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## Research Article

# PHEMA Hydrogels Obtained by Infrared Radiation for Cartilage Tissue Engineering

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Although the exposure of polymeric materials to radiation is a well-established process, little is known about the relationship between structure and property and the biological behavior of biomaterials obtained by thermal phenomena at 1070 nm wavelength. This study includes results concerning the use of a novel infrared radiation source (ytterbium laser fiber) for the synthesis of poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel in order to produce medical devices. The materials were obtained by means of free radical polymerization mechanism and evaluated regarding its cross-linking degree, polymer chain mobility, thermal, and mechanical properties. Their potential use as a biomaterial toward cartilage tissue was investigated through incubation with chondrocytes cells culture by dimethylmethylene blue (DMMB) dye and DNA quantification. Differential scanning calorimetry (DSC) results showed that glass transition temperature ( $T_g$ ) was in the range 103°C–119°C, the maximum degree of swelling was 70.8%, and indentation fluency test presented a strain of 56%–85%. A significant increase of glycosaminoglycans (GAGs) concentration and DNA content in cells cultured with 40 wt% 2-hydroxyethyl methacrylate was observed. Our results showed the suitability of infrared laser fiber in the free radicals formation and in the rapid polymer chain growth, and further cross-linking. The porous material obtained showed improvements concerning cartilage tissue regeneration.

## 1. Introduction

The need to obtain materials with suitable features for tissue engineering leads to the continued research and development of new process and technologies [1–3]. Nowadays, several devices are successfully used in biomedicine. However, with regard to repairing damaged or diseased tissue, there is a lack of proper grafts. Although allogeneic and xenogeneic transplants (using genetically nonidentical individual cells and nonhuman animal species cells, respectively) present as a possible alternative, it is not effective for highly damaged immunogenic tissue (e.g., skin) [4, 5]. In this case, it is necessary to obtain autologous tissues (using patient's own cells) through new engineering approaches.

A viable option is the development of biomaterials that mimic the complexity of tissues and organs, and which can be introduced into living tissues without causing adverse immunological rejection [6]. In this context, the hydrogels get the researchers' attention.

Hydrogels are polymeric networks with high swelling capacity in water because of its three-dimensional structure. This property implies similar features to the human body soft tissue. In addition, hydrogels are biocompatible; they have low immunogenicity and allow a fast mass transfer between cells and the surroundings [7–10]. Currently, hydrogels are used as scaffolds for tissue engineering [11], controlled drug delivery [12], artificial articular cartilage, and intelligent devices (that responds to external stimuli,

such as pH and temperature) [10, 13], and it may provide the initial structural support required to retain cells in the defective area for cell metabolism, growth, differentiation, and new matrix synthesis [14]. We highlight the importance of the PHEMA hydrogels investigation for cartilage tissue engineering. Articular cartilage is made up of an abundant extracellular matrix (ECM) composed of proteins, nutrients, and substances rich in glycosaminoglycans (GAGs), and it cannot be regenerated in cases of tissue integrity and biomechanical functions loss, or traumas associated with injury and congenital diseases. The treatments can include the use of analgesics until autologous chondrocytes implantation [14, 15].

Poly(2-hydroxyethyl methacrylate) (PHEMA) stands out in medical applications among the materials investigated for hydrogel obtainment [8, 16, 17]. It was first studied by Witcherle and Lim [18] in the development of contact lenses. PHEMA is nonbiodegradable, has high hydrophilicity, and can be easily obtained [8]. Cartilage tissue engineering has been studied using several types of hydrogels, made of natural (e.g., chitosan and collagen) or synthetic polymers (e.g., polyvinyl alcohol and polyethylene glycol) [19]. However, PHEMA is remarkable because of the few preparation requirements, suitable biocompatibility, and safety assurance [20]. Karaaslan et al. [21] fabricated PHEMA hydrogels from wood cellulose whiskers coated with chemically modified wood hemicelluloses. The authors showed potential use of them for articular cartilage replacement. Harata et al. [20] observed a significantly increased number of chondrocytes harvested in PHEMA coating and maintenance of the quality of cells, indicating good results of this polymer for use in regenerative medicine.

Many cross-linking methods aiming at hydrogel production have been reported and are available. Some of the techniques used to obtain PHEMA hydrogels are microwave-assisted polymerization [22], polymerization for a polymer linear then followed by cross-linking [12], inverse microemulsion polymerization [23],  $\gamma$ -initiation [24], and ultraviolet radiation [25]. However, there is a particular interest in methods that avoid the use of organic solvents and under mild conditions [7]. In this concern, the radiation-induced polymerization process is very interesting because it provides a product free from toxic solvents and allows the control of physical-chemical properties of the material; it is possible to adjust the intensity and/or absorbed radiation dose [26]. Biofabrication techniques of polymeric devices and hydrogels through laser technology have been reported [27, 28], and their viability for producing 2-D and 3-D grafts is remarkable [28, 29]. However, some of the processing methods have high operating cost, such as UV and femtosecond lasers [30], or only works with transparent materials, as a tightly focused ultrafast laser beam at infrared wavelength [29]. Extreme ultraviolet laser might change the degradation rate of the polymeric biomaterial [31], and some techniques, such as the matrix-assisted pulsed laser evaporation direct writing (MAPLE-DW), imply in using metallic layers that might contaminate the final product [28].

The different synthesis methods produce specific structure on materials. Between the material features for an appropriate appliance in tissue engineering, there are the high porosity

and the possibility to produce 3-D structures with controlled mesh architecture [32]. The porous profile interferes with the water-holding ability of hydrogels. When the pores are not connected and the porosity is high, faster is the water diffusion through the hydrogel network and better is the drug molecules absorption [10, 33]. Thus, the processing technology must concern about the morphology of hydrogels and guarantee the proper surface for cells attachment.

In this study, we uncovered the PHEMA hydrogels synthesis free from toxic solvents by a novel procedure of infrared radiation-induced polymerization using ytterbium laser fiber at 1070 nm wavelength. The process has the advantage of being flexible and allowing local heating. Besides that, little is known about its feasibility for hydrogels processing. The laser fiber promotes a controlled energy flow through the material, causing a cross linking in a defined volume and resulting in specific geometries for use in tissue engineering. Therefore, the aim of this investigation was to demonstrate the potential of IR ytterbium laser to produce PHEMA hydrogels in order to use them in cartilage tissue engineering.

## 2. Materials and Methods

*2.1. Materials.* 2-Hydroxyethyl methacrylate (HEMA, >99%), diethylene glycol dimethacrylate (DEGDMA, 95%), and potassium persulfate (KPS,  $\geq 99\%$ ) were provided by Aldrich. Dibenzoyl peroxide (BPO, 98%) was supplied by Peroxid-Chemie. All chemicals were of analytical reagent grade and were used as received.

*2.2. Synthesis of PHEMA Hydrogels.* The PHEMA hydrogel synthesis was carried out by two distinct approaches: in the first way, the polymer was prepared by adding the cross-linker DEGDMA and potassium persulfate (KPS) (initiator) into the monomer HEMA aqueous solution to improve the control over molecular mass and Trommsdorff effect, typical of radical polymerization reactions, and to enable the formation of a porous structure. Then, diethylene glycol dimethacrylate (DEGDMA) and potassium persulfate (KPS) at 2 wt% and 1 wt% concentrations, respectively (related to the HEMA weight), were added to the 40-wt% (sample A) and 80 wt% (sample B) HEMA/water rate. The mixture was stirred at room temperature for 30 minutes. Afterward, 2 mL of it was poured into glass vials and exposed to the ytterbium infrared laser for  $3 \pm 0.5$  minutes. The infrared laser wavelength and diameter were 1070 nm and 0.8 cm, respectively. The laser fiber power was kept constant at  $30 \pm 0.5$  W, allowing an utmost polymerization temperature of 399 K, as shown in our previous study [34]. The distance between the laser focus and center point of the solution was 9.5 cm. The materials were taken out from the vials after irradiation, washed with distilled water to remove residual monomers, and dried at room temperature. Residual initiator will remain in aqueous solution, and the desirable polymer was precipitated as solid. In the second approach, the samples C, D, and E were prepared without water and at several cross-linker contents. DEGDMA at 1, 2, and 3 wt% was added into HEMA to evaluate the influence of the cross-linker concentration over mechanical

and chemical properties of the materials. Benzyl peroxide (BPO) was used as a soluble-free radical initiator to the purely organic systems at the concentration of 1 wt%, based on the monomer weight, to improve the solution homogeneity. Then, 2 mL of the mixture was poured into 50 mm diameter Petri dishes and polymerized by infrared laser radiation for 5 minutes at the most. Possible organic impurities were solubilized in the unreacted organic monomer phase and eliminated by extraction sol-gel of materials in alcohol for 12 hours.

The approaches show similar characteristics to the thermosetting system, as demonstrated by Huang et al. [35]. The polymerization and cross linking of PHEMA hydrogels were performed in a single step under atmospheric conditions. All reagents are summarized in Table 1.

**2.3. Characterization.** Differential scanning calorimetry (DSC) measurements were performed in a Mettler Toledo DSC 823e instrument to evaluate the glass transition temperature of materials ( $T_g$ ). From this thermal property, it is possible to understand the influence of the initial composition on the mechanical and swelling behavior of samples. First, the samples were dried in an oven for 24 hours at  $40 \pm 5^\circ\text{C}$ . Amounts of 10–15 mg of the previously dried samples were placed in standard aluminum pans, stuck upside down. To eliminate the thermal history of the material, a heating ramp from  $25^\circ\text{C}$  to  $250^\circ\text{C}$  at a rate of  $20^\circ\text{C}/\text{min}$  was performed, followed by a cooling to  $25^\circ\text{C}$ . Afterward, the samples were reheated to  $250^\circ\text{C}$  at  $20^\circ\text{C}/\text{min}$ , under a nitrogen atmosphere as a purge gas with a flow rate of  $50 \text{ mL}/\text{min}$  [16]. Calibration was made with indium standards.

Unreacted materials content and cross-linking degree were investigated by extraction of soluble polymers in water and methanol (extraction sol-gel). The data were evaluated by gel fraction measurements. For this, the weight change of the specimens was observed before and after immersion in the solvent. First, the extraction sol-gel was done using distilled water at  $85 \pm 5^\circ\text{C}$  for 24 hours. After drying, the samples were immersed in methanol for 12 hours at  $40 \pm 5^\circ\text{C}$ , and the solvent was replaced several times to extract soluble species (low molecules weight chains). Gel fraction ( $F_g$ ) (insoluble cross-linking fraction) was determined by Equation (1), where  $m_s$  is the initial weight of dry hydrogel (before immersion test) and  $m_{\text{gel}}$  represents the weight of the samples after unreacted materials removal:

$$F_g = \left( \frac{m_{\text{gel}}}{m_s} \right) \cdot 100\%. \quad (1)$$

Swelling experiments were performed using the modified standard ASTM D570 (Equation (2)). Gravimetric measurements were determined at  $37^\circ\text{C}$  (physiological temperature) and 24 hours.  $w_s$  and  $w_d$  are, respectively, the weight of the swollen and dried hydrogel. The water excess in the swollen state was removed with paper towels. Averaged values for hydrogels swelling rate were calculated from triplicates:

$$\text{Swelling (\%)} = \left( \frac{w_s - w_d}{w_d} \right) \cdot 100. \quad (2)$$

TABLE 1: Composition of the samples used in the infrared radiation-induced synthesis.

| Sample ID | HEMA/water (% wt) | DEGDMA (% wt)* | KPS (% wt)* | BPO (% wt)* |
|-----------|-------------------|----------------|-------------|-------------|
| A         | 40/60             | 2              | 1           | NA          |
| B         | 80/20             | 2              | 1           | NA          |
| C         | 100/0             | 1              | NA          | 1           |
| D         | 100/0             | 2              | NA          | 1           |
| E         | 100/0             | 3              | NA          | 1           |

NA: not added; \*relative to monomer HEMA weight.

Scanning electron microscopy (SEM) was used to investigate the morphology of cross section. Dry samples were fractured in liquid nitrogen, attached to a metallic support with a double-sided tape, and coated with gold for 120 seconds (SC762 Sputter Coater). 2000 magnification images were taken at 50 and  $250 \mu\text{m}$  by a scanning electron microscope (SEM, model LEO, 440i).

In indentation fluency experiments, all hydrogels were immersed in distilled water at  $37^\circ\text{C}$ . Ballpoint indenter of 1.6 mm radius was used. The load applied was 0.5 kgf for 180 seconds. The indentation height ( $h$ ) over time ( $t$ ) was measured two seconds after load application. It is one of the methods used to determine properties material of cartilage.

#### 2.4. Cell Culture

**2.4.1. Chondrocyte Culture.** Bovine ear chondrocytes were obtained by chemical, mechanical, and enzymatic digestion of the cartilage. After harvesting the cartilaginous tissue, the perichondrium was carefully dissected away and minced into small fragments. Then, a protease solution with sodium chloride at  $2 \text{ mg}/\text{mL}$  was added to the explants and it was incubated for 2 hours at  $37^\circ\text{C}$ . Afterward, the protease solution was removed and it was added collagenase B solution dissolved in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum (FBS) and 1% gentamicin-amphotericin B at  $37^\circ\text{C}$  (final concentration  $1.5 \text{ mg}/\text{mL}$ ). The digested cartilage was sterilized by passage through a  $0.22\text{-}\mu\text{m}$  filter. Cell counting was assessed by trypan blue dye using a hemocytometer. The harvested cells were seeded into  $175 \text{ cm}^2$  flasks ( $3500 \text{ nc}/\text{cm}^2$ ) in DMEM with 20% FBS and incubated in a humidified incubator at  $37^\circ\text{C}$  with 5%  $\text{CO}_2$ . 24 hours later, the cells were washed with saline solution and the culture medium was replaced every three days.

**2.4.2. Hydrogel Preparation.** Hydrogel samples (with and without alginate) with a mass ranging from 12 to 15 mg were washed for 24 hours in distilled water and sterilized at  $160^\circ\text{C}$  for 90 minutes. The specimens were placed in a 96-well plate ( $n = 4$ ), and chondrocyte cells were inoculated onto the samples ( $4 \times 10^7 \text{ cells}/\text{mL}$ ). After 2 hours, it was added 0.5 mL of DMEM supplemented with 20% fetal bovine serum (FBS) and 1% gentamicin-amphotericin B, followed by incubation for 1 and 10 days at  $37^\circ\text{C}$ . The medium was replaced every two days.

**2.4.3. DMMB Assay.** GAG concentration was estimated at 1 and 10 days to evaluate chondrocytes cultured on several samples. This assay is used in determining GAG contents by using dimethylmethylene blue (DMMB) dye binding [36]. After incubation of DMMB solution to the wells, absorbance was measured at 530 nm in a microplate reader. From the obtained optical density (OD) values, it was possible to quantify GAGs.

**2.4.4. DNA Quantification Assay.** DNA content of cultivated chondrocytes in hydrogels for 10 and 21 days was determined by the ethidium bromide (EB) method. The control assay was prepared exactly like samples, except for the absence of cells.

### 3. Results and Discussion

**3.1. Infrared Radiation-Induced Synthesis and Characterization of PHEMA Hydrogels.** Infrared radiation-induced hydrogel synthesis was investigated on aqueous solutions and bulk polymerization. HEMA/water ratio increases from 40:60 wt% (sample A) to 80:20 wt% (sample B). In these samples, the water behaves as a chromophore, and it was the main energy absorber. The IR laser wavelength directly induces excitation in the OH stretch vibration modes of water and causes its radiolysis. Hydroxyl radicals, active sites in the monomer, and polymer chain macroradicals were formed. A rapid, free radical chain growth and cross-linking took place [37, 38], but the radiation effect was predominantly produced in the optical penetration region. Samples C, D, and E vary from one to another in cross-linking degree, and the absorbed energy was eased by a number of accessible vibrational states in the molecules of the reaction medium. The energy-rich species undergo scission, abstraction, and a sequence of addition reactions, leading to cross-linking structures [39]. The material changed from opaque off-white color (samples A and B) to clear glass (translucent) in water-free solutions. When the water concentration exceeds a certain critical value, a thermodynamic interaction between water and polymer network occurs, and opaque PHEMA hydrogels are obtained [40]. These hydrogels showed noticeable porous structures and sponge behavior.

After 5 min of infrared radiation-induced polymerization, about 94–95 wt% of the monomers in the feed for samples A and B and 75–82 wt% for samples C, D, and E were converted into water and methanol insoluble hydrogels (gel fraction,  $F_g$ ) [41], as shown in Table 2. The gel percentage may be directly related to the cross-linking degree, and it is inversely proportional to the swelling. So, slightly cross-linked polymers do not soluble in any solvent and extraction process allows obtaining the macromolecules amount which is covalently bound. In solvent-free samples,  $F_g$  slightly increased with swelling decreasing. In samples A and B, the  $F_g$  increased with solvent volume fraction decreasing in an aqueous mixture. In general, around 1 wt% diester ethylene glycol dimethacrylate (EGDMA) is present as a by-product in the commercial monomer [16], and it works as a cross-linker [35]. Thus, it is seen that HEMA increases and

TABLE 2: Glass transition temperature ( $T_g$ ), gel fraction, and swelling results.

| Samples ID | $T_g$ (°C) | Gel fraction (wt%) | Swelling (wt%) |
|------------|------------|--------------------|----------------|
| A          | 115        | 94.5               | 71             |
| B          | 115        | 94.7               | 41             |
| C          | 101        | 75.0               | 55             |
| D          | 109        | 78.5               | 53             |
| E          | 110        | 82.3               | 47             |

cross-linker content increased  $F_g$ . It was observed that gel expansion driven by water diffusion decreases with cross-link density and the limited elasticity of the network structure [42]. The swelling values were found around 71 wt% to A, 41 wt% to B, 55 wt% to C, 53 wt% to D, and 47 wt% to E samples (Table 2).

Nonisothermal DSC curves are presented in Figure 1. There is no noticeable endothermic peak for all five samples studied and only one stage was observed, which indicates the heat capacity change and an amorphous polymeric system. Thereby,  $T_g$  is an important parameter to evaluate molecules mobility. A dependence of  $T_g$  on gel fraction was observed in all samples (Table 2). This relation is attributed to the increase of strong chemical bonds and reduction of polymer-free volume. As a consequence, higher temperatures are required to enhance the molecular motion of materials. In similar studies, it also has been observed on cross-linked epoxy polymers [43]. The glass transition temperature ( $T_g$ ) was found to range from 101°C to 115°C (Table 2), in agreement with the values reported in the literature [44–46].

Figure 2 displays the morphology of the samples cross sections. SEM examination of samples A and B shows a matrix consisting of porous structures and polymer particle agglomerated [17, 40]. However, the variation of pores distribution and uniformity depend on the amount of water in the monomer mixture (Figures 2(a) and 2(b)) and cross-linking concentration [40]. By increasing HEMA/water rate and DEGDMA content, the resulted hydrogel becomes more dense and nonporous (more breakable) as samples C, D, and E (Figures 2(e)–2(g)). The morphology observed is typical of glass aspect polymer. Moreover, the effect of IR radiation absorption on polymer morphology has been observed. In general, the absorption of a material depends on the wavelength of light and laser incidence angle [47]. At normal position (zero inclination angle), the ytterbium laser beam provides a local heating, leading to homogeneous and dense surface in the radiation incidence zone (Figure 2(d)). Cross-linking process then starts from the center to the edges through energy transfer, and porous surfaces are observed at the laser beam surroundings (Figure 2(c)).

Fluency assays are shown in Figure 3. The samples were subject to constant strain, and stress was obtained as a function of the time. It was observed the resilience of all samples on the mechanical forces application. Results revealed the strain that occurs over a period when the material is subjected to constant temperature and stress. In general, despite that hydrogels do not have much tear strength, they have good resistance to perpendicular forces. Samples A and B showed similar behavior between

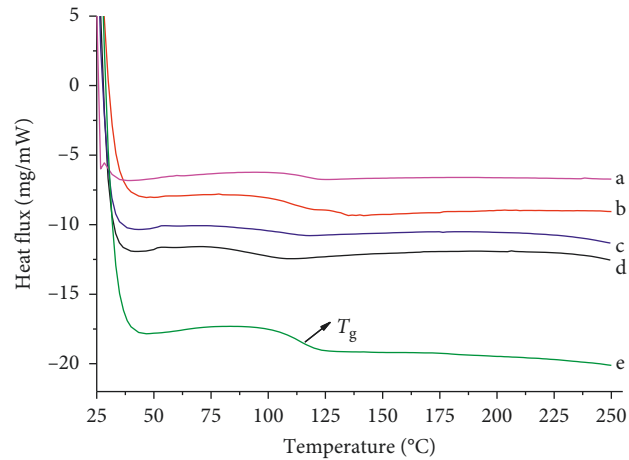


FIGURE 1: Nonisothermal DSC curves of (a) A, (b) D, (c) E, (d) C, and (e) B samples.

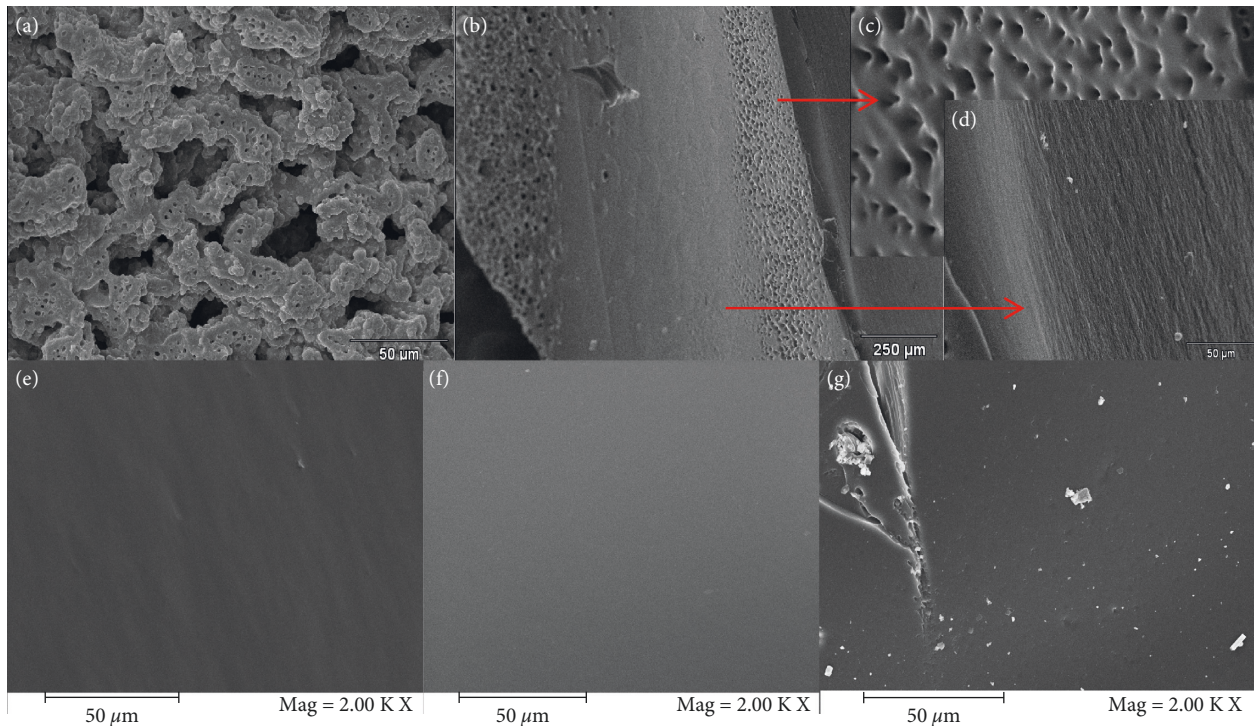


FIGURE 2: SEM images of hydrogels cross section: (a) sample A at  $50\ \mu\text{m}$ : general morphology; (b) sample B at  $250\ \mu\text{m}$ ; (c) porous region magnification of sample B at  $50\ \mu\text{m}$ ; (d) dense region magnification of sample B at  $50\ \mu\text{m}$ ; (e) sample C at  $50\ \mu\text{m}$ ; (f) sample D at  $50\ \mu\text{m}$ ; (g) sample E at  $50\ \mu\text{m}$ .

itself and strain in the range 4–6 mm. It means a strain of 56%–85% to A and 49%–74% to B. There was a polymer continuous deformation with time to support the stress applied. The polymeric chains drain over each other because of its natural mobility [48]. This deformation is influenced by intermolecular bonding force and flow out of the water through hydrogel macromolecular structure. Strain versus time results for articular cartilage are demonstrated by Spiller et al. [49], and it was in the range of 30–40%.

Samples C and E also presented similar behavior, but the larger strain was observed in C because of the lower

cross-linking density, as expected. Sample D did not resist the applied load. It was difficult to measure its mechanical properties because of the easy break and fragility of the material in the first minutes of testing. On this, the Voigt model was used to determine Young's modulus (elastic) of A, B, C, and E samples. It was calculated by curve fitting the slope of the initial unloading curve [50]. The studies demonstrated the elastic modulus values at 0.6 MPa (samples A and B), 6.2 MPa (sample C), and 12 MPa (sample E), according to increased stiffness of polymeric matrix. The mechanic's observations suggested that samples A and B behave like a sponge, characteristic similar to articular

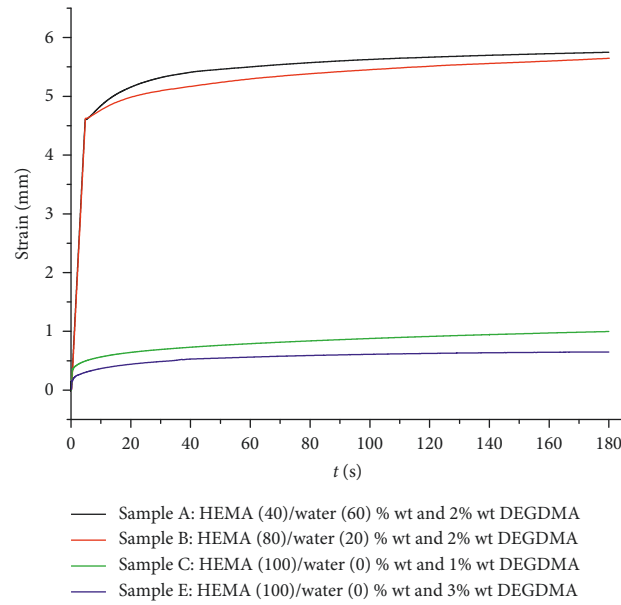


FIGURE 3: Strain versus time to A, B, C, and E samples.

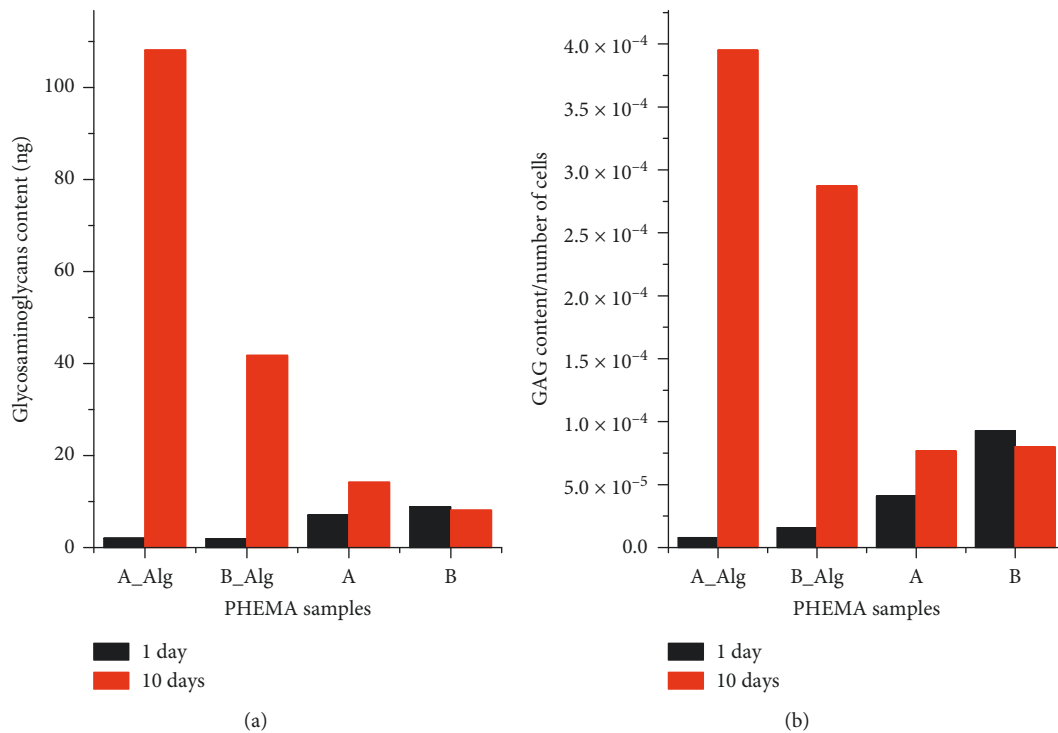


FIGURE 4: (a) Glycosaminoglycans content (OD) expressed by chondrocytes cultured for 1 or 10 days on the A and B samples with and without alginate (Alg); (b) relationship between the number of the cells and levels of glycosaminoglycans (GAGs) expressed by chondrocytes cultured for 1 or 10 days in A and B samples with and without alginate.

cartilage (Young's modulus in the range of 0.45 to 0.80 MPa) [51]. So, they can be most promising for loading applications during movement cycles. Besides that, porous materials improve gliding properties to the maintenance of cell differentiation and can provide a local microenvironment for the diffusion of soluble factors. Samples A and B were selected for the chondrocyte culture tests.

In Figure 4, is observed the GAG quantification from the DMMB assays. Considered one of the major macromolecular components of ECM [52], GAGs play an important role in cell adhesion. The GAG amount harvested after 1 day of culture is minimum but increases after 10 days of culture. Chondrocytes cultured in hydrogels with alginate expressed a higher level of GAGs (Figure 4(a)). On the other

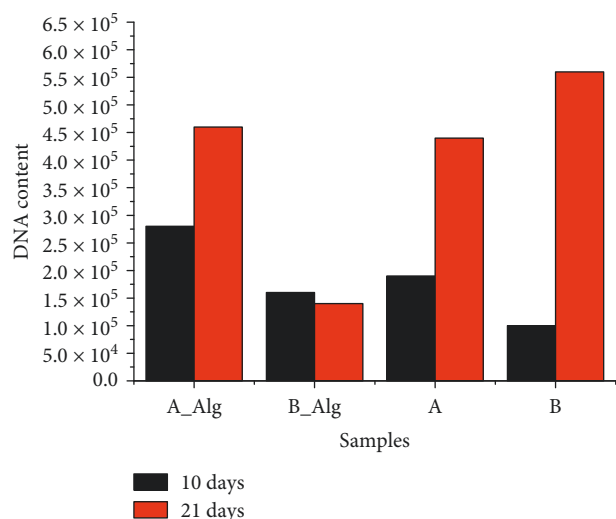


FIGURE 5: DNA content expressed by chondrocytes cultured for 10 and 21 days on the PHEMA samples (A and B) with and without alginate.

hand, after 21 days of culture, the GAG levels were so high that they could not be measured by DMMB assay. This result is justified by dye excess derived from GAG-dye complex, which absorbs all the light emitted at the wavelength of 530 nm and avoids a proper measurement of the glycosaminoglycans content (OD) samples. Figure 4(b) shows the relation between the cells number and levels of glycosaminoglycans. According to the data obtained, GAG amounts are associated with the cell numbers. In this image, it is possible to observe a greater GAG amount and proliferation of cultured chondrocytes in hydrogels with alginate.

DNA content (a chondrocyte amount indicator) for 10 and 21 days is depicted in Figure 5. The samples (with or without alginate) showed a trend of increasing DNA content over the chondrocytes culture period. It was observed, however, that swelling and surface morphology affects cell behavior. Porous structures and larger mesh sizes improve cell growth and nutrients diffusion through the hydrogel structure and resulted in higher GAG/DNA content [53], as sample A.

#### 4. Conclusions

PHEMA hydrogels were obtained by free radical polymerization infrared radiation induced at 1070 nm wavelength. Our results showed the feasibility ytterbium as a laser source and the importance of precursor solution in the final properties of the engineered matrix. The energy absorbed by molecular vibrations modes was predominantly focusing on laser optical penetration region. Cross-linking and macromolecules dissolution was evaluated by gel fraction and swelling measurements. It was observed that chain growth and polymer cross-linking occurred at short processing times and single step. Characterization techniques applied revealed the radiation impact, water content, and cross-linking concentration over hydrogel morphology. It was verified that swelling driven by water diffusion decreases

with cross-link density and limited motion of the network structure. The thermal analysis confirmed the presence of an amorphous region on hydrogels and glass transition temperature between 101°C and 115°C, consistent with previously reported data. Gel fraction and swelling assays displayed a dependence on both solvent volume fraction and DEGMA amount, with 41–71 wt% and 75–95 wt% values, respectively. Mechanical studies regarding the material resilience revealed 49–85% strain. Porous structures improved growth functional proteins and nutrients of the extracellular matrix (ECM), such as GAGs and DNA. Thereby, the PHEMA hydrogel at 40 wt% HEMA (sample A) is pointed out as the optimal composition with regard to swelling rates, mechanical properties (Young's modulus 0.6 MPa), and biological behavior. There were cell proliferation and absence of toxicity, indicating the great potential for using this hydrogel as a template for cartilage construction because of form maintenance in the highlighted tissue.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author on request.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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