ANALYSIS OF EFFICIENCY OF DENTAL IMPLANT OSSEOINTEGRATION CHARACTERIZED BY VARIOUS SURFACE TYPES.

Astashina N.[1], Lugovskoy A.[2], Kossenko A.[2], Lugovskoy S.[2], Zinigrad M.[2], Nakonechny F.[2]

[1]E.A. Vagner Perm State Medical University, Perm, Russia

[2]Ariel University, Scientific Park, Ariel, 40700, Israel

Introduction:

Efficiency of dental implantation and the long-term functioning of intraosseous implants are largely determined by integration conditions. One basic factor that influences osseointegration rate and quality is recipient's bone tissue density. It is clear that as density of bone decreases, its strength and ability to accept foreign bodies, including any implantation systems, also decreases. In "soft" bone types (D4 bone according to Misch's classification [1]) osseointegration processes can be improved by using a layer of an intermediary material that has sufficiently high adhesion both toward the metal and toward the bone tissue. The structures indicated include a coating of hydroxyapatite, employment whereof provides a significant increase in implant/bone contact area and a noticeable improvement in short-range viability indices as compared to 'clean' titanium implants.[2] Coating surfaces of titanium implants with a layer of hydroxyapatite (HA) [3] was shown to increase the rate of adaptation of bone tissue to the implants, ensure sufficient initial fixation rigidity of the implanted structures, and stimulate mature bone tissue growth while, in particular, improving its quantitative and qualitative performance. Furthermore, bone around HA-coated implants is strengthened in comparison to structures that do not have a modified layer. To improve quality and long-term efficiency of dental implantation in type D4bone tissue, researchers recommend using implants that have an osteoinductive hydroxyapatite coating.[4–11]

Experiment.

Comparison of the findings showed the best performance to be yielded by titania-containing coating. This coating demonstrates only minimum microbial colonization of its surface. The largest hydroxyapatite-coated surface (with surface area ~ 2000 times higher than the original sample of untreated titanium alloy) is identical to the original material in colony count.

Colonies grown on hydroxyapatite crystal-containing coating are not retained on the crystals themselves but adhere to uncoated (by oxidation or thermo-hydro-treatment) areas, or to mechanically damaged areas.

Keywords: plasma electrolytic oxidation, hydroxyapatite, oxide layers, biomaterials, osseointegration

Titanium and its alloys are widely used in biomedicine to fabricate various implants, due to biocompatibility and excellent stress-strain performance. However, titanium alloys do not meet certain clinical requirements for an implant as a biomaterial such as antibacterial activity. Major causes of implant rejection after the first year of occlusal load are excessive functional loading [12] and bacterial infections [13].

Bacterial activity related biofilms promoted development of peri-implantitis and rejection of implanted structures. One way of improving antibacterial properties without degrading biocompatibility parameters of titanium is to modify its surface properties.

A technique developed in order to improve titanium implant osseointegration efficiency is based on use of an intermediary material which shows good adhesion both to the metal and to osseous tissue.

The study dealt with medical and biological surface properties of dental implant coatings, in particular, *in vitro* testing was performed on samples made of titanium alloy (a similar material used to fabricate dental implants) with various surface types, to evaluate microbial colonization resistance of the coatings built up.

Samples used:

4 titanium alloy samples were prepared for analysis:

1 – Untreated titanium alloy sample

2 – Titanium alloy sample, after surface oxidation

3 – Titanium alloy sample, after surface oxidation and subsequent growth of hydroxyapatites on the sample surface, at pH = 7

4 – Titanium alloy sample, after surface oxidation and subsequent growth of hydroxyapatites on the sample surface, at pH = 11.

Prior to the *in vitro* test, specific surface areas of all samples as described above were measured.

The main experimental approach to measurement of specific surface area, as one of the fundamental parameters, is low-temperature sorption of nitrogen vapour on solid/gas interface (BET method). Major advantages of the method are as follows: rapidity, comprehensiveness, sample preparation simplicity, precision, and reproducibility. Theoretic concepts underlying this method have been time-tested and proved their high experimental reliability.

That is why the sorption method has actually become conventional for characterization of any nanomaterial. For the BET comparison results, see Table 1.

Table 1

Sample	Bet surface area, m^2	Bet surface area, m^2/gr
Ti-1 (pure titanium)	—	
Ti-2 (post-PEO)	0.0539±0.0055	1.65±0.17
Ti-3 pH = 7 (2 hours)	0.2941±0.0064	11.22±0.62
Ti-4 pH = 11 (2 hours)	56.92±0.73	2013.54±25.56

According to the BET data, the largest surface area was obtained after hydrothermal treatment at pH = 11: 2013.54 m^2/gr. That said, Ca/P ratio remains unchanged throughout the hydrothermal treatment process, only hydroxyapatite crystal shape and size change.

Culture used:

Staphylococcus epidermidis culture (Winslow and Winslow) Evans (ATCC® 35984D-5™). *Staphylococcus epidermidis* was cultivated on a standard agar solution (BHA; Acumedia, USA) for 24 h, then transferred into a standard solution (BH; Ac-

umedia), and cultivated at 37°C, centrifuged at 170 revolutions per minute of shaking. The resulting concentration of the culture used was 3×10^8 cells per ml of concentration.

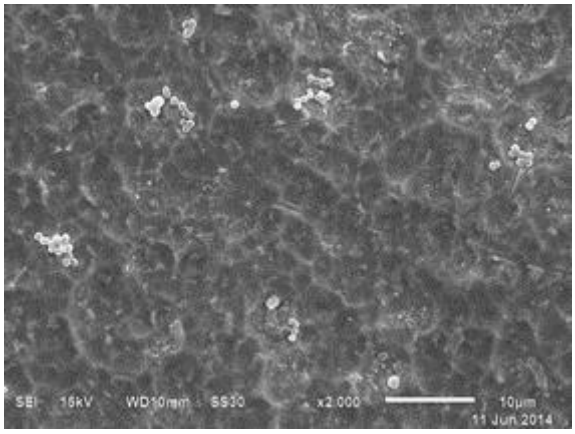
Test sample preparation:

To examine the samples by scanning electron microscopy (SEM) for bacterial colonization rate, the samples were treated as follows: incubated in a liquid BH suspension for 24 h (the initial bacterial concentration was 3×10^8 cells per ml of concentration), at 37°C and at 170 revolutions per minute of shaking, rinsed with 0.01 M PBS buffer at pH = 7.5.

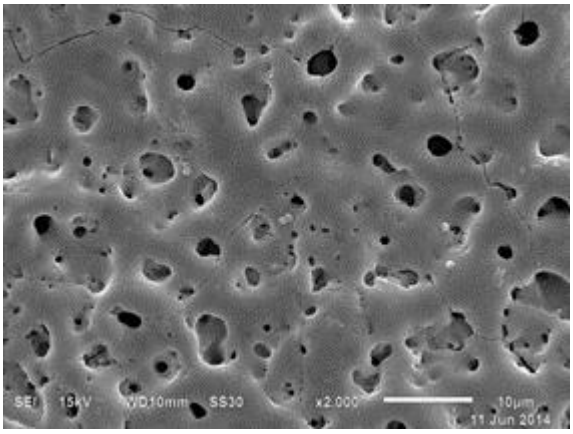
Sample examination:

The sample surface morphology was studied using JSM 6510 scanning electron microscope (SEM) by JEOL (Japan), secondary electron imaging (SEI) and backscattered electron imaging (BSEI).

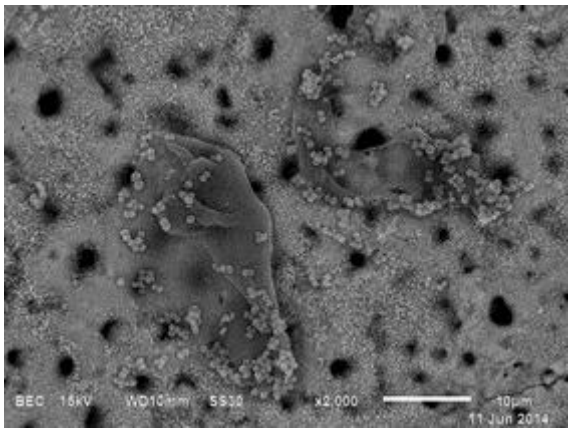
The scanning electron microscopy (SEM) data are as follows:



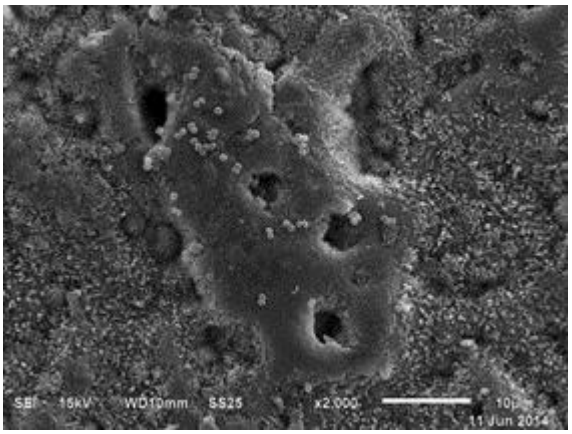
a) Sample 1



b) Sample 2

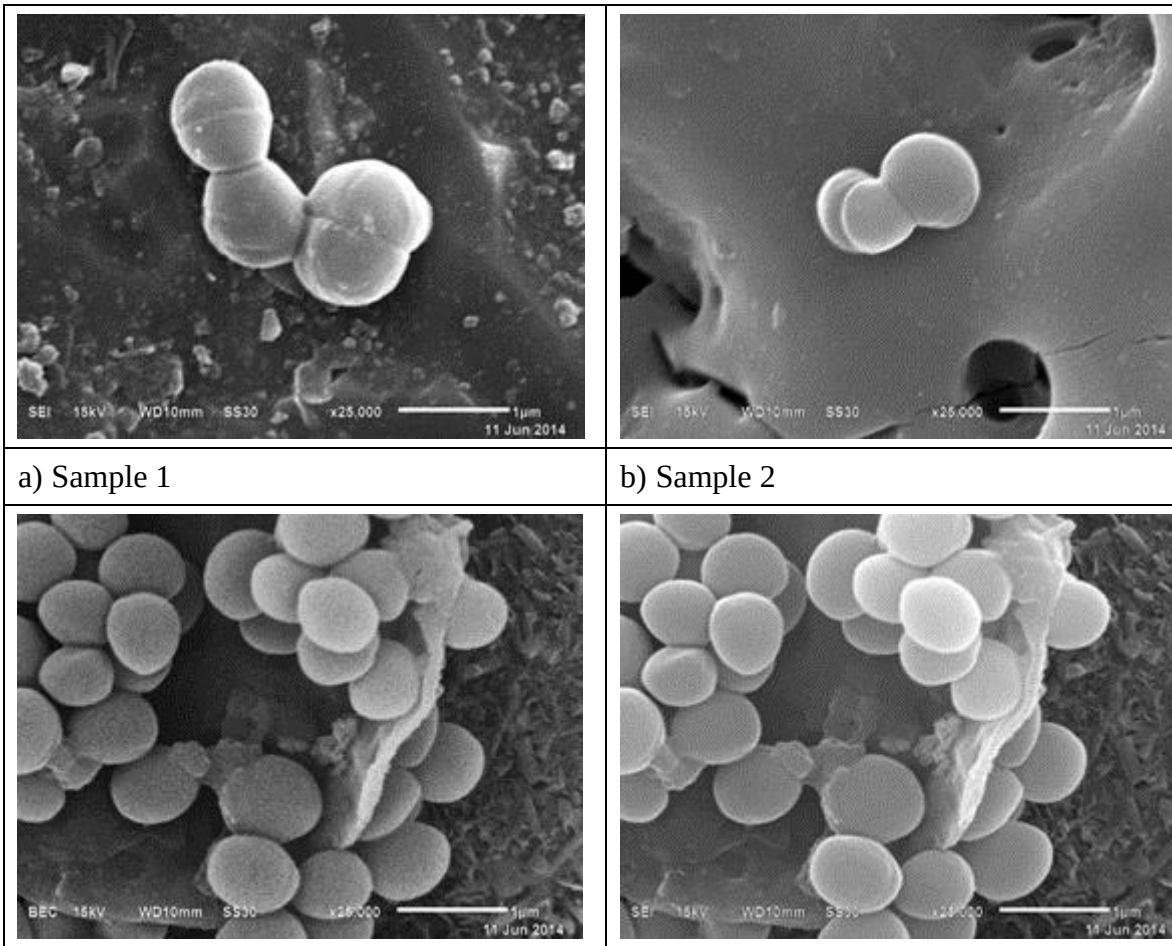


c) Sample 3



d) Sample 4

Fig. 1. SEM images: surface morphology of variously treated samples (×2000)



a) Sample 1 | b) Sample 2

c) Sample 3 | d) Sample 4

Fig. 2. SEM images: surface morphology of variously treated samples (×10000)

Results:

According to the SEM photo (Fig. 1.), the base (uncoated) titanium sample surface demonstrates considerably higher *Staphylococcus epidermidis* colonization rate than the post-PEO titanium sample. This is attributable to antibacterial effect of TiO_2 produced on the sample surface by PEO. The statistics confirmed there was a significant difference between the bacterial counts on the base Ti sample and the post-PEO Ti sample (Tab. 1.)

One can see from the table hydroxyapatite coating increases *Staphylococcus epidermidis* colonization rate of the sample surface (3,4). This is unobvious for comparison samples 1 and 4 (Fig. 2.) since the difference between *Staphylococcus epidermidis* surface counts of samples 1 and 4 is within statistical uncertainty.

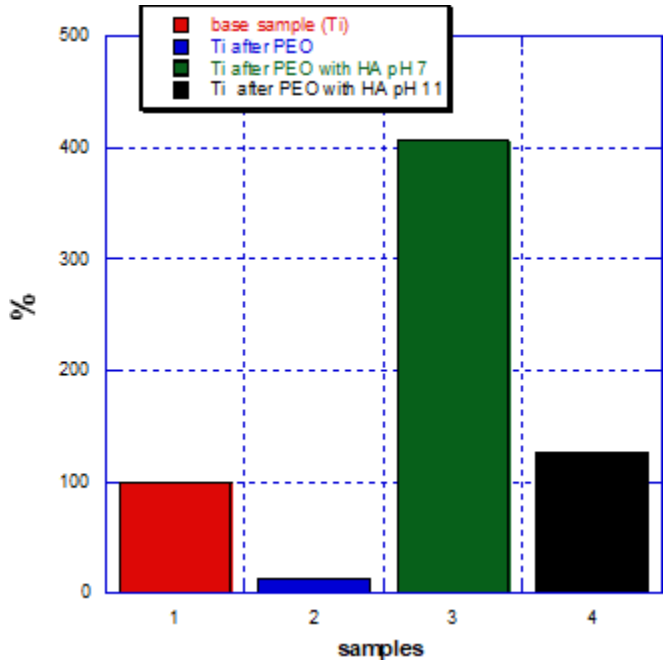


Fig. 3. Quantitative results of *Staphylococcus epidermidis* colonization of various surfaces

Fig. 3. shows *Staphylococcus epidermidis* quantitation data for 812.5-μm² area (counted using ×2000 SEM photo).

Microbial count of sample surface is generally expressed in units per cm² (colony-forming units per cm² (cfu/cm²) (Tab. 2).

Table 2

Microbial count of samples 1 to 4

1-Ti	2-Ti after PEO	3-Ti after PEO with HTT pH 7	4-Ti after PEO with HTT pH 11
60	8	244	76
100%	13%	407%	126%
7.4×10^6 cfu/cm²	1.0×10^5 cfu/cm²	30.0×10^6 cfu/cm²	9.4×10^6 cfu/cm²

Comparison of the findings showed that:

1. The best performance is observed in titania-containing coating. This coating demonstrates minimum microbial colonization of surface.
2. The largest hydroxyapatite-coated surface (with surface area ~ 2000 times higher than the original sample of untreated titanium alloy sample) is identical to the original material in colony count.
3. Colonies grown on hydroxyapatite crystal-containing coating are not retained on the crystals themselves but adhere to uncoated (by oxidation or thermo-hydro-treatment) areas, or to mechanically damaged areas.

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