New Instrument for Real-Time Monitoring of Viscoelasticity of Soft Biomaterials and Engineered Tissues

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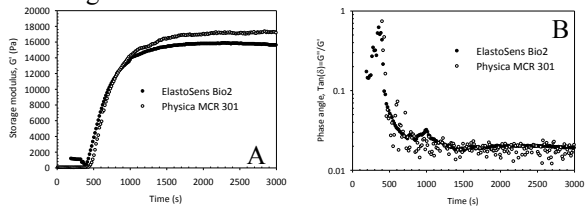
Statement of purpose: The development of biomaterials and engineered tissues are reshaping the future of medicine. Soft and injectable materials are increasingly used to treat pathologies, release drugs and/or seed cells for tissue engineering. Mechanical properties of a biomaterial are critical for its functional efficiency. However, the non-destructive mechanical characterization of soft biomaterials, especially in presence of cells, is still a challenge. The aim of this work was to validate the use of a new instrument, ElastoSens™ Bio2 (Rheolution Inc., Montréal, QC), that measures in real-time, non-destructively and without contact the evolution of rheological properties of viscoelastic biomaterials during reticulation kinetics and tissue culture. This study introduces this new technology and compares it with classical rheometry. The gelation kinetic of various hydrogels was studied by both techniques in parallel. The cytocompatibility of the instrument's sample-holder was verified. Finally, preliminary results of viscoelastic measurements performed on a three-dimensional culture of fibroblasts in hydrogels during culture period are presented.

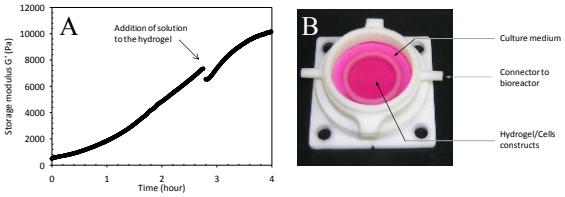
Materials and methods: ElastoSens™ Bio2: The principle of this instrument is to induce the linear resonance of a small amount of material (2mL) contained into a detachable and sterile sample-holder having a soft and flexible bottom allowing the material to vibrate and resonate. A laser measures without contact the resulting vibrational response in order to extract the rheological properties of the sample. A controlled thermal module is used to impose a stable temperature to the sample. The ElastoView™ user interface allows to select experiments settings and to manage measurements. Rheometry: measurements were performed using a Physica MCR 301 rotational rheometer (Anton Paar, Germany) equipped with a plan-plan geometry. Cytotoxicity assays: The cytotoxicity of the flexible silicone membrane of ElastoSens™ Bio2 sample holder was evaluated on 1, 3, 7 and 14 days extracts using L929 fibroblast cells following the ISO 10993-5:2001 standards. After 24 h of exposition to the extracts, the cells were exposed to Alamar Blue VR for 4 hours to estimate cell viability. Hydrogels preparation: Agar was employed for validation phase. 1.4% (w/v) of aqueous solution of agar was heated to 80°C prior to viscoelastic measurements with Elastosens™ Bio2 and the rheometer. Measurements have been performed simultaneously on three samples using the two instruments at the same temperature with a time step of 15s. Cell culture and encapsulation in chitosan hydrogels: Sterile polymer solutions were combined with L929 to encapsulate them at a final density of 20M cells/ml. Constructs were then poured into the sterile sample holder and cultured at 37°C in DMEM-F12 culture medium. The sample holder was then closed with an autoclaved lid to keep the sample sterile for cell culture.

Results: Results of rheometry and ElastoSens™ Bio2 showed that final values and evolution of storage (17133±1365 Pa and 15579±583 Pa respectively) and loss (352±60 Pa and 583±162 Pa respectively) moduli during the gelation kinetic were in very good agreement for both technologies.



Comparison of storage modulus (A) and phase angle (B), during gelation kinetic of agar gel measured by rheometry and Elastosens™.

Cytocompatibility of ElastoSens™ sample holder: Cells exposed to the silicone membrane extracts presented 100±5% of viability compared to the control group. This suggested a high cell compatibility of the device with cell culture condition. Based on these results, three-dimensional cell culture assays were designed by combining mouse fibroblasts and hydrogels. Results show that G' of fibroblasts/chitosan constructs increases during 3 hours and stabilize near the 4th hour.



A. Evolution of storage modulus measured by ElastoSens™ during gelation process of chitosan thermogel seeded with cells (T=37°C). B. Sample holder containing seeded hydrogel.

These results permitted us 1) to characterize gelation behavior of 3D cell/matrix constructs 2) to determine the final rheological moduli and 3) to follow viscoelastic properties at several times of cell culture period.

Conclusions: These preliminary study presents for the first time a new approach to characterize viscoelastic properties of biomaterials in real-time during cell culture period. The non-destructive approach of Elastosens™ Bio2 will permit to characterize, over short and long periods of time, on the same sample, the mechanical evolution of hydrogels seeded with growing cells. A study of viscoelastic properties evolution in hydrogels during mesenchymal stem cells differentiation into chondrocytes is currently under investigation in our group. While further validation is needed, this new tool should permit us to define the best culture conditions and anticipate the time of culture necessary to reach viscoelastic properties matching with those of cartilage. Overall, this new tool will help in developing and monitoring more efficient tissue engineering strategies which aim to replace and repair human tissues.

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