Characterization of Degradation and Bioactive Growth Factor Release for 3D Printed Poly(Propylene Fumarate)-Based Constructs

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Statement of Purpose: Hydrophobic synthetic polymers such as poly(propylene fumarate) (PPF) can serve as mechanically robust scaffolds for biomolecule delivery in bone tissue engineering applications. However, employing these biomaterials in the expanding fabrication methodology of extrusion-based three-dimensional (3D) printing may require subjecting the material to elevated printing temperatures that can potentially inactivate incorporated heat-sensitive biomolecules such as growth factors. In this work, we performed extrusion-based 3D printing of PPF-based constructs loaded with carriers containing bone morphogenetic protein-2 (BMP-2) at physiologic or elevated temperatures to investigate the subsequent *in vitro* bioactivity of released growth factor.

Methods: PPF was synthesized by two-step polymerization from diethyl fumarate (DEF) and propylene glycol. Additionally, a water-in-oil-in-water double emulsion solvent evaporation procedure was used to encapsulate BMP-2 in particulate delivery vehicles (0.7 µg of BMP-2/mg particle) composed of poly(DL-lactic-*co*-glycolic acid) (PLGA; 50:50 L:G, M_w 40 kDa). 2 wt% of BMP-2-loaded PLGA particles and 1 wt% of bisacylphosphine oxide photoinitiator were mixed with PPF, and 10 wt% DEF was added both as a crosslinking agent and for the reduction of printing formulation viscosity. Extrusion-based 3D printing was performed at 37, 60, or 90 °C (physiologic, intermediate, or elevated temperatures, respectively) with a 3D-Bioplotter® (EnvisionTEC; Dearborn, MI) followed by photocrosslinking of printed constructs with an ultraviolet print head and a flash box. Diameters of fabricated PLGA particles and printed PPF-based constructs were quantified by ImageJ analysis of SEM and optical imaging, respectively. Printed constructs (n = 3) and controls of non-printed PLGA particles were then subjected to 28 days of degradation in aqueous buffer solution at 37 °C. pH was measured upon replacement of the buffer solution (pH 7.4) every 3-4 days, and changes in fiber diameter and sample mass loss occurring during the 28-day period were measured. Also, the supernatants incubated with growth factor-loaded printed constructs and particles were collected on days 1, 4, 7, 10, 14, 18, 21, 24, and 28 and frozen at -80 °C. Upon completion of the release study, supernatants were thawed and exposed to W-20-17 mouse bone marrow stromal cells, which upregulate alkaline phosphatase (ALP) expression in the presence of bioactive BMP-2 in a dose-dependent manner. After three days of exposure, the cells were harvested and analyzed for ALP activity and DNA content using absorbance and fluorescence plate reader assays, respectively. Normalized ALP (ALP/DNA) values yielded from known concentrations of BMP-2 (0-100 ng/mL) were used to generate a calibration curve through four-parameter logistic regression, and cumulative release profiles of bioactive growth factor were calculated for each printing temperature.

Results: PPF of 4 kDa M_w and BMP-2-loaded PLGA particles of 1 µm average diameter were successfully synthesized. Prepared printing formulations were extruded at 37, 60, or 90 °C to yield fibers of comparable diameter (0.8-1.4 mm). After 28 days under degradation conditions, none of the experimental groups exhibited a significant decrease in fiber diameter, with mass losses ranging from 3-6%. pH of supernatants varied from 6.1 to 6.9 throughout the incubation period, until reaching a minimum of 5.6 on day 28.

For the bioactivity assay, release supernatants collected from the scaffolds printed at 37 and 60 °C on day 4 yielded higher normalized ALP values than those from the 90 °C and PLGA particle control groups. The other experimental groups and release timepoints did not yield normalized ALP values that were significantly elevated from the baseline enzymatic production measured from non-growth factor-exposed cells. A calibration curve derived from normalized ALP measurements for cells exposed to known concentrations of BMP-2 was fitted to a sigmoidal curve. After correcting for values less than the minimum asymptote, the determined equation was used to calculate the concentrations of bioactive BMP-2 present in each sample, followed by the cumulative release profiles for each printing temperature group (Fig. 1). Significantly higher bioactive BMP-2 was released from the 37 and 60 °C printed groups than the 90 °C printed group for days 4-14, and the cumulative release from PLGA particles became statistically equivalent to that from the 37 and 60 °C printed groups by day 24.

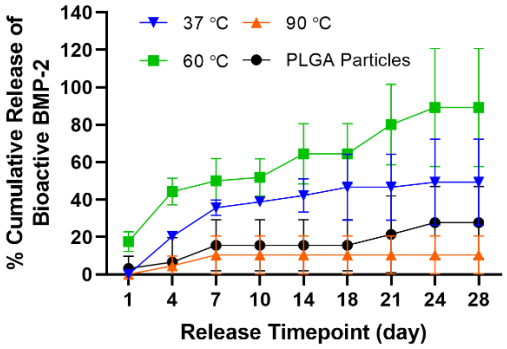

Figure 1. Cumulative release of bioactive BMP-2.

Conclusions: BMP-2-loaded, PPF-based constructs fabricated by extrusion-based 3D printing exhibited a decreased amount of total bioactive growth factor release for the elevated printing temperature relative to physiologic or intermediate printing temperatures. Release of bioactive BMP-2 from the 37 and 60 °C printed groups was accomplished with minimal changes to construct dimensions during 28 days of *in vitro* degradation. These findings can direct the selection of biomaterial carriers suitable for extrusion 3D printing with heat-sensitive biomolecules such as growth factors.

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