SURFACE NANOPATTERNING TO CONTROL CELL GROWTH

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Modifying the structure of materials at the nanoscale frequently allows to tailor and optimize their properties for various applications [1]. In the context of implantology, an important challenge is the design of biomaterials that actively promote functional regeneration of the host tissue while avoiding undesirable tissue responses. The key to designing improved biomaterials lies in controlling the interactions at the material-host tissue interface. This requires selective control of interactions at the tissue/implant interface, the site of complex events that depend on interrelated parameters including surface chemistry, topography and energy. However, specific surface features and properties which optimize cellular reactions are still poorly understood.

Early efforts focused on defining how microtexture influences the molecular and cellular events of tissue repair. However, it has been recently recognized that biological substrates on which cells thrive consist of nanostructured molecular networks, and the sensing apparatus of cells operates at the nanoscale. Many studies have shown that nanometer-scale surface features can influence cellular adhesion and differentiation.

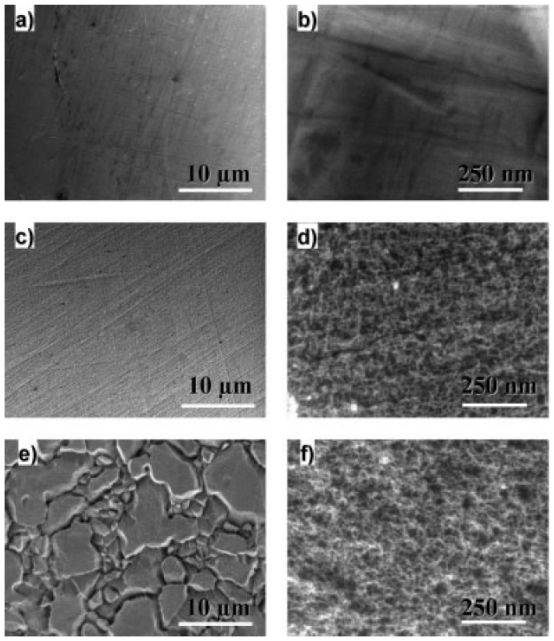

Figure 1. a)–f). Scanning electron micrographs showing low-resolution (a, c, e) and high-resolution images (b, d, f) of untreated (a, b) polished Ti6Al4V surfaces, and surfaces following exposure to H2O2/H2SO4 for 1 h (c, d) and 20 h (e, f).

Our strategy consists in creating molecular and topographic nanopatterns that act as surface cues [2] and affect cell behavior, either by promoting or inhibiting cell growth. In particular, we illustrate a simple chemical treatment for titanium-based materials that produces a unique nanostructured topography [3]. Our work takes an important further step by showing how simple chemical treatment can generate multifunctional nanostructured surfaces that selectively control cell growth. These surface modifications promote the growth of certain cells while inhibiting that of others, without the addition of any exogenous biological or pharmacological agents such as e.g. growth factors. More specifically, nanostructured Ti surfaces selectively inhibit fibroblastic cell growth [4], promote osteogenic cell activity [5] *in vitro* and enhance contact osteogenesis *in vivo* [6].

Our simple chemical etching technique using a mixture of H_2SO_4/H_2O_2 (Piranha solution) can uniquely generate spongelike networks of nanopits within the surface layer of titanium-based metals.

These nanoporous surfaces promote both early and longer-term osteogenic events in cell cultures, thereby imparting bioactive properties to the materials. These intriguing results led us to study in detail how the nanoporous surfaces are created and how they affect various cell types. Here we focused on

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using the Piranha solution and examined the effects of experimental conditions with two objectives: (1) to modulate the formation of nanopores on titanium alloy (Ti6Al4V), and (2) to control the growth of common cell types on this widely used implant metal.

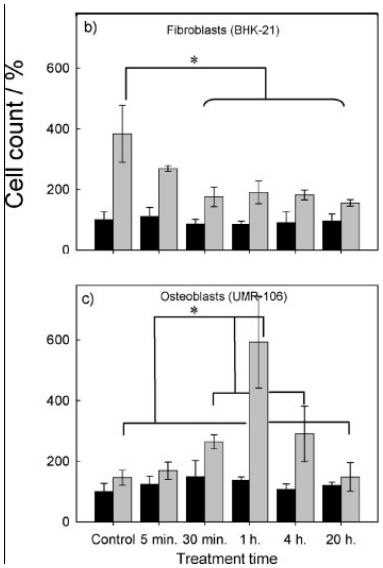

Figure 2. Evaluation of cell count by light microscopy (objective 10 X) after 6 h (black bar) and 3 days (gray bar) on different treated Ti6Al4V substrates (and control) for three different cell lines: smooth muscle cells (SMC) (a), fibroblasts (b), and osteoblasts (c) expressed as % of control count at 6h (* $p<0.05$)

The rationale for using the Piranha solution to nanotexture metals was to first etch the surface with an acid and then reoxidize it in a controlled manner. This chemical etching procedure creates a reproducible spongelike network of nanopits on their surface, with a range of micro- and nanotopographies. When analyzed by scanning electron microscopy (SEM) at low magnification, the machined metal surfaces of untreated controls, polished to a mirror finish, exhibited only shallow grooves resulting from the machining process (Fig. 1a). Moreover, at high magnification, they did not reveal any nanotopographical features (Fig. 1b).

We used these modified surfaces to investigate their ability to influence different cell lines. Osteogenic cells were evaluated because they are critical for the successful integration of implants in bone; Fibroblastic cells were chosen because formation of a fibrous capsule weakens the bone/implant interface, ultimately requiring their replacement.

To determine the influence of nanoporosity on cellular growth, we measured cell adhesion and short-term cell growth for two different cell lines.

The counts obtained revealed that the cell types examined respond differently to nanostructured Ti6Al4V (Fig. 2). After 6 h, the number of fibroblastic cells on treated surfaces was similar to that on controls, but after 3 days of growth it was significantly lower (Fig. 2b). Thus the nanotexture generated by Piranha solution treatment has no effect on the adhesion of fibroblastic cells and in fact limits their growth. This result suggests that nanotextured surfaces can be used to limit the growth of fibroblasts and can thereby retard the undesirable formation of fibrous capsules around implants.

Cell counts at 3 days showed that our treatment promotes the growth of osteogenic cells, with the strongest effect for 1 h of treatment (Fig. 2c). As the treatment time was lengthened, the resulting increase in microscale surface roughness limited cell growth. The inhibitory influence of microtexture was apparent on surfaces treated for 20 h, whereby the benefits of nanotopography were cancelled by microroughness, and cell density returned to the levels of controls at early culture intervals.

The ability to control nanoscale features and further functionalize substrates [7] will lead to a new generation of implantable biomaterials with “intelligent surfaces” that selectively influence cell behavior at the tissue-biomaterial interface.

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