Introduction
Implant surface structure and chemistry determines the contacting cell’s fate. Therefore, the fate of those cells directly affect bone-implant incorporation in clinical practice. However, how these chemical and mechanical signals translating to cellular responses are not yet known. The major drawback is a lack of systematic study of cell-biomaterial interaction in terms of protein expression, specifically, at the attachment interface between the cell and biomaterial (adherence surface, AS). Therefore, we have proposed to unbiasedly identify the biomolecules at the interface by proteomics. This method combines the use of a subcellular fractionation with quantitative mass spectrometry-based proteomics to characterize the biomolecules at cell-material interface in vitro. In the initial study we hypothesize that attachment of cells to a biomaterial in a 2D environment results in the localization of specific proteins at the interface between cells and biomaterial. Through proteomics of the interface, we aimed to discover novel proteins, which are highly localized between the cells and biomaterials.

Materials and Methods
A simple cell-biomaterial attachment model involving Madin Darby canine kidney (MDCK) cells on tissue culture polystyrene was used in this study. To label the proteins at the cell-biomaterials interface, all proteins of cells were labeled isotopically during culture via SILAC (Stable Isotope Labeling of Amino acids in Cell culture).

Results
All imaging suggested that the apical part of adhered cells were removed, retaining its ventral layer on biomaterial. Western-blot suggested that ECM, stress fibers, and focal adhesion proteins were highly enriched in the layer. Proteins identified with high reliability were quantified via SILAC ratio (H/L). Proteins with ratio >1 implicated they were localized at the interface, while <1 implicated it was from the apical portion. Gene ontology analysis confirmed the subcellular location of interfacial proteins were ECM, stress fibers and membrane proteins.

Discussion and Conclusions
Apart from quantifying classical adhesion proteins, proteins not previously known to be cell-substrate interaction related were identified at interface, suggesting possible new linkage to several cellular responses to substrate. Such as Cep 350, which involves in mitosis, centriole growth and maintaining microtubule network, was found. A specific subset of RNA-binding proteins was also highlighted, which agrees with the recent discovery of its association with the “spreading initiation centres”, a novel structure important for the attachment of cells. Their localization at the interface suggests new roles in the regulation of proliferation on the biomaterial surface. Such discovery could be utilized in designing smart implants to guide desirable cell fate. Proteomics study at the interface enables unbiased and high resolution biochemical investigation at interface between cells and biomaterials. While our study is based on a well-characterized mammalian cell line, we confirm it can be adopted on other cell lines, such as osteoblast and mesenchymal stem cells on different substrata.

References List

Disclosures
Authors have nothing to disclose.