Optimization of cell deposition and cellulose nanofiber/alginate bioinks to improve cell survival and proliferation in cell-free 3D-bioprinting

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Abstract

Background: Cell-laden printing is commonly used in 3D-bioprinting. However, the extrusion pressure during the printing causes cell damage and death. To overcome this drawback, cell-free 3D-printing, in which cells are loaded after printing, has been developed. And efficient and homogeneous cell loading is essential in cell-free printing. Our objectives were to improve cell loading and optimize bioink with extracellular matrix (ECM), hyaluronic acid (HA), and collagens for cell-free 3D-printing.

Methods: We prepared basic bioink by mixing cellulose nanofiber and alginate (NFA) at the ratios of 20:10 (NFA20/10) and 20:02 (NFA20/02). The bioink was optimized by adding ECM, HA and collagens namely, NFA20/02-ECM, NFA20/02-HA, NFA20/02-COL-I/III, and NFA20/02-COL-II, and then was used to print scaffolds with dual-porous (DP) and Inherent-porous (IP) designs. hFOB cells were loaded to the scaffolds with self-absorbent (SA) deposition. Cell survival and proliferation were assessed by alamarBlue assay.

Results: NFA bioink showed excellent printability and shape fidelity. Loading cells with SA deposition achieved homogenous cell distribution in scaffolds. Scaffolds printed with NFA20/02 had better cell viability than NFA20/10. Cell survival and proliferation were greatly improved by adding ECM, HA, and collagens to the bioink. The alamarBlue assay indicated that many cells reside in the scaffolds after 30-days of culture. We found DP scaffolds supported cell proliferation better than IP scaffolds, and NFA20/02-COL-II was optimal for cell viability.

Conclusion: NFA20/02-COL-II is an excellent bioink for cell-free 3D-bioprinting. SA deposition can efficiently and homogenously load cells to 3D-printed scaffolds.