



2012 HAWAII UNIVERSITY INTERNATIONAL CONFERENCES
EDUCATION, MATH & ENGINEERING TECHNOLOGY
JULY 31ST TO AUGUST 2ND
WAIKIKI BEACH MARRIOTT RESORT & SPA
HONOLULU, HAWAII

EFFECT OF THE SURFACE DENSITY OF NANOSEGMENTS IMMOBILIZED ON CULTURE DISHES ON EX VIVO EXPANSION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS FROM UMBILICAL CORD BLOOD

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Effect of the surface density of nanosegments immobilized on culture dishes on *ex vivo* expansion of hematopoietic stem and progenitor cells from umbilical cord blood

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Hematopoietic stem and progenitor cells (HSCs) are multipotent cells that have the specific capacity to self-renew and differentiate into all type of mature blood cells. Intravenous infusion of HSCs has been commonly performed to treat patients suffering from hematological disorders and malignant diseases after radiation and/or chemotherapy. Umbilical cord blood (UCB) is an attractive source of HSCs for HSC transplantation. However, the low number of HSCs obtainable from a single donor of UCB limits direct transplantation of UCB to the treatment of pediatric patients. In this study, we investigated the *ex vivo* expansion of HSCs cultured on biomaterials grafted with several nanosegments, i.e., polyamine, fibronectin (FN), RGDS, and CS1 (EILDVPST), at several surface densities. HSCs can expand more rapidly with high pluripotency when they are cultured on materials with amino groups than on materials with carboxylic acid or hydroxyl groups. However, no studies have investigated the effect of the surface density of amino groups on the expansion of HSCs. Therefore, cell culture dishes with various surface densities of amino groups (i.e., PS-AMA dishes) were prepared using ozone treatment to investigate the optimal amino group surface density for the *ex vivo* expansion of HSCs. Fibronectin (FN) and the cell-binding domains of fibronectin, CS1 and RGDS (and negative control, CS1i and RGES) were also covalently immobilized on PS-NH₂ dishes to evaluate the optimal nanosegments on cell culture dishes. The water contact angles of PS-ECM and PS-Peptide (PS-CS1, PS-CS1i, PS-RGDS, PS-RGDS-H, PS-RGES, and PS-RGES-H) dishes were 50–65 degrees, and therefore the dishes are expected to be suitable for the *ex vivo* expansion of HSCs. No direct correlation was found between fold expansion of HSCs and physical parameters of the culture dishes, i.e., surface roughness and water contact angle of the culture dishes. However, a small amount of grafted amino groups, less than 0.8 residual $\mu\text{mol}/\text{cm}^2$, on the dishes was effective for the *ex vivo* expansion of HSCs. This is the first study on the effect of the surface density of nanosegments immobilized on culture dishes on the *ex vivo* expansion of HSCs. HSCs cultured on dishes with a high concentration of CS1 (2.40 residual $\mu\text{mol}/\text{cm}^2$) showed greater expansion of HSCs and more pluripotent colony-forming units (i.e., colony-forming unit–granulocyte, erythroid, macrophage, and megakaryocyte (CFU-GEMM)) than those on fibronectin-grafted and polyamine-grafted dishes. These data suggest that the specific interaction between HSCs and CS1 helps to maintain the pluripotency of HSCs during the *ex vivo* expansion of HSCs. CS1 is one of the cell-binding domains of fibronectin. The presentation of specific binding site number of CS1 is 500 times (440,000/880) higher than that of fibronectin at the same grafting weight density of CS1 and fibronectin on dishes, because of low molecular weight of CS1 (approximately 880 dalton from 110 [average molecular weight of amino acids] \times 8 [No. of amino acids in CS1]) than fibronectin (440,000 dalton). The presentation of specific cell-binding domains of CS1 at high concentrations is important for the signal transduction that promotes the *ex vivo* expansion of HSCs.