FUNCTIONAL TISSUE ENGINEERING OF PERIOSTEUM FOR STRUCTURAL BONE ALLOGRAFT

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Introduction:
The presence of live periosteal progenitor cells on the surface of bone autograft confers superior healing of autograft over devitalized allograft. We have previously demonstrated in a murine segmental femoral bone-grafting model that live periosteum produces robust endochondral and intramembranous bone formation essential for effective healing and neovascularization of large structural bone grafts. To the end of engineering a live pseudo-periosteum that could induce a similar response on devitalized bone allograft, we seeded the mesenchymal stem cells that were genetically engineered to produce human recombinant BMP-2 directly onto devitalized bone allografts. MicroCT, histology and mechanical testing were used to evaluate the healing and repair of bone allografts. Here we showed that the cellularized BMP-2 producing bone allograft healed in a similar fashion to live isograft, as evidenced by formation of bridging new bone callus, extensive neovascularization, bone graft resorption and a marked gain of biomechanical strength.

Materials and Methods:
Femoral bone grafting model: Bone grafting was performed in ten-week-old C57BL/6 mice as described previously (1). Briefly, a 4mm mid-diaphyseal segment was removed from the femur by osteotomy using a saw. A 4mm cortical bone graft was then inserted into the segmental defect and stabilized by a 22-gauge metal pin placed through intramedullary marrow cavity. The grafting procedures were performed between inbred C57BL/6 mice with identical genetic background (isograft), or mice with genetically different backgrounds (allograft). For live bone isograft transplantation, the graft was carefully dissected out of muscles, and immediately transplanted into the mice. For devitalized allograft transplantation, allografts were extensively washed, and fresh frozen at −70°C for at least 1 week prior to transplantation. To prepare cells for bone allograft, BMP2 producing mouse mesenchymal cells (C9) derived from C3H 10T½ were seeded onto bone allografts at a density of 1.5x10⁵ cells per graft and cultured for additional 1 hour at 37°C in DMEM before being used to repair the defects. The grafted femurs were processed for histological and micro-CT analyses at the end time point of the experiments. Biomechanical testing was performed on an EnduraTec TestBench™ system in torsion at a rate of 1°/sec to determine the stiffness, rigidity, ultimate torque, rotation and strain energy to failure.

Results:

![BMP-2 producing mouse mesenchymal cells](image1)

C9 cells were genetically engineered to produce rhBMP-2 under the control of Tet-off system. These cells were also stably transduced with β-galactosidase (2). When cultured in DMEM these cells could survive and grow on the bovine bone wafers or on the surface of mouse bone allografts for at least one week as determined by X-gal staining (Figure 1). C9 cell-coated allografts were used to repair the segmental defects created in femurs of C57/BL6 mice. Radiographic examination demonstrated a marked induction of bridging bone callus formation around bone allograft as early as day 14 which peaked at day 21, similar to the healing of the control live isografts. Volumetric analyses by MicroCT demonstrated that C9 coated allografts produced 3-fold more new bone around graft at 4 and 9 weeks (n=4) compared to allografts. (Figure 3 and 4A). Histological sections confirmed the presence of a bony callus completely bridging and surrounding the C9-coated allograft accompanied by marrow invasion and resorption of non-vital bone, resembling live isograft healing at 4 weeks (Figure 4A and 4C). At 9 weeks, live isografts were completely resorbed and replaced by new structural cortical bone, whereas in the C9-coated allografts the non-vital portions of the graft persisted (Fig 4D and 4F). Biomechanical testing was conducted at 9 weeks to determine the stiffness of the grafted femurs. Live autografts were used as control for healed bone grafting. As demonstrated in Figure 4, the stiffness, ultimate torque, and torsional rigidity of devitalized allografts were less than 10% of values for autografts (n=9, p<0.05) at 9 weeks. In contrast, C9 coated allografts displayed the same stiffness and ultimate torque as those of the autografts, indicating a marked improvement of allograft incorporation.

Discussion:
Structural allografts can restore the size and shape of the resected bone. However, the use of structural allografts is limited due to the lack of the cellular response that confers osteogenic and angiogenic properties on an autograft. In this study, we demonstrated that by seeding mesenchymal cells expressing recombinant human BMP2 (rhBMP-2) onto bone allograft surface, we could generate a response that resembles periosteal bone formation and subsequent restore the biomechanical strength of grafted femur to that of the healed autograft. The study further indicates that adult stem cell-based and gene enhanced tissue engineering may offer novel and exciting therapeutic approaches to augment bone allograft healing and repair.

References:
1. Tiapatanaputi, P et al; J Orthop Res. 22(6):1234-60, 2004

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