Use of Ce Valence States in Cerium Oxide Nanoparticles to Control Cell Proliferation on Scaffold Surfaces

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Introduction: In tissue engineering, design of scaffold and artificial niche surfaces requires a fundamental understanding of the interaction between cells and biomaterial surfaces. Specific biomaterial surface properties (e.g. surface functionalization and adsorbed proteins) enable the control of cell functions such as adhesion, proliferation and differentiation. Our research focuses on functionalization of scaffold material surfaces using cerium oxide nanoparticles (CNPs). Although it has recently been reported that the presence of CNPs in composite scaffolds enhances cell proliferation [1], the mechanism of interaction between cells and extracellular CNPs is still unclear. On a related note, CNPs have demonstrated therapeutic potential based on their ability to scavenge reactive oxygen species in cells. Ce valence states (Ce^{3+} and Ce^{4+}) of CNPs correlate with superoxide dismutase and catalase mimetic activities, respectively [2-3]. Ce^{3+} in CNPs shows the potential to inhibit redox-dependent apoptosis [4]. Ce valance states in extracellular CNPs may be critical to the control of cell proliferation behavior on scaffold surfaces covered with CNPs. To confirm this hypothesis, this study investigated the effect of different Ce valance states (Ce^{3+} and Ce^{4+}) on cell proliferation. By way of understanding the interaction between adherent cells and CNPs with different valence states, we created CNP layers with dominant Ce^{3+} and Ce^{4+} ions on poly-L-lactide acid (PL) substrates [5], and investigated cell adhesion, migration and proliferation behaviors on the Ce^{4+}- and Ce^{3+}-CNP/PL (Ce^{4+} and Ce^{3+} regions).

Methods: In brief, high concentration of Ce^{3+} ions was created in CNP/PL substrates by Ar ion irradiation [5]. Osteoblast-like cells (MG63) and human mesenchymal stem cells (hMSCs) were cultured onto Ce^{3+} and Ce^{4+} regions of CNP/PL. Cell migration and proliferation behaviors were observed. To evaluate cell adhesion level, cell detachment force from CNP/PL was measured by single cell force spectroscopy. Surface charge, wettability and adsorbed elements on both CNP/PL were also evaluated.

Results: Fig. 1 shows cell proliferation behavior on Ce^{4+} and Ce^{3+} regions of CNP/PL at 3 days after seeding. Survival of cells attached to CNP/PL was confirmed by calcein staining. This result indicates that even spherical shaped cells on Ce^{3+} regions survived. As compared with PL control substrates, rapid cell proliferation was observed on Ce^{4+} regions, while Ce^{3+} regions displayed slow proliferation. To compare cell attachment force, single cell force spectroscopy was carried out on Ce^{4+} and Ce^{3+} regions (Fig. 2). Detachment force from Ce^{4+} regions was clearly higher than that from Ce^{3+} regions in Fig. 2. When cells migrated across the border between Ce^{4+} and Ce^{3+} regions, cell morphology reversibly changed from spindle shape (on Ce^{4+} regions) to spherical shape (on Ce^{3+} regions). These findings suggest that the weak interaction between cells and Ce^{3+} regions induces slow proliferation, while strong interaction between cells and Ce^{4+} regions enhances rapid proliferation. Although phosphorus adsorption was observed only on Ce^{3+} regions immersed in culture medium, the total amount of protein/BSA adsorption on both Ce^{4+} and Ce^{3+} regions was similar. In addition, all CNP/PL samples (with Ce^{4+} and Ce^{3+} regions) immersed in culture medium converted to negatively charged surfaces and became moderately hydrophilic surfaces. This suggests that these characteristics slightly influence specific cell proliferation behavior on Ce^{4+} and Ce^{3+} regions. The above results indicate that Ce valance states of CNP/PL, at least indirectly, influence cell proliferation.

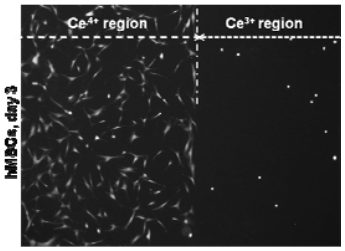

Figure 1 Cell proliferation behavior of calcein-staining hMSCs on CNP/PL with Ce^{4+} and Ce^{3+} regions at 3 days after seeding.

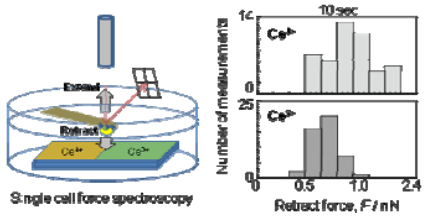

Figure 2 Schematic of detachment force measurement and cell detachment force from Ce^{4+} and Ce^{3+} regions of CNP/PL at retraction time of 10 sec.

Conclusions: Cell proliferation on CNP/PL with different Ce valance states was investigated. Ce^{4+} and Ce^{3+} regions of CNP/PL, at least indirectly, promote and inhibit cell proliferation. Results of this study may be utilized in the design and development of biomaterials to control cell proliferation in tissue engineering.

References:

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