Mechanical and histological significance of Nigella Sativa Oil extract on bone-implant interface

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ABSTRACT

Background: The new trend of coating implants with biological molecules has resulted in improvement in osseointegration. Natural plants can provide biological molecules which are biocompatible, abundant in source, have low cost and can be modified to host properties. The aim of this study is to evaluate the influence of coating implant with black seed oil extract on the strength of bone implant interface mechanically and histologically.

Material & methods: Forty-eight CpTi screw shaped implants (3.75 mm in diameter, 8 mm in length) were placed in the tibiae bone of twelve New Zealand rabbits. Each animal received 4 implant (2 uncoated and 2 coated with black seed oil extract by dip coating technique). Twelve implants removed after each healing period of 2, 6 and 12 weeks using digital torque meter device constructed for the purpose of this study and four implants removed with bone block for histological study at each healing period.

Results: The mean removal torque values for coated implants were higher significantly than the uncoated implants over different time periods. In addition, the histological picture showed improved quality of bone response among the coated screws.

Conclusions: The coated implants seemed to be well tolerated by the bone since no adverse tissue reaction was evident and they have better torque resistance and appear to produce osteophilic surface favoring early osseointegration.

Key words: nigella sativa, dip coating, dental implant, biochemical modification.

INTRODUCTION

The function of modern dental implants depends on the presence of a direct bone anchorage usually called osseointegration(1). The titanium and its alloys are presently the most important materials for biomedical and dental implant application. This is due to the formation on their surface of a passive film, consisting mainly of amorphous titanium dioxide, which is responsible both for its corrosion resistance and its biocompatibility. Moreover, this film favors a very good osseointegration(2). Clinical success is achieved not only because of implant material but also because of other properties, one of the most important is surface quality, which refers to its mechanical, physicochemical and topographic properties(3).

The next evolution in titanium implants is likely to be through biologically inspired specific surface modification. This trend in modifying surfaces with (bio) chemical moieties has the potential to induce cell–selective responses, increase bacterial resistance, reduce the risk of inflammation and improve the long-term performance of the implant.

The easiest way of immobilizing biological molecules of biological nature on an implant surface is by simply dipping the implant into a solution that contains biological molecules(4).

The seeds of Nigella sativa plant have long been used as a natural medicine for the treatment of many acute as well as chronic conditions(5). Its therapeutic use was initiated after the advent of Islam since Prophet Mohammed (peace and pray upon him) mentioned its therapeutic efficacy and potential of cure, when said that (there is cure for every disease in black seed except death)(6,7).

Its chemical composition contains volatile oil, fixed oil, protein, carbohydrates, minerals, alkaloids, saponins and other ingredients(8). The pharmacological investigation of the seed extracts reveals a broad spectrum of activities including immuneopotentiation, antihistaminic, antidiabetic, anti-hypertensive, anti-inflammatory, antitumor,(9) antiparasitic, antibacterial, antifungal and antioxidant(11). In recent study, the black seed oil extract BSO induced bone healing as manifested by faster bone trabeculae formation and mature bone formation(11).

In this study, the black seed oil extract was investigated as a coating material on commercially pure titanium screw by dip coating technique to evaluate the influence on the bone-implant interface.
MATERIALS AND METHOD

Implant description
Forty eight threaded CpTi implants (Pitt-Easy®, Bio-oss, oral tronics, Germany), had titanium plasma spray (TPS) surface with diameter of 3.75mm and pitch height about 1mm were cut to 8mm length, the head of the implant has a slit to fit both the screw driver during insertion and torque meter during mechanical testing (figure 1). The screws were passivated for 1 hour in 28% nitric acid and then rinsed for 5 minutes in each five separate washes of deionized water. The screws were then pressed in plastic sheet were every 2 screws sealed together and sterilized with gamma irradiation.

Experimental animal description
Twelve healthy adults New Zealand rabbits of both sexes weighting 2.5–3.5 kg were kept in standard separate cages in the “National center for researches and drug control” under supervision of the veterinarian. The twelve rabbits were divided into 3 groups of 4 animals for each healing interval (2, 6, 12 weeks). Each animal received four implants, two implants in each tibiae bone (limb), one uncoated (control) and the other coated with Black seed oil extract (experimental).

Method
All instruments and towels were autoclaved at 121°C and 15 bars for 30 minutes. The animals were fasted 12h before surgery. Each animal weighted before operation to determine the required dose of anesthesia and antibiotic. Anesthesia was induced by intramuscular injection of ketamine hydrochloride 50mg (1ml/kg body weight) and xylazine 2% (0.25ml/kg B. W.) Both tibiae were shaved with shaving spray from inner side and skin was cleaned with a mixture of ethanol and iodine. Local anesthesia with 1ml of Medicaine 2% (1:100000 epinephrine) was infiltrated at the surgical site to control bleeding and to provide additional local anesthesia. The surgical towels were placed around the site of the operation, and surgery was performed under aseptic condition.

Incision was made on the lateral side (about 3cm length) to expose the medial side of the tibia. The skin and fascia were reflected (figure 2). Bone penetration was performed with a guide drill of 1.8mm in diameter was first used to make two holes with 10mm apart by intermittent pressure and continuous cooling with normal saline at a rotary speed of 1500 rpm and reduction 16:1 The enlargement of these holes were made gradually with spiral drills from 2.5 till 3.25mm finally the operation site was washed with saline to remove debris. Two screws were then removed from press sealed plastic sheet, one placed in first hole (proximal one) using screw that fit the screw slit and threaded until 5mm of the screw was completely introduced in to the bone tissue and checked for stability. The other screw holds by twizer from the top and dipped in container with black seed oil extract (Cold-pressed black seed oil extract (Le,Sura, Nigoil, Refia Co. with A.U.F–GMBH–Germany)) for 5sec. then brought out and inserted immediately in the distal bed (figure 3). The two implants rinsed with saline. Suturing of muscles followed by skin suturing then sprayed with local antibiotic (oxytetracyclin spray). Postoperative care was performed by giving an antibiotic (local and systemic) for 3 days after surgery. The animals then were followed for 2 weeks, 6 weeks and 12 weeks.

At each interval three animals of each group (six uncoated and six coated implants) scarified for mechanical test by torque removal test, while the remaining one animal (two uncoated and two coated implants) would scarified for histological study.

Digital torque meter
For the purpose of this study and to have accurate measurements of the removal torque of the screws a specially designed digital torque meter was used (figure 4) and routinely tested for accuracy by using standard weight (50-100-200gram) hung to the head of torque measuring handle (12).

RESULT

Mechanical test
From the bar chart (figure 5) it appears there is increase in torque mean values for the coated groups at the three healing period 2, 6 and 12 week (10.382, 18.852, 62.044 N.cm) in comparison to uncoated groups (7.454, 14.053, 41.502 N.cm) in comparison to uncoated groups (7.454, 14.053, 41.502 N.cm).

The equality of variance and t–test for equality of means of torque value between coated and uncoated groups at the three healing periods show a significant difference at p < 0.05. It was obvious that increase in the torque value was
needed to remove implants from the bone bed as the healing period increase.

**Histological test**

After 2 weeks of implantation the microhistological picture of bone around uncoated CpTi implant showed a framework of reticular connective tissue of the bone marrow with primitive osteoid matrix deposition in marrow is obscured while coated implant showed bony trabeculae which ramify and anastomose and enclosed irregular marrow cavities of various sizes.

Twelve weeks after implantation the uncoated CpTi implant showed primitive osteon formation, haversian canal with osteocyte cells scattered around the canal in different manner while coated implant showed well developing mature bone with osteon formation.

**DISCUSSION**

It was shown that there is an increase in the removal torque value with time which may be due to progressive bone formation in bone-metal contact with time and remodeling around the implant during healing period and consequently improved the mechanical capacity \(^{(13,14)}\). The greater increase in the torque value was noticed in this study between 6 and 12 weeks these result may suggest increased holding power and anchorages of dental implant with time due to increase maturation of bone as time proceeded. These results are similar to that found by Sennery et al 1992 who revealed that the large increase in torque values between 6 and 12 weeks may be related to the maturation of woven bone to lamellar bone which takes 6 weeks in rabbits \(^{(15)}\). This study revealed that the coated implants produced a significant increase in torque value when compared to the uncoated ones within different time period. The greater removal force for coated implants can be generally interpreted as an increase in bone healing around the implant and improvement in the strength of bony integration at the bone–implant interface \(^{(16,17)}\).

The BSO extract coating used in this study appears to have several advantages in enhancing the bone response around endosseous titanium implant. It could be attributed to the active components of the black seed oil and there effects which could be summarize as:-

1. **Anti inflammatory effect**: the presence of an aseptic inflammatory zone at the interface has been found to be more responsible than infection through an activation of host cellular and humoral immune system response, for integration failure and prosthetic loosening \(^{(18)}\). The anti inflammatory effect of BSO could be attributed to the i. Inhibition of production of mediators \(^{(19,20)}\). ii. Antioxidant or free radical scavenging \(^{(21-23)}\). iii. Alteration of trafficking of the inflammatory cells into inflammatory lesion \(^{(24)}\).

2. **Protein and amino acid**: N S seed had 22.6 % - 26.7 % protein and amino acid \(^{(25)}\). Glutamic acid (Gla,), Arginine (R) and Aspartic acid (D) were the main amino acids present \(^{(26)}\) in addition to other like glycine(G), leucine, etc \(^{(5)}\). These mentioned amino acid play important role in the formation and function of the following extra cellular matrix proteins:- Osteocalcin, Bone...
Sialoprotein (Bsp) and Osteopontin. Gla residues are critical for the ability of osteocalcin to bind calcium \(^{(27)}\) while Bone sialoprotein and osteopontin are believed to play role in cell adhesion and binding of mineral because they contain Arg–Gly–Asp and poly acidic sequences \(^{(28)}\).

3. **Mineral:** It is believed that integrin function is critically dependent on the concentration of divalent cations. It may be possible that the increased amount of Ca may facilitate integrin-mediated attachment of bone-forming cells through enhanced ligand binding of receptor \(^{(17)}\).

4. **Vitamins:** NS contain vitamins like Thiamine, Pyridoxine, Niacin, Folate, Ascorbate and Retinol \(^{(29,30)}\), so it possesses nutritional value \(^{(31)}\) and has been reported to possess a favorable effect on growth rate and health of human and animal \(^{(32)}\).

Finally it seems that BSO extract play as an organic nonfouling layer or biofunctional surface that prevent undesirable inflammation and improve bone formation and maturation in addition provide vitamins as nutritional value.

**REFERENCES**


