The injured peritoneum:
Consequences of surgery on an organ

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Gevolgen van chirurgie op een orgaan

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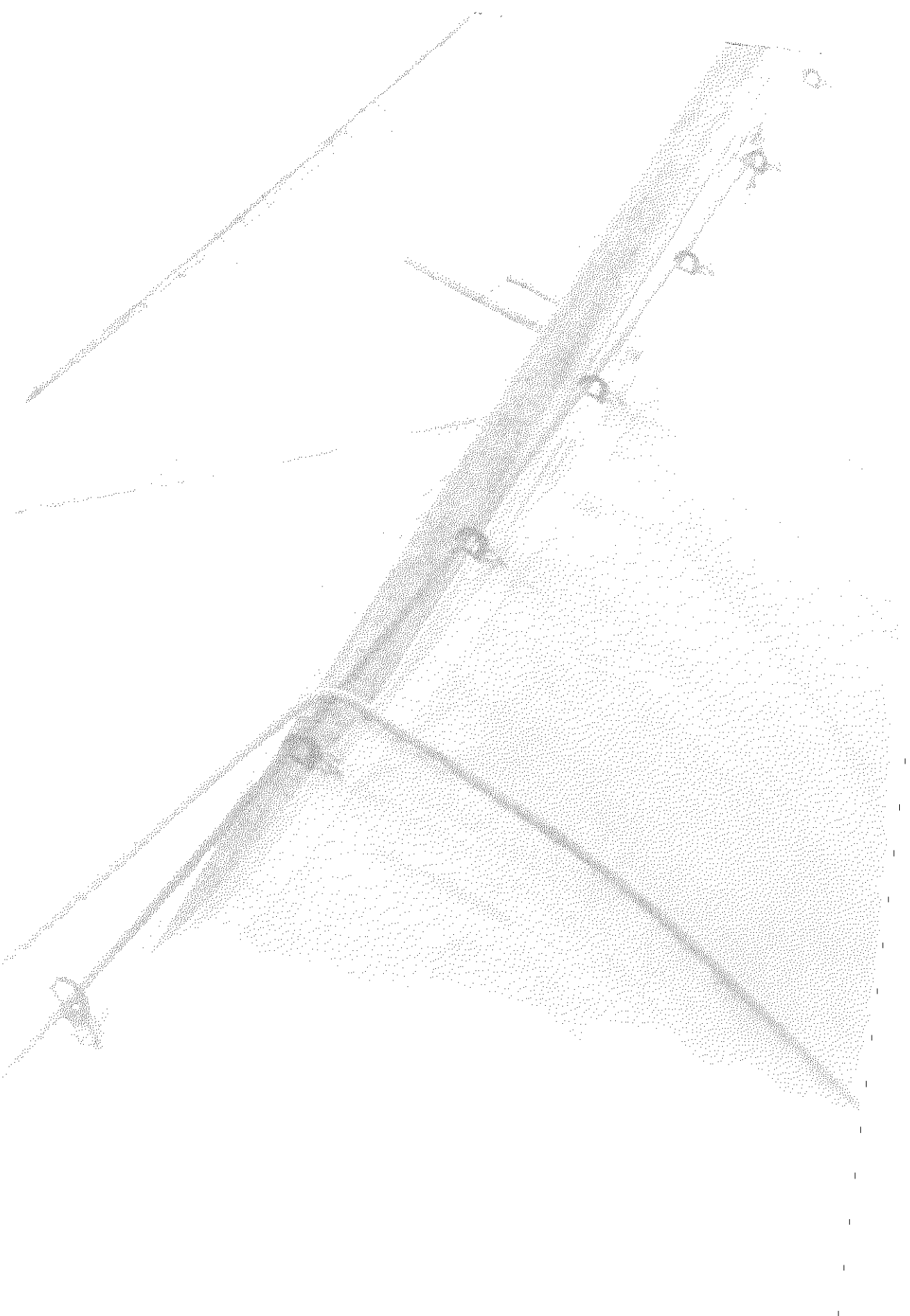
Contents



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Aims and outline of the thesis

Background

Surgical trauma to the peritoneum is inevitable during abdominal surgery, whether performed by laparoscopy or laparotomy. Obviously, entering the abdominal cavity is an essential prerequisite in order to be able to perform any kind of surgical intervention intra-abdominally. However, among surgeons there is only little awareness of the consequences of this essential part of an abdominal procedure.

Postoperative adhesions, responsible for an increased risk of small bowel obstruction, infertility, chronic abdominal pain and considerable difficulties at re-operations, are often taken for granted whereas attempts to prevent them are not being considered. In case of oncological abdominal surgery the traumatised peritoneum may facilitate outgrowth of spilled tumour cells, inducing peritoneal carcinomatosis and hence a worsened outcome for the patient. Increasing the awareness of sequelae of surgical trauma to the peritoneum hopefully leads to a reduction of the amount of peritoneal damage during surgery and a decrease in postoperative morbidity for the patient.

Aims of the thesis

It is almost impossible to address every single aspect of the consequences of surgical peritoneal injury in one thesis, therefore a selection is made.

To be able to treat the consequences of surgical trauma on the peritoneum, understanding of the pathophysiological pathways of adhesion formation and tumour recurrence is essential. **Chapter 2** discusses and explains these sequelae and provides an overview of current (and past) treatment modalities to reduce postoperative adhesion formation and tumour recurrence.

To investigate whether or not the impact of adhesions is confined to general surgery alone, the problem is addressed in the field of kidney-transplantation surgery in **chapter 3.**

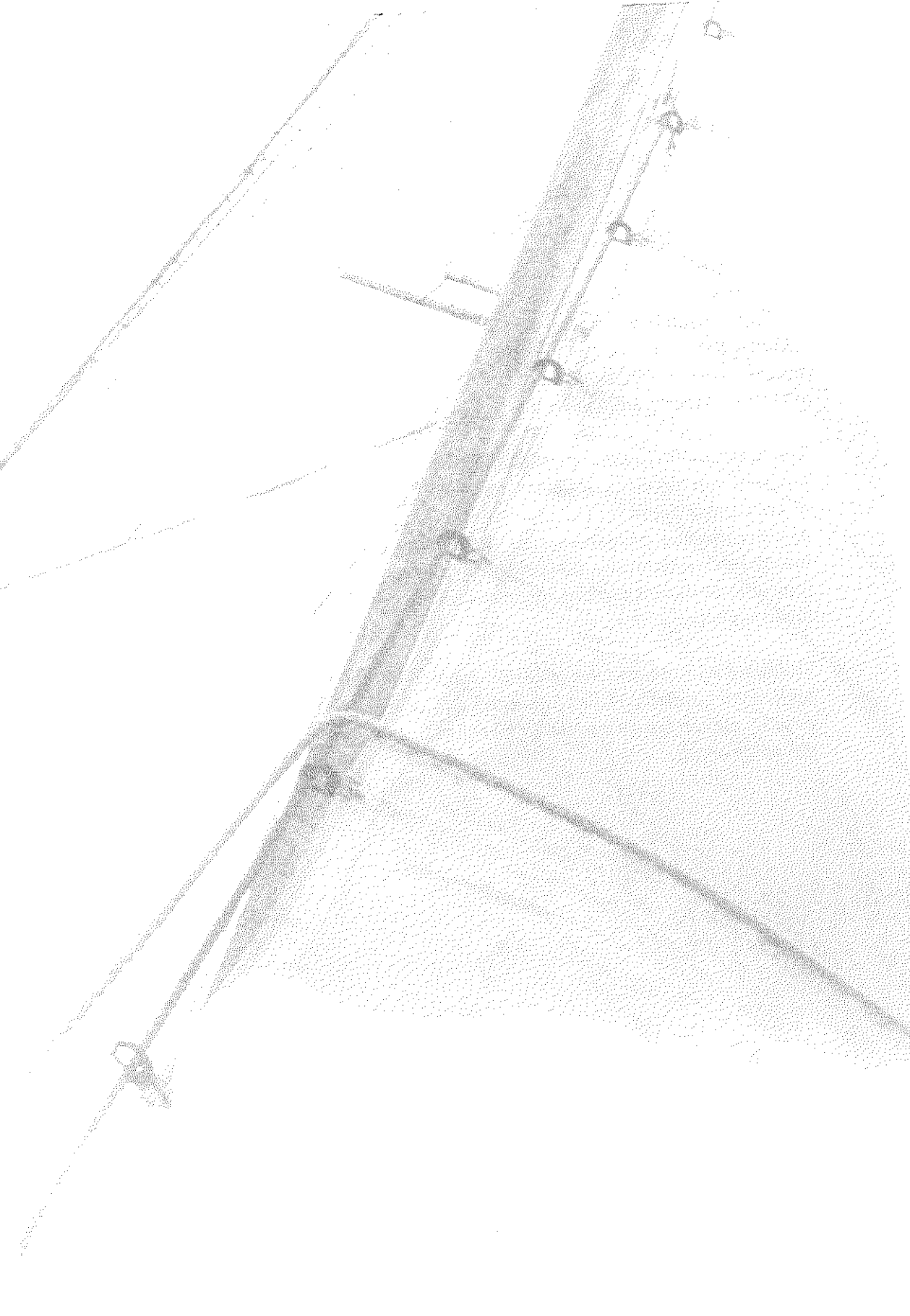
Chronic abdominal pain after abdominal surgery is a known consequence of postsurgical adhesion formation. In the past, surgical adhesiolysis, by laparoscopy as well as by laparotomy, was often performed in an attempt to relieve the patients complaints. However, due to the results of a randomized

controlled trial performed by Swank *et al.* surgical treatment became questionable. **Chapter 4** contains a review of current literature, performed in order to determine whether this discussion is justified.

Adhesion-related complications mostly occur shortly after surgery, however these may also manifestate after several years. Interestingly there is a lack of data concerning the long-term effect of adhesion-prevention strategies. In **Chapter 5** the follow-up of a randomized controlled trial is set out to determine the incidence of adhesive small bowel obstruction and chronic abdominal complaints in patients treated with an anti-adhesion barrier compared to controls.

The inflammatory reaction following surgical trauma to the peritoneum is the common link between adhesion formation and peritoneal metastasis in case of oncological surgery. The main denominator in this reaction are neutrophils and the reactive oxygen species (ROS) they produce. Actual levels of ROS, locally nor systemically, have never been reported and little is known about their role in distant tumour recurrence. **Chapter 6 and 7** attempt to elucidate these aspects.

Chapter 8 summarizes the previous chapters and discusses the results of the performed research. Furthermore the author hypothesizes on future perspectives of preventive strategies with regard to the consequences of surgery on the peritoneum.



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The peritoneum

Partially adapted from:

Van der Wal JBC, Jeekel J. Biology of the peritoneum in normal homeostasis and after surgical trauma. Colorectal Dis 2007; Suppl 2: 9-13

Van der Wal JBC, Jeekel J. The use of statins in adhesion prevention. Ann Surg 2007; 245(2): 185-6

Van der Wal JBC, Ten Raa S, Jeekel J. The impact of irrigants in the peritoneal cavity. Adhesions News and Views 2005; 8: 17-19

Ditzel M, Van der Wal JBC, Jeekel J. Abdominal Adhesions; the search for the perfect solution. Adhesions News and Views 2007;10; 6-7

The peritoneum

The peritoneum is the largest serous membrane in the body. With a surface of 2 m^2 it is equivalent to that of the skin and it covers the visceral organs (visceral peritoneum) and lines the abdominal cavity (parietal peritoneum). The serous membranes of the peritoneal cavity are of the same embryologic origin as the membranes found in the pleural and pericardial cavities.

The peritoneal membrane is composed of a monolayer of mesothelial cells of mesenchymal origin, resting on a continuous basement membrane supported by the submesothelium [1]. The submesothelial layer consists of the extracellular matrix made up of different types of collagen, glycoproteins, glycosaminoglycans and proteoglycans. Vascular structures and lymphatics are found in the subserous space. Diffusion and resorption of fluid occur freely through the mesothelium and submesothelial stroma. Mesothelial cells are loosely attached to the basement membrane and can be readily detached by the slightest trauma [2].

The peritoneum is in constant contact with peritoneal fluid which facilitates normal functioning of the gastro-intestinal tract, the bladder and, in the female genital tract, plays an important role in the motility of the fallopian tubes and oocyte retrieval. The concept of a peritoneal cavity with smooth lubricated surfaces is primordial for normal peristalsis of a long, loop-wise arranged, gastrointestinal tract. The peritoneal fluid circulates within the abdominal cavity and is in continuity, via the lymphatic system, with the pleural fluid in the thoracic cavity and the vascular system. Molecules can enter or exit the peritoneal cavity by transudation, exudation or via the lymphatic system.

The mesothelial cells (diameter approximately 25 µm) are a homogeneous population with either a flattened, stretched and squamous appearance, or are cuboidal. The latter are mostly observed in close proximity to parenchymal organs such as the liver and spleen, the milky spots of the omentum and the diaphragm, situated in the peritoneal cavity overlying the lymphatic lacunae [3-5]. Cuboidal mesothelial cells are also observed within an injured or stimulated mesothelium. Ultrastructural studies have revealed distinct differences between squamous and cuboidal mesothelial cells. Organelles of squamous mesothelial cells are located centrally, close to a round or oval nucleus. These contain few mitochondria, have a poorly developed Golgi apparatus and sparse rough endoplasmic reticulum [6]. In contrast, cuboidal mesothelial cells possess a

nucleus with a prominent nucleolus being endowed with a well defined rough endoplasmic reticulum, Golgi apparatus and numerous smooth-surfaced and coated vesicles, indicative of their dynamic biosynthetic ability and active transmembrane transport [7].

The luminal surface of mesothelial cells has numerous microvilli, increasing the functional mesothelial surface area up to 40 m^2, for exchange between mesothelial cells and the peritoneal cavity. However, these microvilli are labile structures and the number of microvilli expressed on each cell varies under different physiological and pathological conditions [8]. These protect the delicate mesothelial surface from frictional injury by entrapping water and serous exudates, which act as lubricants for the cells [1, 8-10].
Mesothelial cells also possess cilia on their apical surface that are typically five times longer than adjacent microvilli. Cilia are composed of microtubules that contain increased levels of detyrosinated and acetylated a-tubulin. These extend from a single parental centriole located between the Golgi apparatus and the nuclei of the cells. While microvilli are observed in proliferating mesothelial cells, cilia are lacking [11]. The quantity of cilia on the mesothelial surface increases with increasing cell density, which suggests these to play an essential role in mesothelial cell polarity and cell–cell adhesion.

Furthermore, it has been postulated that cilia on transdifferentiated mesothelial cells may direct and coordinate the synthesis of matrix proteins, analogous to that observed in other mesenchymal cells. In mesothelial cells with an epitheloid morphology, cilia may protect the mesothelial surface through their ability to regulate surfactant secretion and contribute to the cellular surveillance system that can identify humoral substances or microbial products within the peritoneal cavity during peritoneal injury or peritonitis [11].

The peritoneum after injury

The peritoneal reaction to injury consists of two different but highly connected pathways which take place simultaneously: inflammation and fibrinogenesis (and subsequently fibrinolysis).

Inflammation

Mesothelial cells proliferate with a limited speed under normal homeostasis; only 0.16% – 0.5% of mesothelial cells are in mitosis at any one time. This rate

increases to 30% – 60% when the peritoneum is injured [12], mostly due to increased levels of growth factors and cytokines.

Injury of the peritoneum, whether of surgical, inflammatory or ischemic origin, causes a desquamation of injured mesothelial cells, leaving a denuded area and causing an inflammatory reaction, characterised by cellular infiltration, formation of serosanguinous exudate and a growth response by the mesothelial cells [13]. Resident cells, as well as the damaged mesothelial cells and invading inflammatory cells in the injured area, produce cellular mediators. Histamine, released by mast-cells, increases vascular permeability and hereby promotes extravasation of fluids, proteins and inflammatory cells such as polymorphonuclear granulocytes (PMN's), monocytes and leukocytes to the site of inflammation. The key-mediators in this acute phase response as mentioned above include chemo-attractants (IL-8, MCP-1), cytokines (TNF-α, Il-1β and IL-6) and growth factors (TGF- β, IGF-1 and PDGF). The first cells to appear in the damaged area are represented by PMN's, which persist at the injured site for 1-2 days. These are followed by monocytes which differentiate into macrophages and then adhere to the wound surface.
In this early postoperative inflammatory reaction, PMN's are responsible for clearing dead tissue and invading organisms by producing and releasing reactive oxygen species (ROS); superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) are formed, as well as lipid peroxide (LPO), which is a downstream product of the oxidation of fatty acids by H_2O_2. Despite their beneficial effect, the oxidative potential can result in additional (peritoneal) tissue destruction [14, 15].
Macrophages are known to produce nitric oxide synthase (NOS) which produces nitric oxide (NO) from the terminal nitrogen group of the amino-acid L-arginine. NO has vasodilatory effects and inhibits platelet aggregation and neutrophil infiltration, hereby modulating the inflammatroy process.

Fibrinogenesis and fibrinolysis

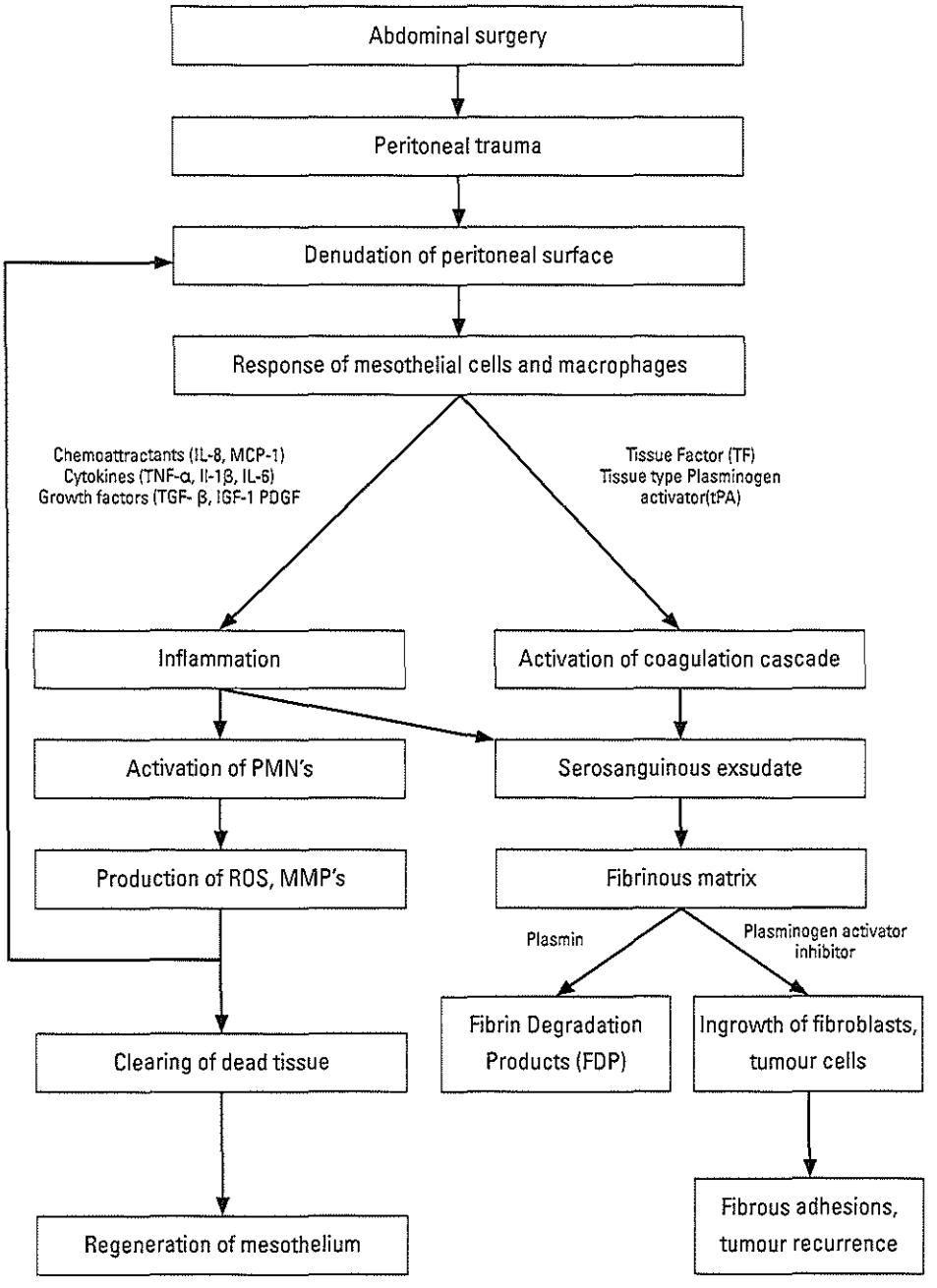
Besides the increased production of the cellular mediators, the expression of tissue factor (TF) by macrophages and mesothelial cells is up-regulated. This leads to activation of the extrinsic pathway of the coagulation cascade, eventually leading to the formation of a transient fibrinous matrix. This fibrin matrix is gradually organized and replaced by tissue containing fibroblasts, macrophages and giant cells. It connects two injured peritoneal surfaces forming fibrin bands, which, under normal circumstances, can be broken down by fibrinolysis into smaller molecules as fibrin degradation products (FDP).

Fibrinogenesis is, just like inflammation, a physiological response of tissue to injury and it is essential for normal wound healing in order to regain tissue strength (scar formation).

The process of fibrinolysis (also physiological; it counteracts fibrinogenesis and prevents excessive formation of scar-tissue) is driven by the enzyme plasmin, produced by macrophages or by mesothelial cells lining the peritoneal cavity [16, 17]. Plasmin is derived from its inactive substrate plasminogen by tissue-type plasminogen activator (tPA) and urokinase-like plasminogen activator (uPA). In its turn, tPA is inhibited in its reaction by plasminogen activator inhibitor-1 (PAI-1), in order to keep the balance. In the abdominal cavity, tPA is responsible for 95% of the plasminogen conversion [18]. However, intra-abdominal surgery disturbs the balance between tPA and PAI-1 resulting in a decreased fibrinolytic activity, an increase in fibrin exudate and eventually an increase in adhesion formation [19].
When the peritoneum is slightly damaged and mesothelial cells are mostly intact, there will be a dynamic balance between fibrinogenesis and fibrinolysis and adhesion-free healing may then take place. When more severe trauma is caused during operation, loss of mesothelial integrity will occur exposing the underlying connective tissue and normal fibrinolytic activity will be lost for at least 48 hours post trauma [20]. The exact mechanism is not quite understood, but it seems that different types of trauma may have a different impact on peritoneal fibrinolysis [21]. When fibrinolysis is inhibited, the fibrinous adhesions will organise into fibrous adhesions due to ingrowth of fibroblasts and endothelial cells which is followed by capillary formation and incorporation of collagen, all stimulated by cytokines and growth factors (day 4 to 10) [20]. However, in case of minor as well as severe trauma, re-epithelisation is complete 5-8 days after the initial trauma [22].

This is a result of the way the peritoneum heals: 4 to 7 days after the peritoneum is traumatised, the predominant cells on the peritoneal surface are mesothelial cells, which proliferate throughout the wound base, forming multiple islands of cells. Because of this formation of islands, large injury to the peritoneal surface heals in the same amount of time as smaller injury.
It has been postulated that peritoneal injury leads to ischemia, either from the inadequate ingrowth of vessels at the base of the wound, or, if adequate ingrowth does occur, from an inadequate blood flow in the vessels [2, 23].

Figure 1. Pathways to adhesion formation and tumour recurrence



It is believed that this ischemia results in a reduction in fibrinolytic activity and thus the persistence of the fibrin bands. Porter *et al.* first described the presence of plasminogen activator activity in the human peritoneum and later work localized plasminogen-activating activity to the mesothelium [24, 25]. Fibrinolytic activity has also been detected in the peritoneal fluid in both humans and animal models. PAI activity has been identified in human peritoneal cells [18]. In the inflamed peritoneal tissue the activity of peritoneal plasminogen activators is significantly reduced, partly because the PAI concentration is increased, ultimately leading to a decreased fibrinolytic capacity resulting in adhesion formation.

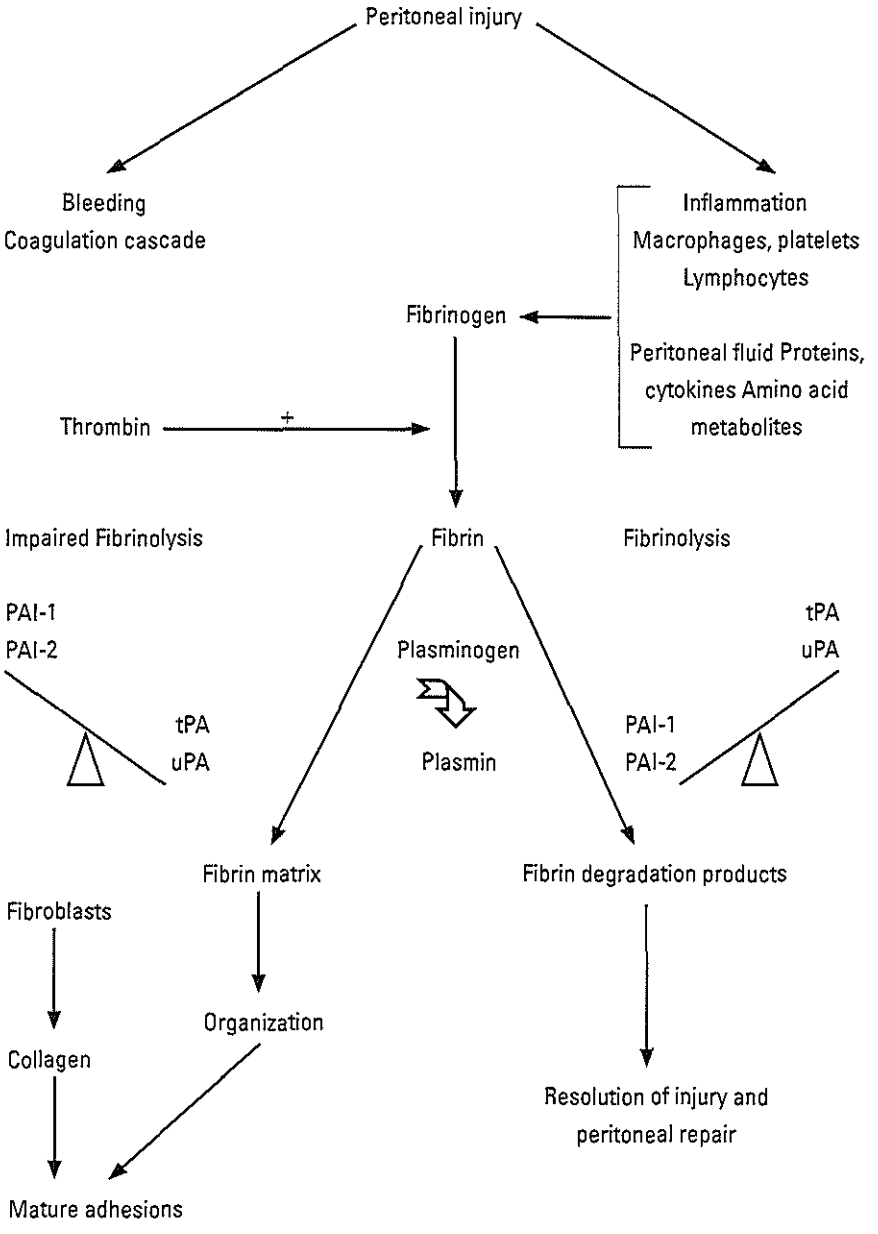
Besides plasmin, PA and PAI, several other factors, such as TGF-β, matrix metalloproteinases (MMP) and cytokines play a role in peritoneal healing, all interacting with each other. TGF-β , found in platelets, macrophages and wound fluid, is activated by plasmin in the acute phase of the inflammatory response to injury. By stimulating fibroblastic production of collagen and fibronectin, it contributes to the synthesis of the extracellular matrix (ECM) but also tissue fibrosis. Overexpression of TGF-β is associated with increased adhesion formation [26-28].

MMP and their inhibitors (tissue inhibitors of MMP, TIMP) are found in the parietal peritoneum and several intraperitoneal organs; especially mesothelial cells and invading PMN's produce these enzymes. MMP's, activated by plasmin, are capable of degrading all components of the (injured) ECM, thus contributing to the wound healing process. However, due to injury, the equilibrium between MMP and TIMP is altered, yet another factor leading to adhesion formation.

As stated before, as a sequence of the acute inflammation of the peritoneum in response to trauma, there is an influx of cells, mainly macrophages, by chemotactic mechanisms. These macrophages, when activated by, again, plasmin, produce IL-1 and TNF-α, important factors in wound healing [29-31]. IL-1 in turn up-regulates the expression of IL-6; together with TNF-α they interact with the fibrinolytic system; these down-regulate tPA activity [32, 33], thereby increasing the tPA/PAI ratio, leading to less fibrinolytic activity and hence contributing to the formation of adhesions.

Figure 2. Perintoneal injury

Adapted from: Jo-Anne P. Attard, Anthony R. MacLean, Adhesive small bowel obstruction: epidemiology, biology and prevention; Can J Surg, vol 50, no 4; 291-300



To summarize, the peritoneum can be considered an organ which has a protective function for the contents of the abdominal cavity. It maintains homeostasis by allowing exchange of molecules and production of peritoneal fluid, thus providing an environment in which intra-abdominal organs can function properly. When traumatised, whether by surgery or due to inflammatory processes, a series of responses come into action in order to regenerate the injured part of the peritoneum. This is represented by the primary inflammatory reaction, causing influx of inflammatory cells but also activating resident mesothelial cells, ultimately leading to a fibrinous exudate. Depending on the severity of the trauma this exudate is transient due to fibrinolysis, or becomes more dense as a result of fibroblasts persisting, leading to fibrinous adhesions. The cytokines and proteinases, produced by invading cells as well as resident cells, also play a part in the regeneration process. A pivotal role is taken by the enzyme plasmin and its promotors and inhibitors; it is mainly the tPA/PAI ratio which determines the rate of fibrinolysis and therefore the rate of adhesion formation. In conclusion, it is the rate of injury determining the rate and extent of the inflammatory response to that injury; the inflammatory reaction in turn determines the extent of adhesion formation and tumour recurrence.

Clinical consequences of peritoneal trauma: postoperative adhesions

Postsurgical adhesions result from the natural wound healing response of tissue to damage that occurs during surgery [34]. This adhesion formation remains a major postoperative surgical problem and is still an almost unavoidable complication of any kind of abdominal surgery [35, 36].

Adhesion formation occurs in an average of approximately 85% (55-100%) of patients undergoing abdominal surgery [37, 38], hence abdominal and pelvic surgery account for up to 90% of all intra-abdominal adhesions [37, 39]; the omentum and the small bowel are the locations most frequently involved [36]. Other causes of adhesions are due to inflammation, endometriosis or are congenital [36, 40].

Adhesions are responsible for an increased risk of small bowel obstruction, chronic abdominal pain, infertility and more difficult access at re-operation [37, 38, 41]. Small bowel obstruction may, if conservative treatment fails, need surgical adhesiolysis. This used to be an option in the treatment of chronic abdominal pain as well, however Swank *et al.* have shown that (laparoscopic) adhesiolysis as treatment for chronic abdominal pain should be abandoned [42].

The economic burden of adhesion-related hospital readmissions and re-operations is enormous, considering the annual costs exceeding $ 1 billion in the US alone [43]. Recently, the results of the third Surgical and Clinical Adhesions Research (SCAR-3) study were published, indicating a readmission-risk of approximately 30% due to adhesions during the first ten years after colorectal surgery [44].

Adhesions are divided in two types [45]:
Type 1 are *de novo* adhesions which are adhesions occurring at sites with no previous adhesion, subdivided into adhesions at sites where no surgical procedure was performed (caused by indirect trauma or inflammation, type 1a), and adhesions at sites of a surgical procedure other than adhesiolysis (caused by direct trauma, type 1b).
Type 2 are called *reformed* adhesions which are adhesions reforming at sites of previous adhesiolysis, occurring at sites of adhesiolysis only or occurring at sites of adhesiolysis, plus sites of another procedure.

Adhesion-prevention strategies

The two main approaches include adjusting surgical techniques, and thereby limiting trauma to intra-abdominal structures, and applying adjuvants, whether pharmacological or as a solid barrier [46].

Adhesion-prevention strategies: surgical technique
Despite of absence of evidence-based guidelines, adjustment of our surgical technique should involve several aspects [47]:

» Tissue injury
During abdominal surgery the peritoneum is susceptible to crush, thermal, electrical, laser, mechanical, hypoxic, and strangulation injury, resulting in denudation of the superficial mesothelial layer. Disrupting the underlying connective tissue and associated microvasculature elicits the inflammatory response, depresses fibrinolytic activity, and promotes adhesion formation [48].
Surgeons should pursue general principles of atraumatic, gentle, and bloodless surgery during either laparoscopy or laparotomy. Forceps, retractors, and clamps should not be placed on structures not intended for dissection, reducing serosal denudation and vascular trauma.

» Peritoneal Suturing

Considerable experimental evidence indicates that peritoneal suturing increases adhesion formation [49]. Grafting or suturing of peritoneal defects increases ischemia, devascularization, and necrosis, predisposing the site to decreased fibrinolytic activity and increased adhesion formation [50]. The presence of suture material and tightening the sutures to the point of ischemia potentiates adhesion formation [36]. The suture materials elicit foreign body reactions of varying degrees. Braided versus monofilament sutures contain microscopic pores that can harbor bacteria and lead to infection. A catgut suture, though rapidly absorbed, leads to greater tissue reaction, whereas polyglycolic acid derivatives and monofilament synthetics are less reactive [48]. Numerous studies show no significant differences in complications, wound healing, and adhesions to the laparotomy incisions with or without parietal peritoneal closure by suturing when evaluated by second-look laparoscopy.

Current data support improved outcome with nonclosure of the peritoneum. Peritoneal closure may induce ischemia and adhesion formation. It is thus unnecessary during closure of abdominal wounds and especially in the presence of intraperitoneal bacterial contamination or infection which may result in postoperative peritoneal adhesions. The practice of omitting the closure of the peritoneum is well supported in the literature [51-55].

» Foreign Materials

Foreign materials such as glove powder (talc and starch), fluff from surgical packs (gauze lint), sutures, and material extruded from the digestive tract cause a peritoneal inflammatory reaction. This inflammatory response potentiates adhesion formation with multiple foreign body granulomas, suggesting a strong relationship between foreign material, foreign-body granulomas, and adhesion formation. Using powder-free gloves should prevent starch granuloma-induced adhesions. Interestingly, powdered gloves when washed can lead to clumping of starch granules, generating a more intense tissue reaction [36].

» Sponges

A recognized association exists between adhesion formation and use of sponges in the peritoneal cavity. Wetting of sponges is performed routinely to prevent de novo adhesion formation when using sponges in the abdominal cavity, but controversy exists over the benefits of this technique [56]. When the bowel needs to be packed outside of the operative field, an atraumatic bag might reduce injury to the serosa [56].

» Intraperitoneal Blood Deposits

The presence of intraperitoneal blood deposits in inducing adhesions is still controversial. In animal models large clots produced adhesions, but small clots did not in the absence of peritoneal injury [57]. Hemostasis is essential, and blood should be aspirated in irrigation solution.

If pinpoint electrocautery cannot provide adequate hemostasis, then the smallest gauged synthetic suture should be used, with special consideration to avoid tissue strangulation [58].

» Minimally Invasive Surgery

It has been hypothesized that laparoscopic surgery, by minimizing peritoneal trauma, may result in reduced adhesion formation following abdominal and pelvic operations. Recent experimental and clinical evidence has demonstrated that postoperative inflammation is less pronounced after laparoscopic procedures than following open surgery [59-61]. This might be due to a more precise tissue handling during laparoscopy: manual manipulation of the small intestine is associated with increased adhesion formation in animal models. This is supported by several animal studies which suggest a reduction in adhesion formation with laparoscopic techniques [62-65]. Clinically, laparoscopic versus open colectomy is associated with reduced adhesion formation, albeit in a study with relatively small numbers [66]. Using minimally invasive/laparoscopic surgical techniques should be encouraged, since de novo adhesion formation occurs more frequently in patients undergoing laparotomy. However, adhesion reformation can occur with laparoscopy.

Adhesion prevention strategies: pharmacological adjuvant therapy

Pharmacological agents can be directed against various causes and components of the inflammatory process (e.g. infection, endotoxin, exudation) and/or adhesion formation (e.g. coagulation, fibrin deposition, and fibroblastic activity and proliferation). A number of obstacles must be surmounted before agents can be used in adhesion prevention. First, ischemic sites are vulnerable to adhesion formation, but are cut off from the bloodstream and, therefore, from systemic drug delivery. Second, the peritoneal membrane has an extremely rapid absorption mechanism, limiting the half-life and efficacy of many intraperitoneally administered agents. Third, any anti-adhesion agent needs to act specifically against adhesion formation and not interfere with normal wound healing processes; these processes of adhesion formation and remesothelialization use the same cascade (exudation, coagulation, fibrin deposition, and fibroblastic activity and proliferation) [46].

» *Nonsteroidal Anti-Inflammatory Drugs (NSAID's)*
NSAID's alter arachidonic acid metabolism by changing cyclooxygenase activities, inhibiting the formation of end products, including prostaglandins and thromboxane. By inhibiting prostaglandin and thromboxane synthesis, NSAID's decrease vascular permeability, plasmin inhibitor, platelet aggregation and coagulation and enhance macrophage function. NSAID's modulate a number of aspects of inflammation and have reduced peritoneal adhesion formation in many, but not all, animal models [46, 67-69].

» *Glucocorticoid and Antihistamine Therapy*
Corticosteroid therapy attenuates the inflammatory response by reducing vascular permeability and liberation of cytokines and chemotactic factors. This therapy is met with mixed results [46]. Corticosteroids, such as dexamethasone, hydrocortisone, and prednisolone, were studied alone or with antihistamines, such as promethazine, by intraperitoneal administration [68-70]. Antihistamines, often used in conjunction with glucocorticoids, inhibit fibroblast proliferation. Potential side effects, initiated by immunosuppression and delayed wound healing (e.g. infection, incisional hernia, and wound dehiscence), argue that these agents should be used with extreme caution [46, 57, 68-71].

» *Progesterone/Estrogen*
Progesterone shows a decreased adhesion formation in animal models. Human studies have either failed to confirm this finding or noted an increase in adhesion formation when medroxyprogesterone acetate was used intramuscularly or intraperitoneally [68, 69]. Estrogen has been associated with increased adhesions in animal models.
In animal studies fat necrosis and fibrotic changes were found less often in anestrogenic subjects. Primates treated with gonadotropin-releasing hormone agonists formed fewer adhesions than untreated animals, implicating a role of estrogen in promoting adhesion formation. It remains unknown whether a hypoestrogenic state leads to less postsurgical adhesions in humans [72].

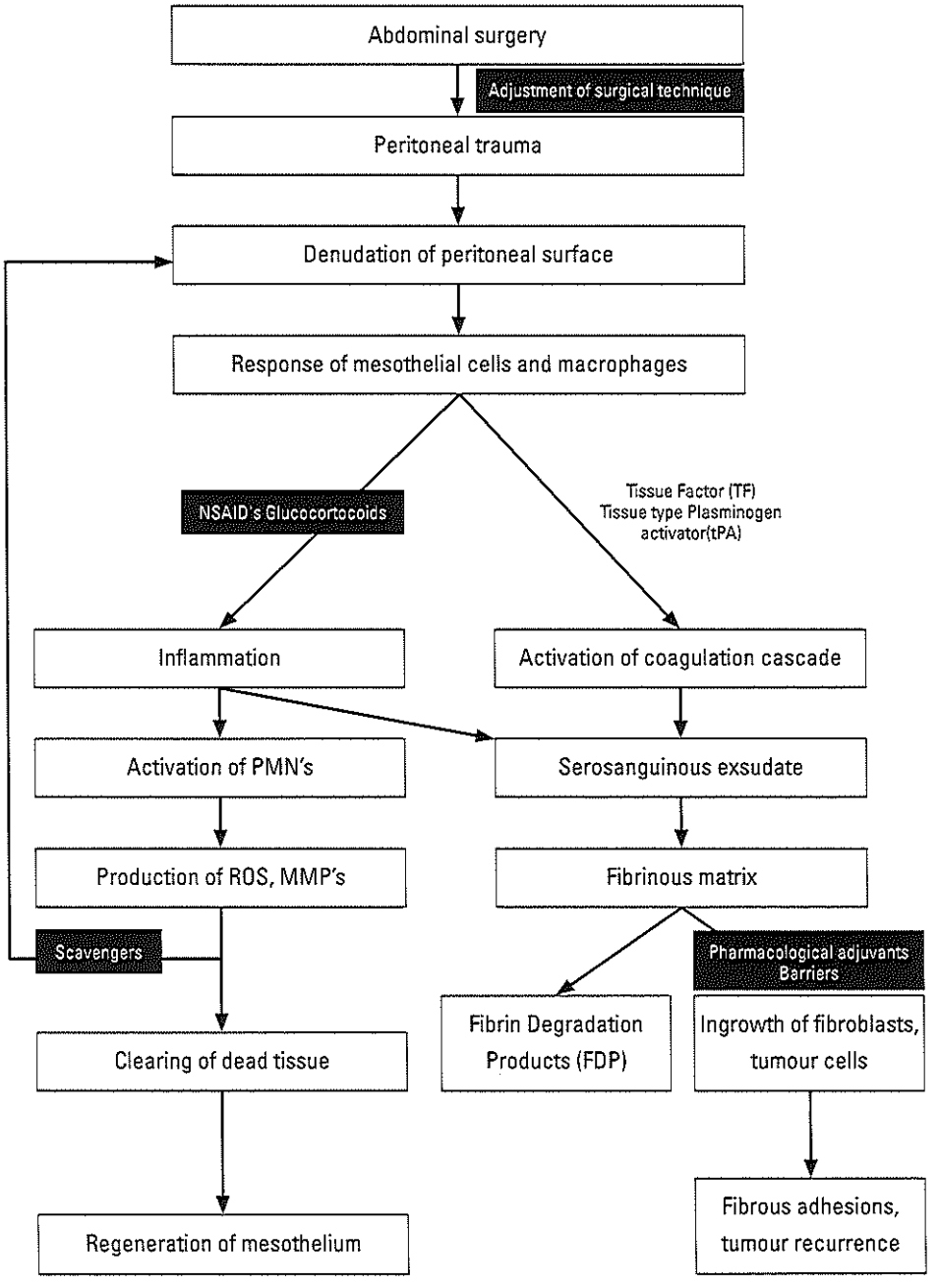
» *Anticoagulants*
Crystalloid isotonic irrigation containing heparin sulfate reduces intra-abdominal adhesion formation by inhibiting fibrin coagulation. However this use of heparin was associated with hemorrhages and delayed wound healing.
Low-dose intraperitoneal heparin irrigation (2,500/5,000 U/l) showed no benefit in adhesion reduction [50, 68, 69, 71].

» *Fibrinolytics*

Fibrinolytic agents caused hemorrhagic complications, although recombinant tPA, when applied locally, reduced adhesions in animal models without increasing complication rates [57, 68, 69, 71]. A promising approach in postsurgical adhesion prophylaxis was described with the use of rtPA. The effectiveness of rtPA with production of tPA by recombinant DNA techniques has been investigated in the prevention of initial as well as recurrent adhesion formation in animal studies. As discussed earlier, decreased plasminogen activator activity is believed to be a possible pathogenic factor in the development of adhesions. In experimental models, this activity has been reduced in the presence of thermal or mechanical trauma, ischemia and inflammatory factors known to lead to adhesion formation.

Although the administration of rtPA succeeded in reducing adhesion formation when studied in a rabbit model, continued research is needed to establish safety and effectiveness of rtPA use in human subjects. The evidence from clinical and animal trials suggests that all of these approaches have had only limited success, impeded by lack of safety, efficacy, and many adverse effects without eliminating the problem of postoperative adhesion formation [39, 73-76].

Figure 3. Prevention strategies



» *Antibiotics*
Broad-spectrum antibiotics are commonly used for prophylaxis against post-operative infections and adhesion formation. Antibiotics in intra-abdominal irrigation fluid actually caused adhesion formation and are not recommended as a single agent for adhesion prevention [68, 69].

» *Scavengers of Reactive Oxygen Species*
Super Oxide Dismutase (SOD) and Catalase, injected intraperitoneally after surgery, significantly reduce adhesion formation in experimental animal adhesion models [77-79]. However, clinical use in humans as of yet has not been investigated.

» *Statins*
Statins (3-Hydroxy-MethylGlutaryl-Coenzyme A reductase inhibitors) antagonize the enzyme HMG-CoA reductase, which catalyzes the rate-limiting step in hepatic cholesterol synthesis. This leads to reduction in the synthesis and secretion of lipoproteins by the liver, as well as upregulation of LDL receptors on hepatocytes, increasing clearance of circulating apolipoprotein E- and B- containing lipoproteins [80]. Clinically, the statins are currently used solely for their lipid-lowering effects in the treatment and prevention of atherosclerosis and cardiovascular disease. However, various experimental studies have shown statins to also have antioxidant-, anti-inflammatory- and pro-fibrinolytic properties [81-84], all of which may play a role in de process of adhesion formation and its prevention. Animal studies have shown an adhesion-reducing effect of statins, but several considerations remain pertinent concerning the practical problems regarding the current use and dose dependent side-effects of statins [85].

Adhesion prevention strategies: barriers
Antiadhesion barriers are basically divided into two main categories: macromolecular solutions and mechanical devices. In recent years both kinds of barriers have demonstrated real progress in adhesion prevention [46, 67]. The ideal barrier, besides being safe and effective, should be noninflammatory, nonimmunogenic, persist during the critical remesothelialization phase, stay in place without sutures or staples, remain active in the presence of blood and be completely biodegradable. In addition, it should not interfere with healing, promote infection, nor cause adhesions.

Barrier Solutions
» *Crystalloids*
Absorption of water and electrolytes from the peritoneal cavity is rapid, with

up to 500 ml of isosmolar sodium chloride absorbed in less than 24 h [86]. Because it takes 5–8 days for peritoneal surfaces to remesothelialize, a crystalloid solution will be absorbed well before the processes of fibrin deposition and adhesion formation are complete. From a theoretical point of view, intraperitoneal crystalloid instillates are not expected to prevent adhesion formation. Studies have shown an adhesion reformation rate of approximately 80% in patients who received crystalloid instillates [56, 87]. Whether used in surgery performed by laparotomy or laparoscopy, the risks of leaving large volumes of fluid in the peritoneal cavity after surgery may substantially reduce the ability of the host to eliminate infection. Increasing the intraperitoneal volume facilitates the accumulation of Escherichia coli by retarding the clearance of E. coli from the peritoneal cavity. Animal studies have shown that increasing the delivery of fluid contaminated with bacteria from 1 to 10 ml in the rat peritoneum increases the lethality from 20 to 60%. Dilution of opsonic proteins and increasing the surface area, on which phagocytes can trap and ingest unopsonized bacteria, and a decrease of the phagocyte-to-bacteria ratio by increasing the intraperitoneal volume are theorized as the basis for this increased morbidity. Decreasing the ratio of phagocyte-to-bacteria or diluting the opsonin source diminishes phagocytosis. Leaving large volumes of crystalloid in the peritoneal cavity after surgery may not benefit the patient's postoperative course [56, 88]. The postsurgical peritoneal cavity is acidic, and consideration should be given to the irrigation solution used in surgery [56, 88].

» Lactated Ringer's solution

Ringer's lactate is safe, inexpensive, readily available, and has a better buffering capacity than normal saline. Intraperitoneal instillation of lactated Ringer's solution in animal models decreases adhesion formation and reformation [89, 90]. The mechanism of action is unclear, but it seems that the presence of a great volume of Ringer's lactate in the abdominal cavity separates raw peritoneal surfaces and prevents adhesion formation. It is also possible that Ringer's lactate cleanses the newly formed fibrin exudate that can serve as a matrix for fibroblast and capillary formation. This initial fibrin, if not removed by fibrinolysis or absorption, produces an inflammatory response, fibroblast proliferation, and adhesion formation. Since most of the LRS is being absorbed within 24 hours after instillation, single instillation is ineffective [91]. The efficacy of Ringer's lactate in clinical situations has not been clinically proven [45, 72].

» Saline solution (NaCl 0,9%)

Irrigation with saline is often used empirically to clean the peritoneal cavity

after contamined operations as well as a means of removing blood, bile and other adjuvants of bacterial infection [92]. It is, just like LRS, being resorbed within 24 hours after administration to the peritoneal cavity [93]. In 1995 Burns *et al.* used Phosphate buffered saline (PBS) in a rat model to study adhesion formation. They found no difference between the control-group (no irrigation) and the PBS-group [34]. In 1999, van Westreenen *et al.* found that irrigation with saline solution in rats caused 150% more adhesions than no irrigation at all [94]. So, when used solitary, (phosphate buffered) saline seems to be ineffective or even contra-productive in preventing adhesion formation.

» 32% Dextran 70

32% dextran 70 (Hyskon®, Pharmacia, Uppsala, Sweden), is a frequently used solution for adhesion prevention. By hydroflotation of intra-abdominal structures with the dextran solution, a physiological separation occurs between peritoneal surfaces [67, 70].

Through dilution, dextran diminishes local fibrin concentration, preserves local plasminogen activators, and interferes with polymorphonuclear neutrophil expression of adhesion molecules [39, 70]. The dextran solution is slowly absorbed, draws fluid into the abdominal cavity and also decreases clot formation [57, 68-70]. Follow-up studies of the initial observation did not show a reduction in adhesions [67, 70]. Moreover, significant side effects, such as ascites, weight gain, pleural effusion, labial edema, liver function abnormalities, and, albeit rare, disseminated intravascular coagulation and anaphylaxis, were noted [67]. Although instillation of high-molecular-weight dextran (32% dextran 70) was popular, the results have been inconsistent [95].

» Hyaluronic Acid (HA)

HA is a naturally occurring glycosaminoglycan and a major component of the extracellular matrix, including connective tissue, skin, cartilage, and vitreous and synovial fluids. HA is biocompatible, nonimmunogenic, nontoxic, and naturally bioabsorbable. Like carboxymethylcellulose, it is negatively charged at physiological pH and freely soluble [56]. HA coats serosal surfaces and provides a certain degree of protection from serosal desiccation and other types of injury. However, its use after tissue injury is ineffective [72, 96].

» HA Combined with Phosphate-Buffered-Saline (HA-PBS)

HA has been combined with PBS into a macromolecular solution to prevent adhesion formation, called Sepracoat® (Genzyme, Cambridge, Mass., USA). HA-PBS is applied intraoperatively, prior to dissection, to protect peritoneal

surfaces from indirect surgical trauma (e.g. abrasion and desiccation) rather than postoperatively to separate surfaces after they are traumatized [34]. In animal models, this solution effectively reduced serosal damage, inflammation, and postsurgical adhesions [34].
In human studies HA-PBS solution safely and significantly decreased incidence, extent, and severity of de novo adhesion in multiple sites indirectly traumatized by complex, multiple gynecologic pelvic procedures via laparotomy [97].

» Carboxymethylcellulose
Carboxymethylcellulose is a derivative of cellulose. Carboxymethylation of the glucosidic hydroxyl groups makes the polymer hydrophilic. It is negatively charged at physiological pH and freely soluble.
Clearance is less clear than that of HA, but it is spontaneously broken down. Carboxymethylcellulose works by separating raw surfaces and allowing independent healing of traumatized peritoneal surfaces [56, 72].

» Icodextrin
Icodextrin is a α-1,4 glucose polymer which, at 7,5% concentration, is widely used as a peritoneal dialysis solution (Extraneal®) in the treatment of chronic renal failure. More recently, 4% (osmotically inert) solutions of Icodextrin have been developed for intraperitoneal delivery of cancer chemotherapy following surgical resection (Deemed®) and for the reduction of adhesion formation and reformation following abdominal surgery (Adept®) [98]. Intraperitoneal administration does not induce fluid accumulation whilst the high molecular weight of the polymer precludes capillary absorption and necessitates its gradual clearance by the lymphatical system. In the bloodstream, Icodextrin is rapidly metabolized by α-amylase to form simple sugars but because of the absence of this enzyme, metabolism does not occur in the human peritoneal cavity. These factors confer a prolonged residence of several (3 to 5) days on instilled 4% icodextrin solution in in humans, in contrast to saline or glucose solutions which are largely cleared within 24 hours [93]. The effects of Icodextrin 7.5% have also been studied in pathways of tumour recurrence and peritoneal metastasis; results showed a reduction of postoperative adhesions and no promotion nor inhibition on both recurrence and metastasis, suggesting Icodextrin also might be useful and safe in oncological surgery [99] .

Solid barriers
» Autologous peritoneal transplants
Experimental studies have demonstrated that covering lesions of the parietal

peritoneum with microsurgically applied autologous peritoneal transplants can completely prevent severe adhesion formation. More significantly was the decrease of visceral peritoneal adhesions with the use of autologous peritoneal transplants, i.e. injuries to the serosa of the uterine horn. This suggests that the risk is higher for adhesion formation stemming from the visceral than from the parietal peritoneum after gynecologic surgery. The visceral peritoneum should be generally covered at the conclusion of surgery, either with autologous peritoneal grafts or a synthetic barrier. The advantage of a synthetic barrier is that the material does not need to be obtained surgically and can be cut to size outside of the abdomen and then applied without sutures [100].

» Synthetic solid barriers

A great number of natural and synthetic graft materials have been employed in an effort to reduce adhesion formation on traumatized surfaces. Natural materials have included peritoneum, omentum, HA, fat, amnion, as well as amnion plus chorion [101-107]. Recently, interest has focused on mechanical barriers placed over traumatized tissues at the conclusion of surgery, in order to separate tissue surfaces.

Such synthetic barriers included Gelfilm® and Gelfoam® paste (Upjohn, Kalamazoo, Mich., USA), Surgicel® (Johnson & Johnson, New Brunswick, N.J., USA), Silastic® (Dow-Corning, Midland, Mich., USA), meshes of polytetrafluorethylene (PTFE, Gore-Tex®; Gore & Associates, Flagstaff, Ariz., USA), Interceed® (TC7) – oxidized regenerated cellulose (ORC; Johnson & Johnson), and Seprafilm® – bioresorbable membrane chemically derivatized sodium hyaluronate and carboxymethylcellulose (HA-CMC; Genzyme) [101, 108-110].

» Gore-Tex®

Expanded PTFE is a nonreactive, antithrombogenic, nontoxic synthetic fabric with small pores that inhibit cellular transmigration and tissue adherence. The use of PTFE is strictly reserved for noncontamination operations. When placed over traumatized tissue it has been shown to reduce adhesion formation [104].

A PTFE barrier prevents adhesion formation and reformation regardless of the type of tissue injury or whether hemostasis is achieved. Expanded PTFE was found to decrease postmyomectomy adhesions and pelvic sidewall adhesions in a randomized study [101]. It was found that expanded PTFE was associated with fewer postsurgical adhesions to the sidewall than oxidized-regenerated cellulose [105].

The use of PTFE in laparoscopy is cumbersome and not easy to handle [67]. PTFE also needs to be secured in place physically and is nonabsorbable.

Therefore, it must be either left in place permanently or removed surgically and this makes it not usable in abdominal surgery.

» Interceed®

Interceed® or Oxidized Regenerated Cellulose (ORC) has been shown in both animal and human studies to reduce adhesion formation by forming a barrier and physically separating adjacent raw peritoneal surfaces, preventing adhesion development between these surfaces.

It appears to decrease adhesion formation-reformation beyond that achieved with meticulous surgical technique. Both raw surface area and the occurrence of adhesion formation-reformation are reduced by a margin of 20%. When applied to a raw peritoneal surface, it becomes gel within 8 h [46, 70, 71, 111].

ORC can be applied easily by laparoscopy, follows the contour of the organ, and does not need suturing. It is essential that complete hemostasis is achieved before ORC is placed on the peritoneal surface, as the presence of intraperitoneal blood negates any beneficial effect [112]. Clinical observation indicates that small amounts of bleeding at the time that ORC is applied results in blood permeating the weave of the material.

Fibroblasts grow along the strands of clotted blood with subsequent collagen deposition and vascular proliferation [56, 70]. This explains the appearance of adhesions despite the use of the adhesion barrier. The most important steps to maximize the efficacy of ORC barriers are to remove intraperitoneal irrigants thoroughly, inspect the operative site to ensure that adequate hemostasis has been achieved, and use a sufficiently large piece of the ORC barrier. If hemostasis has not been achieved, the ORC barrier turns black or brownish black. In these cases the material must be removed, hemostasis achieved, and a new piece of ORC barrier applied [56, 67, 70, 110].

ORC reduces incidence, extent, and severity of postoperative pelvic adhesions, but does not prevent these [110]. ORC has been shown to act in synergy with heparin. In animal models, the application of heparin-treated ORC adhesion barriers significantly reduced adhesion scores. Although adhesion reduction was also seen in human studies, it did not reach statistical significance when compared to nontreated ORC [113]. Rather than support bacterial growth, ORC exhibits antibacterial properties in vivo [114].

» Seprafilm®

Hyaluronic-acid carboxymethylcellulose (HA-CMC) is a nontoxic, nonimmunogenic, biocompatible material effective in reducing incidence and extent of severe postoperative adhesions. It turns to a hydrophilic gel approximately 24 h

after placement and provides a protective coat around traumatized tissue for up to 7 days during remesothelialization. Like ORC, the HA component is completely cleared from the body within 28 days; less clear is the removal of the CMC component. HA-CMC can be used in the presence of blood [115]. HA-CMC reduces the rate of adhesive small bowel obstruction needing surgical intervention [116]. Patients receiving HA-CMC also have less severe adhesions as compared with controls [117]. However, when wrapped around an anastomosis, Seprafilm® causes anastomotic leakage [116].

Clinical consequences of peritoneal trauma: local tumour recurrence and peritoneal metastasis

Despite the introduction of new treatment modalities for gastro-intestinal malignancies during the last decades surgery remains the principal therapy for most gastro-intestinal malignancies, although the recurrence rates after intentionally curative surgery are high [118-121].
Operative trauma in itself may favour development of tumour recurrence. This relation between abdominal surgery and locoregional tumour recurrence was investigated in previous in vivo and in vitro experiments. These studies illustrated that surgical trauma enhanced locoregional tumour recurrence and that this phenomenon involved a dose-response relation, i.e. severe trauma was associated with a higher locoregional tumour recurrence rate compared to mild trauma [122-126].

This indicates that, as well as in adhesion prevention, minimising surgical trauma to the peritoneum is an essential prerequisite in minimising tumour recurrence and peritoneal metastasis. Laparoscopic surgery is associated with less surgical trauma and hence reduced peritoneal tumour recurrence [122].
Further experiments demonstrated that abdominal surgical trauma provoked a local inflammatory reaction with influx of mainly polymorphonuclear cells (PMN), as described earlier in this introduction. These activated PMN produce reactive oxygen species (ROS) which are found to play an important role in the observed enhanced locoregional tumour recurrence in which binding of tumour cells to the mesothelium is an essential step [125, 126].

Several experiments have shown that scavenging these ROS, minimising surgical trauma and modulation of the inflammatory reaction lead to a decrease in tumour recurrence [122, 124-126].

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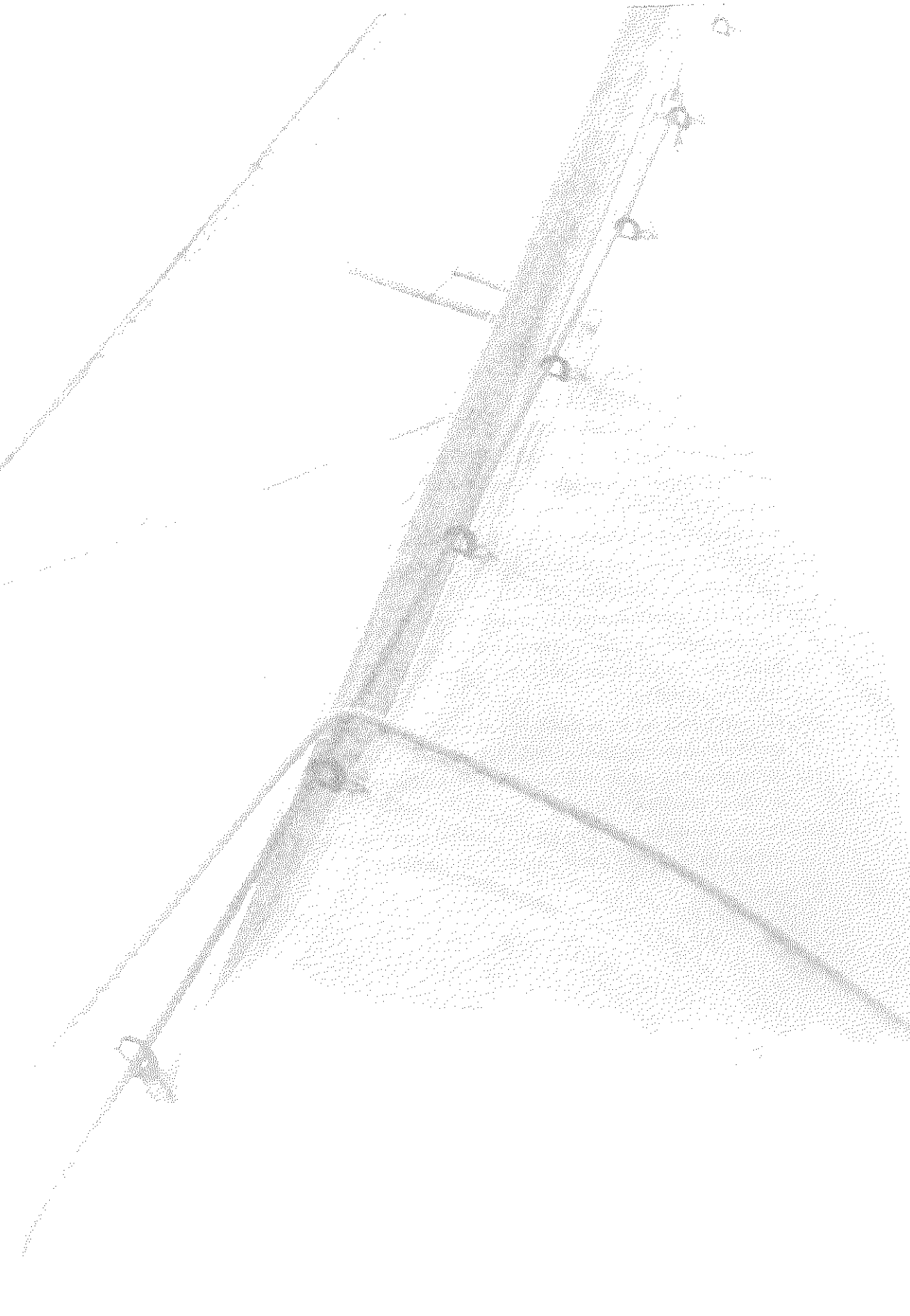

3

Laparoscopic kidney donation: the impact of adhesions

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Abstract

Background: Adhesion formation following abdominal surgery causes substantial burden to society. Laparoscopic donor nephrectomy (LDN) offers an opportunity to study the prevalence of adhesions in healthy individuals. Furthermore we evaluated whether or not adhesions hindered LDN.

Methods: Data of 161 LDNs were prospectively collected. The presence of adhesions was documented. Parameters influenced by the presence of adhesions such as operation time, blood loss and intra-operative complications were documented.

Results: Twenty-eight of 44 donors (64%) who had had prior abdominal surgery presented with adhesions at laparoscopy versus 61 of 107 donors (52%) who had no history of abdominal surgery (P=0.22). Conversion and complication rate, operation times and blood loss did not differ between those with and without a previous history of abdominal surgery. Blood loss and operation time did not differ between donors with and without adhesions. The number of conversions to open was significantly higher in donors with adhesions (9 vs. 0, P=0.005). Three conversions were due to adhesions.

Conclusion: Adhesions are present in a significant number of healthy individuals regardless of a history of previous abdominal operations. As these operations are of no predictive value for the number and complexity of adhesion formation, we advocate starting live kidney donation laparoscopically as the procedure can be most probably conducted successfully by this approach.

Introduction

Postsurgical adhesion formation is an almost unavoidable complication of abdominal surgery; it occurs in approximately 85% (55%-100%) of operations; abdominal surgery therefore accounts for up to 90% of all intra-abdominal adhesions [1-3]. The remaining 10% are caused by inflammation, endometriosis or are congenital [4]. Basically, adhesions are of two types: Type 1, de novo adhesions, occur at sites with no previous adhesions and are subdivided into Type 1a, adhesion formation at sites where no direct trauma to the peritoneum occurred, and Type 1b, adhesions forming at sites of direct surgical trauma. Type 2, reformed adhesions, occur at sites of previous adhesiolysis [5].
Adhesions are considered to be associated with a range of complaints and complications, the most important of which being small bowel obstruction. Others include chronic abdominal pain, infertility and difficulty at reoperation [6]. As a result, the presence of adhesions leads to a substantial burden of readmissions for postoperative related disorders. Approximately 1% of all hospital admissions and 3% of laparotomies are the result of intestinal obstruction due to adhesions, resulting in annual costs in the US as high as $1 billion [1,7].
Recently published, the results of the third Surgical and Clinical Adhesions Research (SCAR-3) study indicated a readmission-risk due to adhesions after colorectal surgery as high as 30% [8]. Postsurgical adhesions are a consequence of injured tissue surfaces fusing together to form scar tissue after incision, cauterization, suturing or other means of trauma. Recently, it was reported that all patients who had undergone at least one prior abdominal operation developed one to more than ten adhesions [8].

Evidence has mounted that transperitoneal, laparoscopic donor nephrectomy (LDN) has become the preferred technique for live kidney donation [9-11]. LDN in asymptomatic healthy individuals offers a unique opportunity to study the prevalence of adhesions. The aim of this prospective registration was to investigate the prevalence of adhesions in asymptomatic people with or without a history of abdominal surgery. Furthermore, we studied whether adhesions hindered LDN.

Methods

From May 2001 until November 2005 161 donors (73 male, 88 female) underwent LDN at the Erasmus MC Rotterdam. Part of the patients participated in a prospective study and a randomized controlled clinical trial both addressing laparoscopic versus open live kidney donation. The medical ethics committee of the Erasmus MC approved these studies. The main outcomes have been published elsewhere [11,12].

An independent research fellow who recorded the presence of adhesions, operation times, blood loss and complications attended all operations. We only scored the presence of adhesions if sharp dissection was required to divide the adhesion. Adhesions to the abdominal wall were also scored as adhesions. LDN was performed as described previously [12]. Briefly, with the patient placed in a lateral decubitus position and the operation table maximally flexed, a 10-mm trocar was introduced subumbillically under direct vision. A 30°-video endoscope was inserted and 3 to 4 additional trocars were introduced. This set-up allowed visualization of possible adhesions within the whole peritoneal cavity. The colon was mobilized and displaced medially. In right-sided kidney donation the liver was displaced cranio-laterally using a babcock fixed to the lateral abdominal wall. Opening of the renal capsule and division of the perirenal fat was facilitated using an ultrasonic device (Ultracision®, Ethicon, Cincinnati, USA). Subsequently the ureter, the renal vein and its branches, and renal artery were identified and dissected. Then, a Pfannenstiel incision was made. An endobag (Endocatch®, US surgical, Norwalk, USA) was introduced into the abdomen. The ureter was clipped distally and divided. The renal artery and vein were divided using an endoscopic stapler (EndoGia®, US Surgical, Norwalk, USA). The kidney was extracted, flushed with 4°-Celsius Eurocollins® (Fresenius, Bad Homburg, Germany) and stored on ice. Then, the incisions were closed in layers.

Time until kidney removal was defined as the time elapsing between incision of the skin and extraction of the kidney. Operation time was defined as time elapsing between incision of the abdomen and tying the last suture at closure of the abdominal wall. The same team that performed the nephrectomy also carried out renal transplantation. Therefore, time spent to flush the kidney after extraction and sometimes even performing direct venous or arterial reconstruction is included in operation time. Intra-operative complications were defined as events unintentionally lengthening the operation or causing potential harm to donor or graft.

Statistical analyses were conducted using SPSS (version 11.5, SPSS Inc., Chicago, USA). First we compared the characteristics of donors with and without a history of previous abdominal surgery. Second we compared the characteristics of donors with and without adhesions.

Categorical variables were compared with the Chi-square test and continuous variables were compared with the Mann Whitney U test. A P-value < 0.05 (two-sided) was considered statistically significant.

Results

None of the 161 donors included in this study preoperatively complained of abdominal pain. During one-year follow-up, none of the donors complained of non-specific abdominal pain. Two donors were re-operated because of an incisional hernia at the Pfannenstiel incision and one donor was suspected of having an incisional hernia of the lumbotomy incision after conversion, but laparoscopy revealed diastasis only.

Forty-four donors had previously undergone abdominal surgery for other reasons, including 21 appendectomies, 19 intra-peritoneal gynecological operations, four abdominal wall corrections, three cholecystectomies, two caesarean sections, one diagnostic laparoscopy, and one duodeno-jejunostomy. The caesarean sections were included because they can result in significant scar formation at the site for the Pfannenstiel incision. Twenty-eight (64%) of these 44 patients had abdominal adhesions. Sixty-one (52%) of the donors who had not undergone previous surgery presented with intra-peritoneal adhesions. The characteristics of both groups are shown in Table 1. Except for a significant higher number of females in the group with previous abdominal surgery, no statistically significant differences were observed. Only one conversion occurred in a donor with a history of previous abdominal surgery.
In Table 2 characteristics of donors without and with adhesions are displayed.

Table 1. Baseline characteristics and outcomes of donors without and with a history of previous abdominal surgery.

	No previous abdominal operation n=107		Previous abdominal operations n=44		p-value
Gender (female)	53	(45%)	35	(80%)	<0.001
Age (years)	50	(18-77)	52	(28-80)	0.38
Body mass index (kg/m²)	26	(17-35)	26	(19-37)	0.09
ASA classification (I)	90	(77%)	35	(80%)	0.83
Relation to recipient					0.61
related	73	(64%)	24	(55%)	
non-related	38	(33%)	18	(41%)	
cross-over or anonymous	6	(5%)	2	(5%)	
Kidney (left)	61	(52%)	16	(36%)	0.08
Renal arteries (>1)	26	(22%)	12	(27%)	0.54
Adhesions (present)	61	(52%)	28	(64%)	0.22
Conversion to open	8	(7%)	1	(2%)	0.45
Warm ischemia time (min)	6	(2-14)	6	(2-17)	0.96
Blood loss (ml)	100	(0-3500)	105	(5-860)	0.55
Time until kidney extraction (min)	183	(99-345)	184	(104-309)	0.87
Operation time	225	(129-395)	234	(135-340)	0.84
Complications	16	(14%)	7	(16%)	0.80
Hospital stay (days)	3	(1-9)	3	(1-10)	0.82

Categorical variables are displayed as number (%) and continuous variables as median (range)

Significantly more conversions occurred in the group with adhesions. In total, nine procedures were converted to open. In three procedures conversion was clearly related to adhesions. These donors had not undergone previous operations. These three conversions included one conversion immediately after introduction of the video-endoscope because of the adherence of the greater omentum to the abdominal wall. In the two other cases the splenic flexure of the colon, the spleen and the left kidney were firmly adhered, which impeded proper vision of the kidney and prompted elective conversion to a muscle-splitting open approach. Other conversions were due to blood loss from the renal vein (n=3), continuous blood loss after extraction of the kidney (n=1) and abundant adipose tissue impeding overview (n=2).

Table 2. Baseline characteristics and outcomes of donors without and with intra-abdominal adhesions.

	No adhesions n=72		Adhesions n=89		p-value
Gender (female)	37	(51%)	51	(57%)	0.53
Age (years)	52	(18-77)	51	(20-80)	0.78
Body mass index (kg/m²)	26	(17-35)	25	(18-37)	0.37
ASA classification (I)	56	(78%)	69	(78%)	1.00
Relation to recipient					0.24
related	48	(67%)	49	(55%)	
non-related	20	(28%)	36	(40%)	
cross-over or anonymous	4	(6%)	4	(5%)	
Kidney (left)	31	(43%)	46	(51%)	0.34
Renal arteries (>1)	15	(21%)	23	(26%)	0.58
Previous abdominal operation(s)	16	(22%)	28	(32%)	0.22
Conversion to open	0		9	(10%)	0.005
Warm ischemia time (min)	6	(3-14)	5	(2-17)	0.003
Blood loss (ml)	100	(0-860)	120	(5-3500)	0.12
Time until kidney extraction (min)	180	(99-345)	185	(104-339)	0.27
Operation time	218	(129-395)	233	(135-390)	0.22
Complications	7	(10%)	16	(18%)	0.18
Hospital stay (days)*	3	(2-10)	3	(1-9)	0.03

Categorical variables are displayed as number (%) and continuous variables as median (range)

*Despite a similar median hospital stay donors without adhesions were earlier discharged from the hospital

Blood loss and operation time did not differ between groups. Warm ischemia time was significantly shorter in the group with adhesions, mainly because 8 of 9 converted procedures had the warm ischemia times of open surgery. For the same reason, hospital stay was significantly longer in the adhesion group despite a similar median hospital stay. Complications in the group without adhesions included a bowel perforation at introduction of the first trocar, transsection of a lower pole artery which was not identified at preoperative imaging with MRI, a splenic hematoma and a splenic laceration and bleeds from the renal vein, the adrenal area and small bleedings from the venous branches.

Except for the bowel perforation that required re-operation, all complications were recognized and treated conservatively without requirement of postoperative treatment. Complications in the group with adhesions included two arterial bleeds, three bleeds from the renal vein, one bleed from the gonadal vein, two bleeds from the adrenal area, three splenic lacerations, a subcapsular hematoma of the kidney, two bowel perforations, a bladder lesion and bleed from the stump of the renal artery after extraction of the kidney requiring conversion to a small open incision to control the bleeding.

Discussion

Adhesions were encountered in 52% asymptomatic kidney donors, who had no history of previous operations. These adhesions must be classified as type 1a adhesions occurring de novo without surgical trauma. Three conversions related to adhesions occurred in this group. This indicates that previous abdominal surgery alone is of no predictive value for the intra-operative course during LDN.

Interestingly, Karayiannakis *et al.* found adhesions in only 2.1% of patients who underwent laparoscopic cholecystectomy without a history of previous abdominal surgery [13]. Weibel *et al.* and Menzies *et al.* reported adhesions in 93% and more than 93% respectively in patients who had undergone a laparotomy [14,15]. We observed adhesions in 64% of the donors with previous abdominal surgery. Possible explanations for the observed discordance with previous studies includes the nature of the procedures. The procedures that these donors underwent before the present study were mainly local. However, the 30°-video endoscope inserted close to the umbilicus allows visualization of the whole peritoneal cavity. The 0° video-endoscope commonly used in laparoscopic cholecystectomy may not always provide sufficient vision to overview the whole abdomen. We acknowledge that it is sometimes difficult to visualize the contralateral hemi-abdomen when the patient is in a lateral decubitus position. However, rotation of the operation table allows investigation of the peritoneal cavity in a more supine position. Furthermore, the prospective design of our study allowed us to even record single adhesions. Probably, retrospective reviewing of operative findings only clarifies severe adhesions. Then the displacement of adjacent organs as the colon, the liver and the spleen during LDN possibly reveals additional adhesions. Finally, the classification of adhesions (i.e. from the colon to the abdominal wall) attributes to a high rate of

adhesions among donors who were not operated on previously. The relatively low incidence in donors who had been operated previously is partly explained by the nature of the procedure. As expected because of the inclusion of healthy individuals, the number of donors with a past medical history revealing midline laparotomies was small. Severe adhesions due to previous midline laparotomies may severely complicate surgery. However, the only donor presenting with massive adhesions preventing further laparoscopic dissection had never been operated on.
The high incidence of adhesions in donors without previous surgery also sheds new light on the ongoing debate on adhesiolysis for chronic abdominal pain. The data of this study question the correlation between adhesions and bowel pain. Of all patients who entered our study 55% had adhesions, but no one preoperatively complained of abdominal pain. These donors may be considered "adhesion formers", but none of the 161 donors complained of abdominal pain during one-year follow-up, which was conducted with visits to the outpatient clinic and administration of quality of life and case-record forms. The placebo effect of diagnostic laparoscopy for adhesions in patients with chronic abdominal pain has been well established by Swank *et al.* [16], who showed alleviation of abdominal pain after both diagnostic laparoscopy and laparoscopic adhesiolysis. The complications found after laparoscopic adhesiolysis stress that this operation should be limited.

Except for a correlation between adhesions and a higher conversion rate we did not assess an adverse relation between adhesions and laparoscopic kidney donation. Possibly, some of the complications such as splenic lacerations and bladder and bowel lesions caused when performing the Pfannenstiel incision occur in higher numbers in donors with adhesions but the number of 161 donors was too small to show a significant correlation.

We acknowledge that the number of complications is relatively high in our series. This is a consequence of our prospective registration and our definition. Surgeons themselves would probably only score significant complications. Most of the complications did not have consequences to the donor. The relatively high number of conversions is the consequence of including donors with complex renal anatomy i.e. multiple arteries and high body mass index.

One of the weaknesses of the present study is that we only classified whether adhesions were present or not. In future studies, the extent and the time to divide these adhesions should preferably be documented.

In conclusion, adhesions in patients without a history of abdominal operations might be far more common than previously thought. Adhesions did not cause abdominal pain in our series and did not necessarily influence the intra-operative course during laparoscopic live kidney donation. We therefore suggest applying a laparoscopic approach first in live kidney donation, regardless of a history of previous surgery.

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4

Chronic abdominal pain: the role of adhesions and benefit of laparoscopic adhesiolysis

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Abstract

Abdominal adhesions can cause bowel obstruction, infertility and chronic abdominal pain. In this review adhesion-related chronic abdominal pain, diagnostic laparoscopy and laparoscopic adhesiolysis as a treatment for chronic abdominal pain are discussed. There is no difference in benefit after diagnostic laparoscopy compared to laparoscopic adhesiolysis. Considering the risk of complications associated with laparoscopic adhesiolysis, it should no longer be recommended as therapy for adhesion-related chronic abdominal pain.

Introduction

Abdominal adhesions, whether caused by (surgical) peritoneal trauma, infection, radiation or of congenital origin, are considered to be associated with a range of complaints and complications, including infertility, small bowel obstruction, difficult re-operation and chronic abdominal pain (CAP) [1]. Approximately 1% of all surgical admissions and 3% of laparotomies are the result of intestinal obstruction from adhesions [2]. The treatment of patients with symptoms caused by adhesions also will generate extra costs; in the US alone the costs of surgery for abdominal adhesions exceed $1 billion annually [3, 4]. The amount of adhesions found at operation is positively correlated to the amount of previous operations a patient has undergone [5].

Concerning CAP, many other organic and functional diseases can be the cause such as irritable bowel disease, functional dyspepsia and various esophageal, biliary and urologic disorders [6]. In this review the focus is on CAP, caused by abdominal adhesions and the place of laparoscopic adhesiolysis in this subgroup.

Chronic abdominal pain after previous abdominal surgery

CAP remains elusive to all known methods of diagnosis and treatment. It is a common disorder both in general and specialized surgical practice and patients may have undergone numerous diagnostic work-ups including surgery [6]. CAP can, just like infertility and small bowel obstruction, be a sequela of adhesions and it may present as continuous or colicky pain. Continuous pain is considered to occur when adhesions retract the viscera without obstructing them, whereas colicky or intermittent pain is suggestive for obstruction.

In 2001, Sulaiman *et al.* [7] found sensory, substance-P containing nerve fibers in human peritoneal adhesions, suggesting the possibility of conducting pain after appropriate stimulation. Although pain physiologic studies were not conducted, it is very well possible that the observed thin, non-myelinated fibers conduct pain stimuli. However, not all patients in this study experienced chronic pelvic pain; therefore, although all adhesions may be able to directly induce pain sensations, there are likely to be other factors to consider, in addition to the innervation, such as peritoneal pathology, organ mobility, and psychosomatic manifestations. Commonly, investigating abdominal pain includes ruling out gastritis, cholecystolithiasis, irritable bowel disease, functional dyspepsia, diverticulosis, pancreatitis, renal concrements, arteriosclerosis of visceral arteries, parasitic disease, or lactase deficiency [8]. In patients with colicky pain,

as mentioned previously, obstruction is more ... y. Auscultation of the abdomen or plain radiographs of the abdomen at th... time of colicky pain can render intestinal obstruction more likely. When bowel obstruction is suspected, enteroclysis combined with either colonoscopy or barium enema may detect serious ailments such as inflammatory bowel disease, tumours or volvulus. Thorough investigations to exclude pathology other than adhesions are of paramount importance to ensure the proper selection of those patients with chronic abdominal pain who may benefit from adhesiolysis.

Nowadays, laparoscopy is most commonly used to assess and take down adhesions, as will be discussed later on. Once adhesions have been found at surgery, it is difficult to determine which adhesions are liable to cause pain. To address this problem, Leidig and Krakamp performed laparoscopy using local anesthesia, enabling the patient to indicate which adhesions were causing the pain upon stretching [9]. After adhesiolysis, 70% of the patients reported an improvement and 29% were free of pain.
In 2004, Demco *et al.* set out to determine the nature and location of adhesions and their relationship to abdominal pain in patients undergoing awake microlaparoscopy [10]. Thirty women, aged 26-49 years, suffering from chronic pelvic pain, were kept awake during their laparoscopy to determine the site and degree of pain when the adhesions were manipulated. Demco *et al.* stated that filmy adhesions between a movable structure, such as an ovary, and the peritoneum produced the highest pain scores, whereas fixed or dense adhesions, no matter where they were located, showed the lowest pain scores.
Mueller *et al.* [11] take it one step further as they state that only adhesions which limit movement of the organs are likely to cause pain. To investigate whether the extent of adhesions is correlated to the pre-operative symptoms, several studies were conducted [12-14].
Freys *et al.* [12] in 1994 found small adhesions to cause recurrent abdominal pain without other symptoms, whereas large adhesions produce recurrent abdominal pain in combination with symptoms indicative of intermittent bowel obstruction. Their results indicate a certain "ideal constellation" for an enduring successful adhesiolysis per laparoscopy: the subjective complaint of recurrent abdominal pain with a localized and reproducible punctum maximum in combination with a circumscribed area of adhesions at that site.

In 1986, Rapkin *et al.* [13] retrospectively reviewed 100 consecutive laparoscopies for chronic pelvic pain and 88 for infertility. Twenty-six of the 100 (26%) chronic pelvic pain patients and 34 of the 88 (39%) infertility patients exhibited

pelvic adhesions as the only abnormal finding. Patients in each group with findings of pelvic adhesions were compared with respect to symptomatology, density of adhesions, and locations of adhesions. Only four of the 34 infertility patients in whom pelvic adhesions were found complained of pain. Comparison of the chronic pelvic pain patients and the asymptomatic infertility patients did not reveal a significant difference in the density or the location of adhesions. In 1991, Stout *et al.* [14] used standardized measures of behavioral and psychosocial factors associated with other chronic pain conditions to interview 102 women scheduled for laparoscopic surgery. Surgeons who were blinded to the patient's self-reported pain data completed the American Fertility Society (AFS) classification for endometriosis and adhesions on the basis of observed physical disease. Although AFS-classification scores were significantly related to self-assignment into pain or no-pain groups, the extent of physical disease evaluated by this procedure was not significantly correlated with ratings of pain levels or a number of indexes of impairment.

The site of CAP correlated well with the location of adhesions according to Stout *et al.* [14], but Rapkin *et al.* [13] failed to find such correlation. The pathophysiology of CAP is still poorly understood [15] and it is very well possible that psychosocial factors play a role in chronic abdominal pain [16].
Recently, the development of tools for brain investigation, such as functional magnetic resonance imaging, has provided new insights on the pathophysiology of chronic pain. These data have shown that plastic changes in the central and peripheral nervous system might play an important role in the maintenance of chronic pain. Therefore, approaches aimed at the modulation of the nervous system, rather than the ones interfering with the inflammatory pathways, may be more effective for chronic pain treatment [17]. As mentioned before [7] adhesions were shown to contain nerve fibers which are likely to conduct pain stimuli, so the assumption that chronic abdominal pain due to adhesions has a psychosomatic origin may be unlikely. Many studies indicate that the results of adhesiolysis deteriorates with time [18-24]. Because the CAP syndrome also has many psychosocial aspects [25], one could assume that the benefit of laparoscopic intervention may diminish during the follow-up period. Since *de novo* formation of adhesions is to be expected after adhesiolysis [26], and the severity of adhesions increases with time [27], this suggests an explanation for the recurrence of pain.
The temporary relief of pain might also be explained by the placebo effect [28]. The highest reported recurrence rate was 26% [20], and the longest pain-free interval was 2 years [18]. According to Mecke *et al.* [29], a longer duration of

preoperative symptoms predisposes for a lower success rate. Unfortunately, no validated pain scores were used in most series, and the duration of follow-up was not given in precise terms by most authors.

Laparoscopy allows surgeons to see and treat many abdominal changes that could not be diagnosed otherwise [30, 31]. In 35% to 50% of the patients with CAP, adhesions may be the only explanation [32, 33] but consensus about the causal association of adhesions with pain is still not achieved. As stated before, intra-abdominal adhesions may be asymptomatic, but in some cases also a significant cause of morbidity, such as infertility, bowel obstruction, and pain [34].

Diagnostic laparoscopy

Consensus exists about the indications for diagnostic laparoscopy for chronic abdominal pain if other pathology has been excluded. In a prospective study of 70 patients suffering chronic abdominal pain, Onders and Mittendorf [30] described the findings during diagnostic laparoscopy. Adhesion (57%), hernia (18%) and abnormal appendices (16%) were the most common diagnoses. In 10 patients no pathology at all was found. These findings correspond with those reported by Salky and Edye as well as Klingensmith [32, 33] (Table 1).

Concerning adhesions, Swank *et al.* [35] found a much higher incidence (96%) at diagnostic laparoscopy, however this was in a patient population mostly having undergone previous surgery (commonly appendectomy, ovary surgery, hysterectomy, bowel and stomach resection, splenectomy and cholecystectomy.) This difference in previous surgery may be held responsible for the high incidence of adhesion in the aforementioned study. It becomes clear that adhesion incidences found at diagnostic laparoscopy may vary considerably.

Table 1. Findings during diagnostic laparoscopy

	Klingensmith *et. al.* (1996) [33]	Salky and Edye (1998) [32]	Onders and Mittendorf (2003) [30]	Swank *et. al.* (2003) [50]	Paajanen *et. al.* (2005) [6]
Number of patients	34	265	70	340	72
Pathology*					
Adhesions	58%	26%	56%	96%	85%
None	30%	24%	14%	2%	8%
Hernia	9%	2%	19%	1%	1.4%
Abnormal appendix	3%	26%	16%	1%	1.4%
Endometriosis	6%	3%	4%	–	–
Abnormal gallbladder	3%	2%	3%	2%	–
Miscellaneous	6%	20%	–	2%	5.5% **

*Some patients had more than one finding **unspecified gynecological disorders

Therapeutic value of diagnostic laparoscopy

It is suggested that even if no pathology, besides adhesions, is found, diagnostic laparoscopy alone may improve pain in 32% of patients [33]. Swank *et al.* supplied definite proof from a double-blinded, randomized controlled trial comparing laparoscopic adhesiolysis and diagnostic laparoscopy. Of the control group of 48 patients, having undergone only diagnostic laparoscopy, 42 percent reported improvement of pain at 12 months follow-up (Table 2) [35]. Hypothetically, the beneficial effect of diagnostic laparoscopy could be a result of peritoneal distension, caused by the pneumoperitoneum; on the other hand a placebo effect cannot be ruled out [35, 36].

Table 2. Outcome of adhesiolysis in patients with CAP for no other cause than adhesions

Author (year) [Ref.]	N	Cured/ improved	Unchanged/ worse	No response	Follow-up (months)	Method
Chan (1985) [51]	43	28 (65.1%)	14 (32.5%)	1 (2.4%)	minimum, 6	laparoscopy
Jung (1986) [52]	27	16 (59%)	11 (41%)	–	unknown	laparotomy
Mecke (1988) [29]	52	23 (44%)	16 (31%)	13 (25%)	6	laparoscopy
Sutton (1990) [22]	65	53 (82%)	10 (15%)	2 (3%)	1-5 yr	laparoscopy
Steege (1991) [21]	30	19 (63%)	11 (37%)	–	6-12 (mean, 8.2)	combined*
Kolmorgen (1991) [18]	153	58 (38%)	42 27%)	54 (35%)	12-96	laparoscopy
Peters (1992) [53]	24	11 (46%)	13 (54%)	–	9-12	laparotomy
Tschudi (1993) [23]	23	15 (65%)	4 (17%)	4 (17%)	5-36 (mean, 18.3)	laparoscopy
Howard (1994) [54]	11	9 (82%)	–	2 (18%)	Mean 10.7 ± 3.8	laparoscopy
Freys (1994) [12]	58	46 (80%)	12 (20%)	–	≤30	laparoscopy
Francois (1994) [43]	35	28 (80%)	5 (14%)	2 (6%)	22 ± 4	laparoscopy
Wipfli-Funke (1995) [24]	105	63 (60%)	35 (33%)	7 (7%)	6	laparoscopy
Mueller (1995) [11]	45	30 (67%)	6 (13%)	9 (20%)	6-36 (median, 10)	laparoscopy
Saravelos (1995) [20]	123	82 (67%)	41 (33%)	–	2-53 (mean, 14)	combined *
Hallfeldt (1995) [55]	16	14 (87%)	2 (13%)	–	4-18	laparoscopy
Miller (1996) [56]	19	16 (84%)	3 (16%)	–	mean, 18	laparoscopy
Nezhat (1996) [57]	48	22 (46%)	24 (50%)	2 (4%)	≤60	laparoscopy
Klingensmith (1996) [33]	19	14 (75%)	5 (25%)	–	3	laparoscopy
Lavonius (1999) [19]	24	17 (71%)	5 (21%)	2 (8%)	4-43	laparoscopy
Nezhat (2000) [58]	48	32 (67%)	16 (33%)	–	2-5 yr	laparoscopy
Schietroma (2001) [59]	45	34 (75%)	7 (16%)	4 (9%)	12-41 (mean, 18.3)	laparoscopy
Schmidbauer (2001) [60]	44	37 (84%)	7 (16%)	–	4-18 (mean, 12)	laparoscopy
Swank (2003) [40]	200	148 (74%)	52 (26%)	–	3	laparoscopy
Swank (2003) [35]	100	22 (43%)	30 (57%)	–	12	laparoscopy
Paajanen *et al.* (2005) [6]	72	57 (79%)	15 (21%)		44	laparoscopy

*combined = both laparoscopic and open adhesiolysis

Laparoscopic adhesiolysis

Adhesiolysis is frequently an integral part of open and minimally invasive abdominal surgery and adhesions can complicate subsequent laparoscopic interventions. Ballesta Lopez *et al.* [37] studied 240 patients who underwent laparoscopic procedures after at least one previous laparotomy resulting in 1.5% conversions to open surgery and a 4% complication rate. Surgery in a previously opened abdomen is described as being difficult. Fathy *et al.* confirmed that adhesions were the most common cause of conversions (57 patients; 2.9%)

in 2000 patients undergoing laparoscopic cholecystectomy [38]. Karayiannakis *et al.* pointed out that previous abdominal surgery is not a contraindication for laparoscopic cholecystectomy per se. However, seventy-eight percent of patients required adhesiolysis and conversion to open surgery was required in 19%. Alternatively, laparoscopic cholecystectomy was converted to an open approach in a virgin abdomen in only 5% of patients [39].

Completeness of adhesiolysis

Swank *et al.* prospectively analysed predictive factors on the results of laparoscopic adhesiolysis for chronic abdominal pain. In this series of 200 consecutive patients with only adhesions as a likely cause of their pain, a complete adhesiolysis was intended, which was possible in 82% of patients. Three months after laparoscopic adhesiolysis 74% of patients were pain free or suffered from less pain, 22% of patients experienced no change in abdominal pain and 4% of patients reported an increase in abdominal pain. Pain relief was found to be unrelated to the completeness of laparoscopic adhesiolysis. Older age, and female gender appeared to be individual factors associated with disappointing pain relief [40]. As mentioned previously, results by Swank *et al.* proved that 42% of patients with adhesions experienced pain relief after sham laparoscopic adhesiolysis (diagnostic laparoscopy) in which identified adhesions were not lysed [35]. On the other hand, Onders and Mittendorf recommend complete adhesolysis if adhesions are the likely etiology of chronic pain. However, their paper did not mention the classification or severity of adhesions, and their technique and results suggest less severe ("friendly") adhesions [30].

Adhesiolysis as treatment for chronic abdominal pain (CAP)

The success rate of laparoscopic adhesiolysis for bowel obstruction, chronic pain and infertility varied from 38 to 87% of patients in 24 publications (Table 2). The number of patients studied varied between 11 and 200 and included a range of follow-ups (at least 3 months, at most 5 years). Abdominal pain recurrence rates of up to 26% are described.

Swank *et al.* performed a prospective study in 224 patients with chronic abdominal pain looking specifically for factors influencing the result of laparoscopic adhesiolysis such as completeness of adhesiolysis, gender and age. After 3 months, 74% of patients were pain free or had less pain. As mentioned earlier, it emerged that younger patients were more likely to become pain free, whereas after previous gynecological operations women were significantly less pain free than men after all other types of intervention. Results of adhesiolysis

were unrelated to the duration of pain, the number and type of previous operations, the technique and (in)completeness of adhesiolysis [40]. In 11 patients (5.5%) bowel perforations occurred during laparoscopic adhesiolysis, leading to laparotomy in all patients. This contributed significantly to the disappointing results in the aforementioned study (unaffected pain or increased pain).

Onders and Mittendorf showed a long-term success rate in 71% of 45 patients with chronic abdominal pain after complete adhesiolysis. Initially, these patients were 100% satisfied. After 6 months however, 29% of patients after adhesiolysis suffered from recurrent abdominal pain. A subsequent follow-up (mean period 129 weeks) showed no further recurrences. The authors hypothesized that adhesion recurrence and de-novo adhesion formation cause recurrent abdominal pain. A placebo effect and the subsequent wearing off was also postulated as a cause for recurrent pain [30].

In the double-blinded, randomized controlled trial mentioned earlier, 116 patients suffering from CAP, likely to have been caused by prior abdominal surgery, and present for at least half a year, were enrolled. All patients underwent diagnostic laparoscopy, and in the case of evident adhesions only, randomized for treatment (adhesiolysis) or continuation of diagnostic laparoscopy. For the period of one year, patients remained unaware of the group they had been randomized for. After 6 months, 52 patients treated by adhesiolysis reported an improvement in the pain (57% of patients), had a reduced VAS pain score (57 versus 38), a reduced MOS SF-36 score, required less analgetics, and felt that their QOL had signifcantly improved. Results at one-year follow-up were no different than results after six months. None of the results in the treatment group were significantly superior to the patient having undergone diagnostic laparoscopy except for the number of complications as is illustrated in Table 2. Complications after laparoscopic adhesiolysis in this study were comparable to those published elsewhere (Table 2) [35].

A recent, prospective study by Pajaanen *et al.* in 72 patients after diagnostic laparoscopy and laparoscopic adhesiolysis reported favourable results (less pain and free of pain) in 79% (n=57 patients) after a mean follow-up of 44 months. In six patients no adhesions were found. It is noteworthy that the diagnostic laparoscopies revealed one umbilical hernia, one chronically inflammated veriform appendix and four patients suffering from gynecological disease not diagnosed earlier (Table 1). The overall complication rate in the aforementioned study was reported to be 13.8% [6].

Complications of laparoscopic adhesiolysis

During laparoscopic adhesiolysis several complications can occur (Table 3). Generally, wound hematoma, hernia and infection are considered to be minor complications, whereas bleeding in the abdominal cavity and bowel perforation are classified as major complications [41]. The incidence of intestinal perforations which occurs during laparoscopic procedures for symptomatic adhesions is reported to occur in 5% to even more than 25% of patients [42-46].

Bowel injuries not recognized at the time of surgery can result from needle introduction (a 0,05% - 0,2% risk according to Bonjer *et al.* [47]), from trocar puncture or from adhesiolysis. The symptoms of peritonitis after a direct perforation are usually clear within 1 or 2 days. Thermal damage to the bowel may be another cause for bowel perforation, in which cases the clinical signs of perforation are usually seen after 4 days [45]. Previous operations (single as well as multiple) are an important factor causing complications during laparoscopic adhesiolysis, and in difficult cases with progressive risk of complications it is better to accept an incomplete adhesiolysis and wait for the possible relief of pain, rather than continue adhesiolysis risking a perforation.
In the end, the goal is an asymptomatic patient instead of an abdominal cavity without adhesions [41].

Regrowth

In 24 patients a second-look procedure was performed as part of a follow-up study of 368 patients after laparoscopic adhesiolysis as treatment of CAP [48]. The indication for second-look laparoscopy was recurrent pain after a mean period of 16 months. New adhesions between the organs had formed and the differences in severity, incidence and extent of adhesions were not significant. A significant reduction of adhesions, however, remained between the organs and the abdominal wall. The incidence, extent and severity of abdominal adhesions was found to be permanently reduced after laparoscopic adhesiolysis, despite de-novo adhesions in 5 patients (20%). Interestingly, three patients were totally free of abdominal adhesions at second-look laparoscopy [48]. It is generally postulated that adhesion formation is progressive the more laparotomies are performed [5, 49]; unfortunately no data are available on the adhesion reformation after adhesiolysis by laparotomy.

Table 3. Complications of laparoscopic adhesiolysis and diagnostic laparoscopy for CAP

Author (year) [Ref.]	N	Pain relief (% of patients)	Follow-up (months)	Complications (%)$	Indication
Shayani *et al.* (2002) [61]	20	78	11	20 major	CAP*; bowel obstruction
Swank *et al.* (2003) [40]	200	74	3	5.5	CAP
Swank *et al.* (2003) [35] **	52	57	12	5	CAP
	48	42	12	–	CAP, diagnostic laparoscopy only
Klingensmith *et al.*	18	73	3	5 minor	CAP
(1996) [33]	9	88	3	–	CAP, diagnostic laparoscopy only
Nezhat *et al.* (2000) [58]	48	64	6–12	10 major	CAP
			44	2 major	CAP

$ major complications: enterotomy, cystotomy; *CAP = Chronic abdominal pain; **RCT

Conclusion

Chronic abdominal pain can be caused by postoperative abdominal adhesions, whether it is by the nerve fibers in the adhesions itself, by traction to the peritoneum or organs or a combination of both, whereas changes in the central nervous system should be considered to play a role as well. All in all the phenomenon is highly complicated and almost always there are several causes to consider. Once other causes than adhesions have been ruled out, (laparoscopic) adhesiolysis is commonly attempted in order to free patients of chronic abdominal pain.

Our randomized study, performed by Swank *et al.*, for the first time described that laparoscopic adhesiolysis was of equal benefit to patients as was diagnostic laparoscopy. Serious complications (i.e. bowel perforations) as a result of laparoscopic adhesiolysis were found to occur in as many as 5% of patients (35). From the results of the randomized study, abolition of laparoscopic adhesiolysis as treatment of choice for chronic abdominal pain is recommended, since adhesiolysis and diagnostic laparoscopy patients differed only in complication rates, not in benefit. All in all the best treatment of adhesions is their prevention.

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5

Adhesion prevention during laparotomy: long-term follow-up of a randomized clinical trial

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Abstract

Objective: The objective of the study was to determine the long-term effect of the use of a Hyaluronic Acid-Carboxymethylcellulose membrane (Seprafilm®) on the incidence of adhesions and subsequent small bowel obstruction and chronic abdominal complaints after colorectal surgery (Hartmann's procedure).
Background: Adhesions occur frequently after abdominal surgery and are the most common cause of bowel obstruction, chronic abdominal pain and infertility. The risk for adhesion related readmission in the first ten years after colorectal surgery is as high as 30%. To reduce the formation of adhesions, a mechanical barrier composed of hyaluronic acid and carboxymethylcellulose was developed, to prevent adherence of tissues after abdominal surgery. Long-term results concerning the incidence of small bowel obstruction and chronic abdominal pain are lacking.
Methods: Between April 1996 and September 1998, 71 patients requiring Hartmann's procedure for sigmoid diverticulitis or obstructed rectosigmoid were randomized to either intraperitoneal placement of Seprafilm® under the midline and in the pelvis or as a control. Direct visual evaluation of the incidence and severity of adhesions was performed laparoscopically in 42 patients at second-stage surgery for restoration of the continuity of the colon. The results of this study were published in 2002. In 2006, the patients' general practitioners were interviewed by means of a questionnaire concerning their patients' health. The patients who were still alive were interviewed and asked to fill out two questionnaires concerning pain and quality of life (VAS-pain score, EQ-5D and SF-36). In 2009 the medical records of the patients were evaluated for adhesion-related hospital re-admissions.
Results: Of the 42 evaluated patients, 35 (16 in the Seprafilm® group, 19 in the control group) could be enrolled in the long-term follow-up. Median follow-up was 126 months (range 41-148) for the Seprafilm®-group and 128 months (range 49-149) months for the control-group. Incidence of chronic (three months or longer existing) abdominal complaints was significantly lower in the Seprafilm® group compared to controls (35.3% vs 77.8% respectively; p=0.018). Incidence of small bowel obstruction showed no significant difference in favour of the Seprafilm® group; no small bowel obstructions occurred in the Seprafilm® group, whereas in the control group two cases of small bowel obstruction were found to have occurred. Evaluation of the quality of life questionnaires did not reveal significant differences between the two groups.
Conclusion: In Hartmann's procedure, Seprafilm®-placement does not provide protection against small bowel obstruction. Incidence of chronic abdominal complaints is significantly lower after use of Seprafilm®.

Introduction

Abdominal adhesions represent an almost inevitable complication of abdominal surgery; nearly every surgical intervention in the abdomen incites the formation of adhesions [1-8]. Although the majority of patients with intra abdominal adhesions experience no or very little symptoms, abdominal adhesions are a well known cause of small-bowel obstruction, chronic abdominal pain and infertility [3, 8-11]. Even though all of these complications may occur shortly after the operation, the manifestation of these complaints several years after operation is no exception [11]. Besides these serious health problems, adhesions may complicate re-operations [8, 12-14]. After colorectal surgery patients have a 30% readmission-risk due to adhesion formation [14, 15]. Given the great burden of abdominal adhesions, it is desirable to prevent the formation of adhesions. Seprafilm® is a bioresorbable membrane developed to prevent the formation of adhesions. It consists of hyaluronic acid and carboxymethylcellulose and acts as a mechanical barrier between two damaged surfaces during the period of peritoneal regeneration [13, 16-19].

In 2002 our research group published the results of a prospective randomized controlled multicentre trial, in which patients were enrolled between 1996 and 1998 [5]. The article discussed the effectiveness of Seprafilm® in preventing abdominal adhesions. Hartmann's procedure with secondary restoration of the continuity of the bowel was chosen to evaluate the effectiveness of the membrane. Seprafilm® was placed under the midline incision and in the pelvic area on the rectal stump. At the time of restorating bowel continuity adhesions were evaluated. The results indicated that the incidence of adhesions did not differ between the Seprafilm®- and the control group. However, the severity of the adhesions was significantly reduced in the Seprafilm®-group.
Only limited data concerning the long-term effectiveness of barriers such as Seprafilm® are available, and most of these studies do not exceed a follow-up period of more than five years [9, 20, 21].

The aim of the present study was to collect data to determine the effectiveness of Seprafilm® in reducing small-bowel obstruction and chronic abdominal pain in the long term.

Methods

In the original study, conducted between April 1996 and September 1998, 71 patients requiring Hartmann's procedure for sigmoid diverticulitis or obstructed rectosigmoid signed an informed consent and were randomized to either intraperitoneal placement of Seprafilm® under the midline and in the pelvis during laparotomy, or as a control. Direct visual evaluation of the incidence and severity of adhesions was performed laparoscopically in 42 patients at second-stage surgery for restoration of the continuity of the colon (Zühlke classification) [5]. The results of this study were published in 2002 [5].

In 2006 the general practitioners of the enrolled patients were contacted and asked to fill out a questionnaire about their patients. The questionnaire contained questions about incidence of chronic abdominal complaints defined as pain, nausea and constipation, as well as questions about wound healing and small-bowel obstruction. Additionally they were asked to note whether their patient was still alive and if not when the patient had died. All patients who participated in the randomized controlled trial 11-13 years ago, and who were still alive and willing to participate, were visited and were asked to fill out a survey focussed on abdominal complaints after Hartmann's procedure and second stage surgery. They were also asked to fill out a Visual Analogue Scale (VAS) and two quality of life questionnaires, the EuroQol-5Dimensions (EQ-5D) and the Medical Outcome Study Short Form-36 (SF36).

The VAS-score which was added, visualized the pain around the midline laparotomy scar, the pain around the stoma-scar and pain elsewhere in the abdomen. The EQ-5D is based on five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). Within each dimension three answers were possible (no problems, some problems and extreme problems). The combination of these five questions results in 243 (35) possible states of health. By giving each question a value and using estimated regression coefficients, each possible health state is valued according to its desirability, where a score of 1 represents perfect health and a score of 0 represents death [22, 23]. The SF-36 is a questionnaire that measures the physical and mental situation based on 36 questions. It includes eight items that measure physical functioning, role limitations due to physical health problems, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health. Possible scores per item ranged from 0 to 100; with higher scores indicating better quality of life [24, 25].

In 2009 the medical records of all patients were reviewed to record hospital re-admissions due to adhesion-related problems.

The results of the questionnaire, the VAS-score, the EQ-5D and the SF-36 were evaluated and calculated with SPSS (Chicago, IL) software. The differences in outcome were compared between the Seprafilm®- and the control-group.

Results

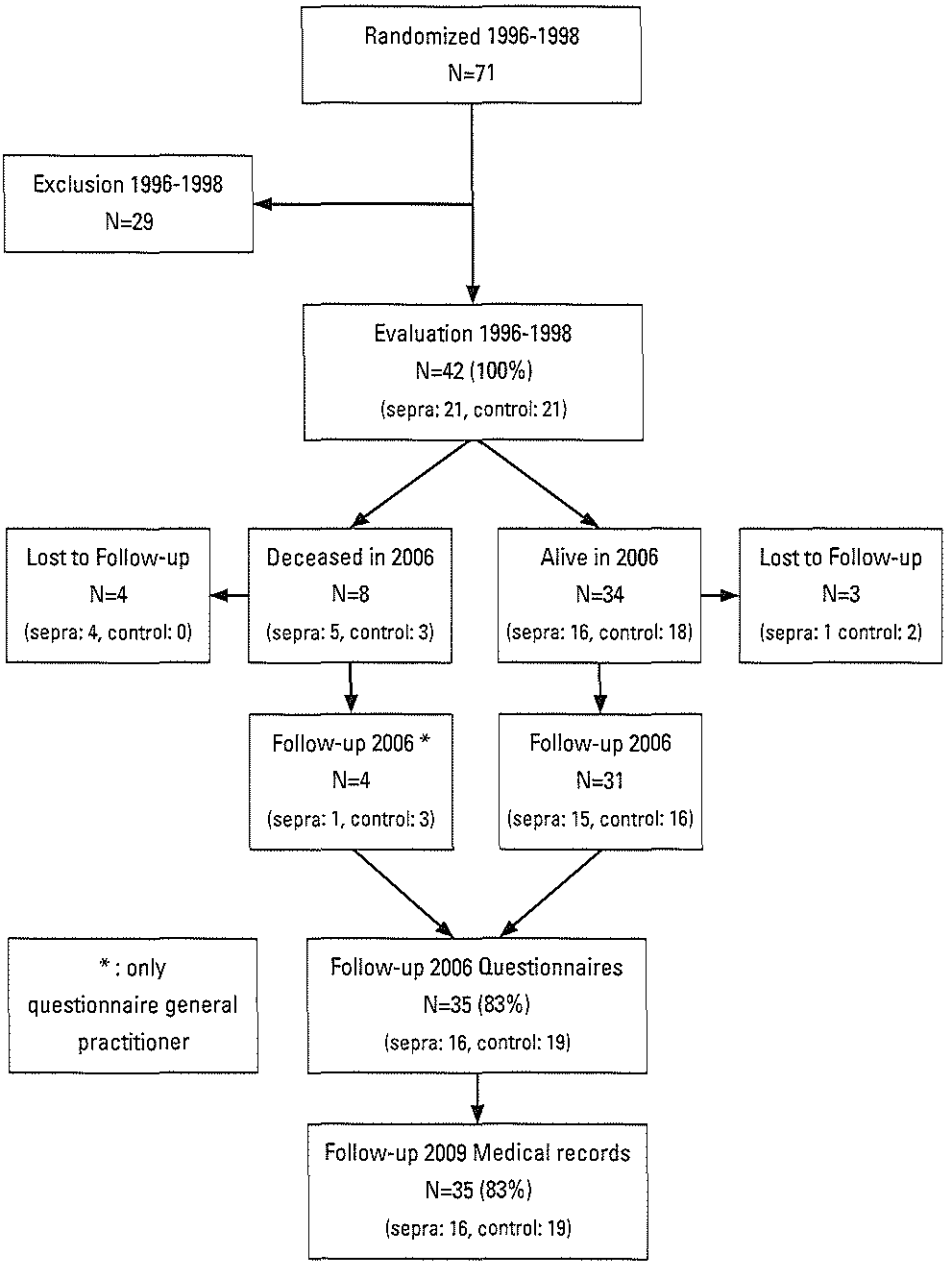
Patients

The 42 patients who were evaluated in the original study in 2002 were all found eligible for participation in the long-term follow-up. Of these 42 patients, eight patients had died of which four were lost to follow-up, either because their general practitioners could not be traced or because their charts could not be retrieved. The questionnaires filled out by the patient's general practitioners could be evaluated in four of the deceased patients (one from the Seprafilm® group and three from the control group). Of the 34 patients still alive, three patients could either not be traced (n=1) or refused to cooperate (n=2) and were therefore declared lost to follow-up. Thus, of the 42 patients included in our study, seven were lost to follow-up, and a total of 35 patients (83%) remained for evaluation, 16 in the Seprafilm® group and 19 in the control group (Figure 1). Baseline characteristics (age, follow-up time, sex) did not differ significantly between the two groups. (Table 1).

Table 1. Baseline characteristics

		Seprafilm® N=16	Control N=19	p-value
Sex	Male (%)	10 (64.7)	9 (44.4)	NS
	Female (%)	6 (35.3)	10 (55.6)	NS
Age (range)		66.91 (48-90)	67.76 (47-90)	NS
Follow-up months (range)		96 (11-118)	98 (19-119)	NS

Figure 1. Flow chart



Abdominal complaints

Analysis of the questionnaire revealed that abdominal complaints (pain, nausea, constipation) occurred significantly less often in the Seprafilm® group than in the control group: six patients (35%) in the Seprafilm® group experienced at least one episode of abdominal complaints of three months or longer, while 14 patients (78%) in the control group went through at least one episode of abdominal complaints of three months or longer (p=0.018).
Of the abdominal complaints constipation was found to be the major complaint: In the Seprafilm® group, two patients (12%) had experienced constipation for at least three months or longer, versus twelve patients (67%) in the control group (p=0.002). As for infections, hernias and abscesses no significant difference was obtained between the two groups.

Hospital re-admissions

Analysis of the medical records, including X-rays of the abdomen, revealed two readmissions due to small bowel obstruction in the control group versus nil in the Seprafilm® group. This was not a significant difference. None of these two patients required re-operation and patients were discharged from the hospital within two days.

VAS

The VAS-score in both groups, visualising the experienced pain in the scar, stoma-scar and elsewhere in the abdomen, revealed no significant difference (Table 2). It should be noted that only the living patients could fill out the VAS-form, therefore the results of the VAS-score contain the answers of 31 patients.

Table 2. VAS-score

Outcome of VAS mean±sd	**Seprafilm® N= 15**	**Control N= 16**	**95% CI mean difference**	**p-value**
Scar pain	0.23 ±0.45	0.29 ±0.60	-0.46 - 0.34	0.76
AP scar pain	0.25 ±0.25	1.08 ±2.05	-1.94 - 0.29	0.14
Pain elsewhere in abdomen	0.93 ±1.81	1.27 ±1.63	-1.60 - 0.92	0.58

EQ-5D

After analyzing the EQ-5D results, the Dutch EQ-5D Tariff for the Seprafilm® group and the control group was 0.85 ± 0.07 and 0.81 ± 0.05 respectively. The difference between the two tariffs, namely 0.04 (-1.39 – 0.21 95% CI of the mean difference), was found not to be significant.

None of the differences between the 5 dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) proved to be significant as well (Table 3).

Table 3. EQ5D

Outcome of EQ5D	**Seprafilm®**	**Control**	**95% CI mean difference**	**p-value**
mean±sd	**N= 15**	**N= 16**		
Mobility	1.33	1.56	-0.65 – 0.19	0.27
Selfcare	1.07	1	-0.08 – 0.21	0.33
Usual activities	1.27	1.44	-0.58 – 0.24	0.4
Pain/Discomfort	1.40	1.50	-0.57 – 0.37	0.66
Anxiety/Depression	1.20	1.25	-0.37 – 0.27	0.75

The last question of the EQ-5D survey was to put a mark on a line from 0 to 100 to symbolize the patient's current health status; nil representing death and 100 representing perfect health. No significant difference between both groups was found (76.33 ± 16.42 in the Seprafilm® group vs 71.0 ± 13.49 in the control group, p=0.53).

SF-36

All eight items of the SF-36 survey (physical functioning, role limitations due to physical health problems, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems and mental health) were analyzed and no significant differences between the two groups were found (Table 4).

Table 4. SF36

Outcome of SF36	Seprafilm® N= 15	Control N= 16	95% CI mean difference difference	p-value
1	15.25	14.07	-1.12 – 2.22	0.58
2	85.00	75.31	-10.59 – 29.97	0.33
3	45.83	46.09	-8.03 – 7.51	0.95
4	76.67	65.63	-21.45 – 43.53	0.49
5	88.89	89.58	-21.43 – 20.04	0.95
6	81.00	68.43	-2.13 – 27.26	0.09
7	85.33	85.50	-11.86 – 11.53	0.97
8	76.87	76.94	-17.62 – 17.48	0.99
9	70.33	60.56	-12.32 – 31.86	0.37
10	48.24	43.87	-5.37 – 14.12	0.36
11	51.62	51.82	-5.86 – 5.46	0.94

Discussion

The follow-up of our randomized controlled trial provides evidence that Seprafilm® is not superior to the control group with regard to incidence of small bowel obstruction in the long term. The incidence, severity and location of adhesions has not frequently been reported, because assessment of postoperative development of adhesions requires invasive techniques. The set-up of the former Seprafilm® trial was appropriate for the assessment of the development of adhesions, given the two stage character of Hartmann's procedure [5, 26]. In the trial significant less severe adhesions in the Seprafilm® group were found. Follow-up of the peroperative placement of Seprafilm® and the assessment of its effect on postoperative complications has been discribed previously [9, 21]. However, mean follow-up did not exceed five years in these studies, whereas the present study has a mean follow-up of over 10 years which provides necessary additional information, especially because complications such as small bowel obstruction sometimes manifest not sooner than 10 years after surgery [5, 11].

Fazio *et al.* found in their follow-up that Seprafilm® significantly reduces the rate of small bowel obstruction requiring re-operation, but it does not lower the overall incidence of small bowel obstruction [9]. Our results, during follow-up twice as long, are in concordance with these findings: two patients in the control group were readmitted to the hospital due to adhesions, both did not need surgical intervention. We found significantly less postoperative chronic abdominal complaints and problems with defecation, especially constipation, in the Seprafilm® group compared with the control group.

One could hypothesize that less severe adhesions provide more mobility for the gastro-intestinal tract, although due to the set-up of our follow-up we were not able to test this hypothesis.
Considering the results from the questionnaire it is noticed that the VAS did not score significantly higher in the control group, whereas the questionnaire reveals significantly more abdominal complaints. All abdominal complaints were scored as abdominal complaints (nausea, pain, constipation). The incongruity between the VAS and the questionnaire may be explained by the fact that in the questionnaire not only pain, but also nausea and constipation were recorded. Furthermore, a VAS-score is an instrument perfectly capable of measuring pain in the recent past, whereas most of the complaints involving the abdomen in our patients occurred in the first postoperative year.

The use of the EQ-5D and SF-36 surveys for measuring and comparing the difference in quality of life between the two groups in our follow-up is unconventional, because in the Seprafilm® trial these forms were not used and hence these outcomes could not be compared to a "baseline". Nevertheless, theoretically, patients suffering from repeated bowel obstruction could experience a reduced quality of life compared to healthy individuals, which in our opinion justified the use of these forms. After thorough analysis of the two surveys, no significant difference was found in difference in quality of life. Given the fact that the two groups could not be distinguished by the EQ-5D nor the SF-36, we conclude that the application of Seprafilm® does not influence the quality of life.

It is remarkable that, even though the control group revealed a significantly higher incidence of abdominal complaints and defecation problems, the EQ-5D and SF-36 were not influenced. A reason for these outcomes might be that both surveys concentrate mainly on the last four actual weeks of a patient's life. It leaves ailments before these four weeks out of consideration.
Our previously published study showed that the application of Seprafilm® in patients undergoing Hartmann's procedure was responsible for a significant reduction in severity of formation of adhesions. The results of the present study indicate that Seprafilm® does not reduce the incidence of small bowel obstruction, which leads to the conclusion that it is not the severity of the adhesion but possibly rather the location of the adhesion which determines whether small bowel obstruction will occur.

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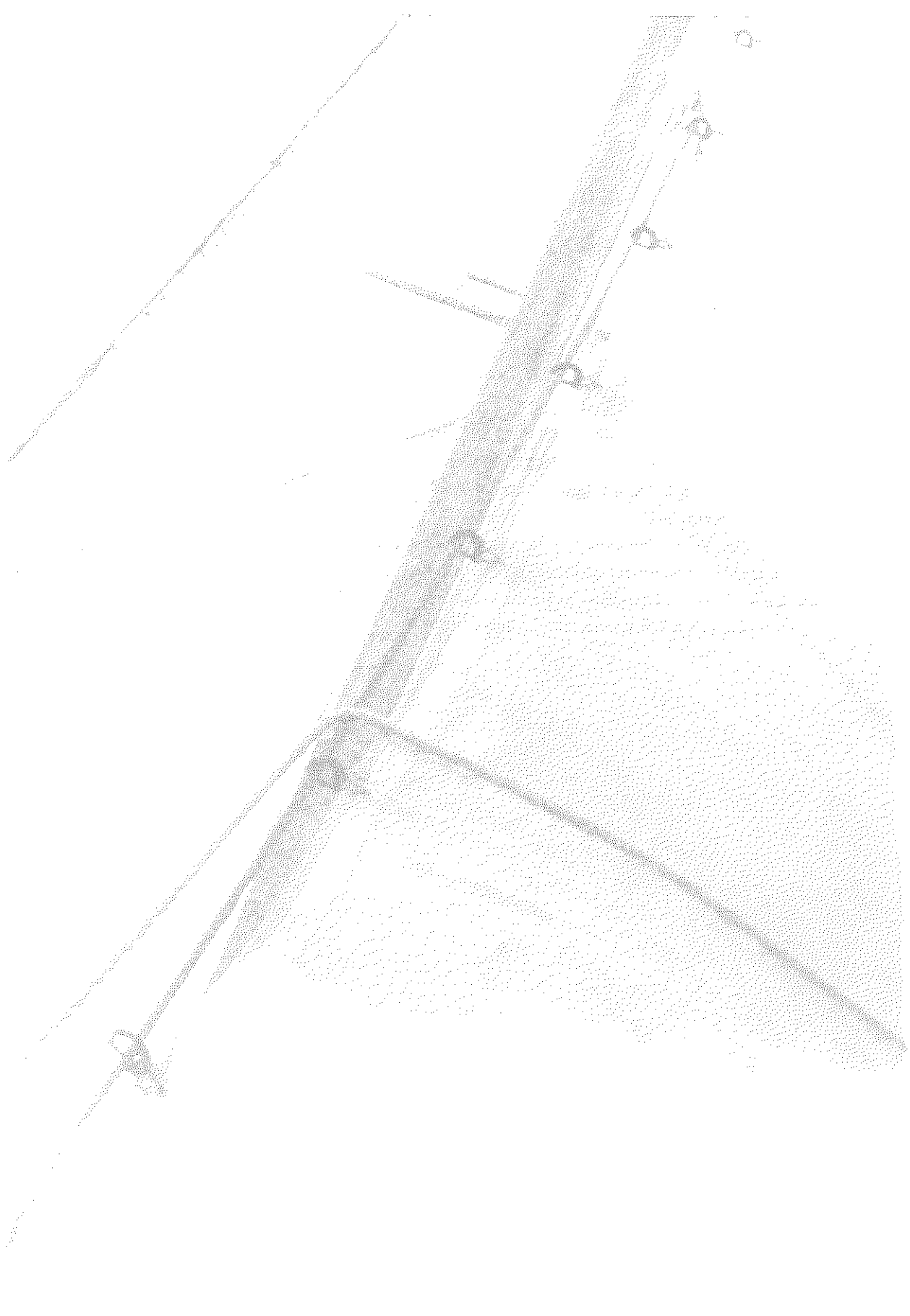


6

Surgical trauma to the peritoneum leads to a locally and systemically transient increase in the levels of hydrogen peroxide

JBC van der Wal, W Sluiter, NAP Komen, J Nieuwenhuizen,
J Jeekel, JF Lange

Abstract

Objective: The objective of this study was to determine whether surgical trauma causes detectable changes in levels of reactive oxygen species (ROS) in vivo.
Background: Polymorphonuclear cells (PMN) play an important role in the healing process after damage of the peritoneum. Indirect evidence shows that ROS produced by PMN are sequelae of the inflammatory reaction caused by surgical trauma. Besides beneficial effects, such as destruction of invading micro-organisms and degradation of damaged tissue, the oxidative potential can result in additional (peritoneal) tissue destruction. The amount of peritoneal damage is positively correlated to postoperative adhesion formation as well as to local tumour recurrence, and various studies have shown that administrating ROS scavengers leads to less adhesion-formation and less tumour recurrence. Surprisingly, the actual levels of the various ROS in vivo have never been reported to exclude the possibility that the beneficial effect of ROS scavengers is an intrinsic effect of these reagents.
Methods: To determine baseline values, before surgical trauma, the peritoneal cavity of 5 animals was flushed with saline and lavage fluid and plasma was collected. After adding the antioxidant butylated hydroxytoluene to prevent any artificial increase in the levels of lipid peroxides (LPO), the levels of hydrogen peroxide (H_2O_2) and LPO were determined spectrophotometrically using the FOX2 assay. Next, animals were operated according to our previously optimised adhesion model and randomized to receive a cocktail of ROS-scavengers Superoxide Dismutase (SOD) and Catalase (CAT) postoperatively or saline, and immediately thereafter and at 5, 12 and 24 hours postoperatively peritoneal lavage was performed and blood samples were taken. H_2O_2 and LPO levels were determined in plasma and lavage fluid.
Results: For H_2O_2 preoperative values in lavage fluid and plasma were 0.97 ± 1.33 nmol/mL and 0.28± 0.39 nmol/mL respectively; as for LPO baseline values were 1.57 ± 1.89 nmol/mL and 1.02 ± 1.44 nmol/mL respectively. Abdominal surgery led to a significant increase in the level of H_2O_2 at between 5 and12 hours post surgery locally and systemically, but the levels of LPO did not change significantly during the observation period of 24 hours.
Animals which had received SOD and CAT had significantly lower levels of H_2O_2 in lavage and plasma compared to operated animals 5 hours after surgery (P=0.011 and P=0.039). At 12 hours after surgery, only the level of H_2O_2 in lavage is lower when compared to untreated animals (P = 0.013). The levels of LPO did not change by the administration of SOD and CAT.

Conclusion: This experiment shows that surgical trauma to the peritoneal cavity led to transiently increased levels of H_2O_2 in lavage fluid and plasma, while the levels of LPO were not affected. The administration of the anti-oxidant enzymes SOD and CAT decreased the H_2O_2 levels that did not return to baseline values until 24 hours postoperatively. This may indicate that previously found beneficial effect of SOD and CAT of preventing adhesion formation after surgical trauma to the peritoneal cavity involves the inactivation of H_2O_2 that is generated shortly after and by surgery.

Introduction

During abdominal surgery, trauma to the peritoneum is inevitable. The degree of this trauma correlates with the degree of postoperative adhesion formation. In case of a cancer-related operation, it is even related to the degree of local recurrence [1-4]. The peritoneum is a delicate serous membrane consisting of a mesothelial monolayer and a submesothelial layer of extracellular matrix. The mesothelial monolayer is loosely attached to the basement membrane and even the slightest trauma causes a denudation of the extracellular matrix, hereby initiating an inflammatory woundhealing response [5-7]. Resident cells, as well as the damaged mesothelial cells and invading inflammatory cells in the injured area, produce cellular mediators, leading to an increased vascular permeability and migration of polymorphonuclear granulocytes (PMN), monocytes and lymphocytes to the site of inflammation [7, 8]. In previous experiments we were able to detect an increased amount of neutrophils up to 96 hours after inducing peritoneal trauma [9]. In the early postoperative inflammatory reaction, PMN are responsible for clearing dead tissue and invading organisms by producing and releasing reactive oxygen species (ROS); especially superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) are formed. Another well known ROS is lipid peroxide (LPO), which is a downstream product of the oxidation of fatty acids by H_2O_2. Despite their beneficial effect, the oxidative potential can result in additional (peritoneal) tissue destruction and possibly underlies the increased adhesion formation and local tumour recurrence [10, 11].

We have previously shown that scavenging these ROS with superoxide dismutase (SOD) and Catalase leads to a decrease in adhesion formation and tumour cell adhesion [9, 12-14]. However, the actual levels of ROS in vivo with and without administration of scavengers have not been reported. This study was conducted to determine the kinetics of ROS levels, and in particular H_2O_2 and LPO, and to find out if SOD and CAT indeed could affect ROS levels in vivo.

Materials and Methods

Animals

Adult female inbred WAG/Rij rats weighing 145 to 190 g were obtained from Harlan-CPB, Austerlitz, the Netherlands. The rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (temperature 20–24°C, relative humidity 50–60%, 12 h light and 12 h

dark cycles), fed with standard rat food and water *ad libitum* and quarantined in our university animal facilities for at least 2 days before use. The experimental protocol was approved by the Animal Experiments Committee under the national Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC.

Antioxidant enzymes

SOD (5000 U/mg) and Catalase (2350 U/mg) from Roche Diagnostics BV, Almere, the Netherlands, were dissolved in phosphate-buffered saline (PBS; pH 7.4) to the appropriate concentration and kept on ice to scavenge the ROS *in vivo*. Per animal a cocktail of 2500 U of SOD and 5000 U of Catalase dissolved in 1.5 mL of PBS were administered during operation to the peritoneal cavity

Surgical Procedure

All animals (except for the animals which were used to determine baseline values of ROS) were operated according to our previously optimized reproducible adhesion-model which leads to an adhesion-rate of 60-70% in controls [9]. In brief: under isoflurane anesthesia and aseptic conditions a laparotomy was performed using a midline incision of 5 cm. A small oval, 2 cm in length and 0.5 cm in width and of 0.3 cm thickness, was then excised on both lateral sides of the parietal peritoneum, simulating surgical trauma, after which the wound was closed using 3 Safyl 5.0 sutures. The abdomen was closed in two layers using Safyl 5.0 continuously.

Measurement of hydrogen peroxide and lipid peroxides

Plasma was separated from heparinized blood obtained by puncture of the abdominal aorta under isoflurane anesthesia, and supplemented with 4 mM butylated hydroxytoluene (BHT) to stop any further lipid peroxidation. The same procedure was performed on the samples obtained by peritoneal lavage. Using spectrophotometry, the concentration of H_2O_2 and LPO in the plasma was determined respectively after incubation in the absence or presence of 35 U/mL Catalase for two minutes at room temperature, by the ferric iron-dependent increase in absorbance of xylenol-orange at 560 nm (hydroperoxide apparent ε = 0.08904 $\mu M^{-1}cm^{-1}$, experimentally assessed using freshly-made reagents only and standard curves of t-butyl peroxide and cumene peroxide) essentially as described by Nourooz-Zadeh *et al.* [15] but using the sample treated by 0.9 mM Tris(2-carboxyethyl)phosphine to reduce any LPO to their respective alcohols as the reference [16].

Experimental design

Determining baseline values and effect of surgery on ROS levels

In order to determine preoperative-values of LPO and H_2O_2, the peritoneal cavity of 5 animals was flushed with 3 mL of PBS of which 1 mL was recovered. Blood samples were taken by left ventricle punction. After adding BHT to the samples they were kept on ice until further use. Next, 30 animals were operated according to the previously described model [9] and sacrificed immediately post surgery, and after 5, 6, 12 and 24 hours. Of all animals, blood samples were taken and peritoneal lavage fluid was obtained. BHT was added to all samples after which they were kept on ice until further use.

Effect of ROS scavengers after surgery

Twelve animals were operated and randomized to either receive a cocktail of the antioxidant enzymes SOD and CAT postoperatively or PBS as control, and sacrificed immediately post surgery and after 5, 12 and 24 hours respectively. Of all animals, blood samples were taken by left ventricle punction. Peritoneal lavage was performed by instilling 3 mL PBS intra-abdominally. The lavage fluid was recovered using a syringe. BHT was added to all samples after which the samples were stored on ice until further use.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 4 for Windows, GraphPad Sofware Inc., USA. Outcomes were compared using a one-tailed, unpaired t-test with Welch's correction, assuming non-equal variances. The criterion for significance was $P < 0.05$ for all comparisons.

Results

Effect of peritoneal-cavity surgery on local and systemic ROS levels

To find out if surgical trauma would lead to increased levels of H_2O_2 and LPO immediately and after some time post surgery, pre-operative levels of H_2O_2 and LPO in lavage fluid and plasma of 5 non-operated animals were determined. For H_2O_2 baseline concentrations in lavage fluid and plasma were 0.97 ± 1.33 nmol/mL and 0.28 ± 0.39 nmol/mL respectively, and for LPO 1.57 ± 1.89 nmol/mL and 1.02 ± 1.44 nmol/mL respectively.

Five hours after surgery onwards, the levels of H_2O_2 in lavage and plasma had increased to 3.70 ± 1.06 nmol/mL (P= 0.026) and 4.52 ± 1.97 nmol/mL (P= 0.038) respectively.
At 12 hours after surgery, the levels of H_2O_2 in lavage and plasma were 4.22 ± 1.22 nmol/mL (P = 0.031) and 2.08 ± 0.77 nmol/mL (P = 0.042) (Figure 1 and 2). After 24 hours, levels in lavage and plasma were returned to the normal pre-operative levels (Figure 1 and 2).

The levels of LPO in lavage and plasma did not change significantly during the observation period of 24 hours after surgery (Figure 3 and 4).

Effect of SOD and CAT on the ROS levels after surgery of the peritoneal cavity

To examine if antioxidant enzymes that prevent adhesion formation and tumour recurrence after surgery [9, 14] could affect the levels of ROS in vivo, SOD and CAT were administered locally during surgery.
As shown in figures 1 and 2, five hours after surgery H_2O_2 levels of SOD/CAT-treated animals in lavage and plasma had decreased to 0.351 ± 0.232 nmol/mL and 0.254 ± 0.254 nmol/mL, both not significantly lower when compared to baseline (P= 0.18 and P= 0.46).
When compared to the operated animals, the value both in lavage and in plasma was significantly lower (P= 0.011 and P= 0.039).
After 12 hours, H_2O_2 levels of SOD/CAT-treated animals in lavage and plasma were 0 nmol/mL and 1.200 ± 0.640 nmol/mL, both not significantly lower when compared to baseline. When compared to operated, untreated animals the level in lavage was significantly lower (P = 0.013) but not in plasma (P = 0.21). After 24 hours, H_2O_2 levels both in lavage and plasma returned to baseline levels.

Although the LPO levels in lavage fluid and plasma did not change after surgery, the administration of SOD/CAT to the peritoneal cavity led to a statistically significant decrease in plasma but not in lavage. At 5 hours after surgery and administration of SOD/CAT, levels of LPO did not change significantly in lavage (Fig.3) (0.792 ± 0.792 nmol/mL; N.S.), but in plasma decreased to 0.002 ± 0.000 nmol/mL (Fig. 4; P=0.031 compared to baseline). Twenty-four hours post-surgery values of LPO in lavage and plasma had returned to the normal level.

Figure 1. **Levels of H_2O_2 in peritoneal lavage after surgery (▲) and after surgery and treatment with scavengers (♦)**

Significant differences between treated and control animals are found at 5 hours (P=0.011) and 12 hours (P=0.013) after surgery

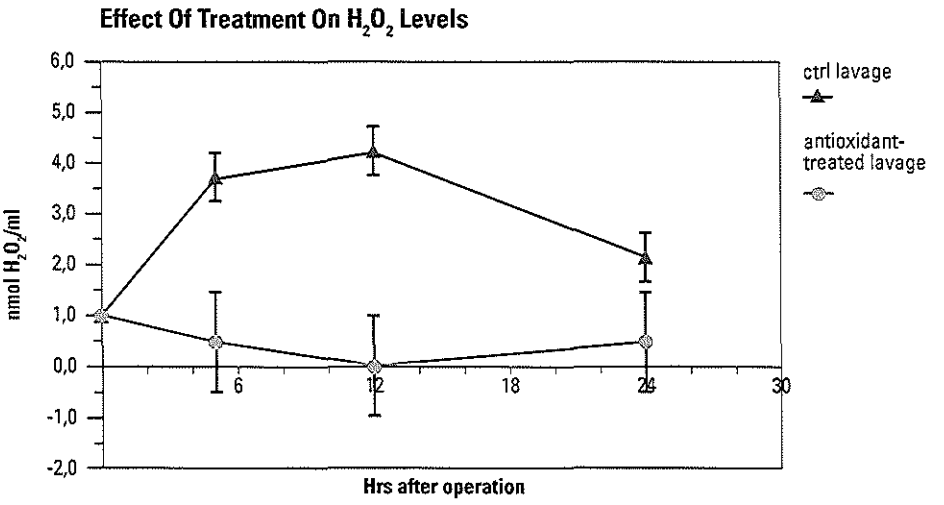


Figure 2. **Levels of H_2O_2 in plasma after surgery (▲) and after surgery and treatment with scavengers (♦)**

Significant difference between treated and control animals is found 5 hours after surgery (P=0.039)

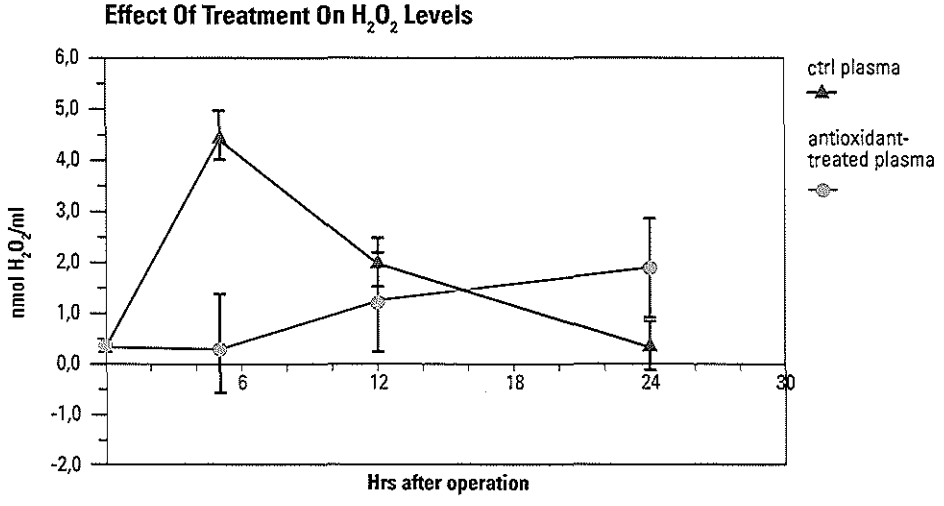


Figure 3. **Levels of LPO in plasma after surgery (▲) and after surgery and treatment with scavengers (♦)**

Significant decrease after treatment with scavengers 5 hours after surgery (P=0.031)

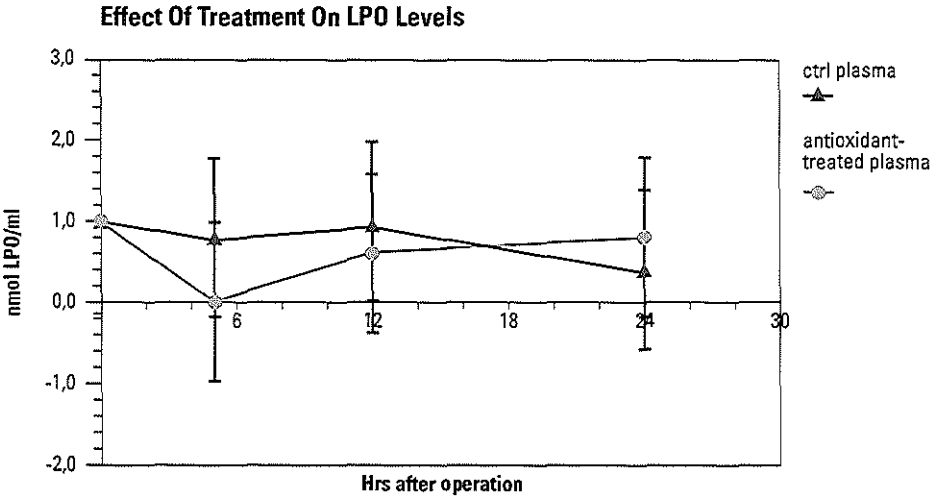
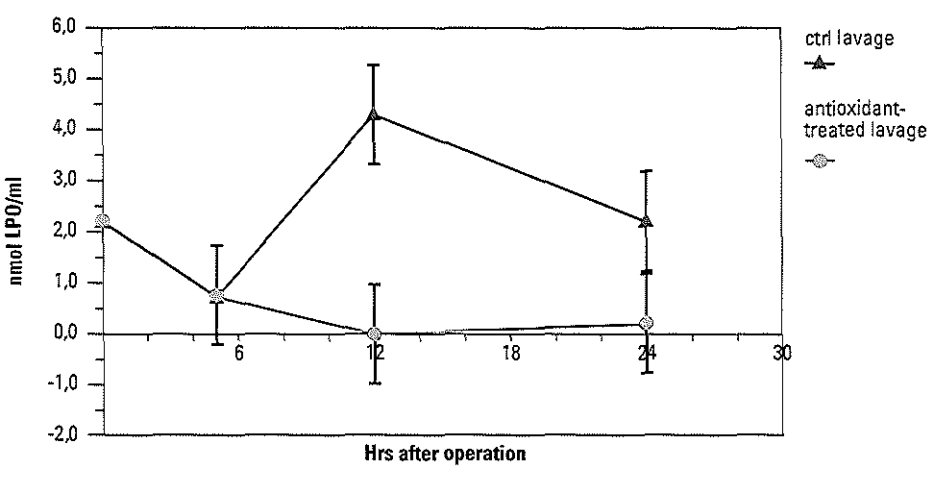


Figure 4. **Levels of LPO in peritoneal lavage after surgery (▲) and after surgery and treatment with scavengers (♦)**

No significant differences are found at any time-point



Discussion

Reactive oxygen species are known to promote postoperative adhesion formation and local tumour recurrence and the administration of antioxidant enzymes immediately after surgery reduced these sequelae, as shown by us and others [9, 12-14]. Surprisingly, actual ROS levels after surgery and the effect of scavengers on those levels have thus far (in rats) not been reported.

Here, we showed that normal levels of H_2O_2 and LPO were low but detectable in vivo by spectrophotometry ranging in lavage and plasma from about 0.3-6 nmol/mL.
Both in lavage and plasma levels, of H_2O_2 increase between 5 and 12 hours after surgery returning to normal level at 24 hours whereas levels of LPO in lavage and plasma do not change much. Based on these observations it seems unlikely that LPO are involved in the induction of the previously reported surgical sequelae of peritoneal trauma [9, 14].

We found that administration of SOD and Catalase led to a decrease in the level of H_2O_2 in the peritoneal cavity and in the circulation returning normal values at 24 hours after surgery. Thus, administration of SOD and Catalase within the first 24 hours after surgery may therefore prevent the detrimental interaction of H_2O_2 with damaged tissue, possibly leading to the release of chemotactic factors attracting granulocytes from the circulation to the lesion site.

As was reported by Ten Kate *et al.*, ROS, besides being involved in local tumour recurrence, also play a role in distant tumour recurrence by increasing binding sites for tumour cells on endothelium [17]. Early scavenging of ROS therefore could be of clinical relevance not only to prevent local tumour recurrence and adhesion formation, but also to prevent distant tumour recurrence.

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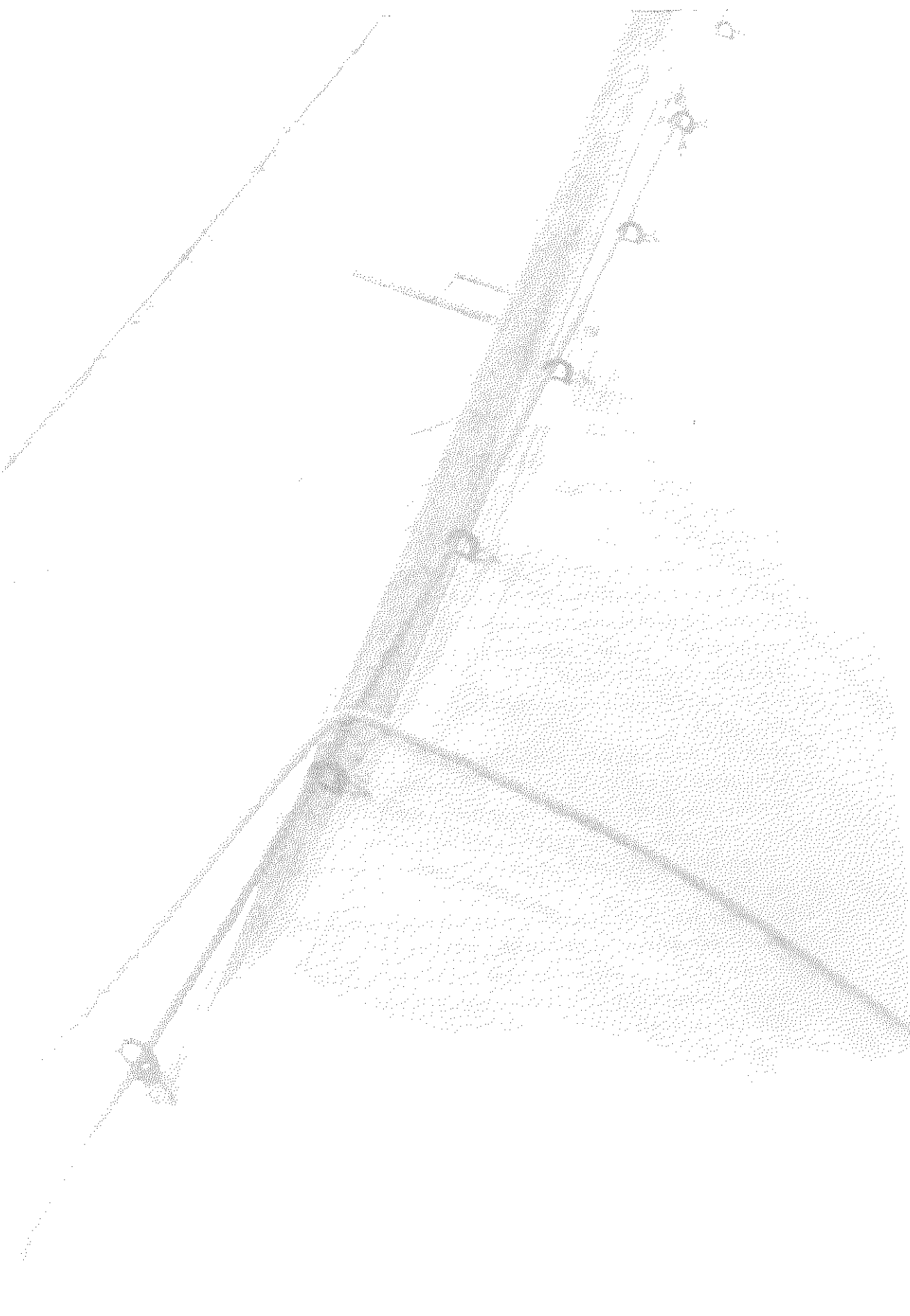

7

The role of superoxide anions in the development of distant tumour recurrence

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Abstract

We hypothesize that reactive oxygen species released from activated polymorphonuclear leukocytes during surgery play a crucial role in enhanced tumour recurrence seen after surgery. Therefore, the effect of reactive oxygen species on adhesion of tumour cells to microvascular endothelium in a reproducible human in vitro model was studied.

Pre-incubation of microvascular endothelial cells with the superoxide anion producing xanthine - xanthine-oxidase complex significantly increased adhesion of the human colon carcinoma cells HT29 (167% vs. control, $p<0.01$), Caco2 (164% vs. control, $p<0.01$) and of the pancreas carcinoma cells PanC1 (180% vs. control, $p<0.01$). Addition of the antioxidant enzymes superoxide dismutase or Catalase significantly decreased tumour cell adhesion ($p<0.01$).

Exposure of endothelial cells to superoxide anions increased the apoptotic rate to 7.9 times the normal rate. Additionally, exposure increased expression of the endothelial adhesion molecules E-Selectin, ICAM-1 and VCAM-1 of maximally 170% vs. control ($p<0.01$).

In conclusion, this study shows that superoxide anions promote the adherence of tumour cells to the microvasculature by inducing endothelial apoptosis that subsequently induces the expression of various adhesion molecules for tumour cells. This indicates that by tackling the production of reactive oxygen species preventing tumour recurrence at distant sites might be feasible.

Introduction

Despite the introduction of new treatment modalities for gastro-intestinal malignancies during the last decades surgery remains the principal therapy for most gastro-intestinal malignancies, although the recurrence rates after intentionally curative surgery are high [1-4]
Operative trauma in itself may favour development of tumour recurrence. This relation between abdominal surgery and locoregional tumour recurrence was investigated in previous *in vivo* and *in vitro* experiments. These studies illustrated that surgical trauma enhanced locoregional tumour recurrence and that this phenomenon involved a dose-response relation, i.e. severe trauma was associated with a higher locoregional tumour recurrence rate compared to mild trauma [5-9]. Further experiments demonstrated that abdominal surgical trauma provoked a local inflammatory reaction with influx of mainly polymorphonuclear cells (PMN). These activated PMN produced reactive oxygen species (ROS) which are found to play an important role in the observed enhanced locoregional tumour recurrence in which binding of the tumour cells to the mesothelium is an essential step [8, 9].

The inflammatory reaction caused by abdominal surgical trauma is not confined to the abdominal cavity, but spreads out systemically [10-16]. So is it found that during and shortly after major surgery, the peripheral blood level of elastase, which is an indicator of PMN activity, is elevated [17-21]. Furthermore, major abdominal surgery results in an elevated PMN concentration at distant sites, for example in the lung leading to a distant inflammatory reaction [22]. Therefore, surgical trauma may not only promote local tumour recurrence, but also tumour recurrence at distant sites.

Cancer dissemination is frequently accomplished via the blood stream. While many circulating tumour cells fail to survive this phase of the metastatic cascade, the establishment of metastases depends upon the arrest of surviving cells and their exit from the circulation, which involves adhering to and crossing the barriers imposed by the microvascular endothelium and extracellular matrix [22, 23].
Based on the previous studies that demonstrate an important role for ROS in locoregional tumour recurrence after surgical trauma, combined with the systemic inflammatory process after surgical trauma, we hypothesize that ROS enhance distant tumour recurrence by increased tumour cell adhesion to the endothelium.

In this study therefore, we investigate the influence of ROS on tumour cell-endothelial cell interactions. The underlying mechanism of the enhanced adhesion by PMN-derived ROS will be further elucidated. Two tumour cell types were used, namely colon and pancreas carcinoma cells to assess the effect of superoxide anions on tumour cell-endothelial cell interactions with focus on the expression of a variety of cellular adhesion molecules and the occurrence of apoptosis of both the tumour and microvascular endothelial cells.

Methods

Cells

Human microvascular endothelial cells of the lung (MEC) were purchased from Cambrex (Verviers, Belgium) at passage 4 and maintained in EGM-2-MV Bullet kit according to the manufacturer's instructions at 37°C, 95% relative humidity and 5 % CO_2. Confluent monolayers were passaged by 0.025% trypsin / 0.01% ethylenediaminetetraacetic acid (EDTA) and cells were used up to passage 8. The human colon carcinoma cell lines HT29 and Caco2 and the human pancreas carcinoma cell line PanC1 were grown in EGM-2-MV Bullet kit as well in order to create similar conditions and maintained by serial passage after trypsinization using 0.05% trypsin / 0.02% EDTA (Gibco, Breda, the Netherlands). Before the adhesion assay, tumour cells were trypsinized and maintained in suspension for 2 hours to regenerate cell-surface proteins.

ROS and scavengers

In this study the xanthine (X) – xanthine oxidase (XO) complex was used in a concentration of 100 µM and 30 mU/ml respectively (Sigma-Aldrich, Zwijndrecht, the Netherlands), to produce superoxide anions.
Superoxide anions were inactivated by the addition of 400 U/ml superoxide dismutase (SOD) (Roche Applied Science, Almere, the Netherlands) that converts superoxide anions into molecular oxygen and hydrogen peroxide. Since hydrogen peroxide may itself affect tumour cell adhesion, 400 U/ml Catalase (Sigma-Aldrich, Zwijndrecht, the Netherlands) was added to the in vitro model alone or in combination with SOD to decompose any hydrogen peroxide.

Ferricytochrome c reduction assay

To assess production of superoxide anions generated by the combination of X and XO in our model we used the ferricytochrome c reduction assay [24]. This assay was performed in phenol red-free and phosphate buffered Hank's

Balanced Salt Solution (Invitrogen, Breda, the Netherlands) with 5% fetal calf serum, since phenol red and pH changes effect the assay. After addition of 75 µM cytochrome *c* (Roche Applied Science, Almere, The Netherlands) the change in absorbance at 550 nm and 540 nm (reference) was continuously recorded by the thermostatted Versamax microplate reader (Molecular Devices) for 125 minutes at 37°C.

Adhesion assay

To quantify tumour cell adhesion to MEC, a standardized cell adhesion assay was developed as described before [25]. Briefly, endothelial monolayers were established in 96 well microtiter plates (Perkin Elmer, Groningen, the Netherlands). To do this, confluent cells were trypsinized and 2×10^4 endothelial cells were added to each well. The plates were incubated at 37°C, 95% relative humidity, 5% CO_2 and medium was daily replaced by fresh medium. MEC reached confluence in 3 to 4 days as determined by light microscopy.

To determine the effect of ROS on tumour cell adhesion, endothelial monolayers were pre-incubated with varying doses of X and XO, during varying times. Untreated monolayers served as controls. Tumour cells were pre-incubated or not with the X - XO complex for 12 hours before the adhesion assay. Appropriate SOD and/or Catalase were added to the model system to assess ROS specificity of the effects.

To quantify tumour cell adhesion, tumour cells (1×10^6 cells/ml) were labelled with calcein-AM (Molecular Probes, Leiden, The Netherlands) and 3×10^4 cells per well were added. Plates were centrifuged for 1 minute at 80 × g and incubated at 37°C for 1 hour. After this, the wells were washed twice with medium. The remaining fluorescence per well was measured on a Perkin Elmer plate reader using a wavelength of 485 nm for excitation and 530 nm for emission respectively.

Enzyme immuno assay (EIA)

Endothelial and tumour cells were grown to confluence as described for the adhesion assays in 96-well flat-bottomed microtiter plates (Becton & Dickinson, Erembodegem, Belgium). Cells were pre-incubated with either cell culture medium alone or combined with X and/or XO.
Next, the cells were washed with phosphate buffered saline (room temperature, pH 7.4) and fixed in ethanol / methanol for 45 minutes and washed again. Subsequently, non-specific binding sites were blocked by incubating the wells for

10 minutes with 1% goat serum (Sigma-Aldrich, Zwijndrecht, the Netherlands). Mouse monoclonal antibody to E-Selectin, intracellular adhesion molecule-1 (ICAM-1) or vascular cellular adhesion molecule-1 (VCAM-1) (ITK, Uithoorn, the Netherlands) in a dilution of 1:500 was added for 1 hour, followed by the addition of biotinylated goat anti-mouse antibody (Sigma-Aldrich, Zwijndrecht, the Netherlands) in a dilution of 1:250. Increased sensitivity was obtained using the ExtrAvidin-Peroxidase system (Sigma-Aldrich, Zwijndrecht, the Netherlands). After washing away any free peroxidase, a substrate solution containing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt in 0.05 M citrate-phosphate buffer with urea hydrogen peroxide buffer with urea hydrogen peroxide was added. Incubation of endothelial cells without the primary antibody served as a negative control. As a positive control, the ExtrAvidin-Peroxidase system was added followed by substrate development without washing away the peroxidase. After 40 minutes the reaction was stopped with sodium fluoride and photometrical evaluation was performed with a computer-controlled ELISA reader at $\lambda = 405$ nm.

Apoptosis

To assess whether superoxide anions caused apoptosis in MEC a cell-death detection ELISA^{plus} kit (Roche Applied Science, Almere, the Netherlands) was used for the detection of cytoplasmic histone-associated DNA fragments. In short, endothelial cells were grown to confluence as described for the adhesion assays in 96-well flat-bottomed microtiter plates. The cells were pre-incubated with X and/or XO for 12 hours and then lysated, whereafter 20 µl of the lysate was transferred into Streptavidin-coated microplate wells. Eighty µl of immunoreagent containing biotinylated anti-histone and peroxidase-labeled anti-DNA antibodies was added into the wells followed by incubation on a plate shaker under gently shaking (300 rpm) for 2 h at 15–25°C. Then the wells were washed thoroughly with incubation buffer and 100 µl of 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) substrate was added. Plates were incubated for 15 minutes on a plate shaker at 250 rpm where after photometric analysis at 405 nm was performed.

Proliferation assay

To establish whether pre-incubation of MEC monolayers with superoxide anions was of influence on MEC cell number, the DNA content was determined using the bisbenzimide fluorescent dye (Roche Applied Science, Almere, The Netherlands) as previously described by Hofland *et al.* [26]. Therefore, 2 ×104 endothelial cells / ml were plated in 24 wells plates and after 1 day X-XO was

added. At day 0, 1, 2 and 3 after the addition of X-XO wells were washed and plates were stored at -20°C until analysis.

Statistical analysis

All data were evaluated using analysis of variance (ANOVA) to determine overall differences between groups. The Dunnett post-test was carried out to compare between groups. $P \leq 0.05$ was considered to be statistically significant. Experiments (n=6) were performed at least twice.

Results

Evaluation of the model

Labelling tumour cells with calcein-AM did not decrease their viability (>95% using trypan blue). Dilution series of labelled tumour cells on endothelial monolayers showed a linear correlation ($r2 > 0.99$) between cell number and the level of fluorescence (Fig. 1). Thus, by using such standard curves it became possible to estimate the number of adherent tumour cells in the experimental wells from the fluorescence intensity.

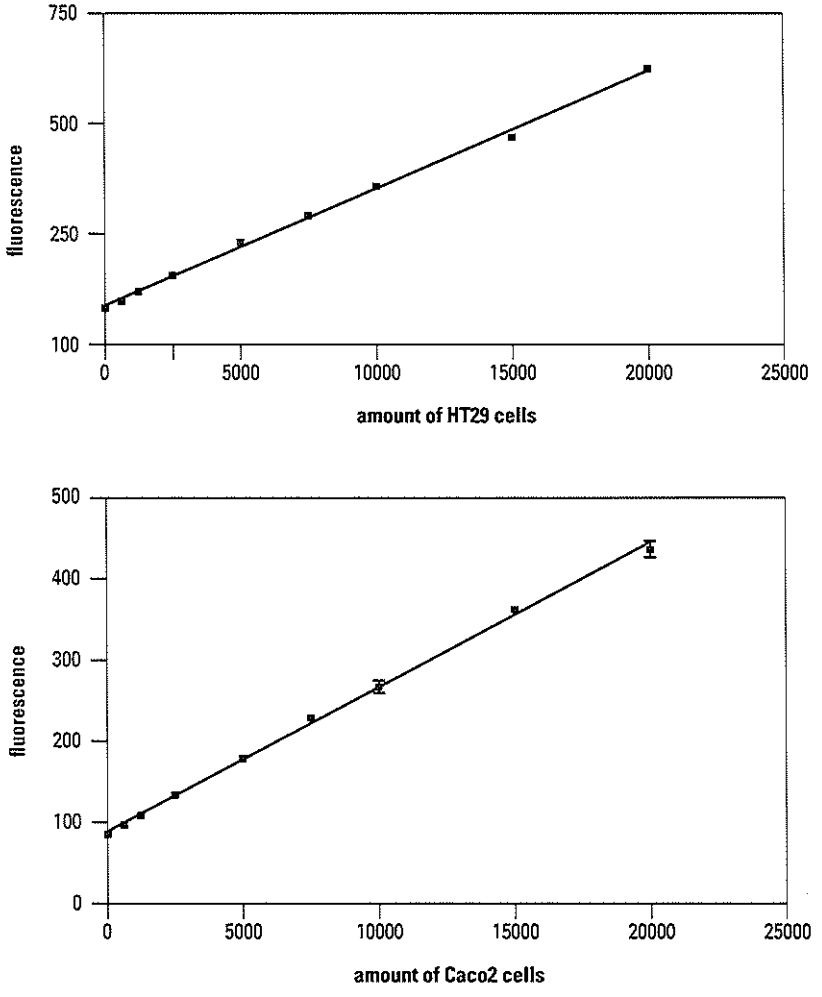
In our model, ferricytochrome c in the wells with X-XO was reduced at a rate of 0.32 nmol/ml/min as can be calculated from the results presented in figure 2 using a molecular extinction coefficient of ferricytochrome c of 13.125 M^{-1} for a light path of 0.625 cm in the microtitre plate. The addition of SOD prevented the reduction of ferricytochrome c completely, indicating that the X-XO system indeed mainly generated superoxide and that 400 U/ml SOD is sufficient in this model to dismutate the formed superoxide anions. Interestingly, in the absence of xanthine xanthine oxidase still generated superoxide, but at a lower rate (Fig.2). In the assay with xanthine oxidase only in the absence of fetal calf serum, we found no superoxide production (data not shown), while in the presence of fetal calf serum, but without the addition of xanthine oxidase and extra xanthine, MEC also were found to produce some superoxide (Fig.2). This made it likely that the fetal calf serum of the medium contained the necessary substrate xanthine, and that MEC contain some endogenous xanthine oxidase (Fig.2).

Adhesion to microvascular endothelial cells

Basal adhesion, i.e. adhesion to non-pre-incubated MEC, was between 20 and 30% of added cells for HT29 and Caco2. For PanC1, basal adhesion was between 10 and 20%.

Pre-incubation of MEC with the X-XO complex enhanced tumour cell adhesion (Fig.3). For PanC1, this enhancement occurred after 12 hours pre-incubation and was increasing with longer pre-incubation times reaching a maximum of 180% vs. control (untreated MEC) after 24 hours of pre-incubation ($p<0.01$). Comparable results were found for Caco2 after X-XO pre-incubation of MEC with a tumour cell adhesion of 164% compared to basal adhesion ($p<0.01$). Maximal adhesion for HT29 occurred already after 12 hours pre-incubation of MEC with X-XO and was 167% vs. control ($p<0.01$).

Figure 1. Linear correlation between number of cells and fluorescence



(Figure 1. Linear correlation between number of cells and fluorescence)

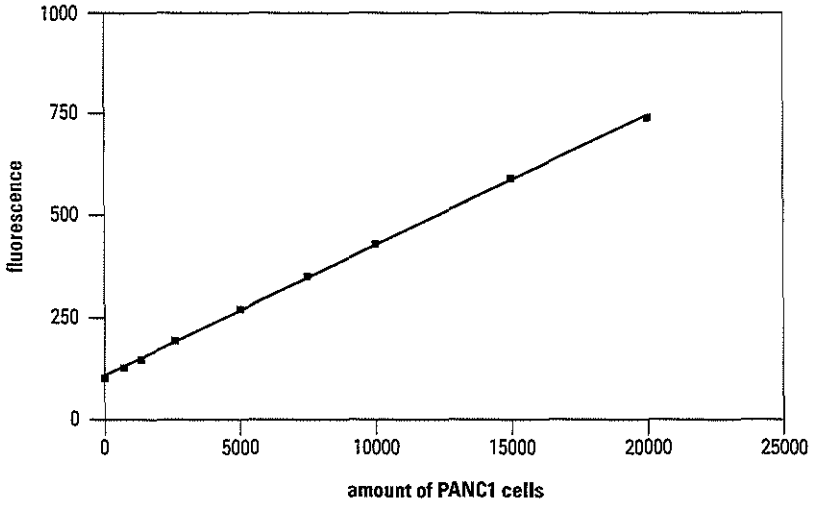


Figure 2. Production of superoxide anions

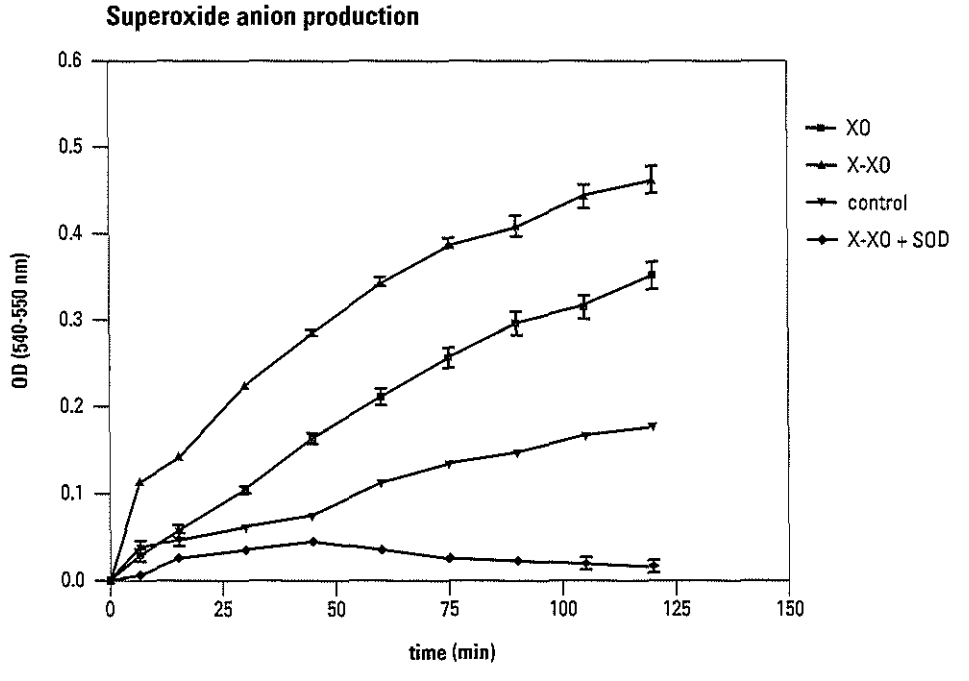
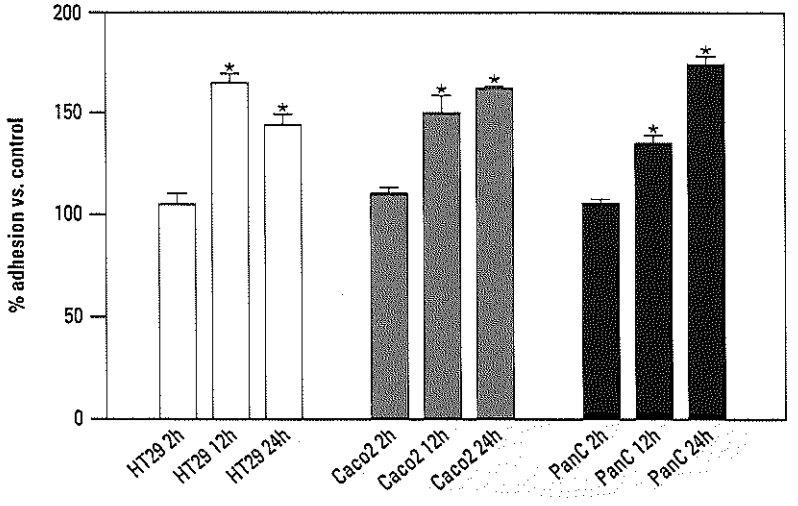
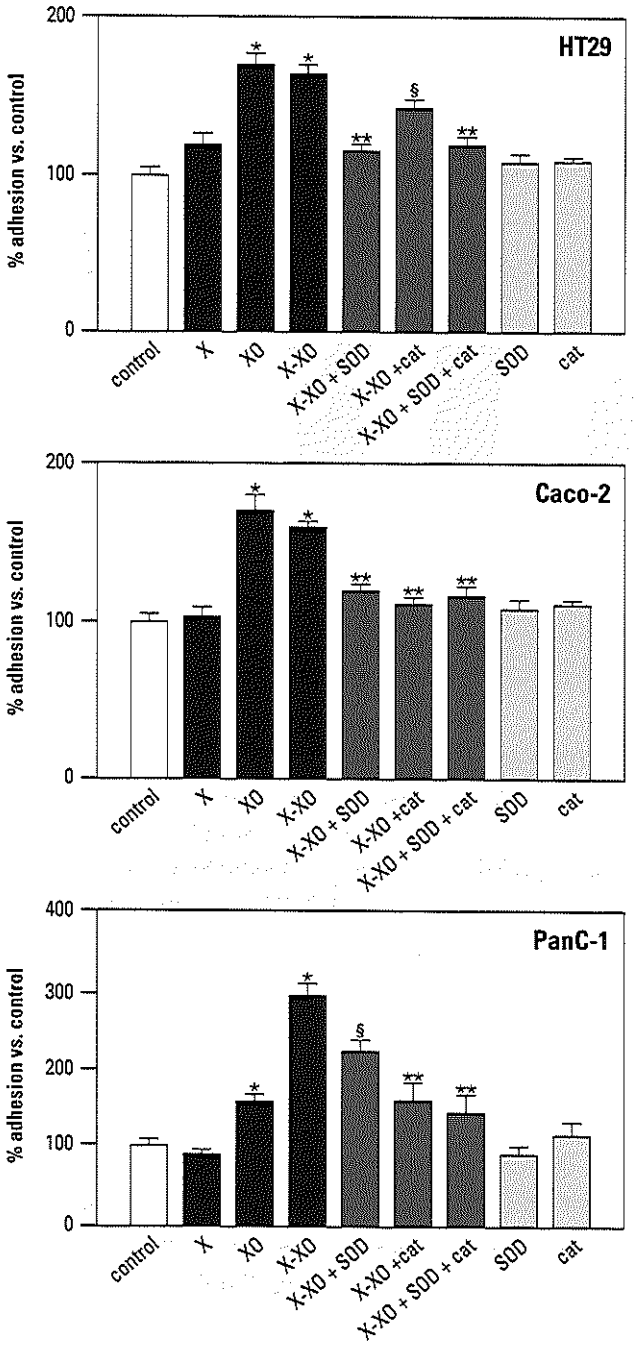


Figure 3. Pre-incubation of MEC with the X-XO complex enhances tumour cell adhesion



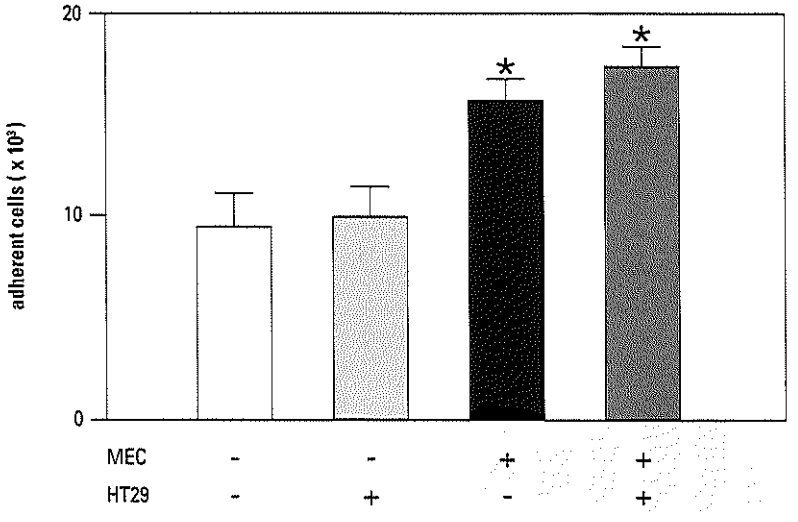
Pre-incubation with X alone did not influence tumour cell adhesion for all three cell lines (Fig.4). However, pre-incubation with XO alone did enhance the adhesion of HT29 to 172%, of Caco2 to 170% and of PanC1 to 128% vs. control (all $p<0.01$) (Fig.4). This was not surprising, since XO in medium alone did produce superoxide anions (Fig.2), probably because fetal calf serum in the medium contains X, acting as a substrate for XO.

Figure 4. Pre-incubation with X and XO



Pre-incubation of HT29, Caco2 and PanC1 with the X-XO complex for 12 hours did not enhance their adhesion to untreated or pre-treated MEC statistically significantly (Fig.5; only data for HT29 are shown).

Figure 5. Adhesion of tumour cells after pre-incubation with X-XO complex



To verify if superoxide is the relevant ROS causing the enhanced adhesion we evaluated the effects of SOD in this model (Fig.4). Addition of SOD did decrease the enhanced adhesion of Caco2 to X-XO – treated MEC to nearly basal levels, from 158% to 116% ($p<0.01$). Comparable results were observed for HT29. SOD also decreased adhesion of PanC1 to X-XO – treated MEC, from 299 to 213% ($p<0.05$). Since superoxide anions spontaneously dismutate into the stronger ROS hydrogen peroxide that may affect MEC on its turn, we studied the effect of Catalase next. The results showed that Catalase inhibited the enhanced tumour cell adhesion after X-XO – pre-incubation effectively as well, i.e. for HT29 adhesion decreased from 167 to 141% ($p<0.05$), for Caco2 from 158 to 113% ($p<0.01$) and for PanC1 from 299 to 163% ($p<0.01$). In combination both antioxidant enzymes did not give an additional effect. The addition of SOD or Catalase to untreated MEC did not decrease basal adhesion alone (Fig.4), indicating that the low level of superoxide production of MEC in culture medium was insufficient to act as an autocrine stimulus (Fig.2).

Mechanism of adhesion

To study if pre-incubation with X-XO influences the number of MEC we determined the course in the amount of DNA during an observation period of 3 days. The results showed a significant decline in the amount of DNA, not until 2 days of incubation with X-XO (Fig.6a). During the first 24 hours of culture in presence or absence of X-XO however, the number of MEC as reflected by the amount of DNA did not change significantly. Photographs of the endothelial monolayers with or without pre-incubation with X-XO for 12 hours also show that the number of endothelial cells is comparable (Fig.6b). On the other hand, pre-incubation with X-XO did lead to an increased apoptosis rate of 7.9 times the normal apoptosis rate ($p<0.01$), while XO only stimulated the apoptosis rate 3.7 fold ($p<0.01$) (Fig.7).

It was previously shown [27-30] that the adhesion molecules E-Selectin, ICAM-1 and VCAM-1 on MEC and the ligands lymphocyte function-associated antigen-1 (LFA-1), very late activation antigen (VLA-4) and CD44 on tumour cells play an important role in tumour cell adhesion to MEC and that the expression of these molecules can be induced by apoptosis [31, 32].

In this model we found that non-stimulated MEC and tumour cells did express E-Selectin, ICAM-1 and VCAM-1 (Fig.8 and 9). After 8 hours of pre-incubation with X-XO, enhanced E-Selectin expression on MEC was observed with a peak expression after 12 hours of 1.66 times the expression on non-stimulated MEC ($p<0.01$). Increased ICAM-1 and VCAM-1 expression on MEC was observed later, namely after 12 hours pre-incubation. While ICAM-1 expression then increased still further to a maximum of 170% vs. control ($p<0.01$), VCAM-1 expression declined after its peak expression of 149% vs. control at 12 hours of pre-incubation (Fig.6). None of the adhesion molecules under study showed enhanced expression on HT29 (Fig.9), Caco2 and PanC1 by X-XO pre-incubation (data not shown).

Figure 6 a. Amount of DNA during 3 days of incubation

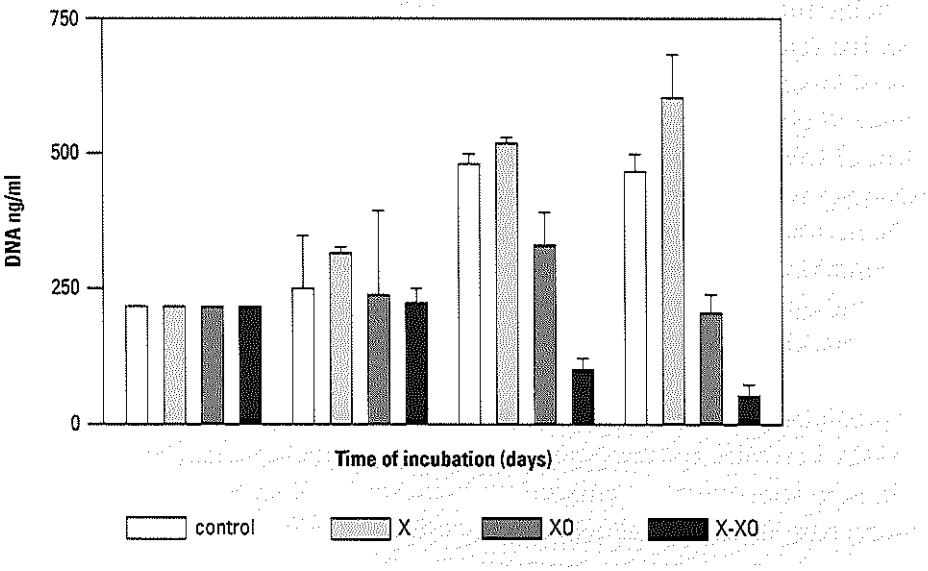
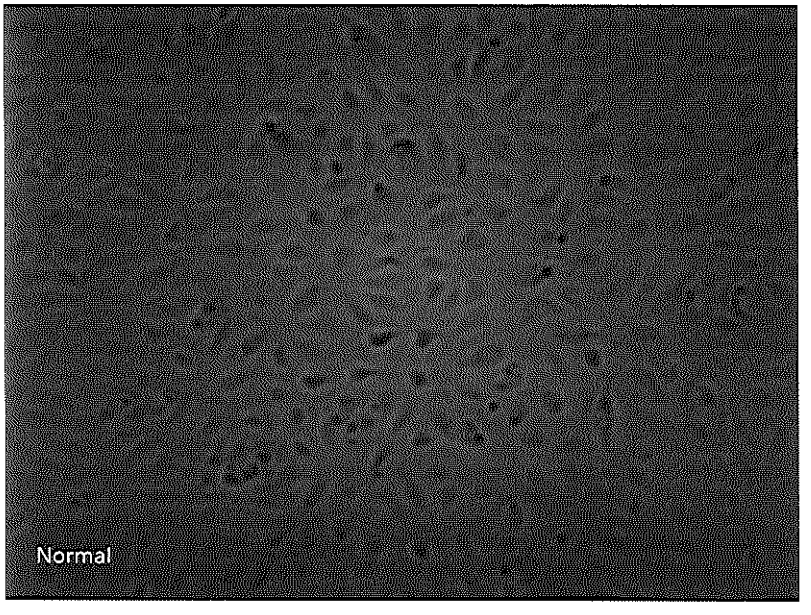


Figure 6 b. Amount of cells after incubation



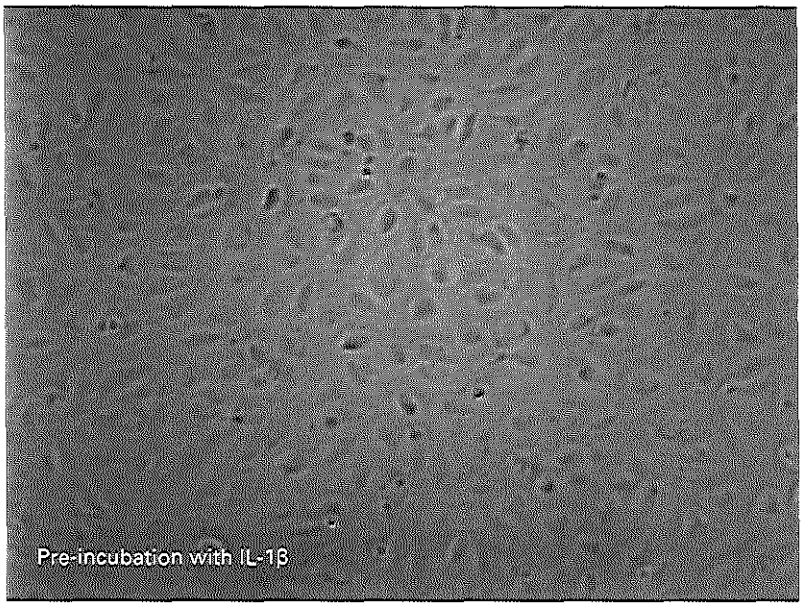


Figure 7. Apoptosis rates

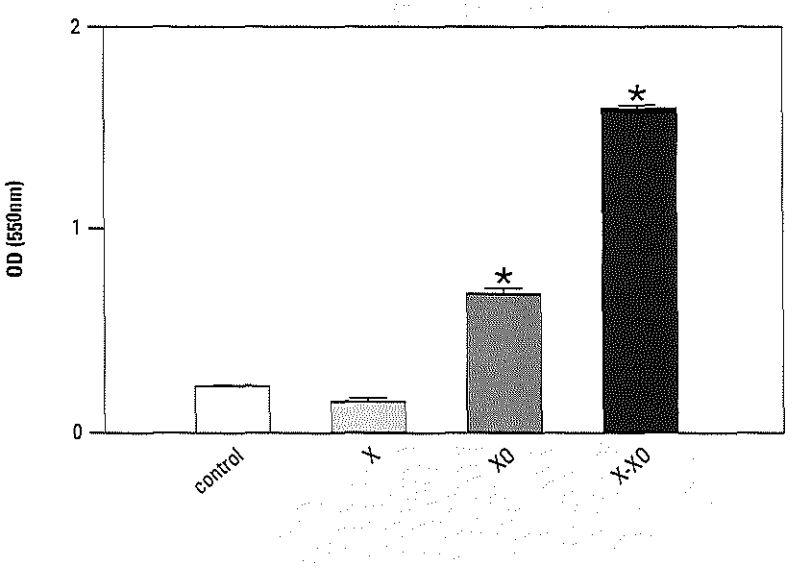


Figure 8. Expression of adhesion molecules

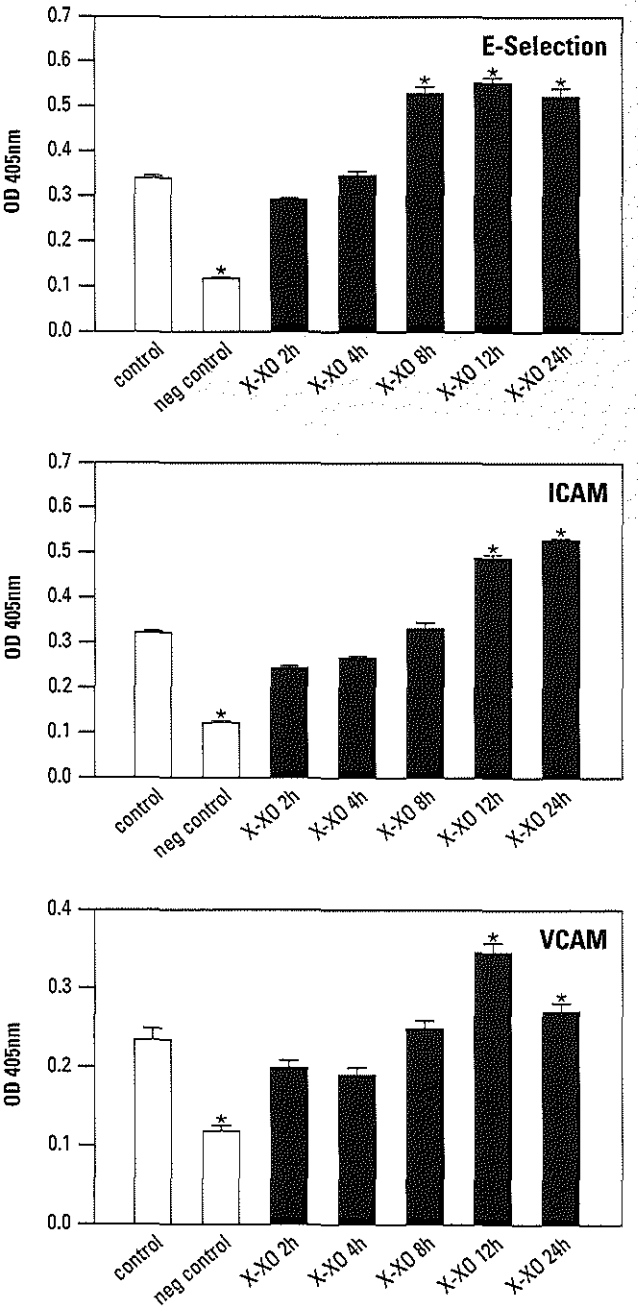
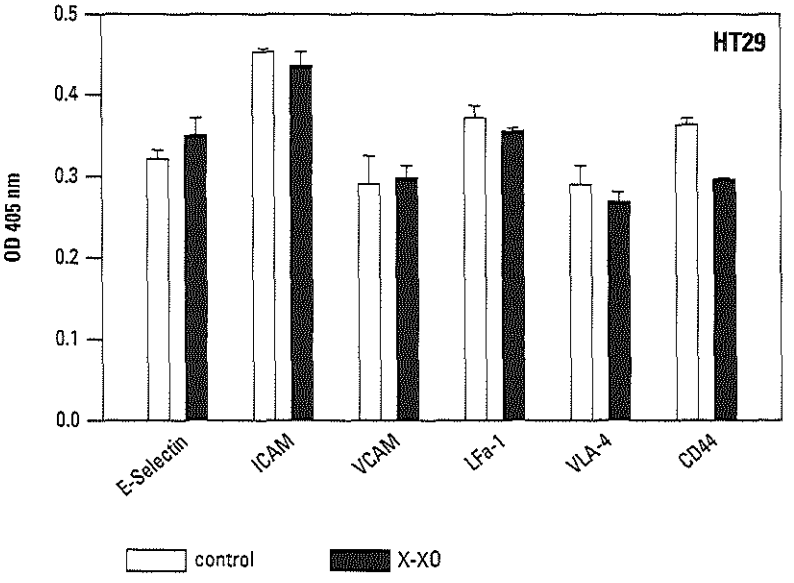


Figure 9. Expression of adhesion molecules on HT29



Discussion

ROS are known to play an important role in loco-regional tumour recurrence after surgical trauma [8, 9]. In preliminary *in vivo* studies, we were able to detect a significant increase of ROS in peritoneal lavage fluid as well as in plasma after surgery, proving that indeed surgery induces not only a local enhancement of ROS, but also systemically (data not shown). So the inflammatory response after surgical trauma does not confine locally, but spreads out systemically and therefore it is interesting to investigate the role of ROS in the development of distant metastases after surgery.

Therefore, the xanthine – xanthine oxidase complex was used to generate superoxide anions and in this way the influence of superoxide anions on tumour cell – endothelial cell interactions was studied. Exposure of microvascular endothelium to superoxide anions gave a substantial enhancement in tumour cell adhesion to the exposed endothelium comparable to the results found with PMN exposure, while exposure of tumour cells to superoxide anions had no effect on their adhesion to untreated endothelial cells.

We found that exposure of the microvascular endothelial cells to superoxide anions led to an up-regulation of the adhesion molecules E-Selectin, ICAM-1 and VCAM-1 on these cells. Enhancement of adhesion molecules on endothelial cells by exposure to ROS was also found by Bradley and Lo [33, 34]. Both found significant increased ICAM-1-expression after pre-incubation with the ROS, although a relation with tumour adhesion was not investigated. Terada et at. [35] did not found an up-regulation of ICAM-1 on the endothelium after a short pre-incubation period of 30 minutes with xanthine oxidase. This period of time is too short for completing synthesis of functional adhesion molecules, which is in accordance to our observation that a continuous exposure of the endothelium to ROS lasting minimally 12 hours is necessary before endothelial cells show any increased expression of cellular adhesion molecules.

Exposure of the endothelium to superoxide anions resulted in a major increase of apoptosis. Apoptosis finally will result in cell death leading to loss of binding sites on the endothelium, but exposure of the underlying extracellular matrix as a substrate for binding sites for circulating tumour cells. However, it does not seem very likely that binding sites on the extracellular matrix contributed to the findings of the present study, because the pre-incubation of the endothelial cells lasted only 12 hours during which the number of endothelial cells did not decrease.

The fact that endothelial cells undergoing apoptosis release interleukin -1 β (IL-1β) that via a paracrine loop in turn stimulates the expression of adhesion molecules on the endothelial cells [31, 32] suggests that the following sequence of events for the recurrence of tumour cells at distant sites occur. Surgical trauma during the excision of a (primary) tumour leads to the activation of PMN. At distant sites these phagocytes by their massive production of ROS induce apoptosis of microvascular endothelium. Subsequently, by the (local) release of IL-1β the endothelial cells stimulate the expression of at least three major cellular adhesion molecules on their own cell membrane to which circulating tumour cells now easily can adhere and next form a metastasis.

The addition of SOD and/or Catalase to ROS-exposed MEC decreased the enhanced tumour cell adhesion significantly. Since both antioxidant enzymes decreased the adhesion to similar levels this means that not only superoxide anions, but also hydrogen peroxide are equally involved in this phenomenon. This indicates that in fact a third kind of ROS, namely the highly reactive hydroxyl radical, is the actual reactant. To generate hydroxyl radicals both superoxide

and hydrogen peroxide are needed in the so-called transition metal catalyzed Haber-Weiss reaction, and thus depleting one or the other ROS completely prevents the generation of the hydroxyl radical. Of note is that the addition of either SOD or Catalase did not decrease the adhesion to basal levels. Incomplete scavenging of ROS by the antioxidant enzymes cannot account for that, because we showed here that adding SOD to the xanthine-xanthine oxidase complex completely inhibited the generation of superoxide. Presumably the local increase in tension of molecular oxygen as a by-product of the inactivation of superoxide and hydrogen peroxide by SOD and Catalase, or xanthine oxidase itself may have contributed to the incomplete reduction in the expression of the adhesion molecules.

In conclusion, the results of the present study suggest that ROS as a result of surgical trauma influence tumour recurrence at distant sites by increasing binding sites for tumour cells on the endothelium. This indicates that by tackling the production of ROS preventing tumour recurrence not only locally, but also at distant sites might be feasible.

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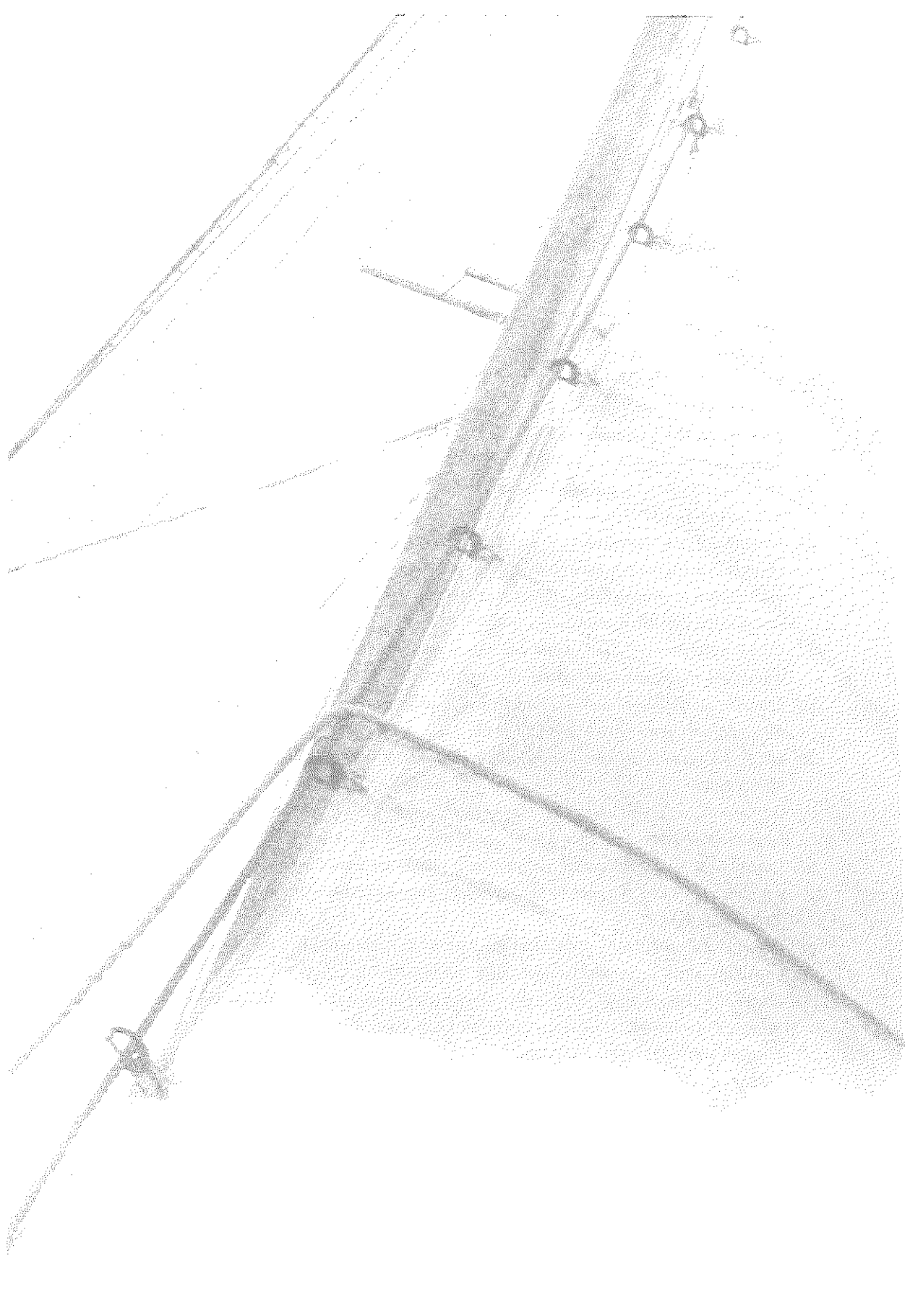

8

Summary and Discussion

The peritoneum is a delicate organ with a thickness of only one layer of mesothelial cells. Abdominal surgery requirers opening it, which causes an inflammatory defence mechanism in order to restore the damage caused by the incision and subsequent manouvres. Although this is a normal response to trauma, it can lead to undesired side effects such as adhesions formation and, in case of an oncological procedure, adherence of spilled tumour cells to the peritoneum causing peritoneal metastasis and local tumour recurrence.

First of all we need to understand the pathways of surgical trauma through inflammation and fibrinogenesis to adhesion formation and tumour recurrence before we can successfully attempt to reduce these complications of abdominal surgery. These pathways are discussed and explained in **chapter 2**. Various strategies are discussed, which have been used to minimise or reduce adhesion formation and tumour recurrence. These strategies include minimising surgical trauma (use of minimally invasive surgery, gentle handling of tissue), irrigation of the abdominal cavity (i.e. saline, Ringer's lactate), the use of liquid and solid barriers (i.e. Interceed®, Icodextrin®, Seprafilm®) and pharmacological adjuvants (i.e. glucocorticoids, NSAID's, fibrinolytics).
Unfortunately as of yet none of these therapies, when used as monotherapy, were completely successfull.

Little is known about the incidence of adhesions in healthy individuals without previous abdominal surgery in their medical history. Adhesions are present in healthy individuals, according to several authors, although reported ranges of incidence vary from only 2% to 28% [1-3]. Since it is unethical to perform diagnostic laparoscopy in a healthy individual (if any person would volunteer), the second best option is to observe and score adhesions in patients undergoing surgery anyway. In our study presented in **chapter 3** we scored adhesions in living kidney-donors.
Adhesions were encountered in 52% of asymptomatic kidney donors, who had no history of previous operations, versus 64% in donors with abdominal surgery in their medical history. Possible explanations for the observed discordance with previous studies includes the nature of the procedures. The procedures that these donors underwent before the present study were mainly local, such as laparoscopic cholecystectomies or appendectomies. Furthermore, the prospective design of our study allowed us to even record single adhesions. Probably, retrospective reviewing of operative findings only clarifies severe adhesions, because these adhesions are mentioned whereas a few filmly adhesions possibly are not reported due to their small impact on the surgical procedure.

Furthermore, the displacement of adjacent organs as the colon, the liver and the spleen during laparoscopic donor nephrectomy (LDN) possibly reveals additional adhesions.
As expected because of the inclusion of healthy individuals, the number of donors with a past medical history revealing midline laparotomies was small. Severe adhesions due to previous midline laparotomies may severely complicate surgery. However, the only donor presenting with massive adhesions preventing further laparoscopic dissection had never been operated on. One of the important results of this study is that adhesions in patients without a history of abdominal operations might be far more common than previously thought.
The high incidence of adhesions found in donors without previous surgery also brings new perspective to the ongoing debate on the correlation between adhesions and chronic abdominal pain. The data of this study question this correlation between adhesions alone and bowel pain. Of all patients who entered our study 55% had adhesions, but no one preoperatively complained of abdominal pain. These donors may be considered "adhesion formers", but none of the 161 donors complained of abdominal pain during one-year follow-up, which was conducted with visits to the outpatient clinic and administration of quality of life and case-record forms. An explanation for this incongruence could be the nature of both previous surgery a well as laparoscopic donor nephrectomy itself. As stated before, previous surgical procedures were mainly local which theoretically causes less peritoneal trauma. In addition, being a minimally invasive procedure, laparoscopic donor nephrectomy is expected to cause less trauma as well, possibly producing less severe adhesions. Nevertheless, it is known from literature that not only severe adhesions but also filmly adhesions can cause abdominal pain [4-6].

To gain more knowledge about the relationship between adhesions and (chronic) abdominal pain, we performed a review of literature on this subject **(chapter 4)**. The placebo effect of diagnostic laparoscopy for adhesions in patients with chronic abdominal pain has been well established by Swank *et al.* [7], who showed alleviation of abdominal pain after both diagnostic laparoscopy and laparoscopic adhesiolysis.

Nevertheless, several studies indicate that chronic abdominal pain can be caused by postoperative abdominal adhesions, whether it is by nerve fibers in the adhesions itself, by traction to the peritoneum or organs or a combination of both, whereas changes in the central nervous system should be considered to play a role as well [4, 8-10].

The phenomenon of postoperative, chronic abdominal pain is highly complicated and almost always there are several causes to consider. Once other causes than adhesions have been ruled out, (laparoscopic) adhesiolysis was in the past commonly attempted in order to treat patients for chronic abdominal pain. From the results of the randomized study of Swank *et al.* it became clear that laparoscopic adhesiolysis for chronic abdominal pain is not the treatment of choice, since adhesiolysis and diagnostic laparoscopy patients differed only in complication rates, not in benefit [7]. From our review we can conclude that this point of view is justified.

The incidence, severity and location of adhesions has not frequently been reported, because assessment of postoperative development of adhesions requires invasive techniques. In 2002, Vrijland *et al.* published the results of a study in which patients with diverticulitis requiring Hartmann's procedure were randomized to receive a solid hyaluronic-acid carboxymethylcellulose barrier (Seprafilm®) or as a control [11]. The set-up of this trial was appropriate for the assessment of the development of adhesions, given the two stage character of Hartmann's procedure in which during the second stage bowel continuity is restored [11, 12]. In the trial less severe adhesions in the Seprafilm® group were found. Follow-up of the peroperative placement of Seprafilm® and the assessment of its effect on postoperative complications has been discribed previously [13, 14], however, mean follow-up did not exceed five years in these studies. Because complications such as small bowel obstruction sometimes manifest not sooner than 10 years after surgery [10, 11], we conducted a follow-up of this randomized clinical trial **(chapter 5)** whith a mean follow-up of over 10 years.

The results of our follow-up did not show an increased incidence of small bowel obstruction in the control group, and no single case of small bowel obstruction requiring re-operation was found. This is in concordance with literature [14, 15]. However, with regard to the questionnaire, we found significantly less postoperative chronic abdominal complaints and problems with defecation, especially constipation, in the Seprafilm® group compared to the control group. This finding is not explained by the outcome of the VAS-score, EQ-5D or SF-36. One could hypothesize that less severe adhesions provide more mobility for the gastro-intestinal tract, although due to the set-up of our follow-up we were not able to test this hypothesis. All abdominal complaints were scored (nausea, pain, constipation). The incongruity between the VAS and the questionnaire may be explained by the fact that in the questionnaire not only pain,

but also nausea and constipation were recorded. Furthermore, a VAS-score is an instrument capable of measuring pain in the recent past, whereas most of the complaints involving the abdomen in our patients occurred in the first post-operative year.

The use of the EQ-5D and SF-36 surveys for measuring and comparing the difference in quality of life between the two groups in our follow up is unconventional, because in the Seprafilm® trial these forms were not used and hence these outcomes could not be compared to "baseline". Nevertheless, theoretically, patients suffering from repeated bowel obstruction could experience a reduced quality of life compared to healthy individuals, which justified the use of these forms. After thorough analysis of the two surveys, no significant difference was found in difference in quality of life. Given the fact that the two groups could not be distinguished by the EQ-5D nor the SF-36, considering quality of life, we concluded that the application of Seprafilm® does not influence quality of life.

The previously published study showed that the application of Seprafilm® in patients undergoing Hartmann's procedure was responsible for a significant reduction in severity of formation of adhesions. The results of the study presented in chapter 5 indicate that Seprafilm® does not reduce the incidence of small bowel obstruction, which leads to the conclusion that it is not the severity of adhesions but possibly rather the location of adhesions which determines whether small bowel obstruction will occur.

To further reveal the pathophysiological pathway of adhesions formation and tumour cell adhesion, various experiments have been conducted in the past. Some of these experiments focussed on the influence of PMN's and their products, reactive oxygen species (ROS). These ROS are now known to play a role in postoperative adhesion formation and local tumour recurrence, and various authors have shown that scavenging these ROS leads to a reduction of both adhesion formation and tumour recurrence [16-19].
However, up to now data concerning actual ROS levels were not available and measurement of actual levels of ROS, before and after surgery or with and without adding scavengers, had never been reported. In the experiments described in **chapter 6** we showed that H_2O_2 and LPO were detectable in vivo by spectrophotometry. Both in lavage fluid and plasma of non-treated animals, levels of H_2O_2 increased after surgery during the first 12 hours. Levels of LPO in lavage and plasma of non-treated animals varied after surgery, but did not differ statistically significant from baseline values. These results for the first

time showed that steady-state levels of ROS can be measured in vivo and are present in the peritoneal cavity and the circulation, and that surgical trauma by itself did affect the level of H_2O_2 in our experiments. Attempts to reduce the levels of ROS both locally and sytemically might still be relevant and show promising results [20].

Furthermore, we showed that the administration of anti-oxidant enzymes decreases the normal ROS levels. Administration of SOD and catalase led to a decrease in the level of H_2O_2 in the peritoneal cavity, and in the circulation. The decreased level of H_2O_2 slowly increased to normal values but not until 24 hours after surgery and administration of SOD and catalase. Therefore administration of SOD and catalase within the first 24 hours after surgery may prevent the detrimental interaction of H_2O_2 with damaged tissue leading to the release of chemotactic factors attracting granulocytes from the circulation to the lesion site. Earlier studies showed that SOD and catalase can diminish the effects of surgical trauma [16, 18], and because these enzymes decrease the levels of H_2O_2, but not of LPO, a role of LPO in postoperative adhesion formation and local tumor recurrence is unlikely.

ROS are known to play an important role not only in postoperative adhesion formation but also in loco-regional tumour recurrence after surgical trauma [18, 21]. In the experiments described in chapter 6, we were able to detect an increase of ROS in peritoneal lavage fluid as well as in plasma after surgery, suggesting that indeed surgery induces not only a local enhancement of ROS, but also systemically. So the inflammatory response after surgical trauma does not confine locally, but spreads out systemically and therefore we investigated the role of ROS in the development of distant metastases after surgery **(chapter 7).** In an *in vitro* model, the effects of ROS on tumour cell adhesion to human mesothelial cells were studied. Exposure of microvascular endothelium cells (MEC) to superoxide anions gave a substantial enhancement in tumour cell adhesion to the exposed endothelium through an increase of binding sites, while exposure of tumour cells to superoxide anions had no effect on their adhesion to untreated endothelial cells. Furthermore, the addition of SOD and/or catalase to ROS-exposed MEC decreased the enhanced tumour cell adhesion significantly, indicating that by tackling the production of ROS the prevention of tumour recurrence might be feasible not only locally, but also at distant sites.

Further Research

Minimising surgical trauma to the peritoneum should be the main goal in adhesion prevention, since this is the origin of adhesion formation. Furthermore, in case of oncological surgery, surgical trauma to the peritoneum promotes adherence of spilled tumour cells, which is a reason to minimise this trauma as well. As of yet no strategy has been discovered causing no trauma at all, however considerable steps forward have been made through minimally invasive surgery and the NOTES (Natural Orifice Transluminal Endoscopic Surgery) technique.

Data on laparoscopic versus open surgery with regard to adhesion formation as well as tumour recurrence is limited. Several experimental and clinical studies indicate that adhesion formation is reduced after laparoscopy, however most of these studies have limited numbers [22-26].
As for tumour recurrence, experimental studies have shown promising results of minimally invasive surgery on tumour implantation [27-29]. In the clinical setting, laparoscopic surgery seems to be of benefit with regard to recurrence and survival as well [30].

Laparoscopy as a single method to minimise peritoneal trauma is not enough. Carbondioxide, used as insufflation gas to establish pneumoperitoneum, causes dessiccation of the peritoneum which leads to peritoneal injury and hence adhesion formation [31]. This is the reason why it should be warmed and humidified [32, 33]. Being a relatively new approach, the NOTES technique has not been extensively evaluated with regard to peritoneal injury and its sequalae but the little data that is available indicate that it leads to less adhesion formation in an experimental model [34]. It is of high importance that comparative studies, preferably randomized clinical trials, are being set out in order to further investigate the effect of these techniques on adhesion formation and tumour recurrence.

Since peritoneal trauma probably always will occur in general surgery, the aftermath of the injury, being inflammation and fibrinogenesis, should be minimised as well. Influencing the inflammatory cascade by blocking PMN influx is hazardous [35] but scavenging the ROS they produce has shown reduced adhesion formation and tumourcell adhesion in experimental models [16, 18]. The use of ROS-scavengers in clinical practice should be evaluated after thorough exploration of possible side effects.

A pivotal role in adhesion formation is being taken by the enzyme plasmin and, through it's effect on the coagulation cascade, the balance between fibrinogenesis and fibrinolysis. Further unravelling of these pathways may lead to a more specific pharmacological treatment.
As is discussed in chapter 2, there is no satisfactory monotherapy available for adhesion prevention and tumour recurrence. Combining a more profound knowledge and understanding of pathophysiological pathways, minimally invasive techniques, pharmacological adjuvants and barriers (whether solid or soluble) is probably the key to success.

Also the ongoing research on human genetics could be of clinical importance. Analysis of pre-operatively taken bloodsamples in order to predict whether a patient is prone to form adhesions, and hence whether preventive strategies during surgery should be used, is not inconceivable in the future.

Clinical implications

For current clinical practice this thesis unfortunately does not provide *the* solution for the consequences of surgical peritoneal trauma. However, several recommendations can be made:
During abdominal surgery, minimising trauma to the peritoneum is of utmost importance. Laparoscopy seems to cause less adhesions and less tumour recurrence and should be the technique of choice if possible. When open surgery is inevitable, the use of gauze and sponges should be minimised and, if possible, be avoided. When the small bowel is moved out of the operating field, it should preferably be packed in an a-traumatic bag instead of gauzes. Suturing the peritoneum during closure of the abdomen should be abandoned.
Adjuvants are at best partially effective, if at all. The most promising irrigation-fluid at this time is Icodextrin (Adept ®), but solid evidence based on randomized clinical trials with appropiate numbers is lacking.
As for solid barriers, Seprafilm® causes less severe adhesions and less small bowel obstruction requiring re-operation. However it does not prevent adhesion formation.
Chronic abdominal pain as a suggested consequence of adhesion formation should not be treated by means of (laparoscopic) adhesiolysis.
Ultimately it is the surgeon who determines the rate of injury to the peritoneum in surgical procedures and who should be aware of that.

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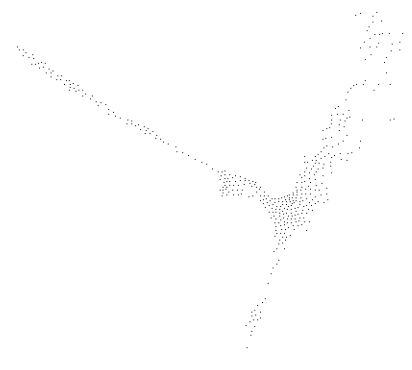
Nederlandse samenvatting



Het peritoneum (buikvlies) is een delicaat orgaan met een dikte van slechts één enkele mesotheliale cellaag. Om chirurgie in de buik mogelijk te maken moet het peritoneum geopend worden, hetgeen een ontstekingsreactie veroorzaakt die tot doel heeft de schade, ontstaan door en tijdens de operatie, te herstellen. Hoewel dit een fysiologische (normale) reactie is, kan dit leiden tot ongewenste neveneffecten zoals het ontstaan van adhesies (verklevingen). In geval van een oncologische ingreep (operatie in verband met een kwaadaardigheid) kan deze reactie leiden tot het ontstaan van metastasen (uitzaaiïngen) op het peritoneum en/of lokaal recidief van de tumor.

De incidentie van adhesies na abdominale chirurgie (buikchirurgie) varieert tussen de 85% en 100%. Vrijwel iedereen die buikchirurgie ondergaat, ontwikkelt dus adhesies. Adhesies kunnen leiden tot het ontstaan van streng-ileus (afklemming van de darm waardoor passage van voedsel onmogelijk wordt), vruchtbaarheidsproblemen bij vrouwen en bemoeilijkte toegang tot de buik in geval van een re-operatie. Er zijn ook aanwijzingen dat ze kunnen zorgen voor chronische pijnklachten na operaties. Na operaties aan het colon (dikke darm) bestaat er een kans van 30% dat een opname in het ziekenhuis in de eerstvolgende 10 jaar noodzakelijk is, ten gevolge van adhesies. Om deze ongewenste effecten van abdominale chirurgie te bestrijden en te verminderen, is het noodzakelijk het onderliggende ontstaansproces van adhesies en peritoneaal metastasen te begrijpen.

In **hoofdstuk 2** wordt beschreven dat adhesies en peritoneaal metastasen een gemeenschappelijke basis hebben, te weten de ontstekingsreactie. Ten gevolge van chirurgie komt het weefsel onder het peritoneum bloot te liggen, waardoor een genezingsrespons in gang wordt gezet. Deze respons wordt gekenmerkt door een toestroom van ontstekingscellen, vorming van een serosanguineus exsudaat (wondvocht) en een groei-respons van mesotheel cellen. Door de productie van cellulaire mediatoren worden ontstekingscellen, met name polymorfonucleaire granulocyten (PMN's), naar de wond getrokken. Deze produceren op hun beurt weer zuurstofradicalen, die betrokken zijn bij het opruimen van beschadigd weefsel. Naast dit positieve effect kunnen deze radicalen ook weer zorgen voor extra schade aan het peritoneum, waardoor de respons in stand wordt gehouden. Tegelijk met de ontstekingsreactie wordt door de macrofagen en mesotheelcellen Tissue Factor geproduceerd. Tissue Factor zorgt er voor dat de stollingscascade wordt geactiveerd. Dit proces behoort tot de normale weefselgenezing en zorgt voor het ontstaan van een fibrineuze matrix, wat uiteindelijk door littekenvorming zorgt voor weefselherstel.

Deze fibrinogenese wordt geremd door fibrinolyse (afbraak); normaal gesproken zijn deze twee processen in evenwicht. Na abdominale chirurgie echter is deze balans verstoord in het nadeel van de fibrinolyse, waardoor het fibrine onvoldoende wordt afgebroken en er uiteindelijk permanente adhesies kunnen overblijven. In het hoofdstuk worden verscheidene methoden beschreven die gehanteerd worden om het ontstaan van adhesies en peritoneale metastasen tegen te gaan.

Over de incidentie van adhesies bij gezonde personen zonder abdominale chirurgie in de voorgeschiedenis is weinig bekend. In de literatuur worden getallen aangehouden tussen de 2 en 28%. Om hierover meer te weten te komen zijn patiënten bestudeerd die geopereerd werden om een nier af te staan, waarbij gedurende deze ingreep de aanwezigheid van adhesies werd genoteerd. De resultaten van deze studie worden beschreven in **hoofdstuk 3.**
De donoren werden verdeeld in twee groepen, te weten een groep mét en een groep zonder abdominale chirurgie in de voorgeschiedenis. Het bleek dat bij 52% van de mensen zonder eerdere operatie adhesies voorkwamen, tegenover 64% in de groep die al wel eens geopereerd was. Opgemerkt dient te worden dat de "voorgaande chirurgie" voornamelijk relatief kleine ingrepen betrof (appendectomie, laparoscopische cholecystectomie), hetgeen een verklaring kan zijn voor de relatief lage incidentie van adhesies in deze laatste groep.
De hoge incidentie van adhesies in de groep zonder voorgaande operaties brengt een nieuw perspectief in de discussie over de relatie tussen adhesies en chronische buikklachten na buikchirurgie. Gedurende 1 jaar follow-up van alle patiënten was er geen verschil in pijnbeleving tussen beide groepen.
Hoewel bij 55% van alle patiënten adhesies werden gevonden had geen van deze patiënten pre-operatief (chronische) pijnklachten.

Om meer inzicht te krijgen in de veronderstelde relatie tussen chronische pijn na buikchirurgie en adhesies, en het nut van het opheffen ervan, werd een review van de bestaande literatuur op dit gebied verricht **(hoofdstuk 4)**. Uit de literatuur is reeds bekend dat laparoscopische adhesiolyse (het via een kijkoperatie opheffen van verklevingen) niet méér vermindering van klachten geeft dan een diagnostische laparoscopie (kijkoperatie waarbij alleen wordt gekeken). Desondanks zijn er eveneens studies die aangeven dat er zenuwbanen kunnen bestaan in adhesies en dat tractie aan het peritoneum door adhesies tot pijnervaring kan leiden. Na bestudering van de bestaande literatuur is de conclusie dat adhesies weliswaar pijnklachten kunnen veroorzaken, maar dat deze niet verbeteren na laparoscopische adhesiolyse.

Incidentie, ernst en locatie van adhesies zijn niet vaak beschreven in de literatuur omdat het beoordelen van deze gegevens een operatie noodzakelijk maakt. Echter, sommige ingrepen bestaan uit twee stappen, wat het mogelijk maakt effecten van anti-adhesie therapieën zonder extra operatie te beoordelen. De procedure volgens Hartmann biedt die mogelijkheid. Hierbij wordt een deel van de dikke darm verwijderd in combinatie met het aanleggen van een tijdelijk stoma. Dit stoma wordt na enige tijd tijdens een tweede operatie weer opgeheven.
Tussen 1996 en 1998 werd een onderzoek uitgevoerd naar het effect van het gebruik van Seprafilm®, een membraan dat in ongeveer 6 weken oplost en dat intra-abdominaal geplaatst wordt om adhesievorming tegen te gaan. Voor de eerste operatie werd geloot of er Seprafilm® achter zou worden gelaten of niet. Vervolgens werd tijdens de tweede operatie beoordeeld wat de ernst en de hoeveelheid van de adhesies was. Seprafilm® bleek voor minder ernstige, maar niet voor *minder* adhesies te zorgen.

Omdat een streng-ileus (darmobstructie ten gevolge van adhesies) soms pas 10 jaar na een operatie optreedt, werd een lange-termijn follow-up van de patiënten uit deze Seprafilm®-studie uitgevoerd **(hoofdstuk 5)**. Streng-ileus bleek niet minder vaak opgetreden te zijn in de groep patiënten waarbij Seprafilm® werd geplaatst. Het aantal patiënten dat aan de follow-up deelnam, was echter relatief laag waardoor uitspraken over deze uitkomst met terughoudendheid gedaan moeten worden. Binnen de controlegroep (dus zonder Seprafilm®) kwamen wel veel meer buikklachten (problemen met de stoelgang, misselijkheid, pijn) voor vergeleken met de behandelde groep. Een eenduidige verklaring voor dit verschil werd echter niet gevonden. Uit deze follow-up zou men kunnen concluderen dat er geen relatie bestaat tussen de ernst van de adhesies en het wel of niet optreden van een streng-ileus.

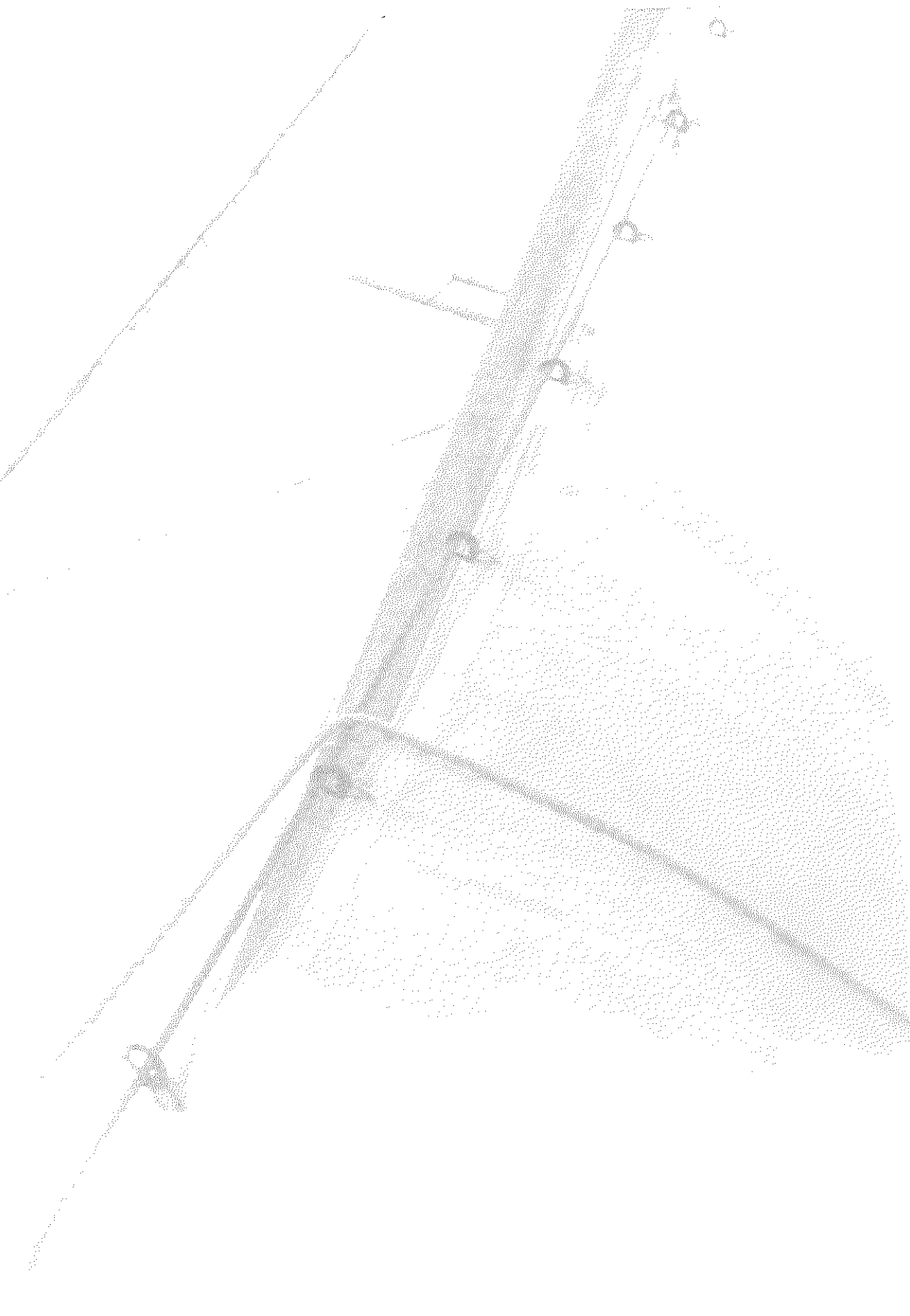
Om meer inzicht te krijgen in de pathofysiologie van het ontstaan van adhesies en tumorceladhesie zijn in het verleden verschillende experimenten uitgevoerd om te onderzoeken wat de invloed is van door PMN's geproduceerde zuurstofradicalen. Het is inmiddels bekend dat chirurgische schade aan het peritoneum zorgt voor een toename van het gehalte aan PMN's en dat het wegvangen van de zuurstofradicalen door zogenaamde scavengers kan leiden tot verminderde adhesievorming en verminderde tumorceladhesie. Getallen omtrent de concentratie van zuurstofradicalen voor en na chirurgie zijn echter niet bekend. In **hoofdstuk 6** wordt een experiment beschreven dat aantoont dat het meten van zuurstofradicalen middels spectrofotometrie (meten middels licht) mogelijk is en dat met name het gehalte aan H_2O_2 (waterstofperoxide) stijgt na chirurgisch

trauma. De metingen werden gedaan in bloedplasma en in peritoneaal lavage van ratten. Bij peritoneaal lavage wordt de buik gespoeld met zout water dat wordt teruggezogen en vervolgens geanalyseerd. In beide media was de stijging meetbaar tot 24 uur na operatie. Het toedienen van scavengers voorkomt deze waterstofperoxidestijging. Conclusie van dit onderzoek is dat pogingen om adhesies en tumorceladhesie te voorkomen middels het wegvangen van zuurstofradicalen moeten worden gedaan binnen 24 uur na de operatie.

Naast hun effect op de *lokale* processen van adhesievorming spelen zuurstofradicalen ook een rol bij het ontstaan van lokaal recidief in geval van oncologische chirurgie. In de experimenten beschreven in hoofdstuk 6 wordt duidelijk dat er een stijging van zuurstofradicalen plaatsvindt zowel in lavage als in plasma. Dit suggereert dat ze ook een rol zouden kunnen spelen bij het ontstaan van metastasen op afstand (uitzaaiingen buiten de buikholte). In **hoofdstuk 7** wordt dit verder onderzocht aan de hand van een *in vitro* model. Menselijk mesotheel werd blootgesteld aan tumorcellen, al dan niet na voorbehandeling met zuurstofradicalen. Deze blootstelling zorgde voor een toename van het aantal bindingsplaatsen op het mesotheel en dientengevolge een toename van aanhechting van tumorcellen, terwijl de met zuurstofradicalen voorbehandelde tumorcellen geen verhoogde adhesie lieten zien. Wanneer vervolgens scavengers werden toegevoegd, verminderde de aanhechting van tumorcellen aan het mesotheel, hetgeen suggereert dat door het bestrijden van de zuurstofradicaal-productie niet alleen een lokaal maar ook een systemisch effect bereikt kan worden.

In **hoofdstuk 8** worden de resultaten van het onderzoek besproken en afgezet tegen de huidige literatuur. De meest effectieve manier om adhesies te voorkomen is door ze niet te maken en dus niet te opereren. Aangezien dit binnen de chirurgie een *contradictio in terminis* is, moet de chirurg zorgen voor zo min mogelijk schade aan het peritoneum. Het lijkt erop dat laparoscopische ingrepen voor minder adhesies zorgen dan "open" procedures. Indien mogelijk moet dan ook een laparoscopische benadering worden toegepast. Als open chirurgie onvermijdelijk is moet het gebruik van gazen geminimaliseerd worden. Het dunne-darm pakket wordt bij voorkeur in een a-traumatische zak geplaatst in plaats van in buikgazen. Het sluiten van het peritoneum moet achterwege worden gelaten. Adjuvantia zijn op hun best deels effectief; als er al voor gekozen moet worden lijkt Icodextrin het meest werkzaam hoewel hard bewijs uit gerandomiseerde klinische studies met grote aantallen patiënten ontbreekt. Binnen de barriers lijkt Seprafilm® te zorgen voor minder ernstige adhesies en voor minder gevallen van ileus die geopereerd moeten worden. Het voorkomt

de vorming van adhesies echter niet. Chronische buikpijn als verondersteld gevolg van adhesies moet niet behandeld worden door (laparoscopische) adhesiolyse.
Tevens worden aanbevelingen gedaan voor toekomstig onderzoek, waarbij het uiteindelijke doel zou moeten zijn patiënten pre-operatief te screenen op hun individuele adhesie-risico. Vervolgens zouden dan passende maatregelen genomen kunnen worden voorafgaand, tijdens en na de operatie; echte maat-chirurgie dus. Dit lijkt nog ver weg, maar gezien de snelheid waarmee de wetenschap voortgaat moet dit binnen afzienbare termijn mogelijk zijn.



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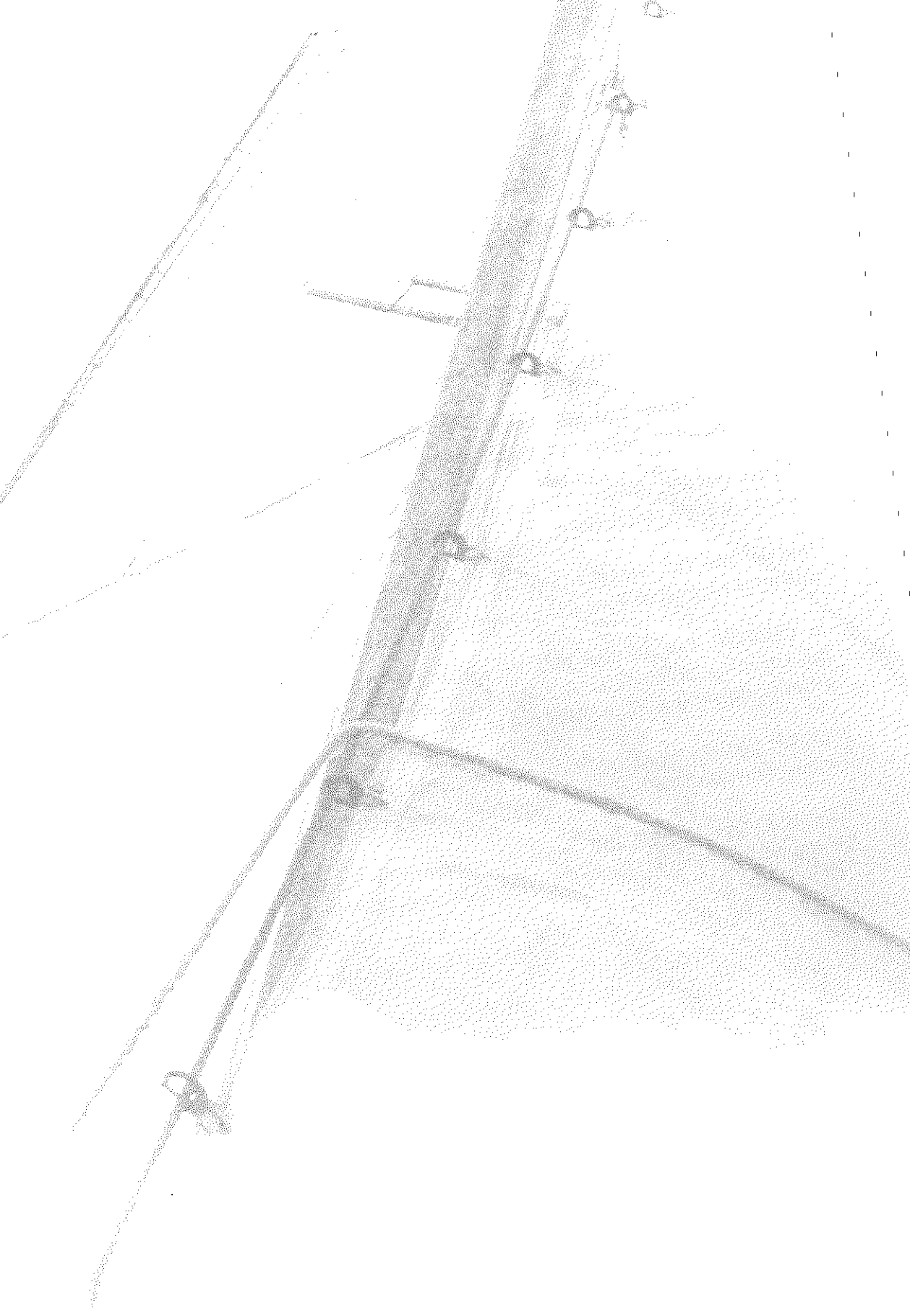
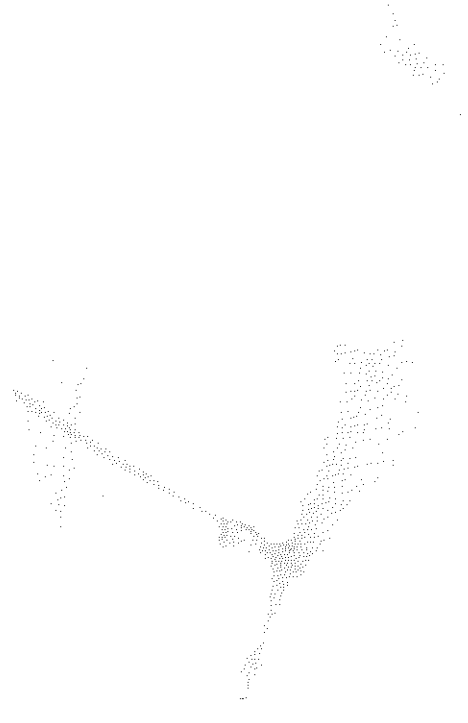


Dankwoord



Geen enkele promovendus staat alleen en ik dus ook niet. Dit proefschrift was niet tot stand gekomen als er om mij heen niet een aantal mensen was geweest die, bewust of onbewust, hun bijdrage hebben geleverd.
Eenieder wil ik daarvoor hartelijk danken, enkelen met name:

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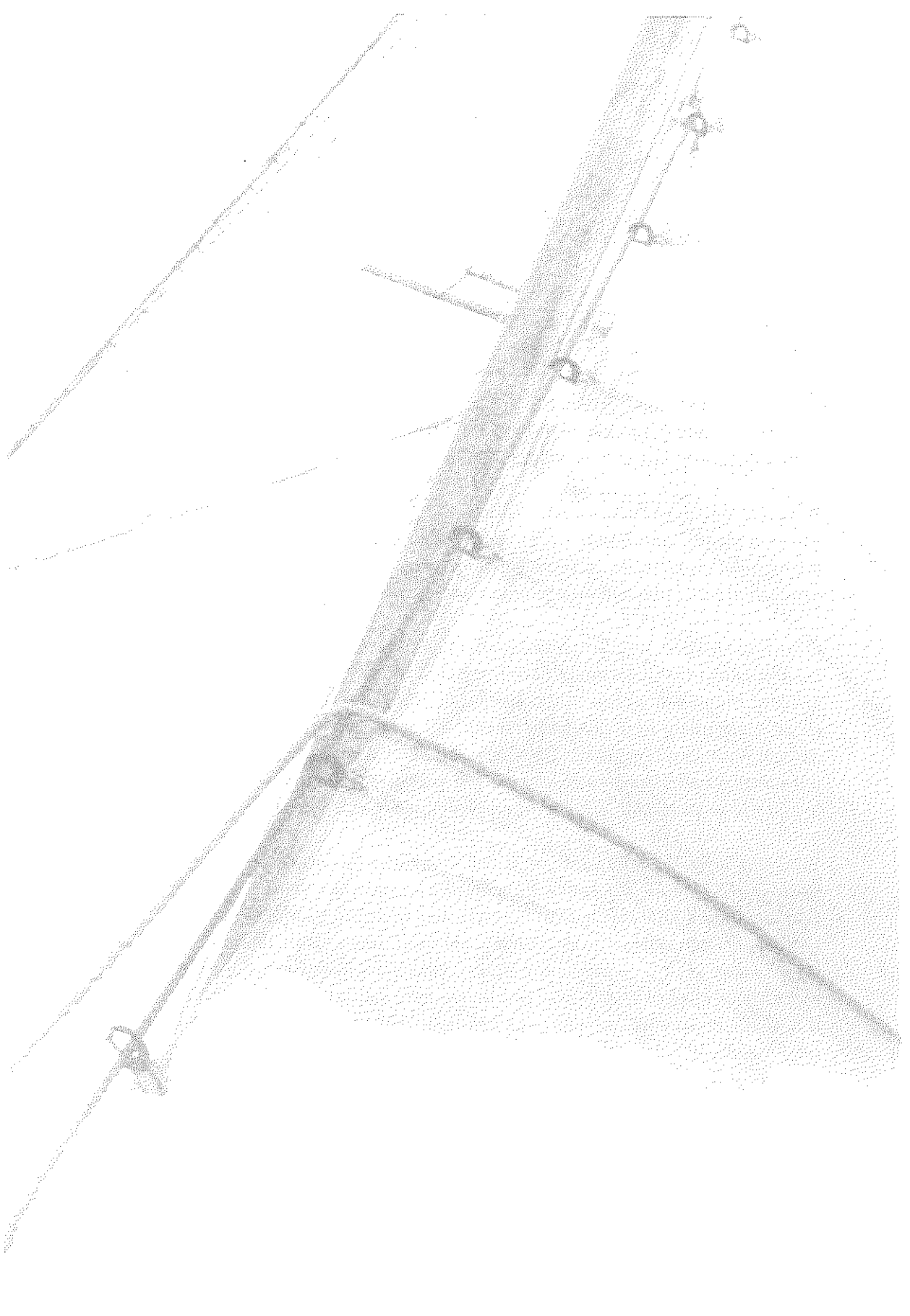
Ik hou verschrikkelijk veel van je en heb heel veel zin in onze toekomst. Je bent mijn meisje.



Curriculum Vitae Auctoris

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Johannes Bernard Christiaan (Hans-Christiaan) van der Wal werd geboren op 26 mei 1976 te Gorinchem. Van 1989 tot 1994 bezocht hij het Rudolf Steiner College (Vrije School) te Rotterdam, waarna hij in 1995 zijn VWO diploma behaalde aan het Mercurius College in Capelle aan den IJssel. Hij werd uitgeloot voor Geneeskunde en besloot Civiele Techniek te gaan studeren aan de Technische Universiteit van Delft. Daar werd hij lid van de Katholieke Studenten Vereniging Sanctus Virgilius. In 1996 werd hij ingeloot voor Geneeskunde aan de Erasmus Universiteit te Rotterdam. Tijdens het collegejaar 1999-2000 onderbrak hij zijn studie om zitting te nemen in het Dagelijks Bestuur der KSV Sanctus Virgilius. Nadien hervatte hij zijn studie en onder leiding van Dr. A. Struijs verrichte hij in 2003 zijn afstudeeronderzoek op de afdeling Anaesthesiologie en Thorax-IC naar *Open Long Concept vs conventionele beademing bij patiënten die CABG ondergingen en/of aan hartklepafwijkingen werden geopereerd en die daarbij afhankelijk waren van een extracorporele circulatie.*
In 2005 werd het artsendiploma behaald waarna hij startte als arts-onderzoeker op de afdeling Algemene Heelkunde van het Erasmus Medisch Centrum te Rotterdam onder leiding van Prof.dr. J. Jeekel en Prof.dr. J.F. Lange. Dat wetenschappelijk onderzoek heeft uiteindelijk geleid tot dit proefschrift.
Sinds juli 2007 is hij in opleiding tot algemeen chirurg in het TweeSteden ziekenhuis te Tilburg (opleider dr. S.E. Kranendonk). Per 1 juli 2011 zal hij zijn opleiding voortzetten in het Universitair Medisch Centrum Utrecht (opleider Prof.dr. I.H.M. Borel Rinkes). Hij woont samen met Anne in Utrecht.