Gradient Surface Wettability Induced by Nanofilms on Titanium Surfaces and Osteoblastic Cell Morphology

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Introduction:

To reduce healing time and enhance osseointegration of bone implants, it is important to engineer and optimize ideal surface properties [1]. Surface properties of implanted materials can directly influence cellular response at the bio-interface between the living tissue and the outermost surface layer of orthopaedic and dental implants [2]. The surface properties that we focused on for this project were: surface roughness and wettability.

The aims of this project were to develop a coating method to control surface wettability without altering the complicated micro- and submicro-scale surface roughness, and to examine how surface wettability influences cell morphology in a time-dependent manner.

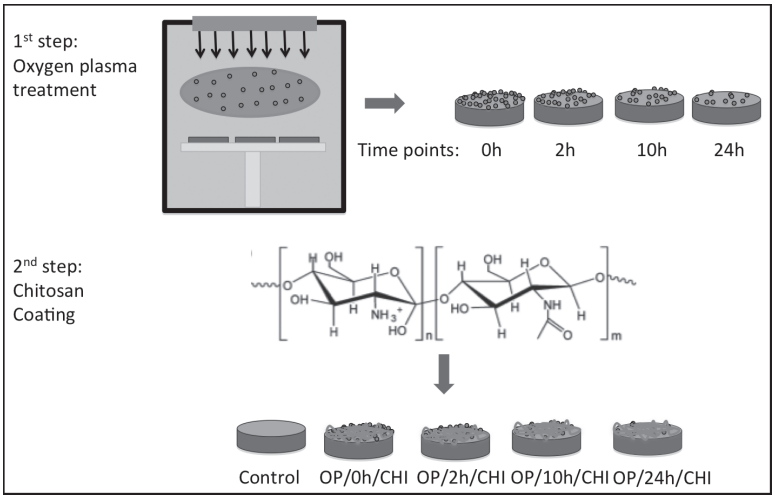

Figure 1: Experimental process for modifying the samples' surface.

Experimental Procedure:

Sand-blasted/acid-etched titanium (SLA) disks were oxygen plasma (OP) treated for two minutes on each side. Right after oxygen plasma treatment, the surface energy is increased and unless the surfaces are coated, they continuously lose surface energy until they are stable again. Using this fact, each disk was coated with a polyelectrolyte called chitosan (CHI) for 30 minutes after oxygen plasma treatment (0, 2, 10, and 24h). This process is shown in Figure 1.

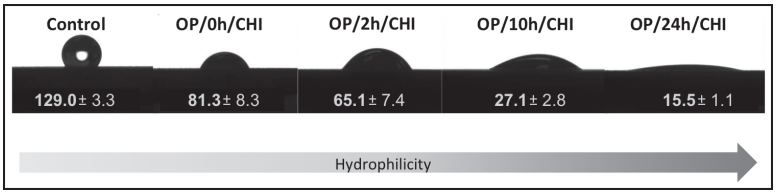

Figure 2: Gradient in contact angle measurements. Values are given in degree measurements.

Results and Discussion:

Surface roughness was measured using confocal laser microscopy (CLM). Oxygen plasma-treated and then CHI-coated SLA surface roughness was not significantly different compared to control SLA surfaces.

Contact angle measurements were made between a drop of water and the surface of the disks to find the wettability of the samples' surfaces. We were able to develop a gradient in surface wettability, increasing the hydrophilicity of the material between each sample, as shown in Figure 2.

Surface chemical composition was analyzed using x-ray photoelectron spectroscopy (XPS). Nitrogen was detected on CHI-coated SLA surfaces except on the control SLA

Groups	Ti2p	O1s	C1s	N1s
Control	20.1	52.9	27.1	-
OP/0h/CHI	11.2	52.5	32.7	3.6
OP/2h/CHI	9.4	43.9	42.0	4.7
OP/10h/CHI	11.4	45.9	36.7	6.0
OP/24h/CHI	11.0	52.1	29.5	6.2

Figure 3: Chemical composition percentage of the samples.

surfaces. The treated and coated groups had a gradient in nitrogen composition. The first group to be coated had the least nitrogen in its composition and the last group that was coated had more nitrogen in its composition. The change in wettability between each sample could be explained as a function of the nitrogen content. The Table of the chemical composition of the surfaces is shown in Figure 3. A high-resolution analysis was done to determine if the wettability gradient was due to the quantity of NH_3^+ in the samples. The results showed that the NH_3^+ content increased as the surface was more hydrophilic.

MG63 osteoblast-like cells were plated on surfaces with different wettability to see the cell morphology on top of each sample's surface. After 1.5, 3, 6, 12, and 24h incubations, cells were fixed, dehydrated, and dried with a critical point dryer. Cell morphology was examined using scanning electron microscopy (SEM).

SEM imaging demonstrated that during the initial cell-material interactions, cell shapes, whether they were elongated or rounded, depended on surface roughness and surface wettability. When the cell morphology was evaluated, it was found that cells in the control group elongated through the surface, while cells in the surfaces that were coated kept a rounder shape in the first 12 hours of cell growth and elongated at 24 hours. This indicates that surface wettability is important for cell morphology in the first 12 hours of cell growth.

At 24 hours of cell growth, all of the groups had elongated cell morphology, demonstrating that as time passes roughness guides cell morphology. The SEM images for the control and one of the groups are shown in Figure 4.

Conclusions:

Our results showed that: we successfully developed a method to control surface wettability with the same polyelectrolyte without modifying the micro-scale surface roughness. MG63 cell shapes are sensitive to surface wettability at an early time point. As time passes, the surface's roughness guides cell growth.

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References:

[1] Park J.H., Schwartz Z., Olivares-Navarrete R., Boyan B.D., Tannenbaum R.; Langmuir. 2011.

[2] Boyan, B.D.; Hummert, T.W.; Dean, D.D.; Schwartz, Z.; Biomaterials. 1996.

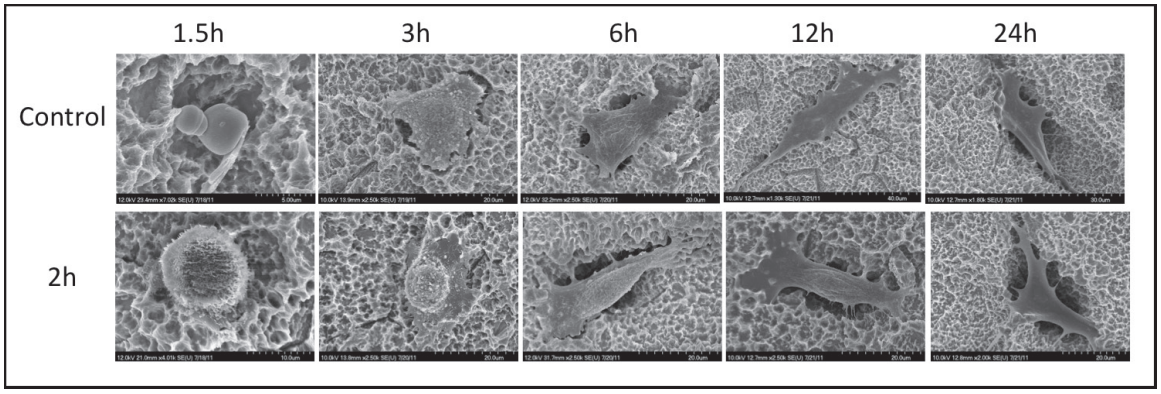

Figure 4: Scanning electron microscopy images of the cell morphology.