Heparin/Poly-L-lysine-coated 3D-printed PLGA scaffolds as drug carriers for local immune modulation in bone regeneration

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Statement of Purpose: Immune responses after injury play a critical role in bone regeneration [1]. Initiation of inflammation at early stages of repair triggers tissue formation and remodeling; however, uncontrolled inflammation underlies a catabolic effect on tissues as commonly seen in arthritis where inflammation breaks down tissues and hinders regeneration [2,3]. Our ultimate goal is to design a novel approach on bone scaffolds for which biodegradable scaffolds are loaded with inflammatory cytokines for local immunomodulation as well as bone regeneration. We employed nanoparticles (NPs) composed of heparin (Hep) and poly-L-lysine (PLL) [4] as cytokine drug carriers adhered on 3D-printed poly(lactic-*co*-glycolic acid) (PLGA) scaffolds. The entire drug delivery system was composed by using a layer-by-layer (LBL) technique. One challenge in the implementation of this technology in clinical drug delivery is the maximization of particle surface concentration on the PLGA surface. Here, we report our approach to create nanoparticle-coated PLGA scaffolds and improve surface coverage.

Methods: PLGA (75:25, Sigma Aldrich) scaffolds were synthesized via 3D printing (3D-Bioplotter, EnvisionTec) (Fig. 1c). Subsequently, various ratios (i.e. 4:1, 2:1, 1:1, etc.) of high (HMW) and low (LMW) molecular weight Hep (MP biomedical, Santa Cruz Biotec) and PLL (Sigma Aldrich) were tested to find stable composition of NPs. This was done through zeta potential measurements (Malvern Zetasizer) and then various coating conditions were carried out to compute coverage. Surface coverage and particle size distribution were determined via scanning electron microscopy (SEM, Zeiss). In order to determine the effect of cytokines on cells, cultures of MC3T3-E1 mouse pre-osteoblast cells were carried out without scaffolds. Cell proliferation and differentiation were measured by MTT assay (Vibrant) and intracellular alkaline phosphatase assay (ALP, Biovision), respectively.

Results and Discussion: Our strategy to improving NP coverage on PLGA scaffolds included (1) formulating a stable NP solution, (2) using higher nanoparticle solution concentration and volume, and (3) performing a higher number of coating layers. Combinations of Hep/PLL ratios at 4:1, 2:1, and 1:1 w/w with both high and low molecular weight polymers indicated that both HMW and LMW Hep/PLL consisting of 5 mg/mL Hep and 2.5 mg/mL PLL remained most stable after 6 days (i.e., HH21 and LL21 in Fig. 1a). Furthermore, the diameter of HH21 NPs were found to remain steady at 150 nm while LL21 showed a slight increase over time. Mixtures of HMW Hep and LMW PLL or vice versa turned out to be unstable for longer term and were not used for coating (data not shown). Fig. 1b demonstrates a surface coverage of 10% by 3 coating layers of 3 mL of NP (HH21) and PEI solution on PLGA scaffold. Separately, it was found that the pro-inflammatory cytokine, TNF-α, significantly decreased cell proliferation after 7 days while the effect of IFN-γ was minimal (solution based administration). The anti-inflammatory cytokines did not adversely impact cell proliferation. TNF-α also adversely affected differentiation.

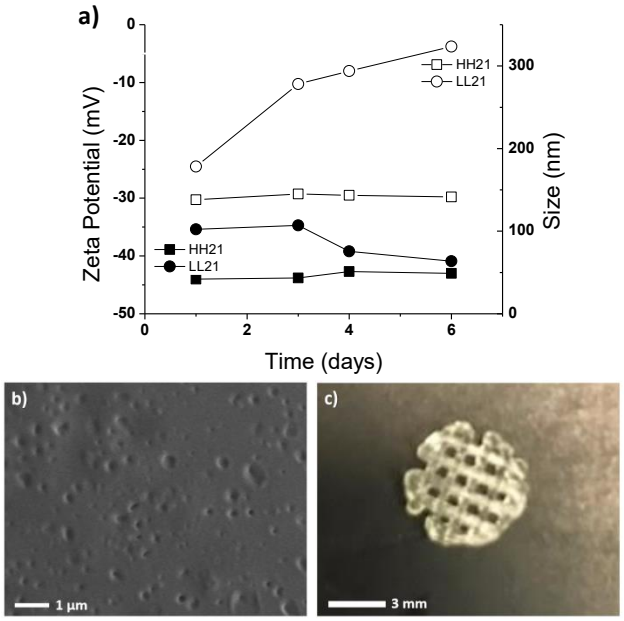

Figure 1. (a) Nanoparticle changes in zeta potential and size for 6 days of duration (HH21= HMW Hep + HMW PLL, 2:1; LL21 = LMW Hep + LMW PLL, 2:1) (b) SEM image of HMW Hep/PLL nanoparticles (HH21) with 3x coating. (c) 3-D printed PLGA scaffold (75:25 copolymer).

Conclusion and Future Direction: We have synthesized Hep/PLL nanoparticle-coated 3-D printed PLGA scaffold with approximately 10% NP coverage. This has implications in carrying a sufficient amount of cytokines as a drug carrier for local immune modulation in bone tissues. We also have obtained preliminary results indicating that proliferation of MC3T3-E1 mouse preosteoblast cells are adversely affected by TNF-α but not by anti-inflammatory cytokines. Our next step is to incorporate cytokines in the Hep/PLL NP system and find out the effect of local delivery.

References

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