PIUMA
NANOINDENTER

Piuma Nanoindenter Application note:

Measuring the mechanical properties of different concentrations fibrin clots

The Piuma Nanoindenter is specifically developed to non-destructively characterize the mechanical properties of soft materials, such as tissues, biomaterials, hydrogels and cells. Using a novel proprietary optical fiber-top technology, the Piuma provides great force sensitiviy, accuracy and precision while remaining very easy to use.

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Fibrin clots, a polymerized form of the fibrinogen protein, naturally occurs in the human body during blood clotting. During wound healing, fibrin clots provide a fibrous scaffold to prevent blood loss and promote tissue formation. As fibrin posesses strain-stiffening properties and withstands the shear stress caused by blood flow, the mechanical properties of fibrin clots are considered a crucial parameter in wound healing. This note describes the mechanical characterization of fibrin clots using the Piuma Nanoindenter.

Introduction

To examine the effect of different fibrin concentrations and their micro-structural organization, three different concentrations of fibrin, 0.5, 1, 2, 3 & 5mg/ml, are measured, and two micro-structural organizations, fine and coarse, are compared.

Methods and materials

The Piuma Nanoindenter is fitted with a 0.059N/m cantilever-based probe with an extending contact tip radius of 102µm. The displacement profile is set to displace 15 micron from the automated near-surface definition, in 2-3-4 second intervals for the loading, holding and unloading sections respectively.

Following the Herz model describing the indentation of elastic bodies, the data in the loading section of the load-displacement curve is used to determine the Youngs Modulus, using a fit of all datapoints from the contact point to the maximum load point [1].

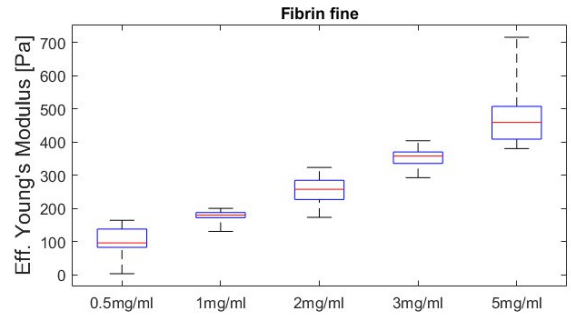
For these experiments, the Piuma Nanoindenter is placed on top of a regular tabletop surface; no special stabilization or dampening is used during the experiments.

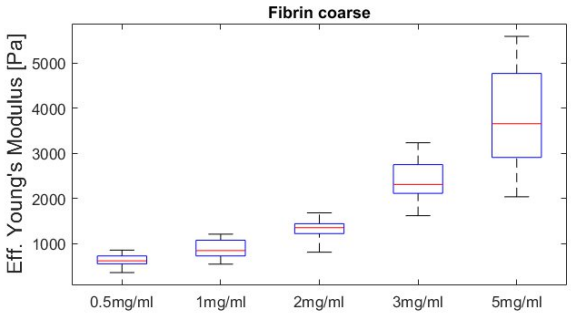
Sample preparation

Fine fibrin clots are formed by addition of 3.2mM Ca^{2+} and 0.5U/ml thrombin to freshly thawed fibrinogen in buffer (50nM Tris-HCL, 400nM NaCl, pH 8.5). For coarse fibrin clot formation at pH 7.4, 5 nM Ca^{2+} is used. Drops of 100-150 µL fibrin solution at different concentrations are pipetted in 35mm petri-dishes and polymerized at 37°C for at least 4 hours.

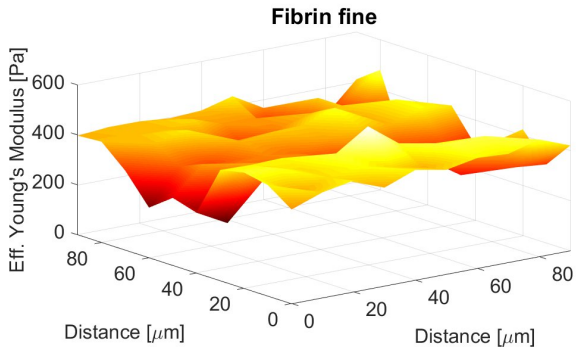
Results

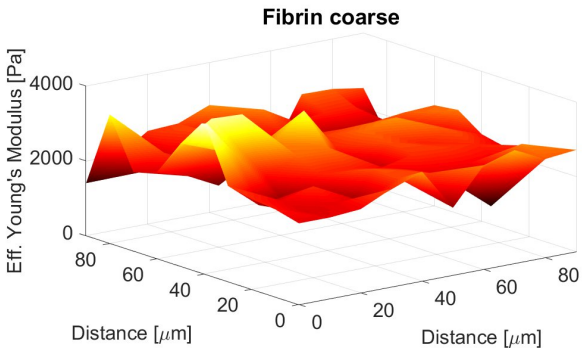
To obtain the elastic modulus, four points are measured with 8 indentations per point.





Additionally the spatial E-distribution of both 3mg fibrin clots are examined with the automated grid scan feature of the Piuma.





As shown in this application note, fibrin concentration and its polymerization microstructure have a significant effect on the mechanical properties of fibrin clots.

[1] Hertz, H. R., 1882, Ueber die Beruehrung elastischer Koerper , in Gesammelte Werke (Collected Works), Vol. 1, Leipzig, Germany, (1895)