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DEVELOPMENT OF 3D INJECTABLE SCAFFOLDS FOR MSC-BASED TISSUE REGENERATION AND IMMUNOMODULATION

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Introduction

Immunomodulation represents a powerful approach that ensures fine-tuned regulation of the immune system and of the inflammatory response. Immunomodulators are crucial to promote tissue repair, minimizing undesired risks. Thanks to the immunomodulation, pro-regenerative, anti-inflammatory, antifibrotic potential of Mesenchymal Stem Cells (MSCs) and their derived secretome, MSCs-based strategies are emerging therapeutic approaches. Currently employed scaffolds for MSCs *in vivo* culture improve cell survival and retention, outperforming cell injections [1]. Our goal is to develop a microscopic scaffold for MSCs delivery, capable of preserving stemness, allowing *in vivo* long-term maintenance of MSCs. This device is designed to enhance tissue regeneration, while addressing the key advantage of injectability, limitation in current systems.

Materials and Methods

We designed the microscaffold structure, using Autodesk Inventor, as a 3D lattice architecture with three concentric interconnected layers of a spherical-like polyhedron. We fabricated the microscaffolds *via* two-photon polymerization (2PP) of SZ2080, a hybrid organic/inorganic sol-gel biocompatible photoresist [2]. Employing a custom-made pulsed laser source ($\lambda = 1030$ nm, rep. rate 1 MHz), we produced structures anchored to a glass coverslip, for preliminary tests. Subsequently, we fabricated the scaffold floating configuration to allow its injectability. We carried out a computational simulation using SIMULIA Abaqus FEA to assess the scaffold's mechanical stability under compression. We used a tetrahedral element mesh (554586 elements of 0.12 minimum size), fixed the bottom surface with an encastre constraint and applied increasing pressure on the top surface, to assess structural stress and displacement. We performed a preliminary biological validation of the scaffolds with the NIH-3T3 cell line, due to their similarity to MSCs in terms of size and morphology. After establishing optimized protocols for scaffold manipulation, we evaluated device colonization and performed nuclear (Hoechst33342) and cytoskeletal (SiR-Actin) staining to observe cell morphology and spatial organization by confocal microscopy.

Results and Discussion

We obtained highly porous microscaffolds (97.97% porosity) with graded volumes, to mimic the complexity of the stem cell niche, scaffold cellularization and soluble factors diffusion. The structure external diameter is 81.4μm, resembling staminal niche dimensions and allowing injectability. We selected a spherically symmetric architecture, to provide isotropic stimuli on cells and preserve stemness [3]. The absence of sharp edges promotes injectability and bio-integrability. By varying scan speed and laser power during fabrication, we identified final optimized parameters (0.1mm/s, 7mW) which guarantee structural stability and integrity (Fig.1). Compression simulations (Fig.2) highlighted structural ultimate stress (1MPa) and displacement (7.94μm). By considering *in vivo* stresses, typically in the order of MPa, we can ensure scaffold performance under physiological stimuli. We assessed scaffold cellularization *via* confocal microscopy (Fig.3): cells appeared vital and showed proper cytoskeletal organization, with the development of the actin cortex.

Conclusions

By exploiting 2PP, we successfully fabricated complex hollow 3D micrometric scaffolds, distinguished by the key property of injectability. This cutting-edge feature will ensure minimally invasive delivery of cellularized constructs in which MSCs stemness is maintained and long-term immunomodulation is promoted, thus favouring tissue repair and regeneration in the host.

Figures

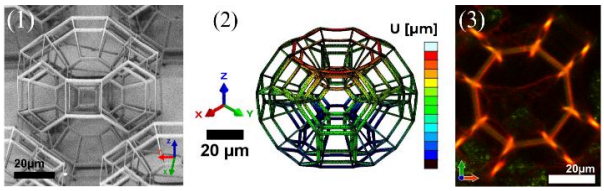

Figure 1: SEM image of microscaffolds fabricated by 2PP. Figure 2: Structural simulation showing scaffold displacement under compression (maximum is 7.94μm). Figure 3: Confocal image illustrating the organization of actin filaments (red) and nuclei (green) in NIH-3T3 cells. The structure exhibits autofluorescence in the red channel.

References

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