Aldehyde-Amine Chemistry Enables Biosealants with Tissue-Specific Adhesion

Natalie Artzi[a,*], Tarek Shazly[a,*], Aaron B. Baker[a], Adriana Bon[b], and Elazer R. Edelman[a,c]
From the [a]Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, [b]Department of Chemistry, Institut Quıimic de Sarria`, Universitat Ramon Llull, Barcelona, Spain 08017; [c]Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115. *-Equal contribution.

Statement of Purpose: Soft tissue surgical sealants provide an ideal material class for assessment of tissue-material interactions. Sealant adhesion can be quantified with functional assays that supplement characterization of tissue reactivity and material fate. Though sealants are routinely used in clinical procedures active questions and limitations force physicians to choose between extremes of adhesion strength and biocompatibility.

We hypothesized that tissue specific adhesion could be achieved by matching material and tissue properties. We used aldehyde-amine chemistry to determine if alterations in tissue surfaces (amine density) could affect interactions with materials of varied (aldehyde content and density) composition. We further examined how judicious changes to materials properties effected material strength by way of cohesion, material-tissue interface by way of adhesion and material-tissue biocompatibility.

Methods: Adhesive mechanics were measured *ex vivo* with rat tissues (heart, lung, liver, and duodenum). Adhesive test elements were created by a 200µL application of PEG:dextran between two uniformly sized tissue biopsies to facilitate uniaxial mechanical testing of tissue–material interfaces. Fluorescently labeled PEG:dextran was applied to tissues and allowed to cure. Tissue samples were cryosectioned and stained with rhodamine and DAPI to identify extracellular matrix and cellular structures against the fluorescent material background (Fig). PEG:dextran morphology at the tissue-material interface was quantified using image analysis techniques to characterize the interface between tissue surfaces and material bulk. The aldehyde affinity of various soft tissues was determined through quantification of the conjugation of aldehyde-coated fluorescent microspheres (f-MS) to soft tissue surfaces. *In vivo* biocompatibility was examined 9 days after a range of material compositions were implanted subcutaneouly and assessed for inflammatory response.

Results: Adhesive strength varied dramatically with material chemistry and is tissue-specific. Cardiac tissue test elements were most and lung least sensitive to interfacial material reactivity. While the modulus of coated heart tissue rose 1785% as dextran aldehyde content increased 2.3 fold, lung displayed a much weaker dependence (283 % increase in modulus for same change in materials). These data support the notion that both tissue type and material chemistry influence aldehyde-mediated adhesive interactions, providing a functional basis for tissue-specific sealant design.

The tissue response to subcutaneous material implants was used to evaluate the *in vivo* biocompatibility of PEG:dextran materials and the influence of material aldehyde content on tissue response. PEG:dextran constructs with varied aldehyde content were implanted into a subcutaneous pocket in mice which were survived for nine days. Inflammation as manifest by thicker fibrous capsule rose with aldehyde content. Inflammatory cell-mediated proteolysis affects wound repair as matrix metalloproteases cleave the extracellular matrix weakening the tissue and surgical anastomoses. The gelatinase activity in the tissues, measured using analytical fluorescent microscopy and *in situ* zymography, also increased with material aldehyde content.

The conjugation of aldehyde-coated fluorescent microspheres to soft tissues is indicative of the aldehyde affinity of soft tissues. Soft tissues display a range of f-MS conjugation metrics, with duodenal tissue possessing the greatest apparent aldehyde affinity. Comparison of tissue conjugation metrics to adhesive mechanical data provides convincing evidence for aldehyde-mediated adhesion, as interfacial moduli correlate strongly ($R = 0.92$, $p < 0.05$) to aldehyde affinity across tested tissue types and material variants.. The tissue-adhesive interfacial regime depicts the intermediate material structure resulting from concurrent dextran aldehyde reactivity with PEG and tissue amines. The morphology of the adhesive regime varied with tissue and reflected the strength of adhesion; appearing fibrillar and discontinuous on cardiac and lung tissues, and more continuous and intact over liver and duodenum.

Conclusions: To date most adhesive materials force a choice between strength of tissue-material interaction and intensity of potential tissue toxicity. The development of materials whose adhesion and biocompatibility can be titrated to a specific tissue would be of immense value.

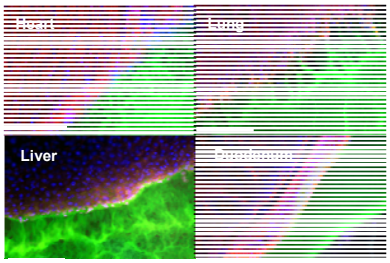

Fig: PEG:dextran interaction with tissues is organ specific. Extracellular tissue material is highlighted with rhodamine phalloidin (actin, red) and cells with DAPI (cell nuclei, blue) against the fluorescent background of applied materials (green).

Amine groups are ubiquitous on tissue surfaces and their density varies from tissue to tissue and with different environmental forces and disease states. Selective modification of adhesive materials presenting aldehyde groups can enable modulated adhesion which optimizes tissue-material physical and biological interaction. There may well be a therapeutic window for optimal tissue–sealant interactions, bounded below by the need for adequate adhesion strength and above by the condition of biocompatibility. The general concept presented herein and tools employed can be employed to create and evaluate a family of selective biomaterials for a range of applications and clinical needs.