Bone Block Allograft Impregnated With Bone Marrow Aspirate

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Adult bone marrow is a rich source of stem cells that can be induced to undergo differentiation into a variety of adult tissues.1 The microenvironment of bone marrow consists of predominantly hematopoietic stem cells (HSCs), as well as nonhematopoietic cells. The HSCs are inactive and quiescent. However, in response to stresses such as infection, exercise, surgery, or injury, the nonhematopoietic cells secrete soluble and insoluble cytokines and growth factors that activate the hematopoietic cells. The hematopoietic cells then proliferate, maintaining their pluripotent progenitor stem cell capability and/or differentiating into osteoclasts, monocytes, macrophages, platelets, red and white blood cells, and other blood cells.2,4

The non-HSCs, which can be roughly classified as "stromal" cells, support and regulate the fate of the hematopoiesis. The nonhematopoietic cells include the following5-7:

1. Mesenchymal stem cells (MSCs) that produce osteoblasts, adipocytes, and fibroblasts.8
2. Endothelial-like cells, which function as a permeable barrier between the marrow cavity and the circulation.9

The endothelial cells line the specialized vascular wall of the marrow called sinusoids. Its structure is sponge-like network giving the marrow support and separating the endosteeum of bone and the bone marrow. The specialized cells participate in supporting the maturation and development of the hematopoietic cells such as the blood cells that can differentiate into white blood cells or red blood cells or megakaryocytes into platelets. Also, they secrete various growth factors such as vascular endothelial growth factors and platelet-derived growth factors.

In recent years, in vivo transplantation of adult stem cells in bone-marrow aspirate has been used successfully to enhance bone regeneration.10 The transplanted cells lay down an initial unmineralized bone matrix (osteon) and start the process of laying down hydroxyapatite, the mineral component of bone in the extracellular matrix.11

Rules for achieving successful bone grafts are discussed in a number of articles.12,13 A simple and effective technique for aspirating bone marrow from patients who are undergoing grafting in preparation for implant

Purpose: To evaluate the influence of bone marrow aspirate when added to bone block allograft to repair osseous defects.

Background: Bone-marrow aspirate has been combined with xenograft and allograft particulate material and has produced a significant quantity of new bone growth. However, the use of allograft bone blocks has advantages in some clinical situations. This article discusses cell-based therapies by means of in vivo transplantation of stem cells derived from bone-marrow aspirate and incorporated into allograft corticocancellous bone block for bone regeneration.

Materials: A technique for combining bone-marrow aspirate with block allografts was developed. To evaluate its influence in repairing osseous defects, a maximum of 3 to 4 mL of bone marrow was aspirated from the anterior iliac crest of 5 patients who had severely atrophic maxillary and mandibular ridges. Five sites were grafted with allograft bone blocks saturated with bone-marrow aspirate and secured with bone screws.

(ACE Surgical Supply Company, Inc. Brockton, MA). At one of the sites a core specimen was taken 4 months after implant placement and submitted for standard histologic and histomorphometric analysis.

Results: After 4 to 8 months of healing, all the grafts had integrated into the recipient bone. Implants were placed at all 5 sites and osseointegrated successfully. Examination of the bone core showed the graft to be well-integrated, with 54% of the core consisting of bone and 46% of marrow. Eighty-nine percent of the bone was vital.

Conclusion: Impregnation of bone-marrow aspirate into allograft bone block activates the body’s ability to form new bone. The bone-marrow aspiration technique is less invasive than harvesting autogenous bone from a second surgical site, offers predictable results, and is cost effective. (Implant Dent 2007;16:329–339)

Key Words: autogenous stem cells, bone marrow aspirate, block allograft, bone graft, tissue engineering
A block of corticocancellous allograft bone was contoured to fit within the prepared recipient-site cavity (Fig. 3). When contouring the bone block, care was taken to avoid removing the cancellous component. All contouring of the block instead was done at the expense of the more dense cortical bone. This is important to enable the bone-marrow aspirate to saturate the cancellous compartment.

Once fitted into the site, the block was stabilized with 1 or 2 bone screws (Fig. 3). A bone mill was used to prepare the pieces removed from the block during the contouring process.

The mucoperiosteal flap was repositioned and examined for passivity during suturing. If primary closure seemed questionable, the nonstretching periosteum was filleted high within the broad-based flap to release this tissue and to ensure a passive position without tension at the sutured incision.

The bone-block was unscrewed and removed from the recipient site and placed in a syringe. Between 1 and 4 mL of bone-marrow aspirate was then aspirated from the patient’s iliac crest, as previously described by Smiler and Soltan. This aspirate was placed in the syringe containing the bone block and the plunger was attached. The end of the syringe was occluded, and the plunger was pulled back to create a vacuum and impregnate the cancellous compartment of the bone block with the bone-marrow aspirate.

The marrow-impregnated bone block was placed in the recipient site again and secured with bone screws. The prepared allograft particles were saturated with the remaining aspirate and mortised around and over the graft. The mucoperiosteal flap was repositioned and sutured without tension at the incision site (Fig. 4).

The specifics of the 5 patients treated were as follows:

**Case 1**

A.M., a 53-year-old woman, presented with bilateral severe resorption of the posterior and anterior maxilla. In the anterior maxilla, the incisive papilla was at midcrest, suggesting a minimum of 10 to 12 mm of horizontal bone loss and a resulting bone width of 2.5 mm (Fig. 5). Two allograft bone blocks (Musculoskeletal Transplant Foundation, University of Michigan Tissue Bank, Michigan) were contoured to fit the deorted anterior maxilla recipient site, saturated with bone-marrow aspirate, and stabilized with 2 bone screws each (ACE Bone Screws, ACE Surgical Supply Company, Inc., Brockton, MA). The right and left posterior max-
illa were prepared for sinus-lift subantral augmentation and grafted with PepGen P-15 (Dentsply-CeraMed-Dental, Lakewood, CO) mixed with bone-marrow aspirate. After 8 months of healing, 12 Xive implants (Dentsply Friadent) were placed (Fig. 6). The implants have all integrated and support a fixed crown and bridge prosthesis.

Case 2

S.H., a 41-year-old woman, presented with a diminished alveolar crest of the anterior maxilla extending from the right second molar to the left canine teeth. Multiple holes were drilled into the buccal region of the bone-graft recipient site to decorticate the diminished cancellous compartment, and a cavity was created. After 2 bone blocks (ACE Surgical Supply, Salt Lake City, UT) were contoured and saturated with bone-marrow aspirate, they were stabilized at the recipient site with screws (Fig. 7) and allowed to heal for 6 months. Seven Nobel Replace Tapered Groovy implants (Nobel Biocare, Yorba Linda, CA) were then placed (Fig. 8). All the implants have integrated and support a fixed crown and bridge restoration.

Case 3

M.B., a 44-year-old man, presented with severe atrophy of the right central incisor region after multiple unsuccessful autogenous bone graft attempts. A computed tomography scan and computer-generated model confirmed the extensiveness of the bone loss (Fig. 9). An allograft bone block (ACE Surgical Supply) was prepared and placed as described above. At 4 months, there was complete healing of the graft to the recipient site. An implant osteotomy was created, and a 5-mm-diameter Replace Speedy implant (Nobel Biocare) was placed (Fig. 10). It has successfully osseointegrated and now supports a single crown restoration.

Case 4

N.G., a 48-year-old woman, required augmentation of the edentulous left first bicuspid site before extraction of the second bicuspid and placement of 2 implants. A fissure bur was used to decorticate the buccal bone at the recipient site, exposing the cancellous compartment. A piece of AlloOss Block Allograft (ACE Surgical Supply) was saturated with bone-marrow aspirate, stabilized with bone screws, and particulate was mortised over the graft (Fig. 11). After 7 months of healing an implant was placed. The implant integrated and supports a fixed restoration.

Case 5

J.B., a 22-year-old woman, presented with a through and through osseous defect of the left maxillary central incisor site (Fig. 12). After piezosurgically removing cortical bone to access the blood supply, a rectangular cavity was prepared within the cancellous compartment at the recipient site. An AlloOss
bone block saturated with bone-marrow aspirate was then positioned and secured with 1 bone screw (Fig. 13). The mucoperiosteal flap was repositioned and sutured without tension, and after healing a single Nobel Biocare Speedy implant was placed. It currently supports a successful provisional crown restoration.

Table 1 summarizes the 5 cases treated with allograft bone block and bone-marrow aspirate.

### Biopsy Procedure

Four months after implant-placement surgery, a core sample 4 mm in diameter was obtained by passing a 4-mm trephine drill through the superior region of the graft placed in patient. Case 3 was selected to show histomorphometric analysis of the grafted site. The patient had critical defect as seen in the computed tomography scan (Fig. 9). Multiple unsuccessful autogenous bone grafts attempted in the past with extensive bone loss, little blood supply available from the cancellous residual ridge and the periosteum, and a thick soft tissue scar present from the previous surgical attempts. The periosteum in the buccal region is thin and compromised lacking in providing the blood supply, the stem cells, and the osteoblasts needed for regeneration. Being a 44-year-old man, there is a natural decrease in the circulation of MSCs in the peripheral blood that normally bring the cells to the surgical sites compared with patients that are younger (Fig. 14).

### Specimen Processing

The biopsy sample was fixed in 10% buffered Formalin and submitted for histologic examination. After dehydration with a graded series of ethanol for 9 days, the sample was infiltrated for 20 days with a light-curing embedding resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany), then embedded in Technovit 7200 VLC, and polymerized by 450-nm light with the temperature of the specimen not exceeding 40°C. The specimen was prepared by the cutting and grinding method of Donath and cut to thicknesses of 150 mm on an EXAKT cutting and grinding system (EXAKT Technologies, Oklahoma City, OK), and then mounted on slides. Each specimen slide was polished to a thickness of 55 μm with a series of polishing sandpaper discs from 800 to 2400 grit (EXAKT microgrinding system) followed by a final polish with 0.3 μm alumina polishing paste. Following final polishing, the specimen slides were stained using Stevenel’s blue and van Gieson’s picric fuchsine and subjected to histological evaluation by light microscopy. The specimen was evaluated using 2 slides to prevent sampling bias.

Microphotographs were obtained, scanned, digitized, and analyzed using a Zeiss Axiolab photomicroscope (Carl Zeiss, Jena, Germany) and Nikon Coolpix 4500 digital camera (Nikon Corp. Tokyo, Japan). All core specimens were photographed at a fixed focal point and ×25 magnification for histomorphometric evaluation. Histomorphometric measurements were completed with a Macintosh G4 computer (Apple, Cupertino, CA) and a public domain image program (NIH Images, US National Institutes of Health) along with Adobe Photoshop (Adobe, San Jose, CA). The data were exported to Microsoft Excel (Microsoft Co., Redmond, WA) for histomorphometric calculations. Histomorphometric analysis was performed and the following parameters were measured in terms of the percentage of the total core area: new bone formation, residual graft material, and marrow space.

### RESULTS

After 4 to 8 months of healing, all the grafts had integrated into the recipient bone. Implants were placed at all 5 sites and osseointegrated successfully. Examination of the bone core for case 3 after 4 months showed the graft to be well-integrated, with 54% of the core consisting of bone and 46% of marrow. Eighty-nine percent of the bone was vital. This patient has had

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### Table 1. A Summary of 5 Cases Treated With Allograft Bone Block and Bone-Marrow Aspirate
Histomorphometric analysis revealed a very solid core with 54% bone and 46% marrow. Eighty-nine percent of the bone was vital; 11% was nonvital allograft.

**DISCUSSION**

What determines the wound healing potential of an injured bone? Throughout our lives we sustain injuries from accidents and surgery from which we recover. This is due to the process of wound healing and the remarkable potential of the body to repair itself.\(^6\)\(^7\) Wound healing and repair involve the recruitment and proliferation of cells capable of restoring tissues to their original form and function. Studies show the involvement of bone marrow in healing following injury, bleeding, or disease. Increased bone marrow activity has been documented, for example, in sites adjacent to osseous fractures.\(^8\)\(^9\)

Surgically to restore the form and function of injured, diseased, or dysfunctional tissues has shifted from being primarily restorative procedures to, increasingly, in vivo regenerative procedures. Studies show that autogenous bone grafts can be replaced by stem cell transplants that are combined with a biocompatible scaffold matrix. This combination repairs and/or restores the form and function of injured or atrophied tissues.\(^10\)\(^20\)\(^22\)

The potential of cell-based therapies for bone regeneration is possible because of advancements in techniques, graft materials, and understanding of the biology of adult stem cells as the regenerative source for bone and other tissues.\(^23\)\(^24\) Cells or tissue act in a variety of ways to restore, maintain, and enhance function. Mechanisms that can enhance bone formation include the following:

1. The use of harvested autogenous cancellous bone.\(^25\)\(^27\)
2. *In vivo* transplantation of stem cells.\(^14\)\(^20\)
3. *In situ* cell activation by means of growth factors and cytokines.\(^28\)\(^34\)
4. Implantation of *in vitro*-generated tissue.\(^35\)\(^36\)
5. Implantation of isolated expanded cultured cells.\(^37\)\(^40\)

The use of stem cells to treat various diseases and reconstruct injured, diseased, or dysfunctional tissue has become a routine procedure in many medical and dental clinical practices. The challenge for dentists is not only to be able to answer the questions of patients who are curious about less-invasive and more-predictable restorative procedures but also to be familiar with the wide range of options available for restoring patients' oral form and function. In addition to the mechanisms listed above, factors that the surgeon must consider in offering options to patients include the following:

1. The patient's needs and requests.
2. The size of the defect and type of proposed reconstruction.
3. The quality and quantity of recipient hard and soft tissues and the amount of blood supply.
4. The patient's physical status, systemic condition, age, and supply of available and viable bone marrow stem cells.
5. Methods and surgical techniques to optimize the matrix scaffold.\(^41\)

Harvesting autogenous bone from the iliac crest and placing it in the maxilla or mandible is a common procedure for treating severely resorbed ridges. However, little attention has been given to the developmental origins of the extraoral donor bone or the intraoral recipient site(s). Many dental practitioners assume that bone is bone. In fact, iliac crest bone is a substantially different entity from alveolar bone, a distinction illuminated by the field of embryology.

Within the third week after fertilization, the human embryo develops 3 distinct germinal layers: the ectoderm, the mesoderm, and the endoderm. Each of these layers later undergoes a complex chain of development, branching off into the myriad components of the fully developed human body.

The iliac crest bone and its marrow have their origin in embryonic mesodermal cells. These cells give rise to the mesenchyme, a loosely organized embryonic connective tissue. In contrast, the alveolar bones (the maxilla and mandible) are ultimately derived from the ectoderm.\(^42\) By the beginning of the fourth week after fertilization, the embryonic neural crest cells, derived from the neuroectoderm,
Bone marrow aspirate is safe and easy to harvest. It is inexpensive to obtain, and it serves a number of functions at bone-grafting sites. It provides cells that can differentiate into bone cells and it influences tissue at, and adjacent to, the recipient site to regenerate bone cells by activating the body’s ability to form new bone. It also supplies growth factors needed both for bone formation and angiogenesis. The technique described here, of using allograft bone blocks in combination with aspirated bone marrow, broadens the options available to dental clinicians seeking to augment patients’ maxillary or mandibular bone as part of an overall strategy for oral rehabilitation.

Disclosure

The authors claim to have no financial interest, directly or indirectly, in any entity that is commercially related to the products mentioned in this article.

Acknowledgments

This study was partially supported by the Platinum Foundation for Research and Education.

References


ZUSAMMENFASSUNG: Zielsetzung: Es wurde darauf abge-geizt, den Einfluss von per Punktion gewonnenem Knochenmark zu ermitteln, das Knocheneckallotransplantat zur Wiederherstellung von Knochendefekten beigegeneinergewhird. 


SCHLÜSSELWÖRTER: Autogene Stammzellen, per Punk tion gewonnenen Knochenmark, Blockallotransplantat, Knochentransplantat, Gewebsbehandlung

SPANISCH / ESPAÑOL

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Alfinjerto con bloque de hueso impregnado con aspiración de médula ósea

ABSTRACTO: Propósito: Evaluar la influencia de la aspiración de médula ósea cuando se agrega a un alfinjerto con bloque de hueso para reparar defectos óseos. Antecedentes: La aspiración de médula ósea se ha combinado con material de partículas de alfinjerto y xenoinjerto y ha producido una cantidad significativa de nuevo crecimiento del hueso. Sin embargo, el uso de alfinjertos con bloques de hueso tiene ventajas en algunas situaciones clínicas. Este artículo explica terapias celulares a través del trasplante in vivo de células madre derivadas de la aspiración de médula ósea e incorporadas a un bloque de hueso cortico-canceloso de un alfinjerto para la regeneración del hueso. Materiales y Métodos: Se creó una técnica para combinar la aspiración de médula ósea con alfinjertos de bloques. Para evaluar su influencia en la reparación de defectos óseos, se aspiró un máximo de 3 a 4 ccs de médula ósea de la cresta anterior ilíaca de 5 pacientes que tenían crestas mandibulares y maxilares severamente atróficas. Se injertaron cinco lugares de alfinjertos con bloques de hueso saturados con la aspiración de médula ósea y asegurados con tornillos para hueso (ACE Surgical Supply Company, Inc. Brockton, MA). En uno de los lugares, se sacó una muestra cuatro meses después de la colocación del imp lante y se la sometió a análisis histomorfométricos e histo lógicos normales. Resultados: Después de 4 a 8 meses de curación, todos los injertos se habían integrado al hueso. Se colocaron los implantes en los cinco sitios y lograron una osteointegración exitosa. El análisis del hueso reveló que el injerto se había integrado bien, con un 54% del núcleo formado por hueso y un 46% de médula. Un ochenta y nueve (89%) del hueso estaba vivo. Conclusion: La impregnación con aspiración de médula ósea en un alfinjerto con bloque de hueso activa la capacidad del cuerpo de crear nuevo hueso. La técnica de aspiración de médula ósea es menos invasiva que cosechar hueso autógeno de un segundo lugar quirúrgico, ofrece resultados predecibles y cuesta menos.

PALABRAS CLAVES: células madre autógenas, aspiración de médula ósea, alfinjerto con bloque, injerto de hueso, ingeniería de tejidos
결과: 치료 4~8개월 후, 모든 이식물과 골절이 합쳐졌고, 골절의 54%와 46%가 각각 골과 조각으로 성장하였다. 골절 표면의 89%가 생합성으로 합쳐졌다.

요약: 골절과 이식물을 합침으로써 신생골이 형성되고, 골절의 54%와 46%가 각각 골과 조각으로 성장하였다. 골절 표면의 89%가 생합성으로 합쳐졌다.