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FORMATION MECHANISMS AND PREVENTION OF POSTOPERATIVE PERITONEAL ADHESIONS

Syventävien opintojen kirjallinen työ
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Abstract:

Postoperative peritoneal adhesions are a common complication after operative treatment and they cause a significant burden to both individual patients and the society. This review's purpose was to examine the mechanisms of postoperative peritoneal adhesion formation and to evaluate different adhesion prevention products that are either already available or being investigated. The reference articles were searched using mainly Primo Central Index.

Adhesions are formed when normal peritoneal healing is somehow impaired. The peritoneal healing is a complicated process and there are multiple stages that need to be completed for normal healing to occur. If this is somehow interrupted, it can lead to poor healing or adhesion formation instead of normal healing. It is known that adhesions can be decreased by using careful surgery techniques, but there are also some risk factors that are related to the patient.

Modern adhesion prevention products can mainly be divided into two groups: 1) barrier products that rely on the effect of keeping wounded tissue surfaces mechanically apart, and 2) immunomodulatory products that attempt to make the healing process occur without adhesion formation by taking part in the immunological pathways. Barrier products include solid membranes, liquids and gels. Immunomodulatory products include specifically targeted pharmacological agents as well as multifunctional products.

Various different products have been investigated, but still the optimal adhesion prevention product remains undiscovered. Barriers are widely used, but immunomodulatory products have shown some interestingly promising results. Whichever type of product is used, it should be effective, affordable, safe and easy to use.

Key words: postoperative, adhesion, prevention

Tiivistelmä:

Leikkauksenjälkeiset vatsaontelon kiinnikkeet ovat merkittävä ongelma sekä yksilölle että yhteiskunnallisesti. Leikkauksenjälkeiset kiinnikkeet ovat hyvin yleisiä ja ne voivat aiheuttaa esimerkiksi suolitukoksia, hedelmättömyyttä, vatsakipuja ja komplikaatioita mahdollisten uusintaleikkausten yhteydessä. Yhteiskunnalle kiinnikkeistä koituvat taloudelliset kustannukset ovat huomattavia, ja kiinnikkeiden ehkäiseminen kohtuullisen edullisella tuotteella olisi kustannustehokasta.

Tämän opinnäytetyön tarkoituksena on tarkastella vatsaontelon leikkauksenjälkeisten kiinnikkeiden muodostumismekanismeja ja arvioida niiden eri ehkäisymenetelmiä, jotka joko ovat jo kliinisessä käytössä tai vasta tutkimusasteella. Jotkut katsauksessa mainituista tuotteista ovat osoittautuneet tehokkaammiksi ja turvallisemmiksi kuin toiset. Viitteinä käytetyt artikkelit on saatu hakutuloksina pääasiassa käyttäen Primo Central Indexiä, jonka Turun yliopisto on tarjonnut käyttöön.

Kiinnikkeitä muodostuu, kun normaali vatsakalvon haavojen paraneminen estyy tavalla tai toisella. Vatsakalvossa sijaitsevien vaurioiden normaali paraneminen muistuttaa ihon haavojen paranemista, mutta niissä on myös merkittäviä eroja, kuten se, että vatsakalvossa olevat haavat ovat jatkuvassa kontaktissa vatsaontelonsisäisen nesteen kanssa. Tämä neste sisältää runsaasti erilaisia sytokiineja, kasvutekijöitä, soluja ja proteiineja. Vatsakalvon paraneminen on monimutkainen tapahtumaketju, jossa on mukana useita eri soluja, proteiineja ja muita osia. Siihen kuuluu useita eri tapahtumavaiheita ja -polkuja, joiden tulee toimia mutkattomasti, jotta vauriot paranisivat normaalisti. Jos jokin näistä tapahtumaketjuista jää suoriutumatta tai muuten häiriintyy, on seurauksena joko huonosti parantunut vatsakalvo tai kiinnikkeiden muodostuminen.

Tiedetään, että leikkauksenjälkeisten kiinnikkeiden muodostumiseen vaikuttaa merkittävästi leikkaustekniikka, ja tähystysleikkauksien jälkeen kiinnikkeiden muodostuminen onkin vähäisempää kuin avoleikkausten jälkeen. Kirurgien tulisi olla tietoisia tekniikoista, jotka suurentavat kiinnikkeiden riskiä. Jotkut riskitekijöistä ovat potilaaseen liittyviä, ja on mahdollista, että tietoa niiden olemassaolosta voitaisiin tulevaisuudessa hyödyntää suunniteltaessa yksilöllistä kiinnikkeidenestohoitoa.

Nykyaikaiset kiinnikkeenestomenetelmät voidaan jakaa karkeasti kahteen eri osa-alueeseen: 1) mekaaniset esteet, jotka pitävät vaurioituneet kudosrajapinnat fyysisesti erillään, ja 2) immunomodulatoriset tuotteet, jotka vaikuttavat paranemisprosessin osiin vähentäen turhaa kiinnikkeiden muodostumista. Mekaanisiin esteisiin kuuluu kiinteitä, kalvomaisia tuotteita, sekä nestemäisiä ja geelimuotoisia tuotteita. Immunomodulatorisiin tuotteisiin taas kuuluu erilaisia, sekä yksittäisiin kohdemolekyyleihin tai -proteiineihin vaikuttavia, että moniin eri immunologisten prosessien kohtiin vaikuttavia aineita.

Kiinnikkeiden ehkäisemistä on tutkittu paljon, ja paljon erilaisia tuotteita on jo markkinoilla tai tutkimusasteella. Ihanteellista vaihtoehtoa kiinnikkeiden ehkäisyyn ei kuitenkaan vielä ole kehitetty ja tutkimusta tarvitaan edelleen. Nykypäivänä käytetään paljon mekaaniseen estevaikutukseen nojaavia tuotteita, mutta myös immunomodulatorisista tuotteista on lupaavia tuloksia. Joka tapauksessa kiinnikkeiden estoon käytettävän tuotteen tulisi olla sekä tehokas että turvallinen, ja lisäksi sen tulisi olla helppokäyttöinen ja kohtuullisen edullinen.

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1. Introduction

Postoperative adhesion formation is the most common complication after abdominal or pelvic surgery [1] [2]. Adhesions appear in up to 94% of patients after laparotomy[3]. Fortunately, most adhesions are asymptomatic, but some will, however, cause problems for a small group of patients. These problems include small-bowel obstruction, female infertility, pelvic and abdominal pain[4], a risk of difficulties and complications at reoperation[1], and 'failure to thrive' in children[5].

Different prevention techniques have been studied but there is a need for further research as postoperative adhesions remain a significant problem causing costs (in the shape of re-hospitalization and further surgery) in addition to individual suffering.

Parker et al. discovered in a cohort study in 2001 that after lower abdominal surgery up to 32.6 percent of patients were readmitted in the subsequent ten years for a potential adhesion-related problem and after open lower-abdominal surgery up to 7.3 percent of readmissions were directly adhesion-related[6]. A population-based study in 2008 showed that in children under 16 years the overall readmission rate due to small-bowel obstruction caused by adhesions in 5 years after open abdominal surgery (excluding appendicectomy) was 5.3%[5]. Patients with prior laparotomy, who therefore have more intra-abdominal adhesions, also seem to have an increased risk of bowel and mesenteric injury after blunt abdominal trauma[7].

In a review article by ten Broek et al. that studied the disease burden of the most important complications of postoperative abdominal adhesions, the incidence of reoperations for adhesive small bowel obstruction was 2.4% and adhesions were found to be the most common cause of postoperative small bowel obstruction (56%)[1] . The same review also revealed that the incidence for adhesive small bowel obstruction depended on the anatomical location of previous surgery, and was highest in paediatric (4.2%) and lower gastrointestinal tract surgery (3.2%) and lowest in abdominal wall surgery (0.5%), upper gastrointestinal tract surgery (1.2%) and urological surgery (1.5%).

In the USA, the total cost for adhesiolysis hospitalizations was 1.44 billion dollars in 1988 and 1.33 billion dollars in 1994. The decrease in the economic burden was thought to have been the result of decrease in the length of stay in hospital.[8] A retrospective study carried out in Finland studied the economic costs of patients hospitalized for intestinal obstruction caused by postoperative adhesions in Varsinais-Suomi region during the year 1999. The total cost was 181 653 UK pounds, and it was estimated that for whole of Finland the figure would have been 2 077 796 pounds. This would have put adhesive intestinal obstruction in 30th place in most costly surgical diagnosis in Finland, roughly corresponding the costs of rectal cancer or gastric cancer that year.[9] In 2007 in the UK, it was estimated that adhesion-related admissions in open lower abdominal surgery would cost the NHS (National Health Service) more than 900 million euros over the following 10 years, and that using a low cost (130€) adhesion reduction product with a 25% reduction in re-admissions would save the NHS up to 42 million euros over the following 10 years.[10]

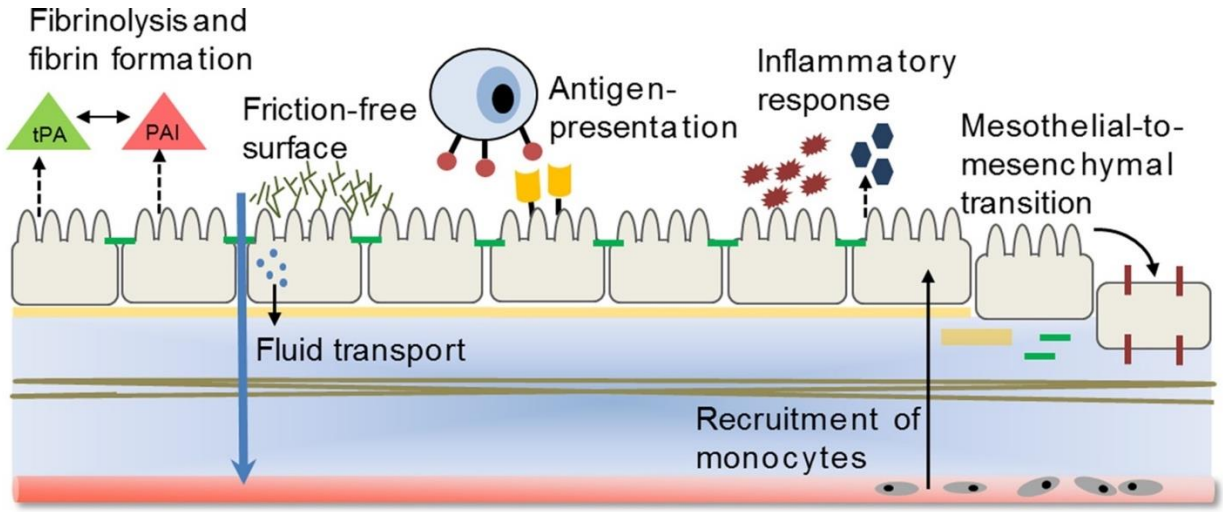
2. Methods

Primo Central Index, which includes databases such as Web of Science, Scopus, JSTOR, PubMed and DOAJ, was used in this article. Search words that were used were "postoperative", "adhesion*", "formation*", "peritone*", and combinations of all these. Additionally, articles were searched using specific product names, names of different cytokines and growth factors, risk factors

etc. combining all the previously mentioned to the search word “adhesion*”. The aim was to create a review of adhesion formation mechanisms and the most important possible ways to prevent them.

3. Peritoneum and normal peritoneal healing

The peritoneum is a thin membrane that consists of two layers; the parietal peritoneum which covers the abdominal wall, and the visceral peritoneum which is attached to visceral organs. These layers are composed of simple squamous epithelial cells called mesothelial cells, which are loosely attached to the basement membrane underneath them.[11] The mesothelium and the basement membrane are supported by a thin layer of submesothelial mature connective tissue containing collagen and scattered elastin fibers. Beneath this there is loose connective tissue with widely spaced collagen fibers, and also occasional fibroblasts, mononuclear phagocytes, lymphocytes and adipose tissue. Small blood vessels, lymphatic vessels and nerves are also present in the zone of loose connective tissue.[12] The basement membrane together with the underlying interstitial matrix, which contains the previously described connective tissues, form the extracellular matrix (ECM). ECM contains many different components that are important in the healing process, including collagen I and collagen III, fibronectin, glycoproteins, fibroblasts, macrophages and blood and lymphatic vessels.[11]



Structure of the peritoneum.[13]

Peritoneal injury, caused by a surgical procedure for example, results in a complicated healing process that includes different phases and many different cells, cytokines, growth factors and other molecules. It can either lead to normal or impaired peritoneal healing or adhesion formation, depending on multiple different processes: migration, proliferation, apoptosis and cell differentiation for example. Various different cells, including inflammatory cells, immune cells, mesothelial cells and fibroblasts, are essential to the healing process as they regulate proteolysis, tissue remodeling, angiogenesis, the synthesis and deposition of ECM, and recruitment of additional cells.[14]

Peritoneal wound healing processes and mechanisms resemble those of dermal wound healing, but there are also important differences: whereas dermal wounds heal from the edges toward the middle, peritoneal wounds are thought to heal throughout the lesion simultaneously[15]. Also, peritoneal wounds are, in contrast to dermal wounds, exposed to the peritoneal fluid, which contains proteins, various different cells, cytokines and growth factors. The peritoneal cavity contains approximately 3-50 ml of peritoneal fluid.[16]

Normal wound healing consists of different phases, including hemostasis/inflammation phase, proliferation phase and remodeling phase. During the hemostasis/inflammation phase, chemokines and growth factors are released and a clot is formed to achieve hemostasis.[17] Injury of the peritoneal surface triggers an exudation of a high-protein fluid that contains fibrin, histamines, monocytes, plasma cells, polymorphonucleocytes, macrophages, mesothelial cells and histiocytes. The fluid coagulates within hours and fibrous bands are formed between the injured surfaces.[11] Neutrophils appear at the injury site to cleanse debris and bacteria and are followed by macrophages[17]. These inflammatory cells release, among other growth factors and cytokines, interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor-alpha (TNF-alpha)[18]. Normally, fibrinolysis within 72 hours inhibits the formation of adhesions. However, if fibrinolysis is somehow ineffective, fibroblasts migrate into the fibrinous mass and deposit extracellular matrix, including collagen and fibronectin, which leads to adhesion formation.[11] Fibrin degradation is regulated by the plasminogen system. Plasminogen activators (PAs) convert inactive plasminogen into active plasmin, and the process is inhibited by plasminogen activator inhibitors (PAIs).[16]

The following phase, proliferation, includes many different cells, cytokines and growth factors, and lasts for days/weeks. The last phase, remodeling, can last for months or years. New cells are being produced, old cells are destroyed by apoptosis, and type III collagen is formed to type I collagen.[17]

Macrophages have an important role in adhesion formation as they are present at the injury site throughout the whole healing interval[19]. They express enormous plasticity and can modify their functional properties depending on the environment. Increasing the number of peritoneal macrophages reduces adhesion formation[20]. Several studies suggest that macrophages have different functional phenotypes depending on the stage of the wound repair progress[21]. Macrophages can acquire a 'classically activated' phenotype (M1 type), in which case they have pro-inflammatory properties, or an 'alternatively activated' phenotype (M2 type), which is described as reparative/growth promoting type[21 22]. An important difference between these types is found in their arginine metabolism: M1 type macrophages metabolize arginine primarily to NO/citrulline, whereas M2 type macrophages metabolize arginine mostly to ornithine/urea[23]. M1 type macrophages produce many different mediators and cytokines, including IL-1, IL-6, IL-12, TNF-alpha and inducible nitric oxide synthase. They have been shown to be common during the early stages of wound healing. M2 type macrophages produce both anti-inflammatory mediators such as IL-1 receptor antagonist and IL-10, and growth factors such as TGF-beta, VEGF and IGF-1. These M2 type macrophages appear to be more common during the later stages of wound repair.[21] However, it seems that these two types more or less represent the extremes of various different phenotypes, and that hybrid-phenotype macrophages, which have characteristics typical to both types, also exist[21 22 24]. Edwards & Mosser (2008) have suggested a different classification based on three different functional properties of macrophages: host defense, wound healing and immune regulation. This model is more like a continuous spectrum where host defense macrophages represent the classically activated (M1) macrophages and the other two groups consist of different types of alternatively activated (M2) macrophages. This classification illustrates that macrophages can evolve to simultaneously express characteristics that are typical to different types, and that the division in two groups M1 and M2 is somewhat problematic.[24]

4. Inflammatory aspects contributing to adhesion formation

Peritoneal wound healing is a complex process that includes multiple phases, various different cells and a vast number of growth factors and cytokines. All of these parts must be optimal for wound

healing to occur normally. If any of the factors are inhibited or over-expressed, or if any other processes that affect wound healing are interrupted, this can either result in poor healing or in adhesion formation.

4.1 Inflammatory cytokines

4.1.1 TNF-alpha

Tumor necrosis factor-alpha (TNF-alpha) is an inflammatory cytokine that stimulates the acute-phase reaction which leads to inflammation. It is expressed in both normal and adhesion peritoneal fibroblasts, but compared to normal fibroblasts, adhesion fibroblasts have an increased quantity of TNF-alpha mRNA.[11] [25] Peritoneal exudate samples contain higher levels of TNF-alpha when gathered from rats with more severe adhesions.[26]

4.1.2 IL-6

Interleukin-6 (IL-6) is also an acute-phase inflammatory cytokine. IL-6 has several different functions, such as acting as a growth and differentiation factor or stimulating the expression of another genes. It acts as a marker of tissue damage of early phase. IL-6 is stimulated by TNF-alpha and also by IL-1 and released by macrophages during peritoneal injury.[11] Similarly to TNF-alpha, IL-6 mRNA levels are also increased in hypoxia and these levels are higher in adhesion fibroblasts compared to normal fibroblasts.

4.1.3 TGF-beta1

Transforming growth factor beta-1 (TGF-beta1) controls cellular proliferation, differentiation, apoptosis, tissue morphogenesis and wound healing. Its levels are increased in response to peritoneal healing. TGF-beta1 has both inactive and active form, of which the active form stimulates extracellular matrix (ECM) deposition and leads to adhesion development. TGF-beta1 may have an effect on adhesion formation by inducing migration of peritoneal fibroblasts as it has a potent effect on macrophage and fibroblast activity during wound healing. Increased levels of TGF-beta1 in hypoxia lead to increased PAI-1 (plasminogen activator inhibitor-1) and uPA (urokinase type plasminogen activator) mRNA expression which in turn leads to enhancement of anti-proteolysis.[11]

What these inflammatory cytokines all have in common with each other is that their levels are increased when adhesions are present and also in hypoxic conditions. They play an important role in adhesion formation as they are involved in regulating coagulation and fibrin formation[25] and inhibition of inflammation is thought to be one of the possible ways to prevent postoperative adhesion formation.

Levels of transforming growth factor beta-1 (TGF-beta1), interleukin 17 (IL-17) and interferon gamma (IFN-gamma) measured from actual patients show that, postoperatively, there are two peak concentrations for TGF-beta1 (12 hours and 6 days), whereas both IL-17 and IFN-gamma reach their peak concentrations in 1-2 days and drop to basal levels in 3-4 days after surgery. Similar results occur also in mice, but within shorter time periods. The first peak apparently results from hemorrhage early after surgery, when TGF-beta1 is released by blood platelets. During the second

peak, TGF-beta1 is released mainly by fibroblasts. In a study by Wang et al., by neutralizing TGF-beta1 at the second peak concentration time by injecting mice with antibodies, it was possible to reduce adhesion formation, while neutralization during the first peak did not have similar results, indicating that only the second peak point affects adhesion formation. Neutralizing IL-17 and IFN-gamma also reduced adhesions significantly, which indicates that both these cytokines may serve as promoters during adhesion formation. IL-17 seems to be secreted by (gamma-delta) T cells whereas IFN-gamma is secreted mainly by Th1 cells.[27]

4.2 VEGF

Vascular endothelial growth factor (VEGF) is a factor that has a remarkable effect on angiogenesis[21], which is essential to wound healing and also adhesion formation. VEGF stimulates endothelial cell proliferation, mediates nitric oxide synthase activity in endothelial cells and therefor induces new blood vessel growth in the wounded area[28]. VEGF-A plays a direct role in adhesion development[29]. It is secreted from various different cells, including platelets. Apparently, mast cells seem to have a role in releasing VEGF to the peritoneal cavity after operation[30] . VEGF production is also stimulated by lactate in macrophages and lactate accumulation might have a significant role in adhesion formation[11].

4.3 Plasminogen activators

Plasminogen activators (PAs) are serine proteases that are secreted by many different cell types and that convert plasminogen to plasmin. Plasmin is an important enzyme that acts, among other things, as a dissolver of blood clots. Two types of PAs have been identified in mammals: tissue type PA (tPA) and urokinase type PA (uPA), which are both inhibited by plasminogen activator inhibitor-1 (PAI-1). Reduced plasminogen activator activity (PAA) results in adhesion formation, whereas in cases where PAA Is not reduced, the fibrinous mass is degraded before fibroblast ingrowth, resulting in healing without adhesion formation. Regulation of PAA resides in both mesothelial cells and in underlying fibroblasts as well. In fibroblasts, the expression of tPA is reduced and PAI-1 levels are increased under hypoxic conditions, both resulting in reduced PAA.[31]

4 Patient-related risks

It is not completely clear whether gender affects adhesion formation rate or not. There are studies that suggest the frequency of adhesions is higher in females, while other studies have found them to be more frequent in males. Similarly, there is no real consensus on how aging affects adhesion formation.[32]

Obesity (BMI greater than 30 kg/m2) affects wound healing in many ways. It is associated with different kinds of wound complications, reduced oxygen delivery to the wound, impaired immune response, and impaired fibrinolysis, which is the result of both environmental and genetic factors; PAI-1 levels are higher in obese individuals. Apparently, PAI-1 levels can, however, be decreased by long-term moderate exercise, resulting in improved fibrinolytic activity. Other factors that improve fibrinolytic capacity are for example a low-saturated-fat diet, coffee consumption, avoiding heavy alcohol consumption and avoiding smoking.[32]

Keloids are a type of excessive wound healing. Their prevalence varies among different ethnicities and especially dark-skinned individuals are more likely to develop excessive scarring and keloids. Keloid scar contains disorganized type I collagen and type III collagen and also more elastin content than normal skin.[17] A prospective cohort study by Stocker et al. discovered that women who had more than one abdominal scar, a palpable scar and/or a longer scar were most likely to have pelvic adhesions, meaning that skin scar characteristics are associated with the degree of pelvic adhesions[33].

Genetics have an important effect on adhesion formation risks. For example, a single nucleotide deletion in PAI-1 promoter causes activated PAI-1 transcription in individuals that are homozygotic for this deletion allele. PAI-1 transcription leads to increased plasma PAI-1 activity and decreased fibrinolytic activity.[34] [35] Another example is the IL-1RN gene, which encodes the proinflammatory cytokine IL-1RN (IL-1 receptor antagonist). A polymorphism of this gene has been identified and mutant allele (IL-1RN*2) carriers seem to have an increased risk of adhesion development.[36] The fibrinogen gene locus has also been shown to have several polymorphisms, which affect the fibrinogen levels and thereby have an impact on fibrinolytic activity, although it seems that environmental factors (smoking, diabetes) have a greater influence on plasma fibrinogen levels than genetics[37].

It has been suggested that in the future it could become a standard to screen special factors prior to surgery to identify individuals that have a larger risk of adhesion formation[38]. These factors include biomarkers, genes, proteins and cytokines. This kind of screening could enable a personalized adhesion-prevention treatment individually for each patient.

5 Surgery techniques and principles

There are some guidelines and principles that should be kept in mind when performing a surgery. Dr. William Halsted was one of the first surgeons to promote “safe surgery”, and the so-called Halstedian principles include asepsis, careful handling of tissues, avoiding tension to the tissues, adequate hemostasis, preservation of blood supply, and obliteration of dead space[39]. Peritoneal damage should be avoided by these principles to prevent adhesion formation. Minimizing tissue dehydration and avoiding use of foreign bodies and unnecessary suturing or clamping is also recommended.[40]

Obviously, peritonitis is always an unfortunate event for the patient. On top of increased mortality and other severe complications, fibrinolytic activity is also reduced in peritonitis, leading to increased adhesion formation. PAI-1, PAI-2, uPA, and tPA/PAI complex levels are significantly higher in peritonitis whereas tPA levels are significantly lower, resulting in reduced plasminogen activator activity, which leads to increased adhesion formation[41]. Bile spillage and gallbladder stone spillage are also related to a significant increase in adhesion formation[42].

Razmaria et al. examined differences in postoperative peritoneal adhesion formation between open ileocystoplasty and robot-assisted laparoscopic ileocystoplasty (RALI)[43]. The study was done on 20 female farm-pigs which were divided into two groups. The pigs were killed on postoperative day 42 and adhesions were visualized and classified by type and tenacity. The results showed that RALI achieved similar functional outcomes (return of bowel function, final weight, bladder capacity etc.) as the open approach. The total mean operating time was longer in the RALI arm, but the pigs in the RALI arm developed significantly less adhesions than those in the open arm.

The incidence of adhesive small bowel obstruction was studied in a review by ten Broek et al. and it seemed that it was significantly lower in laparoscopic cohorts (1.4%) than in open surgery cohorts (3.8%). The same review also revealed that in the 10 studies that directly compared laparoscopic and open surgery, the incidence of adhesive small bowel obstruction was lower after laparoscopic surgery (odds ratio 0.38, 95% confidence interval 0.16 to 0.91).[1]

However, the pneumoperitoneum, induced either by CO2 or helium, which is essential for laparoscopic surgery, creates hypoxia in the tissues which increases adhesion formation. Hypoxia results in a significant increase in TGF-beta1 expression in human macrophages and also in a significant increase in TGF-beta1, VEGF and type I collagen mRNA and protein levels in peritoneal fibroblasts[44]. Hypoxia also reduces the expression of tPA and increases levels of PAI-1, which both lead to reduced plasminogen activator activity and therefor also to an increase in adhesion formation. Hypoxia during surgery can be minimized by keeping the duration of pneumoperitoneum as short as possible and also by adding oxygen[45].

In the 1990s Luijendijk et al. studied the prevalence of foreign body granulomas in intra-abdominal adhesions in patients with a prior abdominal surgery. They discovered that suture granulomas were present in a large percentage of recent adhesions. Intra-abdominal presence of foreign material is a significant cause of adhesion formation and therefore intra-abdominal contamination with foreign material should be minimized.[46]

Starch-powdered gloves have been shown to produce adhesions in the peritoneum[47] and they should thus be avoided when performing intraperitoneal surgery. Tanaka et al. were able to form severe adhesions in mice by injecting a talc suspension intraperitoneally. As many of the adhesion-related studies on animals include a laparotomy to induce adhesions in animals, this could be used in the future as an alternative way to form adhesions.[48]

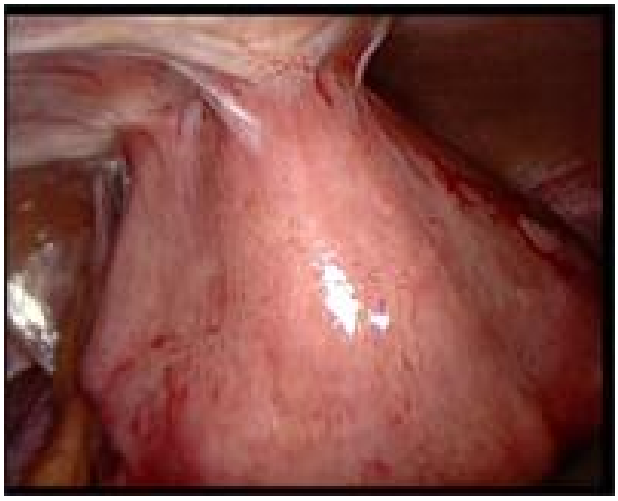
6 Classification of adhesions

There are several ways to classify and score peritoneal adhesions and no official universal classification system exists. It is quite common to use a classification based on the macroscopic appearance and consistency of adhesions. In this sort of classification the different kinds of adhesion types are often described verbally and then scored depending on their appearance. Lower points are often given to thin, filmy adhesions that are easily torn and higher points are given to thicker adhesions that are harder to brake, and that may even have vascularization or nerves.

Adhesions can also be classified and scored depending on their length, quantity, and site.

In some cases, a microscopic evaluation is also used. Samples are taken from the adhesions, examined through a microscope and then scored based on the cells and tissues present in the sample. For example, in an experimental model by Arslan et al., loose connective tissue with fine reticulin fibers had a score of 1 point, whereas old firm granulation tissue with hardly distinguishable serosal layers scored 4 points[49].

In an article published in 2013 Coccolini et al. proposed a standardized classification system, the peritoneal adhesion index PAI (not to be confused with plasminogen activator inhibitor PAI-1 discussed previously). This system would take into account both the macroscopic appearance of the adhesions and their diffusion to different regions of the abdomen, and the adhesions would then be scored from 0 to 30 depending on these criteria.[50]


Adhesions seen in a laparoscopic surgery.[51]

7 Adhesion prevention products currently available / in research

Adhesion prevention techniques are mainly divided into two categories: 1) products that act as a physical barrier between the tissue surfaces, hence inhibiting the forming of fibrous bands during the healing process, and 2) immunomodulatory products that somehow affect the healing process itself.

8.1 Mechanical barriers (membranes, gels, solutions)

Barrier products' main idea is to cover the traumatized tissue surfaces so that they cannot get into contact with each other and form adhesions. There are different forms of barrier products, such as liquids, gels and solid membranes.

8.1.1 Membranes

Hellebrekers et al. evaluated, in rats, the efficacy of five different barrier materials, including Ringer's lactate, PRECLUDE Peritoneal Membrane, Polyactive, Seprafilm and Tissucol[52]. Of these, only Seprafilm and PRECLUDE reduced adhesions significantly. PRECLUDE is a thin, non-absorbable, antithrombotic and non-reactive membrane. Seprafilm is a bioresorbable membrane made of hyaluronic acid and carboxymethyl cellulose that turns into gel within 24 hours of application and is cleared from the body in 28 days.

Seprafilm's efficacy was studied in patients with ulcerative colitis or familial polyposis in a randomized, controlled, blinded, prospective study. The patients went through a restorative proctocolectomy and ileal J-pouch anastomosis with diverting ileostomy followed by second-stage laparoscopy for ileostomy closure. 91 patients were treated with Seprafilm averaging 407 cm2 per patient and 92 patients were in the control group. Seprafilm significantly reduced the incidence, extent and severity of postoperative adhesions.[53]

Interceed is a woven sheet made of oxidized regenerated cellulose that has been proven to reduce postoperative adhesions[54] [55] [56]. A prospective, randomized, controlled study by Naito et al. showed that Interceed is safe and also easy to use in laparoscopic colorectal surgery[57].

A systematic review by ten Broek et al. evaluated that both hyaluronate carboxymethylcellulose (Seprafilm) and oxidized regenerated cellulose (Interceed) can safely reduce clinically relevant consequences of adhesions[58].

In a comparison by Gruber-Blum et al., Prevadh (oxidized bovine atelocollagen, polyethyleneglycol and glycerol) and Seprafilm effectively reduced postoperative adhesion formation in intraperitoneal mesh hernia repair surgery compared to the control group, while the use of SurgiWrap (polylactic acid) was associated with severe adhesion formation[59]. However, there are also results that indicate that polylactic acid (SurgiWrap) is effective in adhesion prevention[60] [61].

A biodegradable composite gauze (N, O-carboxymethyl chitosan/oxidized regenerated cellulose (N, O-CS/ORC) composite gauze) seems to effectively prevent adhesions in an animal model and, in addition, it seems to both have antimicrobial function against E. coli and S. aureus and also have hemostatic effects[62].

Another possible agent to prevent intraperitoneal adhesion formation could be a bioabsorbable polymer film based on polyethylene glycol (PEG) and poly(D,L-lactide) (PLA). PLA-PEG-PLA film's efficacy seems to be equivalent in comparison to Seprafilm's and Hyalobarrier's efficacy in adhesion prevention in rats on 12th postoperative day, with a significant difference to the control group[63]. Apparently, it could be easier to use as it does not appear to adhere to gloves and could be easier to reposition in case of incorrect placement. However, clinical trials to confirm its efficacy are still needed.

8.1.2 Liquids

For vast or geometrically complex tissue surfaces it may be difficult to spread the agent evenly and to cover all the areas needed. That is why preformed solid films and membranes might in some cases be problematic to use and therefore liquid solutions and gels have also been investigated as adhesion-preventing barriers. Liquids are thought to create a 'flotation' effect inside the peritoneal cavity which would keep the tissue surfaces somewhat separated[64]. Mainly, these hydroflotation agents haven't been shown to be quite as effective in preventing adhesions than other barrier products because of their short intraperitoneal residence before being absorbed[65] [66] [67], but some studies show different results[64].

Adept is a solution made of 4% icodextrin that, when administered to the intraabdominal cavity, appears to safely and effectively reduce postoperative adhesions compared to a control group and Ringer's lactate group[68] [69]. It appears to remain in the intraperitoneal cavity for up to 96 hours in contrast to saline solution[70].

Hyaluronic acid has been investigated as one possible adhesion-preventing product. Precoating tissue surfaces with Sepracoat (0.4% hyaluronic acid) solution significantly reduces adhesions[71], but it doesn't seem to be effective in adhesion prevention if administered after lesioning[72]. It is unclear whether hyaluronic acid's effectiveness is due to its pharmacologic activity or rather to limitation of tissue

trauma, since its pharmacological activity may be limited because Sepracoat only persists at the site for approximately 24 hours[71].

Carboxymethylcellulose (CMC) is a polyanionic cellulose derivative and it appears its liquid solution is, in mice, effective in preventing adhesions when injected intraperitoneally[48]. This effect was dose-dependent. Carboxymethylcellulose sponge has also been found to be more effective in adhesion prevention than Interceed[73].

Phospholipids are polar phosphoric acid di-esters arranged as bilayer membranes that can act as a lubricant and a temporary coverage to serosal defects. They have a negatively charged choline branch chain that can bind to the positively charged peritoneal surface, which is why they form a membrane-like structure. In animal models, intra-abdominal dosage of phosphatidylcholine's soluble form of approximately 70 mg/kg seems to be effective in adhesion prevention as long as the exposure time is at least 30 minutes, and dosage increase does not seem to enhance the capacity. However, doses higher than 140 mg/kg resulted in augmented anastomotic leakage rates and therefore caused more deaths due to peritonitis. Phospholipids appear to inhibit bacterial growth and intraperitoneal tumor cell adhesion in addition to adhesion prevention.[74]

Liquid crystals are substances that have some of the structural characteristics of crystalline solids but that flow like liquids. They can be classified into two groups: thermotropic and lyotropic. Lyotropic liquid crystals can form lamellar and nonlamellar structures such as hexagonal and bicontinuous cubic phases. Liquid crystals could form a mechanical barrier between the tissue surfaces by generating a thin membrane and adhering to the tissue surface when in contact with biological tissues. C17 glycerin ester (G17GE) is an amphiphilic lipid of one isoprenoid-type hydrophobic chain. A mixture of C17GE, squalene, pluronic F127, ethanol and water was created and investigated as a possible adhesion-preventing agent that can form non-lamellar structures. Its properties could be modified by altering the lipid compositions, and the product would apparently be ready to use without having to mix ingredients during the surgery. It seems to be effective in preventing intraperitoneal adhesions compared to the control group and even compared to the Seprafilm-treated group in a rat model[75]. In fact, interestingly, in this particular study there was no significant difference between the control group and the Seprafilm-treated group.

8.1.3 Gels

Hydrogels have been investigated as one possible product to be used to separate the tissues from each other. These hydrogels include curdlan (CUR) which is a polysaccharide composed entirely of (1,3)-beta-D-glucose, and gellan gum (GLG), which is a linear anionic heteropolysaccharide composed of tetrasaccharide repeating units of glucose-glucuronic acid-glucose-rhamnose, containing one carboxyl side group[76]. Both of these hydrogels appear to prevent adhesion formation compared to a control group, but as they have different affecting mechanisms, it seems that a mixture of these two is even more effective than either of the two alone[76].

Intergel is a 0.5% ferric hyaluronate (FeHA) gel that reduces postoperative adhesions significantly in comparison to patients treated with Ringer's lactate solution. Adhesion extent and severity were also significantly reduced and Intergel was considered as a

safe adhesion prevention product in a randomized multicenter study by Johns et al. in 2001.[77] However, Intergel was withdrawn from the US market in 2003 because of possible adverse reactions, including pelvic pain, allergic reactions and increased risk of peritonitis[78] [79].

Sakai et al. investigated, in mice, a possibility to form a hyaluronic acid-based hydrogel in situ using a cascade enzyme reaction by contact with body fluid[80], which appeared to be effective. This method could be problematic because of the time needed during an operation for the hydrogel to form in situ, but by altering the concentration of the ingredients it was possible in this study to obtain a gelation time of no more than 5 seconds.

8.2 Immunomodulatory products

Besides mechanically inhibiting tissue surfaces from getting in contact with each other by barrier products, another method in adhesion prevention is immunomodulation. This includes products that can somehow affect the biochemistry behind adhesion formation. Immunomodulatory products can, for example, prevent/reduce inflammation, activate fibrinolysis, inhibit angiogenesis or prevent collagen synthesis. Many of the possible adhesion-preventing immunomodulatory products have multifunctional effects and they can take part in several of the previously mentioned immunological events. There are also specifically targeted pharmacological agents that aim to affect individual growth factors, proteins or cytokines, for example. Some of these immunomodulatory products have been investigated as fluids or solutions that are administered intraperitoneally so that they could have a local effect, and some have been studied as systemic products either administered intravenously or perorally before, during or after the surgery.

8.2.1 Anti-inflammation

Autologous intraperitoneal fat grafting has been studied on rats and results are promising: fat grafting significantly reduced adhesions as well as fibrosis and inflammation. In the study by Cil & Aydogdu, the autologous fat was harvested from rats' inguinal fat pads, chopped and then transferred between cecum and abdominal wall through a needle.[81] A recent study on mice by Laukka et al. also suggests that preperitoneal, instead of intraperitoneal, fat grafting by injection reduces peritoneal adhesion formation both in moderate and extensive adhesion models. The authors also observed that fat grafting increased expression of anti-inflammatory cytokine IL-10 in the extensive adhesions group that received a fat graft in comparison to the non-operated control group. Faster mesothelial healing in the peritoneum was observed promoted by fat grafting. Fat grafting seems to have both anti-inflammatory and anti-fibrotic effects.[82]

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used mainly for pain relieving purposes. They inhibit cyclooxygenase-1 (COX-1) and/or cyclooxygenase-2 (COX-2) isoenzymes. As these cyclooxygenases catalyze the formation of prostaglandins, which are involved in inflammation, inhibiting them also decreases inflammation. NSAIDs have been investigated as a possible adhesion-preventing substance: meloxicam and dexketoprofen seem to not have a significant effect on

macroscopic adhesions[83], but ketorolac tromethamine did significantly prevent adhesions in a mouse model[84].

In a study by Wasserberg et al. the effect of immunosuppression on postsurgical adhesion formation was examined. Rats received a small bowel intestinal transplantation and either tacrolimus as an immunosuppressive medication or no immunosuppression at all. Immunosuppression significantly reduced adhesion formation after intestinal transplantation.[85] Another study by Maciver et al. showed that combining an immunosuppressive drug sirolimus and hydrogel and impregnating them on a prosthetic polypropylene mesh that was implanted operatively into the peritoneal cavity of mice significantly reduced postoperative adhesion formation in comparison to plain mesh[86].

In a randomized controlled trial on mice, interleukin-10 and interleukin-4 (IL-10, IL-4) which are macrophage down-regulating cytokines, and ketorolac tromethamine (non-steroidal anti-inflammatory drug) were evaluated as possible adhesion-preventing products. Both IL-10 and ketorolac separately as well as the two combined significantly inhibited adhesion formation, but IL-4 did not have a significant effect. The authors suggested there to be multiple ways by which the IL-10 could reduce adhesion formation: inhibiting profibrotic cytokine (such as IL-2, TGF-beta and platelet-derived growth factor-B) production and acting on monocytes and macrophages to suppress the synthesis of IL-1 and IL-6. Although IL-4 did not seem to prevent adhesion formation in this study, this lack of significance could be due to limited group size, since IL-4 has been demonstrated to inhibit TNF-alpha and IL-1 production, which are both adhesiogenic cytokines.[84]

Betaglucan is a glucose polymer that binds to the receptors on monocytes and neutrophils. It is a potent macrophage stimulator and it induces production of TNF-alpha and interleukin-1. Although increased TNF-alpha level is in several studies associated with increased adhesion development, Bedirli et al. suggest that the role of TNF-alpha in adhesion formation is not clear. Betaglucan significantly decreased adhesion formation in in their study on rats after ileocolic anastomosis in the setting of peritonitis.[87]

Nigella sativa is a plant that grows in countries bordering the Mediterranean Sea, Pakistan, India, and Turkey. Its seeds contain a lot of oil, which apparently could be effectively used to prevent adhesions when applied onto the surgical surface[88]. N. sativa extract suppresses secretion of IL-6, TNF-alpha and NO, which are all pro-inflammatory mediators.[89] This suggests that N. sativa's anti-inflammatory properties could be the reason why it is effective against adhesions.

Honey is a common household product that has been used in wound healing for ages. It is known to have antioxidant and anti-inflammatory effects. Honey solution sprayed onto the surgical zone seems to decrease adhesion formation in rats and have a significant effect on many adhesion-related factors such as TNF-alpha, IL-6, IL-1-beta, TGF-beta-1, VEGF, NO, GSH, MDA compared to both control group and dextrose-treated group[90].

8.2.2 Fibrinolysis activating

Statins have fibrinolytic activity and intraperitoneally administered lovastatin seems to prevent postoperative adhesions as effectively as Seprafilm[49].

Interferon gamma (IFN-gamma), which is produced, among other cells, by natural killer T cells (NKT cells), seems to have a crucial role in adhesion development. In an experimental mouse model, NKT cell-deficient mice developed adhesions poorly, but developed severe adhesions when after reconstitution with NKT cells from wild-type mice. The study also revealed that IFN-gamma might be in part responsible for regulation of PAI-1 and tPA.[91]

8.2.3 Angiogenesis preventing

Bevacizumab is recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biological activity of human VEGF thereby inhibiting angiogenesis. It has been shown to inhibit tumor angiogenesis in patients with advanced colorectal cancer.[92] Ignjatovic et al. researched bevacizumab's effects on adhesion formation in a study performed on 42 male Wistar rats[93]. All of the rats were operated on and adhesion formation was induced. The rats were divided into two groups: Group A were given NaCl intra-abdominally through a catheter after the surgery whereas Group B received 2.5 mg/kg bevacizumab in a similar way. The rats were killed four weeks after the surgery and the adhesions formed were scored both macroscopically and histologically. The results showed that 97.4% of the animals had formed adhesions. However, the severity of the adhesions was significantly lower in the group that received bevacizumab. This shows that a single dose of bevacizumab given during a surgery seems to hinder the development of abdominal adhesions.

Propolis is a natural, complex product that is obtained by honey bees from different plants and that has many pharmacological and biological properties[94]. Askari et al. discovered that, in rats, oral gavage of propolis solution for 14 postoperative days significantly reduced adhesion formation (macroscopically evaluated), decreased levels of inflammatory cytokines (TNF-alpha, IL1-beta and IL-6), reduced levels of fibrosis and angiogenesis biomarkers (TGF-beta1 and VEGF) and had a significant effect on oxidative biomarkers (NO, MDA and GSH), and all of the effects were dose-dependent[95].

In an experimental model on rats, vitamin C and vitamin E administered intraperitoneally during the surgery effectively prevented adhesion formation. Fibrosis and angiogenesis scores as well as MDA and VEGF immunoreactivity were found to be significantly lower in these vitamin-treated groups compared to non-treated group.[96]

8.2.4 Collagen synthesis preventing

Foreign bodies are often related to adhesion formation.[46] Adhesion prevention to two commercially available polypropylene meshes (polypropylene mesh and titanium-coated polypropylene mesh) using combined icodextrin 4% and dimetindene maleate was evaluated in a study by Bouliaris et al[97]. Icodextrin is an iso-osmolar biodegradable glucose polymer solution initially used for peritoneal dialysis, and it is thought to make a hydroflotation effect in the peritoneal cavity, creating a temporary physical separation of the tissue surfaces, therefore acting as a barrier. Dimetindene

maleate is a potent H1-receptor antagonist of histamine. Histamine is released by mast cells after peritoneal injury or inflammation. The study was done on rabbits, and while icodextrin solution was instilled intraperitoneally during the surgery, dimetindene maleate was infused intravenously before the surgery and also intramuscularly once a day for 6 days following the operation. Results showed that there were significantly less adhesions in treated groups. Hydroxyproline, which is a component of collagen, levels were also measured and it was found that its level was significantly lower in treated groups.[97]

8 Discussion

Adhesion formation is a significant and frequent postoperative complication that causes several different problems including abdominal and pelvic pain, bowel obstruction, infertility and difficulties at reoperation. The costs of these adhesion-related problems to the society and healthcare system are also considerable. Adhesions are formed when normal peritoneal healing is somehow impaired. Normal peritoneal wound healing resembles that of dermal wounds, but there are also important differences, such as the presence of peritoneal fluid which contains several different cytokines, growth factors, cells and proteins. The peritoneal healing is a complicated process that includes various different cells, proteins etc. There are multiple stages and pathways that need to be completed for normal healing to occur. If these pathways are somehow interrupted, it can lead to poor healing or adhesion formation instead of normal healing. It is known that adhesions can be decreased using careful surgery techniques, and laparoscopic operations seem to cause less adhesions than open surgeries. Surgeons should be aware of the techniques that increase the risk of adhesion development. There are also some risk factors that are related to the patient, and in the future, some of them could possibly be taken into account when planning an individual adhesion prevention.

Different prevention techniques and preventive products have been studied and partly the results are promising, but the optimal adhesion-prevention product does not yet exist. The optimal product should be effective in preventing adhesions, be safe to use and have minimal side effects, be easy for surgeons to use in laparoscopic operations as well, and have a reasonably low price. Adhesion-preventing products can mainly be divided into two groups based on the preventive mechanism: 1) barrier products including solid membranes, solutions and gels that are thought to physically separate the damaged tissue surfaces from one another, and 2) immunomodulatory products and pharmacological agents that affect the biochemistry of adhesion formation processes, including locally and systemically (intravenously, perorally) administered products. Immunomodulatory products can be either specifically targeted pharmacological agents or multifunctional substances that have an impact on more than one part of the immunological pathways.

Today, barrier products are more widely used, but immunomodulation seems to have interestingly promising results. In the future, adhesion prevention could possibly be designed more individually and immunomodulatory products might be one way to achieve this. Since inflammation is strongly related to adhesion formation[98], anti-inflammatory products appear as an appealing option in the future of adhesion prevention. Inflammation/hemostasis is the first stage of wound healing[99] and it is a crucial phase when it comes to adhesion formation. Especially specifically targeted pharmacological agents seem an interesting object of development since they have a specific effect during the different pathways of wound healing. Targeting a certain molecule or protein, for instance, might cause less unwanted side effects. To prevent unfortunate adverse effects of adhesion prevention (poor wound healing and anastomotic leakage for example), the impacts and mechanisms of the products must be well known. Further research in the field of immunomodulation regarding adhesion prevention is thus certainly needed.

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