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FIBRIN ADHESIVE SYSTEM (FAS) INFLUENCE ON BONE HEALING RATE

A Microradiographical Evaluation using the Bone Growth Chamber

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A dividable titanium implant containing two canals was inserted into both tibial metaphyses of 15 adult rabbits to test whether the Fibrin Adhesive System (FAS) can accelerate bone regeneration. On one side the implants were pretreated with FAS whereas the implants on the contralateral side were bathed in autologous blood and marrow. After a healing time of 4–5 weeks the assembled implants were removed and then taken apart. The amount of bone which had grown into the canals was quantified by microradiography and microdensitometry. The FAS-treated implants showed a tendency to contain less bone than the medullary cell-treated ones. It is concluded that more evidence should be gathered before recommending FAS treatment to accelerate bone regeneration. It is submitted in addition that the dividable titanium implant, the Bone Growth Chamber, may be useful for quantification of early bone formation under various experimental conditions.

Key words: bone formation; fibrin; microradiography; titanium implants

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Quantification of primary bone ingrowth adjacent to hard tissue implants has mostly been performed with the use of various types of push/pull-out tests. Although such tests provide some numerical data on the incorporation of implants they do not permit histological or ultrastructural analyses of the interface characteristics. Furthermore, push/pull-out tests give less adequate data when applied in the early phases of implantation in comparison with the more significant data obtained after longer implantation times. Various experimental designs aimed at histological description of bone ingrowth into, for example, porous implants have not permitted an easily repeatable quantitative assessment of the ingrowth rate. This paper will present a new experimental model, the Bone Growth Chamber, which facilitates quantification of the bone growth rate during the early phases of implant incorporation. The chamber was used in an experimental investigation of the possible beneficial effects of fibrin (FAS) treatment on implant integration.

A concentrated fibrin clot has adhesive and haemostatic qualities. Bösch et al. (1977a, 1980) have experimented with concentrated FAS in an attempt to facilitate the incorporation of rabbit bone grafts. The authors found an acceleration of vascularity and increased bone density after FAS treatment. Pflüger et al. (1979) have used FAS in an attempt to improve osteogenesis around porous stainless steel implants in the rabbit iliac bone. Skeletal attachment seemed improved at 3 weeks with the presence of FAS. In these and other published experimental studies of FAS in-

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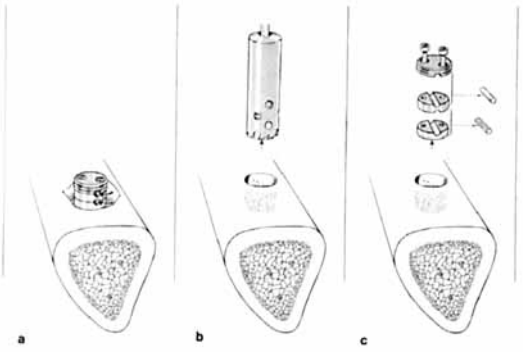

Figure 1a. The titanium Bone Growth Chamber consists of three separate parts held together by two screws. There is one proximal and one distal canal penetrating through the implant at the level of the junctions between the titanium sections. The chamber is inserted in the rabbit tibia using a gentle surgical technique. Bone will then start to grow into the canals (arrows).

Figure 1b. After 4–5 weeks a trephine is used to cut out the chamber with a surrounding bone collar.

Figure 1c. The implant is taken apart and the tissue which has grown into the proximal and distal canals is removed for microradiographical and histological analyses.

fluence on early osteogenesis (Bösch et al. 1977b, Sauer et al. 1977) the controls were left without specific treatment. In the present investigation, on the other hand, care was taken to supply the control implants with an adequate amount of autologous blood and medullary cells, thus providing a natural source of fibrin as well as potentially osteogenic cells.

MATERIALS AND METHODS

Animals

Ten adult lop-eared and five adult New Zealand white rabbits, between 10 and 14 months of age and of both sexes were used for the experiments.

The Bone Growth Chamber

The chamber consists of a titanium implant with an outer diameter of 7 mm which is made up of three sections held together by two titanium screws (Figure 1a). When the titanium sections are assembled, two 1 mm wide canals penetrate through the implant at the level of the junctions between the sections. The principle of this implant is that bone ingrowth occurs in these canals which, after removal of the implant at a predetermined time, can be opened for direct assessment of the bone ingrowth rate. The quality of the ingrown bone may also be assessed by means of microradiographical and histological analyses.

Mode of implant insertion and FAS treatment

Implant insertion was performed under aseptic conditions and with the animals under general anaesthesia maintained by intramuscular doses of Hypnorm (Mecos, Helsingborg, Sweden) at a dose of 0.7 ml per kg body weight. The skin and fascia over the proximal metaphyses on the medial sides of both tibiae were opened via separate curved incisions. The periosteum was removed in the exact area of the bone to be penetrated by the burrs. A trephine with an outer diameter of 6 mm was used to make a hole through the proximal cortex of the bone. This hole was then tapped using a 7 mm wide instrument with a thread pattern exactly corresponding to the outer thread of the top piece of the implant.

Fifteen titanium chambers, representing the test or FAS group, were carefully pretreated with rabbit fibrinogen-cryoprecipitant which was mixed with Thrombin and factor XIII concentrate according to the instructions from the supplier (Immuno AG, Wien & Kemiintressen, Sundbyberg, Sweden). The FAS was placed in the canals and around the test implants. The test chambers were inserted into one tibia of the experimental animals.

Another 15 titanium chambers, representing the control or medullary cell implants, were filled with autologous blood and medullary cells which were collected with a syringe from the tibial marrow of the control bone and distributed in the canals and on the implant surface immediately prior to insertion. The control chambers were inserted into the contralateral tibiae of the same 15 animals.

Group A

In 10 rabbits the implants were inserted in such a manner that the top canal was placed at the level of the cortical bone while the lower canal was left in the marrow space. It was planned to remove these group A implants after 4 weeks.

Group B

In five rabbits the implants were inserted with the top canal in the marrow space close to the proximal cortex and the distal canal deep in the marrow space. Group B implants were to be removed after 5 weeks.

After implant insertion the skin and fascia were sutured in separate layers. No plasters or bandages were used and the animals were allowed full weight-bearing directly after surgery.

Removal of implants and methodological treatment

Four weeks after insertion (group A) or after 5 weeks (group B) the skin and fascia were again opened under Hypnorm anaesthesia and the implants and surrounding bone cortex were removed with a trephine (Figure 1b). The implants were then taken apart by removing the two connecting screws (Fig. 1c). The contents of the canals and the adjacent bone were, after inspection, immediately put into refrigerated Histocon (Histolab, Gothenburg, Sweden). The four bone samples from each animal collected in this way were all trimmed and most of the bone which had formerly been outside the canal was removed. Only the canal bone, i.e. definitely newly formed bone, was later analysed by microradiography. The four samples from each animal were placed on a plate and microradiographed together. The contents of the canals and the remaining tissue adjacent to the outer circumference of the implants were then placed in formaldehyde for fixation, decalcified in formic acid and cut in a microtome for histological analyses. HTX-eosin and toluidine blue stains were used.

Microdensitometry

Glass plates, each with four microradiograms of the test and control bone from one animal were put onto a Joyce-Loebl microdensitometer MK IIIC. The density was measured for each of the bone samples. Care was taken to have a standardized setting in all measurements from one glass plate. Controlled comparisons were thus possible between samples from the same plate, but not between samples from different plates. The aim of the experiment was to investigate the possible beneficial effects of FAS on bone regeneration, not to provide general bone density figures with respect to a standard. The microdensity curves, indicating the overall density and length of the bone samples, were analysed with a planimeter to give a numerical indication of the "bone surface".

RESULTS

The proximal canals of all group A implants were macroscopically found to contain bone in all cases, while the distal canals of the same implants usually contained much less bone and sometimes only connective tissue proper. In two cases the implants of the test group were still surrounded by some FAS at the time of implant removal. The proximal canals of the group B implants did in some cases contain bone, but never to the extent seen in the proximal canals of group A implants. The distal canals of group B implants only rarely contained any bone. Densitometry and further analyses were, therefore, only performed on the 20 group A implants.

Table 1. Bone surface area in proximal canals of test and control samples of group A. A direct comparison between test and control of the same animal is possible but different animals should not be compared with one another

Animal number	Test value	Control value	Ratio
1	101	79	1.28
2	92	102	0.90
3	153	172	0.89
4	78	68	1.15
5	152	156	0.97
6	40	73	0.55
7	157	172	0.92
8	79	154	0.51
9	82	83	0.99
10	90	123	0.73
			$m_x = 0.89$

Densitometry

A comparison between the bone surface areas of test and control specimens is given in Table 1. The corresponding bone surface areas in the distal canals are given in Table 2. Even if exact comparisons between different animals are not re-

Table 2. Bone surface area in distal canals of test and control samples of group A. As several canals contained no bone there is no calculated ratio given. A direct comparison between test and control of the same animal is possible but different animals should not be compared with one another

Animal number	Test value	Control value
1	0	49
2	0	0
3	204	203
4	0	79
5	0	102
6	22	0
7	0	16
8	57	84
9	20	66
10	68	72

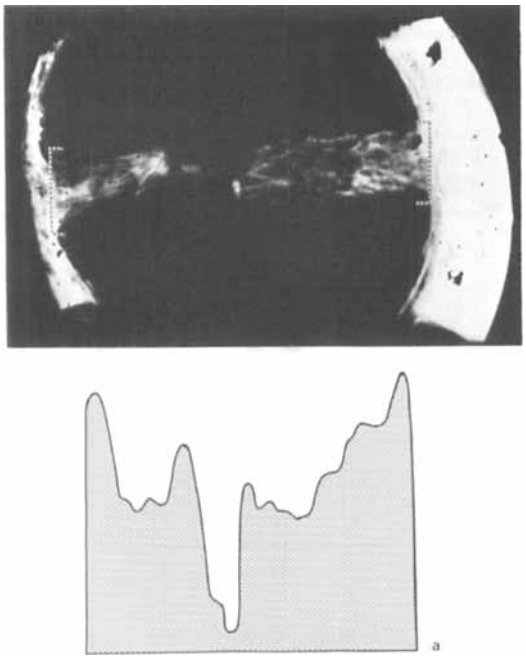

Figure 2a. A test specimen taken from the proximal canal of animal no. 3 (group A). The bone which has grown into the canal is situated between the two lines. The density of the canal bone is measured with microdensitometry as indicated on the sketch from which "bone surface" values can be calculated with planimetry.

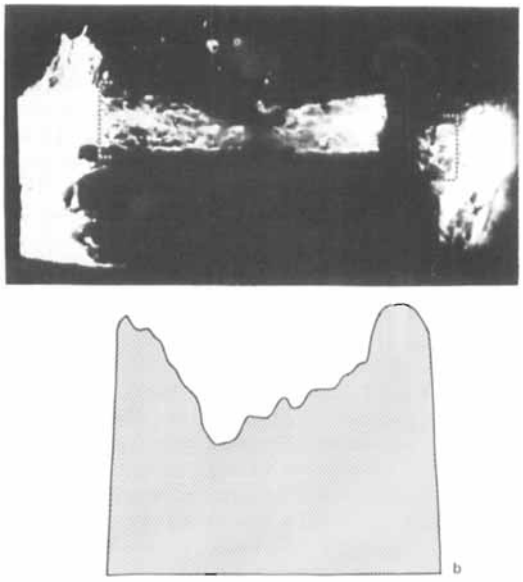

Figure 2b. A control specimen taken from the proximal canal of animal no. 3 (group A). Again only the density of the bone between the two lines is measured. In this case the bone sample was accidently fractured after removal, as indicated by the broken part on the "bone surface" sketch. The ratio test bone: control bone in this case was 0.89, equivalent to the mean value found in the study. There was a tendency for less bone to be formed in the FAS group compared to the controls.

commended, the tendency towards more bone (= higher values) is seen in the samples in Table 1 compared to those in Table 2. In the tables a ratio of more than 1.0 indicates more bone in the test group while a ratio of less than 1.0 indicates more bone in the control canal.

As indicated by the values of Table 1, no clear differences were found between test and control bone when the surface values of the proximal canals were compared. As a rule, the bone in these canals had grown from both sides and was, at 4 weeks, just making contact in the middle. Typical test and control bone microradiograms with corresponding densitometry readings are shown in Figure 2. In the distal canals (Table 2) less bone ingrowth had taken place and several of the implant canals contained only connective tissue proper. The variation in the amount of bone ingrowth was much greater in the distal than in the proximal canals.

Even if no significant differences were found in the proximal canals, there was a tendency towards more bone ingrowth in the controls than in the test group. In the distal canals, on the other hand, there was a statistically verifiable greater bone ingrowth in the control implants compared to the test group. However, the conditions for bone ingrowth into the distal canals on the whole were obviously unfavourable, which was reflected by the low bone surface values recorded in Table 2. Local conditions in the marrow space, depending, for example, on the exact localization of the implant, may to some degree have influenced the results in the distal canals. Therefore it was decided to present the figures in table form, but to avoid a statistical evaluation of the material.

Histology

Histology of the control implants revealed normal bone tissue with osteoblastic activity (Figure

Figure 3. Histological section (HTX-eosin) of a control implant at 4 weeks after insertion of the chamber. Normal bone tissue with osteoblastic activity was seen. The actual diameter of the bone sample is 1 mm.

3). The bone was, as a rule, mostly ordered and of lamellar character. Occasionally, patches of woven bone were seen. The canal bone seemed to be in direct contact with the titanium framework all around its circumference and there were no signs of soft tissue encapsulation. The test bone was in many cases of similar histological appearance. However, a tendency towards an inflammatory reaction was seen in the test samples. In two test bone cases, this inflammatory reaction was very evident (Figure 4a, b).

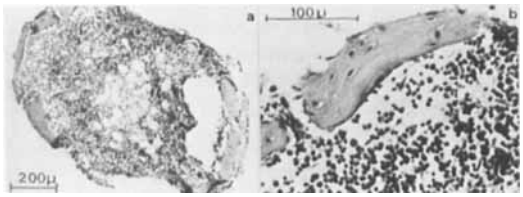

Figure 4a. Histological section (HTX-eosin) of one of the two test specimens showing a strong inflammatory reaction. There was a tendency for there to be more inflammatory cells in the test group than in the controls. However, several of the test specimens were not histologically different from the controls.

Figure 4b. Detail of Figure 4a.

DISCUSSION

A similar dividable titanium implant to that used in this investigation has been applied in a clinical study aimed at the reconstruction of the defective ossicular chain (Tjellström et al. 1978a, b). In this study the implant acted as a mould which was invested by bone. Later, after removing and dividing the implant, this bone was used as a preformed bone graft in the middle ear.

One purpose of the present experiment has been to test whether a modification of this dividable titanium implant can be inserted into the rabbit tibia in so controlled a manner that the subsequent bone ingrowth into the prefabricated canals can be used as a measurement of the bone healing rate under various conditions. After a healing time of 4–5 weeks it was possible to cut out the implant and divide it to gain access to the contents of the implant canals which, during the experimental period, had been buried in the cortical bone and marrow cavity, respectively. Generally the 1 mm wide canal inserted into the cortex had become invaded by growing bone from both sides of the implant. At this time the bone tongues were nearly in contact with each other in the middle of the 6 mm long canal. Furthermore, in a control study Bone Growth Chambers were inserted under similar conditions in both tibial metaphyses of 20 rabbits. The bone contents in the two chambers from the same animal were found to vary only by a few per cent (Albrektsson 1982), which indicates a possible use for this method in estimation of osteogenesis under various experimental conditions. However, if the Bone Growth Chamber is used in comparative studies of osteogenesis it is imperative that surgical technique and animal factors are controlled by letting the same surgeon insert the implants into corresponding sites of the extremities of the same animal.

Effect of FAS treatment

In the present study we were not able to show any positive effects of FAS treatment on the initial osteogenesis within the Bone Growth Chamber. On the contrary, early bone ingrowth seemed to be slightly delayed in the FAS group compared to

the controls. Thus the results of this study do not support those of Pflüger et al. (1979) who found that stainless steel implants in the rabbit tibial metaphysis were incorporated more rapidly after treatment with FAS.

FAS has been reported to facilitate bone healing of cortical defects (Bösch et al. 1977a) and osteotomies (Böhler et al. 1977), as well as the incorporation of autologous (Bösch et al. 1977a, 1979, Rupp & Stemberger 1978) and heterologous bone grafts (Bösch et al. 1980). It has been suggested that the reasons for the improved healing are dependent on FAS prevention of blood clot formation and the inducement of ingrowing capillaries (Bösch et al. 1977a, Zilch et al. 1981). An increased initial stability of the implant due to the gluing effects of the FAS has also been proposed (Pflüger et al. 1979). However, the exact role of the blood coagulum in fracture and bone graft healing is under debate (Albrektsson 1979). A clearly positive influence of blood and autologous marrow on the incorporation of bone grafts has been described by Burwell (1966) and is suggested to depend on factors released from dying cells. Furthermore, where the bone ingrowth of new capillaries, at least into implants, is concerned, the existence of cell debris of mostly red cells and platelets has been regarded as necessary (Zawicki et al. 1981). Actually, in experiments with bone ingrowth into optical, titanium implants the presence of blood and marrow cells in the implant canal seems to be a requisite for reliable bone invasion (Albrektsson 1979). In a recently published experimental study on the rabbit and dog, Zilch & Noffke (1981) also failed to show any positive influence of FAS on bone regeneration.

Earlier studies on the effects of FAS on bone healing have been either clinical (Bösch et al. 1977a, Rupp & Stemberger 1978, Arbes et al. 1981) and thus without a control group, or else experimental studies (Böhler et al. 1977, Bösch et al. 1977b, 1979, 1980, Pflüger et al. 1979) but using control groups without ensuring a rich supply of blood and medullary cells. Whatever the reason, the experimental studies have all conclusively shown the beneficial effects of FAS treatment. However, before applying these results clinically and starting treatment with FAS in various bone healing situations, we feel that FAS should first demonstrate its effectiveness when compared to conventional autologous blood and medullary cell filling. Autologous marrow is an easily accessible source of fibrin as well as of potentially osteogenic cells and should be preferred to homologous FAS administration until any evidence of the advantage of the latter has been presented. In the case of the titanium implant described here, we have a standardized situation where no bone was present in the assembled implant at the onset of the experiment and thus all the bone found afterwards in the dividable implant must have been newly formed. Under such conditions FAS had no superior bone stimulating effect when compared to blood and medullary cell filling.

CONCLUSIONS

1. The dividable titanium implant described above is a newly developed experimental model which allows estimations of early bone ingrowth rates under various conditions.
2. Bone tissue was *not* found to grow more rapidly into FAS-treated implants than into implants treated with autologous blood and medullary cells.
3. Further evidence should be gathered before recommending FAS in the treatment of bone injuries.

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