Hemocompatibility of Crosslinked or Thrombomodulin-modified Decellularized Matrix

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Introduction: Decellularized extracellular matrices have been widely used as biomaterial scaffolds for reconstructing tissues due to their intrinsic topographical and biochemical cues that help coordinate proper cellular function. One decellularized matrix widely used for soft tissue repair is derived from porcine small intestinal submucosa (SIS). SIS can be crosslinked to reduce the degradation rate of the material, and the inflammatory and immune responses to this material in both native and crosslinked forms have been well-documented.[1]

In addition to soft tissue repair, SIS has been utilized in cardiovascular applications including vascular grafting, stent coverings and venous valve replacement.[2,3] In these blood-contacting applications, SIS must serve as a non-thrombogenic surface. Unlike the thorough characterization of the inflammatory and immune responses, the thrombotic response to SIS, in either native or crosslinked states, has not been well-characterized.

Bioactive modifications can be applied to vascular materials to interact with blood proteins and modulate both the local cellular and thrombotic responses. For example, the endothelial cell surface protein thrombomodulin has been applied to biomaterials to facilitate activated protein C (APC) generation in blood.[4,5] APC directly inhibits coagulation factors and also inhibits endothelial apoptosis to sustain endothelium-dependent regulation of thrombosis. This work provides a multi-faceted characterization of plasma coagulation and thrombus formation on SIS, and also determines how either crosslinking or a novel thrombomodulin modification affects these thrombotic processes.

Methods: Materials: Sterile SIS was generously provided by Cook Biotech. Carbodiimide crosslinking of SIS: Crosslinked SIS (cSIS) was prepared with 0.4 mg/mL 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 0.6 mg/mL N-hydroxysulfosuccinimide (NHS). Thrombomodulin modification: SIS was modified with soluble thrombomodulin (TM-SIS, 4 µg/mL thrombomodulin) via adsorption and washed thoroughly prior to experiments. Plasma coagulation times: Following the various treatments, discs of SIS were placed into citrated, pooled platelet-poor plasma, and coagulation was initiated by recalcification with an equal volume of 25 mM $CaCl_2$, or by stimulation with one of the pro-thrombotic agonists: thrombin, tissue factor (TF), or the activated partial thromboplastin time (APTT) reagent HemosIL. To characterize how glycosaminoglycans in SIS affect plasma coagulation, SIS was treated with enzymes to selectively remove glycosaminoglycans, and coagulation times were subsequently measured. Ex vivo arteriovenous shunt: Tubular SIS devices (5 cm long, 4 mm ID) were connected to a baboon chronic arteriovenous shunt loop, and whole blood absent of anti-coagulant and anti-platelet therapies flowed over the devices for 1 hr. The accumulation of ^{111}In-labeled platelets and ^{125}I-labeled fibrinogen were measured over the course of 1 hour. Statistics: For all experiments, a one-way ANOVA with Tukey's post-hoc was performed; differences were considered significant if $p<0.05$ based on the post-hoc.

Results: Plasma coagulation times: SIS significantly prolonged plasma coagulation initiated with HemosIL, and completely inhibited plasma coagulation when recalcified as well as when stimulated with TF (1 pM). Conversely, cSIS did not prolong plasma coagulation times for any assay. TM-SIS generated APC at a rate of 461.818±1.96 $ng \cdot cm^{-2} \cdot hr^{-1}$, but did not significantly prolong coagulation times compared to unmodified SIS. When SIS was enzymatically digested with either heparinase I or heparinase III, but not chondroitinase ABC or hyaluronidase, plasma coagulation was no longer inhibited, suggesting heparin or heparin sulfate in SIS is responsible for the anticoagulant activity. Platelet accumulation and fibrin deposition on SIS and cSIS devices. The number of platelets that accumulated the SIS devices peaked at 35 minutes at 4.16±1.36 x 10^8 platelets per cm^2 (**Figure 1**), and reduced to 2.49±0.52 x 10^8 platelets per cm^2 at 60 minutes. cSIS devices had significantly greater platelet accumulation and fibrinogen deposition than SIS or TM-SIS.

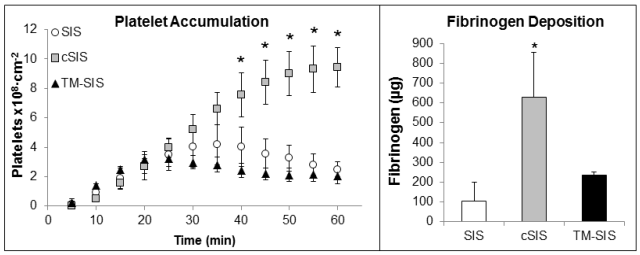

Figure 1: Platelet accumulation and total fibrinogen deposition on SIS devices. "*" $p<0.05$ vs. SIS and TM-SIS via one-way ANOVA and Tukey's post-hoc, n = 3.

Conclusions: SIS inhibited plasma coagulation; this anticoagulant activity is abrogated by either crosslinking or treatment with heparinase I or III. Platelet accumulation and fibrinogen deposition on cSIS were significantly greater than SIS or TM-SIS. These results indicate that SIS possesses good hemocompatibility, and suggest that heparin and/or heparan sulfate in SIS are responsible for the anticoagulant activity and are also susceptible to carbodiimide-mediated crosslinking. In addition, thrombomodulin modification successfully enabled APC generation, but the quantity of APC generated did not result in a measurable additive anticoagulant effect over unmodified SIS. Future work will determine if APC released from TM-SIS can reduce inflammatory cytokine-induced endothelial cell apoptosis.

References:

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