Endogenous Collagen Influences Differentiation of Human Multipotent Mesenchymal Stromal Cells.

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Abstract
Human multipotent mesenchymal stromal cells (hMSCs) are multipotent cells that, in the presence of appropriate stimuli, can differentiate into different lineages such as the osteogenic, chondrogenic, and adipogenic lineages. In the presence of ascorbic acid, MSCs secrete an extracellular matrix mainly composed of collagen type I. Here we assessed the potential role of endogenous collagen synthesis in hMSC differentiation and stem cell maintenance. We observed a sharp reduction in proliferation rate of hMSCs in the absence of ascorbic acid, concomitant with a reduction in osteogenesis in vitro and bone formation in vivo. In line with a positive role for collagen type I in osteogenesis, gene expression profiling of hMSCs cultured in the absence of ascorbic acid demonstrated increased expression of genes involved in adipogenesis and chondrogenesis and a reduction in expression of osteogenic genes. We also observed that matrix remodeling and anti-osteoclastogenic signals were high in the presence of ascorbic acid. The presence of collagen type I during the expansion phase of hMSCs did not affect their osteogenic and adipogenic differentiation potential. In conclusion, the collagenous matrix supports both proliferation and differentiation of osteogenic hMSCs but, on the other hand, presents signals stimulating matrix remodeling and inhibiting osteoclastogenesis.

Specific Fibrinogen and Thrombin Concentrations Promote Neuronal Rather Than Glial Growth When Primary Neural Cells Are Seeded Within Plasma-Derived Fibrin Gels.

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Abstract
Fibrin gels are attractive scaffolds useful for neural tissue engineering applications. The objective of this work was to investigate the apoptotic activity, survival, proliferation, and differentiation of a mixed population of primary neural cells composed of neurons and multipotent precursor cells when cultured in fibrin gels prepared with varying concentrations of fibrinogen (5-25 mg/mL fibrinogen) and thrombin (1-125 U/mL thrombin). Within all fibrin gel formulations tested, the level of apoptosis on day 1 was low and cell survival was equivalent to levels in monolayer culture (67%). Proliferation in gels made from 5 to 12.5 mg/mL fibrinogen was also similar to that observed in monolayer culture, though a lower proliferative response was observed in 25 mg/mL fibrinogen formulations. Relative to monolayer culture, cholinergic and dopaminergic neuronal presence was enhanced, whereas glial cell growth was reduced in fibrin gel cultures. The extent to which levels were altered depended on fibrinogen and thrombin concentration. The findings here suggest the importance of fibrinogen and thrombin concentration in differentially regulating the growth and composition of neural cell populations and are of importance for neural tissue engineering strategies focused on the development of implantable scaffolds for treating neurodegenerative disorders.

Autocrine fibroblast growth factor 18 mediates dexamethasone-induced osteogenic differentiation of murine mesenchymal stem cells.


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Abstract
The potential of mesenchymal stem cells (MSC) to differentiate into functional bone forming cells provides an important tool for bone regeneration. The identification of factors capable of promoting osteoblast differentiation in MSCs is therefore critical to enhance the osteogenic potential of MSCs. Using microarray analysis combined with biochemical and molecular approach, we found that FGF18, a member of the FGF family, is upregulated during osteoblast differentiation induced by dexamethasone in murine MSCs. We showed that overexpression of FGF18 by lentiviral (LV) infection, or treatment of MSCs with recombinant human (rh)FGF18 increased the expression of the osteoblast specific transcription factor Runx2, and enhanced osteoblast phenotypic marker gene expression and in vitro osteogenesis. Molecular silencing using lentiviral shRNA demonstrated that downregulation of FGFR1 or FGFR2 abrogated osteoblast gene expression induced by either LV-FGF18 or rhFGF18, indicating that FGF18 enhances osteoblast differentiation in MSCs via activation of FGFR1 or FGFR2 signaling. Biochemical and pharmacological analyses showed that the induction of phenotypic osteoblast markers by LV-FGF18 is mediated by activation of ERK1/2-MAPKs and PI3K signaling in MSCs. These results reveal that FGF18 is an essential autocrine positive regulator of the osteogenic differentiation program in murine MSCs and indicate that osteogenic differentiation induced by FGF18 in MSCs is triggered by FGFR1/FGFR2-mediated ERK1/2-MAPKs and PI3K signaling.

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Characterization of distinct mesenchymal-like cell populations from human skeletal muscle in situ and in vitro.


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Abstract

Human skeletal muscle is an essential source of various cellular progenitors with potential therapeutic perspectives. We first used extracellular markers to identify in situ the main cell types located in a satellite position or in the endomysium of the skeletal muscle. Immunohistology revealed labeling of cells by markers of mesenchymal (CD13, CD29, CD44, CD47, CD49, CD62, CD73, CD90, CD105, CD146, and CD15 in this study), myogenic (CD56), angiogenic (CD31, CD34, CD106, CD146), hematopoietic (CD10, CD15, CD34) lineages. We then analysed cell phenotypes and fates in short and long-term cultures of dissociated muscle biopsies in a proliferation medium favouring the expansion of myogenic cells. While CD56(+) cells grew rapidly, a population of CD15(+) cells emerged, partly from CD56(+) cells, and became individualized. Both populations expressed mesenchymal markers similar to that harboured by human bone marrow-derived mesenchymal stem cells. In differentiation media, both CD56(+) and CD15(+) cells shared osteogenic and chondrogenic abilities, while CD56(+) cells presented a myogenic capacity and CD15(+) cells presented an adipogenic capacity. An important proportion of cells expressed the CD34 antigen in situ and immediately after muscle dissociation. However, CD34 antigen did not persist in culture and this initial population gave rise to adipogenic cells. These results underline the diversity of human muscle cells, and the shared or restricted commitment abilities of the main lineages under defined conditions.

Cytotherapy. 2010 Apr 29. [Epub ahead of print]

Cancellous bone allograft seeded with human mesenchymal stromal cells: a potential good manufacturing practice-grade tool for the regeneration of bone defects.

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Abstract

Abstract Background aims. Combining autologous bone precursor cells with cancellous bone allograft (CBA) offers an appealing strategy for skeletal regeneration. In this context, multipotent mesenchymal stromal cells (MSC) provide an excellent cell source because they are readily harvested from donors, expanded and differentiated in vitro. The aim of this study was to evaluate the proliferation, morphology, osteogenic differentiation and stem cell-related gene expression during static long-term ex vivo cultivation using human MSC and CBA under good manufacturing practice (GMP)-conforming conditions. Methods. MSC were isolated from healthy donors (n = 5) and cultivated on peracetic acid-sterilized CBA in the presence of 10% human platelet-rich plasma without osteogenic supplements. Total protein content, cell-specific alkaline phosphatase (ALP) activity and osteogenic marker gene expression levels were assessed. Stem
cell-related gene expression was compared with MSC monolayer cultivation using microarray analysis. Furthermore, cellular distribution and morphology within the porous CBA were visualized by histology and scanning electron microscopy. Results. Effective adhesion, spreading, proliferation and intercellular contact of human MSC within the pores of CBA were observed during the study (<42 days). Cell-specific ALP activity peaked after 3 weeks of cultivation. Gene expression of early, intermediate and late osteogenic marker genes was detectable during long-term cultivation. Microarray-based annotation and biologic interaction network data analysis indicated that expression levels of genes encoding crucial differentiation-regulating proteins and extracellular matrix components involved in the process of osteogenesis were induced in CBA-cultivated MSC. Conclusions. MSC-vitalized CBA offers an attractive GMP-grade bone-filling material. Further research is warranted to evaluate its bone-healing potential in vivo.


Chemokines mediate mesenchymal stem cell migration toward gliomas in vitro.


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Abstract
Previous studies have demonstrated the tremendous tropism of mesenchymal stem cells (MSCs) for malignant gliomas, making these cells a potential vehicle for delivery of therapeutic genes to disseminated glioma cells. However, the mechanisms underlying the tropism of MSCs for gliomas remain poorly defined. It has been suggested that malignant gliomas secrete a variety of chemokines, including macrophage chemoattractant protein-1 (MCP-1) and stromal cell-derived factor-1 alpha (SDF-1 alpha). We isolated and cultured MSCs from rat bone marrow and found that these cells express CCR2 and CXCR4, the respective receptors for MCP-1 and SDF-1 alpha. In vitro analysis revealed that MCP-1 and SDF-1 alpha induce the migration of MSCs. Furthermore, neutralization data suggest that MCP-1 and SDF-1 alpha play a role in the mediation of MSC migration toward gliomas. These results highlight the potential of these cells as a tumor targeting strategy for glioma gene therapy.


Mesenchymal stem cell mechanobiology.

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Abstract
Bone marrow-derived multipotent stem and stromal cells (MSCs) are likely candidates for cell-based therapies for various conditions including skeletal disease. Advancement of these therapies will rely on an ability to identify, isolate, manipulate, and deliver stem cells in a safe and effective manner. Although it is clear that physical signals affect tissue morphogenesis, stem cell differentiation, and healing processes, integration of mechanically induced signaling events remain obscure. Mechanisms underlying sensation and interpretation of mechanical signals by stem cells are the focus of intense study. External mechanical signals have the ability to activate osteogenic signaling pathways in MSCs including Wnt, Ror2, and Runx2. It is also clear that intracellular tensile forces resulting from cell-extracellular matrix interactions play a critical role in MSC regulation. Further work is required to determine the precise role that mechanical forces play in stem cell function.


Effect of neural-induced mesenchymal stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model.

Cho HH, Jang S, Lee SC, Jeong HS, Park JS, Han JY, Lee KH, Cho YB.

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Abstract

OBJECTIVES/HYPOTHESIS: The purpose of this study was to investigate the effects of platelet-rich plasma (PRP) and neural-induced human mesenchymal stem cells (nMSCs) on axonal regeneration from a facial nerve axotomy injury in a guinea pig model. STUDY DESIGN: Prospective, controlled animal study. METHODS: Experiments involved the transection and repair of the facial nerve in 24 albino guinea pigs. Four groups were created based on the method of repair: suture only (group I, control group); PRP with suture (group II); nMSCs with suture (group III); and PRP and nMSCs with suture (group IV). Each method of repair was applied immediately after nerve transection. The outcomes measured were: 1) functional outcome measurement (vibrissae and eyelid closure movements); 2) electrophysiologic evaluation; 3) neurotrophic factors assay; and 4) histologic evaluation. RESULTS: With respect to the functional outcome measurement, the functional outcomes improved after transection and reanastomosis in all groups. The control group was the slowest to demonstrate recovery of movement after transection and reanastomosis. The other three groups (groups II, III, and IV) had significant improvement in function compared to the control group 4 weeks after surgery (P < .05). On the electrophysiologic evaluation, there was significantly better performances in groups II, III, and IV when compared to group I with respect to the amplitude and excitation area of the compound motor action potentials (MAPs) 4 and 6 weeks after surgery (P < .05); group IV had the best performance. A Western blot assay showed that group II had marked expression of several neurotrophic factors. Groups II, III, and IV demonstrated better results in axon counts and myelinated thickness compared to group I. Based on quantitative histology analysis, group IV had the greatest myelinated axon fibers compared to the other groups (P < .05). CONCLUSIONS: The use of PRP and/or nMSCs promotes facial nerve regeneration in an animal model of facial nerve axotomy. The use of nMSCs showed no benefit over the use of PRP in facial nerve regeneration, but the combined use of PRP and nMSCs showed a greater beneficial effect than use of either alone. This study provides evidence for the potential clinical application of PRP and nMSCs in peripheral nerve regeneration of an acute nerve injury.

Laryngoscope. 2010 May;120(5):895-901.

Injectable tissue-engineered bone repair of a rat calvarial defect.

Stephan SJ, Tholpady SS, Gross B, Petrie-Aronin CE, Botchway EA, Nair LS, Ogle RC, Park SS.

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Abstract

OBJECTIVES/HYPOTHESIS: Advances in bone repair have focused on the minimally-invasive delivery of tissue-engineered bone (TEB). A promising injectable biopolymer of chitosan and inorganic phosphates was seeded with mesenchymal stem cells (MSCs) and a bone growth factor (BMP-2), and evaluated in a rat calvarial critical size defect (CSD). Green fluorescent protein (GFP)-labeled MSCs are used to evaluate patterns of cell viability and proliferation. STUDY DESIGN: Prospective, controlled trial in an animal model. METHODS: In 30 male rats, 8-mm calvarial CSDs were created, and divided into five groups of six animals each. In the experimental groups, the defects were injected with either chitosan gel, gel loaded with MSCs (0.3 x 10^6 cells/defect), gel loaded with BMP-2 (2 microg/defect), or gel loaded with both MSC and BMP-2. In the control group, the defect was left untreated. At 4 weeks, in vivo microcomputed tomography (micro-CT) analysis was performed. At 8 weeks, calvarial specimens were examined by micro-CT, histology, and immunohistochemistry. RESULTS: New areas of bone growth were seen in the defects of all treated animals. Micro-CT analysis revealed a significant (P < .001) time-dependent increase in the regeneration of bone volume and bone area in defects treated with gel/MSC/BMP-2 as compared to all other groups. Histological analysis confirmed this difference. GFP-labeled TEB was detected within the areas of new bone, indicating cell viability and contribution to new bone growth by the injected MSC. CONCLUSIONS: This study demonstrates that an injectable form of TEB using a chitosan gel, MSC, and BMP-2 can enhance bone formation in a rat calvarial CSD.


Disc Regeneration Therapy Using Marrow Mesenchymal Cell Transplantation: A Report of Two Case Studies.

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Abstract

STUDY DESIGN: Marrow mesenchymal cells (MSCs) contain stem cells and possess the ability to regenerate bone, cartilage, and fibrous tissues. Here, we applied this regenerative ability to intervertebral disc regeneration therapy in an
Human stem cells that can generate, from a single cell, cells with the characteristics of the three germ layers. The cells are stress-tolerant and can be isolated from cultured skin fibroblasts or bone marrow stromal cells, or directly from bone marrow aspirates. These cells can self-renew, form characteristic cell clusters in suspension culture that express a set of genes associated with pluripotency, and can differentiate into endodermal, ectodermal, and mesodermal cells both in vitro and in vivo. When transplanted into immunodeficient mice by local or i.v. injection, the cells integrated into damaged skin, muscle, or liver and differentiated into cytokeratin 14-, dystrophin-, or albumin-positive cells in the respective tissues. Furthermore, they can be efficiently isolated as SSEA-3(+) cells. Unlike authentic ES cells, their proliferation activity is not very high and they do not form teratomas in immunodeficient mouse testes. Thus, nontumorigenic stem cells with the ability to generate the multiple cell types of the three germ layers can be obtained through easily accessible adult human mesenchymal cells without introducing exogenous genes. These unique cells will be beneficial for cell-based therapy and biomedical research.

BMC Neurosci. 2010 Apr 26;11(1):52. [Epub ahead of print]

Intravenous administration of mesenchymal stem cells exerts therapeutic effects on parkinsonian model of rats: Focusing on neuroprotective effects of stromal cell-derived factor-1alpha.


Abstract

ABSTRACT: BACKGROUND: Mesenchymal stem cells (MSCs) are pluripotent stem cells derived from bone marrow with secretory functions of various neurotrophic factors. Stromal cell-derived factor-1alpha (SDF-1alpha) is also reported as one of chemokines released from MSCs. In this research, the therapeutic effects of MSCs through SDF-1alpha were explored. 6-hydroxydopamine (6-OHDA, 20 microg) was injected into the right striatum of female SD rats with subsequent administration of GFP-labeled MSCs, fibroblasts, (i.v., 1x10(7) cells, respectively) or PBS at 2 hours after 6-OHDA injection. All rats were evaluated behaviorally with cylinder test and amphetamine-induced rotation test for 1 month with consequent euthanasia for immunohistological evaluations. Additionally, to explore the underlying mechanisms, neuroprotective effects of SDF-1alpha were explored using 6-OHDA- exposed PC12 cells by using dopamine (DA) assay and TdT-mediated dUTP-biotin nick-end labeling (TUNEL) staining. RESULTS: Rats receiving MSC transplantation significantly ameliorated behaviorally both in cylinder test and amphetamine-induced rotation test compared with the control groups. Correspondingly, rats with MSCs displayed significant preservation in the density of tyrosine hydroxylase (TH)-positive fibers in the striatum and the number of TH-positive neurons in the substantia nigra
pars compacta (SNc) compared to that of control rats. In the in vitro study, SDF-1alpha treatment increased DA release and suppressed cell death induced by 6-OHDA administration compared with the control groups. CONCLUSION: Consequently, MSC transplantation might exert neuroprotection on 6-OHDA-exposed dopaminergic neurons at least partly through anti-apoptotic effects of SDF-1alpha. The results demonstrate the potentials of intravenous MSC administration for clinical applications, although further explorations are required.


Two-photon microscopy for non-invasive, quantitative monitoring of stem cell differentiation.

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Abstract

BACKGROUND: The engineering of functional tissues is a complex multi-stage process, the success of which depends on the careful control of culture conditions and ultimately tissue maturation. To enable the efficient optimization of tissue development protocols, techniques suitable for monitoring the effects of added stimuli and induced tissue changes are needed. METHODOLOGY/PRINCIPAL FINDINGS: Here, we present the quantitative use of two-photon excited fluorescence (TPEF) and second harmonic generation (SHG) as a noninvasive means to monitor the differentiation of human mesenchymal stem cells (hMSCs) using entirely endogenous sources of contrast. We demonstrate that the individual fluorescence contribution from the intrinsic cellular fluorophores NAD(P)H, flavoproteins and lipofuscin can be extracted from TPEF images and monitored dynamically from the same cell population over time. Using the redox ratio, calculated from the contributions of NAD(P)H and flavoproteins, we identify distinct patterns in the evolution of the metabolic activity of hMSCs maintained in either propagation, osteogenic or adipogenic differentiation media. The differentiation of these cells is mirrored by changes in cell morphology apparent in high resolution TPEF images and by the detection of collagen production via SHG imaging. Finally, we find dramatic increases in lipofuscin levels in hMSCs maintained at 20% oxygen vs. those in 5% oxygen, establishing the use of this chromophore as a potential biomarker for oxidative stress. CONCLUSIONS/SIGNIFICANCE: In this study we demonstrate that it is possible to monitor the metabolic activity, morphology, ECM production and oxidative stress of hMSCs in a non-invasive manner. This method therefore represents a powerful tool, which enables researchers to monitor engineered tissues and optimize culture conditions in a near real time manner.

Nat Mater. 2010 Apr 25. [Epub ahead of print]

Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate.

Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ.


Abstract

Stem cells sense and respond to the mechanical properties of the extracellular matrix. However, both the extent to which extracellular-matrix mechanics affect stem-cell fate in three-dimensional microenvironments and the underlying biophysical mechanisms are unclear. We demonstrate that the commitment of mesenchymal stem-cell populations changes in response to the rigidity of three-dimensional microenvironments, with osteogenesis occurring predominantly at 11-30 kPa. In contrast to previous two-dimensional work, however, cell fate was not correlated with morphology. Instead, matrix stiffness regulated integrin binding as well as reorganization of adhesion ligands on the nanoscale, both of which were traction dependent and correlated with osteogenic commitment of mesenchymal stem-cell populations. These findings suggest that cells interpret changes in the physical properties of adhesion substrates as changes in adhesion-ligand presentation, and that cells themselves can be harnessed as tools to mechanically process materials into structures that feed back to manipulate their fate.

Mesenchymal Stem Cells as Therapeutics.

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Abstract
Mesenchymal stem cells (MSCs) are multipotent cells that are being clinically explored as a new therapeutic for treating a variety of immune-mediated diseases. First heralded as a regenerative therapy for skeletal tissue repair, MSCs have recently been shown to modulate endogenous tissue and immune cells. Preclinical studies of the mechanism of action suggest that the therapeutic effects afforded by MSC transplantation is short-lived and related to dynamic, paracrine interactions between MSCs and host cells. Therefore, representations of MSCs as drug-loaded particles may allow for pharmacokinetic models to predict the therapeutic activity of MSC transplants as a function of drug delivery mode. By integrating principles of MSC biology, therapy, and engineering, the field is armed to usher in the next generation of stem cell therapeutics.


Artificial cell microencapsulated stem cells in regenerative medicine, tissue engineering and cell therapy.

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Abstract
Adult stem cells, especially isolated from bone marrow, have been extensively investigated in recent years. Studies focus on their multiple plasticity of transdifferentiating into various cell lineages and on their potential in cellular therapy in regenerative medicine. In many cases, there is the need for tissue engineering manipulation. Among the different approaches of stem cells tissue engineering, microencapsulation can immobilize stem cells to provide a favorable microenvironment for stem cells survival and functioning. Furthermore, microencapsulated stem cells are immunoisolated after transplantation. We show that one intraperitoneal injection of microencapsulated bone marrow stem cells can prolong the survival of liver failure rat models with 90% of the liver removed surgically. In addition to transdifferentiation, bone marrow stem cells can act as feeder cells. For example, when coencapsulated with hepatocytes, stem cells can increase the viability and function of the hepatocytes in vitro and in vivo.

Ann Biomed Eng. 2010 Apr 27. [Epub ahead of print]

Tissue Engineering Strategies for the Regeneration of Orthopedic Interfaces.

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Abstract
A major focus in the field of orthopedic tissue engineering is the development of tissue engineered bone and soft tissue grafts with biomimetic functionality to allow for their translation to the clinical setting. One of the most significant challenges of this endeavor is promoting the biological fixation of these grafts with each other as well as the implant site. Such fixation requires strategic biomimicry to be incorporated into the scaffold design in order to re-establish the critical structure-function relationship of the native soft tissue-to-bone interface. The integration of distinct tissue types (e.g. bone and soft tissues such as cartilage, ligaments, or tendons), necessitates a multi-phased or stratified scaffold with distinct yet continuous tissue regions accompanied by a gradient of mechanical properties. This review discusses tissue engineering strategies for regenerating common tissue-to-tissue interfaces (ligament-to-bone, tendon-to-bone, or cartilage-to-bone), and the strategic biomimicry implemented in stratified scaffold design for multi-tissue regeneration. Potential challenges and future directions in this emerging field will also be presented. It is anticipated that interface tissue engineering will enable integrative soft tissue repair, and will be instrumental for the development of complex musculoskeletal tissue systems with biomimetic complexity and functionality.
Novel in vitro co-culture methodology to investigate heterotypic cell-cell interactions.

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Abstract
Cell-cell interactions are of crucial importance for the formation of tissues, homeostasis and regeneration processes as well as reactions on foreign bodies including implants. So far, however, the importance of heterotypic cell-cell interactions in the in vitro evaluation of implant surfaces has been largely neglected. This work aims to develop an in vitro methodology that enables the in-depth investigation of heterotypic cell-cell interactions in a mixed co-culture system, and to validate it with a primary adult human bone-derived osteoblast cells (HBCs) - abdominal fibroblasts (HAFs) system. The methodology proposed combines a simple live labelling step, semiautomated fluorescence image acquisition and analysis to characterize the interactions between different cell types (cell population dynamics) in co-culture in terms of cell proliferation and cell spatial distribution of each cell type. In this co-culture system, direct cell-cell contacts between the two cell types were permitted while the determination of cell-type specific responses could still be elucidated. We could show that HAF proliferation was reduced in a way negatively correlated with the seeding HBC/HAF ratio, i.e., a high proportion of HBC in the co-culture had an inhibitory effect on HAF proliferation. In all cultures segregation was found after 4 and 7 days of co-culture. HBCs were segregated at low ratios while HAFs were segregated at high ratios. Cell-cell distances depended on the total cell number in the co-culture but the dependence was different for each cell type.

Immunohistochemical comparison of differentiation markers on paraffin and plastic embedded human bone samples.

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Abstract
To assess bone pathologies and bone regeneration immunohistochemistry may provide additional information compared to conventional histology. However, the effectiveness of this technique is limited due to tissue fixation, preparation and embedding. For bone tissue the standard immunohistological procedure includes formalin fixation, followed by decalcification and paraffin embedding. This may lead to a badly preserved trabecular bone structure but allows antibody application. Alternatively, methyl-methacrylate (MMA) resin may be used for embedding, thus circumventing the decalcification procedure. In this study immunohistochemistry of typical bone markers was compared using human bone samples fixed either with alcohol or formalin and further decalcified and embedded in paraffin and decalcified or non decalcified samples embedded in Technovit 9100 New(R). On semi-thin sections immunohistochemistry with bone markers osteocalcin, osteonectin, osteopontin, collagen type I and the cellular markers CD34 and CD68 was performed. Independent of the fixative used, Technovit 9100 New embedded non-decalcified bone yielded a stronger immunostaining for all markers when compared to decalcified bone embedded either in methyl-methacrylate or paraffin. In addition there was a better preservation of the trabecular bone morphology. The immunohistochemical results demonstrate that Technovit 9100 New as a low-temperature acrylic resin embedding method can be favoured over paraffin embedding.

Wnt proteins promote bone regeneration.


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Abstract
The Wnt signaling pathway plays a central role in bone development and homeostasis. In most cases, Wnt ligands promote bone growth, which has led to speculation that Wnt factors could be used to stimulate bone healing. We gained insights into the mechanism by which Wnt signaling regulates adult bone repair through the use of the mouse strain Axin2(LacZ/LacZ) in which the cellular response to Wnt is increased. We found that bone healing after injury is accelerated in Axin2(LacZ/LacZ) mice, a consequence of more robust proliferation and earlier differentiation of skeletal stem and progenitor cells. In parallel, we devised a biochemical strategy to increase the duration and strength of Wnt signaling at the sites of skeletal injury. Purified Wnt3a was packaged in liposomal vesicles and delivered to skeletal defects, where it stimulated the proliferation of skeletal progenitor cells and accelerated their differentiation into osteoblasts, cells responsible for bone growth. The end result was faster bone regeneration. Because Wnt signaling is conserved in mammalian tissue repair, this protein-based approach may have widespread applications in regenerative medicine.

Laryngoscope. 2010 May;120(5):895-901.

Injectable tissue-engineered bone repair of a rat calvarial defect.

Stephan SJ, Tholpady SS, Gross B, Petrie-Aronin CE, Botchway EA, Nair LS, Ogle RC, Park SS.

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Abstract
OBJECTIVES/HYPOTHESIS: Advances in bone repair have focused on the minimally-invasive delivery of tissue-engineered bone (TEB). A promising injectable biopolymer of chitosan and inorganic phosphates was seeded with mesenchymal stem cells (MSCs) and a bone growth factor (BMP-2), and evaluated in a rat calvarial critical size defect (CSD). Green fluorescent protein (GFP)-labeled MSCs are used to evaluate patterns of cell viability and proliferation. STUDY DESIGN: Prospective, controlled trial in an animal model. METHODS: In 30 male rats, 8-mm calvarial CSDs were created, and divided into five groups of six animals each. In the experimental groups, the defects were injected with either chitosan gel, gel loaded with MSCs (0.3 x 10(6) cells/defect), gel loaded with BMP-2 (2 microg/defect), or gel loaded with both MSC and BMP-2. In the control group, the defect was left untreated. At 4 weeks, in vivo microcomputed tomography (micro-CT) analysis was performed. At 8 weeks, calvarial specimens were examined by micro-CT, histology, and immunohistochemistry. RESULTS: New areas of bone growth were seen in the defects of all treated animals. Micro-CT analysis revealed a significant (P < .001) time-dependent increase in the regeneration of bone volume and bone area in defects treated with gel/MSC/BMP-2 as compared to all other groups. Histological analysis confirmed this difference. GFP-labeled TEB was detected within the areas of new bone, indicating cell viability and contribution to new bone growth by the injected MSC. CONCLUSIONS: This study demonstrates that an injectable form of TEB using a chitosan gel, MSC, and BMP-2 can enhance bone formation in a rat calvarial CSD.

Vet Comp Orthop Traumatol. 2010 Apr 26;23(3). [Epub ahead of print]

Enhancing bone healing and regeneration: present and future perspectives in veterinary orthopaedics.

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Abstract
Methods currently used to restore bone defects in human and veterinary orthopaedics are often not satisfactory. This is especially the case in the healing of large, irregular defects which result in the formation of tissues with inferior qualities compared to the original structures. For these reasons, several new approaches are currently being explored to improve bone healing capacities in different situations. This review will examine the different techniques used to enhance bone regeneration, highlighting both experimental and clinically applicable methods with regard to veterinary orthopaedics.


A clinical study on bone formation using a demineralized bone matrix and resorbable membrane.
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**Abstract**

OBJECTIVES: The purpose of this study was to evaluate new bone formation following guided bone regeneration (GBR) using a composite of demineralized cortical and nondemineralized cancellous bone admixed in a poloxamer reverse phase carrier (Orthoblast II) and resorbable collagen membrane (Ossix). STUDY DESIGN: Fourteen patients (14 specimens) participated in this study from January 2006 to May 2006. In all these 14 patients, bone grafting for the regeneration of dehiscence defects around the implants was required. At the 4- and/or 6-month healing period, a biopsy specimen was obtained by one oral and maxillofacial surgeon. The specimens were fixed, demineralized, embedded, and sectioned by a pathologist, and histomorphometric evaluations were performed using a computer-assisted Visus Image Analysis System. RESULTS: A high proportion of new bone formation (12.3%-78.7%) was observed during the 4- and/or 6-month healing period. Although histopathologic findings indicated that the grafted materials did not completely resorb, new bone formation and bone remodeling were observed to increase with healing time. CONCLUSION: It was concluded from this study that the use of GBR consisting of Orthoblast II and Ossix membranes caused favorable bone formation during the 6-month healing period. Additionally, the increase in the woven bone to lamellar bone (LB/WB) ratio and the new bone to residual graft material (NB/GM) ratio observed in this 6-month study also provided evidence of increasing bony remodeling and maturity as well as the continuous resorption of the grafting materials.


The innate osteogenic potential of the maxillary sinus (Schneiderian) membrane: an ectopic tissue transplant model simulating sinus lifting.

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**Abstract**

Maxillary sinus membrane lifting is a common procedure aimed at increasing the volume of the maxillary sinus osseous floor prior to inserting dental implants. Clinical observations of bone formation in sinus lifting procedures without grafting bone substitutes were observed, but the biological nature of bone regeneration in sinus lifting procedures is unclear. This study tested whether this osteogenic activity relies on inherent osteogenic capacity residing in the sinus membrane by simulating the in vivo clinical condition of sinus lifting in an animal model. Maxillary sinus membrane cells were cultured in alpha-MEM medium containing osteogenic supplements (ascorbic acid, dexamethasone). Cultured cells revealed alkaline phosphatase activity and mRNA expression of osteogenic markers (alkaline phosphatase, bone sialoprotein, osteocalcin and osteonectin) verifying the osteogenic potential of the cells. Fresh tissue samples demonstrated positive alkaline phosphatase enzyme activity situated along the membrane-bone interface periosteum-like layer. To simulate the in vivo clinical conditions, the membranes were folded to form a pocket-like structure and were transplanted subcutaneously in immunodeficient mice for 8 weeks. New bone formation was observed in the transplants indicating the innate osteogenic potential within the maxillary Schneiderian sinus membrane and its possible contribution to bone regeneration in sinus lifting procedures.


Osteoblast response to commercially available demineralized bone matrices--an in-vitro study.

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**Abstract**

OBJECTIVE: Reconstruction of lost attachment apparatus is a major goal of periodontal therapy. Although various osteoinductive bone replacement grafts (BRGs) have been used with apparent clinical success, unequivocal evidence of osteoinductivity may be obtained only through the demonstration of increased osteoblastic/osteoclastic differentiation following exposure to these materials. MATERIALS AND METHODS: Bone marrow stem cells (BMSCs) obtained from
rat femur were cultured in Dulbecco's Modified Eagles Medium (DMEM) and 10% fetal bovine serum (FBS). They were then exposed to two demineralized bone matrices (DBM's)--Grafton and Osseograft, and divided into three groups, comprising of a negative control (BMSC + DMEM + 10% FBS), Grafton, Osseograft. An osteogenic medium (OM) (10 hm dexamethasone, 10 hm b-glycerophosphate, and 50 microg/ml ascorbic acid) was added to create three subgroups comprising of a positive control (OM), Grafton with OM, Osseograft with OM. RESULTS: After an initial phase (up to day 5), both Grafton and Osseograft induced an increased proliferative activity in the BMSCs, which reached a plateau after day 10. These grafts also induced increased alkaline phosphatase activity when compared to the control groups and to BMSCs with an OM. CONCLUSION: Both Osseograft and Grafton are capable of inducing osteoblastic proliferation and differentiation.

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**Discovery of a novel unfolded protein response phenotype of cancer stem/progenitor cells from the bone marrow of breast cancer patients.**

**Abstract**

Metastases arise from disseminated tumor cells (DTC) that colonize secondary organs. However, DTC survival strategies to start metastatic outgrowth are unclear. The hostile (hypoxic, hypoglycaemic) microenvironmental conditions of the bone marrow serve as an ideal model environment for investigation of DTC survival strategies under environmental stress. We investigated the breast cancer DTC cell line BC-M1 established from the bone marrow of a cancer patient by 2-D DIGE and MS analysis. We observed specific overexpression of the unfolded protein response (UPR) proteins Grp78, Grp94 and protein disulfide-isomerase in breast, lung and prostate cancer DTC cell lines from the bone marrow. The UPR contributes to survival under adverse environmental conditions including chemotherapy. We show in cellular models that Grp78 expression of the UPR is regulated by tyrosine 1248 of ErbB-2. The breast cancer DTC cell lines shared stem/progenitor cell cancer phenotypes (CD44high/CD24low). Immunocytochemical staining of bone marrow samples from breast cancer patients confirmed in situ high expression of Grp78 and Grp94 in DTC of breast cancer patients, indicating the potential of both proteins as novel markers for DTC detection. Our results suggest the presence of a previously not recognized stress resistant DTC population that combines stem/progenitor attributes with an UPR phenotype.

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**In vivo imaging and monitoring of transplanted stem cells: clinical applications.**

**Rodriguez-Porcel M.**
Abstract
Regenerative medicine using stem cells has appeared as a potential therapeutic alternative for coronary artery disease, and stem cell clinical studies are currently on their way. However, initial results of these studies have provided mixed information, in part because of the inability to correlate organ functional information with the presence/absence of transplanted stem cells. Recent advances in molecular biology and imaging have allowed the successful noninvasive monitoring of transplanted stem cells in the living subject. In this article, different imaging strategies (direct labeling, indirect labeling with reporter genes) to study the viability and biology of stem cells are discussed. In addition, the limitations of each approach and imaging modality (e.g., single photon emission computed tomography, positron emission tomography, and MRI) and their requirements for clinical use are addressed. Use of these strategies will be critical as the different regenerative therapies are being tested for clinical use.


Emerging use of stem cells in regenerative medicine.

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Abstract
Stem cells represent a unique opportunity for regenerative medicine to cure a broad number of diseases for which current treatment only alleviates symptoms or retards further disease progression. However, the number of stem cells available has speedily increased these past 10 years and their diversity presents new challenges to clinicians and basic scientists who intend to use them in clinics or to study their unique properties. In addition, the recent possibility to derive pluripotent stem cells from somatic cells using epigenetic reprogramming has further increased the clinical interest of stem cells since induced pluripotent stem cells could render personalized cell-based therapy possible. The present review will attempt to summarize the advantages and challenges of each type of stem cell for current and future clinical applications using specific examples.