Novel Point-of-Care Wound Diagnostic Devices using Pigmented Thin-Film Substrates

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Statement of Purpose: Excessive protease activity has been implicated in the perpetuation of chronic wounds. Of these, matrix metalloproteinases and neutrophil elastase have been implicated by many as the most dominating in these pathologies. While much attention has been focused on the characterization of chronic wound fluids and the development of bandages capable of entrapping these proteases, little progress has been realized in the development of point-of-care diagnostics for the assessment of chronic wounds. Such devices would allow clinicians to assess and treat chronic wounds on a patient-by-patient basis, advancing the fields of chronic wound care and personalized medicine. To this aim, we have developed a novel protease detection assay based upon the digestion of a pigmented gelatin thin film substrate.

Methods: Pigmented gelatin-based thin films were manufactured by spin-coating on polystyrene Petri dishes. The manufacturing process was optimized using a factorial statistical experimental approach. Films were systematically optimized by screening for high protease detection sensitivities while maintaining low background digestion. Briefly, gelatin solutions were made in aqueous buffers containing Coomassie G-250 as a model pigment. Upon complete dissolution, thin films were cast by dispensing the solutions onto 100mm Petri dishes and spinning above 1500rpm for 30sec. To mimic the predominant proteases present in chronic wounds, standard curves of clostridial collagenase, pronase, and elastase were acquired from EMD Chemicals (Gibbstown, NJ) and prepared in ddH_2O and assay buffer (50mM Tris-HCl, 200mM NaCl, 10mM $CaCl_2$, 1mM $ZnCl_2$, 0.05% $Brij_{35}$, and 0.02% NaN_3). The various thin films were tested for protease detection sensitivity by reacting 5ul protease standards for ten minutes at RT. The reactions were then quenched by rinsing briefly in water. Film "sensitivity" was assessed by the observation of complete digestion at the sample spot, whereas film "specificity" was assessed by the observation of high colorfastness in the peripheral, untested areas following rinsing.

Results: As demonstrated in Figure 1, processing conditions were critical in the manufacture of pigmented gelatin thin films that were specifically sensitive to protease activity. Furthermore, optimized films were capable of detecting elastase, pronase, and collagenase at concentrations as low as 6.3, 6.3, and 9.4ug/ml, respectively within ten minutes.

Conclusions: Until now, a one-size-fits all mindset has been commonplace in the treatment of chronic wounds. However, with the current drive towards personalized medicine and the rapid development of specialized wound treatments, there is an increasing need for simple point-of-care wound diagnostics that would serve the dual purpose of directing clinicians towards a suitable treatment regime while also providing adequate justification to insurance companies for the use of more expensive treatments. We have demonstrated a novel means of assessing protease activity that may meet this need. Our prototypes have proved to be quite economical due to the low cost of raw materials and manufacturing equipment. Finally, not only is the all-or-none nature of these devices simple to interpret, the thin films are readily adaptable to a large number of device implementations.

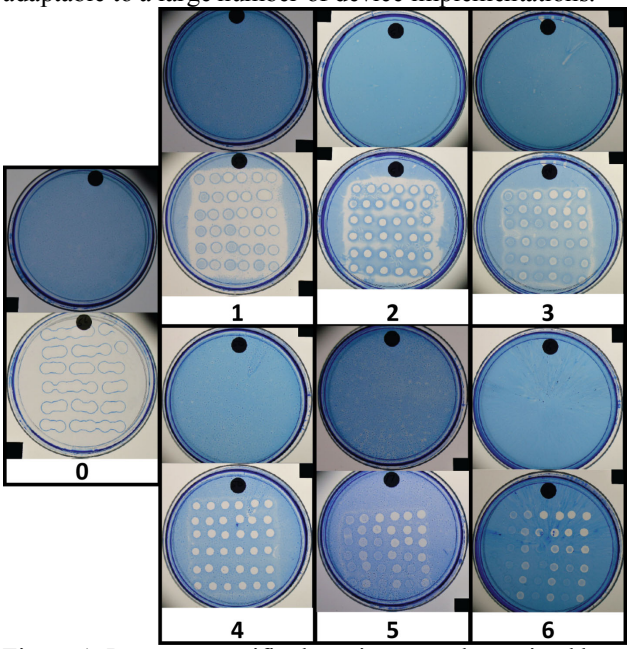

Figure 1. Protease-specific detection was determined by ranking (0-6) the extent of film digestion in the regions peripheral to the sampling spots following rinsing of the films with ddH_2O. The extent of nonspecific film digestion was observed in a process-dependent manner ($p<0.05$). 100, 40, 16, 6.4, 2.6, and 0ug/ml (top-to-bottom) collagenase, pronase, and elastase (right-to-left) were reacted in water and buffer, respectively.

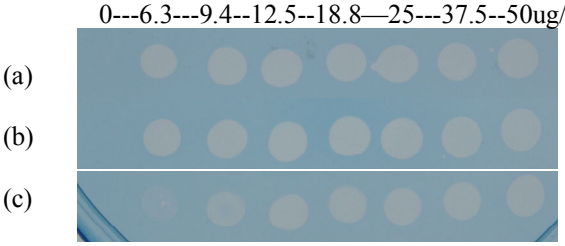

Figure 2. Following optimization of the processing conditions, enzyme detection sensitivities for (a) elastase, (b) pronase, and (c) collagenase were 6.3, 6.3, and 9.4ug/ml, respectively.

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