## Bioactive Glass Coated Titanium Supports as a Novel Cell Delivery System for Regenerative Medicine

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Titanium (Ti) is an exogenous material currently used in medicine in its native form. When coated with a bioactive material, Ti significantly improves its performances and can be used also for tissue engineering applications. In this work, Titanium supports have been coated with RKKP glass-ceramic films (RKKP material developed at the ISTEC-CNR stands for A. Ravaglioli, A. Krajewski, M. Kirsch, A. Piancastelli), for possible applications as biomedical implant materials in regenerative medicine. Pulsed Laser Deposition technique has been applied for the films preparation and the from the sol-gel derived RKKP target have with the following composition: Ca- 19.4, P- 4.6, Si- 17.2, O- 43.5, Na- 1.7, Mg- 1.3, F- 7.2, K- 0.2, La-0.8, Ta- 4.1 (all in wt%). The prepared coatings were compact, and uniform and characterised by a nanometric average surface roughness. The biocompatibility and cell-friendly properties of RKKP glassceramic material have been tested using human colon carcinoma CaCo-2 cells. Metabolic activity and proliferation of these cells seeded on the RKKP films showed the same exponential trend found in the control plastic substrates. The phalloidin fluorescence analysis showed no significant modifications in the actin distribution in the cells grown on the RKKP films. Moreover, in the CaCo-2 cells, a high mRNAs expression of markers, involved in the protein synthesis, proliferation and differentiation, such as Villin (VIL1), Alkaline Phosphatase (ALP1), β-actin (β-ACT), Ki67 and RPL34, was reported. In conclusion, our findings demonstrated that the RKKP coated Ti supports are not only cell-friendly substrates for the CaCo-2 cells adhesion, growth and differentiation, but also represent a new potential cell delivery system that may be used in tissue engineering applications and in the future regenerative medicine protocols.



Figure. 1. AFM image of the RKKP film: 3D image and surface profile.



Figure 3. Real Time-PCR analysis for VIL1, ALP1,  $\beta$ -ACT, Ki67 and RPL34 gene expressions in the CaCo-2 cells seeded on RKKP films compared to control ones.



Figure 2. (A) Human colon carcinoma CaCo-2 cell proliferation, analyzed by BrdU incorporation assay(B) Cell metabolic activity analysis by WST-1 assay. (C) Time evolution of CaCo-2 cells nuclei seeded on RKKP films, revealed by staining with Hoechst 33342.



Figure 4. Actin distribution in the CaCo-2 cells: cells seeded on RKKP films and on plastic Petri dishes (CTR). 20x magnification.