Surgical Mesh as a Scaffold for Tissue Regeneration in the Esophagus

Petra Lynen Jansen^a U. Klinge^b M. Anurov^d Svetlana Titkova^d
P.R. Mertens^c M. Jansen^b

^aInterdisciplinary Center for Clinical Research (IZKF) Biomat, and Departments of ^bSurgery and

^cNephrology, Rhenish Westfalian Technical University, Aachen, Germany; ^dDepartment of Physiology, Russian State Medical University, Moscow, Russia

Key Words

Polyvinylidene fluoride · Polyglactin · Tissue engineering · Foreign body response · Regenerated esophageal tissue, function

Abstract

Background: Textiles in the form of surgical meshes are widely used in hernia surgery. Their porous structure allows tissue infiltration to incorporate the fabric for complete healing and device stabilization. This study was aimed to reconstitute the esophageal wall and to investigate the functional and histological consequences of a new, non-absorbable polyvinylidene fluoride (PVDF) mesh and an absorbable polyglactin 910 (Vicryl®) mesh.

Methods: Semicircular esophageal defects of 0.5 × 1 cm were created 2 cm proximal of the cardia in 10 rabbits. This gap was bridged using either polyglactin 910 or PVDF and additionally covered by omental wrapping. The clinical outcome was observed by clinical observation, regular esophagoscopies and X-ray contrast medium examinations. Local tissue regeneration was verified by light microscopy and immunohistochemistry. **Results:** After an observation period of 3 months we found no anastomotic strictures, complete mucosal regenera-

tion, minimal inflammation reaction and initial regeneration of the muscle layer for the PVDF group. Within the polyglactin 910 group, three patch failures with consecutive anastomotic leakage occurred. **Conclusion:** The results indicate that PVDF mesh structure gives the opportunity of local tissue regeneration in the esophagus. Though re-epithelialization and muscle cell ingrowth could be detected for absorbable polyglactin 910 mesh, this implant was accompanied by a high and early rate of anastomotic leakage.

Copyright © 2004 S. Karger AG, Basel

Introduction

A variety of biomaterials have been proved in the reconstruction of the esophagus [1]. In humans, the repair of small defects after injury using pleura, diaphragm, intercostal muscle, gastric fundus, lung or pericardium [2–6] is performed but can be complicated by anastomotic leakage with a lethality of up to 25% [7]. In animal models, naturally derived macromolecules (collagen, hyaluronic acid) and acellular tissue matrices (e.g. small-intestine submucosa) serve as temporary scaffolds which allow regeneration of local tissue but do not have the

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2004 S. Karger AG, Basel
0014-312X/04/0362-0104\$21.00/0

Accessible online at:
www.karger.com/esr

Petra Lynen Jansen, MD
Interdisciplinary Center for Clinical Research (IZKF) Biomat, RWTH-Aachen
Pauwelsstrasse 30, DE-52057 Aachen (Germany)
Tel. +49 241 808 8842, Fax +49 241 808 2497
E-Mail plynen@ukaachen.de

mechanical strength to provide the reinforcement of the tissue [8]. Artificial materials (Dacron, polytetrafluorethylene, silicon) are used as foils and frequently led to stenosis due to extended development of fibrosis [9]. Though Gonzalez Saez et al. [10] showed that PTFE patches are possible to suture and do not impede epithelialization, only scar tissue occurred and there was no muscle regeneration at the patch areas. Due to their pores, structure meshes combine permission of tissue infiltration and device stabilization. Former studies of our group showed that the extent of foreign body reaction and fibrosis around the mesh clearly depends on the kind of polymer and its structure (filament size, tensile strength, pore size) [11–13].

Polymers in the form of surgical meshes are frequently used in hernia disease. High rates of recurrence hernias led to an increasing number of mesh implantation from 34.7% in 1987 to 65.5% in 1999 [14]. Additionally, meshes have become essential for the temporal closure of the abdominal cavity [15]. In these areas the biocompatibility of mesh implants was proved in detail. Polyvinylidene fluoride (PVDF, Pronova®) is a new, non-absorbable polymer which features superior textile and biostable properties: compared to polyester it shows a higher mechanical stability. The progression of rigidity as seen with polypropylene cannot be found. Klinge et al. [16] analyzed the biocompatibility of a PVDF after implantation in rats in comparison to a heavy-weight polypropylene mesh. Regarding the histological reaction of the mesh (inflammatory infiltration, vascularization, connective tissue ingrowth), the number of infiltrating cells (granulocytes, macrophages, fibroblasts, lymphocytes and foreign giant body cells) and the cellular response (DNA strand breaks, apoptosis, proliferation, cell stress, HSP70), they found that PVDF could be an advantageous alternative to the commonly used materials due to an improved biostability and biocompatibility [16].

Polyglactin 910 (Vicryl®), being absorbable, is completely degraded at 3 months [17]. Though these implants cause only a temporary foreign body reaction, polyglactin 910 leads to a collagen-rich scar tissue and a pronounced inflammatory reaction [11]. The aim of this study was to investigate the functional and histological outcome after implantation of a porous, absorbable polyglactin 910 mesh or a porous, non-absorbable PVDF in the esophageal wall.

Materials and Methods

Animals and Anesthesia

A total of 10 male chinchilla rabbits (mean body weight 2.41 ± 0.23 kg) were included in this study. The animals were kept in single cages under standard laboratory conditions with unrestricted access to water. After operation no oral food uptake was allowed for 24 h. After adaptation, the rabbits were randomly assigned to two groups of equal numbers. The surgical procedure was performed under sterile conditions and general anesthesia by intravenous administration of ketamine (Ketamin 10%; Sanofi-Ceva, Düsseldorf, Germany) and xylazine (Rompun 2%; Bayer, Leverkusen, Germany).

Surgical Procedure

The distal esophagus was exposed and completely mobilized via a midline incision. A semicircular, transmural defect of 0.5×1 cm, according to one third of the circumference, was created 2 cm proximal of the cardia. Technically, the transabdominal approach was the easiest way to reach the esophagus. The transmural defect was performed with scissors. The gap was bridged using either an absorbable polyglactin 910 mesh or a non-absorbable PVDF mesh. The meshes were fixed to the esophageal wall by a running suture of 5-0 Prolene®. Additionally, the mesh area was covered with omentum majus (fig. 1).

Clinical Examination

During the observation period of 12 weeks, esophagoscopies and X-ray contrast medium examinations were performed every 4 weeks. After 3 months, surviving rabbits were sacrificed by a lethal dose of pentobarbital sodium (Narcoren; Rhône Mérieux, Laupheim, Germany). The thorax and abdomen were opened via a full midline incision for complete examination. Finally, the distal esophagus including the cardia region was resected and fixed for further examination.

Morphological Study

Specimens were studied by light microscopy. For light microscopy, tissue samples were fixed in 10% formalin, embedded in paraffin, and 3-µm-thick sections were stained with hematoxylin and eosin (HE). Immunohistochemical investigations were performed on the material embedded in paraffin using the avidin-biotin complex method and diaminobenzidine as chromogen. The following antibodies were used in this study: (1) monoclonal mouse Ki67 (MIB5) for the cell proliferation rate (1:10; Dako, Copenhagen, Denmark); (2) monoclonal mouse SMA for the detection of smooth muscle actin (1:400; Sigma, St. Louis, Mo., USA), and (3) monoclonal mouse desmin to detect smooth muscle cells and other cells of mesenchymal origin (1:10; Euro-Diagnostica, Arnhem, The Netherlands).

Semiquantitative determination of Ki67 was performed by analyzing positive to negative stained cells within the interface of meshes to host tissue ($400\times$, area 100×100 µm) using a digital imaging analyzing software (Image-Pro Plus; Media Cybernetics, Silver Spring, Md., USA). Ten measurements were performed for each mesh.

Collagen Protein Ratio

Specimens of the mesh area were fixed in phosphate-buffered formaldehyde and embedded in paraffin. Three sections, approximately 15 µm thick, were obtained from each sample and placed in individual test tubes. After deparaffinization the slices were stained with Sirius red and Fast green (Polysciences, Eppelheim, Germany).

Fig. 1. Intraoperative setting: **A** esophageal defect, **B** mesh closure, and **C** omental wrapping.

Fig. 2. Macroscopic aspect of **A** PVDF and **B** polyglactin 910. Original magnification $\times 40$.

Table 1. Textile properties of meshes

	PVDF	Polyglactin 910
Filament	Monofilament	Multifilament
Weight, g/m ²	75	53.6
Degradation	No	Yes
Average pore size, μm	500	500

The specimens were rinsed several times with distilled water until the fluid was colorless. The dyes were then eluted from the sections by 0.1 N NaOH in absolute methanol. This fluid was read immediately in a spectrophotometer at those wavelengths corresponding to the maximal absorbance of Sirius red (535 nm) and Fast green (605 nm). The mean value of the three slices of each specimen was taken as the final result and used for the calculations: collagen (μg) = (absorbance

at 535 nm $- 0.33 \times$ absorbance at 605 nm) $\times 1,000/37$ and non-collagenous protein (mg) = absorbance at 605 nm/3. Results are expressed as the ratio of collagen (μg) to non-collagenous protein (mg) to level out the differences in the weight of the slices [18–20].

Results

Material Properties (fig. 2, table 1)

The PVDF mesh is non-absorbable, constructed of monofilaments with a material weight of 45 g/m². Compared to a conventional non-absorbable polypropylene mesh, it appears light weighted and material reduced. The absorbable polyglactin 910 combines glycolide and lactide at a ratio of 9:1. It is constructed of multifilaments and features a weight of 53.6 g/m². Both meshes have an equal pore size of 0.5 cm on average.

Fig. 3. Clinical outcome: **A** PVDF after 4 weeks, X-ray: gastrographin swallow, unimpaired passage, and **B** endoscopy, complete mucosal regeneration.

Clinical Examinations (fig. 3)

Four of 5 animals of the PVDF group survived the observation period without any complications. There were no signs of malnutrition or impairment of deglutition. One animal died because of pneumonia after 4 weeks. Swallow examination with water-soluble contrast medium showed a normal passage into the stomach in all animals (fig. 3A). There was no stricture or perforation. Esophagoscopy showed a complete regeneration of the mucosal layer after 4 weeks (fig. 3B).

Three rabbits of the polyglactin 910 group died during the first week from anastomotic leakage with consecutive diffuse peritonitis. One of these rabbits had a complete intraluminal dislocation of polyglactin 910 mesh. It was fixed to the esophageal wall only by the Prolene® suture. Only 2 animals survived the complete period of 12 weeks. In 1 rabbit we found an abscess within the esophageal wall after 8 weeks, and an esophageal stricture with prestenotic dilatation occurred. The remaining animal had a normal endoscopy and contrast medium passage during all examinations. In both surviving animals the mucosal layer was regenerated after 4 weeks as seen by endoscopy.

Morphological Studies (fig. 4–6)

The complete regeneration of the mucosal layer of PVDF rabbits could be confirmed within the histological specimens (fig. 4A). Though the mucosa was regenerated for 2 surviving polyglactin 910 animals (fig. 4B), we found an ulceration with abscess formation and diffuse inflam-

mation in deeper layers (fig. 4C) in 1 animal. Within the specimens of polyglactin 910 animals which did not survive the postoperative period, we found multiple abscesses and severe inflammation surrounding the implant. Foreign body reaction surrounding PVDF mesh filaments was minimal after 3 months: the foreign body granuloma around the filaments was small with $<40\ \mu\text{m}$ (fig. 5A), the evidence of Ki67-positive cells within the granuloma was not increased compared to the scar tissue (data not shown). At this point of time, polyglactin 910 mesh was completely degenerated (fig. 5B) and the restored esophageal wall revealed a thin but dense and closed fibrotic plate.

Staining with desmin showed an initial regeneration of the muscle layer with islets of muscle cells between PVDF mesh pores (fig. 6A). Muscle cell regeneration could also be observed after implantation of polyglactin 910. Distinctive neoangiogenesis could be detected in all specimens.

Collagen Protein Ratio (fig. 7)

After 3 months, the relative collagen content of the PVDF mesh area was found to be $89.5 \pm 6.4\ \mu\text{g}/\text{mg}$. Tissue samples of the scar area of the polyglactin 910 group registered with a total collagen protein ratio of 58.8 ± 3.5 . Statistic evaluation showed a significant difference between both groups ($p < 0.001$).

Fig. 4. Complete regeneration of the esophageal mucosa 3 months after implantation: **A** PVDF and **B** polyglactin 910 (completely degenerated); **C** ulceration after implantation of polyglactin 910. HE. Original magnification $\times 40$.

Fig. 5. Foreign body reaction: **A** perifilamentary extent of granuloma surrounding PVDF; **B** at this point of time, polyglactin 910 is completely degenerated. HE. Original magnification $\times 400$.

Discussion

Using a porous polymer to close patch defects of the esophageal wall in rabbits, we found local tissue regeneration of both mucous and muscle layers, even in the presence of the alloplastic prosthesis (fig. 4, 6). PVDF is superior to polyglactin 910: no anastomotic leakage occurred and incorporation was better with only minimal foreign body reaction and free esophageal passage. In fact, a primary closure of this defect size would result in a stenosis. The transabdominal approach was chosen because it is

the easiest way to reach the esophagus. However, thoracotomy in rabbits is technically very difficult and is accompanied by high morbidity. The only alternative would be the collar approach, but we could not find any advantages to do so. An endoscopic approach not only gives the opportunity to place the mesh correctly and to fix it with a running suture, but also to wrap the defect with omentum as described in our study.

Though the number of animals used in our study is too small to draw any clinically significant conclusion, we present a new approach to bridge esophageal defects with

Fig. 6. Muscle cell regeneration: **A** muscle cells migrating into the PVDF mesh pores, and **B** after polyglactin 910 implantation.

prosthetic materials. Reviewing literature, a variety of polymers have been investigated for the replacement of the esophageal wall. However, the used materials were foils, not permitting ingrowth of tissue and led to the induction of pure scar tissue, filling out the defect and forming an amotile scar tube. In an experimental study in dogs, the closure of esophageal defects with foils of PTFE or Dacron led to the regeneration of mucosa within 4 months, but in the submucosal wall, foreign body reaction and development of fibrous connective tissue occurred. Submucosal glands as well as a muscle layer were absent [21]. Takimoto et al. [22] succeeded in replacing parts of the esophagus with a prosthesis composed of a collagen sponge and an inner double-layered silicone tube to avoid stricture of the neoesophagus. Regeneration of all esophageal wall layers could be observed, though complete epithelialization was accomplished only after removal of the silicone stent [23, 24]. Mesh as a scaffold for tissue regeneration was successfully described for tracheal reconstruction using a collage-conjugated Marlex® mesh [25]. The authors pointed out the necessity of rapid re-epithelialization and invasion of local tissue into the pores of the mesh as a prerequisite to reduce the formation of stenosis. In our study, epithelialization was complete 4 weeks after mesh implantation (fig. 3B, 4), and the histological investigations revealed the regeneration of the muscle layer (fig. 6). Additionally, we found distinctive neoangiogenesis indicating vessel ingrowth supported by wrapping the prosthesis with omentum. PVDF mesh structure has the mechanical strength to provide reinforcement of the tissue. None of the PVDF animals suffered from regurgitation or malnutrition, and radiographic as well as endo-

Fig. 7. Collagen protein ratio (µg/mg) 3 months after implantation of PVDF/polyglactin 910 mesh (mean $\pm$ SEM, $p < 0.001$).

scopic examinations confirmed the absence of strictures (fig. 3A). In contrast, the use of extracellular matrices as absorbable bioscaffolds for esophageal repair leads to the formation of strictures within 45 days after surgery, though it does not inhibit local tissue regeneration [8]. The reduced inflammatory reaction of PVDF is indicated by minimal inflammation and fibrosis with a minimal collagenous capsule in the perifilamentary region in this study (fig. 5) suggesting superior biocompatibility. Former in vivo studies showed that PVDF is light weight, surface reduced and induces less inflammatory reaction than other polymers [16, 26]. Additionally, PVDF shows a higher mechanical stability and no progression of rigidity as seen with polypropylene. Tough complete re-epithe-

lialization and muscle cell ingrowth can be detected for absorbable polyglactin 910 mesh in our study; this implant was accompanied by a high rate of early leakage. Severe inflammation and high fibrogenic potency was the consequence of early degradation, probably increased by acid reflux. The same problem occurred after reconstruction of a porcine esophagus with a collagen-coated polyglactin 910 tube [27]. Accordingly, the reinforcement of a suture repair of the esophagus with polyglactin 910 mesh showed disappointing results [28]. Inflammatory reaction and fibrogenesis decreases after complete disintegration of polyglactin 910. The lowered amount of collagen measured after 3 months is caused by a smaller scar size compared to PVDF (fig. 7). Seeding the implant with the desired cell population in vitro before implantation is a current method to obtain local tissue regeneration. Marzaro et al. [29] reported that autologous satellite cell seeding improves in vivo biocompatibility of homologous muscle acellular matrix implants. However, cell seeding of implants in vivo is short-lived: van Wachem et al. [30] did not find any muscle regeneration after implantation of a collagen matrix seeded with myofibroblasts. The seeded cells survived for approximately 5 days. The initial regeneration of the muscle layer in our study represents the progressive ingrowth of host cells, likely local adult stem cells. In this context, one may assume that mechanical signals of the polymer will be critical to new tissue development as well. Less is known about how external mechanical signals regulate gene expression, and lead to strategies for tissue engineering and regeneration. The clinical problem of esophageal perforation with local infection is well known. Several studies have shown the

increase of mortality dependent on the time of treatment after perforation. Current studies dealing with mesh implantation are concerned with the problem of local infection by coating the filaments with antibiotics. However, our goal was to show the principal possibility of guided tissue regeneration in vivo. We envisage that the application of optimized mesh materials may be beneficial for high-risk anastomoses in the gut due to synthetic reinforcement, and preliminary investigations are being done in our laboratory. Polymer scaffolds have also been investigated for the reconstruction of heart valves, blood vessels, myocardium and small intestine. Again, in the majority of these grafts, the problem of late stenosis of the prosthesis is difficult to solve. Because of these difficulties, investigators are still in search of the ideal replacement material. Our study shows a new approach for esophageal tissue engineering: PVDF mesh structure gives the opportunity of local tissue regeneration, maintains the esophageal function and can reduce the incidence of stricture in the long term. The results have to be recapitulated with a larger number of animals and make it worthwhile to think about mesh modifications that are designed to specifically interact with the desired cell population and guide the resultant structure and function of the regenerated esophageal tissue.

Acknowledgments

The authors would like to acknowledge the financial support of the project through the Interdisciplinary Center for Clinical Research IZKF-BIOMAT in Aachen, Germany, No. 01KS9503/9 and by the Deutsche Forschungsgemeinschaft, grant KL 1320/2-1 and grant JA 1123/1-1.

References

- 1 Fuchs JR, Nasser BA, Vacanti JP: Tissue engineering: A 21st century solution to surgical reconstruction. *Ann Thorac Surg* 2001;72:577–591.
- 2 Grillo HC, Wilkins EW Jr: Esophageal repair following late diagnosis of intrathoracic perforation. *Ann Thorac Surg* 1975;20:387–399.
- 3 Jara FM: Diaphragmatic pedicle flap for treatment of Boerhaave's syndrome. *J Thorac Cardiovasc Surg* 1979;78:931–933.
- 4 Dooling JA, Zick HR: Closure of an esophago-pleural fistula using onlay intercostal pedicle graft. *Ann Thorac Surg* 1967;3:553–557.
- 5 Hatafuku T, Maki T, Thal AP: Fundic patch operation in the treatment of advanced achalasia of the esophagus. *Surg Gynecol Obstet* 1972;134:617–624.
- 6 Millard AH: 'Spontaneous' perforation of the oesophagus treated by utilization of a pericardial flap. *Br J Surg* 1971;58:70–72.
- 7 Iannettoni MD, Vlessis AA, Whyte RI, Orringer MB: Functional outcome after surgical treatment of esophageal perforation. *Ann Thorac Surg* 1997;64:1606–1609.
- 8 Badylak S, Meurling S, Chen M, Spievack A, Simmons-Byrd A: Resorbable bioscaffold for esophageal repair in a dog model. *J Pediatr Surg* 2000;35:1097–1103.
- 9 Fukushima M, Kako N, Chiba K, Kawaguchi T, Kimura Y, Sato M, et al: Seven-year follow-up study after the replacement of the esophagus with an artificial esophagus in the dog. *Surgery* 1983;93:70–77.
- 10 Gonzalez Saez LA, Arnal Monreal F, Pita Fernandez S, Machuca Santa Cruz J: Experimental study using PTFE (Goretex) patches for replacement of the oesophageal wall. *Eur Surg Res* 2003;35:372–376.
- 11 Klinge U, Schumpelick V, Klosterhalfen B: Functional assessment and tissue response of short- and long-term absorbable surgical meshes. *Biomaterials* 2001;22:1415–1424.
- 12 Klinge U, Klosterhalfen B, Muller M, Schumpelick V: Foreign body reaction to meshes used for the repair of abdominal wall hernias. *Eur J Surg* 1999;165:665–673.
- 13 Klinge U, Klosterhalfen B, Müller M, Anurov M, Ottinger A, Schumpelick V: Influence of polyglactin-coating on functional and morphological parameters of polypropylene-mesh modifications for abdominal wall repair. *Biomaterials* 1999;20:613–623.

- 14 Flum DR, Horvath K, Koepsell T: Have outcomes of incisional hernia repair improved with time? A population-based analysis. *Ann Surg* 2003;237:129–135.
- 15 Klinge U, Schumpelick V, Klosterhalfen B: Functional assessment and tissue response of short- and long-term absorbable surgical meshes. *Biomaterials* 2001;22:1415–1424.
- 16 Klinge U, Klosterhalfen B, Öttinger AP, Junge K, Schumpelick V: PVDF as a new polymer for the construction of surgical meshes. *Biomaterials* 2002;23:3487–3493.
- 17 Laaksovirta S, Laurila M, Isotalo T, Valimaa T, Tammela TL, Tormala P, et al: Rabbit muscle and urethral in situ biocompatibility properties of the self-reinforced *L*-lactide-glycolic acid copolymer 80: 20 spiral stent. *J Urol* 2002;167: 1527–1531.
- 18 Haveman J, James J, Geerdink A: Collagen content in rat liver after experimentally induced cholestasis followed by choledochojejunostomy and X-irradiation. *Liver* 1996;16: 195–200.
- 19 Junqueira LC, Junqueira LC, Brentani RR: A simple and sensitive method for the quantitative estimation of collagen. *Anal Biochem* 1979;94:96–99.
- 20 Lopez-De Leon A, Rojkind M: A simple micro-method for collagen and total protein determination in formalin-fixed paraffin-embedded sections. *J Histochem Cytochem* 1985;33:737–743.
- 21 Freud E, Efrati I, Kidron D, Finally R, Mares AJ: Comparative experimental study of esophageal wall regeneration after prosthetic replacement. *J Biomed Mater Res* 1999;45:84–91.
- 22 Takimoto Y, Nakamura T, Yamamoto Y, Kiyotani T, Teramachi M, Shimizu Y: The experimental replacement of a cervical esophageal segment with an artificial prosthesis with the use of collagen matrix and a silicone stent. *J Thorac Cardiovasc Surg* 1998;116:98–106.
- 23 Yamamoto Y, Nakamura T, Shimizu Y, Matsumoto K, Takimoto Y, Kiyotani T, et al: Intrathoracic esophageal replacement in the dog with the use of an artificial esophagus composed of a collagen sponge with a double-layered silicone tube. *J Thorac Cardiovasc Surg* 1999;118:276–286.
- 24 Leininger BJ, Peacock H, Neville WE: Esophageal mucosal regeneration following experimental prosthetic replacement of the esophagus. *Surgery* 1970;67:468–473.
- 25 Teramachi M, Okumura N, Nakamura T, Yamamoto Y, Kiyotani T, Takimoto Y, et al: Intrathoracic tracheal reconstruction with a collagen-conjugated prosthesis: Evaluation of the efficacy of omental wrapping. *J Thorac Cardiovasc Surg* 1997;113:701–711.
- 26 Klinge U, Klosterhalfen B, Conze J, Limberg W, Obolenski B, Öttinger AP, et al: Modified mesh for hernia repair that is adapted to the physiology of the abdominal wall. *Eur J Surg* 1998;164:951–960.
- 27 Purushotham AD, Carachi R, Gorham SD, French DA, Shivas AA: Use of a collagen coated Vicryl tube in reconstruction of the porcine esophagus. *Eur J Pediatr Surg* 1991;1:80–84.
- 28 Bardaxoglou E, Manganas D, Meunier B, Landen S, Maddern GJ, Campion JP, et al: New approach to surgical management of early esophageal thoracic perforation: Primary suture repair reinforced with absorbable mesh and fibrin glue. *World J Surg* 1997;21:618–621.
- 29 Marzaro M, Conconi MT, Perin L, Giuliani S, Gamba P, De Coppi P, et al: Autologous satellite cell seeding improves in vivo biocompatibility of homologous muscle acellular matrix implants. *Int J Mol Med* 2002;10:177–182.
- 30 Van Wachem PB, Brouwer LA, van Luyk MJA: Absence of muscle regeneration after implantation of a collagen matrix seeded with myoblasts. *Biomaterials* 1999;20:419–426.