Improved MC3T3 cellular growth on nano-grained 316L stainless steel obtained by linear-plane machining severe plastic deformation

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Statement of Purpose: Stainless steel is one of the most widely used biomaterials in orthopedic applications including bone fixation devices (e.g. screws, plates and pins), bone implants and artificial joints. Improvement of surface interaction with surrounding bone tissue increases the success and durability of bone implants. While current surface modification or coating methods enhance the interaction, these methods require multiple steps and sometimes are complicated to be produced. Also, delamination issues always exist during a long-term implantation. Several studies demonstrated that nanostructured surfaces enhance cellular adhesion and viability; therefore, we developed stainless steel samples with nanograin size structure to evaluate the surface adhesion of MC3T3 cells on them. A comprehensive surface characterization was performed on these samples to investigate the effect of grain refinement on wettability, surface roughness, mechanical properties and chemical composition.

Materials and Methods: 316L austenitic stainless steel samples with nano size grains were prepared under severe plastic deformation using linear plane-strain machining technique for three different tool angles (i.e. 0°, 20° and 40°) (Fig.1).

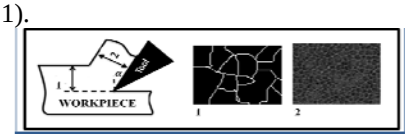

Fig.1. Schematic of plain strain machining technique (α is tool angle), 1) control sample 2) ultrafine grain sized sample produced after machining

Vickers Hardness of all samples was measured using micro-hardness tester. Oxide layer was grown on nanograined samples using chemical treatment technique (5M sulfuric acid+2.5M chromic acid at 70°C). Roughness, wettability, morphology and chemical composition of surfaces were characterized using optical surface profiler, contact angle measurement, scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS), respectively. MC3T3 cells were cultured on all samples for 24hrs and then were stained with Calcein AM. The viability, proliferation and morphology of MC3T3 cells adhered on surfaces were analyzed by both fluorescent optical microscopy and SEM.

Results: Hardness was significantly increased after refining grain sizes; increased from 194.3±21.7 in control sample to 460.5±56 in 0° chip, 383.7± 83 in 20° chip and 310.4±59 in 40° chip. Figure 2 shows representative images for 0° chip. The surface roughness is in the range between 3-10nm (Fig. 2A) and wettability shows 61±3.39° (Fig.2A right). XPS spectra demonstrated similar peaks in both control and nanograined samples indicating existence of the same oxide composition on their surfaces (i.e., four main elements of Cr, Fe, Ni and Mn at 576.7ev, 710.8ev, 641.2ev and 854ev, respectively). Hence, control and nanograined samples showed almost identical surface conditions such as roughness, wettability and chemistry. Chemical surface treatment was conducted in order to compare the grown oxide layer on samples with different grain sizes. The grown oxide layer exhibited a superhydrophilic surface after the chemical treatment and the surface roughness was substantially increased due to the etching process of nanograin boundaries (Fig. 2B).

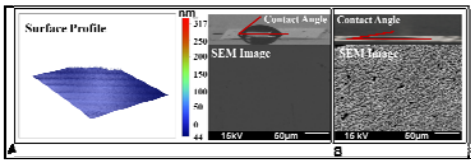

Fig.2. A) Surface characteristics of 0° chip including surface profile, SEM image and contact angle; B) SEM image and contact angle of 0° chip after 1hr chemical treatment

MC3T3 cell adhesion test results showed greatly improved cell adhesion and growth in 0° sample; fluorescent intensities of stained cell as an index of live cells were 420±21, 590±18, 610±5 and 650±45 in the control, 40°, 20° and 0° samples, respectively. For surface treated samples, similar to untreated samples, finest grain size demonstrated the highest cell viability (i.e. 520±30, 620±44, and 650±52 intensities for 40°, 20° and 0°, respectively). Though no significant difference was observed between cell viability after surface treatment, cells on treated samples (i.e. rough surfaces) showed a different morphology; they didn't spread completely and had a polygonal shape with numerous podia on their surfaces (Fig.3B3).

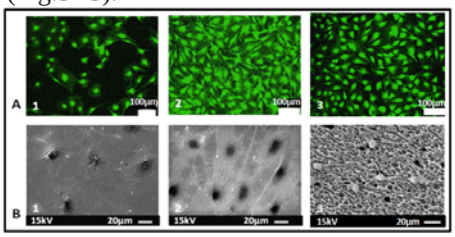

Fig.3. A) Fluorescent images of Calcein AM stained MC3T3 cells, B) SEM images on: 1) control sample, 2) 0° chip surface 3) surface-treated 0° chip

Conclusions: A novel nanograin surface produced by a severe plastic deformation technique showed a significantly improved MC3T3 cellular behavior. The nanograin surface with the grown oxide layer after chemical treatment increased the number of cells adhered on the surface. The improvement of cellular attachment and growth on nanograined samples indicates that this method presents a potential platform technology for the improvement in the biological function of metallic biomaterials.