*Acta Odontologica Scandinavica,* 2013; 71: 626–631

informa
healthcare

**ORIGINAL ARTICLE**

# Effect of osteoblast cell culture on the bone implant contact

F. HOŞGÖR[1], N. YILMAZ[1], Ö. ŞENYURT[1], S. GÜMÜŞOVA[2], B. ÇAM[3], G. CEYLAN[4], C. YARDIMCI[5] & F. ALPARSLAN PINARLI[6]

[1]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Ondokuz Mayıs University, Samsun, Turkey,* [2]*Department of Virology, Faculty of Veterinary, Ondokuz Mayıs University, Samsun, Turkey,* [3]*Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Çukurova University, Adana, Turkey,* [4]*Department of Prosthodontics, Faculy of Dentistry, Ondokuz Mayıs University, Samsun, Turkey,* [5]*Department of Surgery, Faculty of Veterinary, Ondokuz Mayıs University, Samsun, Turkey, and* [6]*Department of Medical Biology, Division of Genetics, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey*

**Abstract**

***Purpose.*** The aim of this study is to acquire an ideal bone implant contact under the cover of osteogenic effect of osteoblasts derived from Mesenchymal Stem Cells (MSCs). ***Materials and methods.*** Thirty dental implants were used for this study. Implants were placed in sheep mandibles and defects were created 4 mm coronally in the dental implants. These defects were filled with Platelet Rich Plasma (PRP) in one group and with PRP + Osteoblast Cell Culture (OCC) in another group. No procedure was conducted on the control group defects (empty defect group). Eight weeks later, osseointegration was investigated with Bone Implant Contact (BIC) measurements histomorphologically. Data were checked statistically. ***Results.*** The variation of BIC rates between Empty Defect Group and PRP groups was significant ($p < 0.05$). The BIC rate of the PRP group was higher than that of the Empty Defect Group. The variation of BIC rates between Empty Defect Group and PRP + OCC groups was significant ($p < 0.05$). The BIC rate of the PRP + OCC group was higher than that of the Empty Defect Group. The variation of BIC rates between PRP and PRP + MSC groups was significant ($p < 0.05$). The BIC rate of the PRP + OCC group was higher than that of the PRP group. At the end of the 8-week healing period, it was observed that the percentage of BIC was highest in the PRP + OCC group. ***Conclusions.*** Implant–bone connection was better in the OCC–PRP group compared with the PRP group and the empty defect group. The use of OCC-PRP combination was effective on healing. The BIC value was increased significantly by OCC.

**Key Words:** *cell culture, dental implant.*

## Introduction

The use of pure titanium implants has been stated in the literature for prosthetic rehabilitations of the edentulous alveolar crest by Branemark et al. [1] in 1969. It has been mentioned that implant materials and surface topography are critical factors which effect Bone Implant Contact (BIC) [2,3].

Anabolic agents such as recombinant bone morphogenic protein (rhBMP2), Mesenchymal Stem Cell (MSC) and parathyroid hormon (PTH) were used in order to increase the quantity of osseointegration and shorten the healing period [4,5]. MSCs originating from bone marrow are the precursor cells of mesenchymal cells. These cells are derived easily. MSCs are differentiated to osteoblasts, chondroblasts, tenocites, adiposites, muscle cells or neural cells in *in-vivo* or *in-vitro* media [6,7]. Much of the current literature points out that the MSCs derived from bone marrow have advantages in treatment of cranial defects and increase osseointegration between the dental implant and bone [8,9].

Platelet Rich Plasma (PRP) is used with MSC as a source for growth factor and structure framework in different tissue engineering procedures [10,11]. PRP contains autologous thrombocyte growth factors and might be promising for acceleration of bone regeneration [12,13].

The aim of this study was to evaluate the effectiveness of Osteoblast Cell Culture (OCC) and PRP on

Correspondence: N. Yilmaz, Associate Professor, Ondokuz Mayıs University, Department of Oral and Maxillo-Facial Surgery, Faculty of Dentistry, Samsun, 55139 Turkey. E-mail: nergizy@omu.edu.tr

*(Received 9 March 2012; revised 25 April 2012; accepted 3 May 2012)*

ISSN 0001-6357 print/ISSN 1502-3850 online 
DOI: 10.3109/00016357.2012.700066

bone healing and osseointegration in the defects experimentally created around the dental implant.

## Materials and method

Thirty dental implants were used for this study. Implants were used in five male sheep, each having a body weight of ~ 40 kg. An essential animal ethic report was received from Ondokuz Mayis University, Animal Ethical Committee. Sedation was performed with a combination of Xylacine hydrocloride Xylazine hydrochloride (Rompun®, Bayer, İstanbul, Turkey) 2 mg/kg IM and Ketamine (Alfamyne®, Egevet, İzmir, Turkey) 20 mg/kg IM. Isoflurane (Forane Liquid Abbott. Illinois, USA) was used for general anesthesia. Six dental implants measuring 3.3 mm in diameter and 8 mm in length (Straumann; Basel, Switzerland ) were placed extraorally to the mandibular corpus bilaterally in each sheep. In order to obtaine primer stabilization, an 8 mm implant socket was prepared with a 2.8 mm diameter final drill. Then, circumferential defects were created coronally 4 mm with a 4.1 mm diameter drill in the same socket.

The study groups were as follows: Group 1: Served as a control group and implants were placed. No procedure was conducted (empty defect group). Group 2: Defects were filled with PRP. Group 3: Defects were filled with PRP + OCC.

Mixtures were prepared to fill defects completely and the neck of the implant inserted was wider than the body of the implant. Because of the mechanical barrier provided by the implant neck, a membrane was not used. After surgery, antibiotherapy was performed with Amoxyciline + Clavulonic acid (Synulox, Pfizer®, Turkey). Eight weeks later, osseointegration was investigated with BIC measurements histomorphologically.

### *Culturation of MSC*

The bone marrow was inspired to 10 ml with a heparin syringe from sheep iliac bone. Then it was diluted in a 1:1 proportion with Phosphate Bufferred Saline (PBS) and Mononuclear cell (MNC) separation was performed in a centrifugation tube with ficoll (1.007 g/ml) according to the density gradient methods. To this end, two units of ficoll were added to one unit content of bone marrow with 45° inclination and centrifugated at 800 g rpm for 20 min. At the end of the centrifugation, the MNC layer was gathered with a pipette on the bottom of the centrifugation tube. The MNCs samples were washed with PBS twice and centrifugated for removal of ficoll from the cell layer. MNCs which were present at the bottom of the centrifugation tube were suspended with 20% of fetal calf sera (FCS) and DMEM with 1% of the antibiotic suspension (Sigma Sıp4333), then MNCs were cultured in a cell culture flask under standard culture conditions (incubation at 37°C, 5% $CO_2$ in air). When MNCs covered ~ 75% of the cell culture flask, cells were removed from the bottom of the flask with trypsinization (SIT4174-Trypsin-EDTA Solution). This method was performed until a significant amount of cells were gathered.

### *Osteogenic differentiation of MSC*

MNCs were cultured in osteogenic differentiation media and included 1 μM dexamethasone (Sigma Sıd6645), 10 μM *β*-glycerophosphate, 0,05 mM ascorbate and FCS (Fluka FL49752) for osteogenic differentiation of MNCs. Approximately 3 weeks after the cultivation, differentiation of MNCs was completed. Osteogenic differentiated cells were analysed for their alkaline phosphatase activites by biochemical assays and calcium oxalate accumulation microscopically.

### *Alkaline phosphatase analyses*

Osteogenic differentiated cells culture medium samples were thawed at 25°C just prior to the assay. Alkaline phosphatase (ALP) activity of the culture medium was measured by the p-nitrophenol method and was based on the principle that the enzymes hydrolyze paranitrophenyl phosphate to paranitrophenol and phosphate in an alkaline medium. This method has been formulated in accordance with the kinetic determination of the ALP. Analysis of the enzyme activity was performed using a biochemistry autoanalyser (Autolab, AMS Srl, Selective Access) by commercial kits (Audit Diagnostics, Ireland) according to the manufacturer's instruction. Analytical sensitivity of this assay was 2 U/L. The absorbance was read at 405 nm [14]. All samples were analyzed in duplicate and results are presented as the average.

### *Preparation of PRP*

The blood taken from the jugular vein of sheep was placed in a tube which included 4.5 ml anticoagulant citrate dextrose phosphate (ACDA). The tubes were centrifugated at 1250 rpm for 15 min and plasma and platelets were separated from the blood sample. This plasma and platelets were centrifugated at 4000 rpm for 10 min in order to separate the platelet from the plasma. Then PRP was obtained. Platelet concentration of PRP was $1.5–2.3 \times 10^9$. PRP was then mixed with CaCl and a gel form was obtained in group II. PRP and OCC were then mixed with CaCl and a gel form was obtained in group III. Gel form was useful for application procedure.

*Histologic evaluation and histomorphometry*

All of the sheep were sacrificed. Dental implants and surrounding bone were obtained. The block of dental implants and surrounding defects were placed in a 4% buffered formalin solution for at least 24 h. For the dehydration procedure all of the samples were placed in alcohol for 1 day at 70%, 80%, 90%, 96% and 99%, respectively. After the dehydration procedure, all samples were filtered under the vacuum and stored in methyl methacrylate resin for 24 h (Technovit 7200 VLC, Kulzer & Co, Wehrheim, Germany). After the filtration procedure, samples were embedded into a clear plastic cast frized with methyl methacrylate with no air bubbles. These casts were polimerized for 8 h at 40°C and 450 nm wave light. The flat surface of the methyl methacrylate blocks was fixed to periglass panel under the vacuum using Technovit 7210 VLC (Kulzer & CO. GmbH, Friedricksdorf, Germany). These samples were cut 300 μm thick using a diamond saw connected to a sensitive cutting device (Exakt 300 CL, Exakt Apparatbau, Norderstad, Germany). This cutting procedure was applied longitudinally through the center of the dental implant. These samples were sliced to a thickiness of 50 μm using 1000, 12 000 and 2500 grid sandpaper with the micro-adhesive system (Exakt 400 CS, Exakt Apparatbau, Norderstad, Germany). Two histologic cross-sections were obtained from each sample. These cross-sections were stained using toluidine blue as suggested by the methods of Donath and Breuner [15]. Stained histologic samples were dried for one night. Then the surfaces of the samples were covered with a lamel using methyl methacrylate. Digital images of the samples were taken using a digital camera (Nikon L-1 DS5M, Tokyo, Japan) connected to a light microscope (Nikon Eclipse E 600, Tokyo, Japan) under 4 × magnification. Then BIC was measured (Wayne Rasband National Institues of Health, Bethesda, Maryland USA). The statistical analysis was performed using SPSS software (version 13.0, SPSS, Chicago, IL). Histological evaluation was carried out and bone-to-implant contact rates of histologic samples were calculated. ANOVA test was used to evaluate the differences between the BIC rates of each group. *P*-values ($\alpha$) were accepted as 0.05 for all statistical analysis.

## Results

During the healing period of the study, all implants osseointegrated successfully.

*Histomorphometric analyses*

Shapiro-Wilk test was used to ensure the data being used had a normal distribution. BIC rates of all groups were calculated.

Mean BIC rate was 31.14 for the Empty Defect Group, 60.82 for the PRP group and 76.64 for the PRP + OCC group.

The variation of BIC rates between the Empty Defect and PRP groups was significant ($p<0.05$). The BIC rate of the PRP group was higher than that of the Empty Defect Group. The variation of BIC rates between the Empty Defect and PRP groups was 29.68.

The variation of BIC rates between the Empty Defect and PRP + OCC groups was significant ($p <0.05$). The BIC rate of the PRP + OCC group was higher than that of the Empty Defect Group. The variation of BIC rates between the Empty Defect and PRP + OCC groups was 45.51.

The variation of BIC rates between the PRP and PRP + MSC groups was significant ($p = <0.05$). The BIC rate of the PRP + OCC group was higher than that of the PRP group. The variation of BIC rates between the PRP and PRP + OCC groups was 15.82.

At the end of the 8-week-healing period, it was observed that the percentage of BIC was highest in the PRP + OCC group.

## Discussion

Bone autogenous, homogenous, xenogene, alloplastic, bone grafts, guided bone regeneration and distraction osteogenesis techniques are used in the maxillofacial region for reconstruction of the bone defects [15–17]. The precise effect of graft material

Table I. BIC rates.

| | Empty Defect group | PRP group | PRP + OCC group |
|---|---|---|---|
| Mean average | 31.14 | 60.82 | 76.64 |
| Confidence interval (95%) | 24.51–37.76 | 58.43–63.20 | 73.16–80.13 |
| Min BIC rate | 16.27 | 51.53 | 67.60 |
| Max BIC rate | 49.47 | 71.69 | 90.93 |
| Min–Max interval | 33.20 | 20.16 | 23.23 |
| *p*-values | 0.246 | 0.999 | 0.051 |

within the osteoinductive as the 'gold standard' is the first choice autogenous bone grafts [18,19]. Even if the iliac bone graft material is considered to be the best, it causes fibrous healing and osteointegration weakness around the implant [20].

Stem cells, found in the human body, can turn into different cells. Development of an *in vitro* culture method previously seen as impossible to replicate in different cell types has now been made routine [21]. Embryonic stem cells are divided into two categoriess, either as developmental or post-natal stem cells (organ-specific, tissue-specific or adult stem cells called) [22]. Recent studies have shown that bone marrow stroma cells can turn into osteoblast, chondrocyte, adipocyte, myoblast, hepatocytes, cardiyomyozit and neural cells [23]. In order to increase the osseointegration, MSC was preferred for the osteoblast cell cultures in our study.

Adult stem cells were obtained from bone marrow, nervous tissue, muscle, skin, bowel, liver, dental pulp, periodontal ligament, alveolar bone and extracted permanent teeth [24–26]. However, organ- or tissue-specific stem cell properties can affect the behavior of stem cells. Thus, it is necessary to be careful in the selection of sources [27,28].

Akintoye et al. [28] stated that MSCs obtained from craniofacial bone marrow could not have been supported by an organized hematopoietic marrow as successfully as cells taken from long bones. Considering the above-mentioned reasons, in order to obtain the high quality and quantity osteoblast, bone marrow aspiration of the sheep iliac bone was preferred in the present study.

According to the literature there are two adult hematopoietic stem cells (HSC) and an MSC population in bone marrow. HSCs can produce all blood cells and they can continue self-renewal capacity thorough life. It has been shown that MSCs sourced from bone marrow can differentiate into a wide variety of cells such as osteoblast, chondrocyte, adiposit, hepatocytes, neurons, astrocytes, tenosit, miyoblast, epithelial and endothelial cells [29,30].

Due to their easy isolation and their large differentiation potential, MSCs have more advantages in terms of clinical use than other cells. Adult MSCs (multi-faceted potential) have lower allogenic immune reaction in addition to a large differentiation potential. They are ideal for tissue repair and regenerations. Adult bone marrow stem cells take place in most existing applications [22]. OCC differentiated from MSCs were preferred for dental implant bone defect repair in the present study.

In bone defects resulting from trauma, osteoradionecrouse or tumors; loaded or injected MSCs can increase osteogenesis in previously identified defects in the scaffold [31–33]. In many medical applications in the oral and maxilllofacial region, the stem cell research has been increasing rapidly [34,35]. Chai et al. [36] studied engineered oral mucosa and suggested that it closely resembled the normal oral mucosa and showed epithelial attachment on a titanium surface.

Under the light of the literature, in the present study we observed that it was not as difficult as we had thought to obtain osteoblasts differentiated from bone marrows MSC. Increasing the numbers of these studies, the major advantage of this application will become routine. Studies have increased using platelet-rich plasma (PRP) since it is easier and cheaper to obtain than bone augmentation use.

The studies in dogs mandibulars, the mixture of PRP and MSC were compared with PRP, autogenous cancellous bone and empty defect by Yamada et al. [10]. As a result implant–bone connection was deemed to be stronger in the MSC-PRP group than in the MSC-PRP and the cancellous bone mixture group. According to these results, bone formation amount was less in the empty defect group than other groups. PRP induces bone formation, but not as much as MSC.

Ohya et al. [37] have identified that the MSC-PRP mixture is superior than an autogenous bone-PRP mixture in osteogenesis.

In the present study, implant–bone connection was better in the OCC-PRP group than in the PRP group and the empty defect group. The results were in accordance with the study of Lucarelli et al. [38]. Rabit mandibular defect repair was better in a mixture of MSC–allogenic demineralized bone group than in the allogenic demineralized bone group [39].

In hard tissue regeneration; stem cell scaffolds such as the bioactive HA, $\beta$-PRP ($\beta$-tricalcium phosphate) ceramics, glass ceramics and aluminum ceramics, calcium carbonate and polilactic acid polymers are used because these materials or artificial cartilage are sufficient for the production and destruction of bone bioadaptation [40–42]. However, due to the the lack of porosities, slow resorption and lack of new bone osteoinductive features of ceramics, it is difficult to invase into the defect [10].

Since a jelly-like consistency of the implementation may be more appropriate in bone defects, stem cells with scaffold PRP have been used to provide bone formation in recent years [10,43]. In these studies, PRP can support MSC's adhesion, proliferation and differentiation to bone when MSCs were used with PRP. MSCs differantiated from bone marrow include receptors for growth factors within the PRP. *In vitro* studies show that adding PRP in MSCs has yet been demonstrated to increase proliferation significantly [38]. In this study, BIC value was high and statistically significant in PRP defects than those with the empty defects. In accordance with the literature, PRP effected the bone regeneration positively in our study.

Carneiro-Campos et al [44] studied the modications on the titanium implant surfaces roughness may

promote differences in the morphology of bone marrow stromal cells. Alves and Wassall [45] concluded that the studied machined implant surface is biocompatible since it preserved the integrity of the cultivated osteoblast-like cells. In the present study, roughened implants were used because of their effectiveness in bone–implant connection [45]. Implant material and surface topography are two of critical factors effecting BIC [2–4]. Clinical and histomorphometric studies observed that surface roughness is important in implants for better BIC values [46,47].

It is a primary prerequisite for stabilization that half of all height implant must be in direct contact with bone [48,49]. In our study, in order to ensure the primary stabilization of implants, in accordance with the literature, implants were placed at half the height of that portion of the apical 4 mm into the bone.

The width of the defect around the implant directly effects BIC values [49]. Ito et al. [5] performed the same surgical methods in dogs and they used the MSCs as regenerative agents in the test group at the $8^{th}$ week. They found the BIC values to be 53% [5]. In a similar test study group, Jung et al. [4] had applied PTH (1–34) as regenerative agents and at the end of the $12^{th}$ week they found the BIC values between 63.5–67.3%. MSCs increased the BIC values significantly, as reported by Ito et al [5]. In our study, using a combination of OCC-PRP was effective on healing. Implant–bone connection was better in the OCC-PRP group compared with the PRP group and the empty defect group. The BIC value was increased significantly by OCC.

In the present study, implant design and surface characteristics were the same because the main gain of the study was to reveal only the osteogenic effect of OCC. In our study, the effect of combination of OCC-PRP on healing was obsereved to be positive. OCCs have increased the BIC value rates significantly.

As a result, we believe that the present study's results will shed light on further studies and current dental treatment augmentation applications will develop tissue engineering in the future.

## Acknowledgements

The present study was presented at the 16th International Association of Turkish Oral and Maxillofacial Surgery Congress, 3–8 November 2009, Ürgüp-Nevşehir, Turkey. This project was supported by Ondokuz Mayıs University scientific project support programme numbered DHF 055.

***Declaration of interest:*** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## References

[1] Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intraosseous anchorage of dental protheses. 1. Experimental studies. Scand J Plast Reconstr Surg 1969;3: 81–100.

[2] Wennerberg A, Albrektsson T, Lausmaa J. Torque and histometric evaluation of c.p. titanium screws blasted with 25- and 75- microns-sized particles of Al2O3. J Biomed Mater Res 1996;30:251–60.

[3] Cochran DL, Schenk RK, Lussi A, Higginbottom FL, Buser D. Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: a histometric study in the canine mandible. J Biomed Mater Res 1998;40:1–11.

[4] Jung RE, Cochran L, Domken O, Seibl R, Jones AA, Buser D, et al. The effect of matrix bound parathyroid hormone on bone regeneration. Clin Oral Implant Research 2007;18:319–25.

[5] Ito K, Yamada Y, Naiki T, Ueda M. Simultaneous implant placement and bone regeneration around dental implants using tissue-engineered bone with fibrin glue, mesenchymal stem cells and platelet-rich plasma. Clin Oral Implants Research 2006;17:579–86.

[6] Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, et al. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105:369–77.

[7] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418:41–9.

[8] Van den Dolder J, Farber E, Spauwen PH, Jansen JA. Bone tissue reconstruction using titanium fiber mesh combined with rat bone marrow stromal cells. Biomaterials 2003;24: 1745–50.

[9] Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, et al. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 2004;75:1281–7.

[10] Yamada Y, Ueda M, Naiki T, Takahashi M, Hata K, Nagasaka T. Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: tissue-engineered bone regeneration. Tissue Eng 2004;10: 955–64.

[11] Yamada Y, Ueda M, Hibi H, Baba S. A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineerin technology: a clinical case report. Int J Periodontics Restorative Dent 2006;26:363–9.

[12] Camargo PM, Lekovic V, Weinlaender M, Vasilic N, Madzarevic M, Kenney EB. Platelet-rich plasma and bovine porous bone mineral combined with guided tissue regeneration in the treatment of intrabony defects in humans. J Periodontal Res 2002;37:300–6.

[13] Lekovic V, Camargo PM, Weinlaender M, Vasilic N, Kenney EB. Comparison of platelet-rich plasma, bovine porous bone mineral, and guided tissue regeneration versus platelet-rich plasma and bovine porous bone mineral in the treatment of intrabony defects: a reentry study. J Periodontol 2002;73:198–205.

[14] Rosalki SB, Foo AY, Burlina A, Prellwitz W, Stieber P, Neumeier D, et al. Multicenter evaluation of Iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. Clin Chem 1993;39: 648–52.

[15] Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. J Oral Pathol 1982;11:318–26.

[16] Kostopoulos L, Karring T. Role of periosteum in the formation of jaw bone. J Clin Periodontol 1995;22: 247–25.
[17] Lynch SE, Genco RJ, Marx RE. In Stream C, editor. Tissue engineering, applications in maxillofacial surgery and periodontics. Quintessence Publishing Co: Illinois, USA; 1999. p71–83.
[18] Nkenke E, Schultze-Mosgau S, Radespiel-Tröger M, Kloss F, Neukam FW. Morbidity of harvesting of chin grafts: a prospective study. Clin Oral Implants Res 2001;12:495–502.
[19] Schultze-Mosgau S, Keweloh M, Wiltfang J, Kessler P, Neukam FW. Histomorphometric and densitometric changes in bone volume and structure following avascular bone grafting in the extremely atrophic maxilla. B J Oral Maxillofac Surg 2001;39:439–47.
[20] Bongso A, Tan S. Human blastocyts culture and derivation of embrionic stem cell lines. Stem Cell Rev 2005;1:87–98.
[21] Otto WR, Rao J. Tomorrows skeleton staff: Mesenchymal stem cells and the repair of bone and cartilage. Cell Prolif 2004;37:97–110.
[22] Grove JE, Bruscia E, Krause DS. Plasticity of bone marrow – derived stem cells. Stem Cells 2004;22:487–500.
[23] Kortesidis A, Zannettino A, Isenmann S, Shi S, Lapidot T, Gronthos S. Stromal-derived factor-1 promotes the growth, survival, and development of human bone marrow stromal stem cells. Blood 2005;105:3793–801.
[24] Marshman E, Booth C, Potten CS. The intestinal epithelial stem cell. Bioessays 2002;24:91–8.
[25] Chen JC, Goldhamer DJ. Skeletal muscle stem cells. Reprod Biol Endocrinol 2003;1:1–101.
[26] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003;100:5807–12.
[27] Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8:191–9.
[28] Akintoye SO, Lam T, Shi S, Brahim J, Collins MT, Robey PG. Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. Bone 2006;38:758–68.
[29] Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875–84.
[30] Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B. Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci USA 2003;100: 1364–9.
[31] Cassiede P, Dennis JE, Ma F, Caplan AI. Osteochondrogenic potential of marrow mesenchymal progenitor cells exposed to TGF-beta 1 or PDGF-BB as assayed *in vivo* and *in vitro*. J Bone Miner Res 1996;11:1264–73.
[32] Kon E, Muraglia A, Corsi A, Bianco P, Marcacci M, Martin I, et al. Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. J Biomed Mater Res 2000;49:328–37.
[33] Oshima Y, Watanabe N, Matsuda K, Takai S, Kawata M, Kubo T. Behavior of transplanted bone marrow-derived GFP mesenchymal cells in osteochondral defect as a simulation of autologous transplantation. J Histochem Cytochem 2005;53: 207–16.
[34] Miyamoto I, Tsuboi Y, Takahashi K, Hyon SH, Iizuka T. Enhancement of bone volume in guided bone augmentation by cell transplants derived from periosteum: an experimental study in rabbit calvarium bone. Clin Oral Implants Res 2004; 15:308–14.
[35] Ohgushi H, Goldberg VM, Caplan AI. Repair of bone defects with marrow and porous ceramic (experiment in rats). Acta Orthop Scand 1989;60:334–9.
[36] Chai WL, Moharamzadeh K, Brook IM, Emanuelsson L, Palmquist A, van Noort R. Development of a novel model for the investigation of implant- soft tissue interface. J Periodontol 2010;81:1187–95.
[37] Ohya M, Yamada Y, Ozawa R, Ito K, Takahashi M, Ueda M. Sinus floor elevation applied tissue-engineered bone. Clin Oral Implants Res 2005;16:622–9.
[38] Lucarelli E, Beccheroni A, Donati D, Sangiorgi L, Cenacchi A, Del Vento AM, et al. Platelet derived growth factors enhance proliferation of human stem cells. Biomaterials 2003;24:3095–100.
[39] Li Z, Li ZB. Repair of mandible defect with tissue engineering bone in rabbits. ANZ J Surg 2005;75:1017–21.
[40] Ohgushi H, Dohi Y, Yoshikawa T, Tamai S, Tabata S, Okunaga K, et al. Osteogenic differentiation of cultured marrow stromal stem cells on the surface of bioactive glass ceramics. J Biomd Mater Res 1996;32:341–8.
[41] Kitamura S, Ohgushi H, Hirose M, Funaoka H, Takakura Y, Ito H. Osteogenic differentiation of human bone marrow derived mesenchymal cells cultured on alumina ceramics. Artif Organs 2004;26:72–82.
[42] Yamada Y, Boo JS, Ozawa R, Nagasaka T, Okazaki Y, Hata K, et al. Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. J Craniomaxillofac Surg 2003;31:27–33.
[43] Dallari D, Fini M, Stagni C, Torricelli P, Nicoli Aldini N, Giavaresi G, et al. *In vivo* study on the healing of bone defects treated with bone marrow stromal cells, platelet-rich plasma, and freeze-dried bone allografts, alone and in combination. J Orthop Res 2006;24:877–88.
[44] Carneiro-Campos LE, Fernandes CP, Balduíno A, Leite Duarte ME, Leitão M. The effect of titanium topography features on mesenchymal human stromal cells' adhesion. Clin Oral Implants Res 2010;21:250–4.
[45] Alves SF, Wassall T. *In vitro* evaluation of osteoblastic cell adhesion on machined osseointegrated implants. Braz Oral Res 2009;23:131–6.
[46] Davies JE. Mechanisms of endosseous integration. Int J Prosthodont 1998;11:391–401.
[47] Veis AA, Papadimitriou S, Trisi P, Tsirlis AT, Parissis NA, Kenealy JN. Osseointegration of Osseotite and machined-surfaced titanium implants in membranecovered critical-sized defects: a histologic and histometric study in dogs. Clin Oral Implants Res 2007;18:153–60.
[48] Polyzois I, Renvert S, Bosshardt DD, Lang NP, Claffey N. Effect of Bio-Oss on osseointegration of dental implants surrounded by circumferential bone defects of different dimensions: an experimental study in the dog. Clin Oral Implants Res 2007;18:304–10.
[49] Botticelli D, Berglundh T, Lindhe J. The influence of a biomaterial on the closure of a marginal hard tissue defect adjacent to implants. An experimental study in the dog. Clin Oral Implants Res 2004;15:285–92.