INTRODUCTION: Scaffold-based tissue engineering strategies have emerged as promising alternatives to autograft, allograft, and prosthetics for treating musculoskeletal disease and injury [1]. Particularly for osteochondral (OC) tissue engineering, multilayer scaffolds aim to simultaneously repair both cartilage and bone. Our approach utilizes biomimetic hydrogels loaded with chondrogenic and osteogenic growth factors (e.g., TGF-β & BMPs) to promote cartilage and bone regeneration, respectively. However, hydrogels are typically softer (E = 10–100 kPa) than articular cartilage (E = 1–10 MPa) and subchondral bone (E = 20–3000 MPa). Thus a structural support is needed to protect hydrogels and encapsulated cells from excessive strains that lead to failure, lack of tissue-implant integration [2], damage to neighboring tissues [3], and to deliver appropriate biomechanical cues for tissue-specific regeneration. We utilize advanced 3D printing via a custom digital light processing (DLP) system to fabricate load-bearing (i.e., micro-truss) structures that recapitulate property gradients of tissues. We previously demonstrated a bi-layer scaffold infilled with biomimetic hydrogels that protected surrounding cartilage from degeneration in an ex vivo model [4]. Here, we sought to regenerate cartilage and bone tissue within appropriate regions of 3D printed composite bi-layer scaffolds infilled with biomimetic hydrogels within OC defects in a porcine model.

METHODS: Scaffold design and fabrication: The design and fabrication of the mechanically-graded composite bilayer scaffold were described previously [6]. In brief, a bilayer OC 3D printed structure was designed by combining a micro-truss structure for the cartilage region and a vertical pillar-based design for the stiffer bone region, with elastic modulus of E = 0.51 MPa and E = 6.5 MPa, respectively (Fig. 1.a). Structures were fabricated using a custom DLP printer and a poly(ethylene glycol) diacrylate-based resin [4,5]. Hydrogel: Infilled around the structure was a degradable poly(ethylene glycol) hydrogel [5]. TGF-β3 and BMP-2 were tethered to cartilage layer and bone layer hydrogels, respectively, to encourage chondrogenesis and osteogenesis.

In vivo Study Design: Four 6 mm diameter x 7 mm depth OC defects were made on the left femoral trochlear groove on 12-week-old Yorkshire pigs (n = 5) that ranged from 30-35 kg in weight. Treatments (Fig. 1.b), including empty defect (n = 3), osteochondral autograft transfer system (OATS) (n = 5), cartilage mimetic gel only (n = 4) and DLP 3D printed bi-layer scaffold with cartilage and bone mimetic hydrogel (n = 8) were performed at random sites on the trochlear groove. All animal use was approved by the IACUC at Massachusetts General Hospital. Imaging and micro-CT (μCT) analysis: Following euthanasia 6 weeks post-surgery, each defect site was harvested and imaged for bone microarchitecteure using μCT (SCANCO Medical AG) and subsequently processed for histology (H&E) (Fig. 1.e). μCT images were reconstructed in Dragonfly (2022.2) and evaluated for bone growth. 6 mm diameter x 4.5 mm depth region of interests (ROIs) represented the original bone region in the surgical defect were created for each group. OATS and hydrogel-only groups. Due to observed implant subsidence, the ROIs for the composite scaffold group were created at the location of cartilage and bone scaffold layers (Fig. 1.d). Bone volume fraction (BV/TV) was calculated after segmentation using Otus thresholding. Additionally, bone infiltration were measured at 25%, 50% and 75% z-plane of each ROI by averaging the distance of bone in-growth to the ROI boundary at x-, y-, z- directions (aligned to the scaffold pores and pillar spaces) (Fig. 2.a).

RESULTS: Structures were fabricated using DLP 3D-printing and the printing accuracy was established using μCT of dry and iodine-stained (for X-ray contrast) scaffolds [6]. Histology of the implant region (Fig. 1.e) following 6 weeks in vivo verified the location of each composite scaffold, with visible ‘pillars’ and new bone within the bone layer and fibrous cartilaginous tissue was observed in the cartilage layer of composite scaffolds. μCT images confirmed bone tissue surrounding the 3D printed pillars in the bone layer with minor intrusion into the cartilage layer (Fig. 2.a). Bone tissue was also present in the scaffold cartilage layer to locate within the subchondral bone. This finding may explain why bone was also present in the scaffold cartilage layer. However, the use of cartilage mimetic hydrogel was associated with lower bone penetration into the scaffolds. Overall, our bilayer composite scaffold showed promising results where soft tissue largely formed in the cartilage region while promoting bone growth in the bone region in an in vivo large animal model. Further investigations are needed to address the implant subsidence and improve strategies to regenerate hyaline-like cartilage in vivo.

SIGNIFICANCE/CLINICAL RELEVANCE: This study addresses a critical need in OC tissue engineering using hydrogels that are stiffened by 3D-printed micro-trusses to deliver region-specific mechanical properties to support the surrounding native tissue while regenerate both cartilage and bone tissue. If successful, this study may improve treatment outcomes for OC-regenerative treatments.


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Figure 1: a) Osteochondral 3D-printed structure, with regional stiffnesses; b) A schematic of four treatment groups of the in-vivo porcine model; c) Histology image of the scaffold treatment region show implant location, with visible ‘pillar’ features in bone region; d) ROIs of μCT μCT analysis.

Figure 2: μCT analysis of bone growth. a) A schematic of bone infiltration measurements into a bilayer composite scaffold; b) BV/TV calculation revealed bone forming in the osteochondral defect bone region of all treatments and in the cartilage layer of composite scaffolds; c) Bone infiltration was significantly higher in the bone layer of composite scaffolds.