Surface modification of Ti by chemical etching and HA sputtering for dental applications

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***Abstract* — This work presents the surface modifications obtained in a two-step chemical etching process consisting of chemical etching and subsequent post etching. The additional post-etching results in an increased cell proliferation of almost 50% compared to the non-post-etched. An additional hydroxyapatite coating (about 160 nm thickness) is highly beneficial to further increase the cell adhesion on the etched Ti surface.**

***Index Terms* — titanium, chemical etching, post-etching hydroxyapatite, cell adhesion.**

I. INTRODUCTION

This paper focuses on the surface modification of titanium (Ti) implant for dental applications. Ti is a widely used dental implant material due to its superior properties such as low toxicity, high biocompatibility, and high corrosion resistance [1, 2]. For utilization of Ti as implants in dentistry or orthopedics, the bone-bonding ability of the Ti should be enhanced to prevent the implants from loosening [3]. Thus, the aim is to change the surface structure of the Ti to increase osseointegration. The success of the Ti implants strongly depends on osseointegration, which is the direct structural and histological connection of the bone and the implants surface [2]. The osseointegration can be promoted by increasing the surface roughness of the Ti implant resulting in a much higher contact area between the implant and bone tissue. In principle, the surface structure can be modified by several techniques, e.g. by mechanical (polishing, blasting, grinding), chemical (etching, anodization) or physical (plasma spraying, sputtering) methods [2, 4, 5]. Purely chemical etching is of special interest, because it is an easy, fast and cheap method. It can be used not only in mass production, but also in small labs. It can be easily integrated in already existing production lines. Chemical etching allows to produce Ti surfaces with various degrees of roughness and various morphologies by varying etchants, concentration of etchants, temperature etc. It has been shown [6 - 8] that a surface coating of the implant with hydroxyapatite (HA) is beneficial for the osseointegration. The present work can be divided in two parts: 1st the Ti surface is modified in a chemical etching step with subsequent post-etching and sputter deposition, and 2nd functionalizing of the Ti surface by sputter deposition of HA.

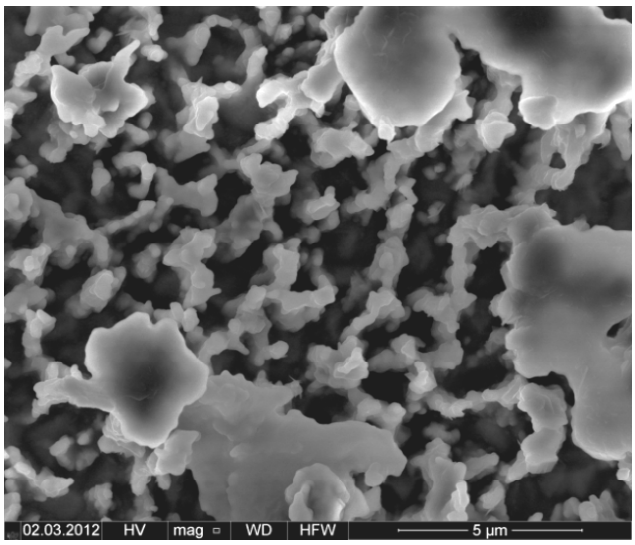
II. EXPERIMENTAL

In the experiments Ti (Goodfellow Ti 007930: annealed, 99.6%) discs were used. The discs were machined to a diameter of 10 mm and a thickness of 1 mm. To remove the sawing marks the Ti discs were polished with SiC grinding paper manually on each side, initially with microgrit P2500 followed by P4000 to obtain relatively smooth surfaces. To remove any oil contamination remaining on the surface, the Ti discs were kept in acetone for 5 minutes and air-dried before they were put into the etching solution. The etching solution is a 1:1 mixture of HCl (37%) and H_2SO_4 (95-97%). The etching is carried out in a plastic beaker at room temperature for 3 h. Afterwards a post-etching in pure H_2SO_4 (95-97%) is performed for 2 h to slightly reduce the surface roughness. After each etching step, the Ti samples are thoroughly rinsed in deionized water and air-dried. The HA coating is done by magnetron-assisted sputter deposition under an incidence angle of 45° at a pressure of $3.4 \cdot 10^{-3}$ mbar and a discharge power of 20 W for 20 h. To find the best surface conditions for cell proliferation and adhesion, three different groups of Ti surfaces were prepared: group 1: chemical etching and HA coating, group 2: chemical etching, post-etching, and HA coating, and group 3: chemical etching and post-etching. For the cell adhesion tests, the Ti samples were sterilized in 70% ethanol and rinsed in a phosphate buffered saline solution (PBS) to maintain a constant pH. At least four samples of each group were prepared an used for the cell adhesion test. The samples were placed into a 12-well culture plate, each having a volume of 1.5 ml standard medium solution and seeded with 20,000 wild type rat embryonic fibroblast cells, followed by an over-night incubation for 17.5 h at 37°C, with 5% CO_2 and 90% humidity. As reference two glass slides were used. For counting the cells under the fluorescence microscope the cells were stained with calcein – a membrane permeable, fluorescent dye labeling living cells.

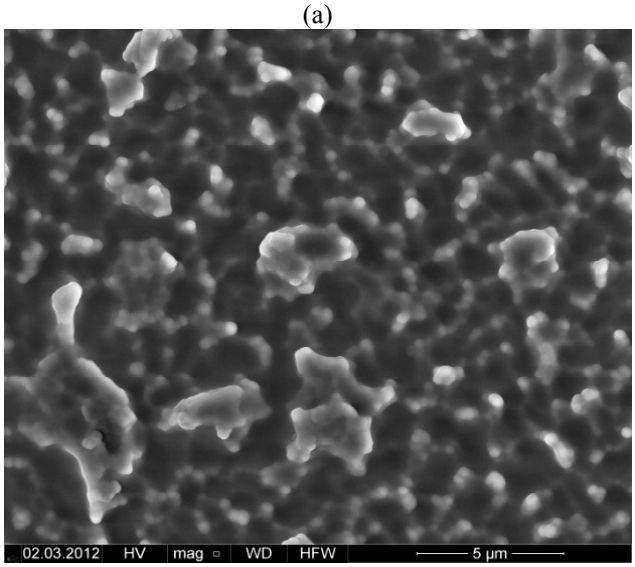
To determine how the cell adhesion is on each Ti surface also fluorescence microscopy images were taken. The surface morphology of the Ti discs was analyzed with a HELIOS D477 SEM. The fluorescence images were taken with an Olympus IX 81 microscope.

III. RESULTS & DISCUSSION

Figure 1 presents a top view on the typical surfaces of the Ti samples after the final processing step, Fig. 2 the corresponding fluorescence microscopy images.



(a)



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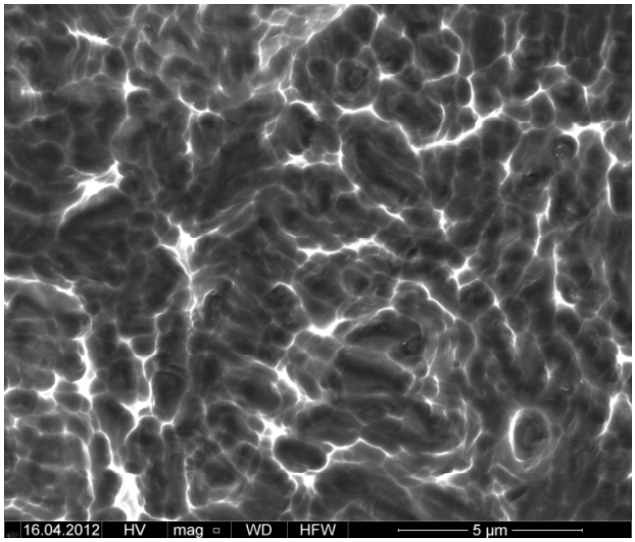
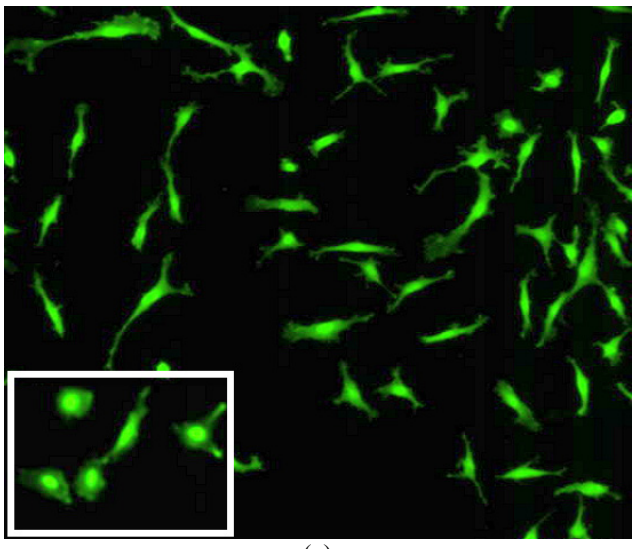
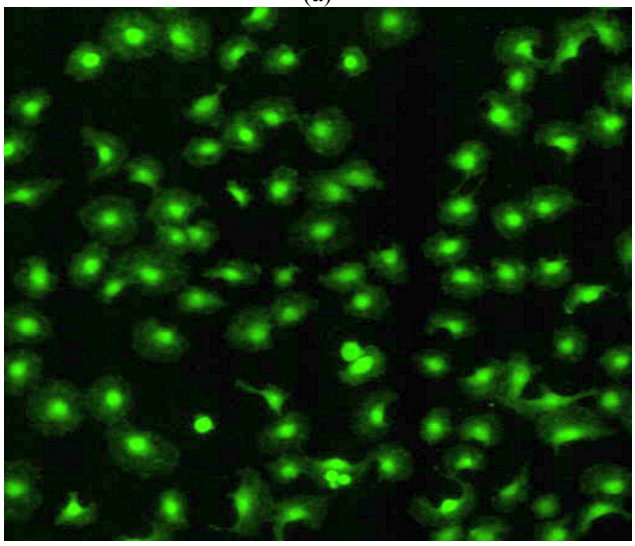


(c)

Fig.1 Top view on surfaces of the Ti samples after the final processing step: (a) group 1 (chemically etched and HA coated), (b) group 2 (chemically etched, post-etched, and HA coated), and (c) group 3 (chemically etched and post-etched). Afterwards, the cell adhesion tests are performed on these samples.



(a)



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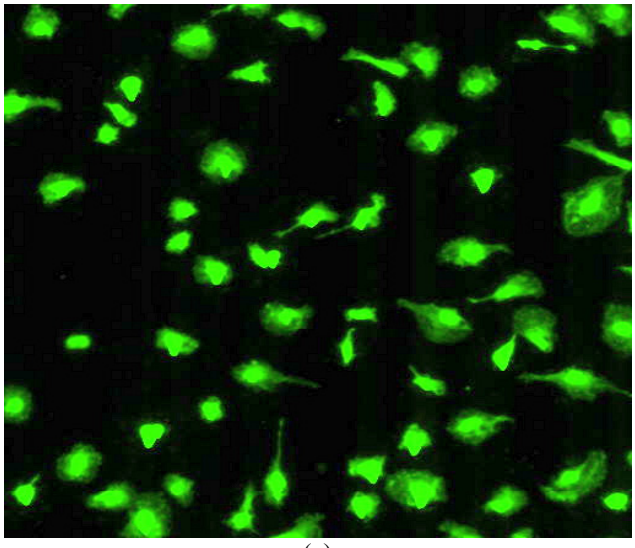

(c)

Fig.2 Fluorescence microscopy images at 10× magnification of wild type rat embryonic fibroblast cells stained with calcein on the surface of (a) group-1, inset: cells on glass slide as reference, (b) group-2, and (c) group-3 Ti samples.

Figure 1 (a) presents the typical surface of the group-1 Ti samples (chemically etched and HA coated). The surface is homogeneous and contains many spike structures that are interconnected. The HA clusters on the surface are visible.

Figure 1 (b) illustrates the typical surface of the group-2 treated Ti samples (etched, post-etched, and HA coated). The surface contains many spikes and narrow hollows, but is less rough compared to the surface of group-1 samples [Fig. 1 (a)]. This is due to the additional post-etching step, which is performed directly after the initial chemical etching, to slightly smoothen the sharp and highly cornered spikes. No large HA clusters are found on the surface. The narrow hollows are almost filled up by the HA coating and the spikes are less pronounced compared to the surface of the group 1 samples [Fig. 1(a)].

In Fig. 1 (c) the typical surface of the group 3 treated Ti samples (etched and post-etched, no HA film) is depicted. The surface has a wavy structure, containing many wide and shallow bowls. It can be seen that the bowls are subdivided into smaller almost round cavities at the bottom of each bowl.

Figure 2 (a) – (c) depict the fluorescence microscopy images of wild type rat embryonic fibroblast cells growing on the three differently processed groups of Ti samples. Each fluorescence microscopy image is given next to the Ti surface it is seeded on. The morphology of the rat embryonic fibroblast cells is a measure for the cell adherence and how compatible the surface is to the fibroblast cells. The cell adherence on the surface is good if the surface is compatible to cells. In this case the fibroblast cell morphology is roundish, while it is elongated and stretched if the surface is incompatible to the cells.

The morphology of the cells presented in Fig. 2 (a) is highly elongated and stretched indicating that the cells try to minimize the contact area to the surface. Compared to the cells on the glass cover slide [see inset of Fig. 2 (a)], the cells on the group-1 Ti sample surface have low cell adherence.

Figure 2 (b) shows the cells grown on the Ti surfaces belonging to the group-2 samples. The number of cells on the surface of group-2 Ti samples is higher compared to the group-1 samples. The cells grown on these surfaces are in average very broadly spread in contrast to group-1 sample surfaces. This indicates that the cell adhesion is increased for the surface of group-2 Ti samples. The cell morphology is quite similar to the cell morphology of the reference sample, although the cells tend to have even spread better on the group 2 surface.

Figure 2 (c) shows the shape of the cells on the surface of the group-3 Ti samples. The cells are broadly spread, and they form filapodia. Compared to the cell shape of the reference sample, there is hardly any difference detectable in the cell shape.

Figure 3 presents the average numbers of cells on the three differently processed Ti samples. The group-1 (chemically etched and HA coated) samples exhibit the smallest number of cells on the surface (about 2100) of all three groups. This is most probably due to the sharp and highly cornered spikes on this surface, so that the cells try to minimize their contact area [see Fig. 2 (a)].

The highest number of adhering cells (about 3200) is found for the group-2 samples, being chemically etched, post-etched, and HA coated. This means the additional post-etching step results in an increase of the cell proliferation by almost 50%. Thus, it seems beneficial for the cell adhesion to smoothen sharp and highly cornered features on the surface, which is well known for other surfaces/materials as well. This result also shows that post-etching is a suitable way to not only increase the cell adhesion, but also to stimulate cell proliferation on the Ti surface.

With about 2700, the mean number of cells on the surfaces of group-3 samples lies in between the results obtained for group-1 and group-2 samples. This means, the HA coating improved the cell adhesion and increased the number of cells on the surface by about 18%.

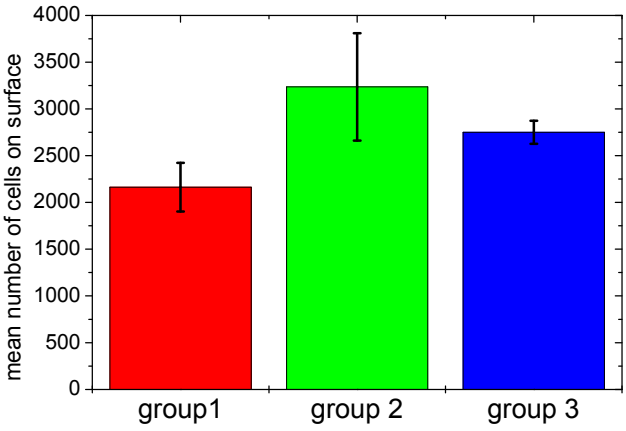

Fig.3 Mean number of cells grown on each group of Ti sample surface (group 1: chemically etched and HA coated, group 2: chemically etched, post-etched, and HA coated, and group 3: chemically etched and post-etched). The standard deviation is given by the error bars.

SUMMARY & CONCLUSION

In this work a two-step etching process consisting of chemical etching and subsequent post-etching of Ti surfaces is presented. The additional post-etching step has a high positive impact on the cell adhesion at the surface. It turns out that a high surface roughness is not beneficial in general, but smoothening of sharp and highly cornered features on the surface is more suitable for a good cell adhesion. On the other hand this also means that by choosing the right etching parameters a rather cell incompatible surface can be turned into a compatible surface. It is also shown that an HA coating of the chemically etched and post-etched surface is highly beneficial for the cell adhesion.

Nevertheless, there are indications that it is still possible to improve the cell adhesion on the Ti surface by further modifications of the etching processes.

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