Supporting information for the publication: "Mechanical properties of soft biological membranes for organ-on-a-chip assessed by bulge test and AFM"

Authors: Pauline Zamprogno[1‡], Giuditta Thoma[1‡], Veronika Cencen[2], Dario Ferrari[1], Barbara Putz[3], Johann Michler[3], Georg E. Fantner[2], Olivier T. Guenat[1,4,5] *

‡ These authors contributed equally to this work.

Affiliations:

[1] Organs-on-Chip Technologies Laboratory, ARTORG Center, University of Bern, Bern, Switzerland.

[2] Laboratory for Bio- and Nano- Instrumentation, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

[3] Laboratory for Mechanics of Materials and Nanostructures, Empa Swiss Federal Laboratories for Materials Science and Technology, Thun, Switzerland.

[4] Department of Pulmonary Medicine, University Hospital of Bern, Bern, Switzerland.

[5] Department of General Thoracic Surgery, University Hospital of Bern, Bern, Switzerland.

*E-mail: olivier.guenat@artorg.unibe.ch

Figure S1: Chip design.

The chip was made of a PDMS top layer and a polycarbonate (PC) bottom layer, between which either A) a collagen-elastin membrane or B) a PDMS membrane supported by a gold mesh was sandwiched. The gold mesh was bonded to the PDMS top part with a double tape (Arcare 90445-5, Adhesives Research, Glen Mark, PA, USA) punched with 2mm in diameter holes.

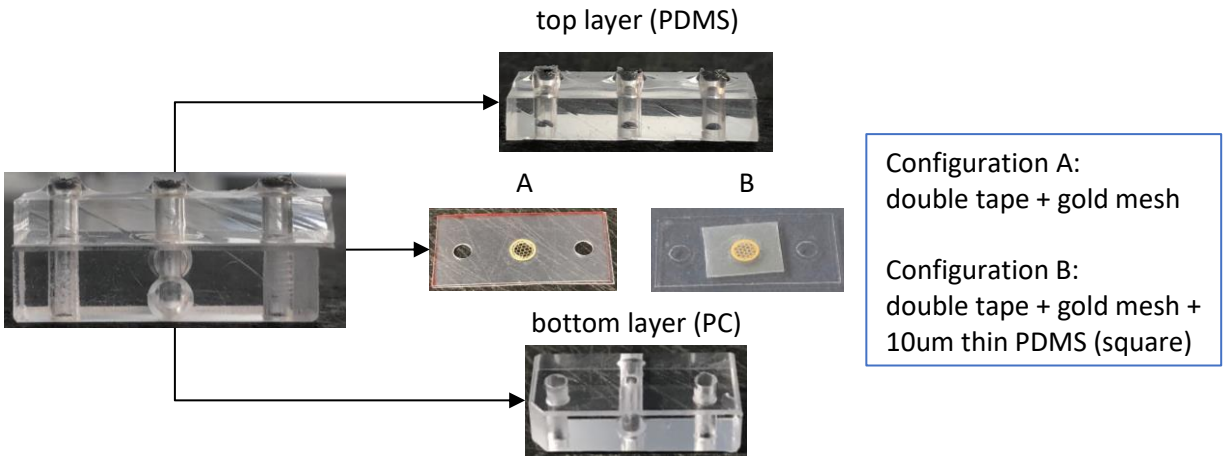


Figure S2: Thickness of the vitrified and of the hydrogel-based biological membranes.

The volume of the collagen-elastin solution pipetted on the gold mesh is 32µl for both membranes (1.6 µL/mm^2). In contrast to the hydrogel membrane, the vitrified membrane is dried following the dispensing of the CE solution. This influences the final thickness of the membrane (Fig. A). For each hydrogel CE-membrane, 3 measurements were taken in the central hexagon. The thickness variation across the hexagon was below 12% (Fig. B).

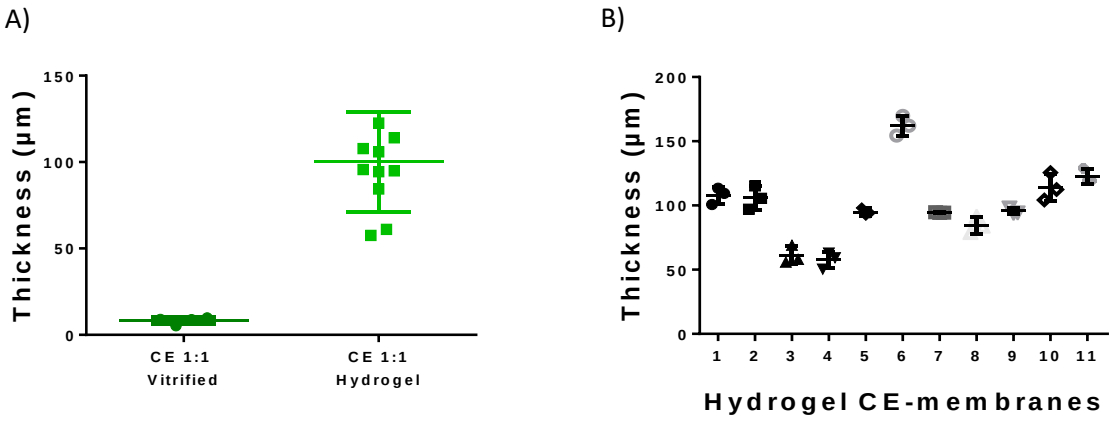
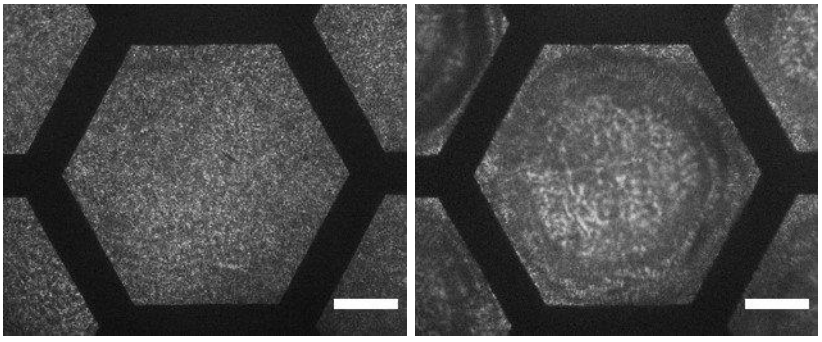


Figure S3: Representative image of a hydrogel CE-membrane at rest and after exposure to a negative pressure of -1.1 kPa.



At rest Stretched

The images were acquired by AxioPlan2 Zeiss Microscope via reflective light. Scale bar: 100 µm.

Figure S4: Determination of the Young's modulus based on the pressure-deflection curve of the hydrogel CE-membrane.

The fitting curve was based on the bulge equation ($P = ah + bh^3$), with *P* and *h*, being the pressure and the bulge height, and *a* and *b* two constants. The least-squares method (MATLAB) was used to fit the experimental pressure-deflection data, resulting in a coefficient of determination R^2 (here R^2=0.9953). The Young's modulus E was then extracted from the constant b from the fitting curve (here $E_{hydrogel}$ = 0.7 kPa).

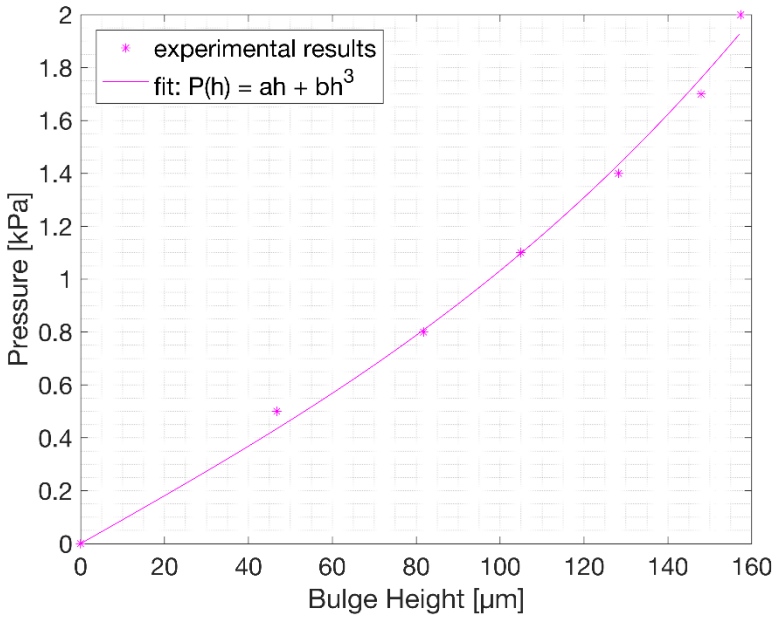


Figure S5: Impact of the storage of the vitrified CE-membrane on its mechanical properties.

The vitrified CE-membrane has been dried for 48h at room temperature and then stored for two weeks. No significant difference of the Young's modulus has been observed between membranes tested before and after storage (173 ± 37 kPa after the production versus 212 ± 84 kPa after two weeks storage).

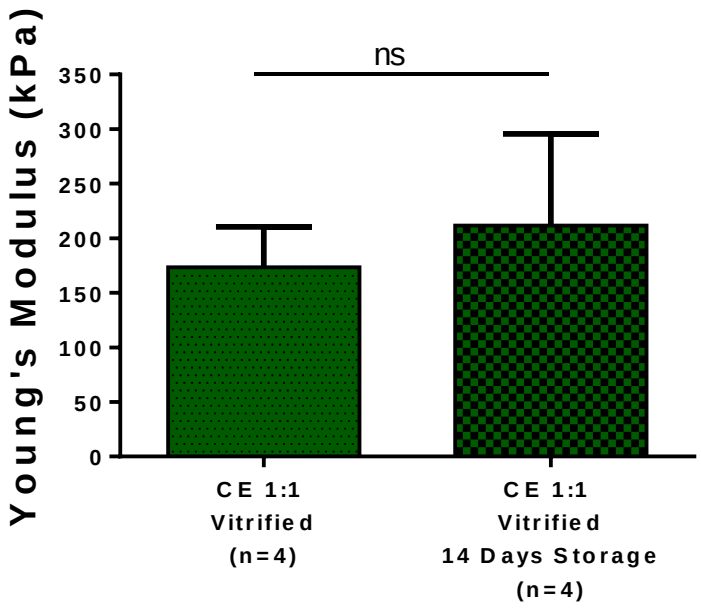


Figure S6: Impact of a 2-weeks immersion in physiological medium on the mechanical properties of the vitrified CE-membrane.

To simulate the impact on the stiffness of a long-term cell culture, the vitrified CE-membrane was immerged during two weeks in physiological medium. The vitrified CE-membrane has been rehydrated with cell culture media, then incubated for two weeks. No significant difference of the Young's modulus has been observed between membranes tested before and after being immerged in physiological medium for two weeks (173 ± 37 kPa after the production versus 172 ± 38 kPa after two weeks immersion).

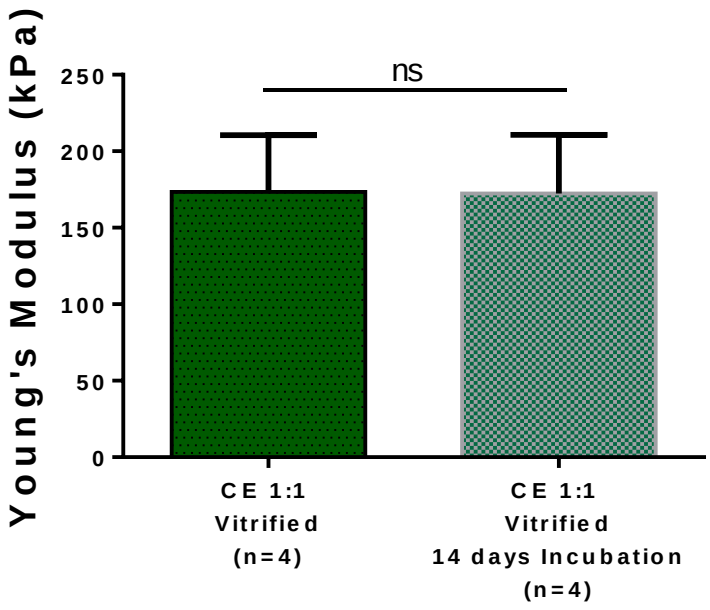


Figure S7: Force-indentation curves and force-volume images obtained by AFM

The modulus of the vitrified CE membrane and of the PDMS membrane were measured using AFM. The figure A and B are the force-indentation curves of PDMS and the CE membrane respectively, whereas figures C and D are their corresponding force-volume images.

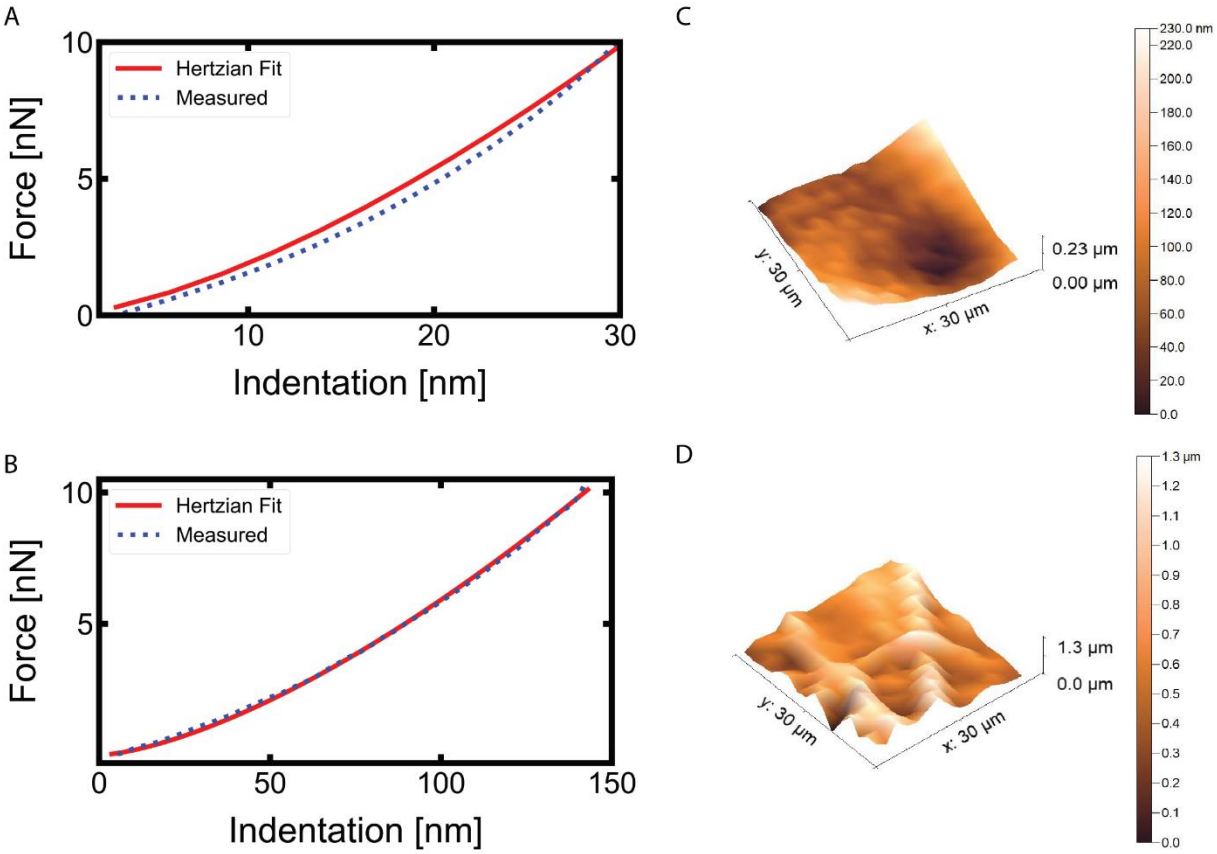
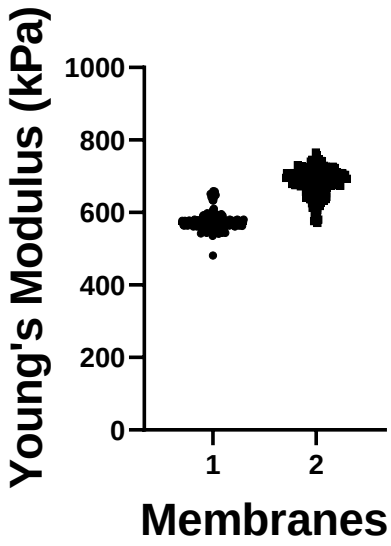


Figure S8: Young's Modulus variance measured by AFM

The AFM method provides the value of the local stiffness. A minimum of 256 measurements was carried out to quantify the stiffness of the membrane. The distribution of the measurements obtained for the PDMS membrane and vitrified CE-membrane are indicated in figures A and B, respectively. The coefficient of variation is smaller than 6% for the PDMS membrane, and is larger than 11% for the CE-membrane.

A)



B)

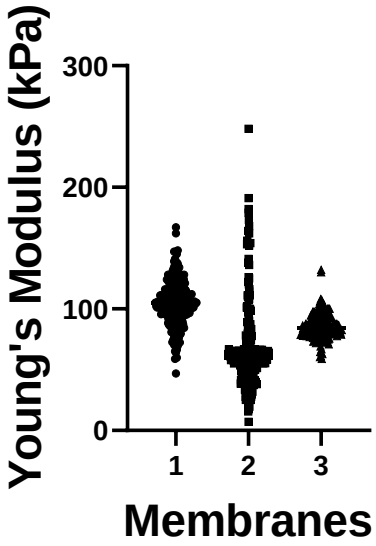


Figure S9: Membrane production: Impact of the gelation temperature on the Young's modulus during the production of the hydrogel CE-membrane.

Following the dispensing of the CE-solution on the gold mesh, hydrogel membranes were produced with different gelation temperatures. The hydrogel solution of the first set of membranes was crosslinked at 37°C for 1h, whereas the second set of membranes had a lower gelation temperature (4°C) and longer incubation time (overnight). At lower gelation temperature, the resulted membrane presented a significantly higher stiffness than at 37°C (0.79 ± 0.36 kPa at 37°C against 2.0 ± 0.54 kPa at 4°C).