THE INTERACTION OF HUMAN OSTEOBLAST-LIKE Saos-2 CELLS WITH STAINLESS STEEL AND Si(100) COATED BY SILICALITE 1 FILMS

IVAN JIRKAa,* , MARTA VANDROVCOVAR, JAN PLšKA, LUCIE BAČÁKOVÁb

a Hegrovský Institute of Physical Chemistry, ASCR, v.v.i. Dolejškova 3, 182 23 Prague 8, Czech Republic
b Institute of Physiology, ASCR, v.v.i. Vídeňská 1083, 142 20 Prague 4, Czech Republic
* corresponding author: ivan.jirka@jh-inst.cas.cz

ABSTRACT: Interaction of osteoblast-like Saos-2 cells with polished stainless steel coupons and Si(100) wafers covered by a film of densely intergrown a, b oriented silicalite-1 crystals is investigated. Due to their unique chemical, mechanical and biological properties these films are promising new anti-corrosive coating for bone replacement. Biocompatibility of silicalite 1 film is affected by its morphology and wettability. The surface properties of silicalite 1 film is controlled by synthesis conditions and post-synthetic modifications. The morphology of the film (number of a oriented crystals) is tuned by time of synthesis. Wettability of the film is tuned by heat treatment in a stream of air at 300 °C. The silicalite 1 film is proved to be biocompatible. The number of adhered Saos-2 cells increases with the number of a oriented crystals observed for the film grown on stainless steel and with increased wettability of heat treated samples. Obtained results demonstrate possible ways of preparation of anti-corrosive silicalite-1 coatings with optimized cytocompatibility.

KEYWORDS: Biocompatibility, silicalite-1 film, outer zeolite surface, wettability.

1. INTRODUCTION

Metals and metal alloys are nowadays the most frequently used implant materials [1]. Their substantial drawback is the evolution of metal species into the body. Great attention has thus been paid to developing suitable surface modifications, which do not lower the biocompatibility of the material and simultaneously suppress evolution of the metal species. Silicalite-1 coating (SC) has been found to be prospective in this area [2].

Various physical-chemical parameters, including wettability and morphology, influence the biocompatibility of prosthetic materials [3]. Possibility the optimization of these parameters by the mode of synthesis and post-synthetic treatments of SC to get its maximal biocompatibility is demonstrated in this paper. Our preliminary data of tuning the wettability and morphology of SC by selection of support and the mode of synthesis are presented.

2. EXPERIMENTAL

The SCs were synthesized in situ on the polished surface of stainless steel foil and Si(100) wafer from reaction mixture of tetrapropyl-ammonium hydroxide (TPA, 1M solution in H2O, Sigma Aldrich), tetraethylorthosilicate (TEOS, ≥ 99.0 %, Aldrich) and deionized water as described in [4]. The reaction mixture was aged for 2 hours. The synthesis proceeded 3 hours (SC on Si(100), referred as to SC-Si(100)) and 6 hours (SC on stainless steel, SC-ss) at 165 °C. The supports and synthesis conditions used enabled the preparation of the samples with substantially different surface morphologies. A portion of the synthesized samples were heated at 300°C in a stream of dry air. The heat-treated samples were abbreviated as SC-Si(100)-300 and SC-ss-300.

Static water drop contact angle θ measurements using the SEE system (Brno, Masaryk University, Czech Republic) were utilized to characterize the wettability of SC. The morphology of the samples was characterized by near-field scanning electron microscopy (FESEM, S 4800-1, Hitachi).

The human osteoblast-like Saos-2 cells were seeded on the sample surfaces as described in our earlier study [5]. Briefly, the samples were sterilized in a hot air sterilizer (2 hours, 120 °C), inserted into 24-well cell culture plates, and seeded with the cells at a density of 15,000 cells/cm² and in 1 mL of McCoy’s 5A medium with 15 % fetal bovine serum and 40 μg/mL of gentamicin.

3. RESULTS AND DISCUSSION

SEM images of the samples SC-Si(100) and SC-ss are depicted in Figure [1] While the SC-Si(100) was mostly composed from a compact layer of intergrown b oriented crystals covered by a small number of a oriented crystals, a high concentration of a oriented crystals on compact layer of intergrown b oriented crystals was observed on the SC-ss. No morphological changes were induced upon heating.

Typical results of water drop contact angle measurements are summarized in Figure [2] The θ values of SC-ss and SC-Si(100) were comparable; i.e. 84°± 3° and 66°± 2°, respectively. Heat treatment induced
Figure 1. SEM image of $SC-Si(100)$ (top) and $SC-ss$ (bottom). Left: a centre of the films; left, right: a periphery of the films.

Figure 2. Water drop contact angle of $SC-Si(100)$ (top) and $SC-ss$ (bottom) before (left) and after treatment at 300°C.
The interaction of human osteoblast-like Saos-2 cells

Figure 3. Immunofluorescence staining of vinculin, a protein of focal adhesion plaques (green) in human osteoblast-like Saos-2 cells on day 3 after seeding on SC-Si(100) (A) or SC-SS (B). The actin cytoskeleton is stained in red with phalloidin-TRITC, and the cell nuclei are stained in blue with DAPI. Leica TCS SPE DH 2500 confocal microscope.

substantial lowering of the $\theta$ value, which was similar for SC-ss and SC-Si(100), i.e., $32^\circ \pm 3^\circ$ and $43^\circ \pm 3^\circ$, respectively.

The morphology of the Saos-2 cells on the sample SC-ss and SC-Si(100) is shown in Figure 3. The cells on both surfaces are of similar morphology, i.e., they are well-spread and polygonal, with well-developed vinculin-containing focal adhesion plaques and F-actin cytoskeletons. The number of Saos-2 cells ($N(Saos-2)$) on the surfaces of SC-ss and SC-Si(100) on day 1 and 3 after seeding is depicted in Figure 4. The $N(Saos-2)$ on the zeolite samples are compared with the cell numbers on polystyrene dish (PS). On day 1 after seeding, the number on both SC samples is equal (SC-Si(100) or higher (SC-ss) than on PS; i.e. the zeolite samples are bioactive. Comparable values of $N(Saos-2)$ are observed on the SC-ss and SC-Si(100) before and after heat treatment of the samples on day 1, after seeding (Figure 4). However, a substantial increase of $N(Saos-2)$ on SC-Si(100)-300 and particularly SC-ss-300 in comparison with non-heated samples and PS was evident in day-3 after seeding.

4. CONCLUSION

Silicalite-1 films are biocompatible materials. Their interaction with Saos-2 cells is influenced by surface wettability and topography. The number of adhered Saos-2 cells depends on synthesis conditions of zeolite coating and on its post-synthetic modification. The cell number increases with increasing numbers of a oriented zeolite crystals on the surface of SC and increasing surface wettability. Obtained results are prospective in the optimization of SC outer surface to reach its maximal cytocompatibility.

ACKNOWLEDGEMENTS

Supported by Grant Agency of the Czech Republic (grant No. 16-02681S).

REFERENCES


