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Original Article

Bone marrow-derived cells homing for self-repair of periodontal tissues: a histological characterization and expression analysis

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Abstract: Periodontitis, a disease leads to the formation of periodontal defect, can result in tooth loss if left untreated. The therapies to repair/regenerate periodontal tissues have attracted lots of attention these years. Bone marrow-derived cells (BMDCs), a group of cells containing heterogeneous stem/progenitor cells, are capable of homing to injured tissues and participating in tissue repair/regeneration. The amplification of autologous BMDCs' potential in homing for self-repair/regeneration, therefore, might be considered as an alternative therapy except for traditional cell transplantation. However, the knowledge of the BMDCs' homing and participation in periodontal repair/regeneration is still known little. For the purpose of directly observing BMDCs' involvement in periodontal repair, chimeric mouse models were established to make their bone marrow cells reconstituted with cells expressing green enhanced fluorescence protein (EGFP) in this study. One month after bone marrow transplantation, periodontal defects were made on the mesial side of bilateral maxillary first molars in chimeric mice. The green fluorescence protein-positive (GFP+) BMDCS in periodontal defect regions were examined by bioluminescent imaging and immunofluorescence staining. GFP+ BMDCs were found to aggregate in the periodontal defect regions and emerge in newly-formed bones or fibers. Some of them also co-expressed markers of fibroblasts, osteoblasts or vascular endothelial cells. These results indicated that BMDCs might contribute to the formation of new fibers, bones and blood vessels during periodontal repair. In conclusion, we speculated that autologous BMDCs were capable of negotiating into the surgical sites created by periodontal operation and participating in tissue repair.

Keywords: Bone marrow-derived cells, bone mesenchymal stem cell, periodontal regeneration, periodontium

Introduction

Periodontitis refer to the inflammatory processes that occur in the tissues surrounding the teeth in response to bacterial accumulations (dental plaque) on the teeth [1]. This disease leads to the formation of deep infrabony defects and soft-tissue crevices between the tooth and its bony socket [2]. Left untreated, periodontitis can result in tooth loss with a significant impact on oral health [3, 4].

In previous studies, labeled bone marrow mesenchymal stem cells (BMMSCs) were transplanted into periodontal defects of animal models [5, 6]. Finally, these labeled BMMSCs were observed in newly-formed periodontal tissues that included alveolar bone, periodontal ligament and cementum. In addition, these cells expressed typical surface markers of osteoblasts or fibroblasts. These studies also demonstrated the effectiveness of BMMSCs transplantation for periodontal repair/regeneration.

The bone marrow is a reservoir of heterogeneous stem/progenitor cells, including BMMSCs, hematopoietic stem cells, osteoblast precursors, fibroblast precursors and endothelial precursors, et al. [7-12]. Bone marrow-derived cells (BMDCs) are capable of homing to many tissues in response to injury signals, where they participate in tissue repair/regeneration [13-15]. The homing and expansion of autologous BMDCs into the brains of murine models with Alzheimer's disease has been hypothesized to contribute to treat Alzheimer's

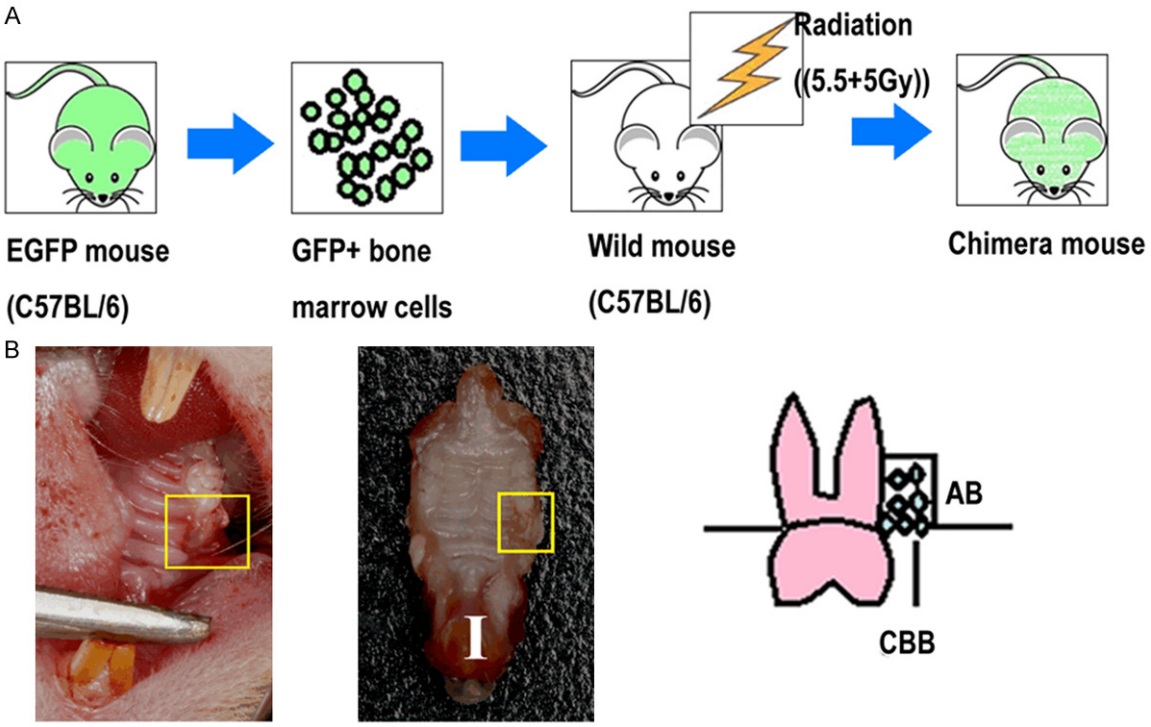

Figure 1. A. The schematic for the establishment of the chimera mice. B. The periodontal defect was prepared on the mesial side of the maxillary first molar (yellow box).

disease through the elimination of amyloid deposits [16]. Osturu et al. implanted bone morphogenetic protein-2 (BMP-2)-containing collagen pellets on the backs of mice, and found that BMP-2 pellets seemed to be able to entrap circulating osteoblast precursors of bone marrow origin, which finally contributed to the formation of ectopic bone [17, 18].

These results prompted our thought that the usage of autologous BMDCs' homing for self-repair/regeneration might be considered as an alternative therapy for repairing or regenerating periodontal defects. Compared with traditional cell transplantation therapies, this method might have merits such as weaker immunereaction from donors and more plentiful resources of stem/progenitor cells which collaboratively contribute to tissue repair/regeneration. However, the knowledge of the BMDCs' homing and participation in periodontal repair/regeneration is still known little.

We established chimeric mice models, whose bone marrow were reconstituted with bone marrow cells from transgenic mice expressing green enhanced fluorescence protein (EGFP). One month after bone marrow transplantation, periodontal defects were prepared on the mesial side of bilateral maxillary first molars in chimeric mice. The green fluorescence protein-positive (GFP+) BMDCs were examined to investigate their involvement in periodontal repair created by periodontal operation. Our study may provide theoretical and experimental basis for the clinical application of autologous BMDCs to periodontal repair/regeneration by taking advantage of their homing characteristic.

Materials and methods

Animals

Four-week-old male EGFP transgenic mice (C57BL/6 background) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). C57BL/6 male mice were purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Animals were reared in a pathogen-free barrier facility at the Animal Center of Tongji University (Shanghai, China). All animal studies were approved by the Institutional Animal Care and Use Committee of Tongji University. The animal experiment approval No. of the study is TJmed-013-31.

Chimera establishment

Twenty C57BL/6 recipients were irradiated with (5.5+5) Gy over a 4-h interval at a rate of 1 Gy/min. Bone marrow cells were obtained from EGFP transgenic mice by flushing the tibia and femur bones with phosphate-buffered saline (PBS) (Gibco, NY, USA) that contained 2% fetal bovine serum (FBS) (Gibco, NY, USA). Erythrocytes were removed by applying 2 ml erythrocyte lysis buffer (eBioscience, San Diego, CA, USA). 5×10^6 GFP+ positive cells were suspended in 500 μl PBS and injected into C57BL/6 recipients via the tail veins (**Figure 1A**) (Duran-Struuck et al. 2009; Cui et al. 2002). Five of the 20 irradiated mice received PBS without GFP+ bone marrow cells by tail vein transplantation to serve as a control group. These 5 mice ended with the death in 2 days after irradiation. The success of chimera establishment was confirmed by demonstrating that GFP+ bone marrow cells in recipients had reconstituted 94% ± 6% of the hematopoietic niche one month after transplantation.

Periodontal defect preparation

One month after bone marrow transplantation, chimeric mice received periodontal operation after anesthesia with pentobarbital sodium (100 mg/kg). Binocular loupes (Heine, Germany) were worn in order to clearly observe surgical area. The gingival and palate mucosae on the mesial sides of bilateral maxillary first molars were flapped to expose the alveolar bone overlying the mesial root. A three-wall defect, approximately 1 mm in diameter and depth, was made using a round dental burr while being cooled with PBS. The defect was then filled with pieces of ceramic bovine bone (CBB) (Research and Development Center for Tissue Engineering, Fourth Military Medical University) as support materials to promote periodontal repair, approximately 0.5mg for each defect (**Figure 1B**). CBB, whose major compositions are HAP [Ca10(PO4)6(OH)2], b-TCP[Ca3(PO4)2], has been shown as an attractive natural mineralized scaffold for dentin or periodontium regeneration [19-23]. Finally, the gingival flaps were repositioned and sutured.

Guided tissue regeneration (GTR), dental surgical procedures that utilize barrier membranes to benefit the achievement of complete periodontal regeneration, were not implemented because of the confined volumes of periodontal defects in mice [24, 25]. Therefore, periodontal repair rather than regeneration might be achieved in our study. The achievement of periodontal repair would be enough for the preliminary investigation into the BMDCs' involvement in periodontal repair/regeneration.

Chimeric mice were sacrificed at 5, 15 and 30 days after operation. The chimeric mice that had not received periodontal surgery were used as 0 day group. Each group was composed of 3 mice.

Bioluminescent imaging

After mice were sacrificed, the maxillae were excised and cleaned with PBS. The maxillae were imaged with NightOWL II LB 983 Imaging System (Berthold, Germany) under a 488 nm excitation light. The intensity of fluorescence in the 3 maxillae of each group was measured by Indigo software (attached to NightOWL II LB 983 Imaging System).

Histology

The maxillae were fixed in 4% paraformaldehyde for 48 hours at 4 centi degree and then decalcified with ethylene diamine tetraacetic acid (EDTA) (sigma, USA) for 10 days at room temperature. Frozen sections that were 10 μm in thickness were prepared for subsequent studies. The sectioning plane is from buccal side to palatal side. Sections were stained with Masson's trichrome (Jancheng, Nanjing, China) and hematoxylin and eosin (Jancheng, Nanjing, China). In Masson's trichrome, frozen sections were stained with Weigert iron hematoxylin solution for 10 minutes, Ponceau solution for 5 minutes, Phosphotungstic-Phosphomolybdic Acid for 1 minute and Aniline Blue solution for 5 minutes [26]. Finally, sections were mounted with neutral balsam (Sigma, USA).

Immunohistochemistry

Sections were incubated in 0.3% triton X-100 for 10 minutes and then blocked in 5% normal goat serum (Gibco, NY, USA) in PBS for 60 minutes at room temperature. Primary antibodies were diluted in PBS, added to sections and incubated overnight at 4°C. Primary antibodies included rabbit anti-mouse fibroblast-specific protein-1 (FSP-1; 1:200, Abcam, UK), rabbit anti-mouse collagen I (COL I; 1:200, Abcam, UK), rabbit anti-mouse Osteopontin (OPN;

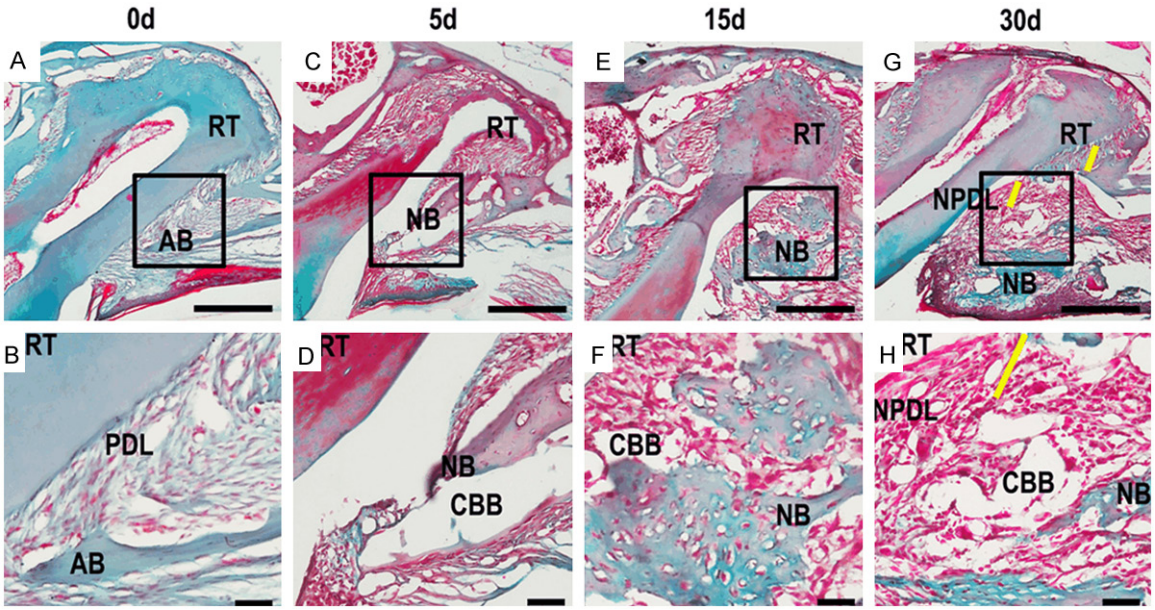

Figure 2. A-H. Masson staining of normal control group and experimental groups. C, D. On the 5th day, the surgical region was invaded with plenty of fibers. E, F. On the 15th day, new bone trabecula had formed in the defect, with osteocytes inside. G, H. On the 30th day, periodontal ligament-like fiber bundles were found to perpendicularly insert into new cementum surface on the damaged root, paralleling the normal periodontal ligament fiber bundles (yellow line). AB, alveolar bone; CBB, ceramic bovine bone; NB, newly-formed bone; NPDL, newly-formed periodontal ligament-like fiber bundles; PDL, periodontal ligament; RT, root of tooth. These images are representative ones. (A, C, E, G ×10, scale bar 500 μm; B, D, F, H ×40, scale bar 50 μm).

1:200, Abcam, UK), rabbit anti-mouse alkaline phosphatase (ALP; 1:250, Abcam, UK), rabbit anti-mouse CD31 (1:200, Abcam, UK), rabbit anti-mouse CD34 (1:200, Abcam, UK). The fluorescent secondary antibody, goat anti-rabbit (1:800, CST, USA), was applied for 60 minutes at room temperature. The nuclei were stained using DAPI (0.5 ug/ml, CST, USA) in PBS for 10 min. Negative controls were applied by replacing the secondary antibody with PBS or rabbit serum (Gibco, NY, USA). The sections were viewed using a laser scanning confocal microscope (Leica TCS SP5, Germany).

Quantification of immunofluorescent staining

To examine the number of the GFP+ cells in the tissue, the images of immunofluorescent staining were quantified by using the Photoshop software (Adobe Systems, San Jose, CA). The number of GFP+ cells counterstained with DAPI in the periodontal defect area was counted. Each group had 6 samples (bilateral sides of the 3 chimeric mice).

Statistical analysis

Statistical analysis was carried out with SPSS 17.0 software (SPSS. San Rafael, CA, USA) for comparison of the BLI values or the number of GFP+ cells in the periodontal defect regions. The results are presented as means ± SD for each group. Means were compared by one-way analysis of variance (ANOVA), followed by Turkey test. A *P* value of less than 0.05 was considered statistically significant.

Results

Histomorphologic analysis

Normal periodontal tissue, including cementum, periodontal ligament and alveolar bone, could be observed in the 0 day group (**Figure 2A**, **2B**). On the 5th day, a plentitude of fibers and newly-formed mineralizing matrix could be observed in the defect area (**Figure 2C**, **2D**). On the 15th days, newly-formed bone trabecula emerged at the surgical site, with osteocytes buried inside (**Figure 2E**, **2F**). Newly-formed periodontal ligament-like fibers were found on the surface of the damaged roots on the 30th day, paralleling the normal periodontal ligament fibers (**Figure 2G**, **2H**). The repair of periodontal tissues approached to be accomplished on the 30th day.

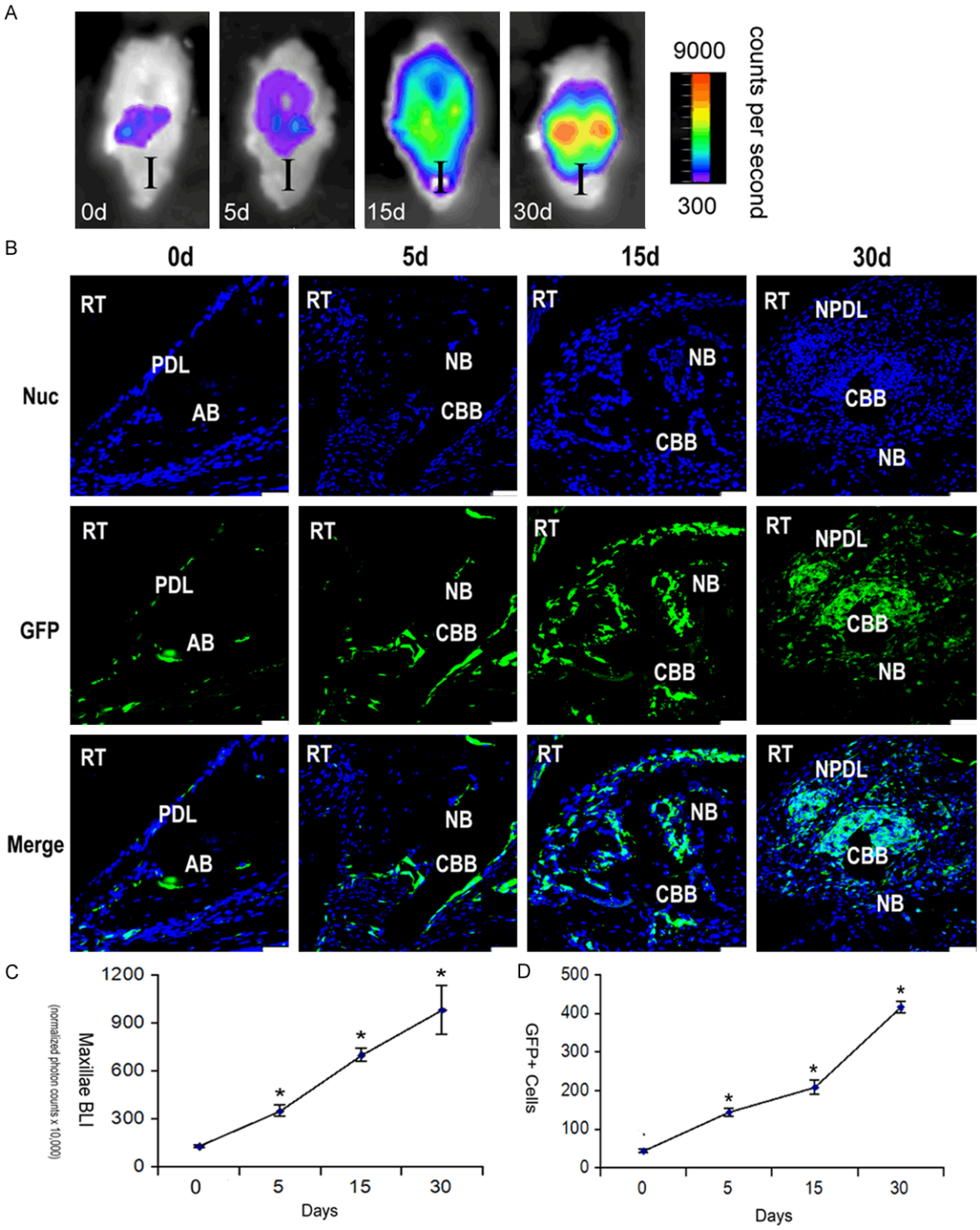

Figure 3. A-D. GFP+ BMDCs migrated into the maxillae. A. The maxillae BLI in normal control group and experimental groups were detected. The luminous intensity gradually increased from purple to red. I, incisor. These images are representative ones. B. Distribution of GFP+ BMDCs in normal and damaged periodontal tissues after the operation was detected. The blue indicates the nucleus of all cells, the green indicates GFP+ BMDCs. On the 15th and 30th day, some cells in the bone trabecula and newly-formed periodontal ligament-like fibers were GFP+. AB, alveolar bone; CBB, ceramic bovine bone; NB, newly-formed bone; NPDL, newly-formed periodontal ligament-like fiber bundles; PDL, periodontal ligament; RT, root of tooth. These images are representative ones. C. The quantitative results for maxillae BLI are shown in the line graph (n=3). Maxillae BLI gradually increased from the 5th to the 30th day after the operation ($P < 0.05$). D. The number of GFP+ cells in the periodontal defect area of all groups are shown in the line graph (n=6, bilateral sides of the 3 chimeric mice). The GFP+ BMDCs gradually increased from the 5th day until the 30th day after the operation ($P < 0.05$). (B ×40, scale bar 50 μm).

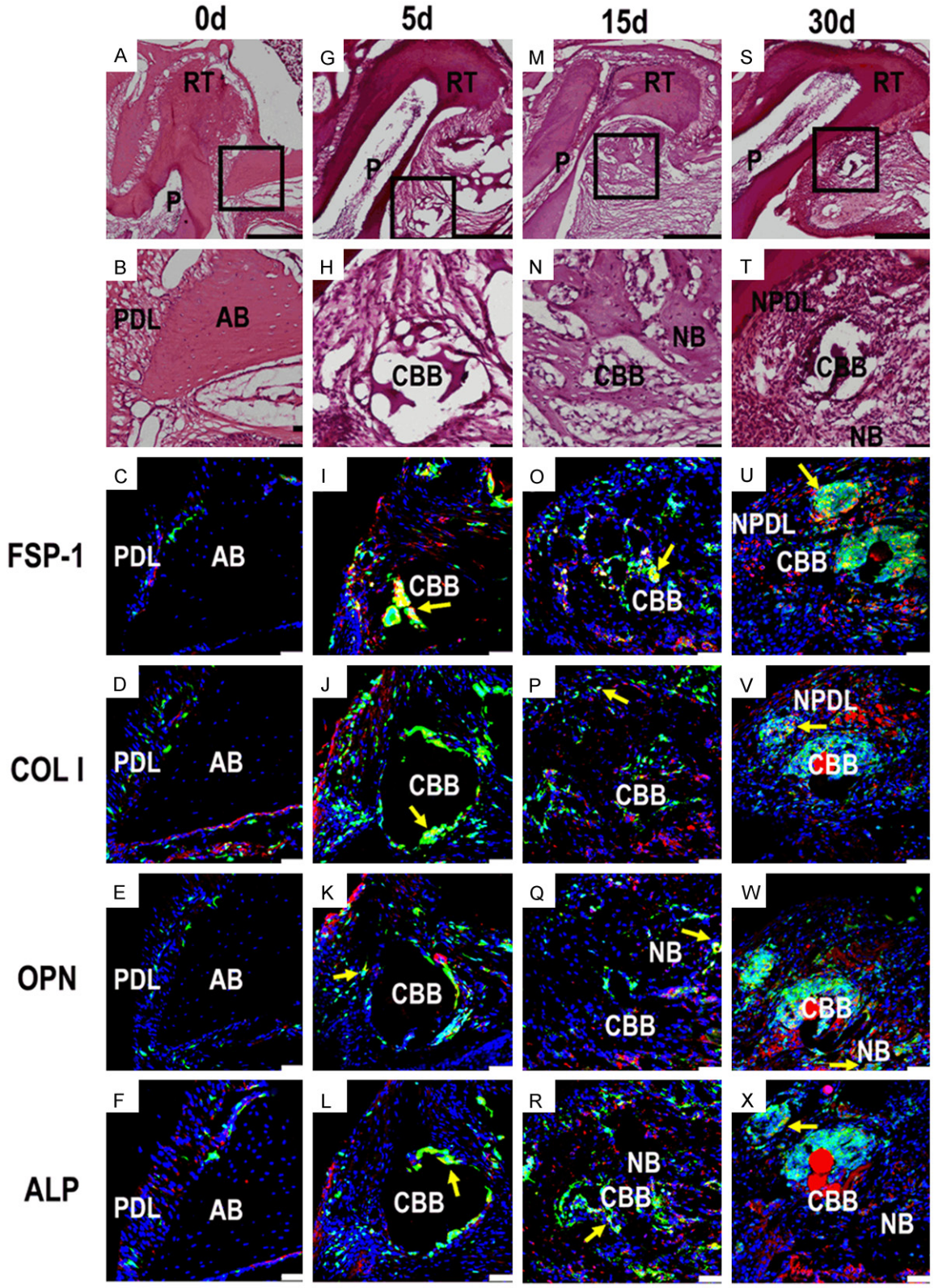

Figure 4. A-X. BMDCs might participate in osteogenesis and fibrogenesis during periodontal repair. A, B, G, H, M, N, S, T. HE staining of normal control and experimental groups. C-F, I-L, O-R, U-X. The immunofluorescence pictures were merged. The blue indicates the nucleus of all cells, the green indicates GFP+ BMDCs, and the red indicates cells positive for FSP-1, COL I, OPN or ALP. GFP+ BMDCs co-expressing either of the 4 markers above were yellow

and partly pointed out by yellow arrows. CBB, ceramic bovine bone; NB, newly-formed bone; NPDL, newly-formed periodontal ligament-like fiber bundles; PDL, periodontal ligament; RT, root of tooth. These images are representative ones. (A, G, M, S ×10, scale bar 500 μm; B-F, H-L, N-R, T-X ×40, scale bar 50 μm).

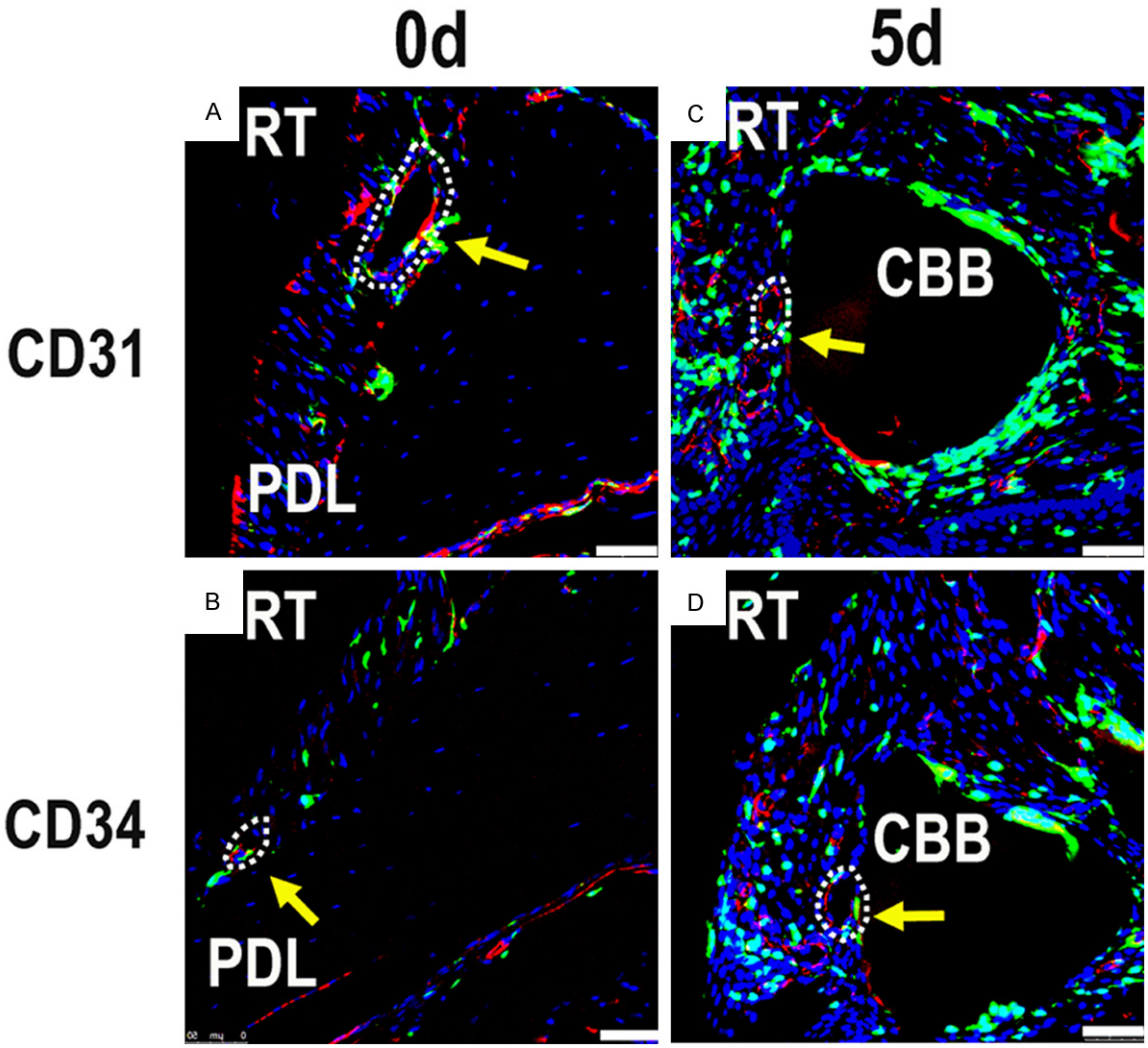

Figure 5. A-D. BMDCs might participate in neovascularization during periodontal repair. The immunofluorescence pictures were merged. The blue indicates the nucleus of all cells, the green indicates GFP+ BMDCs, and the red indicates cells positive for CD31 or CD34. GFP+ BMDCs co-expressing either of the 2 markers were yellow and partly pointed out by yellow arrows. Areas framed by dashed line, vessels; CBB, ceramic bovine bone; RT, root of tooth. These images are representative ones. (A and C ×10, scale bar 500 μm; B and D ×40, scale bar 50 μm).

BLI analysis

The images of BLI showed that GFP signal expressed on the maxillae from the 5th day until the 30th day after operation. The GFP signal expressed highest at the surgical sites, where the strongest luminance was detected (**Figure 3A**). The maxillae BLI values were measured by Indigo software. Means of each group were compared by ANOVA, followed by Turkey test. The result that *P* value was less than 0.05 indicated significant differences. The variation tendency of maxillae BLI values showed that the GFP signal gradually increased from the 5th day until the 30th day after operation (**Figure 3C**).

Distribution of BMDCs in the periodontal tissues

GFP+ BMDCs were detected in both normal and damaged periodontal tissues (**Figure 3B**). GFP+ BMDCs continued aggregating in the

periodontal defect until 30 days after operation, mainly locating around CBB powders. GFP+ BMDCs emerged in the newly-formed bone trabecula on the 15th day and the periodontal ligament-like fibers on the 30th day. The number of GFP+ cells in the periodontal defect area gradually increased from the 5th day until the 30th day after operation (**Figure 3D**).

Cell phenotype analysis of BMDCs in the periodontal tissues

Fibroblasts were labeled using FSP-1 and COL I; osteoblasts were labeled using OPN and ALP; vascular endothelial cells were labeled using CD31 and CD34.

In the normal periodontal tissues, few GFP+ BMDCs co-expressing FSP-1, COL I, OPN, or ALP were found (**Figure 4A-F**). GFP+ BMDCs co-expressing FSP-1, COL I, OPN or ALP emerged in the periodontal defect area (**Figure 4G-X**). Many cells in the newly-formed periodontal ligament-like fibers along the damaged root surface were positive for FSP-1 or COL I on the 30th day, and a part of them also expressed GFP. A few GFP+/OPN+ and GFP+/ALP+ BMDCs were detected in newly-formed bone on the 15th and 30th day.

GFP+/CD31+ and GFP+/CD34+ BMDCs were found to be constituted of or adjacent to the capillaries in the normal periodontal ligament. In the experimental groups, newly-formed capillaries were detected in the periodontal defect area. GFP+ BMDCs co-expressing CD31 or CD34 were also found to be constituted of some newly-formed capillaries (**Figure 5**).

Discussion

This study explored the contribution of autologous BMDCs in periodontal repair by tracing the genetically marked BMDCs in chimerical mice which had received periodontal operation. Our results indicated that autologous BMDCs could home to the periodontal defect area and participate in periodontal repair.

The histology analysis showed that the repair of periodontal tissues approached to be completed on the 30th day after operation, with new bone and new periodontal ligament-like fibers forming. The BLI data revealed that GFP signal expressed especially highly at the points of periodontal defect regions in the maxillae of each experimental group. GFP+ BMDCs were found in the newly-formed bone trabecula and periodontal ligament-like fibers in the defect area. These results confirmed that autologous BMDCs were capable of homing to the periodontal defect regions and participating in tissue repair.

Accompanied with the complement of periodontal repair, both the signal intensity of GFP in the maxillae and the number of GFP+ cells in the periodontal defect regions gradually ascended from the 5th day until the 30th day after operation. These results implied that the amount of GFP+ BMDCs increasingly mounted up during the observation time. There are two reasons that might explain this phenomenon. First, the continuance of periodontal remodeling requires supplemental stem/progenitor cells [27]. A persistent supplemental cell source from the bone marrow might meet the needs. Second, the stem/progenitor cells of bone marrow origin entering into the defect area continued proliferating with the process of periodontal repair/regeneration. Some of them might differentiate into tissue-specific cells [13, 14], while others might participate in tissue repair/regeneration by fusion-dependent mechanism [28-30].

BMDCs are capable of homing to many tissues in response to injury signals, where they exert their effects at multiple levels that include immunomodulation, neovascularization and tissue repair/regeneration [15, 31-33]. In this experiment, we detected the phenotypes of BMDCs in periodontal defect area, which included fibroblasts, osteoblasts and vascular endothelial cells.

As one of the markers of fibroblasts, FSP-1 were speculated to have regenerative effects in injured myocardium and be responsible for keeping periodontal ligament space free of mineralization [34, 35]. Type I and type III collagens, the extracellular matrix proteins produced by periodontal ligament fibroblasts, are major extracellular matrix protein components of periodontal ligament [36, 37]. An elevated ALP level is commensurate with active bone remodeling [38]. High level of OPN expression emerged in preosteoblastic cells early in bone formation and in mature osteoblasts at sites of bone remodeling [38]. GFP+ BMDCs co-expressing FSP-1, COL I, OPN or ALP were

detected in the periodontal defect area in all experimental groups. GFP+/OPN+ and GFP+/ALP+ cells emerged in newly-formed bone on the 15th and the 30th day. Many cells in the newly-formed periodontal ligament-like fibers along the damaged root surface were positive for FSP-1 or COL I on the 30th day, some of which co-expressed GFP. These results indicated that autologous BMDCs might participate in osteogenesis and fibrogenesis during periodontal repair/regeneration.

CD31 and CD34 were always used as one of the specific markers for vascular endothelial cells [39-41]. The strongest expression of CD34 was found in endothelial cells of capillaries, followed by arteries, veins, arterioles, and venules [42]. In this study, GFP+/CD31+ and GFP+/CD34+ BMDCs were found to be constituted of or adjacent to the capillaries in normal periodontal ligament tissues. This result corresponds with the previous speculation that bone marrow might be origin of periodontal ligament stem cells, which are a group of local progenitor cells that mainly locate in the perivascular region of the periodontal tissues [43, 44]. In addition, GFP+ BMDCs co-expressing CD31 or CD34 were also detected to be part of some newly-formed capillaries in the periodontal defect regions. It indicated that BMDCs might contribute to new blood vessels formation in the injured periodontal tissue. Asahara et al. also demonstrated that endothelial progenitor cells of bone marrow origin are responsible for postnatal vasculogenesis in physiological and pathological neovascularization [45].

Some studies have suggested that BMDCs may not be limited to directly participate in tissue repair/regeneration, and they may also have multiple effects on the tissue-resident cells, including enhancement of vascularization and production of growth factors [46, 47]. Therefore, the specific role of autologous BMDCs in periodontal repair/regeneration is worthy of further exploration.

In conclusion, autologous BMDCs were capable of being recruited to a periodontal defect and participating in tissue repair. Acquirement, examination and phenotype analysis of the BMDCs from the periodontal detect regions in the continual study would help further investigate the contribution of BMDCs in periodontal repair/regeneration.

Disclosure of conflict of interest

None.

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References

[1] Loesche WJ and Grossman NS. Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment. Clin Microbiol Rev 2001; 14: 727-752, table of contents.

[2] Monsarrat P, Vergnes JN, Nabet C, Sixou M, Snead ML, Planat-Benard V, Casteilla L and Kemoun P. Concise review: mesenchymal stromal cells used for periodontal regeneration: a systematic review. Stem Cells Transl Med 2014; 3: 768-774.

[3] Gerritsen AE, Allen PF, Witter DJ, Bronkhorst EM and Creugers NH. Tooth loss and oral health-related quality of life: a systematic review and meta-analysis. Health Qual Life Outcomes 2010; 8: 126.

[4] Pihlstrom BL, Michalowicz BS and Johnson NW. Periodontal diseases. Lancet 2005; 366: 1809-1820.

[5] Wei N, Gong P, Liao D, Yang X, Li X, Liu Y, Yuan Q and Tan Z. Auto-transplanted mesenchymal stromal cell fate in periodontal tissue of beagle dogs. Cytotherapy 2010; 12: 514-521.

[6] Yang Y, Rossi FM and Putnins EE. Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. Biomaterials 2010; 31: 8574-8582.

[7] Haudek SB, Xia Y, Huebener P, Lee JM, Carlson S, Crawford JR, Pilling D, Gomer RH, Trial J, Frangogiannis NG and Entman ML. Bone marrow-derived fibroblast precursors mediate ischemic cardiomyopathy in mice. Proc Natl Acad Sci U S A 2006; 103: 18284-18289.

[8] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA and Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41-49.

[9] Koide Y, Morikawa S, Mabuchi Y, Muguruma Y, Hiratsu E, Hasegawa K, Kobayashi M, Ando K, Kinjo K, Okano H and Matsuzaki Y. Two distinct stem cell lineages in murine bone marrow. Stem Cells 2007; 25: 1213-1221.

[10] Kwon O, Miller S, Li N, Khan A, Kadry Z and Uemura T. Bone marrow-derived endothelial

progenitor cells and endothelial cells may contribute to endothelial repair in the kidney immediately after ischemia-reperfusion. J Histochem Cytochem 2010; 58: 687-694.
[11] Oshima Y, Watanabe N, Matsuda K, Takai S, Kawata M and 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: 207-216.
[12] Zhang ZG, Zhang L, Jiang Q and Chopp M. Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. Circ Res 2002; 90: 284-288.
[13] Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K and Fukuda K. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 2004; 104: 3581-3587.
[14] Li S, Tu Q, Zhang J, Stein G, Lian J, Yang PS and Chen J. Systemically transplanted bone marrow stromal cells contributing to bone tissue regeneration. J Cell Physiol 2008; 215: 204-209.
[15] Rankin SM. Impact of bone marrow on respiratory disease. Curr Opin Pharmacol 2008; 8: 236-241.
[16] Simard AR, Soulet D, Gowing G, Julien JP and Rivest S. Bone marrow-derived microglia play a critical role in restricting senile plaque formation in Alzheimer's disease. Neuron 2006; 49: 489-502.
[17] Otsuru S, Tamai K, Yamazaki T, Yoshikawa H and Kaneda Y. Bone marrow-derived osteoblast progenitor cells in circulating blood contribute to ectopic bone formation in mice. Biochem Biophys Res Commun 2007; 354: 453-458.
[18] Otsuru S, Tamai K, Yamazaki T, Yoshikawa H and Kaneda Y. Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. Stem Cells 2008; 26: 223-234.
[19] Guo W, Chen L, Gong K, Ding B, Duan Y and Jin Y. Heterogeneous dental follicle cells and the regeneration of complex periodontal tissues. Tissue Eng Part A 2012; 18: 459-470.
[20] Joschek S, Nies B, Krotz R and Goferich A. Chemical and physicochemical characterization of porous hydroxyapatite ceramics made of natural bone. Biomaterials 2000; 21: 1645-1658.
[21] Ravaglioli A, Krajewski A, Celotti GC, Piancastelli A, Bacchini B, Montanari L, Zama G and Piombi L. Mineral evolution of bone. Biomaterials 1996; 17: 617-622.
[22] Xie H and Liu H. A novel mixed-type stem cell pellet for cementum/periodontal ligament-like complex. J Periodontol 2012; 83: 805-815.
[23] Zhang H, Liu S, Zhou Y, Tan J, Che H, Ning F, Zhang X, Xun W, Huo N, Tang L, Deng Z and Jin Y. Natural mineralized scaffolds promote the dentinogenic potential of dental pulp stem cells via the mitogen-activated protein kinase signaling pathway. Tissue Eng Part A 2012; 18: 677-691.
[24] Pini Prato G, Tinti C, Vincenzi G, Magnani C, Cortellini P and Clauser C. Guided tissue regeneration versus mucogingival surgery in the treatment of human buccal gingival recession. J Periodontol 1992; 63: 919-928.
[25] Wang HL, Modarressi M and Fu JH. Utilizing collagen membranes for guided tissue regeneration-based root coverage. Periodontol 2000 2012; 59: 140-157.
[26] Lv L, Wang Y, Zhang J, Zhang T and Li S. Healing of periodontal defects and calcitonin gene related peptide expression following inferior alveolar nerve transection in rats. J Mol Histol 2014; 45: 311-320.
[27] Grachtchouk M, Liu J, Wang A, Wei L, Bichakjian CK, Garlick J, Paulino AF, Giordano T and Dlugosz AA. Odontogenic keratocysts arise from quiescent epithelial rests and are associated with deregulated hedgehog signaling in mice and humans. Am J Pathol 2006; 169: 806-814.
[28] Fukata M, Ishikawa F, Najima Y, Yamauchi T, Saito Y, Takenaka K, Miyawaki K, Shimazu H, Shimoda K, Kanemaru T, Nakamura K, Odashiro K, Nagafuji K, Harada M and Akashi K. Contribution of bone marrow-derived hematopoietic stem/progenitor cells to the generation of donor-marker(+) cardiomyocytes in vivo. PLoS One 2013; 8: e62506.
[29] Harris RG, Herzog EL, Bruscia EM, Grove JE, Van Arnam JS and Krause DS. Lack of a fusion requirement for development of bone marrow-derived epithelia. Science 2004; 305: 90-93.
[30] Rizvi AZ, Swain JR, Davies PS, Bailey AS, Decker AD, Willenbring H, Grompe M, Fleming WH and Wong MH. Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. Proc Natl Acad Sci U S A 2006; 103: 6321-6325.
[31] Feng W, Madajka M, Kerr BA, Mahabeleshwar GH, Whiteheart SW and Byzova TV. A novel role for platelet secretion in angiogenesis: mediating bone marrow-derived cell mobilization and homing. Blood 2011; 117: 3893-3902.
[32] Kizuka F, Tokuda N, Takagi K, Adachi Y, Lee L, Tamura I, Maekawa R, Taketani T, Tamura H, Suzuki T, Owada Y and Sugino N. Involvement of bone marrow-derived vascular progenitor cells in neovascularization during formation of

the corpus luteum in mice. Biol Reprod 2012; 87: 55.
[33] Koch CA, Geraldes P and Platt JL. Immunosuppression by embryonic stem cells. Stem Cells 2008; 26: 89-98.
[34] Duarte WR, Iimura T, Takenaga K, Ohya K, Ishikawa I and Kasugai S. Extracellular role of S100A4 calcium-binding protein in the periodontal ligament. Biochem Biophys Res Commun 1999; 255: 416-420.
[35] Schneider M, Kostin S, Strom CC, Aplin M, Lyngbaek S, Theilade J, Grigorian M, Andersen CB, Lukanidin E, Lerche Hansen J and Sheikh SP. S100A4 is upregulated in injured myocardium and promotes growth and survival of cardiac myocytes. Cardiovasc Res 2007; 75: 40-50.
[36] Berkovitz BK. The structure of the periodontal ligament: an update. Eur J Orthod 1990; 12: 51-76.
[37] Takayama S, Murakami S, Miki Y, Ikezawa K, Tasaka S, Terashima A, Asano T and Okada H. Effects of basic fibroblast growth factor on human periodontal ligament cells. J Periodontal Res 1997; 32: 667-675.
[38] Ram VS, Parthiban, Sudhakar U, Mithradas N and Prabhakar R. Bonebiomarkers in periodontal disease: a review article. J Clin Diagn Res 2015; 9: ZE07-10.
[39] Lu M, Wu J, He F, Wang XL, Li C, Chen ZN and Bian H. Cell expression patterns of CD147 in N-diethylnitrosamine/phenobarbital-induced mouse hepatocellular carcinoma. J Mol Histol 2015; 46: 79-91.
[40] Tavian M and Peault B. The changing cellular environments of hematopoiesis in human development in utero. Exp Hematol 2005; 33: 1062-1069.
[41] Yoon CH, Hur J, Park KW, Kim JH, Lee CS, Oh IY, Kim TY, Cho HJ, Kang HJ, Chae IH, Yang HK, Oh BH, Park YB and Kim HS. Synergistic neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. Circulation 2005; 112: 1618-1627.
[42] Pusztaszeri MP, Seelentag W and Bosman FT. Immunohistochemical expression of endothelial markers CD31, CD34, von Willebrand factor, and Fli-1 in normal human tissues. J Histochem Cytochem 2006; 54: 385-395.
[43] McCulloch CA. Progenitor cell populations in the periodontal ligament of mice. Anat Rec 1985; 211: 258-262.
[44] McCulloch CA and Melcher AH. Cell migration in the periodontal ligament of mice. J Periodontal Res 1983; 18: 339-352.
[45] Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M and Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999; 85: 221-228.
[46] Chamberlain G, Fox J, Ashton B and Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007; 25: 2739-2749.
[47] Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA and Bhatia M. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotechnol 2003; 21: 763-770.