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Effect of local and systemic inflammation on gingival mesenchymal stem cells

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Abstract

Background. The present study was undertaken to determine the effect of local and systemic inflammation on the quantity and localization of gingival mesenchymal stem cells (gMSCs).

Methods. Gingival samples were collected from 34 systemically healthy (group 1) and 10 consenting adult patients with type II diabetes mellitus (T2DM) (group 2), who had reported for various dental treatments, requiring excision of gingival tissues as part thereof. The tissue samples were further stratified into three categories: healthy gingiva, gingivitis-affected and periodontitis-affected. Samples mounted on slides were stained with hematoxylin and eosin (H&E) while CD105 antibody was used for immunohistochemistry staining. Immuno-positive cells were identified as gMSCs. Three gMSC-populated areas in each sample were selected to determine the density of gMSCs.

Results. The density of gMSCs was significantly higher ($P<0.05$) in sections of gingival tissues affected by gingivitis and periodontitis compared to those of normal gingiva. However, there were no significant differences in the densities of gMSCs in tissues of patients with T2DM and those of healthy subjects.

Conclusion. Local inflammatory status appeared to increase the density of gMSCs. In the presence of periodontitis, an added low-grade systemic inflammation (T2DM) did not appear to affect the density of gMSCs.

Key words: **Type II diabetes mellitus, gingivitis, immunohistochemistry, mesenchymal stem cells, periodontitis.**

Introduction

Mesenchymal stem cells (MSCs) are non-hematopoietic, adherent fibroblast-like cells and are described as multipotent stromal cells that can differentiate into a variety of cell types.[1] MSCs exist in almost every adult tissue, such as bone marrow, neural and oral tissues. They have the intrinsic ability of self-renewal and multi-lineage differentiation. Of those, MSCs derived from umbilical cord blood and adipose tissues are capable of giving rise to at least three cell lineages: osteogenic, chrondogenic, and adipogenic. Mesenchymal stem cells derived from bone marrow may give rise to other li-

neages such as myogenic, neurogenic and tenogenic cells.[2] This makes stem cells crucial in the repair or regeneration of injured tissues; as demonstrated in bone morrow-derived MSCs by displaying mobilization and homing properties similar to immune cells in response to injuries and inflammations.[3] The production of anti-apoptotic molecules and antiinflammatory cytokines at the sites of injury are critical in the MSC-mediated anti-inflammatory activities.[4] The minimum criteria prescribed to identify a cell as MSCs are to: (a) remain plastic-adherent under standard culture conditions; (b) express CD105, CD73, and CD90, and lack expression of CD45, CD34, CD14, CD19, and HLA-DR; and (c) differentiate into osteoblasts, adipocytes, and chondrocytes in vitro.[5] Recent research has identified oral tissues as an accessible and feasible alternative source for MSCs.[6-9] The orofacial region contains a variety of MSC populations, including dental pulp stem cells, periodontal ligament stem cells, apical papilla stem cells, and dental follicle stem cells.[6] In comparison with the well-studied bone marrow-derived MSCs (BMSCs), the characteristics and behavior of MSCs derived from oral tissues remain largely unexplored. Gingiva-derived MSCs (gMSCs) were isolated and characterized as having multi-lineage differentiation capacity and immunomodulatory properties.[4] It was found that gMSCs express CD29, CD44, CD73, CD90, CD105, CD146, and STRO-1.[10] These findings suggested that human gingival tissues act as a unique reservoir for progenitor stem cells.

Gingivitis is a reversible form of inflammation in response to the presence of dental plaque biofilm without the destruction of the periodontium.[11-12] Periodontitis is a microbial-induced inflammatory disorder of the periodontium, characterized by the destruction of the periodontal attachment.[13] Tang et al isolated and characterized gMSCs from normal and hyperplastic gingival tissues and found that they displayed highly clonogenic and long-term proliferative capability, as well as self-renewal and multipotent differentiation properties.[14] Stem cells isolated from inflamed human periodontal ligament (PDL) exhibited enhanced migratory potential when compared to stem cells isolated from healthy human PDL.[8] Studies on PDL stem cells and dental pulp stem cells revealed that inflammation leads to activation of latent stem and progenitor cells. However, no study has investigated the effects of local inflammation on the number of gMSCs.

Type II diabetes mellitus (T2DM) is a common disorder, caused by a combination of genetic and non-genetic factors that result in insulin resistance and insulin deficiency.[15-17] A chronic low-grade inflammation exists in patients with T2DM[18] and epidemiological studies have reported an increase in plasma levels of inflammatory markers like CRP, IL-6 and TNF-α in T2DM patients.[19,20] Hyperglycaemia in diabetes leads to increased non-enzymatic protein glycation and the formation of advanced glycation end products (AGEs) which cause the apoptosis observed in MSCs,[21] which in turn is responsible for the reduced numbers of stem cells found in diabetic subjects.

To date, no studies have evaluated the effect of both local inflammation (gingivitis and periodontitis) and systemic inflammation (T2DM) on gMSCs. The aim of this study was to determine whether local and systemic inflammation affect the number of gMSCs. We hypothesized that more gMSCs would be present in the gingiva of patients with local inflammation when compared to those with healthy periodontium. We also hypothesized that there would be a reduction in the number of gMSCs when there is an added low-grade systemic inflammation (T2DM). We expected that these cells would be localized near the blood vessels of inflamed connective tissues.

Methods

A pilot cross-sectional clinical study was conducted to compare the effect of diabetes and local periodontal inflammation on the quantity of gMSCs. The study was conducted in accordance to standard ethical principles and had the approval of the university's Joint-Committee of Research and Ethics (Research No. IMU264/2012).

Collection of human gingival samples

Human gingival samples were collected from two groups of subjects. Group 1 comprised systemically healthy subjects (n=34) and group 2 consisted of type II diabetes mellitus (T2DM) subjects (n=10). Each group comprised subjects with healthy gingiva, gingivitis and periodontitis. Healthy periodontium was defined as having periodontal probing depths of not more than 2 mm and bleeding on probing in ≤15% probing sites. Gingivitis was defined as having periodontal probing depths of ≤3 mm with bleeding on probing in at least 25% of probing sites. Periodontitis was defined as the presence of at least one site with probing depths of ≥5 mm. T2DM was defined on the basis of: (1) a reported physician diagnosis, and (2) use of medications to control the disease. Subjects with borderline diabetes, immunocompromised subjects and those who were on immunosuppressants were excluded.

All the gingival tissues were obtained during dental procedures based on the treatment plan of each subject. This ensured that the samples were collected as part of their planned treatment and not solely for the purpose of this research. Written consent was obtained from all the participants. The samples collected were fixed in 10% formalin solution.

Laboratory procedure

The samples were embedded in paraffin blocks and serial sectioning was prepared in 4-µm thicknesses. The sections were dewaxed in xylene and hydrated through graded ethanol. Staining with hematoxylin and eosin (H&E) and immunochemistry reactions were carried out to confirm the inflammatory status of every sample and to identify gMSCs, respectively.

Immunohistochemistry

Each immunostaining procedure was carried out at room temperature (32°C). CD105 (Endoglin) was selected as a surface marker to identify gMSCs. The sections were treated with and placed in preheated citrate buffer at pH=6 and then heated at 99°C for 20 minutes. The slides were left to cool to room temperature before rinsing in PBS solution. Peroxidase-blocking solution was used to block endogenous peroxidase activity. A serial dilution of the antibody was carried out for optimization. The 1:1000 ratio of antibodies gave rise to optimum staining and the sections were incubated with this concentration of primary antibody.

The sections were then rinsed with phosphate-buffered saline (PBS) solution and a secondary antibody was applied. The sections were then washed again in PBS solution. Finally, the sections were counterstained with hematoxylin and eosin for light microscope analysis.

Histological analysis

The slides were placed under a light microscope (Nikon Eclipse 80i Microscope) for examination. H&E-stained sections were used to identify the presence of inflammation. The immunohistochemically-stained slides were used to quantify gMSCs. Three areas in each section containing most densely populated immune-positive fibroblast-like cells (gMSC) were selected for quantification. Before quantification of the cells, the examiners were trained and calibrated to achieve reliable statistical results. Intra- and inter-examiner reliability values ≥0.75 were achieved prior to the actual quantification of gMSC. The results were expressed as density of gMSCs (number of cells/300 μm^2). Data were analyzed with SPSS 20, using two-tailed independent t-test and the level of statistical significance was set at $P<0.05$.

Results

Human gingival tissues contain cells expressing mesenchymal stem cells markers

H&E-stained sections revealed that under local periodontal inflammation, tissue samples exhibited thickened and elongated rete ridges with abundant cellularity and extracellular matrix within the lamina propria, as well as increased proliferation of blood vessels when compared to that of healthy gingival samples (Figure 1). Samples of gingival lamina propria from both the healthy and T2DM group exhibited positive staining for CD105 (Figure 2, yellow arrow). The gMSCs exhibited typical fibroblastic morphology with round or oval-shaped, intense hematoxylin-stained nuclei. The distribution of gMSCs was mainly located in the paravascular regions (Figure 2, red arrow).

Locally inflamed gingival tissues showed increased number of gMSCs

More areas of cells with positive staining were observed in the gingival samples from gingivitis and periodontitis sites than that in the healthy gingiva. In the 13 healthy gingival samples studied, the mean gMSCs density was 24.54 cells/300 μm^2. The mean density of the 12 gingivitis-affected tissue samples was 30.22 cells/300 μm^2. In the 9 periodontitis-affected gingival samples, the mean density was 33.74 cells/300 μm^2. The density of gMSCs present

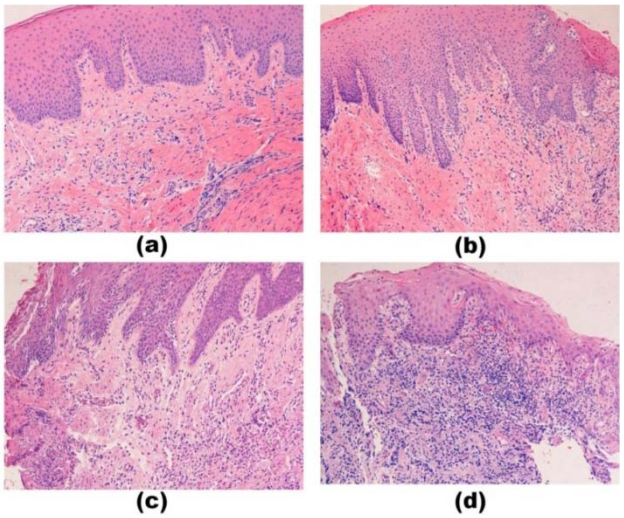

Figure 1. H&E staining of human gingival tissues, magnification ×20; (a) healthy gingival tissues; (b) systemically healthy, gingivitis-affected gingival tissues; (c) systemically healthy, periodontitis-affected gingival tissues; and (d) T2DM, periodontitis-affected sample.

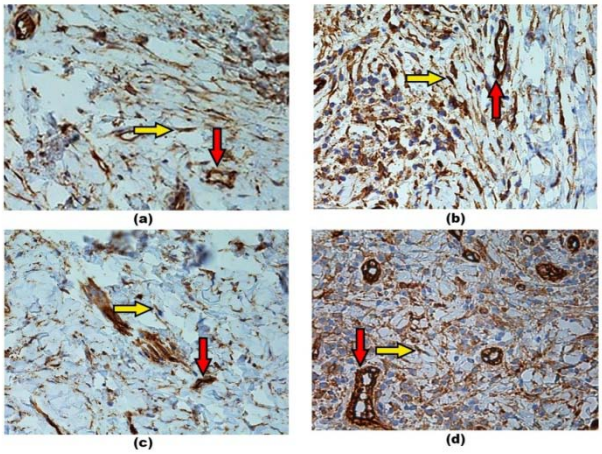

Figure 2. CD105 surface antigen in the lamina propria of human gingival samples, magnification ×40; (a) healthy gingival tissues from systemically healthy patients; (b) systemically healthy gingivitis-affected gingival tissues; (c) systemically healthy periodontitis-affected gingival tissues; and (d) T2DM periodontitis-affected sample.

in diseased tissues was significantly more than that of normal tissues (Tables 1 and 2). Periodontitis-affected samples displayed a greater density of gMSCs when compared to gingivitis-affected samples, but this increase was not significant (Table 3).

T2DM did not affect the number of gMSCs in patients with periodontitis

Due to the small sample size for both healthy and gingivitis subjects of the T2DM group, these subjects were not included in the independent t-test analysis. However, little difference was noticed in the density and distribution of gMSCs in both groups. In the presence of added low-grade systemic inflammation due to T2DM, the density of gMSCs from periodontitis-affected samples in group 2 (27.33 ± 9.10 cells/300 μm^2) was less than that in group 1 (33.74 ± 4.63 cells/300 μm^2). However, this difference was not statistically significant (Table 4).

Table 1. Comparison of gMSCs density expressed as cells/300 μm^2 in samples from healthy periodontium and from those with gingivitis

Gingival health	Healthy	Gingivitis
N	13	12
Mean ± SD (cells/300 μm^2)	24.54 ± 7.56	30.22 ± 4.98
P-value	0.038 (significant)	

Table 2. Comparison of gMSCs density expressed as cells/300 μm^2 in samples from healthy periodontium and from those with periodontitis

Gingival health	Healthy	Periodontitis
N	13	9
Mean ± SD (cells/300 μm^2)	24.54 ± 7.56	33.74 ± 4.63
P-value	0.002 (significant)	

Table 3. Comparison of gMSCs density expressed as cells/300μm^2 in samples from gingivitis and periodontitis-affected samples

Gingival health	Gingivitis	Periodontitis
N	12	9
Mean ± SD (cells/300 μm^2)	30.22 ± 4.98	33.74 ± 4.63
P-value	0.056 (not significant)	

Discussion

In this study, we hypothesized that more gMSCs would be present in the gingiva of patients with local inflammation (gingivitis and periodontitis) and a decrease in gMSC counts when an added low-grade inflammation (T2DM) was present. We confirmed that gMSCs were present within the gingival connective tissues through the expression of CD105 cell surface marker.[10] The identified gMSCs tended to aggregate in the vicinity of blood vessels.

There are currently various sources of dental tissue stem cells with therapeutic uses, such as stem cells obtained from the dental pulp, exfoliated deciduous teeth, periodontal ligament, apical papilla and gingiva. However, most of these sources of stem cells require tooth extraction. The human gingiva has been found to be an excellent but least invasive source for MSCs. They are considered to be genuinely renewable because regeneration of gingiva after injury usually takes place in a short time period. Moreover, the gingiva is easily accessible in the oral cavity and can even be easily obtained as a discarded biosample.

In the present study, we compared the density of gMSCs in localized inflammation with that in a healthy state. In both healthy and diseased gingivae, immunostaining showed paravascular positivity for the CD105 marker. Our results were supported by the findings of Chen et al in 2006, who reported that the progenitor cells are located in areas 0–20 μm from the perimeter of blood vessels.[22] CD105 surface antigen was also expressed by the endothelial cells of blood vessels.[23] This could explain the non-specific positive staining of peripheral vasculature which might be mistaken for gMSCs.

Gingivitis is a reversible form of inflammation in

Table 4. Comparison of gMSCs density expressed as cells/300μm^2 in samples from systemically healthy patients with periodontitis and from T2DM patients with periodontitis

Systemic health	Healthy	T2DM
N	9	7
Mean ± SD (cells/300 μm^2)	33.74 ± 4.63	27.33 ± 9.10
P-value	0.126 (not significant)	

Table 5. ANOVA to compare results between each group (HH= healthy gingiva samples from systemically healthy subjects; HG=gingivitis-affected samples from systemically healthy subjects; HP=periodontitis-affected samples from systemically healthy subjects; DP=periodontitis-affected samples from T2DM subjects)

	Number	Mean ± SD (cells/300 μm^2)	P-value
HH	13	24.54 ± 7.56	
HG	12	30.22 ± 4.98	0.019
HP	9	33.74 ± 4.63	(significant)
DP	7	27.33 ± 9.10	

response to mature dental plaque biofilm without the involvement of the periodontium.[11,12] Periodontitis is an inflammatory condition which induces periodontal tissue connective matrix destruction, results in the loss of fibrous attachment and alveolar bone resorption, and impairs new bone formation.[24] The increase in the number of stem cells during inflammation can be attributed to two reasons: (1) increased migratory capacity and (2) increased proliferative activity.[3,8,25] Currently, it is still questionable which of these two plays a bigger role. In both gingivitis and periodontitis where an inflammatory microenvironment exists, this is always associated with the activation of inflammatory cells like macrophages, neutrophils, and adaptive immune cells. Cytokines that are released include IL-1β, TNF-α, IL-12, IL-1α and many more.[12] The increase in stem cells during inflammation is thought to be due to the migratory activity of cells in response to inflammatory mediators (TNF-α, IL-1β, free radicals, chemokines and leukotrienes) that are produced by phagocytes in response to damaged cells and spilled cell contents.[8] The migratory process of MSCs involves the interaction between released chemokines/growth factors at the site of injury and chemokine receptors on the MSC. Hence, the entire event eventually orchestrates changes in the microenvironment that result in the mobilization and differentiation of MSCs to replace damaged tissue cells. In 2011, Park et al reported that the proliferative potential did not differ between healthy and inflamed periodontal ligament MSCs; however, the migratory capacity significantly increased in the inflamed group.[8] Although the density of gMSCs in periodontitis-affected group was higher than that of the gingivitis group, this was not statistically significant. This could be due to the similar entities of local inflammation between gingivitis and periodontitis, hence affecting the number of gMSCs the same way. However, future studies are needed to understand the exact reason.

In this study, the increase in the number of gMSCs in gingivitis and periodontitis could potentially be due to an increase in migratory capacity of the cells. Subclinical low-grade systemic inflammation and abnormalities of virtually all the systemic indicators of inflammation have been reported in T2DM.[26-30] This includes increases in acute phase proteins, cytokines, mediators associated with endothelial activation and chemokines. It has been reported in previous studies that the presence of a hyperglycaemic-induced oxidative stress in diabetes mellitus subjects impairs the migration of MSCs.[31] Hyperglycaemia in diabetes mellitus generates oxidative stress, which in turn causes damage to the cells. Oxidative stress occurs when free radical production exceeds the body's ability to neutralize them. The imbalance happens due to decreased antioxidant production, and when free radicals are produced in excess. High glucose levels produce reactive oxygen species as a result of glucose auto-oxidation, metabolism and development of advanced glycosylation end products. Apart from that, inflammation is intensified with significant release of pro-inflammatory chemokines and/or cytokines from MSCs. This phenomenon, with diabetic milieu, further damages cellular function, mitigating the therapeutic effects of MSCs in diabetes.[32]

Although a local inflammatory state (periodontitis and gingivitis) increases the number of gMSCs significantly, we found no difference in the number of gMSCs when an added systemic inflammation (T2DM) was present in periodontitis subjects. T2DM could have mitigated the increase in the number of gMSCs due to local inflammation as seen in periodontitis-affected samples. However, the exact mechanism is still unknown and should be addressed in future studies.

One of the strengths of the present study was the quantification of gMSCs when comparing local and systemic inflammation on the periodontium. This has not been carried out in other studies. The use of immunohistochemistry in identifying gMSCs has already been adopted by several studies.[6,10,22] Quantification of gMSCs in our study was achieved by counting the cells in each focus, instead of using flow cytometry which was a more reliable method. However, the density of gMSCs could not be ascertained by the flow cytometry technique which led us to use the present technique for quantification. There were limitations as stem cells in the present study were not cultured as well as stained by additional stem cell markers and the counting procedures were quite subjective to the identification of gMSCs by different examiners. However, these limitations were

minimized by intensive calibration and assessment with adequate agreement among examiners.

Conclusion

Gingival mesenchymal stem cell populations can be localized by using antibodies against cell-surface antigens known to be expressed on these cells (CD105). Our study highlighted the possible changes of gMSCs populations in a state of local inflammation (i.e. gingivitis and periodontitis) and systemic inflammation. Local periodontal inflammation increased while T2DM did not appear to affect the number of gMSCs in patients with periodontitis. Therefore, for therapeutic purposes in the future, this opens up the pool where gMSCs can be harvested from, regardless of the diabetic status of the patient. Gingival MSCs can be harvested from inflamed gingival tissues as an alternative and easily accessible source of multipotent stem cells for clinical and research uses. Studies have already begun to recognize gMSCs as a therapeutic potential for clinical application. Substantial advances in stem cell research have been made in the laboratory, and application of these advances in the clinical practice is a challenge to overcome. Given the limitations of this study, further clarification is necessary to establish the role of the apparent increase in gMSC counts in inflammatory conditions and whether these cells migrate into the inflamed area or necessarily replicate.

References

1. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietictissues. Transplantation 1968; 6:230-247. doi: 10.1097/00007890-196803000-00009
2. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009; 88:792-806. doi: 10.1177/0022034509340867
3. Hannoush EJ, Elhassan I, Sifri ZC, Mohr AA, Alzate WD, Livingston DH. Role of bone marrow and mesenchymal stem cells in healing after traumatic injury. Surgery 2013; 153:44-51. doi: 10.1016/j.surg.2012.06.020
4. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, Le AD. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009; 183:7787-7798. doi: 10.4049/jimmunol.0902318
5. Lv FJ, Tuan RS, Cheung KM, Leung VY. Concise review: the surface markers and identity of human mesenchymal stem cells. Stem Cells. 2014; 32:1408-19. doi: 10.1002/stem.1681
6. Xu X, Chen C, Akiyama K, et al. Gingivae contain neural-crest- and mesoderm-derived mesenchymal stem cells. J Dent Res. 2013; 92:825-832. doi: 10.1177/0022034513497961
7. Marynka-Kalmani K1, Treves S, Yafee M, Rachima H, Gafni Y, Cohen MA, Pitaru S. The lamina propria of adult human oral mucosa harbors a novel stem cell population. Stem Cells. 2010; 28:984-995. doi: 10.1002/stem.425
8. Park JC, Kim JM, Jung IH, et al. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: in vitro and in vivo evaluations. J Clin Periodontol. 2011; 38:721-731. doi: 10.1111/j.1600-051x.2011.01716.x
9. Gao Y, Zhao G, Li D, Chen X, Pang J, Ke J. Isolation and multiple differentiation potential assessment of human gingival mesenchymal stem cells. Int J Mol Sci. 2014; 15:20982-20996. doi: 10.3390/ijms151120982
10. Zhang QZ, Nguyen AL, Yu WH, Le AD. Human oral mucosa and gingiva: a unique reservoir for mesenchymal stem cells. J Dent Res. 2012; 91:1011-1018. doi: 10.1177/0022034512461016
11. Kistler JO, Booth V, Bradshaw DJ, Wade WG. Bacterial community development in experimental gingivitis. PLoS One. 2013; 8:1-13. doi: 10.1371/journal.pone.0071227
12. Boronat-Catalá M, Catalá-Pizarro M, Bagán Sebastián JV. Salivary and crevicular fluid interleukins in gingivitis. J Clin Exp Dent. 2014; 6:175-179. doi: 10.4317/jced.51403
13. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000. 1997; 14:216-248. doi: 10.1111/j.1600-0757.1997.tb00199.x
14. Tang L, Li N, Xie H, Jin Y. Characterization of Mesenchymal Stem Cells from Human Normal and Hyperplastic Gingiva. J Cell Physiol2011; 226: 832-842. doi: 10.1002/jcp.22405
15. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care2013; 36:S67–74. doi: 10.2337/dc13-s067
16. Rosenbloom AL, Joe JR, Young RS, Winter WE. Emerging epidemic of type 2 diabetes in youth. Diabetes Care 1999; 22: 345-354. doi: 10.2337/diacare.22.2.345
17. Harris MI. Diabetes Mellitus: A Fundamental and Clinical Text, 3rd Edn. Definition and classification of diabetes mellitus and the criteria for diagnosis. Lippincott Williams& Wilkins, Philadelphia, 2004: 457–67. doi: 10.2337/diaclin.22.2.71
18. Eschwège E. The dysmetabolic syndrome, insulin resistance and increased cardiovascular (CV) morbidity and mortality in type 2 diabetes: aetiological factors in the development of CV complications. Diabetes Metab 2003; 29: 6S19-6S27. doi: 10.1016/s1262-3636(03)72784-0
19. Pickup JC, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sci2000 67: 291-300. doi: 10.1016/s0024-3205(00)00622-6
20. Mirza S, Hossain M, Mathews C, Martinez P, Pino P, Gay JL, et al. Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. Cytokine 2012; 57: 136-142. doi: 10.1016/j.cyto.2011.09.029
21. Alexandra S, Donna S, Owen L, Andy S. Diabetes Induced Changes in Rat Mesenchymal Stem Cells. Cells Tissues Organs 2010; 191: 453-465. doi: 10.1159/000281826
22. Chen SC, Marino V, Gronthos S, Bartold PM. Location of Putative Stem Cells in Human Periodontal Ligamen. J Peri-

odontal Res 2006; 41: 547-553. doi: 10.1111/j.1600-0765.2006.00904.x
23. Duff SE, Li CG, Garland JM, S. Kumar. CD105 is important for angiogenesis: evidence and potential applications. FASEB J 2003; 17: 984-992. doi: 10.1096/fj.02-0634rev
24. Silva N, Dutzan N, Hernandez M, Dezerega A, Rivera O, Aguillon JC, et al. Characterization of Progressive Periodontal Lesions in Chronic Periodontitis Patients: Levels of Chemokines, Cytokines, Matrix Metalloproteinase-13, Periodontal Pathogens and Inflammatory Cells. J Clin Periodontol 2008; 35: 206-21. doi: 10.1111/j.1600-051x.2007.01190.x
25. Girolamo LD, Lucarelli E, Alessandri G, Avanzini MA, Bernardo ME, Biagi E et al. Mesenchymal Stem/Stromal Cells: A New 'Cells as Drugs' Paradigm. Efficacy and Critical Aspects in Cell Therapy. Current Pharmaceutical Design 2013; 19: 2459-2473. doi: 10.2174/1381612811319130015
26. Cogan DG, Merola L, Laibson PR. Blood viscosity, serum hexosamine and diabetic retinopathy. Diabetes 1961; 10: 393-395. doi: 10.2337/diab.10.5.393
27. Bergstrand CG, Furst P, Larsson Y, Sterky G. Serum haptoglobin in juvenile diabetes. Scan J Clin Lab Invest1962; 14: 629-632. doi: 10.1080/00365516209051294
28. Ganrot PO, Gydell K, Ekelun H. Serum concentration of alpha-2-macroglubulin, haptohglobin and alpha-1-antitrypsin. Acta Endocrinol (Copenh) 1967; 55: 537-544. doi: 10.1530/acta.0.0550537
29. Cleve H, Alexander K, Mitzkat HJ, Nissen P,Salzmann I. Serum glycoproteins in diabetes mellitus; quantitative immunological determination of acid alpha 1-glycoprotein, Gc, alpha 2-machoglobulin and hemopexin in diabetics without angiopath. Diabetoligia 1968; 4: 48-55. doi: 10.1007/bf01241033
30. McMilan DE. Changes in serum proteins and protein bound carbohydrates in diabetes mellitus. Diabetoligia 1970; 6: 597-604. doi: 10.1007/bf00418228
31. Yang K, Wang XQ, He YS, Lu L, Chen QJ, et al. Advanced glycation end products induce chemokine/cytokine production via activation of p38 pathway and inhibit proliferation and migration of bone marrow mesenchymal stem cell. Cardiovasc Diabetol 2010; 9: 1-10. doi: 10.1186/1475-2840-9-66
32. Ponte AL, Marais E, Gallay N, Lagonne A, Delorme B, Herault O, et al. The in vitro Migration Capacity of Human Bone Marrow Mesenchymal Stem Cells: Comparison of Chemokine and growth factor for chemotactic activities. Stem Cells 2007; 25: 1737-1745. doi: 10.1634/stemcells.2007-0054