Colorimetric and Numeric Quantification of Novel MMP/HNE Protease Detection Technology

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INTRODUCTION

CLINICALLY ACTIONABLE BUT HARD TO MEASURE

Certain proteases like MMP2, MMP9 and Human Neutrophil Elastase (HNE) are known to play important roles in the normal wound healing process, while their elevated levels can lead to chronic or non-healing wounds. Current laboratory tests to assess the presence of proteases in wounds include gelatin zymography, measuring enzyme activity using azocoll, and through the use of enzyme-linked immunosorbent assays, all of which are time consuming, cumbersome, and/or expensive, and thus not used in clinical practice.

QUICK, POC, GRADIENT PROTEASE ASSESSMENT

One point-of-care (POC) diagnostic is commercially available in countries outside of the United States, but its results are binary (high/low), and requires several steps over 15 minutes. A newer technology has been developed that requires one step and 3 minutes to achieve a gradient-based colorimetric assessment. This gradient-based colorimetric assay (GBCA) result provides clinical insight regarding the direction of wound heading, helping inform and/or alter therapeutic interventions until elevated levels are more normalized, which could result in significant clinical and cost improvements.

MECHANISM TO ASSESS LEVEL OF ACTVITY

Introducing gelatinases such as HNE, MMP2 and/or MMP9 into the GBCA degrades a layer of gelatin coated, microscopic particles in a visibly pink suspension. As the coating degrades, the inner material is exposed, altering the color of the suspension. The higher the concentration of gelatinases in wound fluid, the darker the color change of the GBCA. Therefore, protease (gelatinase) activity can be proxied by the extent of color change of the GBCA. The degradation of the gelatin coating also causes the suspension to become more cloudy. This allows for the use of a handheld turbidity measurement device to obtain an alternative quantitative assessment of protease activity if desired.

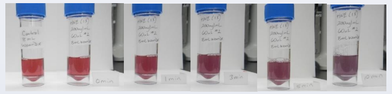
A recent study was conducted to qualify and quantify results using this new technology.

MATERIALS AND METHODS

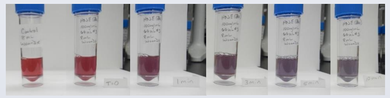
Six serial aliquots of serine proteinase Human Neutrophil Elastase (HNE) (Athens Research, Athens, GA) and one denatured sample were introduced into the novel GBCA to assess colorimetric changes at several time points (T=0, T=1 min, T=3 min, T=5 min, T=10 min). Images were taken to compare to baseline. GBCA analyte flocculation by HNE was also quantified using a handheld turbidimeter (Lutron Electronics Co., LTD, Taipei, Taiwan) for each dilution/time point.

One concentration each of matrix metalloproteinases MMP2 (Abcam, Inc., Cambridge, MA) and MMP9 (Millipore Sigma, Darmstadt, Germany) were introduced into the GBCA to obtain an initial indication of activity.

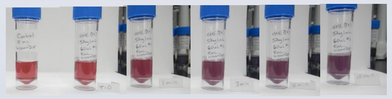
HNE - Dilution #1 - 1X concentration



HNE - Dilution #2 – 0.5X concentration



HNE - Dilution #3 – 0.25X concentration

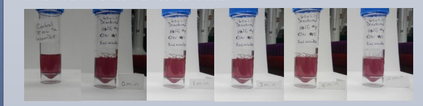


HNE - Dilution #4 – 0.125X concentration

HNE - Dilution #5 – 0.0625X concentration

HNE - Dilution #6 – 0.03X concentration

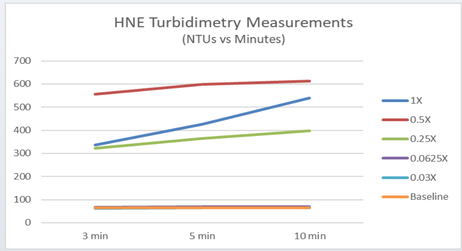
HNE – DENATURED – 1X concentration



Optical Color Assessment of HNE vs Baseline

As would be expected base on the GBCA method of action, as concentrations of HNE decreased, there was less of a color change relative to baseline. The one exception was Dilution #2, and this is believed to have been due to specific interaction with the buffer.

RESULTS

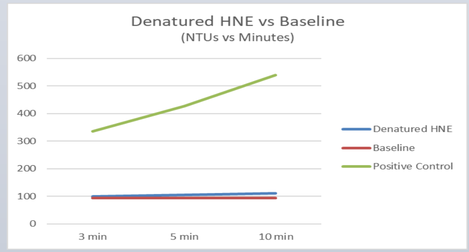


HNE Aliquot Turbidimetry vs. Time

1x is 100% HNE in a buffer solution, and each subsequent aliquot is a 50% reduction of HNE concentration.

Data indicates that the GBCA quantitatively tracks HNE activity (proxied by activated HNE concentration in buffered solution) and that the variation between the 3 and 10 minute interval is small (<<) compared to the measurement at 3 minutes.

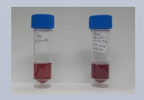
Note: it is theorized that the 1x concentration showed initial and final turbidity less than the 2x due to interaction with the buffer



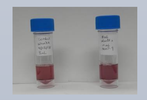
Denatured HNE Turbidimetry vs. Time

Data indicates that the GBCA detects only ***active*** enzymatic activity. This panel of the study attempted to assess whether the GBCA can distinguish between pro-forms or inactive forms of HNE (proxied in this study by denatured HNE).

MMP2



MMP9



Optical Color Assessment of MMP2 and MMP9 vs Baseline

The GBCA showed a slight color change for both MMP2 and MMP9. Based on a literature search, the amount of active MMP2 and/or MMP9 in solution was likely too low to generate a more significant color response.

CONCLUSION

STUDY RESULTS

This study demonstrates both the mechanism of action of a gradient-based colorimetric assay (GBCA) and provides preliminary validation of its capability to assess protease (gelatinase) activity in minutes at point-of-care. Generally, the higher the level of proteases, the darker the color and the higher the turbidimetry score. The one anomaly was the second highest concentration, which resulted in a darker color and higher turbidimetry score than the highest concentration. This is believed to be the result of interaction with the buffer, which at that specific ratio allowed the HNE to reach its highest level of activity within the solution. The denatured HNE aliquot showed no visual difference in color change relative to baseline, and the turbidity measures were in line with the baseline scores as well (some variation in color is believed to be related to insufficient denaturing technique). The GBCA can be observed grossly, visually assessing the extent of color change, or quantitatively, using a handheld turbidimeter.

While only one concentration level was used for each MMP2 and MMP9, the resulting slight color change of the GBCA does indicate a positive result.

Further study in chronic wound fluid is required in order to better characterize GBCA thresholds and gradations and to correlate GBCA results with healing outcomes.

IMPLICATIONS

Since the GBCA color change varies by level of protease activity, the velocity or change in protease activity can be assessed from episode to episode, giving clinicians for the first time ever an immediate potential indication at bedside of efficacy of therapy, the end point of particular therapy (such as protease modulators), and/or clinical insights to indication/contraindication of next therapeutic regimen.