Autologous Adipose-Derived Stem Cells with Platelet-rich Plasma or Fibrin Enhance Healing of Porcine Mandibular Bone Defects

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Disclosures:

Introduction: Patients with critical-size bone defects generally have limited healing without clinical intervention. The autograft is the current standard of care for repair of these defects. However, potential limitations have motivated the development of alternative autologous approaches for the treatment of these defects. Materials used in tissue engineering, such as scaffolds, growth factors and adult stem cells, can be derived from patient blood and adipose tissue and are potential autologous therapeutic options. The objective of this study was to investigate a prospective procedure to improve craniofacial bone healing using fibrin scaffolds and platelet rich plasma (PRP) from patient blood, and adipose-derived stem cells (ASCs) from liposuction. These autologous scaffolds, growth factors and cells were evaluated on their ability to heal critical-size bone defects in a porcine animal model.

Methods: Cell Isolation
Twelve female Yorkshire pigs, aged 1-3 years, were given a sedative cocktail of Telazol, atropine, Rompun, and ketamine, and maintained with isoflurane gas in oxygen. For ASC extraction, liposuction was performed using standard protocols. Following sufficient skin sterilization, pinpoint incisions were made at 4-6 locations on either side of the dorsal midline using a trocar. Sterile 0.9% saline with epinephrine was injected into subcutaneous adipose deposition sites using a 60 mL syringe connected to a Cobra cannula (3 mm diameter, 25 cm length, Shippert Medical, Centennial, CO). A liposuction cannula (Triport, 4 mm diameter, 15 cm length, Shippert Medical) connected to a suction pump (75 kPa) was inserted to collect the lipoaspirate (300-400 mL total). Liposuction time was 60-75 minutes. Collected lipoaspirate was mixed on a shaker plate with an equal volume of type I collagenase (0.075%, Sigma C2674) for 30 minutes at 37°C to digest extracellular matrix. The mixture was centrifuged for 10 minutes at 1400 rpm (547 xg) to separate the higher-density stromal vascular cell fraction, which was then mixed with serum-free DMEM (Sigma D5648). Cells collected were split into two syringes, each having a volume of 5 mL. Processing times for isolation of ASCs was 120-150 minutes.

Blood Product Collection
For PRP, blood was collected from the ear vein using a 21 gauge butterfly needle with 3.3% sodium citrate anticoagulant. An initial centrifugation was performed at 1800 rpm (905 xg) for 15 minutes for removal of RBCs and collection of plasma. A subsequent spin of collected plasma at 3000 rpm (2510 xg) for 10 minutes separated platelets. A total volume of 2-3 mL of PRP was collected for injection (1-1.5 mL per defect). Before injection, PRP was combined with cell/DMEM mixture (20% PRP concentration) and 50 µL of 10% calcium chloride for platelet activation. For fibrin scaffolds, blood was collected from the ear vein in a 10 mL syringe previously filled with 1 mL of cell/DMEM mixture. Blood was drawn to fill syringe up to 4-5 mL total (3-4 mL blood), and then quickly injected into the defect while still in the liquid phase. Calcium hydrogen phosphate (0.1 mL of 0.3 M) was added and stirred in the defect to complete coagulation.

Surgical Procedures
The University of Illinois Institutional Animal Care and Use Committee approved all procedures. Under anesthesia, submandibular incisions were made down to the inferior border of the mandible. The periosteum was reflected to expose the mandibular cortex. One bicortical circular defect measuring 25 mm in diameter was created in the ramus of each hemi-mandible with a trephine bur using a surgical drill with continuous irrigation. Autologous cells and blood products were injected in defect sites, according to treatment allocation. Following injections, the periosteum, muscle, fat and skin were sutured. Pigs were maintained on a soft diet for 7-10 days post-surgery.

Micro-Computed Tomography and Statistical Analysis
At 8 weeks, mandibles were harvested and trimmed using a band saw, and scanned using microCT (Skyscan 1172, Kontich, Belgium). Scan settings included 95% camera gain, 0.4 degree rotation per scan, and frame averaging of 5. A reconstruction algorithm (NRecon 1.1.4, Micro Photonics, Allentown, PA) digitally stacked and aligned these 2-D slices, resulting in a 3-D model of the sample which was used to analyze the three-dimensional morphological parameters of the defect. Quantitative analysis was based on the different attenuations of bone and soft tissue. Each voxel (25 µm resolution), was assigned a gray scale value which was thresholded (Amira 5.0, Visualization Sciences, Burlington, MA).

A one-way ANOVA for the effect of treatment was performed, and Fisher's test was used for pairwise mean comparisons. Comparisons with p < 0.05 were considered statistically significant.
Results: Bone volume fraction, defined as the number of bone voxels divided by total voxels in the defect, increased with injection of ASCs (Figure 1). Bone volume fraction further improved when mixed with fibrin or PRP. During healing, immature woven bone gradually thickened, became denser, and healed from the periphery towards the center (Figure 2). For fibrin treatment, bone was observed throughout, including the center of the defect, likely due to encapsulation of viable cells.

Discussion: Addition of autologous ASCs appears to improve repair of mandibular bone defects. This population of mesenchymal stem cells may enhance bone formation through mechanisms of increased angiogenesis, regulation of inflammation and/or direct differentiation into osteogenic cell types. Fibrin and PRP may modulate these effects through cell encapsulation and increased cell proliferation, respectively. However, these autologous materials cannot bear significant loads; therefore, additional external fixation or a load-bearing scaffold would be required for clinical fractures. Taking into account these limitations, we conclude that addition of autologous ASCs and blood-derived growth factors and scaffolds may be advantageous for the repair of critical-sized bone defects.

Significance: This study details an animal study of a surgical method for collecting and administering autologous products from fat (adipose-derived stem cells) and blood (platelet-rich plasma and fibrin) for the improvement of healing experimental bone defects.

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