J. Biomedical Science and Engineering, 2011, 4, 200-206 **JBiSE**
doi:10.4236/jbise.2011.43028 Published Online March 2011 (http://www.SciRP.org/journal/jbise/).

Cationic polypeptides in a concept of oppositely charged polypeptides as prevention of postsurgical intraabdominal adhesions

Karolin Isaksson, Daniel Åkerberg, Katarzyna Said, Bobby Tingstedt

Department of Surgery, University Hospital of Lund, Sweden.
Email: bobby.tingstedt@med.lu.se

Received 29 November 2010; revised 3 January 2011; accepted 5 January 2011.

ABSTRACT

***Background*: Two differently charged polypeptides, α-poly-L-lysine and poly-L-glutamate, have previously been shown to effectively reduce postoperative intraabdominal adhesions. Though α-poly-L-lysine showed toxicity in doses too close to the lowest therapeutic dose, the aim in the present study was to investigate the possible antiadhesive effect of another four cationic polypeptides. *Materials/Methods*: 125 mice were studied with a standardized and reproducible adhesion model and given epsilon poly-L-lysine, lactoferrin, lysozyme and polyarginine respectively in a combination with poly-L-glutamate. Epsilon poly-L-lysine was also tested in different concentrations and as single treatment. *Results*: All four cationic polypeptides above showed a significantly better anti-adhesive effect than the controls receiving saline ($p < 0.05$). Epsilon poly-L-lysine had the best antiadhesive effect of the new substances tested in the experiment. Single treatment with the epsilon poly-L-lysine showed toxic side effects. *Discussion*: We have shown that epsilon poly-L-lysine, polyarginine, lysozyme and lactoferrin, in descending order, all can reduce postoperative intraabdominal adhesions in mice when combined with poly-L-glutamate. There were side effects of epsilon poly-L-lysine resembling those of α-poly-L-lysine, although less toxic. The antiadhesive effect of epsilon poly-L-lysine did not reach the level of α-poly-L-lysine. Further studies will concentrate on additional investigation, trying to modify the α-poly-L-lysine to lower its toxicity. The less toxic epsilon poly-L-lysine also needs further attention in our research of antiadhesive bioactive polypeptides.**

Keywords: Postoperative Adhesions; Bioactive Polypeptides; Molecular Structure

1. INTRODUCTION

Postoperative abdominal adhesion formation is a significant clinical problem worldwide, especially following lower abdominal surgery [1,2]. Adhesions contain fibrotic tissue that can create bridges between bowels, organs and the abdominal wall, thus creating intraabdominal problems. Abdominal adhesions are not only the leading cause of small bowel obstruction, a common diagnosis often demanding surgical treatment, but also cause abdominal pain and female infertility [3].

The pathogenesis is not completely elucidated but the overall picture is quite clear [4]. The peritoneal surface is very delicate and susceptible to damage. In the process of adhesion formation, the plasmin system plays a crucial part with an imbalance between fibrin formation and degradation at the injured peritoneal site [5-6]. Important factors involved in degradation and formation of local peritoneal adhesions are tPA and PAI-1 [7-10].

The annual cost of adhesion-related diagnosis in Sweden has been estimated at about 6.3 million €/million inhabitants [11].

Various products are available on the market and are used to prevent/reduce the amount of postoperative adhesions [12, for example hyaluronic acid [13] and its derivates and soluble polysaccharides [14] and phospholipids [15]. However, these formulas have not been shown to reduce the risk of small bowel obstruction [12].

In previous experimental studies we have shown very promising results in the reduction of postoperative abdominal adhesions using a combination of differently charged polypeptides, α-poly-L-lysine and poly-L-glutamate [16-18]. The positively charged poly-L-lysine binds to the negatively charged damaged peritoneal surface and then attaches the negatively charged poly-L-glutamate to build a neutral matrix preventing adhesion formation [19]. In a recent study we questioned this combination due to the toxicity observed when the ani-

mals were treated with α-poly-L-lysine alone [20]. The gap between the possible toxicity level of α-poly-L-lysine and the lowest efficient antiadhesive (in combination with poly-L-glutamate) dose is probably too narrow. The aim of this study was to evaluate the possible antiadhesive effect of another four cationic polypeptides, *i.e.* epsilon poly-L-lysine, lactoferrin, lysozyme and polyarginine, together with the negatively charged poly-L-glutamate, based on the concept of using oppositely charged polypeptides for abdominal adhesion protection.

2. METHOD AND MATERIALS

2.1. Animals

One hundred and twenty five (125) female NMRI mice (Scanbur, Stockholm, Sweden) weighing about 25-30 grams were used. The animals were kept in standardized conditions, at a temperature of 22 degrees Celsius and with 12 hours of daylight. The animals had free access to pellets and tap water. The study was conducted with approval of the local ethical committee and the animals received human care in compliance with the guidelines of the Swedish Government and University of Lund, Sweden.

2.2. Chemicals

Osmotic balanced (2.54 w% glycerol) aqueous solutions of the cationic substances epsilon poly-L-lysine (4.7 kDa), lactoferrin (80-90 kDa), lysozyme (14.7 kDa), poly-L-arginine (15-70 kDa), α-poly-L-lysine (> 30 kDa) and the anionic poly-L-glutamat (15-50 kDa) were prepared on the day of the experiment and stored in the refrigerator until used. The chemicals were all purchased from Sigma Aldrich (St Louis, Mo., USA) except for epsilon poly-L-lysine, which was purchased from the Chisso Corporation (Tokyo, Japan).

2.3. Model

The animals were anaesthesized using an intramuscular injection of ketamine (Ketalar, Pfizer, N.Y., USA), 150 mg/kg, and xylazaine (Rompun Vet, Bayer AB, Gothenburg, Sweden), 7.5 mg/kg. A reproducible and standardized model used in our former experiments was adopted for this study [21]. Using an aseptic technique, a 25 mm long midline laparotomy was performed after the abdomen was shaved and disinfected. Parallel on each side, about 10 mm from the midline, a 15 mm long incision, including the peritoneum and underlying mus- cle, was performed. The lateral incisions were occluded with 4 interrupted sutures each of 5/0 PDS with one suture at the end of each incision. The midline incision was enclosed with a running 5/0 PDS in two layers. All the animals were treated with buprenorphine.

After one week, a time interval chosen to match our previous studies, the animals were evaluated concerning intraabdominal adhesions. Anesthesia was induced as described above. The abdomen was opened through a U-shaped incision with its base to the right. Adhesions were considered as tissue (bowels or fat) adherent to the experimental wound or to another intraabdominal organ. The lengths of the incisions as well as the adhesions covering the wound were measured with a caliper up to one-tenth of a millimeter and data were expressed as the percentage of the wound covered by adhesions. The distances were measured at the peritoneal level. Other adhesions between intra abdominal organs were also noticed. The animals received sodium pentobarbital straight after the evaluation for euthanasia, in accordance to the AVMA Guidelines on Euthanasia, 2007.

2.4. Experimental Design

2.4.1. First Part

In the first part of the study the animals were randomly divided into five groups as presented in **Table 1** to examine the antiadhesive effect of different polycations, together with the polyanion poly-L-glutamate. At the end of the operation, just before the abdomen was closed, the treatment substances were installed in volumes and concentrations, as shown in the same table. The different cations were first administered intraabdominally, followed by anion poly-L-glutamate within approximately 10 seconds. Groups number five and six were control groups of 10 animals each, receiving sodium chloride solution (9 mg/ml) and our previous strong antiadhesive formula of poly-L-lysine and poly-L-glutamate respectively.

2.4.2. Second Part

In the second part of the study we examined the antiadhesive effect of the cation with the best antiadhesive proprieties from the first part of the study *i.e.* epsilon

Table 1. Experimental design, part one. Study of different polypeptides.

Group	Animals (N)	Treatment	Concentration (%)	Volume (ml)
1	9	e-PL + PG	0.5 + 0.5	1 + 1
2	10	Lacto + PG	0.5 + 0.5	1 + 1
3	10	Lyso + PG	2.0 + 0.5	1 + 1
4	10	PA + PG	0.5 + 0.5	1 + 1
5	10	NaCl	0.9	2
6	10	αPL + PG	0.5 + 0.5	1 + 1

*e-PL = epsilon poly-L-lysine, PG = poly-L-glutamate, Lacto = lactoferrin, Lyso = lysozyme, PA = poly-L-arginine, αPL = alpha-poly-L-lysine and NaCl = sodium chloride.

poly-L-lysine. Our aim was to investigate the effect with a decreasing amount of the antiadhesive cation-anion complex. The animals were randomly divided into groups as shown in **Table 2**. The substances were installed intra-abdominally and in the same sequence as described above.

2.4.3. Third Part

In the last part of the experiment we studied the possible toxicity of epsilon poly-L-lysine when administered alone, without the neutralizing poly-L-glutamate. Four different concentrations were tested in twenty animals, divided in four groups with five animals in each (**Table 3**). The experiment started with the lowest concentration.

2.5. Statistical Analysis

Values are given as means (SEM). The non parametric Kruskal-Wallis test was used to compare differences in adhesions between the study groups and the Mann-Whitney U-test to determine changes between individual groups. A p-value less than 0.05 was considered statistically significant. For these statistical analyses SPSS® version 17 (SPSS Inc, Chicago, Illinois) was used.

3. RESULTS

In the first part of the study, all animals survived and fared well during the whole experimental period. Epsilon poly-L-lysine, lactoferrin, lysozyme and polyarginine all showed a significant better anti adhesive effect ($p < 0.05$) as compared to the controls receiving sodium hydrochloride (**Figure 1**). As expected, the α poly-L-lysine was significantly more effective ($p < 0.001$) than the control group receiving sodium chloride. As seen in the diagram, the most effective of the four substances tested was epsilon poly-L-lysine, followed by polyarginin, lysozyme and, in last place, lactoferrin. Epsilon poly-L-lysine was equal to α-poly-L-lysine in its antiadhesive effect and both were significantly better than the other three cations.

In the second part of the study, where different concentrations of epsilon poly-L-lysine were tested, all animals fared well and showed no signs of adverse events during the whole experiment. In **Figure 2,** the results are presented and there was a significant anti adhesive effect in every concentration compared to the controls, except

Table 2. Experimental design, part two. Different concentration of epsilon-PL.

Group	Animals (N)	Treatment	Concentration (%)	Volume (ml)
1	10	e-PL + PG	0.05 + 0.05	1 + 1
2	10	e-PL + PG	0.01 + 0.01	1 + 1
3	9	e-PL + PG	0.005 + 0.005	1 + 1
4	10	NaCl	0.9	2

*the abbreviations of the substances are the same as shown in **Table 1**.

Table 3. Experimental design, part three. Toxicity study of epsilon-PL.

Group	Animals (N)	Treatment	Concentration (%)	Volume (ml)
1	5(3)	e-PL	0.5	1
2	5(0)	e-PL	0.1	1
3	5(0)	e-PL	0.05	1
4	5(0)	e-PL	0.01	1
5	7(0)	NaCl	0.9	1

*the number within the parentheses represents the number of animal/s that died. **the abbreviations are the same as shown in **Table 1** above.

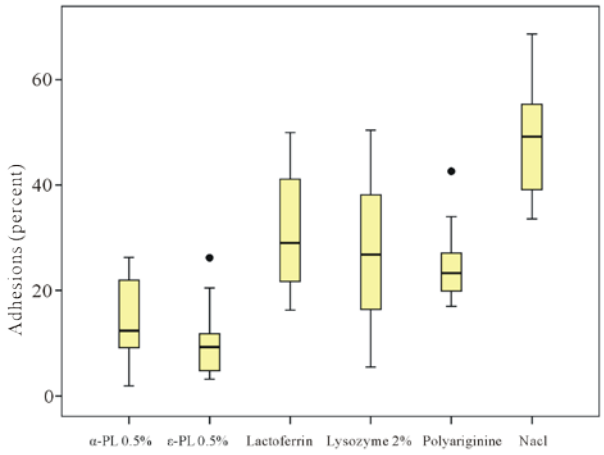

Figure 1. Results of adhesion reduction with various cations in combination with polyglutamate. The dots represent outliers.

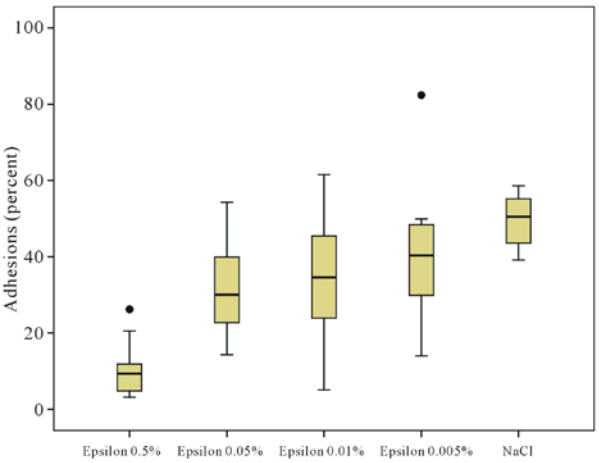

Figure 2. Results of adhesion reduction with different concentrations of epsilon PL in combination with polyglutamate. The dots represent outliers.

for the lowest, p = 0.001, p = 0.002, p = 0.02 and p = 0.103 respectively.

In the third and last part of the experiment we studied the toxicity of epsilon poly-L-lysine. All animals except for three survived and fared well trough the whole study period. Those three that died belonged to the group that received the highest concentration of epsilon poly-L-lysine, at 0.5%. The animals showed distress and inadequate recovery but did not show any convulsions as they did in the toxicity study of α poly-L-lysine [20]. An autopsy of the three animals did not show any signs of intraabdominal bleeding, signs of macroscopic inflammation or intestinal obstruction.

As pointed out above, all animals except for those three mentioned in the third section fared well during the study period. They were observed frequently during their recovery. Food intake did not seem to be changed. The mice were weighed before primary surgery as well as before the evaluation and no changes were observed.

4. DISCUSSION

Polypeptides are a group of macromolecules that are widely used today in biological research as drug carriers and gene vectors and for their antimicrobial properties [22-27]. They are water soluble, biodegradable and often described as non-toxic for humans and the environment [28].

In previous experimental studies we have shown the strong postsurgical intraabdominal anti-adhesive effect of differently charged polypeptides [16]. In our early experiments we noted the optimal effect of the two oppositely charged polypeptides α-poly-L-lysine and poly-L-glutamate, which creates a matrix that serves as a mechanical barrier for adhesion formation [16]. Previous studies have also shown no effect on local immunological functions, *i.e*. peritoneal macrophages, and a local clearance of this biodegradable matrix within 4 weeks [16,19]. The antiadhesive effect of the polypeptides is based on electrostatic binding between the strong cation poly-L-lysine and the negatively charged damaged peritoneum [29], and thereafter electrostatic bonds between the poly-L-lysine and the anion poly-L-glutamate create a mechanical barrier between damaged and adhesion-prone peritoneal tissue. The polypeptide matrix accumulates in areas of damaged peritoneum [16] and has also been shown to aid in intestinal healing as well as decreasing parenchymal bleeding and possibly inflammation [17,18,30].

Due to reports of in vitro and in vivo toxicity using cationic polymers for gene delivery and graft coating [25,31-34] we performed a study on the intraabdominal toxicity of single use of the cation α-poly-L-lysine [20]. This study showed a lethal toxicity in mice with the intraabdominal dose we had previously used. However, in lower doses, the toxicity disappeared but the antiadhesive effect was also diminished. The gap between no toxicity and effect was declared too narrow and we aim to find a less toxic cation to use within the concept of preventing intra-abdominal adhesions with differently charged polypeptides. Poly-L-glutamate, administered alone, has in previous experiments shown that it is non-toxic and even decreased adhesion formation, but is not as promising as when used in complex with poly-L-lysine [19].

In all our previous studies we have used the common form of poly-L-lysine; the α-poly-L-lysine. The alpha form is a long helix-shaped chain that elongates when in contact with the cell membrane. The alpha form carries longer side chains than do most other polypeptides. We hypothesized that due to the long side chains, the alpha form penetrates, interacts and bursts the cell membrane, causing immediate cell cytotoxicity[35-36]. This has support in the literature, suggesting that the toxicity of polypeptides and polycations in particular is not only dose-dependent but also connected to molecular weight and cationic charge density[37-38].

In the present study we have tried to examine other cationic polypeptides with properties that we hypothetically need to create a strong anti-adhesive matrix but still have a non-toxic environment, not only for the combination of polypeptides but also for the polypeptides themselves. These polypeptides would preferably be long enough that a strong matrix can be formed (size) and have a cationic charge sufficient enough to interact with, but not burst, the cell membrane (density). For this test we used two linearly structured substances, *i.e*. epsilon poly-L-lysine and poly-L-arginine, and two globular structured substances, lactoferrin and lysozyme.

Epsilon poly-L-lysine is a natural substance from the metabolism of Streptomyces albus, with the capacity to inhibit growth of both grampositive and gramnegative bacteria. It is widely used as a food preservative and is also reported having an antitumoral effect [28,39-40]. Epsilon poly-L-lysine is of shorter length and has shorter sidechains than α-poly-L-lysine (**Figure 3**), and therefore, we hypothesize, carries less membrane cytotoxicity. Poly-L-arginine is a well-known protein transduction domain used to transport molecules into cells[41]. It is of roughly the same size as α-poly-L-lysine. Lactoferrin is also known as lactotransferrin. It is a globular multifunctional glycoprotein that contains many polycation domains. It has antimicrobial and anti-inflammatory activity and is found in milk and many mucosal secretions such as tears and saliva[42]. Lysozyme is an enzyme that is part of the innate immune system and, like the previous substances, has an antimicrobial effect. It is present in many mucosal secretions like lactoferrin.

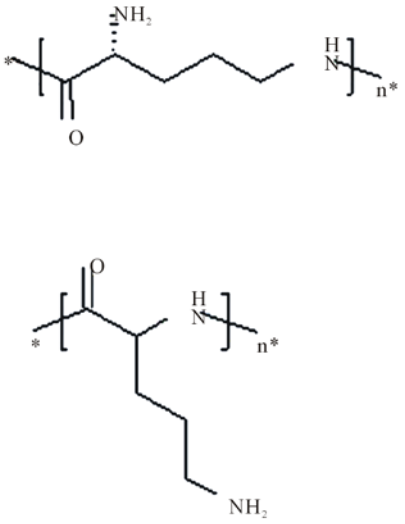

Figure 3. Chemical structure of *epsilon poly-L-lysine* (at the top) and *alpha poly-L-lysine* (at the bottom) with the monomers presented within the brackets. (Isaksson K, Åkerberg D, Said K, Tingstedt B)

In the present study we have shown that all of the new polycationic substances tested with poly-L-glutamate in the experiment reduced the amount of adhesion formation significantly compared to the controls, further strengthening the concept of the use of differently charged polypeptides as a matrix barrier for postsurgical adhesion control.

Epsilon poly-L-lysine was superior to polyarginine, which in turn was more effective than lysozyme and lactoferrin. This indicates that a polypeptide with a linear structure is better than one that is globular, probably due to the fact the globular substances have their ionic charges turned inward. The four-fold increase in the concentration of lysozyme in this experiment is based on its small size, but mostly due to its ball-like structure that does not expose as much charge as the other substances. In previous studies we have also shown a decreased effect of α-poly-L-lysine when using smaller size (shorter chains) [43] and also an effect, however small, of lysozyme in a concentration of 1% [19]. By increasing the concentration of lysozyme we hoped to create enough molecules for strong matrix formation.

In the second and third part of the experiment we focused on the antiadhesive effect of epsilon poly-L-lysine, which turned out to be equal to that of α-poly-L-lysine.

In the latter parts of the study a diminished antiadhesive effect of epsilon poly-L-lysine was shown, as expected, with decreasing doses. However, epsilon poly-L-lysine showed a significant antiadhesive effect in combination with poly-L-glutamate 40-fold below toxicity level as shown in part three of the experiment.

The toxicity of epsilon poly-L-lysine was 10-fold lower compared to α-poly-L-lysine [28], most probably due to the fact that it is smaller in size. Therefore epsilon poly-L-lysine is promising as a polypeptide that could be part of a future antiadhesive treatment, even though an even lower toxicity level would be preferred.

However, there is still room for the development of the concept of bioactive biodegradable oppositely charged polypeptides as antiadhesive treatment.

One possibility might be to construct a premix of alfa poly-L-lysine and poly-L-glutamate with an excess of poly-lysine resulting in a one-dose administration, where the oppositely charged polypeptides are already bound to each other, but with a net positive complex within toxicity levels. Another possible way could be to alter the cationic polypeptide to decrease the cationic charge density, or to alter it spatially.

5. CONCLUSIONS

In summary, we have further proven the antiadhesive effect of using two oppositely charged polypeptides in an experimental mouse model. The use of epsilon poly-L-lysine as the cationic part is promising and needs further attention, and studies along with parallel continuous research for a more atoxic cationic polypeptide in the setting of antiadhesive oppositely charged bioactive polypeptides, preferably of smaller size and of lower ionic density. Studies are ongoing in vitro for cytotoxic evaluation and in vivo to examine the potential direct influence on the fibrosis-fibrinolysis balance. A new model is being developed for testing lower concentra- tions and volumes of the polypeptides used.

This study was performed in parts due to grants from Craaford Stiftelser.

REFERENCES

[1] Ellis, H., Moran, B.J., Thompson, J.N., Parker, M.S., Wilson, M.S. *et al.* (1999) Adhesion-related hospital readmissions after abdominal and pelvic surgery: A retrospective cohort study. *Lancet*, **353**, 1476-1480. doi:10.1016/S0140-6736(98)09337-4

[2] Menzies, D. and Ellis, H. (1990) Intestinal obstruction from adhesions—how big is the problem? *Annals of The Royal College of Surgeons of England*, **72**, 60-63.

[3] Tingstedt, B., Andersson, E., Isaksson, K. and Andersson, R. (2008) Clinical impact of abdominal adhesions: What is the magnitude of the problem? *Scandinavian Journal of Gastroenterology*, **43**, 255-261. doi:10.1080/00365520701708626

[4] Witkowicz, J., (2008) Pol Arch Med Wewn. *Mesothelial Cell Transplantation.* **118**, 307-313.

[5] Liakakos, T., Thomakos, N., Fine PM, Dervenis C and Young, RL., (2001) Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Recent ad-

vances in prevention and management. *Dig Surgery*, **18**, 260-273. doi: 10.1159/000050149

[6] Holmdahl, L., and Ivarsson, ML., (1999) The role of cytokines, coagulation, and fibrinolysis in peritoneal tissue repair. *European Journal of Surgery*, **165**, 1012-1019. doi: 10.1080/110241599750007810

[7] van Goor H, de Graaf JS, Grond J, Sluiter WJ, van der Meer J, Bom VJ and Bleichrodt RP. (1994) Fibrinolytic activity in the abdominal cavity of rats with faecal peritonitis. *British Journal of Surgery*, **81,** 1046-1049. doi: 10.1002/bjs.1800810740

[8] Scott-Coombes, D., Whawell, S. and Vipond, MN. (1995) Human intraperitoneal fibrinolytic response to elective surgery. *British Journal of Surgery*, **82**, 414-417. doi: 10.1002/bjs.1800820346

[9] Reijnen, M.M., Bleichrodt, RP. and van Goor, H. (2003) Pathophysiology of intra-abdominal adhesion and abscess formation, and the effect of hyaluronan. *British Journal of Surgery*, **90**, 533-541. doi: 10.1002/bjs.4141

[10] Ivarsson, M.L., Bergström, M., Eriksson, E., Risberg. B. and Holmdahl, L. (1998) Tissue markers as predictors of postoperative adhesions, *British Journal of Surgery*, **85**, 1549-1554. doi: 10.1046/j.1365-2168.1998.00859.x

[11] Tingstedt, B., Isaksson, J. and Andersson, R. (2007) Long-term follow-up and cost analysis following surgery for small bowel obstruction caused by intra-abdominal adhesions. *British Journal of Surgery*, **94**, 743-748. doi: 10.1002/bjs.5634

[12] Tingstedt, B., Isaksson, K., Andersson, E., Andersson, R. (2007) Prevention of abdominal adhesions--present state and what's beyond the horizon? *European Surgery Research*, **39**, 259-268. doi: 10.1159/000102591

[13] Johns, D.B., Rodgers, K.E., Donahue, W.D., Kiorpes, T.C. and diZerega, G.S. (1997) Reduction of adhesion formation by post-operative administration of ionically cross-linked hyaluronic acid. *Fertility and Sterility*, **68**, 37-42. doi:10.1016/S0015-0282(97)81472-0

[14] diZerega, G.S., Verco, S.J., Young, P., Kettel, M., Kobak, W.A., Martin, D. *et al.* (2002) A randomized, controlled pilot study of the safety and efficacy of 4% icodextrin solution in the reduction of adhesions following laparoscopic gynaecological surgery. *Human Reproduction*, **17**, 1031-1038. doi:10.1093/humrep/17.4.1031

[15] Muller, S.A., Treutner, K.H., Tietze, L., Anurov, M., Titkova, S., Polivoda, M. *et al*. (2001) Efficacy of adhesion prevention and impact on wound healing of intraperitoneal phospholipids. *Journal of Surgical Research*, **96**, 68-74. doi:10.1006/jsre.2000.6031

[16] Nehez, L., Tingstedt, B., Vodros, D., Axelsson, J., Lindman, B. and Andersson, R. (2006) Novel treatment in peritoneal adhesion prevention: protection by polypeptides. *Scandinavian Journal of Gastroenterology*, **41**, 1110-1117. doi:10.1080/00365520600554550

[17] Tingstedt, B., Nehez, L., Axelsson, J., Lindman, B. and Andersson, R. (2006) Increasing anastomosis safety and preventing abdominal adhesion formation by the use of polypeptides in the rat. *International Journal of Colorectal Disease*, **21**, 566-572. doi:10.1007/s00384-005-0053-x

[18] Tingstedt, B., Nehez, L., Lindman, B. and Andersson, R. (2007) Efficacy of bioactive polypeptides on bleeding and intra-abdominal adhesions. *European Surgical Research*, **39**, 35-40. doi:10.1159/000098438

[19] Nehez, L., Vodros, D., Axelsson, J., Tingstedt, B., Lindman, B. and Andersson, R. (2005) Prevention of postoperative peritoneal adhesions: Effects of lysozyme, polylysine and polyglutamate versus hyaluronic acid. *Scandinavian Journal of Gastroenterology*, **40**, 1118-1123. doi:10.1080/00365520510023332

[20] Isaksson, K., Åkerberg, D., Andersson, R. and Tingstedt, B. (2009) Toxicity and dose response of intra-abdominally administered poly-L-alpha-lysine and poly-L-glutamate for post-operative adhesion protection. *European Surgical Research*, **44**, 17-22. doi:10.1159/000258654

[21] Holmdahl, L., Al-Jabreen, M. and Risberg, B. (1994) Experimental models for quantative studies on adhesion formation in rats and rabbits. *European Journal of Surgery*, **26**, 248-56. doi:10.1159/000129342

[22] King, A., Strand, B., Rokstad, A.M., Kulseng, B., Andersson, A. *et al.* (2003) Improvement of the biocompatibility of alginate/poly-L-lysine/alginate microcapsules by the use of epimerized alginate as a coating. *Journal of Biomedical Materials Research A*, **64**, 533-539. doi:10.1002/jbm.a.10276

[23] Takei, Y., Maruyama, A., Ferdous, A., Nishimura, Y., Kawano, S., Ikeijima, K. *et al.* (2004) Targeted gene delivery to sinusoidal endothelial cells: DNA nanoassociate bearing hyaluronanglycocalyx. *The FASEB Journal*, **18**, 699-701.

[24] Geornaras, I., Yoon, Y., Belk, K.E., Smith, G.C. and Sofos, J.N. (2007) Antimicrobial activity of epsilon-polylysine against Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes in various food extracts. *Journal of Food Science*, **72**, M330-334. doi:10.1111/j.1750-3841.2007.00510.x

[25] Billinger, M., Buddeberg, F., Hubbell, J.A., Elbert, D.L., Schaffner, T., Mettler, D. *et al.* (2006) Polymer stent coating for prevention of neointimal hyperplasia. *Journal of Invasive Cardiology*, **18**, 423-426.

[26] Li, C., Yu, D.F., Newman, R.A., Cabral, F., Stephens, L.C., Hunter, N. *et al.* (1998) Complete regression of well-established tumors using a novel water-soluble poly-(L-glutamic acid)-paclitaxel conjugate. *Cancer Research*, **58**, 2404-2409.

[27] Moroson, H. (1971) Polycation-treated tumor cells in vivo and in vitro. *Cancer Research*, **31**, 373-380.

[28] Shih, I.L., Van, Y.T. and Shen, M.H. (2004) Biomedical applications of chemically and microbiologically synthesized poly (glutamic acid) and poly(lysine). *Mini Reviews in Medicinal Chemistry*, **4**, 179-188. doi:10.2174/1389557043487420

[29] Larsson, K. (1994) Lipids-molecular organizations, physical functions and technical applications. The Oily Press, Dundee, Scotland.

[30] Tingstedt, B., Nehez, L., Lindman, B. and Andersson, R. (2007) Effect of bioactive polypeptides on leaking large bowel anastomosis and intestines in the rat. *Journal of Investigative Surgery*, **20**, 229-235. doi:10.1080/08941930701481288

[31] Morgan, D.M., Larvin, V.L. and Pearson, J.D. (1989) Biochemical characterisation of polycation-induced cytotoxicity to human vascular endothelial cells. *Journal of Cell Science*, **94**, 553-559.

[32] Moreau, E., Domurado, M., Chapon, P., Vert, M. and Domurad, D. (2002) Biocompatibility of polycations: In vitro agglutination and lysis of red blood cells and in vivo toxicity. *Journal of Drug Targeting*, **10**, 161-173. doi:10.1080/10611860290016766

[33] Sela, M. and Katchalski, E. (1959) Biological properties of poly-alpha-amino acids. *Advances in Protein Chemistry*, **14**, 391-478. doi:10.1016/S0065-3233(08)60614-2

[34] Kenausis, G., Voros, J., Elbert, D., Huang, N., Hofer, R., Riuz-Taylor, L. *et al.* (2000) Poly(l-lysine)-g-Poly-(ethylene glycol) layers on metal oxide surfaces: Attachment mechanism and effects of polymer architecture on resistance to protein adsorption. *The Journal of Physical Chemistry B*, **104**, 3298- 3309. doi:10.1021/jp993359m

[35] Andrews, D.W. and Ottensmeyer, F.P. (1982) Electron microscopy of the poly-L-lysine alpha-helix. *Ultramicroscopy*, **9**, 337-348. doi:10.1016/0304-3991(82)90094-8

[36] Chittchang, M., Alur, H.H., Mitra, A.K. and Johnston, T.P. (2002) Poly(L-lysine) as a model drug macromolecule with which to investigate secondary structure and membrane transport, part I: Physicochemical and stability studies. *Journal of Pharmacy and Pharmacology*, **54**, 315-323. doi:10.1211/0022357021778556

[37] Fischer, D., Li. Y., Ahlemeyer, B., Kriegelstein, J. and Kissel, T. (2003) In vitro cytotoxicity testing of polycations: Influence of polymer structure on cell viability and hemolysis. *Biomaterials*, **24**, 1121-1131. doi:10.1016/S0142-9612(02)00445-3

[38] Lv, H., Zhang, S., Wang, B., Cui, S. and Yan, J. (2006) Toxicity of cationic lipids and cationic polymers in gene delivery. *Journal of Controlled Release*, **114**, 100-109. doi:10.1016/j.jconrel.2006.04.014

[39] Szende, B., Szokan, G., Tyiha, E., Pal, K., Gaborjanyi, R., Akmas, M. *et al*. (2002) Antitumor effect of lysine- isopeptides. *Cancer Cell International*, **2**, 4. doi:10.1186/1475-2867-2-4

[40] Shih, I.L., Shen, M.H. and Van, Y.T. (2004) Microbial synthesis of poly(ε-lysine) and its various applications. *Bioresource Technology*, **97**, 1148-1159. doi:10.1016/j.biortech.2004.08.012

[41] Fuchs, S.M. and Raines, R.T. (2004) Pathway for polyarginine entry into mammalian cells. *Biochemistry*, **43**, 2438-2444. doi:10.1021/bi035933x

[42] Steijns, J.M. and van Hooijdonk, A.C. (2000) Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. *British Journal of Nutrition*, **84**, S11-17. doi:10.1017/S0007114500002191.

[43] Nehez, L., Tingstedt, B., Axelsson, J. and Andersson, R. (2007) Differently charged polypeptides in the prevention of post-surgical peritoneal adhesions. *Scandinavia Journal of Gastroenterology*, **42**, 519-523. doi: 10.1080/00365520600988204

