Canada Wide Science Fair

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Emily Been and Joshua Thon from Calgary won this year's science fair award for their work on "The Fractal Geometry of Blood Clots."

Emily Been is a senior in the French immersion program at William Aberhart High School in Calgary, Alberta. Several of her interests include piano, choir, dance and volunteering at horseback riding. She is on the school cross country running team, participates in leadership events and organizes blood donation drives through the school. After high school Emily plans to study engineering in the research domain.

Joshua Thon is a grade twelve student from Calgary, Alberta. A self-described debate fiend, he enjoys participating in debates as well as teaching younger students how to debate. Joshua also enjoys playing the trumpet, which he plays in three school ensembles, whose focus ranges from jazz to classical music. He also takes great pleasure in informing himself on matters of world politics and science. After his graduation in June, Joshua plans to study engineering with an eventual specialization in biomedical engineering.

The full article is reproduced below.

The Fractal Geometry of Blood Clots*

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Editor's Note: The submission by Been and Thon was reformatted and edited to fit in to the Journal format.

1 ABSTRACT

We describe a simple method to characterize the fractal dimension of blood coagulation using high-resolution vital light microscopy. We also report that the fractal dimension is a dynamic process that can be influenced by external acoustic vibrations.

2 BACKGROUND INFORMATION

Fractal geometry is a scientific method for measuring, analyzing and categorizing physiological structures. The term fractal refers to a structure that exhibits self-similarity at a range of magnifications. Fractal dimension is a common measurement used to determine the complexity of a fractal shape, which is also how completely the shape fills the plane of view. The software we used, Benoit, uses the box-counting method to determine the fractal dimension. It uses a rotating grid to graph the number of boxes filled to box side length on an exponentially scaled graph. The slope of this graphed line is the fractal dimension.

Presently there are two main ways to assess blood clotting; bleeding time and viscoelasticity. Both are relatively insensitive. The bleeding time provides general information on both the integrity of the blood vessels as well as the coagulation pathways. Measuring viscoelasticity is a highly complicated procedure involving expensive equipment. [1]

Cymatics is the study of the effects of sound on physical substances. It has been shown to change the physical structure of crystals [2] and has several applications in medical practice. In the latter role it is used in diagnostics such as ultrasound imaging as well as therapeutically to fragment renal and gall bladder stones [3].

The proposed model of our experiment was to collect blood, apply a pure frequency as the blood clot develops, and then view and record clotting with a Richardson Light Microscope. We hypothesized that frequency would affect the complexity of the clot. We also examined the evolution of the complexity of the clot as it formed.

3 METHODS/EXPERIMENTS

Blood samples were imaged on a Richardson RTM-3 microscope equipped with a Sony 3CCD Exwave HAD camera [4]. The microscope is used to image living structures in their natural state and has high resolution (~100 nanometers) and suitable for imaging structures such as fibrin crystals. The subjects fasted overnight and were well hydrated. A finger was pricked with a BD GenieTM Lancet. The droplet of blood was transferred directly to a glass slide. Clotting was recorded by video microscopy, an example of which can be viewed online [5]. For the sound studies, control droplets were allowed to coagulate in the absence of sound. Other droplets were exposed to one of two different frequencies (170HZ and 14,846HZ). The sound frequencies were delivered through

Protek B-801 8 ohm speakers, directly mounted onto the stage of the microscope.

Photos were taken of areas of plasma between any sizable gap between red blood cells. (Fig. 1) Videos were used for dynamic fractal testing.

The images were edited using image J software and a custom made macro. To avoid bias all images were processed identically. These images were analysed with Benoit fractal analysis software, using the box counting method.

We then compiled the data into spreadsheets for statistical analysis. Comparison between the FD for different frequencies of sound were analyzed using ANOVA. A p value of <0.05 was considered to be significant.

4 RESULTS

The clots formed from elongate crystals of fibrin, best seen in the areas of plasma/serum between the red blood cells. (Fig 1). The crystals formed a complex mesh with fractal properties. The individual fibres of fibrin were approximately 70 nanometers in diameter at the midpoint. There appeared to be considerable individual variation in the thickness of the fibres as reported by others [6].

In control tests the fractal dimension was dynamic, increasing over 72.7 seconds before reaching a stable state (Fig. 2). The fractal dimension of the fibrin clot in the stable state was relatively constant for this individual. High frequency sound was associated with a higher fractal dimension compared to a low frequency sound (P=0.04) (Fig. 3), indicating that sound could induce changes in the complexity of the fibrin mesh.

5 DISCUSSION

This study used FD analysis to determine changes in the structure of a blood clot induced by sound. Dynamic analysis of blood clotting showed increasing FD over time with an S shaped curve. The presence of sound had a statistically significant effect on the formation of the fibrin mesh. An increase in FD corresponded to a higher frequency of sound. FD is likely to be influenced by fibrin strand width, amount of crossing of the crystals and amount of fibrin present. The FD may be useful for diagnosing clotting disorders, identifying risk for thrombosis and for monitoring the effects of pharmaceuticals that affect blood clotting.

Arguably the fibrin mesh is not a perfect fractal structure however the mesh is too irregular to be described with Euclidean geometry and exhibits self-similarity on a range of scales.

The theory we propose for why the sound affects the fibrin mesh is based on the nucleation of crystals [7]. Crystals start at random nucleation points when the particles of solute concentrate enough to start a crystal. When a vibration is present the likelihood of interactions between molecules should increase, creating multiple nucleation points and increasing the FD. We would also expect smaller crystals. In this study we did not measure the crystal length.

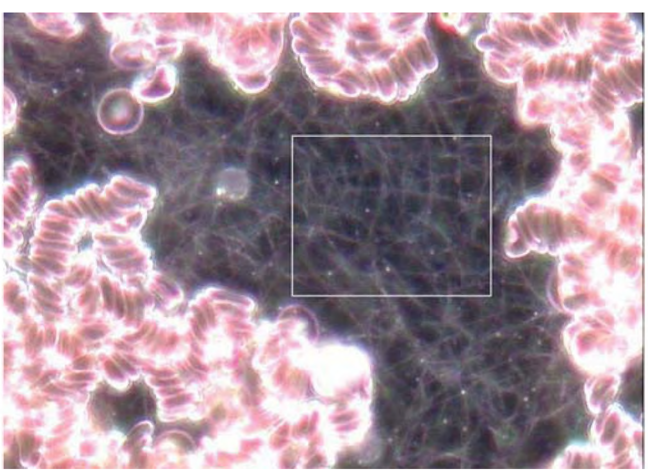

Figure 1. An example of an image used in the study. The inset box indicates how the images were cropped to sample only the fibrin mesh

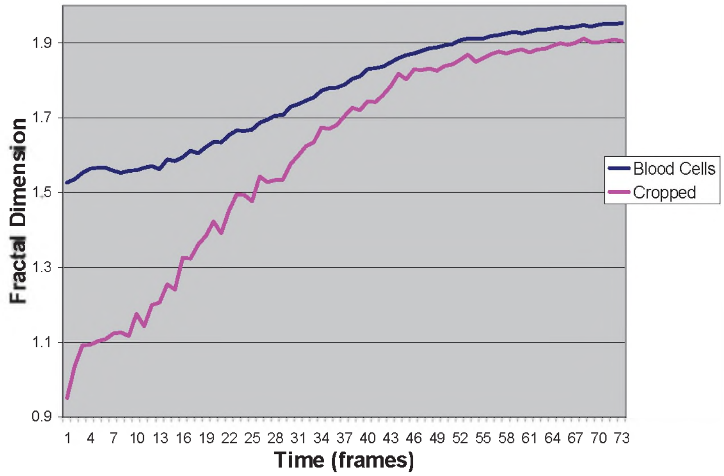

Fig. 2. Time lapse images were taken every second during the blood clotting process. Both the full image (that included blood cells) and a cropped image (see figure 1) of only fibrin were analyzed and their FDs plotted against time

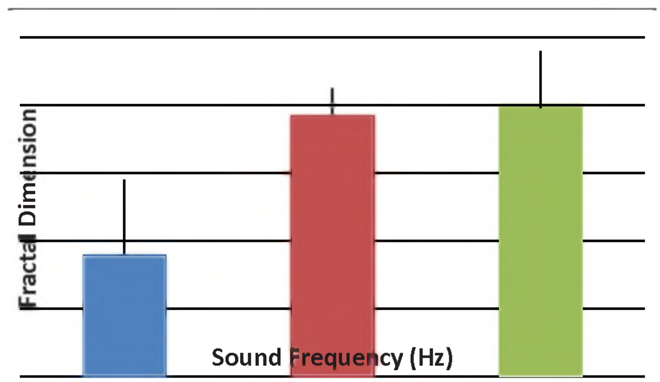

Fig 3. Blood was subjected to two frequencies of sound during the clotting process. Both frequencies increased the FD but only the highest frequency was statistically significant (P=0.04).

The fact that the box counting method was successful in measuring small changes in the blood clot is important. The box counting method is sensitive enough to measure minute changes in the blood clot caused by sound. These changes are not detectable with the naked eye. Because of this newfound possibility to observe exact and detailed changes in the complexity, it opens the door to a completely new type of diagnostic testing.

In summary we have shown that a blood clot has fractal properties and that sound vibrations affect it significantly. We have also shown that using the box counting method to determine fractal dimension is an accurate and effective way to measure changes in the complexity of blood clots. Compared to other methods of measuring clotting, the fractal analysis is relatively non-intrusive and could be developed into a rapid and sensitive test of complexity of the fibrin mesh. It thus has potential to be deployed in the clinical setting.

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