32nd IFSCC Congress
London 19-22 September 2022

A mechanical dimension to evaluate cell traction force based on collagen gel stiffness measurement by atomic force microscopy

biomeca

Jean-André Lapart, Gael Runel, Julien Chlasta
BioMeca SAS, 60F avenue Rockefeller, 69008 Lyon, France

ID-478

Introduction:

Cell traction force plays an important role in both **skin aging** and **wound healing**, where fibroblasts interact with and pull on the collagen fibers in the 3D surrounding ECM to respectively maintain **skin firmness**, mechanical support and to accelerate the **closure of wound**. In turn this interaction allows to maintain cell spreading and a mechanical force.

From Boismal *et al.*, 2020

However, **aging alters fibroblast physiology** where collagen production decreases, protease secretion increases leading to a reduced cellular mechanical force and therefore to a saggy skin and a decrease in the quality of wound healing. Thus, there is a real **need to increase the potential of cell traction** force to accelerate wound speed and maintain a good skin homeostasis

Collagen contraction assay has been widely used to evaluate cell traction force, whose principle relies on the force generated by cells within the gel by interacting with collagen 1 lattice. However, most of the studies mainly evaluate cell traction force by only measuring the gel area after gel contraction and mechanical property analysis were not performed or did not reveal any variations.

Therefore, to better evaluate the cell traction force, we have implemented a mechanical dimension by evaluating collagen gel stiffness by **Atomic Force Microscopy (AFM)**. We show in this study that TGF-beta fibroblasts exhibit a higher cell traction force compared to untreated cells based on the stiffness of collagen gels.

Materials & Methods:

• Biological samples

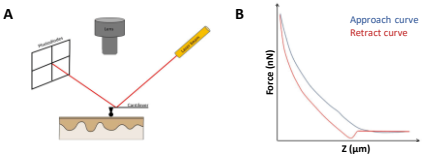
Cryopreserved normal human dermal fibroblast cell line derived from skin biopsies on healthy female from 30 years old were purchased from CTi Biotech (Lyon, Fance). Cells were cultured at 37°C and 5% CO2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% non-essential amino acids. Cells at passage 3-5 were used for experiments. Cells were treated or not by 10ng/mL TGF-Beta for 72 hours.

• Collagen gel formation

The hydrogel solution consists in mixing 2.2mg/mL of rat-tail type I collagen with 10% PBS 10X, 0.1M NaOH, distilled water and cells. Subsequently, solution was then transferred into non-culture-coated 12 well plates (500µL per well) and let polymerizing for 1 hour at 37°C. Then, 5% FBS-containing medium was added to the collagen gels. Gels were dislodged using a spatula 2 hours after TGF-Beta stimulation.

• AFM analysis

Analysis of collagen gel stiffness was evaluated using a Bioscope Resolve (Bruker, USA) equipped with DMi8 epifluorescence microscope (Leica, Germany). Between 10 and 12 AFM areas of 50µm were measured and analyzed per gels.



AFM principle **Force-indendation curve**

A) AFM principle consists in the non-invasive indentation of an AFM tips located at the extremity of a cantilever in a sample. These will generate height variations due to various property of stiffness of the sample. These height variations are recorded using a laser that reflects from the surface of the cantilever to a photodiode. **B)** Raw data correspond to force-indentation curves from which stiffness properties are extracted.

Results & Discussion:

• Collagen gel size is reduced after cell stimulation

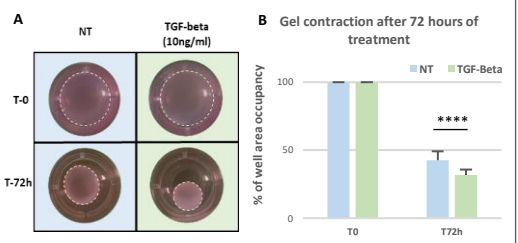


***Figure 1** : **A)** Images illustrating the gel contraction by fibroblasts stimulated or not by TGF-beta for 72 hours. The surface of the gels is delimited by dots*
***B)** Quantification of gel contraction **** : p-value < 0.00005*

• Collagen gel stiffness is significantly increased after cell stimulation

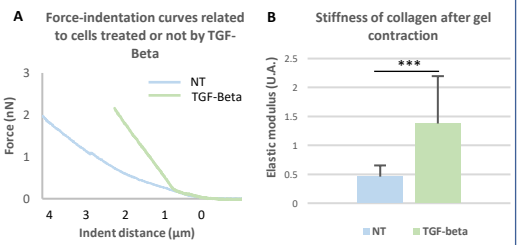


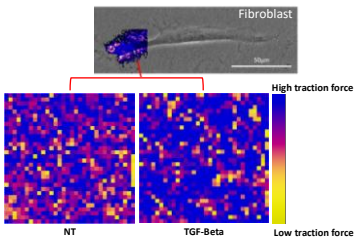
***Figure 2** : **A)** Typical force-indentation curves from AFM analysis, where a stiffer profile of gels containing TGF-Beta stimulated cells is observed. **B)** Quantification of cell traction force by measuring the stiffness (Young's modulus) of collagen gels treated or not by TGF-beta (10ng/ml) by Atomic force microscopy. *** : p-value < 0.0005*

Conclusions:

- **Sustain your anti-aging or healing claim by measuring cell traction force through collagen gel assay:**
  - **Size of collagen lattice gel**: collagen gel size is reduced by fibroblast traction on collagen fibers
  - **Quantification of fibroblast traction**: collagen gel is stiffer after cell stimulation by a protein known to increase fibroblast traction force.
  - **Analysis of fibroblast protein production**: collagen gels provide to fibroblasts an appropriate 3D environment for culture
- **Collagen gel assay can be used for fast screening of active ingredients aiming at increasing cell traction force.**

To go further :

Strain-stiffening mapping assay of collagen matrix close to a fibroblast:



→ **Finer characterization of tensile capacity of fibroblasts**

References:

Boismal et al., 2020 ; Haydont et al., 2019 ; Jin et al., 2015 ; Li et al., 2011 ; Liu et al., 2018 ; Yu et al., 2021