Biofabrication of Tissue Constructs for Craniomaxillofacial Reconstruction

Carlos Kengla[1,2], Young-Joon Seol[1], Hyun-Wook Kang[1], , James J. Yoo[1,2], Anthony Atala[1,2] , and Sang Jin Lee[1,2]
[1]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston Salem, NC, USA, [2]School of Biomedical Engineering and Sciences, Wake Forest University, Winston Salem, NC, USA

Statement of Purpose: The face is composed of a complicated underlying bony/cartilaginous framework that supports muscle, secretory organs, and skin/subcutaneous structures. In the restoration of large defects, autologous techniques (i.e. locoregional and free flaps) are limited in terms of the total tissue coverage they can provide and are associated with donor-site morbidity. These techniques may fail due to unforeseen complications, necessitating future reconstruction. Hence, recent advances in craniofacial surgery and immunotherapy prompted the innovation of composite tissue allotransplantation (CTA), which permits reconstruction with tissue composed of all necessary components. However, CTA is also limited, as it requires lifelong immunosuppression. To overcome these limitations, regenerative medicine strategies have been developed and now provide hope for regenerating missing tissue components while avoiding the need for immunosuppression. We developed a new system based on computer-aided design/computer-aided manufacturing (CAD/CAM) and rapid prototype construction. This system can be manufactured as an “off-the-shelf” product that is intended to enhance the ability of the patient's cells to create tissue components, including bone and cartilage. Taken together, the evidence suggests that the generation of improved "off-the-shelf" bioprinted tissue constructs will lead to successful development of bioengineered complex tissue components. In this study we fabricated clinically applicable bioengineered tissues, including bone and cartilage, using our novel 3-D bioprinting system, which simultaneously delivers a structural biodegradable polymer and a tissue-specific cell-laden hydrogel.

Materials and Methods: Auricular chondrocytes used for printing human ear structures were isolated from New Zealand White rabbits (male, 2.5 - 3.5 kg, Charles River Labs., Inc.) by digesting with collagenase type I. The isolated chondrocytes were cultured in DMEM/F-12 mixture with 10% FBS, 1% penicillin/streptomycin, and 0.25 µg/mL amphotericin B. The printed 3D ear constructs were cultured in DMEM/F-12 with 10 ng/mL transforming growth factor β3 (TGF-β3) (ProSpec-Tany TechnoGene Ltd.), 50 µg/ml L-ascorbic acid 2-phosphate (Sigma), 100 nM dexamethasone (Sigma), 50 mg/mL ITS premix (BD, Franklin Lakes), 1% penicillin/streptomycin, and 0.25 µg/mL amphotericin B.

Human amniotic fluid-derived stem cells (AFSCs) were used for printing the mandible bone structures. Human AFSCs (H1 cell line) were cultured in α-Minimum Essential Medium (α-MEM) supplemented with 15% ES-FBS, 18% Chang B (Irvine Scientific), 2% Chang C (Irvine Scientific), 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-glutamine. For osteogenic differentiation, the 3-D architecture of the mandible bone structure was cultured in low glucose DMEM medium, 10% FBS, and 1% penicillin/streptomycin with osteogenic supplements [100 nM dexamethasone (Sigma), 10 mM β-glycerol phosphate (Sigma), 50 µM ascorbic acid 2-phosphate (Sigma)].

Results: The printed ear-shaped constructs showed 91 ± 8% cell viability at 1 day after printing (n=3). After 5 weeks in the culture medium, the histological staining of the tissue constructs showed the production of a new cartilaginous matrix as confirmed by Safranin O staining. The cells in the newly formed tissue demonstrated similar morphological characteristics to those in native ear cartilage, with cells located within typical chondrocyte lacunae, surrounded by a cartilaginous matrix

Human AFSCs were mixed with the composite hydrogel along with PCL and printed on a Pluronic F-127 temporal structure. At 1 day of culture, 91 ± 2% of cell viability within the printed bone structure was measured (n = 3). After osteogenic differentiation for 28 days (n=5), Alizarin Red S staining indicated calcium deposition on the 3-D bone structure. However, the 3-D constructs without differentiation failed to show any calcium deposition when stained with Alizarin Red S.

Conclusions: 3-D bioprinting system can generate 3-D freeform shapes with multiple types of cells and biomaterials, resulting in various architectures that have the potential to replace human tissues or organs. We demonstrate that the bioprinting can simultaneously deliver a supporting polymeric template and 3-D patterned deposition of cell-laden hydrogels in a precise manner. The printed tissue constructs are able to organize into mature tissues of their specific characteristics *in vitro* and *in vivo*.

Acknowledgments: This work was supported by the Armed Forces Institute of Regenerative Medicine (W81XWH-08-2-0032).

Disclosures: Nothing to disclose