Platelet-Rich Fibrin Versus Albumin in Surgical Wound Repair

A Randomized Trial With Paired Design

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Objective: To study the effects of autologous platelet-rich fibrin (PRF) versus human albumin on incisional wound breaking strength and subcutaneous collagen deposition in patients undergoing laparoscopic cholecystectomy in a randomized trial.

Summary Background Data: Platelet peptidic growth factors may stimulate collagen synthesis and tissue repair.

Methods: One expanded polytetrafluoroethylene (ePTFE) tube was inserted subcutaneously from the edge of each of the two 10-mm trocar incisions in 51 patients. Treatment with PRF prepared from the patient's own blood or human albumin was randomized to respective wound site by concealed allocation. On postoperative day 10, breaking strength of the incisional wounds as well as the collagen concentration, type I procollagen mRNA, type III procollagen mRNA, matrix metalloproteinase-1 mRNA, and fibroblast density in the ePTFE tubes were determined. All analyses were assessor-blinded. The trial was registered in the Current Controlled Trials Registry (ISRCTN34481461).

Results: Local PRF had no significant effect on incisional wound-breaking strength. In the ePTFE tubes, PRF treatment decreased collagen concentration by 24% ($P = 0.046$) and type I procollagen mRNA level by 29% ($P = 0.003$), but had no significant impact on type III procollagen mRNA, matrix metalloproteinase-1 mRNA or fibroblast infiltration. The profibrotic transforming growth factor-β1 level increased ($P < 0.0001$) 2-fold with PRF. Collagen concentration in albumin-treated ePTFE tubes correlated with breaking strength of the skin incisions ($r_s = 0.48$, $P = 0.03$).

Conclusions: PRF did not improve wound strength significantly compared with albumin but suppressed subcutaneous collagen synthesis and deposition during early repair of surgical wounds in humans. Furthermore, deposition of reparative collagen in the subcutaneous ePTFE tube model partly predicted the breaking strength of an incisional skin wound.

Key Words: wound healing, platelets, fibrin, collagen, transforming growth factor, randomized study, wound model

(*Ann Surg* 2010;251: 825–831)

Platelets contain transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and several other wound-healing stimulating factors.[1,2] There are several systems available to produce autologous platelet concentrates for promotion of wound-healing.[3–6] Autologous platelet-rich fibrin (PRF) is a platelet concentrate ($>1000 \times 10^9$ platelets/L)[7] in a biomatrix of autologous fibrin prepared by the automated advanced Vivostat system (Vivostat A/S, Allerød, Denmark).[5,8,9] PRF stimulates proliferation of normal human dermal fibroblasts and their collagen biosynthetic ability.[9] The clinical effect of PRF on wound healing is unknown.

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Supported by Vivostat A/S, Denmark.

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ISSN: 0003-4932/10/25105-0825
DOI: 10.1097/SLA.0b013e3181d3548c

Wound-healing assessments in humans inevitable rely on minimally invasive procedures.[10] Breaking strength analysis of skin incisions reflect mechanical properties of wounds,[11] but the method is invasive.[12] The expanded polytetrafluoroethylene (ePTFE) tube wound healing model was introduced in 1982.[13] In the subcutaneously inserted ePTFE tube new loose repair tissue is deposited in situ. The model allows for biochemical, molecular and cellular analyses.[13–15] Impaired wound healing associated with for example malnutrition is also reflected in reduced collagen deposition in the ePTFE tube.[16] However, to this date there is no investigation in humans that has demonstrated a link between quantitative measures of the tissue deposited in the ePTFE tube model and clinically relevant outcome parameters.

The primary objective of this randomized trial was to assess the effect of PRF on breaking strength of incisional skin wounds, and subcutaneous collagen deposition in subcutaneous ePTFE tubes in patients undergoing laparoscopic cholecystectomy. Determinations of type I procollagen, type III procollagen and matrix metalloproteinase-1 (MMP-1) mRNA, fibroblast density, and TGF-β1 in the ePTFE tubes were secondary outcomes. Moreover, the predictive value of the minimally invasive ePTFE wound-healing model was tested for the first time in humans by correlating ePTFE collagen to the breaking strength of the adjacent incisional trocar wounds. Also, the effect of fibrin alone on breaking strength was studied in a paired rat surgical incision model using a commercial fibrin sealant.

MATERIALS AND METHODS

Participants, Ethics, and Trial Registration

Patients ≥18 years subjected to elective laparoscopic cholecystectomy were recruited. Exclusion criteria were non-Danish speaking, dementia, anemia, coagulation disorder, uncompensated heart or lung disease, pregnancy, and/or ingestion of antiplatelet medication less than 4 days[17] prior to surgery. Participants were enrolled after giving their written informed consent.

The study followed the guidelines of The Helsinki Declaration, and was approved by the local ethics committee (KF 01 264835), and The Danish Data Protection Agency (2005–41–5970).

The trial was registered in the Current Controlled Trials Registry (ISRCTN34481461).

Interventions

Autologous PRF was generated no earlier than 4 hours before surgery. Briefly, 120 mL whole blood was drawn by venipuncture, anticoagulated with acid-citrate solution containing tranexamic acid (about 1 mg/mL) and centrifuged. The resultant platelet-rich plasma (about 60 mL) was converted into an acid-soluble fibrin I polymer by the addition of batroxobin.[18] Batroxobin catalyzes the release of fibrinopeptide A from fibrinogen without activating factor XIII. The platelets and the fibrin I polymer were isolated by centrifugation and dissolved in 0.2 M acetate buffer (pH 4). The fibrin I solution contained 1510 (1130–2020) $\times 10^9$ platelets/L and 19.2 (16.2–21.0) g/L fibrin(ogen). The number of platelets was determined after

centrifugation of a 500 μL-aliquot of the fibrin I solution at 10,000 *g* for 3 minutes in a calibrated platelet tool. Platelet column height was compared with that of a known number of platelets. The fibrin(ogen) concentration was determined in 100 μL of fibrin I solution that was mixed with 400 μL 0.2 M acetate buffer (pH 4.0) containing 24 mM calcium. A 50-μL aliquot was added to 2 mL of fibrinogen assay reagent consisting of 0.88 M ammonium sulfate, 27 mM ethylenediaminetetraacetic acid disodium, 83 mM urea, and 15 mM guanidine hydrochloride (pH 4.9). After 10 minutes of incubation at ambient temperature, the optical density of duplicates of each sample was measured at 400 nm and the concentration in mg/mL calculated from the human fibrinogen (341578; Calbiochem) standard curve. Finally, 5.1 (4.8–5.3) mL PRF was produced by neutralization of 7 parts of the acidic fibrin I solution with 1 part of 0.75 M carbonate/bicarbonate buffer (pH 10).[8] Hydroxyproline was undetectable in PRF analyzed by high-performance liquid chromatography.[19]

Human albumin (ZLB Bioplasma, Bern, Switzerland) was used as control at a final concentration of 14.2 g/L. This concentration matches that of PRF[8] and lacks growth-promoting activity on cultured fibroblasts.[9] Autologous[8] or homologous[20] fibrin are even more immediate controls to account for nonbioactive PRF components but were judged unsuitable due to their growth-promoting activity on cultured cells.[9]

Surgical Procedure, Allocation, and Administration of Interventions

Whole-blood platelet and plasma fibrinogen concentrations were determined before the operation. After completed laparoscopic cholecystectomy, 1 of the 2 lateral trocar wounds (Fig. 1, Wound A) that had been made with a scalpel (number 11) by a senior surgeon was randomized to treatment with either PRF or the human albumin control from codes in individually sealed envelopes. The allocation sequence was computer-generated 1:1 in block sizes of four. Trocar wound B received the opposite treatment of wound A. First, one 8-cm ePTFE tube (International Polymer Engineering, Tempe, AZ) with an outer diameter of 2.5 mm, inner diameter of 1.2 mm, and 90 to 120 μm internodal distance ePTFE tube[14] was filled with 0.3 mL albumin and both ends tied with a 3–0 poliglecaprone (Monocryl; Ethicon, St. Stevens Woluwe, Belgium) suture. Then, with a 3.2-mm cannula, the ePTFE tube was inserted subcutaneously and medially from the edge of the trocar wound. While withdrawing the cannula, albumin was deposited outside the tube that was now positioned in the subcutaneous tissue. Additional albumin was deposited in the trocar wound amounting to a total volume of 3.8 mL albumin (Fig. 1). The trocar incision was closed with one 3–0 nylon suture and covered with a polyurethane film dressing (Stabilon; Coloplast, Humlebæk, Denmark). Subsequently, the second trocar incisional wound was treated similarly but PRF was administered instead of albumin according to the randomized allocation of the wounds. Administration of the interventions and suturing were performed by one of the authors (P.L.D.) in all patients.

Follow-up Postoperative Day 10

The presence or absence of clinical signs of infection was recorded. Sutures were removed and each incisional wound was sampled using a 6-mm punch biopsy under local anesthesia (Fig. 1). Biopsies were kept in phosphate-buffered (pH 7.4) 10% formalin at 4°C for at least 3 months until assessed for breaking strength. The 2 ePTFE tubes were extirpated and divided into 4 standardized pieces (Fig. 1F). About 4 cm was immersed in acetone for the hydroxyproline assay, 1 cm was frozen on dry ice and transferred to −80°C for the TGF-β1 analysis, 1.5 cm of the ePTFE tube was stored in RNA*later* (Ambion, Austin, TX) for 2 months at 4°C for mRNA analyses,[21] and 0.5 cm was fixed in phosphate-buffered (pH 7.4) 4% paraformaldehyde for immunohistochemical analyses.

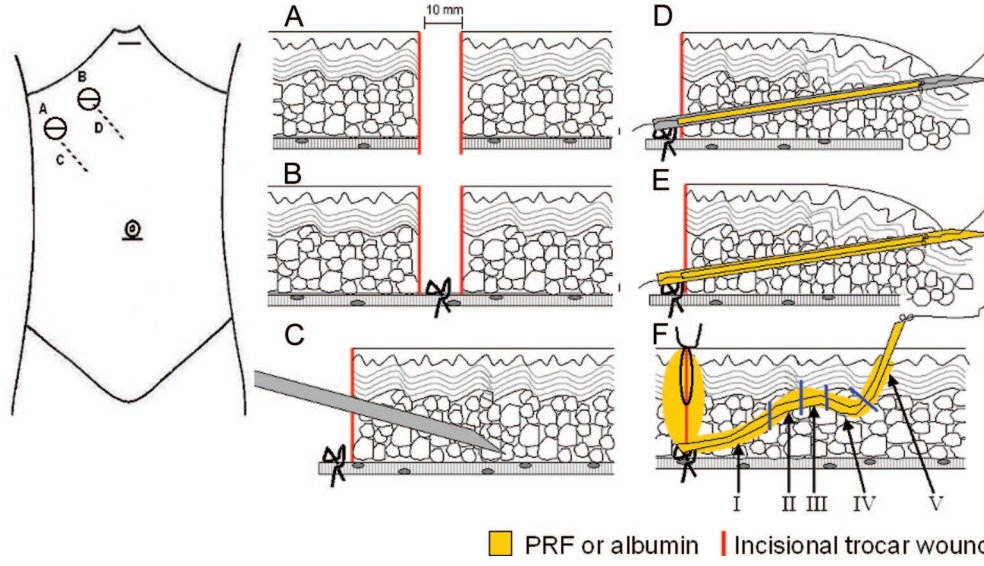

FIGURE 1. Left: Laparoscopic trocar incisions and placement of ePTFE tubes. Dotted lines represent ePTFE tubes implanted subcutaneously. The trocar incisional wounds A and B with accompanying ePTFE tubes C and D were randomized to PRF or human albumin control treatment. Circles represent sites for re-excision of the wound for breaking strength analysis. Right: A, Cross-sectional view of one of the trocar incisional wounds; B, Closure of the external abdominal aponeurosis; C, A subcutaneous channel is created with a cannula; D, The ePTFE tube prefilled with PRF or albumin is inserted through the cannula; E, The cannula is filled with PRF or albumin; F, The cannula is removed and the original incisional wound treated with PRF or albumin before closure. Blue lines indicate standardized divisions of ePTFE tube after harvest. Analyses: I. Hydroxyproline (collagen); II. mRNA; III. Fibroblast density; IV. TGF-β1 protein; V. Discarded.

Breaking Strength Measurements

The biopsies were suspended using 4.0 polyamide suture attached to each side of the wound between the 2 clamps 55 mm apart. Biopsies were stretched vertically at a constant deformation rate of 10 mm/min until rupture by a materials testing machine (LF Plus; Lloyd Instruments, Fareham, United Kingdom) equipped with XLC 10 N load cell. The maximal load (breaking strength) in Newtons was derived from the load-strain curve calculated by the software (NEXYGEN/Ondio; Lloyd Instruments).

Hydroxyproline in ePTFE Tubes[22]

ePTFE pieces were delipidated in acetone, diethylether/acetone, and diethylether. After drying for 24 hours at 100°C delipidated ePTFE tubes were cut into pieces of about 1 mm and weighed. Hydrolysis was performed for 18 hours at 110°C in 1.5 mL 6 M HCl. Acid was evaporated in a vacuum centrifuge overnight at 65°C followed by rehydration in 2.0 mL Milli-Q deionized water and again evaporized. Acid-free residues were dissolved in 2.0 mL acetate/citrate buffer (0.88 M sodium acetate trihydrate, 0.24 M citrate, 0.21 M acetate, and 0.85 M sodium hydroxide, pH 6.0). Hydroxyproline standard in acetate/citrate buffer was produced from a 100 μg/mL 4-hydroxy-L-proline stock (H1637; Sigma-Aldrich, St. Louis, MO) in 1 mM HCl. About 75 μL 0.06 M chloramine-T in 50% (vol/vol) 1-propanol was added to 150 μL sample aliquots in polypropylene tubes. Samples were oxidized for 20 minutes at room temperature followed by addition of 75 μL 1 M Ehrlichs reagent in 3.7 M perchloric acid and 60% (vol/vol) 1-propanol. Colored products were allowed to develop for 25 minutes in water bath (60°C). Tubes were cooled in ice/water bath and 200 μL aliquots added to microcuvette. The optical density was read at 557 nm by a spectrophotometer (Cecil CE2040). Unknown concentrations of hydroxyproline samples were calculated from the linear standard curve. Measurements were made in duplicate. Results are expressed as μg hydroxyproline/mg delipidated tissue including ePTFE tube.

Type I Procollagen, Type III Procollagen, and MMP-1 mRNA Determinations

Preparation of total RNA has been described earlier.[21] First strand cDNA was synthesized with 1 μg total RNA in a total volume of 20 μL using RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, Glen Burnie, MD), following the manufacturers protocol. Poly (dT) oligomer (Fermentas) was used as primer. Copy numbers of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), type I procollagen, type III procollagen, and MMP-1 were determined with real-time reverse transcription polymerase chain reaction (RT-PCR) as described earlier.[23] Primers were designed to cover more than one exon to exclude amplification of genomic DNA (Table 1). A standard sample with 10^5 copies of the amplicon was included. Results were expressed as a ratio between respective amplicon and GAPDH.

Immunohistochemical Quantification of Fibroblasts[19,24]

Fixed ePTFE tubes were embedded in paraffin. About 2-μm thick sections were cut onto capillary glass slides (S2024; Dako), dewaxed and boiled in Tris (10 mM)-EGTA (0.5 mM), pH 9 in a microwave oven. Immunostainings were carried out at room temperature using an automated system (TechMate 500; Dako, Glostrup, Denmark). Endogenous peroxidase was blocked with 3% hydrogen peroxide containing 0.05% Brij 35 (Sigma-Aldrich) for 10 minutes. Sections were washed with 1% bovine serum albumin in Tris-buffered saline (pH 7.6) containing 0.05% Brij 35. ePTFE sections were incubated for 25 minutes with a rat antihuman monoclonal antibody against type I procollagen (MAB1912; Millipore, Billerica, MA) diluted 1:20,000. Sections were then treated with the peroxidase conjugated EnVision system (Dako) and 3-amino-9-ethylcarbazole as chromogen. The slides were counterstained in Mayer's acid hematoxylin for 2 minutes. No unspecific staining was seen in control sections treated identically but devoid of the primary antibody. In 1 cross-section of each ePTFE tube, the area (μm^2) of type I procollagen immunoreactivity was measured in 4 fields at $\times$320 magnification using Image Pro Plus 4.1 (Media Cybernetics, Silver Spring, MD). The mean of the 4 fields from each section at 3, 6, 9, and 12 o'clock was used for the statistical analyses.

TGF-β1 Analysis[22]

Frozen ePTFE tubes were weighed, cut into about 1-mm pieces, and immersed into 20 μL of phosphate-buffered saline (PBS) containing 3 mM Pefabloc SC (Roche Diagnostics, Mannheim, Germany) per mg ePTFE tube. The ePTFE pieces were homogenized using T10 Ultra-Turrax (IKA-Werke, Staufen, Germany) equipped with a 5-mm dispersing tool (S10N-5G) 3 $\times$ 40 seconds at highest speed with intermediate 20-second interruptions on ice. Homogenates were centrifuged at 20,000 g. Total TGF-β1 concentrations in the supernatants were determined with an ELISA kit (DB100B) according to the manufacturer's (R&D Systems, Minneapolis, MN) instructions including activation of latent TGF-β1 with 1 N HCl. Optical density at 450 nm and 540 nm (background) of duplicates was measured using a microplate reader (Multiskan MCC/340, Labsystems, Helsinki, Finland) and concentration in pg/mL calculated from the standard curve. TGF-β1 levels were divided by 50 and expressed in pg/mg wet tube weight.

Animal Experiment

The Animal Welfare Committee (2005/561–1075) approved the study. Nine male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing about 200 g were kept in individual cages under controlled environmental conditions with free access to standard pellets and tap water.

Animals, acclimatized for 7 days, were anesthetized with 15 mg/kg midazolam, 3.3 mg/kg fluanisone, and 0.105 mg/kg fentanyl injected s.c. The dorsum was shaved and skin prepped with 70% ethanol followed by sterile water. Two parallel 6-cm linear incisions were made with a scalpel through the panniculus carnosus paravertebrally in a distance of 2 cm from the spine extending from the prominent seventh cervical spine and caudally. One wound was allocated to treatment with 0.5 mL fibrin sealant (Tisseel Duo Quick, Baxter, Vienna, Austria) and the contralateral control wound with 0.5 mL of 20 mmol/L $CaCl_2$ by randomization. The 1-mL fibrinogen component was diluted with 0.5 mL sterile water at 37°C and the

TABLE 1. Primer Sequences for mRNA Analyses by Quantitative Real-Time RT-PCR

	Forward Primer	Reverse Primer
Type I procollagen	GCCGTGACCTCAAGATGTG	GCCGAACCAGACATGCCTC
Type III procollagen	AGGTCCTGCGGGTAACACT	ACTTTCACCCTTGACACCCTG
MMP-1	TCCACAAATGGTGGGTACAA	GGTGACACCAGTGACTGCAC
GAPDH	AGACAGCCGCATCTTCTTGT	TGATGGCAACAATGTCCACT

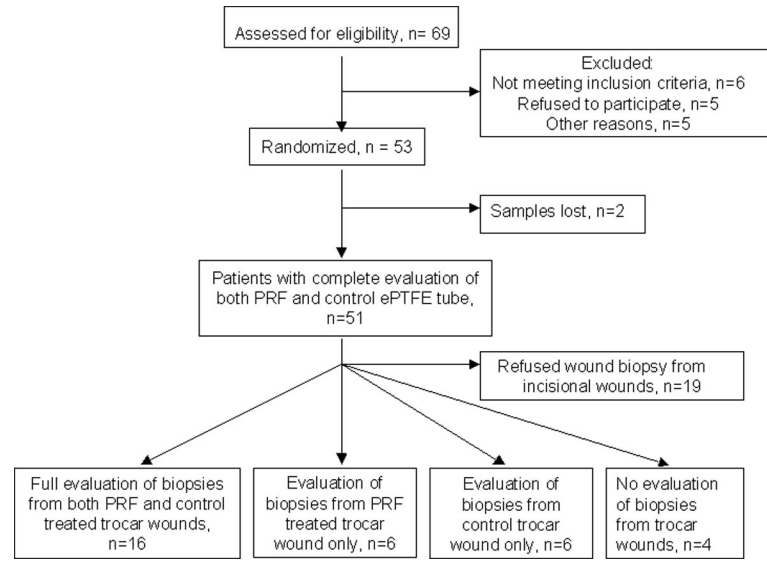

FIGURE 2. Flow of patients. n denotes number of patients.

thrombin component was diluted 125 times with 40 mmol/L $CaCl_2$. The 2 components were mixed during application to the wounds yielding final concentrations of 30 g/L fibrinogen, 2 IU/mL thrombin and 20 mmol/L $CaCl_2$. Wounds were closed with 6 interrupted 4.0 polyamide sutures 8 mm apart and covered with 2 layers of self-adhesive gauze. On postoperative day 7, 4 8-mm wide strips were cut perpendicularly to the incision line through the suture holes and fixed in phosphate-buffered (pH 7.4) 10% formalin for 7 days at ambient temperature to ascertain maximal cross-linking of available collagen.[25] The mean breaking strength (LF Plus) of the 4 strips from each wound was used in the statistical analyses.

Blinding

All patient evaluations, analyses, data entry, interim analyses, statistical analyses, and main conclusions were conducted by assessors blinded to the interventions.

Sample Size Calculation, Interim Analyses, and Statistical Analysis

Provided a type I error of 5%, a type II error of 15%, and standard deviation of 0.81 μg hydroxyproline/mg based on results from previous similar experiments, 49 surgical incisions had to be randomized to each intervention to detect a difference of 19% in the concentration of hydroxyproline. To account for drop-outs it was decided to include 53 patients, each with 2 surgical incisions and accompanying ePTFE tubes.

An Independent Data Monitoring Committee conducted interim analyses. The ePTFE hydroxyproline concentration in the 2 intervention groups was used for these analyses by applying the Peto-Haybittle-stopping rule with $P < 0.001$.[26]

Data were analyzed using the nonparametric Wilcoxon and Spearman rank correlation tests. Significance level was set to 5%. Numerical data are presented as median (25%–75% percentile range). SPSS 15.0 software (Chicago, IL) was used for the statistical analyses.

RESULTS

Clinical Trial

Sixty-nine patients were assessed for eligibility from August 1, 2005 to August 31, 2006. Sixteen patients were excluded; 6 did not meet the inclusion criteria, 5 patients refused to participate, and 5 patients were excluded due to technical problems. Each of the 53 patients was allocated to PRF and albumin control interventions. Due to technical problems ePTFE tubes were lost from 2 patients leaving 51 patients for complete evaluation of ePTFE catheters (Fig. 2). Interim analyses were performed twice during the course of the trial. The first analysis was carried out February 27, 2006 after a complete set of paired ePTFE tubes had been analyzed for hydroxyproline from 21 patients. The second analysis was performed June 29, 2006 after in total 43 patients had been analyzed with respect to ePTFE hydroxyproline concentration. The Data Monitoring Committee recommended continuation of patient enrollment at both occasions.

Accordingly, 47 women and 4 men with a median age of 44.5 (36.0–55.0) years and a median body mass index of 25.1 (21.3–30.5) kg/m^2 completed the trial. Twenty-one patients were smokers and 3 ex-smokers. Whole blood platelet count was within the normal range (150–400 $\times$ 10^9 platelets/L) for all patients with a median of 235 (197–273) $\times$ 10^9 platelets/L. The plasma total fibrinogen level was also normal (6.0–13.0 μmol/L) with a median of 9.5 (8.3–11.2) μmol/L.

No wound infections were observed on the day of wound harvest day 10. Nineteen patients refused to have biopsies taken from the trocar wounds. From the remaining 32 patients, incisions dehisced of 10 formalin-fixed biopsies from each intervention group before being subjected to biomechanical testing (Fig. 2). Breaking strength of the 16 complete sets of incisional wounds revealed no significant ($P = 0.96$) difference between PRF (0.38 [0.26–0.59] Newtons) and control (0.53 [0.28–0.77] Newtons). All these wounds reptured in the scar. There was a weak but statistically significant correlation between breaking strength values in PRF and control wounds ($r_S = 0.47$, $P = 0.045$).

Collagen deposition was assessed by the hydroxyproline concentration in the adjoining implanted ePTFE tubes. The hydroxyproline concentration was reduced by 24% ($P = 0.046$) with PRF treatment (Fig. 3). PRF also decreased type I procollagen mRNA level by 29% ($P = 0.003$), but there was no significant difference in type III procollagen ($P = 0.18$) or MMP-1 ($P = 0.49$) mRNA levels between PRF and control-treated ePTFE tubes (Fig. 4). Fibroblast infiltration was quantified by the immunoreactive intracellular area of type I procollagen (Fig. 5). No significant ($P = 0.83$) difference in fibroblast density was observed between PRF

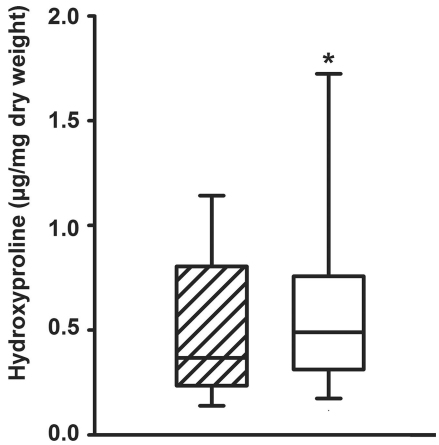

FIGURE 3. Collagen accumulation, measured as hydroxyproline concentration, in ePTFE tubes treated with PRF (hatched) or albumin control (open) postoperative day 10 (N = 51). Boxes represent 25th to 75th percentile, whiskers 5th to 95th percentile and the horizontal lines within the boxes indicate the median value. *$P < 0.05$.

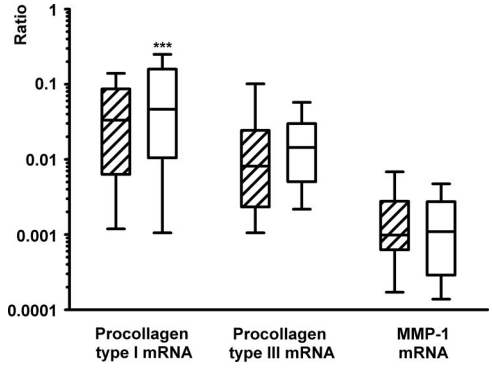

FIGURE 4. Type I procollagen, type III procollagen and MMP-1 mRNA in PRF (hatched) and control-treated (open) ePTFE tubes. Boxes represent 25th to 75th percentile, whiskers 5th to 95th percentile and the horizontal lines within the boxes indicate the median value. ***$P < 0.005$.

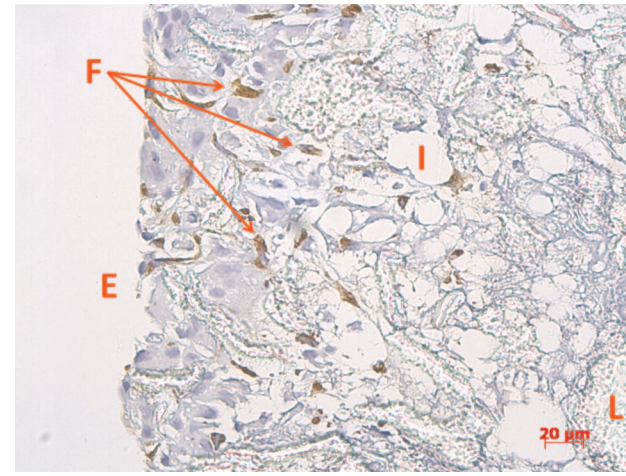

FIGURE 5. Immunopositive type I procollagen-expressing fibroblasts infiltrating the wall of the ePTFE tube. F indicates immunopositive fibroblasts (brown); E, external surface of expanded polytetrafluoroethylene (ePTFE) tube; L, lumen of ePTFE; I, intertrabecular space.

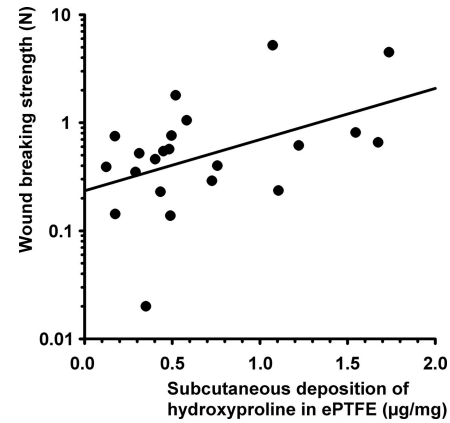

FIGURE 6. Correlation ($r_s = 0.48$, $P = 0.03$) between breaking strength of incisional control wounds and hydroxyproline concentrations in adjacent control ePTFE tubes.

(730 [106–1630] μm^2) and control (437 [90–1840] μm^2). Topical PRF treatment increased ($P < 0.0001$) TGF-β1 concentration in ePTFE 2-fold day 10; 25.0 (17.0–37.9) pg/mg wet weight versus 12.4 (8.7–17.9) pg/mg wet weight for albumin-treated ePTFE. No significant correlation was found in TGF-β1 levels between the PRF and control-treated ePTFE tubes ($r_S = 0.26$, $P = 0.10$).

The hydroxyproline concentration in control ePTFE tubes correlated significantly ($r_s = 0.48$, $P = 0.03$) with breaking strength of the adjacent skin incisions (Fig. 6). The reliability of the ePTFE tube model wound was further demonstrated by the association between the type I procollagen mRNA level and accumulation of hydroxyproline in PRF-treated ($r_S = 0.47$, $P < 0.005$) and control-treated ePTFE ($r_S = 0.47$, $P = 0.005$).

Animal Study

To study the effect of topically applied fibrin alone on wound breaking strength a fibrin sealant was used because sufficient amounts of nonbioactive autologous fibrin could not be produced by the Vivostat system[8] from rat blood. The lowered fibrinogen and thrombin concentrations used on the rat wounds produce a less dense three-dimensional fibrin structure than the commercial product.[27] There was no statistically significant ($P = 0.68$) difference in breaking strength between fibrin-treated (4.07 [3.17–5.64] Newtons) and control wounds (3.84 [2.98–5.32] Newtons).

DISCUSSION

Platelets are rich sources of endogenous growth factors.[1,3–7] In this randomized trial we investigated the effect of a single local application of autologous platelet-rich fibrin, PRF, on surgical wound repair in 51 patients. PRF did not improve breaking strength of incisional wounds, but suppressed collagen synthesis and deposition significantly in implanted subcutaneous ePTFE tubes.

The incisional wounds were formalin-fixed before being subjected to biomechanic testing to eliminate effect of varying cross-linking of deposited collagen molecules.[28] Thus, the influence of PRF on collagen maturation was not assessed in our study.

There are numerous systems for production of autologous platelet-rich formulations.[3–6] Inevitable the products differ with respect to platelet concentration and degree of activation.[3] The

automated Vivostat system achieved a 5.6-fold increase in platelet concentration over baseline. The platelets in PRF are only partially activated which results in a sustained release of growth factors to the wound.[5,9]

We are aware of only 1 randomized clinical trial on acute soft tissue repair that has scrutinized another system. In that trial, Hom et al[29] used a system that relies on autologous thrombin-rich serum, which presumably results in an instant delivery of growth factors. The autologous platelet gel promoted granulation tissue formation and accelerated closure of acute open full-thickness wounds on the thigh in 8 healthy volunteers.[29] The beneficial effect of that platelet product could be ascribed to enhanced epithelialization that was not studied here. Thus, we cannot rule out that PRF promotes healing of excisional full-thickness wounds although PRF did not enhance epithelialization of split-thickness skin donor site wounds.[30]

Endogenous fibrin was increased in PRF by a factor of about 6. Our animal experiment seems to suggest that unless the fibrin concentration exceeds 30 g/L there is no negative effect on breaking strength. This is in accordance with previous studies[31–33] and indicates that the increased fibrin delivered with PRF unlikely masked a positive effect of PRF-derived growth factors.

The optimal temporal profile of growth factors for wound healing is unknown.[2,34–36] TGF-β1 is an important regulator of extracellular matrix synthesis.[34,37,38] Despite a 2-fold elevation of total TGF-β1 and unaltered fibroblast infiltration, PRF treatment inhibited type I collagen synthesis and deposition. We can only speculate on the mechanisms for this apparently contradictory finding. Studies in TGF-β1-deficient mice suggest that endogenous TGF-β1 increases inflammation[39] and elevated levels may then actually be detrimental.[19] Furthermore, raised TGF-β1 alters receptor expression favoring a nonfibrotic response.[40]

It should be emphasized that our measurements did not distinguish between TGF-β1 derived from PRF or from TGF-β1 induced indirectly by PRF. The increased total TGF-β1 levels do not necessarily imply increased levels of bioactive TGF-β1 either.[38] Moreover, PRF supplements the wound with additional growth factors such as PDGF-AB.[5,9] Finally, the impact of the reagents, eg, tranexamic acid[41] and batroxobin,[18] used for preparation of PRF were not accounted for with our design.

Another study examined the effects of growth factors using the ePTFE wound-healing model in rats.[35] Local supplementation with PDGF and TGF-β separately increased the number of cells and granulation tissue in the ePTFE tubes. No synergism between PDGF and TGF-β was observed. The growth factors were administered in a collagen vehicle (25 g/L) intraluminally only.[35] In the present study, growth factors were administered via PRF both intraluminally and extraluminally. Perhaps then, did the extraluminal PRF counterbalance the chemoattractive gradient.

We have previously reported systemic absorption from very low doses of exogenous local granulocyte-macrophage colony-stimulating factor.[19] The lack of correlation between TGF-β1 levels in PRF and control-treated wounds indicates that systemically absorbed PRF-derived factors did not have a significant impact on the control wounds.

In the 1950s, Sandblom et al[12] first introduced a model in humans that measured tensile strength of incisional wounds. They reported reduced biomechanical wound strength in patients with hypoproteinemia.[42] Their model is considerable more invasive than the one we have developed using 6-mm wound biopsies.

To the best of our knowledge this is the first study in humans that has demonstrated a correlation between subcutaneous accumulation of collagen in the ePTFE tube and breaking strength of adjacent incisional wounds. Similar findings have been reported but only in normal and immunocompromised rodents combined[43] or in guinea pigs with subcutaneous cellulose sponge implants.[44] It is also noteworthy that the transcription level of type I procollagen predicted the amount of collagen deposited in the ePTFE model. This new information validates further the usefulness of the ePTFE wound-healing model.

In conclusion, administration of PRF did not promote surgical wound repair significantly, but suppressed subcutaneous collagen synthesis and deposition. The study does not support the use of PRF to accelerate acute wound healing in surgical patients. On the other hand, it may be worthwhile exploring further the use of PRF in the treatment of chronic wounds.[45] Finally, we have demonstrated, for the first time in man that subcutaneous collagen deposition in ePTFE tubes reflects breaking strength of adjacent primary closed skin wounds.

ACKNOWLEDGMENTS

Christian Gluud, Jørn Wetterslev, and Per Winkel, Copenhagen Trial Unit constituted the Independent Data Monitoring Committee and performed the interim analyses. Ulla Hemmingsen, Niels Erik Holm, Elsa Justinussen, Anne-Mette Kjøge, Mikkel Bjerg Kongsbak, Satu Koskinen, Rasmus Lundquist, Rasmus Maltesen, Anette Mørck, Jørgen Olsen and Steen Seier Poulsen also helped with the study.

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