

Engineering Anatomically Shaped Vascularized Bone Grafts with Adipose-Derived Stem Cells and 3D-Printed Polycaprolactone Scaffolds

Joshua P. Temple, Daphne L. Hutton, Ben P. Hung, Pinar Yilgor Huri, Colin Cook, Renu Kondragunta, Xiaofeng Jia, Warren L. Grayson
Johns Hopkins University

Statement of Purpose: The treatment of large craniomaxillofacial bone defects is clinically challenging due to the limited availability of transplantable autologous bone grafts and the complex geometry of the bones. The ability to regenerate new bone tissues that replicate the patient's anatomy would revolutionize treatment options. Advances in the field of bone tissue engineering offer new treatment alternatives using biocompatible scaffold materials and autologous cells. Polycaprolactone (PCL) has emerged as a favorable polymer for scaffold fabrication, as it is biocompatible and biodegradable. With extrusion-based 3D printing, PCL can be melted and extruded to construct scaffolds. Human adipose-derived stem cells (hASCs) are a promising cell source to use in tandem with these scaffolds because of their ability to differentiate down both vasculogenic and osteogenic lineages. We demonstrate the potential to engineer porous, 3D-printed PCL scaffolds with appropriate porosities in the shape of human mandibular and maxillary bones.

Methods: We printed PCL lattices using different temperatures and print speeds and used a custom cross-correlation image analysis technique to select the parameters that yielded the most high-quality prints. Using scanning electron microscopy (SEM), we measured the pores within the scaffold and derived a relationship between specified infill density and measured pore size. hASCs were isolated from lipoaspirate tissue and expanded before aggregating via suspension culture. Aggregates were seeded in fibrin gels into cylindrical scaffolds of infill densities ranging from 20 to 80%. Scaffolds were cultured under either vasculogenic or osteogenic conditions for 14 days. A number of these scaffolds were subcutaneously implanted in rats along with acellular control scaffolds. Samples were stained with hematoxylin and eosin to assess general tissue growth. Osteogenic samples were stained with von Kossa and van Gieson to assess mineral deposition and vasculogenic samples were immunostained for vascular markers. We printed two full-scale anatomical scaffolds, one of the maxilla and one of the mandible, using 3D models generated from CT.

Results: We found that the scaffold quality was highest at the lowest melt temperature, 70 °C. Semi-quantitative analysis of SEM data revealed an exponential relationship between specified infill density and pore size. Though cells were seeded into all scaffolds, fluorescent images of DAPI stains revealed that at lower infill densities (20%, 30%) larger cell-aggregates settled to the bottom of the scaffolds, while at 50% the larger aggregates did not adequately penetrate into the scaffold. The most uniform seeding was achieved using the scaffolds with 40% infill density. After in vitro culture in vasculogenic media, ASCs formed extensive vascular networks throughout the

fibrin-filled pore spaces of the scaffold. Scaffolds cultured in osteogenic media demonstrated dense mineral deposits within the pore spaces of the scaffold, with additional mineral lining the surfaces of PCL fibers.

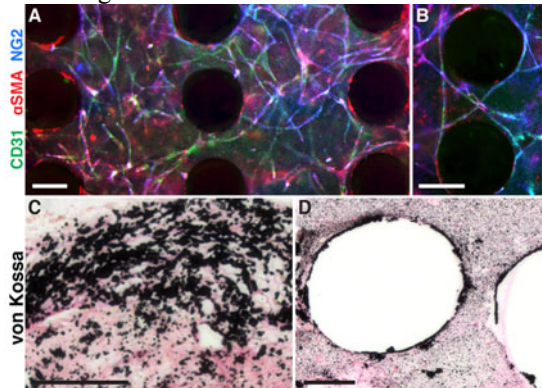


Figure 1. Top: Extensive vascular networks within the scaffold pores. Dark circles indicate PCL fibers. Bottom: Black dots represent mineral deposition throughout the pores and along scaffold. White circles are PCL fibers. Histology from the in vivo samples revealed that pre-vascularized scaffolds contained more lumen containing, pericyte stabilized vessels than those that were uncultured or implanted without cells. The final anatomically shaped scaffolds closely resembled the 3D models from which they were printed. All gross anatomical features were replicated in the scaffolds, with consistent, regular pores maintained throughout.

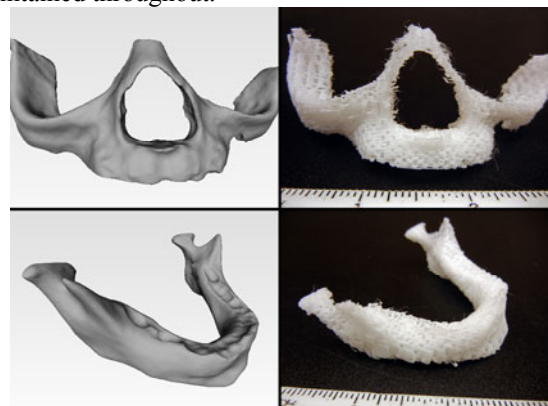


Figure 2. Left: CT-isolated 3D models of maxilla (top) and mandible (bottom). Right: Printed PCL scaffolds.

Conclusions: The findings of this study illustrate the promise of utilizing 3D printed scaffolds to engineer autologous, anatomically shaped, vascularized bone grafts. Our results indicate that pre-seeding cells within scaffolds might provide a benefit to tissue formation within the graft. Future studies will seek to assess the 3D correlation between the 3D models and printed scaffolds via micro-CT and evaluate the potential of anatomically shaped scaffolds for vascularization in vivo, specifically in orthotopic animal models.