# A MECHANICAL STRETCHING DEVICE

## DESIGN AND DEVELOPMENT OF HIGH FREQUENCY UNIAXIAL MECHANICAL CELL STRETCHING SYSTEM AND POLY-DI-METHYL SILOXANE BASED MULTI-WELL CELL CULTRE DEVICES FOR LIVE CELL IMAGING TECHNIQUES

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Mechanical stress exerted at cell-substrate and cell-cell interfacial boundaries is involved in the regulation of a variety of physiological process. In the case of epithelial cells such as skin, it is becoming increasingly apparent that epithelial cell movement and changes in morphology are central to both development and regeneration of epithelial organs. In this paper, we report a new mechanical cell stretching system for live cell stretching and simultaneously to monitor and image the cells under stress. Our aim is to apply the mechanical stress externally using this stretching system on the normal and mutant skin cells and to study the effect of this mechanical stress on these cells under normal physiological environment. Measuring techniques to quantify the extent of mechanical stress on skin are needed for establishing successful treatment strategies to combat skin diseases such as blistering and other processes of skin degeneration. At IMB, We have designed and developed a customized mechanical cell stretching system for live cell stretching, monitoring and imaging with an option to control the spatial stretching length up to 100% and the frequency of stretching up to 10Hz. A multi-well poly-di-methyl-siloxane (PDMS) based cell culture device has also been developed to monitor different kind of cells at once and do the live cell imaging, while stretching. The cells are cultured in the device coated with Collagen-IV and is kept in the built-in incubator attached with the stretching system. A voice-coil based motor is used in this system to stretch the PDMS membrane with maximum operating hours up to 24h of stretching continuously or with an interval of pausing and then stretching according to the program, which can be customized using the software exclusively developed to control this system. To validate and test this system, we have stretched skin epithelial cells such as wild-type NEB1-K14GFP and the mutant NEB1.R125P-K14GFP have been cultured on the flexible substrate coated with Collagen-IV. We have obtained the preliminary data, which shows the wild-type cells can withstand the mechanical stress up to 2h, whereas the keratin filaments in the mutant cells is breaking down into aggregates as early as 5min. after the mechanical stress is applied at the frequency of 4Hz.

*Keywords:* Mechanical Stretching of Cells, Live Cell Stretching, Bioimaging, PDMS Devices, Skin Epithelial Biology, Wound Healing, Mechanical Stress

## 1. INTRODUCTION

Cellular responses to mechanical stimuli are regulated by interaction with the extra-cellular matrix (ECM), which in turn are strongly influenced by the degree of cell stiffness. Living cells can sense mechanical forces and convert them into biological responses. Similarly, biological and biochemical signals are known to influence the abilities of cells to sense, generate and bear mechanical forces. Cells in tissues are living in differently stiff environments and are subjected to different types of mechanical load; such mechanical cues determine cell fate, phenotype and behavior. Blood circulating cells experience fluid flow shear stress cells residing in bone and cartilage are under compressive load and a number of cell types are subjected to stretch. Cardiac myocytes and endothelial and smooth muscle cells of vessels of the intestine and of the airways undergo cyclic stretch. Other cells types, including skeletal muscle cells, connective tissue fibroblasts and epidermal keratinocytes bear gradual stretches of different degrees and of varying rates. It becomes increasingly clear that including a mechanical component, such as stretch and matrix elasticity, significantly improves the physiological relevance of cell culture studies of cell functions. Moreover, stretchable substrates are important tools to study the mechanisms of cell mechanosensing and the consequences of mechanical protein deformation.

Studies into the mechanics of cells, sub-cellular components and biological molecules have rapidly evolved during the past decade with significant implications for biotechnology and human health. Adhesion of cells to extracellular matrix (ECM) through focal adhesion complexes provides both signaling and structural functions. While many tools exist to stretch cells mechanically to manipulate the biochemical adhesiveness of experimental substrates, relatively few approaches have been developed to engineer the stretching substrate and the stretching orientation with high frequency and higher amplitude in order to study the full impact of the mechanical stress on the live cells.

In the case of epithelial cells, the keratins, which are intermediate filaments in the keratinocytes join together and form highly resilient fibers in the lower portion of skin, helping make it durable. If either keratin is defective, they don't mesh and the lower skin tissue becomes unusually fragile and gets damaged from the mildest mechanical stress - leading to blistering pain, a higher risk of infection, and in the most severe cases, death. Epidermolysis bullosa (EB) is a group of inherited bullous disorders characterized by blister formation in response to mechanical trauma. Historically, EB subtypes have been classified according to skin morphology. Recent discoveries of the molecular basis of EB have resulted in the development of new diagnostic tools, including prenatal and pre-

implantation testing. Based on a better understanding of the basement membrane zone (BMZ) and the genes responsible for its components, new treatments (e.g., gene or protein therapy) may provide solutions to the skin fragility found in patients with EB.

In order to apply the mechanical stress directly to the skin cells and study the effect of this stress on these cells to better understand the EB disease, here we propose a new cell stretching system and method, which can be used to stretch the cells up to 10Hz with the maximum stretching length of 100%. By using the microfabrication technology, we have also developed a multi-well stretching device based on poly-di-methyl-siloxane. This device can be used to culture the cells on to this substrate for stretching and simultaneously to monitor the cell behaviour and do the live-cell imaging using an inverted microscope.

## 2. MATERIALS AND METHODS

### *2.1 Design and development of high-frequency mechanical stretching system*

The first step towards designing the experiment for studying the mechanical properties of cells under stress was to design and develop a mechanical cell stretching system in order to apply the external stress on cells. To achieve this, we have proposed a voice-coil based motor in our stretching system instead of stepper motor so as to minimize the heat gen-

eration during the operation. The proposed system can hold our proposed multi-well PDMS based cell culture device with a 3-pin holder to facilitate the transfer of external force to the cell culture device equally on the both direction while stretching. The schematic diagram of the proposed cell stretching system and the multi-well cell culture device is given in Fig. 1(a) and 1(b).

This new stretching technique using voice-coil based motor can provide the maximum frequency up to 10Hz with minimum noise and to provide the smooth pulling of the stretching device attached with the system. There is a built-in heating system available inside the unit with a thermo-couple feedback mechanism to control the temperature and to keep the system at 37°C throughout the experiment. A water reservoir is available inside the stretching unit to keep the system in a moisturized condition to ensure the cells are provided with required humidity. There are two inlets

available in the system to provide the $O_2$ and $CO_2$ supply so that the physiological environment is provided to the cells during the course of the stretching experiment. The entire system is designed in such a way that to fit this stretching unit can be easily fixed on any standard inverted microscopes available in the market such as Olympus IX71, IX81, Zeiss and Nikon. The developed mechanical stretching is

automated and completed controlled by a computer using a Labview based software to set the stretching parameters and control the system accordingly. The software can be programmed based on the requirement whether to stretch the device only one direction and fix the other end; or to stretch the both side of the device simultaneously. It is also possible to do the experiment with stretch and hold to monitor and capture the cell behaviour under mechanical stress. The maximum operating frequency of this system

can be fixed up to 10Hz with the amplitude up to 100% for the continuous operation up to 24h. The snap-shot of the soft-ware screen is shown in Fig.2

### *2.2. Development of PDMS based stretchable multi-well membrane devices*

The schematic diagram of the proposed stretching device is shown in Fig.3. These culture substrates are produced using PDMS silicone elastomer (Sylgard 184, Dow Corning, Wiesbaden, Germany) with 1:10 mixing ratio of curing agent-to-base. We have developed a mold to fabricate the single well, 2-well, 4-well and 6-well stretching devices. To fabricate these multi-well stretching devices, we have first mixed the silicone elastomer with curing agent and removed the bubbles using the vacuum pump based desiccators.

Once the bubbles are removed from the mixed elastomer solution, they are then poured onto the device mold and kept it in the oven for about 4h at 80C. After this baking, the mold is carefully removed and the stretching device is slowly separated from the mold (Fig. 3). For stretching assays, we have fabricated 250μm thick PDMS membranes at the bottom, which can be stretched up to 100%. Fig.5 shows the force vs. elongation graph tested using the fabricated device to confirm the durability of this product.

**Table 1. Comparison of Flexcell and STREX system with IMB stretching system, I-MCS1**

| Parameters | Flex Cell | STREX | I-MCS1 (IMB) |
|---|---|---|---|
| Mode | Cyclic | Static | Cyclic & Static |
| Frequency | 5Hz | 0.5 Hz | 10Hz |
| Stretching length | 20% | 20% | 100% |
| Stretching time | 12h | 2h | 24h |
| Incubation | NO | Separate heating coil | Buit-in heating with $CO_2$ supply |
| Laser Port | NO | NO | YES |
| Live cell Imaging | NO | Need to stop the system | YES |
| Consumable | 6-well plate | Single well device | Multi-well device |
| Automation | NO | NO | YES |
| Maintenance | High | Moderate | Low |

The pre-treated PDMS membranes are kept at 60C for about 1h and treated with plasma oxygen for 1 min at 200 mT and 50 sccm flow of oxygen in a plasma chamber (Harris Plasma, NY, USA) to generate free surface oxygen groups. The comparison of I-STR-v1 stretching system with other commercial available system is given in Table 1.

### *2.3 Coating of PDMS multi-well stretching device with Matrigel / Collagen-IV*

Fabricated PDMS device was first pre-cooled on ice and placed on the sample holder of the spinner (Model WS 656SZ 6NPP/A1/AR1 – Laurell). Then 200ul of 0.12mg/ml of Matrigel (Becton Dickinson) was added on the PDMS before running the spinning program. The spinning program consists of increasing steps to 40000 rpm for 30sec and then decreasing steps to 0 rpm. The coated PDMS was washed once in distilled water before being blow-dried with compressed nitrogen gas. The coated device was left in the 37°C incubator for 1 hour. To confirm the presence of matrigel coating, we have done the immunofluoresence staining using the antibodies. Scotch tape was applied to half of a PDMS surface before coating the entire PDMS with different concentration of Matrigel using the spinning method. This creates a coated and uncoated Matrigel surface side by side when the scotch tape is removed. Immunostaining was then per-

formed on these PDMS surfaces. The coated PDMS was first blocked in 10% goat serum for 30min before incubating with rabbit primary antibody against Mouse Collagen IV (AB756P, Millipore) overnight at room temperature at a concentration of 1:50. After PBS wash, the coated PDMS was incubated for 2 hours in 1:250 diluted goat anti-rabbit secondary antibody conjugated to 488 fluorochrome (Invitrogen) and imaged.

## 3. EXPERIMENTAL

### 3.1 Cell culture on the coated PDMS stretching devices

NEB1.GFP-K14.R125P and NEB1.GFP-K14.WT (from Dundee) were maintained in standard keratinocyte tissue culture medium Dulbecco's modified Eagle's medium with 25% Ham's F12 medium, 10% fetal bovine serum, 0.4 $\mu g\ mL^{-1}$ hydrocortisone, $1.8 \times 10^{-4}$ mol $L^{-1}$ adenine, 5 $\mu g\ mL^{-1}$ transferrin, $2 \times 10^{-11}$ mol $L^{-1}$ lyothyronine, 5 $\mu g\ mL^{-1}$ insulin, 10 $ng\ mL^{-1}$ epidermal growth factor and 1% penicillin-streptomycin. Cells were cultured at 37°C with 5% $CO_2$. Cells at log phase were trypsinised

and seeded on the device at the appropriate cell density, to reach 60 to 70% confluency after two to four days. Seeding density and days in device were optimized to reach desired confluency for the mechanical stretch experiment. For the 4-wells chamber, 40 000 cells of R125P and 60000 cells of WT were seeded for two days before stretch. For the single chamber, 13000 cells of R125P and 22000 cells of WT were seeded for four days before stretch. Culture medium was replaced prior to stretch.

### *3.2. Stretching experiment and optical microscopy for live-cell imaging.*

The stretching device is transferred from the tissue culture room to the microscope attached with built-in incubator for live cell-stretching and imaging experiment. The newly developed stretching system is already fixed with this inverted microscope with high speed camera. Prior to transferring the device with cells to the microscope suite, the microscope incu-

bator is set ready with 37°C and the $CO_2$ supply is connected with the stretching system directly.

Both the stretching system computer and the microscope computer are synchronized to do the stretching and take the time-lapse images. The images were acquired using a fluorescence objective lens with 20X and 40X on an Olympus IX81 inverted microscope equipped with a charged coupled device monochrome camera (QImaging,) and DP71 software distributed by Olympus Japan. Images and figures were processed and assembled with the Image J software (NIH, USA).

## 4. RESULTS AND DISCUSSION

In order to test the working capability and functionality of our newly developed I-MCS1 cell stretching system, we have selected two different cell lines, namely NEB1-K14GFP, a wild type skin epithelial

cells and the mutant cell line, NEB1-R125P.K14GFP to understand the effect of mechanical stress on these cells. Fig. 7 shows the phase contrast image of the wild-type NEB1.K14GFP cells which are grown on the fabricated device and subjected for a mechanical stress using the I-MCS1 system. The mechanical stretch was carried out at a frequency 2Hz and an amplitude of 50% for times varying up to 180minutes at 37C and 5% CO2. We have noticed that the wild-type cells start disintegrating after 2h of stretch.

Fig. 8 shows the phase contrast image of the mutant NEB1.K14GFP cells stretched for 180min at 2Hz with the amplitude of 50% at 37C and 5% CO2. We have observed that the mutant cells not able to withstand the mechanical stress after 15min stretch. This suggests

that the normal epithelial cells can withstand the mechanical stress up to 3h at 2Hz with amplitude of 50%, where as the mutant cells of the corresponding type can not withstand the mechanical stress even for about 15min under the same stretching conditions.

In the epidermis of patients with EBS, keratinocytes break down in response to mechanical stress. This occurs because of the mutation of keratin intermediate filaments, which in some way renders the cells more susceptible to cytolysis on physical trauma. We attempted to reproduce this fragility in the transgenic derived cell lines of NEB1.K14GFP and NEB1.R125P-K14GFP. We have observed the changes in the keratin in-

termediate filament network in both wild-type and mutant keratin EBS cultured keratinocytes. As shown in Fig. 9 (a), a well-formed network of keratin filaments in the control wild-type NEB1.K14GFP cells before stretching. But, we applied the mechanical stress at 4Hz with an amplitude of 50% after 2h of stretching, we have observed the keratin particles or aggregates appeared spontaneously in places close to the cell periphery as indicated by black arrow in Fig.9 (b) and the NEB1 cells started to exhibit thickening of filaments around the cell nucleus as indicated by the red arrow.

Where as in the case of mutant cells as shown in Fig. 10(a), before stretch, clusters of keratin particles or aggregates appeared spontaneously in places close to the cell periphery. Keratin aggregates are diagnostic of

Dowling-Meara EBS (Anton-Lamprecht and Schnyder, 1982) and are seen in situ in both intact and lysed cells nut not in every cell. But, after 5 minutes of cyclic stretch, we have observed that the appearance of keratin particles or aggregates as indicated in red arrow in Fig.10 (a) and (b) in the mutant cells at the cell periphery suggesting that the mutant cells are not able to withstand the mechanical stress even for 5min, when it was applied at 4Hz with an amplitude of 50%. The mutant cells showed increased amounts of filament fragmentations, particularly along free edges.

Fig.11 (a), (b) and (c) shows the breaking down of the cell junction of wild-type NEB1.K14GFP cells, when we applied the mechanical stress for 0min, 1h and 2h respectively at 4Hz with an amplitude of 50%. As the time of stretch was increased, the cell-cell contacts became elongated,

suggesting some elasticity in the cell-cell junctions (Fig.11c). This supports the idea that keratin aggregation begins in areas of the cell rich in junctional proteins such as desmoplakin and that some intrinsic desmosomal elasticity may provide initial resistance to keratin fragmentation in response to stretch.

Mechanical stretch is certain to have more to teach us about the function of intermediate filaments in tissues. The availability of this stretch assay, which reproduces at least part of the pathology of EBS in a tissue culture situation will be useful for analyzing the disease process and any hypothetical measures for disease symptoms of this and related disorders. These cell stretching experiments underlying the need for much further analysis of the role of mechanical forces in regulating biological responses at the cellular level.

## 5. CONCLUSION

In conclusion, the mechanical cell stretching system described here is an easy-to-fabricate and easy-to-use system to apply the mechanical stress on cells either for static or cyclic mode while applying the maximum stretching frequency up to 10Hz and the stretching length up to 100%. Simultaneous live-cell stretching and imaging can be performed using this system in order to study the effect of mechanical stress on live cells and capture the morphological changes taking place in the cells in response to stress. A real-time monitoring of cells is possible using this system, when the cells are treated with drugs and to see the effect of drugs before and after applying the mechanical stress on cells in order to determine appropriate drugs to treat the mutant cells and to find out the optimum drug dosage.

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# Technology Disclosure Form

## SECTION 1: TECHNOLOGY DISCLOSURE DETAILS

| | |
|---|---|
| RI Technology Disclosure No | |
| (1) Title of Technology | Apparatus and method for mechanical cell stretching and live-cell imaging system |
| (2) Keywords relating to your technology (5-10 keywords) | Mechanical cell stretching, live-cell imaging, Mechnosensing, Mechanobiology, force transduction, epithelial cells, polydimethylsiloxane device |
| (3) Indicate the category in which your technology falls under | Biotechniques / Cell-Stretching / Bio-imaging |
| (4) Brief summary of your technology<br><br>Attach also a detailed description of your technology. | This invention is focused on to develop an apparatus to study the effect of mechanical stress on cells and do the live-cell imaging in an R&D laboratory. Mechanical stress exerted at cell-substrate and cell-cell interfacial boundaries is involved in the regulation of a variety of physiological process. Living cells can sense mechanical forces and convert them into biological responses. Similarly, biological and biochemical signals are known to influence the abilities of cells to sense, generate and bear mechanical forces. Cells in tissues are living in differently stiff environments and are subjected to different types of mechanical load; such mechanical cues determine cell fate, phenotype and behavior. For example, cardiac myocytes and endothelial and smooth muscle cells of vessels of the intestine and of the airways undergo cyclic stretch. Other cells types, including skeletal muscle cells, connective tissue fibroblasts and epidermal keratinocytes bear gradual stretches of different degrees and of varying rates.<br><br>In the case of epithelial cells such as skin, it is becoming increasingly apparent that epithelial cell movement and changes in morphology are central to both development and regeneration of epithelial organs. Measuring techniques to quantify the extent of mechanical stress on skin are needed for establishing successful treatment strategies to combat skin diseases such as blistering and other processes of skin degeneration.<br><br>A mechanical component, such as stretchable substrates are important tools to study the mechanisms of cell mechanosensing and the consequences of mechanical protein deformation. Here, we report a new mechanical cell stretching system to do the live-cell stretching and simultaneously to observe and capture the morphological changes of cells under stress using a fluorescence microscope. We have designed and developed this in-vitro cell stretching system for live-cell monitoring and imaging with an option to control the spatial up to 100% and the frequency of stretching up to 5Hz. A multi-well poly-di-methyl-siloxane (PDMS) based cell culture device has also been designed and developed to stretch the 6 different kind of cells all at once and do the live-cell imaging. The cells are cultured |

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| | |
|---|---|
| | in the device coated with either matrigel or collagen-IV and is kept in the incubator attached with the system. A voice-coil based motor is used in this system to stretch the PDMS membrane with maximum operating hours up to 12h for continuous operation. To validate and examine this newly developed system, we have stretched skin epithelial cells such as wild-type NEB1-K14GFP and the mutant NEB1.R125P-K14GFP, which have been cultured on the newly developed flexible stretching device substrate coated with collagen-IV. We have obtained the preliminary data, which shows the wild-type cells can withstand the mechanical stress up to 2h, whereas the mutant cells can breakdown into aggregates as early as 5min. after the mechanical stress is applied at the frequency of 4Hz. |
| (5) Key technical features (excluding advantages such as cost, efficiency).<br>(e.g. A semiconductor structure....<br>An isolated nucleotide sequence ...<br>A method of.... ) | Maximum Stretching length : 100%<br>Current operating time: 12h (man be extended up to 24h later stage)<br>Current maximum stretching frequency: 5Hz ( may be extended up to 8Hz)<br>Number of wells: single / 2 / 4 / 6<br>Live cell imaging: Yes<br>Physiological environment: Yes<br>Mode of stretch: Both oscillating and steady stretch ( Uniaxial)<br>Operation: Automated (computer controlled). |

**Description of Figures:**

Figure 1 (a) The Schematic diagram of the proposed cell stretching system (b) Schematic diagram of the multi-well cell culture device.

Figure 2 (a) CAD design of the proposed IMB Cell Stretching system (b) The prototype version-1 of the IMB Mechanical Cell Stretching ( I-MCS1) system.

Figure 3 (a) The fabricated multi-well cell culture devices to use in the developed IMB cell stretching system. (b) The tensile strength of the fabricated device showing the graph of Elongation Vs Applied force.

Figure 4 The optical microscope image of the PDMS surface before and after polishing and then coated with Collagen-IV

Figure 5 The optical microscope image of the NEB1 and Mutant NEB1 cells cultured on the coated PDMS device.

Figure 6. (a) IMB cell stretching system, I-MCS1 is attached with Olympus IX81 Microscope for direct observation and imaging of cells while stretching (b) The snap-shot view of the operating control software menu and the display of the stretching graph, stretching length Vs time.

Figure 7. Phase contrast image of the wild-type NEB1 cells stretched up to 3 h at various intervals using the IMB stretching system (I-MCS1).

Figure 8. Phase contrast image of the mutant NEB1-R125P.K14GFP cells stretched up to 3h at various intervals using the IMB stretching system (I-MCS1).

Figure 9. The wild-type NEB1.K14GFP live-cell image taken using an IX81 optical fluorescence microscope, (a) No stretch, where the filaments are intact (b) after 2h of stretching at 4Hz the keratin filaments in the cell are breaking down into aggregates and unable to withstand the mechanical stress applied.

Figure 10. The mutant NEB1-R125P.K14GFP live cell image taken using an IX81 optical fluorescence microscope, (a) No stretch, where the red arrow indicates (b) after 5min of stretching at 4Hz, the keratin aggregates are seen as indicated by the red arrow in the same place of the cell, suggesting that the mutant cells not able to withstand the mechanical stress after 5min of stretching.

Figure 11. The fluorescence image of the wild type cell junction (a) no stretch (b) after 1h of stretch and (c) 2h of stretch, indicating that the cell junction is breaking down when the mechanical stress is applied for more than 2 h.

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## Claims

1. A mechanical stretching device, comprising:

- a stretchable substrate comprising at least one stretching material area within which a stretching material is placeable, and two engagement areas being located at opposite ends of the stretchable substrate, respectively;
- two movable elements, each of which comprising an engagement portion, wherein each of the engagement portions is capable of engaging with one of the engagement areas;
- two motors, each of which being configured to drive one of the movable elements;
- wherein the movable elements are movable by the motors such that the engagement portions cause, after having engaged with the engagement areas, either one end or both ends of the stretchable substrate to be stretched, wherein the ends of the stretchable substrate are stretched along opposite directions with respect to each other.

2. The stretching device according to claim 1,
wherein the movable elements are independently movable by the motors such that the ends of the stretchable substrate are independently stretchable.

3. The stretching device according to claim 1 or 2,
wherein the motors are motors which produce none or only little heat when in operation, in particular to maintain the physiological temperature.

4. The stretching device according to claim 3,
wherein the motors are voice coil motors.

5. The stretching device according to any one of the claims 1 to 4,
wherein the motors are drivable such that the stretchable substrate is continuously and/or periodically stretched and/or de-stretched.

6. The stretching device according to claim 5,
wherein the motors are drivable such that a stretching frequency or a de-stretching frequency of the stretchable substrate ranges from 0.5Hz to 10Hz or from 4Hz to 10Hz or from 0.5Hz to 4Hz or is 0.5Hz or is 4Hz or is 10Hz.

7. The stretching device according to any one of the claims 1 to 6,
wherein the movement of the movable elements is controlled such that the stretchable substrate is stretched up to 100% in its length.

8. The stretching device according to any one of the claims 1 to 7,
wherein the motors can be switched between two driving modes, wherein, in a first driving mode, the stretchable substrate is caused to be stretched into opposite directions, whereas, in a second driving mode, the stretchable substrate is caused to be stretched into only one direction.

9. The stretching device according to any one of the claims 1 to 8,
further comprising a temperature controlling unit which controls the temperature of the stretchable substrate and its micro-environment to a predetermined temperature value.

10. The stretching device according to any one of the claims 1 to 9,
wherein the temperature controlling unit comprises a heating unit and/or a fan in particular for the uniform distribution of heat and to maintain the temperature.

11. The stretching device according to any one of the claims 1 to 10,
further comprising a humidity reservoir unit which controls or maintains the humidity of the environment of the stretchable substrate to a predetermined humidity value.

12. The stretching device according to any one of the claims 1 to 11,
further comprising a gas controlling unit which controls the gas parameters of a gas surrounding the stretchable substrate to predetermined gas parameters.

13. The stretching device according to claim 12,
wherein the gas controlling unit comprises a gas inlet adapted to supply gas to the surrounding of the stretchable substrate.

14. The stretching device according to any one of the claims 1 to 13,
further comprising a gas-tight chamber surrounding the stretchable substrate, wherein the movable elements extend from outside of the chamber through walls of the chamber into the

inside of the chamber, and wherein temperature, humidity and gas parameters within the chamber are controlled by the temperature controlling unit, and the gas controlling unit.

15. The stretching device according to any one of the claims 1 to 14,
further comprising an imaging device adapted to image the stretching material during the stretching of the stretchable substrate.

16. The stretching device according to claim 15,
wherein the imaging device comprises a microscope base plate.

17. The stretching device according to any one of the claims 15 to 16,
wherein the imaging device comprises a commercially available microscope.

18. The stretching device according to any one of the claims 1 to 17,
further comprising a motor controlling unit which controls the operation of the motors in a fully automated manner.

19. The stretching device according to any one of the claims 1 to 18,
wherein the stretchable substrate comprises, within the stretching material area, at least one stretching material well into which stretching material can be filled.

20. The stretching device according to any one of the claims 1 to 19,
wherein the stretchable substrate comprises, within each engagement area, at least one engagement cavity into which the engagement portions of the moving elements can be introduced.

21. The stretching device according to any one of the claims 1 to 20, wherein the stretching material and/or the stretchable substrate are made of a stretchable material.

22. The stretching device according to claim 21, wherein the stretchable material is a stretchable polymeric material.

23. The stretching device according to claim 22, wherein the stretchable polymeric material is poly-di-methyl-siloxane (PDMS).

24. The stretching device according to any one of the claims 1 to 23, wherein the stretching material and/or the stretchable substrate are coated with an adhesion layer facilitating binding of the live cells to be analyzed in a stretch test carried out with the stretching device.

25. The stretching device according to any one of the claims 1 to 24, wherein the stretching material and/or the stretchable substrate are coated with matrigel or collagen, such as collagen-IV, or fibronectin.

26. A stretching device, comprising:

- a stretchable substrate area being configured to receive a stretchable substrate comprising: at least one stretching material area within which a stretching material is placeable, and two engagement areas being located at opposite ends of the stretchable substrate, respectively;
- two movable elements, each of which comprising an engagement portion, wherein each of the engagement portions is capable of engaging with one of the engagement areas;
- two motors, each of which being configured to drive one of the movable elements;
- wherein the movable elements are movable by the motors such that, if the stretchable substrate is placed within the stretchable substrate placing area, the engagement portions cause, after having engaged with the engagement areas, either one end or both ends of the stretchable substrate to be stretched, wherein the ends of the stretchable substrate are stretchable along opposite directions with respect to each other.

27. A method of carrying out a stretch test with live cells wherein live cells are placed and cultivated on the stretching material of a stretching device according to any one of claims 1 to 26.

28. The method of claim 27, wherein the stretchable substrate is imaged with the imaging device while stretching the stretchable substrate.

29. The method of claim 27, wherein the stretchable substrate is imaged with the imaging device while stretching the stretchable substrate by holding the substrate in a pre-determined stretched position for a pre-determined period of time.

30. The method of any one of claims 27 to 29, wherein the cells are eukaryotic cells.

31. The method of claim 30, wherein the cells are selected from the group consisting of epithelial cells, muscle cells, cells isolated from the intestines, cells isolated from the airways, cells derived from bone, cells derived from cartilage and cells isolated from the blood stream.

32. The method of any one of claims 27 to 31, wherein the cells are selected from the group consisting of cardiac myocytes, endothelial muscle cells, smooth muscle cells, skeletal muscle cells, connective tissue fibroblasts and epidermal keratinocytes.

33. The method of any one of claims 27 to 32 wherein the live cells are wild type cells or genetically modified mutant cells.

The stretching system is automated in such a way that the stretching substrate can be imaged while stretching by holding the substrate to a pre-determined stretched position for a pre-determined period of holding so that both stretching and imaging can be performed simultaneously.