

## Enhanced Attachment and Proliferation of Fibroblasts on Anodized 316L Stainless Steel with Nano-pit Arrays

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**Introduction:** It is now recognized that surface nanostructures on biomaterials greatly influence cellular behaviors as well as surface biochemical properties<sup>[1,2]</sup>. Nano-featured surfaces might be used as an excellent bioactive interface for implantable materials. Stainless steel is one of the most important alloys for biomedical applications. However, their bioinert surface characteristics are disadvantageous for numerous applications. The aim of this study was to fabricate nano-structured surfaces on 316L stainless steel by an anodization method and examine the effects of these anodized nano-surfaces on the attachment and proliferation of fibroblasts.

**Materials & Methods:** 316L stainless steel (2.5 cm × 2.5 cm × 0.5 mm) (Goodfellow Cambridge Ltd. England) was anodized at 30 and 40V for 10 min in ethylene glycol containing 5 vol. % perchloric acid to create bioactive surfaces. The surface morphology and elemental composition of 316 L after anodization were characterized by scanning electron microscopy (SEM, Hitachi S-4800, Tokyo, Japan) and X-ray photoelectron spectroscopy (XPS). Human fibroblasts (ATCC, CCL-110) were seeded on the samples to evaluate initial cellular responses to the nano-structured surfaces. The anodized surfaces were assessed for their *in vitro* cell-material interactions using fibroblast cells for 4 h, 1 and 3 days. The attachment and proliferation of fibroblasts were determined by a methyl thiazolyl tetrazolium (MTT) assay. Smoothly polished 316L was used as control surface. Light microscopy and SEM were used to observe the morphology of cells. Five specimens of each material were tested for each incubation time and each test was performed in triplicate. Data were analyzed statistically using student t-test.

**Results and Discussion:** Nano-porous structures on 316L stainless steel were prepared by the anodization technique. The pore size could be adjusted and ranged from 0 to 60 nm. After 4 hours of culture, the fibroblasts adhered well on the surfaces of all the stainless steel specimens. SEM images showed that fibroblast exhibited normal morphology and were able to penetrate into pits by long filopodia. MTT tests indicated that the

anodization of 316 L stainless steel significantly promoted a higher degree of fibroblast attachment and proliferation as compared to the polished 316L stainless steel. Specifically, compared to unanodized (that is, nano-smooth,) and smooth surfaces, 50 and 60 nm diameter nano-pit surfaces enhanced dramatically the initial fibroblast attachment and growth for up to 72 hours in culture.

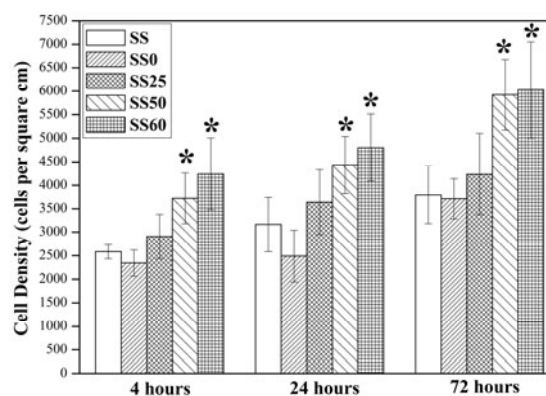


Fig.1 Fibroblast adhesion and proliferation on 316L stainless steel (SS) and anodized 316L stainless steel with different pit sizes. Data were represented as Mean ±SD, n=5. Both substrates were seeded with  $5 \times 10^3$  cells/cm<sup>2</sup>,  $p < 0.05$  compared to the SS at the same time period. (316L stainless steel with tunable pit sizes were denoted as SS0, SS25, SS50 and SS60, respectively, according to the pit sizes on the SS surfaces)

**Conclusions:** In this study, 316L stainless steel (SS) substrates with defined pit sizes ranging from 0 to 60 nm were prepared by an anodization technique. Such anodic surfaces were recognized by fibroblasts. In particular, nano-pit surfaces enhanced fibroblast attachment and proliferation dramatically when the pit sizes were 50 and 60 nm. These results suggest that fibroblast responses may be controlled and optimized by varying the pit size of anodized SS, and the nano-pit surfaces can be designed to support the growth of fibroblasts, and thus, might be designed as bioactive interfaces for numerous percutaneous implantable materials.

### References

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2. Ercan B, Taylor E, Alpaslan E, Webster TJ. *Nanotechnology* 2011; 22: 295102.