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ORIGINAL ARTICLE

Titanium Dioxide as an Osteoconductive Material: An Animal Study

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Abstract The purpose of the present study was to evaluate the biocompatibility and osteoconductive potential of pure and pigment forms of titanium dioxide. Pure and pigment forms of titanium dioxide were incorporated into prepared bur holes in the femur bone of rabbits. Implantation was done on six Albino rabbits which were sacrificed at the end of 3rd, 4th and 5th months after implantation. Radiographic, histologic and scanning electron microscopic evaluations of the implanted sites were performed. Heamatologic and soft tissue response to these materials were also evaluated. The results showed that both pure and pigment forms of titanium dioxide are biocompatible and have good osteoconductive properties. It was concluded that titanium dioxide can be effectively used in the augmentation of osseous defects and inadequate ridge forms.

Keywords Titanium dioxide · Alveolar ridge augmentation · Osteoconduction

Introduction

Satisfactory functioning of a denture to a great extent depends on adequate alveolar ridge height, form and shape. Unfortunately, a large number of patients report to dental clinics with inadequate ridge forms. Sulcus deepening was one of the earliest methods to increase the functional ridge height. However, a minimum of 15 mm alveolar bone height is required for the procedure to be effective [1]. This to a large extent negates the use of this procedure in cases with atrophic ridges which constitute the majority of edentulous cases. To contend with the need, surgical techniques involving ridge recontouring with grafts and implants were popularized. Since the use of autogenous grafts introduces the risk of potential morbidity at the donor site, a variety of implantable materials have been developed as alternatives. Most of these materials are osteoconductive in nature.

The term osteoconduction applies to a three-dimensional process that is observed when porous structures are implanted into or adjoining bone. Capillaries, perivascular tissues, and osteoprogenitor cells migrate into porous spaces and remodel the porous structure with newly formed bone. The observed process is characterized by an initial ingrowth of fibrovascular tissue that invades the porous structure followed by the development of new bone applied directly within it [2].

A number of materials ranging from Plaster of Paris to ceramics have been tried in the past [3, 4]. Of these materials, tricalcium phosphate and hydroxyapatite have been found to be conducive for use in dentistry [5, 6]. The expense of these materials is indeed a cause for concern. Thus, the need to develop a less expensive and locally available alternative for osteoconduction arose. Titanium dioxide was selected for the present study as there was plentiful resource in Kerala and it possessed a favourable pharmacologic and biocompatible profile. The aim of the study was to explore the possibility of using titanium dioxide as an alveolar ridge augmentation material. The objective was to ascertain the osteoconductive potential of pure and pigment forms of titanium dioxide.

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Materials and Methods

Pure and pigment forms of titanium dioxide were developed by M/s Travancore Titanium Products Ltd., Trivandrum in powdered form. The difference between the two varieties of titanium is that the pigment contains certain alkali metal salts to a quantity of 2 % that cause deagglomeration of the titanium dioxide particles. The particle size of both forms was estimated to be 3 μ. Six healthy Albino rabbits weighing between 1.25 and 1.8 kg were chosen for implantation (Fig. 1). They were maintained in the animal house under identical conditions and fed with Hind lever rabbit pellets, grass and a sufficient quantity of water.

The rabbits were sedated by giving intravenous injections of pentobarbitone sodium at a dose of 35 mg/kg body weight through the ear veins. Sterilization of instruments and titanium dioxide was done by autoclaving (120 °C for 15 min under 15 lbs pressure). The femur was selected as the implantation site. Depilation was carried out using depilatory cream and the area was scrubbed with betadine for asepsis. A longitudinal incision was made with a No. 15 Bard Parker blade and the bone exposed through blunt dissection (Fig. 2). In each of the specimens, four bur holes, 2 mm in diameter, were made using an implant placement drill attached to a physiodispenser (Fig. 3). Sterile saline was used as an external irrigant to dissipate heat and to remove the debris. The pure and pigment forms of titanium dioxide were then implanted into the drilled sites (Fig. 4). The pattern of implantation is described in Table 1. A Teflon tube, 0.5 mm in diameter and 4 mm in length filled with TiO_2, was placed subcutaneously to assess the soft tissue reaction. The tissues were then sutured with chromic catgut. Radiographs of the implanted sites were taken with a dental radiograph machine (Philips oralix 65). The rabbit's leg was positioned over an occlusal film at a distance of 16 cm from the tube and an exposure time of 0.4 s was used.

The rabbits were given procaine penicillin injections at a dose of one lakh units per kilogram body weight IM for 5 days. They were fed properly and weighed periodically. Two rabbits were sacrificed at the end of the first 2 months. The soft tissue containing the Teflon tube which had been filled with TiO_2 was carefully excised for testing soft tissue compatibility of TiO_2. Femur bones containing the implanted TiO_2 and the control site were also removed for further evaluation. The remaining animals were sacrificed at the end of 4 and 5 months.

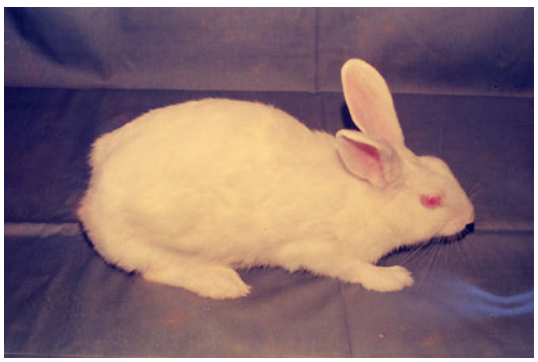

Fig. 1 Albino rabbit

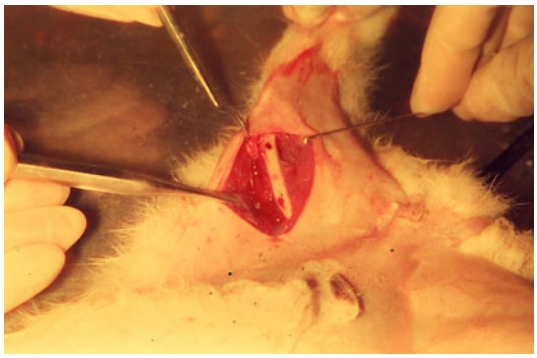

Fig. 3 Bur holes drilled

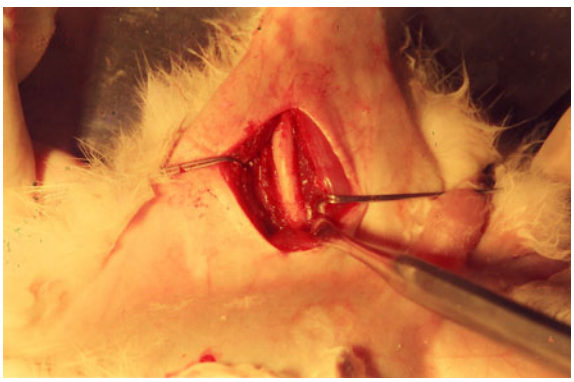

Fig. 2 Femur exposed

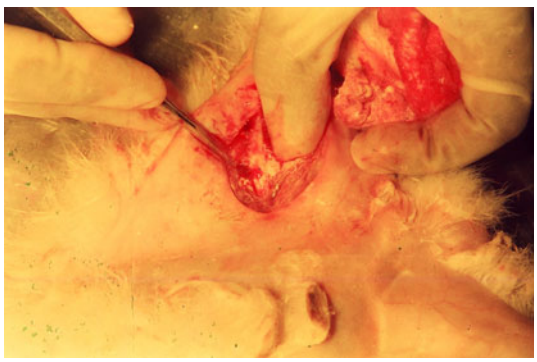

Fig. 4 Bur holes filled with test material

Table 1 Pattern of implantation

Animal used	No. of implanted bone sites			No. of implanted soft tissue sites	
	Pure	Pigment	Control	Pure	Pigment
A	2	1	1	1	–
B	1	2	1	–	1
C	2	1	1	1	–
D	1	2	1	–	1
E	2	1	1	1	–
F	1	2	1	–	1

Radiographic Evaluation of the Implanted Site

The bone with the implanted material was radiographed using specifications similar to that used for the immediate post implantation radiograph.

Scanning Electron Microscopic Evaluation of Bone and the Implanted Specimen

The specimens were fixed using 10 % formalin for 24 h. Thereafter, 20 % hydrogen peroxide was used to remove the soft tissues. They were then dehydrated in absolute alcohol for 24 h. Scanning was performed using the Jeol scanning electron microscope. The specimens containing the implanted material and the control site were attached to metal studs using conductive silver paste. They were sputter coated with gold for 4 min to make them electro-conductive. These sputter coated specimens were placed in the vacuum chamber of the electron microscope, scanned and observed at various magnifications.

Microscopic Evaluation of the Implanted Site

The specimens were immersed in 5 % nitric acid for 8–10 days to achieve decalcification. The decalcified specimens were washed in running water to remove the acid content. Second fixation was done using 10 % formalin for 24 h. The specimens were washed and dehydrated in ascending grades of alcohol (40, 60, 80, 90, 95 % and absolute alcohol) for 2 h each. They were immersed in xylene prior to embedding in paraffin blocks. These specimens were sectioned at 6 μ thickness, washed in running water and mounted on clean slides with the help of Mayer's egg albumin adhesive. The slides were dried in an incubator at 37 °C for 1 h and finally were stained with eosin and haematoxylin, Van Gieson and Mallory's Trichrome stains. A light microscopic evaluation was then done.

Microscopic Evaluation of Soft Tissues

The same procedure as described above was used prior to mounting the specimens on the slides. The slides were examined under the light microscope.

Evaluation of Haemotoxicity

Blood samples were collected before implantation and just prior to sacrificing the animals, in bottles containing EDTA.

The following estimations were done:

1. Haemoglobin estimation by Sahli's method
2. Total WBC count
3. Differential WBC count
4. Erythrocyte sedimentation rate

Results

I. *Health status of the animals* This was ascertained by noting the normal physiologic activity, vigour and body weight of the animals. All the animals were found to be healthy during the entire course of the study. Table 2 shows a comparison of the weight of the rabbits before implantation and just prior to sacrifice.

II. *Macroscopic examination of the implanted site* The soft tissue healing was found to be normal.

III. *Microscopic examination* New bone formation was seen not only surrounding the implanted materials but also between the material masses. An increased number of capillaries suggestive of bone formation were also noticed (Figs. 5, 6). Numerous active osteoblasts with hyperchromatic nuclei were seen in the new bone. Mallory's trichrome showed newly formed mineralized bone as deep blue areas and the unmineralized new bone as blue areas with evident osteoblastic activity (Figs. 7, 8). Under Van Gieson's staining, mineralized bone appeared orange-yellow,

Table 2 Weight of animals before implantation and just prior to sacrifice

Animal weighed	Weight of the animal before implantation (kg)	Weight of the animal at the time of sacrifice (kg)
A	1.8	2.1
B	1.75	2
C	1.75	1.85
D	1.4	1.5
E	1.25	1.3
F	1.6	1.65

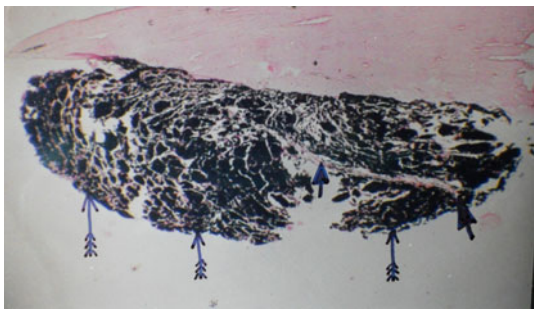

Fig. 5 H&E ×32 showing capillaries and fibrous tissue growing into the masses of TiO_2

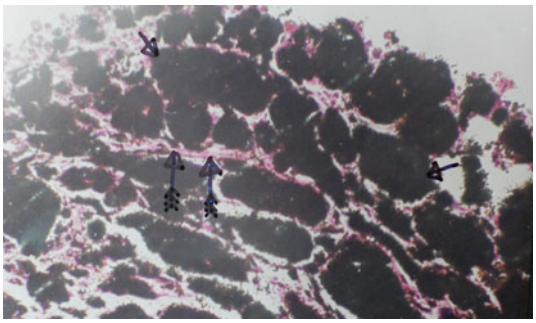

Fig. 6 H&E ×200 showing capillaries and fibrous tissue growing into the masses of TiO_2

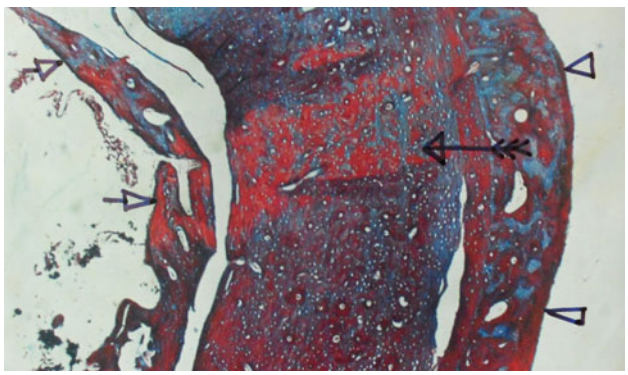

Fig. 7 Mallory's Trichrome ×32 showing mineralized and unmineralized periosteal and endosteal new bone formation

unmineralized bone as pink and implanted material as black (Fig. 9). The absence of both inflammatory cells and fibrous encapsulation of the implanted materials was indicative of soft tissue compatibility.

IV. *Radiographic evaluation* Radiographs of the bone specimens obtained after sacrificing the animals was suggestive of progressive new bone formation. No evidence of osteolysis was noticed. Mild to moderate thickening of periosteum was seen (Figs. 10, 11).

V. *Scanning electron microscopy* Evidence of new bone formation was noticed adjacent to the particles of both pigment and pure forms of titanium dioxide (Figs. 12, 13). In the control sites bone formation was seen to occur with lesser intensity. This difference in bone formation can be attributed to the osteoconductive nature of titanium dioxide.

VI. *Haematologic evaluation* The results of the evaluations carried out with the blood samples are represented in the Tables 3, 4 and 5.

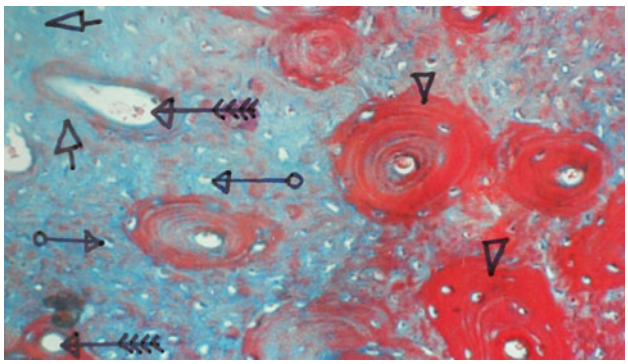

Fig. 8 Mallory's Trichrome ×200 showing mineralized and unmineralized periosteal and endosteal new bone formation

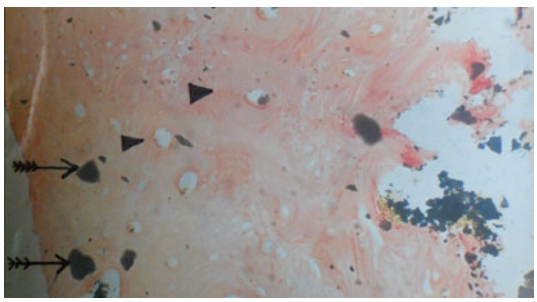

Fig. 9 Van Gieson's stain × 200

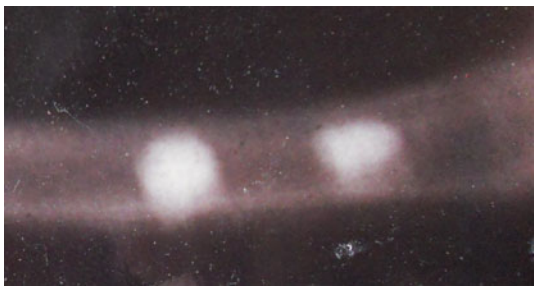

Fig. 10 Radiograph taken soon after implantation with TiO_2

Discussion

Edentulous individuals frequently present with inadequate residual ridges. Sulcus deepening procedures may not be

Fig. 11 Radiograph taken 5 months after implantation showing periosteal thickening

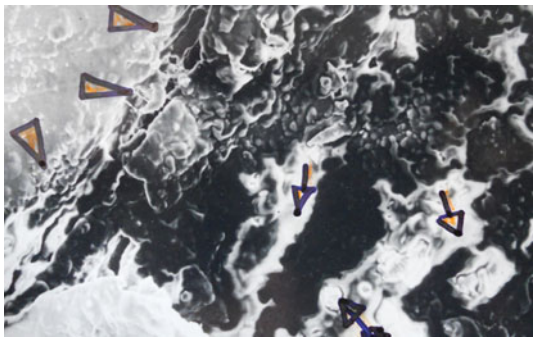

Fig. 12 SEM showing new bone formation near TiO_2 pigment

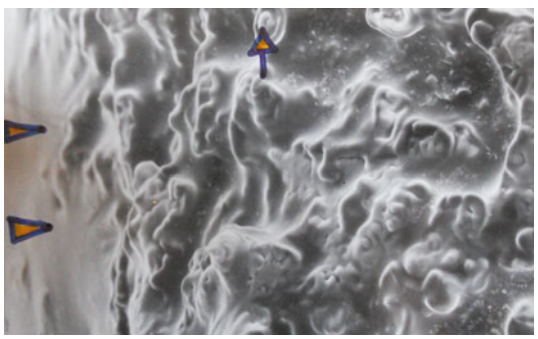

Fig. 13 SEM showing bone formation near pure TiO_2

successful in all clinical situations. Autogenous bone grafts although successful involve extensive surgical procedures and therefore are not very popular now. As an alternative to autogenous bone grafts, synthetic materials have been used in the field of ridge augmentation. One of the earliest synthetic materials used to restore bone defects was plaster of Paris. More recently, Tricalcium Phosphate has been used for alveolar ridge augmentation. Jarcho [7] noticed that the rate of resorption of TCP was variable and unpredictable, thus warranting further search for more suitable osteoconductive materials. A study on the osteoconductive nature of a non resorbable hydroxyapatite called 'Durapatite' was done by John [8]. The alveolar ridge augmented using this material was found to be suitable for supporting dentures [8]. However the material was quite expensive. Hence the need for studies aimed at developing a less expensive osteoconductive material became apparent. So pure and pigment forms of Titanium dioxide manufactured by Travancore Titanium Products Ltd. were studied (Joy, The Travancore Titanium Products Ltd., Thiruvananthapuram, personal communication). The authoritative book "The Extra Pharmacopoeia" by Martindale clearly mentions that Titanium dioxide is non toxic to human tissues and is used to pigment and opacify hard gelatin capsules and tablet coatings [9].

Haematologic evaluation of blood drawn from each rabbit before and after implantation yielded similar values. This finding confirms the non toxic nature of the materials. Soft tissue compatibility of the materials which was studied using Teflon tubes filled with the test materials was found to be excellent. There was no evidence of inflammatory reaction even at the light microscopic level. Microscopic evaluation of the implanted and adjoining sites showed newly formed woven bone penetrating into the implanted materials. Mallory's Trichrome and Van Gieson's stains also demonstrated newly formed mineralized and unmineralized bone. A progressive maturation of new bone was observed in the specimens at the end of 3 and 5 months. The SEM pictures indicated the intimate relation between the implant material and bone. Bone had grown into the scaffold provided by the implanted materials. Thus the SEM pictures supported the histologic finding. Radiographic findings of both forms of TiO_2 were similar in nature.

Radiographs taken after sacrificing the animals showed that:

1. the material was retained in their initial positions throughout the study and no migration had occurred.

Table 3 Animals sacrificed at the end of the 3rd month

Animal sacrificed	Blood count before implantation					Blood count after implantation				
	Total count/cu.mm	Differential count (%)			Hb (g%)	Total count/cu.mm	Differential count (%)			Hb (g%)
		N	E	L			N	E	L	
A	5,200	42	7	51	13	4,900	43	2	55	18
B	4,500	44	9	47	11.5	4,600	51	6	43	11

Table 4 Animals sacrificed at the end of the 4th month

Animal sacrificed	Blood count before implantation					Blood count after implantation				
	Total count/cu.mm	Differential count (%)			Hb (g%)	Total count/cu.mm	Differential count (%)			Hb (g%)
		N	E	L			N	E	L	
C	4,600	56	4	40	12	4,300	54	6	40	12
D	4,300	55	7	38	12.5	5,000	50	10	40	13

Table 5 Animals sacrificed at the end of the 5th month

Animal sacrificed	Blood count before implantation					Blood count after implantation				
	Total count/cu.mm	Differential count (%)			Hb (g%)	Total count/cu.mm	Differential count (%)			Hb (g%)
		N	E	L			N	E	L	
E	3,800	43	7	50	11	3,900	42	2	56	11.5
F	4,400	46	10	44	12	5,000	40	9	51	11

2. an overall increase in the radio opacity in the implanted areas, suggestive of new bone formation and
3. mild to moderate thickening of periosteum, also suggestive of new bone formation.

The results of the present animal study concur with the observations made by Damen et al. in their in vitro study.

Conclusion

Pure and pigment forms of Titanium dioxide are biocompatible and osteoconductive in animal models. They are easily available and inexpensive. Hence, further studies aimed at exploring the possibility of using Titanium di oxide in humans for the purpose of ridge augmentation are warranted.

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