RECORDING OF BASAL CALCIUM LEVELS IN CELLS ON GEOMETRIC STRUCTURES

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Introduction

Topographical surface modifications are of importance for the integration of orthopedic and dental implants in bone tissue. Bone-building cells, the osteoblasts, recognize the underlying topography on an nm and µm scale [1]. However, it has remained unclear how the cells recognize this and why the surface topography influences cell physiology. Stochastically raw metal surfaces, such as those which are produced by corundum blasting, can both change cellular adhesion and alter the receptor expression of the integrins, the important „anchor“ for binding to the extracellular matrix [2]. In order to reduce the complexity of these material surfaces and to be able to better elaborate the cell-physiological phenomena *in vitro,* we used geometrical micro-pillars. Interestingly, we were able to ascertain that osteoblasts attempt to internalize the micro-pillars, which are characterized by sharp edges and ridges [3]. This process causes not only higher energy metabolism (loss of adenosine triphosphate (ATP)) in the osteoblasts, but also the production of reactive oxygen species (ROS) in the cells [3]. Components present in the cell membrane, which otherwise play a role in the cellular intake of micro- and nanoparticles, are involved in these processes, including caveolin-1 as an important membrane-bound protein in the caveolae, cholesterol and CD68 [3]. Intracellular signals are significantly reduced and delayed in cells on micro pillars; this becomes evident by means of the intracellular mobilization of calcium (Ca^{2+}) [4]. The cells attempt at phagocytosis of the micro-pillars is, futile because the pillars are fixed in place. As a consequence of this entire process, the bone-specific cell functions are significantly inhibited, i.e. type I collagen, fibronectin and further proteins designated for the building up of the bone matrix are produced in reduced quantities [3,4]. It raises the question whether the dynamic of intracellular calcium ions (Ca^{2+}) as "second messengers" are important for the cell-material interaction.

Materials and Methods

Silicon wafers with final 100nm titanium coating (Si-Ti, 10 x 10 mm²) with a geometric array of micro pillars 5 x 5 x 5 µm (P-5x5, FIG. 1) were compared to planar surfaces (Ref). The micro pillars were fabricated by Deep reactive Ion Etching (DRIE) [3,4]. Osteoblasts (MG-63, ATCC® CRL-1427™) were cultured in DMEM with 10% FCS (PAA). The calcium imaging were performed with adherent osteoblasts (24 h growth) [4] as well as with cells attached for 10 min. (FIG. 2). For the 24h-adhesion approach the cells were cultured on wafers for 24h and afterwards stained with the calcium indicator Fluo3/AM (5 µM, 40 min incubation). For the 10min-adhesion approach; suspended cells were loaded with Fluo3/AM (5 µM) for 40 min. Afterwards the stained cells were seeded on wafers and cultured for 10 min. The recording of the cell's basal calcium level was done by confocal microscopy (LSM780, Carl Zeiss, Zen2011 (black edition) software) using a time series of 90 cycles each 2 s. For statistical analyses SPSS (15.0) was utilized with Kolmogorov-Smirnov test followed by Kruskal-Wallis test.

Results and Discussion

In 24h-adherent osteoblasts on micro-pillars an altered basal calcium signal could be observed; the cells showed decreased intracellular calcium dynamics and concentration (FIG. 3A) compared to Ref. In contrast, 10min-adherent cells exhibited a weak but stable basal calcium level on pillars comparable to the Ref (FIG. 3B). Our current approaches indicated a low basal calcium level which was independent of the topography within the first minutes of adhesion. It is possible that the proof of the low calcium level occurred on account of the missing contact via gap junctions to other cells [5]. After 24 hours, the influence of the micro-pillars due to weak calcium signal was clearly detectable. In previous studies we showed altered actin organization in short fibers on the top of micro-pillars [3,4]. It is known that an intact actin cytoskeleton affects the calcium dynamic [6].

Conclusions

Adherent osteoblast on micro-pillars with impaired cell showed a reduced basal calcium level. Investigations of the influence of topography on intracellular calcium signaling provide new insights into how external signals from physico-chemical environment affect cell behavior and finally the cell function. The understanding of the biocomplexity of cellular pathways is a challenge and of clinical relevance for the development of bio-functional implant surfaces.

Acknowledgments

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References

[1] J. Rychly & J.B. Nebe, BioNanoMat 14 (2013) 153-60.
[2] F. Luethen *et al,*. Biomaterials 26 (2005) 2423-40.
[3] C. Moerke et al., Biomaterials 76 (2016) 102-14.
[4] S. Staehlke *et al.,* Biomaterials 46 (2015) 48-57.
[5] K. Anselme. Biomaterials 21 (2000) 667-81.
[6] H. Pommerenke *et al*., J Bone Min Res 17 (2002) 603-11.

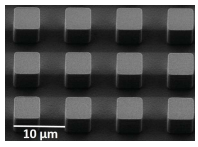

FIG. 1. SEM image of micro-pillars (P-5x5). (FE-SEM Supra 25, Zeiss, 3 kV, magnification = 2,000x, bar 10 µm).

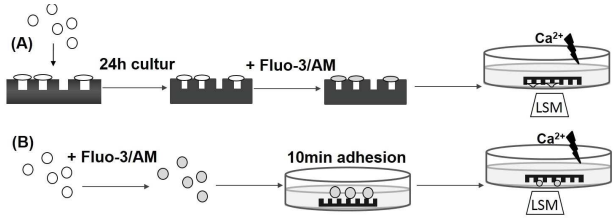

FIG. 2. Schematic illustration of calcium imaging in (A) 24h-adherent and (B) 10min-adherent cells.

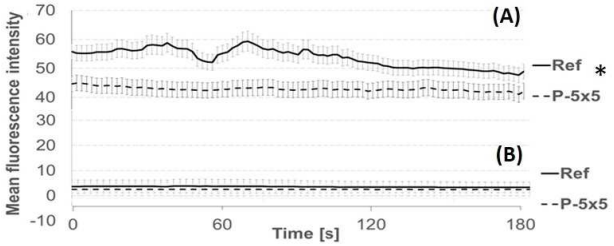

FIG. 3. Basal calcium level in (A) 24h-adherent and (B) 10min-adherent cells on micro-pillars (P-5x5) as well as planar surfaces (Ref). (LSM 780, calcium signal intensity, mean ± SD, n = 140, *p < 0.05, Kruskal-Wallis test).