Thermo-responsive citrate-based nanonets for tissue regeneration: effects of localized SDF-1 release

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Statement of Purpose: Stromal cell-derived factor 1α (SDF-1) is a positively charged chemokine that is involved in stem/progenitor cell mobilization and homing to sites of injury. This chemokine is rapidly degraded by proteases complicating its use as a therapeutic for regenerative medicine applications. Therefore, the development of materials that can be easily delivered to tissue and serve as a depot for the release of active SDF-1 is warranted. Our laboratory has synthesized water-soluble injectable thermo-responsive biodegradable nanonets for the entrapment and controlled delivery of biomacromolecules and cells. This study evaluates the ability of the nanonets to load and release active SDF-1 and promote new tissue formation *in vivo*.

Methods: Nanonets were synthesized by polycondensation of citric acid, polyethylene glycol and glycerol 1,3-diglycerolate diacrylate at 140°C and free radical polymerization with N-isopropylacrylamide at 70°C. The resulting copolymer is referred to as poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN). PPCN was dissolved in PBS at 100 mg/mL and pH adjusted to 7.4. Lower critical solution temperature (LCST) was assessed via circular dichroism spectrophotometry and particle size was measured on a Malvern Zetasizer. SDF-1 was added at different concentrations to obtain PPCN-SDF-1 polyelectrolyte complexes. Entrapment efficiency, in vitro release and bioactivity of SDF-1 were evaluated via radiodetection of iodinated (I-125) SDF-1. 150ul of PPCN (100 mg/ml, pH=7.4) or PPCN-SDF-1 (100mg/ml, pH=7.4, 3ug/ml SDF-1) were injected into the backs of Sprague Dawley rats. The implants and surrounding tissue were harvested and snap-frozen at 3 days and 1 month after injection. Tissue sections were stained with H&E and probed with antibodies against CD68 and CD31.

Results: PPCN nanonets have a lower critical solution temperature (LCST) of 33°C and quickly solidify at 37°C forming a viscoelastic hydrogel. After hydrophilic-to-hydrophobic transition, PPCN at 5 mg/ml had a stable particle diameter of 156.6±9.6 nm at 37°C and 7.0±3.1 nm at 25°C (**Figure 1).** PPCN nanonets entrapped 100% of SDF-1 in solution and slowly released active protein over a period of 2 weeks (**Figure 1**).

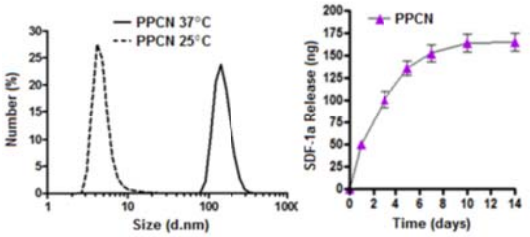

Figure 1. Particle sizes of PPCN nanonets (pH=7.4, 5mg/ml) in water at 25°C and 37°C (left). *In vitro* release of SDF-1 from PPCN nanonets (right, N=4, mean±SD).

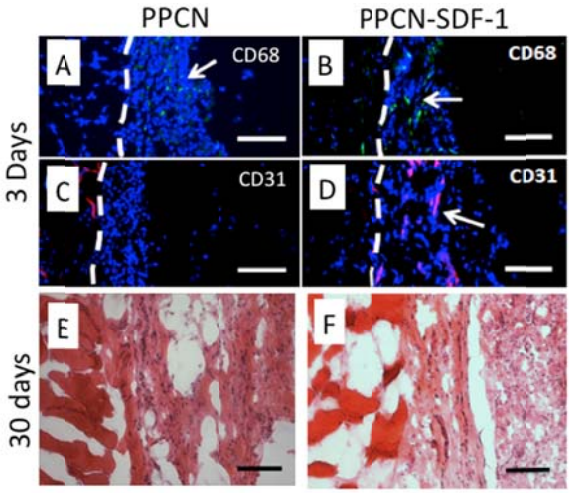

Figure 2. Cell recruitment and new tissue formation within nanonet gels. Macrophage (A&B) and CD31⁺ cells (C&D) within PPCN and PPCN-SDF-1 nanonet gels, respectively, at 3 days. H&E stains show that nanonet gels are replaced by connective tissue at 30 days (E&F).

PPCN and PPCN-SDF-1 nanonets gelled at the injection site as evident by a white lump beneath the skin. Significant tissue infiltration into both materials was observed (**Figure 2**). PPCN-SDF-1 nanonets recruited fibroblasts, macrophages, and CD31⁺ cells into the injection site at 3 days, whereas PPCN nanonets recruited mostly fibroblasts and some macrophages. At 1 month, most of the materials were replaced by connective tissue, suggesting that the nanonets are bioabsorbable.

Conclusions: Thermo-responsive nanonets are biocompatible, effectively entrap and release SDF-1, and promote new tissue formation *in vivo*.