

3D Printed Bioactive Nanocomposite Scaffolds for Osteochondral Regeneration

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Statement of Purpose: Osteoarthritis (OA) is clinically defined as the progressive degeneration of hyaline cartilage within articulating joints leading to structural and functional failure at the interface. Reduced joint mobility and severe pain due to articular cartilage and subchondral bone (collectively known as osteochondral tissue) damage is common to patients suffering from OA. Current treatment methods used to address these defects include autografts, allografts, and mosaicplasties which contain their own inherent limitations, including donor site morbidity, infection, poor tissue integration, and insufficient neovascularization. Therefore, the objective of this work is focused on the manufacture of three-dimensional (3D) bioactive nanocomposite scaffolds for osteochondral tissue regeneration.

Methods: For the current work, a porous and highly interconnected poly(ethylene glycol) diacrylate (PEG-Da) hydrogel scaffold containing graded nanocrystalline hydroxyapatite (nHA) was fabricated via our novel table-top stereolithography 3D printer based on the open-source Solidoodle platform. Various in-fill densities (40% - 80%) of 60 wt% PEG-Da in PEG were evaluated for cell adhesion. Three-layer scaffolds were fabricated with increasing nHA concentration (20%, 10%, and 0%) of the best performing in-fill density. In addition to osteoconductive nHA, transforming growth factor- β 1 (TGF- β 1) was incorporated within the smooth articulating cartilage layer (top) at a concentration of 10 ng/mL. Wet co-axial electrospaying was employed to fabricate poly(lactic-co-glycol acid) core-shell nanospheres for sustained delivery. Human bone marrow-derived mesenchymal stem cells (MSCs) were seeded onto control and graded scaffolds and evaluated for adhesion, proliferation and osteochondral differentiation *in vitro*.

Results: The current work has focused on the development a novel 3D printed bioactive nanocomposite scaffold for osteochondral tissue regeneration. Scanning electron micrographs (Figure 1) of fabricated control and graded nHA scaffolds illustrate good integration between the respective layers producing a highly porous osseous tissue-like porous structure combined with a smooth articular cartilage top layer. Confocal microscopy images of MSCs seeded upon the porous structure illustrate the effectiveness of incorporated nHA in the promotion of cell spreading and adhesion (not shown). In addition, two-week differentiation demonstrated the efficacy of late-stage chondrogenic (Type II collagen) and osteogenic (extracellular calcium) biological markers (Figures 2&3).

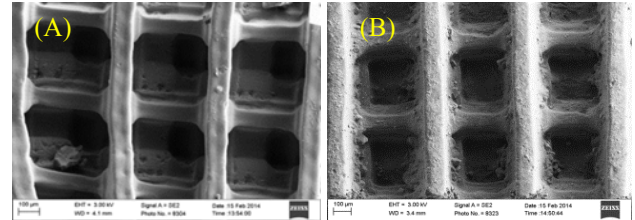


Figure 1. Scanning electron microscopy images of 3D printed scaffolds (A) without nHA and (B) with graded nHA.

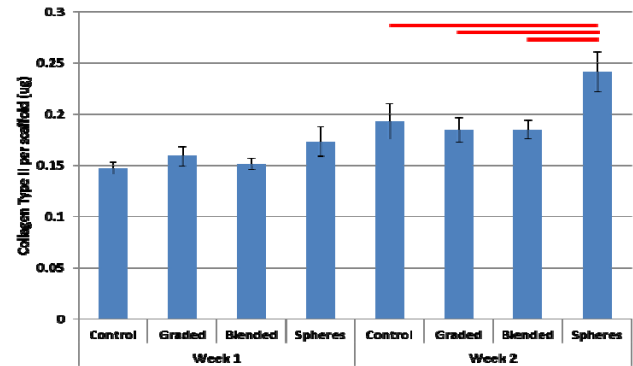


Figure 2. Enhanced type II collagen synthesis on 3D printed nanocomposite osteochondral scaffolds when compared to controls after two weeks of culture. Data are mean \pm standard error of the mean, $p < 0.05$.

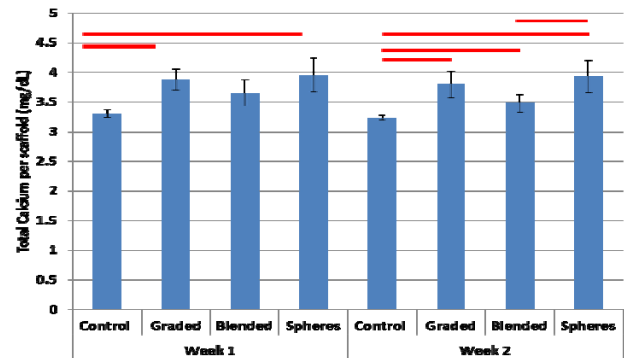


Figure 3. Improved extracellular matrix calcium deposition of MSCs in 3D printed nanocomposite scaffolds. Data are mean \pm standard error of the mean, $p < 0.05$.

Conclusions: The current work illustrates the efficacy of our current 3D printing technology for efficient fabrication of the novel nanocomposite hydrogel materials with good spatiotemporal control of morphogenetic nanomaterials. In addition, tissue-specific growth factors illustrated a synergistic effect leading to increased cell adhesion and directed MSC differentiation.

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