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TISSUE ATTACHMENT ON HYDROXYAPATITE COATED POLYMER-CERAMIC COMPOSITE IMPLANT

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ABSTRACT

Bioactive hydroxyapatite is regularly used as a thin bioactive coating layer over metallic implants to enhance the fixation between implants and bone. Experiment reveals that hydroxyapatite is not only improves the bone formation but also influence the soft tissue attachment over the implants. Hydroxyapatite coated small strips were cut out, sterilized and implanted into the femoral muscle of Sprague Dawli rat for soft tissue attachment. For hard tissue attachment the coated strip was placed in a defect area of bone of a white New Zealand rabbits. After a specific period, the implants were retrieved along with the tissue and examined under scanning electron microscope to observe the new soft and hard tissue growth around the implant via bioactive coating. From the SEM study it was observed that the attachment and regeneration of soft and hard tissue on the coated surface of composite strip was perfectly normal.

KEY WORDS: Bioactive coating, Hydroxyapatite, Biocompatibilty, Implant, Tissue attachment

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INTRODUCTION

The stability and functionality of the coated implants were dependent on tissue response after interaction between materials and the tissues of the body when injured. One of the most important tests to evaluate the biocompatibility of a material is the study of tissue response to the material following implantation in soft tissue, e.g. muscle or hard tissue like bone[1]. The development and improvement of medical devices and artificial organs, has been critically dependant on the realization of the importance of the interactions between materials and the tissues of the body. An assessment of these interactions cannot be made without an understanding of how tissues respond when injured, because the procedure of implantation of prosthesis in the body involves an injury[2]. One of the characteristics of living tissue is its ability to respond to injury[3]. One of the most important tests to evaluate the biocompatibility of a material for use in medical devices or artificial organs is the study of tissue response to the material following implantation in soft tissue or hard tissue[4]. To determine the efficacy of a potential biomaterial or combination of material into a new medical device requires animal experimentation prior administration to human patient[5]. Bioactive materials such as hydroxyapatite and bio-glass are regularly used as thin bioactive coating layer over metallic implants to enhance the fixation between implants and bone[6]. Experiment reveals that micro porous coatings not only exhibit bone cell deposition but also influence the soft tissue attachment over the implants such as orbital implant[7]. The composite material used in this present work is alumina reinforced ultra-high molecular weight polyethylene and the bioactive materials used to coat the composite is hydroxyapatite which is widely used as substitutes for bone augmentation and restoration in orthopaedic, dental and maxillofacial surgery[8, 9] and to assist in tissue engineering[10]. Such materials favour osteointegration and are biocompatible[11, 12].

BIOCOMPATIBILITY STUDY

Biocompatibility of an implant material means when it is implanted into the living tissue on short term or long term basis, it should not produce any toxic, carcinogenic, anaphylactic effect or any other unwanted reaction,. So biocompatibility study is one of the most important and primary criteria for evaluation of any material which is going to be implanted[13]. In the early years, simple test methods, originally intended for closures in intravenous solution containers, were used along with cell culture to screen out potentially harmful materials[14]. Presently this test is carried out in small animal models over a specific period of time such as one to three months. The short-term testing or screening of coated composite materials was conducted in order to assess the effects of the material as well as the attachment property of bioactive material with the animal tissue in which it is implanted. This is an in vivo implantation technique for characterizing the biocompatibility of materials and tissue growing ability of HAP or bio-glass. One of the most important tests to evaluate the biocompatibility is the study of tissue response to the material following implantation in soft tissue. The test is carried out in small animal models over a specific period of time such as one to three months.

a) Surgical Procedure for Soft tissue attachment

The experiment on laboratory animals was performed as per the guideline of the Animal Ethics Committee of the Indian Institute of Chemical Biology, Kolkata, India. Sexually mature Sprague dawley rats having weight of 250-300 gms were indented from the animal house of this institute. They were maintained on a 12 hours light/dark cycle under standard husbandary conditions (temperature- 22 ± 2^{o}C, relative humidity 55±10%) and provided with pellet food and water and in case of rabbits, leafy vegetables, grains were also supplemented. Healthy Sprague dawli rats (weight about 250-300 gm) were used as the host. The animals were maintained and acclimatised in standard polypropylene animal

cages, one per cage in the animal room at Indian Institute of Chemical Biology (IICB), kolkata, for about 2 weeks prior to test. Each rat was immunized with Injection Tetanus Toxoid 0.1ml IM, and prophylactic antibiotic injection Ampicillin and Cloxacillin 250 mg. The corresponding area was shaved with the help of hair removing cream (Anne French) and cleaned with antiseptic lotion (Providon Iodine 10%). General anaesthesia was given to the rat using ketamine (ketamine hydrochloride, I.P.) and local anaesthetic agent, lignocaine HCl 2%. A long 1.5 – 2 cm linear incision was done with the help of a surgical blade. One coated strip was inserted into the femoral biceps muscle on right hind leg in each rat. After insertion of the coated strip, the muscles were sutured by absorbable suture (Chromic Cat-gut, No.-06, Ethicon). The skin was made apposed and held with a mitchel clip. The operated area was dressed first using betadine lotion and then antibiotic skin ointment (Soframycin, Skin). The rat was placed in its cage in the A/C room and kept under strict observation for its behavioral pattern, specially the leg movement. The mitchel clips were removed carefully after 10 days. The surgical procedure for implantation was shown in the following figures, e.g., Fig-1, Fig-2 and Fig-3.

Fig-1: Coated composite strip placed in femoral biceps muscle

Fig-6.2: Suturing of the muscle after implantation

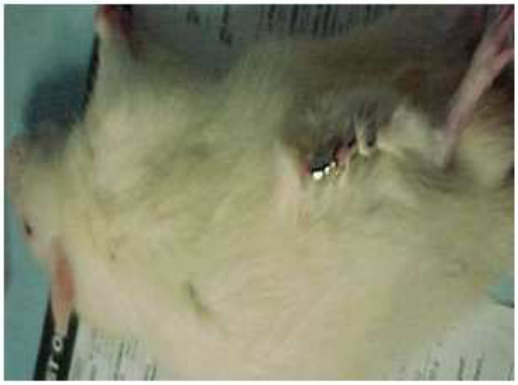

Fig-3: Incised skin was made close together by mitchel clip

b) Surgical Procedure for Hard tissue attachment

The osteoconductive property of hydroxyapatite promotes the bone cell to deposit over it and influence new bone formation[15, 16]. The bone cell deposition over bioactive coating facilitates the joining between implants and bone and enhances the fixation. Therefore coating of such material increases the long-term stability and integrity of the implants. The hard tissue growing ability of hydroxyapatite and bio-glass was assessed by the implantation of coated strip in bone tissue. Healthy white New Zealand rabbits (weight about 3-4 Kg) were used as the host. The animals were maintained and acclamatised in standard stainless steel animal cages, one per cage in the animal

room at Indian Institute of Chemical Biology (IICB), kolkata, for about 2 weeks prior to test. The femur bone of the animal was chosen for the implantation of the test strips. The rabbit was immunized with cephalexine antibiotics. The corresponding area was shaved with the help of hair removing cream (Anne French) and cleaned with antiseptic lotion (Providon Iodine 10%). The surgical procedure was carried out according to the standards of ASTM (American Society for Testing Materials, F 981-99 Standard)[17]. General anesthesia was used with intramuscular application of ketamine and xylocaine (ketamine hydrochloride, I.P. and xylocaine hydrochloride) and local anaesthetic agent, lignocaine HCl 2%. After trichotomy and asepsis of the surgical field with the antiseptic Savlon followed by iodine solution, the lateral and superior aspects of the femur were exposed with a 5 – 6 cm long linear incision. The incision was made with the help of a surgical scalpel blade. Tissue separation was performed with periosteum elevators and molt elevator, so as to obtain a mucoperiosteal flap to expose the bone tissue and allow free access to create osseous defects. [18] In right femur, a rectangular bone defect measuring 10mm X 2mm was prepared with aseptic electrical drilling machine under copious irrigation with 0.9% sterile saline solution throughout the bone manipulation. On the defect area the hydroxyapatite coated composite strip was properly placed to fill the defects in the bone. The skin flaps were closed with silk intermittent sutures and deeper muscles were closed with surgical cat gut suture (Chromic Cat-gut, Ethicon). The operated area was first dressed using betadine lotion and subsequently antibiotic skin ointment (Soframycin, Skin). The rabbit was placed in the cage and kept under strict observation for its behavioral pattern, specially the leg movement.

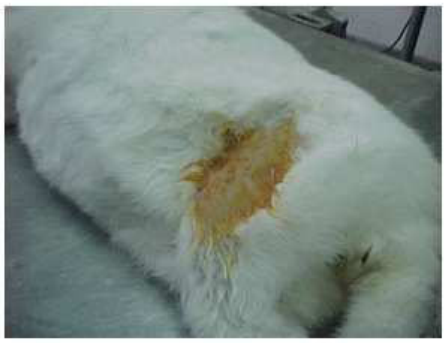

Fig-4: Test sites of the animal were shaved prior to experiment.

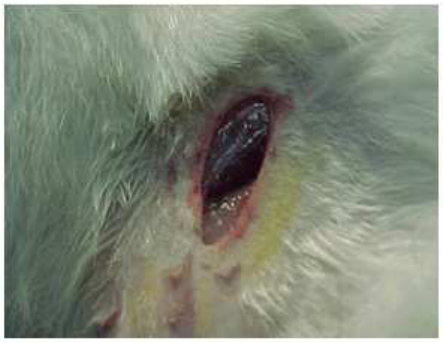

Fig-5: Test site was incised through scalpel blade.

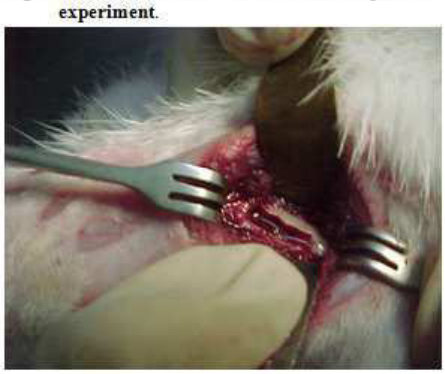

Fig-6: Coated composite strip was placed inside the bone

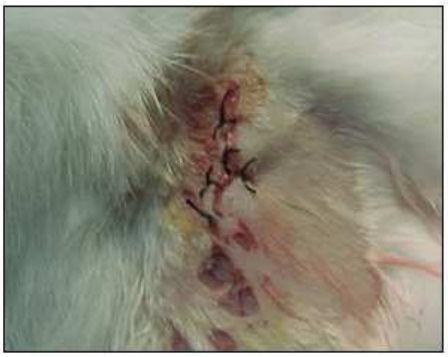

Fig-7: The skin incision was closed by black silk sutures.

c) Implant retrieval and fixation

i) For soft tissue

The animals were sacrificed using an overdose of anesthetic agent (Solvent ether) after 30 days of implantation for accurate characterization of both the test and control group of materials and associated tissue. The femoral muscle along with implant strip was removed and placed in formalin solution for 12 – 24 hours. The tissue containing the implant

was immersed in at least 10 times its volume of fixative (10% buffered formalin). The thickness of the tissue is about 2 to 4 mm for proper permeation of the fixative.

ii) For hard tissue

After one month and three months, the animals were sacrificed with an overdose of anesthetic agent. After removal of soft tissues, bone fragments including the implanted material were obtained and fixed in a 10% formalin solution for at least 48 hours. Fixed samples were cut into a thin sections (10 - 15 µm) using a diamond saw and the sections were examined using JEOL Scanning Electron Microscope.

d) SEM study

The biocompatibility and tissue attachment over the coated implant material was clearly assessed by the examination of the section (tissue along with implant) by scanning electron microscopy[19]. The composite material along with soft and hard tissue is non-conductive in nature. Therefore to view the clear picture all samples were coated with a nano-size (5-20nm) gold film by sputtering and examined under the SEM after mounting in suitable metallic stub.

RESULTS AND DISCUSSIONS

Since tissue attachment over hydroxyapatite coated composite will create a new way for soft and hard tissue replacement, the assessment was made properly through scanning electron microscope**.**

a) Soft tissue attachment

Twenty healthy Sprague dawli rats (weight about 250-300 gm) were used as the host and out of which four was died after implantation. Therefore the success rate was 80%. The SEM images were taken after one and three months of implantation. The tissue regeneration around the composite implant and the tissue attachment on the hydroxyapatite coated surface were shown in Fig-8 and Fig-9.

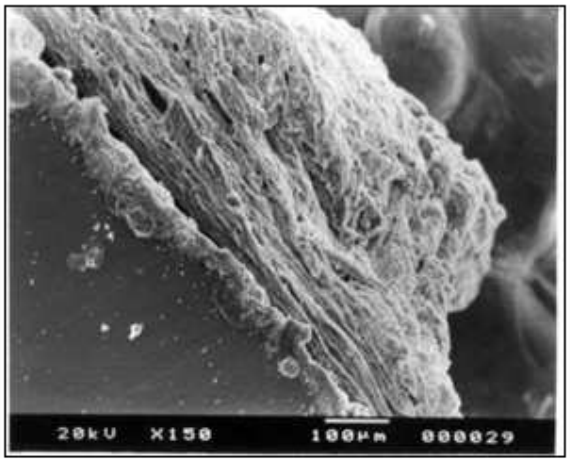

Fig-8: SEM image of soft tissue attached HA coated strip after one month

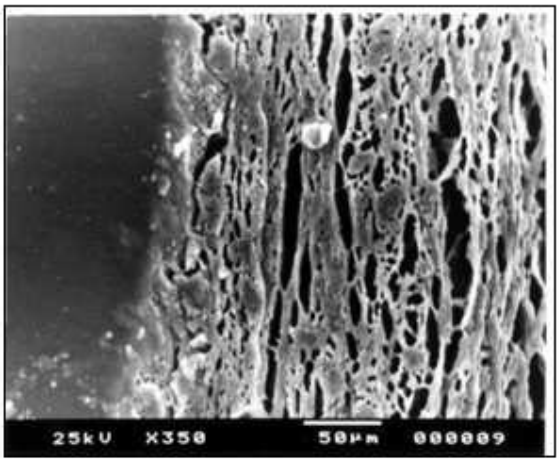

Fig-9: SEM image of soft tissue attached HA coated strip after three month

The Fig-8 shows the soft tissue regeneration around the composite implant and attachment on HA after one month of implantation whereas Fig-9 shows the tissue regeneration after three months. After one month some gap was observed between the coating and the tissue although some tissue was attached but after three months there is no gap and the tissue attachment was much more clear and prominent. From the Fig-8, it was clear that the soft tissue was attached on the coated surface not to the uncoated portion. Some tissue was firmly attached to the coated surface which was shown in the Fig-9 after removing the tissue from that surface.

b) Hard tissue attachment

For hard tissue attachment, three healthy rabbits were used. The success rate is 66.67% as one rabbit was died after implantation. The SEM images of the hard tissue attached composite strips were taken after three months of implantation. The tissue attachment over hydroxyapatite coating was shown in Fig-10 and Fig-11.

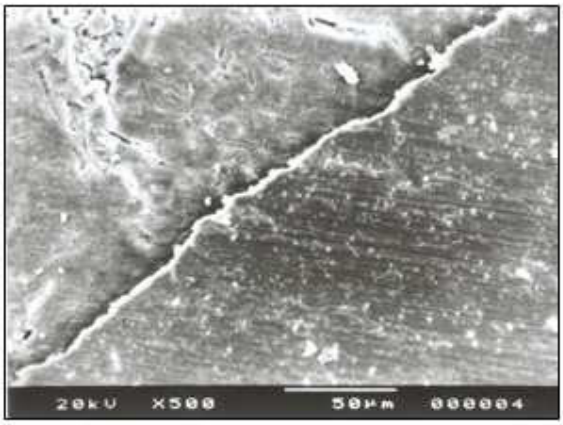

Fig-10: SEM image of hard tissue attached HA coated strip after one month

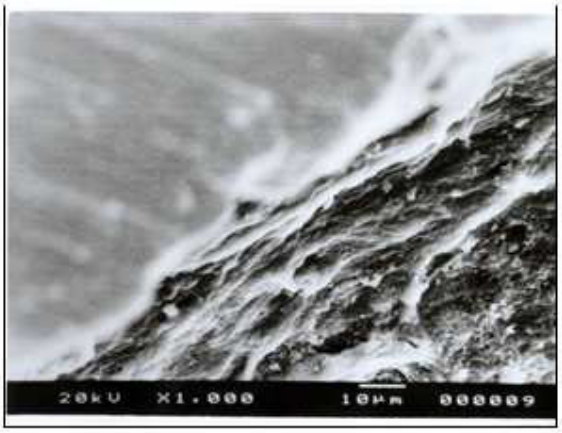

Fig-11: SEM image of bone cell deposition over HA coated strip after three month

The above figures showed the hard tissue regeneration around the composite implant and attachment on hydroxyapatite coating after one month and three months of implantation. The cell deposition over the coating is normal comparable to natural bony materials which are clearly shown in Fig-10. In Fig-11, the hard tissue attachment on bioactive coating was prominent.

CONCLUSION

From soft tissue attachment study, it was found that there were no abnormalities of the muscles and other soft tissues around the composite strip instead of normal tissue regeneration. The SEM images confirm the tissue attachment on porous coating. The tissue regeneration and attachment to the coated surface indicate the biocompatibility of the polymer-ceramic composite implant as well as the soft tissue attachment property of hydroxyapatite. In case of hard tissue attachment, the cell deposition over the coating is much more clear and prominent and it was also observed that the there is no gap between the hard tissue and composite strip. The growth of hard tissue and attachment of soft tissue with polymer-ceramic composite implants through bioactive coating improves the biofriendliness as well as stability of implants and that will create a new way for composite material to be use in soft tissue as well as hard tissue replacement.

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