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A new bioadhesive for *in vivo* bone adhesion

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A new bone adhesive was used to facilitate the union of bone fragments detached and repositioned on the tibia of dogs. The sites were X-rayed and histologically examined 1 and 6 weeks post-operatively. By 6 weeks, the bone fragments were found to be solidly attached in their original sites. Histological examination revealed no signs of inflammation, infection or any other adverse reactions, neither on the operated bone, nor in remote tissues of internal organs (spleen, lung, liver, kidney, and lymph nodes). The tested specimens exhibit, *de novo* bone growth into the adhesive, concomitant with clear signs of biodegradation of the adhesive. Additional *in vivo* experiments were carried out in white rats, whereby the adhesive was implanted subcutaneously and it became evident that the implanted material enabled new bone formation (ectopic), with no adverse effects within the immediate site as well as in the remote areas. Moreover, the biocompatible nature of the material revealed distinct signs of biodegradability.

1. Introduction

The treatment of complicated fractures often includes the utilization of auxiliary devices such as metal screws, wires, plates or nails, which are attached to the bone fragments; in these cases further surgical intervention is usually needed in order to remove these devices at later stages during the course of the healing process. The application of a biodegradable bone adhesive in such complicated clinical cases would simplify the therapeutic course, by saving the patient a second surgical procedure. For the past 20 years, a vast effort has been invested in the development of such an adhesive in order to enable the elimination of metal devices while handling damaged bones [1–3].

A bone adhesive which would be effective and clinically acceptable, must comply with the following basic requirements:

(a) it must tightly adhere to bone fragments at body temperature, and the joint should be formed during a reasonable length of time (a few minutes);
(b) it must possess the durability necessary to enable osseous union;
(c) it must be biodegradable;
(d) it should enable ingrowth of bone cells in order to enhance bone bridging (union);
(e) the adhesive itself, and its biodegraded products, should lack any toxic properties, either at the application site, or at remote organs;
(f) the material should possess neither allergenic nor carcinogenic properties.

The synthetic adhesive used in most studies were alkyl-2-cyano-acrylates [4], fluorinated cyano acrylates, [5] epoxy-type resins [6] and polyurethanes [7]. Most of these substances did not satisfy the clinical expectations, owing to problems connected with polymerization conditions, the toxic activity of some degradation products, and the presence of unpolymerized monomers in the adhesives, thus preventing a wide application of these adhesives in clinical use.

The present report describes the results of a series of experiments performed by use of a new type of adhesive which elicited encouraging results in *in vivo* studies.

2. Materials and methods

A non-elastomeric adhesive designed as A-106, was used in this study [8]. Following polymerization, a network is obtained in the reaction of a polyisocyanate with polyols, of which at least one possesses surface-wetting properties; in the reaction which takes place in the presence of a catalyst participate, also selected compounds containing calcium and phosphorus [8].

2.1. Adhesion of bone fragments in tibia of dogs

Beagle dogs were anaesthetized with Nembutal 30 mg kg^{-1} and the operative site was shaved and scrubbed with Betadine. A 5 cm incision was made in the middle third of the tibia, opening the deep fascia, and the periosteum was separated from the bone. A cortical piece 0.7 cm × 0.7 cm was removed from the anterior surface of the tibia.

The adhesive, prepared *in situ*, was spread over the detached piece of the bone, and the bone was later

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returned to its original site. The wound was closed by layers; the leg was bandaged and placed in a plaster-of-Paris cast. X-rays were taken post-operatively. After 1 week, the operated site was re-exposed; it was evident that the bone fragment had remained in place. The operative site was neither inflamed nor infected. Manual attempts did not succeed in removing the fragment. A specimen and its adjacent tissues were then removed for structural examination. The above procedure was repeated on an additional dog; here, the second operation took place after 6 weeks. X-ray radiograms of the operated region were taken throughout this period, showing that the glued piece of bone remained in place. Bone samples were again taken for histological examination. By that time, blood tests were also performed, together with histological investigation of representative internal organs such as lymph nodes, spleen, liver, kidney and lungs.

2.2. Subcutaneously implanted polyurethane polymers in rats

Rectangular samples (10 mm × 10 mm × 1 mm) of the polyurethane-based polymerized adhesive were implanted subcutaneously in the lower back of rats. Each animal was anesthetized with Ketalar; the shaved area was cleansed with Betadine, prior to incision. White rats, 19 in all, weighing between 140 and 240 g were operated on. One sample was implanted in each animal and the cutaneous incisions were closed with silk sutures.

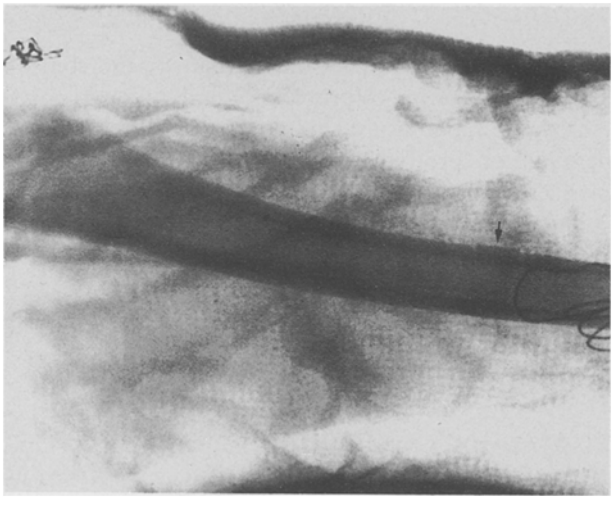

Figure 1 X-ray of the tibia with the glued fragment (arrow), immediately following surgery.

Figure 2 Close-up of an excised tibia containing the glued fragment (1 week post-operatively).

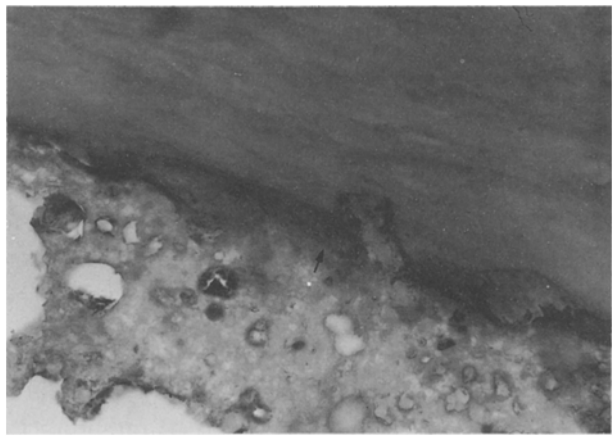

Figure 3 The appearance of a thin layer of the adhesive as seen 1 week after surgery; note the intimate attachment between the adhesive and the host bone (arrow).

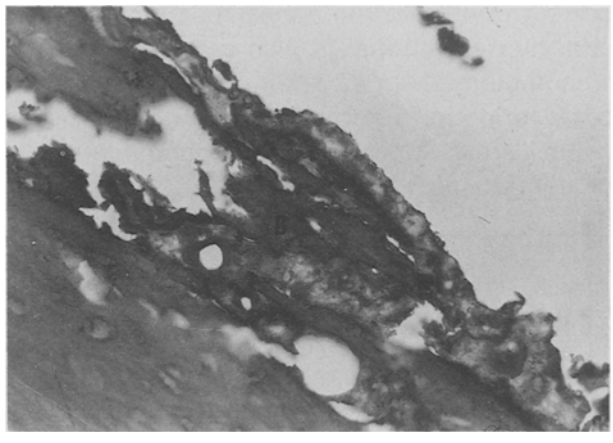

Figure 4 The appearance of the adhesive–host interface, revealing new bone formation (B) within the adhesive material, 6 weeks following the surgical procedure.

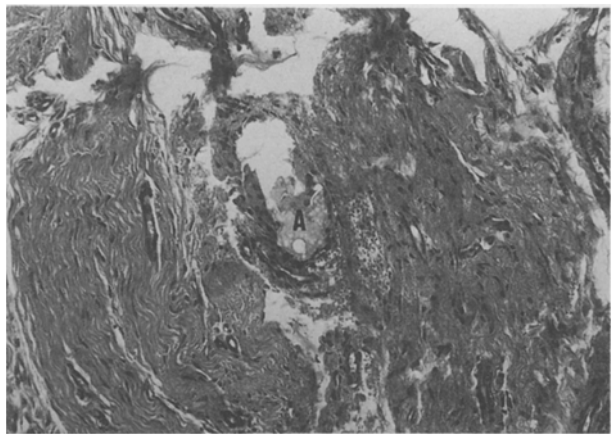

Figure 5 Section through the soft tissues surrounding the graft–host site. A dense layer of collagenous tissue containing blood vessels, fibroblasts and other elements of connective tissues can be identified close to the adhesive (A).

TABLE I Results of blood tests performed on a Beagle dog: before surgery, and 3 and 6 weeks after use of the biodegradable adhesive

Test	Dog 103			Normal range
	Before	After 3 weeks	After 6 weeks	
WBC (10^3 mm^{-3})	9.5	13.4	[a]	4.8–10.8
RBC (10^6 mm^{-3})	6.11	7.22	[a]	4.2–6.2
Hgb (g dl^{-1})	14.2	16.7	[a]	12–18
Hct (%)	40.3	45.5	[a]	37–52
MCV (ft)	66.0	65.7	[a]	82–92
MCH (pg)	23.3	23.1	[a]	27–31
MCHC (g dl^{-1})	35.3	35.2	[a]	32–36
RDW	15.7	15.3	[a]	11–15
PLT (10^3 mm^{-3})	337	321	[a]	200–400
Pct (%)	0.33	0.394	[a]	0.15–0.3
MPV (ft)	9.8	19.3	[a]	6.3–10
PDW	14.7	14.8	[a]	15.5–17.5
Poly	76	54	[a]	[a]
STAB	5	4	[a]	[a]
Lymph	17	21	[a]	[a]
Mono	2	8	[a]	[a]
EOS	–	13	[a]	[a]
Urea nitrogen (mg dl^{-1})		11	[a]	5–20
Glucose (mg dl^{-1})	135	–	[a]	70–110
Creatinine (mg dl^{-1})	0.7	0.8	0.8	0.5–1.3
Calcium (mg dl^{-1})	10.2	10.6	10.5	8.4–10.6
Inorganic phosphorous (mg dl^{-1})	5.1	5.0	5.6	2.5–4.5
Alanine amino transferase (u l^{-1})	45	36	45	4–40
Aspartate amino transferase (u l^{-1})	79	–	36	2–40

[a] Haemolytic blood.

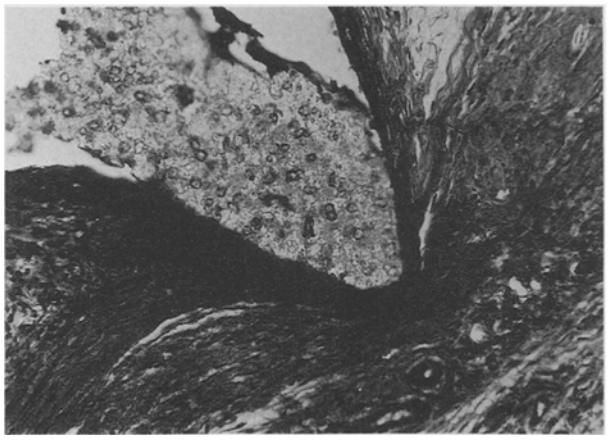

Figure 6 Histological appearance of an implant (polymerized adhesive) introduced subcutaneously in a rat. The implant (A) is surrounded by a dense layer of collagenous connective tissue.

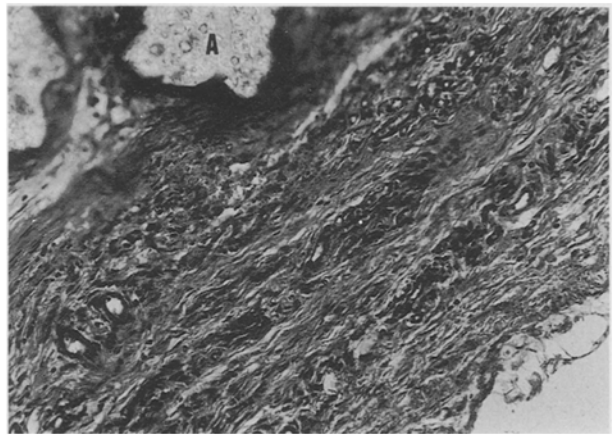

Figure 7 The appearance of a portion of the capsule surrounding the implanted polymerized adhesive (A) in a rat. The host developed a dense layer of collagen fibres hosting blood capillaries as well as multiple fibroblasts. The implanted material shows first signs of disintegration, thereby allowing tissue elements to penetrate in between its fragments.

Three weeks after implantation, nine rats were sacrificed by an overdose injection of nembutal. The implanted polymer was removed and fixed in 4% paraformaldehyde. No adverse reactions were noted in the surrounding tissues. Kidney, liver, lung and spleen samples were also obtained for histological examination.

In the remaining rats the polymer samples were removed after 6 weeks for structural examination.

3. Results

X-ray pictures revealed that immediately after its cementation, and a week later, the bone fragment remained in its original place (Fig. 1). Fig. 2 exhibits a close-up of the cemented bone graft, clearly showing its position in the grafting area.

Histologically, it was apparent that the adhesive remained attached tightly to the host bone, without causing any damage to the underlying bone tissue (Fig. 3). Penetrating blood vessels can be seen in the operative site, and already 1 week after surgery, a new layer of sound bone could be distinguished. Further, the adhesive material allowed continued bone growth in the interphase between the host and the graft.

By 6 weeks, clear signs of new bone formation were noted within the adhesive layer (Fig. 4). No structural signs of toxic, inflammatory or infectious reactions

were encountered in the bone proper, or in the surrounding soft tissues (not shown). The adjacent soft tissues showed that new blood vessels were formed in the adhesive–bone interphase (Fig. 5). The blood picture and the liver function tests of the respective dogs were found to be normal throughout the entire period (Table I). Histological examination of the various internal organs in these dogs did not reveal any atypical reactions or abnormalities.

The polymer that was implanted subcutaneously in rats was found to be encapsulated after 3 weeks (Fig. 6). The immediate neighbouring tissues were composed mainly of collagen fibres and fibroblasts (Fig. 7). In addition, small blood capillaries were encountered throughout the investigated tissue specimens. With increase of the experimental period, the polymer implants revealed signs of disintegration, while new bone tissue penetrated and occupied the newly formed cracks and holes within the implant's bulk (Fig. 4). Figures 5–7 show that within the new tissue (inside the polymer) new blood capillaries developed along side the fibroblasts and other connective tissue cells.

4. Conclusions

The present *in vivo* findings seem to indicate that our new adhesive material enables the re-union of a bone fragment to cortical bone. The adhesive was not found to cause any adverse reactions in the host bone nor in its surrounding tissues. Biodegradation products of the adhesive did not appear to interfere with the *de novo* bone formation or with other functional processes in the organism as a whole. More work, mainly of a quantitative nature, is needed in order to follow the healing capabilities and the degradation processes occuring in this new adhesive.

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