

The Role of Collagen Metabolism in the Formation and Relapse of Incisional Hernia

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Rezumat

Rolul metabolismului collagenului în formarea și recidiva herniilor incizionale

În ciuda progreselor continue în fabricarea plaselor sintetice de protezare și a utilizării lor tot mai extinse, hernia incizională recidivantă apare în 5 până la 20% din cazuri, cu o creștere liniară a incidenței de-a lungul anilor, sugerând astfel un proces multifactorial mai degrabă decât o simplă problemă de tehnică chirurgicală ca și substrat cauzal. Cercetări recente de biologie moleculară au arătat tot mai multe dovezi ale prezenței unor tulburări de țesut conjunctiv, cu deficiențe de vindecare a plăgilor și a cicatrizării la pacienții cu hernii incizionale. Deși există rezultate promițătoare, în prezent, înțelegerea profundă a mecanismelor fiziopatologice și a rolului collagenului în hernia incizională este încă limitată. Scopul acestui studiu sistematic al literaturii este de a evalua mecanismele biochimice implicate în formarea și recidiva herniilor incizionale, punând accentul pe rolul rației collagen tip I – collagen tip III. De asemenea, este discutată și aplicabilitatea acestora în practica chirurgicală.

Cuvinte cheie: hernii, incizionale, metabolism, collagen

Abstract

Despite the continuous development of synthetic prosthetic meshes and their wide use, recurrent incisional hernias still appear in 5 to 20% of cases, with a linear incidence curve over the years, suggesting a multifactorial process rather than a simple failing technical repair as the underlying cause. Recent molecularbiological research provide increasing evidence of connective tissue alterations such as a defective wound healing with impaired scarring process in patients with incisional hernia. Although there are some promising results, at present, in-depth understanding of the pathophysiological mechanisms and of the role that collagens play in the development and recurrence of incisional hernia is rather scarce. The aim of this systematic review is to summarize and evaluate the biochemical mechanisms involved in incisional hernia formation and recurrence, with a primary focus on collagen I to III ratio. Also, the consequences for surgical practice are discussed.

Key words: incisional hernias, metabolism, collagen

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Introduction

Incisional hernia is a common complication after laparotomy, occurring in 10% to 20% of cases (1). Since the introduction of synthetic prosthetic meshes, defect repair with polypropy-

lene mesh or expanded polytetrafluoroethylene has become the mainstay of treatment showing good short- and long-term results (2). However, a significant number of patients still develop multiple recurrences, with estimates from 5 to 20 % (1).

A very disturbing, but well designed retrospective study of Flum et al (3) in 2003, on cumulative incidences for re-operations of incisional hernias in the USA, that analyzed data from 10,822 patients operated by simple suture or by alloplastic procedures, clearly demonstrated that even the use of meshes does not substantially alter the outcome in terms of recurrence but, rather, delays the onset of recurrences for 2-4 years. Even though the use of mesh was associated with a 24% decrease in the hazard of reoperative repair at 5 years postoperatively, the relapse incidence curves tend to equalize in the two groups. The study showed an unexpectedly growing incidence of relapses over the years, not only in the suture group, but in the mesh group as well, the recurrence rate showing a nearly linear curve. (Fig. 1)

This data contradicts the assumption that incisional hernia recurrences occur simply due to technical failures. If that were the case, the recurrence rate should be reflected by an s-shaped curve, with a sharp incline at the beginning, but sooner or later reaching a plateau, respecting the universal rule of causal relationship between one technical component and its failure (Fig. 1). In contrast, in incisional hernia formation the cumulative incidences showed a linear rise over years without any s-shaped deformation suggesting that the etiology and pathogenesis of abdominal wall hernia formation is complex, biological and multifactorial (4,5).

Although risk factors for recurrent incisional hernias have been evaluated, the literature is controversial with regard to many of these, such as obesity, ascites, large hernias exceeding 10 cm in width or length, smoking, occupational lifting, and wound healing disorders (hematoma, seroma, infection) (1).

Abdominal wall hernia formation and recurrence was found to occur more frequently in patients with connective tissue disorders like hemorrhoids, varicose veins, perineal tear, congenital hip dislocation, aortic aneurysms, Ehlers-Danlos Syndrome and polycystic kidney disease (6-8). Retrospective and prospective studies have shown an average risk for incisional hernia after abdominal aortic aneurysm repair of 31.6 %. The association with hernias in these patients can be explained by systemic impairment of connective tissue formation and wound healing, confirming the biological basis underlying the pathogenesis of hernia formation.

Such findings emphasize the importance of mesh repairs and continued research in development of better mesh material, and also on primary tissue healing mechanisms and physiology of scar formation.

Methods

A literature search of the medical databases PubMed and Google Scholar was undertaken covering publications from January 1966 to December 2014 with the key word "incisional hernia", as major topic and also with the subheadings: 'collagen', 'collagen ratio', 'collagen metabolism' 'formation',

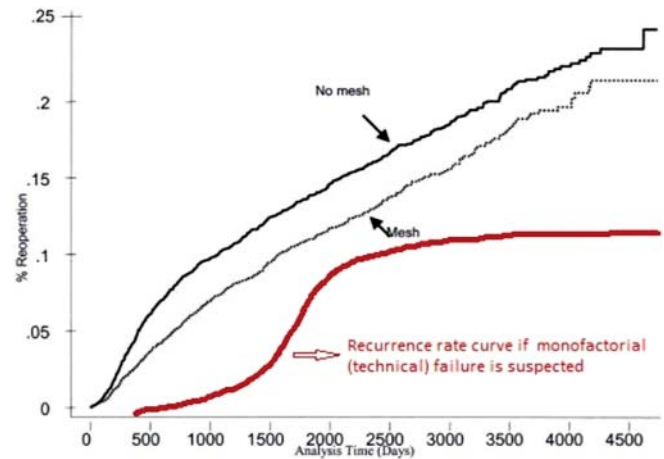


Figure 1. Rate of re-operations for recurrences in 10,822 patients with incisional hernia (3)

'recurrence' 'etiopathogenesis', 'connective tissue'. After the exclusion of case studies, letters to the editor, experimental animal studies and non-English-language papers, 41 relevant papers on hernia formation and collagen metabolism were identified and included in the review. The limited number of articles on collagen disturbances are in high contrast to the more than 3000 publications on "incisional hernia" listed in PubMed. No meta-analyses are so far available.

Collagen metabolism

Collagen, the principal component of the extracellular matrix, consists of 20 types and plays a crucial role in the maintenance of skin tensility and elasticity. The fibrillary collagen molecule is synthesized mainly by fibroblasts and other connective tissue cells. Intracellular hydroxylation reaction forming hydroxylysine and hydroxyproline is essential for both the formation and stability of the collagen molecule assembled into a triple helix. In the extracellular space, Lysyl oxidase enzymes mediate a cross-linking process that will lead to the formation of strong, stable collagen fibrils and fibers. (9)

Types I and III predominate, comprising 95% of the whole. Type I collagen is a strong collagen, widely distributed in the human body, including the fascia, skin, ligaments and fibrous tissue and is responsible for mechanical tissue resistance. Type III collagen is less cross-linked and provides less tensile strength, is found in smaller amounts in the same tissues and during the early days of wound healing and is subsequently replaced by type I collagen in the mature wound/scar. They interact to form the bundle architecture. Type I fibers are mature, strong and thick, whereas type III are thin and flexible. Normally, the former are approximately four times as prevalent as the latter. Increased amounts of type III collagen relative to type I collagen lead to a reduced fibril diameter (10).

The degradation of collagen is realized by the matrix metalloproteinases (MMP-1, MMP-2, MMP-8, MMP-13) (11). In general, MMPs are expressed at very low levels in normal tissues, but are induced as a consequence of pathological mechanisms and in the process of wound healing. (12)

When studying the collagen content in tissue samples, the result is often quantified by the type I : type III collagen ratio. The most common method implies immunohistostaining with goat anti-human IgG for collagen types I and III. Raised levels of type III collagen and consecutively a low collagen I to III ratio, may result in thinner collagen fibrils and higher levels of non-polymeric soluble collagen, because the associative properties of collagen I are relatively diminished (10)

A defective collagen metabolism contributes to a decreased tensile strength and mechanical stability of both the connective tissues and the induced scar tissue. After injury, balanced collagen maturation and degradation is a requirement for normal scar formation. (10,13) Alterations of the collagen composition may occur due to changes in collagen synthesis as well as turn-over. For the latter, matrix-degrading proteases may represent key factors. (11) Therefore these alterations in collagen formation should be of central relevance in the pathophysiology of incisional hernias.

Studies on collagen metabolism in patients with incisional hernia

Prior research has focused on the pathogenesis of incisional hernia as a disorder of collagen regeneration during wound healing.

In 1982, Busuttil, a vascular surgeon, reported that the type I/type III collagen ratio was decreased in the aortic media of patients with abdominal aortic aneurysm (14). A decade later, Friedman et al. (15) described increases in type III collagen gene expression and protein synthesis in cultured fibroblasts obtained from the skin of patients with inguinal herniation. They concluded that the presence of such a change would render an individual liable to herniation, incisional breakdown, and recurrence.

Since then, much of the recent research on the collagen type I/type III index has been conducted by Schumpelick's group at Aachen. Table 1 shows the most important studies on

Table 1. Relevant studies on the collagen metabolism in patients with incisional hernia

Ref. No	Year	No. of patients	No. of controls	Parietal defect	Method	Tissue sample	Findings
16	1999	12	14	Inc H	Western blot IHC	Skin	decreased ratio of type I : III collagen and type I : III procollagen mRNA compared with controls in the skin
17	2001	12	14	Inc H	Western blot IHC	Fascia	decreased ratio of type I : III collagen and type I : III procollagen mRNA compared with controls in the fascia
18	2002	12	10	Inc H, Rec Inc H	Western blot IHC, RT-PCR	Skin fibroblasts	cultured fibroblasts from patients with recurrent incisional hernia exhibit a decreased ratio of procollagen type I/III
19	2003	18	7	Ih Inc H	IHC	Skin scar	patients with recurrent incisional hernias exhibit the lowest types I to III collagen ratios MMP 1 expression is more pronounced in patients with recurrent incisional than in controls
20	2004	78	0	IH Inc H	IHC	Explanted meshes	significantly decreased collagen ratio for recurrent primary and incisional hernias
21	2007	16	9	IH	RT-PCR Western blot	Fascia	The MMP-1 mRNA transcripts were not different in IH versus control, but the MMP-2 level was significantly increased in patients with IH
25	2007	12	11	Rec Inc H	ICR	Non scarred skin, fascia	collagen I/III ratio in non scarred skin biopsies from the recurrent hernia group was significantly less compared with control subjects
22	2008	11	11	Rec Inc H	IHC	Skin scar	proto-oncogene c-myc was significantly raised in skin scars from patients with a recurrent incisional hernia
27	2013	623	0	Inc H Rec Inc H	IHC	Explanted meshes	in the recurrence subgroup of 64% of meshes with small pores and 83% meshes with large pores had a lowered collagen I to III ratio
29	2013	18	15	Rec Inc H	microarray-based analysis	Skin Fascia	bone morphogenetic protein antagonist 1, was under expressed in skin (fold = 0.49, $p < 10^{-7}$), $q = 0.0009$ and fascia (fold = 0.23, $p < 10^{-4}$), $q = 0.095$) of Rec Inc H patients compared with control
30	2014	41	19	IH, Inc H, Rec In H	IHC, Microscopy	Skin Fascia	both skin and abdominal wall fascia of hernia patients, collagen type I/III ratio was lower compared to control collagen type I/III ratio in skin was representative for that in abdominal wall fascia

IH : Inguinal Hernia, IncH : Incisional Hernia, Rec Inc H: Recurrent Incisional Hernia, IHC: Immunohistochemistry; MMP 1: Matrix metalloproteinase-1

collagen metabolism in patients with incisional hernia and their findings.

In 1999, Klinge et al. found that in the skin of patients with recurrent incisional hernia disease the ratio of collagen type I/III is decreased (ratio of controls with healthy skin $n = 7$, 2.1 ± 0.2 ; normal skin scar $n = 7$, $7.1.2 \pm 0.2$; patients with incisional hernia $n = 7$, 1.0 ± 0.1 ; recurrent incisional hernia $n = 5$, 0.8 ± 0.1). (16)

The same team, also found that collagen I/III ratio was also decreased in the fascia and also the scar tissue of patients with recurrent incisional hernias, compared with controls. (17-19)

In 2004, Junge et al, analyzed the collagen formation quantitatively (collagen-protein ratio) and qualitatively (collagen type I/III ratio) 78 prosthetic explants after inguinal and incisional hernia repair. Mean collagen-protein ratio was $45.3 \pm 8.5 \mu\text{g}/\text{mg}$, with significant differences between male ($43.8 \pm 9.1 \mu\text{g}/\text{mg}$) and female tissue samples ($48.1 \pm 6.8 \mu\text{g}/\text{mg}$, $P=0.033$). The mean collagen type I/III ratio of all samples investigated was 2.1 ± 1.4 . Samples explanted for recurring hernias exhibited a significantly decreased ratio (1.3 ± 0.7 , $P<0.05$) compared to samples explanted because of pain (3.4 ± 1.2) or infection (2.9 ± 1.6), suggesting that the composition of scar tissue with a lowered collagen type I/III ratio and, therefore, reduced tensile strength may be a major contribution to hernia recurrence. The same decreased collagen type I/III ratio was revealed even in the peri-mesh scar. (20)

The extent of MMP involvement in the formation of incisional hernia is unclear. One study of skin samples showed significantly more MMP-1 in patients with a recurrent incisional hernia, and only slightly raised MMP-1 levels in patients with a primary incisional hernia compared with controls (19). In contrast, in another study, MMP-1 was significantly decreased in the fascia of both primary and recurrent incisional hernias compared with controls (17). Yet another study found no difference in the MMP-1 level in the fascia transversalis of patients with an incisional hernia compared with controls, but the MMP-2 level was significantly increased (21).

Rosch et al found that the expression of the proto-oncogene c-myc (involved in proliferation and differentiation of fibroblasts and collagen synthesis and degradation) was significantly raised in skin scars from patients with an incisional hernia. (22)

A study of Cheng W. et al, showed that total collagen content in normal skin declined with age, particularly type III collagen which translates to a progressive increase in type I/III ratio (23). However, older age is associated with impaired wound healing and incisional hernia formation (24), leading to the idea that in elderly people, a low collagen I to III does not count for higher risk of developing incisional hernia.

White and colleagues performed a preliminary immunohistochemical trial examining the non scarred skin and fascia of 16 incisional hernia patients for collagen I and III and compared the ratio to normal fascia biopsies from bariatric patients. They found a significant decrease in the ratio in the skin of the hernia patients but found no difference in the fascia (25). Also, samples from the linea alba aponeurosis from

all types of ventral hernia were compared with controls, and showed a lower amount of type I collagen, whereas there was no difference in the amount of type III collagen in the two groups (26)

In 2013 Klosterhalfen, B., and U. Klinge published a 8 year study on 623 polypropylene mesh samples explanted for pain, infection, or recurrence, from patients with abdominal hernia (primary or incisional). Histopathological assessment included morphometry of inflammatory infiltrate, connective tissue and collagen I to III ratio. The study showed a normal collagen ratio in 285 patients (46%), and a considerably reduced ratio in 338 patients (54%). Out of these, the meshes removed for recurrence showed a lowered collagen I/III ratio in 70% of the cases, compared to those removed for pain (27%) or infection (30%). Furthermore, the mesh class seemed to influence the collagen ratio as well. In the absence of recurrence only 18% of the meshes with small pores but 50% of meshes with large pores had a lowered collagen ratio ($p<0.05$), whereas in the subgroup of patients with recurrence, 64% ($n=177$) of meshes with small pores and even 83% ($n=110$), meshes with large pores had a lowered collagen I to III ratio. ($p<0.05$) Also, large pore meshes showed an improved tissue response with less inflammatory side effects. (27)

In a previous study (28), we microscopically analyzed the mesh fragments explanted in 15 recurrent incisional hernia surgical procedures. We found that the mesh fragments explanted after inlay Rieves technique (4 cases), the collagen quantity, and also the width of the collagen fibers was significantly larger than after onlay Chevrel procedures (11 cases), with a ratio of approximately 3 to 1 (Fig. 2 A,B,C). The Rieves mesh explants were found in position, without packing or folding phenomena, and the incorporation of connective tissue was far superior (Fig. 3 A,B). More than 50% of the onlay recurrence had postoperative wound infection history, consistent with the high inflammatory infiltrate found on the mesh fragments.

Discussion

As the literature review showed, there is growing evidence that altered collagen metabolism appears to contribute to incisional hernia formation and recurrence. However, the scientific publications are hampered by certain weaknesses. Many of the studies had few patients or lacked a control group and many treated together patients with primary hernias with incisional or recurrent hernias. In our opinion one should differentiate between primary hernias and incisional hernias, although there are undeniable similarities. The only large study (27) is retrospective. Also, the analyses of collagen metabolism have been carried out on different tissue samples (skin, fascia, scar, explanted mesh) and it is not known which tissue is the most representative, and whether these tissue analyses are comparable.

The collagen metabolism in patients with incisional hernia appears to be altered at three levels: the collagen I to III ratio is decreased (higher content of the weaker type III collagen relative to the stronger type I collagen), the collagen

Figure 2. Microscopic analysis of mesh explants.
Hematoxylin and eosin staining
(A,B - Onlay mesh explant; C - Inlay mesh explant)

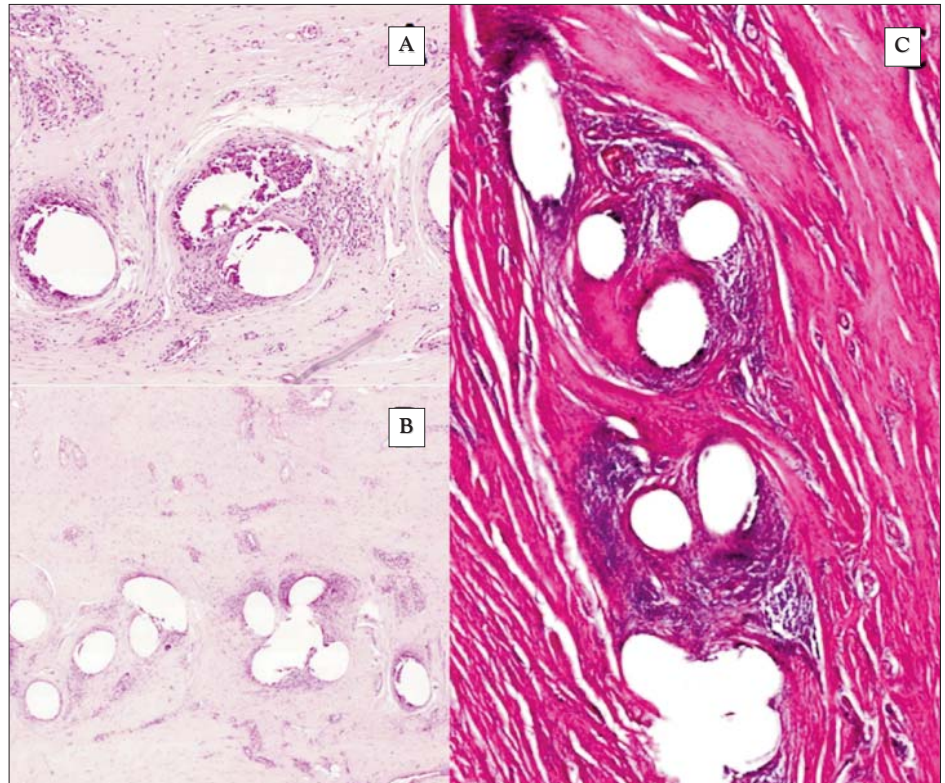
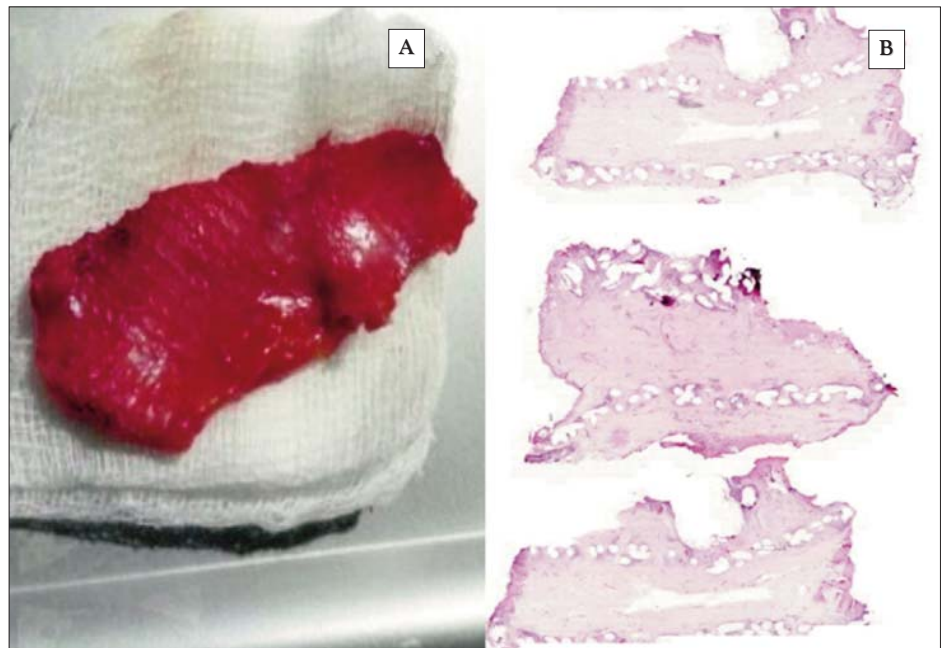


Figure 3. Unintegrated onlay mesh explant
(A. Macroscopic view; B. Microscopic view)



quality is poorer (thinner collagen fibers further contributing to weakness.), and collagen breakdown is increased (increased MMP-2 activity with a greater affinity for type I collagen)

Altogether, the studies concur that immunohistochemical assessment of collagen I to III ratio in skin or fascia biopsies is a useful marker of impaired collagen, metabolism which translates in high risk of developing incisional hernias.

The fact that the low collagen I to III ratio can be detect-

ed by immunohistostaining or mRNA analysis different levels in the non-scarred skin and not just locally at the parietal defect suggest a more severe, even systemic collagen dysfunction in patients with recurrent incisional hernias.

The fact that mesh material characteristics influenced the tissue response (27) confirming several animal experiments (31) unveils future possibilities of personalized approach in terms of selecting the appropriate mesh prosthesis. However,

evaluation of mesh specific reactions will prove rather difficult, considering the more than 200 different mesh devices on the market.

Despite the disappointing results in the study of Flum (3), it should be achievable to delay recurrence lifelong, if the mesh is placed with an extensive overlap of at least 5-7 cm in all directions, away from the margins of the parietal defect.

The apparent superior incorporation of retromuscular meshes (inlay Rieves technique) observed in our study was not statistically relevant due to the small number of cases, but it constitutes an interesting starting point that deserves further research. Unfortunately, to the moment, no other study addressed this topic. If this were indeed confirmed, then mesh placement as posterior as possible at pro or intra peritoneal level (inlay or sublay) should be performed on patients with incisional hernias and impaired collagen metabolism.

Last but not least, we should consider whether mesh repair should be the therapy of first choice in principle after primary laparotomies in patients with confirmed collagen metabolism impairment. However, clinical studies still have to prove such an approach.

Conclusions

Unfortunately, in our opinion, at present, neither skin or fascia biopsies with immunohistochemical assessment of collagen quantity, or collagen I to III ratio, nor RT-PCR of MMP-mARN sustain clinical applicability as a marker of poor outcome in incisional hernia repair, due to the high costs, the low availability of anticollagen antibodies in standard laboratory settings and also the requirement of an invasive procedure that cannot be performed as an intraoperative extemporaneous examination.

However, this review showed us some remarkable results, that should definitely change the perspective in how we treat patients with incisional hernias and most of all patients with recurrent incisional hernias. Knowing that a biological cause and a defective wound healing process is underlying cause of recurrent hernia disease allows us to grasp that simple suture repair is likely to fail and mesh implants are mandatory.

We advocate for the use of extensive overlap and Rieves technique in patients with connective tissue disorders and incisional hernia and in all patients with recurrent incisional hernia.

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