In situ Regeneration of Bioactive Coatings using evolved *Staphylococcus Aureus* Sortase A

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Statement of Purpose: Immobilization of bioactive molecules and drug eluting assemblies onto implantable devices yielded promising products that can abrogate thrombotic cascades or detrimental infections or inflammation. A local or systemic delivery of bioactive therapeutic payloads to the blood-contacting surface of medical implants may facilitate *in situ* regeneration of bioactivity, which can further extend the life-time of medical implants and promote tissue regeneration. Previously, we have reported that immobilization of recombinant thrombomoduline (rTM) onto the luminal surface of prosthetic arterial substitutes[1] or a micelle corona[2] could successfully attenuate thrombi formation. Here, we report a new approach for the regeneration of bioactive surface through a reversible two-step stripping and recharging cycles of a laboratory evolved *Stapylococcus aureus* sortase A (eSrtA), using rTM as a model bioactive compound.

Methods: A recombinant human thrombomodulin (rTM) fragment with a C-terminal LPETG motif (TM_{LPETG}), and wild-type sortase A (WT SrtA) and an evolved sortase A (eSrtA) by directed evolution using a yeast display system were generated[1,3]. A peptaglycine modified surfaces was generated by modifying streptavidin-coated 96 well plate with biotin-PEG5k-GGG or modifying polyurethane (PU) catheter with pentaglycine peptides. Immobilization efficiency of TM_{LPETG} onto GGG-surface via eSrtA or WT SrtA was conducted and repeated charging/ stripping of TM_{LPETG} was performed via eSrtA (charging: 1 μM TM_{LPETG} using 0.1eq. of eSrtA, stripping: 20 μM of eSrtA with 1 mM triglycine). The surface density and enzymatic activity changes upon each reaction were measured by TM ELISA and activated protein C (aPC) assay. For *in vivo* test, pentaglycine-modified catheters were deployed in the vena cava of live mice, eSrtA and either LPETG-tagged biotin or triglycine (GGG) was systemically administered to charge or strip the catheter surface, respectively. After 1hr, catheters were explanted, reacted with streptavidin-Cy3, and imaged with fluorescent microscope, and analyzed using Image J.

Results: Surface immobilization of TM_{LPETG} by eSrtA yielded ~9-fold higher surface TM density than WT SrtA (**Figure 1b**). Near complete removal of immobilized TM films could be achieved with eSrtA in 30 minutes, whereas WT SrtA was substantially less efficient when reacted under identical conditions. Up to ten repetitive recharge cycles were accomplished using eSrtA to reproducibly regenerate a rTM coated layer without any significant loss of enzymatic activity (**Figure 1c and 1d**). Real time fluorescent imaging and analysis of explanted catheters confirmed selective immobilization and removal of LPETG tagged probes from pentaglycine catheters in the presence of eSrtA and GGG within 1 h (**Figure 2**).

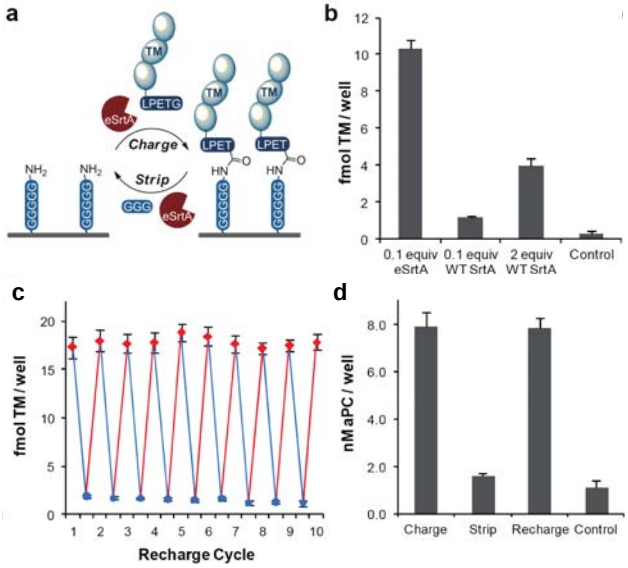

Figure 1. (a) Scheme for two-step rechargeable surface modification by sortase-catalyzed charging/stripping, (b) Immobilization efficiency of eSrtA, WT SrtA, or no sortase for conjugation of 1 μM TM_{LPETG}, (c) Reversible eSrtA-catalyzed charging (red) and stripping (blue) of TM_{LETPG}. (d) aPC generation by immobilized TM. ($n \geq 3$).

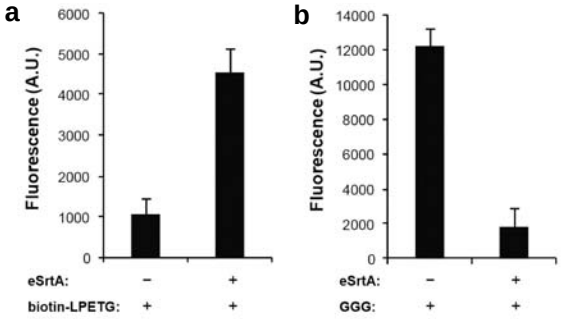

Figure 2. Analysis of explanted catheters after charging (a) or removal of biotin-LPETG probes (b) with intravenous delivery of eSrtA in 1 hr ($n \geq 3$).

Conclusions: We confirmed the capacity of eSrtA to catalyze multiple cycles of rapid conjugation and removal of LPETG tagged biomolecules. Using a mouse model, we demonstrated that such a strategy could also be applicable *in situ*. These studies showed a potential as a rapid, orthogonal, and reversible surface chemistry approach to regenerate selective molecular constitutents in ordert to extend the lifetime of bioactive films in a manner akin to living systems. Regeneration of anti-thrombogenic bioactive films *in vivo* will be further investigated.

References: 1) Qu Z. Adv. Healthcare Mater. 2013;3:30-35. 2) Kim W. Angew. Chem. Int. Ed. 2014;53:1-6. 3) Chen I. PNAS 2011;108: 11399–11404.