BINDING CHARACTERIZATION AND CYTOTOXICITY ANALYSIS OF CELL SURFACE DISPLAY SELECTED TITANIUM BINDDNG PEPTIDES ON TITANIUM IMPLANT SURFACES

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Statement of Purpose: There is a high interest in restorative medicine that relies on finding bio-molecules that can specifically interact with implant materials (e.g. titanium and gold) as well as glass and ceramic-based materials to solve common inorganic-organic phase interaction problems [1,2]. Inorganic Binding Peptides are valuable candidate molecules due to their nano-size and ability to act as bridges among biomolecules and inorganic materials. In the present study, titanium-binding peptides (TiBP`s) were selected against commercially pure (cp) Grade 4 titanium dental implant surface using a biocombinatorial approach. TiBP's are good candidates due to their high affinity to titanium implant materials and they can be linked with many other molecules to create multifunctional constructs to solve possible problems with organic-inorganic phase interactions during implantation.

Methods: Titanium Binding Peptides (TiBPs) were selected against (cp) Grade 4 dental implant surface by applying cell surface display technique using the FliTrx library (Invitrogen). After four successive rounds of biopanning, 60 randomly selected clones were further characterized by fluorescence microscopy (FM) using Syto 9 labeling. The binding activities of selected clones were estimated by averaging the number of adhering cells in at least three randomly selected fields, repeating three times. Binders were grouped as strong, moderate and weak. Following FM characterization, two strong and one weak TiBPs sequences were synthesized by solid-phase synthesis and their adsorption behavior was analyzed via quartz crystal microbalance system (QCM) on titanium To determine the dissociation constant (K_d) of each peptide on titanium and other surfaces, the equilibrium frequency shift caused by peptide binding was measured at several peptide concentrations (0.1 to 15µM in PBS). These values were then fit using the Langmuir adsorption model. Cytotoxicity properties of two strong TiBPs (TiBPS1 and TiBPS2) were evaluated by MTT (Sigma M5655) assay on peptide-functionalized cp Grade 1 and cp Grade 4 titanium dental implant surface with incubation MCT3T3-E1 preosteoblast cells (ATCC, CRL-2593™).

Results: In this study, detailed characterization of three TiBP's (two strong and one weak) selected by cell surface display and characterized by fluorescence microscopy and QCM was performed. The three tested titanium binders showed similar binding affinity on titanium surface that is correlated to their FM characterizations. Cytotoxicity analyses indicated that the two strong TiBP`s have no toxic effect at very high concentrations (200µM), (Figure1).

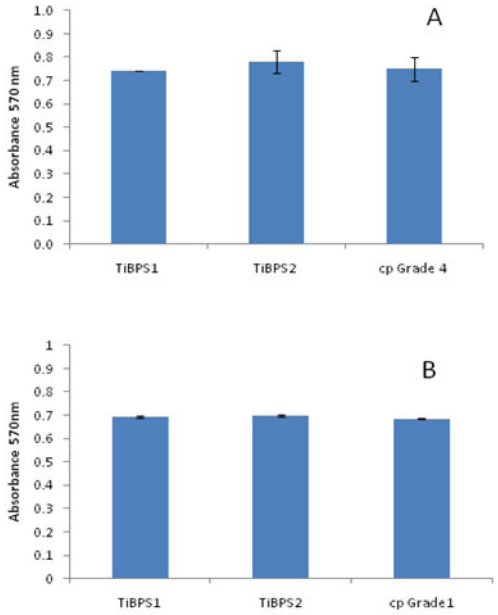

Figure1A: MTT assay on TiBPS1 and TiBPS2 funtionalized cp Grade 4 implant surface C: MTT assay on TiBPS1 and TiBPS2 funtionalized cp Grade 1 implant surface

Conclusions: TiBPs are promising molecules for surface functionalization of the implant materials. Due to their high affinity, they provide robust coating on the surfaces. They can also be conjugated with various molecules to enhance cell attachment and cellular proliferation, all which makes TiBPs a unique utility for tissue engineering studies.

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