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CMC/PEG blended hydrogels for tissue engineering and regenerative medicine

D. Kavaz[1.2]*, N. Aşina[1.2]

[1] *Bioenginering Department, Faculty of Engineering, Cyprus International University, Nicosia 98258, Via Mersin 10, Turkey*

[2] *Biotechnology Research Centre, Cyprus International University, Nicosia 99258, Via Mersin 10, Turkey*

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Hydrogels are ideal drug carrier systems for wound healing in tissue engineering and regenerative medicine applications. Their 3D structure supports the cell binding and tissue forming while treating the extracellular matrix for regulating the cellular functions. Poly (ethylene glycol) dmethacrylate (PEGDMA) based hydrogels hold a prominent place in tissue engineering but their applications are limited due to the low porosity and low biodegradability. Carboxymethyl cellulose (CMC) combined with PEGDMA hydrogels aims to decrease the porosity and cell viability for tissue regeneration with better swelling and biocompatibility features. The hydrogels were prepared with different concentrations of PEGDMA, CMC and Irgacure (2959). Surface morphology, pore size profile, chemical bonds and swelling behavior were investigated by scanning electron microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR), respectively. The results show that the use of CMC improves the porosity along with the swelling behavior. Swelling results are in the range of 90-99.4%.

Keywords: Hydrogel; Tissue Engineering; Regenerative Medicine; PEGDMA; CMC.

INTRODUCTION

Wound healing is a highly complex system that includes many cell types, various cytokines, growth factors and their interactions. The wound healing mechanism plays a key role in tissue regeneration and healing in many enzymatic pathways, apart from cellular and biochemical components [1]. For this reason, natural and synthetic polymer-gel-like structures (hydrogels) which accelerate the process are mainly used in the wound treatment process.

Hydrogels are crosslinked homo- or copolymeric systems that can "swell" by absorbing a high amount of water. They are three-dimensional polymeric networks that don't dissolve in water but swell [2], that is, can take most of the water into their structures, and include both natural and synthetic polymers such as gelatin, agar and alginates [3]. Thus, water lover structure of the hydrogels made them the closest matter of structure to a real human tissue [4]. They swell to a stable volume in water but retain their shape. The amount of water absorbed by a hydrogel is quite large and can even reach 1000 times its own weight. For this reason, they have been used in a wide variety of fields in recent years.

Hydrogels can be classified depending on the method of preparation, ionic charge or physical structure [5, 6]. According to the preparation method, there are 4 types of hydrogels: homopolymer, copolymer, multipolymer and IPN (interpenetrating network) hydrogels. Hydrogels can be prepared by crosslinking with a radiant or a chemical reaction [7]. Radiation reactions take place with electron-emetic, irradiated, X-rays, or UV-rays. Chemical crosslinking occurs in the presence of at least one difunctional, small molecular weight crosslinking agent. This agent binds two long polymer chains through their functional groups.

Polymerization using UV radiation is the safest and cleanest polymerization method as it does not degrade the polymer properties [8, 9]. No chemical additives such as initiators, solvents, protective colloids or surfactants are required for this type of polymerization. As a result, the polymer retains its biocompatibility [10]. In addition, the use of radiation dose depends on the application area of the crosslinked polymer, and radiation dose and time are also important issues that affect the degree of crosslinking of the polymer.

Crosslinked polymer hydrogels exhibit a unique swelling behavior without dissolving in an aqueous environment or when entering a solvent, due to their high water absorption ability and the presence of critical crosslinks in their structure [11]. Their water absorption ability is due to the presence of hydrophilic functional groups such as –CONH, –OH, $–CONH_2$ and $–SO_3H$ in the polymer structure forming the hydrogel. Water absorption by the hydrogel depends on the functional group, the state of the water, and the density of the crosslinking net-

* To whom all correspondence should be sent:
E-mail: dkavaz@ciu.edu.tr

work in the hydrogel [12]. Crosslinks in polymeric networks are achieved by hydrogen bonding, covalent bonds, van der Waals interactions, or physical entanglement. The degree of swelling is often used to describe hydrogels [13, 14]. The degree of swelling also depends on many factors such as mesh density, solvent structure, polymer-solvent interaction parameter.

Photopolymerized poly (ethylene glycol) dimethacrylate (PEGDMA) has been extensively studied for biomedical applications such as cell encapsulation, tissue engineering and drug delivery [9, 15]. PEGDMA is highly hydrophilic and the resulting hydrogel assets are tunable by changing the polymers molecular weight and water content. CMC on the other hand, is water-soluble anionic cellulose derived biopolymer with hydrophilic, pH-sensitive, non-toxic and easy gel-formable behavior [16, 17]. These characteristics show promising features in drug delivery systems and regenerative medicine [18].

Novel hydrogels were created by crosslinking carboxymethyl cellulose (CMC) sodium salt with PEGDMA in this study. The degree of crosslinking and the action of PEG as a network modifier were proven to adjust the mechanical and physico-chemical properties, as well as morphological aspects, in order to mirror the characteristics of real and genuine skin tissue.

EXPERIMENTAL

Materials

Poly (ethylene glycol) and dimethacrylate (PEGDMA) 1000 were purchased from Polysciences, phosphate buffered saline (PBS), carboxymethyl cellulose (CMC) and 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure D-2959) were purchased from Sigma-Aldrich.

Preparation of Polymer Solution

0.2% (w/v) of Irgacure D-2959 was added into 5 mL of 1M PBS solution and heated at 100°C with continuous stirring for 10 min. 20% (w/v) Poly (ethylene glycol) dimethacrylate was added into the Irgacure/PBS solution under constant stirring and left to dissolve. CMC solution was prepared in 1M PBS solution as well, 4% CMC was weighed and added into 30 mL of hot PBS under constant stirring. Remaining 70 mL were stored in a fridge for 30 min to cool down. After all CMC particles were evenly dispersed, 70 mL of cold PBS was added and continued stirring.

Samples were placed 5 cm away from the lamp and 3 mL of solutions were dropped into 60 mm glass petri dishes. All samples were exposed to UV lamp for 5, 10 and 15 min (Table 1).

Freeze-Drying

Freeze-drying (lyophilization) technology is a drying method that can remove all water content without changing the material quality and shape properties of the products. It occurs by freezing the product first, then reducing the pressure in the environment and the water accumulated in the sample evaporates.

Table 1. Photo crosslinking duration and ratios of the hydrogels

	Crosslinking Time	PEGDMA:CMC:Irgacure (w/v)
P1	5 Min	2:1:1
P2	10 Min	2:1:1
P3	15 Min	2:1:1
C1	5 Min	1:2:1
C2	10 Min	1:2:1
C3	15 Min	1:2:1
I1	5 Min	1:1:2
I2	10 Min	1:1:2
I3	15 Min	1:1:2
S1	5 Min	1:1:1
S2	10 Min	1:1:1
S3	15 Min	1:1:1

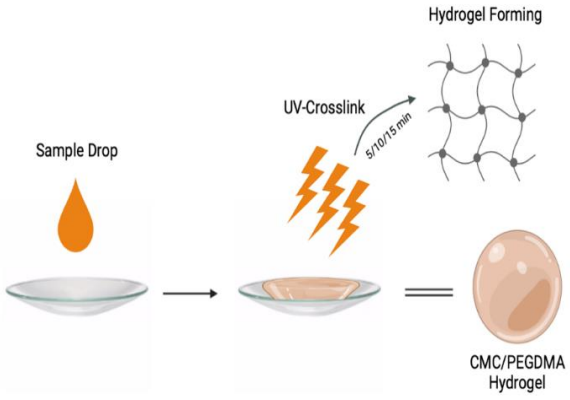

Figure 1. Schematic photopolymerization process of the hydrogels

Most of the physico-chemical properties of the product are preserved safely. Biobase brand freeze-dryer was used for the lyophilization process. Samples were first placed at -20 °C for pre-freezing

for 12 h and then placed at -80 ℃ for 24 h. After pre-freezing, the samples were freeze-dried at -72 ℃ with constant vacuum for 48 h.

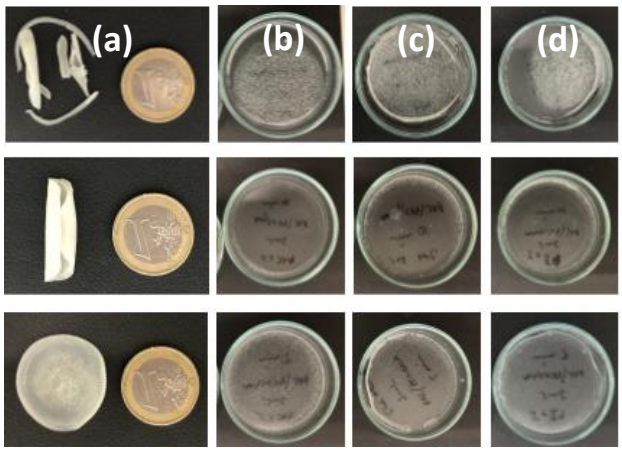

Figure 2. Photo-crosslinked (a) P, (b) C, (c) S and (d) I polymers.

CHARACTERIZATION

Fourier transmission infrared spectroscopy

Shimadzu IR Prestige 21 model FTIR spectroscope was used during the analysis. Organic or inorganic compounds are characterized by the tool called infrared (IR) spectroscopy. In response to the frequencies formed by the vibration of the bonds between the atoms that make up the matter, the IR spectrum shows the corresponding absorption peaks. PEGDMA, CMC and CMC/PEGDMA samples were analyzed by FTIR to conform the bonding between PEGDMA and carboxymethyl cellulose.

SEM and Porosity

Scanning electron microscope (SEM) is a system designed within the framework of electro-optical principles, which enables high-energy electrons to interact with the material to take the sample's surface morphology. The generated electron and photon signals interact with the sample by high-energy electrons. The computer-aided system collects the resulted images as a processing of the detector by the scattered electrons collected from different angles. Secondary electrons, material topography, and backscattered electrons provide information on atomic composition based on atomic number and contrast. Emitech brand SC7620 sputter coater was used for coating the samples. JEOL brand JSM-6610 model scanning electron microscope was used for the analysis. Samples were coated with gold for 90 sec with 5mA current.

For polymer-based hydrogels, the porous structure holds a great importance especially for the cell hosting, cell culture and proliferation. The open structure on the other hand, affects the swelling behavior and mechanical properties of the hydrogel. Different types of materials are available, used for tissue engineering applications, which should provide particular properties to be able to fit for the application. Porosity is one of the main characteristics which affects the cell growth and proliferation. Specific pore size is preferred in the biomaterial production, particularly for cell culture or tissue engineering approaches due to the sufficient oxygen transportation through the ECM, as well as toxic compound removal and cellular growth.

Swelling

Swelling ratio is the water absorption ratio of a polymer as the ratio of wet weight of the sample and dry weight of the sample calculated while conducting the test. Swelling analysis was carried out in PBS solution. Pre-weighed samples (3n) were placed into 3 mL of PBS and kept for 24 h and 48 h at room temperature.

$$(Sk) = \frac{Wt - Wo}{Wo} x100$$

(S*k*) is the swelling ratio, where (W*o*) is the dried mass of the hydrogel and (W*t*) is the wet mass of the hydrogel.

Statistical analysis

The swelling results are presented as mean ±standard error of the mean. SPSS Statistics, SPSS Software version 26 was used during Two-way ANOVA test followed by 3 factor ANOVA, Tukey's multiple comparison test, and factor interaction effect. Significance was considered if the p value was $p \leq 0.05$. All data were repeated three times.

One-way ANOVA was used while SEM pore size data was analyzed, then followed by Tukey HSD from SPSS and ImageJ. The data are presented as mean ±standard error of the mean. Significance was verified convenient if the p value was $p \leq 0.05$ and each sample was analyzed three times to be significant.

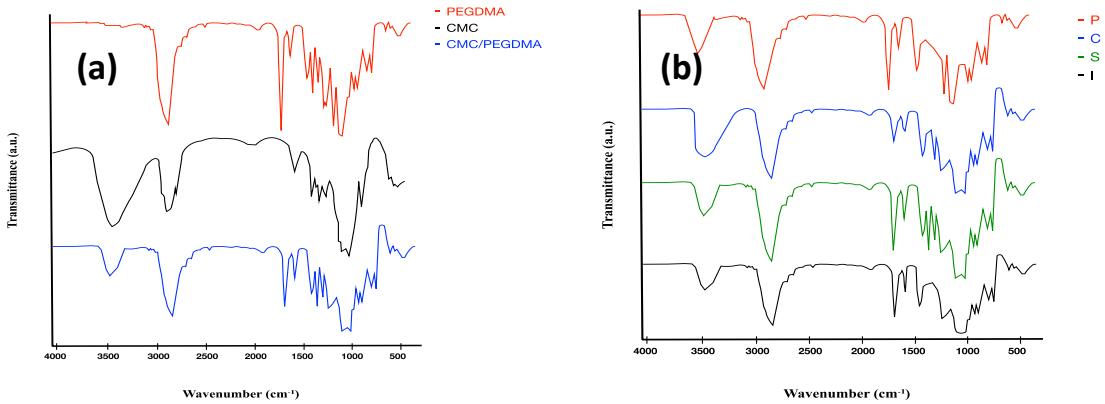

Figure 3. FTIR results of (a) PEGDMA, CMC and CMC/PEGDMA blend, (b) P, C, S and I.

RESULTS AND DISCUSSION

FTIR Results

Figure 3 presents the FTIR spectrum patterns of CMC, PEGDMA, CMC/PEGDMA and photopolymerized hydrogel, respectively. Based on Figure 3 (a), the observed peaks in the range of 3500-2750 cm^{-1} wave numbers represent polysaccharides; O-H and C-H bonding. The single peak in hydrogel formation at 1300 cm^{-1} shows the main chain of PEGDMA hydrogel as Fathi-Achachelouei *et al.* [19] found similar results in their hydrogel studies. For the CMC spectrum, C-H stretching was observed at 3000 cm^{-1}, hydrocarbon groups (-CH_2 scissoring) at 1450 cm^{-1} also peaks around 1400-1100 were observed corresponding to COO^-, OH coupling interactions of the carboxylic group and C-N stretching. C-H, C=C, C-O, amine I, amine II and amine III (stretching and forming) confirmed the binding between CMC/PEGDMA hydrogels. Both PEGDMA and CMC contain absorption peaks of C=C and C=O group chain as Klunklin *et al.* [20] mentioned.

On Figure 3 (b), all hydrogel variations and their bands are compared. Based on the polymerization technique, all showed identical characteristics with CMC/PEGDMA hydrogel. Only few differences were observed depending on the ratio difference. For instance, while P has a similar peak at 1720 cm^{-1} with PEGDMA itself, C showed a similar peak with CMC at 1720 cm^{-1}.

Surface Morphology and Porosity

SEM results helped to characterize and compare the surface morphology of all hydrogel variations. Figure 4 represents the variation of hydrogels along with the PEGDMA hydrogel without added CMC to compare the porosity with/without CMC. Best porosity was observed on C hydrogel, followed by P, S and I, respectively. Figure 4(b) confirms the additional increase in porous structure and the structural change after CMC addition. Figures 4(c) and 4(d) also show similar surface structure but lower porosity when compared with (a) and (b). Figure (e) clearly defines the surface difference when CMC is not added. As Barnett *et al.* [21] mentioned, PEGDMA polymer itself has low porous state without optimum environment (such as thickness and drying method) and plays a critical role on determining the morphology and pore size thereby the hydrogel behavior.

SEM images were used for the pore size distribution, performed on vertical and horizontal cross sections. Average pore diameter of samples imaged spontaneously. All samples were analyzed with average ± standard error. Each sample had 3-5 images during the pore size calculations. C group of hydrogels showed the highest porosity while I group of hydrogels showed the lowest porosity, when compared with P and S. P and S showed similar result with each other which also conforms the effect of carboxymethyl cellulose on porosity. Barnett *et al.* [21] found a pore size range between 5-40% in average. The pore size of the hydrogel must be big enough for the cells to migrate into the structure but small enough at the same time to establish a sufficient surface area. The average size of the fibroblast cells varies between 10-15 μm. In this study, the synthesized CMC/PEGDMA hydrogels showed an average pore size of 20-60 μm, which structure is suitable for cell growth and tissue engineering approaches.

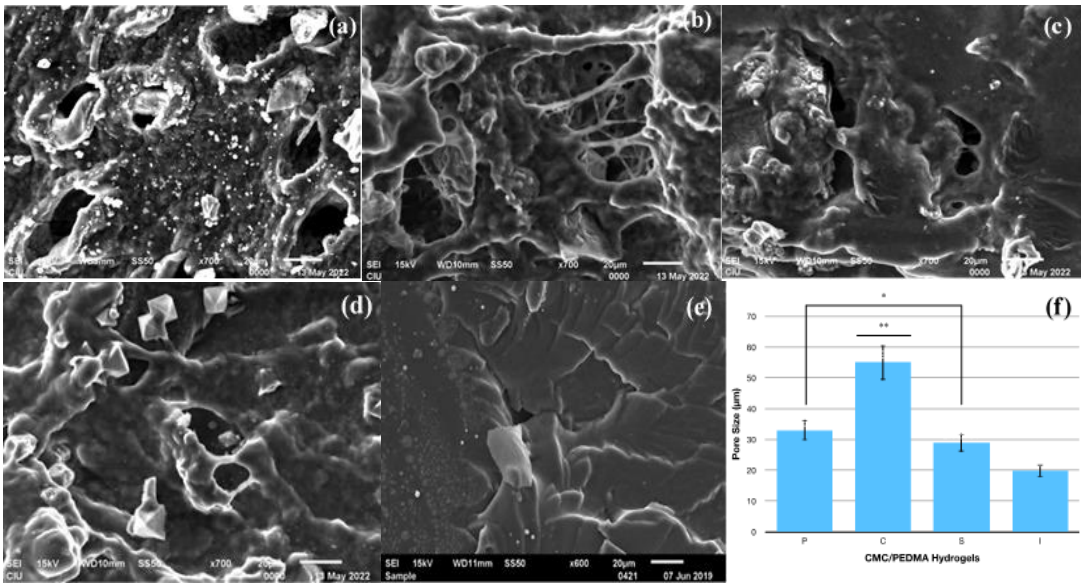

Figure 4. SEM results of (a) P, (b) C, (c) S, (d) I, (e) PEGDMA without CMC and (f) pore size distribution of /CMCPEGDMA hydrogels.

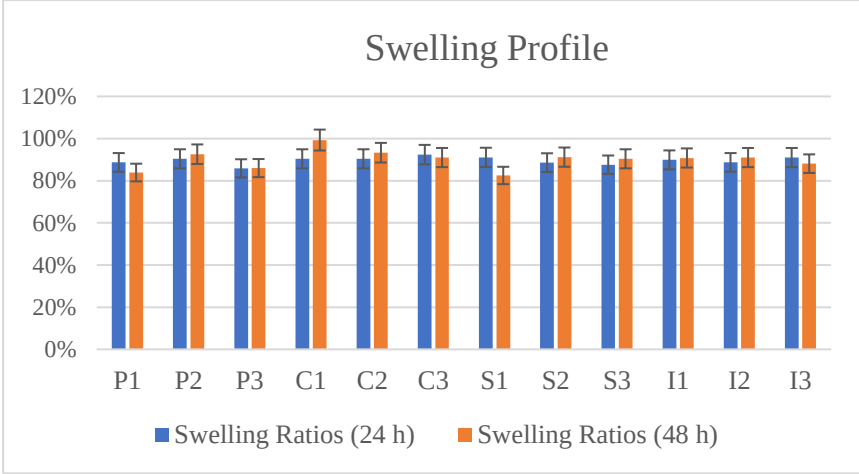

Figure 5. Swelling results of CMC/PEGDMA hydrogels with different ratios, (n=3).

Swelling Results

Swelling profile of the hydrogels is represented on Figure 5. The results were observed as a function of time in PBS over 48 h. Four ratios were compared with three different variations. Swelling profiles for all combination methods were compared with each other. P polymer blend achieved 90.4% over 24 h and 92.6% peak over 48 h while C polymer blend achieved up to 92.4% over 24 h and 99.3% peak over 48 h which recorded the highest swelling ratio out of the four polymer blends. Moreover, S polymer achieved 91.1% over 24 h and 91.2% peak over 48 h, and I polymer blend achieved 91.0% peak over 24 h and 48 h. No significant difference was observed in between the four polymer blends but in conclusion, C polymer blend achieved the highest swelling behavior. Zhang *et al.* [22] and Burke *et al.* [9] studied high-water content and resilience of PEG-based hydrogels to compare the swelling equilibrium of PEG-based hydrogels and their mechanical properties. Zhang and co-workers [22] observed a similar result with 97.5% and 99.4% swelling profile with different PEG ratios whereas, Burke and co-workers [9] found around 40% swelling for their biodegradable PEGDMA hydrogels.

CONCLUSION

An exceptional design methodology for the fabrication of CMC/PEG hydrogels was examined. SEM, FTIR and swelling were employed in the characterization of the CMC/PEG hydrogels. The hydrogels performed exceptionally in terms of moisture absorption and retention and have therefore proven to be compatible in regard to biomedical implementations including wound dressings.

Significant swelling ratios observed over 99.3% ratio. P (1/2/3) and C (1/2/3) showed the best swelling and binding properties. Future work aims to focus on cytotoxicity and anti-cancer studies of the hydrogel samples.

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