Electrochemically-assisted Functionalization of a 316L Stainless Steel Surface: Towards the Minimization of In-stent Restenosis

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Introduction: Occlusive coronary artery disease (CAD) is reported to be the single largest cause of death in developed countries, such as Canada and United States. The disease is characterized mostly by the formation of a plaque, resulting in considerable narrowing and hardening of the vessels. Approximately one third of CAD patients are treated by angioplasty and stenting[1]. However, all current bare-metal stents offer low biocompatibility, which results in occurrence of in-stent restenosis in up to ca. 30% patients, and the need for further medical treatment. Therefore, various stent-surface modification approaches have been employed in order to minimize in-stent restenosis, but none of them have proven to give long-term satisfactory benefits. Thus, the aim of this work was to develop a new method for the stent surface modification in an attempt to decrease the in-stent restenosis rate. We developed a novel electrochemistry-based method for the modification of a 316L stainless steel surface, which allows us to functionalize the surface with a (bio)molecule of choice[2]. The ultimate aim was to functionalize a 316L SS surface with a chemically-bound extracellular matrix protein fibronectin (FN), and to control the protein's surface confromation, and thus endothelial and smooth muscle cells / FN interactions. However, in order to irreversibly immobilize FN on the 316L SS surface, one needs to form a stable and durable chemical "linking" (mono)layer which was the major aim of the work to be presented.

Methods: Binding of FN to 316L SS was done by first electrochemically forming a COOH-terminated self-assembled monolayer (SAM) of mercapto-undecanoic acid on the 316L surface, followed by chemically binding FN to this SAM. Polarization modulation infrared reflection absorption spectroscopy (PM-IRRAS), contact angle (CA), and electrochemical impedance spectroscopy (EIS) were used to characterize the SAM and FN layer. In order to assess the stability of the SAM and FN monolayer, sonication in 0.1M NaOH and long-term immersion in 0.16M NaCl phosphate buffer (PBS), pH 7.4, was performed. To verify the success of the FN-surface modification method in increasing the 316L surface biocompatibility, attachment and proliferation of endothelial cells (ECs) was investigated.

Results: Electrochemical formation of the SAM on a bare 316L surface was monitored *in-situ* by recording current-potential curves. With the increase in SAM surface coverage, the hydrogen evolution current decreased as the result of surface blocking by the SAM. PM-IRRAS spectra showed the presence of peaks at 2922 cm^{-1} and 2850 cm^{-1}, corresponding to the asymmetric and symmetric stretching of methylene groups of the SAM, respectively. The position of the former peak revealed that the formed SAM is relatively ordered. CA measurements showed an initial 20-degree increase in receding contact angle when the SAM was formed on the surface, relative to the bare surface. PM-IRRAS (Fig. 1) and CA measurements demonstrated that the formed SAM remained stable over a period of eight days of constant immersion in corrosive PBS, and that the difference in contact angle increased to 50 degrees.

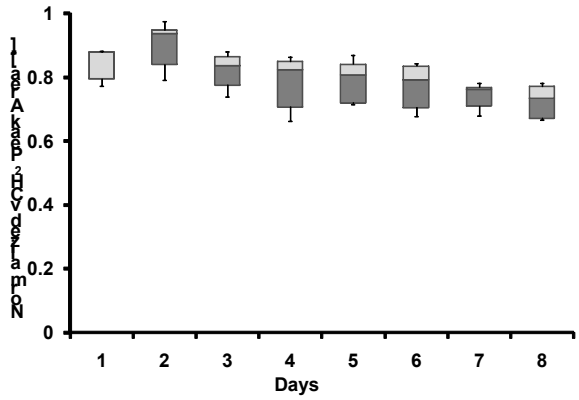

Figure 1. Normalized integrated intensity of methylene spectra of a COOH-terminated SAM formed on a 316L SS surface, monitored over a period of eight days of constant immersed in aqueous PBS, pH=7.4.

Long-term EIS experiments demonstrated that the formed SAM also provides an excellent diffusion barrier for aggressive ions, yielding very high corrosion protection efficiency, ca. 82%. Chemical attachment of FN to this SAM was successful, as evidenced by PM-IRRAS spectra. The formed FN monolayer was stable even after aggressive sonication in 0.1 M aqueous NaOH, while FN physisorbed on the same surface was completely removed by sonication. Preliminary EC/surface attachment experiments revealed that the FN-modified surface promoted the EC attachment by ca. 25%, which indicates that the FN-modified surface is more biocompatible than a bare 316L surface.

Conclusions: A highly stable and chemically reactive COOH-terminated alkanethiol monolayer was formed on a 316L surface using an electrochemical method developed in our laboratory. Further chemical modification of this layer by FN yielded a surface that was demonstrated to be a significantly better substrate for EC attachment than a bare 316L surface. The presented surface modification is suitable for not only FN binding, but for binding of a range of other (bio)molecules (antibodies, drugs, NO-releasing molecules, polymers, etc.).

References:

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