Radiation synthesized acrylamide hydrogel: Preparation, characterization and usability as biomaterial

Radyasyonla sentezlenen akrilamid hidrojeli: Hazırlama, karakterizasyon ve biyomateryal olarak kullanılabilirliği

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Research Article

ABSTRACT

Acrylamide hydrogel was prepared by γ-irradiating ofthe aqueous solution of acrylamide monomer with 4.65 kGy γ-rays. Spectroscopic, thermal and mechanical properties, swelling properties, diffusional behavior of water, diffusion coefficients and network properties of AAm hydrogel are examined.

In vitro swelling and in vivo biocompatibility of acrylamide hydrogel were investigated. The swellings of AAm hydrogelare investigated in distilled water, human serum and some simulated physiological fluids such as phosphate buffer at pH 7.4, glycine-HCl buffer at pH 1.1 physiological saline solution. For the analysis of human sera biocompatibility, acrylamide hydrogel was incubated in 10 different human sera for 24 hours and its biocompatibility with some biochemical parameters have been investigated. No significant difference in values before and after the test procedures has been found. AAm hydrogel was subcutaneously implanted in rats for up to 10 weeks and the tissue response to these implants was studied. Histological analysis indicated that tissue reaction at the implant site progressed from an initial acute inflammatory response characterized. No necrosis, tumorigenesis or infection was observed at the implant site up to 10 weeks. In vivo studies indicated that the radiation induced acrylamide hydrogel was found to be well-tolerated, non-toxic and highly biocompatible.

Key Words
Acrylamide, hydrogel, biomaterial, biocompatibility.

ÖZET

Akrilamidin sulu çözeltisi 4.65 kGy dozunda γ-ışınları ışınlanarak akrilamid (AAm) hidrojeli hazırlandı. AAm hidrojelinin spektroskopik, ısıl, mekanik özellikleri, şişme özellikleri, difüzyon davranışları, difüzyon katsayıları ve ağ özellikleri bulunmuştur.

Amm hidrojelininin vitro şişmesi damıtık su, insan serumu ve fosfat tamponu (pH 7.4), fizyolojik serum çözeltisi, glisin - HCl tamponu (pH 1.1) gibi bazı yapay fizyolojik sıvılarda incelenmiştir. İnsan serumunun bazı biyokimyasal parametreleri ile akrilamid hidrojelinin in vitro biyouyumluluk analizi için, AAm hidrojeli ile 10 farklı insan serumu 24 saat etkileştirilmiştir. Denemeler öncesi ve sonrası biyokimyasal parametrelerinin sayısal değerleri açısından anlamlı bir fark bulunmamıştır. AAm hidrojelinin in vivo biyouyumluluğu için, AAm hidrojeli sıçanları karın derialtı dokusuna yerleştirildi. Bu implantların doku yanıtı 10 hafta boyunca incelendi. İmplant bölgesinde nekroz, tümörogenez ya da enfeksiyon 10 hafta boyunca gözlenmemiştir. İn vivo çalışmalar, radyasyonla sentezlenen akrilamid hidrojelinin iyi tolere edilebildiği, toksik olmadığı ve biyouyumlu olduğu bulunmuştur.

Anahtar Kelimeler
Akrilamid, hidrojel, biyomateryal, biyouyumluluk.

INTRODUCTION

Some polymeric biomaterials such as hydrogels are made of water-soluble molecules, connected usually by covalent bonds, forming a three-dimensional insoluble network. The space between chains is accessible for diffusion of solutes and this space is controllable by the level of cross-linked (connected) molecules. They usually show good biocompatibility in contact with blood, body fluids, and tissues. Therefore, they are very often used as biomaterials for medical purposes, for instance contact lenses, coating of catheters, etc.

Biomaterials are defined as materials that can be interfaced with biological systems in order to evaluate, treat, augment, or replace any tissue, organ, or function of the body. The clinical application of a biomaterial should not cause any adverse reaction in the organism and should not endanger the life of the patient; any material to be used as part of a biomaterial device has to be biocompatible. The definition of biocompatibility includes that the material has to be nontoxic, non-allergenic, noncarcinogenic, and non-mutagenic, and that it does not influence the fertility of a given patient [1-12].

Hydrogels can be synthesized by accomplishing crosslinking via γ-irradiation [12-14]. However, little work is done on the biomedical applications of the hydrogels prepared by crosslinking of a homo-orcopolymer in solution with γ-irradiation. It is well known that the presence of an initiatorand a crosslinking agent affects the macromolecular structure and phase behavior of hydrophilic polymers in solution and contributes to inhomogeneity of the network structure. It is argued that more homogeneous network structures can be synthesized, if crosslinking is accomplished with γ-irradiation in the absence of an initiator and a crosslinking agent. The structural homogeneity of the network affects the swelling behavior and mechanical properties that improved the biological response of materials and subsequently the performance of many medical devices.

Thus, looking to the significant consequences of biocompatibility of biomaterials, we, in the present study, are reporting the results on the biocompatibility with the polymeric hydrogels prepared with acrylamide (AAm) viaradiation technique [15-21]. The selection of AAm as a hydrophilic monomer for synthesizinghydrogel rests upon the fact that it has low cost, water soluble, neutral and biocompatible,and has been extensively employed in biotechnical and biomedical fields.

The aim of the work is the use of acrylamide (AAm) hydrogel, with capacity of absorbing high water content in biocompatibility

Experimental

Preparation of AAm hydrogel

In this study, monomer of acrylamide (AAm) was purchased from BDH (Poole, UK). For the preparation of acrylamide hydrogels, acrylamide weighing 1 g was dissolved in 1 ml of distilled water. These solutions were placed in polyvinyl chloride straws of 3 mm diameter and irradiated at 4.65 kGy in air at ambient temperature in a ^{60}Co Gammacell 220 type γ irradiator at a fixed dose rate of 0.72 kGy h^{-1}. The dose rate was determined by the conventional Fricke dosimeter. Fresh hydrogel rods were cut into pieces of 3-4 mm length. They were washed with distilled water, dried in air and vacuum, and stored.

Spectroscopic, thermal and mechanical analyses of AAm hydrogel

For spectroscopic characterization of the dried polymer, a photoacoustic unit of a Nicolet 520 model Fourier Transform Infrared Spectrophotometer was used, because the dried polymer is too hard for the preparation of KBr disks.

The mechanical tests were performed on freshly prepared hydrogels by usingan Instron 1011 electronic mechanical testing equipment. For tests, 1 cm length of fresh cylindrical hydrogels was elongated with 50 mm min^{-1} elongation in one direction.

To find the glass transition temperature of the

hydrogel, differential scanning calorimeter (DSC) thermogram of the hydrogel was taken from using a Dupont 9900 thermal analyzer. About 10 mg of dry hydrogel sample was heated at 10°Cmin^{-1} under dynamic nitrogen atmosphere.

Dynamic thermal analysis was carried out with a Dupont 990 thermal analyzer. Thermogravimetric analysis was performed with 10 mg samples under nitrogen atmosphere with a nominal gas flow rate of 5 mL. Experiments were made at a heatingrate of 10°C min^{-1} until 600°C.

Swelling study of AAm hydrogel

The swelling of dried hydrogels was followed by immersion in doubly distilled water at 25 ± 1°C in the water bath. The water absorbed was determined by weighing the samples, after wiping, at various time intervals. The radius of cylindrical gel was measured by a micrometer.

In vitro swelling studies

The swelling nature of AAm hydrogel in distilled water, human sera, physiological saline (0.89% NaCl), isoosmotic phosphate buffer at pH 7.4 and simulated gastric fluid at pH 1.1, (gylicine-HCl buffer) was studied at 37 ±0.1°C. Swollen gels removed from the water-thermostated bath at regular intervals were dried superficially with filter paper, weighed, and placed in the same bath.

Cytotoxity of AAm monomer and hydrogel

A mouse connective tissue fibroblast cell line, L929 (ATCC cell line, NCTC clone 929) was cultured in Dulbecco's minimum Eagle Medium (DMEM) (Gibco, Eggenstein, Germany) supplemented with 10% fetal calf serum (Gibco, Germany) and 2 mM glutamine. No antibiotics were added to the cell culture medium. The cell line was cultivated in an incubator at 37°C and 5% CO_2, until cell monolayers attained confluence, which occurred after 7 days. Assays were always performed in the exponential growth phase of the cells.

The overall morphology of the cultures following biomaterial application was monitored throughout the experimental procedure using a phase microscope. Additionally, the individual morphology of the culture cells, as well as the presence of both living and dead cells were analyzed.

The photomicrographs of cell cultures were obtained using a phase inverted microscope (TMS, Nikon, Tokyo, Japan).

A total of 1x10^4 cells were plated in 35 mm culture petri dishes. Three days later, the experimental culture medium was replaced with the saline solution (SS, 0.89% saline) containing testing solution (50 mg mL^{-1}acrylamide monomer solution in SS) or hydrogel, while the control cultures received only SS. The media inside the dishes were discarded and replaced by the solutions diluted in SS. After 1, 3, 6, 12 and 24 h, the number of cells was determined by counting the viable cells in a haemocytometer using the trypan blue dye exclusion assay. For each period, cells from four dishes of each group were harvested using 0.25% trypsin solution, and then those cells in suspension were counted.

In vitro blood compatibility studies

10 different samples of human blood were coagulated, and then human sera were separated by centrifuging at 3000 g. A small volume of human sera was incubated +25°C as control groups.

AAm hydrogel was incubated in 10 different human sera for 24 hours at 25°C as test groups and their biocompatibility with biochemical parameters in human serum such as postprandial blood glucose, triglycerides, cholesterol, blood urea nitrogen, creatine, total protein, albumin, alkaline phosphate, alanine trans aminase, aspartat trans aminase, direct and indirect bilirubin, chlorine, sodium, potassium, phosphorus and calcium have been investigated. Biochemical analyses of control and test groups of human sera were made by TechniconRA 1000 model auto analyzer.

In vivo biocompatibility studies

For implantation study, the animal model used for evaluating the biocompatibility of AAm hydrogels was Wistar Albino rats, weighing 150-280 g. Fifty adult male rats were maintained on a standard diet and water.

Radiation induced AAm hydrogels were sterilized by UV-rays for one day before implantation. Rats were anaesthetized with xylazin (Rampun-Bayer) and ketamin (Parke Davis Ketalar) and prepared for surgery by shaving their abdominal field and then scrubbing with alcohol solution. The dry hydrogels were implanted subcutaneously in the abdominal field of the rats and the incisions were sutured. About 10 mg hydrogel was implanted for each rat at each time point.

Histological analysis

The five rats for each time point were sacrificed periodically at 1, 2, 4, 6 and 10 weeks post-implantation. The surrounded tissue of AAm hydrogels were excised and fixed in 10% buffered formalin. All tissues selected for optical microscopic studies were embedded in paraffin, sectioned at 7 μm thickness. The sections were stained either in Haematoxylin/Eosin or Mallory-Azan stain. Photomicrographs of the stained sections were taken using a Carl Zeiss Jena MET 2 optical microscope (Germany) fitted with a microphotographic attachment.

The connective tissue capsules surrounding the implants were examined for capsule thickness. The capsule thickness was measured in the optical microscope using a micrometer scale.

Results

Preparation and characterization of AAm hydrogel

In this study ionizing radiation processing is used for the preparation of acrylamide and acrylamide hydrogels. When monomers of acrylamide have been irradiated with ionization rays such as γ-rays, free radicals are generated in the aqueous solutions. Random reactions of these radicals with the monomers lead to the formation of polymer of acrylamide. When the irradiation dose has been increased beyond the certain value the polymer chains crosslink and gel is then obtained. The oxygen of air does not affect the polymerization of acrylamide [17-21]. A schematic presentation of possible polymerization reaction between acrylamide monomers is shown in Scheme 1.

It has been reported that gelation of polyacrylamide needs 2.00 kGy of γ-rays irradiation doses at ambient temperature [17-21], so 2.00 kGy of γ-ray irradiation doses were used for the preparation of the hydrogels. The radiation technique is a sterilization technique used in many applications. During polymerization and crosslinking reactions, all monomers reacted together by applied γ-ray irradiation. This process is used for the sterilization of hydrogel systems at the same time. There is no monomer (such as toxic acrylamide) at the end of the polymerization and crosslinking reaction between acrylamide
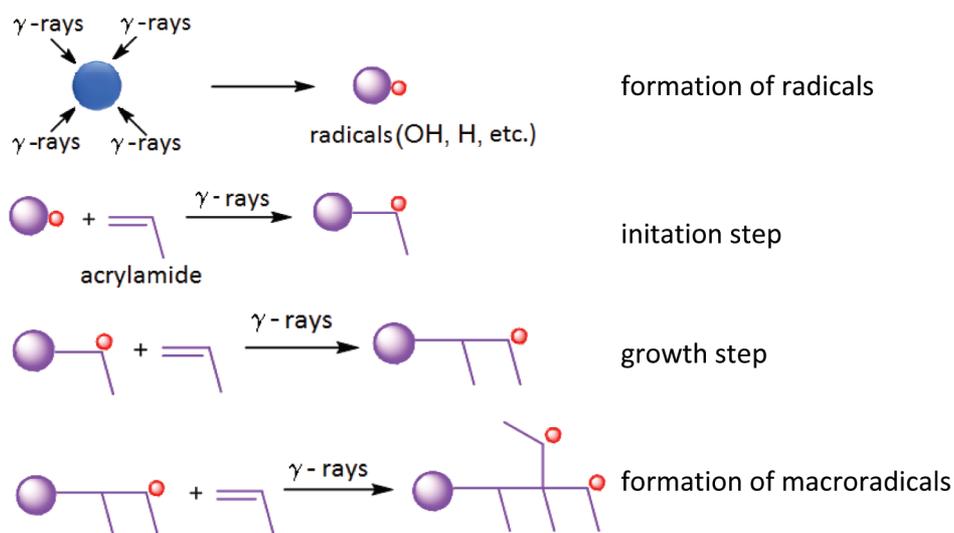

Scheme 1. Possible polymerization reaction of AAm.

and maleic acid because 2.00 kGy is a sufficient dose for 100% gelatin [17-21].

Dried gels areglassy form andtoo hard, but swollen gels are very soft. The hydrogels areobtained in the form of cylinders.Upon swelling the hydrogels retained their shapes.

Spectroscopic Characterization

To understand binding of AAm hydrogels during polymerization, FTIR spectra of the hydrogelwas evaluated and ispresented in Figure 1.
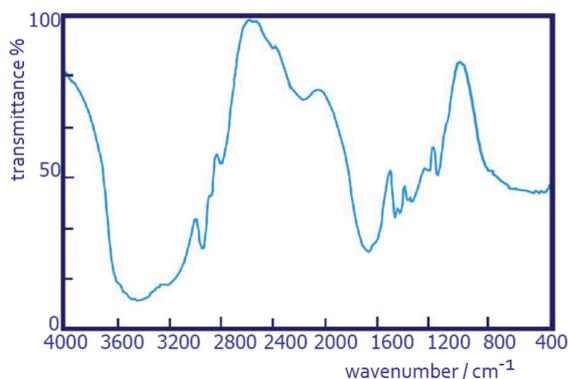
Figure 1. FTIR spectra of AAm hydrogels.

In the FTIR spectrum of the hydrogel, the bands at about 1700 and 3100-3500 cm^{-1} are important. The bands at about 1700 cm^{-1} could be attributed to a shift in stretching vibration associated with hydrogen that is bonded directly to an overtone of the strong carbonyl absorption. The sharp band at 1650 cm^{-1} is the carbonyl group and related to amide groups. The much broader absorption bands in the regions of 3100 and 3500 cm^{-1} are O-H and N-H bands and are related to "polymeric" bands. The broad band at 3500 cm^{-1} is a characteristic band of primary amine. It is thought that the weak bands at 1000 and 1200 cm^{-1} are C-N bands and the weak peaks at 2850 and 1400 cm^{-1} show $-CH_2-$ groups on the chains [17].

It can be seen in Figure 1 that polymerization of AAm hydrogel has advanced from double bonds of acrylamide, because N-H bandis shown in great amounts on the crosslinked structure. Specifically, primary amide band at 3100-3500 cm^{-1} is important for the examining of binding and crosslinking of acrylamide.

Figure 1 shows that there is random polymerization between acrylamide monomers. If the bands at 3100-3500 cm^{-1} had a sharp and narrow band, it is said that the reaction of polymerization between acrylamide monomers also had carried out on the amide group of acrylamide.

Thermal Characterization
DSC Analysis

To examine the thermal properties of AAm hydrogel, it was investigated whether the glass transition temperature (T_g) of the hydrogelschanges when crosslinking has occurred. For this purpose, DSC thermograms of the hydrogels were examined and are shown in Figure 2.
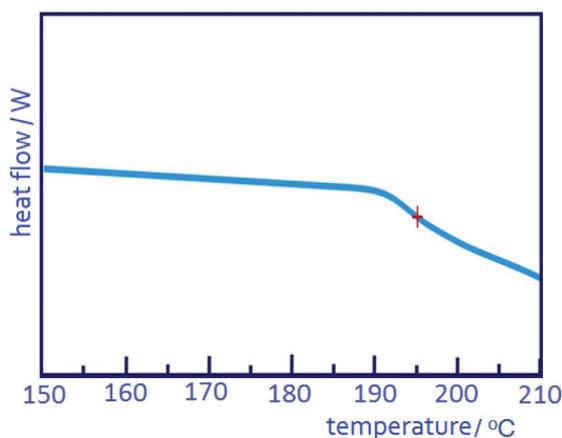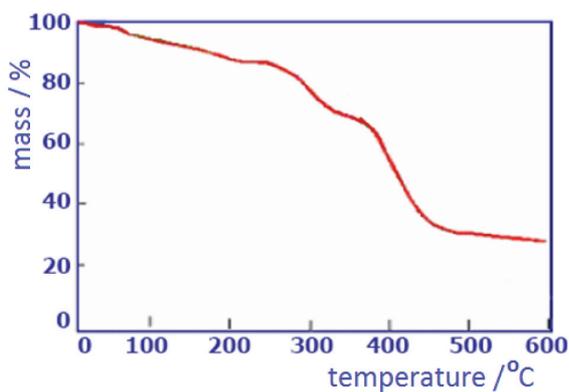

Figure 2. DSC thermogram of AAm hydrogels.

In the thermograms shown in Figure 2, it can be seen that the curves have not returned to base line after glass transition temperatures. So it can be said that the hydrogel systems have not any energy of relaxation. The glass transition temperature of cross-linked AAm hydrogel is 195°C, while T_g of commercial PAAm is 164°C [17]. It can be seen that T_g of crosslinked PAAm hydrogel is higher than commercial PAAm. The process of crosslinking caused this increase at the glass transition temperatures of PAAm. To understsand the transition to the elastic state, the crosslinking density and the number of elastically effective chains of hydrogels are higher than normal and T_g is higher than the uncrosslinked case.

Thermogravimetric Analysis of Hydrogels

Figure 3 shows the thermograms for the dried sample of AAm hydrogel in nitrogen atmosphere a heated at $10^\circ C$ min^{-1} from $0^\circ C$ to $600^\circ C$. There are three types of the decomposition region on the thermograms of the hydrogels. It is reported that the first region (0-$220^\circ C$) correspond to loss bound water, in the second region (220-$340^\circ C$) to loss of NH_3 by imidization (intra- and intermolecular), and H_2O by dehydration. The third region ($>340^\circ C$) represents substantial mass loss, and is normally attributed to main chain break-down.

Figure 3. TG thermogram of AAm hydrogels.

In order to determine the thermal stability of AAm hydrogel, the temperatures for the maximum mass loss (T_{max}/ $^\circ C$) were found directly from its thermogram given in Figure 3. Heating hydrogel at high temperatures than $408^\circ C$ result in rapid decomposition to carbon dioxide and volatile hydrocarbons.

The other thermal degradation parameters of the hydrogel such as initial decomposition temperature (T_i), half-temperature ($T_{1/2}$), final decomposition temperature (T_f), maximum rate (r_{max}), maximum temperature at maximum rate (T_{max}), mass loss at maximum rate (C_{max}), mass loss at final temperature (C_f) were tabulated in Table 1.
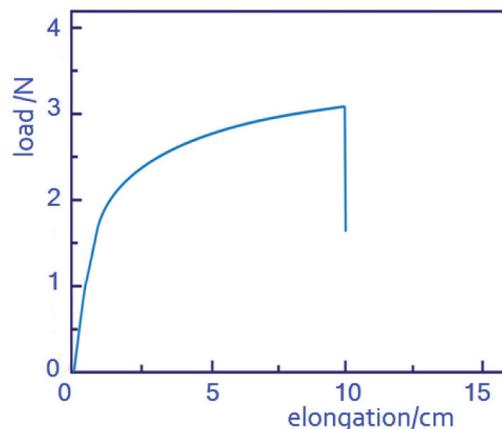
Table 1. Thermal properties of the hydrogels.

T_i/$^\circ C$	T_f/$^\circ C$	$T_{1/2}$/$^\circ C$	T_{max}/$^\circ C$	r_{max}/% min^{-1}	C_{max}/%	C_f/%
125	594	412	282	0.366	64.3	28.8

Mechanical Characterization

To find the mechanical stability of fresh AAm hydrogel, mechanical tests were applied to 1 cm length of the hydrogels. At the end of the mechanical tests, some parameters such as strain percentage at break, stress at break, Young's modulus, energy to break and tensile energy absorption were found [17, 18]. The curves of load-elongation of the hydrogels are shown in Figure 4, and some parameters about mechanical properties are listed in Table 2.

Figure 4. Load-elongation curve of AAm hydrogels.

Table 2. Mechanical properties of the hydrogel.

| Strain at break/%	Stress at break/kPa	Young's modulus/ kPa	Energy to break point / J	Tensile energy of absorption/N mm^{-1}
991	305	519	0.96	109

Swelling Studies

A fundamental relationship exists between the swelling of a polymer in a solvent and the natures of the polymer and the solvent [19]. The percentage swelling S% is calculated from the following relation [17-20].

$$S\% = \frac{m_t - m_0}{m_0} \times 100 \qquad (1)$$

where m_0 is the mass of dry gel at time 0 and m_t is the mass of swollen gel at time t.

The water intakes of initially dry hydrogels are followed for a long time. Swelling curve of the AAm hydrogel is shown in Figure 5.
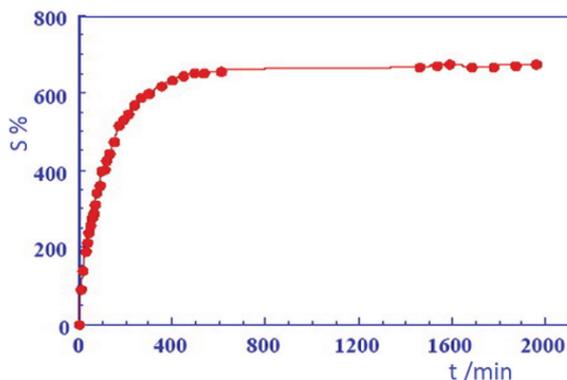
Figure 5. Swelling-time curve of AAm hydrogels.

If Figure 5 is investigated, it can be shown that S% increases over time, but then it shows constant percentage swelling. This value may be named equilibrium percentage swelling (S_{eq}%). The equilibrium percentage swelling of the AAm hydrogel was used at the calculation of some characterization parameters.

Analysis of the mechanisms of water diffusion into swellable polymeric systems has received considerable attention in recent years, because of important applications of polymers in biomedical, pharmaceutical, environmental, and agricultural engineering [21-24]. The following equation is used to determine the nature of diffusion of water into hydrogels.

$$F = kt^n \qquad (4)$$

where F is the fractional uptake at time t, k is a constant incorporating characteristic of the macromolecular network system and the penetrant, and n is the diffusional exponent, which is indicative of the transport mechanism. Equation 4 is valid for the first 60% of the fractional uptake. Fickian diffusion and Case II transport are defined by n values of 0.5 and 1, respectively. Anomalous transport behavior (non-Fickian diffusion) is intermediate between Fickian and Case II. That is reflected by n between 1/2 and 1 [21-24].

For the hydrogel, $\ln F$ vs. $\ln t$ graph is plotted and n exponent and k parameter were calculated from the slope and intercept of the line, respectively.

The number to determine the type of diffusion (n) is found to be over 0.50. Hence the diffusion of water into the hydrogels has taken a *non-Fickian* character [21,25]. This is generally explained as a consequence of the slow relaxation rate of the polymer matrix.

The diffusion coefficient of the cylindrical AAm hydrogel is calculated from the following relations:

$$D^n = \left(\frac{k}{4}\right)(\pi r^2)^n$$

where D is in cm^{-2} sec^{-1}, t in sec and r is the radius of cylindrical polymer sample. The values of diffusion parameters determined for the hydrogels are listed in Table 3.

Table 3. The values of diffusion parameters of AAm hydrogel.

k	n	D x 10⁶ /cm² s⁻¹
0.052	0.62	1.73

Network studies

An important structural parameter characterizing crosslinked polymers is M_c, the average molar mass between crosslinks which is directly related to the crosslink density. The magnitude of M_c significantly affects the physical and mechanical properties of crosslinked polymers and its determination has great practical significance. Equilibrium swelling is widely used to determine M_c. Early research by Flory and Rehner laid the foundations for the analysis of equilibrium swelling. According to the theory of Flory and Rehner, for a network:

$$M_c = -V_1 d_p \frac{v_s^{1/3} - v_s/2}{[\ln(1 - v_s) + \chi v_s - v_s]^2}$$

where V_1 is the molar volume (mL mol⁻¹), d_p is the polymer density (g mL⁻¹), v_s is the volume fraction of polymer in the swollen gel, χ is the Flory-Huggins interaction parameter between solvent and polymer [26].

The swelling ratio (Q) equals to $1/V_s$ Here the crosslink density, q, is defined as the mole fraction of crosslinked units [26].

$$q = \frac{M_0}{M_C}$$

where, M_0 is the molar mass of the repeating unit.

The polymer/solvent interaction parameter, χ, was taken to be 0.494, by analogy to those other acrylamide polymers. The density of the polymer was taken as 1.302 g cm^{-3}. Molar volume of water was taken as 18 cm^3 mol^{-1}. Finally, the swelling of AAm hydrogel was considered ideal. M_c and q of AAm hydrogel are calculated and listed in Table 4.

Another important parameter of networks is gel poresize or mesh size (ε) [26,27]. For determining this parameter, the end-to-end distance in the freely jointed state is determined as

$$r_f = l\sqrt{N}$$

where l = 154 pm and the number of links, N =$\lambda M_c/M_o$ and λ=2. The end-to-end distance in the unperturbed state is calculated through the characteristic ratio C_n=6.32

$$r_0^2 = C_n r_f^2$$

$$\varepsilon = v_s^{-1/3} r_0$$

The porosity (P%) of the hydrogel is

$$P = \frac{V_d}{1 - V_d} \times 100$$

where, V_d is the volume ratio of water imbibed to the gel phase in the equilibrium state.

The values of the number of repeating units between crosslinks, N, the mesh size, ε (nm) and porosity (P) are shown in Table 4.

Table 4. The network values of AAm hydrogel.

M_c	q	N	ε / nm	P%
17 000	0.00417	240	15.2	89.65

### In vitro swelling of the hydrogels in the simulated physiologicalbody fluids

In this stage of the study, the swelling of the hydrogel in the simulated physiological bodyfluids was investigated [24,28-30]. The phosphate buffer at pH 7.4 (pH of cell fluid, plasma, edema fluid, synovial fluid, cerebrospinal fluid, aqueous humour, tears, gastric mucus, and jejunal fluid), glycine-HCl buffer at pH 1.1 (pH of gastric juice), human sera, physiological saline and distilled water intake of initially dry hydrogels were followed for a long time until equilibrium.

The fluid absorbed by the gel network is quantitatively represented by the EFC (equilibrium fluids content), where:

$$EFC\% = \frac{mass\ of\ fluid\ in\ the\ gel}{mass\ of\ gel} \times 100$$

EFCs of the hydrogel for all physiologically fluids were calculated. The values of EFC% of thehydrogel in simulated body fluids are shown in Figure 6.
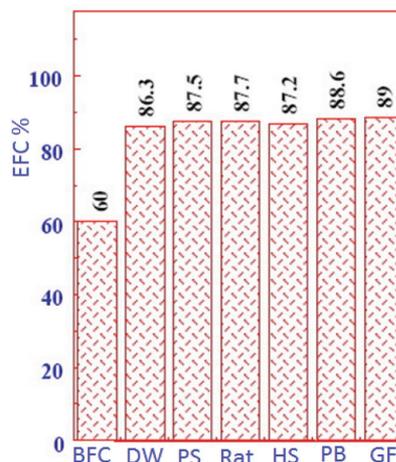
Figure 6. The values of EFC% of the hydrogel, BFC; body fluid content, DW; distilled water, PS; physiological saline, HS; human sera, PB; phosphate buffer, GF; glycine-HCl buffer.

All EFC values of the hydrogel were greater than the percent water content values of thebody about 60%. Thus, the AAm hydrogel was exhibit similarity of the fluid contents with those of living tissues.

In vitro blood biocompatibility

In the second stage of this study, the biocompatibility of the hydrogels was investigated against some biochemical parameters of human sera at 25°C. The mean and standard deviation
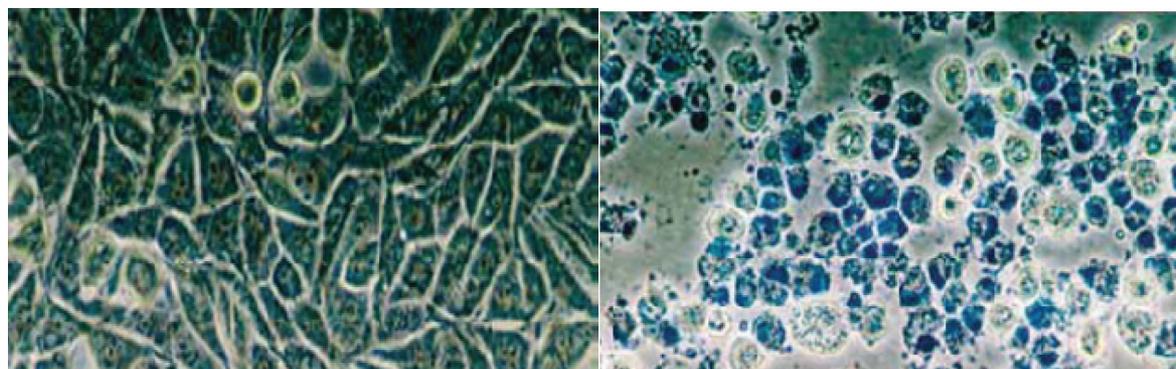
Table 5. Biochemical parameters of human sera.

Parameter	Normal value	control	AAm hydrogel
glucose	70-110 mg dL^{-1}	87.0 ± 8.2	91.0 ± 6.1
triglyceride	40-160 mg dL^{-1}	127.3 ± 24.6	127.0 ± 25.8
cholesterol	135-350 mg dL^{-1}	158.6±10.9	160.6±14.3
blood urea nitrogen	8-25 mg dL^{-1}	14.8±1.27	15.2±4.56
creatine	0.8-1.6 mg dL^{-1}	0.98±0.14	1.06±0.17
total protein	6.0-8.4 mg dL^{-1}	6.52±0.15	6.72±0.15
albumin	3.5-5.6 mg dL^{-1}	4.02±0.15	3.88±0.15
alkaline phosphate	35-125 U	53.6±13.11	54.5±12.27
alanine trans aminase	7-56 U	14.6±2.12	16.0 ±2.63
aspartate trans aminase	5-40 U	16.2±5.33	15.2±3.19
directbilirubin	0.0-0.3 mg dL^{-1}	0.12±0.04	0.12±0.04
indirect bilirubin	0.1-1.1 mg dL^{-1}	0.45±0.05	0.35±0.09
chlorine	95-107 meq L^{-1}	98.5±2.17	98.8±2.30
sodium	137-146 meq L^{-1}	142.7±1.42	142.8±0.92
potassium	3.5-5.5 meq L^{-1}	4.80±0.28	4.68±036
phosphorus	8.5-10.8 mg dL^{-1}	9.40±0.39	9.47±0.28
calcium	2.5-4.5 mg dL^{-1}	3.60±0.41	3.60±0.32

values of control and test groups for biochemical parameters of human sera are listed in Table 5.

Table 5 shows that the values of means of control and test groups are in the range of normal values and there is no significant difference in values before and after contacting these sera with the hydrogels. On the other hand, Student's t-test is applied to control and test groups. No significant difference in values of biochemical parameters was found.

Cytotoxic effects of AAm monomer and hydrogel

Biomaterial suitable for a biomedical application must be biocompatible at least on its surface. We investigated acrylamide used in polymeric biomaterial production had cytotoxic effects [12, 31].

In the photograph by phase inverted microscopy shown in Figure 7, structural defects and deaths were formed in fibroblast cells interacted with AAm monomer.

The number of viable cells (N_{VC}) was obtained by the following mathematical equation:

$$N_{VC} = \frac{UC \times D \times 10^4}{nSQ}$$

where UC, unstained cell count (viable cells); D, the dilution of the cell suspension; and nSQ, number of counted squares of the hemocytometer.

The viability percentage of the cell population (V%) of each petri dish was obtained by applying the following mathematical equation:

a	b

Figure 7. The viability of fibroblast cells, a; control group, b; test group.

$$V\% = \frac{N_{VC}}{N_{TC}} \times 100$$

where N_{VC}, unstained cell count (viable cells); and N_{TC}, total cell count (stained + unstained cells).

The viability curve was plotted for AAm monomer, and shown Figure 8.
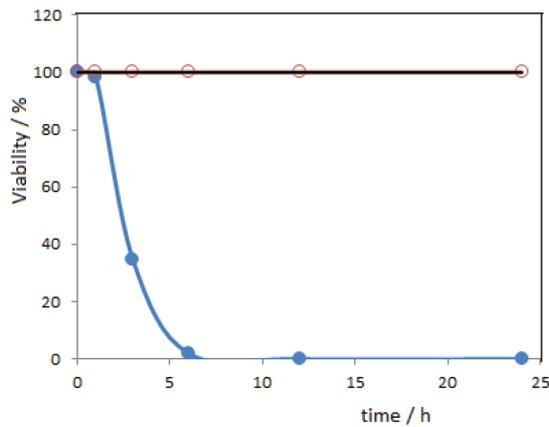

Figure 8. The viability of cell population curve, red; control group, blue; test group.

The viability of cultured fibroblastic cell lines

following the monomer application was found to be decreased in all time intervals (Figure 8).

In the photograph byphase inverted microscopy shown in Figure 9, the viability ofthe fibroblast cells interacting with AAm hydrogel was continued to 10 hours. There is no significant difference in the viability of cultured fibroblastic cell lines following the hydrogel application after contacting with the hydrogel.
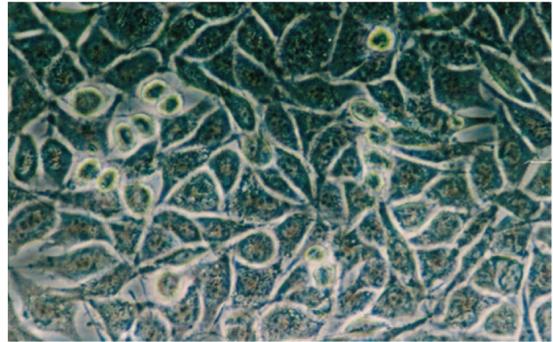
Figure 9. The viability of fibroblast cells interacted with AAm hydrogel.

In vivo tissue biocompatibility

1. week   4. week

6. week   10. week
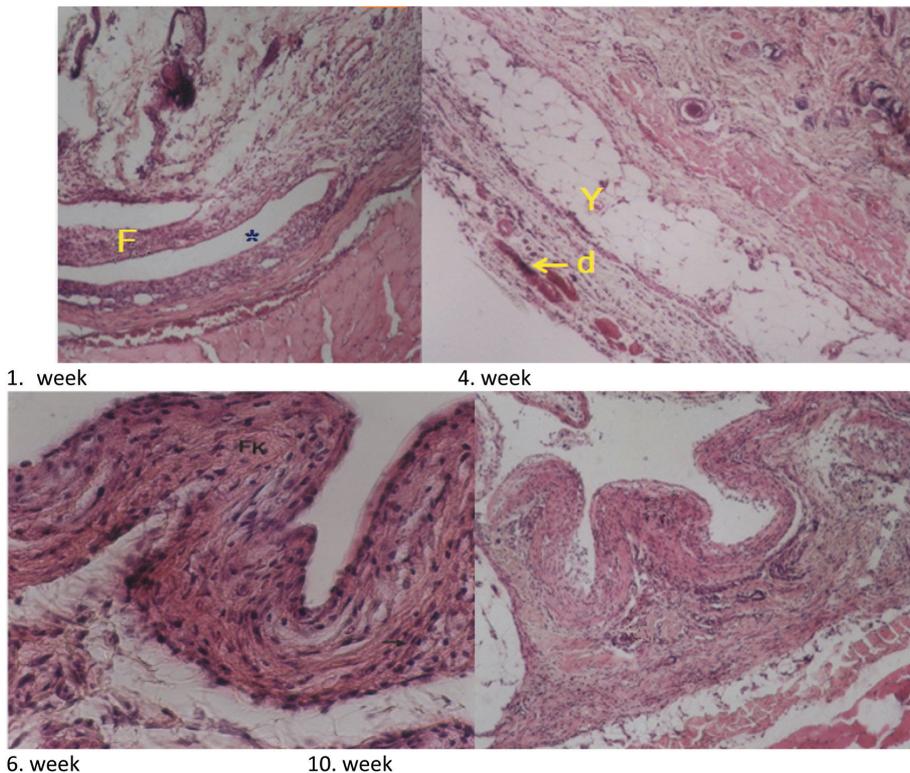
Figure 10. Light microphotographs of post-implantation of AAm hydrogel. Original magnificiations: x40 (Haematoxylin/ Eosin).

In this part, AAm hydrogel with capacity of absorbing high water content in biocompatibility with subcutaneous tissues of rats were examined [32,33]. After one week implantation, no pathology such as necrosis, tumorigenesis or infection was observed in the excised tissue surrounding the hydrogeland in skin, superficial fascia and muscle tissues in distant sites. After 2–4 weeks, thin fibrous capsules were thickened. A few macrophage and lymphocyte were observed in these fibrous capsules consisting of fibroblasts, and a grouped mast cells and lymphocyte were observed between tissues and capsule in the some samples (Figure 10).

After 6-10 weeks, the adverse tissue reaction, giant cells and necrosis of cells, inflammatory reaction such as deposition of foamed macrophage were not observed in the implant site, however, it is observed to increase in the collagen fibrils due to proliferation and activation of fibroblasts (Figure 10).

The thicknesses of the fibrous capsules were measured in the optical microscope using a micrometer scale. The means of five measurements for each the sample and each time point were calculated. The thicknesses of fibrous capsules are gradually increased to 6 weeks, and then these values are becomed a constant value. The thickness of the fibrous capsules was measured in the optical microscope using a micrometer scale. The means of five measurements foreach the sample and each time point were calculated and shown in Figure 11.

The thicknesses of fibrous capsules are gradually increased to 6 weeks, and then these values are becomed a constant value.

On the other hand, Student's t test was applied to the all constant values of thickness of fibrous capsules of the hydrogels and no significant differences ($p > 0.05$) was found. These thicknesses of fibrous capsule indicated well within the critical tissue tolerance range. It was given by the some reporters that the threshold capsule thickness should not exceed 200–250 μm for an implanted biomaterial [34]. Our results clearly indicated that the capsule thickness of the excised tissue was well within

these stipulated threshold limits. On the basis of the findings we can conclude that the biological response against the tested hydrogels was very similar to the biocompatibility of very low swollen of poly(2-hydroxyethyl methacrylate) hydrogel, which considered as a biologically inert polymer [35] However, it is important that the swelling of acrylamide hydrogels are very high than the swelling of poly(2-hydroxyethyl methacrylate) hydrogels for the biomedical uses.
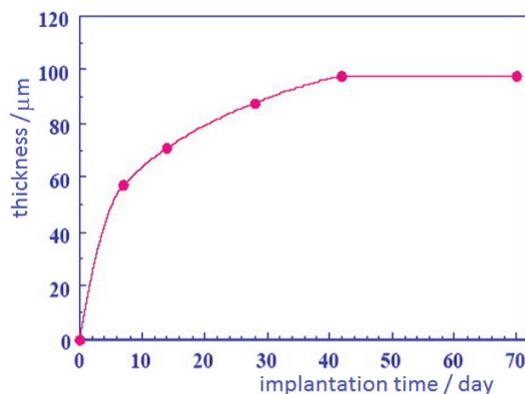
Figure 11. The thickness of the fibrous capsule-time curve of the hydrogel.

Discussion

The use of polymeric materials for medical purposes has a growing interest. Polymers have biomedical application fields such as tissue engineering, implantation of medical devices and artificial organs, prostheses, ophthalmology, dentistry, bone repair, and so on. There are several monomers used in biomaterial production. Polymeric materials suitable for biomedical applications must be biocompatible.

In this study, preparation, characterization, *in vitro* swelling behavior, diffusional properties, and *in vivo* biocompatibility of radiation induced acrylamide hydrogel was investigated.

The biocompatibility studies of AAm hydrogel clearly indicated good tissue tolerance for subcutaneous implantation up to 10 weeks. These histological findings indicated that subcutaneous implantation of hydrogels in rat did not cause any necrosis, tumorigenesis or infection at the implant site during this period.

AAm hydrogel was well tolerated, nontoxic and highly biocompatible.

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