Contrast-enhanced µCT Enables 3D Structural Evaluation of Tissue Engineered Cartilage

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INTRODUCTION: Damaged articular cartilage has poor self-healing capacity. The goal in tissue engineered cartilage development has been to mimic the unique structural and functional complexity of articular cartilage. Biochemical analysis is typically used to characterize extracellular matrix development, yet it mainly reveals the bulk tissue characteristics. Cationic iodine-based contrast agent CA4+, used in micro-computed tomography (µCT), has been shown to correlate with the amount of negatively charged glycosaminoglycans (GAGs), reflecting the proteoglycan distribution in articular cartilage[1] and tissue engineered chondrocyte and mesenchymal stem cell–collagen pellets[2]. Here, we use CA4+ and µCT to reveal the spatial GAG distribution of human chondrocyte-seeded hydrogel constructs that are reinforced with a gradient microfiber scaffold and mechanically stimulated during tissue culture.
METHODS: Microfibre mPCL scaffolds with three equally thick zones of 800 µm (superficial zone), 400 µm (transitional zone) and 200 µm (deep zone) fiber spacing were fabricated using a melt electrowriting device.[3] Human chondrocytes were isolated from articular cartilage explants obtained with informed consent and institutional ethical clearance from donors undergoing total knee arthroplasty surgery. Passage 1 chondrocytes (1×10^7 cells/mL) were encapsulated in GelMA/HAMA (10%/0.5% w/v) hydrogel solution and the constructs were crosslinked in a PTFE mold with biopsy-punched mPCL scaffolds. The cylindrical constructs (d = 5 mm) were first precultured at 37°C under free swelling conditions for 14 days, and then cultured for 28 days in a custom-designed biaxial mechanical stimulation bioreactor[4] where the samples were mechanically stimulated for 1 h per day with various compressive loading protocols (Figure 1A, not all protocols are presented here). In addition to compression, a sliding shear amplitude of 0.5 mm was applied. Four samples from each group were immersed for 16 hours in cationic contrast agent CA4+ (10 mg·I/mL, bath volume of 1 mL per sample). Samples were imaged with Scanco µCT40 scanner (Scanco Medical AG, imaging parameters in Figure 1B), and the reconstructed µCT images were analyzed using MATLAB (R2018b, The Mathworks Inc). The analyses were conducted in a cylindrical volume of interest (VOI, d = 1.2 mm, h = height of the sample). The bulk attenuation values were calculated as the mean attenuation in the VOI. The depth-wise profiles were obtained by calculating the mean attenuation at each depth normalized to the sample height. The groups were statistically compared using one-way ANOVA with Tukey's post hoc test ($p < 0.05$ considered a significant difference).
RESULTS: Compared to the day 1 group, the attenuation (Hounsfield unit, HU) and biochemically obtained GAG content were significantly ($p < 0.05$) higher in free swelling (FS) and increasing compression (IC) groups (Figure 1C). However, significant differences in attenuations or total GAG content were not found between the IC group and the FS group. In depth-wise comparison (Figure 1D), the attenuation in the increasing compression (IC) group on day 42 was significantly higher ($p < 0.05$) than the attenuation in the day 1 group at 0–77% and 95–100% of the total thickness. Additionally, the attenuation in the free swelling (FS) group on day 42 was significantly higher ($p < 0.05$) than the attenuation in the day 1 group at 14–17%, 21–22% and 54–65% of the total thickness. Most notably, on day 42, the IC group had significantly higher attenuation compared to the FS group at the superficial zone, 1–13% of the total thickness.
DISCUSSION: Contrast-enhanced µCT imaging was able to reveal structural differences within the constructs efficiently. Biochemically obtained GAG content of the samples exhibited a similar increase from day 1 to the end of the mechanical stimulation as the bulk attenuation values. However, the strength of the current method is the capability to show the depth-dependent differences between the zones. Unlike biochemical analysis and bulk attenuation values, the depth-dependent analysis showed higher attenuation values at the superficial layer in the increasing compression group, compared to the free swelling group. In the mechanically stimulated constructs, the attenuation was highest in the superficial zone. This is likely due to the gradient structure of the mPCL scaffold: the superficial zone being the softest and thus, experiencing the highest local strains and fluid flow velocities, has led to increased aggrecan biosynthesis[5].
SIGNIFICANCE/CLINICAL RELEVANCE: This is the first time contrast enhanced CT imaging is utilized to follow chondrocyte-seeded fiber-reinforced hydrogel construct development during tissue culture and under various loading conditions. This method enables monitoring the local and depth-dependent matrix accumulation in such constructs.

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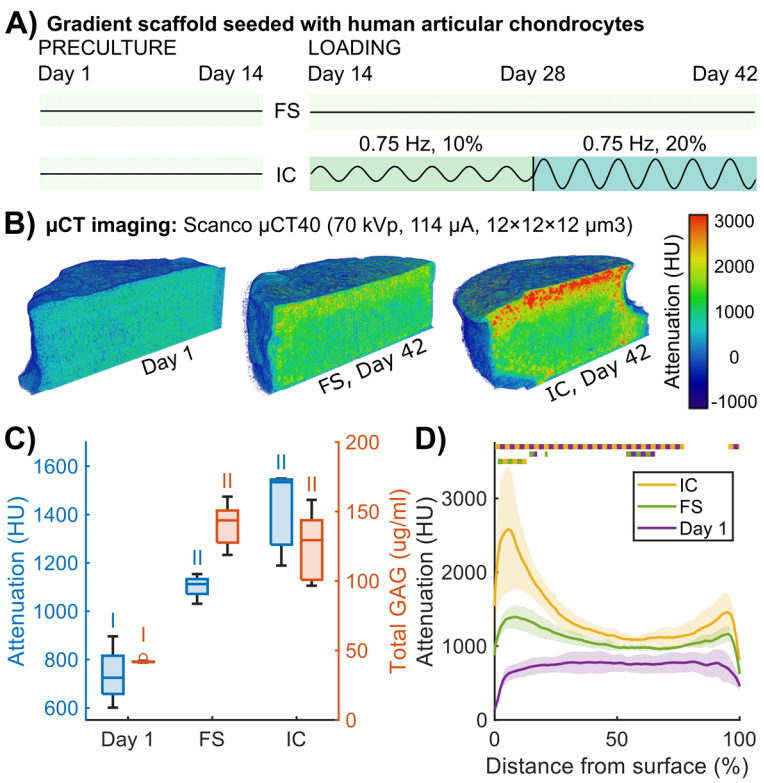

Figure 1. A) After 14-day free swelling conditions preculture, constructs were mechanically stimulated for 1 hour a day for 28 days at 0.75 Hz frequency with increasing compression (IC, from 10% to 20% compression after 14 days of loading). As a control group, free swelling (FS) group culture was continued for 28 days. **B)** The constructs were imaged with Scanco µCT40 after 16 hours incubation time in CA4+ contrast media. **C)** Boxplots of bulk attenuations and GAG contents for day 1 group and free swelling (FS) and increasing compression (IC) groups at day 42. Groups that do not share a Roman numeral are statistically different ($p < 0.05$, for attenuations: n = 3–4 per group, for GAG content: n = 6–11 per group). **D)** Depth-wise attenuation at day 1 and in free swelling (FS) and increasing compression (IC) groups. The line indicates the mean attenuation value of the group, and the shaded area the standard deviation. Bicolored lines at the top indicate statistical difference between the groups of corresponding colors at a certain depth ($p < 0.05$, n = 3–4 per group).