Investigation of the preparation method and its effect on the mechanical properties of fiber-reinforced Hydrogels

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List of Abbreviations

**dECM** decellularized extracellular matrix.

**MedECM** Methacrylate decellularized extracellular matrix able to Photo-crosslink to enhance mechanical properties.

**PBS** Phosphate buffered saline

**PI** Photo-initiator

**UVA** Ultraviolet-A waves have wavelength in the range of $\lambda = [315\text{nm}, 400\text{nm}]$.

**PEGDM** Polyethylene glycol di-methacrylate

**NFC** nanofibrillated cellulose
I. Abstract

This work aims to study the mechanical properties of two kinds of hydrogels, the first is based on natural polymers derived from decellularized bovine cartilage tissue and the second is based on synthetic polymers namely polyethyleneglycole dimethacrylate. In this project, we tried on the one hand to reinforce the hydrogels using fibers and on the other hand to optimize the mechanical properties by studying the influence of the mixing parameters. The first study focused on Polyethylene glycol di-methacrylate hydrogel. We found that higher mixing time resulted in increased strength and stiffness of this hydrogel with a rate of 11%. In a second study we investigated the influence of different NFC fiber concentrations on the young modulus of decellulariated extracellular matrix based hydrogels.

II. Introduction

Hydrogels are soft and wet materials, which are usually composed of a three-dimensional polymer network structure and a large amount of water (50-99%) [1]. From a macroscopic point of view, hydrogels comprise elastic solids: they have a well-defined geometry and do not flow [2]. Although hydrogels are widely used in a wide range of molecular separation techniques (electrophoresis, chromatography), biomedical engineering, cosmetics or agri-food [3]. Hydrogels also possess a degree of flexibility very similar to natural tissue, due to their significant water content [4]. These materials uniquely offer moderate-to-high physical, chemical, and mechanical stability in their swollen state. The structure of a hydrogels can be designed for a specific application by selecting proper starting materials and processing techniques [5].

Hydrogels can be prepared from polymers derived from nature or synthesis. Examples of natural polymers such as collagen, gelatin, fibrin, silk, agarose, hyaluronic acid, chitosan, dextran and alginate have been employed for hydrogel preparations.

However, these natural polymers are often expensive with high batch-to-batch variations and possible chronic immunogenic responses. Furthermore, fine structural modifications of those natural polymers are often limited due to their complex structures and fragile nature. Hydrogels based on synthetic polymers such as poly (ethylene glycol) (PEG), poly (vinyl alcohol) (PVA) and
PEGDM (Polyethylene glycol di-methacrylate) offer great versatility in controlling polymer chemical structure and architecture, which is essential to prepare hydrogels with tailorable network and mechanical strengths. By careful choice of the polymers, well designed and tailored hydrogel materials can be obtained to satisfy specific biomedical applications.

However, most of the synthetic and natural hydrogel suffered from a lack of mechanical strength due to the high level of solvent in their structure and structural defect [6]. This problem could be circumvented by reinforcing the hydrogels with fibers to improve their mechanical behavior.

For this project we used NFC fiber, which are derived from plant tissues. NFC fiber are lightweight, have an elastic modulus as high as that of high-strength aramid fiber, exhibit thermal expansion on par with glass, and presents high barrier properties with regard to oxygen and other gases [7].

In order to increase the mechanical properties of a fiber reinforced hydrogel we focused on the mixing step of polymer and fibers. The parameter of mixing can influence the mechanical behavior of hydrogel. Therefore the aim of this project, on one hand, is to show that NFC fibers can reinforce both natural and synthetic hydrogels, on the other hand is to show the influence of mixing parameters.

![Figure 1 PEGDM Hydrogel](image)
III. Material & Method

All reagents used in the protocols for the hydrogel fabrication and characterization were purchased from Sigma-Aldrich (Buchs, Switzerland) unless stated otherwise.

1) Hydrogel preparation

For the medECM a stock solution of 1% was mixed with different concentrations of NFC (0.3%, 0.5%). For PEGDM lyophilized powder was dissolved in PBS to yield a 10% precursor solution. Then 0.1% photoinitiator I2959 (BASF, Germany) was added and the solution mixed by vortexing (figure 2) briefly. Subsequently 0.3% NFC fiber was added to the solution and mixed by an “Ultra-Turax” mixer at 1240 RPM. The table 1 represents the concentration of each component used in the preparation.

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration %</th>
</tr>
</thead>
<tbody>
<tr>
<td>medECM (%w/w)</td>
<td>1%</td>
</tr>
<tr>
<td>PEGDM (%w/w)</td>
<td>10%</td>
</tr>
<tr>
<td>NFC (v/v%)</td>
<td>0,3% - 0,5%</td>
</tr>
<tr>
<td>PBS (%v)</td>
<td>10%</td>
</tr>
<tr>
<td>I2959 (%v)</td>
<td>10%</td>
</tr>
</tbody>
</table>

*Figure 2 Mixing the NFC fiber for 20 min to homogenate the solution*
The mixing process with “Ultra-Turax” is depicted in figure 3. The solution was covered with aluminum foil to protect the photoinitiator from light.

![Figure 3 Mixing of hydrogel precursor with NFC fibers using an Ultra-Turax.](image)

After mixing the solution it was degassed under vacuum for several minutes. Then it was dispensed into custom-made molds with a diameter of 4 mm and a height of 2 mm. To initiate solidification of the hydrogel it was exposed to UVA light ($\lambda = 365$nm, 1mW/cm²) for 30-45 minutes [8].

![Figure 4](image)
a) Custom-made mold

b) medECM hydrogel before swelling it in PBS
In every experiment, we prepared 3 samples. Figure 5 represents the chemical preparation of the fiber reinforced-hydrogel.

![Figure 5 Representation of preparation method]

2) Mechanical characterization

The mechanical behavior of the natural and synthetic hydrogel was analyzed by determining its elastic modulus. For that we used an unconfined compression test as it is shown in figure 6.

![Figure 6 applying a compression test on hydrogel with a ramp rate of 1 mm/min]

The parameters used in this test was different for each kind of hydrogel:

- For the PEGDM hydrogel we choose a ramp rate of 1 mm/min and strain range 10%-25% [9]
Due to low mechanical properties of the dECM, we used a higher ramp rate of 4mm/min to see the effect of using NFC for this gel clearly and to have a steeper stress-strain curve and we applied a strain range of 15%-35%.

IV. Results

1) PEGDM hydrogel
The first study investigated the effect mixing time of NFC and PEGDM gel on the mechanical properties of the hydrogel. For this, we outlined the mixing step (PEGDM polymers with NFC fibers) which we made 3 experiments; first one we mixed the solution for 20 min, the second for 100 min and for the last 4 hours knowing that for these 3 hydrogels we kept the same concentration of NFC and polymers too we changed only the mixing time.

The graph in figure 7 represents the elastic modulus of the PEGDM hydrogel with these 3 different mixing times.

![Influence of mixing time](image)

We observe in the graph of figure 7, the influence of three different mixing times (20 min, 100 min, 4 h) on the young modulus of the PEGDM hydrogel. We found an increase in Young modulus with increasing mixing time.

The table 2 below represents the increase in % depending on the mixing time.

<table>
<thead>
<tr>
<th>% mixing time</th>
<th>20 min - 100 min</th>
<th>100 min - 4H</th>
<th>20 min - 4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>8%</td>
<td>3%</td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Total increase of young modulus
We obtained, on one hand, a total increase of 11%, on the other hand, we observe from 20 min to 100 min the young modulus increase by 8% but from 100 min to 4h it increases only by 3%. After 4h the solution is mixed and the hydrogel achieve the max of its young modulus with these parameters (table 2).

The second result was to test the same hydrogels after swelling it in PBS for 24h. This test will let us know if the synthetic hydrogel keeps increasing when we mix longer or not.

![Figure 8 Influence of mixing time before and after swelling](image)

Swelling of the aforementioned PEGDM gels in PBS for 24 h increased the mechanical properties by approximately 10% in each condition (Figure 8). We observed, on the one hand, that all hydrogels increase their young modulus after swelling in PBS. On the other hand, we see in this graph that the young modulus still increase when we mix longer.

2) medECM hydrogel

The second study investigated the effect of adding fibers on the mechanical properties of natural hydrogel (figure 9). Different concentrations of NFCs were used to reinforce dECM based hydrogels.
Although we add fibers, the young modulus decreases. We changed the mixing method, we used pipette to resuspend (figure 10) instead of Ultra Turax.

After we changed the mixing method, now we test again natural hydrogel. Figure 11 represents the influence of adding fibers to a natural hydrogel.
We observe in this graph (figure 11) after we add fibers the young modulus increases. This result is different from the first result (figure 9). Then we increase the concentration of fibers (the 3rd bar in the graph) and we observed that young modulus still increases. For the 0,3% NFC fiber concentration we only had 1 sample. The table 3 below represents the increase in % depending adding NFC fibers and also the concentration.

**Table 3 Total increase of young modulus**

<table>
<thead>
<tr>
<th>% increase of young modulus</th>
<th>dECM control 0,5% - + NFC 0,3%</th>
<th>+ NFC 0,3% - +NFC 0,5%</th>
<th>dECM control 0,5% - + NFC 0,5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>40%</td>
<td>11%</td>
<td>47%</td>
<td></td>
</tr>
</tbody>
</table>

We obtained that after adding NFC fiber, the young modulus increased by 40%. Then when we increased the concentration of fibers from 0,3% to 0,5%, we observed a total increased by 47%.
V. Discussion

1) PEGDM hydrogel
In this study, first we have shown that increasing mixing time made the PEGDM hydrogel stiffer because we found that young modulus increase (figure 7). The fact that the mechanical properties increase is that the polymers had more time to be homogenate with the fibers. Furthermore, NFC fibers are well dispersed in the hydrogel making hydrogel stiffer. Although mixing longer increase the mechanical properties, there is a limit which it stayed fix even if we mix longer. Because when polymer and NFC fibers are homogenously mix, the fibers are well dispersed and longer mixing cannot homogenate the gel further, that’s why there is a plateau. After, we found that when we swelled the same PEGDM hydrogel in PBS the young modulus increased (figure 8). That is due to the expansion of fiber which improves their stiffness. Moreover young modulus increases with the increase of mixing time even after swelling.

2) medECM hydrogel
The second study aims to prove that a natural hydrogel derived from decellularized bovine cartilage can be reinforced by adding NFC fibers. We found in figure 9 that when we add NFC fiber the young modulus decrease. That is possibly due to the mixing method that was applied.

![Figure 12 Denaturation of natural polymers under a heat or stress: after applying a stress to natural polymers it loses its structure](image)

Using “Ultra Turax” to mix the solution denature the crosslink of the polymers (Figure 12). This mixing represent indeed a stress that destroy the structure of the collagen fibers in the medECM hydrogel. For that reason we changed the mixing method and resuspended the solution with a pipette instead of mixing with an Ultra-Turrax (figure 10). After changing the mixing method to resuspension with a pipette the young modulus increases by 40%. Above all, adding NFC fibers to natural or synthetic hydrogel increase their mechanical properties.
VI. Conclusion

The purpose of this project was to investigate the preparation method to reinforce the hydrogels based on PEGDM polymers and medECM polymers with NFCs. The first study that concerns a synthetic hydrogel, we found that longer mixing time increases the young modulus of PEGDM hydrogels. Secondly we studied the influence of adding different concentrations of NFCs to a medECM hydrogel. We were obliged to change the mixing method because we destroyed the polymers using an “Ultra Turrax”, so after we changed the method of mixing we conclude that adding NFC fibers to a natural polymer can increase the mechanical properties of hydrogel and even adding the concentration of fibers increases more its mechanical behavior. Nevertheless, it’s not the only possibility to make an hydrogel stiffer. In future work we want to investigate other possibilities to increase the mechanical properties. Another possibility is to investigate the orientation of the fibers in the hydrogel. If we orient fibers in a specific direction we can increase their stiffness. Therefore we thought about using “Electrospinning” [10] as one of the methods that should be investigated in future work.
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