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Nuclear Instruments and Methods in Physics Research B 217 (2004) 281–292
NIM B
Beam Interactions with Materials & Atoms
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In vivo biocompatibility of radiation crosslinked acrylamide copolymers

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Received 7 April 2003; received in revised form 23 September 2003

Abstract

In vitro swelling and in vivo biocompatibility of radiation crosslinked acrylamide copolymers such as acrylamide/crotonic acid (AAm/CA) and acrylamide/itaconic acid (AAm/IA) were studied. The swelling kinetics of acrylamide copolymers were performed in distilled water, human serum and some simulated physiological fluids such as phosphate buffer, pH 7.4, glycine–HCl buffer, pH 1.1, physiological saline solution, and some swelling and diffusion parameters have been calculated. AAm/CA and AAm/IA hydrogels were subcutaneously implanted in rats for up to 10 weeks and the immediate short- and long-term tissue response to these implants were investigated. Histological analysis indicated that tissue reaction at the implant site progressed from an initial acute inflammatory response. No necrosis, tumorigenesis or infection was observed at the implant site up to 10 weeks. The radiation crosslinked AAm/CA and AAm/IA copolymers were found well tolerated, non-toxic and highly biocompatible. However, AAm/IA copolymer was not found to be compatible biomaterials, because one of the AAm/IA samples was disintegrated into small pieces in the rat.

Keywords: Radiation; Acrylamide; Biocompatibility; Biomaterial; Hydrogel

1. Introduction

Hydrogels are macromolecular networks that swell, but do not dissolve, in water. The ability of hydrogels to absorb water arise from hydrophilic functional groups attached to the polymeric backbone, while their resistance to dissolution arise from crosslinks between network chains. Hydrogel networks are useful for applications that require a material has good compatibility with aqueous fluids, yet will not dissolve. Such applications include biomaterials, controlled release devices and electrophoresis gels. Many properties of hydrogels make them suitable for biomedical applications that require contact with living tissue. The ability to absorb and retain aqueous media not only gives hydrogels a strong superficial

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doi:10.1016/j.nimb.2003.09.035

resemblance to living tissue, but also makes them permeable to small molecules such as oxygen, nutrients, and metabolites. The soft, rubbery consistency of swollen hydrogels minimizes frictional irritation felt by surrounding cells and tissue, while the low interfacial tension with aqueous fluids reduced protein adsorption and denaturation. Furthermore, hydrogels can be swollen and washed of undesirable by-products, residual initiators, and monomers, and may be fabricated in variety of shapes and geometries [1–5].

The hydrogels of both acrylamide and acrylamide based copolymers exhibit a very high capability to absorb water, permeable to oxygen and posses good biocompatibility [6]. Alternatively, crotonic or itaconic acid exhibit similarity with the acrylic derivatives, so it can be copolymerized with a large number of monomers, and it has carboxylic groups in its molecule which make it highly hydrophilic.

Hydrogels can be synthesized by accomplishing crosslinking via γ-irradiation [2,7]. However, little work is done on the biomedical applications of the hydrogels prepared by crosslinking of a homo- or copolymer in solution with γ-irradiation [8–11]. It is well known that the presence of an initiator and 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 [11]. Thus, looking to the significant consequences of biocompatibility of biomaterials, we, in the present study, are reporting the results on the biocompatibility with the copolymeric hydrogels prepared with acrylamide (AAm) and crotonic acid (CA) or itaconic acid (IA) via radiation technique. The selection of AAm as a hydrophilic monomer for synthesizing hydrogel rests upon the fact that it has low cost, water soluble, neutral and biocompatible, and has been extensively employed in biotechnical and biomedical fields [8–10]. On the other hand, CA monomer consists of single carboxyl group, while IA monomer is consisting of double carboxyl groups. These carboxylic acids could provide the different functional characteristics to acrylamide-based hydrogels. So, these monomers were selected for the preparation of the hydrogels and their biocompatibility studies.

2. Materials and method

2.1. Materials

All monomers were purchased from B.D.H. (Poole, UK). The samples of human sera were obtained from The Blood Bank in Cumhuriyet University, Turkey.

The suitable mass of CA or IA and, irradiation dose for radiation crosslinked AAm/CA or AAm/IA hydrogels were determined by considering previous experiments [12,13].

2.2. Preparation of the hydrogels

One gram of acrylamide (AAm) was dissolved in 1 mL of distilled water and 40 mg of crotonic (CA) or itaconic acid (IA) was added to this aqueous solution. These solutions were placed in PVC straws of 3 mm diameter and irradiated to 4.65 kGy in air at ambient temperature in a ^{60}Co Gammacell 220 type gamma irradiator source at a fixed dose rate of 12 Gy min^{-1}. Freshly obtained hydrogel rods were cut into pieces of 3–4 mm length. They were washed with distilled water and dried first in air and vacuum, and stored for further use [12,13].

2.3. In vitro swelling studies in simulated body fluids

The swellings of radiation crosslinked AAm/CA or AAm/IA copolymers in distilled water (DW), human sera (HS), and simulated body fluids such as urine (urea) (UR), physiological saline (0.89% NaCl solution) (PS), isoosmotic phosphate buffer in pH 7.4 (IP), simulated gastric fluid, pH 1.1 (glycine–HCl buffer) (GF) [14], and the aqueous solutions of KH_2PO_4 (PP) and KNO_3 (PN) (as the

sources of K ions) were studied at 37 ± 0.1 °C to determine the parameters of swelling and diffusion. Swollen gels, removed from the water-thermostated bath at regular intervals, were dried superficially with filter paper, weighed, and placed in the same bath. The radii of cylindrical gels were measured by a micrometer. The swelling degree, S, was calculated using the following relation [16]:

$$S = \frac{m_s(t) - m_0}{m_0}, \quad (1)$$

where m_0 is the mass of the dry gel at time 0 and $m_s(t)$ is the mass of the swollen gel at time t.

2.4. In vivo biocompatibility studies

2.4.1. Animals and implantation procedure

All procedures were approved and performed under the guidelines of the Animal Care and Use Committee of Cumhuriyet University, Medicine Faculty. The in vivo behavior of the hydrogels was evaluated by inserting radiation synthesized acrylamide based hydrogel cylinders into the abdominal subcutis of adult male Wistar Albino rats, weighing 150–280 g. Rats were maintained on a standard diet and water. The implants were placed in five separate surgical sessions and stayed in situ for periods of 1, 2, 4, 6 and 10 weeks. For every period of implantation, five rats with AAm/CA or AAm/IA hydrogels were used. In total, 50 implants ($2 \times 5 \times 5$) were evaluated.

Before insertion, the AAm/CA and AAm/IA hydrogels were sterilized by UV-rays for one day. Rats were anaesthetized by intravenous injections of ketamin (Parke Davis Ketalar) (90 mg/kg) and xylazin (Rampun–Bayer) (10 mg/kg). The abdominal field of the rats were shaved, depilated, washed with alcohol solution and disinfected with the iodine. The dry hydrogels were inserted 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. To reduce the post-operative infection risk, Mersol® was administered post-operatively. After surgery, the rats were housed in the separate cages and allowed to move unrestrictedly.

2.4.2. Histological evaluation

At the end of the implantation period, the animals were sacrificed. The skin was shaved and the implants with their surrounding tissue were excised immediately and fixed in 10 vol% buffered formalin. After dehydration, excess tissue was removed and the samples were embedded in paraffin. Histological sections of 7 μm thicknesses were prepared a sawing microtome, stained with Haematoxylin/Eosin (H/E) or Mallory–Azan (M–E) 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.

3. Results and discussion

3.1. Preparation of radiation crosslinked hydrogels

When monomers of AAm with CA or IA were irradiated in water with ionization rays such as γ-rays, free radicals from water and monomers are generated. Random reactions of these radicals with the monomers lead to the formation of copolymers of acrylamide. When irradiation dose was increased beyond a certain value, the polymer chains crosslink and then gel is obtained. It has been reported that gelation dose of polyacrylamide is 2.00 kGy at ambient temperature [15]. A total dose of 4.65 kGy is applied for the preparation of AAm copolymers. In dry state, hydrogels gels were hard, and glassy, in swollen state, gels were very soft. The hydrogels are obtained in the form of cylinders. Upon swelling the hydrogels retained their shapes.

3.2. In vitro swelling

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), GF at pH 1.1 (pH of gastric juice), HS, PS, UR, the aqueous solutions of KH_2PO_4

and KNO_3 as the sources of K^+ ions and distilled water intake of initially dry hydrogels were followed until reaching a constant swelling (equilibrium swelling, S_{eq}). Swelling kinetics defined by the change of S versus time. Swelling curves of AAm/CA and AAm/IA hydrogels are shown in Figs. 1(a) and (b), respectively.

Figs. 1(a) and (b) show that the swellings of radiation crosslinked AAm based hydrogels in simulated physiological fluids are different than in distilled water. This is expected due to different interaction parameters of physiological body fluids and AAm based hydrogels. Ions of physiological fluids interacted with the functional groups of hydrogels which are responsible for hydrogel swellings. Solvated ions of the fluids are caused to increase or decrease in the swelling degrees of ionogenic AAm based hydrogels. It is obvious that, ions of physiological fluids interacted with carboxyl groups of acids in AAm based hydrogels. It has been found that, AAm/CA and AAm/IA hydrogels in the simulated body fluids are swollen in the following order: UR > PP > DW > IP > PN > GF > PS > HS and UR > IP > DW > PP > PN > GF > PS > HS, respectively.

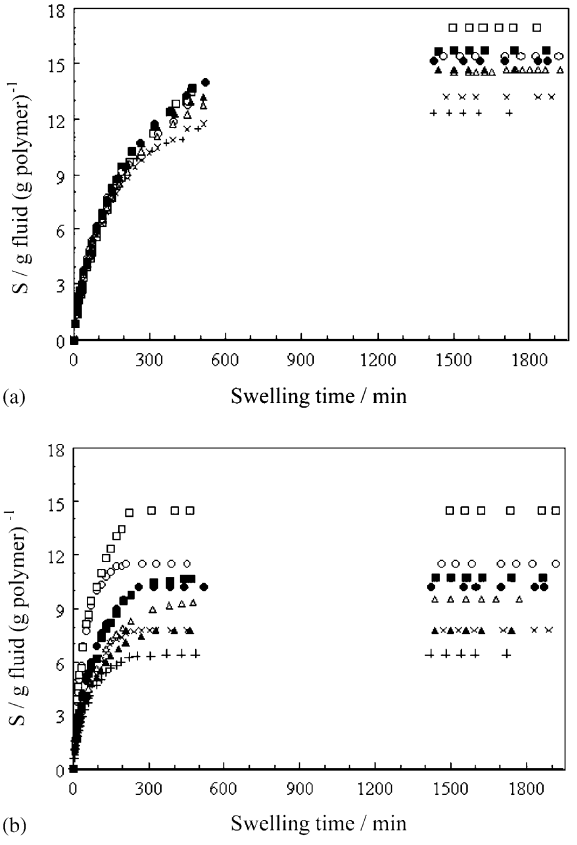

Fig. 1. Swelling curves of the hydrogels in the fluids: (a) AAm/CA; (b) AAm/IA. (□) UR; (■) PP; (○) DW; (●) IP; (△) PN; (▲) GF, (×) PS and (+) HS.

It can be expected that the medical use of the copolymeric acrylamide hydrogels would provide material with a broad range of swellings owing to the non-ionogenic, and ionogenic nature of the AAm based hydrogels [17].

The fluid absorbed by the gel is quantitatively represented by the equilibrium fluid content, EFC [18], where

$$\mathrm{EFC}\% = \frac{\text{mass of fluid in the gel}}{\text{mass of hydrogel}} \times 100. \qquad (2)$$

EFCs of the hydrogels for all physiologically fluids were calculated. The values of EFC% of the hydrogels are presented in Table 1. All EFC values of the hydrogels were greater than the percent water content values of the body about 60%. Thus, the radiation synthesized AAm hydrogels exhibit similarity with respect to the fluid contents of those of living tissues.

In order to examine the control mechanism of the swelling processes, several kinetic models are used to test experimental data. The large number and array of different chemical groups on the AAm chains (e.g. amide, carbonyl, carboxyl or hydroxyl) imply that there are many types of polymer–solvent interactions. It is probable that any kinetic is likely to be global. From a system design viewpoint, a lumped analysis of adsorption rates is thus sufficient for the practical operation.

A simple kinetic analysis is the second-order equation in the form of [19]

$$\frac{dS}{dt} = k_S(S_{max} - S)^2, \qquad (3)$$

where k_S is the rate constant of swelling and S_{max} denotes the degree of the equilibrium or maximum swelling. After definite integration by applying the initial conditions $S = 0$ at $t = 0$ and $S = S$ at $t = t$, Eq. (3) becomes

Table 1
The parameters of swelling of the hydrogels

Fluid	EFC%	S_{eq} g fluid (g polymer)$^{-1}$	S_{max} g fluid (g polymer)$^{-1}$	r_0 mg fluid (g polymer×min)$^{-1}$
AAm/CA hydrogel				
UR	94.4	16.93	18.77	102
PP	94.0	15.79	17.19	117
DW	93.9	15.39	16.65	118
IP	93.8	15.14	16.31	129
PN	93.6	14.65	15.75	117
GF	93.6	14.60	15.77	126
PS	92.9	13.16	14.08	133
HS	92.5	12.33	13.08	142
In rat	93.1	13.43	–	–
AAm/IA hydrogel				
UR	93.6	14.55	14.88	434
PP	91.1	10.20	10.48	255
DW	92.0	11.50	11.64	658
IP	92.2	11.81	12.11	286
PN	91.1	9.42	9.82	175
GF	88.7	7.83	8.07	193
PS	88.7	7.81	7.99	247
HS	86.4	6.37	6.53	195
In rat	91.7	10.05	–	–

$$\frac{t}{S} = \alpha + \beta t, \tag{4}$$

where α and β are two coefficient whose physical sense can be interpreted as follows: At extended treatment time, $\beta t \gg \alpha$ and, according to Eq. (4) $\beta = 1/S_{max}$, i.e. reciprocity of the theoretical equilibrium or maximum swelling. On the contrary, at very short time treatment time, $\alpha \gg \beta t$, in the limit, Eq. (3) becomes

$$\lim_{t\to 0} \frac{dS}{dt} = k_S = \frac{1}{\alpha}.$$

Therefore, intercept, α, represents reciprocity of the initial swelling rate r_0 or $1/k_S S_{max}^2$.

Fig. 2 shows representative graphs obtained by the application of Eq. (4) to the swelling data. In all cases straight lines with excellent correlation coefficients are obtained, which demonstrate that the swelling behavior of these system follows second-order kinetic. The calculated kinetic parameters are tabulated in Table 1. As depicted from Table 1, the results of kinetic model are in agreement with swelling experiment.

Table 1 shows that the values of theoretical maximum swelling of the hydrogels are parallel the results of swelling of the gels. Swelling processes of AAm/CA hydrogel is quicker than the swelling rate of AAm/IA hydrogels in the simulated body fluids.

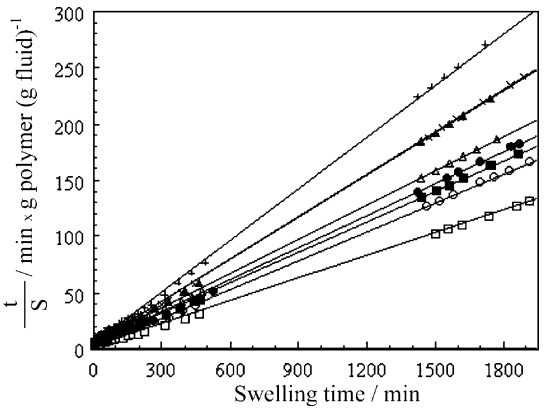

Fig. 2. Swelling kinetics curves of AAm/IA hydrogels in the fluids: (□) UR; (■) PP; (○) DW; (●) IP; (△) PN; (▲) GF; (×) PS; (+) HS.

This kinetic model adequately explains the mechanism of the swelling process according to relaxation of hydrated polymeric chains, by means

of the mobility of polymeric segments by adsorption of water and physiologically fluids.

3.3. Diffusion of simulated body fluids

The following equation was used to determine the nature of diffusion of fluids into hydrogels [20]:

$$F = kt^n, \tag{5}$$

where F denotes the amount of solvent fraction at time t and k is a constant related to the structure of the network and the exponential n is a number indicative of the type of diffusion. Fluid adsorption characteristics of gel exhibited anomalous behavior, ranging between Fickian and Case II extremes depending on experimental temperature and thermodynamic compatibility of the penetrant and the gel. Typically, both the diffused amount and the penetrating swelling front position in Case II transport are completely time dependent in a linear fashion whereas Fickian diffusion is square root time dependent. An intermediate situation, known as non-Fickian or anomalous diffusion occurs whenever the rates of Fickian diffusion and polymer relaxation are comparable [20]. This equation is applied to the initial stages of swelling and some plots of ln F versus ln t are shown in Fig. 3. The exponents were calculated from the slope of the lines and are presented in Table 2.

In Table 2, it is shown that the values of diffusional exponents range between 0.6 and 0.7. For the hydrogels studied here the n values indicating the type of diffusion is found to be over 1/2. Hence, the diffusion of the fluids into the hydrogels is non-Fickian in character [20].

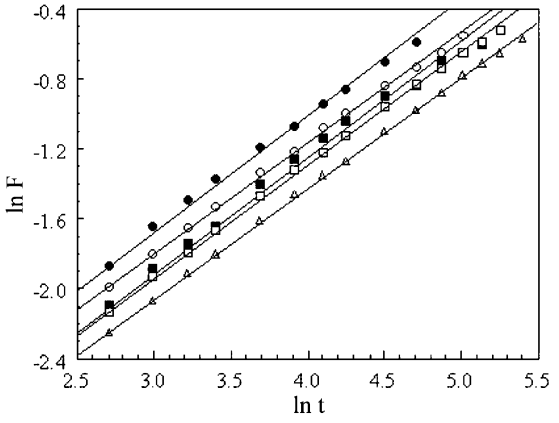

Fig. 3. ln F versus ln t curves of AAm/CA hydrogels in the fluids: (●) HS; (○) PS; (■) DW; (□) PW and (△) UR.

Table 2
The parameters of diffusion of the hydrogels

Fluid	$k \times 10^2$	n	$D \times 10^6$/cm^2 s^{-1}
AAm/CA hydrogel			
UR	1.91	0.63	1.95
PP	2.05	0.65	1.73
DW	1.97	0.67	2.58
IP	2.42	0.62	2.16
PN	2.38	0.62	1.57
GF	2.24	0.65	1.99
PS	2.42	0.64	2.13
HS	2.51	0.67	2.69
AAm/IA hydrogel			
UR	3.45	0.72	9.96
PP	3.49	0.67	4.74
DW	5.17	0.67	9.17
IP	4.85	0.59	2.31
PN	3.88	0.61	3.05
GF	3.90	0.62	3.47
PS	4.63	0.60	3.34
HS	3.25	0.72	5.20

The diffusion coefficients D of the cylindrical hydrogels were calculated from the following relations [21]:

$$D^n = \frac{k}{4}(\pi l^2)^n, \tag{6}$$

where D is in cm^2 s^{-1}, l is the radius of the gel. The values of the diffusion coefficient of the hydrogels are showed in Table 2. Table 2 shows that the values of the diffusion coefficient of the AAm based hydrogels vary from 1×10^{-6} to 10×10^{-6} cm^2 s^{-1}.

3.4. In vivo biocompatibility studies

In this part, novel radiation synthesized hydrogels based on copolymers of acrylamide, crotonic acid (contains one carboxylic group) or itaconic acid (contains two carboxylic groups) with capability of absorbing a high amount of water were used in biocompatibility with subcutaneous tissues of rats.

3.4.1. Macroscopical findings

The animals appeared to be in good health throughout the implantation period. No clinical signs of inflammation or wound complications were observed. An implanted rat was lived to the end of the natural life.

3.4.2. Form of the hydrogels

After all implantations, it has seen that both hydrogels swelled by absorbing of body fluid, and were made a lump in the midline abdominal area of the rats (Fig. 4). The photographs of hydrogels, before and after implantation, are presented in Fig. 5. In Fig. 5, it is shown that AAm/CA and AAm/IA hydrogels are swelled very high in the rat. After implantation, the hydrogels were retained their cylindrical shape and color (Figs. 4–10 are available in color, see the on-line version) after they were excised from the rats.

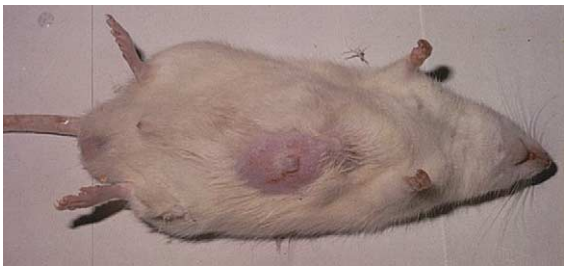

Fig. 4. Wistar Albino rat showing the implantation site of the hydrogel.

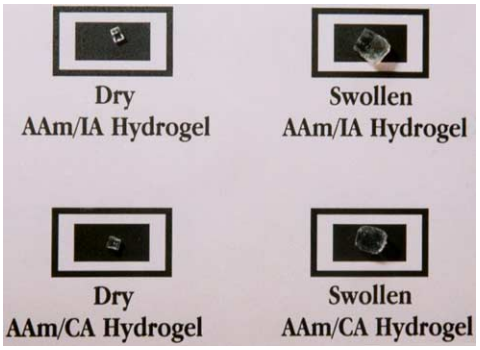

Fig. 5. The photograph of the hydrogels before and after implantation.

3.4.3. Histological evaluation

In the excised subcutaneous tissue surrounding the hydrogel implants, it is shown that the hydrogels were surrounded by fibrous capsules. The capsules contained fibrocytes, collagen and blood vessel and were commonly free inflammatory cells. Some representative micrographs are shown in Figs. 6–10.

After one-week implantation, no pathology such as necrosis or tumorigenesis was observed in the excised tissue surrounding the AAm/CA hydrogel and in skin, superficial fascia and muscle tissues in distant sites (Fig. 6). After six-week, 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 (Fig. 7). 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 was observed an increase in the collagen fibrils due to proliferation and activation of fibroblasts (Fig. 8).

One week after the implantation of AAm/IA hydrogel, the implant was surrounded by a thin, epithelized fibrous capsule, however, there was a rather abundant fibrin accumulation and a development of granulation tissue beneath the capsule. There was also a fibroblast proliferation in the capsule along with a vascular proliferation at the end of the first week (Fig. 9). No pathology was observed in the tissues of straight muscle in the close to implant sites at the end of the tenth week. However, one of AAm/IA samples disintegrated into small pieces in the rat in sixth week. AAm/IA hydrogel particles separated from the hydrogel implant were surrounded by epitheloid cells which were formed by macrophages. Especially in the regions of the formation of granulation tissue, numerous histyocytes migrated out of the capsule and accumulated on the hydrogel surface. There was a foreign body reaction in the implantation region during the sixth week of implantation (Fig. 10).

The thicknesses of the fibrous capsules were measured in the optical microscope using a

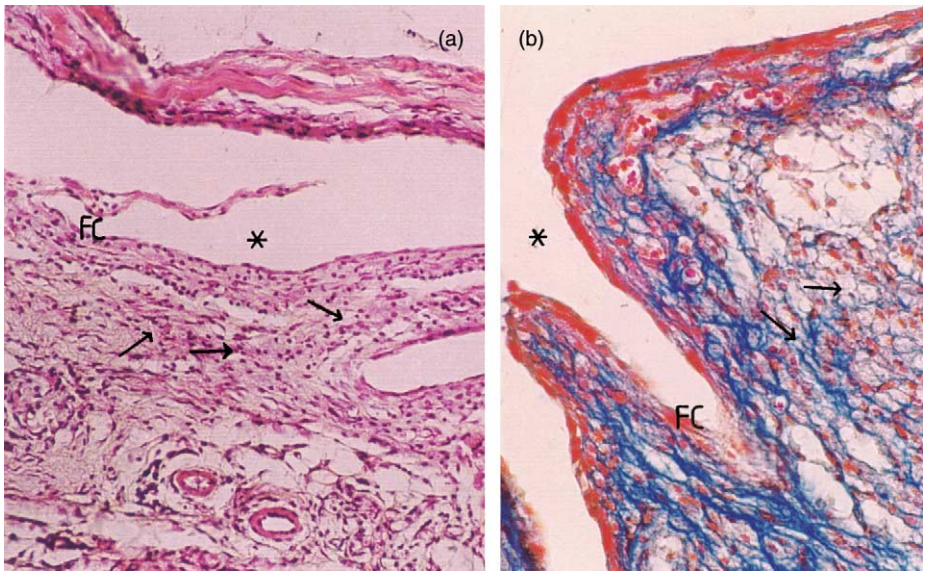

Fig. 6. (a) After one-week, the implantation region of AAm/CA hydrogel (✱) and thin fibrous capsule (FC) and (b) fibroblast, a grouped mast cells and lymphocyte (→) in the fibrous capsule. Original magnification: (a) 20× (Haematoxylin/Eosin) and (b) 40× (Mallory–Azan).

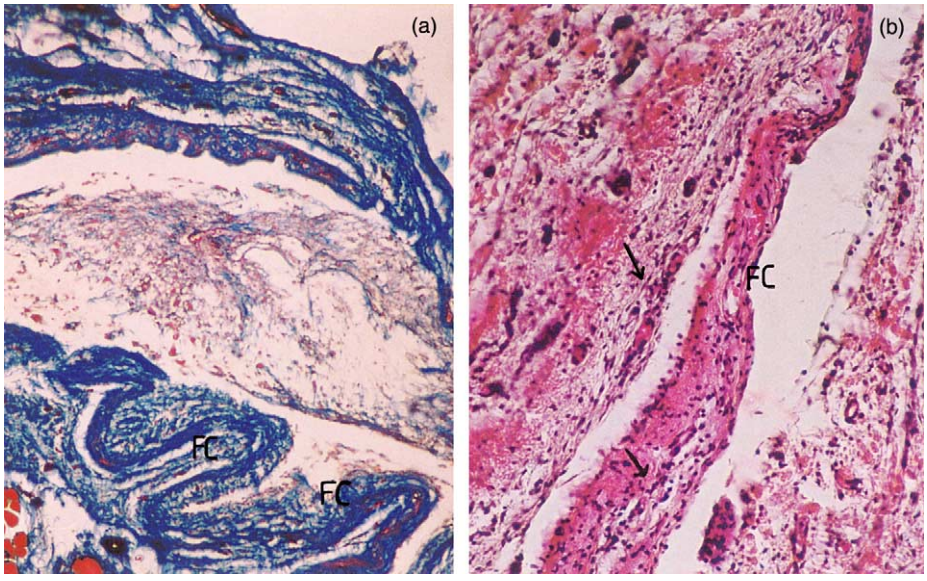

Fig. 7. After six-week, thickened fibrous capsule (FC) contains fibroblast, macrophage and lymphocyte cells (→). Original magnification: (a) 10× (Mallory–Azan) and (b) 40× (Haematoxylin/Eosin).

micrometer scale. The means of five measurements for each of the samples and each time point were calculated. Then, the mean of thickness of fibrous capsules versus implantation time was plotted and

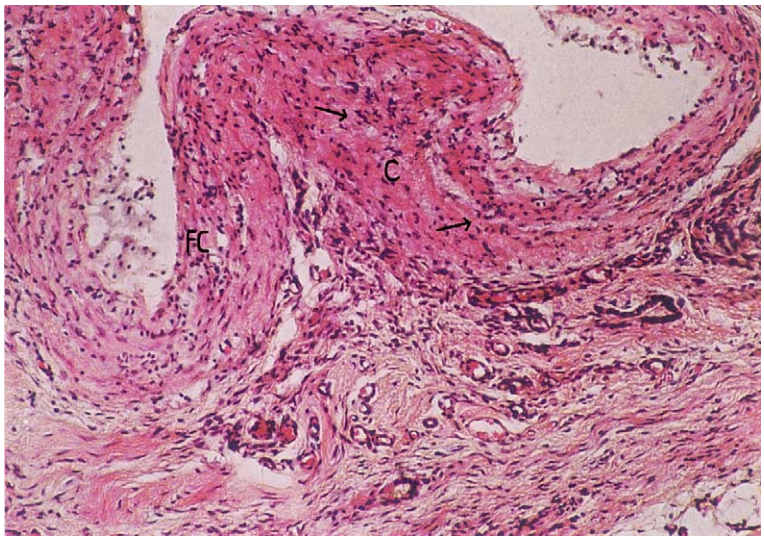

Fig. 8. Light microphotograph of implantation site showing fibrous capsule (FC) collagen (C) and fibroblasts (→) 10 week post-implantation of AAm/CA hydrogel. Original magnification: 20× (Haematoxylin/Eosin).

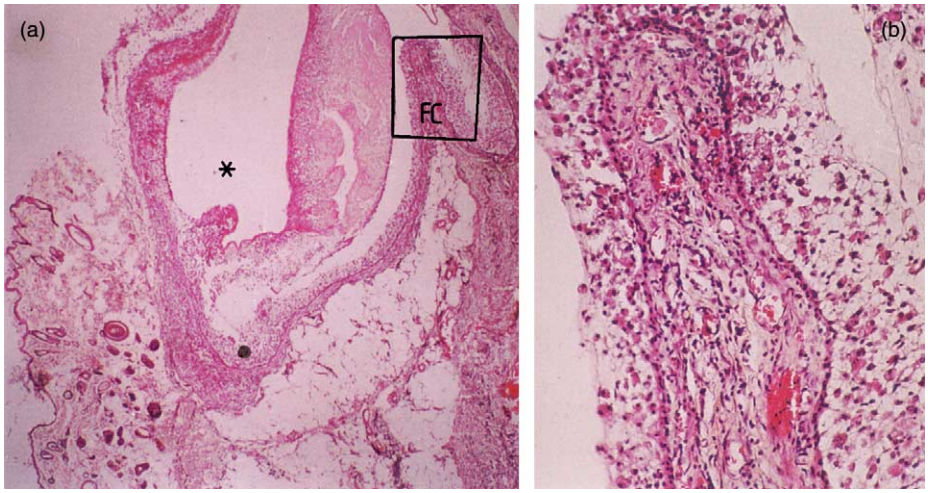

Fig. 9. After one-week, the implantation region of AAm/IA hydrogel (✱) and thin fibrous capsule (FC), abundant fibrin accumulation (●) and granulation tissue beneath the capsule. Original magnification: (a) 3.2× and (b) 20× (Haematoxylin/Eosin).

is presented in Fig. 11. In Fig. 11, it is shown that the thickness of fibrous capsules is gradually increased up to 6 weeks, and then these values reached a constant value. The thickness of fibrous capsule occurred due to AAm/IA hydrogel implant are higher than the thickness of fibrous capsule values of AAm/CA hydrogels. The carboxyl groups in the AAm/IA hydrogels were caused to the high thickness of the fibrous capsule occurred due to the AAm/CA hydrogel [22]. 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 ($p > 0.05$) were found.

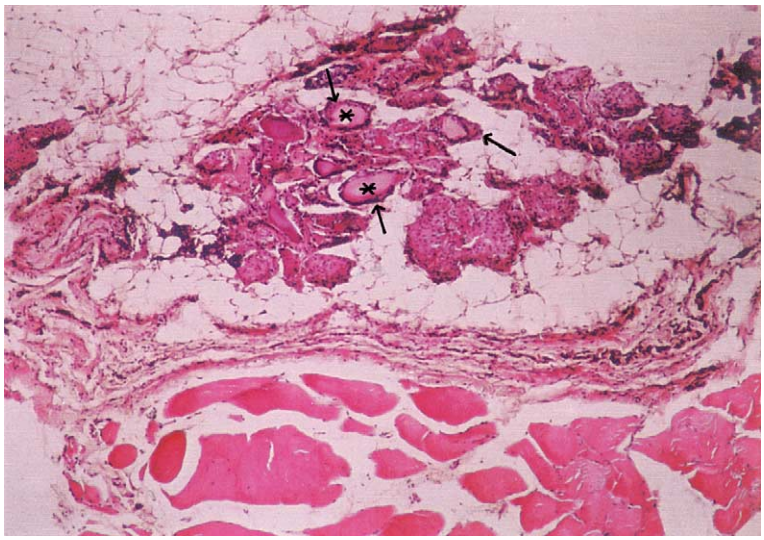

Fig. 10. After six-week, disintegrated hydrogel particles (✱) from the implant are surrounded by epitheloid cells (↓) which are formed by macrophages. Original magnification: 10× (Haematoxylin/Eosin).

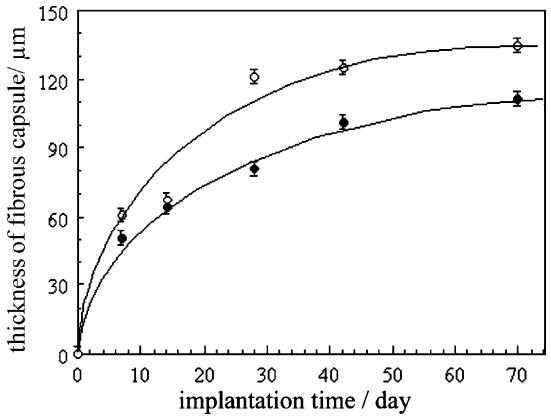

Fig. 11. The curves of thickness of fibrous capsule – implantation time: (○) AAm/CA and (●) AAm/IA.

These thicknesses of fibrous capsules 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 [23]. Our results clearly indicated that the capsule thicknesses of the excised tissue were well within these stipulated threshold limits. These data corroborated with the biological tolerance of the radiation synthesized AAm hydrogels observed histologically.

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-hydroxyethy methacrylate) hydrogel, which considered as a biologically inert polymer. However, it is important that the swelling degree of acrylamide based hydrogels are higher than the swelling degree of poly(2-hydroxyethy methacrylate) hydrogels for the biomedical uses [17].

On the other hand, Greene et al. [24] reported that the literature was replete with controversial evidence linking silicone implants to inflammatory responses as well as other medical disorders. Fibrotic and inflammatory reactions have been observed in the tissues surrounding the implant and in distant sites. The causal link between disease and the presence of silicone breast implants has not definitely established. On the basis the evidence and public concern, the US Food and Drug Administration has banned the use of silicone gel filled silicone breast implants but has allowed the use of saline filled implants. Thus, AAm/CA hydrogels can be used alternative biomaterials against to the silicon implants.

4. Conclusion

In this study, in vitro swelling behavior, diffusional properties, and in vivo biocompatibility of radiation synthesized AAm/CA and AAm/IA copolymers were investigated. Swelling ratio of AAm/IA hydrogel ranged from 6.37 to 14.55, while the value of AAm/CA hydrogel was ranged from 12.33 to 16.93. All EFC values of the hydrogels were greater than the percent water content values of the human body which is about 60%. The fluid diffusion in the hydrogels was *non-Fickian*.

In vitro dynamic swelling study of radiation synthesized acrylamide hydrogels, containing mono- or dicarboxylic moieties, have shown that swellings depend upon the type of fluids as well as the type of comonomers. Related to used specific composition, hydrogels can exhibit different swellings, absorptive capacity, etc. in the various physiological media. However, their medical properties are very close to each other. The technique used in this work known since many years as "clean method" can also be used for synthesis of other hydrogels for biomedical materials. It is possible to obtain hydrogels with a controllable mesh and/or porosity depending on the required properties and places where they are used.

The biocompatibility studies of AAm/CA and AAm/IA hydrogels clearly indicated good tissue tolerance for subcutaneous implantation up to 10 week. These histological findings indicated subcutaneous implantation of hydrogels in rat did not cause any necrosis, tumorigenesis, or infection at the implant site during this period. AAm/CA and AAm/IA hydrogels were well tolerated, non-toxic and highly biocompatible. However, AAm/IA copolymer was found non-suitable for preparation of biomaterial, since the weak mechanical strength of the AAm/IA hydrogels.

The in vitro study in the simulated physiological body fluids and in vivo biocompatibility study are very important on the application of hydrogel as biomaterials. Thus, the prediction of behaviors of radiation synthesized hydrogels provides great advantage to a designer in scientific point of view. In addition, it can be concluded that the use of radiation for hydrogel synthesis is very useful and promising.

Acknowledgements

This research was supported by the Scientific Projects Commission of Cumhuriyet University, F-39. In addition, we would like to thank the members of Polymer group of the Department of Chemistry, Hacettepe University, and Y. Işıkver in Department of Chemistry, Vet. Dr. Y. Yalman in Medicine Faculty, Cumhuriyet University, and Dr. N. Şahiner in Department of Chemical Engineering, Tulane University for technical assistance.

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