The Biology of Adhesion Formation in the Peritoneal Cavity

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Abstract:
Intraperitoneal adhesions are frequently encountered and present significant challenges to the practicing surgeon, including increased operating time, bowel obstruction, pelvic pain and infertility. Until recently, however, our knowledge of the biology of adhesion formation within the peritoneal cavity has been limited, which in turn limits prevention and treatment strategies for surgical patients. Extensive research has now led to an increased understanding of adhesion formation, with hypoxia playing a central role. Hypoxia stimulates a cascade that leads to oxidative stress, anaerobic metabolism, formation of free radicals and ultimately the adhesion phenotype. By understanding the precipitants to adhesion development, we may begin to develop prevention and treatment therapies that will provide clinically significant improvement over the currently available approaches to limit postoperative adhesions.

Key Words: adhesions, hypoxia, oxidative stress, adhesion phenotype
Introduction

Adhesions, defined as abnormal fibrous connections joining tissue surfaces at non-anatomic locations, are generated between adjacent tissues and organs that are injured during surgery, and are a sequel of the healing process. They are encountered frequently and present many challenges for the practicing surgeon. Occurring in up to 55%-100% of women who underwent second look laparoscopies after gynecologic procedures, this rate is similar in men and women who undergo general surgical procedures. It is now clear that adhesions occur after all types of intraperitoneal procedures, regardless of surgical approach (laparotomy vs laparoscopy), and the likelihood of adhesion reformation is high, with sites such as the ovary after laparotomy or laparoscopy recurring as frequently as 65-80%. The clinical consequences of adhesions are significant; these include bowel obstruction, infertility, pelvic pain, increased operating time due to extensive adhesiolysis, and increased morbidity. In addition, the economic burden of adhesions (calculated as hospital cost plus surgeon fees for all adhesion-related admissions) in the United States, as reported by Ray and Colleagues in 1998, was estimated at 1437.1 million per year, but this figure does not include the additional burden of outpatient expenditures and loss of work.

Adhesions can generate de-novo (the development of adhesions at sites that initially did not undergo adhesiolysis), or they can re-form (the redevelopment of adhesions at sites after adhesiolysis). They can be quantified intra-operatively by adhesion scoring systems which takes into account the type, extent and anatomic location of the adhesions. Although adhesion scoring systems provide a basis by which physicians can describe adhesions and correlate management and outcomes, agreement does not always exist. In a study reviewing 13 gynecologic surgical procedures by 11 experienced laparoscopic surgeons, there was only a 64% positive correlation between scoring physicians using the AFS system, but this increased to 89% positive correlation using a more complex scoring system. Agreement is also lacking in the extent to which adhesions cause pain as well as management of these adhesions. In a survey of 13 gynecologic surgeons regarding the likelihood of an adhesion to cause pain at a particular location, surgeons tended to associate more dense adhesions with pain, however, the maximum percentage of patients thought to have pain related to adhesions was 60-70%; thus demonstrating that some patients with dense adhesions have been found to be pain free. Need for lysis of adhesions was also thought to be proportional to the extent of adhesive disease, with 83% of surgeons recommending surgery for sites 80% involved in adhesions. The site of adhesions was also felt to be important with all surgeons recommending surgery for adhesions involving 50% of adnexal structures.

As a means for evaluating adhesions at the time of second look laparoscopies in gynecologic patients, Diamond and Nezhat, developed a classification system for post-operative adhesion development, which recognizes an understanding of adhesion formation and reformation. Adhesions were divided into Type I, de novo adhesions, or Type 2, adhesion reformation. The two types were further divided into two subgroups based on whether surgical procedures were conducted at each site. The likelihood of postoperative adhesion development appeared to be highest at sites with adhesiolysis and surgical treatment of pathology (such as treatment of an ovarian endometrioma in an ovary adhered to the pelvic sidewall) and lowest at sites without surgical procedures or adhesiolysis. The likelihood of recurrence for all sites, from most encountered to least encountered was: 2b- adhesiolysis and treatment of pathology >
2a-sites of adhesiolysis alone > 1b-sites of surgical procedures without adhesions > 1a-de novo formation). These findings of adhesion development provide a basis for understanding the potential efficacy of surgical approaches or anti-adhesion adjuvants to target therapies for post-operative adhesions 1a>1b>2a>2b.11

**Normal Peritoneal Repair**

The peritoneal surface is a serous membrane lined by mesothelial cells loosely attached to the basement membrane, under which lies the extracellular matrix. The extracellular matrix contains many components essential to healing including collagen (specifically collagen I and collagen III), fibronectin, glycoproteins, fibroblasts, macrophages, along with blood and lymphatic vessels.11,17 Normal peritoneal repair is a complex process involving the interplay of several events including inflammation, angiogenesis, cell migration and turnover of the extracellular matrix.2 Once the peritoneal surface is injured, this triggers an exudation of a high protein fluid, known as the provisional matrix, containing fibrin, histamines, monocytes, plasma cells, polymorphonuclearcytes (PMNs), macrophages, mesothelial cells and histiocytes.2,11,18 This fluid coagulates within 3 hours and forms fibrous bands between corresponding surfaces and maintains their contact.2 In response to injury, macrophages exhibit increased phagocytic, respiratory burst and secretory activity; they also recruit new mesothelial and fibroblast cells and are the major components of the leukocyte populations after day 5.11 Normal fibrinolysis inhibits the development of adhesions within 72 hours. If this mass persists during the period of peritoneal repair (usually 3-5 days), then underlying fibroblasts migrate into the fibrinous mass. Fibroblasts deposit extracellular matrix, including collagen and fibronectin, which form the scaffold for sheets of mesothelial cells leading to reepithelialization and thus adhesion formation.19

Essential to the peritoneal healing process is an autocrine/paracrine feedback as the peritoneum is constantly exposed to growth factors and cytokines in the peritoneal fluid (Table 1). These are synthesized by mesothelial cells and activated macrophages within the wound and must be optimal, precise, and synchronized for healing to occur. If these factors are inhibited, interrupted or over-expressed, this can lead to nonhealing or adhesion formation.17 In addition to the feedback system, a variety of other processes affect peritoneal healing including: migration, proliferation, apoptosis, and/or differentiation of many cells types including inflammatory cells, immune cells, mesothelial cells and fibroblasts. These cells then produce molecules which regulate proteolysis, tissue remodeling, angiogenesis, and synthesis and deposition of the ECM.17

The peritoneal lining has intrinsic fibrinolytic activity which modulates fibrin degradation that results from fibrin deposition after injury.11 If this activity is decreased (via hypoxia, trauma or infection), then there is an increased incidence of adhesions.11 Therefore, normal peritoneal healing and adhesion formation can be seen as alternate pathways following peritoneal injury.11

**Factors involved in adhesion formation**

**Plasminogen activators** are serine proteases that convert plasminogen into plasmin, and limit adhesion development of the mesothelial cells lining the peritoneal cavity.19 They are ubiquitous enzymes which are secreted by many cell types and play a central role in regulating proteolysis in a wide variety of processes including tissue remodeling, cell migration, fibrinolysis, tumor metastasis and invasiveness.19
With loss of mesothelial cells and decreased plasminogen activator activity (PAA), underlying fibroblasts are exposed and adhesions result between two adjacent surfaces. In a classic study by Rafferty, free peritoneal grafts with markedly decreased PAA, had resultant adhesion formation. Those grafts in which PAA was not reduced after injury, had degradation of the fibrinous mass prior to fibroblast ingrowth and resultant healing of the peritoneal surface without adhesion development. Thus, if sufficient PAA is present after injury, the fibrinous mass will be degraded by proteolytic activity and the scaffolding required by fibroblasts for invasion will be eliminated, leading to normal healing without adhesion development. In contrast, if PAA is decreased or absent, the fibrinous mass will form a clot which is invaded by fibroblasts, collagen and other proteins from the ECM. Mesothelial cells then reepithelialize and an adhesion develops.

There are 2 types of plasminogen activators: tissue type plasminogen activator (tPA) and urokinase type plasminogen activator (uPA), both of which are inhibited predominantly by plasminogen activator inhibitor-1 (PAI-1), the major plasminogen activator inhibitor in plasma.

**Tissue Plasminogen Activator (tPA)** is the main plasminogen activator in mesothelial cells, and it has also been identified in underlying fibroblasts. Both peritoneal and adhesion fibroblasts have basal tPA levels, but they are 45% higher in normal peritoneal fibroblasts. In comparison to normal fibroblasts, adhesion fibroblasts have decreased tPA and increased PAI-1 which promotes adhesion development. In a hypoxic milieu, fibroblasts demonstrate a decreased ability to degrade the fibrinous mass over injured surfaces. While normal fibroblasts have decreased tPA under hypoxic conditions, it is almost non-existent in adhesion fibroblasts. Also, the PAI-1 is increased in both normal and adhesion fibroblasts during hypoxic conditions. These findings support further the idea that peritoneal healing and adhesion formation can be seen as alternate pathways of peritoneal healing.

**Cytokines**

**Transforming growth factor beta-1 (TGF-β1)** is an inflammatory cytokine that controls cellular proliferation, differentiation, apoptosis, tissue morphogeneis and wound healing. It occurs in mesothelial cells and fibroblasts and is increased in response to peritoneal healing. Having both an inactive and active form, its active form stimulates enhanced extracellular matrix deposition through enhancement of angiogenesis and impairment of both matrix metalloproteases and plasminogen activator. This enhancement of the ECM contributes to adhesion development, and an increase in TGF-β1 has been shown to be associated with adhesion development as demonstrated in peritoneal fluid and adhesions in animal models.

In response to tissue injury, there is a localized increase in TGF-β1. It has a potent effect on macrophage and fibroblast activity during wound activity and has been shown to alter the adhesive properties of cells as well as influencing the expression of integrin subunits and cytoskeletal proteins. TGF-β1 may promote post-operative adhesion formation by inducing migration of peritoneal fibroblasts by altering the expression levels and patterns of specific integrin subunits, vinculin and F actin.

TGF-β1 also increases in response to hypoxia in both normal peritoneal and adhesion fibroblasts. This in turn increases PAI-1 and uPA mRNA expression in endothelial cells with the ratio of uPA:PAI-1 favoring enhancement of anti-proteolysis; there is also a decreased synthesis of collagenase and plasminogen activator. In normal peritoneal fibroblasts, exposure to hypoxia results in an irreversible increase in
TGF-β1 and collagen type I levels to those found in adhesion fibroblasts. In addition, TGF-β1 increases when exposed to superoxide in both peritoneal and adhesion fibroblasts. It is a direct inductor of myofibroblastic differentiation by controlling alpha smooth muscle actin (α-SM actin).

Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine that is involved in the healing process and has been identified in increased concentration in the presence of peritoneal adhesions. It exhibits a more profound effect than lipopolysaccharide (LPS) on the release of tPA, which may be an important mechanism by which inflammatory mediators disrupt fibrin degradation. TNF-α is expressed in both normal and adhesion peritoneal fibroblasts, and TNF-α mRNA is increased by 58% in adhesion fibroblasts compared to normal fibroblasts. When exposed to hypoxia, TNF-α levels are increased in both normal peritoneal and adhesion fibroblasts, but to a greater extent in normal fibroblasts. Additionally, the levels of TNF-α in normal fibroblasts under hypoxia are similar to those expressed in adhesion fibroblasts in normoxic conditions.

Interleukin (IL)-6, is a multi-function cytokine which can behave as a growth and differentiation factor, or stimulate the expression of other genes. It is an early marker of tissue damage and is stimulated by IL-1 and TNF-α in mesothelial cells. Like TNF-α, IL-6 is an inflammatory cytokine that stimulates the acute-phase reaction, leading to a systemic reaction including inflammation, fever and activation of the complement and clotting cascade. Both IL-6 and TNF-α are released by macrophages during peritoneal injury and play an important role in regulation coagulation and fibrin formation, and subsequently, adhesion development. IL-6 levels are significantly elevated in adhesion fibroblasts compared to normal peritoneal fibroblasts. When exposed to hypoxia, IL-6 levels increase in both adhesion and normal peritoneal fibroblasts, but to a greater extent in normal fibroblasts. mRNA levels of IL-6 are also increased in response to hypoxia for both types of fibroblasts, but this effect is less pronounced than the elevations seen under normoxic conditions. When treated with hypoxia, normal peritoneal fibroblasts express IL-6 levels equivalent to those found in adhesion fibroblasts at baseline.

Proteolytic Enzymes

Matrix Metalloproteases (MMPs) are a family of ubiquitous, secreted, zinc-dependent proteolytic enzymes that are important in remodeling the extracellular matrix; they can be found in both the peritoneum and adhesion tissues. MMPs degrade specific proteins within the extracellular matrix and their proteolytic activity is countered by tissue inhibitors of metalloproteases (TIMPs). In vitro, hypoxia inhibits the expression of MMPs and increases TIMPs leading to decreased matrix degradation and increased tissue fibrosis. Specifically, in treatment of peritoneal fibroblasts with hypoxia, there was a 65% decrease in MMP-9 activity, and 80% decrease in MMP-9 mRNA, but no effect on MMP-2. TGF-β1, has a stimulatory effect on MMP activity. When peritoneal fibroblasts were treated with TGF-β1, there was a 180% increase in MMP-9 and a 50% increase in MMP-9 as well as a 37.5% increase in MMP-2 mRNA and a 40% increase in MMP-9 mRNA. When treated with both hypoxia and TGF-β1, fibroblasts had a 160% increase in MMP-2 activity and a 37.5% increase in MMP-2 mRNA but showed a 45% decrease in MMP-9 and a 71% decrease in MMP-9 mRNA. These findings suggest that hypoxia suppresses the stimulatory effect of TGF-β1 on MMP-9 but not MMP-2 and provides further evidence that proteolytic enzymes whose expression is regulated by hypoxia may influence extracellular turnover and adhesions.
Other Factors Important in Adhesion Formation

**Vascular endothelial growth factor (VEGF)** is a potent mitogen for endothelial cells and a critical factor in angiogenesis, which is essential to wound healing and adhesion formation.\textsuperscript{23} It is a dimeric glycoprotein which exists in four isoforms in humans, all of which have been identified in adhesion fibroblasts.\textsuperscript{23} VEGF production is stimulated by lactate in macrophages, and lactate accumulation may have a significant role in adhesion development.\textsuperscript{23} It is also stimulated by NO, although this mechanism is not well understood.

**Cyclo-oxygenase (COX)** is a prostaglandin endoperoxidase synthase which catalyzes the rate limiting step in prostaglandin (PG) synthesis.\textsuperscript{25} It has two isoenzymes, COX-1 and COX-2, both of which perform two enzymatic functions: 1) cyclooxygenase which converts arachidonic acid to PGG\textsubscript{2} and 2) peroxidase which converts PGG\textsubscript{2} to PGH\textsubscript{2}. This is further isomerized and reduced into major biologically active prostanoids PGE\textsubscript{2}, PGA\textsubscript{2}, thromboxane (TXA\textsubscript{2}) and prostacyclin (PGI\textsubscript{2}).\textsuperscript{25} COX-1 is expressed in most tissues, and its levels do not fluctuate in response to cytokines and growth factors. Alternatively, COX-2 is induced by growth factors, phorbol esters and cytokines. It is characterized as an immediate-early gene that is associated with cellular growth and differentiation.\textsuperscript{25}

Expression of COX-2 is increased in response to inflammatory stimuli and causes elevated levels of local prostaglandin.\textsuperscript{25} COX-2 mRNA is elevated in human adhesion tissue but not in normal peritoneal tissue in the same patient. Expression of COX-2 is increased in response to hypoxia in normal peritoneal fibroblasts, but not in adhesion fibroblast, whereas the expression of COX-1 remains unchanged in adhesion and peritoneal fibroblasts under normoxic and hypoxic conditions. It is hypothesized that hypoxia drives normal peritoneal fibroblasts to acquire the adhesion phenotype as manifested by markedly increased COX-2 expression.\textsuperscript{25}

**Role of Oxidative Stress and Adhesion Formation**

**Free Radicals** are atoms or molecules that have one of more unpaired electrons, making it amenable to acquire an electron from other substances; they are highly reactive and can be damaging to tissues through oxidation.\textsuperscript{17} Generated from xanthine oxidase (generates superoxide and is the major producer of superoxide), heme oxygenase, cyclooxygenase and lipoxygenase (produce hydroxyl and peroxyl radicals), free radicals are also generated by neutrophils to destroy intracellular microorganisms during normal cellular oxygen metabolism;\textsuperscript{17} they are necessary for normal physiologic function and act as secondary messengers in several signaling pathways.

Free radicals must be regulated in order to prevent tissue damage and are controlled by the anti-oxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase.\textsuperscript{17} Superoxide dismutase protects against deleterious effects of superoxide (O\textsubscript{2}•) by catalyzing its dismutation to H\textsubscript{2}O\textsubscript{2} and O\textsubscript{2}.\textsuperscript{21} If an imbalance exists between free radical production and the natural defense mechanisms of anti-oxidant enzymes, oxidative stress results.\textsuperscript{17} This is thought to be a predisposition to adhesion formation via both increased free radical activity or decreased scavenger molecules.\textsuperscript{17}

Surgery has been shown to increase free radical activity, with an increase in superoxide anions, xanthine oxidase and malondialdehyde.\textsuperscript{17} During laparoscopy, free radical scavenger (glutathione peroxidase, superoxide dismutase and catalase) levels were negatively correlated with the duration and amount of
CO₂ gas exposure, while shorter duration of gas exposure resulted in a decreased incidence of post-operative adhesions.\textsuperscript{17} It has also been shown in mesothelial cells that exposure to CO₂ decreases free radical scavengers and stimulates oxidative stress, leading to an increase in 8-isoprostaglandin F (2α).\textsuperscript{17}

In addition, hypoxia acutely promotes superoxide generation from disparate intracellular sources including xanthine dehydrogenase oxidase, mitochondrial electron transport chain and NAD[P]H oxidase.\textsuperscript{21} When normal peritoneal and adhesion fibroblasts are exposed to superoxide, this leads to an increase in TGF-β1 and collagen type 1. These are also increased when the normal and adhesion fibroblasts are exposed to hypoxia. However, if normal peritoneal and adhesion fibroblasts are exposed to SOD, the levels of TGF-β1 and collagen type 1 in the adhesion fibroblasts are restored to the levels of normal peritoneal fibroblasts.\textsuperscript{21} Therefore, scavenging of superoxide during hypoxia protects against development of the adhesion phenotype.\textsuperscript{21}

Nitric Oxide (NO), also known as the “endothelium-derived relaxing factor”, is an oxygen-containing radical produced during hypoxia, and serves many roles depending on its bioavailability, timing of release, and the bioavailability of its scavengers. It may act as a ligand or substrate, and oxidant or antioxidant, or an inhibitor or activator.\textsuperscript{26} NO acts on different cell types in a variety of ways including apoptosis through peroxy-nitrite and other metabolites, inhibition of replication, and influencing cell differentiation.\textsuperscript{11} It may affect existing adhesion fibroblasts through apoptosis or decreasing their formation through inhibition of its precursors.\textsuperscript{11}

NO is typically produced in small amounts, but an overproduction or deficiency can lead to numerous diseases including asthma and cardiovascular disease.\textsuperscript{26} NO inhibits the deposition of collagen and has a potent antifibrotic effect under certain conditions.\textsuperscript{14} NO inhibits collagen synthesis in cultured fibroblasts, smooth muscle cells and other cell types.\textsuperscript{11} When NO is low, free radicals accumulate resulting in more collagen synthesis through fibrogenic processes like TGF-β1 and membrane lipid peroxidation.\textsuperscript{17} have demonstrated that long term blockade of NO leads to fibrosis of the heart, lung and kidney.\textsuperscript{11} Alternatively, elevated NO levels through iNOS may result in excess collagen synthesis during wound healing and thus promote adhesion development.\textsuperscript{13}

Nitric oxide synthetase (NOS): Nitric oxide is generated by a family of enzymes called nitric oxide synthases (NOS), which exist in three isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS).\textsuperscript{17} These enzymes catalyze the formation of citrulline and NO from L-Arginine through the formation of N-hydroxy-L-Arg as an intermediate. In order for this conversion to occur, O₂, NADPH and tetrahydrobiopterin (H₄B) as a cofactor are required. In the absence of L-Arg and H₂B, NO binds NOS heme iron at near diffusion rate and generates a five-coordinate Fe(II)-NO complex which inhibits the catalytic activity of the enzyme.\textsuperscript{26} Competitive inhibitors of NOS are methylated analogs of Arg (ADMA- asymmetric dimethylArg) and (L-NMMA, monomethyl Arg).\textsuperscript{17} Of the three NOS isoforms, only iNOS is responsible for synthesis of NO during inflammation.

Molecular O₂ is an essential substrate for iNOS, and hypoxia stimulates upregulation of iNOS expression in normal and adhesion fibroblasts.\textsuperscript{26} In adhesion fibroblasts, stimulation of iNOS expression is associated with attenuation of L-Arg and or H₂B which leads to iNOS uncoupling, therefore, leading to decreased NO in adhesion fibroblasts. Deficiency of L-Arg and H₂B allows iNOS to uncouple and produce the free radical O₂● instead of NO.\textsuperscript{26} Adhesions fibroblasts also have an increase in citrulline compared to normal peritoneal fibroblasts suggesting that there may be a partial disruption in the
transcellular pathway to recycle L-citrulline back to L-Arg. In contrast, normal peritoneal fibroblasts contain: 1) higher H4B and total biopterin, 2) higher levels of L-Arg, 3) lower levels of L-citrulline and 4) lower protein nitration. In a study involving cecal abrasion of rats, those fibroblasts exposed to hypoxic conditions had more dense, vascular and increased frequency of adhesion compared to normoxia fibroblasts. Thus, tilting the balance between NO production and scavenging under hypoxic conditions in peritoneal fibroblasts.

Although iNOS gene expression and iNOS levels are not different in normal and adhesion fibroblasts, normal fibroblasts have increased NO compared to adhesion fibroblasts. NO has been shown to inhibit collagen deposition in animal models, while the decrease of NO in adhesion fibroblasts is associated with overproduction of extracellular matrix molecules, specifically collagen type 1; this combination of decreased NO and increased ECM and collagen production favors adhesion development.

Myeloperoxidase (MPO) is a highly cationic heme protein which uses hydrogen peroxide (H2O2) and chloride ions to generate cytotoxic hypohalous acid and diffusible radical species, and may have a protectant role against the adhesion phenotype. It is traditionally expressed in neutrophils, macrophages and neutrophil precursors, but it has also been identified in normal peritoneal and adhesion fibroblasts. A possible cross talk between MPO and NO in adhesion fibroblasts has been suggested by the finding that the MPO/H2O2 system efficiently consumes NO produced by iNOS during steady state catalysis leading to an increase iNOS activity, production of citrulline and NO production.

Saed and colleagues have further demonstrated that iNOS and MPO are colocalized within the same cell in both normal and adhesion fibroblasts established from the same patients. While iNOS levels in normal peritoneal and adhesion fibroblasts were not different, MPO and NO levels were lower in adhesion fibroblasts, suggesting the MPO may play a protectant role in adhesion formation. They also demonstrated that utilizing a state of the art technology, siRNA, to silence the gene expression of iNOS and MPO, leads to a significant reduction in type I collagen and TGF-β1, which are hallmarks of the adhesion phenotype.

Role of Cellular Metabolism and Adhesion Formation

Oxidative stress and hypoxia play an important role in adhesion formation. Hypoxia triggers a cascade of responses which ultimately lead to adhesion formation. Under normoxic conditions, glucose is catabolized intracellularly to form pyruvate, which is further metabolized to produce adenosine triphosphate (ATP) via the citric acid cycle (TCA cycle). Conversely, under hypoxic conditions, the amount of pyruvate entering the TCA cycle is decreased and pyruvate is converted to lactate. Anaerobic glycolysis is activated by hypoxia-inducible factors (HIFs), which shift metabolism towards anaerobic glycolysis by altering glycolytic enzymes. HIFs inhibit pyruvate dehydrogenase, which converts pyruvate into acetyl CoA, and stimulate lactate dehydrogenase (LDH), which converts pyruvate into lactate; thus leading to increased production of lactate. Lactate may be crucial to adhesion formation through stimulation of other factors involved in adhesion formation such as VEGF and collagen.

Adhesion Phenotype

As a result of extensive research on the pathophysiology of adhesion development, Diamond and colleagues have defined the adhesion phenotype, which is a collection of biologic characteristics of
Adhesions and adhesion fibroblasts as compared to normal peritoneal fibroblasts. These are summarized in Table 2. Adhesion fibroblasts are myofibroblasts, which are activated fibroblasts that exhibit features intermediate between smooth muscle cells and fibroblasts, including expression of α-SM actin and a depleted antioxidant system. Whereas myofibroblasts disappear by apoptosis during normal healing, some pathologic processes can cause persistence of the myofibroblasts resulting in scarring. They are also the source for increased extracellular matrix protein expression as well as TGF-β1, which controls many of the cells’ crucial endpoints.

Compared to normal peritoneal fibroblasts, adhesion fibroblasts demonstrate the following:

1) Decrease in Nitric Oxide (NO) levels
2) Decrease in ratio of tPA/PAI-1
3) Decrease in rate of apoptosis under hypoxic conditions
4) Greater ability to produce TGF-β1 and extracellular membrane molecules

Adhesion fibroblasts also have an increase in basal mRNA of many substances including collagen type 1, fibronectin, MMP-1, TIMP-1, TGF-β1, TGF-β2 and IL-10. Hypoxia further increases the expression of collagen type 1, fibronectin, MMP-1, TIMP-1, TGF-β1, TGF-β2, IL-10, and IFN-γ in peritoneal and adhesion fibroblasts.

This phenotype can be induced, and replicated in vitro with human peritoneal fibroblasts under hypoxic (2% O₂) conditions, and once developed, is irreversible, even after restoration of normoxia. It is hypothesized that hypoxia induces the adhesion phenotype by stimulating high levels of free radicals including superoxide when then initiates a cascade of inflammatory pathways. Additional molecular markers of the adhesion phenotype are increased fibronectin, type I collagen, VEGF, TGF-β1, α-SM1 actin, and COX2, and decreased tPA/PAI-1, and MMP-1/TIMP-1.

**Current Therapies in Adhesion Prevention**

Current therapies in adhesion prevention are limited, as the pathophysiology of adhesion development has not been well understood until recently. These therapies include the tenets of good surgical technique and a variety of adhesion barriers. Good surgical technique was described by Boys “5 Fundamental attacks” directed towards prevention of adhesions which include: 1) limiting initial injury, 2) preventing coagulation of serous exudates, 3) dissolving or removing deposited fibrin, 4) keeping apart the fibrin-coated surfaces, and 5) inhibiting fibroblastic proliferation once established. Although the value of “microsurgical” technique is well accepted, it is clearly not enough in preventing adhesion formation and reformation as demonstrated by the high recurrence rate of adhesions noted on second look laparoscopy.

To supplement surgical technique, three physical barriers are currently FDA approved in the US for prevention of adhesion development.

**Icodextrin wt/vol 4% solution** (Adept; Baxter Healthcare, Deerfield, IL) - Adept is a free-running non-viscous fluid that is iso-osmotic and placed with ease into the peritoneal cavity via the laparoscope. It separates denuded tissues during the critical period of reepitheliazation via hydrofloation and is not site-specific. Adept does not require absolute hemostasis to be effective and remains in the peritoneal cavity.
for up to four days following application. It is currently the only anti-adhesion agent approved for use by the FDA in the United States for adhesion reduction following laparoscopy; it is not approved for use during laparotomy.\(^1\)

In a randomized double blind study of 402 gynecologic patients scheduled for laparoscopic adhesiolysis, 49% of patients randomized to Adept achieved clinical success at second look laparoscopy compared to 38% of patients randomized lactated Ringer’s solution; clinical success was defined as a reduction in adhesions of at least three sites or 30% of sites lysed (whichever was more between the initial surgery and second-look laparoscopy).\(^{13}\) Although clinical success was documented, there was not a statistically significant reduction in the severity and extent of adhesions between the two groups.\(^{23}\) Additionally, a recent European randomized, double-blind study demonstrated the safety of Adept, however, it failed to show a difference in treatment effect between lactated Ringers solution and 4% icodextrin solution for laparoscopic gynecologic patients.\(^{14}\)

**Modified hyaluronic acid and carboxymethylcellulose film** (Seprafilm: Genzyme corporation, Cambridge, MA)- Seprafilm is a bioresorbable membrane composed of modified sodium hyaluronate and carboxymethylcellulose. It is placed directly over damaged tissue and provides a physical separation of these tissues during the early phases of wound repair. It is in part resorbed from the peritoneal cavity within 7 days and is completely excreted within 28 days.\(^{15}\) It is FDA approved only for use at laparotomy but has been used anecdotally during laparoscopy through the formation of a slurry or delivery following rolling up the film and placing through a laparoscopic port.\(^1\)

The safety and efficacy of Seprafilm use during abdominopelvic laparotomy has been well established in randomized controlled trials. In a study of 183 patients undergoing colectomy with ileal pouch anal anastomosis and temporary loop ileostomy, 51% of Seprafilm treated patients had an absence of adhesions to the underside of the abdominal wall noted at time of ileostomy closure, versus only 6% of control patients; the formation of dense adhesion was also noted to be less in Seprafilm treated patients, 58% versus 13% (p<0.00000000001).\(^{16}\) In a multicenter trial of 127 patients undergoing uterine myomectomy via laparotomy, those patients randomized to placement of Seprafilm on the anterior and posterior surfaces of the uterus following myomectomy had a significant reduction in the mean number of sites adherent to the uterus noted at second look laparoscopy compared to controls (4.98 vs 7.88) and a significant reduction in the extent (p<0.01) and severity (p<0.02) of adhesions.\(^{16}\)

**Oxidized regenerated cellulose** (Interceed: Johnson and Johnson, Cincinnati, OH). Interceed is an absorbable adhesion barrier composed of oxidized regenerated cellulose fabric. It is placed over damaged tissue and provides a physical barrier to minimize apposition of serosal surfaces during the critical period of mesothelial repair. It forms a continuous gelatinous protective coat over raw tissue surfaces within 8 hours of surgery and is completely absorbed within 28 days.\(^6\) In addition to providing a physical barrier, Interceed was recently shown to have some biologic effects. Fibroblasts treated with Interceed demonstrated a 25% increase in tPA (tissue plasminogen activator) mRNA as well as an increase in the tPA/PAI-1 (plasminogen activator inhibitor-1) ratio.\(^{12}\)

Interceed has been shown to significantly reduce the occurrence and severity of postsurgical ovarian adhesions. In a multicenter randomized study, 55 patients were treated for bilateral ovarian disease via laparotomy and one ovary was randomly assigned to be wrapped with Interceed, while the other was left uncovered. At second look laparoscopy, adhesion scores were significantly reduced for the treated ovaries
(p=0.02), and significantly more ovaries treated with Interceed were adhesion free (26 of 55) compared to controls (14 of 55). In addition, the treated ovaries had less extensive adhesions compared to controls.\(^6\)

Despite these physical barriers, there is still need for improvement as the incidence of reformation and de novo adhesion formation remains high. Given the limitations of current therapy, much research has been done over the past decade to more completely understand why and how adhesions form. This understanding may lead to treatments that specifically target the precursors to adhesion development and effectively prevent adhesion formation and or reformation.

**Summary**

Adhesions can pose significant morbidity to the patient, however, our current treatment regimens are limited to adhesion barriers, which have varying clinical success rates, and are designed to prevent de novo adhesion development as well as adhesion reformation. Although we still do not completely understand the pathogenesis of adhesion development, it is evident that hypoxia plays an important role. Hypoxia stimulates a cascade that leads to oxidative stress, anaerobic metabolism, formation of free radicals and ultimately the adhesion phenotype. Given recent advances in understanding the pathogenesis of adhesion formation, we may potentially be able to target adhesion precursors and stimulants, and ultimately decrease postoperative adhesions.
References

Legends

Table 1. Adhesion Flow Chart

Table 2. Expression of Biologic Markers in the Adhesion Phenotype of Peritoneal Fibroblasts
Table I. Adhesion Flow Chart

Surgery

Injury

HYPOXIA

Increased Extracellular Matrix:
↑ Type 1 Collagen
↑ Fibronectin
↓ MMP
↑ TIMP

Anaerobic Metabolism:
↑ Lactate

Increased Inflammatory Mediators:
TNF-α
TGF-β

Decreased ratios:
tPA/PAI-1
MMP/TIMP

Increased Extracellular Matrix:
↑ Type 1 Collagen
↑ Fibronectin
↓ MMP
↑ TIMP

Xanthine/Hypoxanthine

Xanthine Oxidase

\[-\text{H4B/ -L-Arg}\]

\[\text{iNOS}\]

\[\text{NO}\]

\[\text{ONOO}^-\]

Protein Nitration

Oxidative Damage
DNA
Proteins
Lipids

Free Radicals \(O_2^+\)

SOD

\[\text{H}_2\text{O}_2\]

GSH Reuctase

\[\text{H}_2\text{O}\]

GSH Peroxidase

\[\text{GSSG}\]

\[\text{GSH}\]

Fe\(^{2+/3}\)

\[\text{H}_2\text{O} + \text{O}_2\]

Oxidative stress

Anaerobic Metabolism:
↑ Lactate

Anaerobic Metabolism:
↑ Lactate

Oxidative stress

Free Radicals \(O_2^+\)

SOD

\[\text{H}_2\text{O}_2\]

GSH Reuctase

\[\text{H}_2\text{O}\]

GSH Peroxidase

\[\text{GSSG}\]

\[\text{GSH}\]

Fe\(^{2+/3}\)

\[\text{H}_2\text{O} + \text{O}_2\]

Oxidative stress

Free Radicals \(O_2^+\)

SOD

\[\text{H}_2\text{O}_2\]

GSH Reuctase

\[\text{H}_2\text{O}\]

GSH Peroxidase

\[\text{GSSG}\]

\[\text{GSH}\]

Fe\(^{2+/3}\)

\[\text{H}_2\text{O} + \text{O}_2\]

Oxidative stress

Free Radicals \(O_2^+\)

SOD

\[\text{H}_2\text{O}_2\]

GSH Reuctase

\[\text{H}_2\text{O}\]

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Table 2. Expression of Biologic Markers in the Adhesion Phenotype of Peritoneal Fibroblasts

<table>
<thead>
<tr>
<th>Biologic Marker</th>
<th>Action</th>
<th>Expression</th>
<th>Summary</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue plasminogen Activator (tPA)</td>
<td>Major plasminogen activator in plasma; converts plasminogen into plasma and limits adhesion development</td>
<td>↓</td>
<td>Decreased basal levels of mRNA compared to NPF*; mRNA levels decreased by 95% under hypoxic conditions</td>
<td>Saed et al, 2003¹⁹</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1 (PAI-1)</td>
<td>Major plasminogen activator inhibitor in plasma</td>
<td>↑</td>
<td>Increased basal mRNA levels compared to NPF*; hypoxia increased mRNA levels by 53%</td>
<td>Saed et al, 2003¹⁹</td>
</tr>
<tr>
<td>tPA/ PAI-1 ratio</td>
<td>Marker for adhesion development</td>
<td>↓</td>
<td>Decreased by 98% in adhesion fibroblasts</td>
<td>Saed, et al, 2003¹⁹</td>
</tr>
<tr>
<td>Nitric Oxide (NO)</td>
<td>Oxygen-containing radical which can stimulate adhesion development in excess or deficiency</td>
<td>↓</td>
<td>Level decreased compared to NPF*</td>
<td>Saed, et al, 2006²⁶</td>
</tr>
<tr>
<td>Inducible nitric oxide synthetase (iNOS)</td>
<td>Responsible for synthesis of NO during inflammation</td>
<td>↑</td>
<td>Upregulated in response to hypoxia</td>
<td>Saed, et al, 2006²⁶</td>
</tr>
<tr>
<td>Myeloperoxidase (MPO)</td>
<td>May protect against adhesion development by consuming NO</td>
<td>↓</td>
<td>Levels decreased compared to NPF*</td>
<td>Saed, et al, 2009²⁷</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Extracellular matrix glycoprotein, important in wound healing</td>
<td>↑</td>
<td>Levels increased compared to NPF; upregulated under hypoxic conditions</td>
<td>Saed et al, 1999²⁹</td>
</tr>
<tr>
<td>Type 1 Collagen</td>
<td>Main structural protein in connective tissue</td>
<td>↑</td>
<td>In response to hypoxia, levels are irreversibly increased in NPF* to those of adhesion fibroblasts</td>
<td>Fletcher, et al, 2008²¹</td>
</tr>
<tr>
<td>Transforming growth</td>
<td>Cytokine that is increased in response to hypoxia, levels are</td>
<td>↑</td>
<td></td>
<td>Fletcher, et al,</td>
</tr>
<tr>
<td>Factor/Protein</td>
<td>Function/Effect</td>
<td>Expression/Regulation</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
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<td>--------------------------------------------------------------------------------------</td>
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<td></td>
</tr>
<tr>
<td>TGF-β1 (TGF-β1)</td>
<td>wound healing; induces migration of fibroblasts</td>
<td>irreversibly increased in NPF* to those of adhesion fibroblasts</td>
<td>2008&lt;sup&gt;21&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Cyclooxygenase2 (COX-2)</td>
<td>Catalyzes rate limiting step in prostaglandin synthesis; increased in inflammation</td>
<td>↑</td>
<td>Saed, et al, 2008&lt;sup&gt;25&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>Potent mitogen for endothelial cells and critical in angiogenesis</td>
<td>↑</td>
<td>Rout, et al, 2000&lt;sup&gt;30&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Matrix metalloproteases/TIMP-1/TIMP</td>
<td>MMPs are proteolytic enzymes important in remodeling extracellular matrix; they are regulated by TIMPs</td>
<td>↓</td>
<td>Saed, et al, 2000&lt;sup&gt;28&lt;/sup&gt;</td>
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<td>Tumor necrosis factor-alpha (TNF-α)</td>
<td>Inflammatory cytokine involved in healing process</td>
<td>↑</td>
<td>Ambler et al, 2006&lt;sup&gt;22&lt;/sup&gt;</td>
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</tbody>
</table>

* NPF (Normal peritoneal fibroblasts)