

BIO-HYDROGELS

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1. Définitions

Gel	Gel is a solid diluted in a fluid that does not flow under its own weight [1].
Hydrogel	Hydrogel is made up of a three-dimensional network of insoluble and hydrophilic polymer with a high water or biological fluid absorption capacity [2].
Précurseur	Precursor is a chemical compound that precedes another following a chemical reaction [3].
Photo-polymérisation	Polymerization is a process that assembles various similar or dissimilar molecules into a more complex compound. Photopolymerization is polymerization triggered by visible or UV light [4].
Photo-initiateur	An initiator is a reactive compound which makes it possible to trigger a reaction such as polymerization. A photoinitiator is sensitive to light and transforms the physical energy of light into chemical energy [5][6].
PBS (Phosphate-buffer saline)	An isotonic buffer solution (at 0.1 Mb) containing a similar concentration of salts to the human body
Trypsine	Trypsin is an enzyme allowing the digestion of membrane proteins
EDTA (Ethylène Diamine Tétracétique)	EDTA and an inhibitor of enzymes and protein hydrolysis
DMEM (Dulbecco's Modified Eagle Medium)	DMEM is a culture medium containing amino acids, salts, glucose and vitamins
FBS (Fœtal bovine serum)	FBS is a fetal serum containing a small amount of antibodies but many growth factors
G (L-Glutamine)	G is an important amino acid that degrades rapidly
PS (Peni-Streptomycine)	PS is an antibiotic limiting external contamination
Chondrocytes	Chondrocytes are cells isolated from the cartilage whose role is the synthesis of the extracellular matrix (eg: collagen)

2. Introduction

Hydrogels have received increasing interest over the past 30 years. In particular, the use of hydrogels in the biomedical field is becoming increasingly important [7](Figure 1).

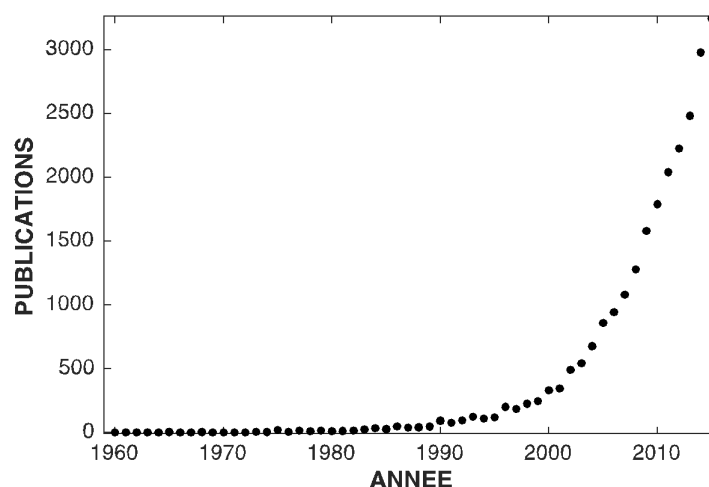


Figure 1 : Number of publications per year concerning hydrogels intended for biomedical applications

Indeed, these materials resemble living tissue by their high water content and high degree of flexibility. In addition, their good biocompatibility as well as the possibility of obtaining a wide range of properties make them promising candidates for various biomedical applications [8][9].

Nowadays, bio-hydrogels are used in particular for contact lenses [10], breast implants [11], dressings [12] or as a matrix for cell culture [13] (Figure 2). Nevertheless, current research is particularly focused in the area of tissue engineering [14] and drug delivery [15]. For example, it is possible to artificially create certain organs such as cartilage [16] or skin [17]. On the other hand, by adjusting the rate of degradation of hydrogels in the human body, it is possible to release drugs at specific times in order to optimize healing [18].

Another interesting feature of hydrogels is that their precursor can be injected directly into the desired location and then polymerized in situ. The use of hydrogels in the biomedical field would therefore allow minimally invasive treatments.

Nevertheless, in situ polymerization is difficult to control. As a result, much research is focused on developing hydrogels that are both biocompatible and light-polymerizable. By means of UV illumination, the polymerization can be precisely controlled [19]. However, this method exposes three major constraints. First, the photoinitiator needed for polymerization to take place must be water soluble. Second, the acceptable concentration of the latter is limited. Indeed, being reactive in nature, the photo-initiator is a potential source of toxicity for living cells. Finally, the human body only tolerates a certain range of UV wavelengths ($>365\text{nm}$), thus considerably limiting the effectiveness of photoinitiators. Therefore, a higher concentration of photoinitiators is often required for complete polymerization. A good number of researchers are working on new photoinitiators in order to find a better compromise between efficacy and tolerated concentration [20].

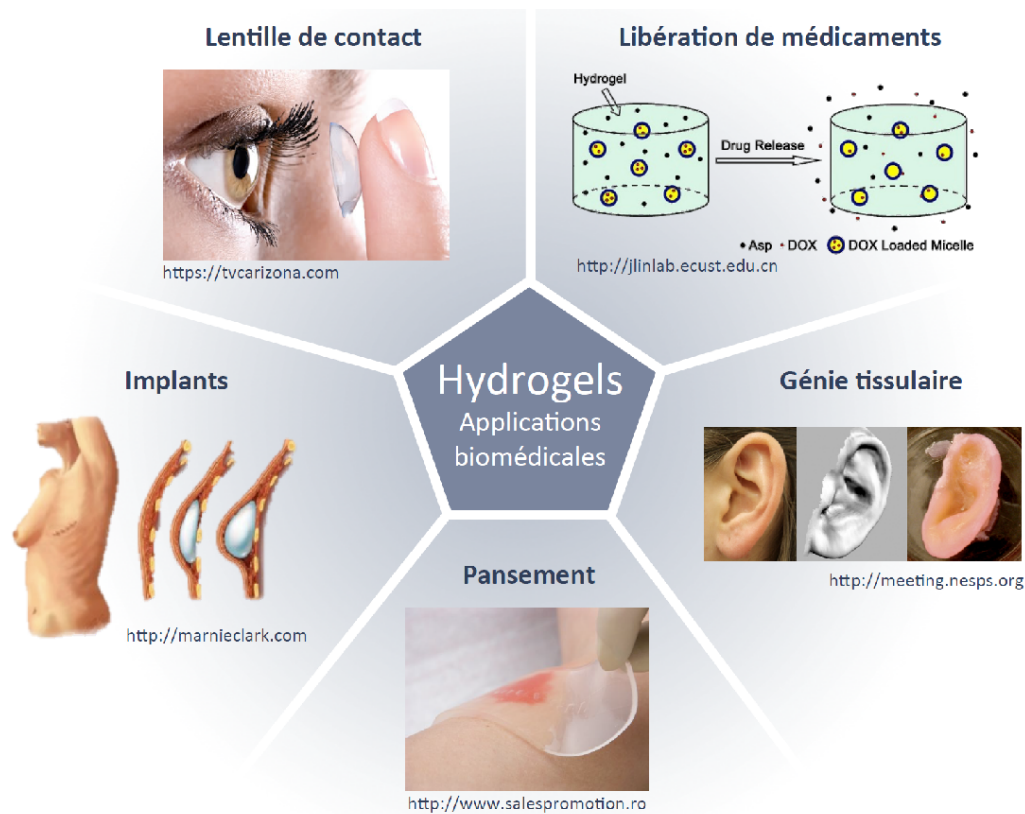


Figure 2 : Example of application of hydrogels in the biomedical environment

Nevertheless, the research does not stop at the biomedical field as illustrated in Figure 3. In particular, the environmental sciences have a particular interest in the great absorption power of hydrogels which would be favorable for cleaning the waters of the oceans [21].

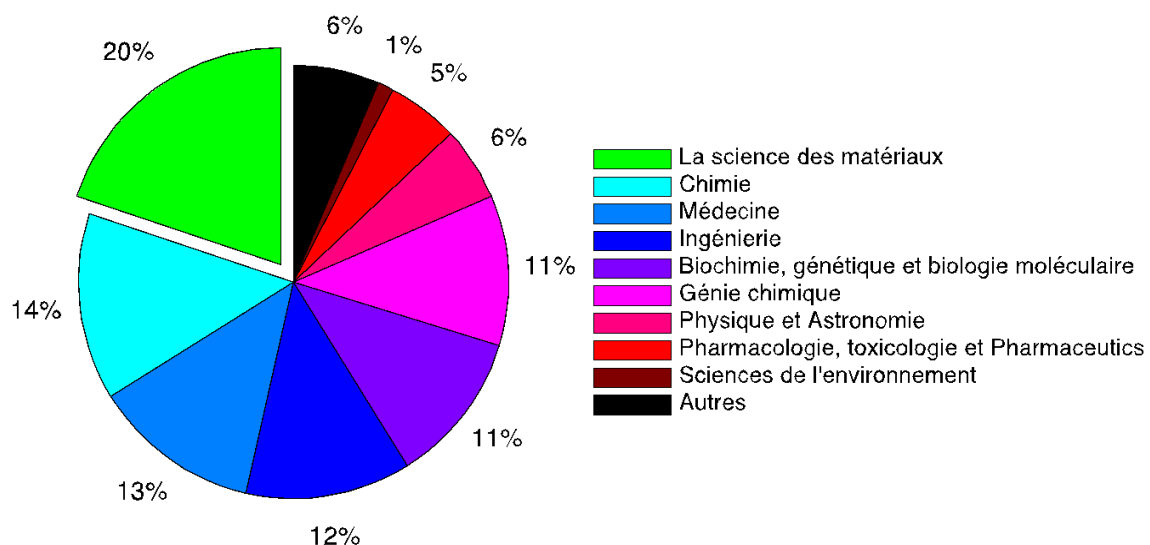


Figure 3 : Domaine de recherches sur les hydrogels

3. Structure and composition of the hydrogel

There is a wide range of different hydrogels. They are mainly distinguished by the type of polymer used, the type of bond, the stability of the system, the swelling behavior and the mesh size (Table 1).

Table 1: Some hydrogel parameters

Descriptions		Exemples	Commentaires
Type de polymère	Synthétique	PEG, HEMA	Les cellules prolifèrent préférentiellement dans les hydrogels composés de polymères naturels.
	Naturel	Cellulose, collagène	PEG : Poly (éthylène glycol) HEMA : (Hydroxyethyl) méthacrylate
Type de liaison	Réversible	Liaison hydrogène ou ionique	Aussi appelé liaison physique
	Permanente	Liaison covalente	Aussi appelé liaison chimique
Stabilité	Dégradable	Alginate	Dans le milieu vivant, les hydrogels naturels ont tendance d'être biodégradable contrairement aux synthétiques.
	Non-dégradable	PEG, PVA	PVA : poly (alcool vinylique)
Taux de gonflement	Gonflé	~ 92 % d'eau*	Le volume peut varier plus d'un facteur 10 entre l'état sec et gonflé (Figure 4)
	Sec	~ 10% d'eau*	
Maillage	Taille	125-160 nm*	La taille du maillage est influencée par la masse molaire, la solubilité (rayon de giration) ou par la conformation des chaînes des polymères et est liée directement à la perméabilité du système.

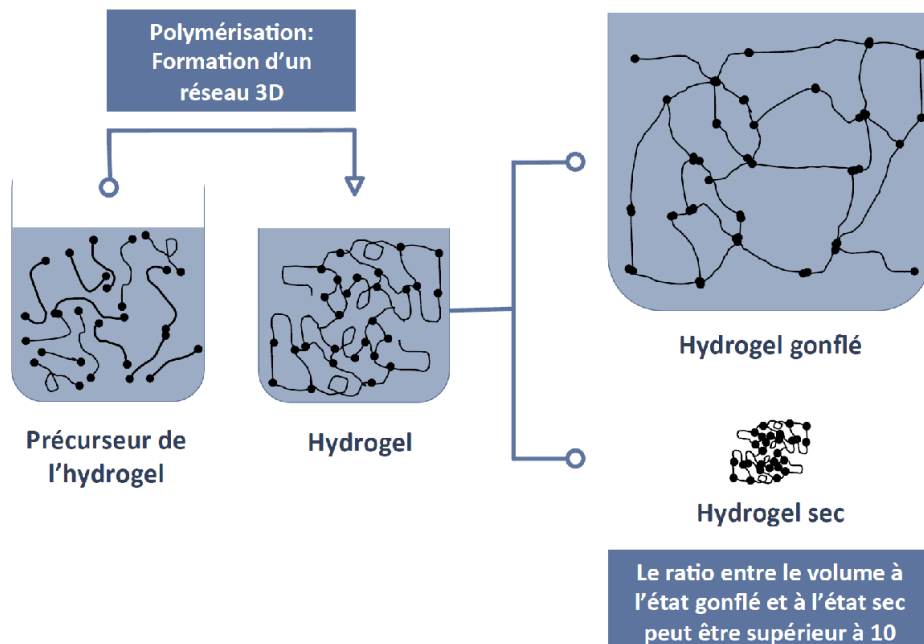


Figure 4 : Illustration of the different states of a hydrogel

4. Méthode

The first step of this practical work is to synthesize polyacrylamide (PAAM) hydrogels with different crosslinking density. Secondly, the elastic modulus, the maximum deformation and the swelling will be characterized.

4.1. Synthèse

The different components and composition of the precursor are listed in Tables 2 and 3 respectively.

Table 2: Components of the hydrogel

Descriptions	Fonction	Commentaires
Solution d'acrylamide à 30 %pds	Polymer of the 3D polymer network of the hydrogel	Acrylamide (AAM) is dissolved in PBS and removed using a 1000 pipette
Solution de méthylènebisacrylamide entre 0.026-2.6 %pds	Participates in network formation by creating chemical bonds between AAMs.	Methylenebisacrylamide (MBAA) is dissolved in PBS and is removed using a 1000 µl pipette
Phosphate Buffered Saline (PBS)	Hydrogel hydration	In liquid form and is collected using a 1000 µl pipette
LAP (5 mg/ml)	Photo-initiateur	In liquid form and is collected using a 200 µl pipette

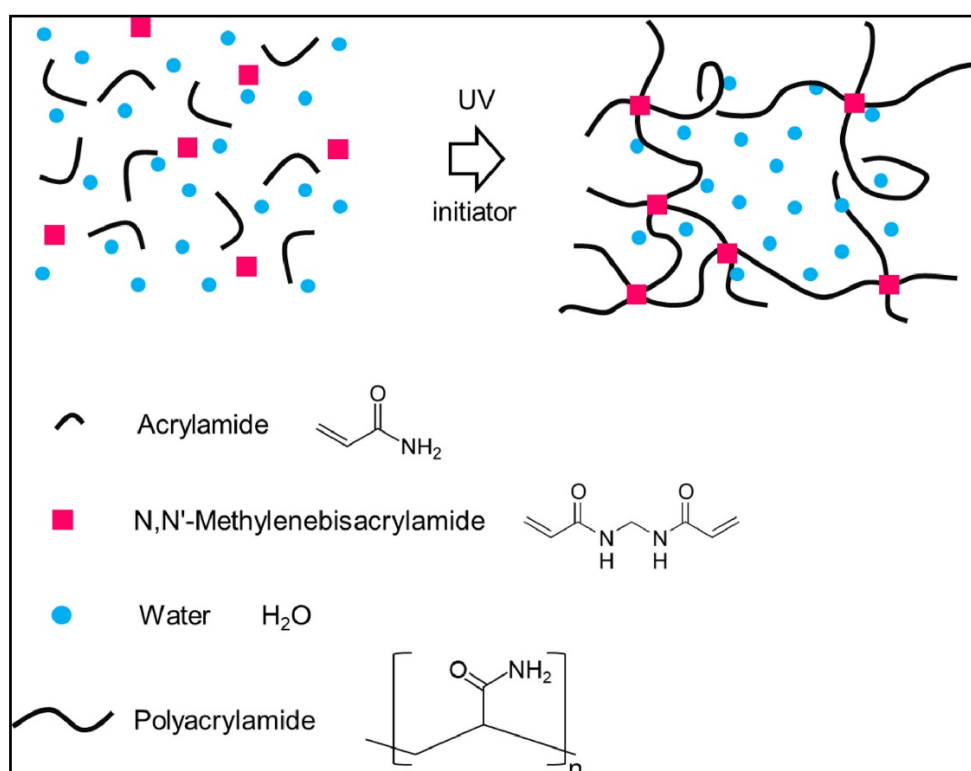


Figure 5. Reaction diagram

Table 3: Composition of the hydrogel precursor

Composition final	Volume requis du précurseur [μl]	Solution d'acrylamide [μl]	Solution de MBAA		LAP	
			% pds	[μl]	% vol	[μl]
Hydrogel 1 : 10% PAAM avec 0,05% MBAA	3000	1100	0.13	1300	20	600
Hydrogel 2 : 10% PAAM avec 0,1% MBAA	3000	1100	0.26	1300	20	600
Hydrogel 3 : 10% PAAM avec 0.5% MBAA	3000	1100	1.3	1300	20	600
Hydrogel 4 : 10% PAAM avec 1% MBAA	3000	1100	2.6	1300	20	600

After adding each component, the precursor is mixed at 900 rpm at room temperature in order to obtain a homogeneous solution then is transferred using a 1000 μl pipette into molds of dimensions Ø8 · 4.5 mm. The molds are then covered with a microscope glass slide and finally illuminated under UV light with a wavelength of 405 nm and a power of 5 mW/cm² for 30 min (Hydrogel-1), 15 min (Hydrogel-2), 10 min for (Hydrogel-3) and 5 min (Hydrogel-4).

4.2. Tests mécaniques

The mechanical tests are carried out with the ZWICK tensile machine equipped with the 100 N load cell and with the parameters summarized in Table 4.

Table 4 : Paramètres su test de compression

Mode	Compression
Déformation	90%
Vitesse de compression	1 mm/s
Pré-charge	0.05N

Given the high degree of flexibility of hydrogels, the real surface A must be considered during compression in order to obtain the real stress σ_r (equation 2). For this, it is assumed that the volume of the hydrogel remains constant (equation 2). The elastic modulus is calculated between 5 and 10% of the applied deformation.

$$A \cdot h = A_0 \cdot h_0$$

respectivement

$$\sigma_r = \frac{F}{A} = \frac{F}{A_0} \cdot \frac{h}{h_0}$$

respectivement

(1) A_0, A : Aire initiale et sous déformation

(2) h_0, h : Hauteur initiale et sous déformation

F : Force appliquée

4.3. Test de gonflement

To evaluate the swelling, the hydrogel is simply weighed just after synthesis (m_{t0}) and after being

Steps		Goals
1	Filter the hydrogels using a syringe and a 0.22 μm filter	Stériliser les hydrogels
2	Wash cells with 5 ml of PBS	Remove all remaining medium and FBS
3	Add 3 ml of Trypsin / EDTA	Take off the cells
4	Wait 5 min	
5	Add 6 ml of culture medium (DMEM / 10% FBS / 1% G / 1% PS)	Stop the effect of trypsin
6	Collect the suspension and transfer it to a 15 ml tube	
7	Centrifuge 5 min at 1200 rpm	Obtain a cell pellet
8	Aspirate the supernatant	
9	Resuspend the pellet in 2 ml of culture medium	
10	Count cells in a Neubauer chamber	
11	Collect $3,10^6$ cells, transfer them to three new tubes at the rate of 1,106 cells / tube	
12	Centrifuge 5 min at 1200 rpm	
13	Aspirate the supernatant	
14	Resuspend the cells in 500 μl of filtered hydrogel and distribute them in the prepared caps	
15	Cover the corks with a glass slide	
16	Polymerize the hydrogels for 30 min under UV	
17	Take the hydrogels out of the caps with a scalpel and distribute them in plates of 24 wells	
18	Wash the hydrogels 1x with PBS	
19	Add 500 μl of medium (DMEM / 10% FBS / 1% G / 1% PS)	
20	Leave in culture for 4 days in an incubator at 37 ° C / 5% CO ₂	
21	On this material cultivated for 4 days, several tests are possible including the viability of the cells by a test called "Life / Dead"	Obtain images and quantify cell viability

immersed in water for 24 hours (m_{t24}). The swelling is then calculated with the following equation.

$$\text{Gonflement } [\% \text{ pds}] = 100 \cdot \frac{(m_{t24} - m_{t0})}{m_{t0}} \quad (3)$$

4.4. Culture of chondrocytes in hydrogels

PBS (Phosphate-buffer saline)

An isotonic buffer solution (at 0.1 M) containing a similar concentration of salts to the human body

Trypsin

Trypsin is an enzyme that allows the digestion of membrane proteins

EDTA (Ethylene Diamine Tetra Acetic)

EDTA and an inhibitor of enzymes and protein hydrolysis

DMEM (Dulbecco's Modified Eagle Medium)

DMEM is a culture medium containing amino acids, salts, glucose, and vitamins

FBS (Fetal bovine serum)

FBS is a fetal serum containing a small amount of antibodies but many growth factors

G (L-Glutamine)

G is an important amino acid that breaks down quickly

PS (Penicillin-Streptomycin)

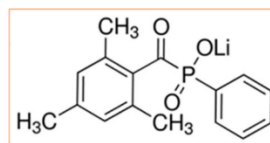
PS is an antibiotic limiting external contamination

Chondrocytes

Chondrocytes are cells isolated from cartilage whose role is the synthesis of the extracellular matrix (e.g.: collagen)

5. Discussions

1. What is the role of the photoinitiator (draw the reaction mechanism)?



LAP

2. Draw stress vs. strain curves for all samples on one or more graphs.
3. Calculate the mean as well as the standard deviation of the elastic modulus between 5-10%, the principal deformation and the swelling.
4. Compare the different hydrogels prepared at the level of:
 - Polymerization
 - Swelling
 - Mechanical properties (elastic modulus and maximum deformation)
 - Cell culture (ideal conditions)
5. What is the influence of the crosslinking rate on the mechanical and swelling properties?
6. What is the influence of swelling on the mechanical properties? In your opinion, why can swelling be problematic for biomedical applications?
7. List 3 mistakes not to make or to avoid when culturing cells under laminar flow
8. What other solutions could be envisaged for:
 - Increase the elastic modulus?
 - Increase deformability?
 - Increase polymerization kinetics?
 - Decrease the rate of swelling?
 - Decrease the viscosity?
 - Improve biocompatibility?

6. Vidéos

DLL-BIO- Culture cellulaire- Comment manipuler des cellules en P1 ?:

<https://tube.switch.ch/videos/112d9c1e>

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