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Basic fibroblast growth factor loaded polypropylene meshes in repair of abdominal wall defects in rats

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Rezumat

Plasele de polipropilenă impregnate cu factor de creștere fibroblastic bazal în repararea defectelor peretelui abdominal la șoareci

Premize și scop: hernia incizională după laparotomie și hernierea recurentă sunt în continuare probleme de actualitate în ciuda perfecționării plaselor. Mecanismul biologic descris poate fi legat de metabolismul colagenului. Recent, câțiva membri ai familiilor factorilor de creștere au fost testați în prevenția dezunirilor de plagă și formării herniilor incizionale. Factorii de creștere pot iniția proliferarea fibroblastică și depunerea de colagen. În acest studiu, am căutat efectele factorului de creștere fibroblastic bazal (bFGF) într-un model de hernie incizională cu plasă pe șoareci.
Metodă: un total de 80 de șoareci Wistar albino au fost împărțiți randomizat în 5 grupuri. O procedură chirurgicală uniformă a fost utilizată în toate grupurile: a fost făcută o incizie tegumentară mediană de 5 cm și a fost excizat în totalitate peretele abdominal pe o arie de 3/2 cm. Peretele abdominal a fost rapid închis cu catgut 3/0 resorbabil. După această procedură standard, 5 procedee diferite au fost aplicate înainte de sutura tegumentară cu polipropilenă monofilament 4.0. Grupul 1, de control, nu a mai suferit nici o procedură suplimentară. Plasa de polipropilenă a fost utilizată în poziția stabilită, fiind fixată cu polipropilenă monofilament 4.0, fire separate la celelalte 4 grupuri. O plasă standard, fără tratament chimic, a fost utilizată în grupul 2. O plasă impregnată cu gelatină a fost utilizată la grupul 3, în timp ce grupurile 4 și 5 au primit plase impregnate cu bFGF în cantitate de 1 μg respectiv 5 μg. Toate grupurile au fost apoi divizate în subgrupuri (n=8 fiecare) de prima lună (precoce: P) și de a doua lună (tardiv: T), în funcție de data sacrificării. S-a făcut evaluare tensiometrică și histopatologică. Probele pentru histopatologie au fost recoltate de la interfața plasă organism și colorate cu hematoxilină-eozină, respectiv tricrom Masson "în orb" de un singur anatomopatolog, urmărind inflamația, vascularizația, activitatea fibroblastică, fibrele colagenice și organizarea țesutului conjunctiv. Metoda avidină-biotină-peroxidază a fost efectuată utilizând anticorpi monoclonali împotriva colagenului tip I și III.
Rezultate: plasele impregnate cu bFGF au prezentat valori de rezistență tensională crescută în comparație cu plasele standard după 2 luni. Studiile histopatologice și imunohistochimice au relevat, de asemeni, oarecare avantaje în favoarea plaselor impregnate cu bFGF față de plasele de polipropilenă standard. Aceste efecte limitate ale bFGF nu pot să fie dependente de doză.
Concluzii: folosirea plaselor impregnate cu bFGF în tratarea peretelui abdominal poate determina valori de rezistență tensională mai mari în comparație cu polipropilena standard. Cu toate acestea, studiile histopatologice și imunohistochimice au arătat numai o vindecare puțin mai bună în favoarea plaselor impregnate cu bFGF față de cele standard.

Cuvinte cheie: factor de creștere, fibroblast, FGF, vindecare plagă, perete abdominal, hernie incizională, cura herniei, plasă

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Abstract

Background and Aim: Incisional hernia following laparotomy and recurrent herniation after its repair are still common problems in spite of mesh augmentation. The underlying biological mechanism may be related to collagen metabolism. Recently, some members of growth factors family have been tested in the prevention of wound failure and incisonal hernia formation. Growth factors may promote fibroblast proliferation and collagen deposition. In the present study, we searched the effects of basic fibroblast growth factor (bFGF) loaded polypropylene meshes in an incisional hernia model in rats.

Methods: A total of 80 Wistar albino rats were randomly divided into five groups. A uniform surgical procedure was employed in all groups: a 5 cm skin incision was made at the midline and a full segment of the abdominal wall sized 3 x 2 cm was excised. Abdominal wall was closed with rapidly absorbable 3/0 catgut. Following this standard surgery, five different procedures were applied to the groups before closing the skin with 4/0 monofilament polypropylene sutures. Control subjects (Group 1) received no extra procedure after abdominal wall suturing. Polypropylene meshes were used in onlay position by fixing 4/0 monofimalent polypropylene interrupted sutures in other four groups. A standard mesh with no chemical treatment was used in Group 2. Gelatin coated meshes were used in Group 3, while Group 4 and 5 received bFGF loaded meshes with 1 microgram (μg) and 5 μg doses respectively. All the groups then divided into 1st month (early: E) and 2nd month (late: L) subgroups (n=8 each) according to sacrification dates. Tensiometric and histopathological evaluations were done. The specimens for histopathology were obtained from the interface area of the meshes and stained with hematoxylin and eosin, and also Masson trichrome. The variables were examined and evaluated by a single blinded pathologist under light microscopy in respect of inflammation, vascularization, fibroblast activity, collagen fibers and connective tissue organization. The avidin-biotin-peroxidase method was performed using the primary monoclonal antibodies against collagen type I and collagen Type III.

Results: bFGF loaded meshes showed higher tensile strength values in comparison with a standard polypropylene mesh after 2 months. Histopathological and immunohistochemistry studies also revealed somewhat better scores in favor of bFGF loaded mesh over a standard polypropylene mesh. These limited effects of bFGF did not seem to be dose dependent.

Conclusions: The use of bFGF loaded polypropylene mesh in the abdominal wall healing may cause somewhat higher tensile strength values in comparison with a standard polypropylene. However, histopathological and immunohistochemistry studies revealed only a slightly better healing in favor of bFGF loaded mesh over a standard polypropylene mesh.

Key words: growth factor, fibroblast, FGF, wound healing, abdominal wall, incisional hernia, hernia repair, mesh

Introduction

Prospective studies revealed that up to 20% of the laparotomies are resulted in incisional hernias (1,2). These hernias today are mostly repaired with prosthetic meshes with lower recurrence rates in comparison with suture repairs (3). However, surgeons still face with early and late recurrences in spite of mesh use (4).

The main cause of early incisional hernia development is technical failure. However, some hernias may appear even a proper wound closing is performed. Current concept in herniology says this kind of hernia formation is a collagen disorder (5). Deficiencies in collagen formation during wound healing may cause incisional hernia and recurrence after its repair. This suggests that a more comprehensive approach to incisional hernia repair than a mere mesh reinforcement is required. Recently, some members of growth factors family have been tested in the prevention of wound failure and incisonal hernia formation (6,7). Several studies have shown that local application of growth factors might lower the incidence of incisional hernia. Nevertheless, a recent experimental study stated that local application of growth factors did not augment the strength of the abdominal wall (8).

Basic fibroblast growth factor (bFGF) is a potent mitogen and chemoattractant that stimulates growth of many cell types including fibroblasts (9-11). It is an angiogenic factor in-vivo and in-vitro. Exogenous bFGF has been shown to stimulate wound healing process and produce a lower risk of experimental incisional hernia (7).

In this experimental study, it was tried to combine the potential effects of bFGF with the benefits of polypropylene meshes in abdominal wound healing. The objective of the study was not only to measure the incidence of incisional hernia, but also observing the healing parameters by tensiometry and microscopy.

Materials and Methods

This experimental study was designed in Diskapi Teaching and Research Hospital, Department of Surgery, and completed in the Animal Laboratory of Ankara University School of Medicine after obtaining ethical committee approvals from both institutions.

Animals

A total of 80 Wistar albino rats were used in the study. All rats were located within the separate cages to prevent cannibalism after rested for 1 week to avoid transport stress. They were fed with regular rat chow and tap water from drinking bottle.

Gelatin coating and FGF loading onto polypropylene meshes

Standard weight monofilament polypropylene mesh was used in the study (Herniamesh®, Italy). Gelatin was obtained from Difco, USA, and glutaraldehyde (50%) was purchased from BDH, UK. bFGF was purchased from Sigma®, Germany. Polypropylene meshes were cut into 20 mm x 20 mm pieces

and placed into Petri dishes. 48 mesh pieces were first coated with gelatin, then, 32 pieces of them were loaded with bFGF.

Aqueous gelatin solution (10% w/v) with glutaraldehyde (0.1 M) was poured onto square mesh pieces and dried at room temperature. Finally, thin layer of gelatin coatings approximately 100 μm in thickness were obtained on PP meshes. On the other hand, bFGF was first dissolved in 50 μL heparin, and then diluted with phosphate buffered saline (PBS, pH 7.4). Aliquots of PBS containing 1 μg or 5 μg FGF were impregnated onto gelatin coated meshes. 100 μL of a FGF solution containing 1 μg or 5 μg FGF was impregnated onto each gelatin coated mesh.

Surgical procedures and grouping

The subjects were randomly divided into five groups. A uniform surgical procedure was employed in all groups: After setting intraperitoneal anesthesia by using 0.09 mg/g ketamine hydrochloride (Ketalar®, Pfizer) and 0.01 mg/g xylazin hydrochloride (Rompun®, Bayer), the ventral abdominal wall was shaved and fully prepped with iodine solution. A 5-cm skin incision was made at the midline and a full segment of the abdominal wall sized 3 x 2 cm. was excised. Abdominal wall was closed with rapidly absorbable 3/0 catgut (Atravmat®, Dogsan). Following this standard surgery, five different procedures were applied to the groups before closing the skin with 4/0 monofilament polypropylene sutures (Prolene®, Ethicon) (*Table 1*). Control subjects (Group 1) received no extra procedure after abdominal wall suturing. Polypropylene meshes were used in onlay position by fixing 4/0 monofimalent polypropylene interrupted sutures in other four groups. A standard mesh with no chemical treatment was used in Group 2. Gelatin coated meshes were used in Group 3, while Group 4 and 5 received bFGF loaded meshes with 1 microgram (μg) and 5 μg doses respectively. All the groups then divided into 1st month (early: E) and 2nd month (late: L) subgroups (n=8 each) according to sacrification dates. Sacrifications were done with intraperitoneal overdose anesthetic injections.

Tensiometric tests

Lloyd LRX 5 K® mechanical test device was used for the assessment of tensile strength (Lloyd Instruments Limited, Hampshire, UK) of mesh applied tissue samples. Tension force was applied with a strain rate and gage length of 20 mm/min and 20 ± 2 mm, respectively. Each tensile test ended when the specimen tore completely. The maximum strain values were recorded as Newton (N). For tensiometric tests, the specimens were excised by leaving free abdominal wall tissue, 1 cm, at the vertical two sides of the mesh-tissue interaction line. Thus, the final dimensions of tensile specimens were 4 cm x 2 cm.

Table 1. *Different surgical procedures used for 5 groups (10 subgroups)*

Gr1E:	Primary closure (control group), 1st month sacrification
Gr1L:	Primary closure (control group), 2nd month sacrification
Gr2E:	Primary closure + polypropylene mesh, 1st month sacrification
Gr2L:	Primary closure + polypropylene mesh, 2nd month sacrification
Gr3E:	Primary closure + gelatin coated mesh, 1st month sacrification
Gr3L:	Primary closure + gelatin coated mesh, 2nd month sacrification
Gr4E:	Primary closure + 1 μg b-FGF loaded gelatin coated mesh, 1st month sacrification
Gr4L:	Primary closure + 1 μg b-FGF loaded gelatin coated mesh, 2nd month sacrification
Gr5E:	Primary closure + 5 μg b-FGF loaded gelatin coated mesh, 1st month sacrification
Gr5L:	Primary closure + 5 μg b-FGF loaded gelatin coated mesh, 2nd month sacrification

Histopathological study

The specimens were obtained from the interface area of the meshes (12) and fixed in 10% formaldehit, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and also Masson trichrome. The avidin-biotin-peroxidase method was performed using the primary monoclonal antibodies against collagen type I (1:100, Santa Cruz Biotechnology Inc, sc59772) and collagen Type III (1:100, Santa Cruz Biotechnology Inc, sc8781).

The variables were examined and evaluated by a single blinded pathologist under light microscopy in respect of inflammation, vascularization, fibroblast activity, collagen fibers and connective tissue organization. Inflammation was studied semiquantitatively according to the intensity of inflammatory cells. To evaluate the vascularization three separate hot fields were identified and examined by x 200 magnification. The vascular structures in these fields were counted and the mean number was calculated. Definition of vascularization was set as "+": 1-3 vessels, "++": 4-6 vessels, "+++": 7-10 vessels, and "++++" > 10 vessels. A similar definition was accepted for fibroblast count. Collagen fibers and connective tissue organization was evaluated semiquantitatively according to the intensity, homogeneity, parallelism to each other, and continuity with peripheral tissue collagen fibers (13).

The intensity and spread of collagen 1 and 3 was recorded by immunohistochemistry study. Areas for the analysis were selected under x10 magnification in a random manner for immunohistochemical scoring. The degree of positive staining was evaluated by semiquantitative scoring on a scale of 1 to 4 for intensity (I) such as inconspicuous (1), mild (2), moderate (3), and strong (4) and for distribution (D) such as perivascular or subepithelial (1), focal (2), patchy (3), and diffuse (4). Tissues with IxD less than or equal to 4 were considered weakly positive, and those with IxD greater than 4 were designated strongly positive (14).

Statistical analysis

SPSS for Windows version 11.5 software program was used for the statistical analysis. Histopathological scores and tensiometric values of 5 groups and each group's early and late results were compared by using Mann-Whitney U test. The effects of bFGF applications and duration were analyzed by using 2-way ANOVA and subsequent post hoc test. A p value < 0.05 was set as statistical difference.

Results

As expected, control group displayed a 92.3% incisional hernia rate, while no herniation was observed in other 4 groups where meshes were used. The values of 5 groups in early and late sacrification subgroups were compared. In addition, each group was evaluated by comparing its own early and late tensiometry and histopathology results.

Tensiometry results

All tears were observed at mesh/untreated tissue junction because of the mechanical strength of the mesh itself, except for the control group subjects. At the first month after surgery, there were no differences among five groups in respect of tensile strength measurements. However, at the end of the two months only Gr4L and Gr5L displayed significant improvements compared with their earlier values. Both groups also had significantly higher tensile strength values in comparison with Gr1L. In addition, Gr4L exhibited a significantly better mean tensiometric value in comparison with Gr2L (*Table 2*). However, neither the dose of bFGF and the duration had any effect on healing measured by tensiometry (p=0.79 and p=0.34).

Histopathologic examination

The histopathological findings and scoring in respect of inflammation, vascularization, fibroblast activity, collagen fibers and connective tissue organization were presented in *Table 3*. All these parameters were similar among early sacrifications groups except for a higher inflammation score in Gr4E in comparison with Gr1E and Gr2E. However, the later scores of the groups showed significant differences. Gr3L, Gr4L and Gr5L had higher vascularization scores than did Gr1L. Fibroblast scores were also better in mesh groups compared with the control subjects. Another striking finding was that only bFGF loaded mesh groups could improve their fibroblast scores between 1st and 2nd months. Lastly, Gr5L had a higher collagen fiber score than Gr2L.

Immunochemistry for type I collagen displayed better intensity scores for bFGF loaded mesh groups in comparison with the control group both in the early and late phases of the study (*Table 4*). Type I collagen distribution was also similarly better in bFGF loaded mesh groups (*Fig. 1 A,B*). Other two mesh groups, where untreated and gelatin coated meshes were

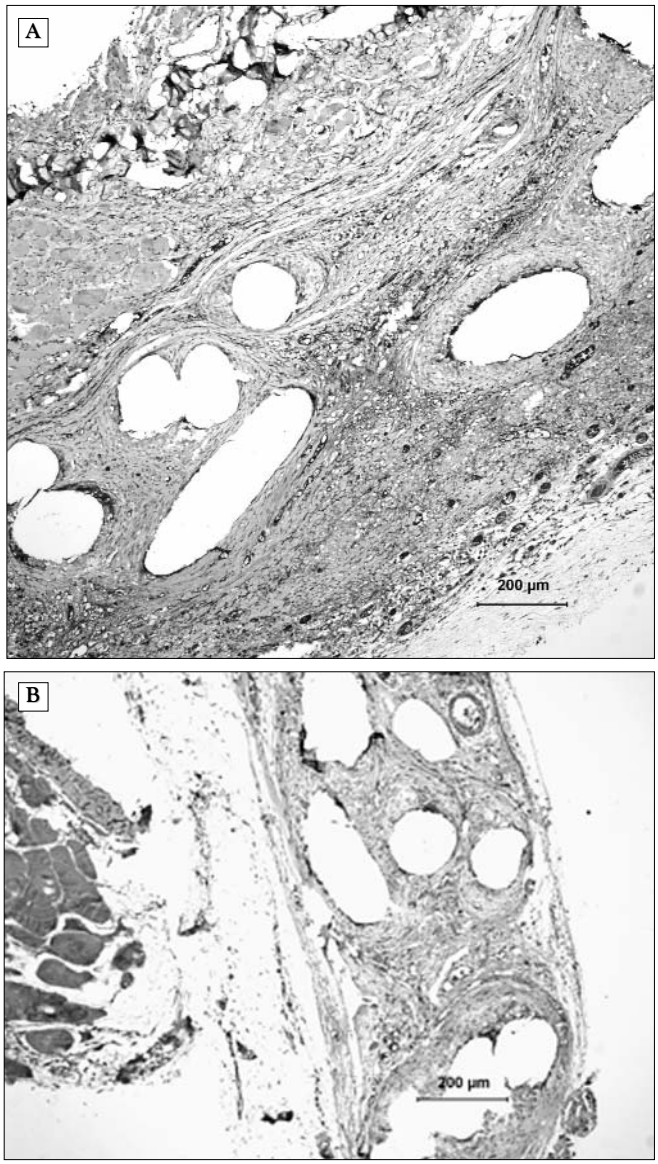

Figure 1. *Staining for type 1 collagen fibers after 2 months. (A). A subject received 5 µg bFGF loaded mesh in Gr5L; intensity:++++, distribution: ++++. (B) Another subject with untreated mesh in Gr2L; intensity: ++, distribution: +++. Immunoperoxidase staining, 100X magnification*

Table 2. *The mean tensile strength values of the groups in 1st and 2nd month tensiometry tests*

	Tensile strength	
	1st month [E]	2nd month [L]
Gr1	9.61 (1.59)	8.99 (1.85)
Gr2	9.82 (1.55)	9.41 (1.83)
Gr3	10.54 (1.50)	10.24 (2.82)
Gr4	9.35 (0.94)	12.18 (1.85)
Gr5	8.90 (1.85)	11.43 (1.50)

Values in parenthesis display standard deviation

Gr1L vs Gr4L : p<0.05	Gr1L vs Gr5L : p<0.05	Gr2L vs Gr4L : p<0.05
Gr4E vs Gr4L : p<0.05	Gr5E vs Gr5L : p<0.05	

Table 3. *The mean histopathology scores of the groups*

	Gr1E	Gr2E	Gr3E	Gr4E	Gr5E	Gr1L	Gr2L	Gr3L	Gr4L	Gr5L
Inflammation*	1.17 (0.41) [1-2 » 1.0]	1.13 (0.35) [1-2 » 1.0]	2.00 (1.00) [1-3 » 2.0]	2.60 (0.99) [1-3 » 3.0]	1.20 (0.44) [1-2 » 1.0]	1.28 (0.48) [1-3 » 2.0]	1.17 (0.41) [1-2 » 1.0]	1.80 (0.83) [1-3 » 2.0]	1.75 (0.71) [1-3 » 2.0]	1.43 (0.53) [1-2 » 1.0]
Vascularization†	2.83 (0.98) [2-4 » 2.5]	2.87 (0.99) [2-4 » 2.5]	3.40 (0.54) [3-4 » 3.0]	3.60 (0.89) [2-4 » 4.0]	3.00 (0.00) [3-3 » 3.0]	2.43 (0.78) [2-4 » 2.0]	3.17 (0.41) [3-4 » 3.0]	3.80 (0.44) [3-4 » 4.0]	3.87 (0.35) [3-4 » 4.0]	3.57 (0.53) [3-4 » 4.0]
Fibroblast‡	2.50 (0.54) [2-3 » 2.5]	2.62 (0.74) [2-4 » 2.5]	3.00 (0.70) [2-4 » 3.0]	3.00 (0.00) [3-3 » 3.0]	2.20 (0.44) [2-3 » 2.0]	2.28 (0.48) [2-3 » 2.0]	3.50 (0.54) [3-4 » 3.5]	3.20 (0.44) [3-4 » 3.0]	3.75 (0.46) [3-4 » 4.0]	3.43 (0.53) [3-4 » 3.0]
Collagen fibers§	2.46 (0.51) [2-3 » 2.0]	2.12 (0.64) [1-3 » 2.0]	2.40 (0.54) [2-3 » 2.0]	2.60 (0.54) [2-3 » 3.0]	2.60 (0.54) [2-3 » 3.0]	2.43 (0.53) [2-3 » 2.0]	2.00 (0.00) [2-2 » 2.0]	2.00 (0.00) [2-2 » 2.0]	2.50 (0.75) [2-4 » 3.0]	2.71 (0.48) [2-4 » 3.0]
Connec. tissue org.	3.16 (0.40) [3-4 » 3.0]	2.37 (0.74) [1-3 » 2.5]	3.00 (0.00) [3-3 » 3.0]	2.80 (0.44) [2-3 » 3.0]	2.60 (0.54) [2-3 » 3.0]	3.00 (0.00) [3-3 » 3.0]	2.83 (0.40) [2-3 » 3.0]	2.60 (0.54) [2-3 » 3.0]	3.00 (0.53) [2-4 » 3.0]	2.71 (0.48) [2-3 » 3.0]

Values in parenthesis display standard deviations. Brackets show ranges and medians

*	Gr1E vs Gr4E; p=0.030	Gr2E vs Gr4E; p=0.019				
†	Gr1L vs Gr3L; p=0.018	Gr1L vs Gr4L; p=0.006	Gr1L vs Gr5L; p=0.017	Gr3L vs Gr4L; p=0.029		
‡	Gr1L vs Gr2L; p=0.008	Gr1L vs Gr3L; p=0.030	Gr1L vs Gr4L; p=0.001	Gr1L vs Gr5L; p=0.007	Gr4E vs Gr4L; p=0.030	Gr5E vs Gr5L; p=0.010
§	Gr2L vs Gr5L; p=0.035					

Table 4. *The mean collagen type I and III scores of the groups in immunohistochemical study*

	Gr1E	Gr2E	Gr3E	Gr4E	Gr5E	Gr1L	Gr2L	Gr3L	Gr4L	Gr5L
Collagen type I										
intensity*	2.00 (0.00) [2-2 » 2.0]	2.37 (0.52) [2-3 » 2.0]	2.80 (0.83) [2-4 » 3.0]	3.60 (0.54) [3-4 » 4.0]	3.20 (1.09) [2-4 » 4.0]	2.00 (0.00) [2-2 » 2.0]	3.00 (0.63) [2-4 » 3.0]	2.80 (0.83) [2-4 » 3.0]	3.62 (0.51) [3-4 » 4.0]	3.29 (0.48) [3-4 » 3.0]
distribution†	2.50 (0.54) [2-3 » 2.5]	3.12 (0.35) [3-4 » 3.0]	3.40 (0.54) [3-4 » 3.0]	4.00 (0.00) [4-4 » 4.0]	3.60 (0.54) [3-4 » 4.0]	2.57 (0.53) [2-3 » 3.0]	4.00 (0.00) [4-4 » 4.0]	4.00 (0.00) [4-4 » 4.0]	3.75 (0.47) [3-4 » 4.0]	4.00 (0.00) [4-4 » 4.0]
Collagen type III										
intensity‡	2.33 (0.51) [2-3 » 2.0]	2.25 (0.46) [2-3 » 2.0]	3.20 (0.44) [3-4 » 3.0]	3.20 (1.09) [2-4 » 4.0]	2.80 (0.83) [2-4 » 3.0]	2.14 (0.37) [2-3 » 2.0]	2.66 (0.51) [2-3 » 3.0]	2.60 (0.89) [2-4 » 2.0]	2.75 (0.46) [2-3 » 2.0]	3.14 (0.37) [3-4 » 3.0]
distribution§	2.50 (0.54) [2-3 » 2.5]	3.12 (0.35) [3-4 » 3.0]	3.40 (0.54) [3-4 » 3.0]	3.40 (0.54) [3-4 » 3.0]	3.60 (0.54) [3-4 » 4.0]	2.57 (0.53) [2-3 » 3.0]	3.33 (0.51) [3-4 » 3.0]	3.40 (0.54) [3-4 » 3.0]	3.25 (0.46) [3-4 » 3.0]	3.71 (0.48) [3-4 » 4.0]

Values in parenthesis display standard deviations. Brackets show ranges and medians

*	Gr1E vs Gr4E; p=0.004	Gr2E vs Gr4E; p=0.011	Gr1L vs Gr2L; p=0.008	Gr1L vs Gr4L; p=0.000	Gr1L vs Gr5L; p=0.001		
†	Gr1E vs Gr5E; p=0.030	Gr2E vs Gr4E; p=0.006	Gr1L vs Gr2L; p=0.001	Gr1L vs Gr3L; p=0.003	Gr1L vs Gr4L; p=0.004	Gr1L vs Gr5L; p=0.001	Gr2E vs Gr2L; p=0.005
‡	Gr2E vs Gr3E; p=0.019	Gr1L vs Gr5L; p=0.004					
§	Gr1E vs Gr5E; p=0.030	Gr1L vs Gr5L; p=0.007					

used, also showed better distribution for type I collagen in the late phase in comparison with the control subjects. The difference between Gr2 and Gr4 at 1st month disappeared at 2nd month as Gr2 displayed a significant improvement by time. On the other hand, in respect the intensity and distribution of type III collagen, only Gr5 reflected better scores in comparison with Gr1.

In 2-way ANOVA and post hoc test, bFGF application had a significant effect on inflammation score ($p=0.026$). Both bFGF and longer time affected fibroblast proliferation positively ($p=0.029$ and $p=0.001$). However, neither factor had ant effect on collagen deposition.

Discussion

Repair of primary abdominal wall hernias, incisional hernias or abdominal wall defects and the prevention of incisional hernias after laparotomies for major intraabdominal pathologies are two different aspects of the same common surgical problem. Apart from early postoperative herniation due do technical failure during wound closure a similar story may affect these processes. Meshes offer better results over traditional suture techniques in treatment of those conditions. However, a recent survey says that meshes do not reduce the risk of recurrence dramatically, but only delay its occurrence (4). Therefore, the researches to find novel methods for making repairs stronger and longer lasting are still going on.

Wound healing is a very complex process; abdominal wound healing is even more complicated because of the dynamics of the abdominal wall. Numerous endogenous substances are naturally involved in healing process, while some exogenous applications have been inserted in clinical or laboratory use. Incisional herniation may be associated with abnormalities in collagen metabolism (5). Therefore better long-term results may be obtained with biological interventions which can ameliorate these disorders in the early phase of the repairs.

The members of the growth factor family are derived primarily from tissue monocytes and macrophages, and promote chemotaxis, angiogenesis, fibroblast proliferation and collagen synthesis (9,15). They may have a potential to restore wound healing process and create a stronger repairs. Indeed, different growth factors have been studied with this purpose since 1980's. Initial studies displayed that these factors shorten the healing time and decrease the size of pressure and diabetic ulcers (16,17). Exogenous bFGF was first used by Robson et al. to decrease the size of pressure sores (18).

In a very first paper on the use of growth factors for incisional healing, published in Science in 1987, Mustoe and colleagues tested the effects of transforming growth factor-β (TGF-β) in rat dorsal skin incisions by the aid of bovine collagen as the vehicle (19). They demonstrated a dose-dependent direct stimulatory effect in breaking strength after a single application.

The first study on the use of growth factors in an incisional hernia model was published in 2001 (6). A research team from Michigan University injected TGF-β2 in an aqueous solution, into the abdominal wound. They observed no incisional hernia in treatment group after 28 days, while an 88% hernia rate was recorded in the control group. Fibroblast number was significantly higher in TGF-β2 treated incisions. Histopathological and immunochemical collagen studies also revealed better results in the treated group. Then, the same team used bFGF loaded polymer rodes with continuous release in a similar model (7). They had a high incisional hernia rate of 90% in non-treated subjects again, while the incidence of incisional hernia was 30% in bFGF treated group, after 28 days. Their breaking strength measurements and qualitative collagen studies were also in favor of growth factor application. In the second step of the same study, all incisional hernias were repaired using the same polymer rods with or without bFGF. Strikingly, only 23% recurrence rate was observed in bFGF loaded rod group, whereas 86% of the placebo rod group developed recurrence after another 28 days. The work of Michigan group, thence, presented a complete promise for both incisional hernia protection and treatment.

One year later, Korenkov and colleagues from Mainz University stated that local application of growth factors did not augment the strength of the abdominal incision (8). They used TGF-β1 in coated absorbable suture and absorbable mesh, and also an intramuscular bolus injection. Interestingly, bolus injection even resulted in a decreased strength. Finally, Aachen group positioned onlay a mesh piece incubated with TGF-β3 in the abdominal wall and observed modification of collagen formation (20). Neither quality nor quantity of collagen was affected by the growth factor manipulation.

The latest study to date on bFGF use in abdominal healing arose from Turkey (21). A 3-day subcutaneous bFGF injection regimen in a dose of 5 μg/kg resulted in better values of tensile strength, hydroxyproline content, and other wound healing parameters in abdominal wall fascia healing.

In the present study, bFGF treated meshes displayed somewhat better tensile strength values after two months. Tensile strength is the eventual consequence of good wound healing. Growth factors including bFGF are expected to promote angiogenesis, fibroblast proliferation and collagen synthesis. Therefore, the mechanism of a possible benefit in wound healing after bFGF treated mesh can be understood when we look at histological parameters of the inflammation and healing. Especially fibroblast proliferation and collagen score is important. In the present study, the apparent findings in microscopy were that bFGF loaded meshes improved fibroblast scores between 1st and 2nd months, and 5 μg bFGF loaded meshes created a higher collagen fiber score than untreated meshes. Nevertheless, histopathological examination and immunohistochemistry for collagen hardly showed an obvious advantage in favor of bFGF application.

Those variable results coming from different studies about the effects of the exogenous growth factors on the abdominal incisional healing might be related to several reasons. In fact, despite a bunch of growth factors have been used in many

experimental and some clinical studies to date there are still unclear points about their optimal use. First, the delivery of the growth factors differs between the studies. The spectrum ranges to various vehicles to direct injections. Vehicle trials include liquids, gels and collagen containing materials. It has recently been shown that a proper delivery method is crucial for ability of growth factor used (22). Growth factors have very short half-life. Therefore, single injections probably could not affect the healing period for a sufficiently long period. Delayed-release materials, like that in Dubay's study and the present one, may create longer lasting effects. Dubay et al documented a sustained delivery of bFGF after the implantation by measuring the serum levels. The same group also displayed that bFGF absorption lasted for at least 3 days after the application. However, this kind of tests is quite expensive and the technology is not available in every center. We did not have a chance to document serum or tissue growth factor levels.

Optimal doses of growth factors to obtain maximum benefit in wound healing are not definite for human being or animals. Furthermore, different doses may be required for healing of different tissues. Various doses from 1 ng to 200 μg have been used in bFGF studies on wound healing (23-26). Not only the application ways and doses but also the results are heterogenous. In addition, whether the effects are dose-dependent is not clear. Two different doses were used in the present study and both resulted in better healing. However, a dose-dependent effect did not appear. This was possibly due to either the short half-life of bFGF or a shorter release from the coated mesh than we had expected during the study planning. It is surely possible to use higher doses of growth factor in this sort of experiments. However these substances are quite expensive, while most of the prosthetic meshes in use for abdominal wall healing are not. An acceptable cost/benefit ratio will be the key element when a clinical use is conasidered. Although we did not exhibit a dose-dependent effect in the present experiment, it is always possible to test different doses in further studies.

Basically, FGF and some other growth factors have been found in the early wounds and body fluids. These factors are naturally indigenous elements of wound healing. Junge et al. found that mesh repair enhanced FGF expression and fibroblast count more than suture repair (12). However, Di Vita and colleagues revealed a decrease in serum level of bFGF production following mesh repair immediately after surgery (27). They speculated that polypropylene meshes might impair the expression of angiogenic factor initially. Therefore, using meshes with built-in FGF may also be useful to cope with this early lack of FGF in the wound.

Besides the lack of serum FGF measurements, the present study has some other shortcomings. The 1st and 2nd month groups received the same procedure in each arm of the study, however the subjects were not the same rats. The design of the study were based on tissue evaluation, therefore sacrification was mandatory contrary to serum measurement protocols that could keep the subjects alive. On the other way, collagen studies were qualitative. A more precise result could be obtained if tissue hidroxyprolene concentrations were obtained as in Fedakar-Senyucel and colleagues' recent study that showed local and sustained release of FGF enhanced the healing of esophageal anastomoses (28). Lastly, unlike previous studies on the effects of growth factors in abdominal wound healing and incisional hernias, we used an unresorbable prosthetic material in the model. It was not possible to measure the strength of the abdominal wound itself because the mechanical strength of the mesh was already strong enough on the linea alba. We, instead, examined the mesh-tissue interference as others did before (12). In fact, most of late recurrences develop at the mesh-tissue interference (4). Therefore, the present model might produce a more reliable and predictive opinion about the duration of the repair.

In conclusion, the use of bFGF loaded polypropylene mesh in the abdominal wall healing may cause somewhat higher tensile strength values in comparison with a standard polypropylene mesh after 2 months. However, histopathological and immunohistochemistry studies revealed only a slightly better healing in favor of bFGF loaded mesh over a standard polypropylene mesh. This potential effect did not seem to be dose dependent. The decision about the use of bFGF loaded polypropylene mesh in clinical setting seemed to need further investigations.

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