Osmotically active hydrogels of acrylics: characterization and application as tissue expander

Ph.D. Thesis

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LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE DISSERTATION

1. László Janovák, **János Varga**, Lajos Kemény, Imre Dékány: Swelling properties of copolymer hydrogels in the presence of montmorillonite and alkylammonium montmorillonite. Appl Clay Sci 2009; 43:260-270. (**IF_{2008}: 2,005**)
2. **János Varga,** László Janovák, Erika Varga, Gábor Erős, Imre Dékány, Lajos Kemény: Acrylamide, acrylic acid and N-isopropylacrylamide hydrogels as osmotic tissue expanders. Skin Pharmacol Physiol 2009; 22(6):305-312. (**IF_{2008}: 2,388**)
3. **Varga János,** Janovák László, Varga Erika, Erős Gábor, Dékány Imre, Kemény Lajos: Akril alapú szöveti expanderek sebészeti felhasználhatóságának vizsgálata. …

PATENT APPLICATIONS RELATED TO THE SUBJECT OF THE DISSERTATION

1. Kemény Lajos, Dékány Imre, **Varga János,** Janovák László: N-izopropil-akrilamid, akrilamid és akrilsav polimerizációjával szintetizált hidrogélek rétegszilikátokkal készült nanokompozitjai, eljárás ezek előállítására és alkalmazásuk ozmotikusan aktív hidrogél szövettágító expanderekben bőr nyerésére. Magyar Szabadalom, bejelentés ideje: 2007. május, Ügyiratszám: P0700384
2. Lajos Kemény, Imre Dékány, **János Varga,** László Janovák: Layer silicate nanocomposites of polymer hydrogels and their use in tissue expanders. International Publication Number: WO 2008/146065 A1

LIST OF OTHER FULL PAPERS

1. László Janovák, **János Varga,** Lajos Kemény, Imre Dékány: Investigation of the structure and swelling of poly(N-isopropyl-acrylamide-acrylamide) and poly(N-isopropyl-acrylamide-acrylic acid) based copolymer and composite hydrogels. Colloid Polym Sci 2008; 286:1575-1585. (**IF_{2008}: 1,736**)
2. László Janovák, **János Varga,** Lajos Kemény, Imre Dékány: Composition dependent changes in the swelling and mechanical properties of nanocomposite hydrogels. Nanopages 2008; DOI: 10.1556/Nano.2008.00002.

3. László Janovák, **János Varga,** Lajos Kemény, Imre Dékány: The effect of surface modification layer silicates on the thermoanalitical properties of poly(NIPAAm-co-AAm) based composite hydrogels. J Therm Anal Calorim 2009; DOI: 10.1007/s10973-009-0311-1. (**IF_{2008}: 1,630**)

1. LIST OF ABBREVIATIONS

AAc:	Acrylic acid
AAm:	Acrylamide
BisAAm:	N,N'-methylenebisacrylamide
d:	Diameter
G':	Storage modulus
G":	Loss modulus
H&E:	Hematoxylin-eosin
KPS:	Potassium persulphate
l:	Length
LCST:	Lower critical solution temperature
m_{dry}:	Dry mass
m_{wet}:	Moisture mass
Na-m:	Sodium montmorillonite
NIPAAm:	N-isopropylacrylamide
S:	Swelling value
TEMED:	N,N,N',N'-tetramethylethylenediamine

2. INTRODUCTION

2.1. BACKGROUND

Gaining of soft tissue for the reconstruction of defects and injuries is a pivotal question of plastic and reconstructive surgery. Thus, many different methods have been developed for the harvesting of tissue for surgical interventions. The subcutaneous balloon is known as the first generation of tissue expanders. After implantation into the subcutaneous layer this device can be distended progressively, hereby leading to skin expansion (**Neumann, 1954**). This method was proven to be effective and it has therefore become accepted and widely used (**Radovan, 1978; 1982**). Tissue expansion has revolutionized plastic surgery in the last 30 years. Burns, trauma and sequelae of previous surgery are the most frequent indications (**Cunha et al., 2002**), but the area of their application is increasing. They are frequently used in breast surgery since it has been shown that tissue expanders allow the harvesting of quantitatively deficient soft tissues and produce breasts with a natural appearance after mastectomy (**Escudero et al., 1997**). Additionally, permanent expandable implants can be very useful in breast aesthetic surgery (**Berrino et al., 1998**). Tissue expanders can be utilized to prevent certain complications in orthopedic surgery (**Gold et al., 1996**) and also have indications in gynecology (**Belloli et al., 1997; Wu et al., 2003**).

Although the application of tissue expanders is accompanied by an acceptable failure rate (**Farzaneh et al., 2006**), it involves the risk of various complications and inconveniences. Regular control is required, which is time and cost-consuming. The overlying skin must be pierced when the balloon is filled; this leads to pain and fear, especially in children. In consequence of the design of the filling valve and the balloon, damage to the expander is frequent. Increasing pressure in the balloon may result in tissue hypoxia (**Pietila et al., 1988**), which may decrease the local perfusion, thereby causing necrosis and perforation. Infection, leakage, migration, flap necrosis and wound separation are further possible complications (**Hurvitz et al., 2005**).

Hence, the development of a new generation of tissue expanders was essential in order to eliminate these disadvantages. The first self-filling expander was described in the early 1980s (**Austad et al., 1982**). This device was a permeable balloon containing hyperosmotic NaCl solution. However, the expansion period was too long and was frequently accompanied by rupture of the balloon, leading to tissue necrosis. Thus, a new design was sought which is independent of hyperosmotic solution. The attention was therefore drawn to the hydrogels as

promising materials in tissue expansion. Hydrogels are 3D lattices containing hydrophilic and hydrophobic parts in appropriate proportions. They consist of two major components; a polymer web of constant quantity and a hydrous phase which changes in volume (**Refojo, 1976**). When hydrogels are placed in aqueous medium, they swell to several times their initial volume without either dissolving or changing their shape to a considerable extent. These substances are also called “intelligent gels”, because – depending on their composition – they react to changes in one or several environmental parameters (temperature, pH, light, magnetic field etc.) and “respond” with a functional reaction (swelling, shrinking, sol-gel conversion). Due to their advantageous properties, they are widely applied in different areas e.g. environmental protection and agriculture (**Kioussis et al., 2005; Liu et al., 2005**). Many properties of hydrogels make them suitable for biomedical applications that require contact with living tissue. Thus, they are also utilized in medicine for controlled drug release, wound treatment, biosensors, etc. (**Benoit et al., 2006; Khetani et al., 2006; Hervas Perez et al., 2006**). Furthermore, osmotically active hydrogels seemed to provide an effective means of tissue expansion (**Wiese, 1993**). Since the dry gel absorbs body fluids, its volume increases and it dilates the tissue without any external intervention. Self-filling osmotic tissue expanders have been used in clinical practice with a success rate of approximately 90%, the cosmetic results are very satisfactory and the expanders are well tolerated by the patients (**Berge et al., 2001; Ronert et al., 2004**). The currently applied tissue expanders contain methyl methacrylate and N-vinyl-2-pyrrolidone. These components allow the expander to swell to 10 times its original mass. Their non-toxicity has been proved as well (**Wiese, 1993; Bacskulin et al., 2000; Wiese et al., 2001**).

Thus, osmotically active tissue expanders possess several advantages as compared to traditional tissue expanding devices. The applied hydrogels can be very small, therefore minor skin incision is required for their implantation. Since the surgical trauma is reduced and external filling is not needed, the duration of hospitalization and the level of patient discomfort are reduced. Moreover, their application is faster and simpler. Nevertheless, the expansion properties of these devices could be improved in order to decrease the period of indwelling and to increase the tissue gain. Recent studies have drawn our attention to materials with high tendency to swell, hereby potentially being able to induce skin growth. The hydrogels of both acrylamide (AAm) and AAm-based copolymers exhibit a very high capability to absorb water. Furthermore, they are permeable to oxygen and possess good biocompatibility (**Güven et al., 1999; Saraydyn et al., 2004**).

In addition to swelling ability, other factors should also be considered in order to regulate the behaviour of the hydrogels and to produce optimal biomaterials. Thermally reversible hydrogels have recently attracted increasing interest in the biomedical field. Poly(N-isopropylacrylamide) [poly(NIPAAm)] is one of the most preferred members of this family in these applications. The thermosensitive behaviour of poly(NIPAAm) gels has been extensively investigated and modelled by different working groups (**Li et al., 2001; Chen et al., 2002; Szilágyi et al., 2005; Guilherme et al., 2006**). Poly(NIPAAm) hydrogel exhibits a lower critical solution temperature (LCST) at around 32 ^{o}C in aqueous solutions. Gels display collapse triggered by an increase in temperature both on the bulk and on the micron scale (**Sierra-Martín et al., 2005 a, b**). At temperatures exceeding the LCST, the state of hydrogels changes from swollen (hydrophilic) to collapsed (relatively hydrophobic). When gels are polymerized at temperatures over the LCST, samples containing heterogeneities of different hydrophilicities are obtained due to the above. As polymerization temperature is increased, the number of hydrophobic sites within the gel matrix will increase (**Hirokawa et al., 1999**). Thus, the hydrophobicity of the gel obtained increases with elevating polymerization temperature. This property of NIPAAm-based materials can be utilized in order to produce thermosensitive tissue expanders with a special behaviour under *in vivo* circumstances.

It is well-known that the properties of gels can be significantly enhanced by the incorporation of inorganic ordered systems, in particular clays, into the gels (**Alexandre et al., 2000; Xia et al., 2003; Shibayama et al., 2004; Sinha Ray et al., 2005; Haraguchi et al., 2006**). As a model system, sodium montmorillonites (Na-m) are widely used as additives to improve the physical properties of plastics (**Churochkina et al., 1998; Yeh et al., 2004; Coughlan et al., 2006; Kumar et al., 2006**).

These findings and data suggested that there would be a need for novel tools for tissue expansion in plastic and reconstructive surgery. They revealed that hydrogels of acrylics seemed to be promising expander-candidates which worth studying. Furthermore, they served as a guideline during design of our experiments.

2.2. AIMS

Our first goal was to synthesize thermo- and pH-sensitive hydrogels, to be tested as skin expanders. We set out to develop copolymer and composite hydrogels that, when implanted under the human skin, swell osmotically thereby leading to tissue expansion. In this respect a series of temperature- and pH-sensitive copolymer gels was prepared by redox polymerization of NIPAAm, AAm and acrylic acid (AAc). Copolymer gels were obtained by varying the initial molar ratios of NIPAAm, AAm and AAc.

Our further aim was to get the possible highest swelling under physiological conditions using the above mentioned materials. The swelling ability of the gels was enhanced by the addition of fillers, Na-m and Na-m organophilized with alkylammonium ions (C_n-m, n=4, 12, 18). The influence of fillers with different hydrophilicity on the swelling of various hydrophilic polymers and copolymers was also studied.

Moreover, our objective was to characterize the swelling rate and expansion kinetics of the hydrogels under *in vivo* circumstances. Another important goal was to examine their biocompatibility and rheological parameters after implantation in order to decide whether these polymers can be used in plastic and reconstructive surgery. In this regard, the protocol was divided into two parts. In the first study the hydrogels were tested *in vitro*. The major aims were:

- to examine the effects of external factors (temperature, pH, electrolyte concentration) on the swelling ability of the polymers;
- to characterize the swelling of copolymers containing different ratios of NIPAAm, AAm and AAc; and
- to study the impact of different fillers on the osmotic properties of the hydrogels.

In addition to this investigation, an *in vivo* study was also designed in an animal (rodent) model. Here the major aims were:

- to observe the swelling of implanted hydrogels as a function of time;
- to determine the changes in their mass after a period of indwelling;
- to assess their rheological parameters; and
- to study the biocompatibility of the implanted polymers and copolymers.

3. MATERIALS AND METHODS

3.1. IN VITRO STUDY

3.1.1. MATERIALS

NIPAAm, AAm and AAc were used as monomers. Monomers and the cross-linking agent N,N'-methylenebisacrylamide (BisAAm) were obtained from Aldrich Chemical Company, Inc., and were used without further purification. Other chemicals used were potassium persulphate (KPS) from Reanal Kft. as an initiator and N,N,N',N'-tetramethylenediamine (TEMED) from Fluka Chemie AG. as an accelerator.

3.1.2. PREPARATION OF POLYMERS

NIPAAm, AAm and AAc polymers and copolymers with various compositions were prepared by radical polymerization. The appropriate amount(s) of monomer(s) were dissolved in 10 ml distilled water and BisAAm, the initiator (KPS) and the accelerator (TEMED) were added to the polymerization medium. The compositions of all reagent used to prepare the hydrogels are summarized in **Table 1**. The monomer/crosslinker molar ratio was 200 in each case and the amount of KPS and TEMED was also constant. KPS and TEMED formed a redox pair for the purpose of radical polymerization. Polymerization was carried out in test-tubes. The reaction was performed at 60 ^{o}C for 30 min under N_2 atmosphere. After polymerization the samples were removed from the thermostated water bath.

For the synthesis of organophilized montmorillonite fillers, 0.01 mol alkylammonium salt with selected carbon chain length (C_nH_{2n+1}-NH_2, n=4, 12, 18) was dissolved in 250 ml ethanol-water mixture (1:1) (pH=4), the solution was added to Na-m swollen in distilled water at a ratio of 100 meq/100 g montmorillonite (10 g montmorillonite in 100 ml distilled water) and the system was stirred for 24h at room temperature. After the completion of ion exchange the suspension was centrifuged, washed and filtered. The hydrophobized filler obtained was dried and ground to 200 μm particle size.

The synthesis of composites was carried out in a similar manner; in the case of Na-m and organophilized Na-m, however, before the addition of monomers and other chemicals, the appropriate amount of montmorillonite was thoroughly suspended in distilled water under ultrasonic irradiation for 1 h. In the course of the synthesis of composites, fillers of various

qualities (Na-m and C_4-, C_{12}-, C_{18}-montmorillonite) and quantities (1, 5, 10, 25 wt.%) were included in the samples listed in **Table 1**. In the case of each composite, the amount in grams of monomers and cross-linkers present in the given solution was first added up and the amount of filler to be added was calculated as a percentage of that amount.

Table 1.

Molar composition of copolymers and other reagents

Sample code	**Amount of monomers**			**Quantity of other materials**		
	NIPAAm (mol)	**AAm** (mol)	**AAc** (mol)	**BisAAm** (mol)	**KPS** (g)	**TEMED** (g)
Poly(NIPAAm)*	0.01	0	0	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(NIPAAm-co-AAm)*	0.005	0.005	0	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(NIPAAm-co-AAm)	0.008	0.002	0	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(NIPAAm-co-AAm)	0.002	0.008	0	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(AAc)*	0	0	0.01	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(NIPAAm-co-AAc)*	0.005	0	0.005	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(NIPAAm-co-AAc)	0.008	0	0.002	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(NIPAAm-co-AAc)	0.002	0	0.008	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(AAm)*	0	0.01	0	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(AAm-co-AAc)*	0	0.005	0.005	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(AAm-co-AAc)	0	0.008	0.002	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$
Poly(AAm-co-AAc)	0	0.002	0.008	$5x10^{-5}$	$2x10^{-3}$	$7.75x10^{-3}$

*: hydrogels produced with fillers, as well

3.1.3. DETERMINATION OF SWELLING

Swelling was determined gravimetrically, using the following formula: swelling value (S)=(m_{wet}-m_{dry})/ m_{dry} [g/g], where m_{wet} and m_{dry} are the mass of the gel in moisture (swollen) and dried state, respectively. The dry gels were placed into thermostated water bath. Swollen gels were removed from the water bath, they were dried superficially with filter paper, weighted with analytical scales (Mettler AE 260, Greifensee, Switzerland) and returned into the same bath. The swelling of hydrogel was investigated in the temperature range of 25-40 ^{o}C. Some samples were placed into physiological saline (pH=7.4) at 36.5 ^{o}C. In some cases the pH of the environment was changed, as well.

3.2. IN VIVO STUDY

Hydrogels displaying outstanding swelling properties according to the results of the *in vitro* study were chosen for *in vivo* testing. Special attention was paid to the remaining monomers and other reagents. Gels involved in the *in vivo* study were washed with distilled water in the end of the polymerization in order to remove unreacted monomers, cross-linker and initiator. The washing period took two weeks and the water was changed three times a day.

3.2.1. ANIMALS

The experiments were performed on 18 male Wistar rats (body weight: 400±25 g). All interventions were in full accordance with the NIH guidelines. The procedures and protocols applied were approved in advance by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged.

3.2.2. SURGICAL INTERVENTION

The animals were anesthetized with Na-pentobarbital (45 mg/kg i.p.). The hair of the dorsal region was removed and a skin incision was made. A small pocket was formed between the dorsal fascia and the panniculus muscle with blunt preparation (**Figure 1**). Cylinder shaped hydrogels were produced with a diameter (**d**) of approximately 10 mm and a length (**l**) of approximately 20 mm (**Figure 2**).

Prior to the implantation m_{dry}, **d** and **l** values were measured. The hydrogels were placed into the preformed pockets and brought into such a position that the cylinders were parallel to the vertebral column. The wound was closed with simple interrupted sutures. The animals were then returned to their cages, where they were provided with free access to food and water and were maintained in a thermoneutral environment (23±2 ^{o}C).

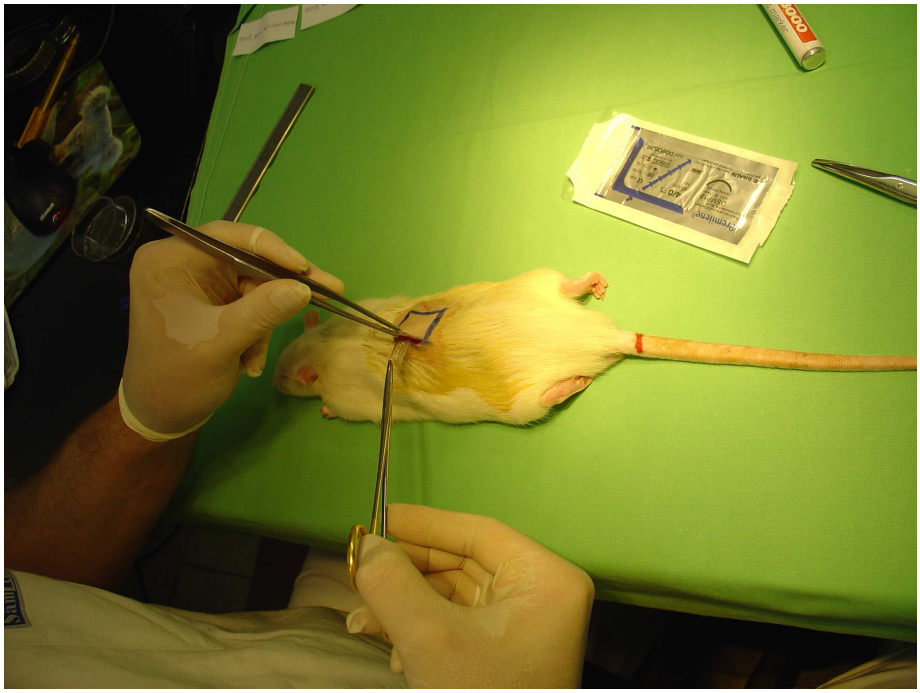


Figure 1. Preparation of animals for hydrogel implantation

3.2.3. EXAMINATION OF EXPANSION RATE

During the postoperative period the animals were carefully observed for the signs of pain. The diameter and the length of the implanted expanders were measured daily with millimetre callipers and photographs of the dorsal region were taken. The rate of expansion was given as a function of time, as a product of **d** and **l** values. The observation period took 18 days. On postoperative day 18 the animals were sacrificed and the expanders were removed. The m_{wet} of the hydrogels was measured immediately. m_{wet} and m_{dry} were determined with analytical scales and S values were calculated by the formula $S=(m_{wet}-m_{dry})/ m_{dry}$, as described above.

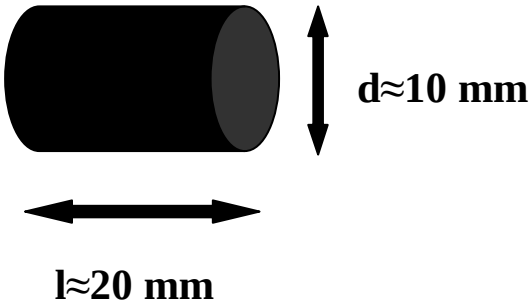


Figure 2. Size of the implanted expanders

3.2.4. RHEOLOGICAL MEASUREMENTS

The dynamic rheological properties of the hydrogels were determined. An oscillatory rheometer (RS 150, Haake, Karlsruhe, Germany) equipped with 20-mm plates in parallel-plate geometry was used for the measurements. The storage modulus (G') was given to characterize the elastic property and the loss modulus (G") to describe the viscosity. Measurements were made as a function of frequency from 0.1 to 1 Hz at a constant shear stress of 1 Pa at 25 ^{o}C. The temperature was controlled with a Haake DC 30/K20 thermostat. G' and G" values are given in Pascal.

3.2.5. HISTOLOGY

Biopsies were taken from the intact skin, the expanded skin and the capsule surrounding the expander. The tissue samples were placed into a 4% solution of formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E). The evaluation was performed in coded sections by a professional pathologist.

3.2.6. STATISTICAL ANALYSIS

Data analysis was performed with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany). Non-parametric methods were used. Friedman repeated measure analysis of variance on ranks was applied within the groups. Time-dependent differences from the baseline were assessed by Dunn's method. Differences between groups were analysed with Kruskal-Wallis one-way analysis of variance on ranks, followed by

Dunn's method for pairwise multiple comparison. The Figures give median values and 75th and 25th percentiles. A p value of <0.01 was considered statistically significant.

4. RESULTS

4.1. IN VITRO STUDY

4.1.1. EFFECTS OF EXTERNAL FACTORS ON SWELLING

We studied the effects of different external factors: temperature, pH and electrolyte concentration on the extent of lattice swelling of polymer and copolymer lattices. **Figure 3A** and **B** represent the swelling of gels as a function of temperature. Maximum swelling of the thermosensitive poly(NIPAAm) was observed at 31 ^{o}C; at higher temperatures the gel collapsed. When the NIPAAm monomer was copolymerized with AAm or AAc at a molar ratio of 50/50, the swelling of the samples continuously increased with the elevating temperature i.e. the copolymer no more exhibited the collapse characteristic of NIPAAm. Hydrogel containing AAm and AAc (molar ratio 50/50) displayed the most extensive swelling of the studied polymers and copolymers (**Figure 3B**). The slope of the curves became steeper with increasing hydrophilicity, indicating that the more hydrophilic the gel is, the larger becomes the increase in swelling brought about by the elevating temperature.

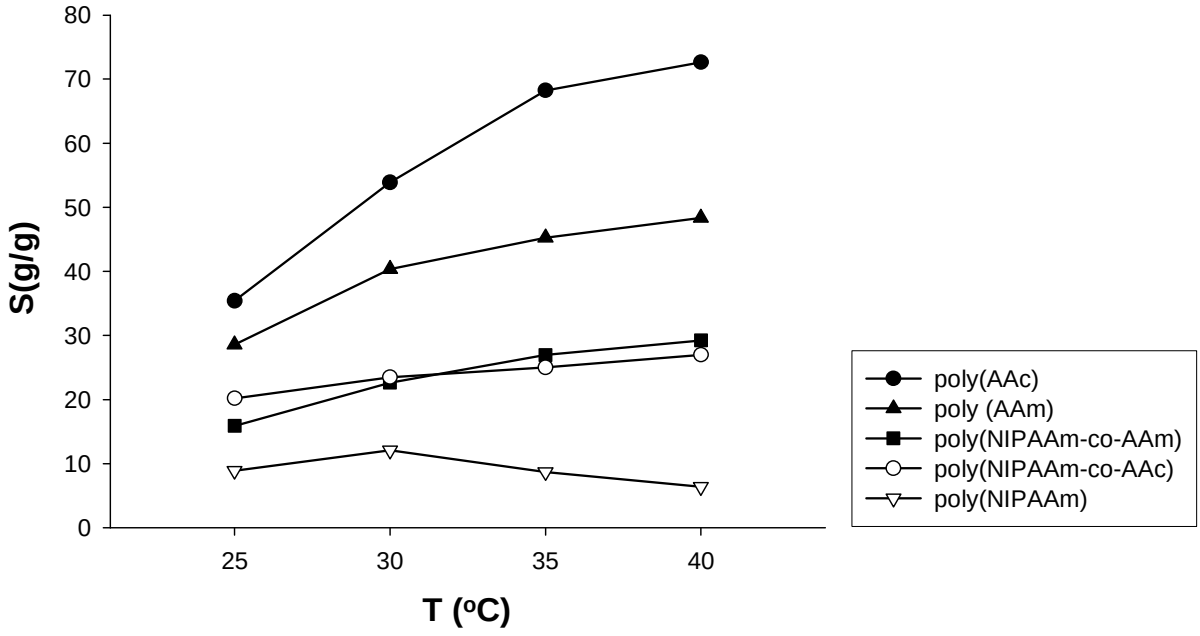


Figure 3A. The temperature-dependence of polymers' and copolymers' swelling

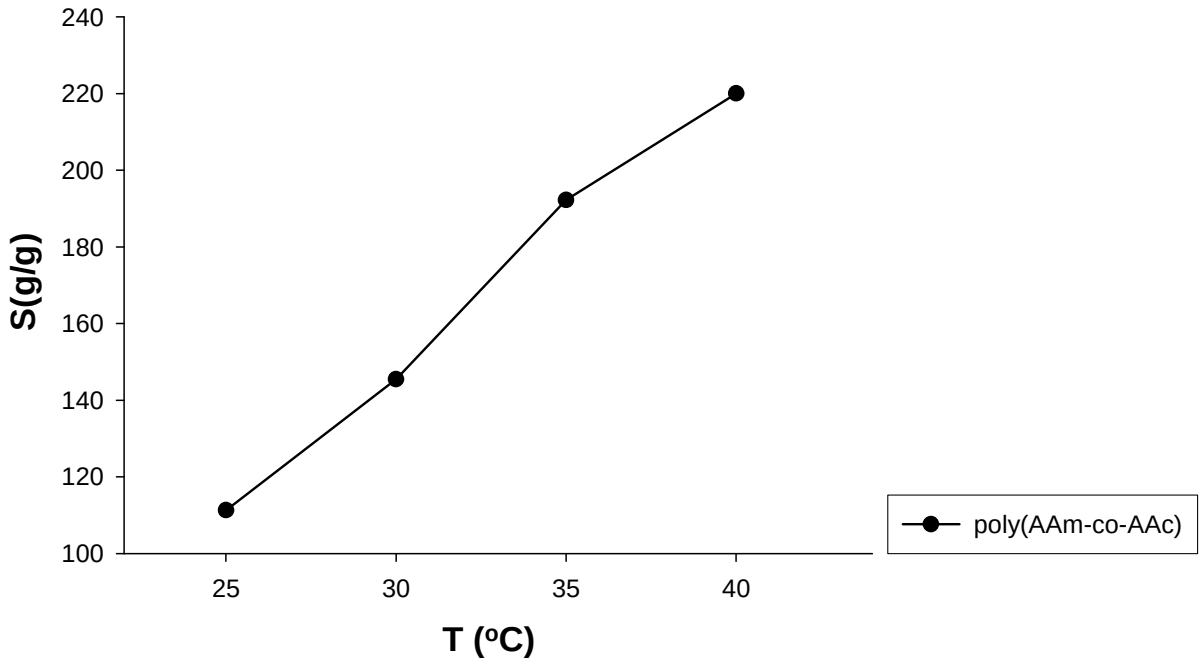


Figure 3B. The temperature-dependence of the swelling of poly(AAm-co-AAc) hydrogel

The swelling of poly(NIPAAm), poly(AAm) and poly(AAc) gels has also been studied as a function of pH (**Figure 4**). Changes in pH are shown to have a diverse effect on the swelling of different polymers. NIPAAm-based samples swelled to 18-20-fold their mass in practically the entire pH range studied, whereas swelling of AAm- and AAc-based gels was markedly dependent of pH. The swelling maximum of AAm polymer was at pH=7-8 and the gel swelled to 58-fold its dry mass at this pH value. The AAc polymer exhibited the highest pH dependence. The peak of swelling was around pH=9, at this pH value 1g of xerogel was capable of absorbing 259g of water.

Table 2 compares the swelling of polymers and copolymers in distilled water and physiological saline solution. A constant pH (pH=7) and temperature (37 ^{o}C) were maintained throughout the experiment. The values measured in saline were lower than those detected in distilled water in the case of all samples. However, the differences depended markedly on the type of copolymer. AAm-based gels were the least sensitive to salt content, while the swelling of NIPAAm- and AAc-based samples was considerably influenced by electrolyte concentration. The former swelled by only 13.78% better in distilled water, whereas swelling of AAc-based hydrogels in distilled water was nearly 7 times higher than the value measured

in saline solution. When the AAm monomer was copolymerized with AAc, the value of the difference approached 17.

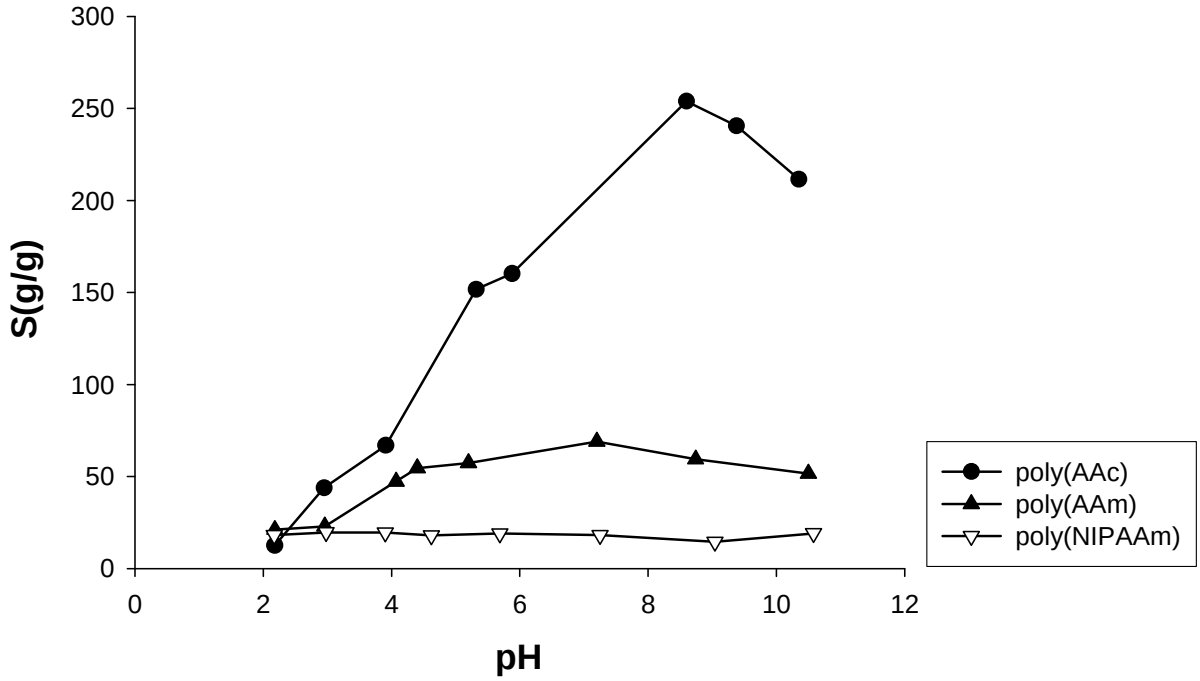


Figure 4. The pH-dependence of the hydrogels' swelling

Table 2.

Swelling of polymers and copolymers in distilled water and in physiological saline solution at 37 ^{o}C (Molar ratio of copolymers was 50/50 of each initial monomer)

Polymer/ copolymer	**Swelling in distilled water [S (g/g)]**	**Swelling in saline [S (g/g)]**	**ΔS* (%)**
Poly(NIPAAm)	7.2	0.5	1440
Poly(NIPAAm-co-AAm)	35.6	31.3	113.74
Poly(AAm)	46.4	33.1	140.18
Poly(NIPAAm-co-AAc)	26.1	0.6	4350
Poly(AAc)	69.5	10.5	661.9
Poly(AAm-co-AAc)	201.5	12.5	1612

*: $\Delta S=(S_{\text{distilled water}}/S_{NaCl})x100$ (%)

4.1.2. EFFECTS OF COMPOSITION OF THE GELS ON SWELLING

In the present study it was also examined whether the changing of molar ratio of the different monomers or the addition of inorganic fillers influence the swelling ability of the hydrogels. **Figure 5-7** demonstrate the effects of monomers with various hydrophilicity on the extent of lattice swelling. 100% AAm-based gels swelled to 30-50-fold their dry mass depending on the temperature. In the AAm/NIPAAm range of 100/0 – 80/20 the extent of swelling decreased by 16.8 g/g depending on the temperature; in the molar ratio range of 20/80 – 50/50 it remained linear. In this range the curves ran parallel; i.e. the swelling of the samples was enhanced by the increased temperature to identical extents. In the next range, between molar ratios of 50/50 – 20/80 the swelling of the gels decreased by a further16.5 g/g. Comparison of the two endpoints of the curves, i.e. swelling of pure AAm and pure NIPAAm based polymers, revealed that AAm-based gels swelled to 30-50-fold their dry mass while NIPAAm-based gels to 6-12-fold their dry mass depending on the temperature. (**Figure 5**).

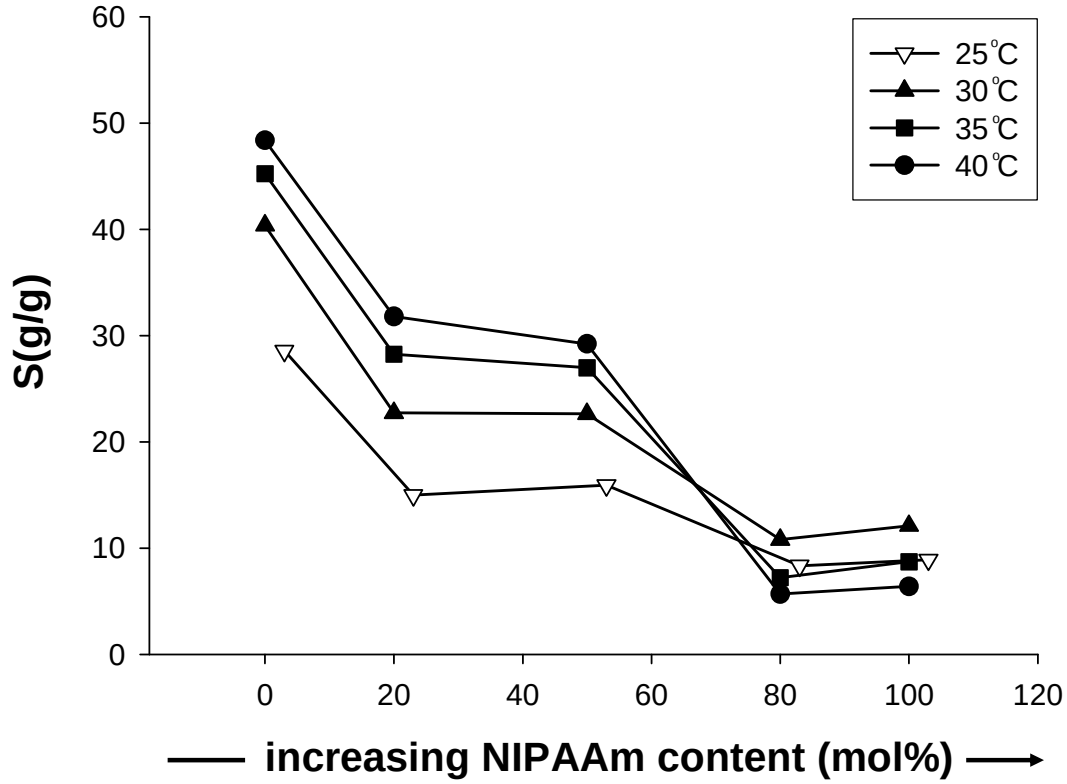


Figure 5. The swelling of poly(NIPAAm-co-AAm) copolymers of different composition in distilled water

In the case of NIPAAm-AAc based copolymers, the 100% AAc-based polymer swelled 35-73-fold, depending on the temperature. Increasing molar ratio of NIPAAm resulted in a decrease in swelling. In the range of AAc/NIPAAm = 100/0 – 80/20 the decrease in swelling of the gels was markedly temperature-dependent: at relatively high temperatures (40 ^{o}C) the extent of decrease was 30 g/g, whereas at 25 ^{o}C it came to 2 g/g. It has been revealed that a higher AAc content (AAc/NIPAAm molar ratio 80/20) has a stronger effect on the temperature dependence of swelling, while at NIPAAm contents over 20% the curves run parallel. Starting from an AAc/NIPAAm molar ratio of 80/20 the decrease is linear and a 10% change in molar ratio in favour of the hydrophobic NIPAAm monomer resulted in an average decrease in swelling of 10 g/g (**Figure 6**). Furthermore, both **Figure 5** and **6** reveal the thermosensitivity of these hydrogels. In case of NIPAAm-AAm based samples the thermosensitivity became pronounced over 70% NIPAAm content: the gels swelled at 30 ^{o}C twice more extensively than at higher temperatures. Concerning NIPAAm-AAc based copolymers thermosensitivity was also found, but the effect of higher temperature on the swelling was not so expressed that in the former case: the difference was only 50%.

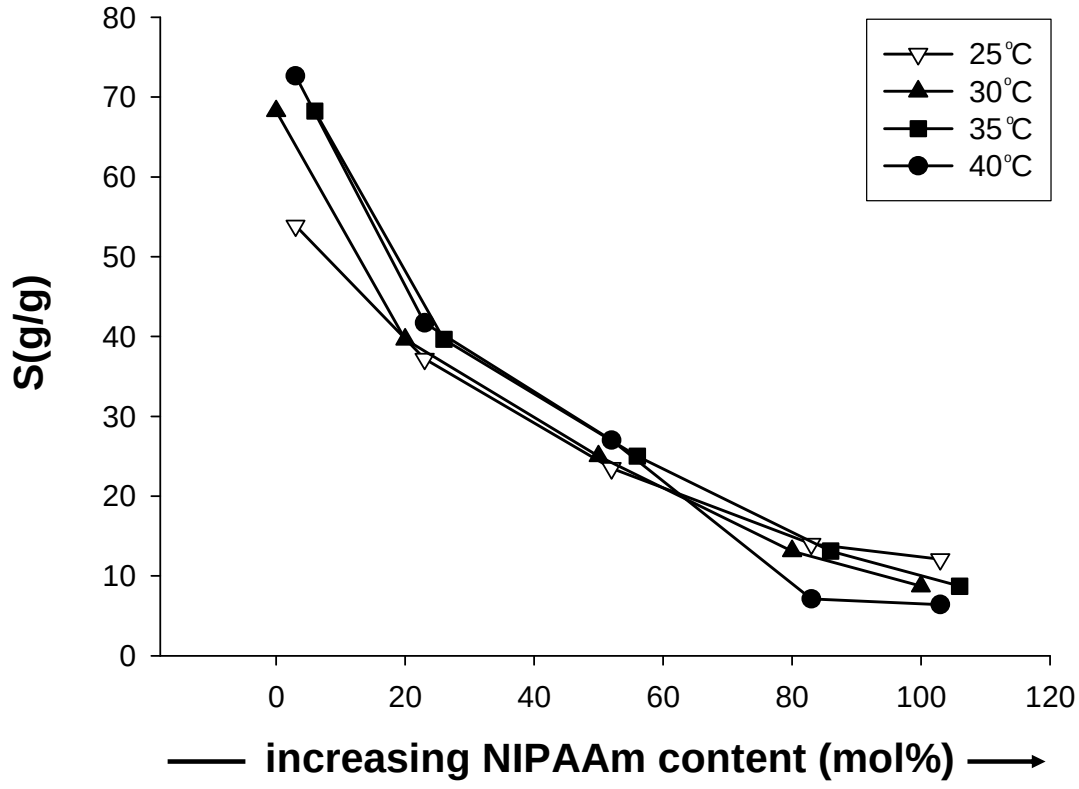


Figure 6. The swelling of poly(NIPAAm-co-AAc) copolymers of different composition in distilled water

Examination of the swelling of samples obtained by copolymerization of the two hydrophilic monomers AAm and AAc [i.e. poly(AAm-co-AAc)] as a function of composition showed that the best swelling gels are those that contain 50% each of AAm and AAc. Swelling of the copolymer of this composition was 110-220-fold the dry mass of the sample depending on the temperature. Increasing the molar ratio of one monomer was accompanied by the reduction of swelling ability. Moreover, the distance between the curves at a molar ratio of 50/50 is increased, i.e. the increase in swelling due to the increased temperature was the largest at this composition. Since the gels did not contain NIPAAm monomer, the curves ran parallel along their full lengths and the extent of swelling increases with elevating temperature (**Figure 7**).

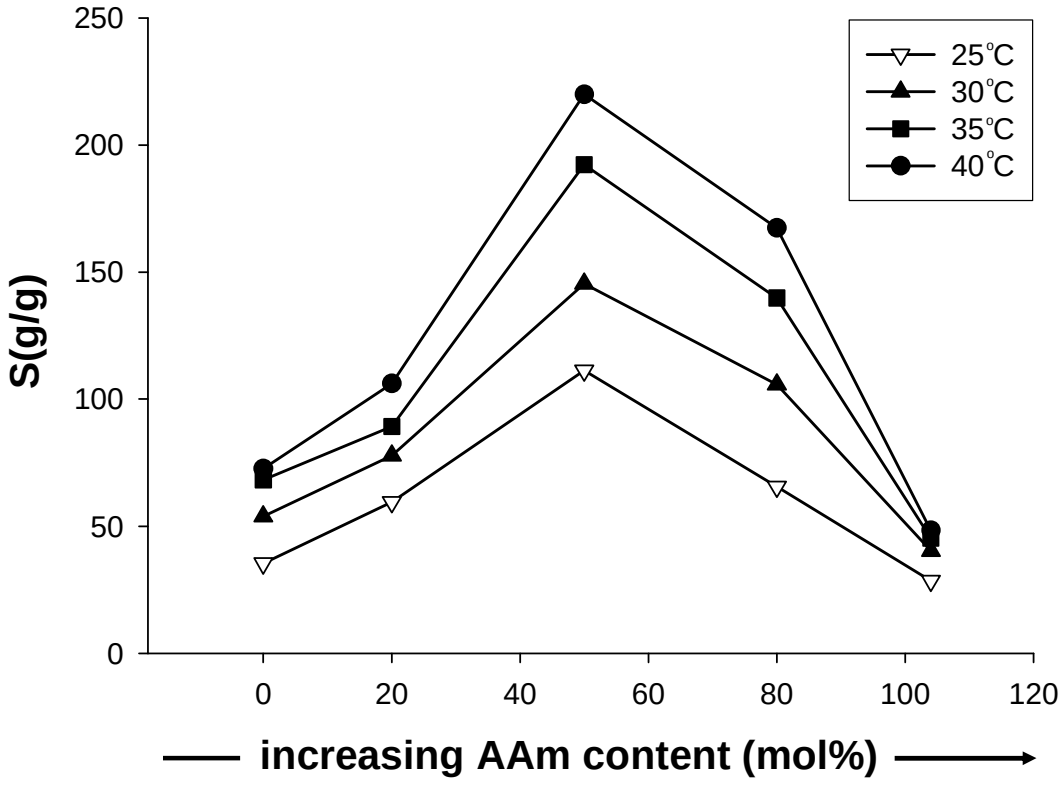


Figure 7. The swelling of poly(AAm-co-AAc) copolymers of different composition in distilled water

Figure 8 demonstrates the swelling of the hydrogels containing Na-m as a function of filler content. Polymers and copolymers composed of hydrophilic AAm or AAc displayed a good tendency to swell. However, hydrophobic NIPAAm decreased the swelling ability. Na-m enhanced the swelling characteristics of the samples, but only at low concentrations. In general, only the samples with 1-5 wt% filler showed better swelling properties than those without filler. Higher filler concentrations interfered with the swelling.

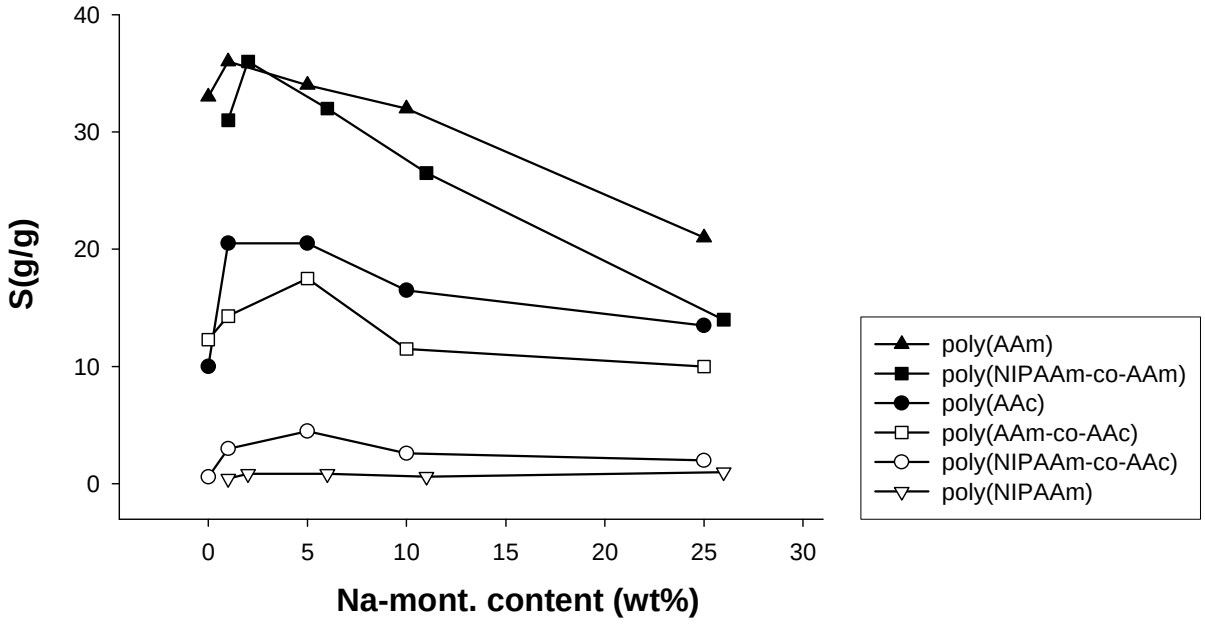


Figure 8. The effect of Na-m on the swelling of the hydrogels (37 ^{o}C, physiological saline)

Figures 9-11 illustrate the swelling of gels containing C_4-, C_{12}- and C_{18}-montmorillonites, respectively. According to our results these materials also enhance the swelling ability of the samples. However, this effect was observed only at lower filler concentrations.

We have found that Na- and C_4-m fillers preferably increased the swelling of hydrogels with hydrophilic composition. E.g. the swelling of poly(NIPAAm-co-AAc) was improved by 445%, that of poly(AAc) by 170% and that of poly(AAm-co-AAc) by 108%. On the other hand, hydrophobic fillers (C_{12}- and C_{18}-m) improved the swelling of hydrophobic NIPAAm: C_{12}-m by 82% and C_{18}-m by 118%. These findings are summarized in **Table 3**.

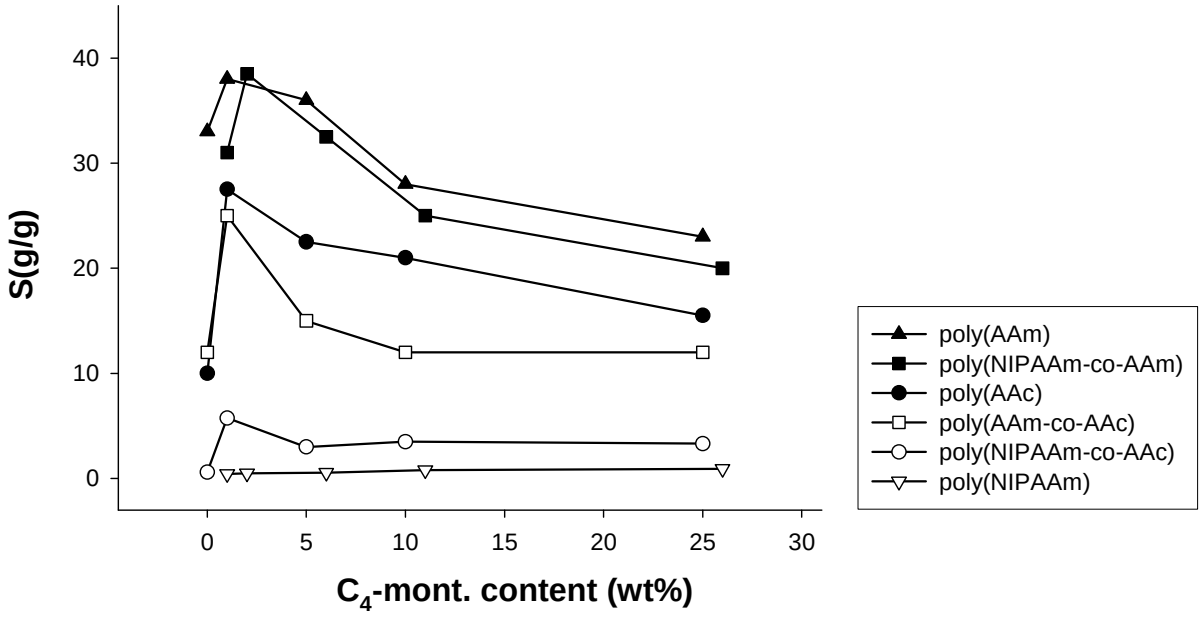


Figure 9. The effect of C_4-m on the swelling of the hydrogels (37 ^{o}C, physiological saline)

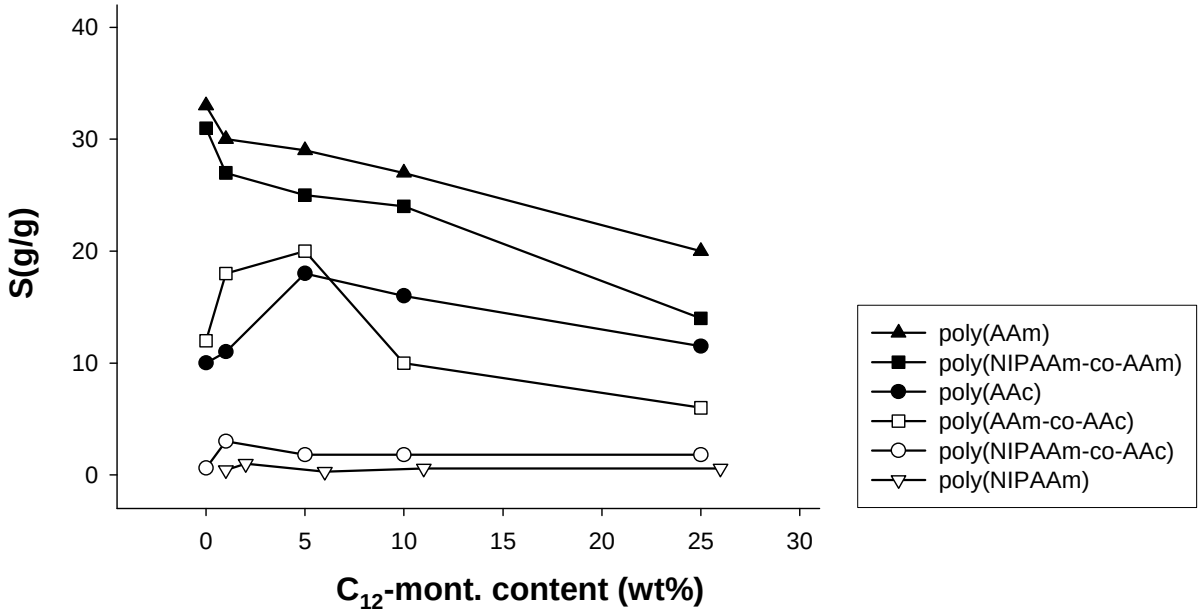


Figure 10. The effect of C_{12}-m on the swelling of the hydrogels (37 ^{o}C, physiological saline)

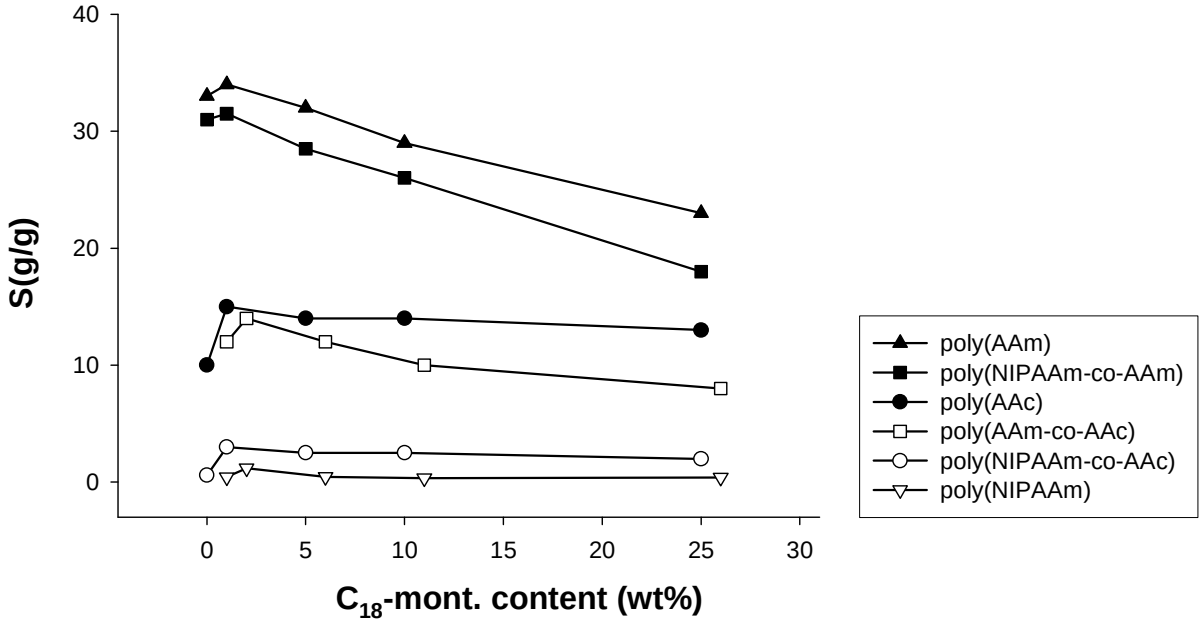


Figure 11. The effect of C_{18}-m on the swelling of the hydrogels (37 ^{o}C, physiological saline)

Table 3.

The effect of fillers on the swelling of the samples at 37 ^{o}C in physiological saline (molar composition of copolymers was 50 mol% of each initial monomer)

Polymer/ copolymer	**Swelling without filler**	**Maximal swelling with filler**	**Filler type**	**Filler concentration ***	**$\Delta S^{\#}$ (%)**
Poly(AAm)	33	38	C_4-m, Na-m	1	15
Poly(NIPAAm-co-AAm)	32	36	C_4-m	1	13
Poly(AAc)	10	27	C_4-m, Na-m	1	170
Poly(AAm-co-AAc)	12	25	C_4-m	1	108
Poly(NIPAAm-co-AAc)	1.1	6	C_4-m	1	445
Poly(NIPAAm)	0.55	1.2	C_{18}-m	1	118

*: filler concentration at maximal swelling

#: $\Delta S=[(S_{\text{with filler}}-S_{\text{without filler}})/ S_{\text{without filler}}]x100$

4.2. IN VIVO STUDY

On the basis of the *in vitro* results three hydrogels were chosen for the *in vivo* experiments: AAm and AAc polymers and a poly(NIPAAm-co-AAm) copolymer. The composition and *in vitro* determined S values of these materials are shown in **Table 4.**

Table 4.
Composition and *in vitro* swelling ability of gels applied during *in vivo* study

Polymer/ copolymer	**Monomer/cross linker ratio**	**Filler (wt%)**	**Swelling in saline [S (g/g)]**
Poly(AAm)	1300	-	36
Poly(AAc)	1500	-	34
Poly(NIPAAm-co-AAm)*	200	Na-m, 1%	38

*: NIPAAm/AAm=50/50

4.2.1. EXPANSION OF THE IMPLANTED HYDROGELS

The photo documentation illustrates the ability of the implanted gels to expand *in vivo* (**Figure 12**). An increase in the size of the cylinders was already apparent in the first few days. After 1 week, this process had resulted in a considerable expansion of the skin. During the postoperative period no sign of pain was observed.
The data of expansion rate are presented in **Figure 13**. The product of **d** and **l** is demonstrated. The expansion of the hydrogels was uneven, the process of swelling was interrupted by short periods of stagnation or decrease. Poly(AAc) showed the highest rate of expansion. These hydrogels achieved a significantly higher value by postoperative day 4 and exceeded the other materials in size throughout the first 2 weeks of the observation period. However, the cylinders of AAc demonstrated a tendency to shrink from postoperative day 14 on. The expansion of poly(AAm) was somewhat slower than that of poly(AAc). Not until postoperative day 9 were the poly(AAm) cylinders significantly larger than the baseline values. Nevertheless, we observed no tendency to shrink in case of this material. The size of poly (NIPAAm-co-AAm) cylinders entered the significantly higher range on postoperative

day 4. The values displayed a slight fluctuation and a tendency to shrink, but only at the very end of the observation period.

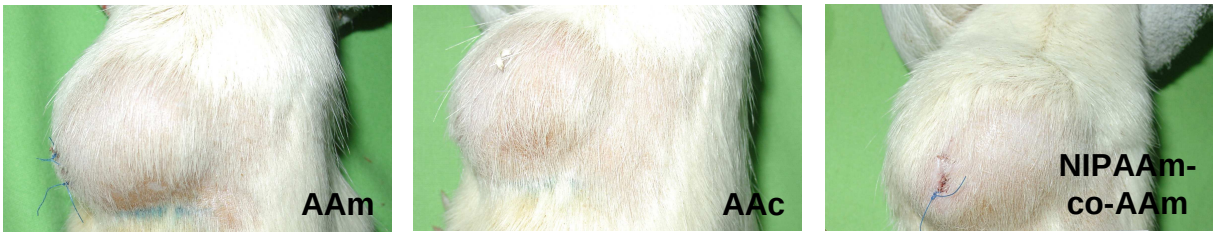
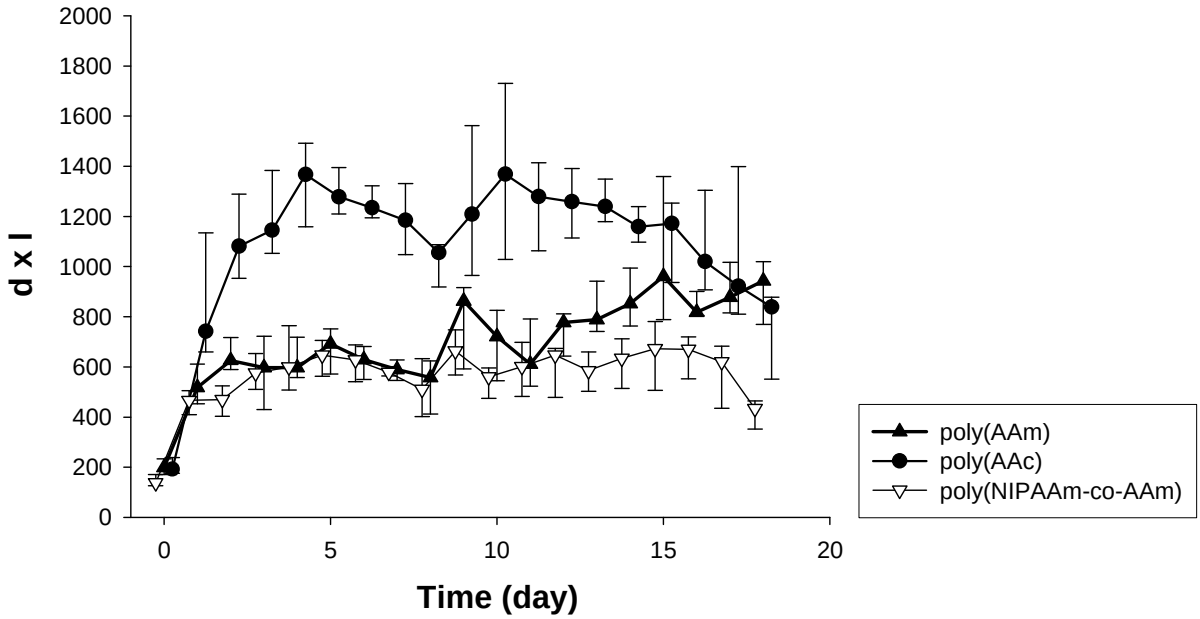


Figure 12. Photo documentation of the implanted expanders



Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
AAm	-	-	-	-	-	-	-	-	a	-	-	a	a	a	a	a	a,b	a,b
AAc	b	b	b,c	a,b,c	a,b,c	a,b,c	a,b,c	b,c	a,b	a,b	a,b,c	a,b	a,b	b	b	b	b	-
NIPAAm -co-AAm	-	-	-	a	a	a	-	-	a	-	-	-	-	a	a	a	-	-

Figure 13. Rates of expansion of the implanted hydrogels. The product of diameter and length is given. The curves display the median values, the 25th and the 75th percentiles. The table under the graph demonstrates the findings of the statistical analysis. a: $p<0.01$ vs. 0-day values; b: $p<0.01$ vs. NIPAAm-co-AAm group; c: $p<0.01$ vs. AAm group.

The changes in mass of the hydrogels are demonstrated in **Figure 14**. The moisture mass of each of the removed hydrogels was considerably higher than the dry mass prior to implantation. At least a 25-fold elevation was observed in each group. The statistical analysis did not reveal mathematically significant differences between the groups.

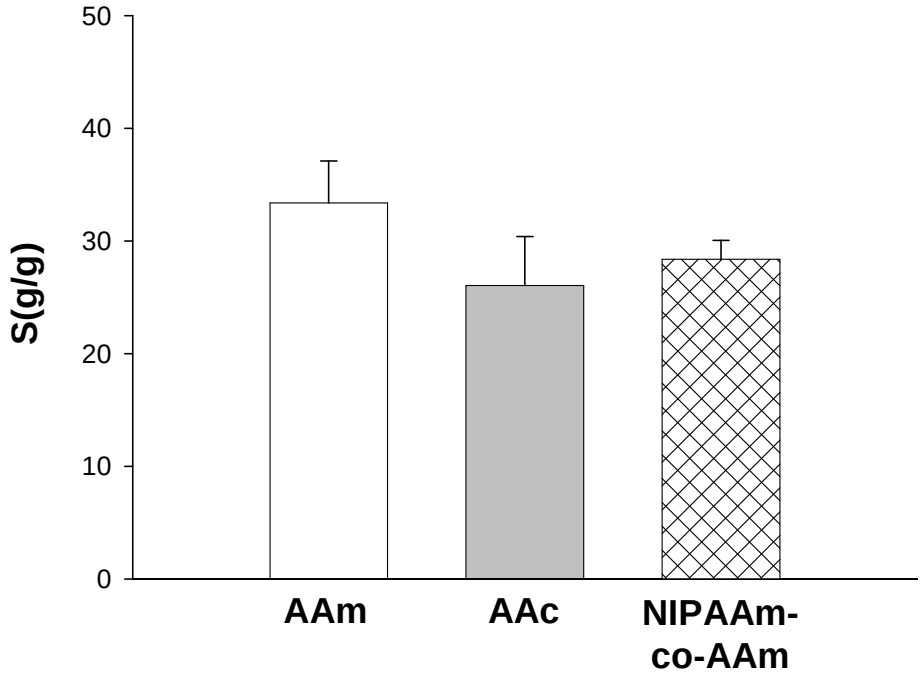


Figure 14. In vivo swelling values of the implanted hydrogels. The bars demonstrate the median values and the 75th percentiles.

4.2.2. RHEOLOGICAL PARAMETERS OF THE HYDROGELS

Figure 15A displays the storage moduli (G') of the gels. These values describe the elastic character of the polymers. The G' values of the AAc expanders were found to be significantly lower than those of the NIPAAm-co-AAm devices. The viscous character of the polymers was characterized by the loss modulus (G") These data are presented in **Figure 15B**. The G" values of AAm samples were significantly higher than those of NIPAAm-co-AAm expanders.

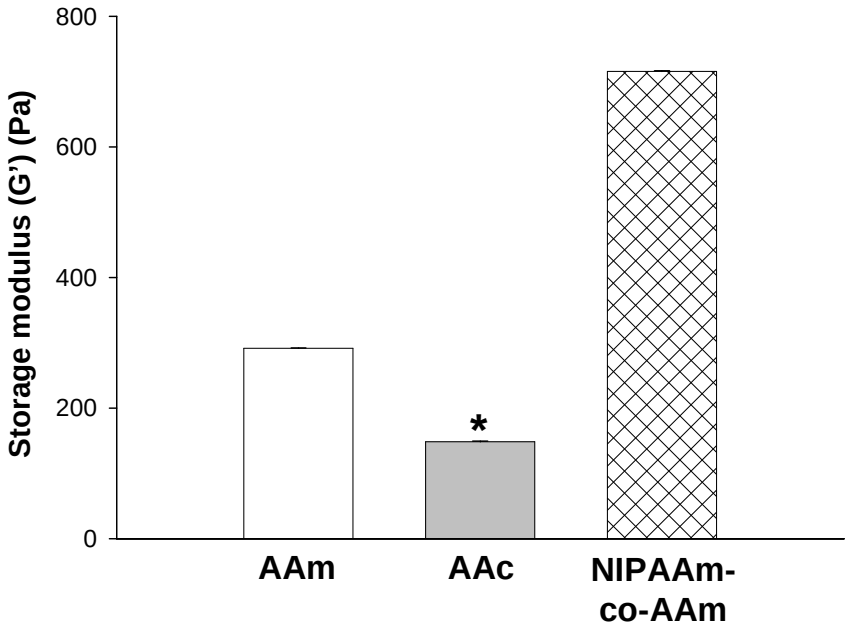


Figure 15A. Storage moduli of the implanted hydrogels. The values are given in pascals. The bars demonstrate the median values and the 75th percentiles. *: p<0.01 vs. NIPAAm-co-AAm group.

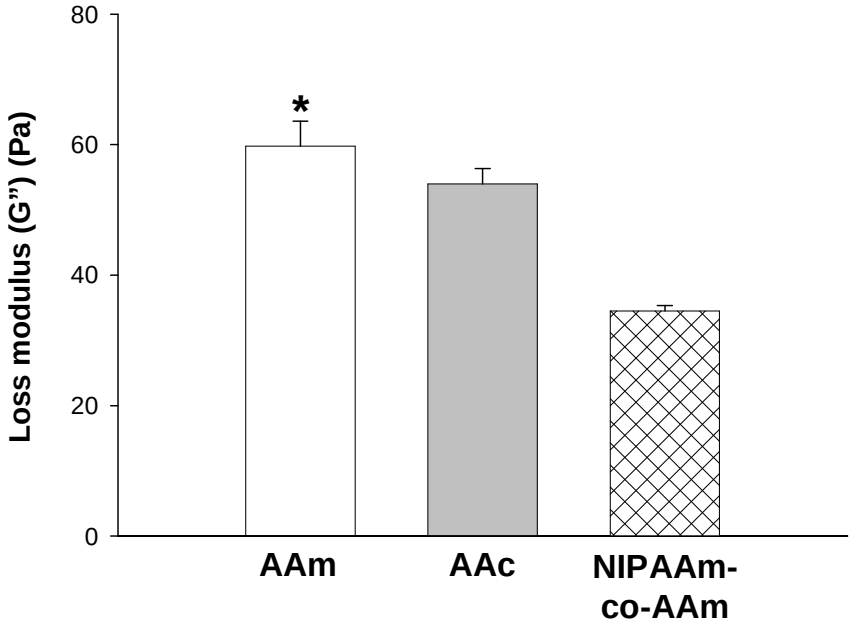


Figure 15B. Loss moduli of the implanted hydrogels. The values are given in pascals. The bars demonstrate the median values and the 75th percentiles. *: p<0.01 vs. NIPAAm-co-AAm group.

4.2.3. HISTOLOGICAL FINDINGS

The histological features of the tissue samples taken at the end of the observation period are demonstrated by **Figure 16**. The histological analysis did not reveal structural changes in the intact skin biopsies taken from the region surrounding the implanted hydrogels. In case of AAm and NIPAAm-co-AAm, the expanded skin did not show any abnormality, macroscopically. However, in 50% of the animals, which received the AAc expander, ulceration of the skin was observed over the implanted material.

Light microscopy revealed a slight to moderate accumulation of macrophages in the expanded skin biopsies when AAm was applied. A few fibroblasts and giant cells could also be seen. Hyperaemia and oedema were apparent under the muscle (**Figure 16A**).

The capsules of the AAm devices were characterized by fibrosis. They demonstrated signs of slight inflammation and significant vessel proliferation (**Figure 16B**). Many mast cells were seen.

When AAc was implanted, ulceration was detected in 3 cases; the histology of one case is presented in (**Figure 16C**).

The implantation of AAc resulted in a significant accumulation of macrophages in the dermis, the subcutaneous layer and the muscle. Severe oedema, hyperaemia, fibrosis and the presence of numerous macrophages were also characteristic of these biopsies (**Figure 16D**), and granulocytes could be observed. The application of the AAc expanders led to fibrosis and significant inflammatory reactions in the capsules. A number of macrophages, and neutrophilic and eosinophilic granulocytes were observed in these biopsies. A homogeneous, eosinophil material too was seen (**Figure 16E**).

Tissue samples taken from the expanded skin of the animals with NIPAAm-co-AAm devices proved to be morphologically normal. Neither inflammation nor destruction was detected (**Figure 16F**).

The histological analysis indicated fibrosis, and the presence of lymphoid cells with a slight degree of hyperaemia in the capsules surrounding the NIPAAm-co-AAm copolymers (**Figure 16G**).

The dermal vessels and the skin appendages did not show significant alterations during the process in any group.

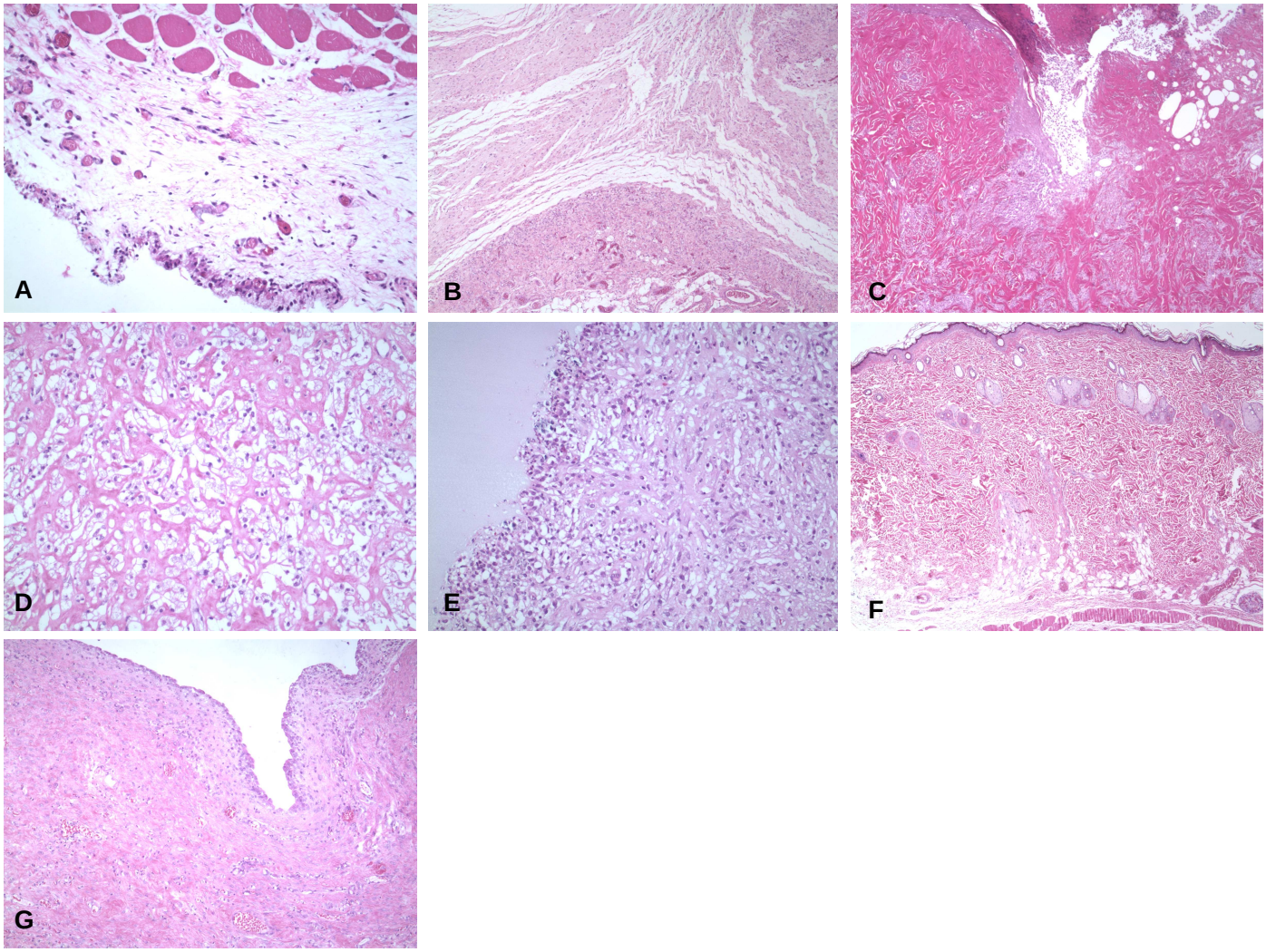


Figure 16. Photomicrographs of expanded skin and capsules from animals which received AAm, AAc and NIPAAm-co-AAm expanders, respectively. Hematoxylin-eosin staining. **A:** AAm, expanded skin. Original magnification X10. **B:** AAm, capsule surrounding the expander. Original magnification X5. **C:** AAc, ulceration. Original magnification X5. **D:** AAc, expanded skin. Original magnification X20. **E:** AAc, capsule surrounding the expander. Original magnification X20. **F:** NIPAAm-co-AAm, expanded skin. Original magnification X5. **G:** NIPAAm-co-AAm, capsule surrounding the expander. Original magnification X10.

5. DISCUSSION

Tissue expanders play an important role in plastic and reconstructive surgery. The implantation of an expander into the subcutaneous layer results in a gradual expansion and provides additional tissue for the reconstruction of tissue defects. During recent decades, many developments have been achieved, with a resulting improved efficacy of these devices. Tissue expanders can be implanted with minimally invasive surgical techniques, thereby reducing the duration of hospitalization and the level of patient discomfort (**Sharobaro et al., 2004**). Osmotically active tissue expanders are free of the side-effects and other disadvantages of the earlier devices, which required external filling. The self-filling tissue expanders cause only relatively slight discomfort and pain for the first few days. Moreover, they can be applied in different size and shape in almost every area of the body (**Ronert et al., 2004**). The modern self-inflating expanders are composed of hydrogels, the properties of which allow considerable expansion. During our *in vitro* and *in vivo* experiments we have studied the characteristic and surgical applicability of polymers and copolymers composed of AAm, AAc and NIPAAm.

It is well-known that many parameters of the surrounding medium can influence the swelling ability, hereby the surgical utilization of these hydrogels. The first studied factor was the temperature, since this parameter has a crucial impact on chemical and biochemical reactions. Moreover, pH also deserves special attention in this way. Since these polymers and copolymers were designed for a future *in vivo* use, the behavior of the hydrogels under physiological temperature and pH values was an important question. Our results revealed not only that the swelling of the gels increases with the elevating temperature, but also that the hydrophilicity of the samples influences the swelling ability. The more hydrophilic the gels are, the more expressed the swelling ability is. The swelling of the hydrogels originates in the water uptake of the materials. AAm and AAc contain hydrophilic amino- and carboxylic groups, respectively. These groups are able to bind a large amount of water, hereby leading to the swelling of the gel. The outstanding swelling ability of poly(AAm-co-AAc) copolymers unraveled that these hydrophilic groups are able to enhance the effects of each other. The explanation is that electrostatic interactions are established between the two types of groups, thus increasing the water content of the gel. On the other hand, NIPAAm, which can be considered relatively hydrophobic, showed a more moderate tendency to water uptake and swelling. The examination of the swelling as a function of temperature led to further interesting results. Elevating temperature induced a more expressed swelling response in

hydrophilic gels. The reason is that rising temperature increasingly extends the 3D polymer lattice, thereby making more and more functional groups accessible to water molecules, resulting in more extensive swelling. Further, the thermosensitivity of NIPAAm gels, which has been described by many authors (see in Introduction) and confirmed by our results, can be eliminated by copolymerization with hydrophilic AAm and AAc. NIPAAm-co-AAm and NIPAAm-co-AAc copolymers did not collapse at higher temperatures until the NIPAAm content achieves approximately 60-70%.

The effects of pH on the swelling of hydrogels can also be explained with the chemical structure of the materials. The changes of pH did not influence the swelling of NIPAAm polymer, while swelling of AAm- and AAc-based gels with dissociable functional groups was markedly dependent on pH. AAm polymer had its swelling maximum at pH=7-8 because of the amino groups, while AAc hydrogel, which is a polycarboxylic acid, achieved the peak of swelling in the basic range, at pH=9.

In order to predict the behavior of the materials after implantation, it was also necessary to examine whether the electrolyte concentration of the surrounding medium changes the swelling ability of the hydrogels. Thus, we studied the swelling both in distilled water and physiological saline at constant pH and temperature. Swelling values measured in saline were lower than those found in distilled water. This difference originates in the coagulating effect of physiological saline solution, but the higher osmotic concentration of this medium may also play a role. Our result revealed that the studied polymers and copolymers displayed different sensitivity to electrolyte concentration. Swelling ability of samples containing AAc decreased markedly as compared to that in distilled water. Thus, AAc-based polymers and copolymers show extensive swelling due to their hydrophilicity and their swelling surpass that of other hydrogels at physiological pH, but the elevation of electrolyte concentration limits the swelling. This property of AAc-based gels is to be considered when planning *in vivo* application of these materials.

Since one of our goals was to produce hydrogels with outstanding swelling ability, we took into consideration that not only the ratio of the applied monomers but also different inorganic fillers may improve the swelling. Depending on the nature of the components used (clay mineral, organic cation and polymer matrix) and the method of preparation, three main types of composites may be obtained when a clay mineral is combined with a polymer. When the polymer is unable to be intercalated, a phase-separated composite is obtained the properties of which stay in the same range as those of traditional microcomposites. Beyond this classical family of composites, two further types of nanocomposites can be recovered. An

intercalated structure in which a single (and sometimes more than one) extended polymer chain is intercalated between the silicate layers results in a well-ordered multilayer morphology built up of alternating polymeric and inorganic layers. When the silicate layers are completely and uniformly dispersed in a continuous polymer matrix, an exfoliated or delaminated structure of is obtained (**Alexandre et al., 2000**). It is known that intercalated structures can be identified by X-ray diffraction (**Smarsly et al., 2003; Lee et al., 2006; Haraguchi et al., 2006**). Our working group has proven that in the course of the synthesis of our clay mineral containing composites, the polymer chains were intercalated and delaminated the clay mineral particles. According to our examination Na-m at lower concentration (1-5%) enhanced the swelling of the samples, but higher filler contents impeded the swelling. The reason may be that at low filler concentrations the lamellae of the filler are well-separated, making the negative surface charges accessible for both the functional groups of the polymer and the incoming water molecules, whereas at higher montmorillonite concentrations the lamellae have no influence on hydrophylicity and, consequently, on the extent of swelling. The same conclusion was drawn by other authors in the case of poly(NIPAAm)-based composites containing Na-m (**Xia et al., 2003**). In addition to Na-m, different organophilized montmorillonites with different hydrophilicity were also studied in order to clarify whether they have an effect on swelling. We have found that swelling is primarily determined by the hydrophilicity of the monomers making up the copolymer and the ratio of the monomers with different hydrophilicity, rather than by the hydrophilicity of the filler, since the curves of polymers with identical compositions and different filler contents are of identical shape. Our results revealed that the swelling of hydrophilic polymers and copolymers was improved by hydrophilic Na-m and C_4-m fillers, whereas that of hydrophobic polymers (e.g. NIPAAm) was increased by hydrophobic C_{12}- and C_{18}-m fillers. The explanation is that poly(NIPAAm)-based gels contain more hydrophobic moieties. If these groups can approach closely to the surface oxygen atoms, van der Waals interaction becomes very strong (**Lagaly, 2001**).

In view of the *in vitro* findings we have chosen two polymers and a copolymer, poly(AAm), poly(AAc) and poly(NIPAAm-co-AAm) (the latest with 1% of Na-m filler) for *in vivo* examination. We have shown that the swelling ability of poly(AAc) at physiological pH and temperature surpasses that of polymers, although physiological electrolyte concentration decreases the swelling. In spite of this, the salt content slightly influenced the water uptake of poly(AAm) and poly(NIPAAm-co-AAm). *In vivo* application of hydrogels produced through the use of Aam, AAc and NIPAAm polymers cannot be considered novelty,

since such materials are extensively used in various biomedical areas (**Fundueanu et al., 2005; Moszner et al., 2006; Oosthuysen et al., 2006; Majekodunmi et al., 2007; Chen et al., 2008**). Although AAm hydrogel has already been tested for the treatment of HIV-related lipoatrophy (**Mole, 2006**), its utilization as a tissue expander has not yet been reported. However, the biocompatibility of these hydrogels required detailed examinations. A crucial question was whether the new expanders are able to generate a pressure sufficient to dilate the surrounding tissue at the expected rate. Although in clinical practice osmotic expanders are implanted in a silicon membrane in order regulate the expansion, we decided not to apply such devices in this study, because our aim was to characterize the free *in vivo* expansion of the hydrogels. We found that the swelling pressure, which is determined by the osmotic pressure and the elastic contractility of the polymers, can overcome the resistance of the adjacent tissues and lead to a considerable dilation. The tested hydrogels gradually dilated. This property of the polymers contributes to the preservation of the normal function and morphology of the skin, as it has been described that the pressure caused by the expander evokes an inflammatory reaction with a tendency to rapid regeneration (**Ratner et al., 1976**). As the periods of increase alternate with periods of decrease or stagnation, the affected tissue is provided with the possibility of recovery and functional normalization before further expansion. The swelling phase of other osmotic tissue expanders is completed in 6 to 8 weeks (**Ronert et al., 2004**). Our new devices achieved the peak of their swelling by the end of the postoperative week 2. Nevertheless, too rapid expansion has the risk of wound separation and tissue damage. With poly(AAc), the rate of the expansion seems to be too high. The histological analysis described serious lesions in animals implanted with poly(AAc). Although the literature underlines that a moderate inflammatory reaction occurs during tissue expansion, morphological changes induced by poly(AAc) expanders should be considered abnormal. Once poly(AAc) is applied *in vivo*, a silicon membrane would be essential in order to limit the rate of expansion. In contrast, the rates of expansion of poly(AAm) and poly(NIPAAm-co-AAm) remained in the safe range (minor histological changes). Poly(AAm) seemed to be prone to further expansion.

Another important property that may be used for the characterization of expanders is the difference between their dry and wet masses. All the tested expanders revealed a strong tendency to water uptake during the preliminary *in vitro* study. Our results confirmed that their swelling ability is similarly outstanding under *in vivo* circumstances. While traditional osmotically active tissue expanders achieve approximately a 10-fold increase in mass, the currently examined devices demonstrated an elevation of at least 25-fold. Thus, poly(AAm),

poly(AAc) and poly(NIPAAm-co-AAm) hydrogels allow the acquisition of more skin for reconstructive interventions. Since no significant difference in swelling ability was found between the three polymers, from the aspect of water uptake all of them seem to be appropriate for surgical application. Other properties of the hydrogels should also be considered when an expander is chosen for a certain intervention.

It is a very important requirement that an implanted tissue expander should retain its preformed shape during the expansion phase. In aesthetic surgery, tissue expanders of different sizes and shapes are utilized in order to dilate the soft tissue in a planned way. The rheological properties of the polymers influence their applicability. Hydrogels are viscoelastic materials. The cross-linked structure is responsible for their elasticity and the viscosity originates in the water taken up by the gels. On inspection, the poly(NIPAAm-co-AAm) expanders exhibited a considerable tendency to retain their original shape, whereas many of the poly(AAc) and poly(AAm) devices were no longer cylindrical by the time of their removal. Rheological measurements confirmed these observations. The **G'** values of poly(AAc) were significantly lower and the **G"** values of poly(AAm) were significantly higher than those of poly(NIPAAm-co-AAm). As **G'** is a marker of elasticity and **G**" describes viscosity, the conclusion can be drawn that, of the three studied polymers, poly(NIPAAm-co-AAm) has the most suitable viscoelastic nature. The elasticity and the viscosity together determine the mechanical behavior of the hydrogels. If higher elasticity values are accompanied by somewhat more moderate viscosity values, the expanders seem to be much more favourable for designed tissue dilation. Thus, the elasticity and the water uptake, which are both characteristic features of a certain material, should be balanced so that the swelling ability and the mechanical properties are also in the optimum range. Our results indicated that the poly(NIPAAm-co-AAm) expanders met these criteria; hence they should be the first choice (within the studied polymers) for plastic and reconstructive interventions.

It is essential that expanders should be free of adverse tissue reactions, so that their implantation can be considered hazardless and healthy skin can be obtained for different interventions. Hence, the question of safety of the applied monomers and polymers has arisen. According to the available Material Safety Data Sheets AAm monomer is proven to be a carcinogenic agent. There is no evidence of carcinogenicity of AAc and NIPAAm monomers. However, AAc monomer is able to damage the skin and NIPAAm has also irritant effects. On the other hand, several studies suggested that polymers of acrylics can be considered safe. It has been described that a poly(AAm)-based soft-tissue filler (Aquamid®) was tested in a clinical trial. The material was proven to be harmless and it has been authorized for sale in

Europe as a medical device (**de Cassia Noves et al., 2003**). Concerning poly(AAc), it is a frequent component of different drug delivery systems. A recent review article summarizes the applicability of poly(AAc). It is a typical component of bioadhesive polymers in vaginal formulations and it is a promising agent for use in controlled drug delivery to pulmonary and nasal sites (**Andrews et al., 2009**). Materials containing NIPAAm are also utilized in biomedical products. An *in vitro* study revealed that islets of Langerhans remained viable and showed no significant decrease of insulin release in a gel matrix of NIPAAm copolymer with AAc (**Bae et al., 1998**). Furthermore, other *in vitro* and *in vivo* toxicity studies with NIPAAm copolymer containing nanoparticles showed the harmlessness of this material (**Kim et al., 2008**). Since polymers can be considered safe, but monomers have side effects, special attention was paid to the remaining monomers. The weights of the dried gels were evaluated. These measurements revealed that polymerization yields were nearly 100% in all cases. Furthermore, in the end of the polymerization the samples were washed with distilled water to remove unreacted monomers, cross-linker and initiator. Hence, there is a good reason to consider that the implanted hydrogels were free of monomers. Moreover, concerning their chemical structure, the applied polymers are known to be non-biodegradable materials.

On use of the NIPAAm-co-AAm polymers, the overlying skin did not show pathological changes. Thus, the animals tolerated the implantation of poly(NIPAAm-co-AAm) well. Although minor lesions accompanied the application of poly(AAm), they were not sufficiently severe to contraindicate its use *in vivo*. However, poly(AAc) induced serious damage in the surrounding tissue. It is possible that the rapid expansion of poly(AAc) leads to inflammation, but the severe lesions suggest that other mechanisms of harmful reactions induced by poly(AAc) cannot be excluded. Our results demonstrated that the application of poly(AAc) expanders cannot be considered safe. However, this question requires further investigation.

The findings of the *in vitro* and *in vivo* studies have shown that modification of the composition of the hydrogels has a significant effect on the swelling ability. This could be used in the design of tissue expanders. Patient of different age and skin status and interventions affecting different parts of the body require tissue expanding devices appropriate for the given situation. In addition to physical methods (e.g. silicon membranes) also chemical ways can get into consideration for the development of tissue expanders with different expansion properties hereby creating a new generation of tools for plastic and reconstructive surgery.

6. SUMMARY AND NEW FINDINGS

We studied the swelling properties of polymers and copolymers composed of AAm, AAc and NIPAAm in distilled water and physiological saline. The investigations have shown that

- Increasing ratio of hydrophilic AAm and AAc monomers considerably improved the swelling of the gels.
- AAc-based polymers and copolymers exhibited the most extensive swelling in distilled water, but the changes of pH and electrolyte concentration markedly influenced the water uptake of the samples.
- Addition of hydrophilic and hydrophobic montmorillonite particles as filler improved the swelling of the gels only when applied at low concentration (1-5 wt. %).
- Swelling of hydrophilic polymers and copolymers was improved by hydrophilic Na- and C_4-m fillers, while that of hydrophobic polymers was increased by hydrophobic C_{12}- and C_{18}-m fillers.

Further study was designed for the *in vivo* behavior and the surgical applicability of poly(AAm), poly(AAc) and poly(NIPAAm-co-AAm) hydrogels. The examination has revealed that

- The implanted samples of poly(AAm), poly(AAc) and poly(NIPAAm-co-AAm) displayed a marked tendency to swell *in vivo*.
- The maximum expansion can be achieved in two weeks.
- According to the observations and the rheological measurements the expanders of poly(NIPAAm-co-AAm) showed the highest tendency to retain their preformed shape.
- Implantation of poly(AAc) devices was accompanied by serious tissue damage, while the other examined hydrogels were safe: local toxicity was not detected.
- In view of its mechanical and biological properties poly(NIPAAm-co-AAm) hydrogel with 1% Na-m seems to be a promising tissue expander-candidate.

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