Hybrid Tissue Engineering Constructs for Distraction Osteogenesis

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INTRODUCTION: Critical size bone defects are those which do not spontaneously heal without surgical intervention. One method for treating critical size defects in the long bone is distraction osteogenesis (DO) or the Ilizarov technique, which involves the use of external fixators to distract and lengthen a segment of bone to bridge the gap at the defect site (Fig. 1a). Although the current clinical application of DO can successfully treat large bone defects, there are significant complications with the procedure, such as prolonged consolidation, docking site nonunion, and pin track infection. In our hybrid tissue engineering approach, we aim to combine 3D printing and bioactive materials to create a Hybrid Tissue Engineering Construct (HyTEC) device to augment the existing DO procedure for improved bone healing and union.

METHODS: Scaffolds were designed using computer-aided design (CAD) and 3D printed using polycaprolactone-β-tricalcium phosphate (PCL-TCP) filament. Each scaffold was hydrophilized with alkaline solution and treated with gelatin methacrylate (GelMA) and calcium to enable the seamless coating of a dual-crosslinked, bone morphogenetic protein-2 (BMP-2)-laden hydrogel onto the polymer substrate (Fig. 1b). The microstructure of the construct was examined under SEM before and after electron beam (E-Beam) sterilization. The implants were evaluated in a large animal model (sheep metatarsus), and four groups were tested (Fig. 1g). Bone volume was quantified via micro-computed tomography (micro-CT), and the *in vitro* release kinetics of BMP-2 was characterized by measuring the protein concentrations (ELISA) and BMP-2 bioactivity (BRITER reporter cell luciferase activity) (Fig. 1h-k). All animal studies were approved by an ethics review board, and all constructs were E-Beam sterilized prior to surgical implantation.

RESULTS: SEM images showed a decrease in the density of interconnected micropores on the E-Beam sterilized surface compared to the control surface (Fig. 1e-f). Micro-CT analysis of the four groups showed a significant, nearly ten-fold increase in bone volume in the test group (DO+HyTEC scaffold) compared to the controls (Fig. 1g-h). The *in vitro* release kinetics of BMP-2 encapsulated in the HyTEC gel showed a sustained, controlled release over at least 7 weeks (Fig. 1i), with a close alignment between BMP-2 concentrations (ELISA) and the bioactive, transcribed BMP-2 reported by BRITER (Fig. 1k).

DISCUSSION: These results indicate that a controlled, sustained release of BMP-2 is crucial for bone healing and union. This sustained release is enabled by the dual crosslinking in the interpenetrating hydrogel network and by the reduced surface pore density after E-Beam irradiation. The BMP-2 released from the HyTEC implants remained bioactive for the duration of the study, and the significant improvement in bone formation with the BMP-2-laden HyTEC implant indicates its efficacy as an adjunctive therapy to the DO procedure.

SIGNIFICANCE/CLINICAL RELEVANCE: The promising *in vitro* and pilot large animal studies indicate that controlling the release rate of BMP-2 in combination with the DO surgical procedure can significantly improve bone healing compared to clinical controls. The FDA-approved biomaterials were terminally sterilized via E-Beam irradiation, increasing the clinical relevance of these results, as the methodology in this study can be carried forward in future clinical studies. Although this study has focused on using the HyTEC technique as an adjunctive therapy to DO, the concept of a pre-sterilized, growth factor-laden implant with a sustained, controlled release profile could also be applied to many other areas of orthopaedics.

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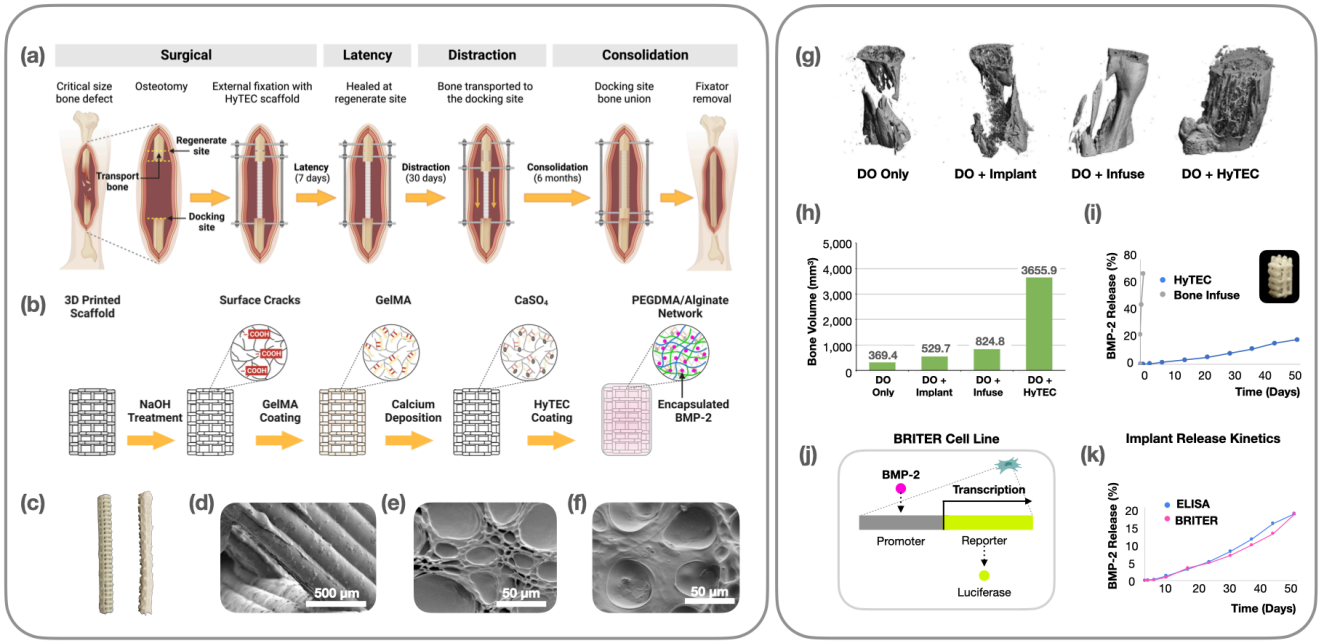

FIG. 1. (a) The scaffold-assisted adjunctive therapy to DO. **(b)** HyTEC surface treatment and coating protocol. **(c)** Coated graft before (left) and after (right) freeze-drying. **(d)** Scaffold surface after calcium deposition. **(e)** Interpenetrating porous hydrogel surface before E-Beam irradiation. **(f)** Reduced hydrogel porous density after E-Beam sterilization. **(g)** Micro-CT after 6-month consolidation. **(h)** Quantified bone volume. **(i)** Sustained BMP-2 release of HyTEC and burst release of the Infuse control group. **(j)** BRITER schematic and **(k)** comparison of bioactive BMP-2 (BRITER) with protein concentrations (ELISA).