Mechanocompatible polymer-extracellular matrix composites for vascular tissue engineering

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Statement of Purpose: Small vessel thrombosis is one of the major hurdles when using decellularized extracellular matrix (ECM) as a tissue/organ engineering scaffold.[1] Heparin is often immobilized onto the ECM to provide anticoagulant activity.[2] However, strategies to immobilize heparin onto ECM often involve chemical crosslinking, which increases the mechanical stiffness and alters the ultrastructure of the ECM. It is important for the ECM to maintain its native mechanical properties and structure for its proper function.[3] Therefore, the goal of this study is to develop a novel strategy to immobilize heparin onto ECM without altering substrate mechanical properties.

Methods: Heparin was immobilized onto ECM using the following strategy: (1) conjugating heparin with (N-[ß-maleimidopropionic acid] hydrazide) (BMPH) as a linker via standard carbodiimide chemistry; (2) coating the ECM with poly(1,8-octanediol citric acid)-co-cysteine (POC-Cys), which was synthesized by reacting 1,8-octanediol, citric acid and L-cysteine (molar ratio 1:1:0.2) at 140°C for 1 hour; (3) reacting heparin-BMPH with POC-Cys-coated ECM, to allow covalent bonds formation between thiols of POC-Cys and maleimides of BMPH, thus linking heparin onto the surface. The strategy was optimized on 96-well plates and then applied to decellularized rat aortas as a model ECM. The structure of POC-Cys pre-polymer and heparin-BMPH was confirmed with H-NMR. POC-Cys (1 wt% in ethanol) prepolymer was coated onto 96-well plates and post-polymerized at 80°C for 4 days, or onto ECM and post-polymerized at 37°C or 45°C for 4 days. The surfaces were then treated with 2-mercaptoethanol (BME) to breakdown disulfide bonds. Free thiol groups were measured with Ellman's reagent. The surfaces with free thiols were then reacted with heparin-BMPH solutions overnight. The heparin bioactivity was measured with a Factor Xa kit. Tensile testing was used to measure changes in ECM elastic modulus due to the modifications.

Results: H-NMR confirmed the structure of POC-Cys (peak at 1.02 ppm, -SH group), and the structure of heparin-BMPH (peak 6.74, -H on maleimide). POC-Cys exhibited blue auto-fluorescence under UV light. (Fig 1) POC-Cys coating provided -SH groups on coated surfaces, which formed di-sulfide bonds during post-polymerization. The di-sulfide bonds were broken down to free -SH groups with BME on both plastic surfaces (Fig 2.A) and ECM (Fig 2.B). Treating decellularized aortas with POC-Cys or BME did not alter the ECM elastic modulus (Fig 3.A) significantly, when compared to EDC/NHS chemistry, which increased ECM stiffness by more than 5 fold (Fig 3. B). Factor Xa activity analysis for heparin-BMPH in solution showed no change in heparin bioactivity due to BMPH conjugation (Fig 4.A). POC-Cys-coated surfaces (80°C plastic, 20mM BME treatment) exhibited an increase in heparin activity with increasing ratio of BMPH (Heparin: BMPH=1:10). (Fig 4.B)

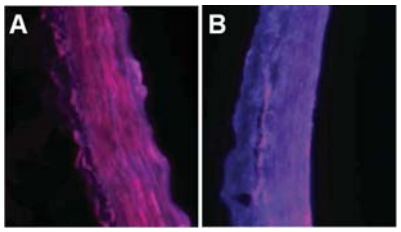

Fig 1. Decellularized aorta without (A) and coated with (B) POC-Cys. The coated aorta exhibits blue florescence .

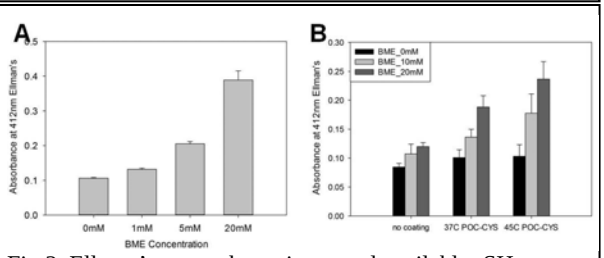

Fig 2. Ellman's assay shows increased available -SH groups with increasing BME concentration for POC-Cys coated 96-well plates (A) and decellularized aortas (B).

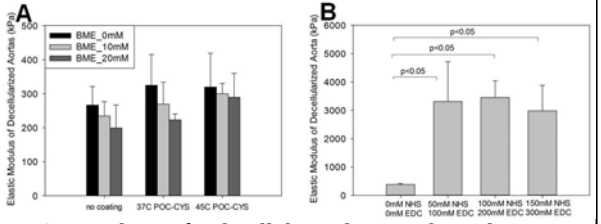

Fig 3. Tensile test for decellularized aortas showed no significant change in elastic modulus with POC-Cys coating and BME treatment (A), as opposed to significant increase in stiffness after NHS/EDC crosslinking (B).

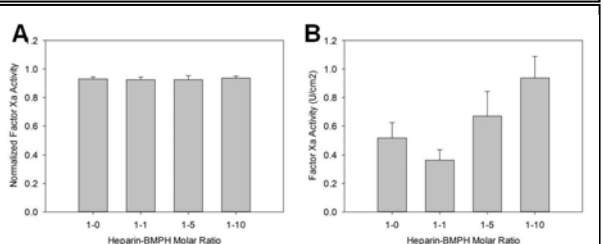

Fig 4. Factor Xa activity analysis for heparin-BMPH in solution (A) and on POC-Cys coated surfaces (B).

Conclusions: Heparin can be conjugated onto aorta ECM via maleimide-thiol chemistry using BMPH and POC-Cys as linkers. This novel strategy allows the immobilization of active heparin without altering ECM mechanical properties.

References:

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