Alveolar Bone Regeneration around Immediate Implants Using an Injectable nHAC/CSH Loaded with Autogenic Blood-Acquired Mesenchymal Progenitor Cells: An Experimental Study in the Dog Mandible

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ABSTRACT

Background: Lack of osseointegration between a dental implant and the walls of the alveolar bone is a common problem in immediate implantation. Injectable tissue-engineered bone (ITB) may be an effective and minimally invasive solution to the problem. In this study, an injectable bone cement, nHAC/CSH, which consists of nano-hydroxyapatite/collagen (nHAC) and calcium sulfate hemihydrate ($CaSO_4.^1/_2H_2O$; CSH) was investigated as a tissue-engineered scaffold material with blood-acquired mesenchymal progenitor cells (BMPC) as seeding cells.

Purpose: The aim of the study was to assess the new bone formation around immediate dental implants using nHAC/CSH loaded with dog blood-acquired mesenchymal progenitor cells (dBMPC) in a canine model.

Materials and Methods: dBMPC were first isolated from peripheral blood of healthy adult dogs. Alizarin red and oil red O staining were then used to evaluate the potential of dBMPC to differentiate into bi-lineage mesenchymal tissues in vitro. Four healthy mongrel dogs were used in this study. The alveolar bone defects around immediate implants of dogs were created. Each defect was randomly assigned to one of the following three groups: (1) the ITB group (dBMPC + nHAC/CSH); (2) injectable bone cement nHAC/CSH; or (3) no materials (controls). Methylene blue staining was used to examine the bone formation after 3 months.

Results: Studies in vitro revealed that dBMPC could be induced to osteoblasts and adipocytes. The ITB group (dBMPC + nHAC/CSH) showed significantly more bone-implant contact and bone density than either nHAC/CSH or control groups in the areas with peri-implant defects 3 months after implantation.

Conclusion: The results indicate that the ITB composed of nHAC/CSH and dBMPC may represent a useful strategy for the clinical reconstruction of bone defects around immediate implantation. However, further investigation is needed involving the use of human BMPC as well as possible use of stem cells.

KEY WORDS: dental implants, injectable bone, peripheral blood, regeneration

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INTRODUCTION

Implant restoration has been used successfully for the replacement of missing teeth. Clinical findings have shown that implant placement is an effective therapeutic procedure for oral reconstruction from a functional and aesthetic point of view.[1] Endosseous implants require adequate osseous height and width at the surgical site, which would be reduced as the result of bone resorption occurring after extraction of teeth.[2]

Immediate placement of implants following extraction yields obvious benefits: (1) a significantly shorter prosthetic treatment time by combining tooth extraction, implant surgery, and restorative treatment; (2) an instant mechanical support to the papillae and midfacial gingival tissues allowing the tissues to be maximally preserved because there is only one surgical procedure, and (3) an optimal aesthetic result as a consequence of correct fixture position and angulation.[2] However, there is often lack of stabilization between the walls of the alveolar bone and the implant after immediate implantation. A previous study showed that there was a smaller amount of direct bone to implant contact when the gaps were wider than 1 mm.[3]

Recently, tissue engineering has become available as a regenerative treatment for bone defects and may offer a sufficient bone regeneration volume at immediate implant sites.[4] Considering the irregular bone defects of extraction sockets, the use of an injectable tissue-engineered bone (ITB) that adapts to the bone defect is of great advantage in immediate implant surgery. In a previous study, a novel injectable bone cement designed as a composite of calcium sulfate hemihydrate ($CaSO_4.^1/_2H_2O$; CSH) and nano-hydroxyapatite/collagen (nHAC) was proved to exhibit satisfactory biocompatibility and favorable bioactivity of bone regeneration.[5] Both calcium sulfate[6–9] and nano-hydroxyapatite[10–12] are reported to be excellent candidates for bone repair and regeneration; collagen[13] can improve the toughness of pure inorganic materials and has exceptional osteoconductivity and osteoinductivity. The combination of their favorable properties may further enhance bone regeneration efficacy.

Peripheral blood-acquired mesenchymal progenitor cells (BMPC) are easily obtained and possess multidifferentiation potential comparable with bone marrow derived stromal cells (BMSC).[14] Consequently, they can act as a new source of seeding cells for bone regeneration and tissue engineering.[15,16] Bajada and colleagues[17] reported that CSH could be combined with BMSC for successful bone tissue engineering. To date, no study has been published that examines novel CSH-based cement as a carrier for BMPC in injectable bone systems. Our hypothesis is that defects around implants treated with injectable ITB constructed with BMPC and nHAC/CSH would have a higher percentage of bone-implant contact (BIC) and a higher percentage of bone fill than defects treated with the carrier only (nHAC/CSH) and defects receiving no treatment (controls). The purpose of this study was to evaluate the bone regeneration of ITB in standardized peri-implant defects around immediate implants placed in dogs.

MATERIALS AND METHODS

Animal Models

Four adult male mongrel dogs with a weight of 15 ± 5 kg were enrolled in the experiments. The protocol in this study was approved by the Ethical Committee on Animal Research of the Chinese PLA General Hospital, and all the experimental procedures were performed according to institutional guidelines. The experimental model previously reported by Boix and colleagues[18] consisted of standardized peri-implant defects in the canine mandible (Figure 1A). This model was used in our study to evaluate the efficacy of injectable tissue engineered bone.

Implants

The implants of β-type titanium alloy Ti-24Nb-4Zr-7.9Sn (T2448)[19,20] were obtained from the Institute of Metal Research, Chinese Academy of Sciences. Twenty-four cylindrical-shaped implants (length 10 mm; diameter 3 mm) were used in this study. Micro-arc oxidation (MAO)[21,22] is an effective and inexpensive method to improve bioactivity. The surface of the T2448 alloy was modified by MAO in this study. All implants were sterilized in an autoclave at 121°C for 30 minutes before use.

Injectable Bone Substitute

The grafting material was a powder composed of CSH and nHAC.[4,23] It was purchased from Beijing Allgens Medical Science and Technology Co. According to the instruction, it was mixed with the liquid in a ratio of 0.8 ml/g when applied. The paste was then transferred to a 5-ml syringe (Figure 1B) to be injected to the bone

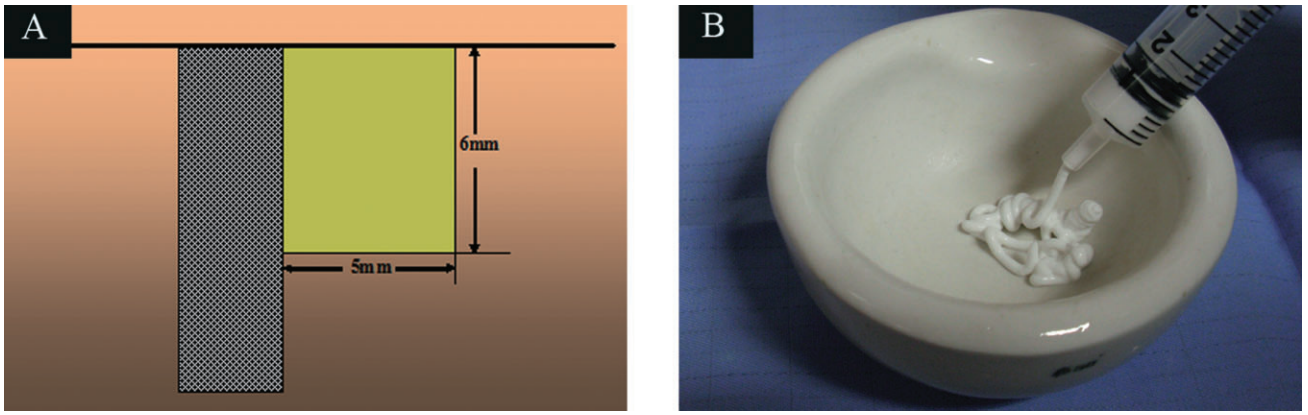

Figure 1 (A) Schematic of the experimental site. (B) Photograph of the ready-to-use injectable cement placed into an injector.

defects during implantation. The powder was sterilized by γ-irradiation at 25 kGy before animal experiments.

Dog Blood-Acquired Mesenchymal Progenitor Cells (dBMPC) Isolation and Culture

Autogenic peripheral blood (PB) was obtained from the four dogs and BMPC were isolated by density gradient centrifugation method. The PB (about 15 ml) was aspirated from the forearm vena through a scalp needle into a 20-ml heparinized syringe at a final concentration of 100 U/ml. The PB was diluted 1:1 in Minimum Essential Medium Alpha (α-MEM; Gibco, Rockville, MD, USA) and was stratified on Ficoll-Hypaque gradient (Sigma, St. Louis, MO, USA) to collect mononuclear cells. Then the mononuclear cells were washed twice in complete medium, resuspended in the fresh complete culture media containing α-MEM, 15% fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA), 2 mM L-glutamine (Gibco BRL), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. The cells and suspension were then transferred to a well of a six-well dish and incubated in a humidified atmosphere containing 5% carbon dioxide at 37°C. After 4 days, nonadherent cells were eliminated by replacing the medium and the fresh complete medium was added. The medium was changed every 3 days. When the fibroblast-like cells at the base of the well almost reached confluence, the adherent cells (mainly BMPC) were detached with trypsin–ethylene diamine tetra acetic acid solution according to standard protocols and subcultured at 1×10^4 cells/ml.

Immunofluorescence Assay

Immunofluorescence staining of cultured dBMPC with monoclonal antibody (mAb) against Vimentin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:100 dilution was performed. Specimens were processed using identical protocols. Specimens without additional primary antibodies were used as the negative control.

Transdifferentiation Assays

The dBMPC differentiation potential along osteogenic and adipogenic lineage was evaluated as follows.

Osteogenesis. The dBMPC were placed in a six-well plate at a density of 50,000 cells per well in base medium for 24 hours to allow cell adhesion. Medium was replaced with the osteogenic inductive medium consisting of 15% FBS, 10^{-8} M dexamethasone, 10 mM β-glycerophosphate, and 50 μg/mL ascorbic acid (all from Sigma). The medium was changed every 3 days after initial plating. Cells were cultured for 35 days and mineralized nodules were revealed by alizarin red staining. Cells were fixed with 95% ethanol for 30 minutes, rinsed three times with phosphate-buffered saline (PBS), treated 30 minutes with 0.1% alizarin red at pH 8.3 to stain the calcium salts, gently washed with PBS for 15 minutes and then observed under a light microscope.

Immunohistochemistry for Collagen type I (Col I) was performed on the osteogenically differentiated cells at 21 days similarly prepared on glass slides. The endogenous peroxidase activity was inhibited with 3% hydrogen peroxide for 10 minutes. After being washed with PBS, they were blocked in 5% normal goat serum at room temperature for 30 minutes. Sides were then incubated overnight at 4°C in humidified chamber with or without mouse mAb against Col I (Acris Antibodies GmbH, Herford, Germany). Sequentially, the slides were rinsed three times with PBS (5 minutes each time),

incubated with reagent 1 and reagent 2 for 20 minutes, respectively, as prescribed by the manufacturer, using Polymer HRP Detection System (Zhongshan Biochemical, Beijing, China). A color reaction was developed using 3, 3′-diaminobenzidine tetrahydrochloride and the slides were lightly counterstained with hematoxylin. A brown precipitate was formed indicating positively stained Col I.

Adipogenesis. Cells were seeded at the same density as used in the osteogenic approach and cultured for 20 days in adipogenic inductive medium containing 15% FBS, 1 μM dexamethasone, 10 μg/ml insulin, 0.2 mM indomethacin, and 0.5 mM 3-isobutyl-methyl-xanthine (all from Sigma). Medium was changed every 3 days. Adipogenic differentiation was shown by cellular accumulation of large lipid drops that were stained red with oil red O.

Construction of ITB

BMPC were induced by osteogenic medium for 2 weeks before in vivo study. The inductive medium was replaced every 3 days and the confluent differentiated BMPC were passaged after the initial induction. Confluent layers formed after 2 weeks were harvested by trypsination and counted. The viability of the cells was detected using the trypan blue exclusion assay. A 0.8 ml suspension of BMPC at final concentration of 2×10^6/ml and powder components of 1 g nHAC/CSH composite were stirred with a spatula in a crucible for about 1 minute to form a homogenous paste – this was the injectable tissue engineered bone. When applied, the mixture was aspirated into a 5-ml syringe with a puncture needle.

Investigation of Alveolar Bone Regeneration at Dental Implant Defects of ITB in the Canine Mandible

The four dogs received no food or water for about 12 hours prior to surgery and were anesthetized by intravenous penobarbital sodium (15 mg/kg). During surgeries, the dogs received lactated Ringer's solution intravenously. Under aseptic conditions, gingival incisions were performed mesially from the first premolar and distally to the molar teeth. Then, a full-thickness mucoperiosteum flap elevation was performed. The second, third, and fourth mandibular premolars (P2–P4) were removed bilaterally after vertical interradicular section and root elevation.

Mesial sockets were then drilled for implant placement under saline irrigation according to a routinely used implant surgical protocol. Thereafter, the interradicular septa were resected with a manual rongeur to create a distal bone defect adjacent to the distal socket at a 6-mm height, 4 mm in the bucco-lingual direction, and 5 mm in the mesio-distal direction (Figure 2A).[18] Therefore, there were three implants sites with distal bone defects on each side. After cylindrical implants were manually inserted and gently tapped into the implant beds (Figure 2B), the three defects on one side were implanted using the following graft materials, respectively (Figure 2C): (1) ITB constructed with dBMPC and nHAC/CSH (dBMPC + nHAC/CSH); (2) injectable bone cement nHAC/CSH; or (3) no materials, leaving the defect only (control). However, implants and grafting materials were placed following the same procedure. The selection of treatments was random. Resorbable collagen membrane (Bio-Gide, Geistlich Pharma AG, Switzerland) was used to prevent connective tissue prolapse in this experiment (Figure 2D). The wounds were closed with an interrupted nonabsorbent suture that was removed under short general anesthesia 15 days after the procedure.

After surgery, penicillin (40,000 IU/kg per day) was given for 4 days by intramuscular injection. Animals were checked daily and fed with a soft diet for 2 weeks. A normal diet was then resumed. Samples were detected by radiographic examination at 12 weeks ($n = 8$) after implantation.

All dogs were sacrificed at 12 weeks postsurgery and the implant containing premolar alveolar parts was prepared for histological interpretations. The tissue blocks were immediately fixed with 10% neutral formalin for 48 hours, dehydrated in graded ethanol, and embedded in methylmethacrylate. These specimens were longitudinally sectioned into 220-μm-thick slices parallel to the axis of the cylinder in a mesiodistal direction, using an Exakt Cutting-Grinding System (Exakt Apparatebau, Norderstedt, Germany). The sections from each implant site were prepared and reduced to a final thickness of about 20 μm by micro-grinding and polishing using a micro-grinding unit (Exakt) and stained with methylene blue for histomorphometric analysis of the tissue surrounding the implant.

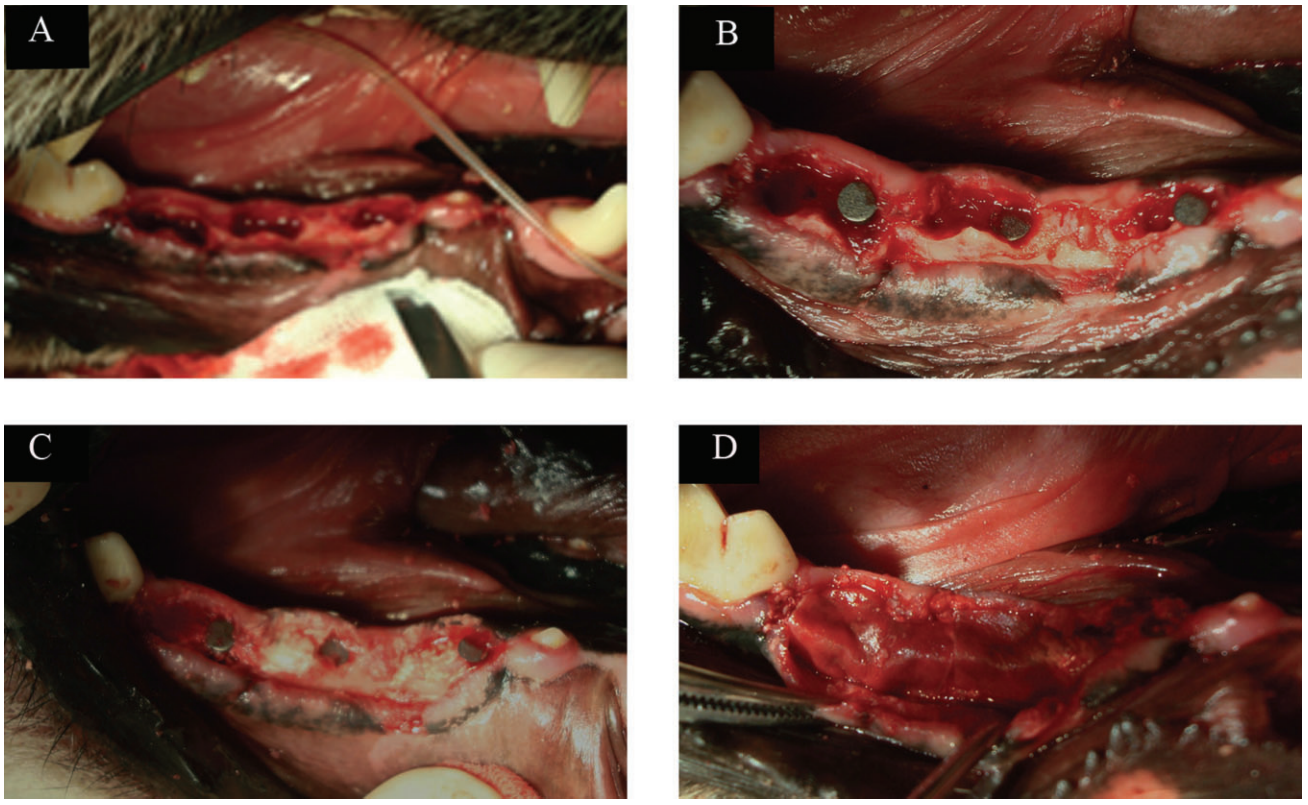

Figure 2 Surgical procedures. (A) Alveolar sockets after teeth extraction and defects preparation. (B) Cylindrical implants were applied in the mesial sockets. (C)The defects were filled with dBMPCs + nHAC/CSH, nHAC/CSH, or nothing. (D) Membranes were placed after implantation. dBMPC = dog blood-acquired mesenchymal progenitor cells; nHAC = nano-hydroxyapatite/collagen; CSH = calcium sulfate hemihydrate.

Two histomorphometric parameters were blindly measured in distal bone defects and distal sites by a single observer with a microscope (DMI3000 B, Leica, Solms, Germany) equipped with a camera and Image Pro Plus analysis software (Media Cybernetics, Silver Spring, MD, USA): (1) new BIC: the percentage of new BIC at the 6-mm cervical height limited to the bone defects; (2) bone density (BD): the percentage of new bone in the defect area (i.e., the new bone area in the defect divided by the defect area).

Statistical Analysis

The results of the histological analysis were expressed as the mean ± standard deviation from three experiments. The data analysis was carried out using a statistical package SPSS 13.0. The one-way analysis of variance was used and the level of significance was set at $p < 0.05$.

RESULTS

Morphology of dBMPC

Mononuclear cells from dog PB were isolated by the Ficoll-Hypaque density gradient centrifugation. At about 5 to 8 days after initial plating, visible symmetric colonies formed by adherent, spindle-shaped fibroblastic cells appeared in PB mononuclear cell cultures as described previously.[24,25] Over the ensuing 6 days, the colonies further expanded, the fibroblast-like cells became the predominant cell type in the culture and were frequently found around the clusters (Figure 3A). The sixth to eighth passage dBMPC with a fibroblast-like morphology were used for preparation of composites in this study (Figure 3B).

BMPC in this study were stained positive for Vimentin by immunofluorescence assay (Figure 3C). The control slide showed negative staining for Vimentin (Figure 3D), indicating that the cells had mesenchymal fibroblast-like properties.

Transdifferentiation Potential of dBMPC

Under the osteogenic inductive conditions, BMPC became osteoblast-like in morphology, with a cuboidal shape and tightly packed arrangement; the monolayer cells aggregated to form mineralized nodules with differentiated cells trapped in an abundant matrix that was

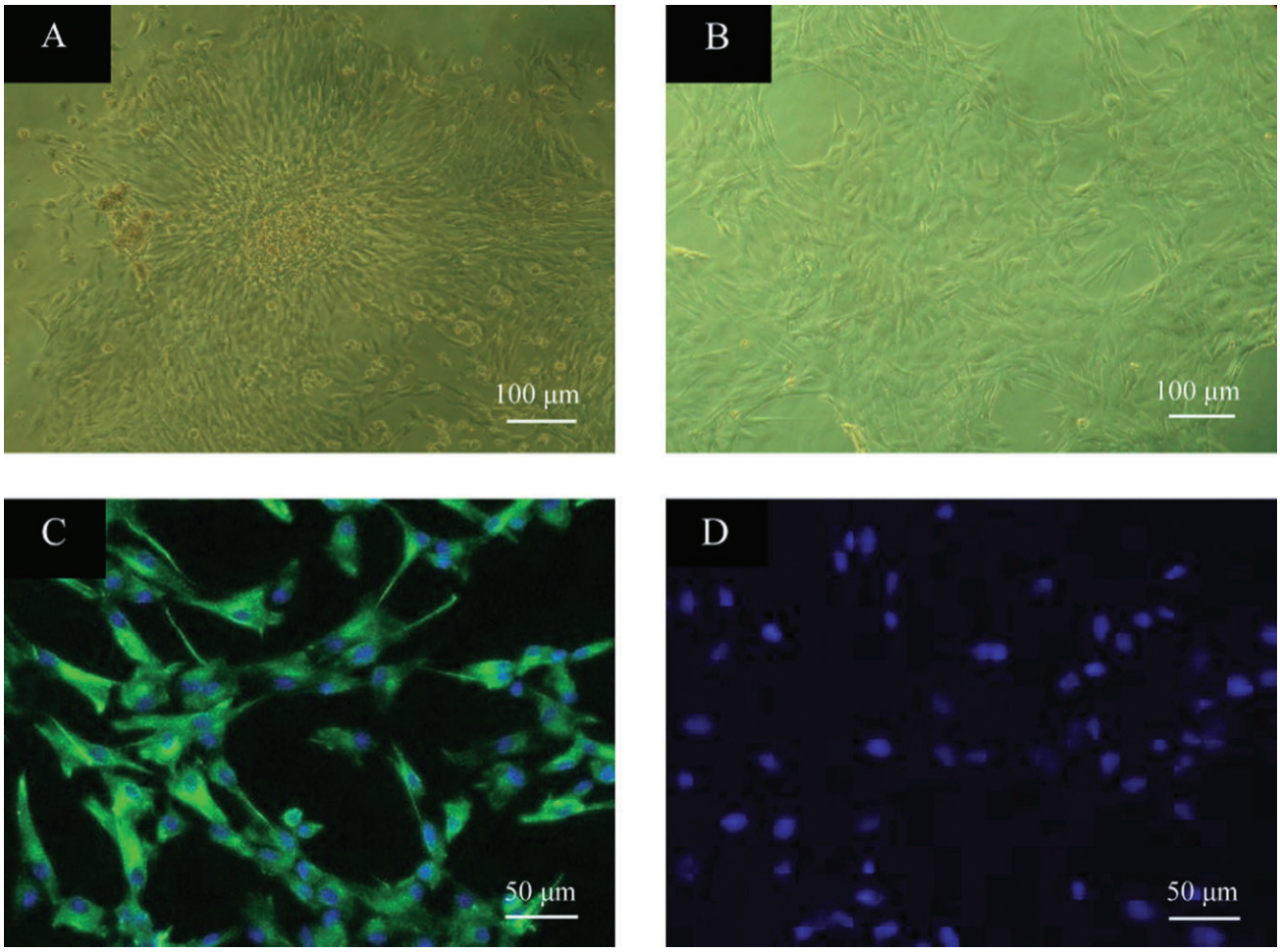

Figure 3 Typical fibroblast-like morphology and source identification of the dBMPC. (A) Cell clone derived from primary peripheral blood monuclear cells. (B) Homogeneous, confluent BMPC in culture at the end of the sixth passage. (C) Immunofluorescence staining of dBMPC was positive for Vimentin. (D) Control slide was negative. (A, B) Bar = 100 μm; (C, D) Bar = 50 μm. dBMPC = dog blood-acquired mesenchymal progenitor cells; BMPC = blood-acquired mesenchymal progenitor cells.

positive in alizarin red (Figure 4A). Col I is one of the most important extracellular matrix macromolecules of osteoblasts and known to be an early osteoprogenitor marker, which is critical for the components and development of bone.[26] Immunostaining against Col I revealed further evidence of osteogenic differentiation. A morphologically distinct subset of large polygonal cells, strongly staining for Col I, was seen 21 days after osteogenic induction (Figure 4B). In contrast, cells that were not incubated with primary antibody showed negative staining for Col I (Figure 4C).

Adipogenic differentiation was induced in the expanded progenitor cell cultures by treatment with adipogenic inductive medium. The presence of many, large lipid droplets positively stained by oil red O was observed 20 days after the induction (Figure 4D). Thus, the differentiation potential displayed by blood-derived MPC resembled very closely the characteristics assigned to bone marrow-derived MPC.

Histometrical Measurements of Peri-Implant Bone Regeneration

All dogs recovered well from surgery. All implants remained submerged and were covered with a clinically healthy mucosa without associated signs of postsurgical complication or infection until sacrifice. Twelve weeks after extraction and grafting, radiologic examination showed no pathology around all implants or defect sites and a more granular type of bone in the dBMPC + nHAC/CSH group than the other two groups.

Ground sections showing implants and adjacent peri-implant tissues of test and control sites after 12 weeks of healing were presented in Figure 5. In the control group, few new bone formations were observed

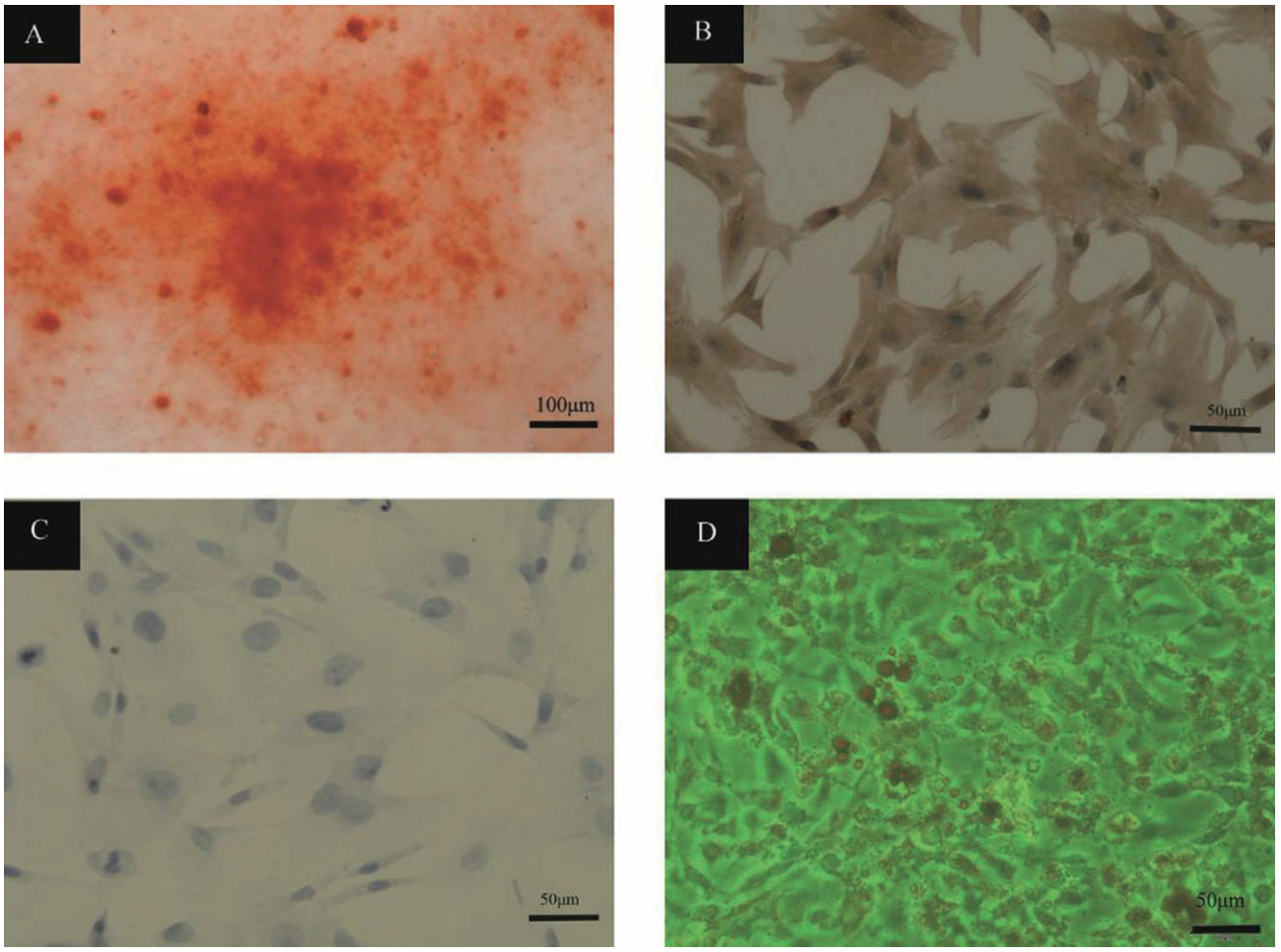

Figure 4 Multilineage differentiation capacity of dBMPC cultured in specific inductive media. (A) Positive alizarin red staining for mineralization; (B) positive staining against type I collage; (C) no positive staining was seen in the nonprimary antibody control; (D) oil red O positive lipid droplets. (A) Bar = 100 μm; (B-D) Bar = 50 μm. dBMPC = dog blood-acquired mesenchymal progenitor cells.

at the peri-implant defect sites. Some newly formed trabecular bone was noted to contact directly with the implant surface (Figure 5, A and B). In the nHAC/CSH group, more new trabecular bone formations were observed on the surface of implants compared with the control group (Figure 5, C and D). In the dBMPC + nHAC/CSH group, more bones were formed in direct contact with the implant surface and the newly formed trabecular bone occupied a substantial part of the defect (Figure 5, E and F).

The implants exhibited varying degrees of BIC and BD. The BIC was 18.27 ± 2.15% (control), 33.13 ± 7.29% (nHAC/CSH), and 65.03 ± 3.13% (dBMPC+nHAC/CSH) (Figure 6A). The density was 12.12 ± 3.08% (control), 28.02 ± 7.48% (nHAC/CSH), and 61.74 ± 3.6% (dBMPC+nHAC/CSH) (Figure 6B). Comparing the groups with each other, there were significant differences observed in the BIC and BD among the three groups. These results suggest that ITB constructed with nHAC/CSH and dBMPC improve bone regeneration and osseointegration of dental implants; however, the nHAC/CSH alone was less effective in achieving BD and BIC.

DISCUSSION

$CD14^+$ fibroblast-like mesenchymal progenitor cells can be isolated from human PB monocytes in primary cultures in the presence of fibronectin.[14,27] In this study, plastic-adherent and colony-forming cells were isolated from canine nonmobilized blood-derived mononuclear cells when cultured in the medium that contains only FBS. In addition, the nature of the adherent cells was found to be consistent in all cases, exhibiting a fibroblast-like morphology.

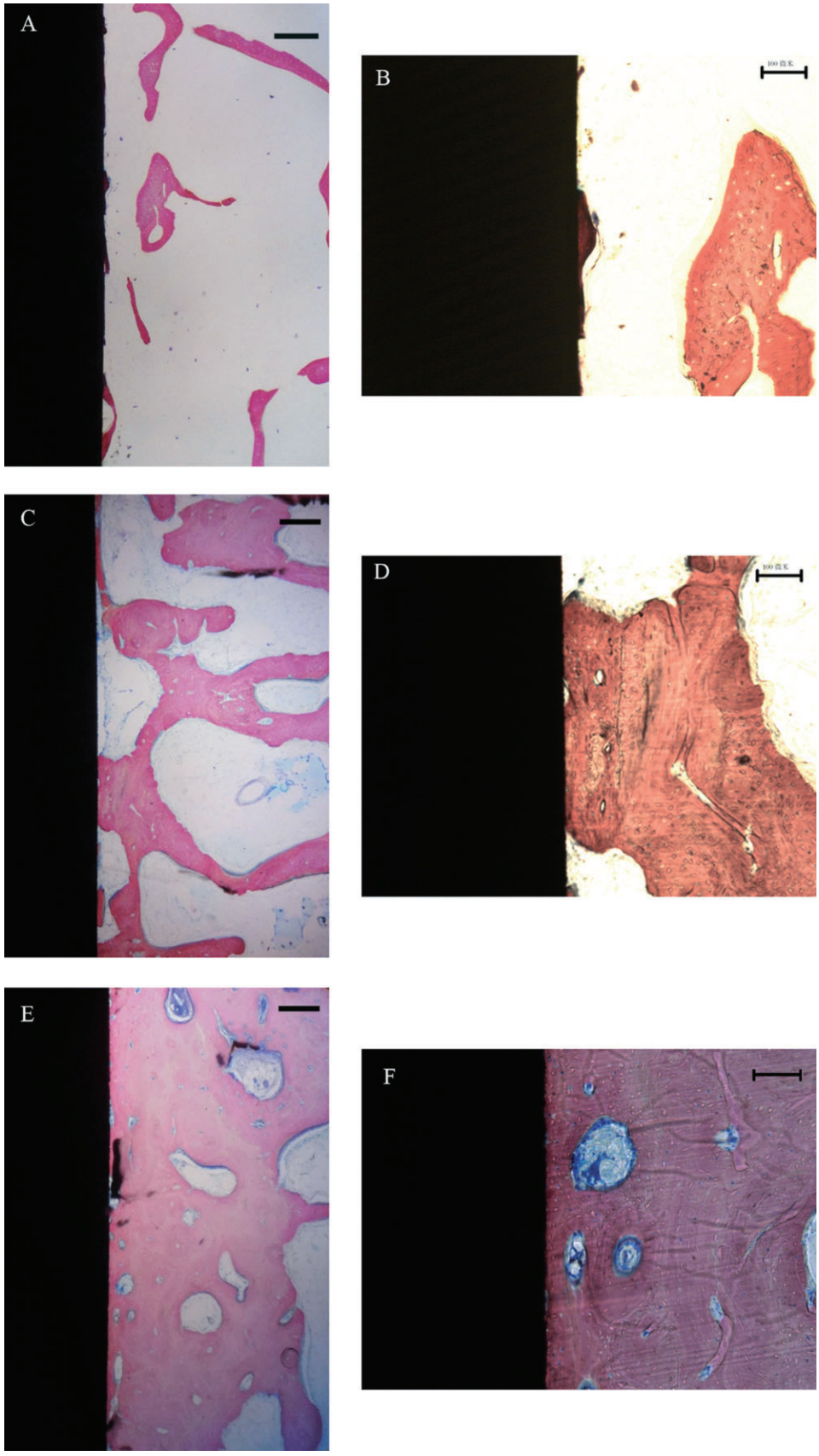

Figure 5 Histology methylene blue staining of peri-implant bone regeneration. (A, B) control; (C, D) nHAC/CSH implants; (E, F) dBMPC + nHAC/CSH implants. (A,C,E Bar = 500 μm; B,D,F Bar = 100 μm). nHAC = nano-hydroxyapatite/collagen; CSH = calcium sulfate hemihydrate; dBMPC = dog blood-acquired mesenchymal progenitor cells.

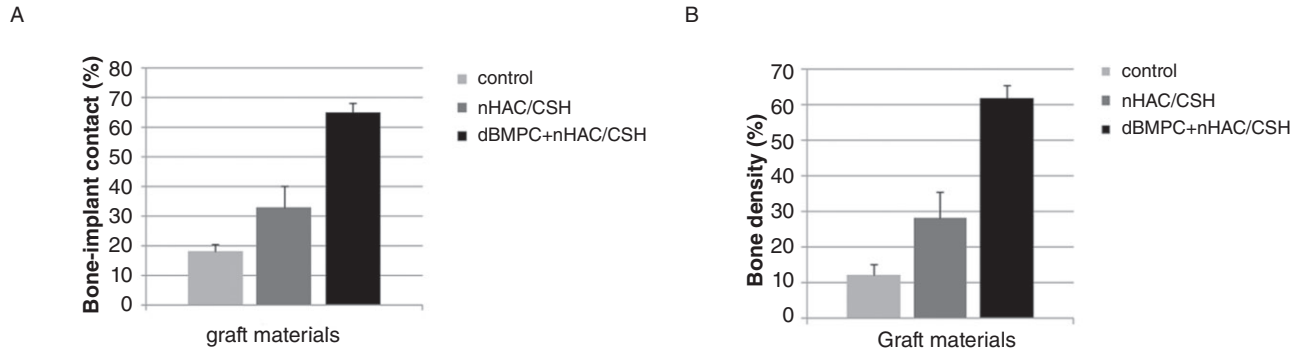

Figure 6 Histomorphometric analyses. (A) Means for bone-implant contact in bone defect height at 12 weeks. (B) Means for bone density by area within defects at 12 weeks.

In a recently published study, BMPC were subjected to flow cytometric surface marker expression analysis.[28–30] It was reported that the human blood-derived adherent cells were negative for the human marrow stromal marker, Stro-1,[30] but positively expressed adhesion molecules CD29,[28,30] CD105,[28] CD106,[28,30] receptor molecule CD44,[28–30] and extracellular matrix protein CD90.[28,29] The cells were negative for hematopoietic and macrophage markers CD45 and CD14,[28,30] which confirmed existence of mesenchymal stem cells in PB. Vimentin is used as a classic marker for mesenchymal cell and mesodermally derived tissues. In this study, dBMPC were stained positive for Vimentin showing that dBMPC had mesenchymal fibroblast-like properties. In addition, they were successfully induced to differentiate towards osteoblasts and adipocytes in vitro. This further confirmed that BMPC were capable of at least bi-lineage differentiation.

The evidence for the isolation of fibroblast-like cells with a mesenchymal phenotype from the PB is conflicting. Lazarus and colleagues[31] and Wexler and colleagues[32] reported no evidence of circulating precursor cells in the PB. The different results may be attributed to variations in methods of cell purification, culture, and characterization.[33]

An ideal bone graft substitute material is one that is biodegradable and completely replaced by new bone formation from host.[34] Functional biomaterial research recently has been directed towards the development of an injectable scaffold. In this regard, several injectable, degradable, biocompatible, and osteoconductive ceramic bone cements or substitutes have been developed for their positive handling characteristics. A new type of composite bone cement was prepared and investigated by adding nHAC to CSH. CSH is a biodegradable material that has been the subject of much research[35,36] and it has often been applied clinically as a bone substitute for many decades.[6–9] Moreover, the nHAC composite is another excellent material because of its biodegradable, biocompatible, and bioactive characteristics. This composite cement can be handled as a paste and easily shaped into any contour within 5 to 20 minutes.[23]

The material was studied both in vitro in simulated body fluid (SBF), and in vivo implanted subcutaneously in mice[23] and into the mandible of rabbits.[5] Degradation rate was carried out by soaking the specimen in PBS.[23] Tissue response, material resorption, and bone ingrowth were studied in the animal models.[5,23] A positive tissue response in both the rat and rabbit without inflammatory reactions and the presence of new woven bone formation in the initial stages were observed. Aging in SBF showed that bone-like apatite precipitated on the surfaces of the material[23] and this might accelerate bone formation. These reports also showed that nHAC/CSH can provide more adequate stimulus for cell adhesion and proliferation, embodying favorable cell biocompatibility.[5,23] When the nHAC/CSH was mixed with liquid, it became an injectable paste (Figure 1B). The paste was injected easily and formed a solid with smooth surface after coagulation. The results suggest that the composite cement has a reasonable setting time, excellent degradability, and suitable mechanical strength and bioactivity.

The main difference between the present and previous studies[5,23] is that we regenerated bones by a tissue-engineering method using the nHAC/CSH as a scaffold loaded with dBMPC. The previous in vitro results[5] indicated that BMSC can be differentiated to osteoblast on the surface of the nHAC/CSH. However, it is unknown whether the nHAC/CSH can be used as an injectable

scaffold loaded with stromal cells and the construct can lead to the formation of bones.

Traditional techniques for the treatment of an insufficient bone volume around implants include bone autografts, allografts, or guided bone regeneration.[37] Few studies[38,39] have been published on the application of tissue engineering for regeneration of osseous defects around implants. The results of this study confirmed that the dog model appears to be very suitable to test osseointegration between dental implants and the ITB. To our knowledge, this study demonstrates for the first time that the use of an injectable tissue engineered bone constructed with nHAC/CSH promotes bone regeneration around dental immediate implants on large animal models.

In this study, more abundant bone formation was found at the bone defect sites implanted with dBMPC + nHAC/CSH at 12 weeks, demonstrating that the injectable dBMPC + nHAC/CSH could activate the new bone formation. The mechanism of action of nHAC/CSH can easily be explained. First, CSH degrades into calcium and sulfur ions when being implanted. Then, the calcium ions combine with phosphorous from body fluids to produce calcium phosphate. When being resorbed, nHAC/CSH provides an osteoconductive surface that stimulates the recruitment of osteoblasts and the development of new bone in the defect. As CSH degrades, it also lowers the ambient pH that encourages angiogenesis and increases release of growth factors such as BMP-2, BMP-7, TGF-β, and PDGF-BB.[40,41] Furthermore, the dBMPC in nHAC/CSH may have the ability to act as a biologic stimulus to repair or regenerate osseous defects around immediate implants. In addition to possible conversion to an osteoblast-like phenotype placed in an osseous defect, cultured dBMPC have other properties that may stimulate or induce bone regeneration, including stimulation of angiogenesis and secretion of extracellular matrix proteins, such as type I collagen and secretion of bone growth factors.

The implants used in this study had micro-arc oxidized surfaces for the whole length. Li and colleagues[22] showed that MAO-treated titanium specimens on rabbits showed a considerable improvement in their osseointegration capability as compared with the pure titanium implant. The biological responses to the physical nature of machined and rough implant surfaces may affect grafting materials differently.[42] Therefore, the surface design of the implant could be created carefully in the clinical situation with peri-implant bone defects.

CONCLUSIONS

The results from this study demonstrated that the injectable nHAC/CSH cement, loaded with dBMPC, showed excellent clinical handling properties combined with a superior bone behavior. In vivo, the injectable ITB enhanced bone regeneration of oral implants surrounded with bone defects. Therefore, this novel injectable ITB is potentially a prime candidate for increasing the reliability of immediate implant therapy in large diameter sockets.

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