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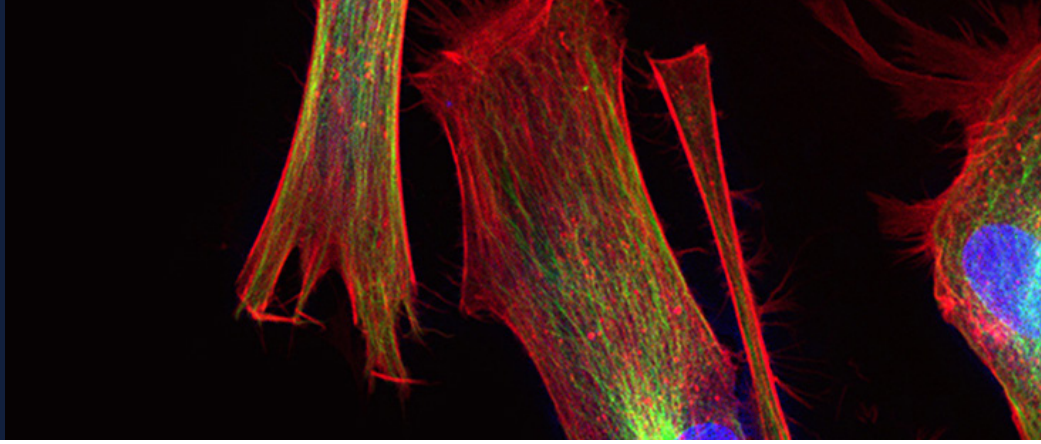
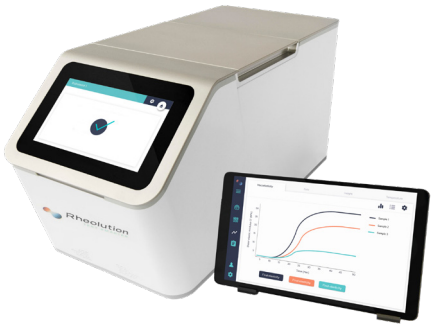
LIFE SCIENCES



Application note | ElastoSens™ Bio

Gel formulation and long term mechanical stability analysis of cellularized gelatin-based hydrogel using ElastoSens™ Bio



ELASTOSENS™ BIO

TISSUE ENGINEERING

HYDROGEL FORMULATION

TIME MEASUREMENTS

BIOLOGICAL MATERIALS

BIO CHEMISTRY

This is a short report of a study performed by Maryam Jahanshahi, David Hamdi, Brent Godau, Ehsan Samiei, Carla Liria Sanchez-Lafuente, Katie J. Neale, Zhina Hadisi, Seyed Mohammad Hossein Dabiri, Erik Pagan, Brian R. Christie and Mohsen Akbari at the University of Victoria (British Columbia, Canada) entitled A*n Engineered Infected Epidermis Model for In Vitro Study of the Skin's Pro-Inflammatory Response* published in 2020 on the Micromachines journal (11(2), 227).

SUMMARY

— The sensitivity of conventional instruments is often an issue to precisely measure the soft nature of hydrogels.

— ElastoSens™ Bio has shown to provide a reproducible and sensitive testing of the gel formation and mechanical stability through the viscoelastic properties of a cellularized gelatin-based hydrogels.

— The fast optimization of a crosslinking agent (5 U/mL of transglutaminase) was performed by evaluating the storage modulus during gelation.

— The fast optimization of the polymer concentration (20 % gelatin) in the hydrogel was performed by evaluating the storage modulus during gelation and culture time using the same set of samples.

— The selected gel formulation was further used for the preparation of an epidermis model.

INTRODUCTION

Cellularized hydrogels have been widely investigated for producing *in vitro* models of tissues such as skin, blood vessels, bone, etc. These models can be a valuable alternative to animal models used in trials for studying physio/pathological processes and for testing new drugs and medical devices [1]. The formulation of hydrogels, including parameters such as polymer concentration and crosslinking agent, needs to be optimized for each specific application. The optimization normally considers the requirements of the manu-

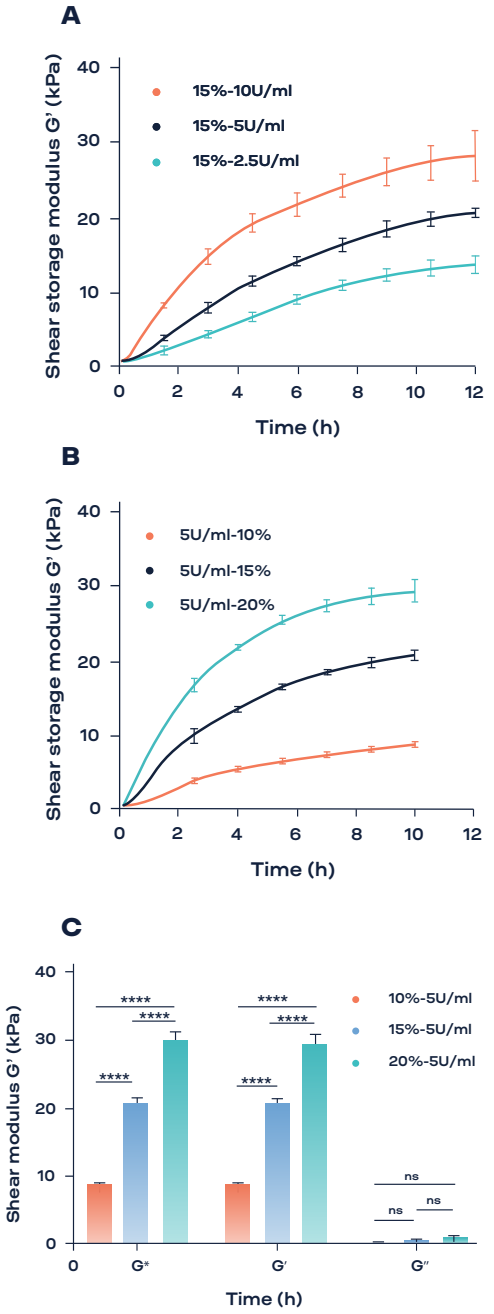


Fig. 1: A) The evolution of the shear storage modulus (kPa) as a function of time of 15% (w/w %) gelatin crosslinked with 2, 5, and 10 U/mL of TG. B) The time evolution of the shear storage modulus (kPa) of 10 %, 15 %, and 20 % (w/w %) gelatin with 5 U/mL of TG. C) G*, G' and G'' of 10 %, 15 % and 20 % gelatin. (n=3).

facturing processes (e.g. 3D bioprinting, electrospinning, biocasting), the cytotoxicity and the mechanical properties and stability of the hydrogel. Conventional techniques used for assessing the mechanical properties of hydrogels such as rheometer, tensile and compression tests are often destructive and prevent the re-use of samples for further characterizations. Furthermore, long term studies over days or weeks require multiple samples which can be a major limiting factor specially when they contain cells.

In this short application note, ElastoSens™ Bio was used to evaluate the crosslinking kinetics of gelatin hydrogels with transglutaminase (TG) at different concentrations of the polymer and the enzyme [2]. Then, the mechanical stability of a set of gelatin hydrogels (with the optimal crosslinker concentration) seeded with keratinocytes was evaluated using the ElastoSens™ Bio under sterile conditions during 14 days of culture. This stability study aimed to identify the optimal hydrogel formulation that served to develop an infected epidermis model and to investigate the inflammatory response of keratinocytes.

MATERIALS AND METHODS

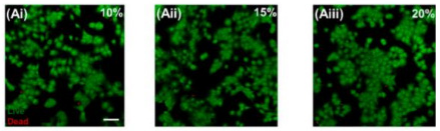
Gelatin powder dissolved in PBS (10 %, 15 %, 20 %, w/w) were enzymatically crosslinked with different concentrations of TG (2.5, 5, and 10 U/mL) at 37 °C for 12 h. Mechanical stability studies were performed with immortalized human keratinocytes seeded on the top of the gels over 14 days of culture. All the gels were prepared in the sample holders of the ElastoSens™ Bio and measurements over time were performed on the same samples under sterile conditions. Gels were maintained between tests in a biological incubator (37 °C, 5 % CO_2) with culture medium.

RESULTS AND DISCUSSION

The amount of enzyme was optimized by real-time measurements of the shear storage modulus over crosslinking time (12 h in total). Fig. 1A shows that the shear storage modulus of 15 % gelatin hydrogels increased with the amount of enzymes (2.5, 5, and 10 U/mL). The condition of 10 U/mL led to the highest storage moduli values; however, it was also responsible for the highest variation in the measurements associated with a fast and inhomogeneous gelation and difficulties in sample manipulation. Therefore, the condition of 5 U/mL was selected for the following experiments. The concentration of gelatin (10, 15 and 20 %) was then varied with the optimized amount of enzyme. Fig. 1B shows that the storage modulus increased with the increasing concentration of gelatin due to the higher densities of carbonyl and amino groups available for bonding to each other. Following the completion of gelation, the complex (G*) and loss (G") moduli (Fig. 1C) of the hydrogels showed dominant solid-like behavior at all concentrations ($\tan(\delta) < 1$).

Keratinocytes were then seeded on top of the different hydrogels (10, 15 and 20 % of gelatin with 5 U/mL of TG) (Fig. 2A) and the storage modulus was measured over 14 days of culture (Fig. 2B). Fig. 2B shows that the 10 % gelatin gel lost its integrity after 7 days of incubation suggesting that degradation was higher than ECM production. For 15 % gelatin, the hydrogel maintained its integrity for 14 days, although its storage modulus decreased by 50 %. On the other hand, the storage modulus of 20 % gelatin remained unchanged over 14 days of culture with respect to the storage modulus of unseeded gelatin. Interestingly, this condition (20 % gelatin) was the one that most matched the human skin storage modulus (40-60 kPa [3]). Due to the more physiological storage modulus and the mechanical stability over time, the concentration of 20 % gelatin was selected for the preparation of the epidermis model. In the study, the model was infected with Escherichia coli to investigate the inflammatory response of keratinocytes by measuring the expression level of pro-inflammatory cytokines. The authors mentioned that the model has great potential for modeling wound infections and drug testing.

A



(Ai) 10 % (Aii) 15 % (Aiii) 20 %

B

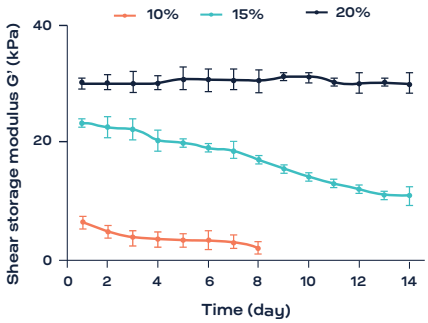


Fig. 2: A) Representative live/dead fluorescence images of HaCaT cells on the top of the hydrogels containing 10% (i), 15 % (ii), and 20 % (iii) of gelatin after 1 day of culture. B) Storage modulus of 10 %, 15 %, and 20 % gelatin during cell culture (n = 3).

CONCLUSION

The optimization of the crosslinking agent (5 U/mL of TG) and gelatin (20 % w/w) concentrations was performed through the analysis of their viscoelastic properties during gel formation. The evaluation of the mechanical stability during 14 days of gelatin hydrogels seeded with keratinocytes with the ElastoSens™ Bio assisted in the selection of the gel formulation used in the epidermis model.

PERSPECTIVES

— ElastoSens™ Bio is a versatile and easy-to-use instrument that measures the viscoelastic properties of bioengineered tissues over short and long periods of time.

— It is now possible to test the same bioengineered tissue over long periods of time without destroying or infecting the sample. The statistical significance of long term studies is greatly improved.

— Testing the viscoelasticity of biomaterials on ElastoSens™ Bio and keeping the samples between tests in a biological incubator allows the easy application of various conditions to the biomaterial in order to simulate *in vivo* environments.

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