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In vivo biocompatibility of radiation induced acrylamide and acrylamide/maleic acid hydrogels

D. SARAYDIN*
Cumhuriyet University, Chemistry Department 58140 Sivas-Turkey
E-mail: saraydin@cumhuriyet.edu.tr

E. KOPTAGEL, S. ÜNVER-SARAYDIN
Cumhuriyet University, Medicine Faculty, Department of Histology-Embryology 58140 Sivas-Turkey

E. KARADAĞ
Adnan Menderes University, Department of Chemistry 09010, Aydin, Turkey

O. GÜVEN
Hacettepe University, Department of Chemistry 06532, Beytepe/Ankara, Turkey

In vitro swelling and *in vivo* biocompatibility of radiation induced acrylamide (AAm) and acrylamide/maleic acid (AAm/MA) hydrogels were investigated. The swelling kinetics of AAm and AAm/MA hydrogels of are 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 and, some swelling and diffusion parameters have been calculated. AAm and AAm/MA hydrogels were 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 week. *In vivo* studies indicated that the radiation induced acrylamide and acrylamide/maleic acid hydrogels were found to be well-tolerated, non-toxic and highly biocompatible. © *2001 Kluwer Academic Publishers*

1. Introduction

In modern medicine, commonly used biomaterials originated from metals, ceramics, carbons, natural tissues and polymers have shown biocompatibility with blood, tissues, cells, etc., in human body [1, 2]. Hydrogels comprise a new family of polymeric materials used for the same purpose [3]. Crosslinked hydrophilic polymers capable of imbibing large volumes of water (i.e. >20%) are termed hydrogels [4, 5]. A great deal of interest upon hydrogels since the pioneering work of Wichterle some forty years ago [4]. Hydrogels have been extensively studied and used for a large number of applications in the medical field as implants [6–8], controlled drug release devices [9–12], for enzyme, protein and cell immobilization [13–17], blood-contacting applications [18] and other uses [3, 19, 20]. A hydrogel can be defined as a polymeric material that is characterized by its capacity to absorb water, other solvents and biological fluids [21].

The water content in the equilibrium of swelling affects different properties of the hydrogels: permeability, mechanical properties, surface properties and biocompatibility. The utility of hydrogels as biomaterials lies in the similarity of their physical properties with those of living tissues. This resemblance is based on their water content, soft and rubbery consistency and low interfacial tension with water and other biological fluids. So, from this viewpoint more hydrophilic hydrogels are better as implants, as long as their mechanical properties are acceptable [21, 22].

The hydrogels of both acrylamide and acrylamide based copolymer exhibit a very high capability to absorb water, are permeable to oxygen and posses good biocompatibility [23].

Alternatively, maleic acid exhibit similarity with the acrylic derivatives, so it can be copolymerized with a large number of monomers, an it has carboxylic groups in its molecule which make it highly hydrophilic.

In our previous works, radiation induced acrylamide based hydrogels [24–27] have been studied in adsorption of protein [28, 29] and biocompatibility with human sera [30, 31], and the influence of amino acids of the swelling behaviour [32, 33]. The aim of the work is the use of a novel hydrogel based on copolymers of acrylamide (AAm) and maleic acid (MA), with capacity of absorbing a high water content in biocompatibility

* Author to whom all correspondence should be addressed.

with subcutaneous tissues of rats. AAm hydrogel was non-ionogenic nature, while AAm/MA hydrogel was ionic character.

2. Experimental

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

A suitable mass of maleic acid and irradiation doses for acrylamide and maleic acid was selected by taking previous experiments into consideration [24].

2.1. Preparation of the hydrogels

One g of acrylamide (AAm) is dissolved in 1 mL of distilled water and 40 mg of maleic acid (MA) is added to this aqueous solution. This solution is 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 γ irradiator source at a fixed dose rate of 0.72 kGy h^{-1}. Fresh hydrogel rods obtained are cut into pieces of 3–4 mm length. They are washed with distilled water and, dried first in air and vacuum, and stored for further use [24].

2.2. *In vitro* swelling studies

The swelling nature of AAm or AAm/MA 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) [34] was studied at $37 \pm 0.1\,^{\circ}$C to determine the parameter swelling, swelling rate 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.

2.3. *In vivo* biocompatibility studies

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

Radiation induced AAm and AAm/MA 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 surgey 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.

2.4. 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 and AAm/MA 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.

Schematic diagram of the hydrogel experiment is presented in Fig. 1.

3. Results and discussion

3.1. Preparation of radiation induced hydrogels

When monomers of AAm and MA have been irradiated with ionization rays such as γ-rays in water, free radicals are generated. Random reactions of these radicals with the monomers lead to the formation of copolymers of acrylamide/maleic acid (AAm/MA). When irradiation dose has been increased beyond a certain value the polymer chains crosslink and then gel is obtained. It is reported that gelation dose of polyacrylamide is 2.00 kGy at ambient temperature [35]. A total dose of 4.65 kGy is applied for the preparation of AAm/MA hydrogels. 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

A fundamental relationship exists between the swelling of a polymer in a solvent and the nature of the polymer and the solvent. Dried hydrogels are left to swell in the some physiologically fluids at 37 ± 0.1°C in a water bath. Swollen gels removed from the water bath at regular intervals are dried with filter paper, weighed and placed in the same bath. The percentage swelling $S\%$, is calculated from the following relation [36]:

$$S\% = [(m_t - m_o)/m_o] \times 100 \qquad (1)$$

Where m_o is the mass of the dry gel at time 0 and m_t is the mass of the swollen gel at time t.

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. Swelling curves of AAm and AAm/MA hydrogels are shown in Fig. 2a and b, respectively.

Fig. 1a shows that the swelling of AAm hydrogels in physiological fluids is higher than in distilled water. The reason of this behavior is the ionic character of physiological solutions. Ions of physiological fluids are placed into the pores of hydrogels instead of the molecules of water. Solvated ions of the fluids are caused to increase swelling of non-ionogenic AAm hydrogel. On the other hand, Fig. 1b shows that the

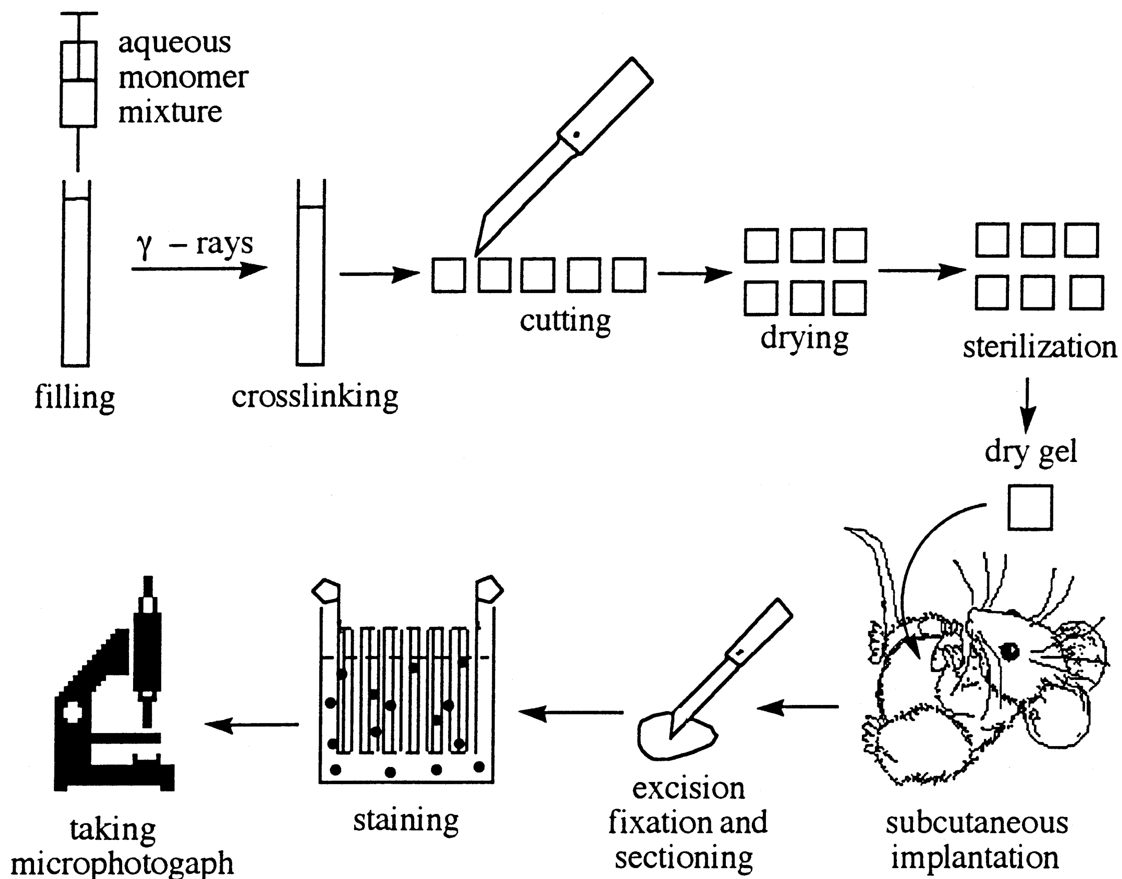
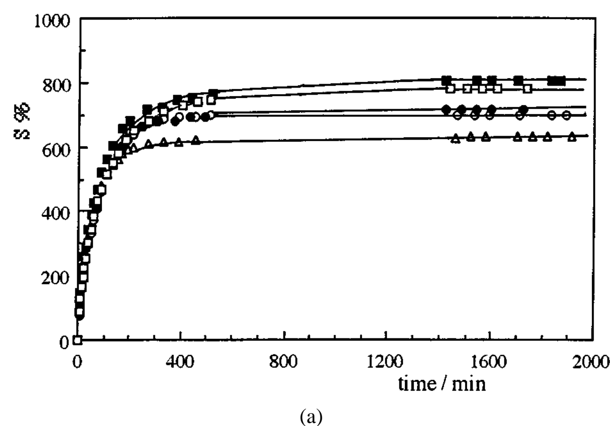
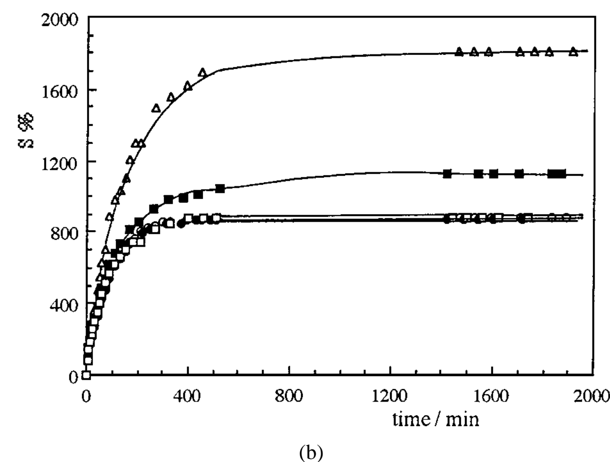


Figure 1 The scheme of the hydrogel experiment.



(a)



(b)

Figure 2 Swelling curves of the hydrogels in the fluids, (a) AAm, (b) AAm/MA. △; distilled water,○; physiologic saline ●; human serum, ■; gylicine-HCl buffer at pH 1.1, ❐; phosphate buffer at pH 7.4.

swelling of AAm/MA hydrogel in distilled water is higher than in physiological fluids. Ions of physiological fluids are interacted with carboxyl groups of maleic acid in AAm/MA hydrogel. So, AAm and AAm/MA hydrogels in the fluids are swollen in the following order: Phosphate buffer > glycine-HCl buffer > human sera ≥ saline solution > water, and water > phosphate buffer > glycine-HCl buffer ≥ saline solution ≥ human sera, respectively.

It can be expected that the medical use of the AAm/MA hydrogel would provide material with a broad range of swelling owing to the non-ionogenic nature of the AAm hydrogel [37].

The fluid absorbed by the gel network is quantitatively represented by the EFC [38], where:

$$\text{EFC\%} = [\text{Mass of fluid in the gel/Mass of hydrogel}] \times 100 \qquad (2)$$

EFCs of the hydrogels for all physiologically fluids were calculated. The values of EFC% of the hydrogels are graphed in Fig. 2. All EFC values of the hydrogels were greater than the percent water content values of the body about 60%. Thus, the AAm and AAm/MA hydrogels were exhibit similarity of the fluid contents with those of living tissues.

For extensive swelling of polymers, it can be written following relation [39, 40];

$$\frac{t}{S} = A + Bt \qquad (3)$$

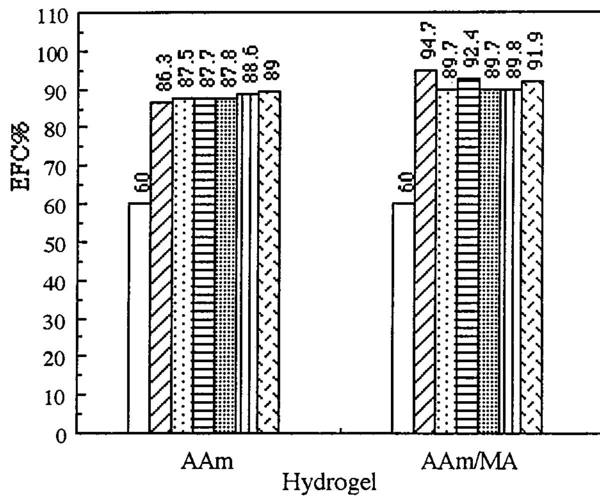


Figure 3 The values of EFC of the hydrogels in the fluids and rat, and the body. □; body, ▨; distilled water, ▤; physiologic saline ▦; human serum, ▤; in rat, ▨; gylicine-HCl buffer at pH 1.1, ▥; phosphate buffer at pH 7.4.

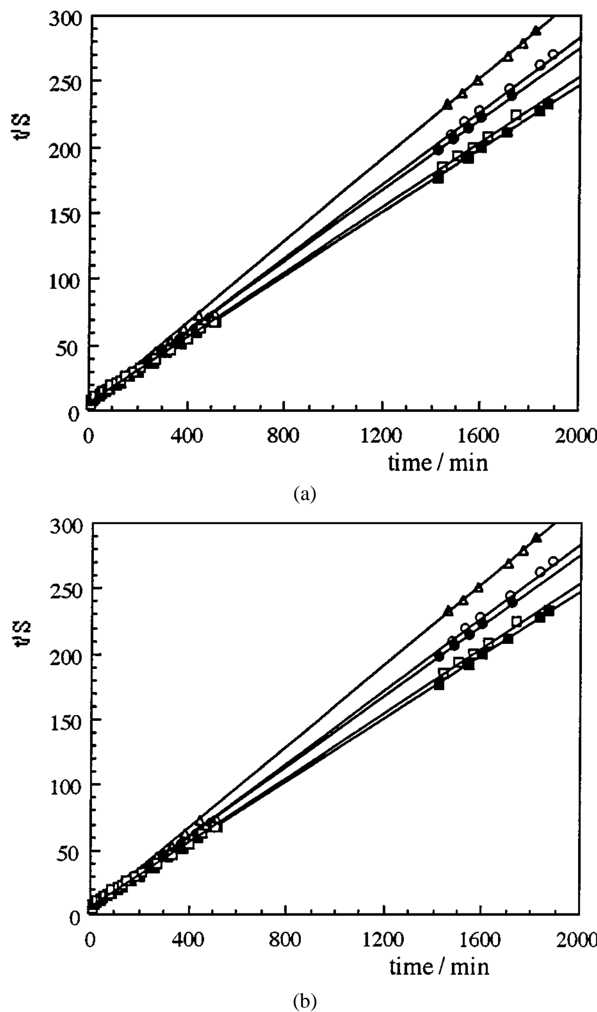


Figure 4 Swelling rate curves of the hydrogels in the fluids, (a) AAm, (b) AAm/MA. △; distilled water,○; physiologic saline ●; human serum, ❒; gylicine-HCl buffer at pH 1.1, ■; phosphate buffer at pH 7.4.

Where $B = 1/S_{eq}$ is the inverse of the maximum or equilibrium swelling, $A = 1/(dS/dt)_0$, is the reciprocal of the initial swelling rate the gel. The relation represents second order kinetics [39].

Fig. 4a and b shows the linear regression of the swelling curves obtained by means of Equation 3. The initial swelling rate and the values of theoretical equilibrium swelling of the hydrogels are calculated from the slope an intersection of the lines and, are presented in Table I.

Table I shows that the values of theoretical equilibrium swelling of the hydrogels are parallel the results of swelling of the gels. Swelling processes of AAm/MA hydrogel is quicker than the swelling rate of AAm hydrogels in the body fluids.

3.3. Diffusion of fluids

The following equation was used to determine the nature of diffusion of water and fluids into hydrogels [41]

$$F = kt^n \tag{4}$$

Where F denotes the amount of solvent fraction at time t, k is a constant related to the structure of the network and the exponential n is a number indicative of the type of diffusion. This equation is applied to the initial stages of swelling and plots of $\ln(F)$ versus $\ln(t)$ are shown in Fig. 5a and b. The exponents are calculated from the slope of the lines and, are presented in Table I.

In Table I, it is shown that the values of diffusional exponent range between 0.59 and 0.74. For the hydrogels studied here the n values indicating the type of

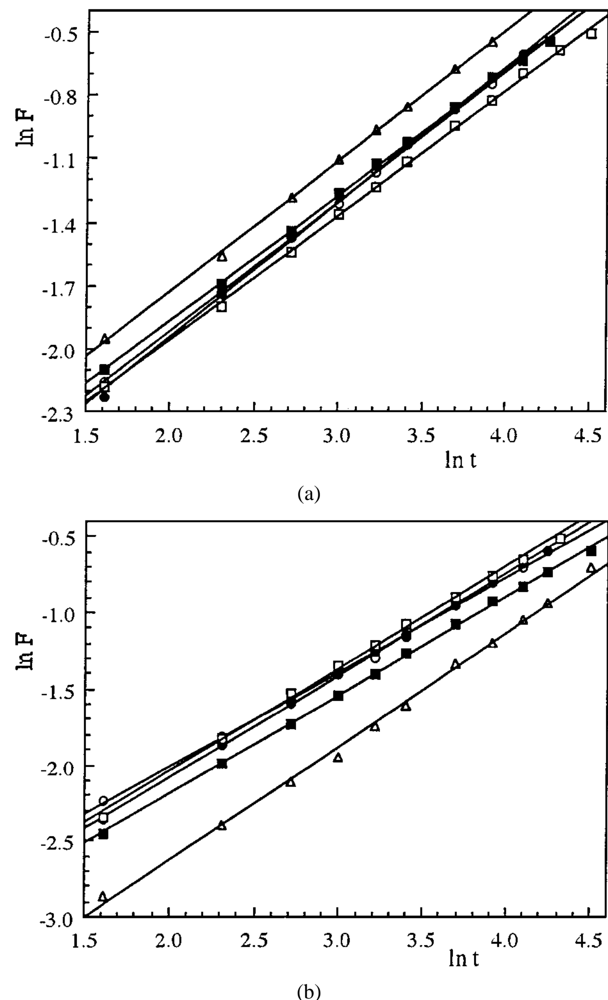


Figure 5 Swelling kinetics curves of the hydrogels in the fluids, (a) AAm, (b) AAm/MA. △; distilled water, ○; physiologic saline ●; human serum, ❒; gylicine-HCl buffer at pH 1.1, ■; phosphate buffer at pH 7.4.

TABLE I The parameters of swelling and diffusion of the hydrogels

Hydrogel Physiological Fluid	AAm						AAm/MA					
	S_{eq}%	$K_2 \times 10^2$	S_{eq^*}	n	$D \times 10^6$/ $cm^2\ s^{-1}$	$\mathcal{D} \times 10^6$/ $cm^2\ s^{-1}$	S_{eq}%	$K_2 \times 10^2$	S_{eq^*}	n	$D \times 10^6$/ $cm^2\ s^{-1}$	$\mathcal{D} \times 10^6$/ $cm^2\ s^{-1}$
Distilled water	630	21.9	6.43	0.62	5.29	5.74	1800	17.8	19.20	0.74	3.99	5.20
Physiologically saline	700	18.3	7.19	0.61	3.63	4.11	875	20.1	9.00	0.62	4.92	5.72
Human serum	720	16.4	7.40	0.64	3.92	4.40	865	18.2	8.98	0.66	3.63	4.19
Glycine-HCl buffer	780	15.0	8.09	0.59	4.53	5.23	875	19.0	9.06	0.67	5.23	5.98
Phosphate buffer	805	14.2	8.30	0.59	4.88	5.59	1130	15.6	11.80	0.65	5.04	6.02

*These S_{eq} values were obtained from Equation 3.

diffusion is found to be over 1/2. Hence the diffusion of the fluids into the hydrogels was taken to be a non-Fickian characteristic. This is generally explained as a consequence of slow relaxation rate of the polymer matrix.

Diffusion coefficients were calculated from the following relation [42].

$$D = 0.049/(t/4l^2)_{1/2} \quad (5)$$

Where D in $cm^2\ s^{-1}$, t is the time at which the swelling is one half the equilibrium value ($V/V_o = 1/2$) and, l is radius of swollen hydrogel rods. The intrinsic diffusion coefficient may be expressed as

$$\mathcal{D} = D(1 - V)^{-3} \quad (6)$$

Where V is volume fraction of solvent penetrating the polymer by the time t defined above [42].

The diffusion coefficients for AAm and AAm/MA hydrogels are listed in Table I. If Table I is examined, it is shown that the values of the intrinsic diffusion coefficient of the hydrogels are bigger than the values of the diffusion coefficient of them. Because, Equation 5 gives a measure not only of diffusion but also of the mass flow of the whole system. Equation 6 gives the intrinsic diffusion coefficient for cases where no mass action effects enter [42].

3.4. *In vivo* biocompatibility studies

In this part, novel hydrogel based on copolymer of acrylamide and maleic acid, with capacity of absorbing a high water content in biocompatibility with subcutaneous tissues of rats was examined. Fig. 6 shows the Wistar Albino rat with the subcutaneous AAm/MA hydrogel implant. After all implantations, it is seen that both hydrogels were swelled by absorbing of body fluid, and were made a lump in the midline abdominal area of the rats The photographs of hydrogels, before and after implantation, are presented in Fig. 7.

In Figs 6 and 7, it is shown that AAm and AAm/MA hydrogels are swelled very high in the rat. After implantation, the hydrogels are retained their cylindrical shape and color after they were excised from the rats.

3.5. Histological analysis

In the excised subcutaneous tissue surrounding the hydrogel implants, it is shown that the hydrogels were surrounded by fibrous capsules no including inflammatory cells in the all test groups (Figs 8–12).

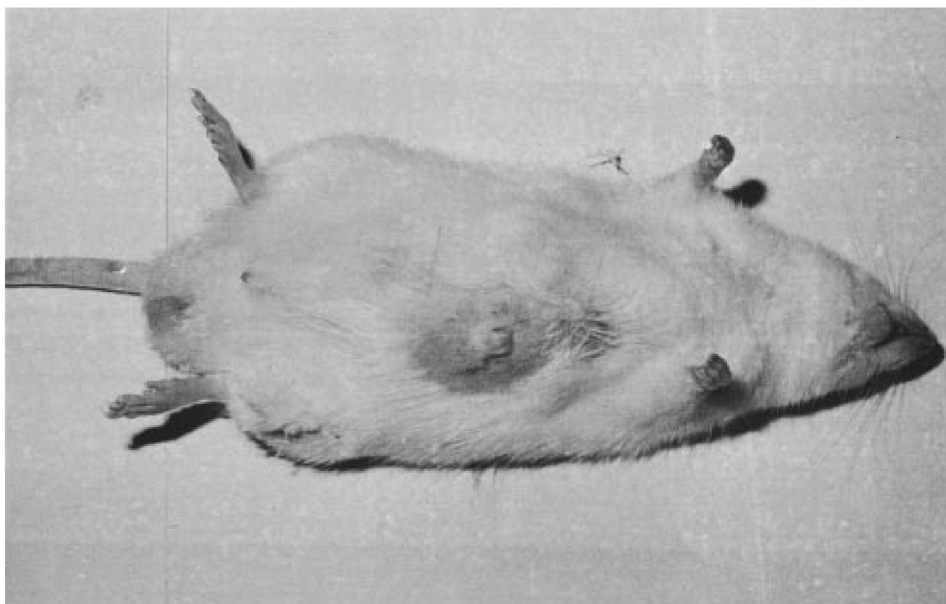


Figure 6 Wistar Albino rat showing the implantation site of the hydrogel.

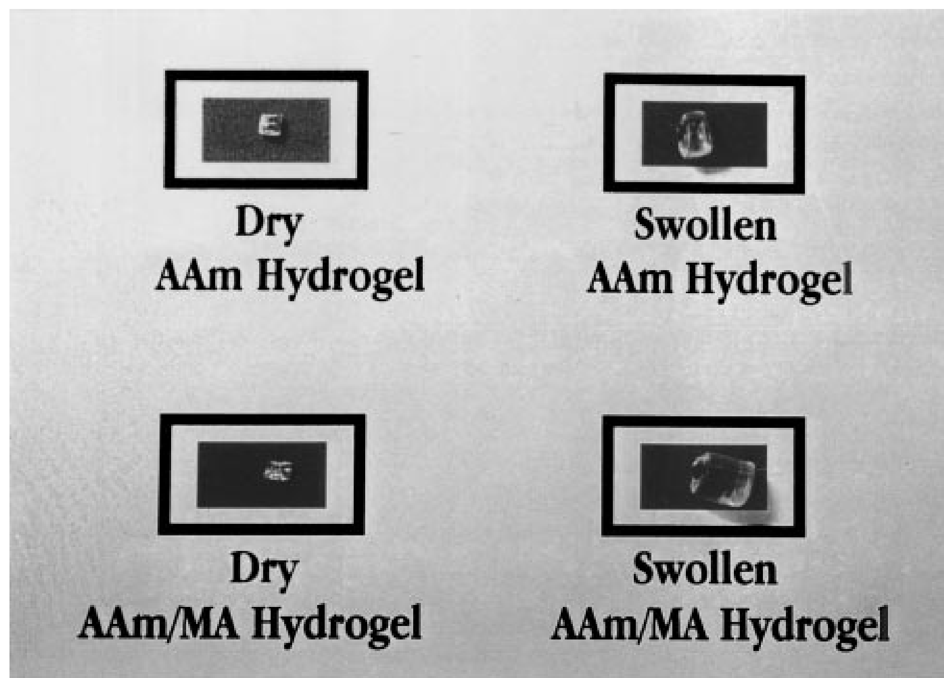


Figure 7 The photograph of the hydrogels before and after implantation.

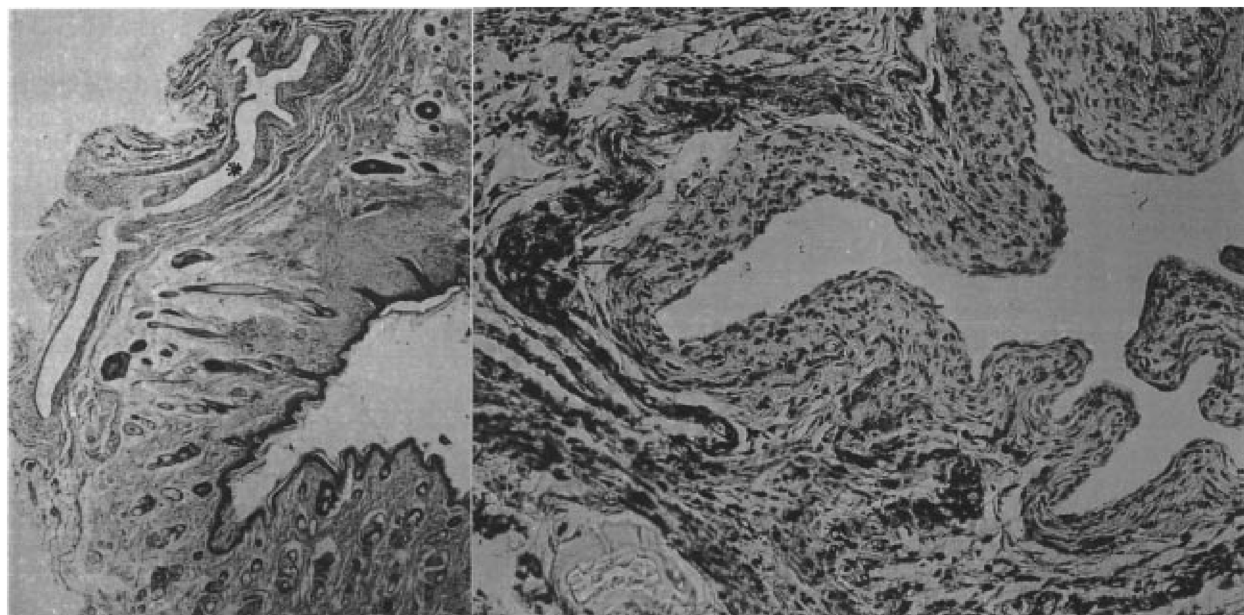


Figure 8 Light microphotographs of implantation site showing thin fibrous capsule (F), mast cells and lymphocyte (→) 2 week post-implantation of AAm hydrogel. Original magnificiations: a; ×40 (Haematoxylin/Eosin), b; ×20 (Mallory-Azan).

After one week implantation, no pathology such as necrosis, tumorigenesis or infection were observed in the excised tissue surrounding the AAm hydrogel and 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 (Figs 8 and 9). 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 (Fig. 10).

After one week implantation of the AAm/MA hydrogel, it is observed an thin fibrous capsules the similar of the test group of AAm hydrogel (Fig. 11). A few macrophage and lymphocyte were observed in the fibrous capsule consisting of fibroblast cells and, a grouped mast cells and lymphocyte were observed between tissues and capsule in the some samples. After 2–10 weeks, vessel proliferation was observed in the capsules and surrounding tissues (Fig. 12). No chronic and acute inflammation, adverse tissue reaction were observed in the all test groups of AAm/MA hydrogel. It is no determination related to the loss of activation and liveliness of cells in the capsule cells and in distant sites. No pathology were observed in the skin and the tissues of straight muscle in the close to implant sites.

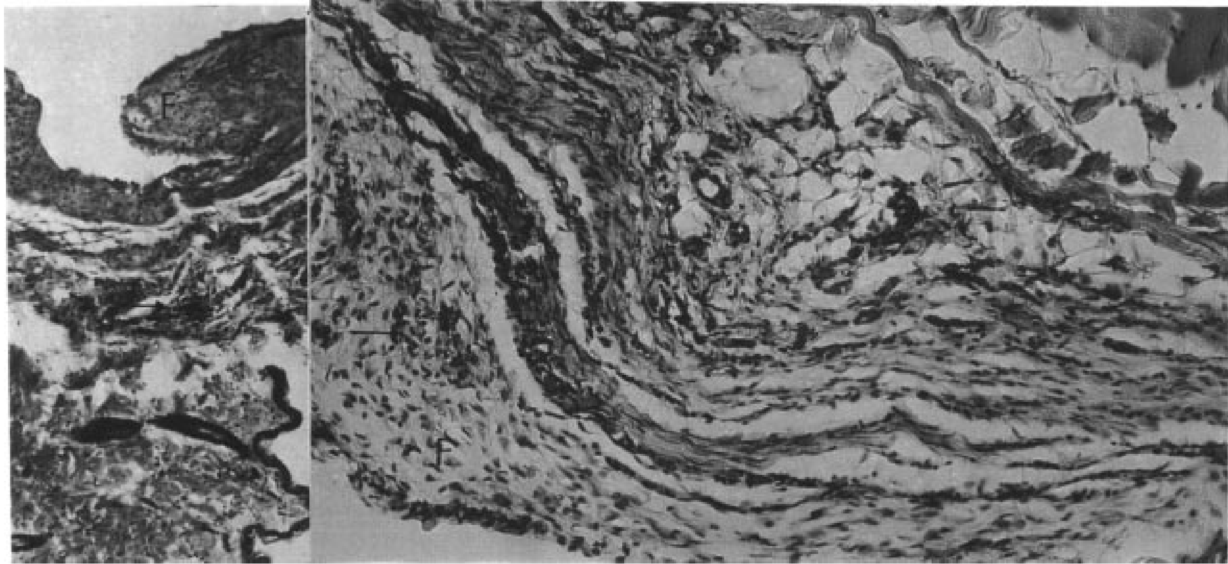


Figure 9 Light microphotographs of implantation site showing fibrous capsule (F), fibroblast, lymphocyte and macrophage cells (→) 4 week post-implantation of AAm hydrogel. Original magnificiations: a; ×20, b; ×20 (Haematoxylin/Eosin).

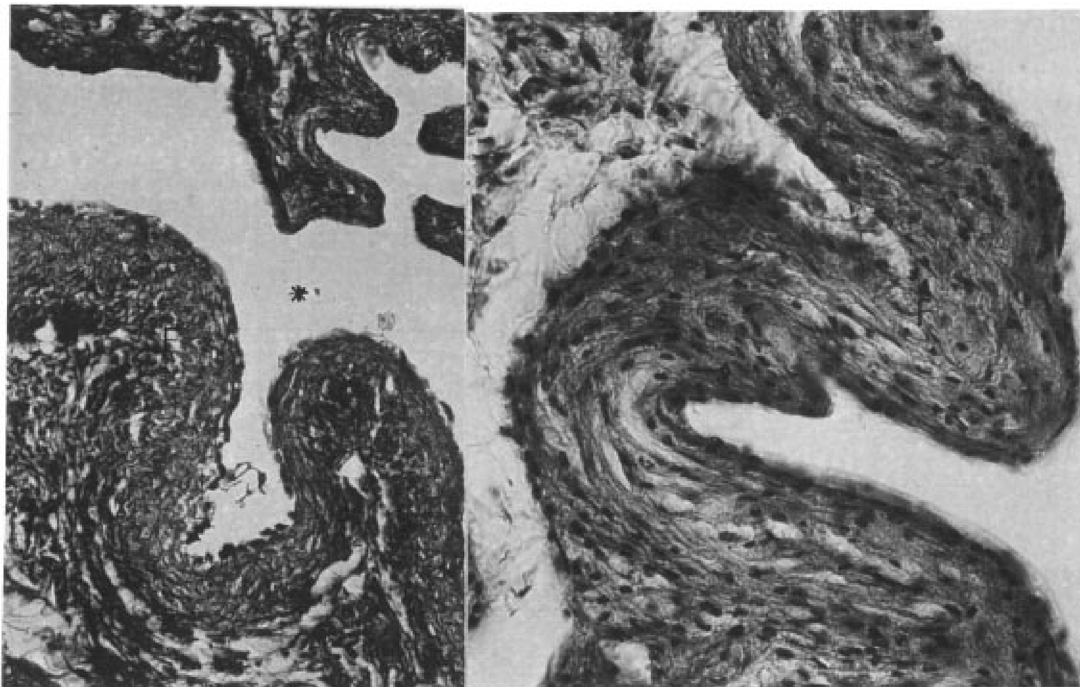


Figure 10 Light microphotographs of implantation site showing fibrous capsule (F) collagen (C) and fibroblasts (→) 6 week post-implantation of AAm hydrogel. Original magnificiations: a; ×20 (Mallory-Azan), b; ×40 (Haematoxylin/Eosin).

The thickness 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. Then, the mean thickness of fibrous capsule versus implantation time were plotted and, are presented in Fig. 13. In Fig. 13, it is shown that the thickness of fibrous capsules are gradually increased to 6 weeks, and then these values are becomed a constant value. The thickness of fibrous capsule occurred due to AAm/MA hydrogel implant are high from the values of AAm hydrogels. The carboxyl groups on the chemical structure and ionogenic character of AAm/MA hydrogel can be caused to the high thickness of the fibrous capsule occurred due to AAm/MA [43]. 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 thickness 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 [21]. Our results clearly indicated that the capsule thickness of the excised tissue were well within these stipulated threshold limits. These data corroborated with the biological tolerance of AAm and AAm/MA 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

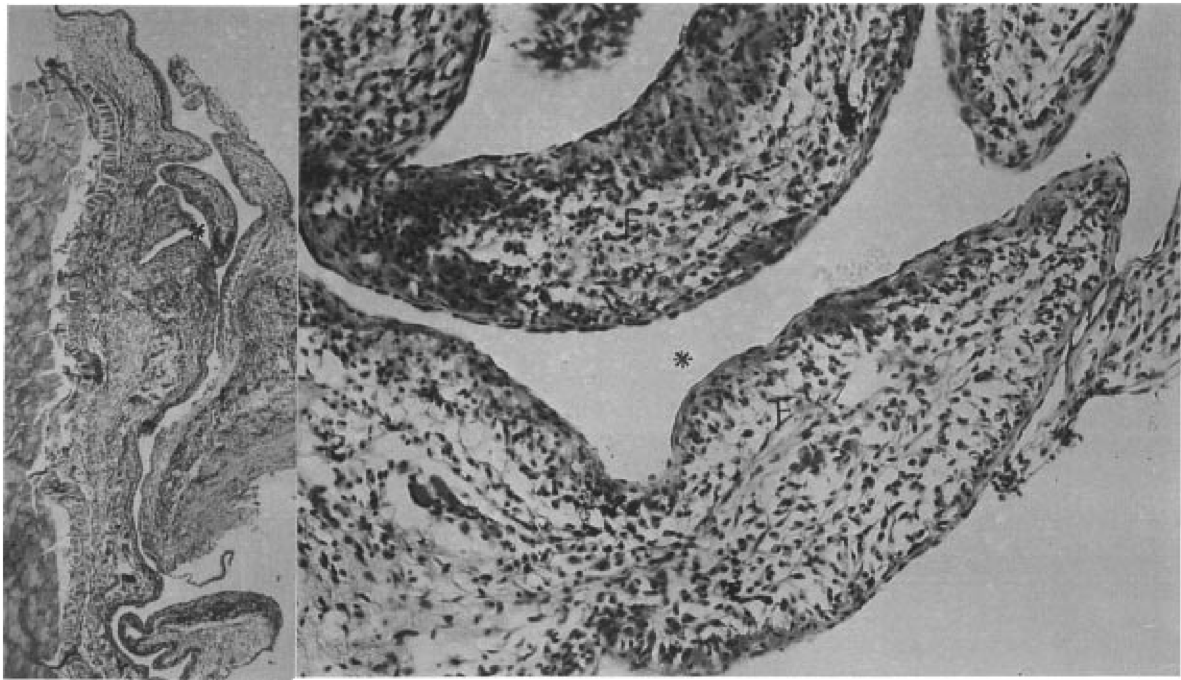


Figure 11 Light microphotographs of implantation site showing implantation area (*) and thin fibrous capsule (F) one week post-implantation of AAm/MA hydrogel. Original magnificiations: a; ×3.2, b; ×20 (Haematoxylin/Eosin).

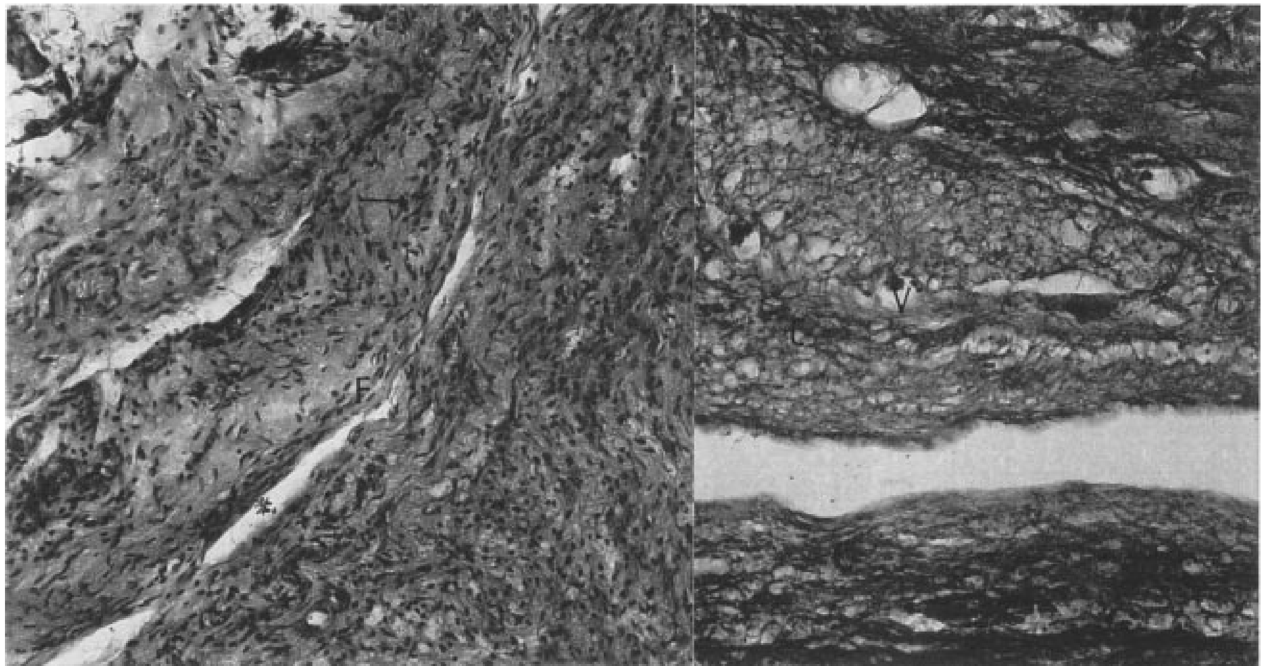


Figure 12 Light microphotographs of implantation site showing implantation area (*) and thick fibrous capsule (F), fibroblasts (→), blood vessels (V) and collagen fibrils (C) 10 week post-implantation of AAm/MA hydrogel. Original magnifications: a; ×20 (Haematoxylin/Eosin), b; ×20 (Mallory-Azan).

swollen of poly(2-hydroxyethyl methacrylate) hydrogel, which considered as a biologically inert polymer [63]. However, it is important that the swelling of acrylamide based hydrogels are very high than the swelling of poly(2-hydroxyethyl methacrylate) hydrogels for the biomedical uses.

On the other hand, it is 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 U.S. Food and Drug Administration has banned the use of silicone gel filled silicone breast implants but has allowed the use of saline filled implants [44, 45]. Thus, AAm and AAm/MA hydrogels can be used an alternative biomaterials against to the silicon implants.

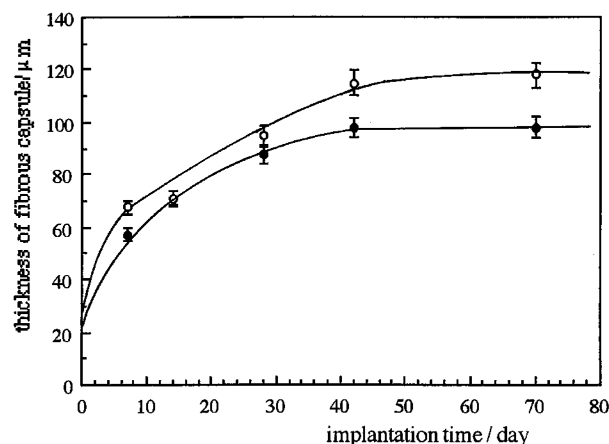


Figure 13 The curves of thickness of fibrous capsule—implantation time. ●; AAm, ○; AAm/MA.

4. Conclusion

In this study, *in vitro* swelling behavior, diffusional properties, and *in vivo* biocompatibility of radiation induced acrylamide and acrylamide/maleic acid hydrogels were investigated. AAm/MA hydrogel was swelled from 865–1800%, while AAm hydrogel was swelled from 630–805%. The EFC values of the hydrogels were greater than the percent water content values of the body about 60%. The fluid diffusion in the hydrogels was *non-Fickian*.

The biocompatibility studies of AAm and AAm/MA hydrogels 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 and AAm/MA hydrogels were well tolerated, nontoxic and highly biocompatible.

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