OSMOTICALLY ACTIVE HYDROGELS OF ACRYLICS: CHARACTERIZATION AND APPLICATION AS TISSUE EXPANDER

Summary of Ph.D. Thesis

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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
m_{dry}	Dry mass
m_{wet}	Moisture mass
Na-m	Sodium montmorillonite
NIPAAm	N-isopropylacrylamide
S	Swelling value
TEMED	N,N,N',N'-tetramethylethylenediamine

1. INTRODUCTION

Gaining of soft tissue for the reconstruction of 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 inflatable subcutaneous balloon has revolutionized plastic surgery in the last 30 years. Nevertheless, the application of this device involves the risk of various complications and inconveniences. Regular control is required; the filling is accompanied by pain, further, infection, leakage and flap necrosis are also possible complications. Hence, the development of a new generation of tissue expanders seemed to be essential. The attention was drawn to the hydrogels which are promising materials in tissue expansion. Hydrogels consist of two major components: a polymer web of constant quantity and a hydrous phase which changes in volume. These materials are able to react to changes of environmental parameters and respond with a functional reaction (e.g. swelling). Due to their advantageous properties many hydrogels are applied in different biomedical areas. Moreover, osmotically active hydrogels seemed to provide an effective means for tissue expansion. The dry gel absorbs body fluid, its volume therefore increases and 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% and the expanders are well tolerated by the patients. However, the expansion properties of these devices could be improved in order to decrease the period of indwelling and to increase tissue gain. Recent studies suggested that acrylamide (AAm) and AAm-based copolymers exhibit a very high capability to absorb water and possess good biocompatibility. Furthermore, polymerized N-isopropylacrylamide (NIPAAm) is a thermosensitive hydrogel. Thermosensitivity can be utilized to regulate the behavior of the expander and to produce optimal biomaterials. It also known that the properties of gels can be significantly enhanced by the incorporation of inorganic ordered systems, in particularly clays into the gels. Sodium montmorillonite (Na-m) is widely used as additive in order to improve the physical properties of plastics.

These data suggested that there would be a need for novel tools for tissue expansion in plastic and reconstructive surgery. They also suggested that hydrogels of acrylics seemed to be promising expander-candidates which worth studying *in vitro* and also *in vivo.*

2. OBJECTIVES

The major aims were:

- To synthesize thermo- and pH-sensitive hydrogels by polymerization of NIPAAm, AAm and acrylic acid (AAc);
- To examine the effects external factors (temperature, pH, electrolyte concentration) on the swelling ability;
- To characterize the swelling of copolymers containing different rations of NIPAAm, AAm and AAc;
- To study the impact of different fillers on the osmotic properties of hydrogels;
- To observe the swelling of implanted hydrogels as a function of time in an animal model;
- To determine the changes in the mass of the expanders *in vivo*;
- To assess their rheological parameters; and
- To study the biocompatibility of the implanted polymers and copolymers.

3. MATERIALS AND METHODS

3.1. Preparation of polymers

NIPAAm, AAm and AAc polymers and copolymers with various compositions were prepared by radical polymerization. KPS was used as initiator, TEMED as accelerator while BisAAm was the cross-linking agent. The reaction was performed at 60 ^{o}C for 30 min under N_2 atmosphere. The composites were synthesized by addition of Na-m or organophilized montmorillonite fillers (C_n-m, n=4, 12, 18).

3.2. 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. The swelling of hydrogel was investigated in the temperature range of 25-40 ^{o}C. Some samples were placed into physiological saline. In some cases the pH of the environment was changed, as well.

3.3. *In vivo* experiments

The experiments were performed on 18 male Wistar rats. The interventions were carried out under anaesthesia and the procedures and protocols applied were approved by the Ethical Committee for the Protection of Animals in Scientific Research at the University of Szeged. Hydrogels displaying outstanding swelling properties according to the results of the *in vitro* study were implanted under the skin of the dorsal region. The observation period took 18 days. The diameter (**d**) and the length (**l**) of the implanted materials were measured daily with calliper and photographs of the dorsal region were taken. On postoperative day 18 the animals were sacrificed and the expanders were removed. The m_{wet} of the hydrogels was measured immediately. The storage modulus (G') and the loss modulus (G") of the removed expanders were determined with an oscillatory rheometer in order characterized their elasticity and viscosity, respectively. Further, biopsies were taken from the intact skin, the expanded skin and the capsule surrounding the expander. The biopsies were embedded in paraffin, stained with H&E and the evaluation was performed in coded sections.

4. RESULTS

4.1. Effects of external factors on swelling

The poly(NIPAAm) hydrogel showed thermosensitivity: the maximum of its swelling was observed at 31 ^{o}C, at higher temperatures the gel collapsed. When the NIPAAm monomer was copolymerized with AAm or AAc the swelling of the samples increased continuously with the elevating temperature and the copolymers did not show the collapse characteristics of pure poly(NIPAAm). Hydrogel containing AAm and AAc displayed the most extensive swelling of the studied materials.

Changes in pH did not influence the water uptake of poly(NIPAAm). The swelling maximum of poly(AAm) was at pH 7-8, while the AAc polymer exhibited the highest pH dependence: the peak of swelling was around pH 9 and the moisture mass of the gel was 250-fold of its original dry mass.

The electrolyte concentration of the environment also had an impact on swelling. The values measured in saline were lower than those detected in distilled water. AAm-based gels were the least sensitive to salt content, while the swelling of NIPAAm- and AAc-based samples were considerably influenced by electrolyte concentration.

4.2. Effects of composition of the gels on swelling

The molar ratio of different monomers and addition of inorganic fillers are also important factors of swelling ability. Higher ratio of hydrophilic AAm and AAc monomers resulted in more expressed water uptake while increasing ratio of the relatively hydrophobic NIPAAm decreased the swelling ability. NIPAAm-containing copolymers displayed thermosensitivity over 60-70% NIPAAm content.

Concerning inorganic fillers, Na-m enhanced the swelling characteristics of the samples, but only at lower concentration. Hydrogels with 1-5wt% filler showed better swelling properties than those without filler. C_4-, C_{12}- and C_{18}-montmorillonites also improved the swelling ability at lower concentrations. Na- and C_4-montmorillonite fillers preferably increased the swelling of hydrogels with hydrophilic composition, whereas hydrophobic fillers (C_{12}- and C_{18}-m) improved the swelling of hydrophobic NIPAAm.

4.3. Results of the *in vivo* study

On the basis of the *in vitro* results the following samples were selected for *in vivo* examination: poly(AAm), poly(AAc) and poly(NIPAAm-co-AAm), the latest material contained also 1

wt% Na-m. We found that the swelling of the implanted hydrogels led to a considerable expansion of the skin. 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. However, these samples demonstrated a tendency to shrink from postoperative day 14 on. The expansion of poly(AAm) was somewhat slower than that of poly(AAc), but no tendency to shrink was observed 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.

The moisture mass of each implanted 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.

Concerning rheological properties of the samples, G' values of the AAc expanders were found to be significantly lower than those of the NIPAAm-co-AAm devices. (G' values describe the elastic character.) Further, the G" values (which shows the viscous character) were significantly lower in case of

poly(AAm) devices than those of NIPAAm-co-AAm expanders.

The histological examination revealed slight inflammatory reaction after the implantation of poly(AAm) cylinders. However, application of poly(AAc) resulted in ulceration in 50% of the cases, severe oedema, hyperaemia and leukocyte accumulation were also found. Tissue samples taken from the animals with NIPAAm-co-AAm devices proved to be morphologically normal.

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. 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. 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. The explanation of our findings originates in the chemical structure 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. On the other hand, NIPAAm, which can be considered relatively hydrophobic, showed a more moderate tendency to water uptake and swelling. The effects of pH on the swelling of hydrogels can also be explained with the chemical structure of the samples: swelling of AAm- and AAc-based gels with dissociable functional groups was markedly dependent on pH. Concerning salt concentration, 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, it may influence their *in vivo* applicability.

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. 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 results of the *in vitro* study served as a guideline for the design of *in vivo* 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. We found that the swelling pressure can overcome the resistance of the adjacent tissues and lead to a considerable dilation.Our 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. In contrast, the rates of expansion of poly(AAm) and poly(NIPAAm-co-AAm) remained in the safe range.

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. 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. The rheological properties of the polymers influence their applicability. 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). If higher elasticity values are accompanied by somewhat more moderate viscosity values, the expanders seem to be much more favorable for designed tissue dilation.

Our results indicated that the poly(NIPAAm-co-AAm) expanders met these criteria.

It is also 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. 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. 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 with different expansion properties hereby creating a new generation of tools for plastic and reconstructive surgery.

6. CONCLUSIONS

We studied the swelling properties of polymers and copolymers composed of AAm, AAc and NIPAAm. 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. %).
- The implanted samples of poly(AAm), poly(AAc) and poly(NIPAAm-co-AAm) displayed a marked tendency to swell *in vivo*.
- 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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LIST OF *IN EXTENSO* PUBLICATIONS RELATED TO THE THESIS

I. 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**)

II. **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**)

III. **Varga János,** Janovák László, Varga Erika, Erős Gábor, Dékány Imre, Kemény Lajos: Akril alapú szöveti expanderek sebészeti felhasználhatóságának vizsgálata. Magyar Sebészet 2010; 63(1):16-22.

PATENT APPLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. 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

II. 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

OTHER PUBLICATIONS

I. 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**)

II. 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.

III. 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**)

IV. Kapitány Klára, Szegesdi Ilona, **Varga János:** Acne Inversa sebészi kezelése. Bőrgyógy Vener Szle, 72: 183-186, 1996.

V. Kapitány Klára, Ágoston Zsuzsanna, Kiss Erika, Mohos Gábor, **Varga János**:Acne inversa sebészi megoldása hónaljban fasciocutan lebennyel.Bőrgyógy. Vener. Szle. 75: 141-143, 1999.

VI. Séra Teréz, Mohos Gábor, Papos Miklós, Osvay Margit, **Varga János**, Lázár Máté, Kiss Erika,

Kapitány Klára, Dobozy Attila, Csernay László, Pávics László: Sentinel node detection in malignant melanoma patients: radiation safety considerations. Dermatol Surg 29: 141-145, 2003 **(IF_{2008}: 2.102)**

VII. Kapitány K, Ágoston Z, Kis E, Mohos G, Szegesdi I, **Varga J:** Szövettágítás a plasztikai sebészetben és dermatochirurgiában. Bőrgyógy Vener Szle 80: 279-281, 2004

VIII. Kapitány K, Kis E, Mohos G, Szegesdi I, **Varga J,** Kemény L: Hónalji defektusok zárása hidradenitis suppurativa miatti exstirpatio után. Coverage for axillary defect resulting from excision of hidradenitis suppurativa. Bőrgyógy. és Vener.Szemle 82: 109-111, 2006

IX. **Varga J,** Pintér S, Mohos G, Kis E, Kocsis Á, Nagy K, Kemény L: Kutyaharapás után kialakult felső ajak hiány rekonstrukciója Kazanjian lebennyel. Bőrgyógy. és Venerol. Szemle 85:83-85,2009

LIST OF ABSTRACTS NOT RELATED TO THE SUBJECT OF THE THESIS

I. Balogh Á, Zöllei I, **Varga J,** Lázár Gy: Kiterjesztett és kombinált vastagbélműtétek. Magyar Sebészet, A 104, 1994.

II. **Varga János**: Die behaarige Kopfhaut als Entnahmestelle. Vereinigung für Operative und Onkologische Dermatologie. Salzburg, 199. szeptember 10-12. Z hautkr. 74: 504, 1999.

III. Oláh Judit, Gyulai Rolland, **Varga János**, Mohos Gábor, Kapitány Klára, Papós Miklós, Varga Erika, Korom Irma, Dobozy Attila.Sentinel nyirokcsomóbiopsia indikációja melanoma malignumban.A Magyar Onkológusok Társaságának 24. Kongresszusa. Budapest, 2001. Magy. Onkol. 45: 286, 2001.

IV. Oláh Judit, Gyulai Rolland, **Varga János**, Mohos Gábor, Papos Miklós, Kapitány Klára, Korom Irma, Varga Erika, Dobozy Attila: Is tumor thickness alone a sufficient criteria for indication of sentinel node biopsy in melanoma?5th International Conference on

Melanoma,Velence, 2001. február 28-március 3. Melanoma Res. 11: Suppl. 1: S78, 2001

V. Papós Miklós, **Varga János**, Séra Teréz, Lázár Máté, Kapitány Klára, Oláh Judit, Korom Irma, Mohos Gábor, Dobozy Attila, Pávics László: Gamma-probe-guided sentinel lymph node biopsy in melanoma patients. Eur J Nuclear Med 28: 1145, 2001.

VI. Zöllei I, Balogh Á**, Varga J:** Urgent surgical cases caused by primer malignant tumors of smal bowels, Barcelona, 1998, A. 74.

VII. Zöllei I, **Varga J:** Different Anastomosis Techniques in Colorectal Suergery. 3rd United European Gastorenterology Week, Oslo, A. 1347.

VIII. Mohos Gábor, Kiss Erika, **Varga János**, Kapitány Klára: Arcon lévő bőrtumorok esztétikus megoldása. .A Magyar Dermatológiai Társulat Nagygyűlése, Budapest, 2001. december 13-15. P.46.

IX. Szegesdi Ilona, **Varga János**, Mohos Gábor: Középsúlyos égett betegek fájdalomcsillapítása. Magyar Égési Egyesület Konferenciája, Szeged, 2001. május 18-19. P.15.

X. **Varga János**, Kiss Erika, Szegesdi Ilona, Mohos Gábor, Kapitány Klára: Szövetexpanderek alkalmazása a dermatochirurgiában. A Magyar Dermatológiai Társulat Nagygyűlése, Budapest, 2001. december 13-15. P. 54.

XI. Szabad Gábor, Koreck Andrea, Kenderessy Szabó Anna, **Varga János**, Dobozy Attila, Kemény Lajos, Bata-Csörgő Zsuzsanna: Hairy scalp, the ideal donor site for keratinocyte transplantation? 2nd World Union of Wound Healing Societies' Meeting, Párizs, 2004. július 8-13. P94-95.

XII. Szabad Gábor, Koreck Ildikó, Kenderessy Szabó Anna, **Varga János**, Dobozy Attila, Kemény Lajos, Bata Zsuzsanna: Hairy scalp as the donor site for keratinocyte transplantation. A Magyar-Német Dermatológiai Társaság (MNDT/DUDG) 5. Tudományos Ülése. Pécs, 2004. augusztus 26-28. P22

XIII. **Varga J**, Szabad G, Kemény L, Dobozy A: Hairy scalp as a donor site. 2nd World Union of Wound Healing Societies' Meeting, Párizs, 2004. július 8-13. P94-95.

XIV. Oláh Judit, Eiler Nóra, Korom Irma, Varga Erika, Tiszlavitz László, **Varga János**, Szőke T, Bata-Csörgő Zsuzsanna, Dobozy Attila:Sarcoidosis resembling metastasis in melanoma patients induced by interferon alfa2b9th Word Congress on Cancers of the Skin, Sevilla, 2003. május 7-10. P 6.

XV. Szegesdi I, Kis E, Kapitány K, **Varga J**, Mohos G, Vimláti L, Kemény L: Analysis of 2949 burn injuries treated at the Burn and Plastic Surgery Unit of the dermatologic Clinic osf Szeged University a 8-year period. 12th Congress of the European Burns association (EBA), Budapest, 2007. szeptember 12-15. P 34

XVI. Varga E, Korom I, Morvay M, **Varga J**, Németh R, Kovács R, Kemény L: Early and late complications due to esthetic procedures. Német-Magyar Bőrgyógyász Kozmetológiai Kongresszus, Budapest, 2008. június 19-21. p. 34.

LIST OF CONGRESS PRESENTATIONS NOT RELATED TO THE SUBJECT OF THE THESIS

I. Kovács Réka, Oláh Judit, Korom Irma, **Varga János,** Dobozy Attila. Merkel-sejtes carcinoma vesetranszplantált betegenAz MDT Vándorgyűlése,Debrecen, 2002. június 13-15.

II. Séra Teréz, Mohos Gábor, Papos Miklós, Osvay Margit, **Varga János**, Lázár Máté, Kiss Erika, Kapitány Klára, Dobozy Attila, Csernay László, Pávics László: Sentinel node detection in malignant melanoma patients: radiation safety considerationsDermatol Surg 29: 141-145, 2003

III. Kapitány Klára, Kis Erika, Mohos Gábor, Szegesdi Ilona, **Varga János**. A szegedi égéskezelés története. A Magyar Égési Egyesület Kongresszusa, Budapest, 2003. szeptember 25-27.

IV. Szegesdi Ilona, Kapitány Klára, Kis Erika, Mohos Gábor, **Varga János**: Fájdalomcsillapító módszerek összehasonlítása égett sérültjeinknél.A Magyar Égési Egyesület Kongresszusa, Budapest, 2003. szeptember 25-27.

V. Kapitány K, **Varga J**, Mohos G, Kiss E, Szegesdi I, Ágoston Zs, Morvay M: Dermatochirurgia és lézersebészet a kezdetektől napjainkig klinikánkon. Tudományos ülés Dr. Dobozy Attila egyetemi tanár, akadémikus 65. születésnapja tiszteletére. Szeged, 2004. május 5.

VI. Papós M, Oláh J, Lázár M, **Varga J**, Kapitány K, Korom I, Varga E, Dobozy A, Pávics L: Őrszem nyirokcsomó tumoros érintettségének prognosztikai értéke melanoma malignumban – három éves követés. SZAB Orvostudományo Szakbizottság tudományos ülése, Szeged, november 29.

VII. **Varga J**, Mohos G, Kapitány K, Dobozy A, Kemény L: Upper lip reconstruction with Kazanjian flap. 6. Tagung der Deutsch-Ungarischen Gesellschaft, Münster, 2006. április 5-6.

VIII. **Varga J:** Új típusú szöveti expander kifejlesztése. Magyar Dermatológiai Orvoskozmetológiai Kongresszus, Debreceni Bőrgyógyász Továbbképző Napok, Debrecen, 2007. június 28-30.

IX. Mohos G, Kis E, Kapitány K, **Varga J:** Orrdefectus pótlása kombinált lebenyek alkalmazásával

(Esteismertetés). A Magyar Sebész Társaság 59. Kongresszusa, Debrecen, 2008. június 18-20.

X. **Varga J,** Kapitány K, Mohos G, Kis E, Pintér S, Nagy K: Felsőajak rekonstrukció Kazanjian lebennyel. A Magyar Sebész Társaság 59. Kongresszusa, Debrecen, 2008. június 18-20.