This project is focused on the functionalization of implant material surfaces through the formation of 3D ECM-analogous biointerfaces on a titanium surface to promote or to avoid bone ingrowth as desired. These biointerfaces are created using lateral chemical structuring and multilayered coatings consisting of biopolymers and proteins.

Lateral structuring of a titanium surface is done by microcontact printing (μCP), where the areas promoting and repelling the deposition of polyelectrolytes are created. Briefly, an elastomeric stamp coated with an “ink” is brought into contact with the substrate and removed after a short time, this leads to the formation of patterns of self-assembled monolayers (SAM) containing different functional groups as shown in Fig.1. Multilayered coatings are created using layer-by-layer deposition of natural polyelectrolytes as shown schematically in Fig.2. A titanium substrate was alternately dipped into an anionic and a cationic solution for 10 minutes, and washed with deionised water. Due to electrostatic forces a stable multilayered system is formed.

The research work includes:
- selection and testing of biopolymers (polysaccharides, gelatin, proteins)
- tests for cytotoxicity
- development and testing of structure size and design affecting cell adhesion and proliferation
- fabrication of ECM-analogous biointerfaces from these biopolymers in view of the target requirements (biocompatibility, biodegradability, stability against sterilization, etc.)

Thus having strong cell repellent properties compared to the control titanium. In contrast to Chi/HA, the proliferation of osteoblasts onto substrates with Chi/Gel multilayer coatings is distinctly higher compared to the control titanium. Thus, it is possible to significantly modify the biologically relevant properties of titanium by applying polyelectrolyte multilayer coatings. Chi/Gel coatings can be used in future for the functionalization of implants, where quick osseointegration is desired (for example, hip implants), and Chi/HA- for implants, which have to be removed (for example, high tibial osteotomy plates).

Laterally structured titanium surfaces were successfully created in IMT, and are at the moment in the biological tests phase. First results on the proliferation of osteoblasts are shown in Fig.5. The layout consists of squares with a size from 50 μm to 200 μm separated by 5-200 μm spaces. Further investigations are needed to define an optimal structure size and layout.

Conclusions

Polypelectrolyte-multilayers on titanium substrates were successfully fabricated at IMT using a wide range of biopolymers. No cytotoxicity was discovered in the first three days of cell culturing. The proliferation of osteoblasts can be significantly altered by varying the chemical composition of multilayers.

Fig. 1: Schematic of microcontact printing (μCP). Elastomeric stamp made of polydimethylsiloxane (PDMS) is coated with an “ink”, brought into contact with the substrate and removed after short time, that leads to the formation of patterns of self-assembled monolayers (SAM).

Fig. 2: Schematic of the formation of polyelectrolyte multilayers using layer-by-layer deposition method. The substrate is alternately dipped into anionic and cationic solution for 10 minutes and washed with deionized water.

Fig. 3: The cytotoxicity of the basic layers from PEI, PLL and AMD.

Fig. 4: Proliferation of osteoblasts on pure titanium substrate and on the titanium coated with multilayers of chitosan/gelatin (Chi/Gel) and chitosan/hyaluronic acid (Chi/HA). Tissue culture polystyrene (TCPs) was used as a control.

Fig. 5: Proliferation of osteoblasts on the laterally structured substrate. The picture captured under high humidity atmosphere for the visualisation of the pattern.