# Histological assessment of titanium and polypropylene fiber mesh implantation with and without fibrin tissue glue

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**Abstract:** Polypropylene (PP) and titanium (Ti) meshes are well-known surgical implants that provoke a relative low foreign body reaction. Firm stabilization of the implant is important to prevent migration and subsequent failure of the operation. Fibrin tissue glues are commercially available adhesives and are widely accepted and applied in the medical field for hemorrhage, surgical bleeding, support of wound healing, wound and tissue gluing, sealing, and closure but also as antiadhesive agent in certain applications. The objective of this study was to evaluate the additional histological effect of fibrin glue application combined with two different types of meshes. Six pieces of mesh of each were subcutaneously implanted for 3, 6, and 12 weeks, with and without fibrin glue. After excision, processing, and staining, light microscopic analysis was performed on the sections, using subjective histological description and histomorphometry. Capsule quality, capsule thickness, interstitial quality, and total score were evaluated. To compare the samples with glue and without glue, analysis of variance (ANOVA) tests were carried out. No complications were observed. In general, the glue remnants remained visible at 3 and 6 weeks of implantation, accompanied by an inflammatory reaction and macrophage activity. At 12 weeks, all samples showed good tissue integration without evidence of glue. Evidently, the samples with glue demonstrated a prolonged inflammatory response and were surrounded by fibrous tissue capsules that were significantly thicker compared with the samples without glue ($p < 0.05$). © 2006 Wiley Periodicals, Inc. J Biomed Mater Res 80A: 372–380, 2007

**Key words:** tissue connector; tissue augmentation; implant fixation; fibrin glue; subcutaneous

## INTRODUCTION

A mesh is a macro- or microporous three-dimensional structure (matrix) that allows or supports adhesion, expansion, encapsulation, or growth of one or more cell types. It can be produced of degradable[1] or nondegradable, rigid or flexible biomaterials in different ways such as knitting, weaving, crocheting, and sintering. A mesh can be used as scaffold for tissue engineering purposes, as a material for tissue augmentation and as tissue repair fabric for defects. Degradable meshes such as polyglycolic acid (PGA), poly-L-lactic acid (PLLA), polyglycolic-polylactic-coplymer (PLGA) and collagens are, especially, used for tissue engineering or guided tissue regeneration. The nondegradable meshes are currently frequently applied in surgery for abdominal hernia repair.[2,3] Combinations of absorbable and nonabsorbable meshes like Ultrapro® (polypropylene (PP) and poligelcaprone 25, Ethicon), Vypro II (PP and polyglactic acid, Ethicon), and Bard® Composix™ (PP and polytretrefluoroethylene) are also commercially available. Finally, meshes or fibered structures can also be used to create a stress-reduction zone to enhance the fixation and to stabilize the tissue around soft-tissue anchored percutaneous implants.[4–6]

Many patients are dependent on medical devices such as catheters,[7] implants, or other aids that need long- or short term fixation to the body. For this rea-

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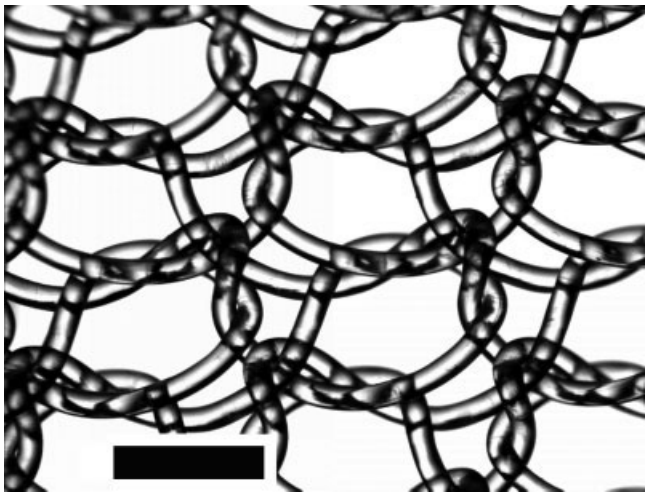

**Figure 1.** Light microscopy photo of polypropylene mesh. Black bar represents 1 mm (2.5×).

son, the devices are provided with a micro-/macroporous cuff or flange to anchor the implant. For example, to obtain better fixation of voice rehabilitation devices, a special tissue connector concept was developed.[5,6] The main component of this tissue connector is a mesh material designed to enhance the fixation of the device by means of soft tissue ingrowth in the subcutis or between the trachea and esophagus.

To improve initial fixation and to prevent migration of such devices, additional techniques are available such as sutures, staples, and spiral tacks.[8,9] Unfortunately, these procedures are regularly related to chronic pain and postoperative increased bleeding, which reduces the chance on successful treatment. As a consequence, alternative methods are developed, such as the use of tissue fibrin glue.[3,10,11]

Fibrin glue is known for its haemostatic properties.[12] In theory, it can reduce the chance on postimplantation infections. Also, it serves as a relative bacteria resistant[13] biological degradable matrix for connective tissue and skin fixation.[14]

Several types of fibrin glue are currently available and are widely accepted in the medical field for hemorrhage, surgical bleeding, support of wound healing, wound and tissue gluing, sealing and closure.[15–19] Besides adhesion, it can also act as antiadhesive agent for tissues. When the glue is applied on two tissue areas and the regular time for the gluing procedure has passed, the substance will be polymerized completely. Subsequently, both separate tissue areas will seal themselves. This seal layer will act as an antiadhesive coating.[15,20] Meek et al. noted that this antiadhesive effect was related to a higher concentration of fibrinogen in the fibrin glue.[20]

From a practical point of view, the application of glue is easy and helpful, as addition to current surgical approaches and can reduce operation time.[15,21] However, studies reporting comparisons of the soft tissue response to mesh implants with and without fibrin tissue glue are scarce.

The objective of this study was to evaluate the additional histological effect of fibrin glue application combined with two different types of mesh that are commercially available. In this article, we report about the histological results of the animal experiments as performed.

## MATERIALS AND METHODS

### Implants

Two types of meshes were tested. First was the knitted monofilament polypropylene mesh (Bard or Marlex® Bard Benelux N.V., Nieuwegein, The Netherlands). This 0.71-mm thick mesh has a specific weight of 90 g/m$^2$, an 800 μm pore size and a fiber thickness of 0.16 mm (Fig. 1). It was provided by the manufacturer in a sterile manner.

Second, the titanium mesh was used. This 1-mm thick mesh was composed of 50 μm diameter sintered fibers with a porosity of 80% and a specific weight of 500 g/m$^2$ (N.V. Bekaert S.A., Zwevegem, Belgium) and is depicted in Figure 2. Before implantation, the mesh was cleaned in trichloroethylene, rinsed in hot water and alcohol (70%), and dried in air. Subsequently, the samples were packed, sealed, and sterilized in an autoclave at 121°C for 20 min. Comparable Ti-mesh was used previously by Paquay et al.[22,23]

Both meshes were implanted with and without fibrin tissue glue (Tissucol Duo, Baxter B.V., Hyland Immuno, Utrecht, The Netherlands). Fibrin glue was chosen because of its relative quick resorption, availability, and already wide use in surgical specialties. The size of all mesh

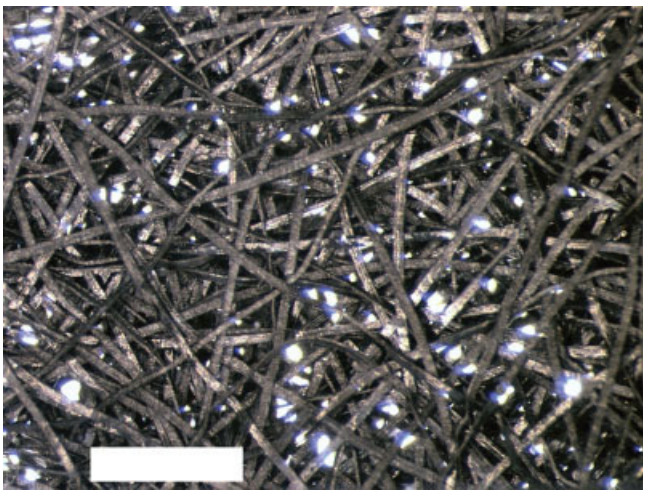

**Figure 2.** Light microscopy photo of Ti-mesh. White bar represents 1 mm (2.5×). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

implants as inserted into the experimental animals was $\sim$1 $cm^2$.

## Experimental animals and surgical procedure

Seven adult female Saane goats were used, weighing about 60 kg. The protocol for the study was approved by the Ethical Experimental Animal Committee of the Radboud University Nijmegen Medical Centre and the studies were performed according national guidelines for the care and use of experimental animals.

Implantation experiments were performed with 24 implants for each follow-up time (6 PP and 6 Ti, both with and without glue) of 3, 6, and 12 weeks. The 3- and 6-week follow-up times were selected as key identifiers for the beginning and later part of the chronic inflammatory phase of the wound healing response. The 12-week period was added to evaluate the chronic fibrous encapsulation state.

For anesthesia, the animals received medetomidine (Domitor®) (A.U.V., Cuijk, The Netherlands) 25 μg/kg, i.m., pentobarbital (Nembutal®) (A.U.V., Cuijk, The Netherlands) 10–20 mg/kg, i.v. Inhalation anesthesia was achieved by tracheal intubation and ventilation with $O_2$ (30%), $N_2O$ (70%), isoflurane $\sim$1.5%.

The surgery was performed under ''full sterile'' conditions in a modern equipped operating theatre. The implantation area was shaved, washed, and disinfected with poviodone solution followed by covering with sterile blankets. Subsequently, skin incisions, about 1.5 cm long, were made with a scalpel and knife. Lateral of the incisions, subcutaneous pockets were created by means of blunt dissection using scissors. Special attention was put to place the mesh not directly under the incision and to maintain distance between the different implants (>3 cm). For the implants with glue, 0.25 mL of glue was applied with a needle on top of the mesh (near the side of the skin) after placing the mesh in the subcutaneous pocket. We had the intention of covering the mesh completely with a homogenous coating. After the implantation, the wounds were closed with two vicryl 2-0 sutures.

## Processing of histological specimens

The animals were killed using an overdose of pentobarbital i.v. The implants were excised with surrounding soft tissue after which they were cleaned in water and all excessive hair was cut off. They were preserved in 10% buffered formalin and consequently dehydrated in mounting alcohol solutions and impregnated with methylmethacrylate (MMA). After a period of 8 weeks, the samples impregnated with the MMA were polymerized in glass jars. The temperature was controlled by placing the jars in a water bath at room temperature to prevent bubble formation in the samples. After complete polymerization, excess PMMA was cut off. After sagittal alignment of the mesh, thin sections of 10 μm thickness were cut using a modified sawing microtome (Leica RM 2165) technique.[24] At least, three regular sections of each specimen were made for histological scoring. The sections were stained with methylene blue and basic fuchsin.

**TABLE I**
**Histologic Grading Scale for Soft-Tissue Implants**

| | |
|---|---|
| **Capsule qualities:** | |
| Capsule tissue is fibrous, mature, not dense, resembling connective or fat tissue in the noninjured regions | 4 |
| Capsule tissue is fibrous but immature, showing fibroblasts and little collagen | 3 |
| Capsule tissue is granious and dense, containing both fibroblasts and many inflammatory cells | 2 |
| Capsule consists of masses of inflammatory cells with little or no signs of connective tissue organization | 1 |
| Cannot be evaluated because of infection or other factors not necessarily related to the material | 0 |
| **Capsule thickness rating:** | |
| 1–4 fibroblasts | 4 |
| 5–9 fibroblasts | 3 |
| 10–30 fibroblasts | 2 |
| >30 fibroblasts | 1 |
| Not applicable | 0 |
| **Interstitial tissue quality:** | |
| Tissue in interstitium is fibrous, mature, not dense, resembling connective or fat tissue in the noninjured regions | 4 |
| Tissue in interstitium shows blood vessels and young fibroblasts invading the spaces; few macrophages may be present | 3 |
| Tissue in interstitium shows giant cells and other imflammatory cells in abundance but connective tissue components in between | 2 |
| Tissue in interstitium is dense and exclusively of inflammatory type | 1 |
| Implant cannot be evaluated because of problems not related to the material tested | 0 |

## Histological evaluation techniques

The histological evaluation consisted of a subjective description of the observed tissue reaction and histomorphometry.

Histomorphometrical analysis was done to quantify the quality of the tissue response inside and around the implants. The used method was adapted from Jansen and van't Hof.[25] Aspects that were scored were capsule quality, capsule thickness, and interstitial tissue quality (Table I). Also, the total score of each sample was calculated. All histomorphometric measurements were performed separately by two researchers. In case of differences, the scores of the senior researcher were retained as conclusive.

## Statistical analysis

The means and standard deviations of the obtained data were calculated. Subsequently, the scores of the implants with and without glue were compared for significant dif-

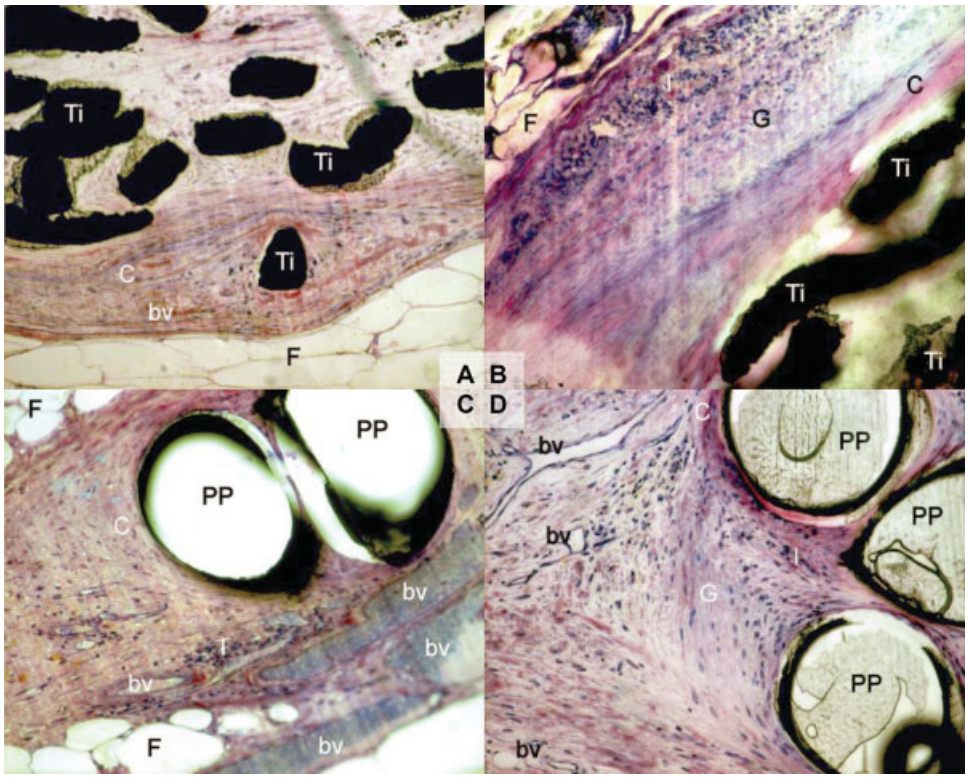


**Figure 3.** Histological sections of mesh implantations during 3 weeks. (A) Titanium mesh, (B) titanium mesh with glue, (C) polypropylene mesh, and (D) polypropylene mesh with glue. Ti, titanium; PP, polypropylene; C, capsule formation; I, inflammatory cells; bv, blood vessel; F, fat cells; G, fibrin tissue glue (×10 with zoom). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ference ($p < 0.05$) for each implant time separately. For comparison of the data, a one-way analysis of variance (ANOVA) test was performed. Statistical analysis was carried out using the software SAS and SPSS version 12.0.1. on a Microsoft Windows XP system.

## RESULTS

### Macroscopic findings

During the operation, the glue flowed into the pores of the mesh, and we considered this as a non-homogenous layer. Postoperative care was without complications. After implantation of the mesh, only mild inflammatory signs of the skin were noted (redness). This initial inflammatory response was more profound in the samples in which fibrin glue was added. The signs diminished gradually during the first 2 weeks. No other adverse effects were seen.

### Descriptive light microscopic evaluation

All but three samples of the 3 weeks (2 PP-glue, 1 PP), 3 of the 6 weeks (2 PP-glue, 1 Ti-glue) and 4 of the 12 week (2 PP-glue, 1 PP, 1 Ti-glue) implantation time were retrieved. After excision of the implantation area, some samples showed implant folding. This implant folding occurred in varying degrees and more for the PP-mesh ($n = 8$ of which six with glue) than the Ti-mesh ($n = 2$, both with glue).

The overall picture of the tissue after implantation was that, in time, a foreign body response with many inflammatory cells (especially in the samples with glue) changed into an increasingly organized tissue with fibrous connective tissue. All implants were well incorporated.

For compiled morphometric data we refer to the section ''histomorphometry''.

### Three weeks

Evaluation of the prepared sections of Ti-mesh demonstrated a thin to medium-thin fibrous capsule. Inside the mesh, the porosities were filled with early-stage connective tissue with capillaries, larger blood vessels, and only few macrophages.

The PP-mesh showed a medium thick capsule consisting of many inflammatory cells and little collagen. Tissue in the interstitium showed many giant cells and lymphocytes and also connective tissue components in between. Around one PP-implant, a haematoma was seen.

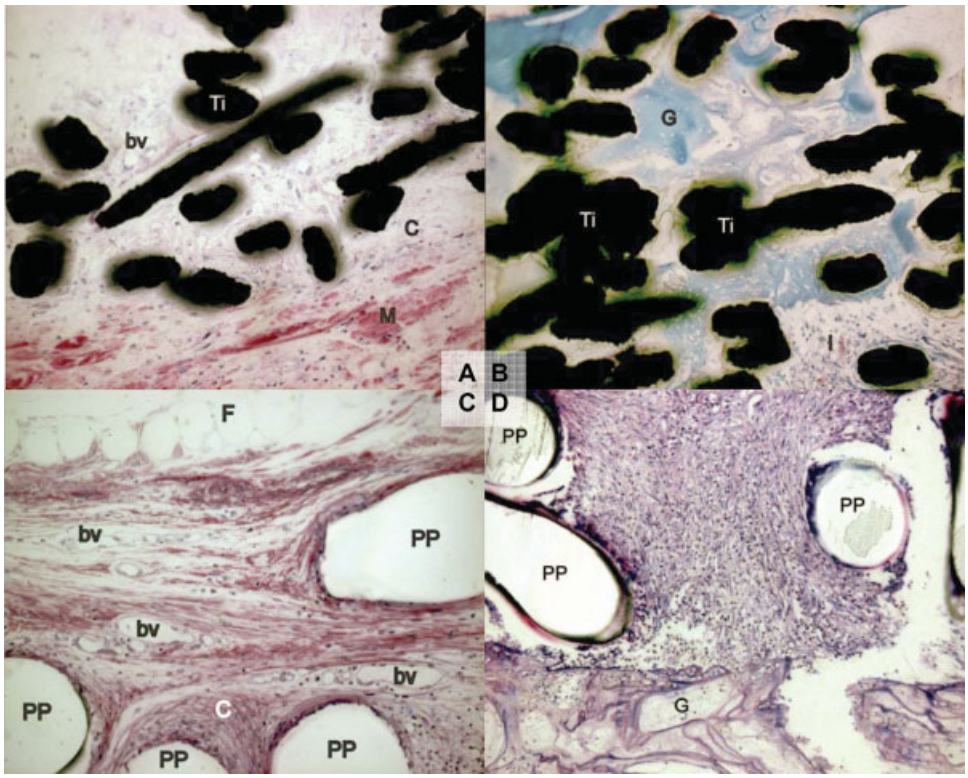


**Figure 4.** Histological sections of mesh implantations during 6 weeks. (A) Titanium mesh, (B) titanium mesh with glue, (C) polypropylene mesh, and (D) polypropylene mesh with glue. Ti, titanium; PP, polypropylene; C, capsule formation; I, inflammatory cells; bv, blood vessel; F, fat cells; M, muscle fibers; G, fibrin tissue glue (×20). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Ti-mesh and PP-mesh samples with glue demonstrated a comparative uniform reaction. Clear fibrin glue residues with an inflammatory reaction of polymorphonuclear granulocytes, macrophages, and increased neovascularisation was seen in nearly all samples [Fig. 3(A–D)].

### Six weeks

The tissue in and around the Ti-mesh showed a similar reaction compared with that in 3 weeks.

Most of the PP-mesh fibers were surrounded by a tissue capsule containing 4–9 layers of fibroblasts. Between the fibers, the porosity was filled with immature connective tissue, blood vessels, and some inflammatory cells. Mainly, the amount of collagen fibers increased and the tissue became more organized in one direction.

Ti-glue and PP-glue, both, were surrounded by a thick fibrous capsule that contained large accumulations of inflammatory cells. Around the remaining glue clots, clear macrophage activity was observed. We estimated that 10–15% of the original amount of glue was not completely degraded at this time [Fig. 4(A–D)].

### Twelve weeks

Thin mature fibrous capsules were observed around the Ti-mesh, comparable with those of 6 weeks. Also, the histological observations of the tissue surrounding the PP-mesh were comparable with those of the implantations of 6 weeks.

Around the samples of Ti-mesh combined with fibrin glue, medium thick immature capsules with little collagen were noted. In general, the samples showed a decrease of inflammatory cells around the mesh fibers compared with the Ti-glue of 6 weeks. No glue remnants were observed any more.

The capsule and interstitial tissue around the PP-mesh fibers with glue also showed less inflammatory cells compared with that in 6 weeks. There were no remaining parts of fibrin tissue glue visible [Fig. 5(A–D)].

### Histomorphometry

Means and standard deviations of capsule quality, capsule thickness, interstitial quality, and total score for all implantation periods were calculated and are depicted in Figure 6. After 3 weeks, no significant differences were seen between PP and PP-glue. How-

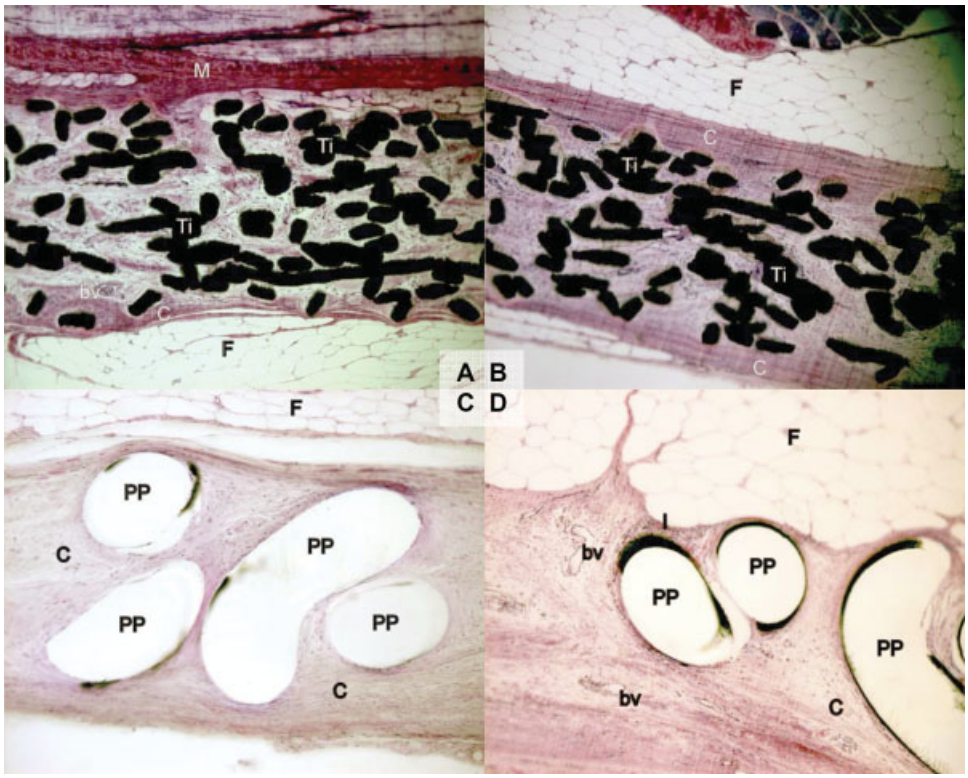


**Figure 5.** Histological sections of mesh implantations during 12 weeks. (A) Titanium mesh, (B) titanium mesh with glue, (C) polypropylene mesh, and (D) polypropylene mesh with glue. Ti, titanium; PP, polypropylene; C, capsule formation; I, inflammatory cells; bv, blood vessel; F, fat cells; M, muscle fibers (×10 with zoom). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ever, Ti scored significantly better compared with Ti-glue on capsule quality, capsule thickness, interstitial quality, and on the total score.

After 6 weeks, PP-meshes showed significant higher scores concerning capsule quality, capsule thickness, and the total score compared with PP-glue. For interstitial quality, the difference was nonsignificant.

Overall, the total scores of the nonglued meshes scored always higher than the glued meshes.

## DISCUSSION AND CONCLUSION

The aim of the present histological study was to investigate the additional effect of fibrin glue on the soft tissue healing of titanium and polypropylene mesh after 3, 6, and 12 weeks of implantation.

Evaluation of our histomorphometrical data demonstrates that the application of fibrin glue with Ti- and PP-mesh induces a significantly thicker fibrous capsule surrounding the implants, which is probably the consequence of the extended initial inflammatory response as evoked by the fibrin glue. Nevertheless, our histomorphometric results of Ti confirm the results of Paquay et al. concerning the interstitium quality.[23,26] Capsule quality and thickness scores were just slightly higher in our series. Also, the results of PP were comparable with previous results of Geertsema et al.[5,6]

The standard deviation of the capsule quality in the group PP-glue of 3 weeks was high compared with the others because of the inclusion of the sample that presented the haematoma and may also be due to nonhomogenous glue distribution and sampling error.

We think that the subcutaneous mesh implantation experiments relate quite closely to the clinical relevant anatomical locations. The devices for improving voice rehabilitation, catheter fixation aids, and hernia repair mesh are embedded under similar circumstances (near fatty tissue and muscular tissue).

At retrieval of the implants, also some meshes were found to be folded. This process can be attributed to mechanical trauma and capsule contracture in combination with the properties of the mesh, which was also described previously.[7] The total number of folded meshes of PP versus Ti can be explained by the rigidity. PP-mesh was considered flexible compared with the quite rigid Ti-mesh. We think that in our series the numbers of folded meshes associated with the application of glue was due to coincidence.

Application of mesh needs secure initial fixation at the time of implantation to prevent migration and failure. To improve the chance on successful treat-

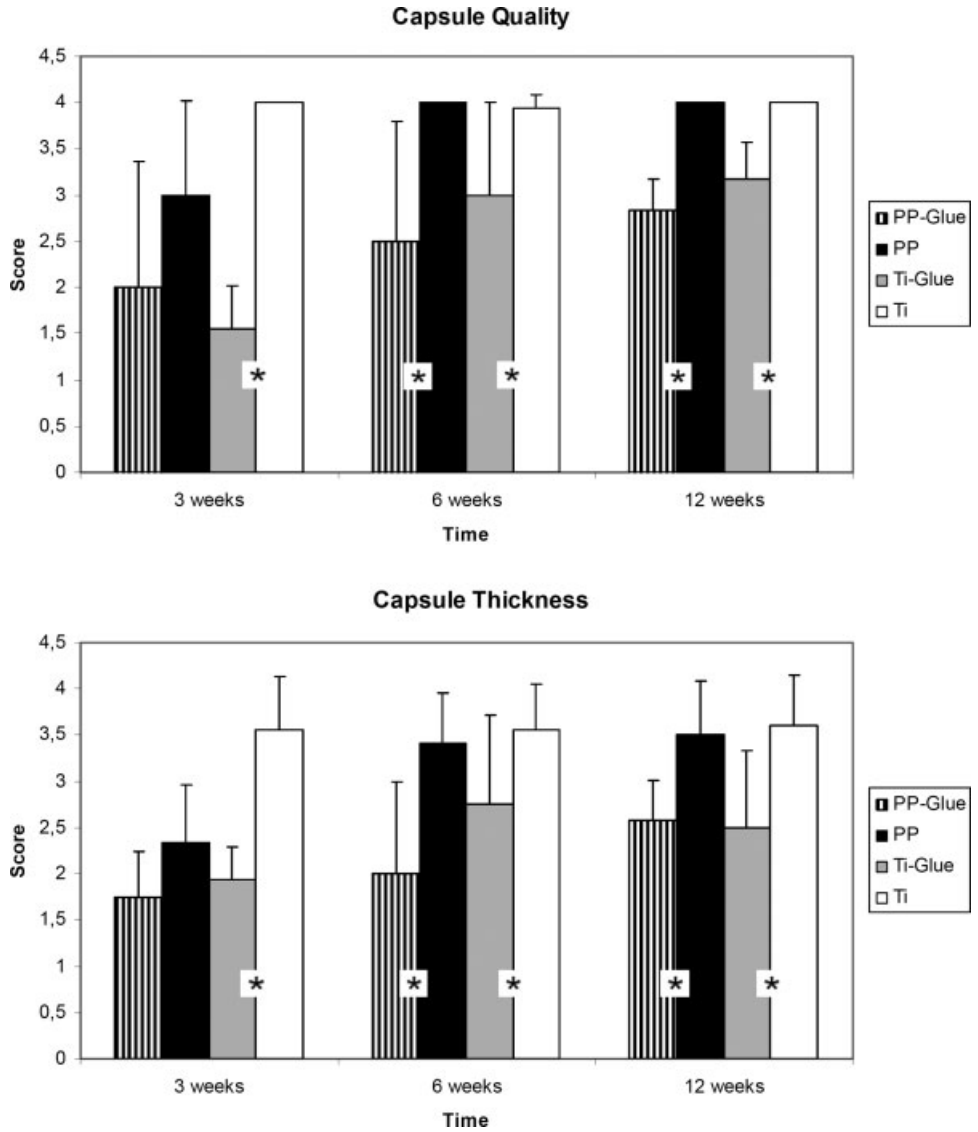


**Figure 6.** Results (mean and SD) of the histomorphometrical evaluation, according to the table. Significant difference ($p < 0.05$) is marked with *.

ment, different types of tissue glue are investigated as an alternative to or a reduction of sutures.

Recently, Scheidbach and coworkers compared four different PP-meshes. One of the meshes was coated on both sides with a 30-nm thin titanium layer. Concerning the markers for B-lymphocytes, macrophages, and monocytes, this so-called *Ti-Mesh Extralight* scored significantly better than a heavy weight PP-mesh after a 3-month implantation period.[2] However, Junge et al., found significant less inflammation in PP-mesh compared with the Ti-coated PP-mesh after implantations of 84 and 182 days in rats.[27] If a medical implant gets infected, it may have drastic consequences such as the need for reoperation and removal of it. Implant infection can result in life-threatening sepsis. Usually, infection rates after mesh implantation are low. However, in "nonsterile" or "contaminated" areas (such as the tracheo-esophageal fistula) that need reconstruction or another surgical procedure, these rates can be much higher. If PP-mesh is infected with *S. aureus* or *S. epidermidis*, the biomechanical behavior is not affected.[28] Junge et al. recently succeeded in antibiotic surface modification on a polyvinylidenfluoride mesh. If the mesh used for implants in relative nonsterile operating areas maybe an antibiotic-loaded mesh, it can provide long-term bacterial infection prevention.[29] Mesh without nodes should be used because the internodal areas of the mesh are mainly colonized by bacteria and allow difficult access for antibiotics.[30]

Our results indicate that addition of fibrin glue to PP- or Ti-mesh induces a thicker connective tissue capsule and an extended inflammatory reaction in time. Our results, especially, of the 3-weeks implantation time show that the glue induces a profound inflammatory reaction. The tissue glue appeared to have a relatively higher influence on the scores in the

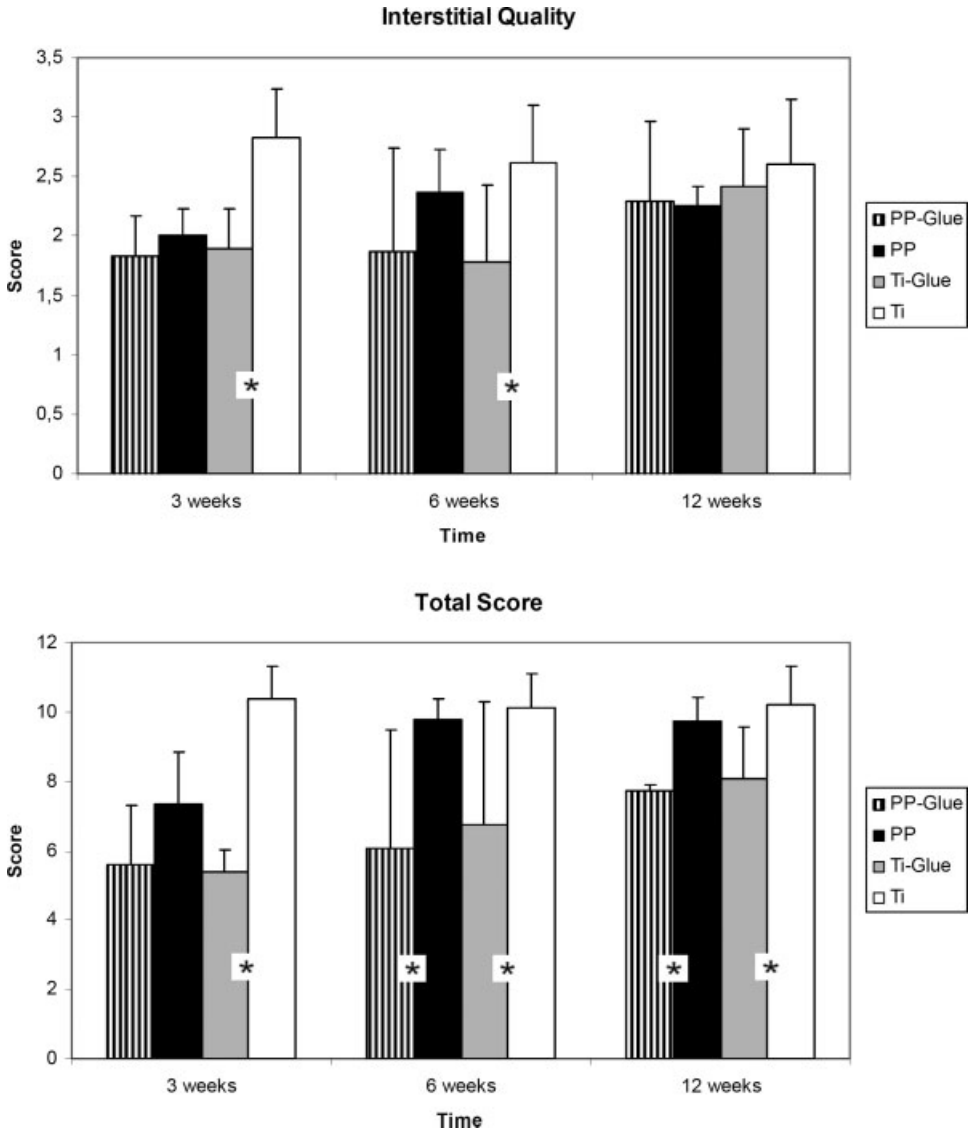


**Figure 6.** (*Continued*)

Ti-group compared with the scores of the PP-group. The observations of the prolonged inflammatory reaction do not corroborate with the results of others. Park et al. did not find any "undesirable" tissue change after 3, 7, 14, or 21 days of a 0.1 mL injection of fibrin glue subcutaneously in rats.[18] Siedentop et al. described that after the injection of 0.05 mL glue and the same follow-up terms, there was "no evidence of tissue reaction".[11] Romanos and Strub studied the influence of fibrin glue on the connective tissue matrix. Subcutaneous implantation of 1 mL of Tissucol Duo® for 4, 7, 14, 21, or 28 days did not reveal inflammatory reactions in the lamina propria.[14] This discrepancy in tissue response can be due to different types of experimental animals used in the various studies. Both species (*Capra hircus*) and skin/soft tissue structure are known to influence the length and height of the wound healing response.

Also, a lot of glue remnants were still visible after implantation of 6 weeks [Fig. 4(B,D)]. The application of a clot of glue instead of a thin sprayed layer may have contributed to the duration of complete resorption. However, we applied the glue with the standard application needle, because the spray device is mainly suitable for larger areas than those used in our experiment.

The effects of larger fiber diameter (increased capsule thickness and higher macrophage density)[31] were not the subjects of our experiments. Although we did not test different fiber thickness of PP and Ti, our findings confirm this theory. By the addition of tissue glue to our mesh implants, the total area of tissue that is exposed to foreign material is increased. We think that this may have contributed to lower scores of the capsule thickness and capsule quality in our study.

Further, we have to note that some of the implants appeared to be lost at retrieval. Apparently, these mesh implants migrated out of the wound bed almost immediately after installation. Evidently, the

fibrin glue could not prevent this or the goats were able to manipulate the implants.

On the basis of our findings, we conclude that fibrin glue cannot be regarded as a replacement for a current surgical technique like a suture. We think that, in selected cases, fibrin glue application is a valuable addition for implant and mesh fixation and repair in surgical specialties with many additional possibilities as delivery medium or matrix for tissue engineering towards guided and controlled cell/tissue growth and repair. Besides, the initial inflammatory reaction and thicker fibrous capsule includes a risk of which the final implications are currently not clear. For example, additional research is required to determine whether the connection between the implant and the tissue is indeed sustainable stronger under these conditions when fibrin glue is used.

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