Validation and application of a nondestructive and contactless method for rheological evaluation of biomaterials

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Abstract: Hydrogels are extensively used for tissue engineering, cell therapy or controlled release of bioactive factors. Nondestructive techniques that can follow their viscoelastic properties during polymerization, remodeling, and degradation are needed, since these properties are determinant for their in vivo efficiency. In this work, we proposed the viscoelastic testing of bilayered materials (VeTBiM) as a new method for nondestructive and contact-less mechanical characterization of soft materials. The VeTBiM method measures the dynamic displacement response of a material, to a low amplitude vibration in order to characterize its viscoelastic properties. We validated VeTBiM by comparing data obtained on various agar and chitosan hydrogels with data from rotational rheometry, and compression tests. We then investigated its potential to follow the mechanical properties of chitosan hydrogels during gelation and in the presence of papain and lysozyme that induce fast or slow enzymatic degradation. Due to this nondestructive and contactless approach, samples can be removed from the instrument and stored in different conditions between measurements. VeTBiM is well adapted to follow biomaterials alone or with cells, over long periods of time. This new method will help in the fine tuning of the mechanical properties of biomaterials used for cell therapy and tissue engineering. © 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2016.

Key Words: mechanical characterization, hydrogels, viscoelastic properties, nondestructive methods, VeTBiM


INTRODUCTION
Hydrogels are increasingly used to treat various pathologies and to regenerate tissues. Their biocompatibility makes them particularly appealing as drug and cell delivery systems, materials for bioprinting or injectable scaffolds for tissue engineering. In vitro characterization of mechanical properties is needed due to high cost of biomaterials development, and to predict in vivo behavior. More specifically, the characterization of mechanical properties is important since they play a key role in device functionality. For example, the rate of release of entrapped drugs or cells from hydrogels is strongly dependent on their molecular weight cutoff, their crosslink density, and thus, their mechanical properties. In tissue engineering and bioprinting, matching the mechanical properties of the replaced tissue is important to foster its regeneration and to ensure sufficient resistance to in vivo stress. Moreover, the mechanical properties of hydrogels are susceptible to change due to their degradation induced by enzymes and/or their remodeling due to cell invasion, growth, and extracellular matrix deposition. In all these cases, it is often essential to characterize the evolution of a biomaterial’s mechanical properties over time, possibly in the presence of encapsulated cells. Several techniques (rheometry, quasi-static or dynamic tensile or compression tests, indentation, and so forth) can evaluate the mechanical properties of hydrogels and tissues. Unfortunately, these techniques are destructive, due to the extent of mechanical stimulation being applied to the sample during the test. Sterility also remains a challenge with long term cell culture studies due to direct contact

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**EXPERIMENTAL**

**VeTBiM: principle and characteristics**

VeTBiM is a measurements method that characterizes in real time, without contact, and nondestructively, the viscoelastic modulus of gels as a function of time or temperature. It has been implemented in the analytical instrument Elasto-Sens™ Bio (Rheolution, Montréal, Canada). More specifically, the instrument measures the storage ($G'$) and loss ($G''$) moduli of the complex linear shear modulus of gels. Materials may be mechanically tested using VeTBiM in three configurations:

1. During a liquid-to-solid phase transition (case of a hydrogel during gelation or polymerization kinetics);
2. In steady solid state (case of a solid viscoelastic hydrogel evolving in time);
3. During a solid-to-liquid phase transition (case of a melting soft material or a degrading hydrogel).

Unlike rotational rheometry, compression, confined compression, tension, indentation, and dynamic mechanical analysis, VeTBiM uses low amplitude vibrations as mechanical stimulants to characterize materials. A laser is used to carry out a contactless measurement of the dynamic response of the material to vibrations (Fig. 1). These two important characteristics of VeTBiM allow the testing of soft and fragile materials without contact and nondestructively.

The sample to be tested has to be poured in a liquid or viscous state (before gelation/polymerization) into a detachable sample holder having a flexible elastomeric membrane.
at its bottom. The mechanical flexibility of the membrane allows the sample to remain strictly in contact to form a composite (bilayered) and soft body capable of reacting to external vibrations once material is poured into sample holder. The membrane has two functions: (1) to prevent any leakage of the material in its liquid or soft solid state and (2) to confer a relatively low flexural rigidity to the sample, allowing its vibration.

When a test is started, a low frequency and amplitude vibration is conveyed to the sample holder which is attached to a vibrating unit. The composite body formed by the sample/membrane system will freely vibrate as a consequence of the dynamic excitation and its inertia. A one-dimensional (1D) charged couple device (CCD) reflective laser allows the measurement of the displacement of the membrane from its free bottom boundary during the vibration of the composite body. The measured temporal displacement signals are then processed in the Fourier (spectral) domain to extract the mechanical resonance characteristics of the bi-layered system which are closely related to the viscoelastic properties of the gel. Knowing the geometry and dimensions (i.e., volume) of the sample, the mechanical and dimensional characteristics of the flexible membrane, as well as the excitation signal, the vibration of the composite body is affected by the only unknown parameter: the viscoelasticity of the sample. A theoretical model is then used to calculate the storage and loss moduli of the material.17

Sample holders can be stored in an incubator for long-term studies. To avoid dehydration of the sample or to induce its enzymatic degradation, liquid solutions can be poured on the top of the sample during storage (between mechanical tests). During long-term degradation studies supernatant and fluid degradation products have to be removed from the sample holder before each measurement in order to prevent any biased measurement.

The dimensions of the cylindrical sample holder are 19.0 mm in height and 22.1 mm in inner diameter. The sample to be tested has to be pourable and have a volume ranging from 0.5 to 7.0 mL. The VeTBiM method implemented on ElastoSens™ Bio allows storage moduli measurement from 10 Pa to 100 MPa. Typically, the composite body’s response to the stimulus is affected by the changes in the material’s elasticity and viscosity. This physics is exploited in VeTBiM to measure the sample’s viscoelasticity at each measurement point during a phase transition kinetic or at a steady physical state. However, the initial height and, consequently, volume of the sample may vary depending on the available amount of material and possible shrinkage or swelling during gelation or material loss during degradation. Using a ultrasonic probe, the system enables to measure the actual height of the sample in real time at each measurement point during kinetics in order to take into account the effect of this important and changing dimensional property on the mechanical measurements. It is assumed that the sample remains roughly cylindrical and well coupled to the holder boundaries during long term degradation studies. In this kind of study, every sample is uniquely labeled to be identified by the instrument software when reloaded in the instrument for testing. This traceability allows the precise monitoring of height (volume) changes over long periods of time.

Materials and methods

Experimental steps. Two different hydrogels were used to study the ability of VeTBiM to characterize the viscoelastic properties of hydrogels, their gelation kinetics and their degradation process: a) agar gels, and b) various chitosan-based thermosensitive hydrogels prepared with different gelling agents known to influence their mechanical properties and gelation kinetics.18 As a validation step, we first compared the rheological properties of agar gels obtained with rotational rheometry and with VeTBiM during their gelation process. This part of the work was done with agar hydrogels since they have the advantage of having an easily reproducible preparation and gelation during cooling. Secondly, the shear storage modulus, \( G' \), of various chitosan-based hydrogels were studied and data were compared with unconfined compression test data. Finally, the degradation of these hydrogels with papain (rapid degradation, 1 h) and with lysozyme (slow degradation, over 1 week) was performed to evaluate the ability of VeTBiM to follow the fast and slow kinetics of mechanical changes over time.

Hydrogels preparation. Preparation of agar hydrogels. Agar hydrogels were prepared by solubilizing agar powder (Sigma, St. Louis, USA) in milli-Q water at 1.4% (w/v). The solution was heated to 80°C under agitation with a magnetic stirrer for 10 min before being used for viscoelastic measurements in both instruments.

Preparation of chitosan hydrogels. Preparation of chitosan solution: A chitosan solution (3.33% w/v) was prepared by solubilizing chitosan powder in 0.1M hydrochloric acid. Two different sources of chitosan were used: (1) HDDA MMw: Chitosan with high degree of decacylation (DDA, 94%) and medium molecular weight, Kitomer (ref PSN 326–501, MarinardBiotech, Gaspe, Canada). (2) LDDA MMw: Chitosan with low DDA (70–85%) and medium molecular weight (ref 448877, Sigma, St. Louis, USA).

The chitosan solutions were homogenized overnight with a magnetic stirrer, sterilized by autoclave at 121°C in a sealed container for 20 min, and stored at 4°C before use.

Preparation of gelling agent (GA): B-glycerophosphate (BGP), sodium hydrogen carbonate (bicarbonate, NaHCO₃, hereafter SHC) and phosphate buffer (PB, pH = 8, prepared with Na₂HPO₄ and NaH₂PO₄ at a ratio of 0.932/0.068 in milli-Q water) were combined at different concentrations to obtain seven different formulations containing the following final concentrations of gelling agents: 1) BGP 0.4M, 2) BGP0.1M + SHC0.05M, 3) BGP0.1M + SHC0.075M, 4) PB0.04M + SHC0.05M, 5) PB0.04M + SHC0.075M, 6) PB0.08M + SHC0.05M, 7) PB0.08M + SHC0.075M.

Preparation of hydrogels: Chitosan and gelling agents were filled in separate syringes connected by a Luer lock connector for mixture at a volume ratio of 3:2, respectively. Immediately prior to use, the contents of the syringes were...
mixed and tossed from side to side for 15 repeats to get a homogenous solution, as already described in our previous work.18 Hereafter, these chitosan hydrogels (containing 2% (w/v) chitosan) are named according to their final concentration in gelling agent, that is, BGP0.4, BGP0.1:SHC0.05, BGP0.1:SHC0.075, PB0.04:SHC0.05, PB0.04:SHC0.075, PB0.08:SHC0.05, PB0.08:SHC0.075.

**Mechanical testing.** VeTBIM: 2.0 mL of hydrogel solution was poured in the detachable sample holder especially designed for VeTBIM. To avoid perturbations due to the presence of liquid, supernatant was removed before testing when present on the top of the hydrogel. The sample holders were firmly attached to the instrument (ElastoSens™-Bio, Rheolution, Montréal, Canada) before measuring \( G' \) and \( G'' \). Free resonances of the samples in the linear viscoelastic regime were observed between 20 and 200 Hz. For long-term experiments (more than one day), the sample holders were detached from the instruments and incubated at 37°C between measurements.

Rotational rheometry: Rotational rheometry was performed using a Physica MCR 301 (Anton Paar, Ostfildern-Scharnhausen, Germany) equipped with a parallel plate geometry (P25/P2). It was used to compare viscoelastic properties during gelation of agar (1.4% w/v, preheated at 80°C) with results obtained from VeTBIM. Agar is a thermo-sensitive hydrogel, liquid at high temperature, which is able to form a gel when cooled down, with a sol-gel transition between 32 and 47°C, depending on its concentration.19 As the cooling rate can have a strong impact on polymer network formation (and therefore on mechanical properties), we applied exactly the same temperature profile in both instruments when comparing rotational rheometry with VeTBIM. To that end, we first monitored the evolution of temperature in agar reference samples in VeTBIM using a thermocouple. The recorded temperature profile was then applied in the rotational rheometry. Preheated agar was introduced by pipetting directly in the gap of the geometry (1.00 mm) and excess matter was removed. Measurements of \( G' \), \( G'' \), and Tan \( \delta \) were performed in the linear-viscoelastic region, at 1 Hz, at a 0.1% strain rate, and at different repetition measurements rates (only at the end of gelation, every 15 s, or every 3 min). Results were recorded with the Rheoplus software (Anton Paar, Ostfildern-Scharnhausen, Germany).

Compression tests: Unconfined compression tests on chitosan hydrogels were also performed using the Physica MCR 301 equipped with a parallel plate (P25/P2). 2.0 mL of chitosan hydrogel was introduced into cylindrical containers (14 mm diameter) and incubated at 37°C (corresponding to sol-gel temperature transition of chitosan hydrogels). To avoid drying, 2.0 mL of PBS was added on the top of each hydrogel after 1 h of gelation. Samples were incubated for 24 h until complete gelation. Before the test was performed, supernatant was removed and the samples were gently taken out of the container. Compression was applied at a constant rate of 0.5 mm/s (corresponding to 4.5%/s), until reaching 50% deformation. The corresponding secant Young’s modulus \( (E) \) and \( G' \) were calculated at 5% deformation using the following formulas20:

\[
E \text{ (Pa)} = F/S \times 1/D \quad (1)
\]

\[
G' \text{ (Pa)} = E/3 \quad (2)
\]

where \( F \) is the force in Newton, \( S \) is the surface in m², and \( D \) is the deformation in mm/mm.

**Enzymatic degradation of chitosan hydrogels.** Kinetics of gelation and papain degradation: 2 mL of chitosan hydrogel solution (formulation: PB0.04:SHC0.075) was poured into the sample holder and placed in the prewarmed (37°C) thermal chamber of Elastosens Bio. A thin film of distilled water was added to cover the sample and avoid drying. Measurements of rheological properties started 10 min after the mixing of chitosan and gelling agent, and were taken for 60 min. Next, a papain enzymatic solution (from papaya latex, 0.5 U/mL in 0.1M acetate buffer at pH 4 supplemented with 5 mM of L-cysteine, all from Sigma, St. Louis, USA) was added on the surface of the gel and removed after 10 min, enough time for the solution to diffuse and to induce the degradation. \( G' \) was then measured again for another 60 min. In a reference sample, water was used instead of papain solution. Experiments were performed on three different samples and controls.

Lysozyme degradation experiments: Two different chitosan lots, respectively with high (94%) and low (70–85%) degrees of deacetylation, were used to prepare PB0.04:SHC0.075 hydrogels. Their degradation was then evaluated (1) by measuring their rheological properties and (2) by weighing samples over time. To that end, 2.0 mL of each hydrogel were introduced in the sample holder and kept at 37°C for gelation. After 1 h, 2.0 mL of PBS was added on the top of each hydrogel to avoid drying. The hydrogels were again incubated at 37°C. After 24 h, the PBS supernatant was removed, and measurements were done by VeTBIM to obtain their initial \( G' \) at day 1. The samples were randomly divided into two groups, which received either PBS (control groups) or lysozyme (human lysozyme, Sigma, 1 mg/mL in PBS). The samples were incubated at 37°C for 7 days. VeTBIM measurements were performed every day, with the supernatant carefully removed before measurements. Fresh PBS or lysozyme solution was added after measurements, and the sample holders were replaced in an incubator at 37°C between tests. In parallel, weight loss during degradation was measured using an analytical balance (Mettler Toledo, model ML104, Toronto, Canada), with a readout accuracy of 0.0001 g. Each VeTBIM sample holder was weighed before and after adding chitosan hydrogels, and every day to monitor mass changes.

**Statistics.** Results are expressed as mean ± standard deviation (SD). A statistical analysis of the data was performed using GraphPad Prism software (Version 5, GraphPad Software, La Jolla, USA) with a two-way ANOVA and post-
Bonferroni’s test for comparison of more than two groups. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION
Validation of VeTBiM measurements
VeTBiM was first validated by comparison with rotational rheometry. Both instruments were used to follow the viscoelastic properties of agar during cooling.

During rotational rheometry testing, shear deformation is induced at each measurement. This may influence the polymer network formation, as is reported in the literature. To evaluate this effect, and prevent it as much as possible, we tested the effect of measurement repetition frequency on agar gelation in the Physica MCR 301 rheometer.

As a control, agar was introduced in the gap of the rheometer geometry and allowed to gel without measurement, and thus, without mechanical stimulation, during the entire gelation process. Measurements were then performed only during the last 10 min (Fig. 2, with cross). The experiment was then repeated, but measurements were carried out every 15 s (dash line) or every 3 min (black circles). Interestingly, $G'$ was lower when measurements were taken every 15 s. On the other hand, results after complete gelation were similar when the agar was measured every 3 min or only during the last 10 min of gelation. Thus, we assumed that a time step of 3 min avoids any mechanical influence on hydrogel network formation during measurements, and this condition was used for the comparison with VeTBiM.

Figure 3 shows the superposition of $G'$ (Panel A), $G''$ (Panel B), and tan $\delta$ (Panel C) obtained by VeTBiM and rotational rheometry during agar gelation. The evolution of $G'$, $G''$, and tan $\delta$ were in very good agreement for both instruments. For example, after 50 min of gelation, viscoelastic data measured by VeTBiM and rheometry at the plateau were respectively, $G'$: 15.6 ± 0.6 kPa and 17.1 ± 1.4 kPa, $G''$: 0.4 ± 0.1 kPa and 0.6 ± 0.2 kPa, and tan $\delta$: 1.9 ± 1 and 2 ± 0.3, without any significant difference between the two techniques. Both methods showed excellent reproducibility as attested by standard deviations ($n = 3$), which tend to be lower with VeTBiM than with rotational rheometry for $G'$ measurements (0.6 and 1.4 kPa, respectively, after 50 min of gelation), and slightly higher for $G''$ (0.2 and 0.06 kPa, respectively) and tan $\delta$ data (1 and 0.3, respectively).

Evaluation of VeTBiM sensitivity
Measurement of shear storage modulus after gelation. VeTBiM performance was also evaluated with seven formulations of thermosensitive chitosan hydrogels known to present different mechanical properties and gelation kinetics. Figure 4 presents the shear storage modulus $G'$ obtained by VeTBiM after 1 and 24 h of gelation at 37°C. Significant differences in terms of $G'$ were observed among these hydrogel formulations. Moreover, the data provides an interesting insight into the evolution of the mechanical properties over time, indicating whether or not gelation is complete. Thus, polymer gelation was not completed after one hour for the BGP01:SHC005, BGP01:SHC0075 and PB004:SHC0075 formulations, according to the statistical
differences of $G'$ measured after 1 and 24 h of gelation. Conversely, $G'$ of BGP0.4, PB004:SHC005, PB008:SHC005 and PB008:SHC0075 were not statistically different between 1 and 24 h of gelation, suggesting that gelation was already nearly complete after 1 h. These data are in agreement with our previous studies.\textsuperscript{18}

Comparison with compression tests. The same chitosan hydrogels, after 24 h of gelation, were used to compare $G'$ values obtained by VeTBiM with those calculated from unconfined compression tests ($G = E_5/3$) (Fig. 5). Results are in relatively good agreement, with no statistical differences, excepted for the PB004:SHC0075 formulation [Fig. 5(A)]. There is a clear correlation ($R^2 = 0.8317$) between the results obtained by the two methods [Fig. 5(B)].

Gelation and degradation kinetics of biopolymers

Real-time measurement of rapid gelation and degradation kinetics. The ability of VeTBiM to follow the kinetics of gelation or degradation of hydrogels was evaluated by measuring $G'$ of chitosan hydrogels (formulation PB004:SHC0075) during gelation at 37°C (Fig. 6, Curve A) and degradation after immersion in papain, an enzyme capable of cleaving chitosan molecular chains (Fig. 6, Curve B). PBS was used as a control (Fig. 6, Curve C). A continuous and progressive increase of the hydrogel $G'$ was observed during gelation, reaching 3200 ± 300 Pa after 60 min at 37°C (Curve A). After immersion in papain, $G'$ of the hydrogels slowly decreased to 2700 ± 900 Pa (Curve B), while $G'$ of the controls immersed in PBS continued to increase to 7600 ± 300 Pa (Curve C) after 60 min.

Long-term mechanical monitoring of hydrogels degradation. To evaluate the potential of VeTBiM to follow the mechanical properties of hydrogel on long-term course studies, we monitored viscoelastic changes occurring in the presence of lysozyme, and compared the data with results obtained by sample weighting. SHC0075PB004 hydrogels made with chitosan having a high (HDDA) and low (LDDA) degree of deacetylation were tested, since they are supposed to have different degradation kinetics. The evolution of their $G'$ and weight over 7 days are presented in Figure 7(A). HDDA and LDDA hydrogels were stable over 7 days in PBS according to their constant $G'$ during the time course (between 9750–9850 and 10,300–11,500 Pa, respectively).
When immersed in lysozyme, they presented a continuous degradation over time according to a progressive decrease of $G'$. The degradation kinetics between chitosan types slightly differed, with HDDA hydrogels being more stable initially, but then degrading faster than LDDA ($3090 \pm 569$ and $4829.9 \pm 523$ Pa at 7 days, respectively). The appearance and volume of samples in the holder was not affected despite the decrease of $G'$. In parallel, weight measurements did not show weight loss during the experiment [Fig. 7(B)]. After 7 days, all hydrogels were recovered from the sample holders and observed macroscopically. HDDA and LDDA immersed in PBS conserved their original shape after removal from the sample holders, while those immersed in lysozyme were difficult to handle, and were clearly degraded, as suggested by VeTBiM data [Fig. 7(C)].

**DISCUSSION**

The goal of the present study was to evaluate the potential of VeTBiM as a new method for the characterization of soft biomaterials such as hydrogels. First, we validated the method by comparing data obtained during agar gelation with that obtained from rotational rheometry. The direct comparison showed similar data and standard deviations for the two instruments. We also measured the mechanical properties of different thermosensitive chitosan hydrogels that possess various mechanical properties and gelation kinetics. VeTBiM was able to discriminate their mechanical properties and was in accordance, though not perfectly so, with the results from compression tests after 24 h gelation. The difference seen could be explained by the nonlinear behavior of hydrogels and/or their variability. VeTBiM provided an interesting insight into the evolution of mechanical properties with time, and thus on gelation kinetics. Due to its nondestructive nature and the possibility to remove sample holders from the instrument between measurements, it was used to study the evolution of the shear elastic modulus of various formulations between 1 and 24 h of gelation at $37^\circ$C, as measured on the same samples. This ability is particularly interesting in the context of biomaterials and tissue engineering. Indeed, a detachable sample holder can dramatically reduce the number of samples in comparison to most conventional characterization methods, which require producing samples for each time point. For example, Huang et al. evaluated the effect of mesenchymal stem cell culture on the mechanical properties of agarose hydrogels during cell growth and extracellular matrix deposition. To

**FIGURE 7**. *In vitro* degradation of SHC0075PB004 hydrogels prepared with 94% DDA chitosan (HDDA, full lines) and 85% DDA chitosan (LDDA, dotted lines), after immersion in PBS (black lines) or lysozyme solution (gray lines): (A) shear storage modulus, $G'$; (B) weight (mean ± SD, $n = 5$; curves were obtained with polynomial interpolation, degree 4); (C) pictures of hydrogels removed from sample holders, after 7 days in lysozyme (+) or PBS (−).
that end, they prepared between 168 and 240 samples (4 time points \(\times 7–10\) replicates \(\times 6\) conditions) and tested 42 of them at each time point by unconfined compression tests.\(^{15}\) With a nondestructive method such as VeTBiM, the number of samples could be reduced fourfold, while the number of time points could be increased to allow a well-defined kinetics study. Moreover, by following the same samples over the entire time period, the statistical strength is increased as compared to testing independent samples through different time points. This new technique can be used to monitor the degradation of mechanical properties over short and long periods of time, as shown here with chitosan gels subjected to papain and lysozymes, respectively.

Furthermore, the rate and extent of biodegradation are critical design considerations for hydrogels in tissue engineering. While short-term degradation by papain could be studied by other methods, such as rheometry, monopolarizing a rheometer for a degradation test over one week is not practical.\(^{13}\) Interestingly, VeTBiM was able to detect the progressive loss of mechanical properties of chitosan hydrogels due to lysozyme degradation over one week, while no weight loss was detected. This could be explained by the fact that, at this initial stage of degradation by hydrolysis, these physical hydrogels keep their cohesiveness and hydrophilicity despite the decrease in molecular weight and chain interaction.\(^{22}\) While weight loss is a common and interesting method for following the biodegradation of biomaterials,\(^{23–25}\) it does not necessarily correlate with mechanical properties. Most applications in tissue engineering emphasize following global mechanical properties than simple weight loss, since the rate of scaffold degradation should mirror the rate of new tissue formation.\(^{26}\)

The use of VeTBiM for tissue engineering studies is possible due to the possibility of introducing liquids, such as culture media, in the sample holder and moving them in incubators or other controlled environments. This opens the perspective of monitoring the mechanical properties of 3D scaffolds containing cells. Such techniques are still needed in laboratories in order to optimize their long-term efficiency. To the best of our knowledge, only a few systems currently exist; these include the Biodynamic bioreactor chambers (Texas Instruments), which combine mechanical simulation, flow perfusion and measurements of mechanical properties during cell culture in incubators, or custom-made systems using indentation tests specifically adapted for the characterization of thin hydrogel films.\(^{27,28}\) However, these systems are booked during the whole period of the study, while VeTBiM enables the testing of a large number of samples from various experiments in parallel. VeTBiM also presents some limitations. The sample has to be liquid or of limited viscosity during its introduction into the sample holder to ensure good contact with the flexible membrane, which is responsible for transmitting the vibrations. This excludes the testing of precast gels. Another minor limitation concerns the need to remove excessive liquids from the sample holder before measurements since they can disturb signals, and may affect the final results. More generally, any defective mechanical contact between the sample and the sample holder or the membrane may induce unreliable measurements due to an affected vibrational response. Finally, at this point, the sample holders are adapted only for static culture media, which is not optimal for 3D cell culture. Investigations are in progress to optimize the system for cell culture, in order to allow the sample holders to remain sterile and to be plugged into a bioreactor to allow dynamic cell culture conditions.

**CONCLUSION**

In this work, we have evaluated the performances of VeTBiM in measuring and following the mechanical properties of hydrogels over short- and long-term periods of time, without contact and in a nondestructive way. This new tool could help both fine-tune the mechanical properties of biomaterials, and evaluate their gelation or degradation rates, possibly in the presence of entrapped proliferating cells for tissue engineered constructs.

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