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PROCEEDINGS

Cryogenic 3D Printing: A New Approach to Produce Hard Polyester-Based Tissue Engineering Scaffolds with In Situ Dual Delivery of Growth Factors and Cells

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ABSTRACT

Please Creating mechanically robust tissue engineering scaffolds capable of delivering growth factors and stem cells in situ for hard tissue repair remains a significant challenge. Inspired by the spiral structure of ice cream, our group developed an advanced 3D printing technique known as cryogenic 3D printing to fabricate polyester-based scaffolds with embedded growth factors. This method utilizes water-in-oil (w/o) polyester emulsions containing growth factors as the printing ink, which is patterned onto a cryogenic substrate. The resulting scaffolds feature a hierarchically porous structure, allowing mesenchymal stem cells (MSCs) to easily attach and proliferate. Additionally, the biological activity of the growth factors is well-preserved throughout the printing process, promoting efficient MSC differentiation. To further enhance the scaffold's properties, a collagen hydrogel containing an angiogenic peptide can be coated onto cryogenic 3D printed bone scaffolds loaded with osteogenic growth factors. This modification endows the scaffolds with both osteoinductive and angiogenic properties, improving bone formation and vascularization. Beyond the in situ delivery of growth factors, incorporating MSCs directly into the scaffolds is highly desirable, as conventional post-cell seeding strategies often lead to low cell seeding density and uneven distribution. To address this, a hybrid cryogenic 3D printing approach was developed. This method involves the alternating deposition of polyester emulsion ink and MSC-laden GelMA/gelatin hydrogel bioink onto the cryogenic substrate, resulting in scaffolds that simultaneously deliver growth factors and MSCs. The GelMA/gelatin hydrogel acts as a protective barrier, shielding MSCs from the toxic effects of organic solvents present in adjacent polyester struts until the solvents evaporate. Furthermore, the dissolution of gelatin within the hydrogel enables the rapid release of MSCs, facilitating their migration and integration into the surrounding polyester structures. By combining this hybrid cryogenic 3D printing strategy with sequential multi-material 3D printing, bi-phasic MSC-laden osteochondral scaffolds with a heterogeneous structure can be generated. When different material matrices are employed to construct zonal microenvironments, MSCs adopt distinct cellular organizational structures in each zone, forming MSC microspheres in the cartilage region and fusiform MSCs in the subchondral zone. Additionally, the spatial delivery of TGF-β1 and osteogenic peptides independently enhances the chondrogenic and osteogenic differentiation of MSCs within their respective zones.

KEYWORDS

Cryogenic 3D printing; polyester; in situ loading; growth factor; stem cells

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