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(54) **TSM BIOSENSOR TO EX-VIVO CHARACTERIZE THE VISCOELASTIC PROPERTIES OF SKIN**

TSM-BIOSENSOR ZUR EX-VIVO-CHARAKTERISIERUNG DER VISKOELASTISCHEN
EIGENSCHAFTEN DER HAUT

BIOCAPTEUR TSM POUR CARACTÉRISER EX VIVO LES PROPRIÉTÉS VISCOÉLASTIQUES DE
LA PEAU

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- **FANG LI, JAMES H.-C. WANG: "An Acoustic Wave Biosensor for Probing the Viscoelastic Properties of Living Cells", CHEMICAL AND BIOLOGICAL SENSING VII, vol. 6218, 19 May 2006 (2006-05-19), XP040224519, BELLINGHAM DOI: 10.1117/12.668256**
- **EHSSEIN CO ET AL: "Kinetic reaction monitoring of acidified milk gels with a quartz resonator : effect of temperature and gdl quantities", 2004 IEEE ULTRASONICS SYMPOSIUM : 23 - 27 AUGUST 2004, PALAIS DES CONGR GBP ES, MONTRÉAL, CANADA, IEEE OPERATIONS CENTER, PISCATAWAY, NJ, vol. 1, 23 August 2004 (2004-08-23), pages 319-322, XP010784098, DOI: 10.1109/ULTSYM.2004.1417729 ISBN: 978-0-7803-8412-5**

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Description**FIELD OF THE INVENTION**

[0001] The invention concerns a TSM biosensor to ex-vivo characterize the viscoelastic properties of skin.

STATE OF ART

[0002] The skin which comprises dermis, epidermis and the stratum corneum, is a complex biological material similar to layers based on fibers, and on collagen and elastin membranes intermixed and immersed in a fluid, itself complex, in a gel phase (hyaluronic, fibroblasts, proteoglycans, fat and water). The orientation of these fibers and the very thin thickness of these layers imply that the viscoelastic properties of the the skin are often seen as non-linear and anisotropic, and are difficult to characterize either in vivo or ex vivo.

[0003] Most commonly used skin samples ex vivo test is the tensile test. Using this method, it is difficult to have homogeneous and reproducible measurements particularly because of non-linear mechanical effects involved, the heterogeneity and variability of the skin.

[0004] Document titled "An Acoustic Wave Biosensor for Probing the Viscoelastic Properties of Living Cells" by Fang Li et al, discloses a biosensor according to the preamble of claim 1.

[0005] Document US 2010/0075347 a biosensor for detecting an analyte.

[0006] Document titled "Kinetic reaction monitoring of acidified milk gels with a quartz resonator" by Ehseein C. et al, discloses a biosensor for studying acidified milk gels that can be found in soft cheese and yoghurts

DESCRIPTION OF DRAWINGS OF THE INVENTION

[0007]

- Figure 1 describes the TSM biosensor of the invention in an exploded view; the different elements of the bio sensor is not to scale.
- Figure 2 describes the TSM biosensor of the invention in a sectional view.
- Figure 3 shows the modified electrical model used to characterize the viscoelastic properties of skin.
A) TSM Biosensor 1 used for ex-vivo skin characterization (untreated tired skin after dehydration process monitoring): the biosensor 1 consist of two elements: the transducer made of an AT-cut quartz resonator 3C and an ex-vivo skin explant specially designed. B) The electrical model: two parallel branches can independently represent both electrical and mechanical effects. The static branch of impedance Z_0 (in gray) includes the dielectric properties of the quartz as a function of the electrical field distribution between the electrodes 4A, 4B; the dynamic branch (motional branch) is representative of the mechanical properties of the quartz Z_m^0 and skin (Z_{load}).
- Figure 4 shows typical fit of the total admittance of the TSM biosensor 1 corresponding to the 3rd order theoretical electrical complex function corresponding to the electrical circuit.
- Figure 5 describes the Mass effect skin explant 2 vs nutritional BEM: The total admittance X is compared for different samples. The mass effect is principally due to the liquid stored in the dermis 2A and epidermis 2B.
- Figure 6 is a comparison of R load for the 36 yo donor: for BEM load (left) and skin load (right).
- Figure 7 describes the kinetic of dehydration at 37°C, 22% Hygrometry for Negroid (points only) and Caucasian (line style) treated skins: A) Relative variation of imaginary part of Z_{load} ; B) 44yo Caucasian skin donor.
- Figure 8 describes the complex shear modulus $G = G' + j G''$, representative of skin viscoelasticity at micro-scale, function of time, for the nutritional medium alone without skin explant (left part of the figure), and with the skin explant (right part of the figure) at 37°C; the skin explant comes from a 44 years old Caucasian skin donor and the effect of delipidation (dotted line) versus standard skin (solid line) is shown.

GENERAL DESCRIPTION OF THE INVENTION

[0008] The present invention concerns a Thickness Shear Mode (TSM) biosensor 1 which comprises:

- an ex vivo living skin explant 2,
- a TSM transducer 3 which comprises: an AT cut quartz resonator 3C which has two opposite exterior surfaces 3A,3B, and two conducting electrodes 4A, 4B, each conducting electrode being deposited on one of the two exterior surfaces 3A, 3B.

[0009] The word 'living' means that the skin explant 2 lives when the measures are realized and has biophysical behavior comparable to the in-vivo skin behavior.

[0010] The skin explant can be extracted from a person or an animal with plastic surgery or can be reconstituted with biological cells in an extracellular matrix.

[0011] Here the conducting electrodes are in metal, for instance in gold.

[0012] In other words, the AT cut quartz resonator 3C represents all the existing quartz crystal piezoelectric substrate used as a shear wave generator and as a thickness shear mode sensor.

[0013] The shear waves generated are volume waves.

[0014] The wave mode generated is then Thickness Shear Mode (TSM).

[0015] The thickness of the quartz resonator 3C is chosen to obtain a frequency resonance at RF range and to generate ultrasound shear waves.

[0016] For instance, the fundamental frequency of the quartz resonator 3C used here for the figures is 5 MHz.

[0017] The dimensions of the quartz resonator 3C can be determined so that the quartz resonator 3C can have a quality factor Q higher than 10^5 .

[0018] The electrodes shape 4A, 4B can be designed in order to optimize the generation wave mode. Here on the figure 1, the quartz resonator 3C is a piezoelectric disc with a lateral surface. The shape of electrodes placed at both surfaces of the quartz resonator 3C is also a disc, and advantageously can have the same dimensions and can cover the entire surface of the AT cut quartz resonator 3C.

[0019] But the quartz resonator 3C and the electrodes 4A, 4B can have any form.

[0020] The electrodes can be for example spiral or other form, in order to be controlled by induction, as described in the patent application WO2012/172253.

[0021] Advantageously, the skin explant 2 covers the entire surface of the electrode on which it is deposited.

[0022] The TSM transducer 3 allows to measure and convert electrical parameters for determining mechanical characteristics of the living skin explant 2, and can be connected to a computer and a network analyser.

[0023] The TSM transducer 3 presents:

- measuring means 30,
- monitoring and calculating means 31 which monitor an evolution in time of an electrical response of the living skin explant, and which calculate, in time, from the electrical response, micro rheological characteristics of the living skin explant 2.

[0024] The skin explant 2 can comprise at least one of the skin layers among: hypodermis, dermis 2A, epidermis 2B and the stratum corneum 2C.

[0025] Advantageously, the skin explant 2 comprises a stacking of layers which comprises dermis 2A, epidermis 2B and the stratum corneum 2C, the dermis 2A being in contact with the TSM transducer 3, the stratum corneum 2C being in contact with air.

[0026] The layers can comprise a product or can be pretreated before measuring electrical response of the skin explant 2, function of shear waves of the TSM.

[0027] Advantageously, the dermis 2A does not need to be glued or stick on the TSM transducer 3, and naturally adheres properly to the TSM transducer 3. Thus, both skin explant 2 and TSM transducer 3 can be considered as a unique device called the TSM biosensor 1. The invention allows to realize an instrumented skin.

[0028] It is particularly the case when the electrodes 4A, 4B are in gold and when the dermis 2A is disposed on the electrode just after the preparation and the deposit of the electrode on the substrate resonator.

[0029] A bottom surface of the skin explant 2 is in contact with the TSM transducer 3, a top surface of the skin explant 2 being in contact with air, considering a vertical axis perpendicular to the TSM and the skin explant that are horizontally maintained together.

[0030] When the skin explant 2 comprises dermis 2A, epidermis 2B and the stratum corneum 2C, a bottom surface of the dermis 2A being in contact with the TSM transducer 3, a top surface of the stratum corneum 2C being in contact with air.

[0031] The skin explant 2, in particular the stratum corneum 2C can be treated by a product which is tested.

[0032] The biosensor 1 is modeled by the modified equivalent electrical lumped elements Butterworth-van-Dyke circuit (BVD) which comprises:

- a static branch modeling the electrical behavior of the quartz resonator 3C, and
- a dynamic branch parallel to the static branch and modeling the mechanical behavior of the quartz resonator 3C and the mechanical behavior of the living skin explant.

5 **[0033]** The static branch comprising a resistor and a capacitor connected in parallel.

[0034] The dynamic branch comprising in series:

- a capacitor, a self-inductance and a resistor modeling the mechanical behavior of the quartz resonator 3C, and
- an additional equivalent electrical impedance denoted $Z_{load} = R + jX$. This impedance is linked with the complex shear modulus $G = G' + jG''$, representative of the living skin explant (2) viscoelasticity at micro-scale, using following equations:

$$15 \quad R = \frac{h^2}{4Ae_q^2} \sqrt{\rho_{skin} \left(\frac{|G(\omega)| + G'(\omega)}{2} \right)}$$

$$20 \quad X = \frac{h^2}{4Ae_q^2} \left(\sqrt{\rho_{skin} \left(\frac{|G(\omega)| - G'(\omega)}{2} \right)} + \omega h_{eff} \right)$$

25 where h is the thickness of the TSM transducer (excluding the explant thickness), A the surface of the metal electrodes, e_q the piezoelectric constant, ρ_{skin} is the skin density, ω is the resonance pulsation, G' , G'' are respectively the storage modulus and the loss modulus corresponding to the real and imaginary parts of the complex shear modulus G, and h_{eff} is the depth of living skin.

Z_{load} is the electrical response of the living skin explant 2 and is measured and monitored by the means 30 and 31.

30 **[0035]** The complex shear modulus G characterizes the micro rheological behaviour of the living skin explant 2.

[0036] The Figure 8 shows the capacity of the biosensor 1 to measure the skin viscoelasticity (comparison between experiments with nutritional medium only -left part of the figure- and with the living skin explant -right part of the figure) and to observe the effect of skin pretreatments.

35 **[0037]** Actually the loss modulus G'' is identical in presence of nutritional medium whether the living skin explant is delipidated or not delipidated, whereas the storage modulus G' which is representative of the skin elasticity is clearly higher for the standard skin than for the lipid deficient skin (right part of the figure).

[0038] In a first embodiment, the TSM transducer 3 comprises a culture medium 5 in contact with at least one of the surfaces of one of the two electrodes 4A, 4B and on which the hypodermis or dermis 2A of the skin explant 2 is in contact with its bottom surface, in order to guarantee the survival of the skin explant 2 on the TSM transducer 3 at least several hours.

40 **[0039]** In fact, the survival of the skin explant 2 can be ensured several days (more than 10 days, for instance 16 days), depending upon the contact between the culture medium 5 and the skin explant 2, the exterior conditions (temperature...) and the nature of the culture medium 5.

[0040] The culture medium 5 is a liquid, a gel, an aerogel, a cream and/or a functionalized layer.

45 **[0041]** In a first variant of the first embodiment, the culture medium 5 is a gel, an aerogel, or a cream deposited on the surface of one of the two conducting electrodes 4A, 4B.

[0042] In a second variant of the first embodiment, the culture medium 5 is a functionalized layer disposed on one of the two conducting electrodes 4A, 4B, and on which the bottom surface of the skin explant 2 is in contact, in order to guarantee the survival of the skin explant 2 on the TSM transducer 3 at least several hours.

50 **[0043]** In a third variant of the first embodiment, the TSM transducer presents at least one opening which is filled with culture medium 5, allowing the survival of the skin explant 2 during at least several hours or at least several days.

[0044] It is possible to realize many openings in the electrode and in the quartz of the TSM transducer and to form like a grid, each opening containing some culture medium 5.

55 **[0045]** In a second embodiment, the skin explant 2 is moved and removed between the TSM transducer 3 and a receptacle in which there is the culture medium 5. In this second embodiment, the skin explant 2 remains on the TSM transducer only for the measure for instance 15 minutes and is then reintegrated in the receptacle to feed the skin explant 2 and to maintain it alive.

[0046] In a third embodiment, the invention concerns also, as drawn on figure 2, a device which comprises a TSM biosensor 1 as defined above, and a receptacle in which is deposited the TSM biosensor 1,

the TSM biosensor 1 being surrounded by culture medium 5,
the TSM transducer 3 being immersed in the culture medium 5 so as to maintain the bottom surface of the dermis 2A in the culture medium 5 and the top surface of the stratum corneum 2C in contact with air, allowing the survival of the skin explant 2 during at least several hours or at least several days.

[0047] Thus, the TSM biosensor 1 and the receptacle can be sold together, the skin explant 2 being able to survive at least several days.

[0048] The invention also concerns a method for determining mechanical characteristics of an ex vivo living skin explant 2, the living skin explant 2 comprising at least one layer among: hypodermis, dermis 2A, epidermis 2B, and the stratum corneum 2C.

[0049] The method comprises the following steps:

- (a) withdraw a living skin explant 2 from a culture medium 5,
- (b) deposit the living skin explant 2 on the TSM transducer 3, a bottom surface of the skin explant 2 being deposited on the TSM transducer 3, a top surface of the skin explant 2 being in contact with air,
- (c) measure and monitor electrical parameters for determining and calculating mechanical characteristics of the living skin explant 2.

[0050] For instance, the hypodermis or dermis 2A is deposited on the TSM transducer 3, the stratum corneum 2C being in contact with air.

[0051] In a step (d) after the step (c), the living skin explant 2 is withdrawn from the TSM transducer 3 and is deposited in the culture medium 5.

[0052] In a step (e) after the step (d), the steps (a), (b), (c), (d) are realized again.

[0053] In particular, the steps (a), (b), (c), (d) are realized several times during several days.

[0054] The living skin explant 2 is pretreated, for instance hydrated or delipidated.

[0055] The living skin explant 2 is treated with at least one product, prior to or after its deposition, on the Thickness Shear Mode (TSM) transducer, for monitoring products-to-skin effects.

[0056] In the step (b), in a first embodiment, the living skin explant 2 is disposed in contact with one of the two conducting electrodes 4A, 4B.

[0057] In the step (b) in a first variant of the first embodiment, the living skin explant 2 is deposited on a gel which comprises culture medium 5, the gel being deposited on one of the two conducting electrodes 4A, 4B.

[0058] In the step (b) in a second variant of the first embodiment, the living skin explant 2 is deposited on a functionalized layer with culture medium 5, the functionalized layer being deposited on one of the two conducting electrodes 4A, 4B.

[0059] The invention also concerns a use of the TSM biosensor 1 to monitor ex vivo mechanical behavior of the living skin explant 2 and thus to follow its structural evolution function of time, by measuring its viscoelastic characteristics.

[0060] In particular, it concerns a use to detect and/or follow skin diseases.

[0061] In particular, it concerns a use to analyse and/or study the effect of a product applied to the skin explant 2 or specific treatment.

[0062] EXAMPLE OF A NEW ONLINE SKIN INVESTIGATION TECHNIQUE FOR TRACKING PRODUCTS-TO-SKIN MECHANICAL EFFECTS WHICH CONCERNS DELIPIDATED SKIN AND HYDRATED SKIN WITH A SPECIAL TREATMENT CREAM-BASED APPLICATION.

[0063] Ex-vivo abdominal skin explants 2 from plastic surgery and kept alive are used for this study. Considering the skin as a complex fluid made of membrane and fiber structures immersed in liquid, its mechanical response of a bulk thickness shear wave excitation (ie stress-strain analysis) involves both a viscous component associated with energy dissipation and an elastic component associated with energy storage. A tight monitoring of these two components from the response of a TSM sensor (based on an AT cut quartz resonator 3C at 5 MHz) in contact to the dermis 2A of the ex-vivo explant give us access to the complex dynamic shear moduli (G' and G'') evolution of the skin; The appropriate mechanical model describing the sensor response vs shear waves/matrix interactions allows investigating the impact of the product (or treatment) to the viscoelastic properties of the skin.

[0064] The complex study of the TSM response in time domain permits a control a) of the dehydration evolution at 37°C due to interpenetrated intercellular lipid membranes matrix including the first step of permeation process from dermis 2A to Stratum corneum 2C (SC); b) the impact on the kinetics of the permeation process by a product applied at the SC surface. This information includes the structure and properties evolution of the collagen and elastic fibers and the proteoglycans located in the skin. A comparison of mechanical results with other techniques in the literature confirms the validity of the model. These preliminary results show that our TSM technique can be an appreciable new way for ex-vivo skin investigation for test and optimization of new cosmetic products.

[0065] Skin is a material of four main complex biological layers (epidermis 2B, dermis 2A, hypodermis or stratum corneum 2C) principally based on interconnected collagen and elastin fibers embedded in a gel-like mixture of fluids (hyaluronic, fibroblasts, proteoglycans, fat and water) [1]-[3]. From a mechanical point of view, skin can be considered

as a viscoelastic complex fluid based on membranes and fibers immersed in a liquid [4], [5]. On these conditions, its properties can be characterized as nonlinear and anisotropic soft hybrid complex material or elastomers [6]-[11]. On one hand, the skin viscoelasticity can be mainly attributed to interaction between the interpenetrated elastin - collagen fibers and the liquid mixture. On the other hand the anisotropy behavior is the result of the preferential orientation of collagen beams and residual in-vivo skin stress [12]-[14]. Different non-invasive testing are used to characterize and macroscopically detect skin diseases [15]. Using motional stress on ex-vivo sample, the linear viscoelasticity measurement can be more easily reached. The most commonly used for ex vivo skin samples test is the tensile test. Using this method, the nonlinear effects and viscoelastic behavior of animal skin have been mostly pointed out [16]-[19]. Many other difficulties to perform homogeneous measurements exist because skin structure heterogeneity and the large variability between samples. To date other experimental methods based on stress-strain measurement are largely described in the literature. Some of them are based on measurements of torsion and deformation generated by ultrasound wave propagation. A recent review describe the relevant results available in the literature [20]. They can give access to the linear viscoelasticity of the skin at different scale depending on the wavelength.

[0066] A high frequency rheological technique added to a suitable model is used to monitor the complex shear modulus G (related to viscoelasticity) of skin. The technique uses a biosensor 1 made of a Thickness Shear Mode (TSM) resonator (as a transducer) in contact to the dermis 2A of skin. Both transducer and material are then considered as an equivalent lossy TSM biosensor 1. Using an original approach based on Wheeler analysis for lossy micro-strip, this model gives access to mechanical evolution of skin considered as a heterogeneous complex fluid [21], [22].

[0067] In order to determine the impact of the skin dehydration to ultrasonic shear wave interactions, the responses of the TSM resonator loaded by different skins and treatments are studied.

THE TSM EXPERIMENTAL SETUP FOR EX-VIVO SKIN MONITORING

Ex-vivo samples

[0068] Ex-vivo human skin samples of 5mm thickness were prepared from abdominal biopsies (healthy plastic surgeries) of two male donors of respectively 36 years old with Negroid skin and 44 years old with Caucasian skin. In order to study the 3 first layers (from stratum corneum 2C (SC) to dermis 2A layer) the subcutaneous fat is removed from the skin.

[0069] Circular explants of 10 mm of diameter are cut out in order to embed each sample with a quartz crystal piezoelectric substrate (described in the following section) used as a shear wave generator and thickness shear mode sensor. Human skin explants 2 were maintained in survival on grids, in plates containing 2mL special BIO-EC medium (BEM: medium from BIO-EC Laboratory, France). BEM maintain explants in survival for several days, about 20 days [24].

[0070] Circular explants preparation: A) circular cut out for embeddeed biosensing, B) Human skin maintained in survival ready for treatment.

[0071] The samples from each donor are prepared in order to study the sensitivity of the new biosensor 1 versus skin hydration treatment. 3 type of treated skin are then studied: A) untreated skin used as the reference; B) delipidated skin; C) Hydrated skin with a special treatment cream-based application. Experiments were performed in triplicate

[0072] The tested products and treatments were applied on explants as soon as their preparation. Treated and untreated skin explants 2 were incubated at 37°C in a normal atmosphere conditions. To monitor the dehydration process, a protective film is deposited at the surface of skin. This film ensures a standard skin hydration. As long as the film is present the hydration conditions remain unchanged.

TSM biosensor 1 for skin characterization

[0073] The TSM biosensor 1 consists of a thin disk of AT-cut quartz with metal electrodes 4A, 4B deposited on both sides (the resonator) in contact on one side with the dermis 2A of the ex-vivo human skin sample. The diameters of the crystal and the electrode are respectively 14 mm and 5 mm. The quartz thickness of 270 μm provide a resonance frequency about 5 MHz. Considering the piezoelectric properties of the quartz disk, its crystal orientation, the shape of electrodes 4A, 4B, and the electric field applied between the electrodes 4A, 4B, a specific deformation of the crystal surface can be induced. The AT cut of 35°10' involves maxima shear displacements at the surfaces [25].

[0074] Electro-mechanical models already describe the complete oscillations process of the resonator. They are derived from piezoelectric constitutive equations. A general one dimensional acoustic wideband model has been suggested by Mason [26]. In particular cases, such as low load, the Mason model can be easily simplified near the resonant frequencies into an equivalent electrical lumped elements Butterworth-van-Dyke circuit (BVD) [27].

[0075] When the sensor is loaded by a rigid layer or a liquid, the mass of the layer in contact involves a frequency shift [28], [29]. Moreover the mechanical boundary conditions including skin loading are modified by the rheological properties of skin. These conditions induce an additional frequency shift and a damping of the resonance corresponding to an attenuation and a delay of the propagated ultrasonic shear waves [30]-[32].

[0076] $Z_{load} = R + j X$ is representative of these changes. The variation of the electrical response can then accurately extract G, using the following expression [33]-[35].

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{skin} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{skin}}} + \omega h_{eff} \right) \quad (1)$$

where K is the electro-mechanical coupling factor, ρ_{skin} is the skin density (1.1 g/cm³), ω is the angular resonance frequency and G', G" are respectively the storage modulus and the loss modulus corresponding to the real and imaginary parts of the complex shear modulus G and h_{eff} is the depth of living skin. Note that the mass effect (at the resonance frequency) only depends on the product skin density - effective thickness of wave penetration.

Experimental setup

[0077] The experimental set-up consists of an impedance analysis of the biosensor 1 during skin dehydration. In order to study the effect of treatments each sample is monitored at controlled temperature (37°C) using a laboratory oven. Considering the high Q factor of TSM resonator, the admittance measurement is preferred. A span of 7 kHz around the resonance frequency is then analyzed to measure the total admittance response $Y_e(\omega)$ of loaded biosensor 1 using the HP4195A network analyzer. 400 spectral points are then regularly measured in less than 50 seconds. An online fit of this response is performed in the complex plan to extract the real and the imaginary part of Z_{load} . The viscoelastic characteristics of skin can be then extracted.

[0078] In order to observe the temporal evolution, the frequency response of the admittance is then sampled every minute.

SKIN DEHYDRATION MONITORING USING MICRO-RHEOLOGY TESTING

[0079] In order to study the sensitivity of the biosensor 1 and to validate the proof of concept of a new technique to monitor the hydration of ex-vivo skin explants 2, 3 type of treatment are explored: untreated skin, hydrated skin, and delipidated skin.

Mass effect and rigid structure sensitivity

[0080] To accurately monitor R and X variation due to the viscoelastic effects during the dehydration, the mass effect due to the liquid embedded in the epidermis 2B and dermis 2A is firstly studied. Transducers (without skin explant 2s) are firstly characterized to extract the unloaded motional elements. This measurement gives access to characteristic parameters of unperturbed transducers. A measurement of these transducers loaded by nutritional BEM liquid is after performed. Considering that this liquid is a Newtonian liquid ($G' \approx 0$), the mass effect can be directly extracted from the two first measurements (variation of unperturbed transducer admittance and transducer loaded with BEM liquid). Assuming equation 1, the real part of Z_{load} allows the extraction of the viscosity from viscous modulus G". As a consequence, the following substitution gives the mass effect (X_m):

$$X_m = \text{Im}[Z_L] - \text{Re}[Z_L] = K \rho_{skin} \omega h_{eff}$$

Comparing the real and the imaginary part of Z_{load} for each measurement the typical mass effect can be estimated to $X_m = 10 \Omega$. This first result shows that the total reactance is principally due to viscoelastic variation ($X_m/X < 1\%$). The figure 5 shows the variability of the total reactance and the effect of skin fibers in comparison with BEM.

[0081] At the initial conditions, the total reactance X for skin load is similar to the BEM load. In agreement with the literature of skin tribology, skin can be considered as a viscous complex fluid having a viscosity mainly due to the liquid

characteristic. The density of skin can be considered close to liquid density. This result is in concordance with the fact that the dermis 2A is continuously supplied with water mixture by way of the bloodstream. In the dermis 2A, a part of the liquid mixture is present in gel phase, attached to many hydrophilic macromolecules. Despite the large capacity of these molecules to capture water mixture, a part of it remains mobile and diffuses to upper layers of skin. Note that the reactance of the transducer loaded by the skin is slightly lower in proportion to the surface in contact with the load.

[0082] In contrast, while the imaginary part remains constant, the real part of impedance load R increases significantly as shown in the figure 6.

[0083] Considering the equation 1, this variation is due to the interactions of the macromolecules forming a rigid ultrastructure based on elastin and collagen fibers immersed in fat and liquid. The variation of R gives us access to elasticity of skin during dehydration. It can also be observed that allows for better interpenetration of layers increasing the rigidity as shown in figure 1.

Dehydration kinetic monitoring

[0084] The intercellular SC lipid membranes play a crucial role in regulating the loss of intrinsic liquid from the skin. This process is in fact directly correlated with SC membranes permeability to this aqueous liquid. SC monitoring using X-ray scattering spectroscopy and NMR studies generally this process [36]-[38]. In order to preliminary study the capability of our technique to monitor the dehydration process, different treatments of ex-vivo human skin are tested. The figure 7 shows typical variation of X for treated an untreated skin from the two donors.

[0085] The initial time ($t=0$) is the moment from which the protective film is removed. As a general shape, we note an already known exponential decrease due to the diffusivity of liquid vapor in the lipid membrane. In agreement with one-dimensional diffusion model, this result is also observed by an indirect relative humidity measurement in the QCM chamber as a function of time [39]. The figure 7A) shows the repeatability of the technique (untreated skin). The kinetic characteristic time is clearly modified as a function of skin treatment. The long kinetic of dilapidation skin shows the role of lipids in dehydration. In addition the real part of Z_{load} (figure 7B) for this treatment shows the skin stiffening due to the interactions of macromolecules. Comparing the effect of different treatments our technique can detect their structure modification and their efficiency. A systematic study should allow cosmetic product optimizing and effectiveness evidence.

[0086] The TSM biosensor 1 allows a monitoring of ex-vivo human skin for treatment testing with a large dynamical range. The first results show that it is possible to completely follow the viscoelastic parameters evolution for dehydration monitoring or structure monitoring of skin from stratum corneum 2C to dermis 2A. From the temporal evolution of real and imaginary part of impedance load the effect of skin treatment to the skin layers structure and mechanical properties can be detected. This preliminary study shows that this technique can be suitable to accessing to the elastic G' and viscous G'' moduli by micro-rheology. This new biosensor 1 added with our model should become a sensitive ex-vivo technique for cosmetic product efficiency and skin objective testing.

REFERENCES

[0087]

[1] R. F. Rushmer, K. J. K. Buettner, J. M. Short, and G. F. Odland, "The Skin," Science (80-.), vol. 154, no. 3747, pp. 343-348, 1966.

[2] G. Odland, "Structure of the skin," in Physiology, Biochemistry, and Molecular Biology of the Skin, 1991, pp. 3-62.

[3] C.W. Oomens, D. H. van Campen, and H. J. Grootenboer, "A mixture approach to the mechanics of skin.," J. Biomech., vol. 20, no. 9, pp. 877-885, 1987.

[4] F. Xu and T. Lu, "Introduction of Skin Biothermomechanics," in Introduction to Skin Biothermomechanics and Thermal Pain, 2011, pp. 209-220.

[5] F. Xu, T. J. Lu, and K. A. Seffen, "Biothermomechanical behavior of skin tissue, "Acta Mech. Sin. Xuebao, vol. 24, no. 1, pp. 1-23, 2008

[6] F. Xu and T. J. Lu, "Chapter 3 Skin Biothermomechanics: Modeling and Experimental Characterization," in Advances in Applied Mechanics, vol. olume 43, 2009, pp. 147-248.

[7] F. Xu, T. J. Lu, and K. A. Seffen, "Biothermomechanics of skin tissues," J. Mech. Phys. Solids, vol. 56, no. 5, pp. 1852-1884, 2008.

[8] A. Delalleau, G. Josse, J.-M. Lagarde, H. Zahouani, and J.-M. Bergheau, "Characterization of the mechanical properties of skin by inverse analysis combined with the indentation test.," *J. Biomech.*, vol. 39, no. 9, pp. 1603-1610, 2006.

[9] M. J. Koehler, T. Vogel, P. Elsner, K. Konig, R. Bückle, and M. Kaatz, "In vivo measurement of the human epidermal thickness in different localizations by multiphoton laser tomography," *Ski. Res. Technol.*, vol. 16, no. 3, pp. 259-264, 2010.

[10] C. Sanchez, L. Rozes, F. Ribot, C. Laberty-Robert, D. Grosso, C. Sassoie, C. Boissiere, and L. Nicole, "Chimie douce': A land of opportunities for the designed construction of functional inorganic and hybrid organic-inorganic nanomaterials," *Comptes Rendus Chimie*, vol. 13, no. 1-2, pp. 3-39, 2010.

[11] S. Hsu, A. M. Jamieson, and J. Blackwell, "Viscoelastic studies of extracellular matrix interactions in a model native collagen gel system.," *Biorheology*, vol. 31, no. 1, pp. 21-36, 1994.

[12] X. Liang and S. A. Boppart, "Biomechanical properties of in vivo human skin from dynamic optical coherence elastography," *IEEE Trans. Biomed. Eng.*, vol. 57, no. 4, pp. 953-959, 2010.

[13] B. J. Bell, E. Nauman, and S. L. Voytik-Harbin, "Multiscale strain analysis of tissue equivalents using a custom-designed biaxial testing TSM biosensor," *Biophys. J.*, vol. 102, no. 6, pp. 1303-1312, 2012.

[14] R. B. Groves, S. A. Coulman, J. C. Birchall, and S. L. Evans, "An anisotropic, hyperelastic model for skin: Experimental measurements, finite element modelling and identification of parameters for human and murine skin," *J. Mech. Behav. Biomed. Mater.*, vol. 18, pp. 167-180, 2013.

[15] J. Serup, G. B. E. Jemec, and G. L. Grove, *Handbook of Non-Invasive Methods and the Skin*, Second Edition -, CRC Press. 2006.

[16] J. M. Pereira, J. M. Mansour, and B. R. Davis, "Dynamic measurement of the viscoelastic properties of skin.," *J. Biomech.*, vol. 24, no. 2, pp. 157-162, 1991.

[17] Z. Del Prete, S. Antonucci, A. H. Hoffman, and P. Grigg, "Viscoelastic properties of skin in Mov-13 and Tsk mice," *J. Biomech.*, vol. 37, no. 10, pp. 1491-1497, 2004.

[18] H. G. Vogel, "Directional variations of mechanical parameters in rat skin depending on maturation and age.," *J. Invest. Dermatol.*, vol. 76, no. 6, pp. 493-497, 1981.

[19] O. Lokshin and Y. Lanir, "Viscoelasticity and preconditioning of rat skin under uniaxial stretch: microstructural constitutive characterization.," *J. Biomech. Eng.*, vol. 131, no. 3, p. 031009, 2009.

[20] S. Derler and L. C. Gerhardt, "Tribology of skin: Review and analysis of experimental results for the friction coefficient of human skin," *Tribol. Lett.*, vol. 45, no. 1, pp. 1-27, 2012.

[21] H. A. Wheeler, "Transmission-Line Properties of Parallel Strips Separated by a Dielectric Sheet," *IEEE Trans. Microw. Theory Tech.*, vol. 13, no. 2, pp. 172-185, Mar. 1965.

[22] C. Ould Ehssein, "Système instrumental pour la rhéologie ultrasonore," Université de Cergy-Pontoise, 2006.

[23] E. Lopez, E. Latie, P. Gasser, and M. Rousseau, "Calcium improves skin health," *Househ. Pers. Care Today*, vol. 3, pp. 32-35, 2008.

[24] P. Gasser, F. Arnold, L. Peno-Mazzarino, D. Bouzoud, M. T. Luu, E. Lati, and M. Mercier, "Glycation induction and antiglycation activity of skin care ingredients on living human skin explants.," *Int. J. Cosmet. Sci.*, vol. 33, no. 4, pp. 366-70, Aug. 2011.

[25] P. Leclaire, J. Goossens, L. Martinez, N. Wilkie-Chancelier, S. Serfaty, and C. Glorieux, "Study of the bending modes in circular quartz resonator 3Cs," *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, vol. 53, no. 10, pp. 1934-1943, Oct. 2006.

[26] W. Mason, "A Dynamic Measurement of the Elastic, Electric and Piezoelectric Constants of Rochelle Salt," *Phys. Rev.*, vol. 55, no. 8, pp. 775-789, Apr. 1939.

[27] R. W. Cernosek, S. J. Martin, A. R. Hillman, and H. L. Bandey, "Comparison of lumped-element and transmission-line models for thickness-shear-mode quartz resonator 3C sensors," *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, vol. 45, no. 5, pp. 1399-1407, Sep. 1998.

[28] G. Sauerbrey, "Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung," *Zeitschrift für Phys.*, vol. 155, no. 2, pp. 206-222, Apr. 1959.

[29] K. Keiji Kanazawa and J. G. Gordon, "The oscillation frequency of a quartz resonator 3C in contact with liquid," *Anal. Chim. Acta*, vol. 175, no. C, pp. 99-105, 1985.

[30] H. L. Bandey, S. J. Martin, R. W. Cernosek, and A. R. Hillman, "Modeling the Responses of Thickness-Shear Mode Resonators under Various Loading Conditions," *Anal. Chem.*, vol. 71, no. 11, pp. 2205-14, Jun. 1999.

[31] A. Arnau, Y. Jiménez, and T. Sogorb, "Thickness-shear mode quartz crystal resonators in viscoelastic fluid media," *J. Appl. Phys.*, vol. 88, no. 8, p. 4498, 2000.

[32] S. J. Martin, V. E. Granstaff, and G. C. Frye, "Characterization of a Quartz Crystal Microbalance with Simultaneous Mass and Liquid Loading," *Anal. Chem.*, vol. 2281, no. 26, pp. 2272-2281, 1991.

[33] C. O. Ehssein, S. Serfaty, P. Griesmar, E. Caplain, L. Martinez, and M. Gindre, "Kinetic reaction monitoring of acidified milk gels with a quartz resonator 3C: effect of temperature and gdl quantities," in *IEEE Inter. Ultrasonics, Ferroelectric, and Frequency Controls, 50th Conf.*, 2004, vol. 1, pp. 319-322.

[34] E. Caplain, C. Ould Ehssein, S. Serfaty, L. Martinez, J.-Y. Le Huérou, and M. Gindre, "Toward embedded viscosimeter using quartz crystal resonator," in *3rd Meeting IEEE Sensors, Vienna, Austria, 2004*, p. octobre-octobre.

[35] C. Ould Ehssein, J. Y. Le Huerou, L. Martinez, E. Caplain, N. Wilkie-Chancellier, M. Gindre, G. Guedard, and P. Figuiere, "Kinetic study of silica gels by a new rheological ultrasonic investigation," *Ultrasonics*, vol. 44 Suppl 1, pp. e881-5, Dec. 2006.

[36] A. C. Rowat, N. Kitson, and J. L. Thewalt, "Interactions of oleic acid and model stratum corneum membranes as seen by 2H NMR," *Int. J. Pharm.*, vol. 307, no. 2, pp. 225-231, 2006.

[37] N. Y. Ryabova, M. A. Kiselev, S. Dante, T. Hauss, and A. M. Balagurov, "Investigation of stratum corneum lipid model membranes with free fatty acid composition by neutron diffraction," *Eur. Biophys. J.*, vol. 39, no. 8, pp. 1167-1176, 2010.

[38] X. Chen, S. Kwak, M. Lafleur, M. Bloom, N. Kitson, and J. Thewalt, "Fatty acids influence 'Solid' phase formation in models of stratum corneum intercellular membranes," *Langmuir*, vol. 23, no. 10, pp. 5548-5556, 2007.

[39] D. Lee, J. N. Ashcraft, E. Verploegen, E. Pashkovski, and D. A. Wertz, "Permeability of model stratum corneum lipid membrane measured using quartz crystal microbalance," *Langmuir*, vol. 25, no. 10, pp. 5762-5766, 2009.

Claims

1. A Thickness Shear Mode (TSM) biosensor (1) which comprises:

- a culture medium,
- a TSM transducer (3) which comprises: an AT cut quartz resonator which has two opposite exterior surfaces (3A,3B), and two metal electrodes (4A, 4B), each metal electrode being deposited on one of the two exterior surfaces (3A,3B),

characterized in that the TSM biosensor (1) further comprises an ex vivo living skin explant (2) in contact with the culture medium, the skin explant (2) comprising dermis (2A), epidermis (2B) and stratum corneum (2C), the TSM

transducer (3) allowing to measure and to convert electrical parameters for determining mechanical characteristics of the living skin explant (2), and being configured to generate TSM waves into the ex vivo living skin explant (2), and **in that** the dermis (2A) is in contact with the TSM transducer (3), and the stratum corneum (2C) is in contact with air.

2. TSM biosensor (1) according to claim 1, and a receptacle in which is the TSM biosensor (1), the TSM biosensor (1) being surrounded by culture medium (5), the TSM transducer (3) being immersed in the culture medium (5) so as to maintain the dermis (2A) in the culture medium (5) and the stratum corneum (2C) in contact with air, allowing the survival of the skin explant (2) during at least several hours.
3. TSM biosensor (1) according to claim 1, wherein the culture medium (5) is disposed on one of the two electrodes (4A, 4B), and wherein the dermis (2A) of the skin explant (2) is in contact with said electrode, in order to guarantee the survival of the skin explant (2) on the TSM transducer (3) at least several hours.
4. TSM biosensor (1) according to claims 2-3, wherein the culture medium (5) is a gel.
5. TSM biosensor (1) according to claim 3, wherein the culture medium (5) is a functionalized layer.
6. TSM biosensor (1) according to one of claims 1-5, wherein the electrode has at least one opening which is filled with the culture medium (5), allowing the survival of the skin explant (2) during at least several hours.
7. TSM biosensor (1) according to one of claims 1-6, wherein the TSM transducer (3) comprises a monitoring system with monitoring and calculating means (31) which give access to an additional equivalent electrical impedance of the TSM biosensor (1) loaded by the living skin explant (2), denoted $Z = R + j X$, Z being linked with the complex shear modulus $G = G' + j G''$, representative of skin viscoelasticity from the modified equivalent electrical lumped elements Butterworth-van-Dyke circuit (BVD), using following equations:

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{skin} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{skin}}} + \omega h_{eff} \right)$$

where K is the electromechanical coupling factor, ρ_{skin} is the skin density, ω is the resonance pulsation, G' , G'' are respectively the storage modulus and the loss modulus corresponding to the real and imaginary parts of the complex shear modulus G , and h_{eff} is the depth of living skin.

8. TSM biosensor (1) according to claim 7, wherein the monitoring and calculating means (31) integrate an electrical model of the biosensor by an electrical circuit, the biosensor (1) being modeled by an electrical circuit which comprises:
 - a static branch modeling the electrical behavior of quartz, and
 - a dynamic branch parallel to the static branch and modeling the mechanical behavior of quartz and the mechanical behavior of skin,

the static branch comprising a resistor and a capacitor connected in parallel,
the dynamic branch comprising in series:

 - a capacitor, a self-inductance and a resistor modeling the mechanical behavior of quartz, and
 - the additional equivalent electrical impedance Z modeling the mechanical behavior of the skin.
9. TSM biosensor (1) according to one of claims 1-8, wherein the electrodes (4A, 4B) cover the entire surface of the

AT cut quartz resonator.

10. TSM biosensor (1) according to one of claims 1-9, wherein the skin explant (2) covers the entire surface of one electrode on which it is deposited.

11. TSM biosensor (1) according to one of claims 1-10, wherein the electrodes (4A, 4B) are made of gold.

12. Method for determining mechanical characteristics of ex vivo living skin explant (2) being in contact with a culture medium, the living skin explant (2) comprising dermis (2A), epidermis (2B), and stratum corneum (2C), using a TSM transducer (3) which comprises: an AT cut quartz resonator which has two opposite exterior surfaces (3A,3B), and two metal electrodes (4A, 4B), each metal electrode being deposited on one of the two exterior surfaces (3A,3B), the method being **characterized in that** it comprises the following steps:

- (a) putting the dermis in contact with the TSM transducer (3), and the stratum corneum (2C) in contact with air,
- (b) generating, by the TSM transducer, TSM waves into the ex vivo living skin explant (2),
- (c) measuring and converting, by the TSM transducer, electrical parameters for determining mechanical characteristics of the living skin explant (2).

13. Method according to claim 12, wherein the living skin explant (2) is pretreated, for instance hydrated or delipidated.

14. Method according to one of claims 12-13, wherein, the living skin explant (2) is treated with at least one product, prior to or after its deposition on the TSM transducer (3), for monitoring products-to-skin effects.

15. Method according to one of claims 12-14, wherein the TSM transducer (3) comprises a monitoring system with monitoring and calculating means (31) which give access to an additional equivalent electrical impedance of the TSM biosensor (1) loaded by the living skin explant (2), denoted $Z = R + jX$, Z being linked with the complex shear modulus $G = G' + jG''$, representative of skin viscoelasticity from the modified equivalent electrical lumped elements Butterworth-van-Dyke circuit (BVD), using following equations:

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{skin} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{skin}}} + \omega h_{eff} \right)$$

Where K is the electromechanical coupling factor, ρ_{skin} is the skin density, ω is the resonance pulsation, G' , G'' are respectively the storage modulus and the loss modulus corresponding to the real and imaginary parts of the complex shear modulus G , and h_{eff} is the depth of living skin.

the biosensor (1) being modeled by an electrical circuit which comprises:

- a static branch modeling the electrical behavior of quartz, and
- a dynamic branch parallel to the static branch and modeling the mechanical behavior of quartz and the mechanical behavior of skin,

the static branch comprising a resistor and a capacitor connected in parallel,
the dynamic branch comprising in series:

- a capacitor, a self inductance and a resistor modeling the mechanical behavior of quartz, and
- the additional equivalent electrical impedance Z modeling the mechanical behavior of the skin.

16. Use of a Thickness Shear Mode (TSM) biosensor (1) according to one of claims 1-11, to test ex vivo mechanical behavior of living skin explant (2) and to determine viscoelastic characteristics of the living skin explant (2).

17. Use of a Thickness Shear Mode (TSM) biosensor (1) according to one of claims 1-11, to detect and/or follow skin diseases or to analyse the effect of a product on the skin explant (2).

Patentansprüche

1. Dickenschermmodus (TSM)-Biosensor (1), der Folgendes umfasst:

- ein Kulturmedium,
- einen TSM-Wandler (3), der Folgendes umfasst: einen AT-Schnittquarzresonator, der zwei gegenüberliegende äußere Flächen (3A, 3B) und zwei Metallelektroden (4A, 4B) aufweist, wobei jede Metallelektrode auf einer der zwei äußeren Flächen (3A, 3B) abgelegt ist,

dadurch gekennzeichnet, dass der TSM-Biosensor (1) weiter ein lebendes ex vivo-Hautexplantat (2) in Kontakt mit dem Kulturmedium umfasst, wobei das Hautexplantat (2) Dermis (2A), Epidermis (2B) und Stratum Corneum (2C) umfasst, wobei der TSM-Wandler (3) ermöglicht, elektrische Parameter zu messen und umzuwandeln, um mechanische Eigenschaften des lebenden Hautexplantats (2) zu bestimmen, und konfiguriert ist, um TSM-Wellen im lebenden ex vivo-Hautexplantat (2) zu erzeugen, und dadurch, dass die Dermis (2A) in Kontrakt mit dem TSM-Wandler ist (3) und das Stratum Corneum (2C) in Kontakt mit der Luft ist.

2. TSM-Biosensor (1) nach Anspruch 1 und Behälter, in dem sich der TSM-Biosensor (1) befindet, wobei der TSM-Biosensor (1) von Kulturmedium (5) umgeben ist, wobei der TSM-Wandler (3) in das Kulturmedium (5) eingetaucht ist, um die Dermis (2A) im Kulturmedium (5) und das Stratum Corneum (2C) in Kontakt mit der Luft zu halten, wodurch ermöglicht wird, dass das Hautexplantat (2) während mindestens einigen Stunden überlebt.

3. TSM-Biosensor (1) nach Anspruch 1, wobei das Kulturmedium (5) auf einer der zwei Elektroden (4A, 4B) angeordnet ist, und wobei die Dermis (2A) des Hautexplantats (2) in Kontakt mit der Elektrode ist, um das Überleben des Hautexplantats (2) auf dem TSM-Wandler (3) mindestens mehrere Stunden zu garantieren.

4. TSM-Biosensor (1) nach den Ansprüchen 2 bis 3, wobei das Kulturmedium (5) ein Gel ist.

5. TSM-Biosensor (1) nach Anspruch 3, wobei das Kulturmedium (5) eine funktionalisierte Schicht ist.

6. TSM-Biosensor (1) nach einem der Ansprüche 1 bis 5, wobei die Elektrode mindestens eine Öffnung aufweist, die mit dem Kulturmedium (5) gefüllt ist, wodurch das Überleben des Hautexplantats (2) während mindestens einigen Stunden ermöglicht wird.

7. TSM-Biosensor (1) nach einem der Ansprüche 1 bis 6, wobei der TSM-Wandler (3) ein Überwachungssystem mit Überwachungs- und Berechnungsmitteln (31) umfasst, die Zugang zu einer zusätzlichen äquivalenten elektrischen Impedanz des TSM-Biosensors (1) geben, geladen durch das lebende Hautexplantat (2), bezeichnet als $Z = R + jX$, wobei Z mit dem komplexen Schermodul $G = G' + jG''$ verbunden ist, das die Viskoelastizität der Haut aus der modifizierten äquivalenten elektrischen Butterworth-van-Dyke (BVD)-Schaltung mit konzentrierten Elementen darstellt, unter Verwendung der folgenden Gleichungen:

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{skin} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{skin}}} + \omega h_{eff} \right)$$

wobei K der elektromechanische Kopplungsfaktor ist, ρ_{skin} die Hautdichte ist, ω die Resonanzpulsation ist, G' , G'' jeweils das Speichermodul und das Verlustmodul sind, die dem tatsächlichen und imaginären Teil des komplexen Schermoduls G entsprechen, und h_{eff} die Tiefe der lebenden Haut ist.

8. TSM-Biosensor (1) nach Anspruch 7, wobei die Überwachungs- und Berechnungsmittel (31) ein elektrisches Modell des Biosensors durch eine elektrische Schaltung integrieren, wobei der Biosensor (1) durch eine elektrische Schaltung modelliert ist, die Folgendes umfasst:

- einen statischen Zweig, der das elektrische Verhalten von Quarz modelliert, und
- einen dynamischen Zweig parallel zum statischen Zweig, und der das mechanische Verhalten von Quarz und das mechanische Verhalten der Haut modelliert,

wobei der statische Zweig einen Widerstand und einen Kondensator umfasst, die parallel geschaltet sind, wobei der dynamische Zweig in Reihe Folgendes umfasst:

- einen Kondensator, eine Selbstinduktivität und einen Widerstand, wodurch das mechanische Verhalten von Quarz modelliert wird, und
- wobei die zusätzliche äquivalente elektrische Impedanz Z das mechanische Verhalten der Haut modelliert.

9. TSM-Biosensor (1) nach einem der Ansprüche 1 bis 8, wobei die Elektroden (4A, 4B) die gesamte Oberfläche des AT-Schnittquarzresonators abdecken.

10. TSM-Biosensor (1) nach einem der Ansprüche 1 - 9, wobei das Hautexplantat (2) die gesamte Oberfläche einer Elektrode abdeckt, auf der es abgelegt ist.

11. TSM-Biosensor (1) nach einem der Ansprüche 1 - 10, wobei die Elektroden (4A, 4B) aus Gold hergestellt sind.

12. Verfahren zum Bestimmen der mechanischen Eigenschaften des lebenden ex vivo-Hautexplantats (2), das in Kontakt mit einem Kulturmedium ist, wobei das lebende Hautexplantat (2) Dermis (2A), Epidermis (2B) und Stratum Corneum (2C) umfasst, unter Verwendung eines TSM-Wandlers (3), der Folgendes umfasst: einen AT-Schnittquarzresonator, der zwei gegenüberliegende äußere Flächen (3A, 3B) und zwei Metallelektroden (4A, 4B) aufweist, wobei jede Metallelektrode auf einer der zwei äußeren Flächen (3A, 3B) abgelegt ist, wobei das Verfahren **dadurch gekennzeichnet ist, dass** es die folgenden Schritte umfasst:

- (a) In-Kontakt-Bringen der Dermis mit dem TSM-Wandler (3) und In-Kontakt-Bringen des Stratum Corneum (2C) mit der Luft,
- (b) Erzeugen, durch den TSM-Wandler, von TSM-Wellen im lebenden ex vivo-Hautexplantat (2),
- (c) Messen und Umwandeln, durch den TSM-Wandler, von elektrischen Parametern, um die mechanischen Eigenschaften des lebenden Hautexplantats (2) zu bestimmen.

13. Verfahren nach Anspruch 12, wobei das lebende Hautexplantat (2) vorbehandelt ist, z. B. hydriert oder entfettet.

14. Verfahren nach einem der Ansprüche 12 bis 13, wobei das lebende Hautexplantat (2) mit mindestens einem Produkt vor oder nach seiner Ablage auf dem TSM-Wandler (3) behandelt wird, um Produkt-Haut-Effekte zu überwachen.

15. Verfahren nach einem der Ansprüche 12 bis 14, wobei der TSM-Wandler (3) ein Überwachungssystem mit Überwachungs- und Berechnungsmitteln (31) umfasst, die Zugang zu einer zusätzlichen äquivalenten elektrischen Impedanz des TSM-Biosensors (1) geben, geladen durch das lebende Hautexplantat (2), bezeichnet als $Z = R + j X$, wobei Z mit dem komplexen Schermodul $G = G' + j G''$ verbunden ist, das die Viskoelastizität der Haut aus der modifizierten äquivalenten elektrischen Butterworth-van-Dyke (BVD)-Schaltung mit konzentrierten Elementen darstellt, unter Verwendung der folgenden Gleichungen:

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{\text{skin}} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{\text{skin}}}} + \omega h_{\text{eff}} \right)$$

wobei K der elektromechanische Kopplungsfaktor ist, ρ_{skin} die Hautdichte ist, ω die Resonanzpulsation ist, G' , G'' jeweils das Speichermodul und das Verlustmodul sind, die dem tatsächlichen und imaginären Teil des komplexen Schermoduls G entsprechen, und h_{eff} die Tiefe der lebenden Haut ist,

wobei der Biosensor (1) durch eine elektrische Schaltung modelliert wird, die Folgendes umfasst:

- einen statischen Zweig, der das elektrische Verhalten von Quarz modelliert; und
- einen dynamischen Zweig parallel zum statischen Zweig, und der das mechanische Verhalten von Quarz und das mechanische Verhalten der Haut modelliert,

wobei der statische Zweig einen Widerstand und einen Kondensator umfasst, die parallel geschaltet sind, wobei der dynamische Zweig in Reihe Folgendes umfasst:

- einen Kondensator, eine Selbstinduktivität und einen Widerstand, die das mechanische Verhalten von Quarz modellieren, und
- wobei die zusätzliche äquivalente elektrische Impedanz Z das mechanische Verhalten der Haut modelliert.

16. Verwendung eines Dickenschersmodus (TSM)-Biosensors (1) nach einem der Ansprüche 1 bis 11, um das mechanische Verhalten *ex vivo* des lebenden Hautexplantats (2) zu testen und die viskoelastischen Eigenschaften des lebenden Hautexplantats (2) zu bestimmen.

17. Verwendung eines Dickenschersmodus (TSM)-Biosensors (1) nach einem der Ansprüche 1 bis 11, um Hautkrankheiten nachzuweisen und/oder nachzuverfolgen oder die Wirkung eines Produkts auf das Hautexplantat (2) zu analysieren.

Revendications

1. Biocapteur à mode de cisaillement d'épaisseur (TSM) (1) qui comprend :

- un milieu de culture,
- un transducteur TSM (3) qui comprend : un résonateur à quartz de coupe AT qui a deux surfaces extérieures (3A, 3B) opposées, et deux électrodes de métal (4A, 4B), chaque électrode de métal étant déposée sur l'une des deux surfaces extérieures (3A, 3B),

caractérisé en ce que le biocapteur TSM (1) comprend en outre un explant de peau vivante *ex vivo* (2) en contact avec le milieu de culture, l'explant de peau (2) comprenant un derme (2A), un épiderme (2B) et une couche cornée (2C), le transducteur TSM (3) permettant de mesurer et de convertir des paramètres électriques pour déterminer des caractéristiques mécaniques de l'explant de peau vivante (2), et étant configuré pour générer des ondes TSM dans l'explant de peau vivante *ex vivo* (2), et **en ce que** le derme (2A) est en contact avec le transducteur TSM (3), et la couche cornée (2C) est en contact avec l'air.

2. Biocapteur TSM (1) selon la revendication 1, et un réceptacle dans lequel se trouve le biocapteur TSM (1), le biocapteur TSM (1) étant entouré par un milieu de culture (5), le transducteur TSM (3) étant immergé dans le milieu de culture (5) de façon à maintenir le derme (2A) dans le milieu de culture (5) et la couche cornée (2C) en contact avec l'air, permettant la survie de l'explant de peau (2) pendant au moins plusieurs heures.

3. Biocapteur TSM (1) selon la revendication 1, dans lequel le milieu de culture (5) est disposé sur l'une des deux électrodes (4A, 4B), et dans lequel le derme (2A) de l'explant de peau (2) est en contact avec ladite électrode, afin de garantir la survie de l'explant de peau (2) sur le transducteur TSM (3) au moins plusieurs heures.

4. Biocapteur TSM (1) selon les revendications 2 à 3, dans lequel le milieu de culture (5) est un gel.

5. Biocapteur TSM (1) selon la revendication 3, dans lequel le milieu de culture (5) est une couche fonctionnalisée.
6. Biocapteur TSM (1) selon l'une des revendications 1 à 5, dans lequel l'électrode a au moins une ouverture qui est remplie du milieu de culture (5), permettant la survie de l'explant de peau (2) pendant au moins plusieurs heures.
7. Biocapteur TSM (1) selon l'une des revendications 1 à 6, dans lequel le transducteur TSM (3) comprend un système de surveillance avec des moyens de surveillance et de calcul (31) qui donnent accès à une impédance électrique équivalente additionnelle du biocapteur TSM (1) chargé de l'explant de peau vivante (2), notée $Z = R + j X$, Z étant lié au module de cisaillement complexe $G = G' + j G''$, représentatif de la viscoélasticité de la peau à partir du circuit de Butterworth-van-Dyke (BVD) à éléments localisés électriques équivalents modifiés, à l'aide des équations suivantes :

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{skin} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{skin}}} + \omega h_{eff} \right)$$

où K est le facteur de couplage électromécanique, ρ_{skin} est la masse volumique de la peau, ω est la pulsation de résonance, G' , G'' sont respectivement le module de conservation et le module de perte correspondant aux parties réelle et imaginaire du module de cisaillement complexe G , et h_{eff} est la profondeur de la peau vivante.

8. Biocapteur TSM (1) selon la revendication 7, dans lequel les moyens de surveillance et de calcul (31) intègrent un modèle électrique du biocapteur par un circuit électrique, le biocapteur (1) étant modélisé par un circuit électrique qui comprend :

- une branche statique modélisant le comportement électrique du quartz, et
- une branche dynamique parallèle à la branche statique et modélisant le comportement mécanique du quartz et le comportement mécanique de la peau,

la branche statique comprenant un résistor et un condensateur connectés en parallèle, la branche dynamique comprenant en série :

- un condensateur, une induction propre et un résistor modélisant le comportement mécanique du quartz, et
- l'impédance électrique équivalente additionnelle Z modélisant le comportement mécanique de la peau.

9. Biocapteur TSM (1) selon l'une des revendications 1 à 8, dans lequel les électrodes (4A, 4B) couvrent la surface entière du résonateur à quartz de coupe AT.

10. Biocapteur TSM (1) selon l'une des revendications 1 à 9, dans lequel l'explant de peau (2) couvre la surface entière d'une électrode sur laquelle il est déposé.

11. Biocapteur TSM (1) selon l'une des revendications 1 à 10, dans lequel les électrodes (4A, 4B) sont faites d'or.

12. Procédé de détermination de caractéristiques mécaniques d'un explant de peau vivante *ex vivo* (2) qui est en contact avec un milieu de culture, l'explant de peau vivante (2) comprenant un derme (2A), un épiderme (2B), et une couche cornée (2C), à l'aide d'un transducteur TSM (3) qui comprend : un résonateur à quartz de coupe AT qui a deux surfaces extérieures (3A, 3B) opposées, et deux électrodes de métal (4A, 4B), chaque électrode de métal étant déposée sur l'une des deux surfaces extérieures (3A, 3B), la Procédé étant **caractérisée en ce qu'elle** comprend les étapes suivantes :

- (a) la mise en contact du derme avec le transducteur TSM (3), et de la couche cornée (2C) en contact avec l'air,

- (b) la génération, par le transducteur TSM, d'ondes TSM dans l'explant de peau vivante *ex vivo* (2),
- (c) la mesure et la conversion, par le transducteur TSM, de paramètres électriques pour déterminer des caractéristiques mécaniques de l'explant de peau vivante (2).

13. Procédé selon la revendication 12, dans laquelle l'explant de peau vivante (2) est prétraité, par exemple hydraté ou délipidé.

14. Procédé selon l'une des revendications 12 à 13, dans laquelle, l'explant de peau vivante (2) est traité avec au moins un produit, avant ou après son dépôt sur le transducteur TSM (3), pour surveiller des effets de produits sur la peau.

15. Procédé selon l'une des revendications 12 à 14, dans laquelle le transducteur TSM (3) comprend un système de surveillance avec des moyens de surveillance et de calcul (31) qui donnent accès à une impédance électrique équivalente additionnelle du biocapteur TSM (1) chargé de l'explant de peau vivante (2), notée $Z = R + j X$, Z étant lié au module de cisaillement complexe $G = G' + j G''$, représentatif de la viscoélasticité de la peau à partir du circuit de Butterworth-van-Dyke (BVD) à éléments localisés électriques équivalents modifiés, à l'aide des équations suivantes :

$$R \cong K \rho_{skin} \sqrt{\frac{(|G(\omega)| + G'(\omega))}{2 \rho_{skin}}}$$

$$X \cong K \rho_{skin} \left(\sqrt{\frac{(|G(\omega)| - G'(\omega))}{2 \rho_{skin}}} + \omega h_{eff} \right)$$

où K est le facteur de couplage électromécanique, ρ_{skin} est la masse volumique de la peau, ω est la pulsation de résonance, G' , G'' sont respectivement le module de conservation et le module de perte correspondant aux parties réelle et imaginaire du module de cisaillement complexe G , et h_{eff} est la profondeur de la peau vivante, le biocapteur (1) étant modélisé par un circuit électrique qui comprend :

- une branche statique modélisant le comportement électrique du quartz, et
- une branche dynamique parallèle à la branche statique et modélisant le comportement mécanique du quartz et le comportement mécanique de la peau,

la branche statique comprenant un résistor et un condensateur connectés en parallèle,
la branche dynamique comprenant en série :

- un condensateur, une induction propre et un résistor modélisant le comportement mécanique du quartz, et
- l'impédance électrique équivalente additionnelle Z modélisant le comportement mécanique de la peau.

16. Utilisation d'un biocapteur à mode de cisaillement d'épaisseur (TSM) (1) selon l'une des revendications 1 à 11, pour mettre à l'épreuve le comportement mécanique *ex vivo* d'un explant de peau vivante (2) et pour déterminer des caractéristiques viscoélastiques de l'explant de peau vivante (2).

17. Utilisation d'un biocapteur à mode de cisaillement d'épaisseur (TSM) (1) selon l'une des revendications 1 à 11, pour détecter et/ou suivre des maladies de la peau ou pour analyser l'effet d'un produit sur l'explant de peau (2).

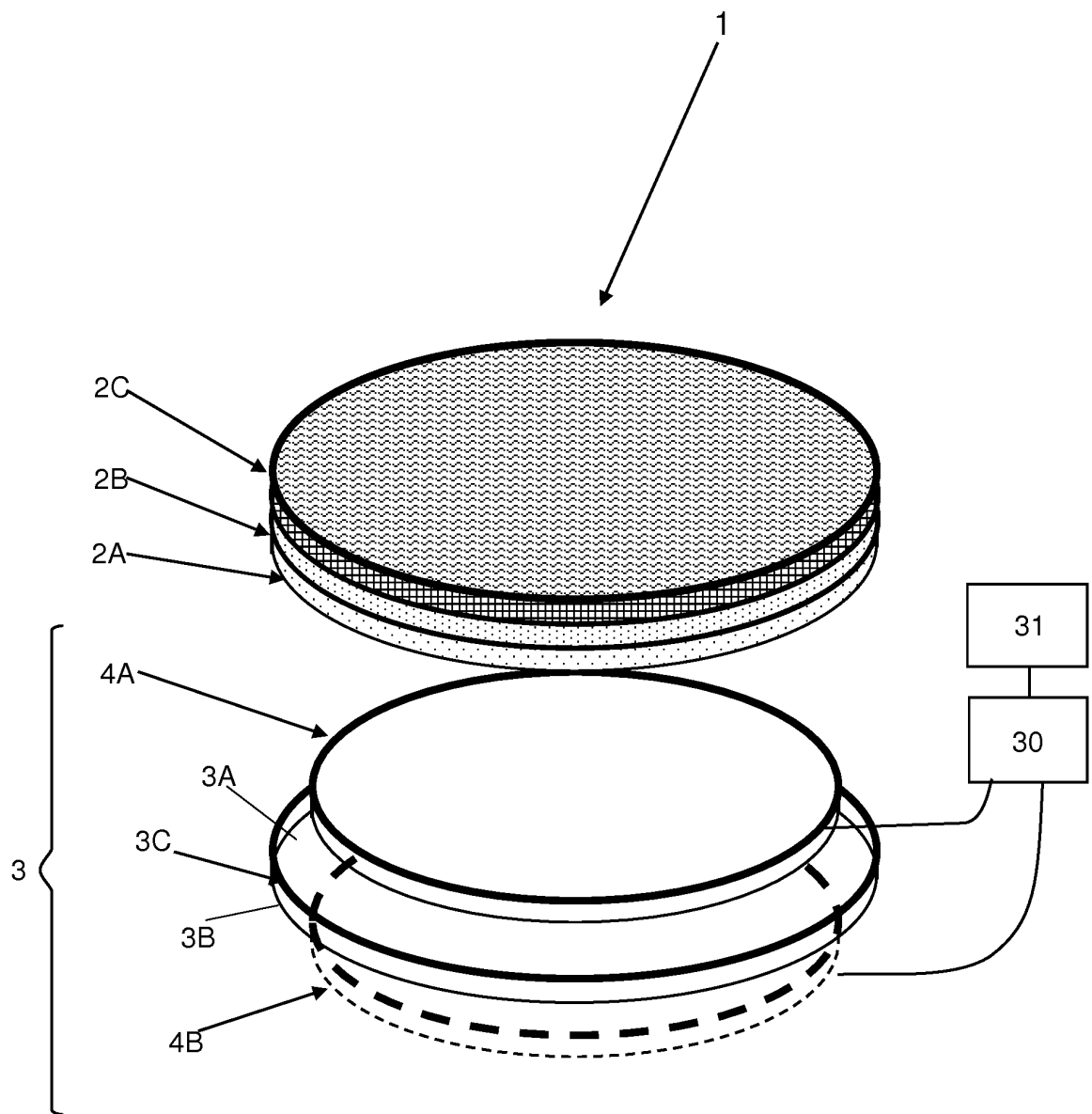


Figure 1

Figure 2

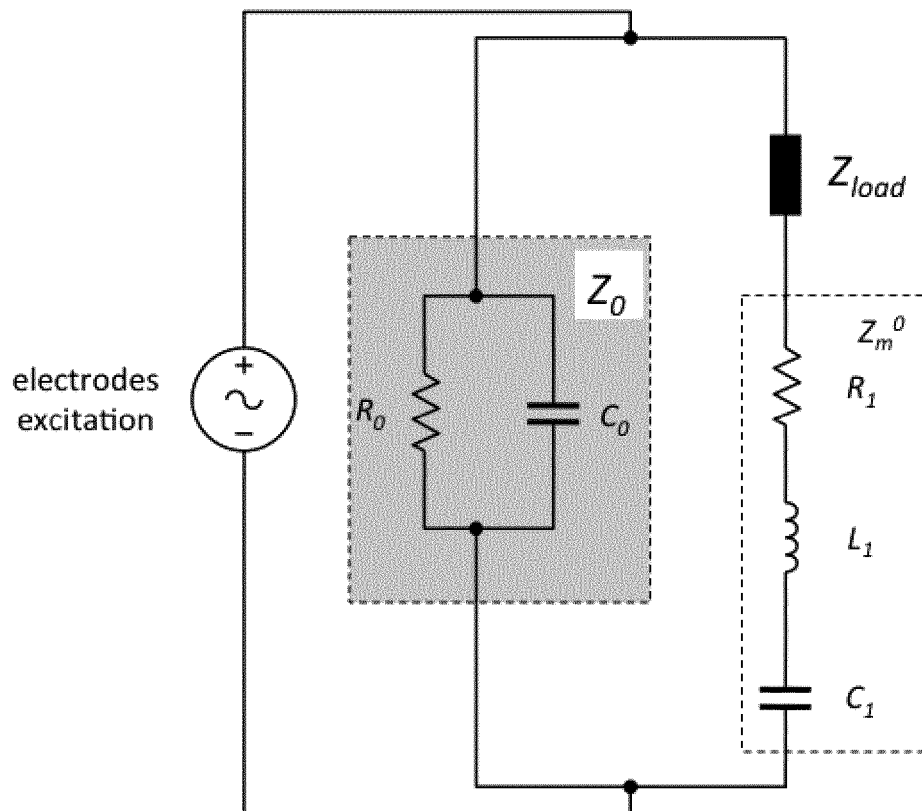
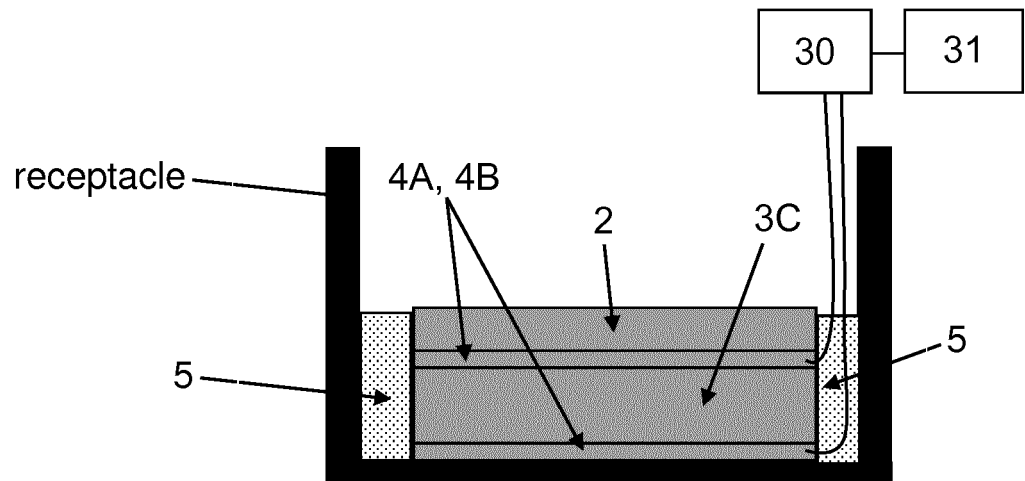


Figure 3

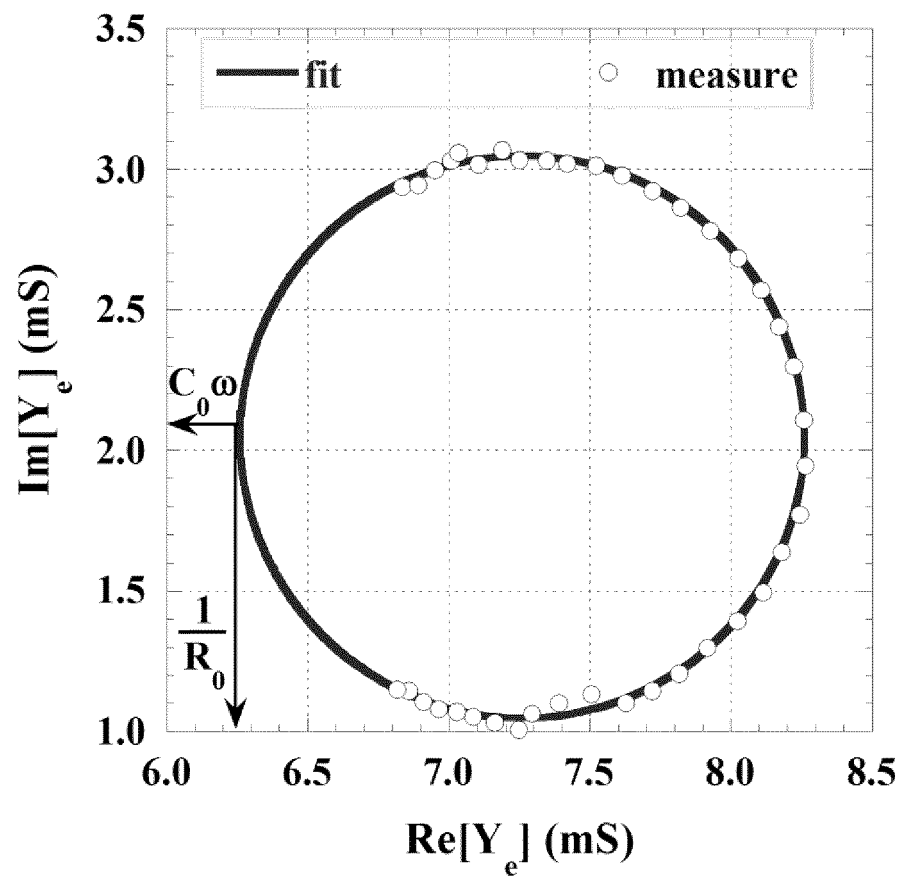


Figure 4

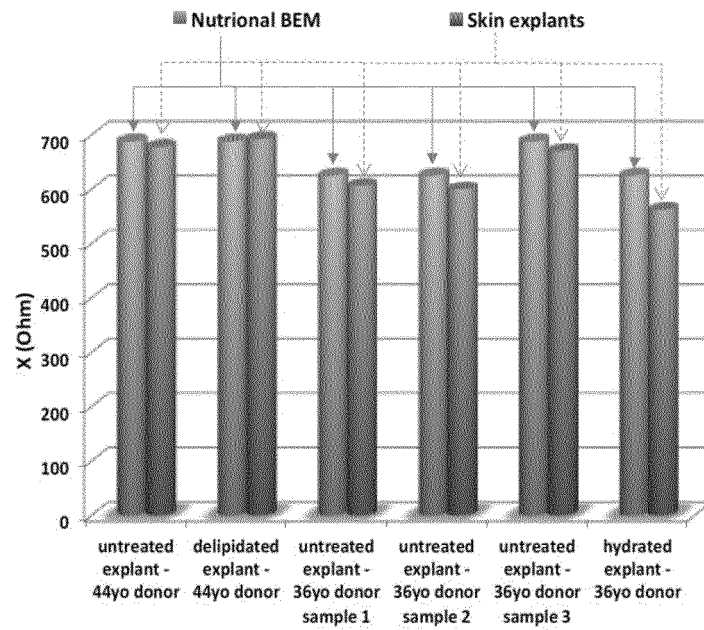


Figure 5

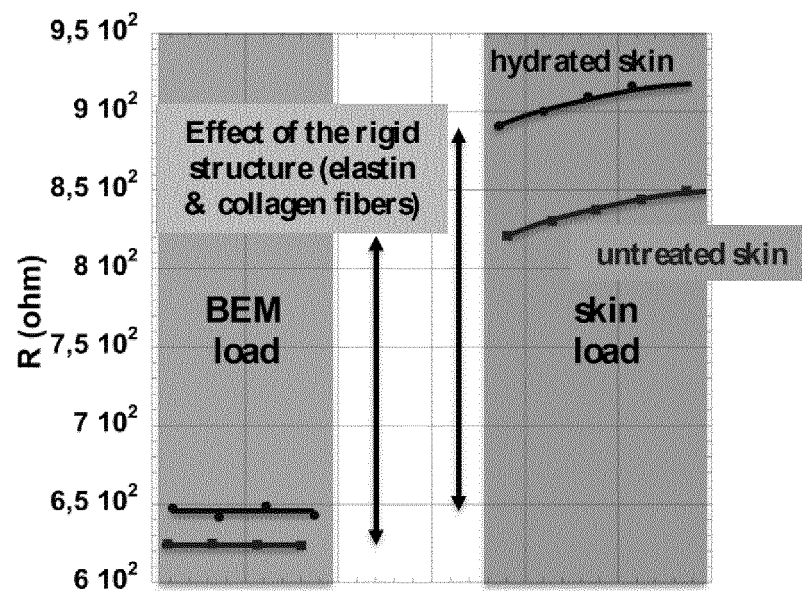


Figure 6

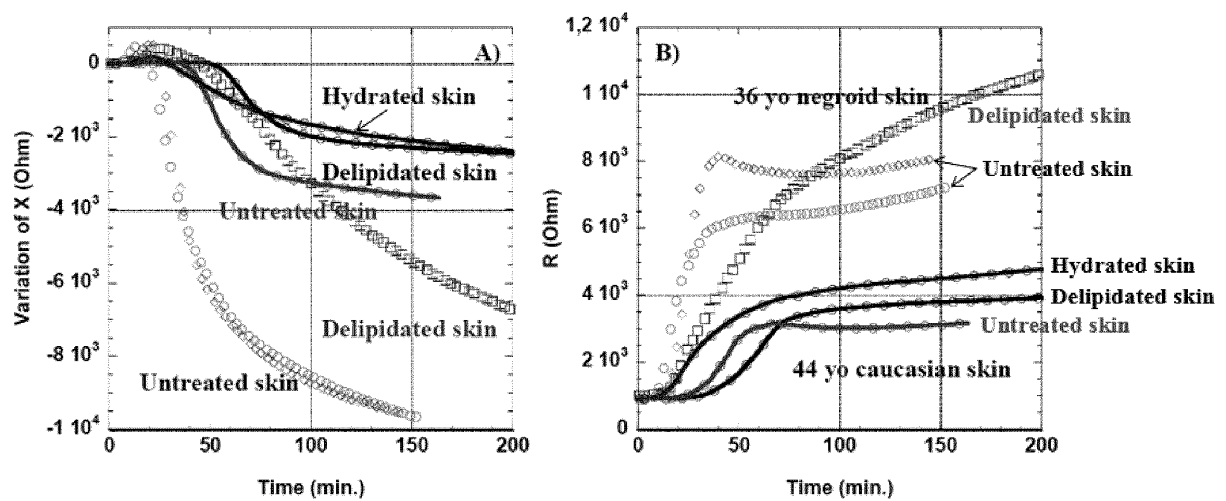
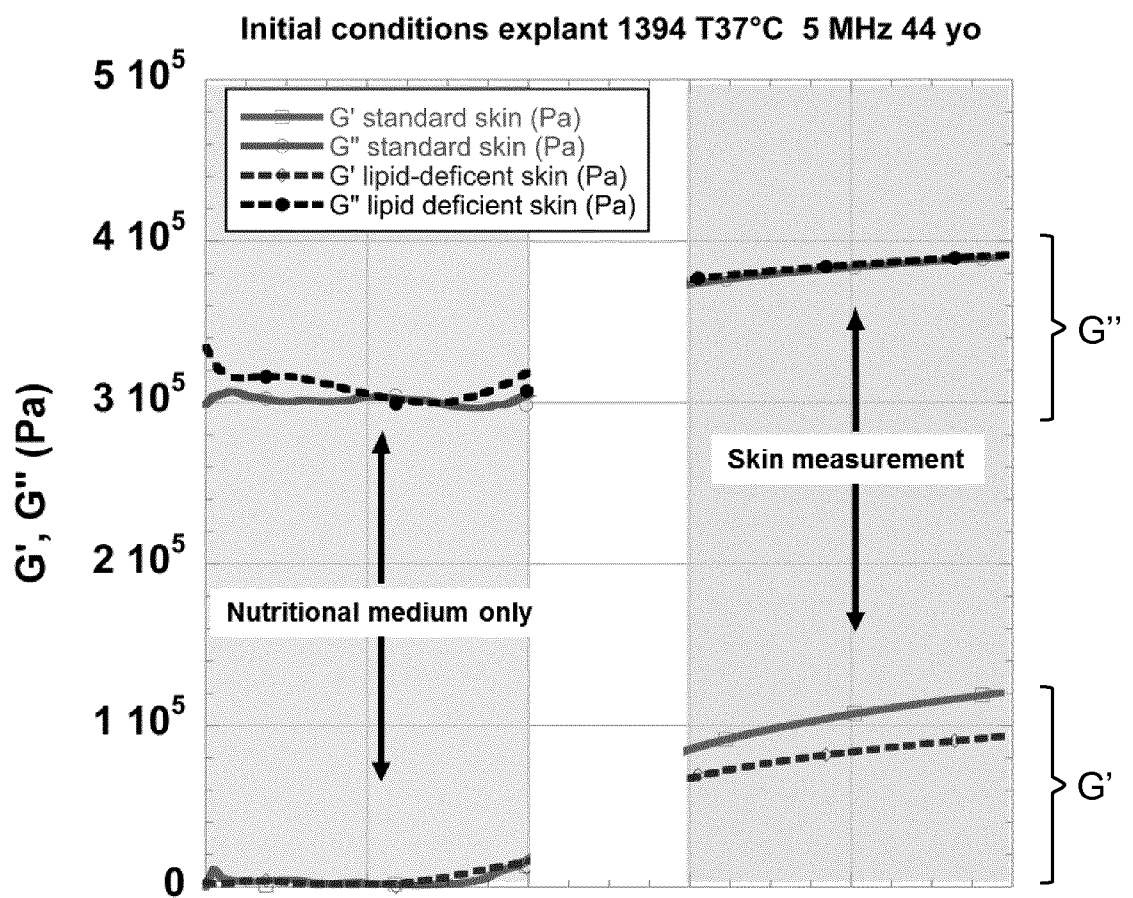


Figure 7

Figure 8



REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 20100075347 A [0005]
- WO 2012172253 A [0020]

Non-patent literature cited in the description

- **FANG LI.** *An Acoustic Wave Biosensor for Probing the Viscoelastic Properties of Living Cells* [0004]
- **EHSEIN C.** *Kinetic reaction monitoring of acidified milk gels with a quartz resonator* [0006]
- **R. F. RUSHMER ; K. J. K. BUETTNER ; J. M. SHORT ; G. F. ODLAND.** *The Skin. Science*, 1966, vol. 154 (3747), 343-348 [0087]
- **G. ODLAND.** *Structure of the skin. Physiology, Biochemistry, and Molecular Biology of the Skin*, 1991, 3-62 [0087]
- **C.W. OOMENS ; D. H. VAN CAMPEN ; H. J. GROOTENBOER.** *A mixture approach to the mechanics of skin. J. Biomech.*, 1987, vol. 20 (9), 877-885 [0087]
- **F. XU ; T. LU.** *Introduction of Skin Biothermomechanics. Introduction to Skin Biothermomechanics and Thermal Pain*, 2011, 209-220 [0087]
- **F. XU ; T. J. LU ; K. A. SEFFEN.** *Biothermomechanical behavior of skin tissue. Acta Mech. Sin. Xuebao*, 2008, vol. 24 (1), 1-23 [0087]
- **F. XU ; T. J. LU.** *Chapter 3 Skin Biothermomechanics: Modeling and Experimental Characterization. Advances in Applied Mechanics*, 2009, vol. 43, 147-248 [0087]
- **F. XU ; T. J. LU ; K. A. SEFFEN.** *Biothermomechanics of skin tissues. J. Mech. Phys. Solids*, 2008, vol. 56 (5), 1852-1884 [0087]
- **A. DELALLEAU ; G. JOSSE ; J.-M. LAGARDE ; H. ZAHOUANI ; J.-M. BERGHEAU.** *Characterization of the mechanical properties of skin by inverse analysis combined with the indentation test. J. Biomech.*, 2006, vol. 39 (9), 1603-1610 [0087]
- **M. J. KOEHLER ; T. VOGEL ; P. ELSNER ; K. KONIG ; R. BÜCKLE ; M. KAATZ.** *In vivo measurement of the human epidermal thickness in different localizations by multiphoton laser tomography. Ski. Res. Technol.*, 2010, vol. 16 (3), 259-264 [0087]
- **C. SANCHEZ ; L. ROZES ; F. RIBOT ; C. LABERTY-ROBERT ; D. GROSSO ; C. SASSOYE ; C. BOISSIERE ; L. NICOLE.** *'Chimie douce': A land of opportunities for the designed construction of functional inorganic and hybrid organic-inorganic nanomaterials. Comptes Rendus Chimie*, 2010, vol. 13 (1-2), 3-39 [0087]
- **S. HSU ; A. M. JAMIESON ; J. BLACKWELL.** *Viscoelastic studies of extracellular matrix interactions in a model native collagen gel system. Biorheology*, 1994, vol. 31 (1), 21-36 [0087]
- **X. LIANG ; S. A. BOPPART.** *Biomechanical properties of in vivo human skin from dynamic optical coherence elastography. IEEE Trans. Biomed. Eng.*, 2010, vol. 57 (4), 953-959 [0087]
- **B. J. BELL ; E. NAUMAN ; S. L. VOYTIK-HARBIN.** *Multiscale strain analysis of tissue equivalents using a custom-designed biaxial testing TSM biosensor. Biophys. J.*, vol. 102 (6), 1303-1312 [0087]
- **R. B. GROVES ; S. A. COULMAN ; J. C. BIRCHALL ; S. L. EVANS.** *An anisotropic, hyperelastic model for skin: Experimental measurements, finite element modelling and identification of parameters for human and murine skin. J. Mech. Behav. Biomed. Mater.*, 2013, vol. 18, 167-180 [0087]
- **J. SERUP ; G. B. E. JEMEC ; G. L. GROVE.** *Handbook of Non-Invasive Methods and the Skin.* CRC Press, 2006 [0087]
- **J. M. PEREIRA ; J. M. MANSOUR ; B. R. DAVIS.** *Dynamic measurement of the viscoelastic properties of skin. J. Biomech.*, 1991, vol. 24 (2), 157-162 [0087]
- **Z. DEL PRETE ; S. ANTONIUCCI ; A. H. HOFFMAN ; P. GRIGG.** *Viscoelastic properties of skin in Mov-13 and Tsk mice. J. Biomech.*, 2004, vol. 37 (10), 1491-1497 [0087]
- **H. G. VOGEL.** *Directional variations of mechanical parameters in rat skin depending on maturation and age. J. Invest. Dermatol.*, 1981, vol. 76 (6), 493-497 [0087]
- **O. LOKSHIN ; Y. LANIR.** *Viscoelasticity and preconditioning of rat skin under uniaxial stretch: microstructural constitutive characterization. J. Biomech. Eng.*, 2009, vol. 131 (3), 031009 [0087]
- **S. DERLER ; L. C. GERHARDT.** *Tribology of skin: Review and analysis of experimental results for the friction coefficient of human skin. Tribol. Lett.*, 2012, vol. 45 (1), 1-27 [0087]
- **H. A. WHEELER.** *Transmission-Line Properties of Parallel Strips Separated by a Dielectric Sheet. IEEE Trans. Microw. Theory Tech.*, March 1965, vol. 13 (2), 172-185 [0087]

- **C. OULD EHSSEIN.** Système instrumental pour la rhéologie ultrasonore. Université de Cergy-Pontoise, 2006 [0087]
- **E. LOPEZ ; E. LATIE ; P. GASSER ; M. ROUSSEAU.** Calcium improves skin health. *Househ. Pers. Care Today*, 2008, vol. 3, 32-35 [0087]
- **P. GASSER ; F. ARNOLD ; L. PENO-MAZZARINO ; D. BOUZOUZ ; M. T. LUU ; E. LATI ; M. MERCIER.** Glycation induction and antiglycation activity of skin care ingredients on living human skin explants. *Int. J. Cosmet. Sci.*, August 2011, vol. 33 (4), 366-70 [0087]
- **P. LECLAIRE ; J. GOOSSENS ; L. MARTINEZ ; N. WILKIE-CHANCELIER ; S. SERFATY ; C. GLORIEUX.** Study of the bending modes in circular quartz resonator 3Cs. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, October 2006, vol. 53 (10), 1934-1943 [0087]
- **W. MASON.** A Dynamic Measurement of the Elastic, Electric and Piezoelectric Constants of Rochelle Salt. *Phys. Rev.*, April 1939, vol. 55 (8), 775-789 [0087]
- **R. W. CERNOSEK ; S. J. MARTIN ; A. R. HILLMAN ; H. L. BANDEY.** Comparison of lumped-element and transmission-line models for thickness-shear-mode quartz resonator 3C sensors. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control*, September 1998, vol. 45 (5), 1399-1407 [0087]
- **G. SAUERBREY.** Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. *Zeitschrift für Phys.*, April 1959, vol. 155 (2), 206-222 [0087]
- **K. KEIJI KANAZAWA ; J. G. GORDON.** The oscillation frequency of a quartz resonator 3C in contact with liquid. *Anal. Chim. Acta*, 1985, vol. 175 (C), 99-105 [0087]
- **H. L. BANDEY ; S. J. MARTIN ; R. W. CERNOSEK ; A. R. HILLMAN.** Modeling the Responses of Thickness-Shear Mode Resonators under Various Loading Conditions. *Anal. Chem.*, June 1999, vol. 71 (11), 2205-14 [0087]
- **A. ARNAU ; Y. JIMÉNEZ ; T. SOGORB.** Thickness-shear mode quartz crystal resonators in viscoelastic fluid media. *J. Appl. Phys.*, 2000, vol. 88 (8), 4498 [0087]
- **S. J. MARTIN ; V. E. GRANSTAFF ; G. C. FRYE.** Characterization of a Quartz Crystal Microbalance with Simultaneous Mass and Liquid Loading. *Anal. Chem.*, 1991, vol. 2281 (26), 2272-2281 [0087]
- **C. O. EHSSEIN ; S. SERFATY ; P. GRIESMAR ; E. CAPLAIN ; L. MARTINEZ ; M. GINDRE.** Kinetic reaction monitoring of acidified milk gels with a quartz resonator 3C: effect of temperature and gdl quantities. *IEEE Inter. Ultrasonics, Ferroelectric, and Frequency Controls, 50th Conf.*, 2004, vol. 1, 319-322 [0087]
- **E. CAPLAIN ; C. OULD EHSSEIN ; S. SERFATY ; L. MARTINEZ ; J.-Y. LE HUÉROU ; M. GINDRE.** Toward embedded viscosimeter using quartz crystal resonator. *3rd Meeting IEEE Sensors*, 2004 [0087]
- **C. OULD EHSSEIN ; J. Y. LE HUÉROU ; L. MARTINEZ ; E. CAPLAIN ; N. WILKIE-CHANCELLIER ; M. GINDRE ; G. GOUEDARD ; P. FIGUIERE.** Kinetic study of silica gels by a new rheological ultrasonic investigation. *Ultrasonics*, December 2006, vol. 44 (1), e881-5 [0087]
- **A. C. ROWAT ; N. KITSON ; J. L. THEWALT.** Interactions of oleic acid and model stratum corneum membranes as seen by 2H NMR. *Int. J. Pharm.*, 2006, vol. 307 (2), 225-231 [0087]
- **N. Y. RYABOVA ; M. A. KISELEV ; S. DANTE ; T. HAUSS ; A. M. BALAGUROV.** Investigation of stratum corneum lipid model membranes with free fatty acid composition by neutron diffraction. *Eur. Biophys. J.*, 2010, vol. 39 (8), 1167-1176 [0087]
- **X. CHEN ; S. KWAK ; M. LAFLEUR ; M. BLOOM ; N. KITSON ; J. THEWALT.** Fatty acids influence 'Solid' phase formation in models of stratum corneum intercellular membranes. *Langmuir*, 2007, vol. 23 (10), 5548-5556 [0087]
- **D. LEE ; J. N. ASHCRAFT ; E. VERPLOEGEN ; E. PASHKOVSKI ; D. A. WERTZ.** Permeability of model stratum corneum lipid membrane measured using quartz crystal microbalance. *Langmuir*, 2009, vol. 25 (10), 5762-5766 [0087]

专利名称(译)	Tsm生物传感器用于离体表征皮肤的粘弹性		
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申请号	EP2016732958	申请日	2016-06-08
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优先权	2015020088 2015-06-08 EP 2015306357 2015-09-03 EP		
其他公开文献	EP3302237A1		
外部链接	Espacenet		

摘要(译)

本发明涉及厚度剪切模式 (TSM) 生物传感器 (1) , 其包括离体活皮肤外植体 (2) , 皮肤外植体 (2) 包括皮下层中的至少一个 : 皮下层 , 真皮层 (2A) , 表皮层 (2B) 和角质层 (2C) , TSM换能器 (3) , 包括 : AT切割石英谐振器3C, 其具有两个相对的外表面 (3A , 3B) 和两个导电电极 (4A , 4B) , 每个都导电电极沉积在两个外表面 (3A , 3B) 中的一个上 , TSM换能器 (3) 允许通过使用剪切波的压电换能器确定活皮外植体 (2) 的微流变特性 , TSM换能器 (3) 呈现 : 测量装置 (30) , 监测和计算装置 (31) , 其监测活皮外植体 (2) 的电响应的时间演变 , 并且从电响应 , 生活的微流变特性及时计算皮肤外植体 (2) , 其底面kin外植体 (2) 与TSM换能器 (3) 接触 , 皮肤外植体 (2) 的顶表面与空气接触。

$$R = \frac{h^2}{4Ae_q^2} \sqrt{\rho_{skin} \left(\frac{|G(\omega)| + G'(\omega)}{2} \right)}$$