Generation of Heterogeneous Three-Dimensional Cellular Constructs using Ink Jet Technology

Tao Xu, Weixin Zhao, Anthony Atala, James Yoo. Wake Forest Institute for Regenerative Medicine
Wake Forest University School of Medicine, Winston Salem, NC 27157

Introduction

Living tissues maintain an inherent multi-cellular heterogeneous structure. Rebuilding of such complex structure requires subtle arrangements of different types of cells and extracellular matrices (ECM) at their specific anatomical target sites. To achieve tissue reconstitution, an effective method for a precise delivery of cells and biomaterials is needed. The inkjet printing technology has been applied to address this endeavor. Although the capability of inkjet printing of viable single cells has been verified, the possibility of simultaneously printing multiple cell types to build viable heterogeneous cellular constructs has not been demonstrated to date. We hypothesize that distinct cell types can be mixed with collagen gels and printed into the target areas to form a 3-dimensional tissue structures. Further, basic physiological functions and properties of each cell type within the structure can be maintained.

Materials and Methods

Three distinct cell types were used in this study: human amniotic fluid-derived stem cells (hAFSC) transfected with lacZ, bladder smooth muscle cells (BSMC), and GFP labeled MS1 (mouse pancreatic islet endothelial cell line). Each cell type was grown separately, trypsinized, collected and resuspended in Type I collagen solution. Different mixtures of collagen and cells were loaded into different ink cartridges. Each cell-collagen mixture was printed layer-by-layer into the pre-designed target locations using a modified HP 550 printer. A solution containing NaOH was subsequently printed in order to neutralize the pH. The printed constructs were placed in the incubator for 3-5 hours. Once the collagen gel was set, 3-D viable multi-cellular constructs with a specific shape were formed. After 2 days of culture, the printed multi-cellular constructs were fixed and characterized using cell specific markers (α-actin, X-gal).

To examine the function of each cell type within the printed constructs, hAFSC cells were induced to differentiate into osteogenic lineage followed by evaluation of calcium production using Alizarin red staining. Smooth muscle cell function was assessed by measuring the resting membrane potentials and K^+ currents using a patch clamp system (Axopatch 200B).

Results and Discussion

Fabrication of multi-cellular structures. All three printed cell types were confirmed by their corresponding cell identification methods, as shown in Figure 1. The GFP labeled MS1 cells exhibited green fluorescence and smooth muscle cells emitted red under UV. The X-gal staining confirmed the lacZ transfected hAFSC cells in blue under bright field microscopy. All three cell types were present in an organized fashion within the printed construct. A 3-D collagen "pie" with different color dyes was shown in Figure 1E, demonstrating the capability of the inkjet printers to print different biomaterials as well as multiple cell types.

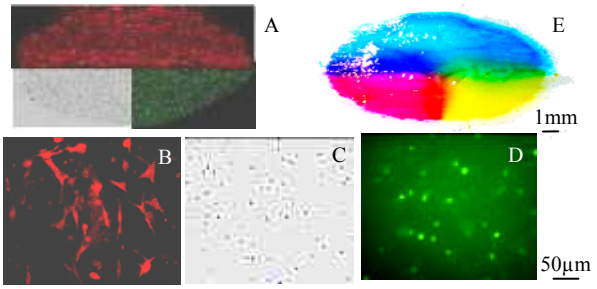
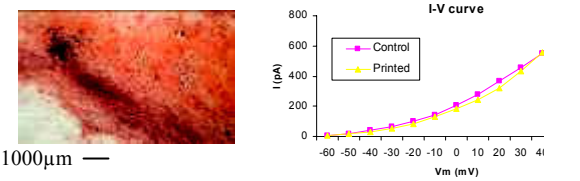


Figure 1. (a) Multi-cellular "pie" structure. Morphologies of 3 distinct cell types in the "pie" structure: (b) SMC, (c) hAFSC, and (d) MS1. (e) Printed collagen scaffold with different color dyes.

Functional evaluation. Alizarin red staining showed the production of calcium in the osteogenic differentiation culture of hAFSC (Figure 2a), which suggests that hAFSC in the collagen constructs retain their capability to differentiate into specific cell lineages under appropriate conditions. The whole cell patch clamp recording showed the average resting membrane potential of the printed BSMC (-58.5±5.8mV), which is similar to normal non-printed smooth muscle cells (-54.7±7.5mV). There was no significant difference on the K^+ I-V relationship between the printed cells and the normal controls (Figure 2b). These findings demonstrate that smooth muscle cells in the printed collagen constructs maintained their normal basic electrophysiological properties.



Fgure 2. (a) Alizarin red staining of printed hAFSC within the 3-D collagen constructs after 25 days of culture. (b) I-V relationship of BSMC printed in the collagen constructs.

Conclusions

This study shows that viable three-dimensional heterogeneous constructs with multiple cell types can be generated by printing multiple cells and collagen gels layer-by-layer. These distinct cells are able to survive and proliferate within the 3-D constructs, and maintain normal basic cellular properties and function in their spatially registered regions. These findings demonstrate the possibility of building complex tissues that require multiple cell types and ECM materials by using the bio-printing technology.