Acta Orthop Scand 57, 19–24, 1986

Fibrin sealant in bone transplantation

No effects on blood flow and bone formation in dogs

To study bone formation and regional blood flow following the use of fibrin sealant in autologous cancellous bone transplantation, a dog model was developed. In 18 dogs, a standardized defect in both tibiae was filled with an autologous iliac crest graft. On one side, the bone chips were mixed with fibrin sealant while the other side served as control. After 1, 2 and 3 weeks the blood flow of the transplant was calculated and the new bone formed evaluated histomorphometrically. Generally, the highest blood flow rates and most intensive new bone formation were observed at 2 weeks postoperatively. Fibrin sealant did not alter blood flow or new bone formation, but a tendency to diminished new bone formation was found in some grafts. Our study does not support the application of fibrin sealant in ordinary cancellous bone grafting.

Correspondence: Ulf Lucht, Mirabellevej 7, DK-8240 Risskov, Denmark

Ulf Lucht
Cody Bünger
Jens T. Møller
Frederic Joyce
Hanns Plenk, Jr.[1]

Institute of Experimental Clinical Research, University of Aarhus, Aarhus, Denmark and [1]Institute of Histology and Embryology, University of Vienna, Vienna, Austria

In bone transplantation, fibrin sealant (FS) has been reported to improve a homogeneous incorporation of the implant due to an accelerated vascularization (Bösch et al. 1977, 1979, 1980, Arbes et al. 1981, Bösch 1981). Special indications for adding FS to the graft have been claimed to be poor osteogenic potency of the recipient site, chronic osteomyelitis and implantation of an allograft (Bösch 1981). However, the beneficial effect of FS on bone formation has been questioned in other studies (Zilch & Noffke 1981, Albrektsson et al. 1982).

Our study was carried out in order to contribute to the clarification of the role of FS in bone transplantation.

Material and methods

Eighteen adult mongrel dogs (19–33 kg) were used. The FS was a two-component glue, Tissel® (Immuno, Austria) for dogs, which was used without aprotinin.

After bilateral autologous bone transplantation of standardized tibial defects where the right and left transplants were alternately mixed with FS, the dogs were divided into three groups of six each. On the 7th, 14th and 21st postoperative day, each of the three groups was analysed by measurement of regional blood flow rates, histology and histomorphometry. The selection of these investigational periods was based on pilot studies.

After premedication with Combelin® 10 mg i.m., the dogs were anaesthetized with Immobilon® (1.2 ml/20 kg i.v.), and intubated. Additional Immobilon (0.5 ml) was given as necessary. The dogs were not mechanically ventilated, but supplementary oxygen (10 l/min) was given. After completion of the operation, they were awakened with the antidote Revivon® (2 ml/20 kg i.v.).

Prior to measurement of blood flow, the dogs were premedicated with Diazepam (20 mg i.v.). Anaesthesia was induced as described above. The dogs were mechanically ventilated and Pavulon® (1 mg/20 kg i.v.) was given to ensure total and constant relaxation. The dogs were in steady state before each flow determination and were monitored by means of mean arterial pressure, central venous pressure and blood gases including pH.

Surgical procedures. The dogs were placed on their right or left side, depending on which hip was used as cancellous bone donor. An incision was made over the iliac crest. The fascia was divided, and the muscles attached to the posterior side of the ileum were loosened subperiostally. The cortical bone was removed with a gouge and the cancellous bone taken. Haemostais was assured with bone wax. The wound was closed with Dexon® sutures in the fascia and Prolene® sutures in the skin.

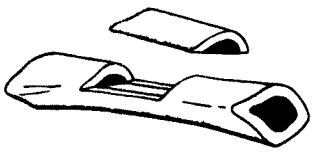

Figure 1. The standardized tibial defect used for the bone graft.

The dogs were then placed in the supine position. A lengthwise incision over the anterior tibia was made, exposing the bone. An 1.5-cm-long area was marked on the middle of the tibia, and the periosteum from this area was removed. At the proximal and distal border of the marked area, holes were drilled through the tibia from its lateral border medially. A Gigli saw was introduced through each hole and the tibia was sawed posterioanteriorly so that a half cylinder could easily be removed with an osteotome (Figure 1).

The bone marrow in the 1.5-cm-long area was removed, and the central bone artery was cauterized in all cases.

The cancellous bone removed from the iliac crest was kept in a dish at room temperature covered with isotonic saline-saturated gauze. Prior to use, the graft was divided into two parts and cut into chips. The two components of the FS were preheated to 37°C. Half of the chips were soaked with 1 ml of the mixed FS and pressed into the tibial defect. Bone stability was ensured with an AO 7-screw plate. Only six screws were used, the middle screw hole lying over the transplants being left empty. The incision was closed like that over the iliac crest described previously.

The same procedure was performed on the contralateral leg, except that the cancellous bone was not treated with FS.

One gram Pentrexyl® (ampicillin) was given intravenously at the start of each operation.

The dogs were able to walk immediately after awakening from the operation. It was necessary to give analgetics in only a few cases in the first days postoperatively. All wounds healed without complications.

Blood flow measurement. The anaesthetized dog was placed in the supine position, with the hind legs supported in 90° flexion at the hip and knee joints. A polyethylene catheter (Venflon®) was inserted into the right brachial artery and a central venous line was introduced through the right brachial vein. The catheters were connected with a pressure-recording system consisting of Portex® polyethylene manometer tubes, Siemens strain gauge tranducers model 746, Siemens pressure amplifier model 863 and Siemens Mingograph model 805. To prevent clotting, a constant perfusion system was established with heparin-saline solution at a rate of 5 ml/h using a Unita I heavy Duty Pump (Braun Melsungen AG). The catheter in the brachial artery was also used for blood gas analyses. The left carotid artery was exposed and a pigtail polyethylene catheter (Cook 7 F) was introduced into the left ventricle and was later used for injection of the radioactive microspheres. Another cathter (Cook 7 F, no end-curve) was introduced through the right carotid artery to the abdominal aorta and used for reference blood sampling via a reversed Unita I heavy duty pump.

NEN-TRAC microspheres (New England Nuclear) with a diameter of 15 μm, labelled with ^{113}Sn, were used to measure the regional blood flow. The spheres were suspended in 6 ml 10% Dextran with 0.01% Tween 8 added. Before injection, the batch was agitated for 5 min on a Whirlimixer®. The spheres were injected through the catheter in the left ventricle over a period of 30 s followed by flushing with 10 ml 37° heparin-saline. Reference blood sampling from the abdominal aorta was started 30 s before sphere injection and continued until 4 min after injection.

After flow measurements, the dogs were killed. The hind legs were dissected and the AO Plates removed. The cancellous bone graft was cut out and divided into a superficial and a profound part of equal size. Then, the samples were placed in preweighed plastic vials which, after reweighing were covered with Schaffer's fixing solution (Romeis 1968).

Gamma radiation counting and calculations of regional blood flow rates were performed as described earlier (Bünger et al. 1983).

Histology and histomorphometry. Undecalcified graft samples were embedded into methylmethacrylate, and 5-μm-thick sections were cut on a heavy duty microtome (Type K, Jung- Heidelberg, FRG). Then, trichrome-Goldner stain, toluidine blue, modified von Kossa stain and PAS-Alcian blue were applied (Schenk 1965). In addition, for the demonstration of fibrin, the phosphotungstic-acid-haematoxylin (PTAH) method was used (Lendrum et al. 1962).

The trichrome-Goldner stained section were analysed morphometrically, employing the Merz-grid at a magnification of 70 ×. The following parameters were calculated according to Merz & Schenk (1970): volume densities (per cent) of transplanted bone and newly formed mineralised bone and osteoid and surface densities (mm^2/mm^3) of newly formed bone and vessels in the marrow cavities. The surface densities of the new bone were divided into remodelling and resting bone. Because only bone fragments were seen in some of the samples, at least 2 fields of view had to be available for evaluation to include the sample in the study.

Table 1. Blood flow rates in bone grafts; mm per min per 100 g (Mean±SEM).

		Day 7	Day 14	Day 21
Superficial	FS	17.9±5.65	36.2± 9.81	12.1±2.46
	C	19.0±7.55	33.6± 9.22	12.8±1.31
Profound	FS	25.4±7.61	33.0±12.84	6.5±0.66
	C	22.3±6.74	23.1± 7.23	9.7±1.79
Total	FS	20.4±7.03	34.8±10.08	10.0±1.72
	C	20.4±6.05	29.4± 8.35	11.2±1.24

FS Fibrin adhesive system added to the graft. C Control.

Statistics. Mean values and stard error of the means (S.E.M.) were calculated from the recorded parameters. Comparison of test and control values was performed by a paired *t*-test. A P-value below 0.05 was regarded as significant.

Results

The blood flow in the transplanted cancellous bone increased from 1 to 2 weeks postoperatively, after which it decreased to reach the lowest level after 3 weeks (Table 1). Comparison of the the blood flow rates in the superficial bone grafts, the profound bone grafts as well as the total bone grafts, did not show any differences between FS-treated grafts and controls at any time.

Histology. One week after the operation, new bone formation had started. However, in the superficial FS-treated grafts, many transplanted bone chips were stille enveloped in fibrin clots (Figure 2A) and showed no signs of cellular reaction or new bone formation. A clear borderline was visible where cellular and vascular invasion had started into the fibrin clots and below which new bone formation was found (Figure 2B).

Small superficial fibrin clots were also observed in one of the controls, but in this sample "normal" new bone formation was ongoing. Two weeks after the operation, there was distinctly more newly formed bone and active remodelling in the superficial grafts of both groups (Figure 3,4). The grafted bone chips were always recognizable by their empty lacunae and, in some cases, new bone formation filled the spaces between the chips. Bone formation started from either the surfaces of the bone chips or directly in the cell-rich, vascularized tissue in between. In some places, formation of hemopoetic cells could be seen around wide sinusoidal vessels. In the profound FS-treated grafts, reactions seemed less pronounced than in the superficial FS-treated grafts.

Three weeks after the operation, the bone samples from the superficial region seemed more dense, in some cases having a corticalis-like structure. The grafted bone chips were still recognisable but fully incorporated into new bone which showed mainly lamellar structure. A "tunnelling resorption" of the grafted dead bone occurred in some places. In areas with less active remodelling, the marrow cavities were filled with hemopoetic marrow. There was no obvious difference between FS-treated and control grafts.

Histomorphometry. The volume density of grafted bone was very uniform i all the grafts examined. No difference between FS-treated grafts and controls was found.

One week after the operation, there was a lower volume density (Table 2) and surface density (Table 3) of new bone formation in the superficial FS-treated grafts compared to controls, but the differences were not significant. The same applied to the surface density of remodelling zones and vessels in the superficial graft. In the profound FS-treated grafts and controls, the corresponding values were very uniform.

Two weeks after the operation, there was a trend toward diminished new bone formation, remodelling activity and surface density of vessels in the profound FS-treated grafts com-

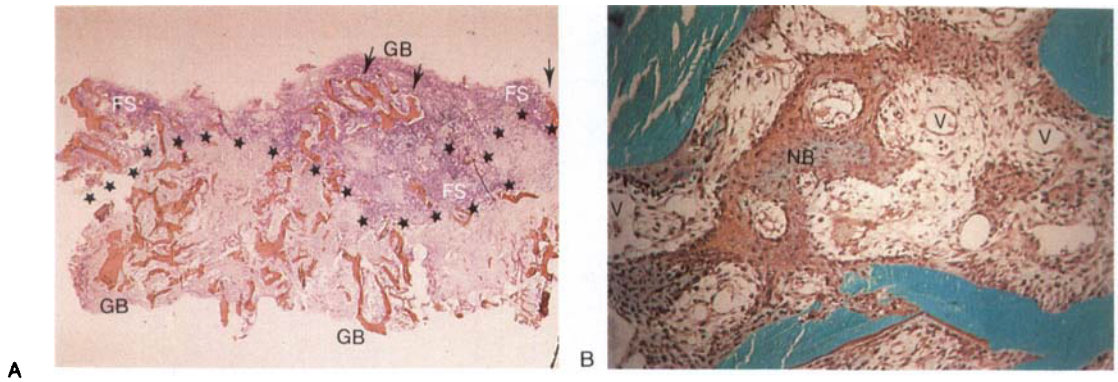

Figure 2. Undecalcified section from the superficial portion of a FS-treated graft 7 days after operation.
A: Within the superficial clots of fibrin sealant (FS) there is no reaction around the grafted bone chips (GB, arrows), while beyond a borderline (asterisks), granulation tissue and initial new bone formation are visible (PTAH-fibrin stain, × 7).
B: Upon the dead grafted bone chips (GB, note the empty lacunae!) bone formation zones (BF) can be seen. New bone (NB) is also formed in the surrounding granulation tissue (V = vessels), (Trichrome-Goldner stain, × 110).

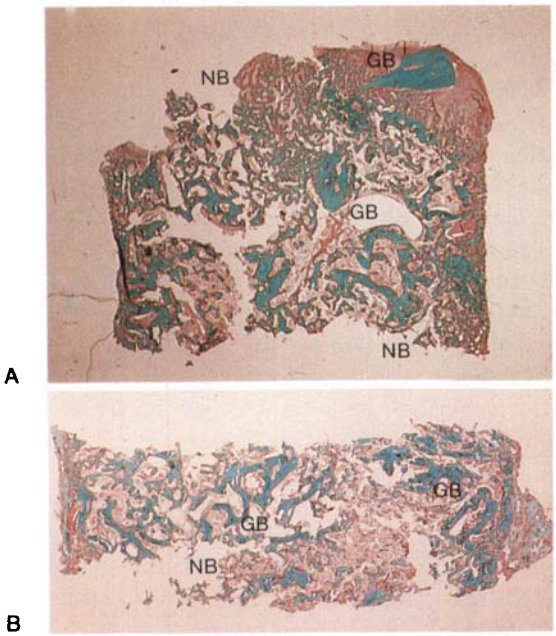

Figure 3. Undecalcified sections from the superficial, A, and the profound portion, B, of a FS-treated graft 14 days after operation (Trichrome-Goldner stain, × 7). Dense new bone formation (NB) is mainly found in the superficial portion of the graft, A. The grafted bone chips (GB) are still recognisable.

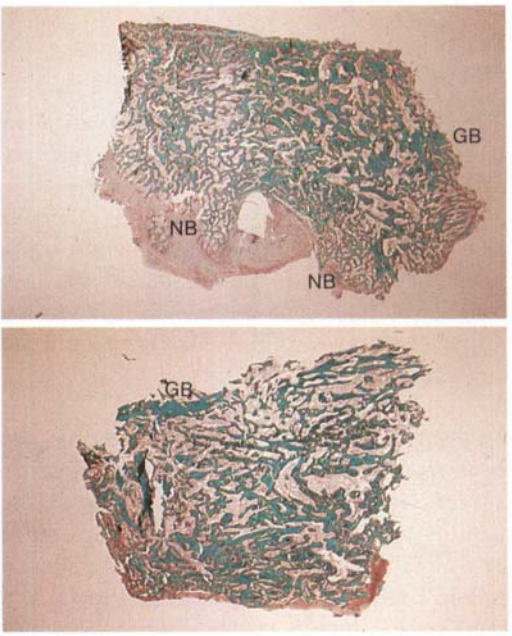

Figure 4. Undecalcified sections from the superficial, A, and the profound portion, B, of an untreated control graft 14 days after operation (Trichrome-Goldner stain, × 7). More equal new bone formation (NB) is seen around and between the grafted bone chips (GB) in both portions of the grafts.

pared to controls, but again the differences were not significant. No differences were observed in the superficial grafts either.

Three weeks after the operation, new bone formation, remodelling activity and surface density of vessels were equal in the FS-treated grafts and controls.

Discussion

Although the most favourable results from bone grafting come from the transfer of autologous cancellous bone, problems with the incorporation of the graft may arise, one being due to reduced vascularization of the bone bed.

Table 2. New bone formation in bone grafts. Volume density in per cent (Mean±SEM).

		Day 7	Day 14	Day 21
Superficial	FS	6.7±3.07	39.2±7.28	28.2±5.61
	C	12.2±4.22	30.5±7.93	36.3±2.23
Profound	FS	11.5±3.99	14.0±0.62	27.1±3.49
	C	11.4±4.05	21.2±7.63	27.1±4.99
Total	FS	9.1±2.50	26.6±5.83	27.6±1.65
	C	11.8±2.71	25.8±5.39	31.7±3.07

FS Fibrin adhesive system added to the bone graft. C Control.

In our experimental model, the tibial blood supply was reduced by cauterization of the tibial artery in order to standardize the model, but simultaneously to create suboptimal conditions as often seen in humans. The experimental model may, thus, be regarded as appropriate for the study of a possible favourable effect of FS. The attainment of uniform volume density of grafted bone and stable haemodynamic values prior to blood flow measurements are important conditions for the comparison of FS-treated grafts and controls.

The histological study showed that FS did not accelerate new bone formation. On the contrary, there was a tendency to diminished bone formation in the FS-treated superficial bone grafts on the 7th day and the FS-treated profound grafts on the 14th day. On the 7th day, this may be explained by the observation that many of the transplanted bone chips were still surrounded by fibrin. The blood flow of the grafts was not affected by the FS-treatment but it should be emphasized that the increase of blood flow from the 7th to the 14th day coincided with increased bone formation in both FS and control groups.

These quantitative data are in contrast to previous reports of improved bone formation and accelerated vascularization as a results of FS-treatment (Bösch et al. 1977, 1979, 1980, Arbes et al. 1981).

In rabbits, Zilch & Noffke (1981) filled tibial bore holes with FS and found no effect on bone formation. Albrektsson et al. (1982) studied bone formation in the canals of a titanium implant and found that FS-treated implants contained less bone than those treated with marrow cells. This study was criticized by Schlag & Redl (1983) who claimed that the implant did not qualify as a model for testing FS for its osteogenic potential because FS in that model was not allowed to undergo biological degradation and formed a barrier to natural tissue ingrowth. This aspect was supported by Zilch (1981) who in an experimental study, using histology and microangiography, found that FS accelerated the revascularization of the bone grafts only in cases of a small border of the adhesive between the grafts and the host.

We used the technique for bone grafting with FS as recommended by Bösch (1981). The chips with FS were pressed into the bone bed and excessive FS was removed. Thus, the present model fulfils the demand of a thin layer of FS, and there should be no impediment for the biological degradation of fibrin. In spite of this,

Table 3. New bone formation in bone grafts. Surface density in mm per ml^3 (Mean±SEM).

		Day 7	Day 14	Day 21
Superficial	FS	1.61±0.71	6.54±0.53	4.56±0.28
	C	3.06±1.09	5.40±1.03	4.10±0.51
Profound	FS	3.53±1.14	2.69±0.48	3.11±0.80
	C	3.59±0.89	4.33±1.67	3.28±0.58
Total	FS	2.57±0.72	4.57±0.79	3.83±0.48
	C	3.32±0.66	4.87±0.93	3.69±0.39

FS Side with fibrin adhesive system added to the bone graft. C Control.

the histology after 1 week still showed many bone chips in the superficial FS-treated graft surrounded by non-degraded fibrin.

We conclude that there seems to be no indication for FS in ordinary transplantation of cancellous bone. If used as a haemostatic agent, or as a glue in situations where it is difficult to keep the transplant in place, it should be applied in a thin layer.

References

Albrektsson, T., Bach, A., Edshage, S. & Jönsson, A. (1982). Fibrin adhesive system influence on bone healing rate. *Acta Orthop. Scand.* **53**, 757–763.

Arbes, H., Bösch, P., Lintner, F. & Salzer, M. (1981). First clinical experience with heterologous cancellous bone grafting combined with the fibrin adhesive system. *Arch. Orthop. Traumat. Surg.* **98**, 183–188.

Bösch, P. (1981). Die Fibrinspongiosaplastik. Experimentelle Untersuchung und klinische Erfahrung. Wien. Klin. Wochenschr. **93**, Suppl. 124, 1–26.

Bösch, P., Braun, F., Eschberger, J., Kowac, W. & Spängler, H. P. (1977). Die Beeinflussung der knochenheilung durch hochkonzentriertes Fibrin. *Arch. Orthop. Unfall-Chir.* **89**, 259–273.

Bösch, P., Lintner, F. & Braun, F. (1979). Die Autologe Spongiosatransplantation under Anwendung des Fibrinklebesystem im Tierexperiment. *Wien. Klin. Wochenschr.* **91**, 628–633.

Bösch, P., Lintner, F., Arbes, H. & Brand, G. (1980). Experimental investigation of the effect of the fibrin adhesive on the Kiel heterologous bone graft. *Arch. Orthop. Traumat. Surg.* **96**, 177–185.

Bünger, C., Hjermind, J. & Bülow, J. (1983). Hemodynamics of the juvenile knee in relation to increasing intra-articular pressure. *Acta Orthop. Scand.* **54**, 80–87.

Lendrum, A. L., Fraser, D. S., Sliders, W. & Henderson, R. (1962). Studies on the character and staining of fibrin. *J. Clin. Pathol.* **15**, 401–413.

Merz, W. A. & Schenk, R. K. (1970). Quantitative structural analysis of human cancellous bone. *Acta Anat.* **75**, 54–66.

Romeis, B. (1968). *Mikroskopische Technik*. 16th edn., p. 228. R. Oldenburg Verlag, Munich-Vienna.

Schenk, R. K. (1965). Zur histologischen Verarbeitung von unentkalktem Knochen. *Acta Anat.* **60**, 3–19.

Schlag, G. & Redl, H. (1983). Letter to the Editor. *Acta Orthop. Scand.* **54**, 655–658.

Zilch, H. (1981). Der Einfluss des Fibrinklebers auf die Revaskularisierung des Knochentransplantates. *Unfallheilkunde* **84**, 353–362.

Zilch, H. & Noffke, B. (1981). Beeinflusst der Fibrinkleber die Knochenneubildung. *Unfallheilkunde* **84**, 363–372.