

In-Situ Crosslinkable Osteoinductive Poly(lactide) Scaffold for Bone Regeneration

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Background in scaffolds for bone regeneration: Current clinical methods of treating skeletal defects involve bone transplantation or the use of other materials to restore continuity¹. Autologous bone graft has been the gold standard of bone replacement because it provides such essential elements as osteogenic cells, osteoinductive factors, and an osteoconductive matrix for healing. However, the limited supply of autograft bone, donor site morbidity, and the long recovery time for segmental defects restrict its use in bone repair. Allograft bone, although available in abundant supply, has drawbacks that include reduced rates of graft incorporation compared to autograft bone. Furthermore, the long recovery time for segmental defects or partial recovery in the case of non-unions has prompted researchers to look for alternative bone grafts to accelerate the rate of fracture healing.

rhBMP-2 delivered in a biodegradable carrier has been demonstrated to accelerate the repair of bone defects². Currently the most appropriate carrier for rhBMP-2 delivery is not determined. Recent studies demonstrate that implants made from collagen/gelatin or PLGA sponge soaked in rhBMP-2 have faster rate of fusion compared to autologous iliac bone graft³. The drawback of collagen/gelatin sponge is soft tissue compression which prevents bone induction at standard rhBMP-2 doses. On the other hand, proteins can not be immobilized in PLGA sponge because the sponge is fabricated by casting from organic solvents. Since proteins such as rhBMP-2 have significant solubility in organic solvents, a large fraction of the protein is lost or deactivated. We propose a novel carrier based on stabilization of rhBMP-2 in biodegradable poly(lactide-ethylene oxide-fumarate) (PLEOF) hydrogel microspheres and embedding of the microspheres in the *in-situ* crosslinkable poly(lactide fumarate) (PLA) scaffolds.

Rational to use *in-situ* crosslinkable poly(lactide) scaffold: It is well established in preclinical or clinical studies that rhBMP-2 delivered in a biodegradable carrier accelerates the repair of bone defects. Implants made from PLGA sponge soaked in rhBMP-2 solution have faster rate of healing compared to autologous iliac bone graft. However, rhBMP-2 can not be immobilized directly in PLGA sponge because organic solvents which deactivate the protein are used in the fabrication of the sponge. Biodegradable *in-situ* crosslinkable hydrogels, due to their high water content, are ideal for immobilization of proteins⁴. A logical question is: Can injectable *in-situ* crosslinkable PLAF embedded with rhBMP-2 loaded hydrogel microspheres be used as an osteoinductive scaffold for repairing bone defects?

The hypotheses of this study include 1) that rhBMP-2 encapsulated in degradable PLEOF hydrogel microspheres retains its activity, 2) rhBMP-2 is released from the microspheres into the pore volume of the PLAF scaffold in therapeutic concentrations, and 3) the rhBMP-2 released from the scaffold promotes differentiation of BMS cells to osteoblasts and formation of mineralized matrix *in-vitro* and *in-vivo*.

Recently, in our laboratory, we have developed a novel method to encapsulate rhBMP-2 in hydrogel microspheres by emulsion crosslinking of a gel phase in mineral oil. The hydrogel microspheres are then lyophilized to a free-flowing dry powder. Since proteins do not have appreciable solubility in mineral oil, very high encapsulation efficiency can be obtained with this method. Subsequently, the dry hydrogel microspheres are embedded in a poly(lactide fumarate) (PLAF) based matrix, developed in our laboratory, to form biodegradable *in-situ* crosslinkable osteoinductive scaffolds for bone regeneration. The PLAF macromer is synthesized from poly(lactide) which is approved by FDA for certain clinical applications and fumaric acid (a substance that occurs naturally in the Krebs's cycle). We hypothesize that rhBMP-2 encapsulated in hydrogel microspheres retains its activity and is released in therapeutic concentrations to promote differentiation of bone marrow stromal (BMS) cells to osteoblast lineage and to accelerate the formation of mineralized tissue. rhBMP-2 is encapsulated in degradable poly(lactide-ethylene oxide-fumarate) hydrogel microspheres, embedded in PLAF scaffold, and its release kinetics is measured by enzyme-linked immunosorbent assay (ELISA). Porous PLAF scaffolds with well-defined pore geometry are seeded with bone marrow cells isolated from rats. The constructs are cultured *in-vitro* or implanted in segmental femur defects of rats to assess the extent of mineralization and bone formation. The *in-vitro* effect is evaluated using cell count, alkaline phosphatase activity, and mineralization. The *in-vivo* effect is evaluated radiographically and histologically.

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