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*Abstract*

# In vitro cytocompatibility and first in vivo results of additively manufactured titanium implants with polymer multilayer coatings

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Titanium and its alloys are extensively used materials in surgical fields such as oral surgery following trauma, tumors, or malformations due to their high biocompatibility, excellent bone integration, and favorable mechanical properties. Ensuring a stable healing process requires effective tissue interaction and osseointegration, where the surface structure and implant modifications play a crucial role in tissue regeneration. Surface modifications can influence wettability, cell proliferation, and growth.

The aim of the DFG Research Group 5250 “Permanent and bioresorbable implants with customized functionality” is the development and validation of an innovative approach for the manufacturing, characterization and simulation-based design of additively manufactured implants for various surgical disciplines.

Additively manufactured titanium discs (Ti-6Al-4V ELI) with three different roughnesses and surface coatings (PAA/PAH, PAA/PSS, HA/Chi) underwent cytocompatibility testing to the standard protocol DIN EN ISO 10993-5:2009. Cell proliferation (BrdU-assay), viability (XTT-assay) and cytotoxicity (LDH-assay) were assessed indirectly by exposing L929 mouse fibroblasts and MC3T3 mouse preosteoblasts to extracts of the specimens. Surface coating and roughness compatibility with cell adhesion was evaluated directly by staining adherent cells on the test materials. Based on the in vitro tests, the favored implants were implanted subcutaneously in rats for 10 and 30 days according to the 3R principle. After euthanization, the samples were processed histologically in accordance with DIN EN ISO 10993-6:2017 to demonstrate the hisopathological and inflammatory processes.

All extracts of the different surface coatings and roughnesses indicated no inhibitory effect on cell proliferation and cell viability nor a cytotoxic activity on the cells. Additionally, no restriction on cell attachment ability on the materials could be observed. Initial in vivo results showed promising outcomes compared to untreated titanium control.

Excellent cyto- and promising biocompatibility was demonstrated by all materials and coatings in accordance with the 3R principle.

**AUTHOR’S STATEMENT**

Authors state no conflict of interest. Animal models: Approval was obtained by the Local Ethical Committee based on the Veterinary Directorate of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia (decision number 323-07-01762/2019-05/9, Date: 01 March 2019)

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