Assessment in rats of a new bioerodible bone-wax-like polymer

Bjørn Sudmann[1], Ole-Gunnar Anfinsen[1], Gisle Bang[2], Rolf Koppang[4], Steinar Ørbeck Stølen[5], Hanna Strømme Koppang[5] and Einar Sudmann[3]

Holes drilled in rats' skull, iliac crest, and tibia were filled with beeswax or with a new, wax-like, bioerodible polyorthoester (Alzamer®). Empty drill-holes served as controls. In addition, beeswax and polyorthoester were deposited between the left and the right oblique abdominal muscles, respectively. In muscle, both the beeswax and polyorthoester elicited a transient foreign body reaction. The beeswax was not resorbed in bone or muscle, whereas the polyorthoester was. Bone healing was inhibited in the iliac crest and the tibiae filled with beeswax, whereas holes filled with polyorthoester healed readily.

[1]Surgical Research Laboratory, [2]Department of Oral Pathology and Forensic Odontology, School of Dentistry, and [3]Hagavik Orthopedic Hospital, University of Bergen, Bergen, [4]Department of Preclinical Techniques and Material Sciences, and [5]Department of Pathology, Dental Faculty, University of Oslo, Oslo, Norway
Correspondence: Dr. B. Sudmann, Hagavik Orthopedic Hospital, N-5220 Hagavik, Norway. Tel +47-5 308010. Fax -5 308204
Submitted 92-02-05. Accepted 92-09-06

Bone wax, made on the basis of Horsley's original recipe (Horsley 1892, Parker 1892), has been used for 100 years to control bleeding from bone. The bone wax used today mainly consists of refined beeswax (88%) and isopropylpalmitate (12%). It is not resorbed, and it can induce chronic inflammation and marked foreign body reaction (Howard and Kelly 1969, Brightmore et al. 1975). In addition, beeswax may lower the bacterial clearance in cancellous bone and give rise to wax embolization as well as form a mechanical barrier to bone regeneration (Rockwood et al. 1968, Wolter et al. 1975, Johnson and Fromm 1981, Robicsek et al. 1981, Aurelio et al. 1984, Sorrenti et al. 1984, Rodrigues and Carvalho 1986). Thus, a hemostatic bone wax without these shortcomings is needed.

We report the effect on bone healing and the inflammatory reaction between 2 muscle layers of a novel wax-like, bioerodible polyorthoester in rats, compared to beeswax.

Material and methods

42 male Wistar rats (Møllegaard, Eiby, Denmark) weighing 351 (322–401) g were randomly allocated to Groups A and B, and another 20 male rats weighing 257 (240–274) g were allocated to Group C. The rats were caged 3 or 2 together and given standard animal food pellets and water ad libitum (Table 1).

Design of experiment

On the left side drill-holes in skull, iliac crest and tibia were plugged with ordinary bone beeswax (Ethicon Ltd, Edinburgh, Scotland). On the right side the holes were filled with a new, wax-like, bioerodible polyorthoester (Alzamer®, Alza Corporation, Palo Alto, California, U.S.A.), which is hydrolyzed at body pH. The polyorthoester is available hard or soft; a wax-like mixture of these was used in this study. Thus, the rats served as their own controls for comparison of the effect of beeswax vs polyorthoester (Table 1). Bees-

Table 1. Design of experiment. Beeswax and polyorthoester were deposited on the left and on the right sides, respectively, and empty burr holes in one animal in each subgroup served as controls. Figures are number of rats

Group	Localization of implant	N	Observation time in weeks											
			0	1	2	3	4	5	6	8	16	32	48	52
A	Skull and iliac crests	21	1[a]	3	3	3	3	3	5	0	0	0	0	0
B	Abdominal mm and tibiae	21	2[a]	3	3	3	3	3	4	0	0	0	0	0
C	Abdominal mm	20	0	0	0	0	0	0	0	4	4	4	4	4

[a] Died during anesthesia.

wax and polyorthoester were also deposited between the oblique abdominal muscles (Group B, Table 1). To observe any long-time tissue reaction, 20 other rats received abdominal implants only (Group C).

The polyorthoester appeared bone-wax-like, but more grainy than beeswax; kneading rendered it soft and equally usable as beeswax. Hemostasis in bone was effective whether using beeswax or polyorthoester. The polyorthoester could more easily be removed from the surface of surgical instruments and gloves than beeswax.

Surgical procedure

Group A. Under neuroleptic anesthesia (Hypnorm Vet®, Leo, Helsingborg, Sweden), and non-sterile conditions, the frontal and parietal bones of 21 rats were subgaleally exposed through a median incision. 4 holes were drilled with a 2 mm spherical, dental burr in each skull, 1 hole through each frontal and parietal bone onto the dura and filled with beeswax on the left side and polyorthoester on the right side. One empty burr hole in each parietal bone in every third rat in each subgroup served as controls. The aponeurotic galea was loosely adapted and the skin sutured.

Furthermore, the rostral part of the iliac crest was exposed through a muscle-splitting incision. With a 2 mm ordinary drill-bit, a hole was drilled through the crest from the posterolateral side. The left and the right side holes were filled with beeswax or polyorthoester. One empty burr-hole in each iliac crest in every third rat in each subgroup served as controls. The wounds were closed in layers.

Group B. The abdominal muscles in 21 other rats were exposed through a median incision. Standardized and equal volumes of beeswax and polyorthoester each weighing about 35 and 45 mg, respectively, were inserted between the external and the internal oblique abdominal muscles, on the left and the right sides, respectively. Wounds were closed with skin sutures only. Furthermore, the lateral, proximal surface of both tibiae were exposed through a longitudinal incision and, with a drill-bit, a 2-mm-diameter cavity was prepared in each metaphysis. The left cavity was filled with beeswax, and the right with polyorthoester. Two empty burr-holes in every third rat in each subgroup served as controls. The wounds were closed in layers.

Group C. For long term observations, beeswax and polyorthoester were deposited in the abdominal muscles of 20 rats, as described for Group B. A tiny dash of medical carbon was added to every second polyorthoester implant to localize the deposition site after possible resorption.

1 rat in Group A and 2 rats in Group B died during initial anesthesia (Table 1).

Evaluation

3 random rats in Groups A and B and 4 in Group C were killed at intervals of 1–16 weeks (Table 1). Dissected rat skulls, iliac crests, abdominal muscles and proximal parts of tibiae were fixed in 4 percent buffered formaldehyde. The bone specimens were decalcified in Decalc (Histolab), and all specimens were embedded in paraffin, sectioned and stained with hematoxylin, eosin and saffron.

Results

Macroscopically, at Day 7, the beeswax had evoked marked inflammation in the abdominal muscles, whereas no such reaction could be detected adjacent to the polyorthoester. All bony cavities filled with beeswax remained plugged after 6 weeks, whereas no polyorthoester was seen in corresponding sections (Figure 1). In muscle, solid deposits of beeswax were easily located throughout the experiment, whereas the polyorthoester could hardly be detected in the abdominal muscles after 5 weeks.

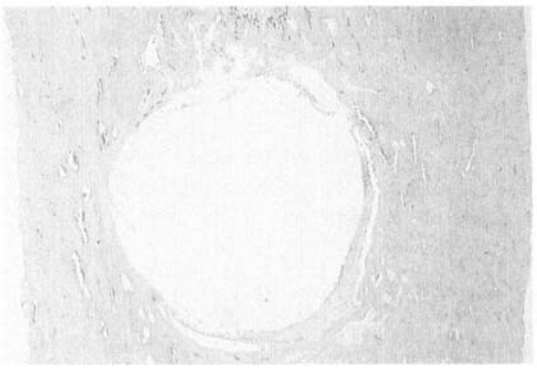
Histology

Beeswax elicited only a minor inflammatory reaction in the rat skull. But, acting as a mechanical barrier, it impeded filling of all the burr holes by fibrous and osseous tissue. In contrast, all holes initially plugged with the polyorthoester were filled with granulation tissue after 2 weeks, and were increasingly filled with new bone from the endosteal side. All control holes were filled with granulation tissue after 2 weeks, and after 6 weeks more endosteal bone formation had occurred here than in holes initially filled with the polyorthoester.

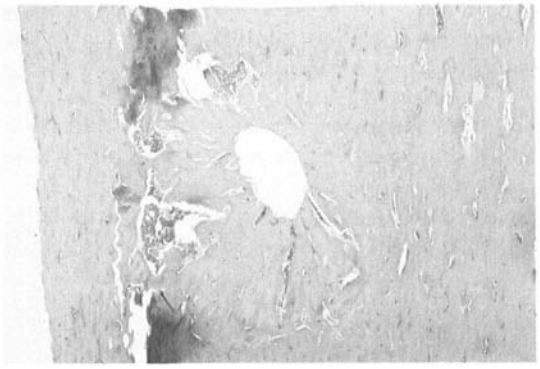
In the iliac crest, beeswax evoked moderate inflammation with only a minor increase in fibrous encapsulation during the experiment. No bone healing was revealed. All control burr holes and all burr holes plugged with the polyorthoester were filled with new bone tissue after 3 weeks. No bone formation was seen in any burr cavities in the proximal tibiae filled with beeswax (Figure 1). All holes initially plugged with the polyorthoester were filled with new bone tissue after 4–5 weeks, and in the controls after 2 weeks.

Between the oblique abdominal muscles beeswax elicited a marked inflammatory reaction after 1 week, with accumulation of polymorphonucleated leucocytes. The number of polymorphonucleated leucocytes subsequently decreased, but an increasing amount of fibrous tissue appeared (Figure 2). In muscle, the foreign body reaction elicited by the polyorthoester was

Figure 1. Tangential section of a decalcified section of rat tibia at Week 4 postoperatively. HE and saffron. Field width 4 mm.

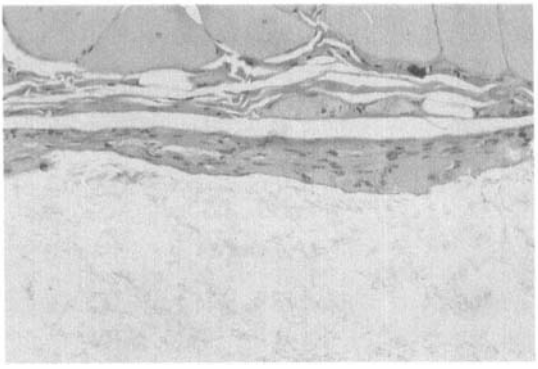


No fibrous tissue or bone is seen in the 2 mm cavity initially plugged with beeswax.

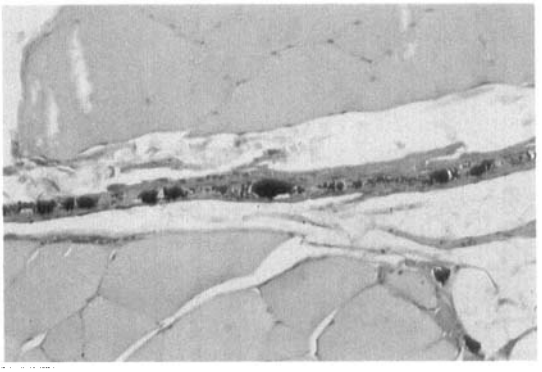


The polyorthoester has been resorbed and the 2-mm defect is almost filled with new bone.

Figure 2. Histological section of rat abdominal muscle 1 year postoperatively. HE and saffron. Field width 0.55 mm.



The beeswax (lower half of figure) is not resorbed, leaving a space, encapsulated by fibrous tissue, after histological preparation.



The polyorthoester is resorbed and no inflammatory reaction is evident, only scattered black coal particles are seen where the polyorthoester was deposited. Open spaces are due to artefacts during the preparation.

maximal during the second week, and more intense than in corresponding beeswax specimens. Polymorphonucleated leucocytes and multinucleated giant cells were identified. However, after 1 year only medical carbon was seen.

Discussion

Until now no hemostatic bone sealant except beeswax has been adopted into regular clinical practice. The polyorthoester used in our study easily gained malleable consistency like wax. It stuck slightly to gloves and instruments used for its application. The hemostatic effect was as effective as using beeswax. Platelets play an important role in normal hemostasis, but the polyorthoester does not promote platelet aggregation (Solheim et al. 1991).

Differences in consistency and time of resorption can be obtained by varying the composition of the Alzamer® polyorthoesters. Finally, since the polyorthoester may release a drug of choice at a predictable rate (Capozza et al. 1978, Heller et al. 1981), unlike beeswax, it can be used as a local, sustained drug release system, e.g., for antibiotics, antiinflammatory drugs and bone-inducing substances (Pinholt et al. 1991, Engesæter et al. 1992, Solheim et al. 1992). These polymers thus seem promising as a resorbable device, a hemostatic sealant, and as a vehicle for drugs in bone surgery.

Acknowledgements

The authors would like to thank Gunnvor Øijordsbakken for providing the histological sections and Alza Corporation,

Palo Alto, California, U.S.A for provision of the polyorthoester (Alzamer®).

Funds were received from the Norwegian Research Council for Science and the Humanities.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References

Aurelio J, Chenail B, Gerstein H. Foreign body reaction to bone wax. Report of a case. Oral Surg Oral Med Oral Pathol 1984; 58 (1): 98–100.

Brightmore T G, Hayes P, Humble J, Morgan A D. Haemostasis and healing following median sternotomy. Langenbecks Arch Chir (Suppl) 1975: 39–41.

Capozza R, Sendelbeck L, Balkenhol W. Preparation and evaluation of a bioerodible naltrexone delivery system. In: Polymeric Delivery Systems (Ed. Kostelnick, R. J.) Gordon and Breach Science Publishers, New York 1978: 59–73.

Engesæter L B, Sudmann B, Sudmann E. Fracture healing in rats inhibited by locally administered indomethacin. Acta Orthop Scand 1992; 63 (3): 330–3.

Heller J, Penhale D W H, Helwing R F, Fritzinger B K. Release of norethindrone from poly (ortho esters). Polym Eng Sci 1981; 21: 727–31.

Horsley V. Antiseptic wax. Brit Med J 1892; 1: 1165.

Howard T C, Kelley R R. The effect of bone wax on the healing of experimental rat tibial lesions. Clin Orthop 1969; 63: 226–32.

Johnson P, Fromm D. Effects of bone wax on bacterial clearance. Surgery 1981; 89 (2): 206–9.

Parker R. Aural pyaemia successfully treated by removing putrid thrombus of jugular vein and lateral sinus. Brit Med J 1892; 1: 1076–7.

Pinholt E M, Solheim E, Bang G, Sudmann E. Bone induction by composite of bioerodible polyorthoester and demineralized bone matrix in rats. Acta Orthop Scand 1991; 62 (5): 476–80.

Robicsek F, Masters T N, Littman L, Born G V. The embolization of bone wax from sternotomy incisions. Ann Thorac Surg 1981; 31 (4): 357–9.

Rockwood C A, Perkins J C, Roberts L, Dixon D F. Reaction of bone wax on bone and muscle. J Bone Joint Surg (Am) 1968; 50: 837–8.

Rodrigues S O, Carvalho A C P. "Bone wax" implantation into subcutaneous connective tissue and into surgical cavity in mandible of rats. Histological study. Rev Odontol Unesp (Brazil) 1986–7; 15–16: 39–51.

Solheim E, Anfinsen O G, Holmsen H, Sudmann E. Effect of local hemostatics on platelet aggregation. Eur Surg Res 1991; 23 (1): 45–50.

Solheim E, Pinholt E M, Bang G, Sudmann E. Inhibition of heterotopic osteogenesis in rats by a new bioerodible system for local delivery of indomethacin. J Bone Joint Surg (Am) 1992; 74 (5): 705–12.

Sorrenti S J, Cumming W J, Miller D. Reaction of the human tibia to bone wax. Clin Orthop 1984; 182: 293–6.

Wolter D, Mohr W, Kinzl L. Die Reaktion des Knochen und Weichteilgewebes auf implantiertes Knochenwachs beim Schaf. Chirurg 1975; 46 (10): 459–62.