Investigation of biomimetic hydroxyapatite formation on titania nanoparticles and lobed nanotubes

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Abstract

An anodizing method was used to produce TiO_2 nanotubes (TNTs) on Ti metal bases. These tubes were transformed into lobed nanotubes (LNTs) by heating at 600^0C as scanning electron microscopy (SEM) images showed. A rapid break down anodizing (RBA) technique was utilized to produce TiO_2 nanoparticles (TNPs). These particles were deposited on LNTs by the electrophoretic deposition (EPD) method. An X-ray diffraction (XRD) test revealed the polycrystalline composition of the formed LNTs and the amorphous structure of TNPs. The bioactivity of LNTs and the deposited TNPs on the LNTs were tested by immersing them in simulated body fluid (SBF) for one month. For the two samples, XRD patterns showed the appearance of small peaks of hydroxyapatite (HAp), which indicates the bioactivity of these samples. SEM images show that the HAp layer on LNTs was in the form of protrusions, while on TNPs it was in the form of crossed filaments.

Keywords: Anodizing technique; bioactivity**;** hydroxyapatite; lobed nanotubes; titania.

1. Introduction

Many researchers are interested in accelerating the formation of new bone on new medical implants. This is done by two procedures, the first is concerned with the production of new alloys with improved specifications, and the second with the surface modification of these implants (Mustafa, 2014). For both types, blood flow to the bone implant must be considered, because the process of new bone construction necessitates blood reaching this implant. One of the ways to facilitate the passage of blood to medical implant surfaces is to cover them with separated nanoparticles, as we did in this research.

Despite the long history of producing bioactive medical implants, obtaining complete specifications is difficult. Requirements like corrosion resistance, building strong bonds in a short time and biocompatibility should exist in good implants at the same time (Dabing, 2013). Covering medical implants with bioactive nanoparticles meets the requirements for rapid and efficient building of strong bonds between them and bone (Boutinguiza *et al*., 2011). Nanomaterials have better cellular compatibility than conventional micron scale materials. When compared to conventional materials, the nanostructure features of the nano materials promote new bone formation (Zhang *et al*., 2008); this is due to the large ratio between the areas of a nano material and its volume.

One of the well-known properties of TiO_2 nanotubes (TNTs) is their large surface area, and the deposition of nanoparticles of the same material on them will increase this surface area. This will increase the potential bonds of organic and inorganic materials that will form after the implantation process of the covered Ti with TiO_2 nano particles (TNps) decorated TNTs inside the bone (Lory, 2011).

The heat treatment for TNTs usually affects their morphologies; for example Naduvath *et al*. converted TNTs to faceted nanoparticles at low temperatures and heating rates (Johns *et al*.,2015) . As the report by Baiju *et al*. confirmed, TNTs begin to collapse to form nanorods when the temperature reaches 400 °C. (Baiju *et al*., 2010). In current work, there is different titania morphology after heat treatment, as the following paragraphs show.

There are numerous studies that demonstrate the bioactivity of titanium oxide (Mustafa & Reem, 2020; Sang *et al*., 2007; Chun *et al*., 2012), but none that demonstrate this activity for lobed nanotubes (LNTs) and deposit TNPs on the LNTs.

The purpose of this post is to test the bioactivity of nano titanium oxide in two different forms: LNTs and deposited TNPs on the LNTs.

2. Experimental part

The procedure of production of TNTs was mentioned in detail by (Mustafa, 2014). The chemical solution of the anodic process is composed of NH_4F:H_2O and glycerol in a weight ratio of 1:20:7. The cathode (Ti) and anode (Ti) were immersed in the solution for one hour. The voltage difference between them was kept at 30V. The transformation of these TNTs to LNTs was done by heating at 600^0C for one hour.

The method of preparing TNPs by rapid break down anodizing (RBA) method is described in detail by (Reem & Mustafa, 2020). The Ti foil (0.1 mm thick) with a rectangular form (1 x 2 cm) was submerged in 0.1 M $HClO_4$ electrolyte after being cleaned with alcohol. Two Ti pieces were utilized in the anodization procedure, one as a working electrode and the other as a counter. The distance between the two electrodes was 0.5 cm, and the applied voltage was 20 volts between them.

The electrophoretic deposition (EPD) method was utilized to deposit TNPs on the Ti base. Deposition details are explained fully by (Dalal, 2018). Two Ti rectangular pieces (1 cm x 2 cm) serve as the cathode and anode in the EPD technique cell. A 0.5 cm gap existed between the two electrodes. Artificial ethanol was used to fill the space between the electrodes. TNPs were added to ethanol to be deposited on the Ti base. A thin Ti oxide layer covered the Ti base after applying 50V between the electrodes for 30s.

To investigate the formation of HAp, coated plates with TNPs were immersed in a concentrated five times simulated body fluid (SBF) for one month. The composition of SBF is shown in Table 1.

Table 1. The composition of SBF and the concentration of (SBF × 5) (Helebrant,2002)**.**

ITEM	SBF*5 (g m/l)
NaCl	40.18
$NaHCO_3$	3.176
KCl	1.125
K_2HPO_4	1.15
$MgCl_2 .6H_2O$	1.555
$CaCl_2$	1.465
$NaSO_4$	0.36

The purity and sources of the used chemicals are listed in Table 2.

Table 2. Sources of chemicals and their purity

ITEM	Purity	Source
NH_4F	98%	Riedel-de Haen-Germany
glycerol	98%	Phywe-Germany
NaCl	99.5%	Romil-England
$NaHCO_3$	98%	Riedel-de Haen-Germany
KCl	99%	Thomas Baker -India
K_2HPO_4	98%	Chem Supply-Australia
$MgCl_2 .6H_2O$	98%	Riedel-de Haen-Germany
$CaCl_2$	98%	Chem Supply-Australia
$NaSO_4$	98%	Riedel-de Haen-Germany

The phases of the materials produced were examined using the X-ray diffraction (XRD) technique. A scanning electron microscope (SEM) was used to examine the shapes and sizes of particles. The energy dispersive X-ray spectroscopy (EDS) technique was used to specify the compositions of the used and processed materials.

3. Results and discussions

Figure.1 shows the XRD patterns of LNTs and TNPs. Produced TNPs had an amorphous structure. This result is in agreement with that obtained by (Reem & Mustafa ,2019). After heat treatment, LNTs had a polycrystalline structure with a dominant peak (110). Also, LNTs had a pure rutile phase and there was no trace of an anatase or brookite phase. This result is in agreement with that obtained by (Yu & Anchun, 2018).

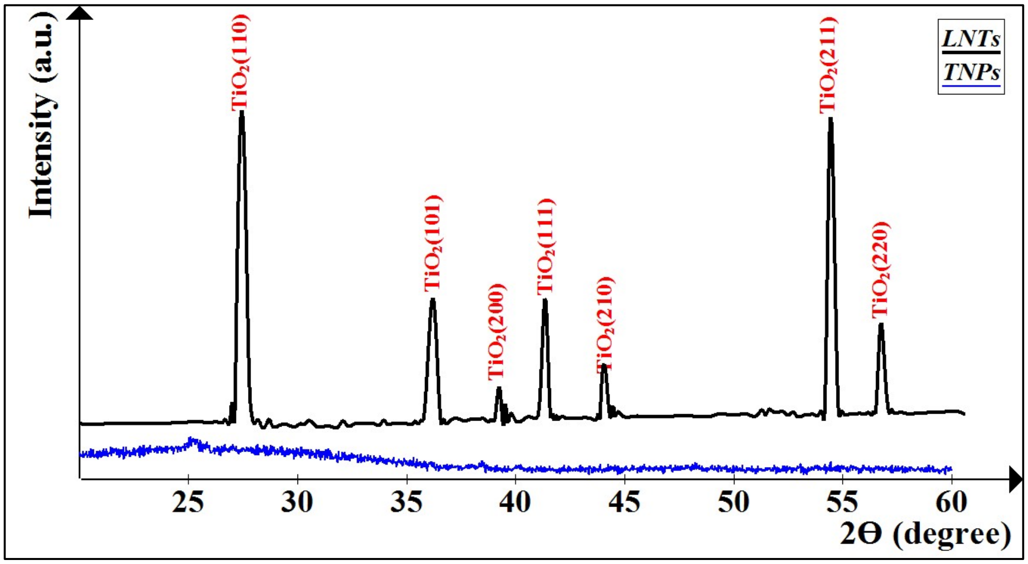


Fig. 1. XRD patterns of TNR and TNPs

After heat treatment, SEM images in Figure. 2 show the disintegration of TNTs into lobed tubes consisting of rings arranged on top of each other, and each ring consisting of a belt of nanoparticles with an average diameter of 32.96 nm (standard deviation 3.32). To our knowledge, this result is not similar to any transformation of TNTs after the heat treatment process.

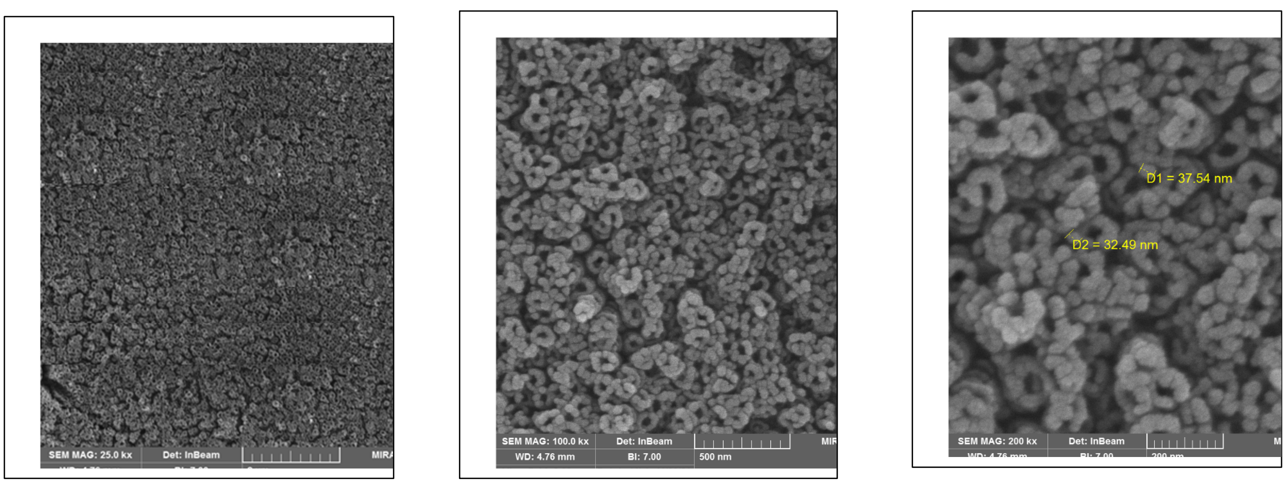
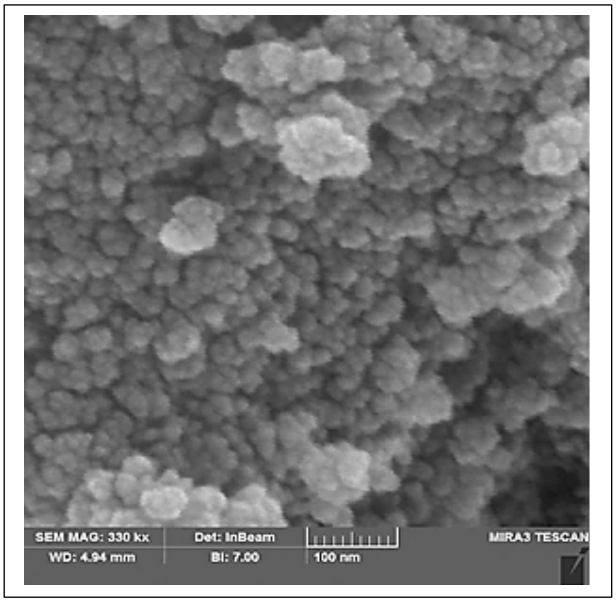
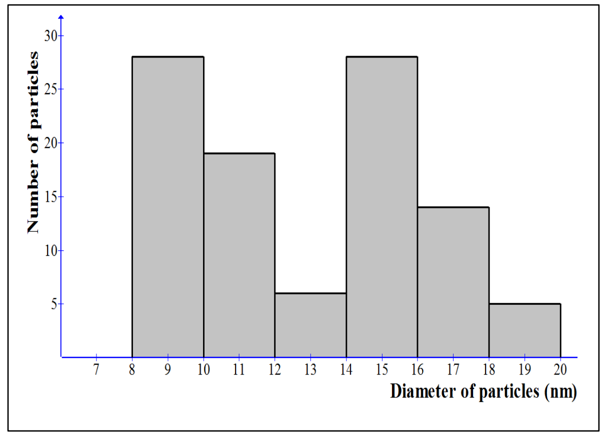


Fig. 2. SEM images of LNTs.

The second anodic process (RBA) produced TNPs as SEM in Figure. 3 illustrates. These nanoparticles were, in general, semi spherical nanoparticles with an average diameter of 13 nm (standard deviation 3.44) . The result is similar to that obtained by (Mustafa & Reem, 2020).

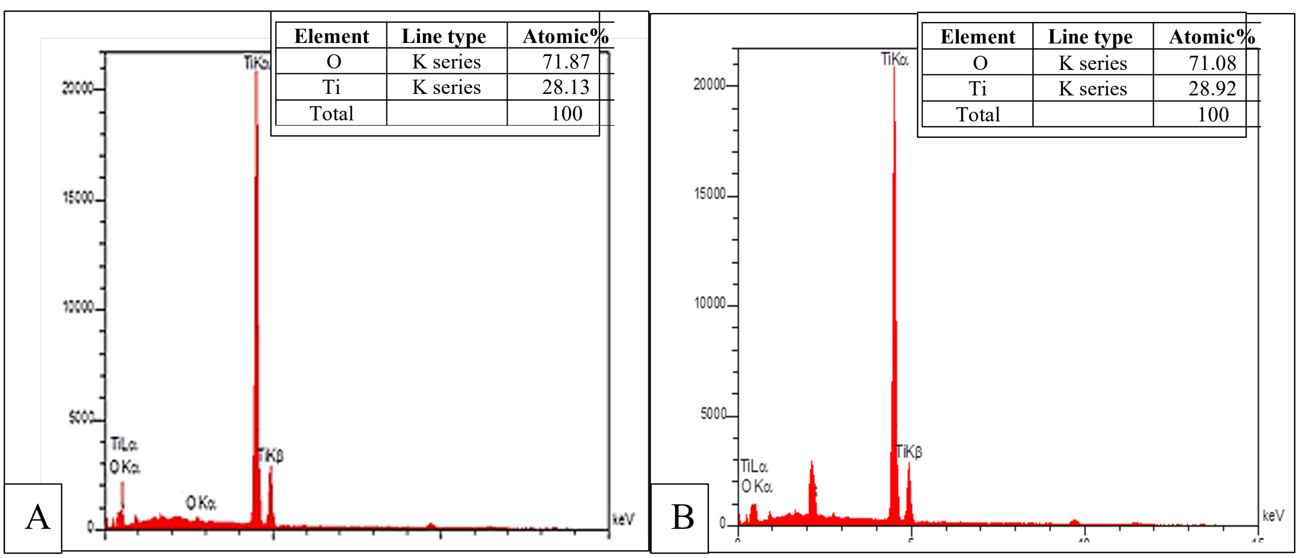




Average diameter	13 nm
Standard deviation	3.44
Minimum size	9.161nm
Maximum size	20 nm

Fig. 3. SEM image of TNPs and some statistical parameters**.** .

To know the compositions of TNPs and LNTs, the EDS technique was used. The inset tables inside Figure.4 show the elements of the two samples. For the two nanostructures, the atomic percentage of oxygen is approximately twice that of titanium, which indicates the complete transformation of Ti to TiO_2.



Element	Line type	Atomic%
O	K series	71.87
Ti	K series	28.13
Total		100

Element	Line type	Atomic%
O	K series	71.08
Ti	K series	28.92
Total		100

Fig. 4. Results of EDS test for A:TNPs & B:LNTs.

After soaking in SBF, hydroxyapatite (HAp) was formed on two samples (LNTs and the deposited TNPs on the LNTs) as XRD patterns in Figure.5 confirmed. The insets show small peaks for HAp with orientation [211] and [112] in both patterns. Relatively small intensities for these both

HAp peaks might be due to a thin layer of this material. The SBF-based biomimetic approach frequently results in an amorphous, smooth, and homogeneous HAp layer. However, like with (Yaser *et al*., 2016), this layer was partly crystallized after heat treatment; our results are in agreement with these findings. SEM images and EDS spectra for the two samples confirm the formation of HAp.

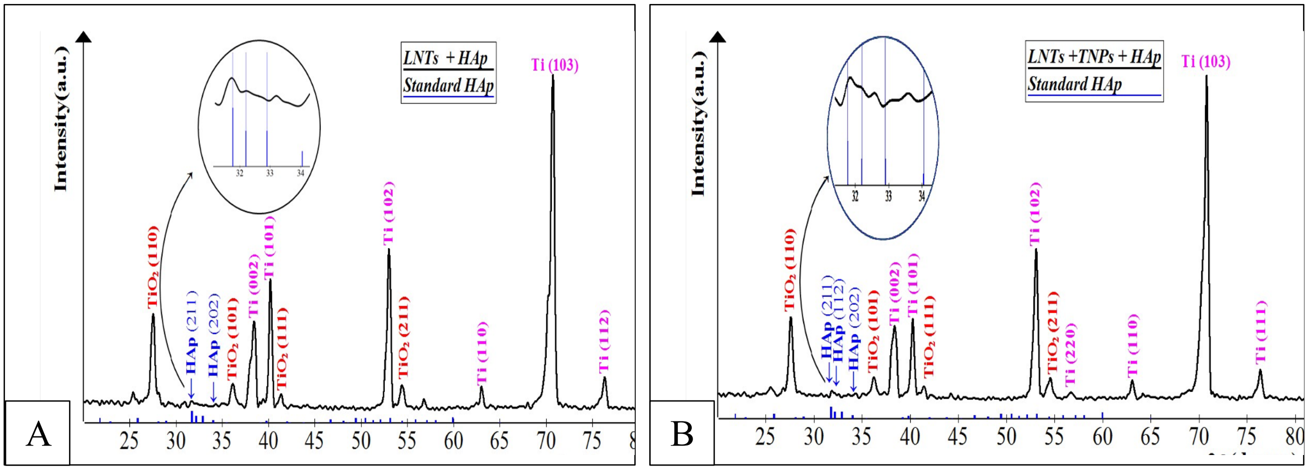


Fig.5. XRD patterns of A: LNTs and B:coated LNTs with TNPs after soaking in SBF.

After taking out the samples from SBF; SEM images in Figure.6 show that the formed HAp has a different morphology on both samples.

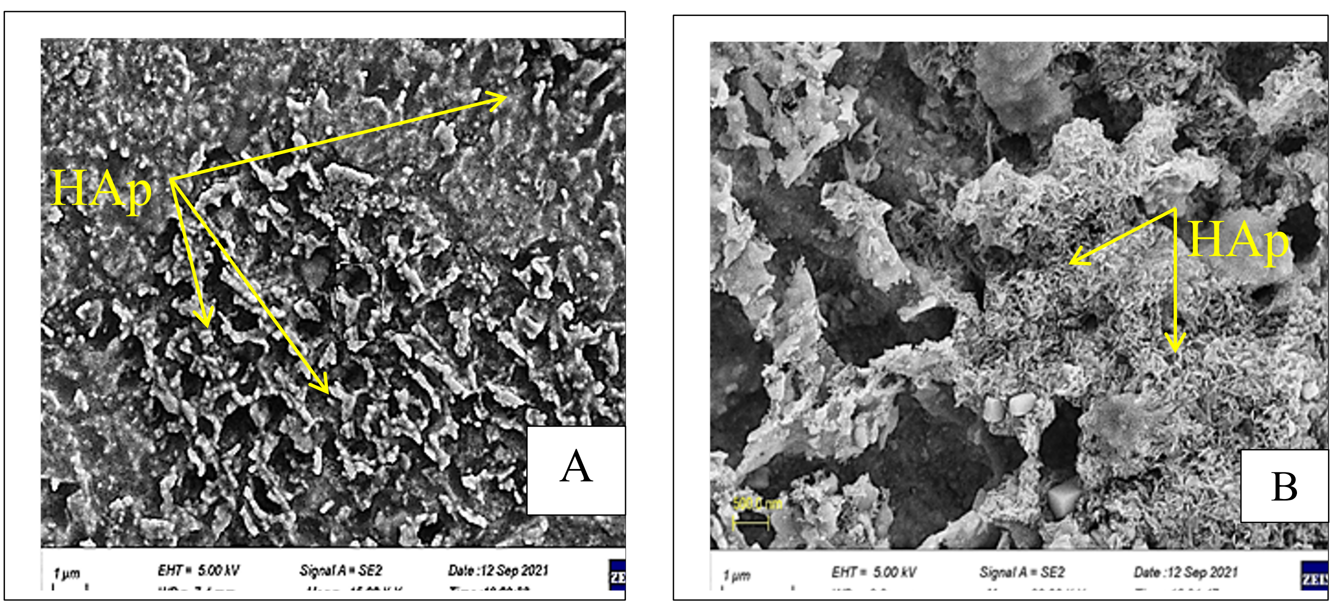


Fig. 6. SEM images of the formed HAp after biomimic process**.**

A: On LNTs, B:On coated LNTs with TNPs.

The growth of HAp is affected by the layer under it. Above LNTs the formed HAp has protrusions distributed uniformly. This distribution may be due to the growth of HAp on a semi uniform distribution of the LNTs layer. On the other hand, the deposited HAp on TNPs has a network of interlocking filaments.

EDS spectra of the two samples after soaking inside SBF are displayed in Figure.7. Beside Ti and O peaks there are peaks belong to Ca and P indicating the formation of the HAp layer. The low intensities of HAp inside XRD patterns correspond to the low intensities of calcium and phosphorous in the EDS spectrum.

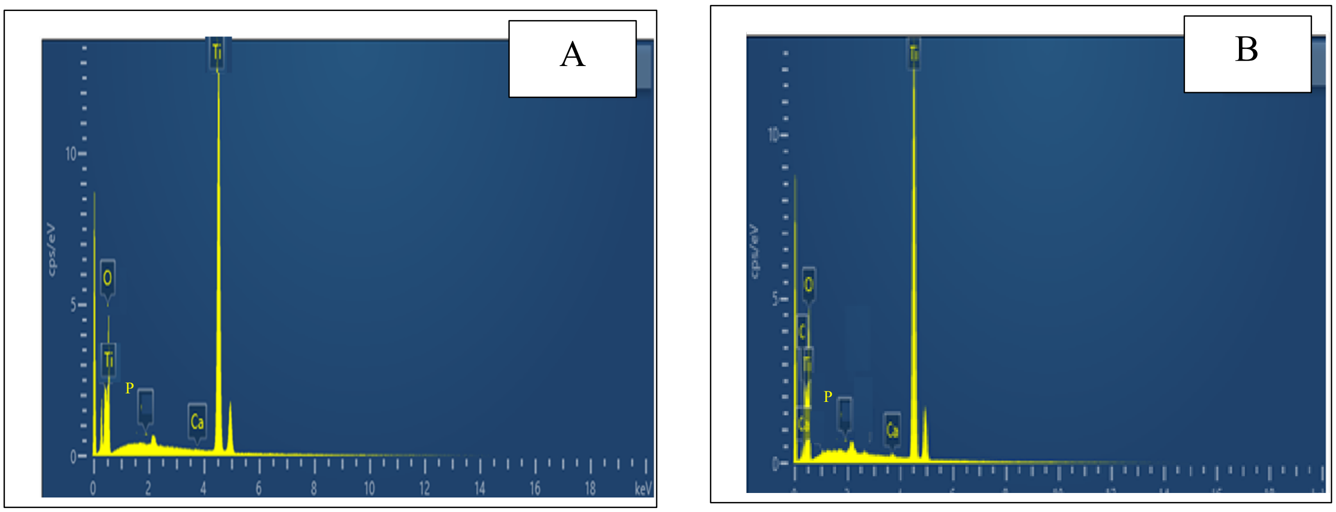


Fig. 7. EDX of A: LNTs + HAp B: coated LNTs with TNPs + HAp.

4. Conclusions

Both TiO_2 nanoparticles (TNPs) and lobed nanotubes (LNTs) are bioactive, and the formed hydroxyapatite (HAp) layers on them have similar XRD patterns and EDS spectra. But these HAp layers have different topographies. According to SEM images, the HAp layer on LNTs was in the form of protrusions, whereas it was in the form of crossing filaments on TNPs.

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