Open-source cell extension system assembled from laser-cut plates (Kurata et al., 2019)

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Introduction

The cells have the ability to respond to a large amount of signals in order to adapt their activity to the environment. Those signals have different origins and cause a specific response. One of them is the mechanical stimulation that is fundamental to regulate the proliferation, differentiation and functional activity of the cells, specially for the bone tissue.

In fact, this tissue can change its load-carrying ability by regulating the orientation, mass and physical properties of the matrix, according to the functional mechanical environment. So the mechanical input is recognized by mechanosensitive modules that convert it to biochemical signals (Hippler et al., 2020). Thanks to those signals the process of modeling and remodeling mediated by the osteoblasts and osteoclasts is regulated (Kadow-Romacker et al., 2009).

Osteocytes are only one example, but there are many cells that present an important response to mechanical inputs that researchers want to characterize. Commonly the tests for mechanical stimulation consist of stretching, fluid shear, and hydrostatic pressure. The first one is the easiest to perform and is based on the deformation by an electric motor or pneumatic pressure pump of an elastic substrate where cells are cultured (Kurata et al., 2019).

Even if there are commercially available devices for stretching to cells, the costs of them are too high. Therefore the motivation of the authors of the article is to generate a cheaper device, self-assembled, that researchers can easily download and build. The dispositive that they create uses an electric motor and its structure is based on laser-cut acrylic plate, so the design is easily replicable.

The reason why I chose this publication is that it fits very well with all the things we learned about during the semester. The first point that caught my attention was the “Open-source” in the title, although the device is not that open source as the title suggests, the paper and documentation are very complete and open to everyone. Then they use some tools named in the course, like the laser-cut, the process of mold and the implementation of a controller based in a Raspberry Pi. Finally the motivation of the paper is the same as the course, to use prototyping like an implement to more affordable bio-research.

Main characteristics of the device

The hardware has three fundamental parts: the mold for casting the silicone culture container, the cell extension device and the controller. The idea is to create a culture container of silicon rubber that is elastic, transparent and minimally toxic to cells, in order to be stretched and to permit to observe by microscopy the cells grown on its bottom. Then the cell extension device has a mechanism to extend the silicone culture container using an electric motor converting rotational motion to linear motor. Finally the controller, consisting of a stepping motor driver circuit, a single board computer, and a touch screen, allow the researchers to control the device.

Next I will detail every part of the hardware.

- Silicone culture container:
  The authors included instructions to build a mold for the silicone culture container made of 5-mm thick acrylic plates. Then they indicate to pour PDMS resin into the mold and, after curing and taking the product from the mold, insert a pair of stainless steel rods at the both ends of the container, which will be used to stretch the container while testing the cells. This silicone culture container gives a 900 mm^2 growth area to the cells.

- Loading Unit:
  The structural members of the loading unit are also made by 5-mm thick acrylic plates and assembled with the other mechanical elements like screw shaft and resin nut, used in the conversion of rotation to linear motion, guide rails and sliders, to guarantee the straight movement in the direction of mechanical loading, and the stepping motor, that moves a movable flam hooked to a basement flame and the stainless steel rods of the container.
- Controller:
  The rotation of the stepping motor is driven by the open source hardware Easy Driver, then a single board computer, Raspberry Pi, is connected to the Easy Driver and a touch screen. For the generation of the electric pulses that control the stepping motor and for the input/output interface on the touch screen, the developers use a house-written software by Python programming language. The software is able to apply 0.5-5% elongation to the culture container at a frequency of 0.1-2Hz, with the possibility to choose between three different wave motions like sinusoidal, trapezoidal and triangular.
  The stepping motor driver circuit and connectors are installed in a housing made of 5 mm and 2 mm thick acrylic plates. Then the software is copied into a micro SDHC card that is inserted in the Raspberry Pi, which is later mounted on the circuit board of the touch screen. Finally the housing is also mounted on the back of the touch screen.

To start using the hardware it is fundamental to sterilize the silicone culture container and the loading unit, then put 2 ml of cell suspension in the container and incubate it for cell attachment and proliferation. After the incubation, it is possible to mount the container on the loading unit and to put a lid on the container. By rotating the screw shaft the starting position is determined.
By connecting all the cables the software will be automatically started. The researcher can set the hardware parameters as “Device Settings” if they didn’t change the design, and set the operation parameter on the “Wave parameters” to select the shape of the motion. Finally they have to press “RUN” to start the cycle or “STOP” to stop the device.
To validate the device, the authors evaluated the reciprocating motion of the loading unit by a laser displacement meter and they found that the motion agreed well with the designed one, giving an error smaller than 3.6%. They also characterized the magnitude of the surface strains in the loading and orthogonal directions.
Finally they conducted a cell experiment using NIH3T3 fibroblasts to examine the cell extension system, the cell culture container was then stretched by a sinusoidal waveform with a 5% starin at 1Hz. After 24 h they observed the cells using a phase contrast microscope comparing them with another culture that wasn’t mechanically stimulated. The result was that the cells without loading were distributed in random direction, while the others showed an alignment perpendicular to the tensile direction, which was the expected result.

Context and state of the art

As I said before, there are some commercial available devices that have the same purpose. One of them is *Flexcell Cell Stretching Bioreactors* (https://www.flexcellint.com/), which has different dispositives like tension systems, compression systems and fluid shear systems. For the tension system they use a pneumatic stretching protocol instead of a motor-driven system to avoid vibrational strain. The device that’s more similar to the designed by Kurata et al. (2019) is the *FX-6000T Tension System*. *Flexcell* permits stretching more than one culture container at the same time and has more waveforms, but is really expensive.
Another one is *ShellPa* (https://www.menicon-lifescience.com/english/shellpapro.html). The *Pro* version uses an electronic motor and has more waveforms and parameters of control than the dispositive that the authors propose. It also permits tu analize between 6 and 12 chambers at the same time. Though it is cheaper than *Flexcell*, this device still has an elevated cost.
On the other hand, there are a lot of researchers that tried to do the same as the authors. For example, Kah et al (2021) propose an open-source cell stretcher built from parts of an 3D printer,

controlled by an open-source software and that's able to stretch up to six substrates at the same time. The device can be built for less than 400 Euros and uses a stepper motor. The hardware has an CC 4.0 License, that means that people can share, copy and modify the dataset as long as they give appropriate credit, even for commercial purposes. So it seems like this article presents a cheaper option and more open-source.

Evaluation of openness of the design project

The authors provide a fully documentation of the design project downloadable in the site *OSF.io*, that includes the planes design for the laser cutter in *pdf*, the software for the *Raspberry Pi*, an editable bill of materials (with prices and links to buy them) and the instructions for assembling and use of the device. The access to the paper and those files are completely free. The article is published by Elsevier and is available under the *Creative Commons CC-BY-NC-ND* license.

It's not possible to say that this is an Open-source hardware, because it's not allowed to adapt or alter the design and it's only permitted to use it for non-commercial purposes. However it´s allowed to read, print and download the article, republish it or reuse portions in other works. For commercial reuse it´s necessary to ask for permission. Then the community can´t participate in the edition and improvement of the design. In *OSF.io* it's possible to see that all the changes in the repository were made by the author, also nobody has duplicated the project.

Potential application

There is a rising interest in the use of scaffolds to improve the regeneration of a tissue. A scaffold is a material that is used to give a support structure for cell culture and grown in the process of repairing tissues or organs. This help is temporary, then the scaffold suffers a gradual biodegradation leaving the new repaired tissue in the site. It's fundamental to the scaffold to meet the specific requirements of the tissue in order to provide a correct cell diffusion and differentiation (Eltom et al., 2019), so for the case of cells that have an important response to mechanical stimulation the scaffold has to permit the alignment of them and the other consequences of mechanical inputs.

For that reason is that it can be interesting to study the comportament of cellularized scaffolds with those types of cells under mechanical stimulation as stretch, in the way that it will permit the researchers to understand if the scaffold will work well in the site that it will be implanted. The device proposed by the authors can maybe be used to perform this other type of test, adapting the loading unit to stretch a scaffold instead of a cell culturing.

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