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Histopatological Effect Characteristics of Various Biomaterials and Monomers Used in Polymeric Biomaterial Production

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1. Introduction

When a synthetic material is placed within the human body, tissue reacts towards the implant in a variety of ways depending on the material type. The mechanism of tissue interaction (if any) depends on the tissue response to the implant surface. In general, there are three terms in which a biomaterial may be described in or classified into representing the tissues responses. These are bioinert, bioresorbable, and bioactive.

Biomaterials are often used and/or adapted for a medical application, thus comprises whole or part of living structures or biomedical devices which performs, augments, or replaces biological functions. Biomaterials are used in dental and surgical applications, in controlled drug delivery applications. A biomaterial may be an autograft, an allograft or a xenograft used as a transplant material.

Biomaterials are mostly polymers produced by monomers, and are used in artificial organ production in contemporary medicine. They are prepared by the polymerization reaction of certain monomers.

In several previous studies, we investigated whether acrylamide, methacrylamide, N-isopropylacrylamide, acrylic acid, 2-hydroxyethyl methacrylate, 1-vinyl-2-pyrrolidone and ethylene glycol had cytotoxic effects and induced apoptosis or not in spinal cord. Immunolocalization of glial fibrillary acidic protein (GFAP) was also determined, and it was evaluated by using semi-quantitative morphometrical techniques. The cytotoxicity of monomers on cultured fibroblastic cell lines was also examined *in vitro*.

Acrylic acid had the most cytotoxic effect when compared to the methacrylamide and the ethylene glycol groups. GFAP immunoreactivity was found to be rather stronger in the methacrylamide than the other monomers application groups. The methacrylamide, acrylic acid, N-vynil pyrrolidine, acrylamide, N-isopropylacrylamide and 2-hydroxyethyl methacrylate application groups had TUNEL positive cells when compared to the other groups. While some monomers used in biomaterial production seemed not to affect the cell viability and GFAP immunoreactivity, some other monomers had adverse effects on those features. This in turn may contribute to the pathological changes associated to the monomer type.

In our previous other works, *in vitro* swelling and *in vivo* biocompatibility of radiation crosslinked acrylamide and its co-polymers such as acrylamide (AAm) and acrylamide/crotonic acid (AAm/CA), acrylamide/itaconic acid (AAm/IA), and acrylamide/maleic acid (AAm/MA) hydrogels were investigated.
The radiation crosslinked AAm, AAm/CA, AAm/IA and (AAm/MA) co-polymers were found to be well tolerated, non-toxic and highly biocompatible.
On the other hand, calcium phosphate ceramics and xenografts have been used in different fields of medicine and dentistry. We demonstrated the effects of calcium phosphate ceramics (Ceraform) and xenograft (Unilab Surgibone) in the field of experimentally created critical size parietal bone defects in rats. Although Ceraform was less resorptive and not osteoconductive properties, it could be considered as a biocompatible bone defect filling material having a limited application alternative in dentistry and medicine. However, xenograft seems biocompatible, osteoconductive, and could be used in a limited manner as a filling material in osseous defects in clinical practice.

2.Toxicological effect of the water-soluble monomers

2.1 Monomers

Monomer is a molecule of any of a class of compounds, mostly organic, that can react with other molecules of the same or other compound to form very large molecules, or polymers. The essential feature of a monomer is polyfunctionality, the capacity to form chemical bonds to at least two other monomer molecules. Bifunctional monomers can form only linear, chainlike polymers, but monomers of higher functionality yield cross-linked, network polymeric products. Toxicological effects of the monomers are changing from very low (zero) to very high.
Some polymeric biomaterials such as hydrogels are produced by the effect of initiator such as chemical initiator, heat, light or high energy radiation from the water soluble-monomers.

2.2 Cytotoxic effects

Biomaterial suitable for a biomedical application must be biocompatible at least on its surface. In several previous studies, we investigated whether acrylamide, methacrylamide, N-isopropylacrylamide, acrylic acid, 2-hydroxyethyl methacrylate, 1-vinyl-2-pyrrolidone and ethylene glycol used in polimeric biomaterial production had cytotoxic effects (Unver Saraydin et al., 2011). The cytotoxicity of xenograft (one of the alternative graft materials) was also examined in vitro (Unver Saraydin et al., 2011).
The viability of cultured fibroblastic cell lines following all monomer applications except for the ethylene glycol group were found to be decreased in all time intervals (Figure 1, 2), and differences were statistically significant ($p<0.05$). In addition, the cell viability was significantly ($p<0.05$) lower in the acrylamid application group when compared to the control group. Acrylic acid demonstrated the maximum cytotoxic effect when compared to the methacrylamide and ethylene glycol groups. On the other hand, the ethylene glycol group showed no cytotoxicity for cells (Graphic 1).
In our study of the xenograft cytotoxic activities, the xenograft showed no cytotoxicity for the cells (Figure 3). There was no decolorization zone around the samples. Although the cells were directly in contact with the xenograft in the culture media, they did not show any signs of injury and preserved their morphological characteristics and wholeness like those seen in the controls.

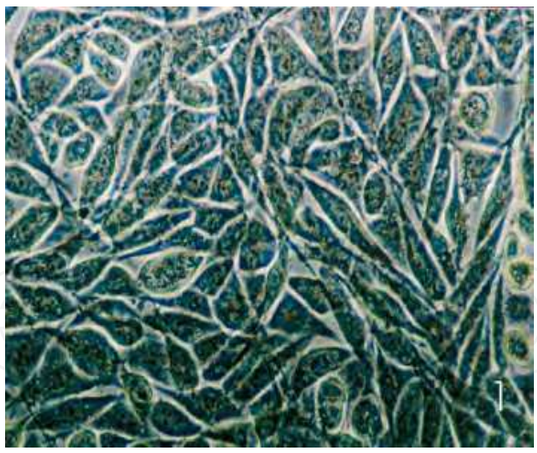


Fig. 1. Fibroblast viability %100 after 12 h incubation period with ethylene glycol

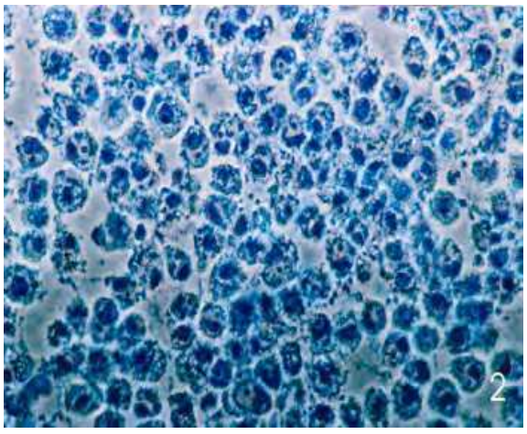
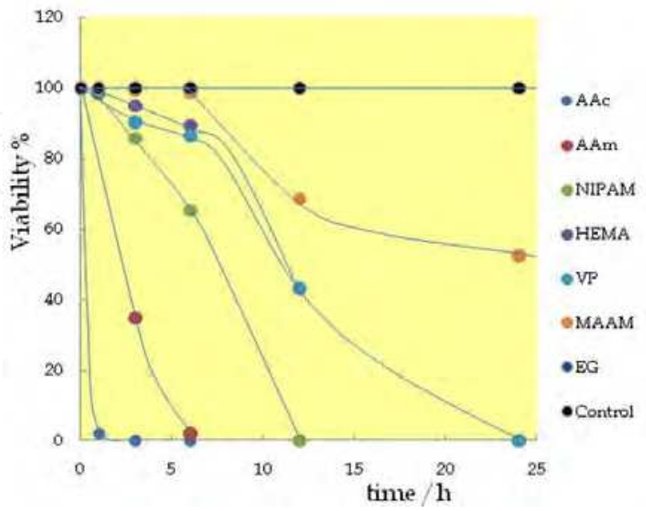


Fig. 2. Fibroblast viability % 0 after 12h incubation period with N-isopropyl acrylamide



Graphic 1. Shows the cell viability alterations between groups in the fibroblastic cell lines by the time.

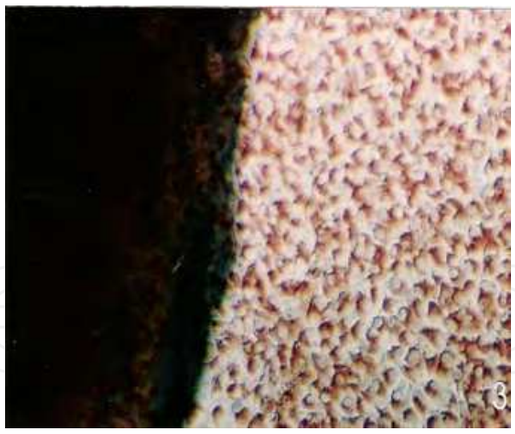


Fig. 3. There is no cytotoxicity for the cells.

2.3 Neurotoxic effects

Several studies revealed neurotoxic effects as well as ataxi and muscle weakness caused by biomaterials on humans and on laboratory animals. It has been suggested that they cause axonal degeneration in central and peripheric nervous system (Barber et al., 2001).

Astrocytes are the stellate glial cells in the central nervous system, which play a major role in supporting neurons, scar formation and development and maintenance of the blood-brain barrier. The physiological and metabolic properties of astrocytes indicate that those cells are involved in the regulation of water, ions, neurotransmitters, and pH of the neuronal milieu (Montgomery 1994). They are also implicated in protection against toxic insults such as excitotoxicity and oxidative stress (Lamigeon et al., 2001). Glial fibrillary acidic protein (GFAP) is an intermediate filament protein found predominantly in astrocytes (McLendon 1994). Therefore it is important to determine the glial fibrillary acidic protein (GFAP) immunoreactivity in astrocytes for the evaluation of biomateials.

In our study, immunolocalization of glial fibrillary acidic protein (GFAP) was determined, and it was evaluated by using semi-quantitative morphometrical techniques (Unver Saraydin et al., 2011). GFAP immunoreactivity was found to be very strong in the methacrylamide, N-isopropylacrilamid, ethylene glycol and N-vinyl pyrrolidine application groups whereas it was weak in acrylic acid, acrylamide and 2-hydroxyethyl metacrylad applied groups (Table 1, Figure 4-10). Changes in GFAP immunoreactivity could be due to following conditions; astrocyte dysfunction, astrocyte loss accompanied by astroglial cell proliferation, de-differentiation, and changes in functional state of neuronal cell types, thus altering the neuron-glial homeostasis. The over-expression of GFAP could probably indicate the protective strategy of these tissues.

Although the neurotoxicity of acrylamide and many monomers has been known since 1950s, its' mechanisms have remained obscure (Lee et al., 2005, Gold and Schaumburg, 2000). Acrylamide increases p53 protein (Okuno et al., 2006), recent studies indicate that it plays a role in apoptotic cell death in neurons (Morrison et al., 2003). Acrylamide can activate caspase- 3 and cause apoptosis in neuronal cells (Sumizawa and Igisu, 2007). The cellular process of apoptosis is an important component of tissue and organ development as well as the natural response to disease and injury (David et al., 2003). DNA fragmentation in neurons was characterized by double staining with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) (Bao and Liu, 2004). To our knowledge, however, it has not been determined whether acrylamide and other

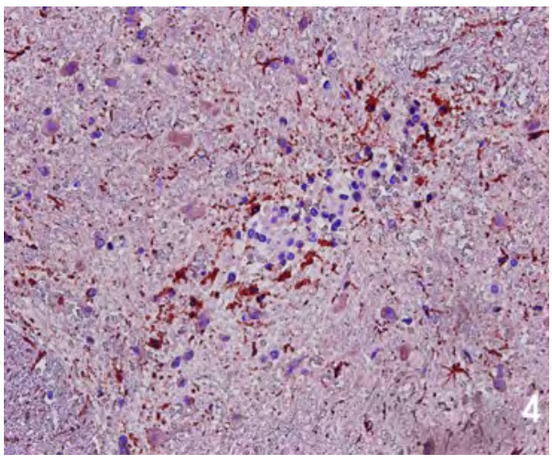


Fig. 4. Control group GFAP immunoreactivity. GFAP40X

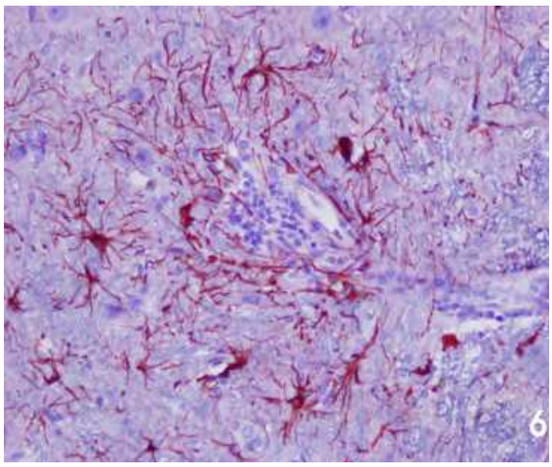


Fig. 5. GFAP immunoreactivity 6 week after Acrylic acid exposure. GFAP 40X

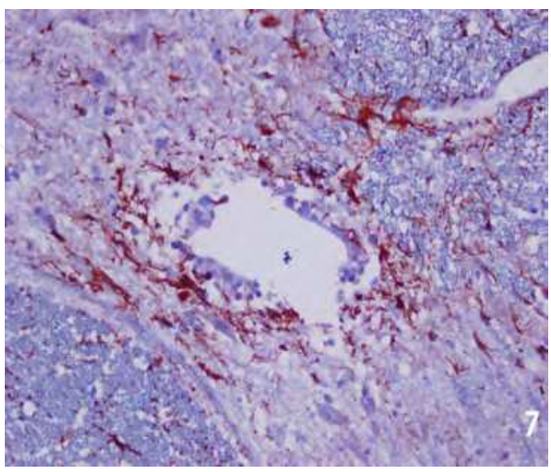


Fig. 6. GFAP immunoreactivity 2 week after Acrylamide exposure. GFAP 40X

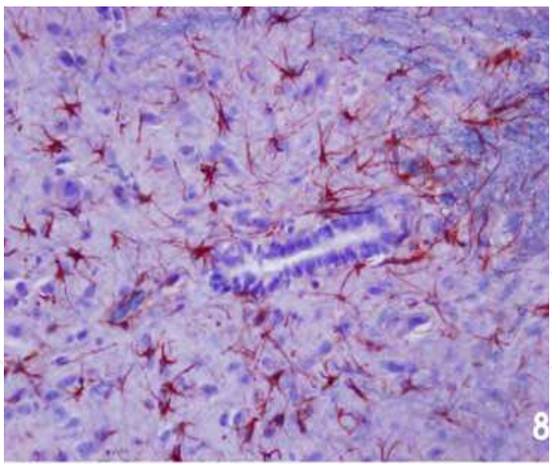


Fig. 7. GFAP immunoreactivity 6 week after 2-hydroxyethyl methacrylate exposure GFAP 40X

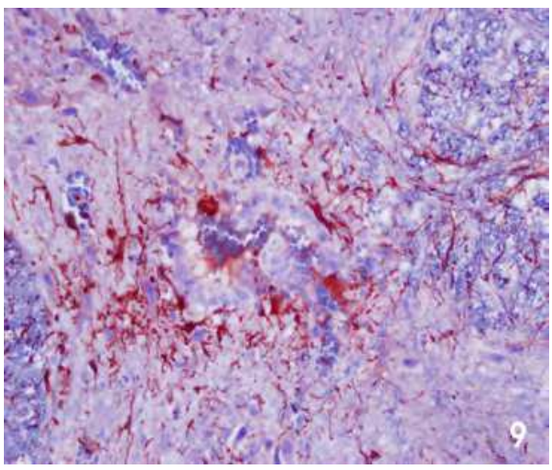


Fig. 8. GFAP immunoreactivity 4 week after methacrylamide exposure GFAP 40X

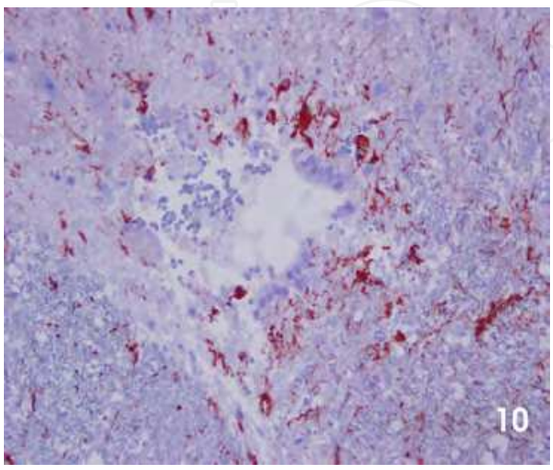


Fig. 9. GFAP immunoreactivity 6 week after N-isopropylacrylamide exposure GFAP 40X

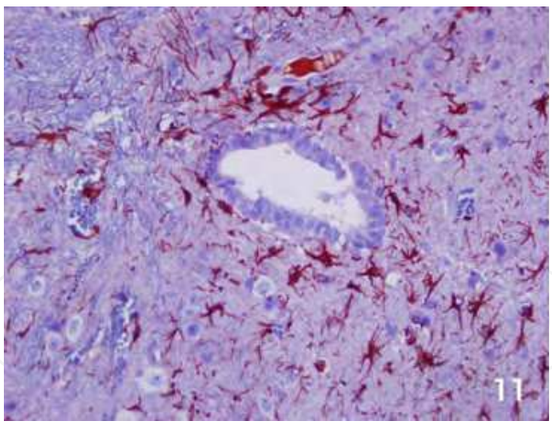


Fig. 10. GFAP immunoreactivity 4 week after N-vinyl pyrrolidone exposure. GFAP 40X

Monomer	1st week	2nd week	4th week	6 th week	12 th week
Ethylene glycol	++	++	+++	++	++
N-vinyl pyrrolidone	+++	+++	+++	+++	++
2-hydroxyethyl methacrylate	+	++	+++	++	++
Acrylamide	++	++			
Methacrylamide	+++	+++	++	++	+
N-isopropylacrylamide	+++	+++	+++	+++	
Acrylic acid	+	++		++	++
Control	+++	+++	+++	+++	+++

Table 1. Demonstrates the semi-quantitative scoring findings of GFAP immunolocalization in rat medullaspinalis following 1, 2, 4, 6 and 12 weeks of particular monomer applications

monomers cause apoptosis in neuronal cells. We therefore examined apoptosis by using terminal deoxynucleotydil transferase dUTP nick and labelling (TUNEL) method in spinal cord (Unver Saraydin et al., 2011).

While TUNEL positive cells has been detected rarely in the control and in the ethylen glycol application groups, numerous TUNEL positive cells were intensively observed in the spinal cord of the methacrylamide, acrylic acid, N-vinyl pyrrolidine, acrylamide, N-isopropylacrylamide and 2-hydroxyethyl metacrylate application groups (Figure 11-14).

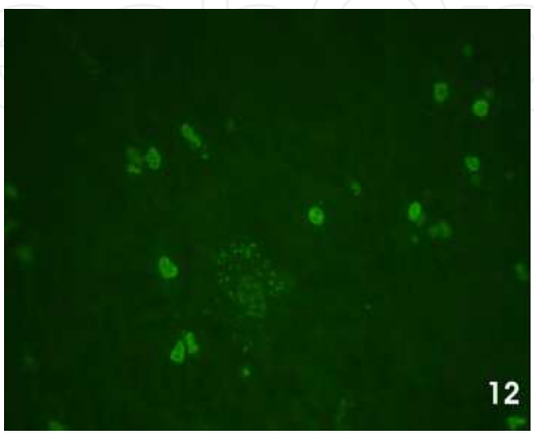


Fig. 11. TUNEL-positive apoptotic cells in the control group. TUNEL 100X

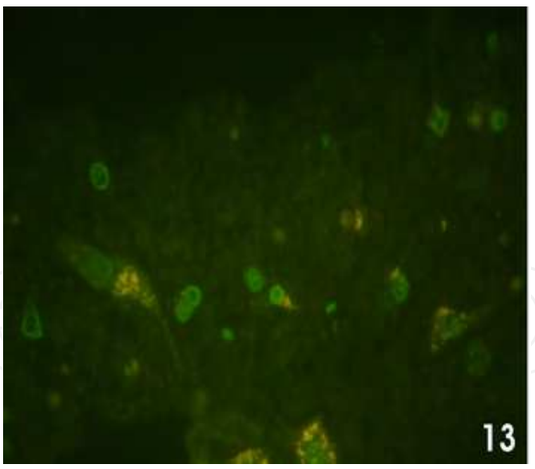


Fig. 12. TUNEL positive cells 6 week after 2-hydroxyethyl methacrylate. TUNEL 100X

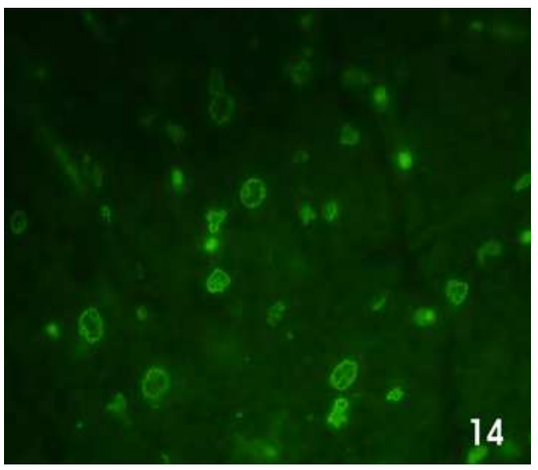


Fig. 13. TUNEL positive cells 6 week after N-isopropylacrylamide. TUNEL 100X

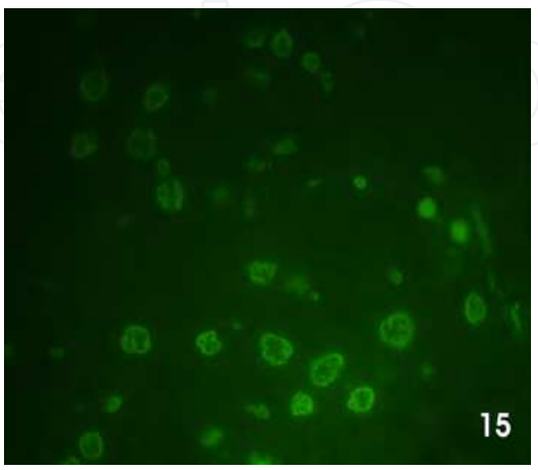


Fig. 14. TUNEL positive cells 6 week after N-vinyl pyrrolidine. TUNEL 100X

3. Polymeric biomaterials

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. Preliminary use of in vitro methods is encouraged as screening tests prior to animal testing. In order to reduce the number of animals used, these standards use a step-wise approach with review and analysis of test results at each stage. Appropriate in vitro investigations can be used for screening prospective biomaterials for estimations of toxic effect. Cytotoxicity in vitro assay is the first test to evaluate the biocompatibility of any material for use in biomedical devices (Rogero et.al. 2003).
Hydrogels can be synthesized by accomplishing crosslinking via γ-irradiation (Guven, O; et.al. 1999, Saraydın et.al. 1995, 2002, Karadağ et. al. 2004). 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. 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 (Benson 2002). 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) or maleic acid (MA) 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. On the other hand, CA monomer consists of single carboxyl group, while IA and MA monomers are 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.
In our previous other works, *in vitro* swelling and biocompatibility of blood *in vivo* biocompatibility of radiation crosslinked acrylamide co-polymers such as acrylamide (AAm), acrylamide/crotonic acid (AAm/CA), acrylamide/itaconic acid (AAm/IA) and

acrylamide/maleic acid (AAm/MA) hydrogels were investigated (Saraydin et al., 1995, Karadağ et. al. 1996, Saraydin et al., 2001, 2004).

3.1 In vitro swelling of the hydrogels in the simulated body fluids

In this stage of the study, the swelling of the hydrogels in the simulated physiological body fluids was investigated (Saraydin et al., 1995, Karadağ et. al. 1996).

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 (Saraydin et al., 2001, 2002).

The fluid absorbed by the gel network is quantitatively represented by the EFC (equilibrium body fluids content), where: EFC% = [mass of fluid in the gel/mass of hydrogel] x 100. EFCs of the hydrogels for all physiologically fluids were calculated. The values of EFC% of the hydrogels are tabulated in Table 2.

Simulated body fluid	AAm	AAm /CA	AAm /MA	AAm /IA
Distilled Water	86.3	93.9	94,7	92.0
Isoosmotic phosphate buffer	87.5	93.8	89,7	92.2
Gastric fluid	87.7	93.6	92,4	88.7
physiological saline	87.8	92.9	89,7	88.7
Human Sera	88.6	92.5	89.8	86.4
In rat	89.0	93.1	91.9	91.7

Table 2. EFC values of the hydrogels

All EFC values of the hydrogels were greater than the percent water content values of the body about 60%. Thus, the AAm and AAm/CA, AAm/MA and AAm/IA hydrogels were exhibit similarity of the fluid contents with those of living tissues.

3.2 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 ^{O}C.

The mean and standard deviation values of control and test groups for biochemical parameters of human sera are listed in Table 3.

Table 3 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.

3.3 In vivo tissue biocompatibility

In this part, hydrogels based on copolymer of AAm, AAm/MA, AAm/CA and AAm/IA with capacity of absorbing a high water content in biocompatibility with subcutaneous tissues of rats were examined. After one week implantation, no pathology such as necrosis, tumorigenesis or infection were observed in the excised tissue surrounding the hydrogels 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

Biochemical parameters of human serum / Unit	Normal values	Control	AAm	AAm/CA	AAm/MA	AAm/IA
Glucose/mg dl^{-1}	70-110	87.0±8.2	91.0± 6.1	88.1±3.99	88.8±6.0	88.4±5.30
Triglyceride/mg dl^{-1}	40-160	127.3±24.6	127.0±25.8	130.6±19.9	127.2±25.1	125.6±20.7
Cholesterol/mg dl^{-1}	125-350	158.6±10.9	160.6±14.3	159.8±11.3	157.8±10.8	160.6±14.3
BUN/mg dl^{-1}	8-25	14.8±1.27	15.2±4.56	14.6±3.73	15.2±4.10	15.6±3.84
Creatinin/mg dl^{-1}	0.8-1.6	0.98±0.14	1.06±0.17	1.02±0.14	0.98±0.18	1.00±0.18
Total protein/g dl^{-1}	6.0-8.4	6.52±0.15	6.72±0.15	6.70±0.13	6.60±0.22	6.48±0.30
Albumin/mg dl^{-1}	3.5-5.6	4.02±0.15	3.88±0.15	3.98±0.18	3.96±0.20	3.94±0.10
Alkaline phosphatase/U	35-125	53.6± 13.1	54.5±12.3	54.0±14.9	52.6±12.6	52.6± 10.3
Alanine transaminase/U	7-56	14.6±2.12	16.0±2.63	15.7±3.23	15.9±2.47	16.0±2.63
Aspartate transaminase/U	5-40	16.2±5.33	15.2±3.19	16.5±3.03	17.2±5.16	15.2±3.19
Direct bilirubin/mg dl^{-1}	0.0-0.3	0.12±0.04	0.12±0.04	0.11±0.03	0.11±0.03	0.12±0.04
Indirect bilirubin/mg dl^{-1}	0.1-1.1	0.45±0.05	0.35±0.09	0.35±0.09	0.45±0.05	0.40±0.07
Chlorine/meq dl^{-1}	95-107	98.5±2.17	98.8±2.3	98.6±2.12	97.8±1.75	98.2±2.10
Sodium/meq dl^{-1}	137-146	142.7±1.4	142.8± 0.9	143.0±1.6	142.7±1.4	142.0±1.6
Potassium/meq dl^{-1}	3.5-5.5	4.80±0.28	4.68±0.36	4.94±0.39	4.87±0.35	4.70±0.35
Calcium/mg dl^{-1}	8.5-10.8	9.40±0.39	9.47±0.28	9.42±0.28	9.63±0.42	9.47±0.28
Phosphorus/mg dl^{-1}	2.5-4.5	3.60±0.41	3.60±0.32	3.68±0.42	3.60±0.36	3.56±0.38

Table 3. Means and standard deviations of biochemical parameters of human sera

these fibrous capsules consisting of fibroblasts, and a grouped mast cells and lymphocyte were observed between tissues and capsule in the some samples (Figure 15, 16).

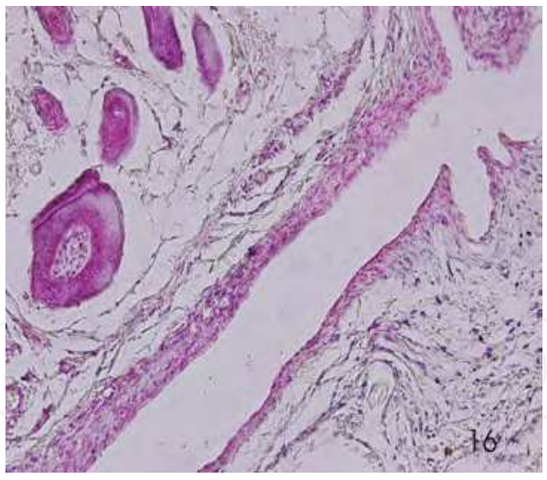


Fig. 15. After one week, the implan-tation site of AAm hydrogel, H-E, 20X

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. 17). No chronic and acute inflammation, adverse tissue reaction were observed in the all test groups. 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.

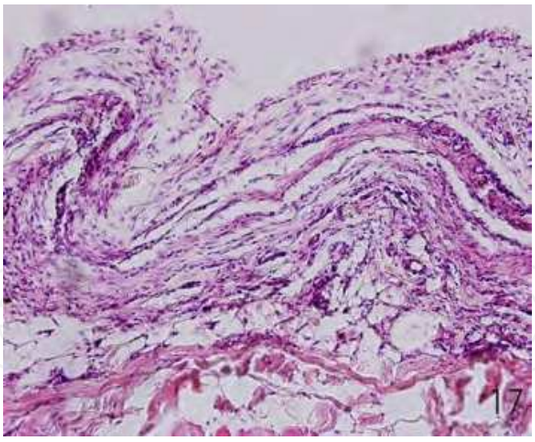


Fig. 16. After 4 week, the implantation site of AAm hydrogel, H-E, 20X

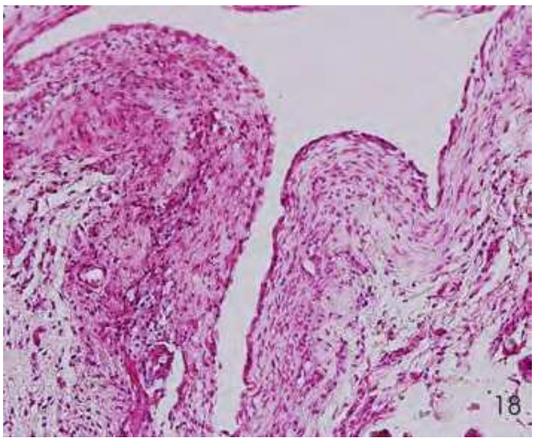
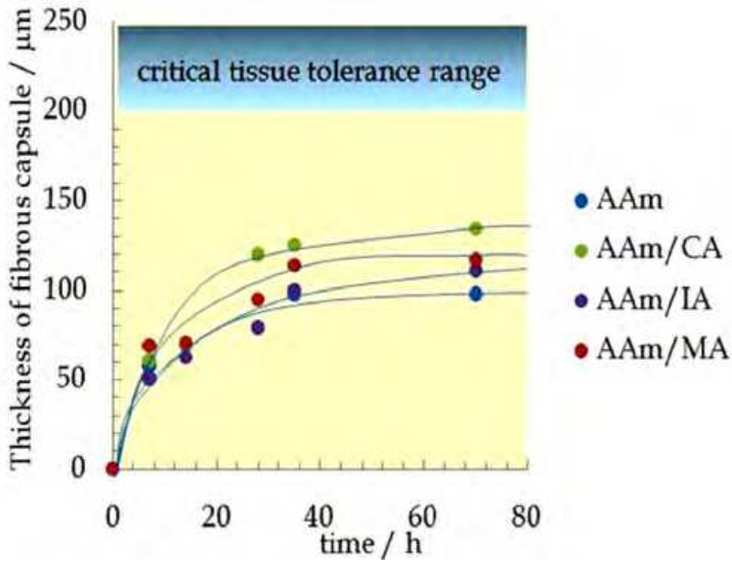


Fig. 17. 10 week postimplantation of AAm/CA hydrogel. H-E, 20X

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. 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/CA, AAm/MA and AAm/IA hydrogels implant are high from the values of AAm and hydrogels. The carboxyl groups on the chemical structure and ionogenic character of AAm/CA, AAm/MA and AAm/IA hydrogels can be caused to the high thickness of the fibrous capsule (Smetana et al., 1990). 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 and shown in Graphic 2. 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/CA, AAm/MA and AAm/IA hydrogels implant are high from the values of AAm and hydrogels. The carboxyl groups on the chemical structure and ionogenic character of AAm/CA, AAm/MA and AAm/IA hydrogels can be caused to the high thickness of the fibrous capsule (Smetana et al., 1990). 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 (Jeyanthi and Rao, 1990). Our results clearly indicated that the capsule thickness of the excised tissue were 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 (Smetana et al., 1990). 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.



Graphic 2. The curves of thickness of fibrous capsule—implantation time.

4. Bioactive ceramic biomaterials

Bioactive refers to a material, which upon being placed within the human body interacts with the surrounding bone and in some cases, even soft tissue. This occurs through a time – dependent kinetic modification of the surface, triggered by their implantation within the living bone. An ion – exchange reaction between the bioactive implant and surrounding body fluids – results in the formation of a biologically active carbonate apatite (CHAp) layer on the implant that is chemically and crystallographically equivalent to the mineral phase in bone. Prime examples of these materials are synthetic hydroxyapatite, glass ceramic and bioglass.

Calcium phosphate ceramics and xenografts have been used in different fields of medicine and dentistry. We demonstrated the effects of calcium phosphate ceramics (Ceraform) and xenograft (Unilab Surgibone) in the field of experimentally created critical size parietal and mandibular bone defects in rats (Develioglu et al., 2006, 2007, 2009, 2010).

Many researches are currently conducted to find out the ideal material to support bone repair or regeneration. The limitations of autogenous grafts and allogeneic bankbone have led to a search for synthetic alloplast alternatives. Calcium phosphate ceramics have been

widely used because the mineral composition of these implants materials does fully biocompatible (Rey C. 1990, LeGeros. 2002). The porous structure of the ceramics is claimed to enhance bone deposition and implant stabilization in the recipient bone. The optimal pore size is still debated to be ranging from 50 and 565 µm (Gauthier et al., 1998, Chang et al., 2000). However, porosity of the material is inversely proportional to the mechanical stability of these calcium phosphate based ceramics (Le Huec et al., 1995). This loss of stability is often cited as a limitation in the use of calcium phosphate-based ceramics in clinical practice. A convenient compromise to overcome this problem is to use a biphasic ceramic, which maintains its mechanical resistance until the resorption is achieved (Gauthier et al., 1998).

Various types of xenografts are used in medicine, dentistry, and also in periodontology. One of the xenografts is Unilab Surgibone, which is currently being used succesfully in medicine and implantology. Moreover, osteoconductive properties are also known (Zhao et al., 1999). Unilab Surgibone is obtained from freshly sacrificed calves which is partially deproteinized and processed by the manufacturers. It is available in varius shapes like tapered pins, blocks, cubes, granules, circular discs and pegs (Balakrishnan et al., 2000). Xenograft materials, bovine bones have been the most preferred ones, basically because they are easily obtainable and there are no great ethical considerations. Additionally they have the great advantage of practically unlimited availability of source/raw material. Partially deproteinized and defatted preparations (e.g.Unilab Surgibone) was indicated reduce antigenity and mild immune response (William et al., 2008).

Generally, xenografts are one of the alternative graft materials used in different fields for filling osseous defects Slotte and Lundgren, 1999, Salama 1983). Nonetheless, an interesting alternative to xenografts is Biocoral® (natural coral), which has been shown to exhibit osteoconductive and biocompatible properties whereby gradual replacement with newly formed bone occurred after its resorption (Guillemin et al.,1989, Doherty et al., 1994, Yılmaz and Kuru, 1996, Yukna Ra and Yukna CN, 1998).

Another xenogeneic, bone-derived implant material is Bio-Oss, which is similar to the xenograft investigated in our studies (Develioglu et al.2009, 2010). Bio-Oss has been proposed as a biocompatible graft material for bony defects for it has shown osteoconductive properties — that is, it was replaced with newly formed bone after grafting (Yıldırım et al, 2001, Sculean et al., 2002, Carmagnola et al., 2002). However, regarding the resorption of Bio-Oss, contradicting reports have emerged. On one hand, a previous study revealed that the bovine bone mineral underwent resorption (Pinholt et al., 1991). On the other hand, numerous researchers claimed that the resorption process of Bio- Oss® was very slow (Skoglund et al., 1997, Jensen et al 1996, Klinge et al., 1992).

In our previous studies with Ceraform (calcium phosphate ceramics) and xenograft (Unilab Surgibone), multinuclear giant cells (MNGC) were observed in the implantation region on 1st, 3rd, 6th ve 18th months.

The observed MNGCs are featured morphologic characteristics of foreign body giant cell (FBGC). These cells are osteoclast-like cells. Both cell types develop from a common precursor (Anderson, 2000) Since foreign body giant cell (FBGC) are the fusion products of monocytic precursors, which are also the precursors of macrophages, (Brodbeck at al., 2002, Matheson et al., 2004) the presence of such leukocytes in the wound healing compartment may be of central importance in driving the tissue reaction to the material. No necrosis, tumorigenesis, or infection was observed at the implant site up to 18 months (Figure 18-20).

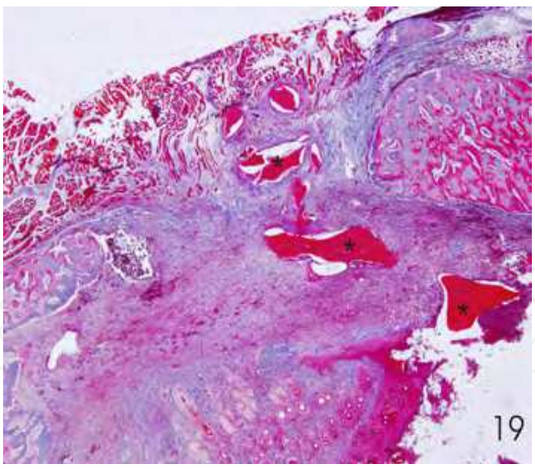


Fig. 18. Remnants of the Xenograft (*) surrounded by fibrous tissue at 30 days. A-T 4X.

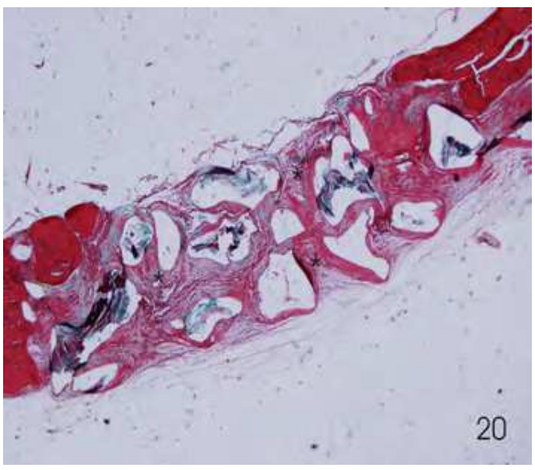


Fig. 19. A dense, fibrovascular tissue (*) in the side of ceraform implantation at 12th month M-T, 4X.

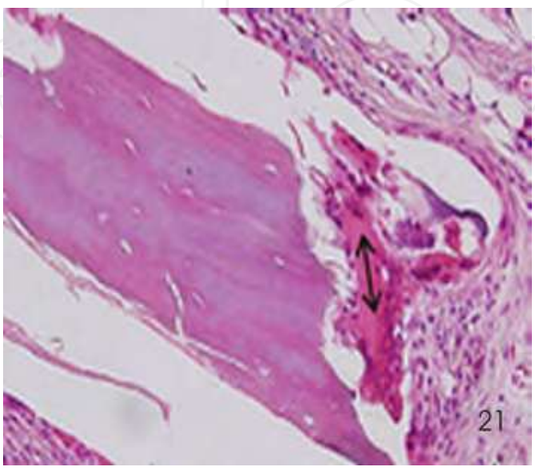


Fig. 20. Multinuclear giant cell (↔)in the implantation site. H-E, 40X

A long-term study would be useful to evaluate the biological degradation behavior of the material utilized in this study. BCP ceramics are well known to be biodegradable due both to body fluid dissolution and bio resorption cellular activity Nery et al., 1990, Piatelli et al., 1996). It might indicate that the implants utilized in our studies are progressively resorbed, but the size of the particle might be big (Handschel et al., 2002) The studies reveal that Ceraform and xenograft are biocompatible. However, the materials did not promote bone formation.

5. Conclusion

In conclusion, while some vinyl monomers had cytotoxic effects on tissues, their polymers, Ceraform and Unilab Surgibone were found to be biocompatible in soft and hard tissues and they seem only to be beneficial bone filler materials in treatment of the bone defects. Unilab Surgibone and xenograft could be used as bone filler materials in the treatment of traumatic and post-traumatic skeletal complications (e.g delayed unions, non-unions), defects due to bone removal (e.g. bone tumors, congenital diseases) or low bone quality (e.g. osteoporosis, osteopenia) and in other medical fields.

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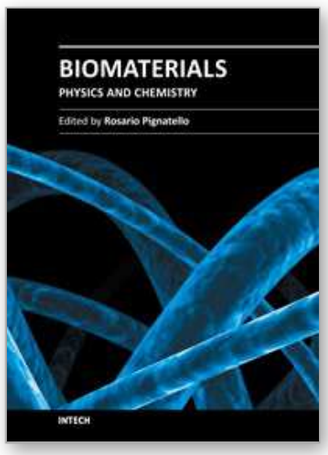

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These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentialities of different synthetic and engineered biomaterials. Contributions were selected not based on a direct market or clinical interest, but based on results coming from very fundamental studies. This too will allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessarily imposed by industrial or profit concerns. The chapters have been arranged to give readers an organized view of this research area. In particular, this book contains 25 chapters related to recent researches on new and known materials, with a particular attention to their physical, mechanical and chemical characterization, along with biocompatibility and hystopathological studies. Readers will be guided inside the range of disciplines and design methodologies used to develope biomaterials possessing the physical and biological properties needed for specific medical and clinical applications.

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