Natural bone inspired 3D printed composite biomaterial-ink composed of hyaluronan, collagen and calcium phosphate particles to promote bone regeneration

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Introduction: Bone has the intrinsic capacity to regenerate after injury. However, in large bone defects caused by trauma, tumor resection, skeletal abnormalities or infections, the self-healing capacity of bone is insufficient and leads in 10-20% of the cases to non-union. The clinical gold standard treatment autologous bone grafting, where bone is harvested from a donor site in the same patient, has limitations, since to harvest the bone another injury is created and the amount of bone that can be harvested is limited. Besides, current tissue engineered constructs lack spatial control over scaffold architecture to anatomically match complicated bone defect sites. This represents an extensive clinical challenge and a need to develop new treatment options for large bone defects. 3D printing can overcome the above-mentioned limitations, by enabling the fabrication of patient specific bone graft substitutes that are customized to fit the bone defect site. This technology also facilitates control over the microarchitecture of scaffolds, including the pore size, porosity, and shape. Additionally, 3D printing facilitates the fabrication of materials composed of multiple components to resemble the heterogenous composition of bone. Therefore, this study aims to develop a 3D-printable composite biomaterial-ink to fabricate patient-specific bone graft substitutes that provide control over shape, architecture, and composition to promote bone regeneration. Inspired by the natural composition of bone, the biomaterial-ink consists of inorganic osteoinductive calcium phosphate particles (CaP) in a biopolymer matrix made of tyramine modified hyaluronic acid mixed with Collagen (THA-Col) of tailorable printability properties.

Methods: Biomaterial-ink was composed of CaP that were incorporated in THA or THA-Col, supplemented with horseradish peroxidase (HRP) and Eosin Y, for crosslinking. Pre-crosslinking, by addition of H_2O_2 and NaOH, was carried out to form a gel that can be extruded using 3D-printing and keeps its shape after extrusion. After the desired 3D structure was printed, scaffolds were further cured with green light (505 nm) for 30 minutes. Biomaterial-ink formulations were characterized by printability (continuous strut, line spacing, lattice, and an overhanging strut on a pillar structure), cohesion, swelling, *in vitro* degradability, and compressive modulus. Compositions were assessed *in vitro* using a metabolic activity assay. Additionally, human mesenchymal stem cells (hMSCs) will be cultured on different scaffold compositions to evaluate for new bone formation potential by differentiating hMSCs into osteoblasts assessing mineralization, alkaline phosphatase (ALP) production, and gene expression.

Results: Both THA alone and THA-Col are 3D printable, it is possible to extrude via a nozzle and when extruded it forms a continuous strut without waviness and hold its shape. Swelling of the formulations reached a plateau after 24 hours. The increasing addition of CaP into the organic matrix resulted in increasing compressive modulus and decreasing *in vitro* degradation rate. *In vitro* evaluation, without the addition of CaP, revealed that THA-Col showed both higher metabolic activity of hMSCs and higher ALP content after 14 days of osteogenic differentiation, compared to THA alone.

Conclusion: Here, a 3D printable composite biomaterial-ink of THA-Col with CaP was biofabricated. This biomaterial-ink overcomes current treatment limitations and holds great potential as bone graft substitute to promote regeneration in large bone defects. This work, inspired by the natural composition of bone, is significant since it can aid in the treatment of bone defects without the necessity to harvest bone from a donor site.

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