Biosensors and Bioelectronics 198 (2022) 113831

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Ultrasonic microrheology for *ex vivo* skin explants monitoring: A proof of concept

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ARTICLE INFO

Keywords:
Ex vivo studies
Soft matter
Biophysics
Skin viscoelasticity
Ultrasonic microrheology

ABSTRACT

As an answer to alternative non-animal testing, biosensors dedicated to the *ex vivo* skin explants monitoring are a challenge to study physiological-like behavior and optimize new topical products. Because of the skin viscoelastic behavior, mechanical tests are commonly based on macroscopic measurement and give global descriptors of its state. Other techniques, including photoacoustic ones, are more focused on the molecular scale. There is a gap to fill in the mesoscopic range to get information about the microstructure of the skin. This article presents the proof-of-concept of a biosensor coupling a thickness shear-mode transducer with human skin explants kept in life-like state for a week. Thanks to a multifrequency analysis of the transducer impedance, this biosensor is able to monitor the viscoelastic properties of the skin. To extract the complex shear modulus and the microstructural evolutions, a mechanical model based on fractional calculus is used. As a preliminary results, the sensitivity of the sensor to probe the skin viscoelasticity in lifelike state and the impact of its culture medium are presented. A suitable microstructural coefficient is also extracted in order to identify mechanical breaches in the skin barrier after the application of peeling products.

1. Introduction

The development of innovative materials in contact with humankind requires a tight control to prove its harmlessness regarding health conditions. This is particularly true for chemicals, according to the REACH regulation enforced since 2007 (Parlement Européen, 2007). For dermocosmetics, animal testing is banned (European Comission, 2013a) and clinical tests are strongly regulated. In addition, cosmetic product claims must be proved in terms of efficiency, disclosure, and natural-based formulation (COLIPA, 2008; European Commission, 2013b). Therefore, innovation in designing products requires non-animal alternative tests to control physicochemical properties and evaluate the risk during their elaboration process. There is a need for non-invasive sampling and measurement (Berekméri et al., 2019).

Ex vivo skin explants stemmed and kept-in-life from surgical tucks can be suitable. Indeed, skin models make possible a wide range of measurements (Suhail et al., 2019). Such a method is useful to perform tests closer to physiological conditions (Lebonvallet et al., 2010). As an example, they keep cellular differentiation properties (Holbrook and Hennings, 1983) and allow to guess the *in vivo* skin behavior (Gasser et al., 2011). The *ex vivo* samples can be then studied with histological, chemical, and structural tests (Dhandapani et al., 2020; Gasser and Bouzoud, 2008; Labroo et al., 2021).

The skin can be considered as a viscoelastic multilayered complex material, made of an elastic layer (the epidermis) and a viscoelastic one (the dermis) (Groves et al., 2013; Hsu et al., n.d.; Koehler et al., 2010; Xu and Lu, 2009). The usual techniques to characterize the skin are based on quasistatic methods with indentation tests, torsion and suction tests (Constantin et al., 2020; Firooz et al., 2012; Joodaki and Panzer, 2018; Monteiro Rodrigues and Fluhr, 2020; Pailler-Mattei et al., 2008) or surface wave propagation (Vexler et al., 1999). Rheological techniques can also be used to characterize such viscoelastic materials (Holt et al., 2008) These mechanical characterizations give global indicators about the skin physiology (hydration mostly) based on Young's modulus, shear modulus and skin overall deformations at macroscopic scale. The results obtained limit the comprehension of the skin behavior because of its microscopic heterogeneity (Lipsky and German, 2021). On the other hand, photoacoustic techniques give information close to the molecular scale (collagen and elastin protein) (Karlas et al., 2019; Labroo et al., 2021; Mercuri and Fernandez Rivas, 2021). They present limitations

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https://doi.org/10.1016/j.bios.2021.113831
Received 8 July 2021; Received in revised form 13 October 2021; Accepted 19 November 2021
Available online 27 November 2021

regarding signal-to-noise ratio and penetration depth (Hindelang et al., 2019). In addition these experimental set-up and their inverse problem associated remains complex (Ghorbel-Feki et al., 2019; Luo et al., 2015).

This paper presents a biosensor made of an *ex vivo* skin explant coupled with a thickness shear-mode (TSM) transducer in order to investigate the skin mesoscopic mechanical behavior. Firstly used as quartz crystal microbalances (Johannsmann, 2015), this kind of transducer is also highly sensitive to viscoelastic effects evolutions (Granstaff and Martin, 1994; Lucklum and Hauptmann, 2000). Seen in microrheological context, the TSM sensor has been proved to be relevant to investigate complex fluids such as sol-gel materials (Ould Ehssein et al., 2006), cosmetic emulsions (Desplan et al., 2017), and even to monitor conformational disease process (Didier et al., 2017). Given a surface functionalization, TSM transducers can also be used for the design of biosensors (Bhattarai and Hameed, 2020; Janata, 2009; Kanazawa and Cho, 2009; Perumal and Hashim, 2014; Wegener et al., 2000).

The new experimental set-up associated with a fractional calculus model is presented and tested on a rough skin. Then, a qualitative study of skin/product interactions regarding peeling products is discussed to validate the biosensor sensitivity to surface modifications. The coupling between the transducer and the skin has been achieved thanks to a measurement device designed in respect with the constraints implied by the *ex vivo* context. Its proof-of-concept has been demonstrated in previous works (Besse et al., 2016) but has not been not exploited until now. The sensor ability to monitor skin properties, in a physiological-like situation and as early as a topical application, should give access to relevant biological information.

2. Material and methods

In this section, the experimental set-up including the biosensor preparation and the whole measurement environment is presented. Then, the mathematical model based on fractional calculus and how the extraction of viscoelastic parameters is discussed. Finally, the skin explants and the chemicals applied to test the skin-product interactions are introduced.

2.1. Experimental set-up

2.1.1. Biosensor preparation

The biosensor is made of an *ex vivo* skin explant coupled with a thickness shear-mode quartz piezoelectric transducer. The transducing element of the biosensor is classically made of an AT-cut piezoelectric quartz, which is able to generate shear wave in the skin explant, supplied by the *Bio-EC* company (Peno-Mazzarino et al., 2018). The investigated explants are constituted of the dermis and the epidermis including *stratum corneum*. They are packed to be fully and uniformly nourished with a dedicated culture medium supplied under the dermis, and then kept in life-like state for around ten to twelve days. Usually, the optimized survival is achieved by using little grids made of polylactic acid (PLA) plastic, covering a well full of culture medium known as the *Bio-EC Medium* (BEM). Half of the culture medium amount must be renewed every two days to ensure the explants survival. Besides, this storage implies a mechanical stress-free state of the explant. The explants have a nominal diameter of 14 mm and must be kept at $T_0 = 37\ ^\circ\mathrm{C}$ in an incubator set at a carbonation rate of $\tau_{CO_2} = 5\ \%$.

The adaptation of this skin model to the proposed microrheological technique requires an effective adhesion of the skin on the TSM transducer without adding any pre-constraints. The explants are then strapped, with O-rings seals, over PLA plastic mounts, and disposed in free boundary condition on the TSM quartz whose gold electrodes foster the skin adhesion. Furthermore, this packing prevents the natural shrinking following surgical extraction, in order to be closer to real physiological conditions. The quartz diameter of 14 mm implies an internal diameter of 17 mm and an external one of 19 mm for the PLA disk to maintain its mechanical strength. Therefore, the skin explant has a useable area of 20 mm for a total diameter of 24 mm. This is the best dimensions possible regarding the nominal one 14 mm. A detailed of the explant assembly is shown on Fig. 1.

To ensure the survival for several days, an automated circulation of the culture medium, in closed loop, nourishes the skin by capillarity. This prevents the renewal of the fluid which could create a bias of the ultrasonic measurement. As displayed in Fig. 1, the transducer is bonded to a printed circuit board (PCB) with silver paint. This PCB serves as coupling electrodes. It is glued to a well, made of polyether ether ketone (PEEK) plastic, with epoxy resin. The well is the culture medium tank in which the explants edges are soaking. It is supplied and flushed with peristaltic pumps. The well dimensions are 15 mm of inner diameter and 28 mm of external diameter. The permanent amount of culture medium contained in the well during the experiment is equivalent to $4.5\ 10^{-6}\ \mathrm{m}^3$ (4.5 mL), corresponding to 10.3 mm of height. To prevent the explant and the culture medium to be in contact with the epoxy resin, the TSM/PCB/PEEK double junction is covered with polydimethylsiloxane (PDMS) seal. Therefore, all the materials in contact with the explant (PEEK, PDMS, gold of electrodes) are biocompatible for, at least, the experiment duration.

2.1.2. Complete experimental set-up

The biosensor is settled in a sterilisable incubator *(Binder* CB170*)* set to $T_0 = 37\ ^\circ\mathrm{C}$ and $\tau_{CO_2} = 5\ \%$. The peristaltic pumps (*Boxer 9000 – 24 V, 33 RPM, 3 rollers*) ensure a slow continuous flow of the culture medium and are controlled with a dedicated motor driver (Fig. 3). The flow $1.5 \pm 0.1\ \mathrm{cm}^3{\cdot}\mathrm{min}^{-1}$ at "standard laboratory conditions" ($T = 25\ ^\circ\mathrm{C}$, and atmospheric pressure) is set to prevent the detachment of the explant from the quartz and have been experimentally determined. The complete experimental set-up is shown in Fig. 1.

A network analyzer (*Keysight E5061B*) including a HF-bridge performs the impedance measurements by time-domain reflectometry. In order to investigate the viscoelasticity of the skin at different scales, a multifrequency analysis is realized by probing the 3rd, 5th, 7th and 9th odd harmonics of a TSM *Q-Sense QSX-301* sensor frequency response (fundamental frequency $f_0 = 4.95$ MHz). The power of the signal source is set to $P = 0$ dBm. The measurements are performed continuously over time every 5 min. A computer collects and processes data. Up to four explants can be monitored at the same time to allow replication tests.

2.1.3. Multifrequency viscoelastic parameters extraction

The electrical impedance of the biosensor can be modelled with a lumped element circuit around the resonance frequencies of the transducer (Granstaff and Martin, 1994). Fig. 2 represents the lumped element circuit around the nth resonance frequency, the viscoelastic model of the skin, and the bare frequency response of the TSM.

At the nth resonance frequency, it is possible to identify two parallel branches: a static one which represents the capacitive effect between the electrodes $C_{0,n}$, and a motional branch standing for the impedance due to the quartz layer properties $Z_m^{0,n}$ and the electrical load $Z_{load,n}$. Considering the skin as a continuous viscoelastic semi-infinite load, we can retrieve its mechanical impedance Z_m with (1) (Cernosek et al., 1998; Granstaff and Martin, 1994). Z_m is experimentally obtained from the identification of $Z_{load,n}$ in the electrical model. In (1), ω is the angular frequency, R_m and X_m are the real and imaginary parts of the skin mechanical impedance, ρ is the skin equivalent density, and $\widetilde{G}^*(\omega)$ its complex shear modulus.

$$Z_m(\omega) = R_m(\omega) + jX_m(\omega) = \sqrt{\rho\widetilde{G}^*(\omega)} \tag{1}$$

The stress-strain frequency dependance of the skin is linked to its structure and mechanical interactions between dermis and epidermis layers. Regarding constitutive equations, $\widetilde{G}^*(\omega)$ depends on the integration of local shear rates of the investigated volume. The mechanical impedance at the electrode surface is then biased because of the local

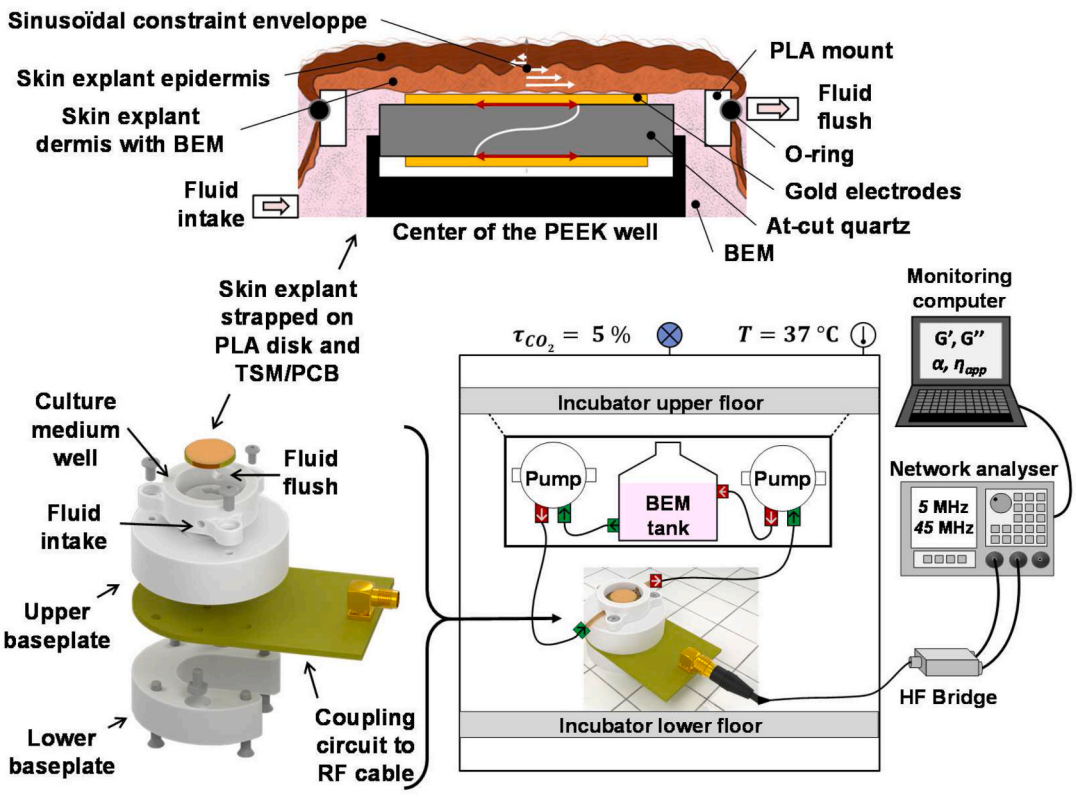

Fig. 1. Complete experimental set-up.

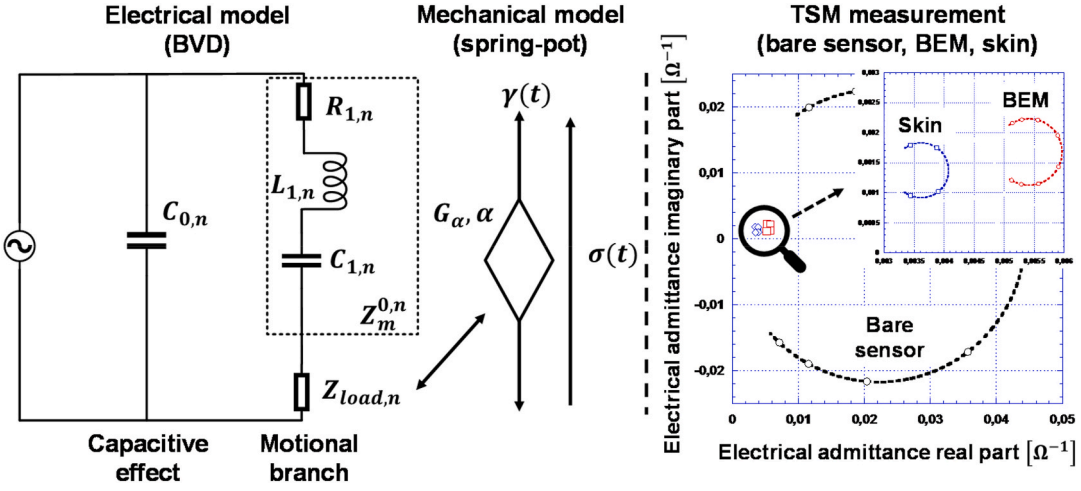

Fig. 2. Equivalent model of the loaded TSM. The lumped element circuit is displayed on the left; the spring-pot model associated with the electrical load and corresponding to the skin viscoelastic parameters is shown in the middle; the bare frequency responses are displayed on the right.

shear rates misestimations. One way to take into account this spatio-temporal bias implied by the viscoelastic multilayer structure of the skin is to introduce a fractional derivative formalism (Bagley and Torvik, 1986; Debnath, 2003; Kearney et al., 2015). The complex shear modulus can be then expressed by (2).

$$\widetilde{G}^*(\omega) = G'(\omega) + jG''(\omega) = \eta\tau^{\alpha-1}(j\omega)^\alpha \tag{2}$$

The real part, $G'(\omega)$, is the elastic modulus, relevant for the stored energy in the material and mostly representative of its solid dimension. The imaginary part, $G''(\omega)$, is the viscous modulus and stands for the energy losses; thus, it roughly describes the soft material liquid dimension. The fluid viscosity is denoted η. The latter is linked to the investigation scale regarding the mean relaxation time of stored constraints associated to it, τ. Finally, the spatiotemporal bias is represented by the fractional order α. This fractional order can be determined from both elastic and viscous moduli nonlinear frequency dependance. α can also be spelled out from the modulus (3) and the phase (4).

$$|\widetilde{G}^*(\omega)| = \eta\tau^{\alpha-1}\omega^\alpha \tag{3}$$

$$\arg(\widetilde{G}^*(\omega)) = \frac{\pi\alpha}{2} \tag{4}$$

According to Hooke's law the material can be considered as a solid at the investigated scale when α tends to zero. On the contrary when it tends to one, the material behaves like a Newtonian fluid.

Using the microrheological technique, it is finally possible to extract the mechanical impedance of the skin from the biosensor. The viscoelastic parameters and the structural coefficient α are then obtained from (5).

$$Z_m(\omega) = R_m(\omega) + jX_m(\omega) = \sqrt{\rho \widetilde{G}^*(\omega)} = \left(\rho\eta\tau^{\alpha-1}\omega^{\alpha}\right)^{\frac{1}{2}} \exp\left(\frac{j\pi\alpha}{4}\right) \quad (5)$$

2.2. Materials used for the proof-of-concept

Two studies are presented in this paper. The first one deals with different kept-in-life skin explants. It aims to prove the sensitivity of the biosensor to skin mechanical behavior monitoring. The second one concerns *ex vivo* studies with active product deposit to evaluate the system sensitivity to tissue mechanical modifications over time, even in the case of topical applications. Active product implying desquamation phenomenon have been selected because they create breaches in the superficial skin layer.

Both must prove the exploitability of the experimental set-up, including the explants survival, and the microrheological model associated validity.

2.2.1. Skin explants used for the two measurement series

For the first measurement series, three female stemmed explants are compared. Two of the benevolent donors are approximately 50-years old while the other one is 23-years old. These measurements are extracted from two measurement campaigns.

Note that in the second one, all the tests are performed on explants stemmed from 44 and 46-years old females. All the measurements are performed at once.

All the explants are characterized by a phototype ranging between 1 and 3 (usual terms refer to “Caucasian” or “European”). A particular attention has been given to select explants in good physiological conditions. Note that, the reproducibility study with systematic replications (explant stemmed from the same surgical tuck) has been verified and is not presented in this paper.

2.2.2. Active products for validation

For the second measurement campaign, two active peeling products were tested: the Sodium Lauryl Sulfate (SLS), a mild surfactant used in clinical rash tests (Welss et al., 2004), and the salicylic acid, known for acne treatments (Arif, 2015).

The first one was self-formulated in an aqueous solution with a ratio of 10%, while the salicylic acid was an aqueous solution available on the market, containing 2% of active ingredient (*The Ordinary – DECIEM*).

2.2.3. Characterization protocol

The settling protocol is the following:

- Measurement of the culture medium for a half-day to check the proper functioning of the experimental set-up.
- Strap of the explant on PLA disks with the O-ring at their receipt.
- Settling of the strapped explant on the TSM quartz in stress-free state
- Waiting period of approximately one day before product application. This is necessary because of the explant stress relaxation due to the disk mounting. During this time, it is mandatory to check the adhesion of the explant at a half day by a softly press.

Regarding the first measurement series, two experiment stop times (after four days – D4, and eight days – D8, of measurement) have been chosen to check the physiological state of the 50-years old explants thanks to histological techniques.

Concerning the second measurement campaign, after observing the explant settling protocol, the peeling product has been applied. A first drop of $m = 20$ mg of product, in accordance with the usual surface dose of $m_s = 2\ \mathrm{mg.cm^{-2}}$ used for clinical tests, is applied with a micropipette which ensures no mechanical contact at the deposit. If it is possible, a second drop is deposited after a delay of 72 h.

3. Results and discussion

Firstly, the ability of the biosensor to differentiate the skin viscoelastic effect from its culture medium is evaluated, as well as the baseline of the sensor with a nearly Newtonian fluid. Then, the viability of the explant in measurement conditions is assessed. Usual trends of skin explants evolution are also identified. Finally, the sensitivity of the biosensor to topical application is highlighted.

3.1. Validation of the skin measurement

Previous studies using the biosensor have shown its sensitivity to monitor defrosted skin evolutions (Besse et al., 2016). Its ability to differentiate non-survival skin explant given their hydrations, their ages and their phototypes, have been proved. Under these conditions, the measurement do not consider the effect of the culture medium.

In the new measurement device, the culture medium (BEM) is renewed continuously. Fig. 3 represents the shear and loss moduli for the first instants of an *ex vivo* measurement in survival with the biosensor described in this article. The concerned skin explant is the 23-years old one. Two areas are highlighted: in the red one, before $t = 0$, the biosensor measures only the culture medium; in the white one, after $t = 0$, the skin is disposed on the transducer. The typical observed variability for explants stemmed from the same donor is estimated to 10%.

Firstly, the comparison between the red (BEM only) and white (skin explant soaked in BEM) areas, shows that skin viscoelastic effects can be detected, even if culture medium is contained in the first layers of the dermis.

When the skin is deposed on the TSM surface, its adaptation is observable through the rapid evolutions of both $G'(\omega)$ and $G''(\omega)$. This adaptation, which corresponds to the explant tensioning process when it has been strapped on the PLA disk, lasts for around 4 h as is can be seen with $G'(\omega)$. The second peaks that are observable at $t = 6$ h match with the viscoelastic response due to the soft press used for the adhesion check. One can note the rapid return to initial values of both $G'(\omega)$ and $G''(\omega)$. This proves the robustness of the skin adhesion to gold electrodes as well as the impedance measurement relevance.

In the red area, the value of the shear modulus $G'(\omega) = 0$ Pa explains that the culture medium is close to a Newtonian fluid at the investigated scale. Furthermore, the computation of the BEM apparent viscosity gives $\eta_{BEM} = 7.69\ 10^{-4} \pm 9.41\ 10^{-8}$ Pa·s which is consistent with the water viscosity value at $T = 37\ ^{\circ}$C. The difference of less than 10% is due to the additives. The comparison of $G'(\omega)$ and $G''(\omega)$ values with the patent highlights the effect of the culture medium with a sensitivity loss of less than one magnitude order for skin viscoelastic parameters measurement (Besse et al., 2016). The culture fluid presence does not significantly interfere with the measurement even if $G''(\omega)$ is less sensitive than $G'(\omega)$ to tissue physiological variations. However, to achieve a complete study of the skin, both parts of the complex shear modulus will be presented for several days.

3.2. Testing the viability duration of the biosensor

After the first 24 h of measurement validity shown previously, the explants sustainability in contact with the TSM transducer have been evaluated over a period of eight days. Fig. 4 illustrates the complex shear modulus and structural coefficient evolutions over the time.

To evaluate the viability domain regarding explants survival, one of them (the 50yo explant referred in graphic by G'_{old} *D4 and* G''_{old}D4) is stopped at day 4 to compare with histological imaging. The other samples are kept in measurement for eight days and then compared. The

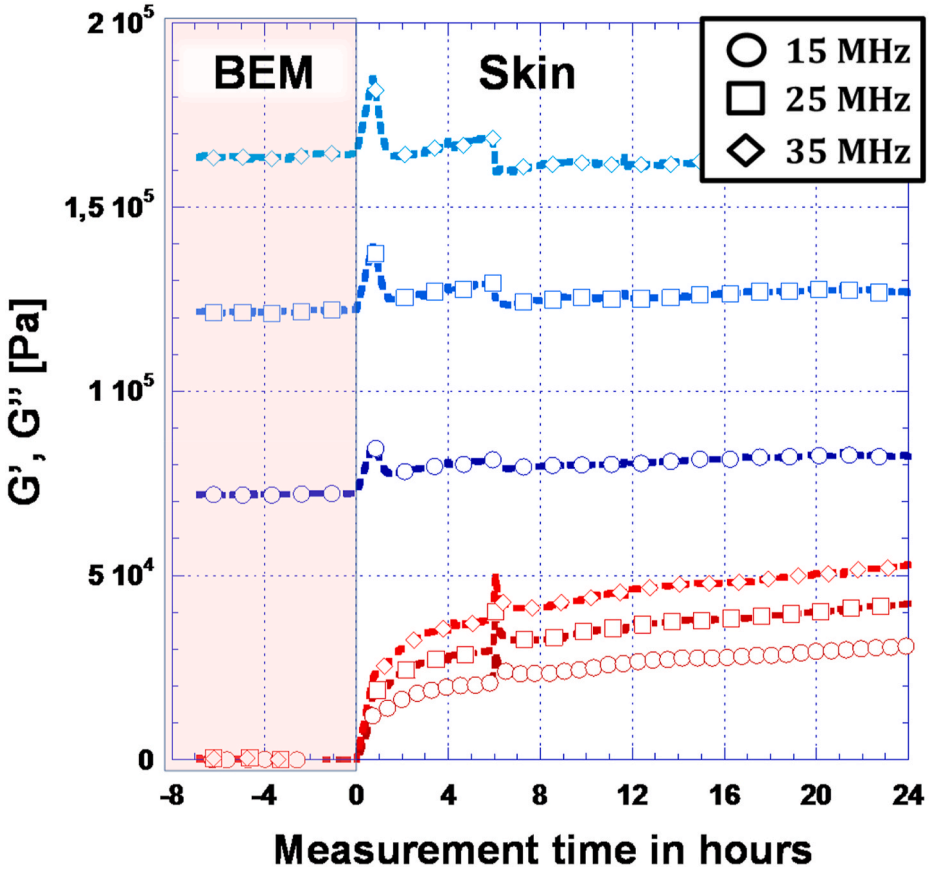

Fig. 3. BEM and skin complex shear modulus parts at the first instants of a measure for the third ($f = 15$ MHz), the fifth ($f = 25$ MHz) and the seventh ($f = 35$ MHz) transducer harmonics.

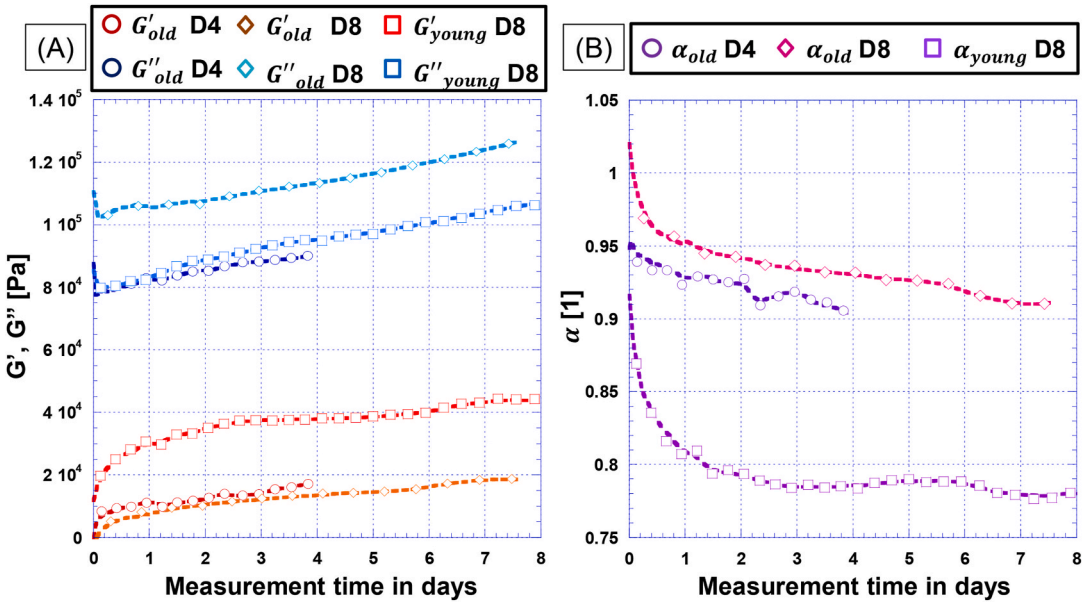

Fig. 4. Skin viscoelasticity evolutions at $f = 15$ MHz for 3 *ex vivo* explants: 2 samples aged of around 50yo (denoted "old" in the legend) and one sample aged of 23yo (denoted "young"). (A): $G'(\omega)$ and $G''(\omega)$ (B).α

biochemical analysis from histology (performed by Bio-EC Laboratory) confirms a "really good" survival until four days, and "moderate" alteration at day eight.

All these curves present the same kinetics regardless of the explant donor. For viable explants, the typical complex shear modulus curves are described by a short exponential response followed by a mild increase, nearly linear, over time. As discussed previously, over 24 h, the curve corresponds to the explant adaptation. After this period, the

evolution is linked to the natural dehydration and deterioration of the sample. This trend is also noticed for the structural coefficient, with an exponential decay and a nearly linear decrease.

Considering absolute values, $G''(\omega)$ is highly dependent of the skin adhesion on the TSM because of the amount of soaking culture medium at the interface between the dermis and the electrode. Only its variation can be relevant. Moreover, the age effect of the donor is consistent with the literature: the younger skin has an elastic modulus higher than the older ones, which implies a greater firmness of this skin (Rawlings, 2006). This is particularly significant by observing the structural parameter α. It drops from values corresponding to fluidic behavior to solid-like ones, the absolute values for the younger explant being substantially lower than older ones. Considering (3), one can extract an effective viscosity of 4.5 mPa·s^{-1}. From these kinetics, it is possible to identify a typical behavior singularly, interesting to compare with topical application. It is noted *control* in the following study.

3.3. Sensitivity to topical applications

To study the effect of topical products applied at the surface of *stratum corneum*, two aqueous solutions are studied. The Sodium Lauryl Sulfate (SLS) and Salicylic Acid (SA) solutions are known to be peeling test products. The first one has been applied twice (here at day 0.75 and at day 3.5). The second one is applied only once because of its high impact. To stay in "really good" survival, the treated explants are monitored for only four days. The curves of Fig. 5 present the elastic and viscous moduli evolutions for the experiments implying the SLS and the salicylic acid.

The desquamation process is clearly detected for both treated explants. As of the first application, this implies an increase of $G'(\omega)$. Besides, in accordance with physiological observations attested in literature (Lee and Maibach, 1995; Loffler and Happle, 2003), the SLS has a cumulative effect: this kinetic growth is more important after the second deposit and seems to start to follow a power rise. However, no significant evolution of $G''(\omega)$ is noted until the second application. The application of SLS does not impact the structural parameter α. Its curve follows the control one.

Concerning the salicylic acid, the impact is dramatically higher: after only one application both curves of $G'(\omega)$, $G''(\omega)$ and α start to follow a power law. This growth can be explained by a complex combination of bio-physical phenomena. As the desquamation process involves a weakening of the cutaneous barrier, this growth can be associated with the explant dehydration. The power value amplitude rate may be representative of the dehydration intensity. At the investigation scale, the dehydration singularly impacts the skin elasticity by the fiber tensioning as shown in $G'(\omega)$ curves. The dehydration implied by the weakening of the cutaneous barrier is also explained by the evolution of the structural coefficient which is consistent with a rigidification of the material.

In addition, the power law can also be a signature of the explant survival in concordance with histological measurements. Indeed, the explants viability is respectively slightly and substantially altered by SLS and SA.

These preliminary results prove the ability of the biosensor to measure physiological evolution due to viability and dehydration for instance. The modification of the skin surface can also be detected. By this proof of concept, the obtained results have shown that this novel technique should be useful to monitor skin/product interactions, even in topic application cases.

4. Conclusions

A new biosensor, based on ultrasonic microrheology allied with kept-in-life human skin explants, has been proposed to study skin/product interactions. This measurement technique usually allows retrieving viscoelastic parameters of soft matter but has already been proved to be useful for biological sensing. As skin can be considered as a viscoelastic multilayered material, a skin explant is placed on a TSM quartz to retrieve its mechanical parameters, the complex shear modulus, and the microstructural parameter, over the time.

This biosensor can monitor skin-product interactions over seven days approximately, the effective possible duration being dependent on the skin explant initial condition. The comparisons between microrheological data and histology measurements allow us to say that the explants are in good survival until four days and can be pushed toward eight days but with a little morphological deterioration. Then, the obtained values are relevant regarding the subjects ages: the elastic modulus is higher for the younger skin than the older ones, while the opposite is true for α coefficient, as this kind of skin is firmer. The viscous modulus is impacted by the slight skin structural difference which can exist, and this still need to be correlated with histological data.

Besides, the typical evolution curve seems to be composed of an exponential decay and a nearly linear decrease for the structural coefficient, or the opposite for the complex shear modulus, until significant tissue deterioration occurs. The first part of the curve corresponds to the matrix tensioning due to the disk strapping, and the remaining behavior should be due to the usual dehydration and deterioration of the explant in *ex vivo* conditions.

This proposal is fostered by the results of the experiments based on a

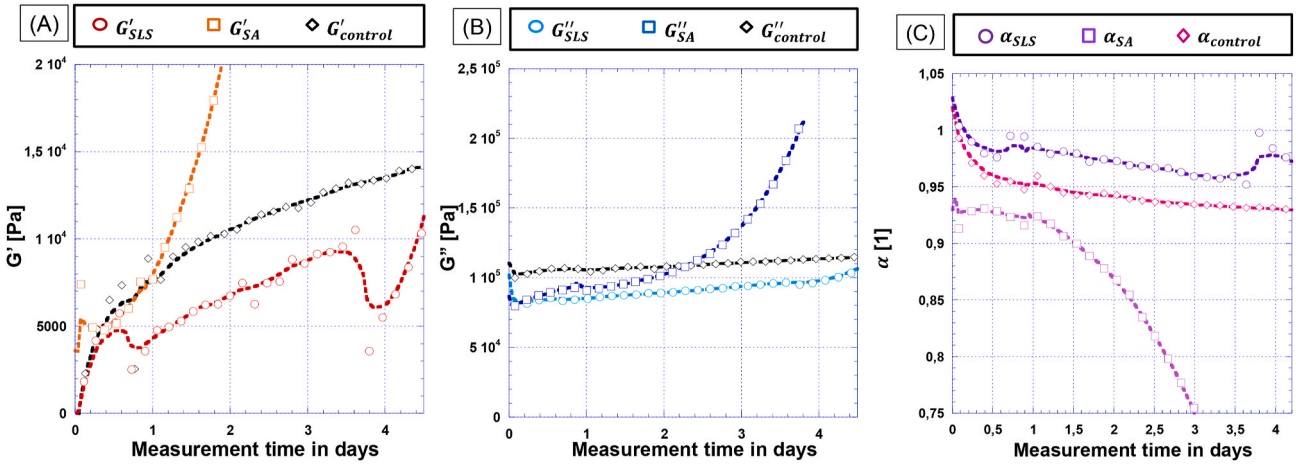

Fig. 5. Skin viscoelasticity evolution for 3 *ex vivo* explants, one subject to SLS, another to salicylic acid (SA), and a control specimen from Fig. 2 (A): $G'(\omega)$ (B): $G''(\omega)$ (C).α

chemical desquamation enforced by SLS or salicylic acid deposit: the desquamation implies an intensified dehydration due to a skin superficial layer weakening, and the curves show an increase in $G'(\omega)$ and $G''(\omega)$ kinetics, which depends on the desquamation intensity. Furthermore, the viability of the explants seems to be appreciable with the complex shear modulus curve shape: when the skin tends to be strongly damaged, in the salicylic acid case for example, it tends to a power law. However, the comparison to literature is quite difficult for now: there is too few data about the viscoelasticity of human skin explant kept in life-like state. In (Geerligs et al., 2011), the shear modulus values ranges from 4 kPa to 12 kPa, but samples are prepared from skin explants but they are sliced with a dermatome, treated with chemical, and dried. Similar values can be found *in vivo* on the fore-arm (Kearney et al., 2015; Luo et al., 2015). Even the α coefficient differs substantially from (Kearney et al., 2015) who found values closes to 0.3. Once more, this can be explained by the differences in the experimental protocol and the presence of the culture medium. As the evolution of α is due to the structure evolution regardless of the culture medium, a mathematical transformation of this parameter could help to get closer to the literature results.

In addition, some limits are to be noted. The results have not been systematically reproduced on several explants, and they are to be confirmed on reconstructed skin. Even if a strictly statistical study cannot be performed, it would be necessary to verify these conclusions with large measurement campaigns. Even more, these conclusions are only qualitative for now and it would be relevant to extract quantitative physical and biological characteristics. Then, improvements must be made on the biosensor concerning the contact between skin/quartz, and the uniform supply of the culture medium under the dermis. A solution would be to functionalize the quartz with sticking proteins to improve the adhesion of the explant in free condition and change the electrodes shapes to create small channels (Didier, 2017). Another point would be to find a way to produce the homemade cell measurements without handcrafting to prevent even more reproducibility bias. Finally, a system able to handle skin tension would be fundamental to test the skin in the same experimental conditions from one measure to another – for now this tension is only checked with a cutometer.

Considering the validity domain explained in this paper, the ability to sense mechanical modifications induced by topical applications validates the relevance of the technique to get a mesoscopic monitoring of skin conditions. The mesoscopic investigation scale of the biosensor completes the information given by macroscopic mechanical and microscopic photoacoustic techniques (Labroo et al., 2021; Lipsky and German, 2021; Monteiro Rodrigues and Fluhr, 2020). As the system is sensitive enough to perceive slight variations happening in the most superficial part of the explant, it is, up to now, possible to use it to characterize topical applications. It would be useful to evaluate the safety of chemical and the efficiency of dermo-cosmetics. The ability of this technique to characterize the skin and the product at mesoscopic scale and its links with macroscopic characteristics could foster a new way of instrumented sensory analysis considering the skin/product interactions.

CRediT authorship contribution statement

Vincent Gauthier: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – review & editing, Writing – original draft, Visualization. **Alice Lemarquand:** Conceptualization, Methodology, Validation, Formal analysis, Investigation. **Emmanuel Caplain:** Conceptualization, Writing – review & editing, Supervision. **Nicolas Wilkie-Chancellier:** Writing – review & editing, Supervision, Project administration. **Stéphane Serfaty:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the BIO-EC Company for their supply and knowledge-sharing of *ex vivo* skin explants.

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