Investigation of allogeneic mesenchymal stem cell-based alveolar bone formation: preliminary findings

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Abstract: This study was designed to evaluate mesenchymal stem cell (MSC)-based alveolar bone regeneration in a canine alveolar saddle defect model. MSCs were loaded onto hydroxyapatite/tricalcium phosphate (HA/TCP) matrices. Scanning electron microscopic (SEM) evaluation demonstrated greater than 75% MSC coverage of the HA/TCP porous surface prior to placement regardless of MSC donor. Matrices, 6 mm × 6 mm × 20 mm, with and without cells, were implanted for 4 and 9 weeks, then removed for histological evaluation of bone formation. Cell-free control matrices were compared with MSC-loaded matrices post implantation. Histomorphometrical analysis showed that equivalent amounts of new bone were formed within the pores of the matrices loaded with autologous MSCs or MSCs from an unrelated donor. Bone formation in the cell-free HA/TCP matrices was less extensive. There was no histologic evidence of an immunological response to autologous MSCs. Surprisingly, allogeneic MSC implantation also failed to provoke an immune response. Analysis of circulating antibody levels against MSCs supported the hypothesis that neither autologous nor allogeneic MSCs induced a systemic response by the host. Analysis of dye-labelled MSCs in histological sections confirmed that the MSCs persisted in the implants throughout the course of the experiment. At 9 weeks, labelled cells were present within the lacunae of newly formed bone. We conclude that autologous and allogeneic MSCs have the capacity to regenerate bone within craniofacial defects.

Dental and craniofacial surgeons must often restore aesthetics and function to their patients, despite an insufficient amount of bone. For this reason, bone regeneration procedures are an integral part of dental and craniofacial therapy. Various procedures and materials are used to promote osteogenesis beyond the existing skeletal boundary. (Nasr et al. 1999) Although alloplastic materials including synthetic materials and natural bone-derived materials are used, autogenous bone remains the standard of care for regeneration of significant volumetric defects of the alveolar bone, basal maxilla or mandible (Block & Kent 1997). Due to the limited amount of autogenous bone available for reconstructive use, other therapies are being explored [Hollinger et al. 1996].

Beyond current guided bone regeneration using autogenous bone with alloplastic materials, molecular, cellular, and material engineering strategies can be applied to bone regeneration [Hollinger et al. 2000]. The molecular approach is best exemplified...
by the bone morphogenetic proteins [BMP] (Reddi 1998). Their discovery as the osteoinductive component of bone matrix and subsequent cloning has allowed for the production of recombinant BMPs for use in directing bone formation in vivo. On-going clinical experimentation seeks to apply the known osteoinductive function of BMPs to clinical methodologies for bone regeneration. [Sailer & Kolb 1994; Boyne et al. 1997] However, limitations of this approach include controlling both the delivery and function of these powerful growth factors in vivo (Groeneveld & Burger 2000).

Material engineers have fabricated matrices suitable for the replacement of bone in orthotopic defects [Peter et al. 1998]. New polymeric scaffolds have yet to reach clinical investigation. Current hydroxyapatite/ CaPO4 materials serve a space-maintaining function and are osteoconductive, but they are also slowly resorbed. Their behaviour has been shown to vary with respect to patient, defect size and defect position.

A promising alternative bone regeneration approach was established by the identification of certain multipotent cells among the stromal cells of the bone marrow. [Friedenstein et al. 1987; Ohgushi et al. 1990] These cells have been termed mesenchymal stem cells [MSCs] [Caplan 1991; Bruder et al. 1997; Pittenger et al. 1999] or marrow stromal cells [Bianco et al. 1999]. By culturing, they may be expanded many fold to provide nearly unlimited starting material for cellular therapeutic applications. The MSCs can differentiate into stroma, bone, cartilage, fat and muscle in vitro under defined cell culture conditions [Pittenger et al. 1999]. In vivo evaluation has demonstrated MSC differentiation along these lineages under local stimuli in many pre-clinical models [Bruder et al. 1998a; Bruder et al. 1998b]. Bone marrow aspirates [9 ml] were drawn from the ilioc crest of the canines involved in the study. Marrow samples were washed with saline solution, followed by centrifugation for 20 min over a 1.072-g/ml Percoll density cushion. The interface layer was removed, washed with saline solution, and counted. Nucleated cells were plated in 75 mm2 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. Colonies formed during a 14–17-day period of primary culture. When the tissue culture plates were near confluence, MSCs were trypsinized and passaged. At the end of the first passage, MSCs were then trypsinized and cryopreserved until needed.

MSC isolation and expansion is briefly described [Bruder et al. 1998a; Pittenger et al. 1999]. Bone marrow aspirates [9 ml] were drawn from the iliac crest of the canines involved in the study. Marrow samples were washed with saline solution, followed by centrifugation for 20 min over a 1.072-g/ml Percoll density cushion. The interface layer was removed, washed with saline solution, and counted. Nucleated cells were plated in 75 mm2 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. Colonies formed during a 14–17-day period of primary culture. When the tissue culture plates were near confluence, MSCs were trypsinized and pas saged. At the end of the first passage, MSCs were then trypsinized and cryopreserved until needed.

Material and methods

Canine MSC cultivation and manipulation

MSC isolation and expansion is briefly described [Bruder et al. 1998a; Pittenger et al. 1999]. Bone marrow aspirates [9 ml] were drawn from the iliac crest of the canines involved in the study. Marrow samples were washed with saline solution, followed by centrifugation for 20 min over a 1.072-g/ml Percoll density cushion. The interface layer was removed, washed with saline solution, and counted. Nucleated cells were plated in 75 mm2 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. Colonies formed during a 14–17-day period of primary culture. When the tissue culture plates were near confluence, MSCs were trypsinized and passaged. At the end of the first passage, MSCs were then trypsinized and cryopreserved until needed.

Allogeneic pair determination

An extensive major histocompatibility complex [MHC] mismatch between donor and recipient canines was assured, based on a combination of three techniques: pedigree, mixed lymphocyte reactions [MLR], and dog leukocyte antigen [DLA] typing. The pedigree mismatch was addressed by obtaining the first group of animals from one colony [eight males] and the second group [eight females] from a separate colony.

Blood samples were drawn from all canines prior to study onset. Peripheral blood mononuclear cells [PBMC] were isolated and cryopreserved until analysis. For PBMC isolation from whole heparinized blood, a buffy coat was prepared by centrifuging the blood for 30 min at room temperature over a Percoll density cushion. The upper layer containing plasma, platelets and mononuclear cells was transferred into a separate tube, pelleted and washed with DMEM containing 10% FBS. The number of PBMCs was determined, and the cells were cryopreserved using 90% FBS/10% DMSO until use.

Mixed lymphocyte reactions represent an in vitro model of alloreactivity [Lang et al. 1972]. In the MLR, T lymphocytes from a potential recipient [responder] are mixed with similar cells from the potential donor [stimulator]. A proliferative responder T-cell response is indicative of an allogeneic mismatch between the donor-recipient combination. MLRs were run with all possible donor-recipient combinations, limiting the combinations to intercolony pairs. Stimulator cell division was prevented by treatment with Mitomycin C [50 μg/ml] for 20 min at 37 °C, then washing with an excess of DMEM four times. Stimulator cells were mixed with responders in 96-well tissue culture plates at 1 x 105 cell/well each. Control wells contained responder or stimulator cells alone, with or without mitogen stimulation. At day 3, the T cell mitogen phytohemagglutinin [PHA, 5% in DMEM] was added to appropriate wells and, at day 5 cultures were pulsed with [3H]-thymidine [5 Ci/ mmol, 1 μCi/well in 50 μl], Wells were harvested and counted at day 6 using a Betaplate Trilux β counter [Wallac Inc., Gaithersburg, MD, USA]. Positive T-cell proliferation over that of the non-stimu-
lated controls was considered indicative of sufficient mismatch between the donor and recipient pair.

An additional blood sample was removed prior to surgery and used for DLA typing. DNA was isolated using a Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN, USA), and samples were then sent for microsatellite analysis of DLA groups (Wagner et al. 1996) to an external facility. Loci mismatches were identified for potential donor-recipient pairs. The pedigree, MLR and DLA data were then assembled and used to designate allogeneic donor-recipient pairs.

Implant preparation

Porous hydroxyapatite/β-tricalcium phosphate [HA/TCP] ceramic matrices were manufactured to specifications by Biomatante (Vigneux de Bretagne, France). The matrices were 6 mm × 6 mm × 20 mm (w × t × h), with a 3-mm diameter longitudinal central canal. The material consisted of 60% HA and 40% TCP with a mean pore size of 300–500 μm. The implants were sterilized via depyrogenation at 250°C for 4 hours and aseptically transferred to 3 ml Leur-lok syringes fitted with three-way stop-cocks prior to loading with cells.

Loaded matrices were prepared using cryopreserved MSCs 1 day prior to implantation. MSC vials were thawed in a 37°C water bath and the cells were transferred to conical tubes containing serum-free DMEM. MSCs were then washed and labelled with Di-I [CellTracker CM-Dil], chloromethylbenzamido 1, 1-dioctadecyl-3,3,3', 3'-tetramethylindocarbocyanine, Molecular Probes, Eugene, OR, USA] according to the manufacturer’s recommended method. After a final wash, the MSCs were resuspended in DMEM at 10^6 cells/ml for matrix loading. Using a pipetor, 1.5 ml of cell suspension was added to each matrix via the 3-way stop-cock, using autologous or allogeneic MSCs as determined by the experimental design. Additional matrices were loaded with DMEM alone, serving as cell-free controls. All matrices were placed in a 37°C incubator for 3 hours, followed by subsequent addition of serum-free DMEM. Matrices were then packaged in an insulated box for ambient temperature shipment to the surgical site. Temperature probes were included in the shipment packaging to monitor the shipping conditions.

Scanning electron microscopy

Implants were removed from shipping containers, placed into 10 ml of PBS and directly transferred to 1 ml of 3.7% paraformaldehyde. After 1 h of fixation, implants were rinsed in water and stained with OsO4. After dehydration in graded alcohols and critical point drying, implants were sputter-coated with gold-palladium. Scanning electron microscopic (SEM) images were obtained and digitally recorded using a Jeol 660 instrument (Jeol USA Inc., Peabody, MA, USA).

Implantation of cMSC constructs in canine mandible defects

Adult beagle dogs in good systemic health were housed in individual cages and provided with canned dog food for 10 days prior to initiation of the study. Under general anaesthesia induced with Nembutol and maintained using isoflurane, the second, third and fourth premolar teeth were extracted bilaterally by bisection and elevation. Following extraction, each dog received 1 million units of Betamax [penicillin G].

Seven weeks following extractions, a mid-crestal incision was made and full thickness mucoperiosteal flaps were elevated from the first premolar to the first molar teeth. Saddle defects (6.5 mm deep × 20 mm long) were created bilaterally in the mandible of each dog under general anaesthesia. HA/TCP matrices, machined and loaded as described above, were placed in the saddle defects and secured using figure-of-eight wire ligatures. A PTFE membrane was placed over each matrix, extending 2 mm beyond all surgical margins. The surgical site was closed using vertical mattress 4–0 silk sutures. Systemic antibiotic coverage was provided for 7 days post operatively.

At 4 and 9 weeks post implant placement, blood samples were drawn and the dogs were killed with a lethal dose of Nembutal. Each mandible was dissected, fixed in 10% buffered formalin, and processed for histologic evaluation. Decalcified 5 μm sections were prepared and stained using haematoxylin and eosin or modified aniline blue stains. Ground sections were prepared by embedding in methylmethacrylate [Technovit 7200, Kulzer]. Sections were cut parallel to the coronal plane and parallel with the frontal plane and stained with Goldner’s tri-chrome, providing representation of tissues formed within the entire matrix.

Assessment of humoral antibody response to allogeneic cells following implantation

The humoral allo-antibody response to implanted MSC-loaded matrices was evaluated by flow cytometric analysis, similar to methods used to evaluate human panel reactive antibody [PRA] levels [Braun 1997]. Target cells (donor PBMCs and MSCs) were incubated with multiple dilutions of recipient serum (1:10, 1:100, 1:1000), from both pre-operative and post-operative [taken at killing] blood samples in the presence of 0.2% bovine serum albumin [BSA] in PBS for 1.5 h at room temperature. Following washing, FITC-labelled secondary antibodies specific to canine IgG and IgM (Serotec, Raleigh NC, USA) or isotype controls were applied at 5 μg/ml in the same buffer for 1 h at room temperature. Cells were washed and resuspended in PBS/BSA containing 5 μg/ml of propidium iodide (PI, Molecular Probes) to identify dead cells in the analysis. To ensure secondary antibody reactivity, targets of canine IgG- and IgM-coated polystyrene beads were used as positive controls. Flow cytometric analysis was performed with a FACSCalibur (Becton Dickinson, San Jose, CA, USA).

Results

To explore the use of allogeneic MSCs for tissue engineering of bone in this preliminary study, DLA typing and MLR evaluation were performed to confirm unrelated MSC donors and recipients. To ensure disparate pedigree, animals were ordered from distinctly separate breeding colonies. MLR assays demonstrated significant levels of proliferative response, indicating mismatch between the donor-recipient pairs. Finally, these pairings were confirmed by the DLA typing to be significantly mismatched, our third level of confidence that true allo-
Generic pairings had been determined. Two dogs showed DLA matching, however, engraftment of these donor/recipient pairs was not evaluated in this report. The results collated in Table 1 were used to indicate implant donor cell origin for each recipient.

Canine MSCs were isolated and expanded readily from small-volume bone marrow aspirates drawn from the iliac crest of each animal. MSCs were cryopreserved at the end of the first passage to simulate product preparation and storage conditions. There were a sufficient number of cells harvested at the end of the first passage to load at least eight devices from each donor. SEM evaluation demonstrated that MSCs attached to each matrix (Fig. 1). The number of MSCs loaded and the cell spreading/attachment on the matrix surface and throughout the pores as illustrated (Fig. 1) was consistent.

Animals were killed at 4 and 9 weeks post implantation. Samples were processed.

Table 1 DLA typing of dogs enrolled in the study

<table>
<thead>
<tr>
<th>Dog</th>
<th>Class I marker</th>
<th>Class II marker</th>
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<tr>
<td>3</td>
<td>H/1</td>
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<td>4</td>
<td>J/K</td>
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<td>5</td>
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<td>H/L</td>
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<td>7</td>
<td>G/H</td>
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<td>8</td>
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<td>16</td>
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<td>17</td>
<td>P/M</td>
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<td>18</td>
<td>P/Q</td>
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Patterns describing class I markers were labelled alphanumerically and patterns describing class II markers were labelled numerically. Patterns describing two dogs with matching DLA typing are italicized.

The MSC-loaded matrices were successfully implanted in the canine mandibular defect model described, testing the feasibility of MSC-based bone regeneration for alveolar and craniofacial surgery. Following extraction of premolars P2, P3, and P4, all animals remained healthy during a 7-week healing period prior to implantation with cell-loaded or cell-free matrices. Critically sized saddle defects (6.5 mm deep × 20 mm long) were created bilaterally in the extraction sites of the mandible of each dog, and the HA/TCP matrices were placed in these defects, secured in place with wires as described (Fig. 2). A PTFE membrane was used to minimize periosteal cellular contributions to bony healing. All dogs recovered from the anaesthesia and surgical procedure rapidly and without complication. During the first 12–14 days, no animals displayed signs of surgical complication, infection, swelling or graft site dehiscence. At 12–14 days, graft site dehiscence occurred in select animals, irrespective of cell-free, allogeneic or autologous MSC-loaded matrices. A habit of cage chewing was considered to predispose animals to dehiscence, which in two instances, lead to early killing of the animals.

Animals were killed at 4 and 9 weeks post implantation. Samples were processed.
through paraffin and methylmethacrylate histological methods to allow assessment of bone formation and matrix persistence. Bone formation was not observed at the early time point; host cell infiltration and inflammatory processes were indicative of normal granulation, neovascularization and osteoconduction processes. There was no evidence of any detrimental immunological response to the implanted cells, independent of autologous or allogeneic cMSC source, clearly demonstrating implant acceptance (Fig. 3).

Increased bone formation was observed at the 9-week time point with continued normal host immunological responses. Coronal sections made through the central region of the matrix demonstrated a significant amount of new bone formation in the hollow central canal (not shown). Within the matrix, distant from the host bone surgical margins, new bone formation was observed in the majority of the pores in the superior aspects of all MSC loaded matrices (coronal sections, Fig. 4). Sagittal sections displayed the relationship of host bone and the MSC loaded matrices. Positive host tissue response at the allogeneic MSC loaded matrices was indicated by osteoconduction at the bone/construct interface (Fig. 5a). Normal bone formation was also observed in surgical gaps adjacent to the allogeneic MSC loaded matrices (Fig. 5c). Higher magnifications confirmed the lack of inflammatory cell infiltrate in response to either allogeneic or autologous MSC implantation (Figs 5b and d). Comparison of cell-free and MSC loaded matrices demonstrated a clear difference in bone formation, confirming the osteogenic potency of MSC-loaded constructs. Essentially, every pore space of the MSC-loaded matrices exhibited new bone. In contrast, many pores of the cell-free matrices were devoid of new bone formation (Fig. 6). Pores within the centre of the cell-free matrices were most often filled with connective tissue, and only pores on the periphery contained mineralized tissue due to osteoconduction from the host.

Quantification of the data was approximated from Goldner’s Trichrome stained ground sections representing coronal sections made centrally through each construct. Counting of available pores of each construct that were filled with bone or osteoid revealed that both autologous- and allogeneic-MSC-loaded constructs had a high percentage of pores filled with bone (83% of pores for autologous vs. 85% of pores for allogeneic). In contrast, only 60% of pores were filled with bone in the cell-free constructs. Qualitatively, the Goldner’s Trichrome staining indicated that the tissue formed at 9 weeks in the cell-free
constructs was largely osteoid and relatively immature when compared with the histological appearance of woven bone formed in either the autologous or allogeneic MSC-loaded constructs. There was no apparent difference observed in the osteogenesis of autologous cMSC versus allogeneic cMSC matrices. Further qualitative evaluation of these histological sections was performed using fluorescence microscopy. For both the allogeneic and the autologous matrices, numerous fluorescently labelled cells could be identified within the pores of the implanted matrices [Fig. 7]. Importantly, many arrays of fluorescently labelled cells were observed adjacent to osteoid in known osteoblastic formations and within the lacunae of the newly formed bone, strengthening evidence that implanted MSCs participate in the surrounding mineralization.

The host response to placement of allogeneic cells of the bone-engineered construct was measured by evaluation of allogeneic antibodies in the serum of dogs at 4 and 9 weeks following construct placement. No differences between pre- and post-operative alloantibody levels were observed using either MSCs [DLA class I positive] or PBMCs [DLA class II positive] from each donor as targets, in either allogeneic or autologous recipient animals [Fig. 8].

Discussion

The primary finding of this study was that allogeneic MSCs loaded onto an HA/TCP matrix formed bone in a critically sized mandibular defect. To our knowledge, this is the first report of successful bone regeneration using allogeneic MSCs without the use of immunosuppressive therapy. Histological analysis demonstrated equivalence in the extent of bone formation between autologous and allogeneic MSCs, both of which formed a greater amount of mature woven bone than cell-free implants. The MSC-loaded implants were well integrated with the surrounding host bone. In most cases, the cell-free implants showed slight evidence of osteoconduction from the host tissue, but were loose in the defect site (particularly at the 4 week time point) and had not formed significant bonding with the surrounding bone.

It is known that HA/TCP is an osteoconductive matrix [Cornell & Lane 1998] and when implanted alone will allow for limited bone formation. While evidence of this behaviour was observed in the cell-free matrices, the histological data indicated the enhancement in bone formation upon MSC delivery. Still, there was osteoconduction from the host into the matrices. This is especially encouraging in the allogeneic implants, as they did not inhibit a positive response from the host. It is clearly beneficial for any tissue-engineered device to allow the host cells to contribute to
regeneration of the desired tissue. It is possible that one important effect of the MSC/matrix is the encouragement of regional or local osteogenesis. Molecular analysis of the MSC indicates that it produces a broad range of cytokines and several bone morphogenetic proteins in vitro (Oreffo et al. 1999) that may be enhancing the host response in vivo, leading to the robust bone formation seen here. In addition, the MSC-based constructs supported vascularization and peripheral osteoinduction by the host, key features required for integration of the construct and its eventual structural integration in the host skeleton.

Bone was formed by an intramembranous pathway exclusive of evidence of chondrogenesis at 4 or 9 weeks. Under the current conditions, MSC osteogenesis in orthotopic sites occurs by a direct conversion of mesenchymal cells into osteoblasts rather than by an endochondral sequence. (Breitbart et al. 1998b) The fate of metabolically labelled cells was determined by direct microscopic identification and confirmed their persistence. The labelled MSCs were identified both within the lacunae of newly formed bone and along the calcification front. Labelled cells with the distinct morphology of osteoblastic cells positioned along osteoid seams in newly formed bone implicated their role in osteogenesis. Other investigations using human cells in immunocompromised hosts defined the newly synthesized bony matrix as human in origin, therefore derived from the viable implanted cells. (Cooper et al. 2001)

A central observation concerning the allogeneic MSC implants was the generalized absence of significant mononuclear cellular infiltrate at either 4 or 9 weeks. When isogenic and xenogenic materials were previously evaluated, treatment with the anti-inflammatory drug FK506 was required for increased bone formation and repair. A role for the T lymphocytes was suggested in mediating xenogeneic cell survival in these models (Voggneretter et al. 2000). By contrast, this experiment showed strong bone repair in allogeneic implants without the use of immunomodulatory treatments. Local inflammation within the matrix or surrounding bone was absent and regional submandibular and cervical lymph nodes were found to be normal upon histological evaluation (data not shown).

Occasionally, inflammation was observed adjacent to the PTFE membranes at the superior aspect of the surgical site. Inflammation associated with membrane exposure has been shown to result in bacterial contamination and minimal bone regeneration, as was seen in this study (Nevins & Jovanovic 1997). Here, membrane exposure and graft site dehiscence was attributable to cage chewing, a problem previously reported in other canine alveolar bone regeneration studies (Schliephake et al. 2000). One advantage of cell-based bone regeneration and the associated enhanced bone healing is the suggestion that barrier membranes (with their associated infection risks) may be excluded from bone regeneration protocols.

The systemic response to placement of allogeneic or autologous cells for repair of mandibular saddle defects was examined at the level of circulating allo-antibodies. The failure to elicit a strong humoral antibody response is a positive indication that allogeneic MSCs do not sensitize recipient animals in the small defect model studied here. Combined with the histologic results showing positive bone formation without significant inflammatory host response, these results begin to solidify a role for allogeneic MSCs in a variety of bone repair concepts.

This initial investigation of allogeneic MSCs employed for bone regeneration contributes to a growing body of literature that illustrates the promising future for osteoinductive bone regeneration products. The current experimental use and testing of BMPs demonstrates the potential value and illustrates the clinical challenges presented by technology transfer. Groeneveld et al. 1999, Mori et al. 2000, Yudell & Block 2000) A clinically relevant advantage of the cell-based tissue engineering constructs used here is the delivery of sufficient cells to promote vascularization and bone formation within the central core regions of large grafts.

Implantation of culture-expanded autologous MSCs offers the advantage of directly delivering the cellular machinery responsible for synthesizing new bone and circumventing the otherwise slow steps leading to natural or enhanced bone repair. By incorporating living cells with specifically designed matrices, the shortcomings of osteoinductive factors alone to effect permanent bone repair may be overcome.

Other cell sources have been considered for their osteogenic potential. One such cell type, the embryonic stem cell, although capable of giving rise to bone progenitor cells, also give rise to many ethical issues of critical importance. A distinct advantage of using MSCs is that they are adult stem cells, isolated from donors capable of informed consent. Another potential source for osteogenic cells may be periosseum. Periosteum, while a source of chondrogenic and osteogenic progenitor cell populations [Breithart et al. 1998] is un-

![Fig. 8. Flow cytometry analysis of humoral allo-antibody levels. Represented here are pre- and post-operative serum samples (1:100 dilution) from five animals each killed at 4 and 9 weeks post implantation with allogeneic MSCs, using MSCs as the targets to test the specific donor-recipient pairings. Results are presented as mean fluorescence intensity of the cell population. Recipient serum dilutions tested were 1:10, 1:100, 1:1000, no differences were observed in any of the dilutions when either donor MSCs or PBMCs were used as targets.](Image 86x602 to 331x736)
likely to provide an ample number of cells to direct bone repair of large defects.

While MSCs make up an extremely small number of cells within bone marrow, their expansion is remarkable under appropriate culture conditions. MSCs may be isolated by a relatively simple clinical procedure of bone marrow aspiration, culture-expanded over one billion-fold and cryopreserved without a loss in their osteogenic potential [Bruder et al. 1998b]. Initiating cultures with larger marrow volumes from intentional donations and expanding the cells to further passages makes allogeneic cell-based tissue engineering products a possibility.

In conclusion, the successful formation of bone using allogeneic cMSCs to repair critically sized mandibular defects in the canine model was accompanied by an absence of systemic or local response to the foreign cells. The use of allogeneic MSCs for clinical repair of bone broadens the applicability of tissue engineering for large bony defects. Detailed evaluation of the remarkable absence of immunological responses to implanted allogeneic MSCs for bone regeneration and repair represent a new and important direction for tissue engineering.

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Zusammenfassung

Resumen
Este estudio se diseñó para evaluar la regeneración de hueso alveolar basado en células madre mesenquimales (MSC) en un defecto alveolar en silla de montar en un modelo canino. Se cagaron MSCs en matrices de fosfato de hidroxiapatita/tricalcica (HA/TCP). La evaluación por SEM demostró que una cobertura de MSC mayor del 75% de la superficie porosa de HA/TCP previa a la colocación sin relación con el donante de MSC. Se implantaron matrices de 6×6×20 mm, con o sin células, durante cuatro a nueve semanas, retirándose posteriormente para evaluación histológica de la formación ósea. Se compararon tras la implantación las matrices de control libres de células con matrices cargadas de MSC. Los análisis histomorfométricos mostraron que se formaron cantidades equivalentes de nuevo hueso entre los poros de las matrices cargadas con MSC autólogo o MSC de donante desconocido. La formación de hueso en las matrices libres de células HA/TCP fue menos extensa. No hubo evidencia histológica de respuesta inmune frente a MSCs autólogos. Sorprendentemente, la implantación alógénica también fracasó en provocar una respuesta inmune. El análisis de los niveles de anticuerpos circulantes contra MSCs apoyaron la hipótesis de que ni los MSCs autólogos ni los autógenos indujeron una respuesta sistémica del huésped. El análisis en las secciones histológicas de los MSCs marcados con tinción confirmaron que los MSCs persistieron en los implantes a lo largo de todo el experimento. A las nueve semanas, las células marcadas estaban presentes dentro de las lagunas del hueso noformado. Nosotros concluimos que los MSCs autólogos y alógenos tienen la capacidad de regenerar hueso dentro de defectos craneofaciales. 

Résumé
Cette étude a été effectuée pour évaluer la régénération osseuse alvéolaire aidée par des cellules souches mésenchymateuses (MSC) dans un modèle de lésion en selle de l’alvéole chez le chien. Les MSC étaient chargées sur des matrices de phosphate tricalcique/hydroxyapatite (HA/TCP). L’évaluation au microscope électronique à balayage a démontré plus de 75 % de recouvrement MSC de la surface porueuse HA/TCP avant le placement quelque soit le donneur MSC. Des matrices de 6×6×20 mm, avec ou sans cellule, ont été implantées pour quatre et neuf semaines et ensuite enlevées pour l’évaluation histologique de la formation osseuse. Les matrices contrôles sans cellule ont été comparées aux matrices chargées de MSC après l’implantation. L’analyse histomorphométrique a montré que des quantités équivalentes de nouvel os s’étaient formées dans les pores des matrices chargées avec MSC autogènes ou MSC d’un donneur sans relation. La formation osseuse dans les matrices HA/TCP sans cellule était moins importante. Il n’y avait aucune évidence histologique d’une réponse immunitaire au MSC autogène. L’implantation MSC allogène ne provoquait également pas de réponse immunitaire. L’analyse des teneurs en anticorps circulants contre les MSC supporte l’hypothèse que ni les MSC autogènes ni les allogènes n’induisaient une réponse systémique par l’hôte. L’analyse des cellules marquées dans les coupes histologiques a confirmé que les MSC persistaient dans les implants durant le cours de l’expérience. A neuf semaines, les cellules marquées étaient présentes dans les lacunes de l’os néoformé. Les MSC autogènes et allogènes ont la capacité de régénérer l’os dans les lésions craniofaciales.
References


