The Use of Micro-CT in 3D Printing and Tissue Engineering

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Aims
Sufficient long-term treatment of musculoskeletal defects remains an ongoing clinical challenge. Large mandibular defect repair remains technically intensive, while the gold standard autograft for non-union bone defects results in donor site morbidity among other issues. Finally, cartilage and osteochondral defects have limited ability for self-repair. Three-dimensional printing has emerged as an attractive technique in tissue engineering, as the high fidelity over spatial and material control allows for the development of complex tissue repair scaffolds. Here, we report the use of Micro-CT techniques in four different 3D printing experiments to evaluate new bone formation, characterize scaffold properties, observe material distribution within constructs, and demonstrate physical property relationships of printed scaffolds.

Method
In a sheep model, 3D-printed bioreactors were filled with either morcellized bone or commercially available ceramic materials and implanted against rib periosteum for 9 weeks while a 3D-printed porous space maintainer was implanted in a mandibular defect. At the end of the culture period, newly-formed bone was removed from bioreactors and implanted in place of the space maintainer for an additional 12 weeks. At the end of the \textit{in vivo} experiment, the reconstructed mandible was harvested and analyzed for clinical scoring, radiology, and histology. For radiographic examination, micro-CT was used to determine the ratio of bone volume to total volume (BV/TV), trabecular number (Tb.N.), trabecular spacing (Tb.Sp.), and trabecular thickness (Tb.Th.) for comparison between week 0 and week 9 (implantation against rib periosteum), and week 9 and week 21 (reconstruction of mandible).

In a series of 3D-printed scaffolds experiments, constructs of poly(\epsilon-caprolactone) (PCL), hydroxyapatite (HA), and \beta-tricalcium phosphate (\beta-TCP) were analyzed using micro-CT imaging to characterize scaffold architecture, including fiber size, fiber spacing, and total porosity, in order to evaluate scaffold fidelity to original design. In an \textit{in vitro} experiment, scaffolds with a ceramic composition gradient, an architecture gradient, or a dual composition-architecture gradient were cultured for 3, 14, or 28 days and evaluated via biochemical assays to determine the extent of osteogenic differentiation of seeded rabbit MSCs. Separately, scaffold architecture measurements were used to demonstrate physical property relationships in a uniaxial compression experiment. Scaffolds with a composition gradient, an architecture gradient, or a dual composition-architecture gradient were subjected to unconfined compression up to 20\% strain, and compressive moduli, yield stresses, and changes in architecture post-compression were evaluated. Finally, the distribution of ceramic materials within a polymer matrix were visualized. Specifically, scaffolds with radial gradients in composition and architecture were developed using a novel segmented fiber printing technique. In addition to architectural analysis and compressive property determination, selective thresholding was using to visualize spatial distribution of ceramic particles.
Results

Figure 1. Micro-CT measured parameters taken at the superior border of the mandibulocortex of the hemi-mandibles for autograft scaffold before implantation (Week 0), after nine weeks of implantation against the periosteum (Week 9), and after 12 week reconstruction with bioreactor generated tissues (Week 21), as well as the native mandibulocortex in the contralateral mandible (Native). Error bars represent standard deviation. Groups which do not share the same letter are statistically significantly different. Tissues generated within the bioreactors and then transferred to the mandible continued to remodel and evolve. Comparison of Week 21 and Native groups demonstrates that repair of the mandibles with bioreactor-generated tissue resulted in similar bone volume/total volume and trabecular spacing, but had not yet reached native levels of trabecular number and trabecular thickness. Adapted from Tatara, et al. PNAS (2019).
Figure 2. (Top left) Architectural, compositional, and dual-gradient representative schematics for use in micro-CT characterization and later in vitro osteogenic differentiation. (Top right) Average fiber diameters evaluated using micro-CT were consistently within a ±15% quality control window of the original CAD model across all scaffolds. (Bottom) Total porosity of top, middle, and bottom thirds of uniform, gradient, and dual gradient scaffolds was evaluated using micro-CT. Groups not sharing the same letter are significantly different (n=3, p < 0.05). All scaffold sections of the same fiber spacing (e.g. pink bars in all groups, orange bars in groups PoreG, TCP10PoreG, TCP0PoreG) had statistically similar porosities. Adapted from Smith, et al. Tissue Engineering Part A (2020).
Figure 3. (Top left) Representative micro-CT images of uniform porosity scaffolds of different compositions and fiber spacings (PCL-only/0.2mm, 15% HA/0.5mm, 30% HA/0.9mm). (Top right) Compressive moduli for uniform and gradient porosity scaffolds organized by fiber spacing. Scaffolds with gradient porosity demonstrated similar compressive moduli to medium and large fiber spacing scaffolds. (Bottom) Compressive moduli for uniform porosity scaffolds on a continuous basis on log-log scale. The compressive modulus-porosity relationship for these scaffolds was readily modeled and consistent with prior theoretical literature for porous polymer constructs. Adapted from Bittner et al. Acta Biomaterialia (2019).
Conclusion

Micro-CT is a powerful tool for use in 3D printing and bone tissue engineering applications. Radiographic imaging of reconstructed mandible explants allowed for analysis and evaluation of newly formed bone relative to native mandibular tissue. Additionally, architectural analysis of printed scaffolds allowed for quality control of constructs relative to the original design and the demonstration of mechanical property relationships after compression testing. Finally, micro-CT imaging enabled the visualization of ceramic distribution within a radial gradient scaffold as well as architectural analysis within each section independently. These techniques enable researchers to design and fully characterize constructs for use in tissue engineering and later evaluate the formation of new tissues.

References: