Bone Tissue Engineering by Way of Allograft Revitalization: Mechanistic and Mechanical Investigations Using a Porcine Model

Christopher M. Runyan, MD, PhD, Samantha T. Ali, BS,† Wendy Chen, MD,‡ Bennet W. Calder, MD,§ Aaron E. Rumburg, BS,‖ David A. Billmire, MD,¶ and Jesse A. Taylor, MD#*

Purpose: "Allograft revitalization" is a process in which cadaveric bone is used to generate well-vascularized living bone. We had previously found that porcine allograft hemimandibles filled with autologous adipose-derived stem cells (ASCs) and recombinant human bone morphogenetic protein-2–soaked absorbable collagen sponge (rhBMP-2/ACS) were completely replaced by vascularized bone, provided the construct had been incubated within a periosteal envelope. The present study sought to deepen our understanding of allograft revitalization by investigating the individual contributions of ASCs and rhBMP-2 in the process and the mechanical properties of the revitalized allograft.

Materials and Methods: Porcine allograft hemimandible constructs were implanted bilaterally into rib periosteal envelopes in 8 pigs. To examine the contributions of ASCs and rhBMP-2, the following groups were assessed: group 1, periosteum alone; group 2, periosteum+ASCs; group 3, periosteum+rhBMP-2/ACS; and group 4, periosteum+ASCs+rhBMP-2/ACS. After 8 weeks, the allograft constructs were harvested for micro-computed tomography (CT) and histologic analyses and 3-point bending to assess the strength.

Results: On harvesting, the constructs receiving rhBMP-2/ACS had significantly greater bone shown by micro-CT than those receiving periosteum only (51,463 vs 34,310 mm^3; $P = .031$). The constructs receiving ASCs had increased bone compared to group 1 (periosteum only), although not significantly ($P = .087$). The combination of rhBMP-2/ACS with ASCs produced bone (50,399 mm^3) equivalent to that of the constructs containing rhBMP-2/ACS only. The 3-point bending tests showed no differences between the 4 groups and a nonimplanted allograft or native mandible ($P = .586$), suggesting the absence of decreased strength of the allograft bone when revitalized.

Conclusions: These data have shown that rhBMP-2/ACS significantly stimulates new bone formation by way of allograft revitalization and that the revitalized allograft has equivalent mechanical strength to native bone.

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Autologous bone grafting became the standard for mandible reconstruction after reports of successful application in wounded soldiers during World War I and II.[1,2] After World War II, oral cancer and odontogenic tumors became the predominant sources of critical mandibular defects. For smaller defects, cancellous bone grafts will

*Resident, Division of Plastic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

†Medical Student, Division of Plastic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

‡Resident, Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA.

§Resident, Department of Surgery, Medical University of South Carolina, Charleston, SC.

‖Medical Student, Division of Plastic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

¶Attending Surgeon, Division of Plastic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

#Attending Surgeon, Division of Plastic Surgery, Children's Hospital of Philadelphia, Philadelphia, PA.

Address correspondence and reprint requests to Dr Taylor: Perelman School of Medicine at the University of Pennsylvania, Division of Plastic Surgery, Children's Hospital of Philadelphia, 3501 Civic Center Boulevard, 9th Floor, Philadelphia, PA 19104; e-mail: Taylorj5@email.chop.edu

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often be sufficient. For larger segmental deficiencies, cortical bone grafts, including allogeneic cribs, have frequently been used to provide structural integrity during bone healing. Complex cases, including those with radiated beds, soft tissue deficiencies, and large anterior defects, have been particularly challenging to repair using bone grafting. Marx[3] has shown excellent results in these instances using hyperbaric oxygen therapy and soft tissue flaps as an adjuvant to bone graft-based mandible reconstruction. With the advent of microsurgery, vascularized bone flaps have largely supplanted the use of bone grafts for the reconstruction of large mandible defects.[4] Comparisons of the 2 approaches have shown that bone grafting results in a much greater failure rate, in particular, with larger defects (>10 cm) and requires more operations to achieve union.[5-7] By carrying their own blood supply, vascularized bone flaps will be resistant to radiation-related complications and more predictable than bone grafts for restoring function and form.

However, vascularized bone flaps are not without limitations. Fibula flaps have had a 30% complication rate, including wound healing difficulties, hardware exposure, poor shape match, and donor site morbidity, including leg weakness, decreased ankle mobility, gait disturbance, and great toe contracture.[8,9] An ideal reconstructive approach would provide a predictable outcome and minimize donor morbidity.

Bone tissue engineering (BTE) has the potential to produce an alternative to vascularized bone flaps for repair of critical bony defects.[10-13] BTE involves the use of osteogenic growth factors (eg, bone morphogenetic protein [BMP]-2), mesenchymal stem cells, and a scaffold to guide bone growth and provide support. Preclinical, large-animal studies have shown promise for healing critical defects using either bone marrow-derived stem cells (BMSCs)[14-16] or BMP-2,[17,18] or a combination of the 2.[19,20] The results from early clinical reports have been mixed. Herford and Boyne[21] reported bony union using a BMP-2–soaked collagen sponge to repair defects in 14 of 14 nonirradiated patients. Clokie and Sandor[22] found similar success in 10 of 10 patients using BMP-7 with demineralized bone matrix. However, others have reported high rates of BMP-containing graft failure due to infection and fracture.[10,23] One factor limiting the success of BTE-based approaches has been a lack of consensus on the best scaffold to use. Perioperative swelling with the use of BMPs will only worsen pocket collapse because of the lack of a rigid scaffold support.[23] However, tenting open a soft tissue pocket with mesh or plates will increase the risk of exposure and infection.

Mandible allografts have excellent potential as a scaffold for BTE. Structural allografts will have equivalent strength to native bone and can be selected to perfectly fit a defect, without donor morbidity. Numerous reports have been published of using auto- or allograft mandible as cribs filled with cancellous bone for the repair of large defects; however, these have frequently failed owing to a lack of incorporation.[24-28] Similar to others, we believed that were large allografts able to be revascularized, they would be the ideal scaffold for BTE.[29-31] We, therefore, conducted an experiment comparing a periosteal envelope and a vascularized muscle flap for revascularizing an allograft hemimandible crib construct in pigs.[32] All constructs were filled with recombinant human BMP-2/absorbable collagen sponge (rhBMP-2/ACS) and autologous adipose-derived stem cells (ASCs) as a mesenchymal stem cell source. The allografts that had incubated for 2 months within a periosteal envelope had been completely replaced by large amounts of well-vascularized bone. We concluded that large bony allografts could be an effective scaffold for BTE and that vascularized periosteum would be necessary in this process.

The purpose of the present study was to more fully understand this "allograft revitalization" process, preparatory to its clinical application. We sought, using our described porcine hemimandible allograft model, to determine whether ASCs and rhBMP-2 are necessary for the process, and whether the bone produced by allograft revitalization would be mechanically sound. We hypothesized that both ASCs and rhBMP-2 would contribute to allograft revitalization and that the bone produced would be mechanically equivalent to native bone.

Materials and Methods

ALLOGRAFT PREPARATION AND ANIMAL HUSBANDRY

Mandibles were harvested from 6-week-old Yorkshire/Hampshire pigs. The soft tissues were removed, the teeth extracted, and the mandibles bisected at the symphysis. The hemimandibles were then sent for allograft processing, including detergent and alcohol washes,[33] sterility validation, packaging, and freeze drying (Veterinary Transplant Services, Kent, WA). The processed hemimandibles were stored at −20°C until implantation. Eight additional female Yorkshire/Hampshire pigs were used as the allograft recipients. The pigs were housed in a standard 12-hour light/dark-cycle room, with twice daily swine chow and ad lib access to water. The pigs were fasted for at least 12 hours before all surgical procedures. The Cincinnati Children's Hospital Medical Center Institutional Animal Care and Use Committee approved and monitored all procedures (approval no. 1D03029).

LIPOSUCTION

At 6.5 weeks of age, select pigs (n = 4) were anesthetized with intramuscular ketamine (30 mg/kg) and intramuscular acepromazine (1.1 mg/kg) and