3D bioprinted constructs for bone tissue regeneration

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Bioprinting is one of the most versatile 3D printing techniques in the field of tissue engineering, combining cells and biomaterials into constructs with desired geometry. The success of a bioink is governed by many factors such as its biocompatible nature, its mechanical properties and its capacity to promote cell proliferation. This in turn leads to an increasing demand for the development of new combinations of biomaterials and biological constituents in order to produce biomimicking devices, optimal for tissue regeneration [1,2]. In this work, we prepared two different compositions of bioinks comprising gellan gum (GG) and polyvinyl alcohol (PVA) [3,4] with the osteoinductive compound nanohydroxyapatite (HAP) [5], to assess their mechanical properties, and their osteogenic potential when mixed with MC3T3-E1 pre-osteoblasts.

By mixing a 10% w/v PVA, a 4% w/v GG and a 15% w/v HAP solution in different ratios, four compositions were prepared in total: (i) 50-50 GG/PVA, (ii) 70-30 GG/PVA, (iii) 50-50 GG/PVA with 2.5% w/v HAP (50-50 GG/PVA/HAP) and (iv) 70-30 GG/PVA with 2.5% w/v HAP (70-30 GG/PVA/HAP), with the 50-50 and 70-30 indicating the ratios of the gellan gum to PVA solutions volume. The solutions were mixed with MC3T3-E1 pre-osteoblastic cell suspensions at a ratio of $5x10^6$ cells/ml, formulating the bioink constructs and crosslinked by using a 1% w/v $CaCl_2$ solution. The biological evaluation of the bioinks includes the visualization of the living cells by the Live/Dead assay, the morphological characterization of cells and extracellular matrix (ECM) by the nuclei/actin cytoskeleton stain using DAPI/rhodamine phalloidin, and ECM collagen and glycosaminoglycans, as well as alizarin red staining to determine the levels of calcium mineralization. Moreover, the bioinks have been mechanically characterized by examining their biodegradation rate and their rheological properties by employing protocols such as dynamic strain sweep (DSS), dynamic frequency sweep (DFS), calculation of tanδ, recovery rate after applying extreme stress and viscosity levels.

At day 1, the cells were visibly round and stressed but on day 3 they had an increased proliferation in all compositions, with the HAP scaffolds having a more prominent effect. At day 7, bioinks had evident extracellular matrix formation, with the HAP containing bioinks depicting greater levels. Regarding the biodegradation rate, the 50-50 GG/PVA bioink retained values between 14% and 28%, the 70-30 GG/PVA, 7% and 23%, the 50-50 GG/PVA/HAP, 9% and 17% and finally the 70-30 GG/PVA/HAP, 5% and 16% for days 7 and 21, respectively. The rheological analysis showcased that the presence of HAP only slightly affected the examined quantities. Specifically, the yield points ranged between 11 and 28 kPa for the various compositions, with the DFS analysis validating the G' and G'' values. Tanδ which is indicative of the ratio between the elastic and the viscous part of the material, ranged between 0,11 and 0,71. Finally, all bioinks retained a recovery rate of at least 89% of their viscosity levels after the application of a 200% strain.

Novel bioinks containing gellan gum, PVA, and hydroxyapatite were constructed to assess their role in bioinks' biomechanical traits. The 3D bioprinted constructs were mixed with pre-osteoblasts to form bioinks for bone tissue growth. Their rheological properties support their excellent bioprintability, while their biocompatible attributes promote bone regeneration.

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