3D Bioprinting Biomimetic Blood Vessels

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Introduction

Blood vessel repair and regeneration represent a significant clinical challenge worldwide, with the issue being primarily complicated by the inherent complexities of vascular tissue structure. Currently, the standard surgical procedures for treating blood vessel injuries or defects are autologous and allogenic vascular graft transplantations. However, successful applications of these techniques for vascular tissue repair are often impeded by insufficient donor sources. In recent years, 3D bioprinting technologies have shown great promise for the fabrication of complex and customizable artificial tissue matrices. The primary objective of the study was to create novel biomimetic blood vessels with three distinct cell layers using vascular endothelial cells (VECs), smooth muscle cells (SMCs), and mesenchymal stem cells (MSCs), by means of an advanced co-axial bioplotter platform. The printing materials used in our study were composed of gelatin methacrylate (GelMA), alginate (Alg), alginate lyase, and the photoinitiator (I2959). An SMC-laden GelMA-Alg matrix was used as a supportive medial layer, while the VECs and MSCs were seeded respectively into the inner and outer layers of the vessel-like matrix. The alginate lyase was used to gradually degrade the alginate of the construct in order to provide space for SMC stretching and proliferation.

Overview

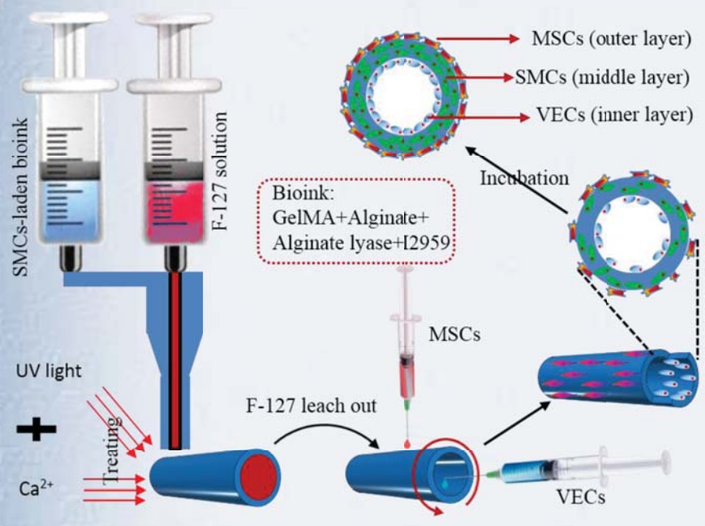

Figure 1. Schematic diagram of 3D bioprinted biomimetic blood vessels

GelMA was synthesized as a photocurable derivative of gelatin. 1% aginate was blended with 10% GelMA as the main components (GelMA-Alg) of our bioink, and was supplemented with 1% photoinitiator I2959 and 0.02% alginate lyase. Subsequently, SMCs were encapsulated into the GelMA-Alg bioink to generate the medial layer of our matrix, and a Pluronic F127 solution was used as a sacrificial material. The blood vessels were fabricated using a coaxial bioplotting platform and were cross-linked with both Ca^{2+} ions and UV light. The F127 was leached out of the printed construct, leaving a hollow lumen after placing the matrix in 4 ^{o}C for 20 mins. VECs and MSCs were then seeded into the lumen and on the surface of the vessels to form the inner and outer layers of the biomimetic blood vessels.

Results

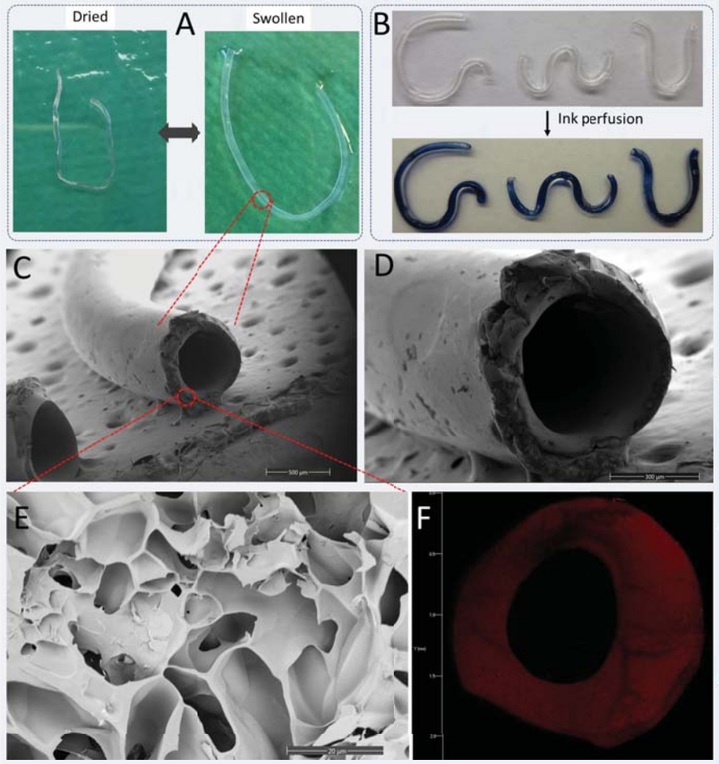

Figure 2. (A) Dried and swollen blood vessels. (B) Vessel with blue ink perfusion. Scanning electron microscopy (SEM) images of the vessel at (C) low magnification, (D) high magnification, and (E) vessel wall. (F) Fluorescent image of the biomimetic blood vessel in cross-sectional view. The vessel was stained with Texas Red-X phalloidin.

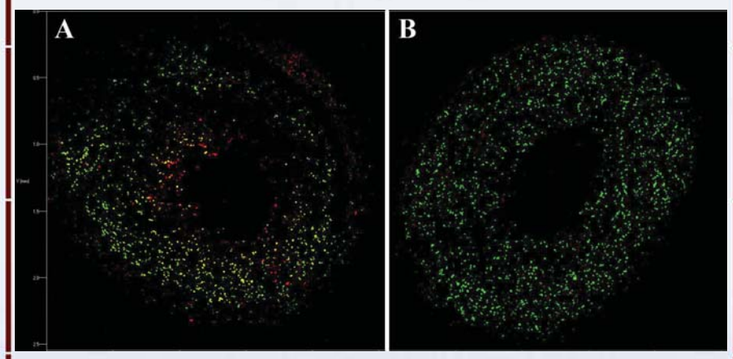

Figure 3. Confocal micrographs of SMC laden biomimetic blood vessels after (A) 1 day and (B) 7 days. The live and dead cells were stained by live-dye (green) and propidium iodide (red), respectively.

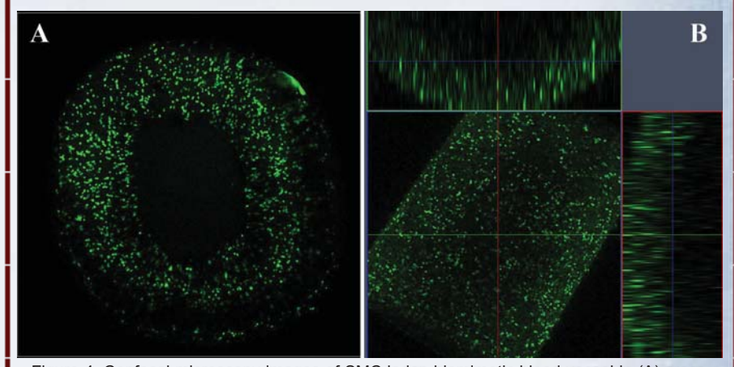

Figure 4. Confocal microscopy images of SMC laden biomimetic blood vessel in (A) cross-section and (B) side view. The cells were marked by CellTracker™ Orange CMTMR dye (green).

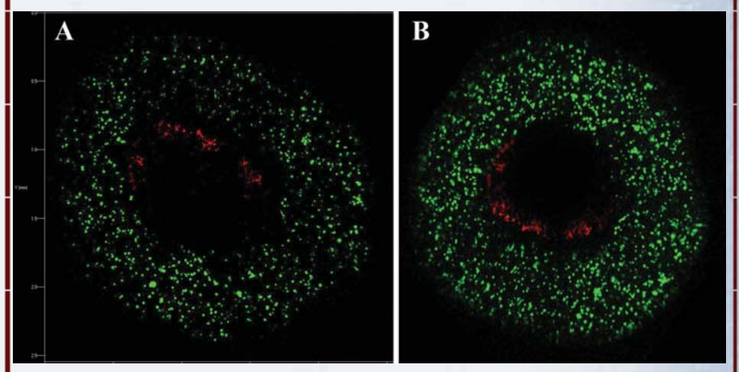

Figure 5. Confocal microscopy images of the bioprinted blood vessel with SMCs and VECs seeded in lumen after (A) 1 day and (B) 7 days. The SMCs and VECs were stained with CellTracker™ green CMFDA dye (green) and red CMTPX dye (red), respectively.

Conclusion

The lumen diameter of the 3D bioprinted blood vessel was approximately 500-600 μm and the thickness of the wall was ~400 μm. SMCs encapsulated in GelMA-Alg with alginate lyase grew fast in the vessel wall. This observation could likely be explained by the net generation of space yielded by the gradual degradation of alginate in the construct, which allowed SMCs to grow and proliferate more freely. Additionally, VECs also grew faster in the lumen of vessel with time and formed two distinct cell layers. The study was further expanded through the seeding of MSCs on the surface of the biomimetic blood vessel. It shows that our 3D bioprinting platform can be used to create small diameter blood vessels with biomimetic, three cell layer structures. We believe that the bioprinted vasculature produced in this study shows considerable promise for future application in both blood vessel repair and therapeutic discovery.

Acknowledgments

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