NANOSCALE-TEXTURED Ti6Al4V SURFACE ENHANCED THE FRICTION FORCE AND MINERALIZED NODULE FORMATION OF RAT BONE MARROW CELLS

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INTRODUCTION
Acid etched grit-blasted titanium (Ti) has been shown to improve bone apposition and push-out strength in vivo [1]. More interestingly, hot acid etched (Ti) beads coated implants was found to increase the bone apposition by more than 50% compared to the smooth beads [2]. This indicates that the added microtextuere onto the individual bead enhances the bone formation. We are able to achieve nanoscale texture on Ti Porocoat® via mild chemical etch. It is known that Ti Porocoat® increases the friction force due to the macrotextuere presented by the beads. However, it is unknown that if the nanoscale texture can further improve the friction (or initial implant stability) of Ti Porocoat®. Ti beads are in general more complex coating and may demonstrate variability. It is thus prudent to start with flat substrate in order to extract mechanical and biological effects associated with nanoscale-textured surface.

The purpose of this study was to investigate the effect of the nanoscale texture (flat Ti6Al4V surface) on the mineralized nodule formation in vitro and the static friction coefficient.

MATERIALS AND METHODS

Cell culture
Polished Ti6Al4V disks (1” diameter and 0.125” thick) were chemically etched in an acidified sodium fluoride solution. The disks were Gamma irradiated before cell culture. Bone marrow cells from the femora of Wistar rats were prepared according to established protocols. 20mL of the cell suspension was added to a large cell culture dish (3.35” in diameter) and stored in a humidified incubator (37°C and 5% CO2). The medium was refreshed every 2-3 days. 7-day cell cultured disks were characterized by scanning electron microscopy (SEM) for cell morphology. Disks were also stained (Von Kossa) at two days from 10 to 16 days.

Friction test
Polished Ti6Al4V sleds (0.75” × 0.75” × 0.25”) were chemically etched under the same condition as the cell culture disks. Roughness of the polished and etched surface was characterized both by atomic force microscopy at 10 µm × 10 µm area (tapping mode, DI) and profilometer (Taylor Hobson Ltd.) with 4mm data length and 0.25mm cutoff. Centrally to the end of each sled specimen a 6-32 hole was drilled and tapped to allow a custom eyetlet to be screwed into the sled for the pulling apparatus. 5N, 30N and 50N of dead weight were applied to the sled. A 10” length of Modified CDA 274 EDM wire (Electrodes Incorporated, Milford, CT 06460) threaded through the eyetlet screw and the ends clamped into the gripping fixture attached to the 250 N load cell. The sleds were pulled by a MTS Tyton 250 machine at 47.2”/min (120mm/min) for 0.1” according to ASTM 1894-01. The supporting base was made of Last-A-Foam® (General Plastics Manufacturing Co., Tacoma WA, 98409) blocks as cancellous bone analogue. The static friction coefficient (µs) was calculated according to the equation µs = f/N, where N is the sum of dead weight and the sled; f is the static friction force defined by the first maximum load recorded in the load-displacement chart (arrows in Fig. 3A).

RESULTS AND DISCUSSION

Figure 1 shows the AFM images of the polished (A) and etched surface (B) at 10 µ in length (width) and 2µ in height. AFM pit analysis shows that most etch pits distant from each other from 0.3µ to 0.7µ with average peak spacing about 0.5 µ. AFM can obtain high-resolution 3-dimensional (3d) topography of a surface at 10µ to 50µ range. Characterization of surface at this scale with 3d information is of great interest since it is commensurate to the size of an osteoblast (Fig. 2A and B). There is more than a ten-fold increase of surface roughness after etch, as measured by AFM (polished: 9 ±4 nm; etched: 180 ± 39 nm) and contact mode profilometer (Fig. 1C). Though measured at micron scale (10 µm), the surface roughness estimated by AFM seemed to match the roughness measured by profilometer at a larger scale (4mm data length) pretty well. Verification of AFM with known standards show that Ra obtained by AFM agrees with the certified standards measured by 0.25µ-stylus within one standard deviation (standard values in µ: 0.038, 0.150; AFM in µ: 0.036±0.014, 0.168±0.033). This means that AFM can be a valid tool to obtain topographical information at micron scale.

Murrow culture study showed significant differences with respect to cell morphology and mineralized nodule formation dependent on topography (Fig. 2). The cells on the polished surface usually appear flattened with widely spread lamellipodia (Fig. 2A). The cells on the nanoscale textured surface are more spherical in shape with shorter filopodia (Fig. 2B). This unique difference in cell morphology may indicate different cellular activity dependent upon the topography of the substrate. Fig. 2C and D shows the Von Kossa staining on samples cultured for 12 days. The etched surface has a higher number of mineralization nodules (as indicated by black color) than the polished samples. The etched samples also show larger area of mineralization with higher intensity compared to the polished ones. Similar difference of Von Kossa staining was observed at 10 and 14 days, which are consistent with our previous observation.

Fig. 3A shows a typical friction force-distance curve of an etched sled (#1) and a polished sled (#2). Fig. 3B shows that the etched sled has more than 200% increment in static force compared to polished sled under various vertical load (5N, 30N and 50N). The increase of friction force is directly related to the increased surface roughness by chemical etch. The increased friction force may be beneficial to improve the initial implant stability.

CONCLUSIONS
A mild chemical etching in an acidified dilute sodium fluoride could effectively add nanoscale texture on polished Ti6Al4V surface and increased surface roughness (Ra) from 0.01 µm to 0.18 µm. The nanotextured surface with average peak spacing of around 300 nm could enhance the mineralized nodule formation by rat bone marrow cell and increase the static friction coefficient by more than 200%.

REFERENCES