Peri-implant Alveolar Bone Augmentation Using Allogeneic Marrow-Derived Stem Cells: A Pilot Study in the Canine Mandible

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Abstract

Vertical bone augmentation at endosseous implants is a technically challenging procedure that, when successful, can contribute to the biomechanical and esthetic value of implant-supported dental rehabilitations. One approach is the creation of a protected space for host cell ingrowth and subsequent bone formation. The aim of this pilot study performed in the beagle dog model was to examine the feasibility of an alternative approach for concurrent implant placement and vertical bone augmentation using an allogeneic marrow-derived stem cell-based construct without a barrier membrane. Allogeneic marrow-derived stem cells were obtained from iliac crest aspirations from donor animals and subsequent cell culture expansion. Alveolar defects (7 mm long x 4 mm deep) were created six weeks following tooth extraction. Central to the defects, 3.5 x 8 mm endosseous implants were placed at a depth of 4.5 mm. The 3.5 mm of exposed implant was either a) left exposed to the forming blood clot, b) covered with a HA/TCP matrix, c) covered with an allogeneic-stem cell-loaded matrix or d) covered with an autogenous bone graft. After 6 weeks, block sections of the mandible were prepared for histological evaluation of healing. For each implant (2 per test group), three sections were made parallel with the long axis of the implants. The histological appearance of bone was scored for each dental implant at both the implant-host bone interface and the implant-regenerated bone interface. The results demonstrated that allogeneic stem cell–loaded devices supported the formation of a bone–to-implant interface along the entire vertical augmentation surface of the implants and along the bone-to-graft interface. Vertical augmentation was also achieved using autogenous bone. However, in the absence of allogeneic stem cells or in the absence of any matrix graft, bone regeneration failed to occur. This initial success with vertical alveolar bone augmentation at dental implants using an MSC-based tissue engineering approach suggests a number of avenues to improve or simplify current regenerative therapies in dentistry.

Keywords: Mesenchymal stem cell; Tissue engineering; Alveolar bone regeneration; Dental implant

Introduction

The development of guided bone regeneration for dental implant procedures has expanded the opportunities for endosseous implant placement [1–4]. This approach dictates that a space should be created extra-skeletally and protected by a barrier membrane to stabilize the blood clot and to exclude non-osteogenic cells [5–6]. Within this space, the slowly migrating osteogenic cells that come from the underlying marrow form new bone. Additional requirements include prevention of acute inflammation and mechanical stability of the wound [7]. This barrier membrane approach to Guided Tissue Regeneration has been extended to formation of bone around titanium dental implants [8]. However, limitations to the use of barrier membranes alone exist, such as a lack of osteogenic cells within the vicinity of the defect [9].
Pragmatic limitations are revealed in challenging vertical alveolar bone augmentation. Previous investigations reveal limitations in different clinical methods for obtaining clinically significant alveolar bone augmentation [10]. In a systematic review focusing on vertical bone augmentation, the authors considered GBR, distraction osteogenesis and onlay bone grafting. Vertical bone gain was reported to be 2–8 mm, but relatively high percentage of treatment complications was revealed. A general conclusion was that the generalizability of the approach is limited at this time [11]. In an earlier review, Aghaloo and Moy (2007) [12] indicated that existing evidence suggested that implant survival may function of the residual bone rather than grafted bone. Continued research is needed for clinical improvement [13].

The advances in bone–tissue engineering using marrow-derived mesenchymal stem cells (MSCs) offers the clinical opportunity to directly place appropriate numbers of osteogenic cells in desired extra-skeletal spaces to direct bone formation [14]. MSCs are rare cells resident among the bone marrow that can be selectively isolated from an aspirate and expanded several million-fold to generate tissue engineering devices containing relatively high numbers of cells [15]. Based upon the matrix environment, the MSC can be intentionally differentiated in vitro to adipose, tendon, muscle, cartilage or bone [16]. These developments in cell biology have led to pre-clinical evaluations of potential uses for diverse orthopedic indications [17-19]. An additional focus of MSC-based regenerative therapy is in the dental arena.

Vertical alveolar bone augmentation is a challenging clinical scenario of significant importance in dentistry. The simplification and expansion of this therapy might involve the direct circumferential application of a solid or semi-solid matrix at the exposed implant without need for an exclusionary membrane. To direct bone formation, an osteoinductive stimulus (e.g. BMPs) or an osteogenic precursor (e.g. MSCs) may be included. It was the aim of this introductory study in the beagle dog model to determine whether or not allogeneic (donor-derived) MSCs adherent to a hydroxyapatite/tricalcium phosphate (HA/TCP) matrix could direct vertical bone augmentation concurrent with placement of a cpTitanium endosseous implant without the application of an exclusionary membrane.

Materials and Methods

This pilot study using the beagle dog model was designed as a randomized, controlled investigation with four treatment arms; MSC allograft plus HA/TCP matrix, HA/TCP matrix only, autogenous bone, or no graft treatment. The research proposal was approved by the Institutional Animal Care & Use Committee (IACUC), at the University of North Carolina. All surgical procedures were performed under general anesthetic according to the guidelines of the IACUC and the Department of Laboratory Animal Medicine.

MSC harvest and expansion - Bone marrow aspirations were drawn from animals used in another study. The marrow donor animals were from a colony different from the recipient animals; this method has been shown in parallel studies to give a high probability of mismatched donor–recipient pairs, employed in the allogeneic construct group [20].

Bone marrow aspirations from the posterior ilium were performed using standard procedures with a biopsy needle. Nine milliliters of marrow were drawn into a 10 cc syringe containing 1000U heparin and transferred to the laboratory for MSC isolation and expansion. Marrow samples were washed with saline, followed by centrifugation over a 1.073 g/ml Percoll density cushion. The interface layer was removed and the cells were washed in phosphate buffered saline solution, counted and were plated in tissue culture flasks in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal bovine serum (FBS). Non-adherent cells were washed from the culture during twice weekly feedings [17]. When the plated MSCs were near confluence (3–4 weeks), they were trypsinized, passaged, and cryopreserved for use in alveolar bone grafting experiments.

Bone tissue engineering construct preparation – HA/TCP (60%/40%) was obtained in a porous block form (Biomatlante, Vigneux de Bretagne, France). Twelve (12) cylinders, 5.5 mm in diameter and 3 mm thick with a 3.5 mm central hole, were prepared. The machined matrices were then sonicated to remove particulate material and sterilized by heating at 250°C for 4 hours. Allogeneic MSCs (passage 1 following cryopreservation) were suspended at a concentration of 10 x 10⁶ cells/ml in DMEM (without serum) and were applied to six of the prepared cylinders in a closed, sterile system using negative pressure to assure loading within the matrix pores. The constructs remained in the MSC suspension for 48 hours. To remove non-adherent cells, the constructs were dispensed into 10 ml of sterile saline solution just prior to engraftment.

Bone autograft preparation – A 5.5 mm diameter core of autogenous cortical bone was excised from the buccal plate of the mandible lateral to the first molar roots. Central to this core, a 3.5 mm diameter osteotomy was prepared to create 6 grafts for placement circumferentially around the dental implant. The graft was immediately placed at the selected dental implants.

Animal surgery – The P2 and P4 bilateral mandibular teeth were extracted in six (6) beagle dogs. Six weeks after extraction, saddle defects (3 mm deep x 7 mm long) were created in the P2 and P4 regions of the mandible via a mid-crestal incision (Figure 1a, 1b). 4.5 mm deep osteotomies were prepared to 3.35 mm diameter for implant placement using sequential drilling and copious irrigation. Twenty four (24) 3.5 mm diameter x 8 mm long implants (Microthread; AstraTech AB, Waltham, MA) were placed to the 4.5 mm depth leaving 3.5 mm of the implant exposed above the alveolar defect (Figure 1c). The 3.5 mm of exposed implant was either a) left exposed to the forming blood clot, b) covered with an HA/TCP matrix, c) covered with an allogeneic–stem cell–loaded HA/TCP matrix or d) covered with an autogenous bone graft. Six (6) cylindrical constructs or autogenous bone grafts were placed directly onto the implant (Figure 1d) and (Table 1).

The twenty-four (24) surgical sites were then closed using vertical mattress 4.0 chromic gut sutures. The planned post–surgical relationship of the graft materials, the implants, and existing alveolar bone is illustrated in figure 2. Animals were provided antibiotic (Trimox), received a daily oral antimicrobial mouthwash (0.2% Chlorhexidine Gluconate) and a soft diet for 28 days. After a total of 6 weeks, the 6 dogs were euthanized and block sections of the mandible were made and fixed in 10% formalin. Four of the six constructs were evaluated for both the autogenous, MSC + HA/TCP and empty treatment groups. One of the six HA–TCP grafts remained intact in tissue culture flasks in Dulbecco’s Modified Eagle’s Medium (DMEM)

Histological analyses – Fixed tissues were processed for embedding in acrylic resin [21]. The embedded tissues were sectioned in the buccolingual direction to allow for assessment of the implants as well as the devices. Implants were bisected and additional sections were made from each tissue half. At least three sections were made through each implant and were evaluated after staining with Toluidine Blue by light microscopy.
a) Condition of residual alveolar bone and mucosal following tooth extraction.  b) Representation of surgical defect created in residual alveolar bone preceding implant placement and augmentation.  c) Condition of implants after placement in a supracrestal position bisecting the surgical defect.  d) Representation of autogenous graft (P4 site; left) and MSC-loaded matrix graft (P2 site; right).

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Table 1: Implant distribution
Results

All 6 beagle dogs survived the surgery successfully and did not reveal clinical signs of infection or undue or extended discomfort associated with the procedures. The placement of implants was achieved without buccal or lingual bone dehiscence and primary stability was achieved for all implants. One construct (cell-free) was fractured upon placement. All cpTitanium implants were present at the 6 week time point. Implants associated with allogeneic MSC-containing grafts or with autogenous bone remained in a submucosal location. All implants that were not grafted and the implants grafted with cell-free constructs were in a transmucosal condition following 6 weeks of healing. There was no apparent inflammation of the mucosa at these implants.

Radiographic evaluation of the mandibles following healing showed that the autogenous bone and MSC-matrix engrafted sites were intact and that bone formation occurred along the implant superior to the alveolar surgical margin (Figure 3a). In contrast, the cell-free matrix engrafted sites were devoid of the matrix and resembled the situation for the ungrafted implants (Figure 3b, 3c). There were no radioluencies present at the bone interface formed against the 4.5 mm of the implant placed within alveolar bone. Other pathologic signs were not identified.

Diagrammatic representation of model to evaluate MSC – loaded graft material for vertical alveolar bone augmentation simultaneous with implant placement. 8 mm implants were placed 4.5 mm into alveolar bone. The extra-skeletal 3.5 mm implant body was surrounded by a 3.5 mm inside diameter / 5.5 mm outside diameter x 3.5 mm high tissue engineered matrix loaded with adherent canine MSCs. During the six week period, osteoconduction between the alveolar bone and the MSC-loaded HA/TCP matrix was expected to occur (Black arrows). Additional osteoconduction may occur over a short distance at the implant alveolar bone crest (pink arrow). Normal bone healing would occur at the implant interface within alveolar bone. The main criterion for success in this model is osteoinduction and osteogenesis (green arrowheads) that would contribute to the formation of bone at the implant surface 2 – 3.5 mm from the surgical alveolar bone margin (dotted white line).
Histological evaluation of implants that were not grafted revealed the absence of osteoconduction from the superior surgical margins along the extra-skeletal implant surfaces (Figure 4a, 4b). A dense collagenous connective tissue formed at the implant-tissue interface (Figure 4c). There was limited cellular infiltrate located in the peri-implant connective tissue above the superior surgical margins. A direct bone-to-implant contact resulted at the implant along surfaces of the implant placed into the alveolar osteotomy (Figure 4d).

The evaluation of implants that were grafted with the cell-free constructs revealed a similar absence of bone formation extra-skeletally along the exposed implant surface. The gap between the implant and the matrix displayed no bone formation on the implant surface and one or two bone spicules (b) forming near the matrix (m) (Figure 5a, 5b). The peri-implant connective tissue in this region was largely devoid of polymorphonuclear lymphocytes or large numbers of mononuclear cells, or phagocytic macrophages (Figure 5c). The implant in host alveolar bone displayed a direct bone to implant contact (Figure 5d).

Photomicrographic evaluation of ground section histology of healing at implants without grafts. a) Absence of new bone formation at the extraskeletal portion of the implant. Arrows indicate isolated surgical margin / alveolar crest (b) Osteocondution along the implant surface was limited beyond the surgical margin (arrow). c) Higher power magnification of the intervening gap reveals dense connective tissue with collagen fibrils running parallel to the threaded implant surface with an absence of inflammatory cellular infiltrate. Dense bone was formed against the implant placed into host alveolar bone. Note the presence of a large multinucleated giant cell at the implant surface.

Photomicrographic evaluation of ground section histology from cell-free matrix grafts. a) Absence of new bone formation at the grafted portion of the implant. Arrows indicate isolated islands of new bone near the surgical bone crest (b). Connective tissue is surrounding the matrix (b). b) The gap between the implant and cell-free matrix was typically free of bone. c) Higher power magnification of the intervening gap reveals dense connective tissue and an absence of inflammatory cellular infiltrate. Opaque particulate material represents matrix particles that were displaced from the bulk graft upon placement. Isolated islands of osteogenesis were infrequently observed near the matrix. d) Dense bone was formed against the implant placed into host alveolar bone.
Histological evaluation of the bone-to-implant interface formed at the implants grafted with autogenous bone revealed successful osseointegration and vertical augmentation. The extra-skeletal region of the implant was opposed by the grafted bone or new bone formation. Because the autogenous bone was press fit around the threaded implants at surgery, any bone in the threads represents new bone formation following grafting (Figure 6a, 6b). Within host alveolar bone, new bone formation occurred within the threads and was observed along the majority of the endosseous implant surface (Figure 6c, 6d).

Photomicrographic evaluation of ground section histology of healing at implants with autogenous bone graft. a) The interfacial region between the host alveolar bone and graft bone is well healed and new bone formed within the threads located extrasakletally is revealed. b) A second implant indicates the successful integration of the implant with the autogenous bone graft at the most superior aspect of the implant located approximately 3.5 mm from the surgical margin of alveolar bone. c) High power examination of bone forming at the host alveolar bone – implant interface reveals rich cellularity and complete bone – implant interface. d) The implant bone interface at these implants was generally complete within the endogenous alveolar bone.

The histological evaluation of MSC-loaded HA/TCP engrafted sites revealed the maintenance of the HA/TCP matrix and the formation of new bone between the matrix and the extra-skeletal region of the implant (Figure 7). In the gap between the implant and the matrix, evidence of osteogenesis was observed in many locations spatially distinct from the surgical bone margin (Figure 8a, 8b). The implant surfaces were largely covered with newly formed bone both along the portion of the implant placed within alveolar bone and along the portion of the implant placed extrasakletally. Higher magnification revealed many sites where bone formation and supporting vascularization between the cpTitanium implant and the hydroxyapatite/tricalcium phosphate matrix (Figure 8d).

Photomicrographic evaluation of bone formation between implants and allogeneic MSC loaded grafts. The opaque matrix is filled with new bone tissue and opposed by dense connective tissue beneath the alveolar mucosal and gingival. At the superior aspect of the implant and in the gap between the implant and the graft, new bone tissue is present. This location is 3.5 mm above the surgical margin of host bone.
Photomicrographic evidence of ground section histology of healing at implants with allogeneic MSC-loaded grafts. 

a) The opaque matrix obscures much of the field, however, much of the gap between the implant threads and the matrix is filled with new bone tissue. 
b) High power examination of this region reveals rich cellularity of the forming bone not unlike the host bone healing. 
c) Bone formed at these implants within host alveolar bone was richly cellular and the bone – implant interface was generally complete. 
d) Higher magnification of the gap region between the MSC-loaded matrix and the implant reveals a rich vascular supply and densely cellular bone matrix formed within 6 week healing period.

As expected, osteoconduction was not restricted to the MSC-loaded HA/TCP sites. Osteoconduction occurred within the region of all HA/TCP matrices opposing the surgical bone margin (Figure 9a). At the allogeneic MSC-loaded matrices, inflammation was not noted. Moreover, regions of new bone formation were observed superior to the MSC-loaded HA/TCP matrix beneath the alveolar mucosa (Figure 9b).

Photomicrographic evidence of osteoconduction and osteoinduction at the HA/TCP and MSC-loaded HA/TCP matrices. 

a) Ingrowth of bone at this cell-free matrix / alveolar bone interface is representative of healing that occurred at all HA/TCP matrices which approximated the surgical margins. 
b) High magnification of a single island of osteogenic activity displays osteoblastic cells organized along an osteoid front only on the side of this island of newly formed bone which faces the HA/TCP matrix carrier. At this location in the sample the formed tissue is not congruent with the matrix. The forming tissue is surrounded by a relatively dense and fibrous connective tissue that is devoid of inflammatory cell types.
Reference


