COMPOSITE COATING USING (HAP-NANO SILVER) BY MACRO ARC OXIDATION PROCEDURES WAS USED TO STUDY THE IN-VIVO PROPERTIES OF TITANIUM SUBSTRATE

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***ABSTRACT**: Bone is a one-of-a-kind tissue that can mend itself after being damaged. Certain fractures and abnormalities, on the other hand, necessitate therapeutic intervention for appropriate alignment and healing. As with any implant, the material utilized to make the implants to treat these issues must be carefully considered. In study a preparation and an investigation of composite coating (HA/ nAg) on commercial pure titanium using micro-arc oxidations at constant voltage(200 V), different time(30sec, 45sec, 60sec) and alter the nano silver proportion(0.05, 1, 1.5, 2) g/L. The greatest outcomes on Ti groups were composite coatings (HA / 60sec, HA / 0.5g/L nAg/ 60sec, and HA/1 g/L nAg/ 60sec.*

***KEYWORDS**: Composite coating; Hap-nano silver; macro arc oxidation; titanium substrate*

1 INTRODUCTION

Any manufactured or modified natural materials that come into touch with biological tissues or fluids are referred to as biomaterials. The goal of biomaterials research is to provide the biomedical community with the best materials possible to aid in the restoration of biological functional appearance (Horowitz & Torgesen, 1975; Nabaa S. Radhi et al. Nabaa S. Radhi et al., 2018; N.S. Radhi & Al-Khafaji, 2018; Nabaa Sattar Radhi, 2018).

Metallic materials (Ali et al., 2022), ceramics (Marshdi et al., 2021), polymers, and composites are the materials utilized to make biomedical Researchers have successfully developed a variety of biomaterials and technology for joint replacement in the human body, including metallic materials (Ti Alloys, co-cr Alloys and stainless steel).devices (orthopedic, dental, bone cements, and so on) (Abed Janabi et al., 2021; Al-Zubaidy et al., 2019; Dawood et al., 2020; Hussein et al., 2015; N.S. Radhi & Al-Khafaji, 2018).

Researchers have successfully developed a variety of biomaterials and technology for joint replacement in the human body, including metallic material (Ti Alloys, co-cr Alloys and stainless steel). These materials were chosen primarily for their bulk mechanical properties, which are almost identical to those of human bone, as well as their inertness to bodily tissues and fluid (Chakraborty et al., 2017).

The most often used implants in biological applications are Ti-6Al-4V and commercial pure (CP)-Ti extremely low interstitial (Al-khafaji et al., 2018; Kaur & Singh, 2019). However, when they're used in implants, they cause a slew of issues: (1) Stress shielding is one of the detrimental implications of the difference in mechanical characteristics between Ti and natural bone (Kuroda & Okido, 2012). (2) Although Ti's inert behavior is a positive trait, it is difficult to bind to bones since it does not react with human tissues (Oldani & Dominguez, 2012).

When implants are not surface modified to improve osteoconductive, it requires a long time for fixing the metal implantation to the bone in a stable manner. The osteoconductive of Ti and its alloys can be improved in a variety of ways (Kuroda & Okido, 2012). Coating the metal surface with a bioactive substance that promotes the development and adherence of hydroxyapatite (Ca10 (PO4)6(OH)2, the normal bone's inorganic composition, is one way to extend implant life (Oldani & Dominguez, 2012). The MAO process created a porous and rough morphology that improved mechanical interactions and cell adhesion of the tissue and implantation. Furthermore, the calcium and phosphorus sources (Li et al., 2005).

While bone-implantation osteointegration was necessary for the effectiveness and long-term life of such kinds of implantation, it also is equal crucial to avoid bacterial infection following implantation placement (Miranda et al., 2010). Modifying the implant surface with antibacterial coatings while keeping high biocompatibility is one strategy to prevent such infections (Ionita et al., 2011). The rapid development of nanotechnology has aided the fight against bacteria, enabling for more efficient usage of antimicrobial compounds. In this vein, consumer items make extensive use of silver nanoparticles' antibacterial properties (Marambio-Jones & Hoek, 2010).

In 2020 Awham et al. (Salman et al., 2020) studied the design, production, and characterization of a cylindrical Alumina/Zirconia functionally graded material for dental implants are presented in this study. Fabrication was done using an electrophoretic deposition method (EPD). The samples are made out of a strong Zirconia core that is homogenous; Alumina surface layer with a graded composition and a robust, wear-resistant biocompatible surface layer. The effect of suspension parameters (powder and additives) as well as process factors were investigated (voltage, time and electrodes). Four rabbits were implanted with four FGM implants (2 porous and 2 nonporous) in their femur bones for an in vivo research. Both sets had new bone growing around the implantation after three weeks, but the porosity set's implantation took 6 weeks to fully develop and osseointegrate because of the existence of haversion lamellae. This refers to alumina's biocompatibility, which allows for faster osseointegration between the implant and the bone.

Medically pure titanium (Ti) has high mechanical and chemical stability in clinical applications, however early attachment with host tissue upon implantation is often postponed due to the bio-inerts Ti surface. Kien et al., (Kien et al., 2021), manufacture the titanium with a hydroxyapatite (HA) coating using three responses: (1) Submerging Ti substrate in an alkaline solutions, including such NaOH, to create a functionalized O2 layer; (2) Ca-O-Ti bond formation, where such O serves as a bridge material between Ca and Ti material; and (3) hydrothermal converting of Ca-O-Ti specimens into HA-coated Ti specimens by submerging Ca-O-Ti specimens in Na2HPO4 2 M for three days at 180 degree centigrade. Utilizing the optically microscope, thin layer XRD, and SEM/EDX, it may also be explored how various phosphate solution 2 M for every(Na2HPO4 and NaH2PO4 solutions) and hydrothermal remidation times (one and three days) affect the characteristics of the hydroxyapatite coating on titanium substrate. The titanium substrate was covered with fibers of HA with a diameter ranging from 0.1µm to 0.3 m in the HA-coating Ti specimens created by immersion in Na2HPO4 2 M at 180 degree centigrade for three days. The Ti specimens with HA coatings are potentially useful multi-function bio-materials.

2 EXPERIMENTATION

2.1 Micro-Arc-oxidations (MAO)

A kind of oxidations that utilizes little energy was known as micro-arc oxidations. "MAO" seems to be a ground-breaking, environmentally responsible coating technique that enables the one-step sculpture of ceramics coatings on alloys such as aluminum, magnesium, and titanium (Nasab et al., 2010). The anodic surface is defined by guaranteed bright electrical arcs (Chen & Thouas, 2015). Voltages greater than the breakdown voltage of oxygen characterize the (O)gas around the anode surface, anode, and stratum. On voltages larger than the oxygen breakdown voltage, micro-arc-oxidation functioning was agreed upon (Park & Bronzino, 2002). Instances of this are shown in Fig.s 1 and 2 (Hamza et al., 2021).

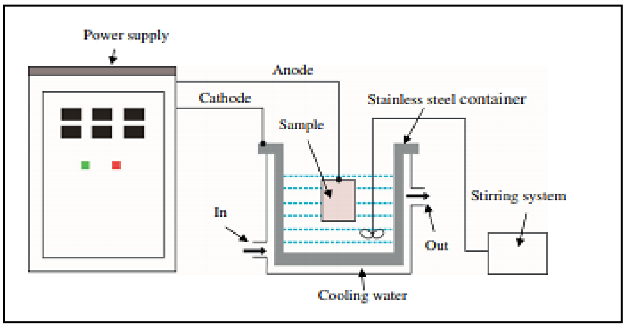

Fig. 1. A schematic of a micro-arc-oxidation apparatus

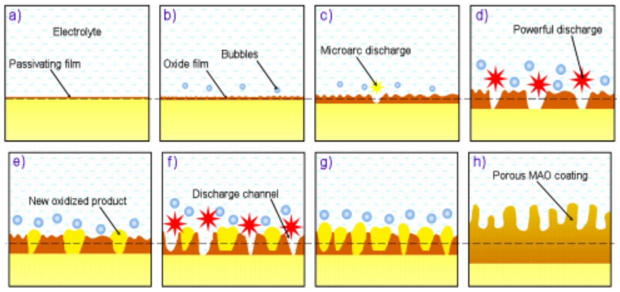

Fig. 2 .The (MAO) mechanism for porous coating formation is depicted in this diagram. Initially, it's a passive-aggressive film (a) and an irritating oxide film that is porous (b) The new spawned oxide coating is produced (e) Under the influence of spark discharges, it is then produced and thickened (c) and explosive arc discharge (d). The twisted ceramic oxide covering is leaking (h) as the oxide covering forms and breaks down inexorably (g) big discharge canals (f) (Hamza et al., 2021).

A titanium plate with a thickness of (0.1mm) has been cutting into (1 cm,1 cm). The titanium plate pureness was (99.8%). Using an ultrasonic cleaning system, samples have been cleaned ultrasonically by ethanol for 15 minutes prior to coating. The following materials were utilized to create composite coatings (HA, HA/nAg) on titanium substrates: -

1. Preparation of hydroxy-apatite electrolyte the hydroxyapatite electrolyte in the electrolyte cell was a distilled water with 0.06 mol/L sodium biphosphate dihydrate $NaH2PO4.2H2O2$ and 0.13 mol/L calcium acetate monohydrate ((CH3-COO) 2.8Ca.H2O) and a PH of 2.3.
2. In quantities of 0.5, 1, 1.5, and 2 g/L, nano silver was applied to hydroxyapatite electrolytes.

As indicated in table 1, various parameters must be established to finish the coating (HA/nAg, HA) on a titanium plate, including constant voltage200 V, time(30sec,45sec,60sec), and nano silver is 0.5 g/1 L.

Table1. shows parameter of MAO process

No	Specimen	time	Proportion Ag (g/L)
	HA	30	---
		45	---
		60	---
	HA/ nAg	30	0.5 g/L
	HA/ nAg	45	0.5 g/L
	HA/ nAg	60	0.5 g/L
	HA/ nAg	30	1 g/L
	HA/ nAg	45	1 g/L
	HA/ nAg	60	1 g/L
	HA/ nAg	30	1.5 g/L
	HA/ nAg	45	1.5 g/L
	HA/ nAg	60	1.5 g/L
	HA/ nAg	30	2 g/L
	HA/ nAg	45	2 g/L

2.2 Tests

2.2.1 Surgical procedure and implantation test

Six adult male rabbits of local origin weighing between 2 and 3 kg were used. These animals were given conventional pellets and veggies in a regular cage with free access to tap water. Before the surgery, they were kept in the same environment for three days.

As a preliminary step, implantations and instruments should be sterilized pre-surgery. The intramuscular xylazine and ketamine injection has been used to induce anesthesia. The surgery was carried out in a sterile environment using a meticulous surgical technique. Each rabbit's left leg was shaved on all sides with a shaver. The surgical towel was draped over the operative site.

Ethanol and then iodine were used to clean the skin. A medical cloth was used to wrap the leg. The femur bone was exposed through a (3.5 cm) long incision, and the fascia and skin were reflected. The periosteum (vascular connective tissue) was accurately portrayed in Fig 3.

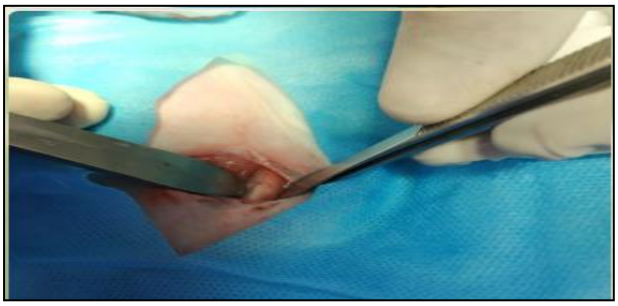

Fig. 3. Around the surgery site, surgical clamps and towels, reflection of skin and reflection fascia and muscles

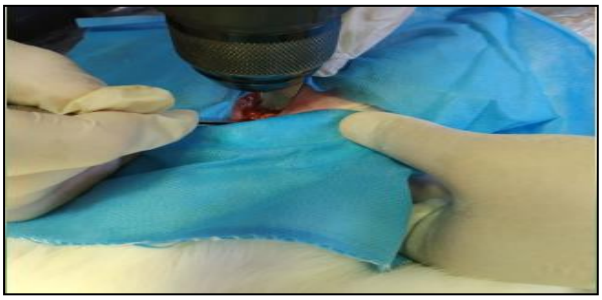

Fig. 4. Preparation of holes

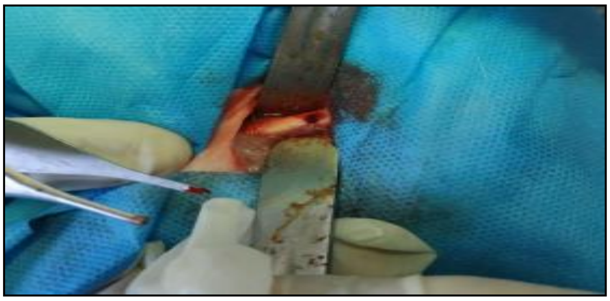

Fig. 5. The hole

Figures (4) and (5) depict a drilling process using a round bar and an interrupted pressure. A drill with a diameter of 2 mm was used to gradually enlarge the hole.

The implants, which were 5mm in length, were placed in the hole with a small pressure to ensure that they were completely inserted into the bone, as shown in Fig. (6).

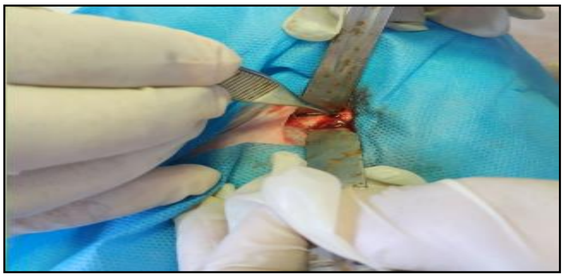

Fig. 6. Insert the implants

Fig. 7. Stitching of skin

The fascia was stitched with absorbable Suteon suture, then the skin was sutured, as shown in the diagram (7). An antibiotic and a voltaren injection were given three days following surgery as part of post-operative care.

2.2.2 Histological testing

Bone sectioning was conducted after the animal was sacrificed with an overdose of general anesthesia at 40 days interval. Following that, an optical microscope was used to examine the implant's histology. To obtain the bone–implant block, a cutting instrument was employed. The bone–implant block was placed in 10% newly produced formalin and kept for 3 weeks.

The histological sample fixative in 10% formalin for 48 hours at room temperature and then washed with tap water for two hours, after that decalcification of specimen by used formic acid-sodium citrate method, until decalcification is complete washed the specimen with tap water 4 hours (Luna, 1968; Suvarna et al., 2018). The specimen send to processed by routine histological processing methods including: Dehydration, Clearing, Infiltration, Embedding, Sectioning, Mounting, Staining by Hematoxylin & Eosin and Cover slipper and labeling (Suvarna et al., 2018).

Dehydration utilizing series spiraling of alcohol starting with 50, 60,70, 80, 90 and 100% for 2 hrs for each amount.

Clearing utilizing Xylen twice 10-50 minutes for each solution. Infiltration by placing the tissue samples in paraffin wax on (56.5-58°C) for overnight. Embedding the specimens within paraffin wax for to made blocks. Sectioning the specimens to 5-7µm by using rotary microtome. Rehydration utilizing alcohol abdicable sequence starting by 100, 90, 80, 70, 60 and 50%. Finally staining the specimens by Mayer's hematoxylin and eosin and massons trichrome stains. Mounting was the process of adding the amount of DPX and then putting the cover slide on the sections strip on glass slides to cover it [17]. Finally examine the histological slides under the light microscope.

3 RESULTS AND DISCUSSION

3.1 Histological results

The current study's histological results from the Hematoxylin and Eosin stain (H&E) under a light microscope revealed the creation of new bone trabeculae in all of the groups tested (control and experimental) as show in Figures below.

Histological changes in the bone treated with titanium at 40 day.

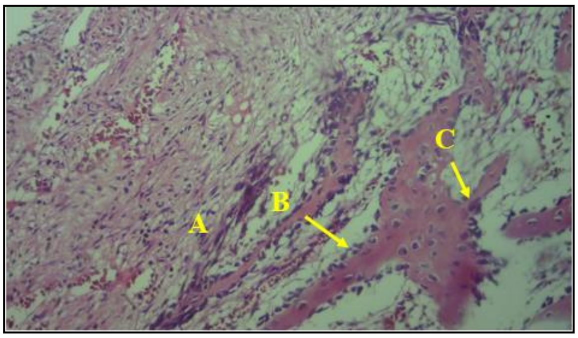

Fig. 8. A section in the bone treated with titanium group at 40 day show A- collagen fibers in the woven bone B- Metabolically active osteoblasts line woven bone C- Osteoclast (H & E stain 40X).

Titanium group histological slice revealed new bone trabeculae bordered by osteoblasts and filled by osteocytes.

Histological changes in the bone treated with coating (HA/ 60 sec) on Ti at 40 day

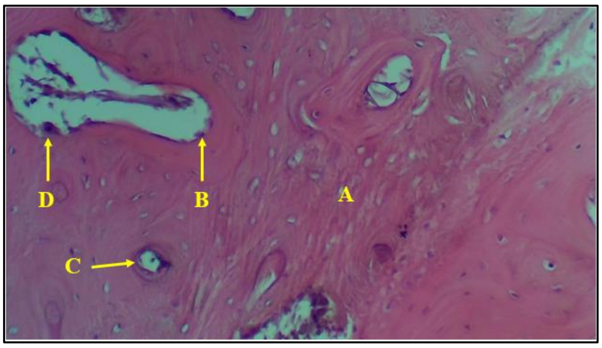

Fig. 9. A section in the bone treated with coating (HA/ 60 sec) on Ti group at 40 days show A- The Woven bone that characterized by haphazard organisation of collagen fibers B- osteoblasts C- Haversian canals, D- Osteoclast. (H & E stain 40X).

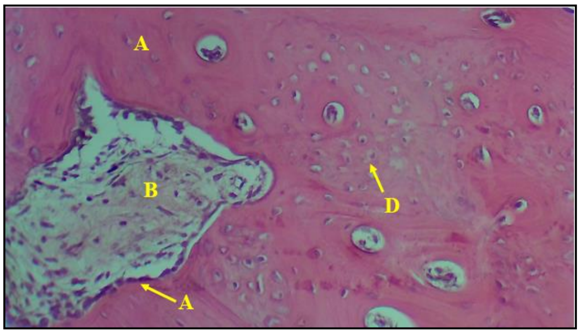

Fig. 11. A section in the bone treated with coating (HA/ 60 sec) on Ti group at days show A- lamellar bone organization with B- Area of bone remodeling and activity of C-osteoblasts and D- osteocytes (H & E stain 40X).

Histological changes in the bone treated with composite coating (HA/0.5 g/L nAg/ 60 sec) on Ti at 40 day.

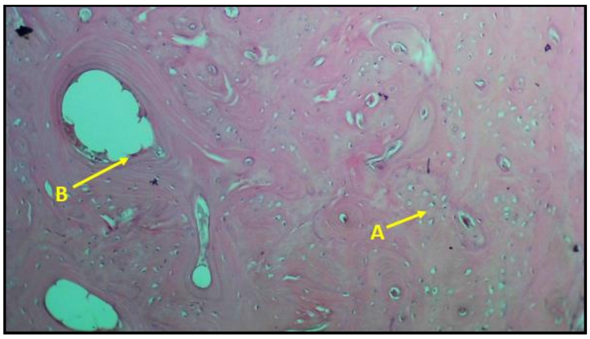

Fig. 12. A section in the bone treated with composite coating (HA/0.5 g/L nAg/ 60 sec) on Ti group at 40 day show The compacting bone was composed of calcified extracellular material, the bones medium and 2 main cell kinds A- Osteoblast B- Osteocytes that detected in cavities (lacunae) between layers of bone matrix (H & E stain 40X). Histological changes in the bone treated with composite coating (HA/1 g/L nAg/ 60 sec) on Ti at 40 day.

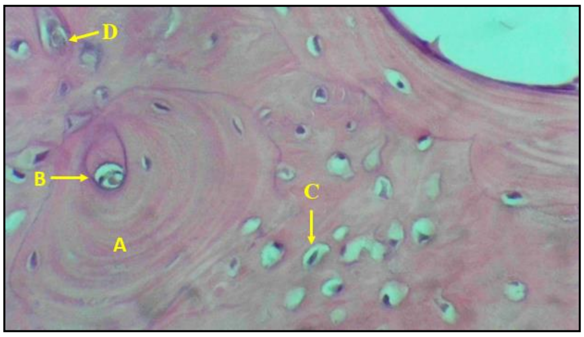

Fig. 13. A section in the bone treated with composite coating (HA/1 g/L nAg/ 60 sec) on Ti group at days show A- The compacting bone was composed of calcified extracellular material, the bones matrix B- (HC), Haversian canals C- lacuna and lamellar bone D-Osteoclast. (H & E stain 40X).

Histological changes in the bone treated with composite coating (HA / 1.5 g/L n Ag / 60) on Ti / 3 days.

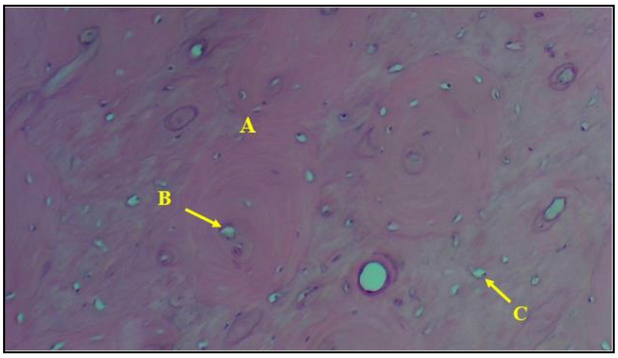

Fig. 14. A section in the bone treated with composite coating (HA/1.5 g/L nAg/ 60 sec) on Ti group at 3 days show A- the compact bone is composed of calcified extracellular material, B- Haversian canals, C- Osteocyte within lacuna. (H & E stain 40X).

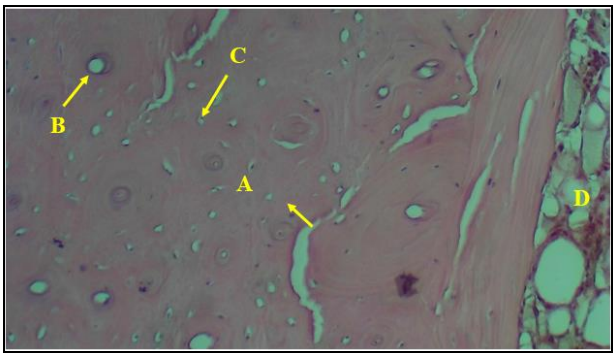

Fig. 15. A section in the bone treated with composite coating (HA /1.5 g/L n Ag / 60 sec) on Ti group/ 3 days show A the compact bone B- Haversian canals, C- Osteocyte within lacuna and D- marrow (H & E stain 40X).

Histological changes in the bone treated with composite coating (HA/2 g/L nAg/ 60 sec) on Ti at 3 days.

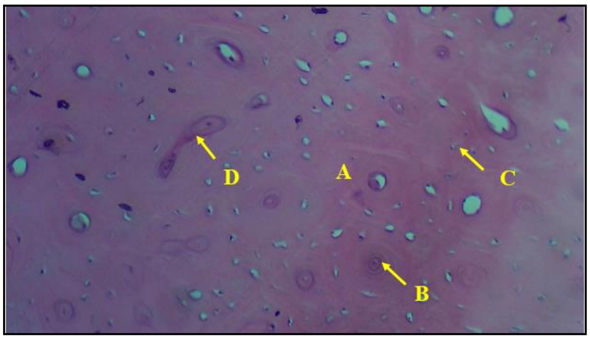

Fig. 16. A section in the bone treated with composite coating (HA / 2 g/L n Ag / 60 sec) on Ti group/ 3 days show A- The matrix of the central portion of the wall are arranged concentrically around B- Haversian canals C- Osteocyte within lacuna D- Volkmann's canal (H & E stain 40X).

Even though AgNPs have several features that make them ideal for cutting-edge and difficult biological applications [19] However, the growing use of AgNPs in medicine and food raises concerns regarding their safety, particularly the potential health effects of human exposure. For thousands of years, bulk silver has been employed in sanitary and medical uses. However, as compared to their bulk counterparts, AgNPs have unusual physicochemical features that result in a substantial increase in toxicity due to their unique cell-contactable surface areas and high surface-to-volume ratio [20] The toxic effect of AgNPs was evident when used composite coating {(HA / 1.5 g/L nAg/ 60 sec) on Ti & (HA / 2 g/L nAg / 60) on Ti } groups, which the high percentage of AgNPs caused the death of the rabbit after 3 days of implantation process.

4 CONCLUSION

A titanium plate surface was treated by micro arc oxidation (MAO) with hydroxyapatite coating and composite coating (hydroxyapatite / nano silver), and the coating time and silver percentage were varied in this study. Following the surgical process and implantation test for 40 days, as well as histological tests, the following significant points can be drawn:

- Hydroxyapatite coating (HA / 60 sec) on titanium plate and composite coating (HA / 0.5 g/L nAg/ 60, HA / 1 g/L nAg / 60) on titanium plate were best results.
- The toxic effect of AgNPs was evident when used composite coating {(HA/1.5 g/L nAg/60) on titanium plate & (HA/2 g/L nAg/60) on titanium plate}, this mean that if the percentage of silver is more than 1 g/L, the solution becomes toxic and does not apply in the human body.

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