A New Instrument to Quantitatively Measure the Effect of Hemostatic Agents on Blood Clot Formation

Hemostatic agents (e.g. powders, adhesives and sealants) have been utilized for decades to control bleeding. The demand for these agents has recently grown as their application in many surgical procedures becomes the new standard-of-care. With that, the need for development of these agents also grows. However, progress, in terms of the capacity of state-of-the-art instruments and technologies to fully characterize these varied materials as they are developed, has not matched this need. The state-of-the-art instruments and test methods typically used to study these agents (e.g. rheometer, TEG, ROTEM, compression instruments, visual inspection; See Table 1), while they do allow for some analysis, do not provide for a comprehensive evaluation. Insufficiency of research tools for the testing of hemostatic agent prototypes and for the comparison of products currently on the market ultimately slows the technology development phase, and thus the time-to-market.

Full characterization of hemostatic agents includes analysis of 1) the general interaction of the agent with blood, 2) hemostatic effectiveness (i.e. time to form a clot and the mechanical strength of the formed clot), 3) the mechanism of action of the applied agents (i.e. understanding what leads to the formation of a clot and/or blood component aggregation), 4) blood uptake profiles (if applicable to test polymer), 5) biocompatibility (i.e. safety, immune response, healing response of the agent in the body), 6) clot swelling (i.e. volume change), 7) biodegradation profiles (i.e. duration of the material once exposed to physiological conditions, resorption).

ElastoSens™ Bio²: real-time, non-destructive and contactless instrument for mechanical monitoring of gelation and degradation kinetics of soft biomaterials.

Such a comprehensive evaluation is key to bringing products to market sooner, with decreased risk, and in a cost-effective way.

Here we introduce the ElastoSens™ Bio² as the new state-of-the-art tool for quantitative measurement of the effect of hemostatic agents on blood. Outlined are the test methods, applications specific to the ElastoSens™ Bio² (compared to the current state-of-the-art equipment), and how it offers a more effective solution to hemostatic agent characterization overall. This document also includes examples of how we used the ElastoSens™ Bio² to test and compare two commercially available hemostatic products.

HOW ARE HEMOSTATIC AGENTS CURRENTLY TESTED IN VITRO?

Visual inspection and manual time monitoring: limited technique and qualitative assessment

Traditional material testing instruments like rheometers and compression testers (e.g. DMA, indenters, etc.) can be difficult to use when testing certain forms of hemostatic agents and almost impossible in the case of hemostatic powders, which are widely used. Visual inspection of coagulation is thus the typical method used to qualitatively assess and compare hemostatic agents.

Visual inspection, while simple, does not provide for the comprehensive evaluation of hemostatic agents described above. Furthermore, this method only allows for estimated clotting times, qualitative comparisons of formed clots (e.g. strength), and qualitative predictions on mechanism of action of the agent. In addition, this method does not allow for analysis of the clotting profile over time or after exposure to simulated physiological fluids. Visual inspection can also be quite time consuming and may not allow for controlled comparative testing of replicate samples. Challenges in handling of samples (in test tubes etc.) may also limit the ability to use this method to answer dosing questions or confidently test comparator products head-to-head.

HOW DOES THE ELASTOSENS™ BIO² WORK?

ElastoSens™ Bio² is a benchtop instrument that measures the mechanical properties of soft materials (such as blood under the action of hemostatic agents) without contact, without destroying the sample and in real time. The instrument measures and displays the change in strength (shear elastic modulus, G') and viscous behavior (shear loss modulus, G'') of a clot as a function of time during its formation. The patented technology behind this instrument uses mechanical vibrations to interrogate the sample. The hemostatic agent/blood mix sample is placed into a detachable cylindrical well that has a flexible bottom. At each measurement point, a low amplitude vibration is applied to the well containing the sample. The sample displacement is remotely measured by an optical probe and processed to obtain the viscoelastic properties of the agent/blood mix (i.e. G' and G''). This process is sequentially repeated to characterize the mechanical evolution of the test sample. An air-coupled sensor measures the sample height in real time in order to precisely quantify the swelling of the hemostatic agent/blood mix sample. Temperature can be adjusted to match physiological conditions or to simulate extreme conditions for accelerated aging studies. The well that contains the sample can be easily detached from the instrument without handling the sample and reloaded at any time into the instrument. This allows for a re-measurement of the mechanical properties of the hemostatic agent/blood mix sample at any time. This feature is especially useful to study long term degradation of clots or the stability of the agent/blood mixture over time when exposed to simulated test conditions.

WHAT MAKES ELASTOSENS™ BIO² THE CHOICE INSTRUMENT FOR ANALYSING HEMOSTATIC AGENTS?

	Rheometer, TEG, ROTEM	Compression Instruments	Visual inspection	ElastoSens™ Bio²	How the ElastoSens™ Bio² does it?
Can measure rate of clotting, final clotting time and clot strength?	YES	NO	NO	**YES**	Elastic modulus of the hemostatic agent/blood product or clot is measured as a function of time.
Can precisely measure clotting kinetics on hemostatic powders?	NO	NO	NO	**YES**	The clotting reaction can be performed in the well with precise start times and progression monitored.
Is sample volume representative of real conditions?	NO	NO	NO	**YES**	ElastoSens™ Bio² may contain up to 7 mL of blood/agent mixture.
Except TEG/ROTEM, can measure multiple samples simultaneously?	NO	NO	NO	**YES**	ElastoSens™ Bio² can measure up to 3 different samples simultaneously.
Can measure long-term mechanical stability of fragile hemostat/blood gels?	NO	NO	NO	**YES**	The well containing the sample may be detached, stored out of instrument and re-tested over hours or days. Testing is non-destructive.
Can measure effect of simulated physiological fluids and time on hemostatic agent/blood clot?	NO	NO	NO	**YES**	Removal of the sample well allows for incubation of gels/clots in simulated physiological fluids (testing specific temp, pH, enzymes, etc.)
Can quantitatively test whole blood, plasma, PRP and PFP?	YES	NO	NO	**YES**	Clots are formed into the wells and tested during coagulation.

	Rheometer, TEG, ROTEM	Compression Instruments	Visual Inspection	ElastoSens™ Bio²	How the ElastoSens™ Bio² does it?
Can samples be tested non-destructively?	NO	NO	YES	**YES**	No contact with sample is required for measurements.
Can take measurements while maintaining sterility of test samples for long time?	NO	NO	YES	**YES**	Sample wells may be sterilized and instrument kept into a laminar flow hood.
Can measure clot swelling?	NO	NO	YES	**YES**	Sample height (volume) is measured in real time.
Can be easily operated with minimal training?	NO	NO	YES	**YES**	Test set-up an execution are simple and do not require expertise in mechanical testing.

HOW DOES THE ELASTOSENS™ BIO² MEASURE THE EFFECT OF HEMOSTATIC AGENTS ON BLOOD?

The tables below illustrate the measurements that can be obtained on hemostatic agents and blood using the ElastoSens™ Bio². The clotting of blood alone or of blood in the presence of the hemostatic agent can be compared side-by-side. Typical resultant profiles are shown (see below) and yield the following data:

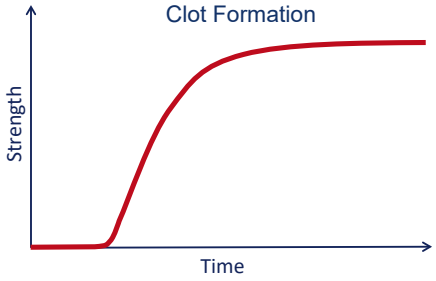
Clot formation: this graph gives the estimated clotting initialization (in seconds), the rate of clot formation (in Pascal/seconds), the final clot strength (in Pascal) and the estimated clotting time (in seconds);

Clot swelling: this graph displays the changes of sample height during the clot formation. It gives a precise measure of the initial height, the percentage of height change (if it changes), and how long it takes to complete swelling (in seconds);

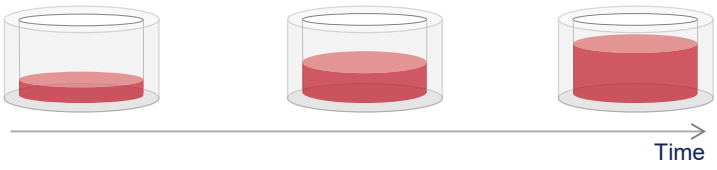
Clot degradation: It's possible to use the ElastoSens™ Bio² to non-destructively measure the long term evolution of clot mechanical properties and thus how stable the clot is over hours and days. The effect of exposure to simulated physiological fluids at different conditions (pH, Temp, enzymes) can be tested to predict resorption rates and/or general stability of the resultant clot/polymer over time.

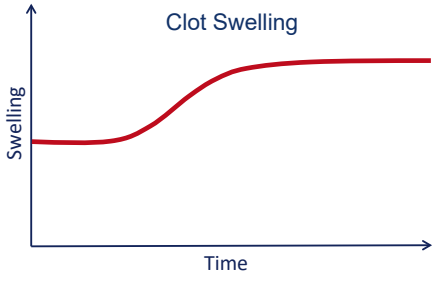
These valuable data couldn't be easily obtained with traditional tools and methods, if at all.

How Quickly the Fibrin Network Formation or the Polymerization is Initiated and Completed?

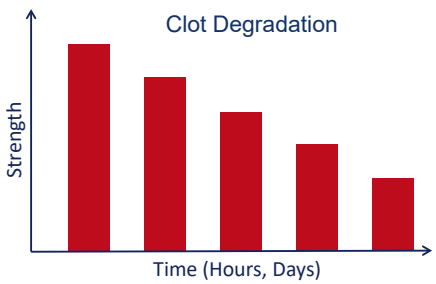


How the Clot Swells during Formation?



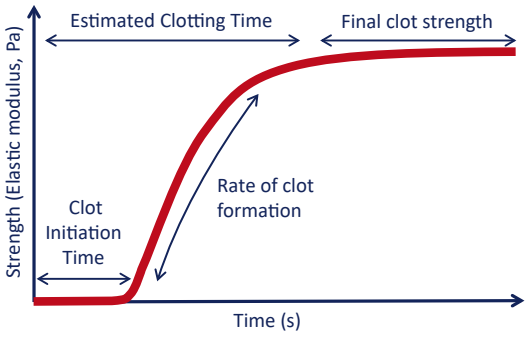


How Long Network Degradation Takes during Degradation?



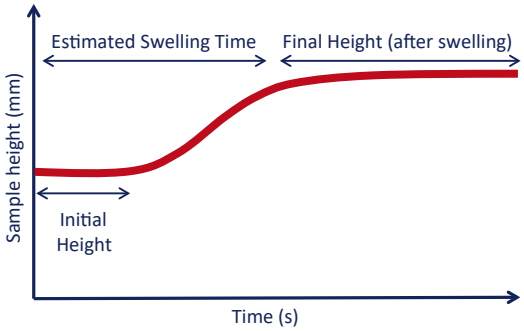
WHAT QUESTIONS THE ELASTOSENS™ BIO² IS ANSWERING?

CLOT FORMATION



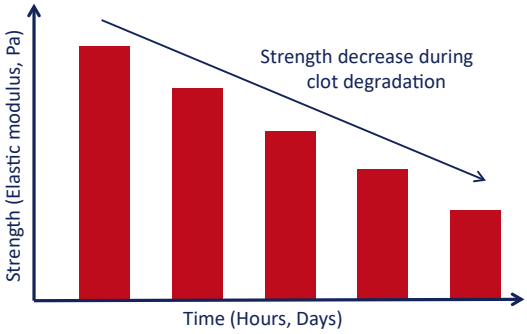
MEASUREMENTS	ANSWERED QUESTIONS
Clot Initiation Time	How many seconds before blood coagulation is initialized?
Rate of Clot Formation	How fast the clot is forming under the action of the hemostatic agent?
Estimated Clotting Time	How long it takes for the hemostatic agent to complete the clot formation?
Final Clot Strength	How strong and stable is the formed clot?

CLOT SWELLING



MEASUREMENTS	ANSWERED QUESTIONS
Final Height	What is the percent height increase of the blood/hemostatic agent mix due to swelling?
Estimated Swelling Time	What is the time that the blood/hemostatic agent volume maxes out?

DEGRADATION



MEASUREMENTS	ANSWERED QUESTIONS
Strength Decrease During Clot Degradation	How mechanically stable is the clot over time? How is the mechanical stability of the clot affected under simulated physiological conditions? How long does it take for the clot to mechanically degrade?

EXAMPLES: TESTING COMMERCIAL HEMOSTATIC AGENTS AND WHOLE BLOOD USING ELASTOSENS™ BIO²

Two commercially available hemostatic powders CELOX™ and QuikClot™ have been tested using the ElastoSens™ Bio². CELOX™ (MedTrade Products Ltd., Crewe, UK,) is a chitosan-based hemostatic powder used to treat bleeding wounds. QuikClot™ (Z-MEDICA, LLC, Wallingford, CT, USA) is a kaolin-based hemostatic agent. The hemostatic agents were disposed into the sample holders of the ElastoSens™ Bio² following different powder/blood weight dosages: 0%, 5% and 10% (w/w) for CELOX™ and 5%, 10% and 15% (w/w) for QuikClot™. Sample holders and hemostatic agents were pre-heated at 37°C into the thermally regulated chamber of the ElastoSens™ Bio². In the two examples, whole sheep blood in anticoagulant (Sodium Citrate) from Cedarlane (Burlington, ON, Canada) was first heated at 37°C in a water bath and then re-calcified by mixing with $CaCl_2$. A volume of 5 mL of re-calcified blood was than pipetted and introduced into the sample holders of the ElastoSens™ Bio² and the test was initiated. The instrument recorded the evolution of the shear elastic modulus (G') as a function of time during 40 minutes for CELOX™ and 20 minutes for QuikClot™.

The resultant clot formation curves provide important insights into action of the hemostatic agents and their effects on clotting kinetics (see curves and table below). The data suggest the following:

• Clot Initiation Time (reaction of fibrinogen polymerization) is quantitatively measured by the ElastoSens™ Bio². It decreases when the dosage of CELOX™ increases while it is relatively stable when the dosage of QuikClot™ increases.

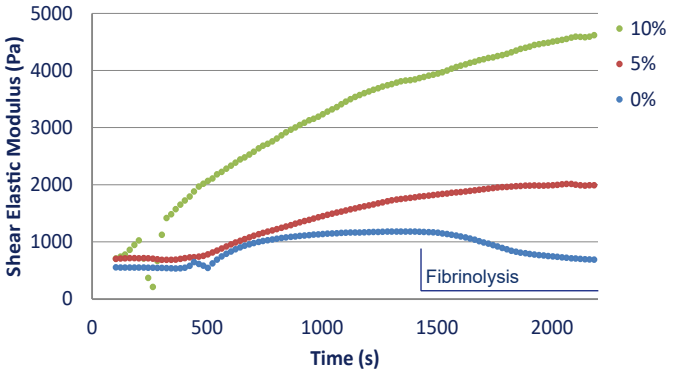
• Increasing the ratio of hemostatic agent to blood (w/w) results in increased clot strength for both hemostatic agents.

• The ratio of hemosatic agent to blood (w/w) affects the maximum rates of clot formation for CELOX™ but does not seem to significantly change those of QuikClot™.

• Testing the coagulation of re-calcified whole blood with no hemostatic agent provides a baseline (blood only control) to compare to sample containing the hemostatic agent. The coagulation kinetics of blood with 0% CELOX™ exhibits fibrinolysis (breaking down of fibrin network) after 1,500 s.

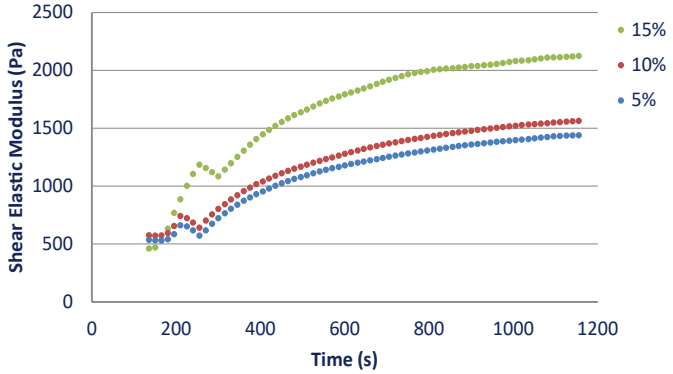
• These two hemostatic agents do not yield changes in sample height, suggesting no significant clot swelling of the

CELOX™

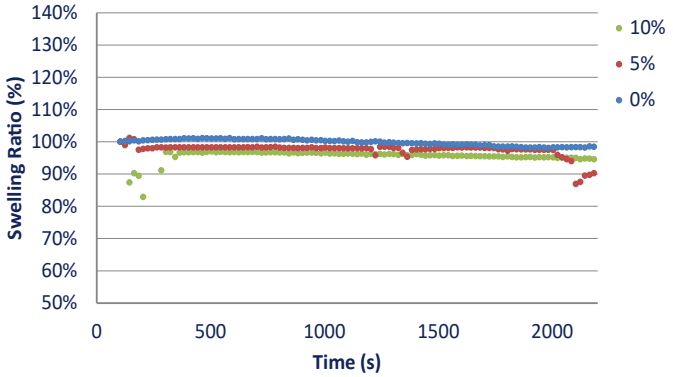


Elasticity profiles of blood clots as a function of time at different concentrations of CELOX™ hemostatic agent.

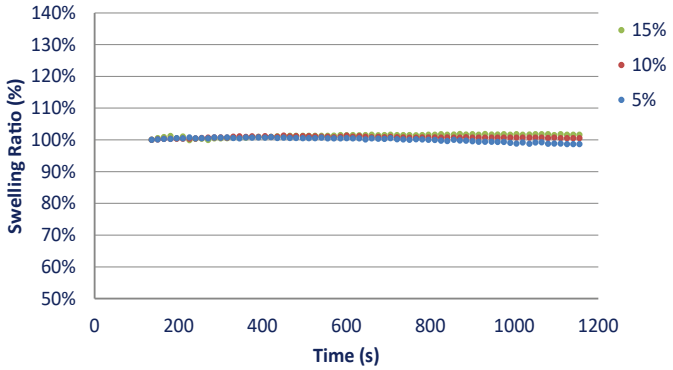
QUIKCLOT™



Elasticity profiles of blood clots as a function of time at different concentrations of QuikClot™ hemostatic agent.



Evolution of swelling ratio of blood/agent mix samples as a function of time at different concentrations of CELOX™ hemostatic agent.



Evolution of swelling ratio of blood/agent mix samples as a function of time at different concentrations of QuikClot™ hemostatic agent.

	CELOX™			QUIKCLOT™		
Ratio of hemosatic agent to blood (w/w)	**0%**	**5%**	**10%**	**5%**	**10%**	**15%**
Clot Initiation Time (s)	442	404	225	226	210	255
Maximum rate of clot formation (Pa/s)	3.4	1.8	20.7	3.0	3.0	3.6

Notice: The data reported in this document are presented to illustrate the capabilities of the ElastoSens™ Bio² using commercially available hemostatic agents. The experiments performed to obtain these data have note been designed to perform a comparative study.

APPLICATIONS OF THE ELASTOSENS™ BIO²

In the context of hemostatic agents, the ElastoSens™ Bio² may be used in three main business sectors:

1) R&D & Product Development: superior quantitative data to better evaluate product prototypes and accelerate research;

2) QC & Manufacturing: improve quality control processes, strengthen documented traceability, optimize costs and qualify suppliers;

3) Preclinical Studies: simulate *in vivo* conditions to better predict outcomes and adapt to clinical research conditions. All kind of animal blood as well as human blood can be tested on the instrument.

ElastoSens™ Bio² has the potential to help streamline and build on each business phase to ultimately optimize benefits offered to patients.

R&D and Product Dev.

- Understand and optimize the mechanism of action (MoA) of hemostatic agents
- Quantitatively compare formulations and define dose effects (ratio of agent to blood)
- Optimize the clotting time and rate of clot formation
- Optimize blood/agent clot strength to meet application requirements
- Measure clot formation and cross-linking kinetics
- Evaluate MoA of competing products and assess comparator products
- Generate data for regulatory pre-submission and marketing
- Simulate *in vivo* conditions
- Assess blood coagulation under varied blood chemistries

QC & Manufacturing

- Transfer testing protocols from R&D to QC labs
- Quantify coagulation parameters to yield documented product specs
- Improve traceability and documentation of processes
- Ensure batch-to-batch consistency, conformity and quality
- Qualify ingredients and suppliers
- Establish performance criteria
- Adjust dosage of ingredients and reagents to meet required performances
- Optimize costs and production processes
- Troubleshoot production issues

Preclinical Studies

- Correlate with *in vivo* conditions
- Compare the action of different hemostatic agents
- Assess efficacy of hemostatic agents in coagulation-deficient blood (e.g. coagulation disorder patients)
- Study hemostatic agent efficacy under different anti-coagulant regimens
- Compare *in vitro* testing of clot stability and degradation with *in vivo* data
- Perform testing using patient derived blood (and pre-op, pre-surgery donors)
- Perform research for hemhorrage treatment

Benefits to Patients

There are 3 Easy Ways to Test the ElastoSens™ Bio²

1 On-site demo of ElastoSens™ Bio² in your lab

2 Mail us your samples and we will use our Application Lab

3 Visit us and lets test your samples together in our Application Lab

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