

Bioengineered Device Imaging in the ORION® PLUS

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Application

The development and study of engineered surfaces that are designed to promote tissue compatibility in the body.

ORION® PLUS Capabilities

High resolution imaging, charge neutralization, non-destructive imaging.

Background

Biomedical devices are designed to replace lost functionality of the human body. A consideration in creating such a device is that it must be incorporated successfully alongside tissues in the body. Biomimetics addresses this need by the application of function and design found in nature to engineering technology. By mimicking and even improving upon the chemical and morphological features of the naturally occurring cellular environment, tissue engineers are able to fabricate structures which elicit a positive cellular response and promote tissue growth. Charged particle microscopy serves in biomimetics studies by providing high magnification images of engineered structures and surfaces.



Challenge

There are three main hurdles to overcome in order to successfully capture surface and structure information. The first is to get high resolution, including surface sensitivity, in order to investigate the nano-scale features that mimic real tissues. The second is the need to overcome the serious charging that occurs with the insulating polymers, minerals, and cellular material that comprise the devices under investigation. Conductive coatings are often used to circumvent this problem, but these obscure the very surface that is under study. Variable Pressure SEM offers charge neutralization, but at the cost of resolution, for the gas environment causes scattering in the primary beam. Low energy SEM can reduce charging, but it is difficult and sometimes impossible to find a stable beam energy at which all the materials and surfaces are in charge balance. In addition, low energy operation sacrifices resolution. The third challenge is the electron beam sensitive nature of many polymers, which causes them to degrade, sometimes drastically, during imaging.

ORION® PLUS Solution

The helium ion microscope (HIM) can address all three of these challenges. With a demonstrated 0.25 nm probe size performance, the tool provides the highest surface resolution of any microscope. Charge neutralization is provided through the use of a low energy electron flood gun. This device can maintain charge balance on the sample during the imaging process. This procedure is possible because of consistent positive charging: when imaging with an ion beam, the incoming particles are positively charged and the outgoing secondary electron (SE) signal is negative. .



One can reliably use an electron flood beam to reduce surface potential variations, thus obtaining consistent image intensity. Third, sample sensitivity is often observed to be lessened for a primary helium ion beam as compared to an electron beam. The physics of the sample interactions are very different, as can be observed in the images. We illustrate these capabilities through an example, as laid out below.

We are looking at an application related to bone tissue engineering. The work here is part of the ongoing study by Ian Smith and co-workers at the University of Michigan's Biologic and Materials Sciences department. They have been developing biomimetic structures that support bone mineral uptake. By combining a polymer scaffold with a ceramic component like hydroxyapatite (HA), they mimic the mineralization of collagen in the extra cellular matrix (ECM) and create a bio-composite scaffold for bone tissue engineering. In the case at hand, this polymer scaffold was composed of poly(L-lactide) (PLLA). The scaffold is mineralized by soaking it in simulated body fluid, which mimics the ionic concentrations in the body. The imaging task at hand is to see the earliest stages of uptake of HA onto the PLLA.

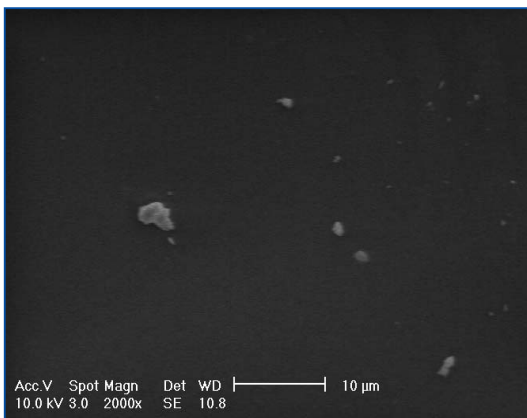


Figure 1: SEM image of PLLA/HA surface after 3 hours of mineralization. 10 keV beam energy.

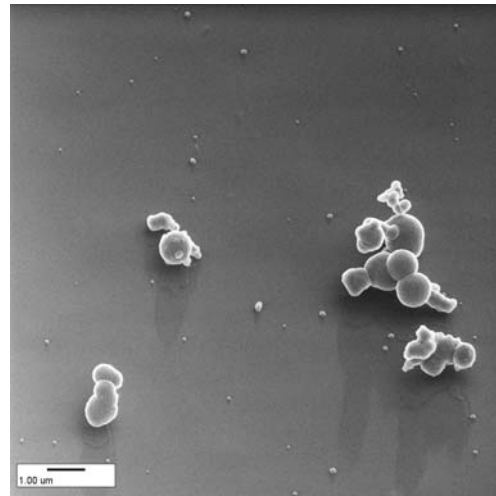


Figure 2: HIM image of PLLA/HA surface after 3 hours of mineralization.

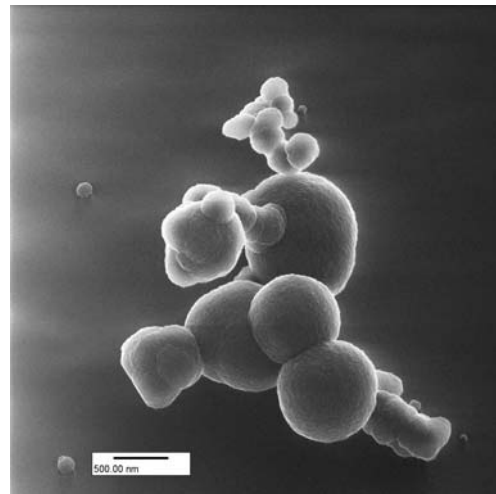


Figure 3: HIM image of PLLA/HA surface after 3 hours of mineralization; 25 kX magnification.

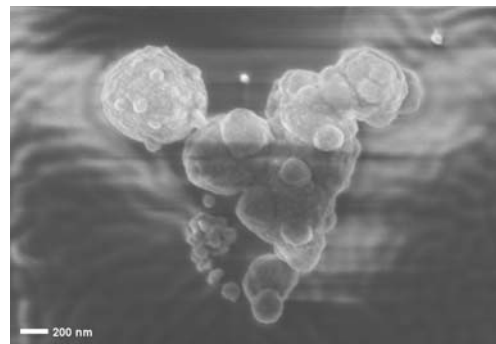


Figure 4: Low voltage (2.5 keV) SEM imaging of PLLA/HA

PLLA/HA samples were prepared by spin-coating a polymer solution onto glass and then treating them in the simulated body fluid for a period of either one hour or three hours. Initial inspection by high voltage SEM after three hours of treatment (Figure 1) was not promising. It was not possible to get high magnification imaging. A contrasting HIM image at 8.8 kX is shown in Figure 2. The surface detail is very clear, both for the PLLA surface and the deposited material. These deposits are likely HA and further analysis is underway to verify this. One can obviously see the smaller nucleation sites of the mineral all around the four larger agglomerations that are captured. In Figure 3 we see an image at 25 kX magnification, centered on one large cluster. The growth of HA spheres one upon the other can be observed in detail, and even the fine surface texture on the mineral surface shows clearly. We also tried to optimize SEM imaging, utilizing a low energy SEM. An example is seen in Figure 4. There are brightness fluctuations across the image, horizontal streaks, and a loss in resolution – the surface appears soft. The polymer surface in particular looks poor and has taken on topography. This last artifact is seen more clearly in Figure 5, which shows the polymer surface now with severe erosion. This is understood by realizing that one phase of the polymer is being damaged more heavily by the electron irradiation. Since the electron dose per area increases quickly at higher magnification, it would not be feasible to attempt high resolution inspection of the surface or the HA nucleation process. The PLLA, however, is more resilient under helium ion beam imaging. We can see this in Figure 6, which shows the early nucleation stage of HA. The surface of the polymer has remained completely flat during the image acquisition. We can see that this 70 nm particle does not grow with maximum contact area to the substrate, but there is about a 30 nm wide contact area. Such information could not be obtained if the surface were textured by imaging. We can look even earlier into the mineralization process by imaging samples that had been treated for just one hour. No large spheres of mineral are found at this early stage of the process. Figure 7 reveals many 400-500 nm crystallites but also the continuing formation of new nucleation sites. Figure 8 shows growths down to about 60 nm in diameter.

We can see from this illustration how the combination of high resolution, charge control, and damage-free imaging enables studies such as this in bioengineering. This trio of qualities is unique to helium ion microscopy and makes it possible to learn new information about the formation of biomimetic structures. We thank Ian Smith and his colleagues for the samples and descriptions discussed here.

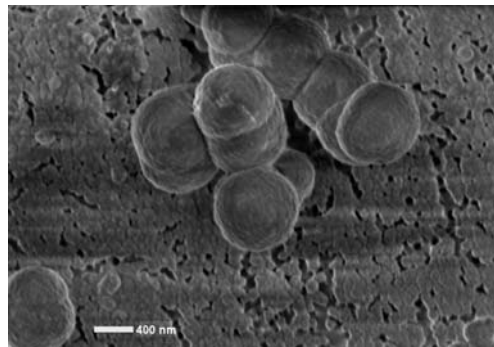


Figure 5: Low voltage (1 keV) SEM imaging of PLLA/HA.

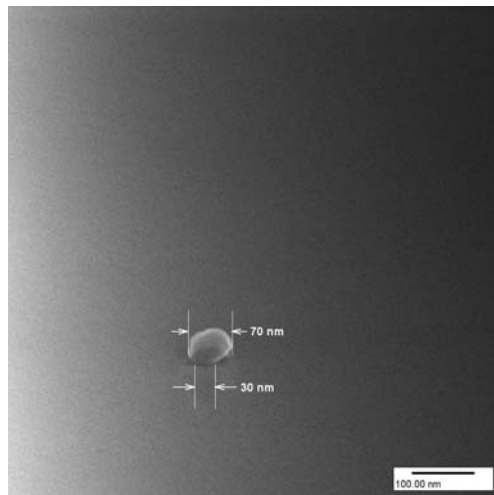


Figure 6: HIM image of early HA nucleation on PLLA surface after 3 hours of mineralization; 143 kX magnification.

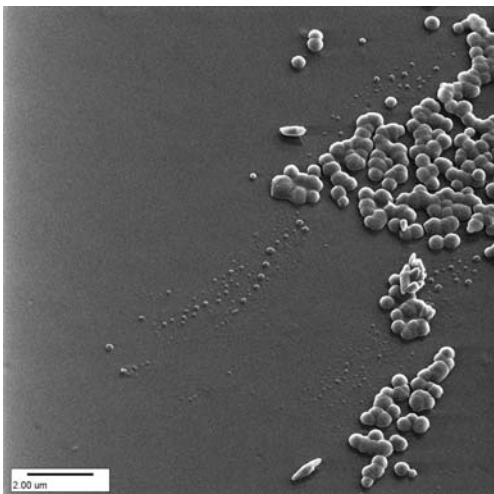


Figure 7: HIM image of PLLA/HA surface after 1 hour of mineralization.

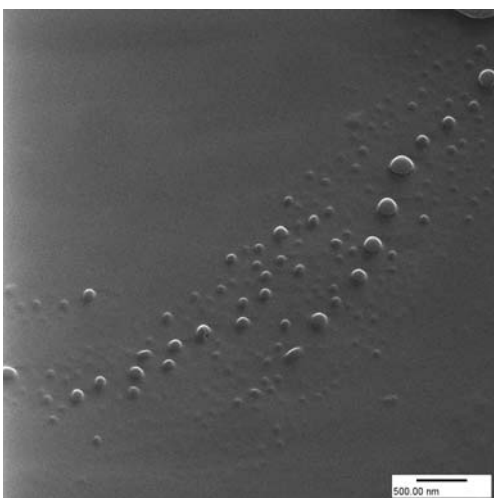


Figure 8: HIM image of PLLA/HA surface after 1 hour of mineralization, 23 kX magnification.



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