Role of the peritoneal cavity in the prevention of postoperative adhesions, pain, and fatigue

Philippe R. Koninckx, M.D., Ph.D.,[a,b] Victor Gomel, M.D., Ph.D.,[c] Anastasia Ussia, M.D.,[b] and Leila Adamyan, M.D., Ph.D.[d,e]

[a] Department of Obstetrics and Gynecology, Catholic University Leuven, University Hospital, Gasthuisberg, Leuven, Belgium; [b] Gruppo Italo Belga, Villa del Rosario and Gemelli Hospitals Università Cattolica, Rome, Italy; [c] Department of Obstetrics and Gynecology, University of British Columbia, Women's Hospital, Vancouver, British Columbia, Canada; [d] Department of Operative Gynecology, Federal State Budget Institution V. I. Kulakov Research Center for Obstetrics, Gynecology, and Perinatology, Ministry of Health of the Russian Federation, Moscow, Russia; and [e] Department of Reproductive Medicine and Surgery, Moscow State University of Medicine and Dentistry, Moscow, Russia, Russian Federation

A surgical trauma results within minutes in exudation, platelets, and fibrin deposition. Within hours, the denuded area is covered by tissue repair cells/macrophages, starting a cascade of events. Epithelial repair starts on day 1 and is terminated by day 3. If repair is delayed by decreased fibrinolysis, local inflammation, or factors in peritoneal fluid, fibroblast growth starting on day 3 and angiogenesis starting on day 5 results in adhesion formation. For adhesion formation, quantitatively more important are factors released into the peritoneal fluid after retraction of the fragile mesothelial cells and acute inflammation of the entire peritoneal cavity. This is caused by mechanical trauma, hypoxia (e.g., CO_2 pneumoperitoneum), reactive oxygen species (ROS; e.g., open surgery), desiccation, or presence of blood, and this is more severe at higher temperatures. The inflammation at trauma sites is delayed by necrotic tissue, resorbable sutures, vascularization damage, and oxidative stress. Prevention of adhesion formation therefore consists of the prevention of acute inflammation in the peritoneal cavity by means of gentle tissue handling, the addition of more than 5% N_2O to the CO_2 pneumoperitoneum, cooling the abdomen to 30°C, prevention of desiccation, a short duration of surgery, and, at the end of surgery, meticulous hemostasis, thorough lavage, application of a barrier to injury sites, and administration of dexamethasone. With this combined therapy, nearly adhesion-free surgery can be performed today. Conditioning alone results in some 85% adhesion prevention, barriers alone in 40%–50%. (Fertil Steril® 2016;106:998–1010. ©2016 by American Society for Reproductive Medicine.)

Key Words: Surgery, adhesion prevention, peritoneum, conditioning, endometriosis

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Intraperitoneal adhesions after surgery occur in 80%–90% of women (1). They are a major cause of postoperative pain, infertility (2), small bowel obstruction (3, 4), and surgical reinterventions which cause a huge cost for the society (5–10), as recently confirmed (11–13).

The pathophysiology of adhesion formation has been considered to be a local healing process of opposing surgical lesions (14–17), and prevention was based on barriers. Over the past decade the important role of the entire peritoneal cavity in this process became apparent, and the combined treatment of the peritoneal cavity during surgery together with application of a barrier after surgery resulted in nearly adhesion-free surgery, which provides the additional benefits of less postoperative pain and a faster recovery (18).

This prompted us to review the role of the peritoneal cavity in the pathophysiology and in the prevention of postoperative adhesion formation.

MATERIALS AND METHODS

The United States National Library of Medicine (www.pubmed.com) from 2000 to May 2016 was searched for "postoperative adhesions" OR "peritoneal adhesions" (n = 4,826) together with factors in peritoneal cavity by adding "peritoneal cavity" (n = 257 of which seven relevant), "cooling" (n = 5), "temperature" (n = 39), or "N_2O" (n = 2). Because relevant articles could not be identified the 2,058 articles on adhesions since 2010 were hand searched.

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Reprint requests: Philippe R. Koninckx, M.D., Ph.D., Vuilenbos 2, B 3360, Bierbeek, Belgium (E-mail: pkoninckx@gmail.com).

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THE PERITONEAL CAVITY IS AN ORGAN

The peritoneum has a large surface of 1.7 m^2 and is composed of a single layer of mesothelial cells, a basal membrane, and some loose connective tissue resting on the underlying tissues with blood vessels and lymphatics. The mesothelial cells facilitate gliding of bowels by means of microvilli, glycosaminoglycans, and surfactant.

The intact peritoneal cavity is a virtual cavity similar to the inside of mouth and bowels. The peritoneum used to be considered a semipermeable membrane with fast diffusion of fluid and small molecules but limited diffusion of larger molecules. Indeed, the concentrations of smaller blood proteins, such as albumin, LH, and FSH, are 40% lower than in plasma, whereas larger molecules, such as factors V and VIII, are virtually absent, preventing thrombin activation. Locally secreted macromolecules as CA125 and glycodelins accumulate with concentrations much higher than in plasma (19–24). Mesothelial cells also actively regulate the exchange with vessels and extracellular spaces by means of gap junctions and vesicular transport. Surprisingly, the intact mesothelial layer also actively inhibits the diffusion of gases such as CO_2 (25, 26) and N_2O (unpublished observations). In women, the peritoneal fluid also contains transudation from growing ovarian follicles, increasing the volume and the concentrations of sex steroid hormones. The peritoneal cavity thus is a microenvironment with specific concentrations of hormones, cytokines, growth factors, cellular components such as macrophages, natural killer cells, and lymphocytes, and a specific immune system. Peritoneal fluid circulates clockwise, explaining a higher incidence of endometriosis on the left side. The peritoneum also has specific pain receptors (27, 28). The peritoneal cavity therefore has to be considered as a separate active organ and not a passive container (29, 30).

The large flat mesothelial cells react within seconds to minor trauma, such as exposure to air, by retraction resulting in bulging of cells and consequent direct exposure of the basal membrane (31). This retraction is so rapid that in vivo fixation is necessary to study the intact mesothelial layer. This retraction increases over time (25, 26). Identified traumas are mechanical trauma, exposure to a CO_2 pneumoperitoneum (32, 33), desiccation, infection, and chemical irritants. Normal saline solution detaches the mesothelial cells after 30 minutes with a loss of fibrinolytic activity (34). This "toxicity" was confirmed recently in vitro (35–37) and in vivo (38). Within hours transforming growth factor β increases and tissue plasminogen activator decreases. By retraction, the contiguous mesothelial cells are transformed into individual cells which causes passive diffusion through the exposed basal membrane (31, 39–42) and initiates acute inflammation (43). The peritoneal cavity thus becomes part of the body, which is an efficient defense mechanism against infection via recruiting immunoglobulins and macrophages. Similarly the decreased bowel motility helps to keep an infection localized. This retraction and the subsequent passive diffusion also explains the increasing resorption of CO_2 (25, 26) requiring increasing ventilation during laparoscopic surgery.

PATHOPHYSIOLOGY OF ADHESION FORMATION

Repair and Adhesion Formation after Surgical Trauma of the Peritoneum

The repair of a peritoneal injury with damage of the basal membrane and the subendothelial connective tissue is a strictly timed process (Fig. 1). Within minutes, with onset of acute inflammation and activation of the coagulation cascade, platelets attach and coalesce over the lesion. The increased blood flow, dilation of arterioles, increased permeability of the capillaries, and migration of neutrophils and macrophages onto the lesion forms a fibrin mesh. Within hours, the lesion is covered with macrophages and/or "tissue repair cells" which acquire enhanced fibrinolytic activity (34). These cells start to remove cell debris while platelet-derived growth factors activate migration and proliferation of fibroblast/mesothelial cells. Visible mesothelial cell growth starts after 24 hours, followed by fibroblast proliferation on day 3 and angiogenesis on day 5. It is unclear whether these cells are fibroblasts, macrophages, or stem cells (44–47) and whether they originate from the peritoneal fluid, the mesothelium, the submesothelial connective tissue, the vascular endothelium, or blood cells (47–51). Mesothelial cells and the endothelial and hematopoietic cells are derived from a common progenitor cell originating embryologically in the splanchnic mesothelium (52).

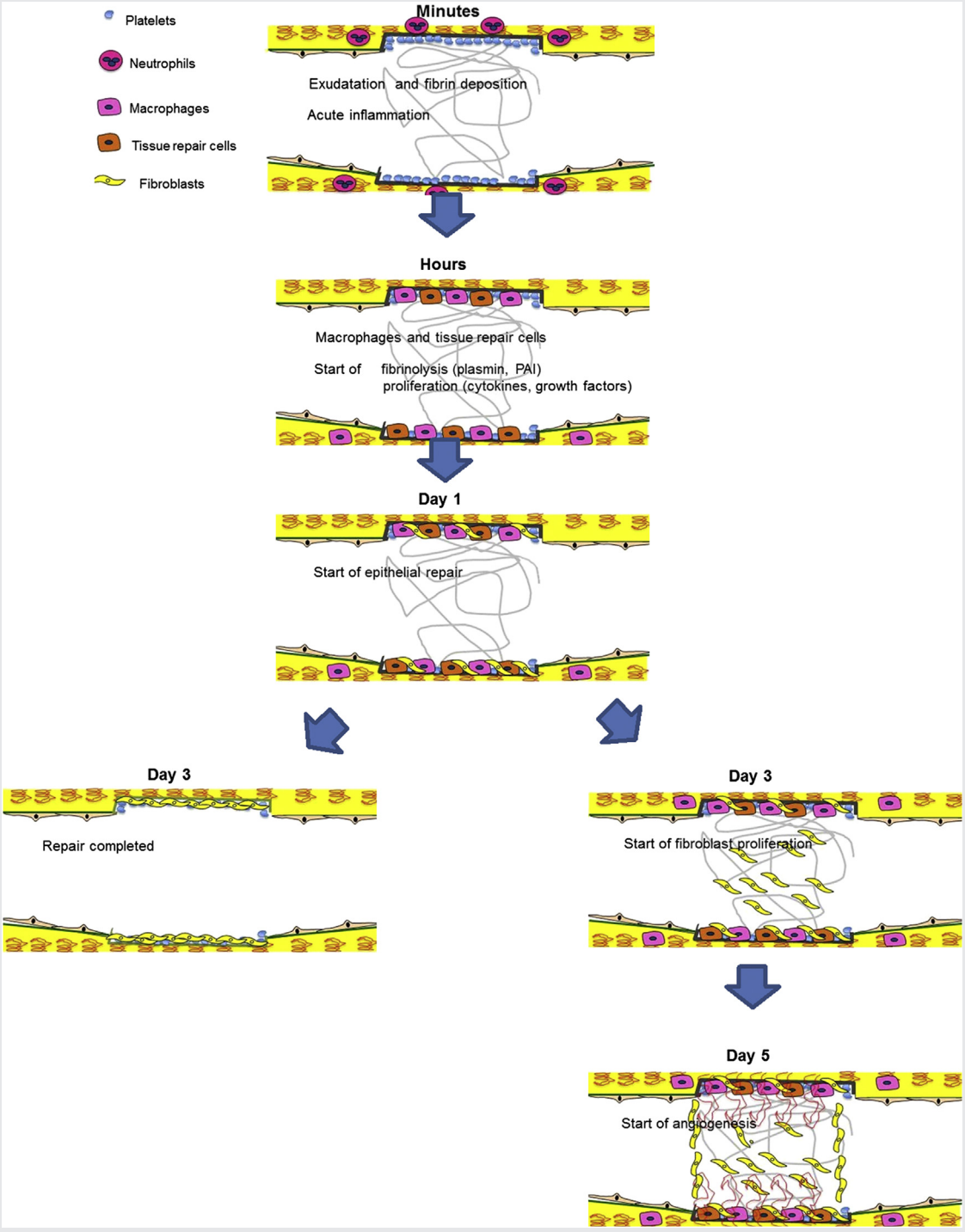
Opposing lesions attach with a fibrin mesh. In the absence of bowel movements, nonopposing lesions can attach as well, because the inflammatory process damages the fragile mesothelial cells of opposing organs. The time courses of repair and cell proliferation explain why the speed of fibrinolysis (53) that breaks this fibrin attachment determines whether the healing results in repair (54) or adhesion formation. If fibrinolysis is fast, the mesothelial repair, starting on day 1 from numerous small islands (55), is completed within 3 days, i.e., before the fibroblast and angiogenetic proliferation processes become fully activated. This also explains why repair of small and large areas is equally rapid (56).

If this repair mechanism is not completed by day 3, the proliferating fibroblasts invade the fibrin scaffold, which together with angiogenesis starting on day 5 leads invariably to adhesion formation. Repair can be delayed by local factors such as a decreased fibrinolysis, presence of necrotic tissue, tissue ischemia, and oxidative stress secondary to vascular damage or sutures, and by infection (57). Furthermore, the injured area may be shielded from the blood stream through vascular damage, and from the peritoneal fluid by the fibrin plug. A drug therefore would have difficulty reaching the injured area. This explains why the effect of tissue plasminogen activator, administered intraperitoneally after a minor surgical trauma, varies from poor (58) to 40% effective (59).

Factors in Peritoneal Fluid Enhance Adhesion Formation at Surgical Lesion Sites

The mesothelial cell retraction (31, 39–42) and the acute inflammation (43) in the entire peritoneal cavity release substances into the peritoneal fluid that delay the local

FIGURE 1



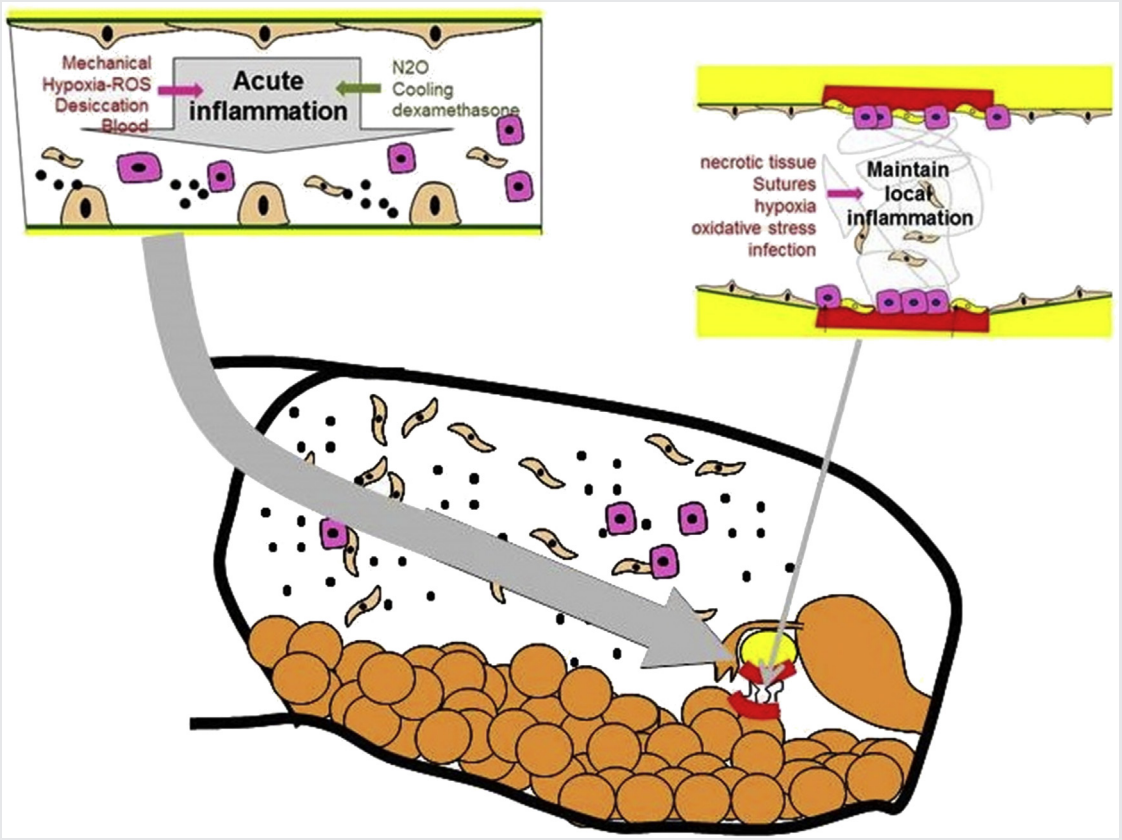
Time course of peritoneal repair and adhesion formation. Within minutes, exudation, fibrin deposition, and acute inflammation starts; within hours, the surgical lesion is covered by "macrophages," and day 1 repair starts to be completed by day 3. If repair and/or fibrinolysis are delayed, fibroblast proliferation and angiogenesis lead to adhesion formation.

Koninckx. Peritoneal cavity and adhesion prevention. Fertil Steril 2016.

repair and enhance adhesion formation (Fig. 2) (60). In a laparoscopic mouse model, adhesions between opposing lesions in the lower abdomen increase proportionally to the duration of bowel manipulation in the upper abdomen (61, 62).

The severity of the acute inflammation in the entire peritoneal cavity and the associated enhanced adhesion formation is the sum of the duration and intensity of all the factors that damage the mesothelial cells. The mechanical trauma caused by manipulation explains the decrease in the rate of adhesions

FIGURE 2



Pathophysiology of adhesion formation. Repair can be delayed by factors that maintain local inflammation, such as necrotic tissue, sutures, hypoxia, oxidative stress, and infection. Even more important are factors derived from retraction and bulging of mesothelial cells and acute inflammation in the entire peritoneal cavity. Bad factors are mechanical trauma, hypoxia, reactive oxygen species (ROS), desiccation, and blood. Beneficial factors are N_2O, cooling, and dexamethasone.

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with increased surgical experience, as shown in learning curves in rabbits and in mice (63, 64). CO_2 pneumoperitoneum causes superficial mesothelial hypoxia (32, 33, 65–67). Indeed, the CO_2 pneumoperitoneum–enhanced adhesion formation is absent in mice deficient of one or both of the two hypoxemia-inducible factors, HIF-1α and HIF-2α. The severity of the effect increases exponentially when partial oxygen concentrations are below 7 mm Hg (or 1% of oxygen), which is consistent with the affinity of O_2 for the HIF receptor. The effect increases with the intraperitoneal pressure (68) and with duration of exposure (33, 65). The mechanism of mesothelial hypoxia was indirectly confirmed by the beneficial effects of increasing partial oxygen tension in blood either by increasing the oxygen concentration during ventilation (69) or by adding 4% O_2 to the CO_2 pneumoperitoneum (32), which is similar to the physiologic partial oxygen pressures of ~30 mm Hg in peripheral cells. At low insufflation pressures this effect is minimal or absent (70). Acidosis (71) has a minor role only. It is unclear to what extent the effect of hypoxia is also mediated by oxidative stress, because hypoxia decreases oxygen scavengers. Partial oxygen pressures higher than 70 mm of Hg are deleterious (72, 73), and the effect increases with the duration and height of partial oxygen tensions (74). This mechanism involving ROS was confirmed by the preventive effect of ROS scavengers (75), such as superoxide dismutase, catalase, melatonin, and ascorbic acid. Desiccation (76) enhances adhesion formation, and the effect increases with the severity of desiccation. Red blood cells and/or fibrin (77) are strongly adhesiogenic, probably by increasing (acute) inflammation.

The severity of this enhanced adhesion formation increases with temperature, especially above 37°C, and decreases exponentially at lower temperatures down to 25°C (78). In mice, 80% of the beneficial effect of cooling is obtained at 31°C. The beneficial effect of cooling was confirmed by irrigation with the use of cool saline solution (79), with a maximal effect at 15°C (80). A lower intra-abdominal temperature slows down metabolism of the mesothelial cells as well as retraction, ROS damage, and acute inflammation.

In addition, angiogenic factors are important because CO_2 pneumoperitoneum–enhanced adhesion formation is absent in placental growth factor (PlGF) or vascular endothelial growth factor (VEGF) knockout mice and can be prevented with the use of monoclonal antibodies (67, 81). CO_2 pneumoperitoneum–enhanced adhesion formation is also strongly decreased in plasmin activation inhibitors 1 and 2 knockout mice (82). These effects vary with the genetic constitution of mouse

strains (83), which is consistent with genetic differences in adhesion formation in the human (84, 85).

It is important to note that these factors do not damage the basal membrane, because in none of these experiments were de novo adhesions observed outside of surgical lesion sites. In the human, it is unclear whether the acute inflammation of the peritoneal cavity is present in the entire peritoneum or is limited to the areas in direct contact with the noxious stimuli.

Integrated Model of Adhesion Formation

Surgical trauma results in local inflammation, with fibrin deposition; if fibrinolysis occurs rapidly, the lesion will be repaired within a few days. If repair is delayed, either by local factors or by factors associated with the acute inflammation of the entire peritoneal cavity, adhesions will be formed.

In animal models with mild surgical lesions, the severity of adhesion formation results mainly from currently unknown factors in the peritoneal cavity. This is quantitatively most important and is some 20 times more important than the surgical lesion itself. It is unclear whether this remains true for more severe surgical traumas with more necrotic tissue, local devascularization, and oxidative stress.

The relative importance of the immune system in adhesion formation remains unclear. The immune system may be depressed after difficult and/or extensive surgery (86). In animal models, adhesion formation may be enhanced by transferring activated macrophages (87). Also, the role of ischemia-reperfusion trauma causing oxidative stress at the lesion site and in the entire peritoneum is unclear (88).

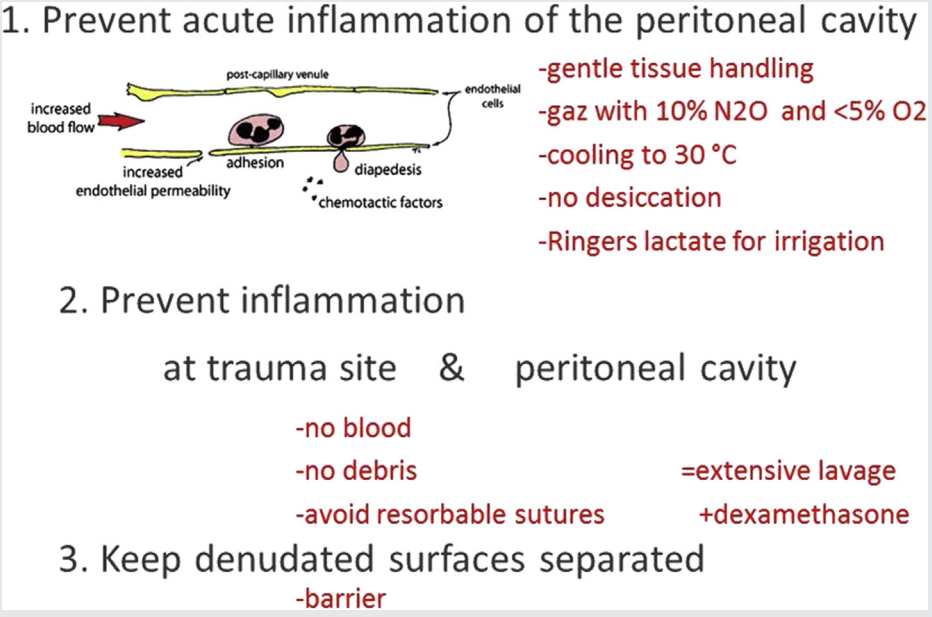
Little is known about the mechanisms of adhesion maturation and the nature of adhesions that result: velamentous or thick vascularized, and/or innervated (89–91).

Prevention of Postoperative Adhesion Formation Based on Pathophysiology

Keep opposing lesions separated for five days. The milestone observation that adhesion formation decreased when injured areas were kept separated by silastic membranes for 30 hours (92) resulted in a series of solid and semisolid barriers for human use (54, 93). They reduce adhesion formation by 40%–50% but with high interindividual variability. Efficacy for clinical end points such as pain, infertility, bowel obstruction, and reoperation rate has not been proven. Because these trials were performed in rather simple surgical procedures, such as ovariectomy and cystectomy, with a limited peritoneal cavity–enhanced adhesion formation, it is unclear to what extent the available results of efficacy can be extrapolated to more extensive procedures. It also remains unclear to what extent these barriers are effective by preventing peritoneal fluid from reaching the surgical lesions and to slow down repair. Moreover, it is unclear to what extent they increase the inflammatory reaction, as evidenced in mice (94) and suggested by increased C-reactive protein concentrations in humans (unpublished observation).

Separation of denuded or damaged surfaces and temporary ovarian suspension for ~10 days after adhesiolysis to avoid

FIGURE 3



Prevention of adhesion formation based on pathophysiology. Steps 1 and 2 result in 85% adhesion prevention. Together with step 3, adhesion prevention becomes close to 100%.

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adherence was practiced already in 1968 (95) and was one of the tenets of microsurgery (96). More recently, temporary ovarian suspension after ovarian or deep endometriosis surgery was demonstrated to reduce ovarian adhesion formation (97).

The peritoneal cavity is also important to understand the role of flotation agents. Lactated Ringer solution is slightly but significantly effective in a mouse model (98) of CO_2 pneumoperitoneum–enhanced adhesion formation. In human surgery, the efficacy of lactated Ringer solution remains debated, but this might have to be reconsidered because retention time of lactated Ringer solution is much longer in women with minimal mesothelial damage (99). Also, the use of Adept should be critically reconsidered. Efficacy was low in both a Cochrane review (100) and the GENEVA trial (101). This is not surprising, given that Adept has an exponential clearance from the peritoneal cavity, with little volume remaining after 24 hours, similarly to lactated Ringer solution (99); the claimed long peritoneal retention time is probably not correct because it was observed in only nine patients undergoing intraperitoneal 5-fluorouracil treatment for colorectal cancer (102).

Increase bowel motility. It seems logical, but is without direct experimental evidence, that bowel motility during the first days after surgery would break weak fibrin attachments and thus reduce adhesion formation.

Decrease the duration and severity of local inflammation at surgical lesion sites. Resorbable sutures, which maintain inflammation for more than 5 days, increase adhesion formation (101). Clinical observation suggests that local bleeding enhances adhesion formation, although this may also be the result of blood leaking into the peritoneal cavity. Necrotic tissue will obviously prolong inflammation, and decreased vascularization due to excessive coagulation or sutures will increases oxidative stress and thus adhesion formation (16).

Prevent mesothelial cell trauma and acute inflammation of the entire peritoneal cavity. N_2O in concentrations higher than 5% is the single most effective prevention of peritoneal cavity–enhanced adhesion formation in a laparoscopic mouse model (77). A half-maximal effect at 2.5% (unpublished results) and a maximal effect from 5% onward suggest an unknown drug-like effect of N_2O. In humans, the use of 100% of N_2O for the pneumoperitoneum instead of CO_2 was known to be less painful during surgery, permitting laparoscopy under local anesthesia (103, 104) After surgery pain was much less when pure N_2O had been used (105, 106). In the human we confirmed that the addition of 10% of N_2O to the CO_2 pneumoperitoneum was equally effective as 100% N_2O in reducing pain during laparoscopy under local anesthesia (107). The use of 10% N_2O and 4% of O_2 in CO_2 also reduced postoperative pain and adhesions in a recent myomectomy trial (108).

The second most effective prevention is a lower intraperitoneal temperature. Cooling to 30°C is suggested because at that temperature in mice 80% of the benefit is obtained (75, 78, 109). In humans, the abdomen can be cooled to 30°C without side effects and without affecting the core body temperature (110), as applied in a trial discussed subsequently.

The quantitative effects of the other factors on adhesion prevention are less pronounced although significant. The beneficial effect of gentle tissue handling as demonstrated in animal models (61, 64) is expected to be more important in human surgery, which is of longer duration. In mice, prevention of desiccation with the use of humidification decreases adhesion formation even in the absence of the associated cooling (76). In humans, the effect of desiccation is difficult to interpret, because all studies compare humidified CO_2 at 37°C with cold and dry CO_2. The addition of 4% of O_2 to the CO_2 pneumoperitoneum prevents mesothelial hypoxia and slightly reduces pneumoperitoneum-enhanced adhesion formation in mice (33). The decrease of oxidative stress with the use of ROS blockers, such as ascorbic acid, is logical but has been demonstrated in animal models only (71).

Blood and remaining fibrin should be removed from the peritoneal cavity by means of lavage, because blood, both red blood cells and plasma (77), are highly adhesiogenic. The efficacy of heparin in the irrigation fluid during surgery to decrease fibrin deposits has not been experimentally tested. For lavage during and after surgery, a more cell-friendly fluid, such as lactated Ringer solution, instead of saline solution seems preferable, given the toxicity of saline.

Dexamethasone, given at the end of surgery, decreases adhesion formation by 30%, at least in mice with minimal peritoneal trauma or full conditioning. The mechanism could be a direct effect upon acute inflammation or by slowing down fibroblast proliferation (75). Surprisingly, cyclooxygenase 1 and 2 inhibitors and anti–tumor necrosis factor α were ineffective in preventing acute inflammation–enhanced adhesion formation (75).

Other factors. The administration of LHRH agonists before surgery in women was observed to decrease adhesions (111), but this was probably a consequence of reduced bleeding during surgery.

In nonhuman animal models, several drugs were described to reduce adhesion formation, although the mechanism of action is unclear. These include calcium channel blockers (75), cholesterol-lowering drugs such as atarvastin (112), losartan and angiotensin II receptor blockers (112), and pirfenidone, which has antiinflammatory effects (113). Adhesion formation can be reduced by 30% by adding mesothelial cells at the end of surgery (114, 115), or phospholipids (116–120), which facilitate gliding of bowels. Prevention of angiogenesis also reduces adhesion formation, as demonstrated by the administration of anti-VEGF and anti-PlGF monoclonal antibodies (67, 121). Viscous liquids (122) decrease adhesion formation, especially if applied before surgical manipulation (123), likely as a result of a decrease in mechanical trauma.

ADHESION PREVENTION STRATEGY IN HUMANS

Prevention of Adhesion Formation

Given the different mechanisms involved, adhesion prevention should address all of the factors involved (Fig. 3) (124).

During surgery I: good surgical practice with minimal direct trauma, little bleeding, and no infection. Adhesion

prevention starts with gentle tissue handling, precise hemostasis with minimal manipulation and grasping, and a short duration of surgery, such as skilled surgeons do. Liberal use of coagulation should be avoided, because it leaves more necrotic tissue.

Sterile conditions during surgery are obviously required, given the strong adhesiogenic effect of infection. The beneficial effect of prophylactic antibiotics can not be proven, because infections are rare. Common sense, however, suggests that it is wise to use them whenever the risk of infection is increased. This, and the use of surgical masks, also applies to laparoscopic surgery, which erroneously is often considered to be a semisterile intervention.

Bleeding during surgery and remaining blood or fibrin should be minimal (77) by means of a combination of surgical skill, knowledge of anatomy, and meticulous hemostasis. Saline solution for irrigation should be avoided (34–38) and replaced by a fluid as lactated Ringer solution. It is unclear whether adding heparin to the solution (96) is beneficial.

During surgery II: peritoneal conditioning to prevent mesothelial cell retraction and acute inflammation. In nonhuman animal laparoscopic models, the severity of acute inflammation and of adhesion formation is minimal when, in order of importance, more than 5% of N_2O is added to the pneumoperitoneum, the abdominal cavity is cooled to 30°C, and desiccation is prevented. The addition of a 2%–4% of O_2 is not or at best marginally beneficial.

In humans, the peritoneal cavity can be cooled to 30°C by sprinkling 2–3 mL/min lactated Ringer solution at room temperature. The gas used for insufflation can not be used for cooling, because a gas at lower temperature will be heated in the cavity, causing unavoidable desiccation (110). Ideally, use of a slightly warmer humidified gas, at 32°C, would cause condensation on entrance into an abdomen at 30°C, which would completely prevent desiccation.

A shorter duration of surgery is preferable because adhesions increase with the duration of surgery (101). It is unclear whether the duration of surgery alone remains important when mesothelial damage and acute inflammation can be completely prevented.

At the end of surgery I: lavage to remove debris, infection, and blood. Meticulous lavage to remove blood and foreign material is suggested with the use of a fluid such as lactated Ringer solution. It is suggested that lavage should include the upper abdomen if it is contaminated with blood or debris and continued until the liquid is clear. Indeed, after full-thickness resection or deep endometriosis, significantly lower postoperative C-reactive protein concentrations were found after lavage with 8 L versus 0.5 L, suggesting less inflammation (125). This obviously also applies to all situations with an increased risk of infection, such as when the vagina has been opened, when the bowel is entered, or in the presence of an abscess.

Evidence also indicates that lavage with the use of disinfectants should not be used, because these are invariably toxic to the peritoneal cells.

At the end of surgery II: dexamethasone. Dexamethasone at the end of surgery is suggested because it is effective in nonhuman animal models (126) and because of the absence of negative side effects in humans. Because effectiveness could be demonstrated only in mice in which mesothelial damage had been prevented, it is not surprising that in human surgery without peritoneal conditioning, effectiveness is difficult to prove. Since, after conditioning, intraperitoneal retention time of Ringers lactate is longer and similar to Adept (99), use of 300–500 ml of Ringers may be considered instead, as practiced in microsurgery.

At the end of surgery III: application of a barrier. Barriers alone decrease postoperative adhesions by 40%–50% in humans. In nonhuman animal models, conditioning alone reduces adhesion formation by ~85%, and if in addition a barrier is used, adhesion prevention reaches close to 100%.

Proof-of-concept randomized controlled trial in humans. To test the concept that peritoneal conditioning during surgery, use of a barrier, and administration of dexamethasone at the end of the intervention would significantly decrease postoperative adhesions, a randomized controlled trial was performed in women who underwent laparoscopic excision of deep endometriosis, excluding full-thickness bowel resection (18). The surgical technique was as described previously (127). In the control group (n = 11), humidified CO_2 at 37°C was used for the pneumoperitoneum. In the treatment group (n = 16), the insufflation gas consisted of a mixture of 86% CO_2, 10% N_2O, and 4% O_2; the abdomen was actively cooled to 30°C by means of sprinkling 2 mL/min saline solution at room temperature; and desiccation was prevented with the use of humidified gas at 32°C, which caused some condensation on entrance into the abdomen. At the end of the surgery, Hyalobarrier gel was applied over the surgical site and 5 mg dexamethasone was given intramuscularly. At second-look laparoscopy, the control group had important adhesions, whereas the treated group had virtually no adhesions. Because of the unprecedented efficacy, this trial confirmed the hypothesis and benefits of using conditioning together with the other cited measures. The relative importance of each factor, however, remains to be explored. Moreover, because saline was used in the trial, results might even be better with lactated Ringer solution.

Conclusion. Prevention of adhesion formation starts with a proper indication, a skilled and knowledgeable surgeon, a short duration of surgery, little bleeding, little tissue hypoxia, little oxidative stress, and strictly aseptic conditions. For laparoscopic surgery, 5%–10% N_2O should be added to the CO_2 pneumoperitoneum; during surgery, the abdomen should be cooled to 30°C as described to prevent desiccation. At the end of surgery, extensive lavage with lactated Ringer solution, eventually with some heparin, should be performed, a barrier should be applied, and 5 mg dexamethasone should be administered to the patient. With this approach, surgery may become virtually adhesion free. This also conveys to the patient the additional benefits of less pain and faster recovery. In addition, the patient will have less CO_2 resorption during surgery (18) and eventually less tumor cell implantation, as demonstrated in mice (128).

During open surgery in mice the same principles were found to be valid (unpublished observation) as suggested previously for humidified CO_2 (129). To avoid ROS caused by the 20% O_2 in ambient air, humidified CO_2 with 5%–10% N_2O at

room temperature should be instilled deep into the abdomen. Because both gases are heavier than air they can progressively flood the operating field, like operating in an aquarium.

Prevention of Adhesion Reformation after Adhesiolysis

The rate of adhesion reformation after adhesiolysis is high (130), and the reduction of pain is not significant (131). It has been recommended to excise adhesions instead of simply dividing them, because fibroblast cultured from adhesions are different from fibroblasts from a normal peritoneum (16, 57, 132–135). Specific products to prevent adhesion reformation are being explored but are not yet clinically available (136).

The Broader Picture: Prevention of Postoperative Pain, Ileus, and Postoperative Fatigue Syndrome

Pain after surgery consists of somatic pain from the skin and abdominal wall incision(s) and visceral pain via specific nociceptors and specific neurotransmitters (27, 28). At rest, these nociceptors are minimally reactive to a stimulus. Acute inflammation of the pelvic cavity results in a rapid recruitment and activation of the >90% dormant nociceptors which become more reactive to any stimulus. Thus, bowel movements start hurting and paralytic ileus may be considered to be a defense mechanism to reduce pain. The time to first flatus after surgery was suggested to reflect the severity and duration of the peritoneal inflammatory reaction. It therefore becomes logical to postulate that conditioning would decrease the duration of hospitalization, as demonstrated for a shorter duration of surgery (137). The intraperitoneal administration of local anesthetics is not a solution, because it reduces pain for only 6 hours (138, 139), although it may facilitate earlier ambulation.

Postoperative fatigue (140–142) after an abdominal intervention can last for up to 3 months in ~30% of patients. The mechanism is poorly understood but most authors relate postoperative fatigue to a prolonged peritoneal inflammation (143).

Therefore all evidence today points to an interrelationship between duration of surgery and postoperative ileus, pain, depression of fibrinolysis (144), prolonged recovery, adhesion formation, and postoperative fatigue with "acute inflammation and/or inflammation of the peritoneal cavity" as the common denominator.

DISCUSSION AND CONCLUSION

The power of clinical observation and judgment, is emphasized by the striking similarity between our actual understanding of the prevention of adhesion formation and the empirical recommendations made by the pioneers of microsurgery in the mid-1970s. The concept was based on common sense and clinical observation and comprised use of delicate instruments, an atraumatic technique, and glass rods to manipulate. Minimal tissue damage by electrical or laser energy was the aim, and pinpoint hemostasis with a microelectrode or micro-bipolar forceps was introduced. Continuous irrigation with the use of heparinized lactated Ringer solution at room temperature prevented desiccation and caused cooling. Adhesions were excised (145–147), and suturing was performed without traction and with fine nonreactive sutures. A thorough pelvic lavage with the use of heparinized lactated Ringer solution was performed, and 300–500 mL lactated Ringer solution with 500–1,000 mg cortisone succinate was left in the peritoneal cavity at the end of surgery. Additionally one or two doses of Dexamethasone were administered postoperatively.

This illustrates how easily knowledge is forgotten if the mechanisms are not well understood. Laparoscopic fertility surgery initially used the same microsurgical principles (147–149). During the subsequent expansion of laparoscopic surgery to almost all indications, these principles were largely forgotten with the belief that laparoscopic surgery was "minimally invasive" surgery and that postoperative adhesions would become a minor problem (55, 150). Simultaneously, reconstructive surgery for infertility with the use of laparoscopy became mainstream surgery (150, 151). In addition, the improved results of IVF further contributed to the decrease in fertility surgery. Another illustration is the use of saline solution for irrigation. Already in 1973 (34), saline was demonstrated to be toxic for the mesothelial cells, leading to the use of lactated Ringer solution in microsurgery. Saline solution, however, continued to be widely used up to the present day, and this despite the toxicity being reconfirmed recently (35–37).

Clinical data on the prevention of adhesion formation in humans are and will always be limited because a second-look laparoscopy is needed to score adhesions. In addition, in the human many surgical aspects, such as blood and infection, can not be investigated experimentally for ethical reasons. Given the importance of good clinical practice and gentle tissue handling, it is suggested that adhesion prevention trials in the future should use video registration (152) to document quality of surgery, and that duration of surgery and minor incidents such as bleeding should be monitored and used as covariables in the final analysis.

The strength of the proposed model of adhesion prevention is that the importance of each factor enhancing or preventing acute inflammation in the entire peritoneal cavity has been solidly proven in nonhuman animal models, and that in the human the combined use of preventing acute inflammation together with a barrier resulted in virtually adhesion-free surgery in a small ($n = 27$) proof-of-concept trial. To date, all experiments have confirmed the concept of acute inflammation of the peritoneal cavity without exception.

Although in nonhuman animal models with strictly standardized conditions efficacy was demonstrated for many products, the numbers required to reach statistical significance in humans are prohibitive. It is unclear whether and when these treatments should be introduced in human surgery, at least those with proven safety, such as calcium channel blockers, lipid-lowering drugs, and vitamin C to reduce oxidative stress. Others will need prior safety evaluation, such as the addition of phospholipids intraperitoneally at the end of surgery. Given the importance of oxidative stress at the surgical injury site (57), in the entire peritoneal cavity,

and in ischemia reperfusion (153), at least administration of vitamin C should be considered.

Laparoscopic surgery increases oxidative stress in the pelvic and splanchnic organs; the effect increases with the duration of surgery and the insufflation pressure. Systemic oxidative stress increases in both laparoscopic and open surgery. Currently it is unclear whether this is more pronounced in laparoscopic than in open surgery or vice versa (154, 155).

The transplantation of cultured mesothelial cells into the peritoneal cavity may become effective for human use. In nonhuman animal models it is effective in decreasing adhesion formation (156, 157). Also, mesothelial cells are being investigated as transplantable tissue-engineered artificial peritoneum, with research focusing on the use of mesothelial progenitor cells (158). For the same purpose, under current active investigation are the following: addition to the peritoneal fluid of factors known to stimulate resident mesothelial proliferation, mobilization, or differentiation (159); activation and multiplication of mesothelial cells (50, 160, 161); and the potential of mesothelial stem cells derived from muscle (162).

Prevention of fibroblast proliferation and angiogenesis is another target. The use of dexamethasone to reduce adhesion formation has been debated and questioned. In our laparoscopic mouse model, especially under conditions of minimal trauma to the peritoneal cavity, the effectiveness was important and clear. This was surprising, because other antiinflammatory agents, such as cyclooxygenase 1 and 2 inhibitors, were not effective. Therefore, it is suggested that dexamethasone works through a specific pathway of acute inflammation or by inhibiting fibroblast proliferation. This is consistent with the observations that dexamethasone reduces cell proliferation, collagen deposition, and lung fibrosis (163). Hepatocyte-derived growth factor also inhibits fibroblast proliferation, prevents peritoneal fibrosis (164, 165), and reduces adhesion formation via transplantation of transfected mesothelial cells (166).

The importance of fibrinolysis and inflammation was recently confirmed. Blocking thrombin-activated fibrinolysis inhibitor reduces adhesions (167). Dexmedetomidine, a selective α_2-adrenergic with secondary antioxidant and antiinflammatory effects (168), bromelain, a pineapple extract with fibrinolytic and antiinflammatory properties (169), clinoquinol, which limits inflammation and fibroblastic activity (170), vitamin C and vitamin E (171), ellagic acid, with antioxidant and antiinflammatory properties (172), and biodegradable micelles (173) all reduce adhesion formation.

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