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Application of purified bone morphogenetic protein (BMP) in cranio-maxillo-facial surgery

BMP in compromised surgical reconstructions using titanium implants

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SUMMARY. **After a review of the clinically relevant literature on early modified whole bone products (demineralized bone; AAA bone (*Urist* et al., 1975)) – predecessors of purified bone morphogenetic protein (BMP) – and a summary of the only published clinical experience with purified human BMP (in orthopedic surgery; from the group of *Urist; Johnson* et al., 1988–1992), an introductory overview of our experience with our own preparations of BMP from bovine bone in 271 procedures on the face and cranium in 145 patients is presented.**

In this first article of a series of three, major preprosthetic reconstructions using iliac bone grafts and titanium screw implants are described. All patients are examples of compromised bone and/or soft tissue conditions and cannot be considered routine indications for the operations performed.

The most endangered implants became osseointegrated after 6 to $8\frac{1}{2}$ months as judged from clinical examination and CT imaging. These results demonstrate the efficacy of purified, concentrated BMP preparations, able unequivocally to induce bone even in areas with seemingly lost implants.

INTRODUCTION

Bony healing is the crowning response to all surgical measures on the skeleton. It involves two major mechanisms: ingrowth of osteoblasts from severed bone into a gap or onto and along an inert surface of an implant material (the latter being known as osteoconduction) with subsequent deposition of bony substance; or the recruitment of undifferentiated mesenchymal cells into the pathway of osteogenic differentiation, called osteoinduction. The first mechanism pertains to uncomplicated fractures or osteotomies and to the osseointegration of implants such as titanium screws used in cranio-maxillofacial and in preprosthetic surgery. The second mechanism is mediated by bone morphogenetic proteins present in bone matrix and can be artificially induced in orthotopic or ectopic sites; however, its relative importance in spontaneous bone modelling, remodelling and repair is unknown.

Historically, bone inducing mechanisms originating in osseous transplants or implants were already suspected in the last century by orthopedic surgeons (for a review see *Mulliken* et al., 1984). In pioneering, conclusive experiments in the late 1960s *Urist* showed that demineralized bone contained a major bone inducing principle. He and his associates (*Urist* et al., 1979), and also the group of *Reddi* and co-workers (*Sampath* and *Reddi*, 1981, 1987 *Sampath* et al., 1987; *Luyten* et al., 1989) developed methods to isolate such local bone growth and differentiation factors, named bone morphogenetic proteins (BMPs) by *Urist* or *Osteogenin* (corresponding to BMP-3) by *Reddi*, respectively.

Up to now, 7 BMP components have been identified, 6 of them homologous to transforming growth factor beta.

Orthopedic and cranio-maxillofacial surgeons have always been eager to have at their disposal bone inducing materials, for the following situations and with these intentions: to speed up healing in all major operations, especially on elderly patients or when rigid fixation is impossible, or in cases prone to or with already impending infections, or in patients with known compromised healing potential after irradiation or extensive posttraumatic scarring. However, the search for healing adjuvants seemed mandatory in patients with pseudarthroses or osseous defects to be bridged by autologous transplants or various types of implants, whose success depended on rapid osseointegration.

Early attempts to induce bone: demineralized human bone; partially deproteinized human bone

For mechanical and physiological reasons the ideal material for bridging osseous defects is autologous, viable bone. Allogenic bone in chemically unmodified form, on immunological grounds, is unsuitable for this purpose. Usually it is rapidly resorbed, walled in by dense connective tissue or eliminated by sequestration and discharge. On the other hand, experimentally at least, demineralized and defatted allogenic bone is osteoinductive in ectopic (*Urist* et al., 1967) and orthotopic sites (facial bones and cranium; *Mulliken* and *Glowacki*, 1980) in the rat, although in facial and calvarial reconstruction where the recipient bone and the implant are in primary contact,

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Editorial

Beginning in 1994 there will be a change in the number of Cranio-Maxillo-Facial Surgery journals published each year. There will be a decrease from eight to six issues, however, the number of pages will remain the same. Therefore, there will be no decrease in the overall size or content of our Journal. The sequence of the distribution from 1994 will be: February, April, June, August, October and December. The advantage of this change will be a 'perfect bound journal'. This change will not influence the cost of the new publication. Along with the change in binding we have made the cover of the journal a darker blue. This together with the perfect binding will meet the expectations and ideas of our readers.

It has been my intention to change the citation style of the references beginning in 1994. As mentioned in a previous editorial, most of the scientific journals utilise the so-called 'Vancouver style' for the citation of references. As a rule, in this system the cited authors are given numerical code numbers which are then listed consecutively in the order of references at the end of the article. Undoubtedly, this method has the advantage of saving space in each article and also the reader does not have to go over a long list of names before he can continue reading the text. On the other hand, the reader must compare the number in the text with the numerical code in the reference list at the end of the article, and thus, he must repeatedly turn pages back and forth. Other medical journal editors share my opinion that it is easier for the reader if the cited authors and the year of publication can be found immediately in the text. In order for this idea to be most effective, authors must refrain from citing too many references in the article and from repeating these citations over and over. Otherwise, if too many names and references are cited, the article becomes difficult to read and could become boring.

We do hope that the members of our Association and all our other readers are in agreement with the proposed amendments in the publication of our Journal.

E. W. Steinhäuser
Editor-in-Chief

Table 1 – Reports on application of modified bone preparations in patients (demineralized or AAA bone)

Authors and year of publ.	Number of patients	Diagnosis	Implant type used	Time of observation	Results and comments
Sharrard and *Collins* (1961)	3	Scoliosis	Demineralized autologous bone matrix	long term	good
Mulliken, *Glowacki* et al. (1981)	44	Congenital cranio-maxillo-facial malformations (37), acquired lesions (7)	Demineralized bone matrix (human)	up to 1 year	good
Urist and *Dawson* (1981)	40	Spondylolisthesis or degenerative joint and disk disease	AAA bone (human)	5-9 years	good. Results equal to autologous controls
Sonis, *Kaban* et al. (1983)	21	Periodontal defects	Demineralized bone matrix (human)	5-18 months	Pocket depth decreased in more than 50% of patients
Ousterhout (1985)	25	Cranio-maxillo-facial defects, congenital and acquired	Demineralized bone (human) from commercial source	6-18 months	Significant resorption in operations for contour augmentation
Toriumi et al. (1990)	53	Reconstructions of the nose (51 cases)	Demineralized bone (human) from commercial source	1-3 years	50-82% resorption
Total	186				

osteoconduction cannot be excluded as an additional factor. The same is probably true in comparable clinical surgery.

Sharrard and *Collins* (1961) and 20 years later, *Glowacki* and *Mulliken* (1981) used demineralized human bone of autologous or allogenic origin, respectively, in orthopedic or cranio-maxillofacial surgery. The former group stated that demineralized autologous bone struts in 3 children treated with spinal fusion for scoliosis provided a good scaffold for 'appositional growth of new reparative bony callus' (judged by histology and X-ray examination)—another way of describing osteoconduction.

Because of the close and extensive contact between implants and recipient bone—the implants were put into a wide host cancellous bed—osteoconduction was undoubtedly the predominant mechanism of healing in these cases.

On the other hand, *Glowacki* et al. (1981) and *Mulliken* et al. (1981) applied allogenic demineralized bone (in the form of blocks, chips or powder) with the intent and in the belief of providing osteoinduction in 44 cranio-maxillofacial operations on 37 patients with congenital malformations and 7 with acquired lesions, mostly by onlays on facial bones, or by small interpositional implants into osteotomy gaps. In one patient, a 6-year-old child with extensive craniectomy in a craniosynostosis of cloverleaf appearance, discs of demineralized autologous calvarial bone pieces were prepared and placed in a mosaic-like fashion onto the dura. Consolidation of this flaccid construct occurred after 4 months. But even in this situation, osteoinduction by the demineralized bone could not be proven unequivocally, although the individual discs were separated from each other and the surrounding bone, since the dura, like the periosteum, may produce bone spontaneously. In the other cases too, differentiation between osteoinduction and osteoconduction from the underlying or adjacent bone was hardly possible. On a more practical scale, the authors reported that the short term (less than 1 year) results were good in most cases, but the fate of the still persisting demineralized bone, that is, the degree of its later resorption, could not be evaluated.

Bone bridging and stabilisation with another type of modified allogenic implant was reported by *Urist* and *Dawson* (1981). They applied antigen extracted, autolyzed, allogenic (AAA) bone blocks (*Urist* et al., 1975) to induce spinal fusion (transverse process arthrodesis) in 40 patients and compared them with a control group who received autologous bone blocks for stabilisation. The results were equivalent, lasting consolidation was obtained in both groups. Clearly, vertebral bridging here was the result of implant incorporation (bone conduction) into callus originating from the opened spongiosa of the recipient bone bed. The authors did not mention the possibility of bone induction by the implants, although in theory, this was no less likely than in the series of *Mulliken* et al. (1981).

In 1983, *Sonis* et al. from *Mulliken*'s group applied demineralized allogenic bone powder in the treatment of 21 patients with periodontal defects. The results showed a significant reduction in pocket depth after 4 to 18 months. Again, no long term results were reported.

Another publication on the use of demineralized bone (*Ousterhout*, 1985), this time from a commercial source (powdered or cancellous form), in 25 patients with cranio-maxillofacial defects with a follow-up period of 6 to 18 months states that contour augmentation with demineralized bone of large areas (e.g. the forehead) was fraught with early resorption. The correction of smaller defects was more successful. In cases with extensive resorption no new bone was ever found. In more favourable cases, early deposition of bone around, but not within the implant was seen. Best results (early healing) were observed in Le Fort I reconstructions treated with autologous bone pieces and demineralized bone powder applied to the cut bone surfaces. Sometimes, in some implants, there was concomitant bone formation in one area and resorption in another. In conclusion, the results were unpredictable.

Table 2 – Purified BMP plus autologous bone or AAA bone

Authors and years of publication	Number of patients	Diagnosis	BMP implant type	Additional materials implanted	Healing time	Results and comments
Johnson et al. (1988)	6	Traumatic tibial defects, 3-17 cm long	BMP within PLA/PGA onlay strip	Autologous cancellous bone; metal fixation (int. or ext.)	$5\frac{1}{2}$	Good
Johnson et al. (1988a)	12	Femoral shaft nonunions	BMP within PLA/PGA onlay strip or within gelatin capsule onlay	Autolog. (4) or AAA bone (4). No bone (4). Metal appliances for fixation (12)	4.7	Solid union
Johnson et al. (1990)	4	Nonunions of distal tibial end	BMP within PLA/PGA onlay strip (2), BMP in gelatin caps. in fracture. gap (1) or as onlay (1)	Autol. bone (1). Autol. + AAA bone (1) imbibed with BMP. No bone (2)	4.4	Solid union
Johnson et al. (1992)	25	Nonunions of tibia (7), femur (12) or humerus (6) with or without defects (10; 2-9 cm long)	BMP within and on the surface of AAA bone	AAA bone alloimplant imbibed with BMP alone (18), plus autol. bone chips (7). Metal fixation (int. or ext.) in all cases	6	Solid union in 24 cases. No union in 1 case due to recurrent infection (not received autologous bone)
Total	47					

A more recent report (*Toriumi* et al., 1990) on the use of commercial demineralized bone, deals with 53 cranio-maxillofacial cases (51 with corrections of the nose, dorsum and columella). Resorption of the implants was about 50% after 1 year, 64% after 2 years and 82% after 3 years or more. One implant to the tip of the nose showed 73% resorption. Operations on the columella or for small nasal defects were more successful. Infection (2/53 cases) led to complete resorption of the implant. The authors speculated that insufficient bone-inducing activity in the implant material and/or inadequate contact between implant and surrounding tissue were the main reasons for the poor results.

The details and results of the above clinical applications of demineralized bone matrix or otherwise modified bone are summarized in Table 1.

Only the isolated, refined BMP allows defined and promising applications

Purified human BMP

The clinical use of purified, concentrated BMP for bone induction has only been reported recently, in 4 papers from 1988 to 1992 by *Urist*'s group (Table 2; *Johnson* et al., 1988, 1988a, 1990, 1992). Purified human BMP was available to these authors since 1983. BMP preparations were used here exclusively to treat complications in orthopedic cases—pseudarthroses or osseous defects. In all patients, autologous or AAA bone (*Urist* et al., 1975) was used. These bony substitutes were supplemented by BMP as an onlay within a strip of polylactic-polyglycolic acid, or as a coating of lyophilized AAA bone, rehydrated with a BMP-containing buffer solution. In the series of 1992, solid union was achieved in 24 out of 25 cases 6 months after operation, while an average of 3 earlier operative attempts at promoting union had been unsuccessful. In spite of the remarkable effect of concentrated, partially purified BMP, this material has hardly been used by other groups, probably due to the inadequate availability of suitable donor bones. This situation could change if BMP recently produced by recombinant cDNA technology (*Wozney* et al., 1988; *Celeste* et al., 1990; *Sampath* et al., 1992) should prove to be equally efficacious.

Purified bovine BMP

Because of the problems of procurement of human bone, we have purified BMP from fresh, young bovine bone. Our purification scheme was based on *Reddi*'s and co-workers' procedures (*Sampath* et al., 1987; *Luyten* et al., 1989) used by them for commercial bovine bone powder.

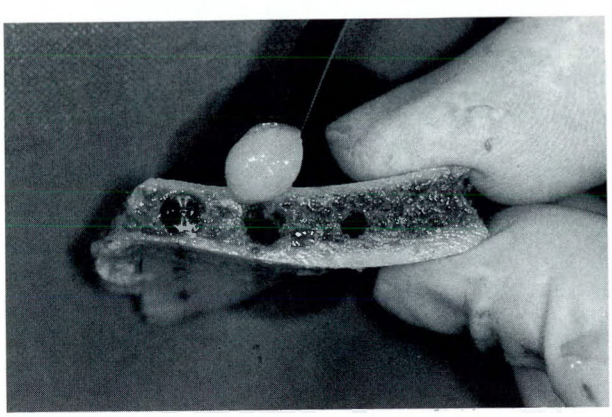
The hope that such preparations could be suitable for clinical surgery grew out of several experimental observations showing that BMP induced bone after ectopic or orthotopic application across species, provided a homologous carrier was used; this meant that BMP itself was not species specific. The final significant impetus to the clinical use of our BMP preparations derived from determinations of the primary structure of human and bovine BMP preparations which showed identity for the 2 major BMP components, BMP-2 and BMP-3, respectively, from human and bovine sources (*Wozney* et al., 1988; *Luyten* et al., 1989; *Wozney*, 1989).

After preliminary application in human volunteers of our partially purified BMP preparations had demonstrated their innocuity, we used the material (mainly as a paste; often together with lyophilized cartilage, more rarely with lyophilized bone) on a larger scale in a variety of surgical procedures on the face, jaws and cranium. Many of our patients and all of those presented below received titanium implants (*Brånemark* type) during reconstructive procedures on the maxilla or mandible (*Sailer*, 1991). In general, osseointegration of titanium screw implants occurs uneventfully when the recipient bone is wide, has a

dense structure and a normal blood supply and if it is not significantly traumatized mechanically or by heat during implant insertion. Normally, after positioning of the implant there is a very narrow zone of osteonecrosis around it which is rapidly bridged by outgrowing osteoblasts, without the formation of a fibrous scar. In all of our 4 representative complex surgical cases described, one or more of the important prerequisites for success were lacking. Above all, in 3 of the 4 patients the implants were inserted partially into transplanted autologous bone, when reconstruction of jaws by sandwich-type or graft inlay procedures were done (horizontal sandwich on the mandible, 1 case; vertical sandwich of the maxillary alveolar ridge, 1 case; bone inlay onto the maxillary floor, on both sides, 1 case). This meant that these implants were partially or totally surrounded by bone of reduced vitality. In addition, the bone bed of the screws was incomplete in some cases. Applying BMP to these implants in artificial beds or in other compromised conditions seemed to be a good test of the efficacy of BMP. Thus, the question was: can BMP compensate for missing favourable factors and promote implant osseointegration even if the bone bed is incomplete or its condition is less than optimal?



A



B

Fig. 1 – Intraoperative application of BMP. **(A)** BMP paste is mixed with chips of lyophilized cartilage. **(B)** Autologous iliac bone graft being prepared for vertical sandwich type operation. Drill holes are filled with BMP paste before implant insertion.

MATERIALS AND METHODS

BMP preparation

BMP was purified from fresh bovine bone (femoral diaphysis of 4–8-wk-old calves) with a modification (*Kolb* and *Sailer*, 1992, 1993) of the procedure of *Luyten* et al. (1989). Briefly, batches of 1.5 kg of freeze-dried bone particles (up to 0.5 mm in diameter), after defatting and demineralization, were extracted at room temperature with a dissociating buffer (50 mM Tris, pH 7.4, 6.0 M urea, 1.0 M sodium chloride). The protein extract was applied to a hydroxyapatite column and eluted with a phosphate gradient (0.01–0.2 M sodium phosphate, pH 7.4). The fractions were analyzed with SDS-polyacrylamide gel electrophoresis without reduction. Those predominantly containing proteins of the molecular weight range around 30–40 kD were pooled. Usually 3 pools were made.

The purest pool was applied to a Sephacryl-S-200 (Pharmacia) column and run in 50 mM Tris, 4.0 M guanidine hydrochloride, pH 7.4. The latest fractions of this elution profile were almost completely reducible with dithiothreitol (100 mM) and mercaptoethanol (5%) to a protein band of about 20 kD.

BMP activity could also be recovered from the less pure hydroxyapatite pools by subjecting them to High Pressure Chromatography (FPLC, Pharmacia): Mono S HR with a gradient of 10–500 mM sodium chloride in 50 mM sodium acetate, pH 5.0; reverse phase silica (C18) column, eluted with a gradient of 10–60% acetonitrile in 0.1% trifluoroacetic acid.

BMP preparations were tested for bone inducing activity in a mouse assay, with a BMP-free mouse collagen preparation as a carrier (adapted from Muthukumaran and Reddi, 1988). Of the purest preparations 100 μg induced cartilage and bone after 12 days.

The recovery of BMP as estimated from protein determinations (Bio Rad Kit) and reducibility was 10 to 20 mg per kg dry bone.

BMP in clinical surgery was mainly applied as a paste (Fig. 1A) containing 1 mg of purified BMP per cm^3 of paste and granular human bone matrix (made from demineralized femoral bone) gamma-ray sterilized with 25 KGy. In some cases, BMP was added to the rehydrating buffer of lyophilized cartilage or lyophilized bone (*Sailer*, 1983). (Fig. 1B).

Surgical procedures with intraoperative BMP application

So far, BMP has been used in maxillofacial reconstructions including pseudarthroses, in cranial reconstructions (contour augmentation) (*Sailer*, 1983) and in implantology for cases with impaired healing capacity due to various imponderabilities of the implant bed. Table 3 shows the types and number of operations and the number of patients in each treatment group.

In the following text our results achieved in compromised conditions of implantology are described. The results obtained in maxillofacial reconstructions and in craniofacial surgery will be published

Table 3 – Operations performed using BMP

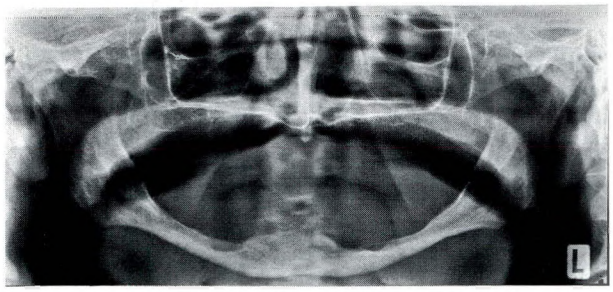
Diagnosis/Procedure (145 patients)	Number of operations
Pseudarthrosis (mand. + max.)	7
Le Fort I (+lyobone)	14
Le Fort I (+iliac bone)	6
Le Fort I (edentulous) (+iliac bone + implants)	4
Vertical maxill. sandwich + implants	4
Sinus inlay + iliac bone + implants	5
Sag. splitting	12
Le Fort III + I	2
Alveol. reconstr.	14
Genioplasty	2
Mandibular sandwich + implants + lyobone	3
Mandibular reconstruction + autol. bone	16
Mandibular reconstr. (plates + lyocartilage)	4
Cadaver mandible	1
Maxillary reconstruction + autol. bone	4
Calvarial reconstr. (lyocartilage)	6
Contour corrections (lyocartilage)	5
Mandibular/maxillary fractures	12
Implants	139
Cysts	11
Total	271

in subsequent issues of the Journal of Cranio-Maxillo-Facial Surgery.

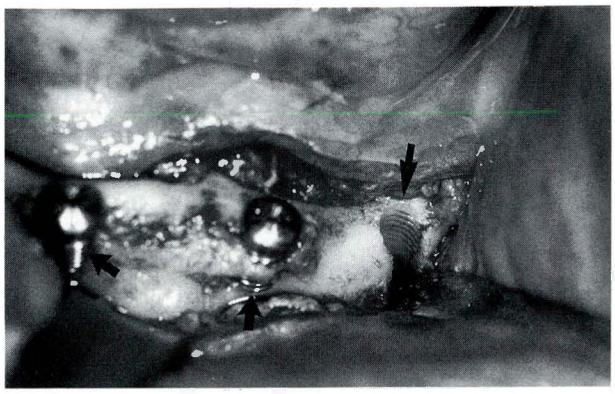
Results obtained in compromised conditions of implantology

Patient number 1 (Figs 2A–D), male, 77 years of age, edentulous for decades, with severe mandibular atrophy, was to be provided with 4 mesially placed implants. Three screws, 15, 15 and 13 mm in length, respectively, could be successfully inserted in positions 43 and 41, and 31. However, an attempt to place an implant in position 33 had to be abandoned because the buccal mandibular wall broke completely away after drilling. All implants were partially denuded because the bone was very brittle everywhere. BMP paste was put into all osseous defects. A polyglycolide foil (Biofix R) was adapted to the site and placed over the alveolar ridge, holding the BMP paste in place, but sparing the heads of the screws. Four and a half months after insertion the implants were uncovered. They were all osseointegrated. CT images 4 months later showed that all implants, at all levels, were surrounded by bone.

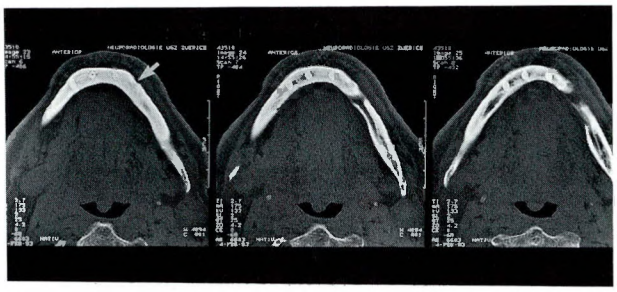
Patient number 2 (Figs 3A–D). In this 47-year-old man a partial resection of the anterior half and upper two thirds of the right mandibular body had been performed 5 years earlier because of an adenoid cystic carcinoma, with a neurolysis of the alveolar nerve. In January 1992 reconstructive operation of the mandible was carried out whereby the whole length of the residual mandible was split horizontally and an iliac bone graft was interposed (horizontal sandwich type reconstruction, *Sailer*, 1991). The 3 bone layers were fixed together with 5 implants (*Brånemark*) of 20 mm (4) and 18 mm (1) in length, respectively, placed in positions 42, 43, 45, 46 and 47. Two months later, infection along the whole length of the



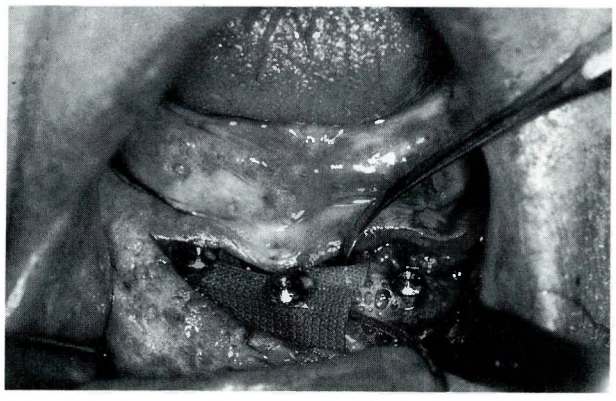
A



B



C



D

Fig. 2 – Case 1. (A) Orthopantomogram $3\frac{1}{2}$ months after implant insertion into the atrophic mandible. Arrows show formation of new bone. (**B**) (**C**) Intraoperative views. (**B**) Abandoned drill site with large anterior wall defect (long arrow). Partially denuded left and middle implants (short arrows). (**C**) Middle and right implants with polyglycolide foil covering BMP placed on denuded implant areas. (**D**) Computerized tomographs in 3 horizontal planes (uppermost on the right) 8 months after operation showing bone formation around all implants on all levels. The abandoned drill hole, filled with BMP paste intraoperatively, has become filled with bone (arrow).

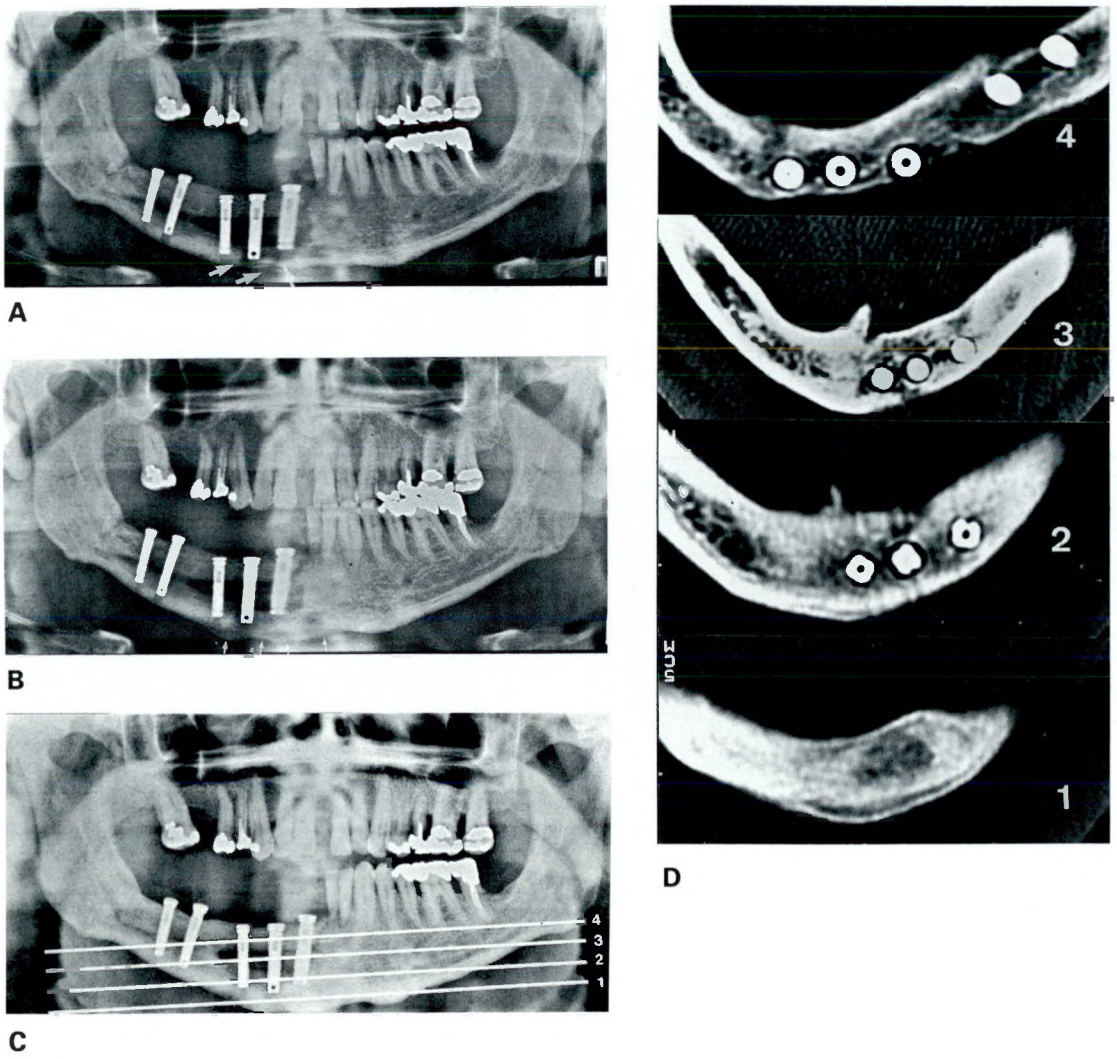


Fig. 3 – Case 2. Horizontal sandwich type reconstruction of the right mandible using iliac bone interposition 5 years after tumour resection. Development of massive infection 2 months after the sandwich procedure. **(A)** OPT showing osteolysis at the tips of the 2 mesial implants (arrows). **(B)** 6 weeks later osteolysis is seen to be reduced and a periosteal reaction has developed around the 3 mesial implants (arrows). (C) $8\frac{1}{2}$ months after revision the cover and the base of the sandwich are joined by new bone and the osteolytic areas described above have disappeared. The width of the mesial half of the mandible has increased considerably. Lines 1-4 show the planes of the horizontal CTs taken $10\frac{1}{2}$ months after revision. **(D)** CT images, planes 1-4: 5 implants, covered by bone at all levels.

autologous osseous transplant developed, originating around the most mesial implant, but also involving the neighbouring one, both of which showed osteolysis around the tip. Intra- and extraoral fistulae were found. An operative revision was done, revealing extensive granulation and suppuration along the bone transplant and the implants and buccal denudation of the 2 most mesial implants. The whole buccal area was cleared of granulations and secretions and the cavity filled with chips of lyophilized cartilage mixed with BMP. The lyocartilage was rehydrated in an antibiotic solution and acted as a local antibiotic deposit (*Sailer*, 1983).

The infection subsided under antibiotic treatment and hyperbaric oxygen therapy. The patient's course was followed with monthly OPT's which showed unequivocal bone formation increasing on each X-ray film.

The implants were uncovered $10\frac{1}{2}$ months after revision, all of them were integrated, as proven by CT imaging.

Patient number 3 (Figs 4A–D). This 25-year-old woman suffered a serious accident (dragon flight crash) 2 years earlier with multiple fractures of the facial bones (Le Fort II type fracture, compound fracture of both zygomas, fracture of both orbital floors, fracture of the right maxillary suture, paramedian fracture of the mandible). A left paramedian maxillary ridge defect remained after the fractures had healed. This ridge remnant (from region 22–25) was split in a vertical sandwich type operation and the bone transplant carrying the implants was wedged in between. Three titanium implants (*Brånemark*) were inserted into the graft after BMP application to the drill holes and the transplant surfaces. Ten months after insertion the implants were all firmly fixed as

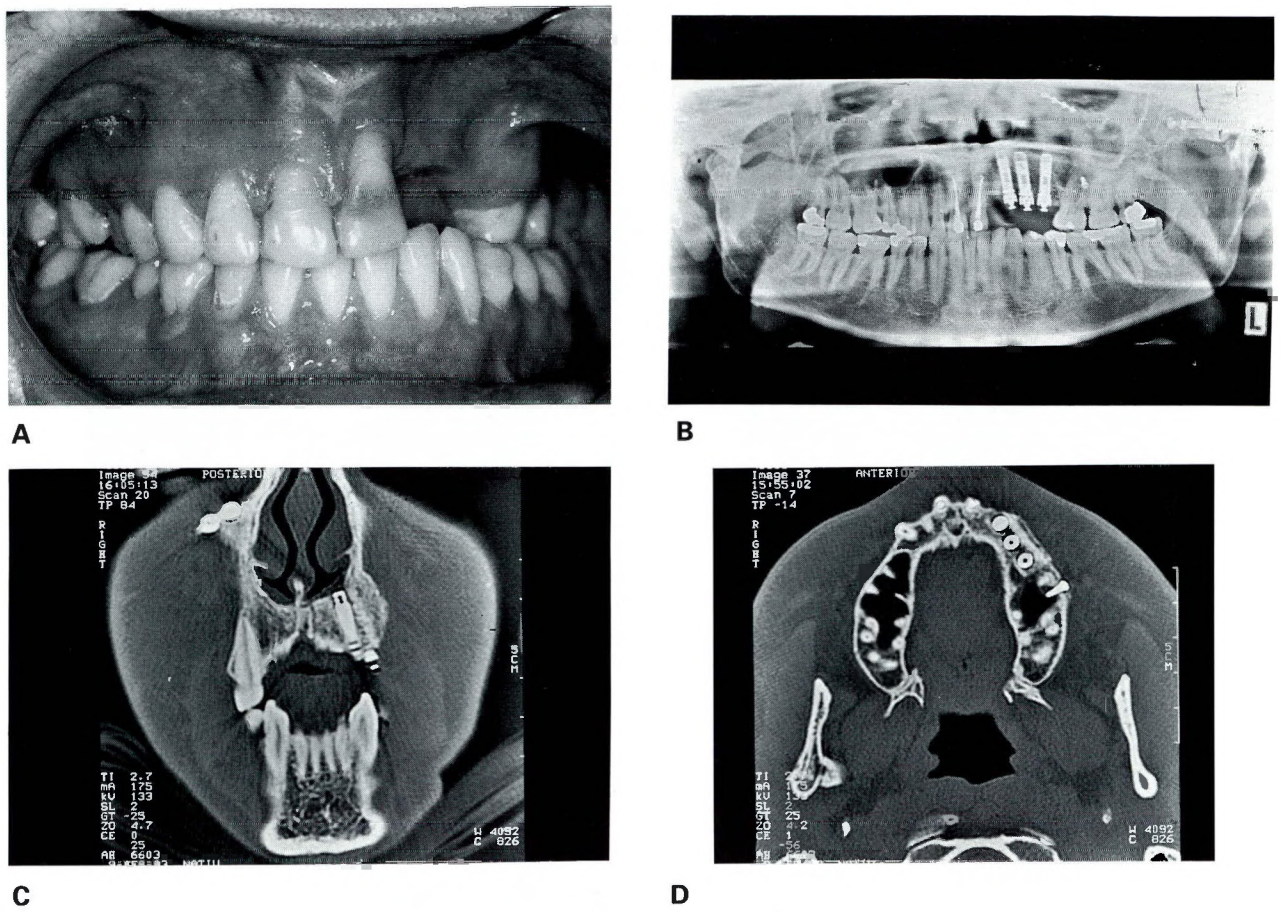


Fig. 4 – Case 3. **(A)** Preoperative view, showing maxillary alveolar defect in region 22–25. **(B)** Orthopantomogram 12 months after ridge reconstruction with a vertical sandwich type operation, using an iliac bone transplant (as shown in Fig. 1A) and after uncovering the implants. BMP was applied to all the cut bone surfaces. **(C)** Vertical computerized tomography (CT) 12 months postoperatively showing anterior **(C)** implants surrounded by bone. **(D)** Horizontal CT of high plane, showing the 3 implants sitting within the intact transplanted bone wedge.

judged from clinical examination and CT imaging (images taken in vertical and horizontal planes).

Patient number 4 (Figs 5A–E). This 50-year-old woman suffered from progressive parodontolysis of the upper and lower jaws. In the maxilla there was only an anterior residual dentition left. The floor of the maxillary sinus almost reached the alveolar crest. The bone deficiency did not allow direct implantation. Transplants from iliac bone were adapted to serve as inlays onto the floor of the sinus (sinus inlay method, *Sailer*, 1989, 1991). The transplanted bone was fixed with titanium implants, 4 on the right and 3 on the left. Seven months later the implants were uncovered: all of them were osseointegrated as shown by horizontal serial CT imaging.

DISCUSSION

A critical interpretation of early attempts—at a time when BMPs had not yet been identified—to induce osteogenesis by demineralized or partially deproteinized bone pieces leads to the conclusion that in most cases from the literature on reconstructive surgical procedures using bone derived implants, both osteoconduction and osteoinduction were probably operative, except perhaps in cases where the bone donor was a young child. This is not surprising since the content of BMP in the matrix of adult bones—the usual starting material of demineralized bone preparations—is extremely small and decreases with age (*Urist* et al., 1985).

While early results in cases with cranio-maxillofacial surgery using hospital-made demineralized bone were reported to be good (*Glowacki* et al., 1981; *Mulliken* et al., 1981), a more detailed and critical intermediate term (up to $1\frac{1}{2}$ years) evaluation (*Ousterhout*, 1985) in a similar group of patients who had received commercial demineralized bone preparations showed a wide range of behaviour of the implants (0–100% resorption). Best results were obtained in Le Fort I reconstructions when demineralized bone powder was applied to the surfaces of small autologous bone transplants placed into the osteotomy gaps—observations of little weight concerning the putative bone inducing ability of demineralized bone powder.

The most recent report on the use of commercial demineralized bone on a selected group of patients (51 out of 53 with constructive/reconstructive procedures of the nose) with short- and long-term follow-up examinations states that resorption on average amounted to 50% after 1 year, to 64 and 82% after 2 and 3

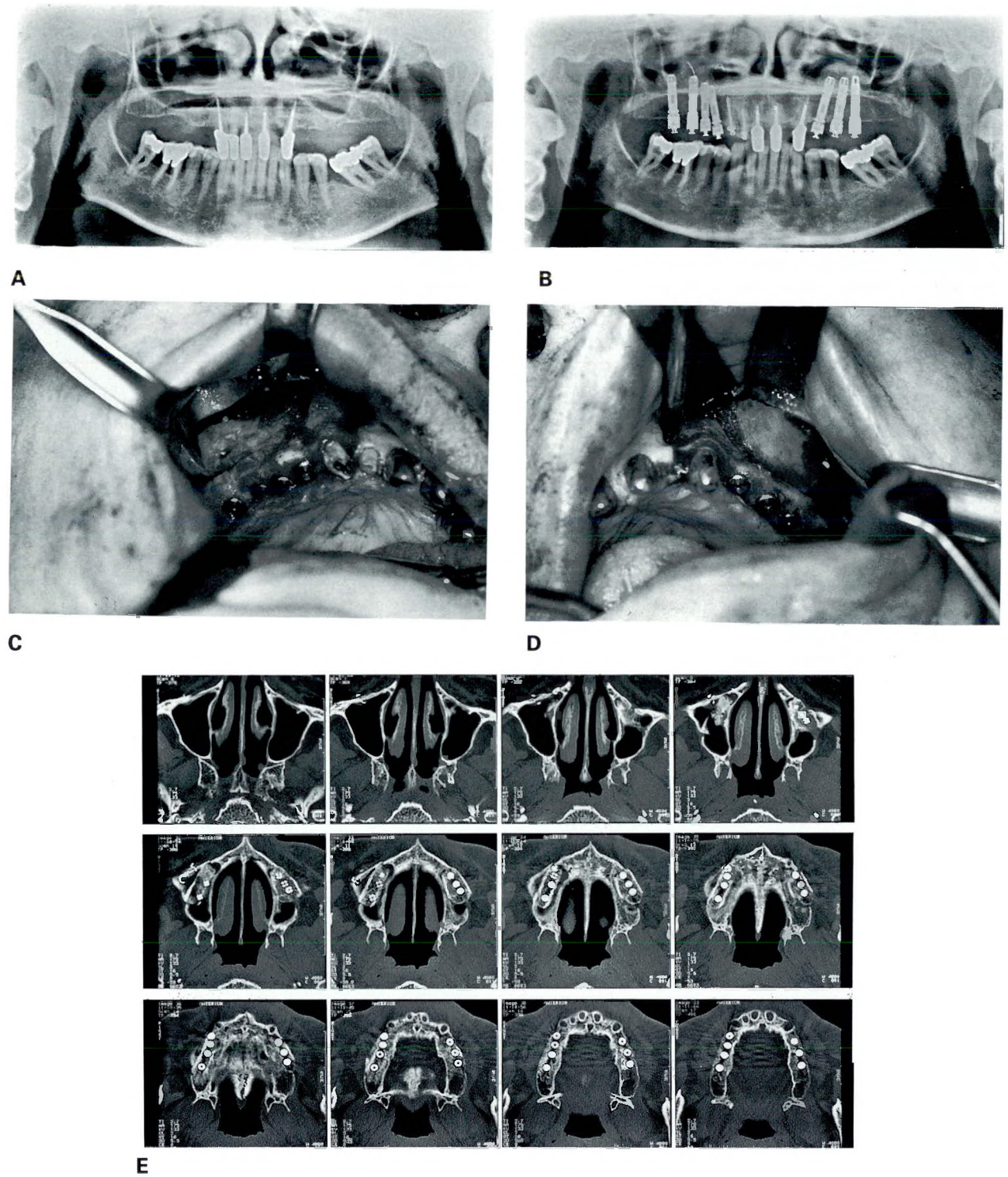


Fig. 5 – Case 4. (**A, B**) Orthopantomograms before and 7 months after reconstruction (sinus inlay method with iliac grafts). Note the increased bone mass within the maxillary sinus in (**B**). (**C, D**) Operation site, right and left, after 7 implants have been inserted through the thin maxillary ridge into the iliac bone transplants. (**E**) Computerized tomography in successive horizontal planes, moving cranio-caudally. All implants at all levels are covered by bone.

years, respectively (*Toriumi* et al., 1990). The reasons for these rather poor results are unclear.

In theory, positive aspects of demineralized bone are: it is a good scaffold for invading cells (osteoblasts or mesenchymal cells) from the neighbourhood to settle and eventually lay down new bone; it does not attract osteoclasts (in contrast to some mineralized bone preparations), thus it may prevent bone resorption as a primary reaction, and it provides mass at least temporarily in situations of bone loss. However, when defect filling is not the major treatment problem, but rather a limited number of BMP responsive cells

has to be dealt with, then the amount of BMP in an implant (endogenous or added) is critical. In this case, purified and concentrated BMP should be applied, together with a minimal or larger amount of inert matrix as a substrate for mesenchymal cells, since the extent and speed of bone formation are dependent on the local concentration of BMP (*Wang* et al., 1988; *Muthukumaran* and *Reddi*, 1988).

As we understand it today, these two factors—extent and speed of bone induction—determine the fate of bone derived implants. Good late results can be expected when the implanted material is rapidly invaded in many places by bone producing cells resulting in an early mosaic of dead matrix and living new bone. In this situation bone formation and not resorption will be the first step in a row of locally dispersed repetitive cycles of formation and resorption characteristic of bone remodelling involving both the old matrix and the new bone. This sequence will lead to a gradual disappearance of the implant material without manifest resorption, the highly desirable process called creeping substitution (*Barth*, 1893; *Axhausen*, 1912, both cited in the review by *Mulliken* et al., 1984).

In our patients, all with *Brånemark* type implants, the two most critical aspects were an incomplete bed of bone surrounding the titanium screws, bone, although totally or partly autochthonous, mostly of poor quality, or bone of questionable vitality in the cases with autologous grafts in sandwich type procedures or maxillary inlays (*Sailer*, 1991).

In cases 1 and 2, one or more implants were partially denuded, that is, the natural osteogenic bone scaffold was partially missing, and in these areas the screws were in direct contact with soft tissues, which, on their own, are not osteoinductive. The application of BMP onto the denuded screws most probably restored the osteoinductive potential of mobile, undifferentiated cells in the neighbourhood and allowed osseointegration of the implants to proceed in the whole circumference (as shown by computerized tomography examinations). This could hardly have happened without BMP.

In case 2, a phenomenal triple response was seen: the restoration of bone at the tips of 2 infected implants, the formation of bone on partially denuded implants and the massive growth of bone between and around the sandwich-like components of the reconstructed body of the mandible wherever lyocartilage chips, mixed with BMP, had been applied—all of this within only $8\frac{1}{2}$ months.

The remaining 2 cases demonstrate that in the presence of BMP, bone induction around implants within free autologous bone grafts inserted into or onto an atrophic maxillary base can proceed normally although the number of viable, BMP-responsive cells may be small. The bone transplants themselves, covered with BMP paste, became firmly fixed to the atrophic bone of the maxilla in both cases, although in case 3 the scarred mucoperiosteal cover probably could not respond normally to BMP.

As an answer to the original question at the end of the introduction, we may make these tentative statements, based on our clinical observations: BMP seems to be able to make up for an insufficient number of BMP responsive cells by stimulating their division; it apparently can induce the transformation of adult periosteal or other soft tissue cells into osteoblasts, thereby promoting the formation of a bony cover when implants are partially denuded, and it can induce the formation of new bone around an infected implant if the infection is kept under antibiotic control. The latter two observations are strong circumstantial evidence that our purified BMP preparations were the decisive factor for implant osseointegration. This was proven definitively by CT imaging in all cases described.

CONCLUSION

1. The demonstrated great potential of purified and concentrated BMP is in sharp contrast to the variable or questionable effects of undefined demineralized bone materials used and reported on earlier in the literature.
2. Because the structures of the major homologous BMPs (BMP-2 and BMP-3) from human and bovine sources, respectively, are identical, purified bovine BMP is tolerated without any adverse reactions. This eliminates the problems of bone procurement for BMP purification experienced with human bone of sufficient quantity and quality.
3. From preliminary experiments in our laboratory we have justified hopes that freeze-dried allogenic human bone and cartilage treated with purified, concentrated bovine BMP can substitute for fresh autologous bone grafts in the future.

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Cellular and molecular biological events at the implant interface

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SUMMARY. **Since implants attain and maintain contact with the body environment through their surfaces, the interfacial interaction between implant bed and implant surface could be a determinant of the implant's performance. This study used osteoblasts to test the ability of different ceramic and metallic surfaces to bind fibronectin and to support cell attachment, adhesion, migration and synthesis of extracellular matrix.**

The results showed active molecular and cellular interaction between the cells and the surfaces. Extracellular matrix synthesis was demonstrated on surfaces that did not subject the cells to toxicity embarrassment. It is concluded that the surfaces of implants should not be designed to be mere bystanders in the interfacial scenario. Instead they should be conceived with a view to enabling them to interact positively with the molecules responsible for would healing, interfacial tissue morphogenesis and repair.

INTRODUCTION

An implanted material attains and maintains contact with interfacial tissue through its surface. Since reactions in vivo are governed by molecular mediators, it makes biological sense to suppose that such mediators could be playing a role in the interfacial reactions leading to implant integration, encapsulation, or rejection. When a substrate or an implant is inserted into the body environment, it is exposed to cells and a host of ionic and molecular species that ultimately determine the course of interfacial events (Fig. 1). One of the first things to happen is the adsorption of proteins onto the substrate. According to *Horbett* and *Schway* (1988) and *Reddi* (1985), the adsorption takes place within the first 10 to 60 s of contact, long before the cells get access to the surface. This means that any cells which interact with the alloplast surface can only do so indirectly, through the adsorbed protein layer.

Regarding the protein species interacting with alloplastic materials in an in vivo milieu, it is known that the nature and amount of protein adsorbed is specific to the alloplast (*Breemhaar* et al., 1984; *Uniyal* and *Brash*, 1982). The protein contingents adsorbed seem to depend on the physico- and electrochemical properties of the given surface. It is conceivable that the adsorbed protein contingent could determine what kind of cells interact with the alloplast surface. One class of compounds that will be crucial in the post-implantation events is comprised of the adhesion proteins. At the molecular level, adhesion proteins are responsible for cell anchorage, attachment, spreading, adhesion and migration, a range of events termed phenomenology.

Cell phenomenology is itself partly governed by the interaction between fibronectin on the cell surface and actin of the cellular skeleton. It is known that these interactions are a necessary condition for cell function. Cell contact with the substrate is maintained by the formation of subcellular spatially and morphologically defined adhesion sites called focal adhesions (Fig. 2). Focal adhesions are within 15 to 30 nm proximity of the substrate (*Izzard* and *Lochner*, 1976) and are about 2 to 10 μm long and 250 to 500 nm wide (*Burridge* et al., 1988). Actin is diffusely distributed in rounded up cells. In spreading cells it is organized in bundles (called stress fibres) across the cell or parts thereof, through the action of adhesion proteins. The actin fibres are fixed at each end to the cell membrane in focal adhesion sites.

Against this background, different metallic and ceramic surfaces of implantologic and dental interest were tested in regard to their ability to interact with fibronectin.

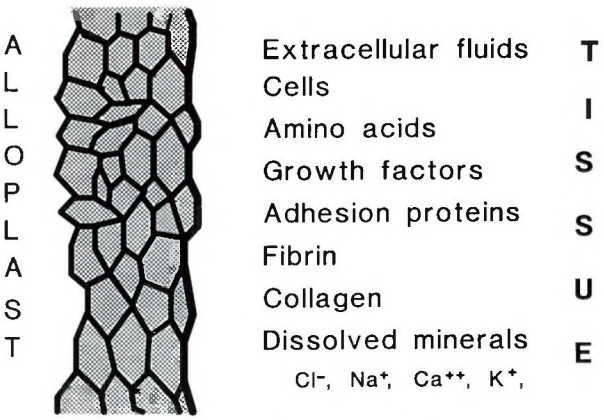


Fig. 1 – Schematic representation of some of the tissue components with which the surface of an implanted alloplast is confronted.