

Disease State Affects Tissue Microenvironment and Material Performance

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Statement of Purpose: Biomaterials therapeutic outcome and clinical performance are increasingly unpredictable due to the lack of consideration of target tissue microenvironment as manifested by a disease. Soft-tissue surgical sealants provide an ideal material class for assessment of tissue-material interactions under different microenvironmental conditions. We designed a new class of adhesive materials based on dendrimer and dextran^{1,2} and investigated the effect of inflammatory and neoplastic bowel diseases on tissue properties and tissue:material interactions. Adhesive material biocompatibility was shown to be contextual and not only a constitutive property of the material, owing to the abundance of immune cells in the diseased inflammatory environment. Furthermore, our findings demonstrate that our adhesive induces tissue healing (data not shown), seemingly through modification of local immune response under compromised diseased state. We show that tissue surface chemistry is modified by the disease, necessitating strategic variation of material composition to allow optimal biocompatibility and adhesion strength for specific clinical scenarios.

Methods: Dendrimer:dextran solutions were mixed to form a cross-linked network. Tissue amine density was quantified by applying fluorescently labeled aldehyde-coated microspheres that are able to interact with tissue amines. Collagen and inflammation levels were measured by immunostaining using antibodies against COL-I and TNF- α , respectively. Stainings were performed either on 8 μ m cryosections and quantified using fluorescence microscopy (images) or *en face* and quantified using the IVIS.

Results: Inflammatory bowel disease reduces tissue surface amine density, as evident by lower microsphere conjugation (Figures 1 a - b), as opposed to cancer, which increases surface amines compared to healthy states (data not shown). As amine groups interact with material aldehydes, reduction in amine density affects material adhesion strength (Figure 1 c - d). Such alteration in tissue surface chemistry mandates specific material formulation to compensate the loss of tissue locks (tissue amines), by providing more chemical keys in the form of tethered aldehydes to improve adhesion. To understand the source for tissue amine density modification, we hypothesized that collagen, being the most abundant protein in tissues, would correlate with tissue surface amines and, therefore, with material adhesion. We induced inflammation of varying severity to rabbit colon and showed that inflammation causes basement membrane collagen degradation (Figure 2) that linearly correlates with disease severity (Figure 3). Additionally, serosal collagen content is affected by the disease, and correlates with

surface tissue amine density measured by aldehyde coated microspheres conjugation (Figure 4).

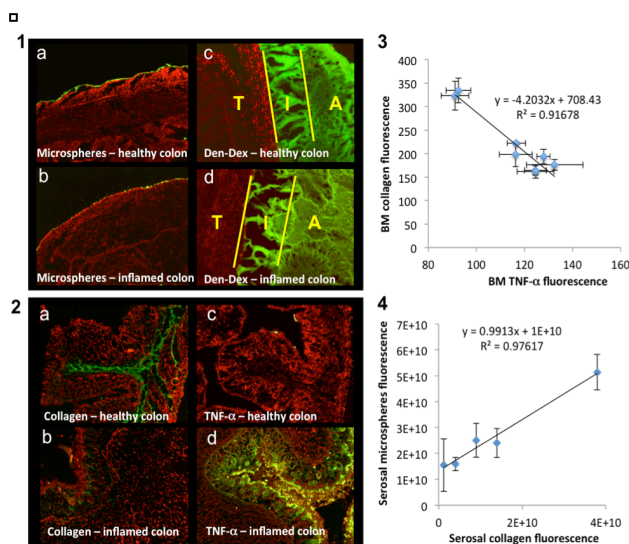


Fig. 1: Impact of disease on serosal layer assessed by fluorescent aldehyde-coated microspheres (green) on healthy (a) and diseased tissue (b). Material (green):tissue (red) interaction in healthy (c) and diseased tissue (d). The three regions of interest are delineated as T (tissue), I (interface) and A (adhesive). Fig. 2: Basement membrane (BM) collagen immunostaining (green) on healthy (a) and diseased (b) tissue. Basement membrane (BM) TNF- α immunostaining (green) on healthy (c) and diseased (d) tissue. Fig. 3: Correlation between basement membrane collagen and inflammation. Fig. 4: Correlation between serosal amine density and serosal collagen content.

Conclusions: Material performance, tissue:material interaction and thus therapeutic outcome, are contextual. Hence, it is critical to assess tissue microenvironment and develop materials in the settings of clinically relevant conditions, to enable predictable and favorable material performance. Disease type and severity give rise to diverse effects on the target organ. While inflammatory disease resulted in lower collagen content and correlated with the severity of the disease, colorectal cancer increased the collagen level- reiterating the importance of examining materials in light of their target microenvironment. Collagen levels can be used to predict disease severity and can inform on the right material formulation to be used.

References:

1. Artzi et al, US Patent 2012/0263672.
2. N. Oliva, S. Shitreet, E. Abraham, B. Stanley, E. Edelman and N. Artzi, *Langmuir* 2012, 28 (43), 15402.