# Towards Innovative Nanostructured Surfaces with Targeted Biological Responses

Ita Junkar[1], Metka Benčina[1,2], Tatjana Filipič[1], Katja Lakota[3], Polona Žigon[3], Valentina Puca[4], Rossella Grande[4], Vittoria Perrotti[5], Aleš Iglič[2]

*[1]Department of Surface Engineering, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia*
*[2]Laboratory of Physics, Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, 1000 Ljubljana, Slovenia*
*[3]Department of Rheumatology, University Medical Centre Ljubljana, Vodnikova 62, 1000 Ljubljana, Slovenia*
*[4]Department of Pharmacy, University "G. d'Annunzio" Chieti-Pescara, Via Dei Vestini 31, 66100 Chieti, Italy*
*[5]Department of Innovative Technologies in Medicine & Dentistry, University "G. d'Annunzio" Chieti-Pescara, Via Dei Vestini 31, 66100 Chieti, Italy.*
*E-mail: ita.junkar@ijs.si*

Implant-associated infections (IAIs) are often identified as a leading cause of implant failure, resulting in increased medical costs and posing serious risks to patient health. These infections usually stem from bacterial colonization, which leads to the formation of biofilms on the implant surface. Nanostructured surfaces have shown great potential in preventing bacterial adhesion due to their unique surface nanotopography and inherent antibacterial properties. Changes in surface topography influence various physicochemical properties such as surface chemistry, morphology, wettability, and surface charge, which can significantly impact biological responses. Three different titanium (Ti) surfaces were developed: micro flowers produced by plasma-induced electrochemical anodization, nanotubes created through electrochemical anodization, and nanocubes formed by the hydrothermal (HT) method. Additionally, low-pressure gaseous plasma was used to fine-tune the surface chemistry for the desired biological response. These nanostructured Ti surfaces were studied for potential use in vascular stents or dental implants. Their morphology, surface chemistry, and wettability were assessed using scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and water contact angle analysis. The interaction with endothelial and smooth muscle cells was examined through in vitro biological response, with cell proliferation analyzed by fluorescence microscopy.

The antibacterial efficacy of the synthesized nanostructures was tested using the Escherichia coli bacterial strain and a multispecies oral biofilm comprising Fusobacterium nucleatum ATCC 25586 and saliva microorganisms from a periodontitis patient. Anti-biofilm activity was measured by Colony Forming Units (CFU) count and the Live/Dead assay, followed by analysis with fluorescence microscopy. Bacterial adhesion and biofilm formation on the titanium (Ti) surfaces were also examined using a scanning electron microscope (SEM). Our findings demonstrate that alterations in the titanium oxide layer and surface nanotopography significantly impact specific cell-surface interactions. This could provide new insights for designing multifunctional biomaterial surfaces that promote the growth of one cell type over another while simultaneously reducing bacterial infections.