Hydroxyapatite Electret suppresses proliferation of vascular smooth muscle cells *in vitro* by modulation of their phenotypic conversion

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Purpose:

Vascular smooth muscle cells (VSMCs) normally exist in a differentiated state in the adult arterial media. The cells are capable of phenotypic change from a differentiated to a dedifferentiated state in response to changes in local environment. In response to mechanical injury of artery, medial VSMCs undergo a phenotypic conversion, and induce intimal hyperplasia by proliferation and migration from the media into the subendothelial spaces. This phenotypic modulation of VSMCs plays a major role in the pathogenesis of cardiovascular diseases such as atherosclerosis and restenosis after balloon angioplasty.

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) used as an artificial bone material, is one of the best biocompatible materials. HA has various biological performances, such as protein adsorption and cell growth in vitro. We have shown that HA can be an excellent electret.[1] HA electrets are made by the polarization of HA ceramics, in which ion dipole moments are aligned in a direction for a considerable period due to an external electric force. In simulated body fluid (SBF) test, crystal growth on HA electret was accelerated compared to HA. Moreover HA electret has abilities to increase adsorption of protein, such as fibrin and cofactor, and to stimulate bone formation with angiogenesis *in vivo*. Recently we showed that HA electret attenuates intimal hyperplasia occurring after endothelial removal of the rabbit carotid artery. [2] In this study, we examined the effects of HA electret to the phenotypic conversion of VSMCs *in vitro*.

Methods:

HA was precipitated from calcium hydroxide aqueous suspension and phosphoric acid solution and then characterized by X-ray diffraction and IR adsorption. HA electret was made by sintering and electrical polarization in a DC field of 5kV/cm^2 with a pair of Pt electrodes. Polarization was confirmed by thermal stimulated depolarization current mesurement. The negatively and positively charged surfaces on the HA electret were designated the N-surface and P-surface, respectively, while non-polarized surfaces were referred to as 0-surfaces.

Rat aortic VSMCs were isolated by enzymatic dispersion, and were grown in DMEM supplemented with 5% heat-inactivated FBS, 20mM L-glutamine, 50mg/ml penicillin, and 50mg/ml streptomycin in a humidified 5% CO_2 atmosphere at 37°C. Cells used in experiments were from passages 4-7. Cells were cultured on HA and HA electret and were collected after they were confluent.

The MTT assay was used to measure cell viability. Molecular biological analyses of VSMC marker genes were performed using the collected cells.

Results:

VSMCs cultured in vitro belong to the dedifferentiated phenotype. To determine the effect of the polarization, viability was measured using the MTT assay. There was no significant difference in VSMCs 6 hours after seeding, but decrease in VSMCs cultured on HA electret compared to HA after 48 hours.

We used immunocytochemistory and RT-PCR analysis and showed that SMA, that is one of markers of differentiated VSMCs, is significantly increased in cells cultured on HA electret compared to cells on HA. These results suggested that HA electret could modulate the state of VSMCs.

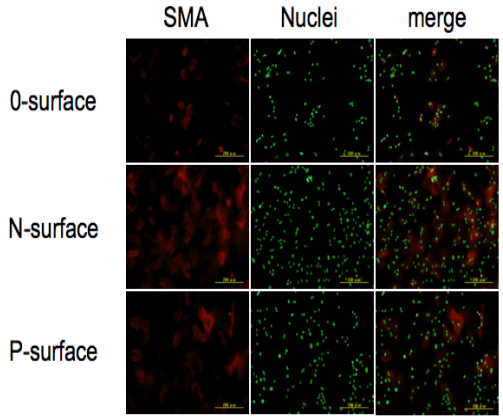

Figure 1. Immunocytostaining

Conclusions:

HA electret can suppress proliferation of VSMCs and induce them from dedifferentiated to differentiated state *in vitro*. This finding suggest the surface charge of HA electret is involved in phenotypic conversion of VSMCs.

References:

1. Yamashita K. Chem. Mater. 1986; 8: 2697-
2. Nagai A. Life Sci. 2008; 82: 1162-1168.