

(b)

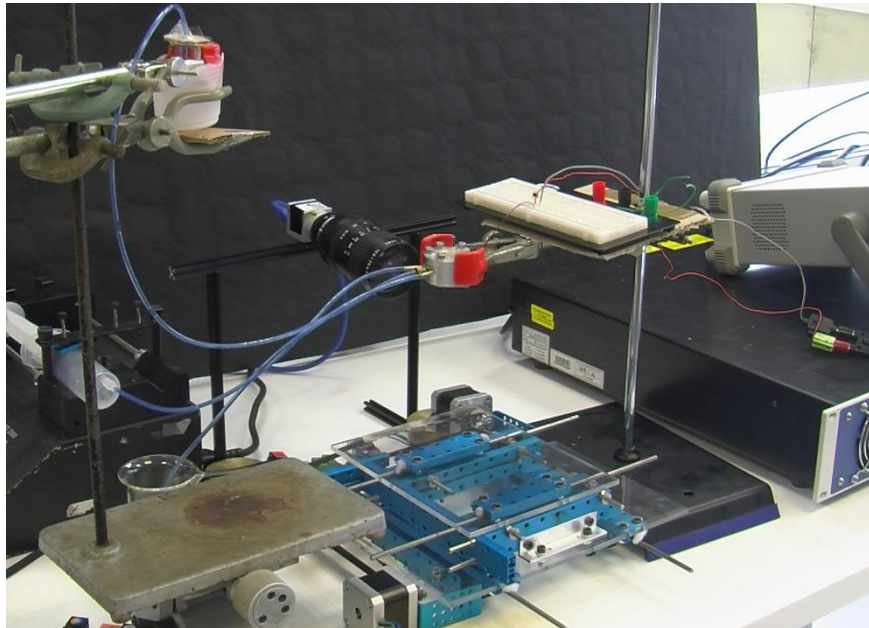
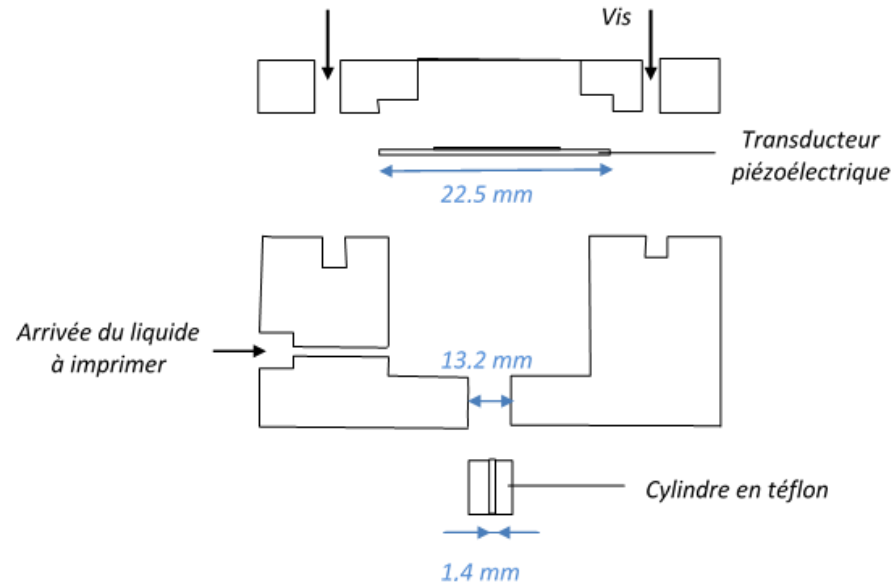


Figure 1: Schematic of the bioprinter.

The bioprinter is composed of a droplet generator with a fluid chamber, a piezoelectric transducer and a 300 μm diameter nozzle (described in figure 2), a height-adjustable fluid reservoir, an XY platform controlled by an Arduino Uno board, and a voltage generator. The reservoir contains the bioink (a collagen solution at 0.9 mg/mL). Thanks to a vertical translation stage, the height difference between the reservoir and the nozzle can be adjusted to reach the maximum height difference where the collagen solution doesn't flow through the nozzle. This adjustment must be made very precisely. The reservoir is immersed in ice water and the temperature is maintained near 0 °C in the printer head with a cooling system operating with a syringe pump (Figure 2) to prevent the formation of collagen fibers. A function generator sends positive voltage pulses of frequency 5 Hz and duty cycle of 37% to an amplifier which amplifies these waveforms so as to reach an amplitude of 40 V. These pulses are applied to the piezoelectric transducer and each pulse causes the deformation of the transducer disk, the generation of a pressure pulse in the fluid chamber and the expulsion of a single droplet from the nozzle (Figure 2). The droplets of collagen solution fall on a Petri dish positioned on the XY platform. To print patterns of particular symmetry, the Petri dish can be moved by controlling the motors of the XY platform with an Arduino board. After printing the pattern, the Petri dish is placed at 37°C under humid atmosphere for 30 minutes to allow the polymerization of the collagen solution (figure 3).

(a)



(b)

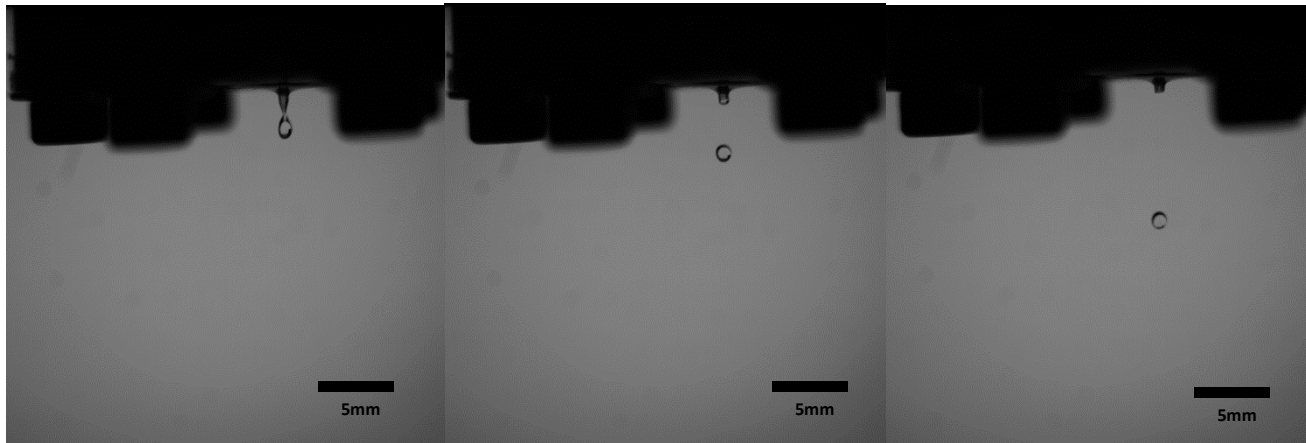


Figure 2: Schematic of the droplet generator of the bioprinter.

The droplet generator of the bioprinter is composed of a piezoelectric transducer, a nozzle of 300 μm intern diameter, a cooling system and a chamber containing the fluid to print (collagen for us) connected to a reservoir (figure 2a, schematic without the cooling system). The piezoelectric transducer is excited by a pulse of frequency 5Hz and duty cycle 37%, amplified by an amplifier to a voltage of 40V. The pulse generates the transducer deformation leading to a sufficient pressure in the chamber to expulse a single droplet through the nozzle for each pulse. The nozzle is made of Teflon and its length is about 2 cm but in order to successfully eject a droplet, the nozzle has to appear outside the generator only about 2-3 mm. The Teflon nozzle, since it is hydrophobic, allows the droplet to be spherical from the beginning and therefore she can fall down easily. The droplets (with a diameter of about 1 mm) fall at the same frequency that the pulse sent to the piezoelectric transducer (figure 2b). The cooling system is a channel, where we inject water at 0°, thanks to a syringe pump, surrounding the chamber to keep the fluid at 0° in order to avoid the polymerisation of the collagen. In order to keep the different part of the droplet generator sealed, we use a polymer (PVS). To allow his polymerisation, we add a crosslinker with the same mass proportion and we mix it. It takes around 20 minutes to polymerise and during this time we dispose it where we want the sealing to be done. After this time, it becomes hard and thus it avoids the leaks. We use it to seal the edge of the nozzle, the edge of the piezoelectric and the fluid intake of the chamber.

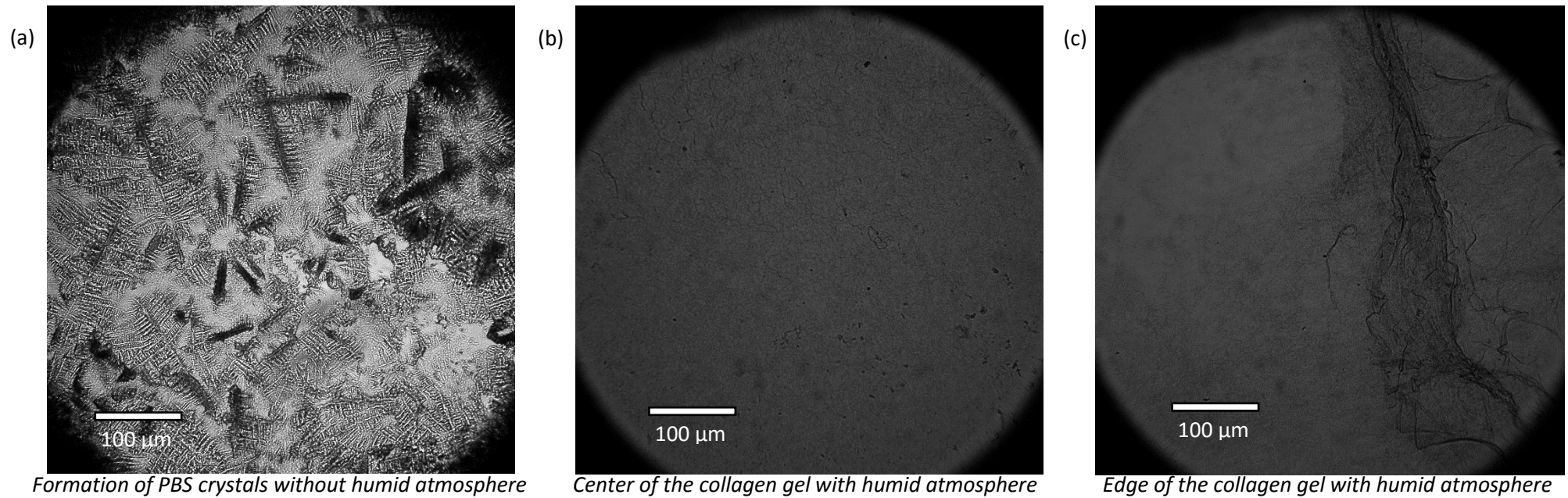


Figure 3 : Realisation of the matrix of collagen.

The sample obtained without humid atmosphere (figure 3a) and the gel of collagen obtained under humid atmosphere with Picrosirius (in the center of the gel (figure 3b) and at the edge (figure 3c)) are observed with a basic light microscope at magnification x40. The solution used to obtain a matrix of collagen is a solution of type I collagen from rat tail in 1x PBS (phosphate buffer saline) at a concentration of 0.9 mg/mL. The application of the solution is made with a micropipette on a glass slide at ambient temperature. The glass slide is put in a Petri dish in which humidified papers are placed and the box is sealed with parafilm. This allows the sample to be in a humid atmosphere to avoid the formation of PBS crystals (figure 3a, result with no humid atmosphere and the same gelling protocol). The Petri dish is then immediately placed in a water bath heated at 37°C until the solution forms a gel (50 minutes for a volume of 50 μ L). It is then cooled at ambient temperature for 10 minutes. To reveal the collagen fibers, the Petri dish is opened and Picrosirius is added in order to cover all the gel of collagen. Parafilm is put in order to restore the humid atmosphere. This bath lasts 25 minutes and then the gel is rinsed several times with demineralized water until the red color disappears.