Investigating Substrate Mechanics Effects in Combination with TiO₂ Thin Layer Coated by Atomic Layer Deposition (ALD) for Dental Pulp Stem Cell (DPSC) Proliferation and Differentiation

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Introduction and Objectives

Adult DPSC that are harvested from the pulp of deciduous teeth were shown to be able to differentiate into odontoblasts, osteoblasts, myocytes, chondrocytes, adipocytes, and even neural and cardiac cells. Stem cells are extremely sensitive to their environmental signals, such as hormones, growth factors (GFs), and mechanical loading are able to induce their multi-genesis differentiation. Previously, we have shown that DPSC can sense differentially small changes of the substrate moduli and adjust their moduli to the same functional dependence, while the biomineralization existed when the substrate modulus is higher than a critical value.

In order to improve the differentiation behaviors of DPSC by only mechanics factor, we modified surface properties of the substrates with TiO₂ coating by Atomic Layer Deposition (ALD) method, which is allowed to precisely control the homogeneous thin layer deposition without changing underlying substrate mechanics.

Cell Culture

- Human DPSC strain AV3 were cultured on hard and soft PB films, both coating and non-coating samples, with a density of 5000 cells/cm².
- Cells were grown in an incubator at 37°C with 5% CO₂. Cultured medium was refreshed every alternate day.
- The cell number was counted on day 1, 3, 5, and 7. For the differentiation experiments, samples were cultured for 28 days, with data points taken weekly.

DPSC Adhesion and Proliferation at Week 1

- Better actin stretching were observed on substrates with TiO₂ coating, indicating TiO₂ promotes cell adhesion.
- On the hard substrate, more cells adhered on the substrates with TiO₂ coating, while the population doubling time are the same.
- On the soft substrate, cells adhesion and proliferation have been improved much more distinctly with TiO₂ coating, that the doubling time decreased to a comparable value as on the hard.

Characterization of mineral deposits at week 4

- Without TiO₂ coating, biomineralization were observed on the hard but not on the soft substrate.
- Particle-like mineral deposits were presented on the hard; while with TiO₂ coated, mineral deposits templated on banded fibers were found on both hard and soft substrates, which structure is similar to in vivo hard tissue (ex. dentin or bone).
- EDS spectra showed Ca and P peaks for mineral deposits, indicating hydroxypapatite crystals formation.

Conclusions

- Coating ~3.5 nm TiO₂ onto PB substrates doesn’t affect its film moduli.
- Substrates coating with TiO₂ improves early stage cells adhesion and proliferation.
- Thin layer of TiO₂ coating onto soft substrate promotes biomineralization and differentiation of DPSC, that mineral crystals and OCN protein expressions were observed on coating soft substrate but absent on the noncoating soft substrate.
- On the hard substrate, mineralized deposits templated on fibers and more evenly spreading OCN protein were observed on the substrate with TiO₂ coating, while particle-like deposits without fibers presented on the hard PB substrate without coating.
- The surface coating of TiO₂ by ALD may support collagen forming banded fiber structures, allowing hydroxypapatite crystals nucleate and grow between gap area, thus we observed fibers templated minerals on both coating substrates, which structures are close to in vivo hard tissue formation.
- Substrate mechanics also plays an important role to regulate differentiation lineages.

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