

Mechanical Monitoring of the Degradation of Biomaterials by ElastoSens™ BIO²

How to control the mechanical degradation of biomaterials?

ElastoSens™ BIO²: the first contactless & non-destructive mechanical testing instrument

To monitor degradation rate for drug delivery and tissue engineering



degradation of hydrogels, biomaterials rheology, non-destructive & contactless testing

SUMMARY

Functional and stimuli-responsive hydrogels are promising materials in biomedical applications. They can be used as scaffolds to fill tissue defects, as cell carriers in tissue engineering and as delivery systems of bioactive molecules. They have several advantages as biomaterials. Their mechanical properties are comparable to those of soft tissues in the body and are highly biocompatible. These features can reduce dramatically the risk of rejection and improve the efficiency of treatments.

The mechanical degradation or dissolution of hydrogels is as important as their gelation kinetics. For example, in tissue engineering, the biodegradation rate of the hydrogel matrix has to be tailored to correspond to the rate of new tissue formation (i.e., production of extracellular matrix and growing cells). Slow degradation rate can eventually reduce the formation of new tissue. Similarly, the degradation rate in drug delivery has to be modulated according to the treated disease, the pharmacokinetics of the bioactive molecule and the type of stimulus-responsive hydrogel. The development of modulated and slowly degrading hydrogels as drug delivery vehicles is necessary for the treatment of chronic diseases, such as diabetes and cardiac dysrhythmia.

Drug release and degradation processes change the mechanical properties of hydrogels. Thus, measuring the evolution of mechanical characteristics quantitatively and precisely during the degradation is important for the



ElastoSens™ BIO²: real-time, non-destructive and contactless instrument for mechanical monitoring of gelation and degradation processes of hydrogels.

development of efficient and bioacceptable biomaterials. Conducting the mechanical measurements *in vitro* simultaneously with the stimuli is also important. These stimuli can be chemical (pH, enzymes, specific molecules,..) or physical (temperature, light, electric field,...).

Currently, soft hydrogels are mechanically characterized mainly by two techniques: rotational rheometry and compression tests. These techniques have the same main limitations:

- Measurements are destructive.
- Long term measurements cannot be done on a same sample.
- Hydrogel samples are in contact with sensors thus it is very challenging to ensure sterile testing conditions.
- Coupling mechanical measurements with stimuli is difficult and costly.
- Interpreting the results of compression tests (force/displacement curves) in biomedical context is hard.

To overcome these limitations, Rheolution Inc. has developed ElastoSens™ BIO², the first mechanical testing instrument allowing:

- To characterize in real time and non-destructively the viscoelasticity of biopolymers during the gelation and degradation.
- To measure the mechanical properties of biomaterials without contact and under sterile conditions.
- To use removable sample holders to follow the evolution of the same sample over long periods of time.
- To immerse the hydrogel sample within the sample holder in physiological liquids to simulate chemical stimuli.
- To couple physical stimuli with mechanical testing.

This application note presents the use of ElastoSens™ BIO² to characterize the viscoelastic evolution of a chitosan-based hydrogel during enzymatic degradation (by papain).

ELASTOSENS™ BIO²: PRINCIPLE & COMPARISON

ElastoSens™ BIO² is a benchtop instrument that measures without contact, non-destructively and in real time the shear storage (G') and loss (G'') moduli of gels as function of time and temperature. The patented technological principle behind this instrument is based on an original approach using propagative mechanical waves. The sample is contained into a cylindrical cuvette having a flexible bottom part. A small and gentle vibration of a few micrometers is applied to the sample and its response is measured using a laser probe without contact. Finally, the material response is processed to obtain the viscoelastic properties of the gel (i.e. shear storage and loss moduli). This process is repeated to characterize the mechanical evolution of the studied material. In addition, the instrument allows connecting probes to perform simultaneous and complementary measurement (such as pH) on control samples.

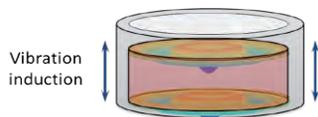


Fig. 1 Principle of ElastoSens™ BIO²: Non-contact and non-destructive measurement of few micrometers amplitude vibrations

ElastoSens™ BIO² delivers viscoelastic information that is identical to classical rheometry, the storage and loss shear moduli, but without contact. Figure 2 shows a comparison of the results of the gelation of agar (Noble Agar, Sigma, A5431, 1.4% (w/w)) measured by ElastoSens™ BIO² and a rotational rheometer.

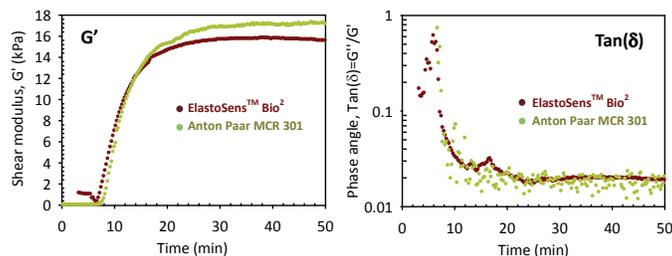


Fig. 2 Comparison of ElastoSens™ BIO² with rotational rheometry.

EXPERIMENTAL PROTOCOL AND RESULTS

A chitosan-based hydrogel was prepared and tested during the gelation and degradation using ElastoSens™ BIO². Degradation was induced by the hydrolytic action of papain enzyme to simulate the gastrointestinal digestion of hydrogel matrices. Such studies are useful for pharmaceutical applications since it helps to formulate biomatrices with controlled degradation rates.

An aqueous acid solution of chitosan (Marinard Biotech, Rivière-au-Renard, QC, Canada) was mixed with a gelling agent to induce gelation of the polymer (final concentration 2% w/w). After rapid mixing, the solution (2 mL) was poured into the ElastoSens™ BIO² sample holder, which was then placed in the thermal chamber of the instrument set to 37°C. A thin film of distilled water was added to cover the sample and avoid drying. The measurement of viscoelastic properties was started 10 min after the addition of the gelling agent and continued for 60 min. After this, the digestion solution (acetate buffer at pH 4 containing 5 mM L-cysteine and 0.5 U/mL papain) was added and then removed after 10 minutes. Then, the mechanical measurements were continued for another 60 min. A reference sample was studied using the same protocol but adding distilled water instead of digestion solution.

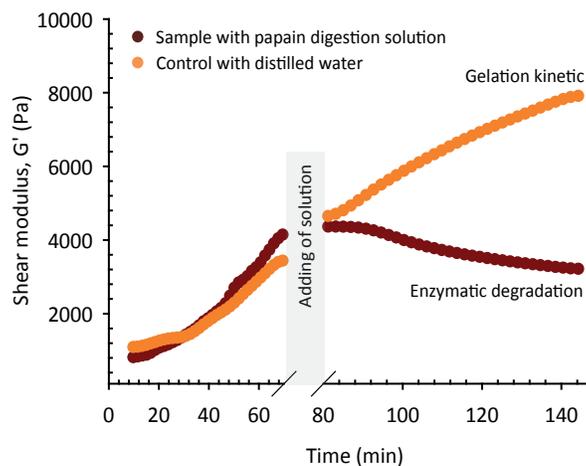


Fig. 3 Gelation and enzymatic degradation kinetics of a chitosan hydrogel measured by ElastoSens™ BIO²

Figure 3 shows the temporal evolution of the shear storage modulus (elastic modulus) of the two chitosan hydrogel samples, measured by ElastoSens™ BIO². The gelation kinetics during the first 60 min is nearly identical for both samples. Adding distilled water does not change the gelation process and storage modulus of the reference sample continued to increase (increase in G' by 70 % in 1 h). However, when papain solution was added, the sample started to lose its mechanical strength

(decrease in G' by 25 % in 1 h). Chitosan, which is a copolymer of glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) linked by beta-1,4-glycosidic bonds, can be degraded by nonspecific enzymes. Among such enzymes, papain depolymerizes chitosan efficiently, mainly on GlcN-GlcNAc bonds, yielding low-molecular-weight chitosans and chitoooligosaccharides. As a result, the solubility of chitosan increases and its viscosity decreases. In a hydrogel, this leads to poorer mechanical properties and eventually dissolution of the gel. We can conclude that the decrease in mechanical properties measured by ElastoSens™ BIO² is directly related to the degradation initiated by the action of the enzymatic solution. The instrument was able to measure this effect without altering the micro-structure of the hydrogel.

CONCLUSION AND APPLICATIONS

This interesting application shows the capability of ElastoSens™ BIO² to monitor the mechanical evolution of hydrogels over time under the action of chemical stimulus. Measurements were conducted non-destructively and without contact. The instrument has an innovative feature of removable and sterilizable sample holder that can be stored in a controlled environment (e.g. incubator, under cell growth medium) to induce long-term physical and biochemical changes. The sample can then be mounted in the instrument at any time and as often as desired to periodically perform new mechanical characterization. Moreover, the ElastoSens™ BIO² can be coupled to external probes that simulate physical stimuli during the mechanical testing of the biomaterial.

ElastoSens™ BIO² is particularly suitable for mechanical characterization of gel formation and degradation kinetics. Its versatility and unique technical features make it a very useful research and quality control tool in pharmacology, tissue engineering and regenerative medicine. In tissue engineering, one of the most interesting applications of the instrument is the *in vitro*, real-time and non-destructive measurement of the mechanical evolution of hydrogels seeded with cells.

In the context of the characterization of hydrogels degradation, ElastoSens™ BIO² is a unique, precise and non-destructive tool for the design and optimization of biomaterials for drug delivery and tissue regeneration. Moreover, an accessory designed to test simultaneously three samples is available to significantly improve the statistical significance of measurements.

All these capabilities are made possible by the new technology of ElastoSens™ BIO². An instrument designed to overcome challenges in the development of biomaterials and their medical implementation.

ACKNOWLEDGMENT

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Spec. of ElastoSens™ BIO²

Mechanical & Physical	
Shear Elastic Modulus	0 Pa to 100 MPa
Precision	0.1 Pa
Simultaneous	3 samples (optional)
Swelling measurement	YES
Temperature	
Temperature range	15°C to 50°C (optional 5° to 50°C)
Precision	0.5°C
Programmable ramps	YES
Temporal	
Temporal resolution	1 second to 120 minutes
Measurements duration	10 seconds to 1200 hours
ElastoView	
Interface	Simple interface with touchscreen
Software	Test settings, post-processing, real time results display, archiving and data transfer



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