# BIOMATERIALS BASED ON CHITOSAN AND ITS DERIVATIVES TO PREVENT ADHESION FORMATION

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**Abstract**

*An increase in the number and volume of surgical interventions leads to an increase in the frequency of postoperative adhesions. The development of the adhesion process in the abdominal cavity causes pain, a decrease in the quality of life of patients, a violation of the reproductive function of women as well as acute adhesion intestinal obstruction. Recently, polymer biomaterials, including those based on chitosan, have been widely used for the prevention of adhesions. Due to their biocompatibility and biodegradation ability, they do not require repeated operations to extract the material. It is believed that these materials act as barriers, physically separating the damaged surfaces. The molecular mechanism of their action is still poorly understood. In this review, the main mechanisms of adhesion formation, as well as ways to prevent them with the help of materials based on chitosan and its derivatives, are discussed.*

***Keywords:*** *prevention of adhesions, biomaterials, polymers, chitosan, derivatives of chitosan*

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## 1. Introduction

Adhesion formation is a common process that often occurs after surgical procedures, such as operations on the abdominal and pelvic organs, tendon repair, plastic surgery, and others. Postoperative adhesions are the main cause of complications, many of which can occur several years after surgery. These complications include intestinal obstruction, pelvic or abdominal pain and infertility. The frequency of adhesions after the first surgical intervention varies from 10.4% to 67%, and it increases to 90%-100 % of cases after repeated operations. Despite the progress in medicine and surgery, the number of patients with adhesive disease continues to increase proportionately to the number of operations.

In response to the damage caused by surgical intervention, an inflammatory reaction develops, which is accompanied by a significant release of cytokines, expansion of the vascular lumen and release from the capillaries of exudate rich in fibrinogen. As a result of the coagulation cascade activation, thrombin is formed, which triggers the conversion of fibrinogen to fibrin. In this case, the fibrinolytic system is activated and, with normal healing, any intra-abdominal fibrin deposits are lysed. However, after surgery, the balance between coagulation and fibrinolysis shifts towards the coagulation system. Fibrin deposits form matrix for the ingrowth of fibrous tissue. Fibroblasts penetrate the fibrin matrix, and extracellular matrix proteins are formed and deposited. The extracellular matrix can be completely destroyed by matrix metalloproteinases, which would lead to normal healing. However, this process can be inhibited by tissue inhibitors of matrix metalloproteinases. As a result, the extracellular matrix proteins collagen and fibronectin form a framework for the mesothelial cell layer, which leads to re-epithelisation and the formation of adhesions.

To create anti-adhesion biomaterials, polymers of natural origin are often used, including chitosan and its derivatives, the main mechanism of action of which is considered to be the physical separation of damaged surfaces. In preclinical studies and clinical trials, biomaterials reduced the formation of adhesions to varying degrees; however, none of them achieved 100% prevention of adhesion formation. An improved understanding of the pathophysiology of the adhesion development and changes in molecular biological mechanisms compared with the restoration of the peritoneum will allow the creation of anti-adhesion agents with high efficiency. This review examines the main mechanisms of the adhesion formation after surgical interventions, as well as ways to prevent them with the help of chitosan, its derivatives and chitosan-based biomaterials.

## 2. Anti-adhesion Agents Based on Chitosan and Its Derivatives

Currently, anti-adhesion agents are mainly developed from natural polymers: proteins (collagen, gelatine) and polysaccharides (hyaluronic acid, cellulose, dextran, chitosan, etc.). Natural polysaccharides from various sources have long been studied and are widely used in various fields of medicine and pharmacy. There is an increased interest in the use of biologically active polysaccharides in biomedicine due to their biocompatibility, biodegradability, non-toxicity, as well as some specific biological properties. In particular, biologically active polysaccharides are used in tissue engineering, wound healing, as well as for drug delivery and the development of anti-adhesion materials [1]. The main mechanism of anti-adhesion agent action is considered to be the physical separation of damaged surfaces. However, materials that effectively prevent the formation of adhesions, in addition to the barrier function, can affect the mechanism of adhesion formation.

**2.1. The Mechanisms of Adhesion Formation**

The molecular mechanisms of adhesion formation are being actively investigated. The classic concept of adhesion formation is a violation of the balance between the production of fibrin and fibrinolysis. In response to damage to the peritoneum caused by surgery, infection or irritation, an inflammatory reaction develops, accompanied by a significant release of cytokines, dilation of the lumen of the vessels and the release of exudate from the capillaries. Exudate rich in fibrinogen accumulates inside the peritoneum. Fibrin occurs as a result of the activation of the coagulation cascade, which is activated in the abdominal cavity and leads to the formation of thrombin, an enzyme that converts fibrinogen to fibrin. Fibrinolysis is important to repair the damage without adhesions that occurs under the action of the enzyme plasmin. Plasmin, a serine protease that acts on fibrin, is produced in the liver, where it is stored as an inactive precursor called plasminogen. Plasmin is converted into an active form by tissue plasminogen activator (t-PA) or urokinase-type plasminogen activator (u-PA) [2]. Plasminogen activator inhibitors (PAI) form inactive complexes with t-PA and u-PA [3]. A violation of the balance of the fibrinolytic pathway occurs during surgical interventions [4]. Fibrin deposits predispose to the ingrowth of fibrous tissue due to the production of extracellular matrix proteins by fibroblasts migrating to the fibrin matrix. Plasmin also activates matrix metalloproteinases (MMPs), which specifically hydrolyse the main proteins of the extracellular matrix. MMPs belong to the family of zinc metalloproteinases, as they contain $Zn^{2+}$ [5] in the active centre. Fibrin is a substrate for MMP-9; it decomposes fibrin directly, independently of plasmin [6]. The concentration of MMP-9 in peritoneal exudate in women with pelvic adhesions was found to be significantly lower than in women without adhesions [6]. The specific MMP-9 substrate, type IV collagen, was found to be increased more than 2 times in the fibroblasts of the adhesions compared to normal peritoneal fibroblasts [7]. In addition, elevated serum levels of MMP-2 and MMP-9 have been suggested as a marker for peritoneal adhesions [8]. The extracellular matrix can be completely destroyed by MMPs, which would lead to normal healing. However, this process can be inhibited by tissue inhibitors of MMPs (TIMPs), a phenomenon that leads to the formation of adhesions [9]. Chegini *et al*. [10] demonstrated that the adhesion tissue had increased levels of TIMP-1 and a lower ratio of MMP-1 to TIMP-1 compared with an intact peritoneum in the same patient. It has also been shown that the levels of TIMP-1 in the abdominal fluid in people with extensive adhesions are higher compared with patients without adhesions [10]. Xin Liu *et al*. [11] showed that intraperitoneal administration of berberine – an inhibitor of TIMP-1 – led to a decrease in adhesion formation in rats.

It has been shown that if the fibrinolysis carried out by plasmin or MMPs does not occur within 5-7 days after the peritoneal injury, the temporary fibrin matrix is preserved and gradually organised with the help of collagen-secreting fibroblasts. Fibroblasts located in the extracellular matrix form the framework for the mesothelial cell layer, alongside collagen and fibronectin. Together, these molecules lead to the formation of adhesions and the growth of new blood vessels mediated by angiogenic factors [12, 13].

It has been proven experimentally that many of the cytokines found in the peritoneal fluid after trauma play an important role in the subsequent formation of adhesions. Profibrotic transforming growth factor beta (TGF-β1), which is associated with increased adhesion formation and fibrosis, has been characterised best. TGF-β1 is expressed mostly by mesothelial cells and adherent fibroblasts. The peritoneal fluid and tissues of patients with adhesions contain an increased level of TGF-β1 [14]. Hypoxia induces the expression of TGF-β1 messenger RNA (mRNA) and type I collagen by peritoneal fibroblasts [15]. TGF-β1 in turn stimulates angiogenesis during peritoneal recovery [14].

Interleukin 1 (IL-1) and tumour necrosis factor alpha (TNF-α) are among the earliest cytokines present in the damaged abdominal cavity. Intraperitoneal administration of IL-1 after caecal injury in rats increased adhesion scores after 10 days, findings that confirm the role of IL-1 in the adhesion formation [16]. TNF-α is also a marker of adhesion formation: the higher the level of TNF-α in peritoneal fluid and plasma, the more severe the subsequent adhesions are formed [17]. IL-10 reduced the formation of adhesions in the model of peritoneal wall damage in mice [18].

Interferon gamma (IFN-γ) is another cytokine that plays a significant role in the development of adhesions. After injury, intraperitoneal levels of IFN-γ and IL-17 increased both in animals and humans [19]. Neutralisation of IFN-γ and IL-17 reduced the formation of adhesions. Repeated injections of IL-17 into the peritoneum of mice led to inflammation and fibrosis [20].

Other cell populations also play a role in the inflammatory recovery phase. Peritoneal macrophages cause immune responses that underlie the formation of adhesions. Macrophages appear on the damaged areas within 24 h, adhere to the site of damage and persist even after the complete restoration of the mesothelial layer [21]. In response to damage, macrophages increase phagocytic and secretory activity; they also attract new mesothelial cells and fibroblasts [22]. Macrophages release chemokine (C-C motif) ligand 1 (CCL1) and its receptor chemokine (C-C motif) receptor 8 (CCR8) in response to damage. Experimentally, a decrease in the CCL1-CCR8 interaction reduced peritoneal macrophage migration and adhesion formation [23].

Mast cells degranulate at the repair sites, releasing vascular endothelial growth factor (VEGF) and other angiogenic and vasoactive molecules that play a role in adhesion formation [24]. VEGF is directly involved in early inflammatory processes and wound healing, affecting fibroblast function. In an animal model, intraperitoneal treatment with a VEGF antibody resulted in a lower rate of late-stage adhesion formation [25]. The stabilisation of mast cells to prevent degranulation reduced the formation of adhesions in the cecum abrasion model [26].

### 2.2. Required Properties of Anti-adhesion Materials

Anti-adhesion agents prevent the formation of adhesions to varying degrees in animal models and/or clinical studies, but none of them is effective in all cases [27]. The main disadvantages of the existing materials are: short residence time at the injection site [28], loss of activity in the presence of blood, the need for fixation to tissues as well as the difficulty of use in laparoscopic operations [29]. Anti-adhesion materials can have various forms: solid (membranes, films, powders), gel-like (gels, micelles) and liquid (solutions, sprays) [30]. Membranes or pre-formed gels are placed and fixed directly on the damaged surfaces where the formation of adhesions is potentially possible. Fixing with threads can cause increased adhesion formation, so to obtain anti-adhesion agents, it is necessary to use materials that adhere to the damaged surfaces without additional fixation. Another disadvantage associated with the use of membranes or pre-formed gels is the relative difficulty in determining the size and shape of the area to be covered. The surgeon should know in advance the possible location, size and shape of potential areas of adhesions [31]. During laparoscopic surgery or in a case of inaccessibility of damaged areas, it is more convenient to use injectable gel forms of anti-adhesion materials. They are easily applied with a syringe or form a gel when in contact with a damaged surface. Thus, an ideal anti-adhesion material should be safe and effective, adhere to damaged surfaces without additional fixation, remain active in the presence of blood, be completely biodegradable without the need for removal and should not interfere with the wound healing [32]. The most promising among anti-adhesion agents are materials based on biocompatible,

biodegradable polymers, because they meet all the requirements for anti-adhesion agents, and could also contribute to wound healing due to their biological properties.

### 2.3. Chitosan-Based Anti-adhesion Agents

Chitosan is a cationic polysaccharide, a deacetylated derivative of the natural polysaccharide chitin, which is the main component of the exoskeleton of arthropods or the cell walls of fungi and yeasts. Chitosan consists of D-glucosamine and *N*-acetyl--D-glucosamine units connected by β-1,4-glycosidic bonds. Chitosan, either in solution or in solid form, has a haemostatic effect. In the early 2000s, researchers had found that chitosan not only causes platelet aggregation, but also increases the concentration of calcium ions ($Ca^{2+}$) in platelets and the expression of glycoprotein IIb/IIIa on platelet membranes, increasing the haemostatic effect [33]. Chitosan also promotes wound healing and repair of mesothelial cells [34], inhibits the growth of fibroblasts and reduces the synthesis of collagen fibres [35]. The listed biological properties of chitosan affect the healing process of damaged tissues, helping to prevent the formation of adhesions. Unmodified chitosan often has low anti-adhesion efficiency, a factor that may be due to its high positive charge, which causes interaction with negatively charged cell compartment. Lin Long-Xiang *et al*. [36] compared the efficacy of several anti-adhesion agents such as polylactic acid film (PLA), Seprafilm®, a medical solution containing polyethylene glycol and berberine (PEG), medical sodium hyaluronate gel (HA) and medical chitosan. The authors showed a decrease in the efficacy of anti-adhesion materials as follows: Seprafilm > PLA >> HA > chitosan > PEG [36]. Yeo *et al.* [37] investigated the effect of ultraviolet (UV)-crosslinked and unmodified chitosan on postoperative adhesions in the abdominal cavity of rabbits. Unmodified chitosan caused a granulomatous reaction with the formation of adhesions in several animals, while chitosan cross-linked by UV radiation did not lead to adhesion formation [37]. Wang *et al*. [38] compared the prophylactic effect of mitomycin-C and chitosan on intra-articular adhesions after knee surgery in rabbits. Mitomycin-C and chitosan prevented adhesion formation by inhibiting fibroblast proliferation and reducing the formation of collagen fibres [38]. Chitin can also serve as the basis for the development of anti-adhesion barrier materials, as it has been shown to prevent epidural fibrosis [39]. One of the possible mechanisms of chitosan action has been shown in a model of flexor tendon repair in rabbits and was associated with the transmission of sirtuin 1 (SIRT1) signals. In addition, chitosan has been shown to inhibit inflammation and protect tendocytes from apoptosis by suppressing nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activating p53 via SIRT1. The increased regulation of SIRT1 as a result of chitosan treatment of damaged tendons may be useful in the development of future therapeutic strategies for the treatment of tendon injuries [40].

### 2.4. Anti-adhesion Agents Based on Chitosan Derivatives

Due to the presence of reactive amino groups, chitosan is easily modifiable, which makes it possible to create biomaterials with different properties. Water-soluble chitosan derivatives, such as *N*-carboxymethylchitosan, *O*-carboxymethylchitosan and *N*,*O*-carboxymethylchitosan, prevent adhesion formation; they do not require their removal after surgery, because they are decomposed *in vivo* by macrophages [41]. Among chitosan derivatives, *N*,*O*-carboxymethylchitosan is the most widely used. It inhibited peritoneal adhesions in the fallopian tubes of rats and in a model of small intestinal lacerations, making it a more effective anti-adhesion agent than hyaluronic acid [42]. In addition, the effectiveness of the gel and the *N*,*O*-carboxymethylchitosan solution has been studied in three surgical models: abdominal aortic anastomosis, intestinal anastomosis and abdominal wall incision.

*N*,*O*-carboxymethylchitosan was more effective and it did not interfere with postoperative tissue healing in rats at doses that prevent the formation of peritoneal adhesions [43]. *N*,*O*-carboxymethylchitosan significantly reduced fibrosis and the formation of adhesions without side effects in cardiac surgery in animals [44]. In addition, mouse fibroblasts and macrophages do not adhere to the surface of *N*,*O*-carboxymethylchitosan-coated culture plates even in the presence of serum [45]. Lopes *et al.* [46] reported that the combined use of keratinocyte growth factor and *N*,*O*-carboxymethylchitosan produced a synergistic effect in inhibiting postoperative pericardial adhesions. Zheng *et al.* [47] showed that the anti-adhesive effect of *N*,*O*-carboxymethylchitosan was associated with the inhibition of inflammatory cells accumulation and a reduced expression of TGF-β1. Another chitosan derivative, *O*-carboxymethylchitosan, also effectively prevented the formation of postoperative adhesions by reducing the accumulation of inflammatory cells and fibroblasts, and it also reduced collagen synthesis [48].

**2.5. Anti-adhesion Biomaterials Based on Chitosan Derivatives and Other Polymers**

Often, several polymers are used to develop anti-adhesion materials, permitting a change in the physical characteristics of composites (strength, adhesiveness, porosity), as well as in their biological activity (degradation time in the body, antibacterial, haemostatic properties). In combination with chitosan and its derivatives, natural polymers (gelatine, collagen, starch, dextran, cellulose derivatives, hyaluronic acid) and synthetic polymers (copolymers of polylactic and glycolic acid, polyethylene oxide, polypropylene, polyester, etc.) are used. Based on the photo-induced crosslinking reaction of imines, Yun long Yang *et al*. [49] developed a hydrogel that consisted of modified carboxymethylcellulose (CMC) and chitosan-glycol. There was a significant reduction in the adhesion formation (20% of animals with adhesions) in the hydrogel-treated group compared with the control group (100% adhesions) [49]. On the basis of *N*,*O*-carboxymethylchitosan and oxidised regenerated cellulose, a composite gauze (*N*,*O*-CS/ORC) was obtained. This *N*,*O*-CS/ORC material has antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, haemostatic activity and was biodegradable. Modelling of the adhesive process in rats showed a high efficiency of the resulting gauze, and thus it has possible clinical application in surgery [50]. An anti-adhesion membrane based on cross-linked transglutaminase (TGase) carboxymethylchitosan, carboxymethylcellulose and collagen in the ratio of 25/25/50 significantly prevented peritoneal adhesions with negligible immunogenicity. Histological examination of the damaged tissues showed that the membrane reduced the synthesis of collagen [51].

Zhou *et al*. [52] found that gelatinised chitosan films effectively prevented peritoneal adhesions induced by wounds, ischaemia and infection. A composite mesh with a three-dimensional macroporous structure, formed by polyester, oxidised collagen and chitosan, was used to repair abdominal wall defects in a rabbit model. The composite caused good peritoneal regeneration and reduced formation of postoperative adhesions [53]. Lin Long-Xiang *et al*. [54] developed a chitosan-gelatine hydrogel obtained by crosslinking with carbodiimide (CD-CS-gelatine); they used a chitosan solution was used as a reference drug. The authors modelled adhesion formation by using a damage to the abdominal wall and cecum. The use of a CD-CS gelatine gel significantly reduced the number of adhesions in rats from 100% to 50% compared with the control material. The chitosan solution reduced the formation of adhesions only to 88% [54].

Lauder *et al.* [55, 56] reported that chitosan-dextran gels significantly reduced the formation of abdominal adhesions without side effects. At the same time, treatment with a spray-sealant based on chitosan and starch reduced adhesion formation by 76% after endoscopic sinus surgery in sheep in a model of chronic sinusitis [57].

Chitosan membranes bound to a polypropylene mesh were able to reduce peritoneal adhesions in rats, as they did not cause tissue reactions and did not increase inflammation [58]. In addition, Jayanth *et al*. [59] reported the effectiveness of polypropylene mesh coated with chitosan. Li *et al*. [60] showed that poly-L-glutamic acid and chitosan-based barriers effectively inhibited epidural fibrosis and peridural adhesions in the rabbit model. Jae Eok Ko *et al.* [61] developed a nanofibre mat by electrospinning moulding from chitosan, a copolymer of polylactic and glycolic acid (PLGA), and polyethylene oxide (PEO). In a model of abdominal wall and cecum damage in rats, the mats effectively prevented adhesions.

In most of the relevant studies conducted, the authors have not analysed the molecular mechanisms of action of anti-adhesion agents. An exception is Tian Lin *et al.* [62], who examined a biomaterial based on chitosan, cellulose and seaweed polysaccharide (CCS). The material significantly reduced the formation of intraperitoneal adhesions in rats. Specifically, the composite suppressed the expression of TGF-β1, plasminogen activator inhibitor 1 (PAI-1) and smooth muscle alpha-actin. As a result, the production of t-PA was significantly enhanced, and fibrinolysis was increased accordingly. In addition, CCS reduced the activity of kinases – transformative growth factor-activated kinase 1 (TAK1), N-terminal c-Jun kinase (JNK)/stress-activated protein kinase (SAPK) and p38 – in the mitogen-activated protein kinase (MAPK) signalling pathway of inflammation transmission, changes that underscored the anti-inflammatory effect of the material. Histological studies further confirmed the role of CCS in inhibiting fibrosis, collagen deposition, inflammation and vascular proliferation. The results obtained on the expression of key genes in this study were confirmed by enzyme-linked immunosorbent assay analysis of proteins [62]. The use of a 'phase transition' barrier obtained by copolymerisation of *O*-carboxymethylchitosan and $CaCl_2$ and the addition of cyclosporine A reduced the formation of adhesions due to a significant increase in the expression of MMP-9 seven days after surgery [63].

## 3. Conclusions

This review has examined the causes, the mechanism of adhesion formation and materials developed for the prevention of the development of adhesive disease. Special attention has been paid to the materials based on chitosan and its derivatives, which prevent adhesion formation and are biocompatible and biodegradable, resulting in elimination of the material post-surgery. In addition, chitosan and its derivatives have their own biological activity that helps to reduce the formation of adhesions (bioadhesiveness, wound healing, antibacterial activity). Thus, anti-adhesion materials based on chitosan and its derivatives, in addition to the mechanical separation of damaged surfaces, can modulate the mechanism of adhesion formation, reducing the adhesion formation process.

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## 5. References

[1] Liu J, Willför S, Xu C.; (2015) A review of bioactive plant polysaccharides: Biological activities, functionalization, and biomedical applications. Bioact. Carbohydrates Diet. Fibre. 5, 31–61. **DOI**:10.1016/j.bcdf.2014.12.001.

[2] Hellebrekers B.W., Trimbos-Kemper G.C., van Blitterswijk C., Bakkum E., Trimbos J.B., Emeis J., Kooistra T.; (2000) Use of fibrinolytic agents in the prevention

of postoperative adhesion formation. Fertil. Steril. 74, 203–212. **DOI**:10.1093/humrep/15.6.1358.

[3] Hong G.-S., Schwandt T., Stein K., Schneiker B., Kummer M. P., Heneka M. T., Kitamura K., Kalff J.C., Wehner S.; (2015) Effects of macrophage-dependent peroxisome proliferator-activated receptor γ signalling on adhesion formation after abdominal surgery in an experimental model. Br. J. Surg. 102, 1506–1516. **DOI**:10.1002/bjs.9907.

[4] Fometescu S.G., Costache M., Coveney A., Oprescu S.M., Serban D., Savlovschi C.; (2013) Peritoneal fibrinolytic activity and adhesiogenesis, Chir. 108, 331–340.

[5] Ярмолинская М., Молотков А., Денисова В.; (2012) Матриксные металлопротеиназы и ингибиторы : классификация , механизм действия. Журнал акушерства и женских болезней. LXI, 113–125.

[6] Lelongt B., Bengatta S., Delauche M., Lund L.R., Werb Z., Ronco P.M.; (2001) Matrix Metalloproteinase 9 Protects Mice from Anti–Glomerular Basement Membrane Nephritis through Its Fibrinolytic Activity. J. Exp. Med. 193, 793–802. **DOI**:10.1084/jem.193.7.793.

[7] Rout U.K., Saed G.M., Diamond M.P.; (2005) Expression pattern and regulation of genes differ between fibroblasts of adhesion and normal human peritoneum. Reprod. Biol. Endocrinol. 3, 1. **DOI**:10.1186/1477-7827-3-1.

[8] Christodoulidis G., Tsilioni I., Spyridakis M.-E., Kiropoulos T., Oikonomidi S., Koukoulis G., Tepetes K.; (2013) Matrix Metaloproteinase-2 and -9 Serum Levels as Potential Markers of Intraperitoneal Adhesions. J. Investig. Surg. 26, 134–140. **DOI**:10.3109/08941939.2012.730599.

[9] Cheong Y.C., Laird S. M., Li T.C., Shelton J.B., Ledger W.L., Cooke I.D.; (2001) Peritoneal healing and adhesion formation/reformation. Hum. Reprod. Update. 7, 556–566. **DOI**:10.1093/humupd/7.6.556.

[10] Chegini N., Kotseos K., Zhao Y., Ma C., McLean F., Diamond M.P., Holmdahl L., Burns J.; (2001) Expression of matrix metalloproteinase (MMP-1) and tissue inhibitor of MMP in serosal tissue of intraperitoneal organs and adhesions. Fertil. Steril. 76, 1212–1219. **DOI**:10.1016/S0015-0282(01)02875-8.

[11] Liu X., Wei Y., Bai X., Li M., Li H., Wang L., Zhang S., Li X., Zhao T., Liu Y., Geng R., Cui H., Chen H., Xu R., Liu H., Zhang Y., Yang B.; (2020) Berberine prevents primary peritoneal adhesion and adhesion reformation by directly inhibiting TIMP-1. Acta Pharm. Sin. B. 10, 812–824. **DOI**:10.1016/j.apsb.2020.02.003.

[12] Arung W., Meurisse M., Detry O.; (2011) Pathophysiology and prevention of postoperative peritoneal adhesion. World J. Gastroenterol. 17, 4545–4553. **DOI**:10.3748/wjg.v17.i41.4545.

[13] Braun K.M., Diamond M.P.; (2014) The biology of adhesion formation in the peritoneal cavity. Semin. Pediatr. Surg. 23, 336–343. **DOI**:10.1053/j.sempedsurg.2014.06.004.

[14] Chegini N.; (2008) TGF-β system: The principal profibrotic mediator of peritoneal adhesion formation. Semin. Reprod. Med. 26, 298–312. **DOI**:10.1055/s-0028-1082388.

[15] Saed G.M., Diamond M.P.; (2002) Hypoxia-induced irreversible up-regulation of type I collagen and transforming growth factor-β1 in human peritoneal fibroblasts. Fertil. Steril. 78, 144–147. **DOI**:10.1016/S0015-0282(02)03146-1.

[16] Wiseman D., Lyachovetsky Y., Keidan I., Trout J.R., Nur I.; (2004) The effect of tranexamic acid in fibrin sealant on adhesion formation in the rat. J. Biomed. Mater. Res. Part B Appl. Biomater. 68, 222–230. **DOI**:10.1016/0002-9378(91)90326-M.

[17] Kaidi A., Gurchumelidze T., Nazzal M., Figert P., Vanterpool C., Silva Y.; (1995) Tumor necrosis factor-alpha: a marker for peritoneal adhesion formation. J. Surg. Res. 58, 516–8. **DOI**:10.1006/jsre.1995.1081.

[18] Holschneider C.H., Cristoforoni P.M., Ghosh K., Punyasavatsut M., Abed E., Montz F.J.; (1997) Endogenous versus exogenous IL-10 in postoperative intraperitoneal adhesion formation in a murine model. J. Surg. Res. 70, 138–43. **DOI**:10.1006/jsre.1997.5124.

[19] Wang G., Wu K., Li W., Zhao E., Shi L., Wang J., Shuai X., Cai K., Lu X., Tao K., Wang G.; (2014) Role of IL-17 and TGF-β in peritoneal adhesion formation after surgical trauma. Wound Repair Regen. 22, 631–639. **DOI**:10.1111/wrr.12203.

[20] Rodrigues-Díez R., Aroeira L.S., Orejudo M., Bajo M.-A., Heffernan J. J., Rodrigues-Díez R.R., Rayego-Mateos S., Ortiz A., Gonzalez-Mateo G., López-Cabrera M., Selgas R., Egido J., Ruiz-Ortega M.; (2014) IL-17A is a novel player in dialysis-induced peritoneal damage. Kidney Int. 86, 303–15. **DOI**:10.1038/ki.2014.33.

[21] Haney A.F.; (2000) Identification of macrophages at the site of peritoneal injury: Evidence supporting a direct role for peritoneal macrophages in healing injured peritoneum. Fertil. Steril. 73, 988–995. **DOI**:10.1016/S0015-0282(00)00490-8.

[22] Saed G.M., Diamond M.P.; (2004) Molecular characterization of postoperative adhesions: the adhesion phenotype. J. Am. Assoc. Gynecol. Laparosc. 11, 307–14.

[23] Hoshino A., Kawamura Y.I., Yasuhara M., Toyama-Sorimachi N., Yamamoto K., Matsukawa A., a Lira S., Dohi T.; (2007) Inhibition of CCL1-CCR8 interaction prevents aggregation of macrophages and development of peritoneal adhesions. J. Immunol. 178, 5296–304. **DOI**:10.1016/S0090-3671(08)79025-6.

[24] Cahill R.A., Redmond H.P.; (2008) Cytokine orchestration in post-operative peritoneal adhesion formation. World J. Gastroenterol. 14, 4861–4866. **DOI**:10.3748/wjg.14.4861.

[25] Maciver A.H., McCall M., James Shapiro A.M.; (2011) Intra-abdominal adhesions: Cellular mechanisms and strategies for prevention. Int. J. Surg. 9, 589–594. **DOI**:10.1016/j.ijsu.2011.08.008.

[26] Langer G.J., Liebman J.C., Monk S.M., Pelletier P.K.; (1995) Mast Cell Mediators and Peritoneal Aadhesion Formation in the rat. J. Surg. Res. 59, 344–348.

[27] Wiseman D.M.; (2000) Adhesion Prevention: Past the Future. In: Perit. Surg., Springer New York, New York, 401–417. **DOI**:10.1007/978-1-4612-1194-5_35.

[28] Grainger D.A., Meyer W.R., DeCherney A.H., Diamond M. P.; (1991) The use of hyaluronic acid polymers to reduce postoperative adhesions. J. Gynecol. Surg. 7, 97–101.

[29] Liu Y., Li H., Shu X.Z., Gray S.D., Prestwich G.D.; (2005) Crosslinked hyaluronan hydrogels containing mitomycin C reduce postoperative abdominal adhesions. Fertil. Steril. 83, 1275–1283. **DOI**:10.1016/j.fertnstert.2004.09.038.

[30] Trew G.; (2006) Postoperative adhesions and their prevention. Rev. Gynaecol. Perinat. Pract. 6, 47–56. **DOI**:10.1016/j.rigapp.2006.02.001.

[31] DiZerega G.S.; (2000) Use of Adhesion Prevention Barriers in Pelvic Reconstructive and Gynecologic Surgery, in: Perit. Surg., Springer New York, New York, 379–399. **DOI**:10.1007/978-1-4612-1194-5_34.

[32] Cheung J. P. Y., Tsang H. H. L., Cheung J. J. C., Yu H. H. Y., Leung G. K. K., Law W. L.; (2009) Adjuvant therapy for the reduction of postoperative intra-abdominal adhesion formation. Asian J. Surg. 32, 180–186. **DOI**:10.1016/S1015-9584(09)60392-4.

[33] Chou T.C., Fu E., Wu C.J., Yeh J.H.; (2003) Chitosan enhances platelet adhesion and aggregation. Biochem. Biophys. Res. Commun. 302, 480–483. **DOI**:10.1016/S0006-291X(03)00173-6.

[34] Patrulea V., Ostafe V., Borchard G., Jordan O.; (2015) Chitosan as a starting material for wound healing applications. Eur. J. Pharm. Biopharm. 97, 417–426. **DOI**:10.1016/j.ejpb.2015.08.004.

[35] Chatelet C., Damour O., Domard A.; (2001) Influence of the degree of acetylation on some biological properties of chitosan films. Biomaterials. 22, 261–268. **DOI**:10.1016/S0142-9612(00)00183-6.

[36] Lin L.-X., Yuan F., Zhang H.-H., Liao N.-N., Luo J.-W., Sun Y.-L.; (2017) Evaluation of surgical anti-adhesion products to reduce postsurgical intra-abdominal adhesion formation in a rat model. PLoS One. 12. e0172088. **DOI**:10.1371/journal.pone.0172088.

[37] Yeo Y., Burdick J.A., Highley C.B., Marini R., Langer R., Kohane D.S.; (2006) Peritoneal application of chitosan and UV-cross-linkable chitosan. J. Biomed. Mater. Res. Part A. 78A, 668–675. **DOI**:10.1002/jbm.a.30740.

[38] Jingcheng W., Lianqi Y., Yu S., Daxin W., Shanhe D., Tangyun Y., Jiaxiang G., Baichuan J., Xinmin F., Hansheng H., Qiang W., Bangliang Y., Guohua L.; (2012) A Comparative Study of the Preventive Effects of Mitomycin C and Chitosan on Intraarticular Adhesion after Knee Surgery in Rabbits. Cell Biochem. Biophys. 62, 101–105. **DOI**:10.1007/s12013-011-9266-5.

[39] Keskin F., Esen H.; (2010) Comparison of the Effects of an Adhesion Barrier and Chitin on Experimental Epidural Fibrosis Deneysel Epidural Fibroziste Adezyon. Turk. Neurosurg. 20, 457–463.

[40] Chen Q., Lu H., Yang H.; (2015) Chitosan prevents adhesion during rabbit fexor tendon repair via the sirtuin 1 signaling pathway. Mol. Med. Rep. 12, 4598–4603. **DOI**:10.3892/mmr.2015.4007.

[41] Higham P.A., Posey-Dowty J.D.; (1992) Use of derivatives of chitin soluble in aqueous solutions for preventing adhesions, USA Patent #5093319.

[42] Kennedy R., Costain D. J., Mealister V. C., Lee T. D. G., Scotia N.; (1996) Prevention of experimental postoperative peritoneal adhesions by N, O-carboxymethyl chitosan. Surgery. 120, 866–870.

[43] Costain D. J., Kennedy R., Ciona C., McAlister V.C., Lee T.D.G.; (1997) Prevention of postsurgical adhesions with N, O-carboxymethyl chitosan: Examination of the most efficacious preparation and the effect of N,O- carboxymethyl chitosan on postsurgical healing. Surgery. 121, 314–319. **DOI**:10.1016/S0039-6060(97)90360-3.

[44] Krause T.J., Zazanis G., Malatesta P., Solina A.; (2001) Prevention of Pericardial Adhesions with N, O-Carboxymethylchitosan in the Rabbit Model. J. Investig. Surg. 14, 93–97.

[45] Zhou J., Liwski R.S., Elson C., Lee T.D.G.; (2008) Reduction in postsurgical adhesion formation after cardiac surgery in a rabbit model using N, O-carboxymethyl chitosan to block cell adherence. J. Thorac. Cardiovasc. Surg. 135, 777–783. **DOI**:10.1016/j.jtcvs.2007.09.033.

[46] Lopes J.B., Dallan L.A.O., Moreira L.F.P., Campana Filho S.P., Gutierrez P.S., Lisboa L.A.F., De Oliveira S.A., Stolf N.A.G.; (2010) Synergism between keratinocyte growth factor and carboxymethyl chitosan reduces pericardial adhesions. Ann. Thorac. Surg. 90, 566–572. **DOI**:10.1016/j.athoracsur.2010.03.086.

[47] Zheng Z., Zhang W., Sun W., Li X., Duan J., Cui J., Feng Z., Mansour H.M.; (2013) Influence of the carboxymethyl chitosan anti-adhesion solution on the TGF-β1 in a postoperative peritoneal adhesion rat. J. Mater. Sci. Mater. Med. 24, 2549–2559. **DOI**:10.1007/s10856-013-4981-7.

[48] Wang D., Mo J., Pan S., Chen H., Zhen H.; (2010) Prevention of postoperative peritoneal adhesions by O-carboxymethyl chitosan in a rat cecal abrasion model. Clin. Investig. Med. Médecine Clin. Exp. 33, E254-60.

[49] Yang Y., Liu X., Li Y., Wang Y., Bao C., Chen Y., Lin Q., Zhu L.; (2017) A postoperative anti-adhesion barrier based on photoinduced imine-crosslinking hydrogel with tissue-adhesive ability. Acta Biomater. 62, 199–209. **DOI**:10.1016/J.ACTBIO.2017.08.047.

[50] Cheng F., Wu Y., Li H., Yan T., Wei X., Wu G., He J., Huang Y.; (2019) Biodegradable N, O-carboxymethyl chitosan/oxidized regenerated cellulose composite gauze as a barrier for preventing postoperative adhesion. Carbohydr. Polym. 207, 180–190. **DOI**:10.1016/j.carbpol.2018.10.077.

[51] Cai X., Hu S., Yu B., Cai Y., Yang J., Li F., Zheng Y., Shi X.; (2018) Transglutaminase-catalyzed preparation of crosslinked carboxymethyl chitosan/carboxymethyl cellulose/collagen composite membrane for postsurgical peritoneal adhesion prevention. Carbohydr. Polym. 201, 201–210. **DOI**:10.1016/j.carbpol.2018.08.065.

[52] Zhou X., Chen S., Liao G., Shen Z., Zhang Z., Sun L., Yu Y., Hu Q.; (2007) Preventive effect of gelatinizedly-modified chitosan film on peritoneal adhesion of different types. World J. Gastroenterol. 13, 1262–1267.

[53] Pascual G., Sotomayor S., Rodríguez M., Bayon Y., Bellón J.M.; (2013) Behaviour of a new composite mesh for the repair of full-thickness abdominal wall defects in a rabbit model. PLoS One. 8, 1–16. **DOI**:10.1371/journal.pone.0080647.

[54] Lin L.X., Luo J.W., Yuan F., Zhang H.H., Ye C.Q., Zhang P., Sun Y.L.; (2017) In situ cross-linking carbodiimide-modified chitosan hydrogel for postoperative adhesion prevention in a rat model. Mater. Sci. Eng. C. 81, 380–385. **DOI**:10.1016/j.msec.2017.07.024.

[55] Lauder C.I.W., Strickland A., Maddern G.J.; (2011) Use of a Modified Chitosan-Dextran Gel to Prevent Peritoneal Adhesions in a Rat Model. J. Surg. Res. 171, 877–882. **DOI**:10.1016/j.jss.2010.06.028.

[56] Lauder C.I.W., Strickland A., Maddern G.J.; (2012) Use of a modified chitosan-dextran gel to prevent peritoneal adhesions in a porcine hemicolectomy model. J. Surg. Res. 176, 448–454. **DOI**:10.1016/j.jss.2011.10.029.

[57] Medina J.G., Das S.; (2013) Sprayable chitosan/starch-based sealant reduces adhesion formation in a sheep model for Chronic sinusitis. Laryngoscope. 123, 42–47. **DOI**:10.1002/lary.23583.

[58] Paulo N.M., De Brito E Silva M.S., Moraes Â.M., Rodrigues A.P., De Menezes L.B., Miguel M.P., De Lima F.G., Faria A.D.M., Lima L.M.L.; (2009) Use of chitosan membrane associated with polypropylene mesh to prevent peritoneal adhesion in rats. J. Biomed. Mater. Res. - Part B Appl. Biomater. 91, 221–227. **DOI**:10.1002/jbm.b.31393.

[59] Jayanth S.T., Pulimood A., Abraham D., Rajaram A., Paul M.J., Nair A.; (2015) A randomized controlled experimental study comparing chitosan coated polypropylene mesh and ProceedTM mesh for abdominal wall defect closure. Ann. Med. Surg. 4, 388–394. **DOI**:10.1016/J.AMSU.2015.10.002.

[60] Li C., Wang H., Liu H., Yin J., Cui L., Chen Z.; (2014) The prevention effect of poly (l-glutamic acid)/chitosan on spinal epidural fibrosis and peridural adhesion in the post-laminectomy rabbit model. Eur. Spine J. 23, 2423–2431. **DOI**:10.1007/s00586-014-3438-0.

[61] Ko J.E., Ko Y.-G., Il Kim W., Kwon O.K., Kwon O.H.; (2017) Nanofiber mats composed of a chitosan-poly(d,l-lactic-co-glycolic acid)-poly(ethylene oxide) blend as a postoperative anti-adhesion agent. J. Biomed. Mater. Res. Part B Appl. Biomater. 105, 1906–1915. **DOI**:10.1002/jbm.b.33726.

[62] Tian L., Li H., Li Y., Liu K., Sun Y., Cong Z., Luan X., Li Y., Chen J., Wang L., Ren Z., Cong D., Wang H., Pei J.; (2018) A combination of chitosan, cellulose,

and seaweed polysaccharide inhibits postoperative intra-abdominal adhesion in rats. J. Pharmacol. Exp. Ther. 364, 399–408. **DOI**:10.1124/jpet.117.244400.

[63] Li X.-D., Xia D.-L., Shen L.-L., He H., Chen C., Wang Y.-F., Chen Y.-P., Guo L.-Y., Gu H.-Y.; (2016) Effect of "phase change" complex on postoperative adhesion prevention. J. Surg. Res. 202, 216–224. **DOI**:10.1016/j.jss.2015.12.033.