and fibrocytes. **(g)-** Alveolar bone with reversal lines, many empty osteocytes lacunae and some with pyknotic nuclei. AC, acellular cementum; CC, cellular cementum; AB, alveolar bone; PDL, periodontal ligament; MC, marrow cavity; Cb, cementoblast; Ob, osteoblast; Ocy, osteocyte; Fb, fibroblast; Fcy, fibrocytes; Ccy, cementocyte; *, acellular areas of PDL; L, empty osteocyte lacuna; R, reversal line; X, areas of absent cementoblasts; Y, areas of absent osteoblasts **(H&E, Original magnification a & d x200, b, c, e, f & g x400)**.

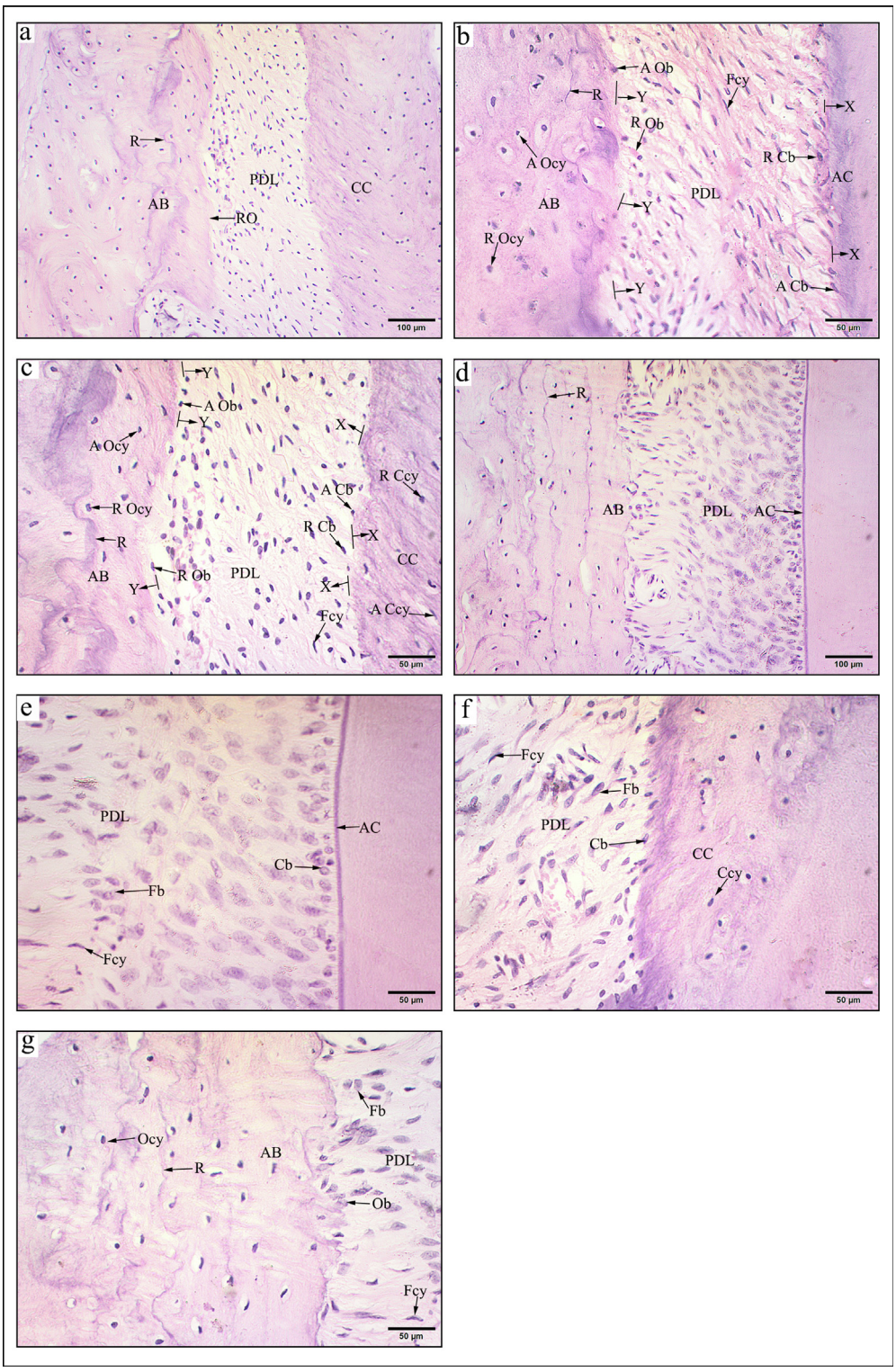

Fig. 2 Photomicrographs of dento-alveolar complex showing; Group III: **(a)-** Alveolar bone with relatively regular outline and reversal lines. Dense PDL with regularly arranged fibers. **(b)-** Acellular cementum, **(c)-** Cellular cementum showing; Some regular shape or flattened or absent cementoblasts and osteoblasts. Some ordinary cementocytes and osteocytes, while others shrunken and altered. Spindle or flattened fibrocytes. Group IV: **(d)-** Both cementum and alveolar bone with regular outline. **(e)-** Acellular cementum, **(f)-** Cellular cementum, **(g)-** Alveolar bone showing; Multiple cementoblasts. Regular cementocytes and osteocytes. Many reversal lines in the alveolar bone. Dense and regularly arranged PDL with fibroblasts and fibrocytes. AC, acellular cementum; CC, cellular cementum; AB, alveolar bone; PDL, periodontal ligament; Cb, cementoblast; Fb, fibroblast; Fcy, fibrocytes; Ccy, cementocyte; Ob, osteoblast; Ocy, osteocyte; R, reversal line; RO, regular outline; R Cb, regular cementoblast; A Cb, altered cementoblast; R Ob, regular osteoblast; A Ob, altered osteoblast; R Ocy, regular osteocyte; A Ocy, altered osteocyte; R Ccy, regular cementocyte; A Ccy, altered cementocyte; X, areas of absent cementoblasts; Y, areas of absent osteoblasts **(H&E, Original magnification a & d x200, b, c, e, f & g x400)**.

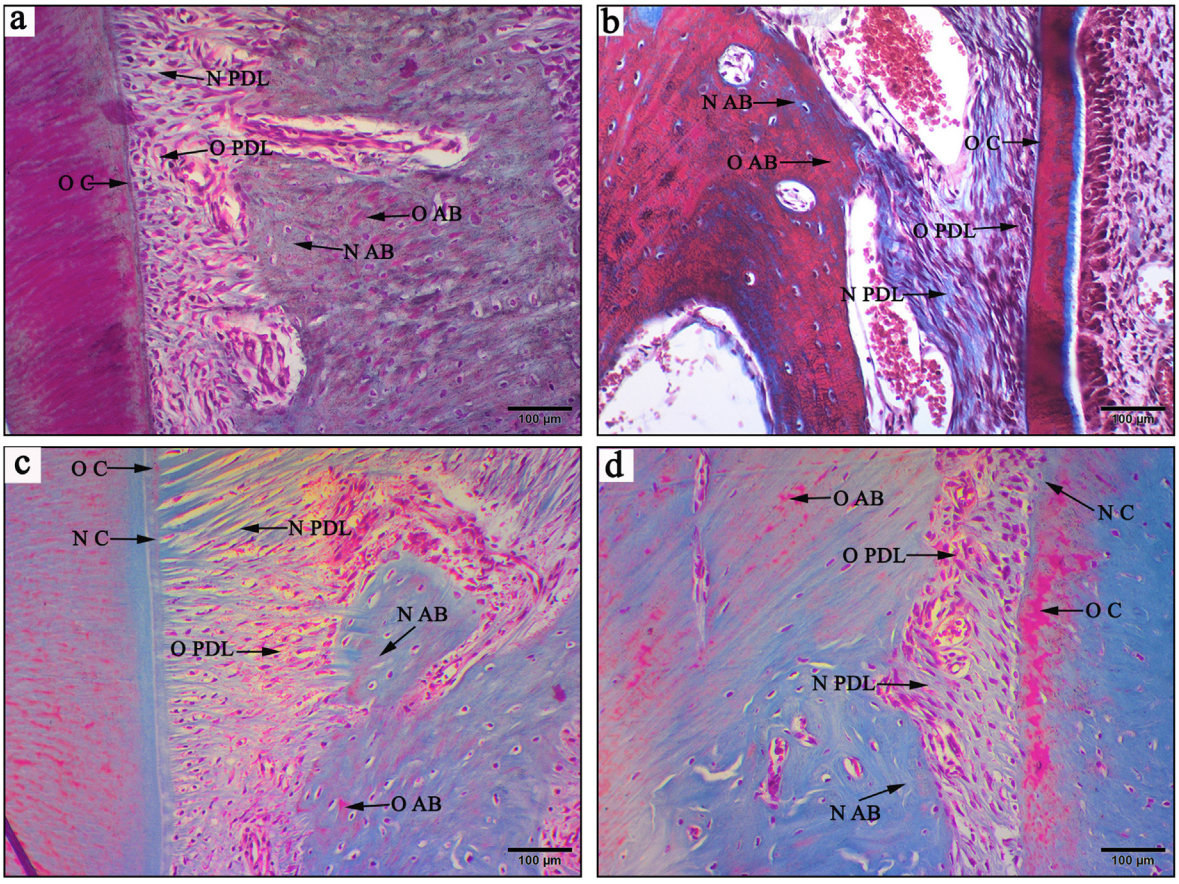

Fig. 3 Photomicrographs of dento-alveolar complex showing: **(a)-** Group I: PDL with wide areas of new collagen and few areas of old collagen. Cementum with old collagen. Alveolar bone with markedly large areas of old collagen and small areas of new collagen. **(b)-** Group II: Alveolar bone and PDL with areas of both old and new collagen. Cementum with only old collagen. **(c)-** Group III: Newly formed collagen in PDL, cementum and alveolar bone with very few areas of old collagen. **(d)-** Group IV: PDL with newly formed collagen. New collagen in cementum with minute layer of old collagen. Alveolar bone with new collagen except for some areas of old collagen. O PDL, Old collagen in periodontal ligament; N PDL, New collagen in periodontal ligament; O C, Old collagen in cementum; N C, New collagen in cementum; O AB, Old collagen in alveolar bone; N AB, New collagen in Alveolar bone **(Masson's trichrome, Original magnification x200)**.

3.2.3. Group III (BM-MSCs treated)

This group demonstrated newly formed collagen in PDL, cementum and alveolar bone with detection of very few areas of old collagen (Fig. 3c).

3.2.4. Group IV (Insulin treated)

The PDL showed newly formed collagen. Cementum illustrated new collagen with minute layer of old collagen. Alveolar bone revealed new collagen except for some areas of old collagen (Fig. 3d).

3.3. β-catenin immunohistochemical results

Group I showed noticeably positive β-catenin immunoreactivity in dento-alveolar complex tissues with positive cytoplasmic and nuclear immunoreactivity in tissue cells "PDL fibroblasts, cementoblasts, osteoblasts and osteocytes" (Fig. 4a). Group II represented nearly negative β-catenin immunoreactivity in dento-alveolar complex tissues and cells except for very few positive PDL fibroblasts (Fig. 4b). Group III and IV demonstrated positive β-catenin-immunoreactivity in the dento-alveolar complex tissues and cells (Fig. 4c & d).

3.4. Morphometric and statistical results

3.4.1. Masson's trichrome area%

Groups III and IV showed the significantly highest values of newly formed collagen with non-significant difference between them, followed by Group I. Group II represented the significantly lowest value (Table 1 & Fig. 5a).

3.4.2. β-catenin area%

Groups I, III and IV revealed the significantly highest values, with non-significant difference between them. Group II showed the significantly lowest value (Table 1 & Fig. 5b).

4. Discussion

Uncontrolled hyperglycemia raises two to three times the possibility of developing periodontal disease and subsequently

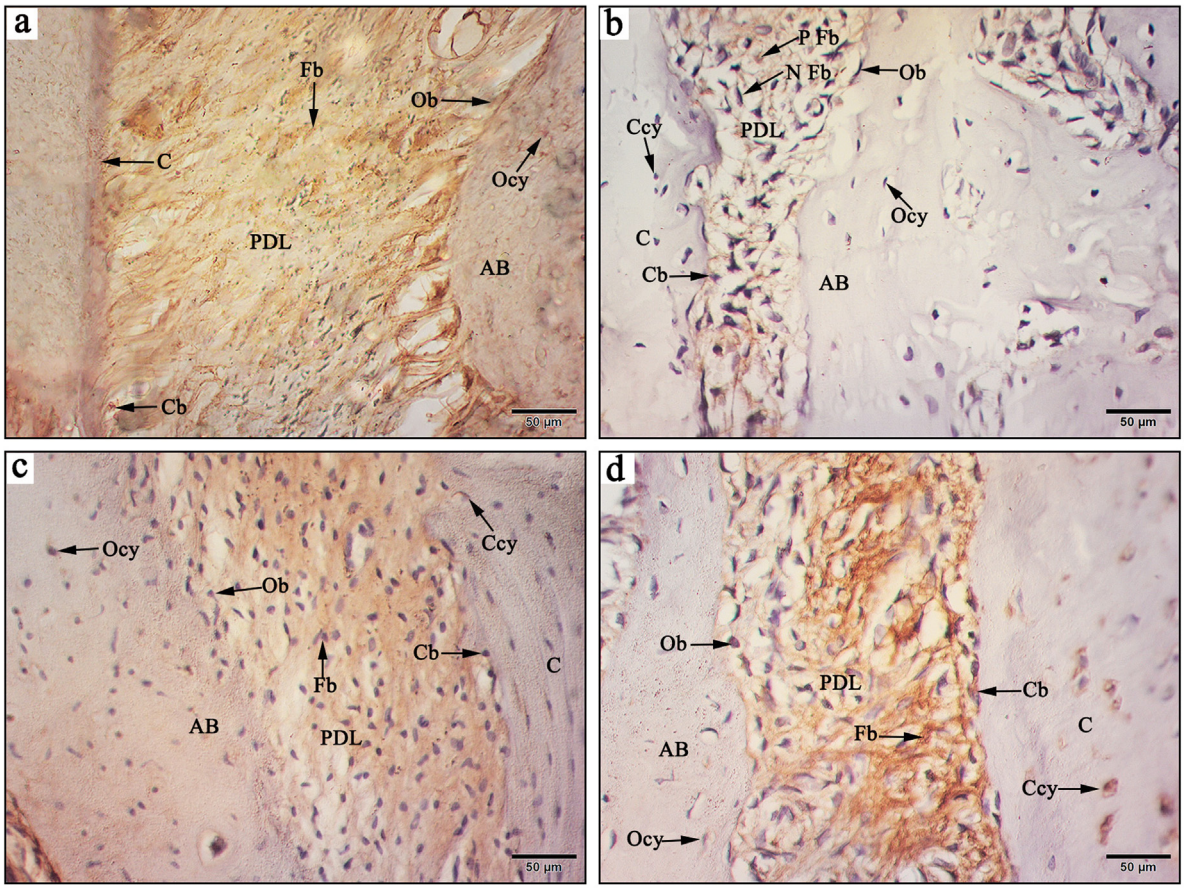

Fig. 4 Photomicrographs of dento-alveolar complex showing: **(a)-** Group I: Positive β-catenin immunoreactivity in dento-alveolar complex tissues with positive cytoplasmic and nuclear immunoreactivity in the tissue cells. **(b)-** Group II: Negative β-catenin immunoreactivity in dento-alveolar complex tissues, negative cytoplasmic and nuclear immunoreactivity in the tissue cells. Very few positive PDL fibroblasts. **(c & d)-** Group III & IV respectively: Positive β-catenin-immunoreactivity in the dento-alveolar complex tissues as well as positive cytoplasmic and nuclear immunoreactivity in the tissue cells. PDL, periodontal ligament; C, Cementum; AB, alveolar bone; Fb, fibroblast; P Fb, positive fibroblasts; N Fb, negative fibroblasts; Cb, cementoblast; Ccy, cementocyte; Ob, osteoblast; Ocy, osteocyte **(Anti-β-catenin antibody, Original magnification x400)**.

Table 1 Showing comparison between the groups according to Masson's trichrome and β-catenin area%.

		Group I (n = 7)	Group II (n = 7)	Group III (n = 7)	Group IV (n = 7)	F-test	p-value
Masson's trichrome area%	Mean ± SD	39.158 ± 7.820^{B}	20.323 ± 7.110^{C}	63.265 ± 14.871^{A}	70.260 ± 13.913^{A}	27.879	< 0.001**
	Range	28.740–51.848	11.660–26.591	42.548–82.510	52.820–85.080		
β-catenin area%	Mean ± SD	29.986 ± 10.865^{A}	7.880 ± 3.821^{B}	28.810 ± 10.324^{A}	34.146 ± 13.848^{A}	9.008	< 0.001**
	Range	14.189–44.502	2.522–14.100	16.973–43.877	16.673–53.300		

Using: F-One Way Analysis of Variance; **p-value < 0.001 is highly significant.
Values in each row which have different superscripts are significantly different at (P < 0.05) using Least Significant Difference test.

teeth loss (Demmer et al., 2012). Administration of exogenous insulin can ameliorate hyperglycemia; however, application of stem cells is considered a new treatment modality as it can regulate blood glucose level through preserving β-cells function (Chen et al., 2020).

The reported destructive changes of Group II in current study may be a result of increased blood glucose level that causes advanced glycation end products formation, vascular dysfunction, and collagen synthesis reduction (Ido et al., 2001; Santana et al., 2003; Nassar et al., 2007). Furthermore, hyperglycemia impairs immunological responses, enhances inflammation by increasing levels of inflammatory cytokines and C-reactive protein, reduces the production of anti-inflammatory cytokines and transforming growth factor β,

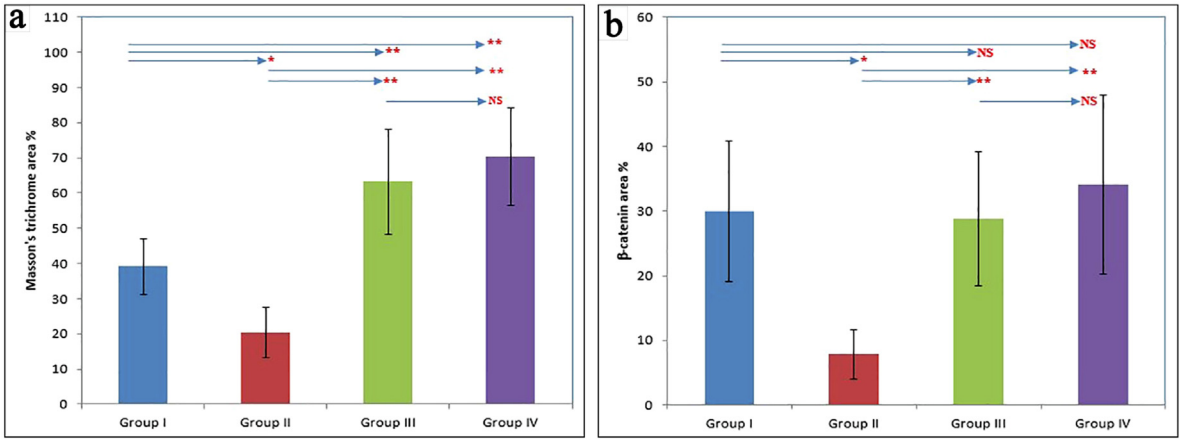

Fig. 5 Bar charts representing **(a)-**comparison between the groups according to Masson's trichrome area%. **(b)-**comparison between the groups according to β-catenin area%. p-value > 0.05 is non-significant (NS); *p-value ≤ 0.05 is significant; **p-value ≤ 0.001 is highly significant.

and influences tissue-degrading enzymes expression causing tissue damage (Knight et al., 2016; Liu et al., 2016; Bastos et al., 2017; Graves et al., 2020).

The detected histological changes of PDL and cementum of Group II could be linked to hyperglycemia that inhibits proliferation and differentiation of PDL fibroblasts, and increases production of matrix metalloproteinase-1 resulting in collagen fibers and other extracellular matrix components destruction (Ejeil et al., 2003; Kim et al., 2006; Yaras et al., 2008), beside significant decreases of both calcium contents and cementoblast activity (Balint et al., 2001; Amro et al., 2011). Yet, changes in the alveolar bone could be attributed to the diabetic affection on Wnt signaling pathway that impairs bone metabolism and stimulates bone loss (Hie et al., 2011; Reni et al., 2016). The reported cellular changes were considered as one of diabetic complications due to mitochondrial dysfunction, formation of pro-apoptotic factors and up-regulation of apoptosis regulating genes leading to increase in different periodontal cells apoptosis (Graves et al., 2007; Graves et al., 2020).

The reported improvement in Group III may be related to ability of injected BM-MSCs to stimulate proliferation of host progenitor cells, regulate expression of different chemokines, plus their ability to differentiate into multi-lineage tissues in vivo (Kawaguchi et al., 2004; Hasegawa et al., 2006; Wang et al., 2008). In addition to anti-inflammatory and immunomodulatory effects of BM-MSCs which contribute to tissue repair (Du et al., 2014).

The recorded regeneration in Group IV may be related to direct effects of insulin on lowering blood glucose level by promoting glucose uptake in muscle and adipose tissue, and suppressing liver gluconeogenesis (Saltiel & Kahn, 2001; Maekawa et al., 2017), to its immunosuppressive effect, and its ability to increase osteoblastic differentiation (Oh et al., 2015; Bilotta et al., 2018), also to anti-inflammatory effect on periodontal tissues (Nishikawa et al., 2020).

Masson's trichrome stain and statistical results Group II may be owing to diabetic status that decreases gene expression and growth factors in osteoblasts, and limits new bone extracellular matrix production (Lu et al., 2003). Additionally, it decreases collagen synthesis and increases collagen degradation in periodontal tissues as a consequence of hyperglycemia (Zhang et al., 2011).

The recorded Masson's trichrome area% of Group III is related to BM-MSCs ability to be differentiated into osteoblasts which synthesis immature collagen during early bone regeneration and remodeling (Wang et al., 2015). Furthermore, BM-MSCs can differentiate into fibroblasts and cementoblasts that produce immature collagen fibers of PDL, and immature principal collagen fibrils of newly formed cementum (Rezaei et al., 2019).

Masson's trichrome and statistical results of Group IV may be linked to insulin efficacy in maintaining structure and function of mitochondria, alongside with antioxidant potential of insulin against diabetic oxidative stress which contributes to progressive destruction of periodontal tissues (Song et al., 2018; Matsuzaki et al., 2021).

Negative β-catenin expression of alveolar bone in Group II could be attributed to reduction of insulin-like growth factor-1 receptor in osteoblasts that inhibits phosphorylation of glycogen synthase kinase 3β "a key regulator of Wnt signaling" causing degradation of β-catenin in osteoblasts and impair bone formation (Hie et al., 2011; Yee et al., 2016). Moreover, oxidative stress caused by hyperglycemia reduces differentiation and viability of periodontal tissue cells as well as decreases matrix production, alkaline phosphatase activity and mineralization (Kook et al., 2016; Houschyar et al., 2019).

The reported results of β-catenin-immunoreactivity in Group III could be related to BM-MSCs ability to secrete angiogenic and antioxidants factors, and to trigger several signaling pathways such as Wnt/β-catenin (Prockop, 2017; Pal & Das, 2017). Whilst, using insulin treatment in Group IV can activate Wnt/β-catenin signaling pathway (Cabrae et al., 2020). Activation of Wnt/β-catenin signaling allows tissue progenitor cells to be differentiated into various types of dentoalveolar complex cells helping in tissue repair and regeneration (Rezaei et al., 2019).

More studies are needed to evaluate the therapeutic effect of insulin and BM-MSCs on STZ-induced hyperglycemia on different dental tissues.

5. Conclusions

Streptozotocin-induced hyperglycemia has degenerative effect on structure of dento-alveolar complex, reduces the amount of newly formed collagen and decreases β-catenin expression. Both BM-MSCs and insulin have reparative role against destructive effect of STZ on periodontium, and statistically, they enhance new collagen production and increase β-catenin expression in all periodontal tissues.

6. Recommendation

It is recommended to investigate combined insulin and MSCs therapy which may give more pronounced reversal of morphological changes in dento-alveolar complex in diabetes Type I.

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This research is self-funded.

Ethical statement

Research Ethics Committe of Faculty of Dentistry, Ain Shams University, Cairo, Egypt, approved this study protocol under the approval number: FDASU-Rec IR092201.

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

Amro, S.O., Othman, H.I., El-Hamidy, S.M., 2011. Ultrastructure and microanalysis of root cementum in diabetic patients versus healthy patients with periodontitis. J. King Abdulaziz Univ. Med. sci. 18, 69–82.

Balint, E., Szabo, P., Marshall, C.F., Sprague, S.M., 2001. Glucose induced inhibition of in vitro bone mineralization. Bone 28 (1), 21–28.

Bancroft, J.D., Suvarna, K., Layton, C., 2013. Bancroft's Theory and Practice of Histological Techniques. Elsevier, Churchill Livingstone.

Bao, J., Yang, Y., Xia, M., Sun, W., Chen, L., 2021. Wnt signaling: an attractive target for periodontitis treatment. Biomed. Pharmacother. 133, (2021) 110935.

Bastos, M.F., Tucci, M., de Siqueira, A., de Faveri, M., Figueiredo, L. C., Vallim, P.C., Duarte, P.M., 2017. Diabetes may affect the expression of matrix metalloproteinases and their inhibitors more than smoking in chronic periodontitis. J. Periodontal Res. 52, 292–299.

Bilotta, F.L., Arcidiacono, B., Messineo, S., Greco, M., Chiefari, E., Britti, D., et al, 2018. Insulin and osteocalcin: further evidence for a mutual cross-talk. Endocrine 59, 622–632.

Cabrae, R., Dubuquoy, C., Caüzac, M., Morzyglod, L., Guilmeau, S., Noblet, B., et al, 2020. Insulin activates hepatic Wnt/β-catenin signaling through stearoyl-CoA desaturase 1 and Porcupine. Sci. Rep. 10, 5186.

Chen, S., Du, K., Zou, C., 2020. Current progress in stem cell therapy for type 1 diabetes mellitus. Stem Cell Res. Ther. 11 (275), 1–13.

Chen, A.C.H., Lee, Y.L., Fong, S.W., Wong, C.C.Y., Ng, E.H.Y., Yeung, W.S.B., 2017. Hyperglycemia impedes definitive endoderm differentiation of human embryonic stem cells by modulating histone methylation patterns. Cell Tissue Res. 368, 563–578.

Demmer, R.T., Holtfreter, B., Desvarieux, M., Jacobs Jr, D.R., Kerner, W., Nauck, M., Völzke, H., Kocher, T., 2012. The influence of type 1 and type 2 diabetes on periodontal disease progression: prospective results from the Study of Health in Pomerania (SHIP). Diabetes Care 35 (10), 2036–2042.

Du, J., Shan, Z., Ma, P., Wang, S., Fan, Z., 2014. Allogeneic bone marrow mesenchymal stem cell transplantation for periodontal regeneration. J. Dent Res. 93, 183e8.

Ejeil, A.L., Igondjo-Tchen, S., Ghomrasseni, S., Pellat, B., Godeau, G., Gogly, B., 2003. Expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in healthy and diseased human gingiva. J. Periodontol. 74, 188–195.

El Qashty, R.M.N., Mohamed, N.N., Radwan, L.R.S., Ibrahim, F.M. M., 2018. Effect of bone marrow mesenchymal stem cells on healing of temporomandibular joints in rats with induced rheumatoid arthritis. Eur. J. Oral. Sci. 126, 272–281.

Forbes, J.M., Cooper, M.E., 2013. Mechanisms of diabetic complications. Physiol. Rev. 93 (1), 137–188.

Graves, D.T., Liu, R., Oates, T.W., 2007. Diabetes-enhanced inflammation and apoptosis: impact on periodontal pathosis. Periodontology 2000 (45), 128–137.

Graves, D.T., Ding, Z., Yang, Y., 2020. The impact of diabetes on periodontal diseases. Periodontology 2000 (82), 214–224.

Hasegawa, N., Kawaguchi, H., Hirachi, A., Takeda, K., Mizuno, N., Nishimura, M., et al, 2006. Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. J. Periodontol. 77, 1003–1007.

Hie, M., Iitsuka, N., Otsuka, T., Tsukamoto, I., 2011. Insulin-dependent diabetes mellitus decreases osteoblastogenesis associated with the inhibition of Wnt signaling through increased expression of Sost and Dkk1 and inhibition of Akt activation. Int. J. Mol. Med. 28, 455–462.

Houschyar, K.S., Tapking, C., Borrelli, M.R., Popp, D., Duscher, D., Maan, Z.N., et al, 2019. Wnt pathway in bone repair and regeneration–what do we know so far. Front. Cell Dev. Biol. 6 (170), 1–13.

Ido, Y., Chang, K.C., Lejuene, W.S., Bjercke, R.J., Reiser, K.M., Williamson, J.R., Tilton, R.G., 2001. Vascular dysfunction induced by AGE is mediated by VEGF via mechanisms involving reactive oxygen species, guanylate cyclase, and protein kinase C. Microcirculation 8, 251–263.

Kawaguchi, H., Hirachi, A., Hasegawa, N., Iwata, T., Hamaguchi, H., Shiba, H., et al, 2004. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J. Periodontol. 75, 1281–1287.

Kim, H.S., Park, J.W., Yeo, S.I., Choi, B.J., Suh, J.Y., 2006. Effects of high glucose on cellular activity of periodontal ligament cells in vitro. Diabetes Res. Clin. Pract. 74, 41–47.

Knight, E.T., Liu, J., Seymour, G.J., Faggion Jr., C.M., Cullinan, M. P., 2016. Risk factors that may modify the innate and adaptive immune responses in periodontal diseases. Periodontology 2000 71 (1), 22–51.

Kobayashi, Y., Uehara, S., Udagawa, N., Takahashi, N., 2016. Regulation of bone metabolism by Wnt signals. J. Biochem. 159, 387–392.

Kook, S.H., Lee, D., Cho, E., Heo, J.S., Poudel, S.B., Ahn, Y., et al, 2016. Activation of canonical Wnt/b-catenin signaling inhibits H_2O_2-induced decreases in proliferation and differentiation of human periodontal ligament fibroblasts. Mol. Cell. Biochem. 411, 83–94.

Kotz, S., Balakrishnan, N., Read, C.B., Vidakovic, B., 2006. In: Encyclopedia of Statistical Sciences,, Volume 1. John Wiley & Sons.

Lim, W.H., Liu, B., Mah, S., Yin, X., Helms, J.A., 2015. Alveolar bone turnover and periodontal ligament width are controlled by Wnt. J. Periodontol. 86, 319–326.

Liu, C., Feng, X., Li, Q., Wang, Y., Li, Q., Hua, M., 2016. Adiponectin, TNF-alpha and inflammatory cytokines and risk of type 2 diabetes: a systematic review and meta-analysis. Cytokine 86, 100–109.

Liu, X., Zhang, Z., Pan, S., Shang, S., Li, C., 2018. Interaction between the Wnt/β-catenin signaling pathway and the EMMPRIN/MMP-2, 9 route in periodontitis. J. Periodontal. Res. 53 (5), 842–852.

Lu, H., Kraut, D., Gerstenfeld, L.C., Graves, D.T., 2003. Diabetes interferes with the bone formation by affecting the expression of transcription factors that regulate osteoblast differentiation. Endocrinology 144, 346–352.

Maekawa, S., Katagiri, S., Takeuchi, Y., Komazaki, R., Ohtsu, A., Udagawa, S., Izumi, Y., 2017. Bone metabolic microarray analysis of ligature-induced periodontitis in streptozotocin-induced diabetic mice. J Periodontal Res. 52, 233–245.

Matsuzaki, S., Eyster, C., Newhardt, M.F., Giorgione, J.R., Kinter, C., Young, Z.T., et al, 2021. Insulin signaling alters antioxidant capacity in the diabetic heart. Redox Biol. 47, 102140.

McBey, D.P., Dotzert, M., Melling, C.W.J., 2021. The effects of exercise training versus intensive insulin treatment on skeletal muscle fibre content in type 1 diabetes mellitus rodents. Lipids Health Dis. 20 (64), 1–12.

Mohsen, R.O.M., Halawa, A.M., Hassan, R., 2019. Role of bone marrow-derived stem cells versus insulin on filiform and fungiform papillae of diabetic albino rats (light, fluorescent and scanning electron microscopic study). Acta Histochemica 121, 812–822.

Nassar, H., Kantarci, A., van Dyke, T.E., 2007. Diabetic periodontitis: a model for activated innate immunity and impaired resolution of inflammation. Periodontology 2000 (43), 233–244.

Nishikawa, T., Suzuki, Y., Sawada, N., Kobayashi, Y., Nakamura, N., Miyabe, M., et al, 2020. Therapeutic potential for insulin on Type 1 diabetes-associated periodontitis: analysis of experimental periodontitis in streptozotocin-induced diabetic rats. J. Diabetes Investig. 11, 1482–1489.

Oh, J.H., Lee, J.Y., Joung, S.H., Oh, Y.T., Kim, H.S., Lee, N.K., 2015. Insulin enhances RANKL-induced osteoclastogenesis via ERK1/2 activation and induction of NFATc1 and Atp6v0d2. Cell Signal 27, 2325–2331.

Pal, B., Das, B., 2017. In vitro culture of naïve human bone marrow mesenchymal stem cells: a stemness based approach. Front. Cell Dev. Biol. 5, 1–19.

Pari, L., Rathinam, A., 2016. Anti-diabetic effect of myrtenal on plasma and tissue glycoproteins components in stz induced experimental diabetic rats. J. Dis. Medicinal Plants 2, 11–16.

Pinheiro, L.S., Melo, A.D., Andreazzi, A.E., Júnior, L.C.C., Costa, M. B., Garcia, R.M.G., 2011. Protocol of insulin therapy for Streptozotocin-diabetic rats based on a study of food ingestion and glycemic variation. Scand. J. Lab. Anim. Sci. 38, 117–127.

Preshaw, P.M., Alba, A.L., Herrera, D., Jepsen, S., Konstantinidis, A., Makrilakis, K., Taylor, R., 2012. Periodontitis and diabetes: a two-way relationship. Diabetologia 55 (1), 21–31.

Prockop, D.J., 2017. The exciting prospects of new therapies with mesenchymal stromal cells. Cytotherapy 19, 1–8.

Rabea, A.A., 2020. Histological, histochemical and immunohistochemical evaluation of the role of bone marrow-derived mesenchymal stem cells on the structure of periodontal tissues in carbimazole-treated albino rats. Arch. Oral Biol. 119, 104887.

Rebuelto, M., Ambros, L., Montoya, L., Bonafine, R., 2002. Treatment-time-dependent difference of ketamine pharmacological response and toxicity in rats. Chronobiol. Int. 19 (5), 937–945.

Reni, C., Mangialardi, G., Meloni, M., Madeddu, P., 2016. Diabetes stimulates osteoclastogenesis by acidosis-induced activation of transient receptor potential cation channels. Sci. Rep. 6, 30639.

Rezaei, M., Jamshidi, S., Saffarpour, A., Ashouri, M., Rokn, A.R., 2019. Transplantation of bone marrow–derived mesenchymal stem cells, platelet-rich plasma, and fibrin glue for periodontal regeneration. Int. J. Periodontics Restorative Dent. 39 (1), 32–45.

Rieppo, L., Janssen, L., Rahunen, K., Lehenkari, P., Finnilä, M.A.J., Saarakkala, S., 2019. Histochemical quantification of collagen content in articular cartilage. PLoS One 14 (11), e0224839.

Salem, M.A., Budzynska, B., Kowalczyk, J., El Sayed, N.S., Mansour, S.M., 2021. Tadalafil and bergapten mitigate streptozotocin-induced sporadic Alzheimer's disease in mice via modulating neuroinflammation, PI3K/Akt, Wnt/beta-catenin. AMPK/mTOR signaling pathways. Toxicol Appl Pharmacol. 429, 115697.

Saltiel, A., Kahn, C., 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414, 799–806.

Santana, R.B., Xu, L., Chase, H.B., Amar, S., Graves, D.T., Trackman, P.C., 2003. A role for advanced glycation end products in diminished bone healing in type 1 diabetes. Diabetes 52, 1502–1510.

Song, Y., Ding, W., Bei, Y., Xiao, Y., Tong, H.D., Wang, L.B., Ai, L. Y., 2018. Insulin is a potential antioxidant for diabetes-associated cognitive decline via regulating Nrf2 dependent antioxidant enzymes. Biomed. Pharmacother. 104, 474–484.

Valenta, T., Hausmann, G., Basler, K., 2012. The many faces and functions of β-catenin. EMBO J. 31 (12), 2714–2736.

Wan, X.X., Zhang, D.Y., Khan, M.A., Zheng, S.Y., Hu, X.M., Zhang, Q., Yang, R.H., Xiong, K., 2022. Stem cell transplantation in the treatment of type 1 diabetes mellitus: from insulin replacement to beta-cell replacement. Front Endocrinol. 18, (13) 859638.

Wang, Y., Deng, Y., Zhou, G.Q., 2008. SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model. Brain Res. 1195, 104–112.

Wang, L., Qing, L., Liu, H., Liu, N., Qiao, J., Cui, C., et al, 2017. Mesenchymal stromal cells ameliorate oxidative stress-induced islet endothelium apoptosis and functional impairment via Wnt4-β-catenin signaling. Stem Cell Res. Ther. 8 (188), 1–13.

Wang, Y., Zhou, L., Li, C., Xie, H., Lu, Y., Wu, Y., Liu, H., 2015. Bone marrow-derived cells homing for self-repair of periodontal tissues: a histological characterization and expression analysis. Int. J. Clin. Exp. Pathol. 8 (10), 12379–12389.

Yaras, N., Sariahmetoglu, M., Bilginoglu, A., Aydemir-Koksoy, A., Onay-Besikci, A., Turan, B., Schulz, R., 2008. Protective action of doxycycline against diabetic cardiomyopathy in rats. Br J Pharmacol. 155, 1174–1184.

Yee, C.S., Xie, L., Hatsell, S., Hum, N., Murugesh, D., Economides, A.N., Loots, G.G., Collett, N.M., 2016. Sclerostin antibody treatment improves fracture outcomes in a Type I diabetic mouse model. Bone 82, 122–134.

Zhang, L., Li, X., Bi, L.J., 2011. Alterations of collagen-I, MMP-1 and TIMP-1 in the periodontal ligament of diabetic rats under mechanical stress. J. Periodontal Res. 46, 448–455.