Migration of endothelial cells on polyurethane-gold nanocomposites

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Statement of Purpose: The nanocomposites from polyurethane containing small and various amounts of gold (Au) were prepared. The nanocomposites (PU-Au) exhibited greater proliferation of endothelial cells, especially on PU-Au 43.5 ppm. PU-Au also showed enhanced cell migration rate. In addition, cells had higher level of eNOS expression on PU-Au than on PU. The enhanced cell proliferation and migration were closely associated with greater levels of eNOS. The induction of eNOS by PU-Au was simultaneously abolished by LY294002. This suggested that the signaling pathway for the cellular events on PU-Au was via PI3K/Akt.

Methods: The preparation of PU-Au composites was previously described (Hsu SH, Tang CM, Tseng HJ. JBMR: A, in press.). The material was coated on coverslips and placed in the 24-well tissue culture plate where bovine carotid arterial endothelial cells (EC, 1×10^4 cells/well) were seeded. The cell number was counted after 48 h. RT-PCR and Western blotting were used to analyze the eNOS mRNA and protein expression. The cell migration into a gap was monitored real-time under the microscope. The average rate of cell migration in the period was calculated using Image J (NIH) software. LY294002 (30 μM) was used as a specific inhibitor for PI3K to verify the eNOS expression via the PI3K pathway. AVONA was used to assess the statistical significance. $p < 0.05$ was considered significant.

Results/Discussion: The number of EC at 48 h was greater on all naocomposites than on PU, and greatest on PU-Au 43.5 ppm. The calculated migration distance up for 8 h on PU-Au 43.5 ppm was about 2 times over PU (Figure 1).

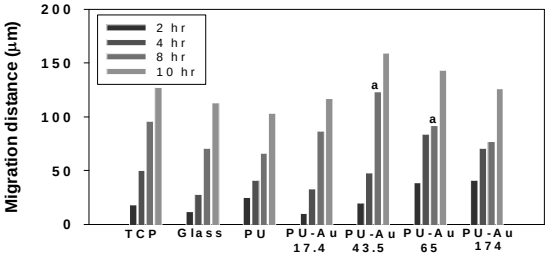

Figure 1: Migration distance of EC. ($P<0.05$): a: greater than PU.

RT-PCR and Western blotting results showed the highest levels of eNOS mRNA and protein expression on PU-Au 43.5ppm (Figure 2).

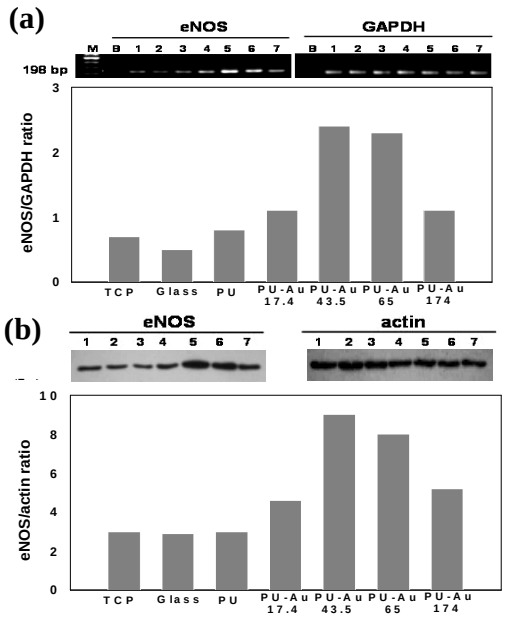

Figure 2: The eNOS (a) mRNA and (b) protein expression of EC on materials for 48 h. Lane1-7: TCPS, glass, PU, PU-Au at 17.4-174 ppm.

NO stimulates EC migration and up-regulate VEGF. The increased eNOS expression was thus closely associated with the cellular events such as enhanced proliferation and migration. The eNOS inductions by PU-Au and PU were significantly abolished by LY294002 (Figure 3). This demonstrated that the enhanced EC proliferation and migration by nanocomposites involved in eNOS via PI3K mechanism (Hashimoto A, et al. Atherosclerosis 2006;189:350-357).

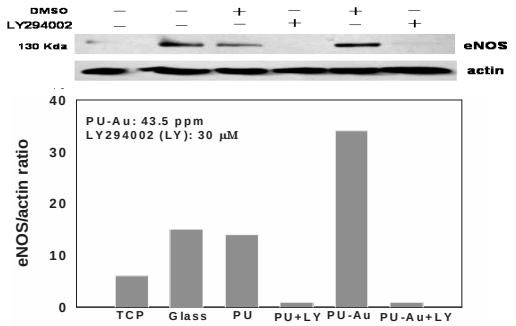

Figure 3: eNOS protein expression was abolished by addition of LY294002.

Conclusions: The greater rates of growth and migration of EC on the PU-Au were believed, at least in part, to be attributed to the up-regulation of eNOS expression via PI3K pathway of the cells cultured on the nanocomposite surfaces.