Peritoneal repair and post-surgical adhesion formation

Gere S. diZerega¹ and Joseph D. Campeau

Department of Obstetrics & Gynecology, University of Southern California Keck School of Medicine, Department of Obstetrics and Gynecology, Livingston Reproductive Biology Laboratories, 1321 North Mission Road, Los Angeles, CA 90033, USA

¹To whom correspondence should be addressed. E-mail: GSD1270@aol.com

It was shown in 1919 that peritoneal healing differs from that of skin. When a defect is made in the parietal peritoneum the entire surface becomes epithelialized simultaneously and not gradually from the borders as in epidermalization of skin wounds. While multiplication and migration of mesothelial cells from the margin of the wound may play a small part in the regenerative process, it cannot play a major role, since new mesothelium develops in the centre of a large wound at the same time as it develops in the centre of a smaller one. Development of intraperitoneal adhesions is a dynamic process whereby surgically traumatized tissues in apposition bind through fibrin bridges which become organized by wound repair cells, often supporting a rich vascular supply as well as neuronal elements.

Key words: mesothelial cells/peritoneal repair/post-surgical adhesion/wound repair

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Post-surgical peritoneal repair

General agreement exists between investigators on the time taken for regeneration of the mesothelial layer (diZerega, 2000a). Ellis et al. and Hubbard et al. reported that healing occurs in 5–6 days in the case of parietal peritoneum (Ellis et al., 1965; Hubbard et al., 1967). Peritoneal defects 2×2 cm and 0.5×0.5 cm were both entirely covered by a continuous sheet of mesothelium 3 days after wounding (Ellis et al., 1965). Glucksman reported that the visceral mesothelium covering the terminal ileum heals in 5 days (Glucksman, 1966), while Eskeland demonstrated that regeneration of the mesothelial layer of parietal peritoneum is not complete until 8 days (Eskeland, 1966). Raftery confirmed that parietal peritoneum of the rat is healed within 8 days (Raftery, 1973).

Mesothelial regeneration

diZerega summarized the cellular sequence of repair in the parietal peritoneum after traumatic injury (diZerega, 1996) (Figure 1). Raftery studied the regeneration of parietal and visceral peritoneum using evaluation by scanning electron microscope of healing peritoneal defects in the rat (Raftery, 1973). Twelve hours after injury, numerous polymorphonuclear leukocytes (PMN) were seen entangled in fibrin strands. Very little cellular infiltrate was found in the depths of the wound compared to the wound surface. At 24–36h after wounding, the number of cells in the superficial part of the wound was greatly increased; most of the increase in cell number was due to infiltration by macrophages. The macrophages were intertwined with the filaments of fibrin projecting from the wound surface. The base of the wound remained relatively acellular.

At 2 days, most of the wound surface was covered with a single layer of macrophages supported by a fibrin scaffold. Two additional cell types were also seen on the wound surface: a cell which looked like a primitive mesenchymal cell which was also seen in small numbers at the base of the wound, and islets of mesothelial cells which were interconnected by desmosomes and tight junctions.

Three days after injury, the number of primitive mesenchymal cells on the wound surface increased, although macrophages were still the most prevalent cell type present. The base of the wound contained scattered mesenchymal cells and some proliferating fibroblasts. The cells on the wound surface at 3 days were similar in appearance to cells in the deeper layers of the wound and were similar to primitive mesenchymal cells.

At 4 days, cells resembling primitive mesenchymal cells or proliferating fibroblasts on the wound surface were in contact with one another. In some areas, healing appeared complete at 5 days since a single layer of mesothelial cells was present on the wound surface interconnected by desmosomes and tight junctions. No basement membrane was found beneath the mesothelial cells of visceral peritoneum or caecum at this stage, although one was often present beneath those covering the liver. Thus, peritoneal healing of parietal peritoneum was associated with basement membrane formation at this time in contrast to visceral...
peritoneum, which, although similar in appearance on the surface, did not contain a basement membrane.

At days 5–6, the number of macrophages was clearly decreased from the wound surface while most of the wound surface was covered by mesothelial cells. At day 7 after surgery, the appearance of the wound resembled that on day 6 except that a discontinuous basement membrane was now evident beneath the mesothelial cells lining the parietal peritoneum and covering the caecum. At day 8, a continuous layer of mesothelial cells was present over the wound surface. A single layer of mesothelial cells resting on a continuous basement membrane was seen at day 10. Fibroblasts in the base of the wound were arranged with their long axis parallel to the wound surface, and bundles of collagen were present between the fibroblasts.

Normal parietal mesothelial surface of the rat peritoneal cavity contained a mat of microvilli which obscured the contour of the cells to which they were attached. New cells were seen in the surface at 30 min after injury; at 8 h, most of the surface contained new cells with a variety of morphologies.

**Visceral versus parietal peritoneum**

Visceral peritoneum appears to differ little in its healing properties from the parietal peritoneum (diZerega and Rodgers, 1990; diZerega 1996, 2000a). Light microscopy indicates that the liver acquires a new mesothelial covering 1 day earlier than either caecum or parietal peritoneum (Raftery, 1973). A discontinuous basement membrane is present beneath mesothelial cells covering the liver at 5 days. In contrast, discontinuous basement membrane does not form beneath the mesothelial cells of the parietal peritoneum or caecum until 7 days after surgery. Raftery postulated that the liver (viscera) provides a firmer substrate for mesothelial regeneration (Ellis et al., 1965). On day 3, the entire defect was covered by a sheet of cells. Trypan Blue was not detected by microscopy in any of the cells covering the wound. By day 5, the new surface mesothelium achieved continuity with the surrounding edges of the previously undamaged mesothelium. By 7–10 days, no evidence of mitosis was evident within the wound base or surface nor along the margins of the previously uninjured peritoneum. A similar experiment was performed on another group of rats. A polyethylene sheet was placed over the peritoneal defect after injury and sutured in place. Up to 2 weeks after injury, the surface of the polyethylene was covered by macrophages without appreciable numbers of fibroblastic or mesothelial cells. The cells which did cover the polythene were separated by large areas of fibrin. At 3–4 weeks after injury, the wound surface became covered with mesothelium. Thus, new peritoneal cells do not arise to any significant degree by the centripetal spreading of the mesothelial cells surrounding the wounded area, since they are distributed rather uniformly at an early stage over the wound surface (diZerega and Rodgers, 1990).

Some investigators consider that metaplasia of fibroblasts within the loose connective tissue beneath the surface of the peritoneum leads to mesothelial regeneration (Ellis et al., 1965; Lucas et al., 1996). Electron microscope observations identify undifferentiated primitive mesenchymal cells in the perivascular connective tissue, suggesting that these cells may also contribute to the new mesothelial cells. Further experimental evidence for this hypothesis was provided by Ellis et al. (1965) who postulated that peritoneal reformation results from transformation of subperito-
neal fibroblasts into an intact mesothelial layer. Ellis’s work was confirmed by Raftery, who further noted that peritoneum appears to arise by differentiation of subperitoneal fibroblasts (Raftery, 1973). Direct support for the role of mesenchymal stem cells (MSC) arising from within the adjacent peritoneum to form mature mesothelial cells was provided by transplantation of MSC into the abdominal cavity of rats at different times after surgery. MSC given 4–5 h after surgery increased formation of post-operative adhesions, perhaps due to direct implantation on pre-existing fibrin strands (Lucas et al., 1996). However, when MSC were injected immediately after surgery, a significant reduction in adhesion formation occurred, with half of the rats being adhesion-free (Figure 3). These results suggest that stem cells contribute to postsurgical mesothelialization as well as adhesions once fibrin bridges are available for their implantation.

**Effect of cauterization**

At the sites of peritoneal cauterization and suture repair, two studies have reported deep submesothelial haemorrhage and necrosis which prolonged the duration of inflammation and associated delay in collagen deposition (Bellina et al., 1984; Elkins et al., 1987). The sites of cautery contained tissue necrosis and inflammation 3 weeks after surgery. Cautery of peritoneal injury induced more tissue damage than other types of wounding. Early collagen deposition was noted at 5 days after surgery. However, at 3 weeks these lesions contained PMN, tissue necrosis, and granulation tissue, with no fibroblasts and minimal collagen formation. Thus, healing at the cauterized site was not completed by 3 weeks after surgery. Elkins et al. found that mesothelial regeneration was also delayed after peritoneal damage by electrocautery (Elkins et al., 1987). Peritoneal repair after cauterization by electrical current or laser is complicated by carbonization: the black–brown tissue rests in the area of the wound. Carbon induces an inflammatory reaction leading to giant cell formation which is required to phagocytize the foreign body thereby further delaying mesothelial repair.

Filmar et al. compared the histology of uterine horn repair in rats after incision with the CO2 laser or microcautery (Filmar et
Incisions were reapproximated with 10-0 nylon sutures. Although the general appearance of the scars and the amount of collagen which accumulated over a 21 day observation period were similar, foreign body reaction as measured by histocyte and giant cell infiltration was significantly greater in the electrocautery group. Carbon particles which formed in response to cautery may lead to formation of foreign body granulomas. Cutting with the CO2 laser caused significantly more necrosis and foreign body reaction than cutting with microscissors. Sharp mechanical transection was followed by the least amount of tissue reaction, necrosis and an absence of particulate carbon. Montgomery et al. compared the healing patterns of canine uterine peritoneum and myometrium after injury by CO2 laser, scalpel or electric knife standardized to a 3 cm incision (Montgomery et al., 1983). Their observations confirmed those of other investigators in that necrosis was less with the scalpel than either CO2 laser or electric knife.

Summary

New mesothelial cells may have multiple sources: (i) transformed peritoneal cells, (ii) metaplasia of subperitoneal connective tissue cells, (iii) maturation of mesenchymal stem cells, or (iv) adjacent normal peritoneum. Primitive mesenchymal cells identified on the wound surface in the early stages of healing may differentiate into mesothelial cells. Whether these cells are differentiated fibroblast or multipotential mesenchymal stem cells is unclear. Thus the origin of new mesothelium remains circumspect because of difficulty in distinguishing between primitive mesenchymal cells and proliferating fibroblasts in the later stages of healing. It is possible that the former give rise to the latter, but definitive evidence for this is lacking. Substantial evidence exists for a role of cell adhesion molecules including integrins and other fibronectin interacting proteins in the process of peritoneal repair and adhesion formation (Rodgers et al., 1998; Witz et al., 1998; Rodgers, 2000).

Adhesion formation

A major clinical problem relating to peritoneal repair is the formation of intra-abdominal and pelvic adhesions. Although the term ‘adhesions’ is used in reference to ophthalmic, orthopaedic, central nervous systems, cardiovascular and intrauterine repair processes, the formation of peritoneal adhesions is unique and specific to the peritoneal response to injury.

Morphogenesis of adhesion formation

Adhesion formation typically occurs when two injured peritoneal surfaces are apposed (Lamont et al., 1992; Haney and Doty, 1994). The initiation of adhesion formation begins with formation of a fibrin matrix which typically occurs during coagulation (Figure 4) in the presence of suppressed fibrinolysis (diZerega, 2000b). Surgical injury of tissue reduces or eliminates blood flow, thereby producing ischaemia, which leads to local persistence of fibrin matrix (Figure 5). This matrix is gradually replaced by vascular granulation tissue containing macrophages, fibroblasts and giant cells. The clots are slow to achieve complete organization. In the process, they consist of erythrocytes separated by strands or condensed masses of fibrin which are covered with two or three layers of flattened cells and contain a patchy infiltrate of mononuclear cells. Eventually the adhesion matures into a fibrous band, often containing small nodules of calcification. The adhesions are often covered by mesothelium and contain blood vessels and connective tissue fibres, including elastin. Even at 6 months, collections of haemosiderin-filled macrophages are present in many adhesions (diZerega and Rodgers, 1990).

Nerve fibres were found in pelvic adhesions from 17 patients, 10 of whom had a history of pelvic pain (Kligman et al., 1993). There was no significant correlation of pelvic pain with the number of adhesions containing nerve fibres nor in the presence of mesothelial proliferation, calcification, oedema, vascularization, inflammation, fibroblastic proliferation, or collagenization.
These results were confirmed and extended by Tulandi et al. who additionally noted the presence of inflammatory cells in adhesions concomitant with endometriosis but not in adhesions associated with other disease states (Tulandi et al., 1998).

diZerega and Rodgers summarized the histological and morphological features of post-surgical adhesion formation in rats using light and electron microscope techniques (diZerega and Rodgers, 1990).

At day 1–3, the adhesion was characterized by a variety of cellular elements encased in a fibrin matrix. The cells were primarily PMN but also included macrophages, eosinophils, red blood cells and tissue debris as well as necrotic cells presumably desquamated from the peritoneal injury. By day 4, macrophages were the predominant leukocyte in the fibrin mesh which primarily contained large strands of fibrin associated with a few fibroblasts. A few mast cells were seen at day 5 and unorganized fibrin was not apparent. In contrast many fibroblasts were lying together, assuming the formation of a syncytium together with macrophages. Distinct bundles of collagen were evident, as were scattered foreign body granulomas. At 7 days, collagen and fibroblasts were the predominant components of the adhesion. However, small vascular channels containing endothelial cells were present. The number of mast cells slightly increased between 2 weeks and 2 months. During this interval, the cellularity of the adhesion was replaced almost entirely by collagen fibrils associated with macrophages. Occasional macrophages and lymphocytes persisted for ~2 weeks.

The minimum post-operative interval required for the use of an impermeable barrier to prevent adhesion formation was established (Harris et al., 1995). By removing a silastic sheet 6, 12, 18, 24, 30, 36, 72 or 96 h after peritoneal injury, the incidence of adhesions dropped from 100 to 0% during the first 36 h (Figure 6).

**Fibrin**

Fibrinous exudate is a necessary precursor for adhesions (Figures 4 and 7). Highly mobile intraperitoneal structures will not permanently adhere to each other unless held in continuous, close apposition until fibroblast invasion leads to collagen deposition beginning on the third post-operative day. Thus, the crucial consideration is the factor which determines whether the fibrin bridge is absorbed, or persists and is organized (Ellis, 1971). Further evidence for the role of fibrin in the formation of adhesions comes from the observation that use of defibrinated blood or blood products is less frequently associated with adhesion formation. Serosal injury, though relatively innocuous per se, readily leads to adhesions if combined with blood products. Peritoneal injury sufficient to produce adhesions requires removal of the mesothelial surface: not only drying but
even prolonged moistening is sufficient to denude the surface of peritoneal mesothelium (Richardson, 1911; diZerega, 1996). Plasma alone on dried areas creates fibrinous attachments, most of which disappear within a few days. Fibrin provides the initial bridge between two surfaces; when the bridge is made of fibrin only, it is amenable to lysis by fibrinolytic mechanisms; but when it contains cellular elements (erythrocytes, leukocytes, platelets etc.) within the fibrin it will probably undergo organization into an adhesion. Under these experimental conditions, (i) desquamation of mesothelial cells appears to be the critical event in adhesion formation, and (ii) adhesions apparently develop only when two denuded surfaces are involved.

**Predisposing factors**

In two large surveys, post-surgical adhesion formation did not appear to be age-dependent (Perry et al., 1955; Weibel and Majno, 1973); however, no prospective evaluation of the effect of age on adhesion formation is available. Weibel and Majno (1973) reported a slightly higher frequency of ‘spontaneous’ adhesions (i.e. those adhesions which form without any apparent cause) after age 60 years.

There does not appear to be a sex bias in the development of post-operative adhesions. Weibel and Majno (1973) reported a slightly higher frequency of adhesions among male patients. After excluding adhesions resulting from gynaecological procedures, Raf (1969) reported that the incidence of intraperitoneal adhesions was 47% in male patients and 53% in female patients.

The omentum is particularly susceptible to adhesion formation. In Weibel and Majno’s studies (1973), the omentum was involved in 92% of patients with post-operative adhesions. The omentum was also the predominant organ involved in ‘spontaneous’ adhesions (i.e. those with no prior history of surgery); 100% of the 126 spontaneous adhesions examined by Weibel and Majno involved the omentum (Weibel and Majno, 1973). These reports raise the question of omentectomy during pelvic surgery where post-operative adhesion formation is likely to occur. With the exception of the omentum, the internal organs involved in post-operative adhesions may vary as a function of the surgical procedure. The small intestine was involved in 21% of the adhesions present after appendectomy but in only 6% of those formed following gynaecological laparotomy; 47 and 19% of adhesions which form after appendectomy and gynaecological laparotomy, respectively, involve the colon (Turunen, 1933). The ovary, due to its close proximity to the other peritoneal surfaces and the fragility of the coelomic epithelium which covers the ovarian surface, is the most common site for adhesions to form after reconstructive surgery of the female pelvis [Adhesion Study Group, 1983; Diamond et al., 1987; Interceed (TC7) Adhesion

**Figure 5.** Summary of normal tissue repair and adhesion formation following surgical trauma. After trauma to the peritoneum, increased vascular permeability, mediated by histamine, produces an inflammatory exudate and formation of fibrin matrix. As with other parts of the body, this fibrin matrix is normally removed by fibrinolysis. Under normal conditions, where fibrinolytic activity is allowed to occur, fibroblast proliferation results in remesothelialization. However, under the ischaemic conditions present in surgical trauma, fibrinolytic activity is suppressed and fibrin is allowed to persist. Once the fibrin bands are infiltrated with fibroblasts, they become organized into adhesions. (Permission to publish as in Figure 1.)

**Figure 6.** Kinetics of adhesion formation. Removal of silastic barrier from between two wounded surfaces at various times after injury documented the susceptibility of wounds to form adhesions as a function of time. (Permission to publish as in Figure 1.)
Ovarian adhesions were found at second-look laparoscopy in >90% of cases after ovarian surgery (Pittaway et al., 1985).

**Blood**

The role of blood in the peritoneal cavity in the formation of adhesions is controversial. Hertzler reported that large volumes of clotted blood could be completely absorbed by a normal peritoneum within 48 h (Hertzler, 1919). Jackson found that 100 ml of free blood and a well-formed clot were absorbed from the peritoneal cavity within 8 days (Jackson, 1958). Nisell and Larsson suggested that trauma to the serosa rather than blood was the instigator of adhesion formation (Nisell and Larsson, 1978). Ryan et al. showed that blood may play an important part in the

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Figure 7. Schematic representation of fibrin gel matrix formation. Fibrinogen deposited on the surface of injured peritoneum interacts with thrombin to become the soluble polymer fibrin, which is further modified to an insoluble form. Together with fibronectin, the insoluble fibrin polymer and cellular debris form the fibrin gel matrix, which provides the scaffolding for intraperitoneal adhesion formation. Frequent irrigation during surgery can remove the soluble polymer; delays in irrigation reduce the removal of deposited fibrin because it is converted into an insoluble form. (Permission to publish as in Figure 1.)

Figure 8. The level of fibrinolytic activity of the human peritoneum from biopsies. Biopsies of serosal peritoneum were obtained from various sites during peritoneal surgery. Fibrinolytic activity of human serosal peritoneum is primarily caused by plasminogen activator. (Permission to publish as in Figure 1.)
pathogenesis of adhesions (Ryan et al., 1978). Addition of fresh blood to an otherwise uninjured peritoneal cavity resulted in omental adhesions while preformed clots produced widespread adhesions even without peritoneal injury. When 0.2–2 ml of fresh blood was dripped onto a dried peritoneal surface and allowed to clot, adhesions formed at the site of injury. If peritoneum was excised, the degree to which the clot induced adhesion formation was markedly enhanced. When addition of fresh blood was delayed, adhesions formed provided blood was added to the injured site. The addition of blood alone without caecal drying led to more limited adhesion formation. Serosal damage, no matter how mild, may lead to adhesions in the presence of blood. Clotted blood may constitute a fibrinous network upon which fibroblasts may proliferate, resulting in adhesions (Pfeiffer et al., 1987). Golan and Winston confirmed the findings of Ryan et al., reporting that blood in conjunction with trauma to the serosa is more important in adhesion formation than either trauma alone or trauma and serum (Ryan et al., 1978; Golan and Winston, 1989).

Fibrinolysis

Fibrinolytic activity of peritoneum is present on all mesothelial surfaces (Figure 8) and is dependent primarily on the balance of and tissue plasminogen activator (tPA) and plasminogen activity inhibitors (PAI) (Thompson et al., 1997; Holmdahl et al., 1998). The plasminogen-activating activity of the peritoneal exudate is reduced as early as 6 h after surgery and disappears at 24–48 h (Scott-Coombes et al., 1995). Although there is a transient reduction in the concentration of tPA following surgery, the principal cause of reduced fibrinolytic activity appears to be an increase in concentration of both PAI-1 and PAI-2. The levels of PAI-1 and PAI-2 in cell lines, including cultured human mesothelial cells, can be increased by the presence of bacterial lipopolysaccharide and inflammatory mediators such as interleukin-1 and tumour necrosis factor. Such molecules are present in the peritoneum following inflammation and may stimulate PAI-2, thereby inhibiting lysis of fibrinous deposits within the abdominal cavity and promoting adhesion formation (Whawell et al., 1994).

There is a wide variation in the volume of peritoneal exudate among patients which may affect the concentrations of individual fibrinolytic parameters leading to a reduction in functional fibrinolytic activity. Concentrations of both PAI-1 and PAI-2 are increased in inflamed peritoneum during appendicitis, in association with a reduction in peritoneal plasminogen activating activity. Thus, there appears to be a biphasic response to surgery by the peritoneum; the early reduction in peritoneal plasminogen-activating activity may be secondary to a reduction in tPA concentrations, whereas the subsequent loss of fibrinolytic activity probably arises from the dramatic increase in PAI-1 and PAI-2 concentrations (Holmdahl, 2000). Further, differences in endogenous PAI (especially PAI-2) may lead to individual differences in tPA activity resulting in individual susceptibility to adhesion formation (Thompson, 2000).

Summary

Development of intraperitoneal adhesions is a dynamic process whereby surgically traumatized tissues, in apposition bind through fibrin bridges which become organized by wound repair cells, often supporting a rich vascular supply as well as neuronal elements.

These events begin at the time of surgical incision and include fibrinous exudate, cytokine production, cell migration, vascular oedema, and suppression of fibrinolytic activity. Following surgery, PMN predominate with some contribution from macrophages, eosinophils, and red blood cells. This profile shifts by day 4 to predominately macrophages while islands of mesothelial cells reperitonealize the damaged surfaces including unresorbed fibrinous bands. Fibroblasts contribute collagen which further stabilizes the adhesions and promotes vascular in growth.

References


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