Investigation of a Thermoplastic Polymeric Carrier for Bone Tissue Engineering Using Allogeneic Mesenchymal Stem Cells in Granular Scaffolds

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Purpose: **The purpose of this project was to compare alveolar bone repair by allogeneic mesenchymal stem cells using bioglass or synthetic hydroxyapatite (HA)/tricalcium phosphate (TCP) granular scaffolds delivered in a thermoplastic polymeric carrier.**

Materials and Methods: **Canine mesenchymal stem cells were obtained from iliac crest bone marrow of beagle dogs and expanded without differentiation. Cells were resuspended at a final concentration of 5×10^6 cells/ml in a thermoplastic polymeric carrier (30% w/v Pluronic F-127) and mixed with an equal volume of synthetic HA/TCP or bioglass scaffold and placed into surgically created 5 mm cylindrical defects in the edentulous premolar region of beagle dogs. After 4 weeks or 7 weeks, tissue healing was evaluated by standard histomorphometric methods (Bioquant Nova, Bioquant Image Analysis Corporation, Nashville, TN) by measurement of bone formation within five random sites from each biopsy.**

Results: **After 4 weeks, sites treated with or without mesenchymal stem cells contained 58.25 ±18.43% or 43.35 ± 17.68% bone area ($p = 0.049$), respectively. After 7 weeks, sites treated with or without mesenchymal stem cells contained 62.73 ± 19.10% or 60.39 ± 21.32% bone area. Bone formation occurred without inflammation in defects treated using Pluronic F-127 carrier with and without mesenchymal stem cells. There was no difference in percent bone area when bioglass or HA/TCP scaffolds were compared at either time point.**

Conclusions: **The thermoplastic polymeric carrier did not limit alveolar bone repair in the canine mandible. The combination of a thermoplastic, viscous carrier with a granular scaffold allowed for the delivery of allogeneic mesenchymal stem cells in a clinically manageable form that enhanced bone formation at early stages of alveolar repair.**

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INDEX WORDS: tissue engineering, mesenchymal stem cell, scaffold, osteogenesis, alveolar bone repair

ADVANCEMENTS IN the prevention of dental disease have been paralleled by innovation in treatment techniques and materials to replace missing teeth. Trauma, periodontal disease, congenital and acquired defects, or surgical resection of cancer also result in reduced volume of bone with inferior physical properties.[1] For these patients, tooth replacement frequently necessitates alveolar bone repair. Future advances in implant dentistry may be linked to innovation in bone regeneration. Predictable, simple, economical bone grafting procedures are desired.

Bone repair and regeneration is dependent upon osteogenesis, osteoinduction, and osteoconduction.[2] Grafting materials invoke one or more

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of these biologic processes. Currently, autogenous bone harvested from extraoral or intraoral sources is considered the best grafting material and has no existing replacement for larger defects.[3] Allografts have been widely investigated[4-6] and used with satisfactory results; however, bone formation is slower and the volume is smaller.[7] Additional risks of host-donor incompatibility or disease transfer must be acknowledged. Finally, a great variety of synthetic bone substitutes are currently used and include human or bovine-derived bone mineral, bioactive glasses, synthetic or natural polymers, and synthetic calcium phosphate or apatite ceramics. They are largely regarded as osteoconductive materials dependent on host tissue ingrowth and host-dependent osteoinduction.

Bioceramics include inert (e.g., alumina), surface active (e.g., Bioglass®), and resorbable materials (e.g., β-TCP, beta-tricalcium phosphate). Bioactive glasses are hard, solid (nonporous) materials consisting of calcium, phosphorus, and silicon-dioxide (silicate, the main component). By varying the proportions of sodium oxide, calcium oxide, and silicon dioxide, a range of forms can be produced, from soluble to non-resorbable. They possess both osteoinductive and osteoconductive properties. Both TCP-ceramic and hydroxyapatite (HA) are highly biocompatible. Synthetic HAs are relatively non-resorbable, while tricalcium phosphates are resorbed by solution- and cell-mediated actions. When attached to healthy bone, osteoid is produced directly onto the surfaces of the ceramic in the absence of a soft-tissue interface. Consequently, the osteoid mineralizes, and the resulting new bone undergoes remodeling. The use of bioglasses and resorbable calcium phosphates as scaffolds for bone repair may permit formation of osteoid and resorption of the synthetic scaffold.

Tissue engineering[8] targets tissue regeneration by using molecules, cells, or tissues alone or in combination with biocompatible materials that support and augment the body's natural healing process.[9] Both bone and cartilage formation represent intense areas of tissue engineering research.[10] The potential advantages of bone tissue engineering include reduced negative host responses, reduced surgical interventions, and enhanced bone repair.[11]

Tissue engineering of bone employs growth factors to modulate innate cellular activities. Growth factors exert pleiotropic effects and are involved in the repair of all tissues,[12] including alveolar bone, periodontal ligament, and cementum.[13] The bone morphogenetic proteins (BMPs) represent key osteoinductive signaling molecules involved in complex regulation of bone physiology. At the simplest level, BMPs are abundant in bone, they are produced by several cell types, including osteoblasts,[14] and their hallmark action is to commit undifferentiated pluripotential cells to differentiate into cartilage and bone.[15] Delivery of BMPs or other growth factors in a scaffold is a common experimental or clinical strategy for tissue engineering of bone.

Another tissue engineering strategy involves the use of progenitor or mature cells in a 3D biocompatible material scaffold with the appropriate signaling molecules or growth factors.[11] The bone marrow contains a population of undifferentiated pluripotent stem cells known as bone marrow stromal cells or marrow-derived mesenchymal stem cells[16] (MSCs). The MSCs may differentiate into osteocytes, chondrocytes, adipocytes, tendonocytes, myocytes, and bone marrow stem cells,[17] and may produce several connective tissues, including bone (Fig 1). MSCs can be isolated from many tissues (including bone marrow) expanded in vitro, and directed in a controlled manner to a desirable cell lineage.[18-20] Implantation of in vitro expanded MSCs within the appropriate scaffold resulted in bone regeneration in various animal models.[19,21,22] Potential allogeneic implantation may allow bone from a donor to be used for many recipients.

In bone-tissue engineering, the scaffold plays a key role, because osteoblasts are anchorage-dependent cells.[23] An "ideal" matrix should be biocompatible, easy to handle, possess osteoconductive and osteoinductive properties, and at the same time degrade at a rate that permits ingrowth of new bone. Additionally, it should allow rapid angiogenesis and provide the interface to respond to biological changes and to remodel the extracellular matrix as it integrates with the surrounding tissue. A wide range of grafting materials have been used as scaffolds. They are often the same materials used for bone repair and include allograft bone products (FDBA, DFDBA), natural bone minerals (bovine bone) calcium phosphate materials (HA, TCP), bioactive glasses, and polymer materials (PLA, PLAGA).

The Pluronic polymers (generic name: Poloxamers) are another family of materials largely used as drug carriers and controlled release

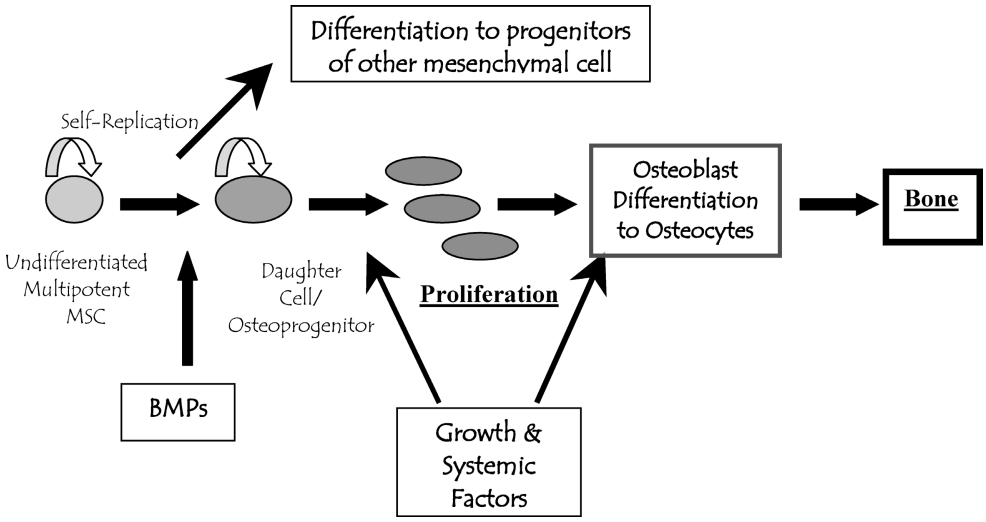

Figure 1. Summary of critical steps in differentiation and proliferation of MSCs toward the osteoblastic lineage (modified from Rose and Oreffo[11]).

agents. More recently, they have been examined as carriers for cells or BMP.[24] They are synthetic polymers of oxypropylene and oxyethylene in a basic A-B-A structure (EOx-POy-EOx). Interestingly, some copolymers exhibit the unique property of temperature-dependent reversible gelation. At room temperature, Pluronic F-127 exists as a viscous liquid, which is transformed into a semisolid gel at body temperature (37°C). The sol-gel transition can be controlled by creating various concentrations of polymers with different physical properties. For example, a 30% (w/v) solution of Pluronic F-127 is liquid at low temperature, but is a gel at 37°C.[25] While this is a useful property for clinical manipulation, these materials are soluble and may have limited stability in vivo. Pluronic F-127 increases early fibroblast attachment, early collagen formation, and microcirculation, thus promoting wound healing.[26] There is evidence that Pluronic F-127 has very low toxicity, suggesting it is a good candidate as a carrier of MSCs for bone grafting.[27] These characteristics suggest that Pluronic F-127 could be used in conjunction with granular scaffold materials to form a tissue engineering construct. The primary goal of this investigation was to evaluate the potential use of granular grafting materials in combination with a thermoplastic polymeric matrix as a moldable construct for MSC-based tissue engineering. The specific aim was to assess histologically the viability and the efficacy of allogeneic MSCs delivered within a polyaxomer polymer (Pluronic F-127) in a canine alveolar defect and to compare the bone repair observed between different matrices at 4 and 7 week intervals.

Materials and Methods

In this investigation, the canine mandible model was used in a split-mouth design with four treatment groups: MSCs plus polyaxomer carrier in an HA/βTCP matrix; MSCs plus polyaxomer carrier in a Bioglass matrix; Polyaxomer polymer with HA/βTCP only; and Polyaxomer carrier with Bioglass only. Moreover, two sites were left untreated for spontaneous healing and served as controls. Two more sites were treated with polymer plus MSCs in a Bio-Oss matrix (Table 1).

Six male and two female 1-year-old beagle dogs were used in the study and were donated by GlaxoSmith Kline (Research Triangle Park, NC). The animals were housed and treated in accordance with the policies established by the IACUC and the UNC Department of Laboratory Animal Medicine. Four weeks prior to the engraftment, the P2 and P4 teeth from the mandible of each animal were extracted bilaterally. Atropine 0.54 mg/20 kg was used for pre-surgical sedation. Induction was achieved by intravenous sodium pentothal at a dose of 13.2 mg/kg. One ml of lidocaine containing 1:100,000 epinephrine was provided for every site. Two to three 4-O resorbable gut sutures were placed per site. Postoperatively, the dogs were provided butorphanol 0.2 mg/Kg and amoxicillin 4 ml twice a day for 7 days. Daily mouth rinses with 5 ml of 0.12% chlorhexidine gluconate were performed daily, and a soft diet was provided for 2 weeks.

Table 1. Distribution of Cell-Loaded and Cell-Free Matrices in the Corresponding Post-Extraction Sites

		Grafting Material / Site			
Dog Number		*P2L*	*P4L*	*P2R*	*P4R*
3430677	1	A + BG	A + TCP	B + BG	B + TCP
3429024	2	A + BG	A + TCP	B + BG	B + TCP
3363279	3	A + BG	A + TCP	B + BG	B + TCP
3440338	4	A + BG	A + TCP	B + BG	B + TCP
3366367	5	A + BG	A + TCP	B + BG	B + TCP
3367167	6	A + BG	A + TCP	B + BG	B + TCP
3436951	7	B + BO	Empty	B + BG	B + TCP
3364381	8	B + BO	Empty	B + BG	B + TCP

A = cell-free matrices; B = cell-loaded matrices; BG = bioglass granules; TCP = β tricalcium phosphate granules; BO = Bio-Oss.
The numbers in the first column correspond to the dog number marked by tattoo on the ear.

Four weeks after extractions, the grafting procedure was performed. Canine MSCs had been previously obtained from allogeneic donor beagles and expanded without differentiation in culture as described by Bruder et al.[22] The cryopreserved MSCs were transferred rapidly at 37°C and immediately rinsed in 10 ml of sterile phosphate buffered saline (PBS). Cells were counted and suspended at concentration of 5×10^6 cells/ml. Two ml of suspension was mixed with 30% w/v Pluronic F-127 polymer carrier and loaded in 1 cc syringes. Additionally, cell-free Pluronic F-127 polymer was loaded in syringes. The carrier, which was in a liquid phase at room temperature, was mixed by sterile transfer with equal volume of granules of the appropriate grafting materials (2 ml) 30 minutes prior to the surgery. The grafting material was then transferred to the surgical room at 4°C.

Under general anesthesia, buccal full thickness mucoperiosteal flaps were elevated to expose the underlying bone. Eight mm deep osteotomies were prepared in the center of the extraction sites, using surgical drills (AstraTech Inc., Waltham, MA) to a final diameter of 5 mm under copious irrigation. Following site preparation, each loaded syringe was immersed in a water bath at 37°C for 1 minute, and the grafting material obtained a gel consistency. The osteotomies were filled completely with grafting material, and 4-O chromic gut sutures were placed to stabilize the flaps. Each of the four extraction sites of every animal was grafted with one of the four grafting combinations. The left side was assigned to receive cell-free grafts and the right side was assigned to receive cell-loaded grafts with either Bioglass (US Biomaterials, Jacksonville Beach, FL) or synthetic HA/TCP scaffold. Two sites were randomly assigned to receive Bio-Oss (Geistlich Biomaterials, Wolhuser, Switzerland) grafts, and two other sites were left empty for spontaneous healing (Table 1). Post-surgical care was provided as described above.

Four weeks after grafting, three randomly selected animals were anesthetized, full thickness mucoperiosteal flaps were elevated, and cores from the center of the grafted sites were removed using trephine burs with 3.5 mm external diameter (ACE Dental Implant Systems, Brockton, MA) to 8 mm depth. The cores were separated from the burs and were inserted in plastic bottles containing 10 ml of 10% buffered formalin for fixation. The flaps were sutured, and the same post-surgical care was provided for all animals. Identical surgical procedures were performed on the remaining 5 animals at 7 weeks after the engraftment (Fig 2). After the surgical sites were healed, the animals were included in a donation program.

All fixed tissue samples were decalcified with 5% ethylenediaminetetraacetic acid (EDTA) (pH 7.2) for 3 weeks, dehydrated, cleaned, and embedded in paraffin. The embedded cores were sectioned in a buccolingual direction parallel to the saggital plane in five micron sections. Four to five sections were obtained from each core. Sections were stained with hematoxylin and eosin and subsequently mounted on glass slides for histomorphometric analysis.

Quantitative histomorphometric analysis was performed using the Bioquant Nova Image Analysis System (BIOQUANT Image Analysis Corporation, Nashville, TN). Digital images from the histological sections made at 4× and 10× magnification were used for histomorphometry. The surface area of the new bone was calculated from five randomly selected sites per biopsy and expressed as percentage of the overall surface for further statistical evaluation.

Two sample *t*-tests were performed for inter-group comparisons in bone formation between sites grafted with MSC-loaded matrices and sites grafted with cell-free matrices for 4 and 7 week intervals. Intra-group comparisons were also made to test for evaluation of the effect of each matrix in bone regeneration. The level of significance was set at $p \leq 0.05$.

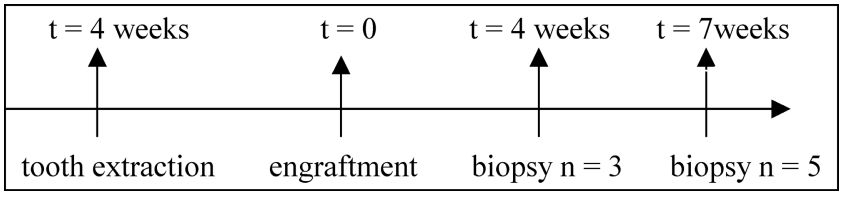

Figure 2. Chronological order of performed surgical procedures.

Table 2. Average Bone Formation (% area) Following 4 Weeks of Healing

	Matrix		
	TCP + BG	*TCP*	*BG*
MSC-loaded	58.25 ± 18.43	57.07	59.43
MSC-free	43.35 ± 17.68	44.29	42.42

TCP = tri-calcium phosphate; BG = bioglass; MSC = canine mesenchymal stem cell.

Results

Post-extraction and post-grafting healing occurred without clinical signs of infection, bleeding, or any significant discomfort for any of the animals. During the osteotomies, small buccal dehiscences in two P2 sites were created as a result of the small size of the mandible of the female animals. Early loss of sutures was observed in two sites of one animal after the grafting without any clinical consequence for bone healing. There was an absence of marked inflammatory cell infiltrate surrounding the implanted allogeneic cell containing constructs at either 4 or 7 weeks.

Histomorphometric analysis revealed that the bone formation for the 4 week period was higher for the sites grafted with cell-loaded matrices (mean = 0.5825, SD = 0.1843) than from those grafted with cell-free matrices (mean = 0.4335, SD = 0.1768) (Table 2). A paired *t*-test analysis revealed statistically significant differences between the two groups ($p = 0.04916$) (Fig 3). Statistical analysis between different matrices for the same healing period did not reveal any significant statistical difference. In 7 weeks, the average bone formation was relatively similar for cell-loaded (mean = 0.6273, SD = 0.1910) and cell-free matrices (mean = 0.6039, SD = 0.2132) (Table 3). Statistical analysis did not reveal any difference between cell-loaded and cell-free matrices, or between matrices (Fig 4).

Discussion

Bone repair and regeneration has been the aim of intensive orthopedic and dental research, and emphasis has been given to the development of the appropriate scaffold. The aim of this study was to evaluate the healing of alveolar bone defects when grafted with bioglass or HA/βTCP matrices loaded with allogeneic MSCs in a thermoplastic polymeric carrier.

After 4 weeks of healing, the sites that received allogeneic MSC-loaded scaffolds demonstrated higher bone regeneration, increased vascularization, and improved density (Figs 5 and 6). The direct implantation of a large number of osteoprogenitor cells (MSCs) within the appropriate matrix accelerates the process of bone formation and reduces the need for chemotaxis and massive proliferation of the osteoblast progenitor cells into the defect. Only a single concentration of cells was used in this study (5×10^6/ml). The 4-week time point for analysis was selected as an opportunity to view woven bone formation at an early time point. This was based on earlier studies using canine MSCs in a critical size defect.[28] In other preliminary studies, using fewer than $0.5–1.0 \times 10^6$ cells/ml to load HA/TCP scaffolds did not support reproducible bone formation (data not shown). While higher cell numbers may be effective, 1.0×10^6/ml was successful in regenerating bone in 8 weeks by use of a polyglycolic acid scaffold.[29]

Given the nature of the defects created, spontaneous bone repair for the untreated sites was anticipated. The rationale for choosing this defect size was based on replicating 3 wall defects

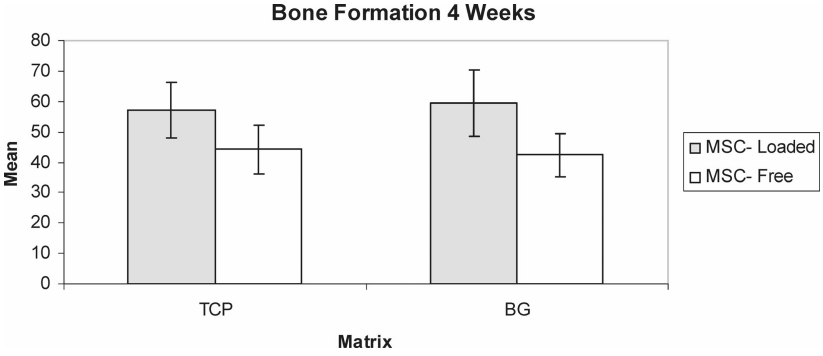

Figure 3. Percentage of bone formation at 4 weeks for sites grafted with cell-loaded and cell-free matrices (HA/TCP + BG) and percentages for each matrix separately.

Table 3. Average Bone Formation (% area) Following 7 Weeks of Healing

	Matrix		
	TCP + BG	*TCP*	*BG*
MSC-loaded	62.73 ± 19.10	60.06	65.40
MSC-free	60.39 ± 21.32	60.48	60.31

TCP = tri-calcium phosphate; BG = bioglass; MSC = canine mesenchymal stem cell.

commonly encountered in dental implant therapy. Therefore, it was important to evaluate both an early (4 week) and later (7 week) healing period. In 7 weeks of healing, there was a small tendency for larger volumes of newly formed bone to be present in the cell-loaded matrices (Figs 7 and 8). This preliminary investigation demonstrated the potential of bone repair by allogeneic cells delivered with a polyaxomer carrier. The advantages of grafting procedures are more distinct in situations where larger defects are present, or if the host healing mechanism or the number of precursor cells is deficient due to age, disease, or local factors[30,31] where spontaneous healing may result in deficient form and function.[32] Future studies involving critical size defects may reveal the efficacy of this allogeneic MSC-based tissue engineering construct.[33]

The construct tested included culture-expanded, undifferentiated allogeneic MSCs, an osteoconductive granular scaffold, and a thermoplastic polymeric carrier. The allogeneic MSC approach offers significant advantages. Allogeneic cells would be immediately available. The concept of an off-the-shelf, cell-based grafting material prepared and stored under strict quality and safety control protocols, with the appropriate modification for each clinical scenario may represent a significant advantage for the clinician. The surgical procedure for harvesting autogenous bone or autogenous MSCs would be eliminated. Allogeneic cells may overcome innate limitations of healing among patients who have been irradiated or have undergone chemotherapy. Risks for allogeneic MSC cells include disease transmission risks associated with all other allogeneic materials, as well as risks of rejection or development of graft versus host disease. Allogeneic, culture-expanded cells derived from bone marrow have been given in large numbers to supplement bone marrow of chemotherapy patients,[34] or in combination with allogeneic hematopoetic stem cells,[35] and were well tolerated; however, debate continues regarding the immunoprivileged status of the MSC.[36,37]

Allogeneic MSCs may form bone after engraftment. De Kok et al[28] demonstrated that allogeneic stem cells repaired 20 × 6 mm defects in canine mandibles. When allogeneic stem cells adherent to HA/TCP scaffolds were implanted in extraction sockets, bone repair occurred without inflammatory response to the allogeneic cells.[38] The treatment of patients with high numbers of allogeneic bone marrow-derived MSCs may be beneficial to some inflammatory disorders. For example, allogeneic MSCs may reduce the severity of Graft-Versus-Host disease (GVHD);[39] however, additional animal studies provided contradictory information and suggested that allogeneic cell MSCs are not intrinsically immunoprivileged and cannot serve as universal donors.[37] Further study of allogeneic MSCs for clinical bone repair is necessary.

The modeled clinical approach to bone repair used granular scaffolds in a carrier that imparted stability to the engrafted construct. There is

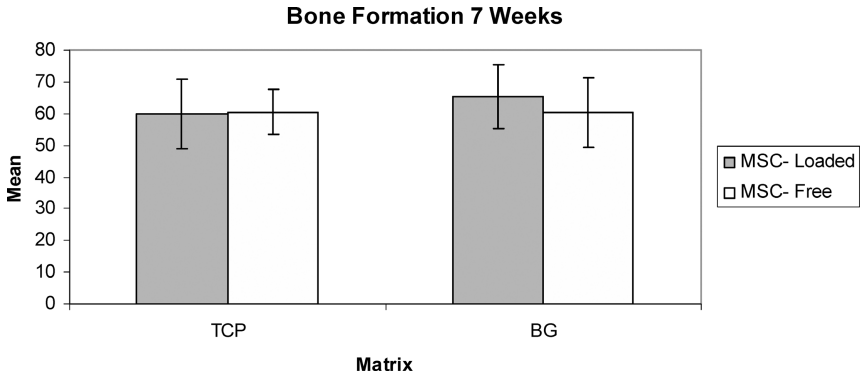

Figure 4. Percentage of bone formation at 7 weeks for cell-loaded and cell-free site or for different matrix separately. Similar results for all treatment groups are observed at this time interval.

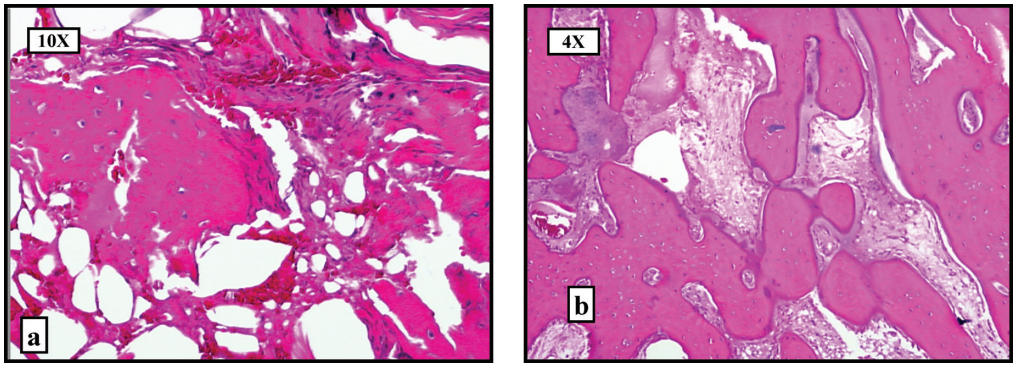

Figure 5. Histological evaluation of tissue formation in alveolar defects 4 weeks after grafting with or without MSCs in F-127/Bioglass scaffolds. Hematoxylin and eosin stained, demineralized sections of tissue formation for the cell-free sites (*a*) and cell-loaded sites (*b*). Increased early bone formation is evident in the grafted site, characterized by an increased number of osteoblasts and consolidating islands of woven bone (*b*).

evidence of advantage in bone formation when the scaffold is in the form of particles.[40,41] Mankani et al[42] observed a peak bone formation for scaffold particles between 0.1 and 0.25 mm, while smaller size particles probably impeded the vascularization due to close packing. Practically, the use of a granular material provides the advantage of shape adaptation to a specific defect. Biologically, granules may promote vascular and tissue ingrowth due to appropriate spaces between the inorganic matrix. Also, the use of granular material, due to increased surface for osteoclastic activity, accelerates the process of matrix resorption and replacement of anorganic material with newly formed bone. β-TCP materials display more rapid resorption, which is advantageous for small- or medium-sized defect regeneration; however, in this investigation, differences between the matrices were not significant.

The use of Pluronic F-127 (generic name Poloxamer 407) as a carrier merits further consideration. This carrier provided the construct with mixing and handling properties that facilitated placement. The Pluronic F-127 used in this study possessed a critical solution temperature (LCST) below 37°, and it exists in gel state in the body.[43] Others have suggested that salt concentration can alter the solution temperature, requiring higher polymer concentration.[24] For larger constructs, this would facilitate the complete fill of the defect area, and increased adaptation on the defect walls, as well as offer early stability of the graft after placement. The present results of enhanced bone repair are in contrast with the results of studies

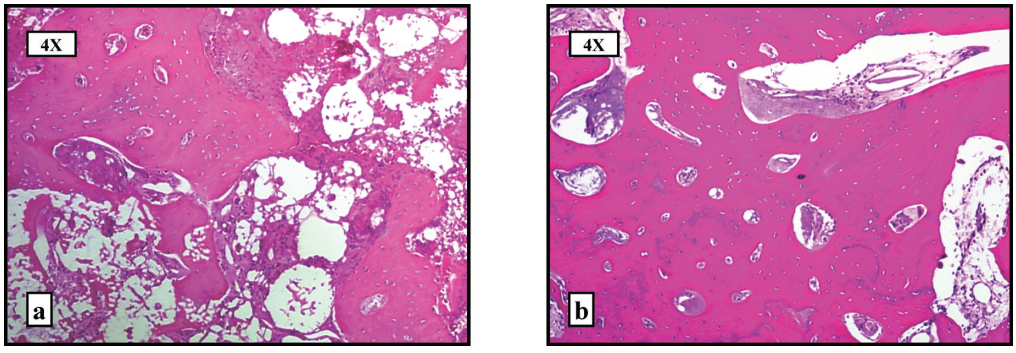

Figure 6. Histological evaluation of tissue formation in alveolar defects 4 weeks after grafting with or without MSCs in HA/TCP scaffolds. Hematoxylin and eosin stained, demineralized sections of tissue formation for the cell-free sites (*a*) and cell-loaded sites (*b*). The cell-free HA/TCP grafted site reveals woven bone formation and regions of loose connective tissue (*a*). Increased bone formation and resorption of inorganic matrix is evident in cell-loaded sites (*b*). Areas with new lamellar bone are observed.

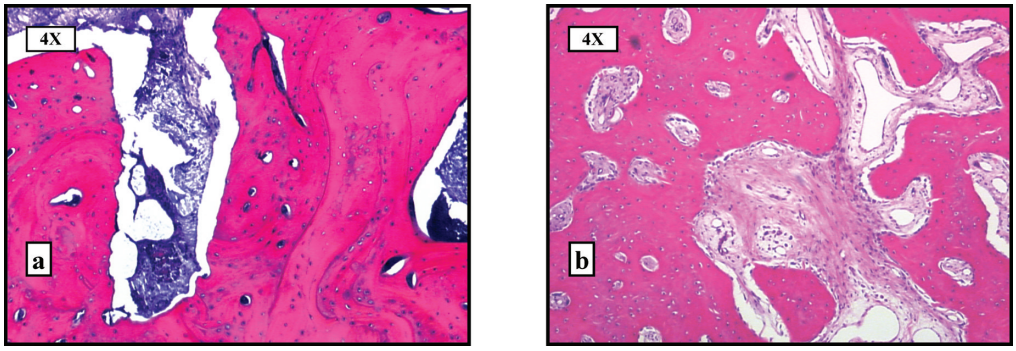

Figure 7. Histological evaluation of tissue formation in alveolar defects 7 weeks after grafting with or without MSCs in F-127/Bioglass scaffolds. Hematoxylin and eosin stained, demineralized sections of tissue formation for the cell-free sites (*a*) and cell-loaded sites (*b*). Significant bone formation with areas of lamellar bone and increased number of osteogenic cells are evident in all sections of defects grafted without (*a*) or with MSCs (*b*).

in which other products, such as fibrin glue or adhesive[44-46] or osteocalcein/osteonectin[47] used to form a moldable grafting material impaired bone formation.

The findings of this study are limited to this specific defect size and cannot be generalized. The proposed grafting combination may not have the expected results in critical size defects or in vertical augmentation procedures, since the mechanical environment, which is a critical factor for bone healing,[48,49] is different. The application of a moldable thermoplastic matrix in a vertical augmentation scenario may lack the necessary rigidity and stability to withstand the multidirectional mechanical stresses applied to an unprotected site[50] unless rigid space holders are used. The merging of alloplastic technologies with biological approaches to regenerate tissues provides a dynamic possible solution to significant problems in clinical prosthodontics.

Conclusion

Within the limitations of this study, it was observed that the use of ex-vivo culture-expanded allogeneic canine MSCs loaded in a polyaxomer carrier with granular scaffolds increased bone formation at the early stages of bone healing of the defect. The presence of the polyaxomer carrier did not limit bone formation, induce an inflammatory response, or result in other complications. Moreover, the use of a moldable scaffold that consisted of polyaxomer polymer and a granular osteoconductive matrix improved the handling properties of the cell-based tissue engineering constructs.

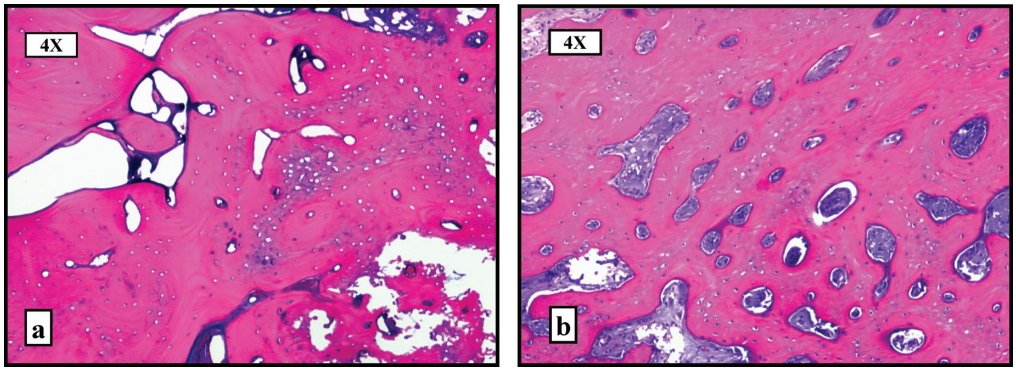

Figure 8. Histological evaluation of tissue formation in alveolar defects 7 weeks after grafting with or without MSCs in HA/TCP scaffolds. Hematoxylin and eosin stained, demineralized sections of tissue formation for the cell-free sites (*a*) and cell-loaded sites (*b*). Bone formation is observed in defects grafted with cell-free (*a*) HA/TCP and cell-loaded HA/TCP (*b*). Areas with dense bone and lack of residual matrix can be observed, and significant numbers of osteoblastic cells are apparent.

The efficacy and the safety of allogeneic MSC-loaded granular constructs for bone repair merit further investigation.

Acknowledgments

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References

1. Nevins M, Jovanovic SA: Localized bone reconstruction as an adjunct to dental implant placement. Curr Opin Periodontol 1997;4:109-118
2. Lynch SE, Genco RJ, Marx RE: Tissue Engineering: Applications in Maxillofacial Surgery and Periodontics. Chicago, IL, Quintessence, 1999
3. Schallhorn RG: Long term evaluation of osseous grafts in periodontal therapy. Int Dent J 1980;30:101-116
4. Brugnami F, Then PR, Moroi H, et al: Histological evaluation of human extraction sockets treated with demineralized freeze-dried bone allograft (DFDBA) and cell occlusive membrane. J Periodontol 1996;67:821-825
5. Harris RJ: A clinical evaluation of an allograft combined with a bioabsorbable membrane versus an alloplast/allograft composite graft combined with a bioabsorbable membrane: 100 consecutively treated cases. J Periodontol 1998;69:536-546
6. Simion M, Jovanovic SA, Trisi P, et al: Vertical ridge augmentation around dental implants using a membrane technique and autogenous bone or allografts in humans. Int J Periodontics Restorative Dent 1998;18:8-23
7. Misch CE, Dietsh F: Bone-grafting materials in implant dentistry. Implant Dent 1993;2:158-167
8. Langer R, Vacanti JP: Tissue engineering. Science 1993;14;260:920-926
9. Devin JE, Attawia MA, Laurencin CT: Three-dimensional degradable porous polymer-ceramic matrices for use in bone repair. J Biomater Sci Polym Ed 1996;7:661-669
10. Lysaght MJ, Reyes J: The growth of tissue engineering. Tissue Eng 2001;7:485-493
11. Rose FR, Oreffo RO: Bone tissue engineering: hope vs. hype. Biochem Biophys Res Commun 2002;292:1-7
12. Ripamonti U, Reddi AH: Periodontal regeneration: potential role of bone morphogenetic proteins. J Periodont Res 1994;29:225-235
13. Giannobile WV: Periodontal tissue engineering by growth factors. Bone 1996;19(1 Suppl):23S-37S
14. Urist MR, Nakata N, Felser JM, *et al.* An osteosarcoma cell and matrix retained morphogen for normal bone formation. Clin Orthop 1977;124:251-266
15. Paralkar VM, Nandedkar AK, Pointer RH: Interaction of osteogenin, a heparin binding bone morphogenetic protein, with type IV collagen. J Biol Chem 1990;265:17281-17284
16. Haynesworth SE, Goshima J, Goldberg VM, et al: Characterization of cells with osteogenic potential from human marrow. Bone 1992;13:81-88
17. Friedenstein AJ, Chailakhyan RK, Gerasimov UV: Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet 1987;20:263-272
18. Caplan AI: Mesenchymal stem cells. J Orthop Res 1991;9:641-650
19. Kadilaya S, Jaiswal N, Bruder SP: Culture-expanded, bone marrow-derived mesenchymal stem cells can regenerate a critical-sized segmental bone defect. Tissue Eng 1987;3:173-185
20. Jaiswal N, Haynesworth SE, Caplan AI, et al: Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 1997;64:295-312
21. Bergman RJ, Gazit D, Kahn AJ, et al: Age-related changes in osteogenic stem cells in mice. J Bone Miner Res 1996;11:568-577
22. Bruder SP, Kurth AA, Shea M, et al: Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. J Orthop Res 1998;16:155-162
23. Malik MA, Puleo DA, Bizios R, et al: Osteoblasts on hydroxyapatite, alumina and bone surfaces in vitro: morphology during the first 2 h of attachment. Biomaterials 1992;13:123-128
24. Fowler EB, Cuenin MF, Hokett SD, et al: Evaluation of pluronic polyols as carriers for grafting materials: study in rat calvaria defects. J Periodontol 2002;73:191-197
25. Matthew JE, Nazario YL, Roberts SC, et al: Effect of mammalian cell culture medium on the gelation properties of Pluronic F127. Biomaterials 2002;23:4615-4619
26. Hokett SD, Cuenin MF, O'Neal RB, et al: Pluronic polyol effects on human gingival fibroblast attachment and growth. J Periodontol 2000;71:803-839
27. Khattak SF, Bhatia SR, Roberts SC: Pluronic F127 as a cell encapsulation material: utilization of membrane-stabilizing agents. Tissue Eng 2005;11:974-83
28. De Kok IJ, Peter SJ, Archambault M, et al: Investigation of allogeneic mesenchymal stem cell-based alveolar bone formation: preliminary findings. Clin Oral Implants Res 2003;14:481-489
29. Tatebe M, Nakamura R, Kagami H, et al: Differentiation of transplanted mesenchymal stem cells in a large osteochondral defect in rabbit. Cytotherapy 2005;7:520-530
30. Tsuji T, Hughes FJ, McCulloch CA, et al: Effects of donor age on osteogenic cells of rat bone marrow in vitro. Mech Ageing Dev 1990;51:121-132
31. Hollinger JO, Winn S, Bonadio J: Options for tissue engineering to address challenges of the aging skeleton. Tissue Eng 2000;6:341-350
32. Mecall RA, Rosenfeld AL: Influence of residual ridge resorption patterns on implant fixture placement and tooth position. Int J Periodontics Restorative Dent 1991;11:8-23
33. Bruder SP, Jaiswal N, Haynesworth SE: Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem 1997;64:278-294

34. Koc ON, Day J, Nieder M, et al: Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). Bone Marrow Transplant 2002;30:215-222
35. Aggarwal S, Pittenger MF: Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815-1822
36. Lazarus HM, Koc ON, Devine SM, et al: Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 2005;11:389-398
37. Eliopoulos N, Stagg J, Lejeune L, et al: Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. Blood 2005;106:4057-4065
38. De Kok IJ, Drapeau SJ, Young R, et al: Evaluation of mesenchymal stem cells following implantation in alveolar sockets: a canine safety study. Int J Oral Maxillofac Implants 2005;20:511-518
39. Le Blanc K, Rasmusson I, Sundberg B, et al: Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439-1441
40. Krebsbach PH, Kuznetsov SA, Satomura K, et al: Bone formation in vivo: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. Transplantation 1997;63:1059-1069
41. Shapoff CA, Bowers GM, Levy B, et al: The effect of particle size on the osteogenic activity of composite grafts of allogeneic freeze-dried bone and autogenous marrow. J Periodontol 1980;51:625-630
42. Mankani MH, Kuznetsov SA, Fowler B, et al: In vivo bone formation by human bone marrow stromal cells: effect of carrier particle size and shape. Biotechnol Bioeng 2001;72:96-107
43. Oh SH, Kim JK, Song KS, et al: Prevention of postsurgical tissue adhesion by anti-inflammatory drug-loaded pluronic mixtures with sol-gel transition behavior. J Biomed Mater Res 2005;72:306-16
44. Carmagnola D, Belglundh T, Linhe J: The effect of a fibrin glue on the integration of Bio-Oss with bone tissue: an experimental study in Labrador dogs. J Clin Periodontol 2002;29:377-383
45. Kalebo P, Buch F, Albrektsson T: Bone formation rate in osseointegrated titanium implants: influence of locally applied haemostasis, peripheral blood, autologous bone marrow and fibrin adhesive system (FAS). Scand J Plast Reconstr Surg Hand Surg 1988;22:53-60
46. Lucht U, Bunger C Moller JT, et al: Fibrin sealant in bone transplantation: no effect on blood flow and bone formation in dogs. Acta Orthop Scand 1986;57:19-24
47. Becker W, Clokie C, Sennerby L, et al: Histological findings after implantation and evaluation of different grafting materials and titanium micro screws into extraction sockets: case reports. J Periodontal 1998;69:414-421
48. Rubin CT, Lanyon LE: Osteoregulatory nature of mechanical stimuli: function as a determinant for adaptive remodeling in bone. J Orthop Res 1987;5:300-310
49. Carter DR, Orr TE: Skeletal development and bone functional adaptation. J Bone Miner Res 1992;7:389-395
50. Jovanovic SA: Bone rehabilitation to achieve optimal aesthetics. Pract Periodontics Aesthet Dent 1997;9:41-51