Supplementary materials

Direct Measurements on CD24-Mediated Rolling of Human Breast Cancer MCF-7 Cells on E-selectin

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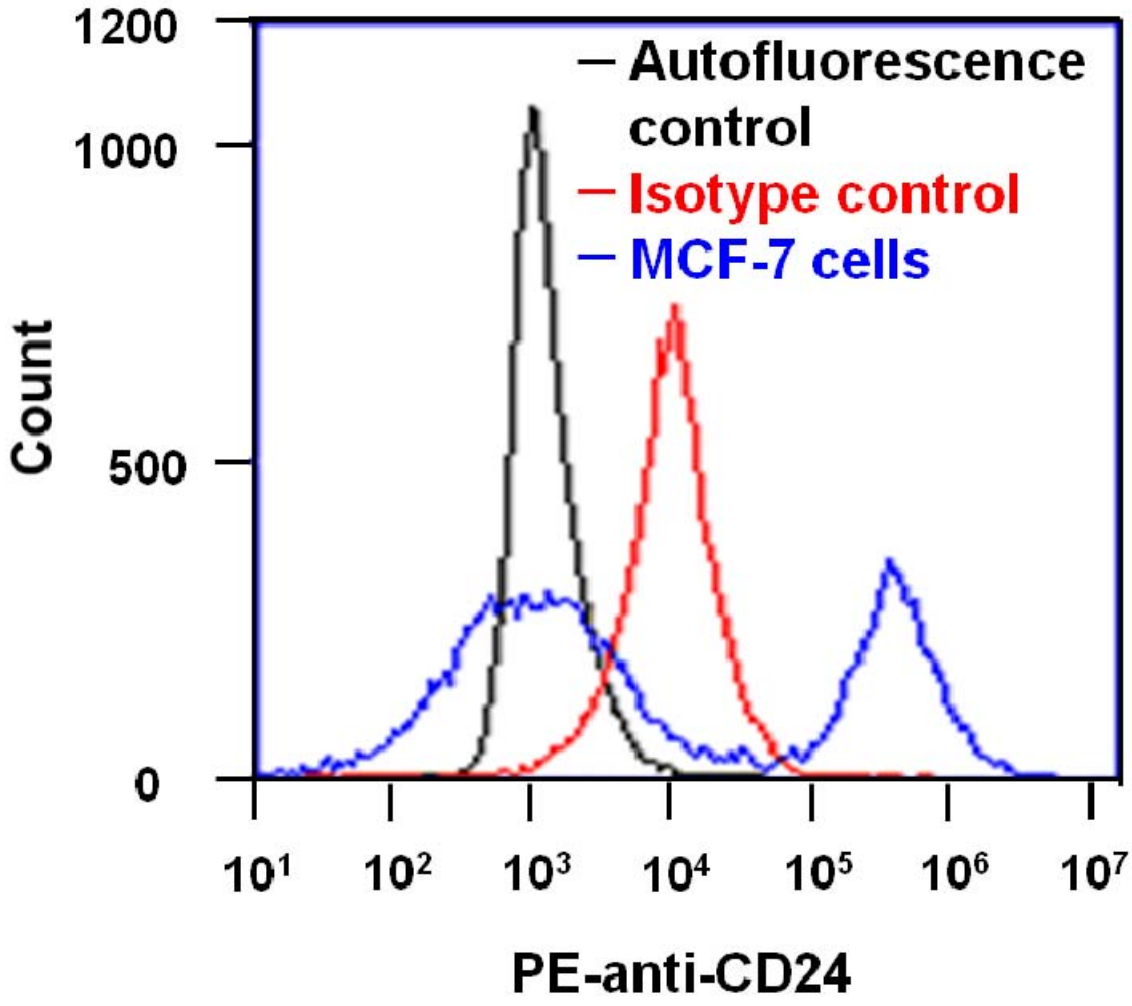
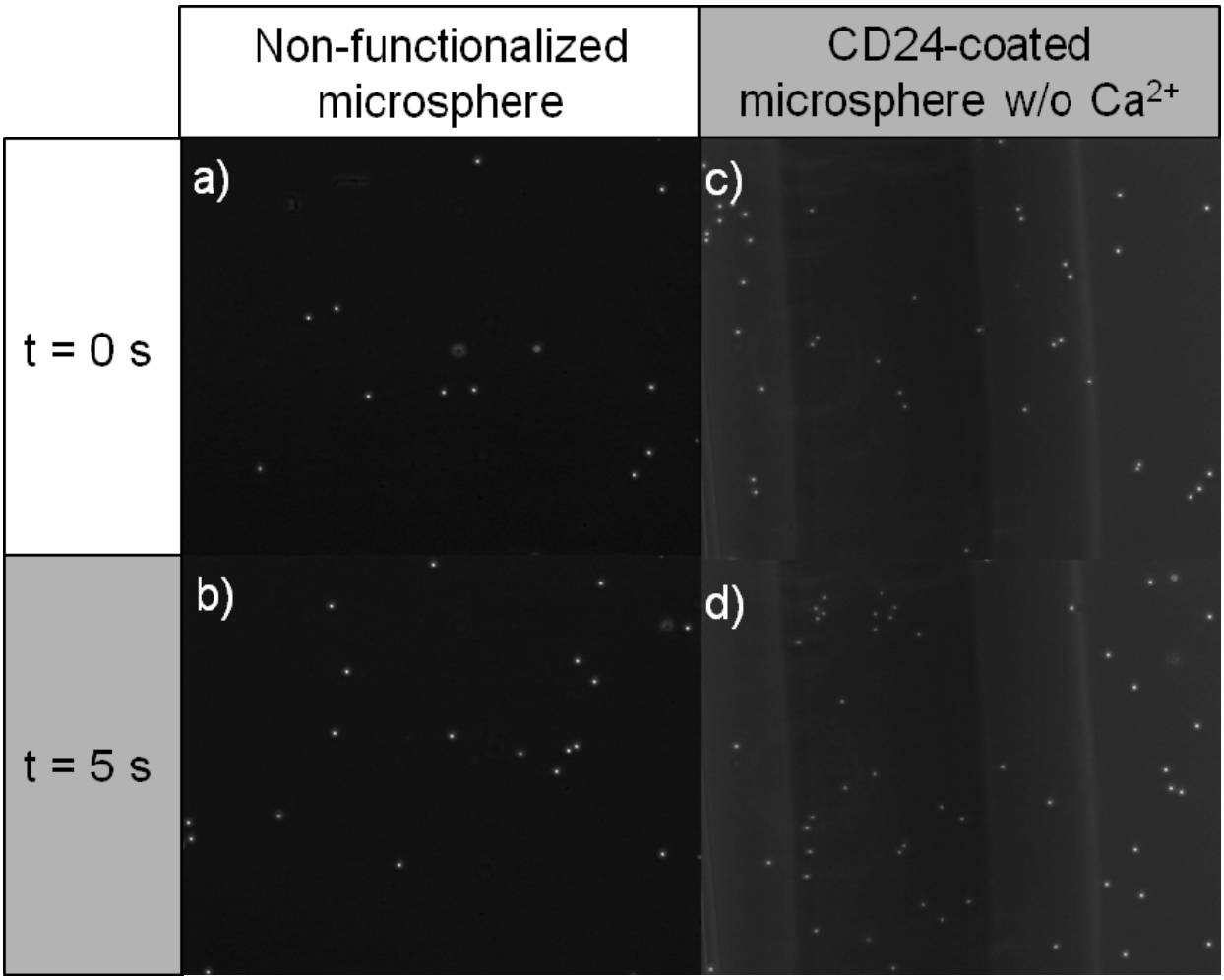
Flow Cytometry Measurements of CD24 expression

CD24 expression of MCF-7 cells was measured using a fluorescence activated cell sorter (FACS, Beckman Coulter EPICS Elite ESP, Miami, FL). Trypsinized MCF-7 cells (50 μL of 1×10^7 cells/mL solution in PBS with 1% FBS) were incubated with 10 μL of PE-conjugated anti-CD24 on ice for 15 min, centrifuged at 1800 rpm for 5 min, and resuspended in the FBS-containing PBS for the FACS measurements. The control groups include the untreated cells as a negative control, the cells treated with 10 μL of anti-$IgG_{2a,\,k}$, an isotype of anti-CD24, as an isotype control. The FACS histograms of the PE fluorescence intensities are shown in Supplementary Figure 1. MCF-7 cells were divided into two groups based on CD24 expression; CD24-positive and CD24-negative subpopulations, which is consistent with a previous report.[31] The CD24-positive subpopulation showed the strongest fluorescence intensity, indicating high expression of CD24. The fluorescence intensity of the $CD24^-$ subpopulation was similar to that of the non-treated cells. The anti-$IgG_{2a,k}$-treated MCF-7 cells exhibited a lower intensity than $CD24^+$ group but higher than the untreated cells.

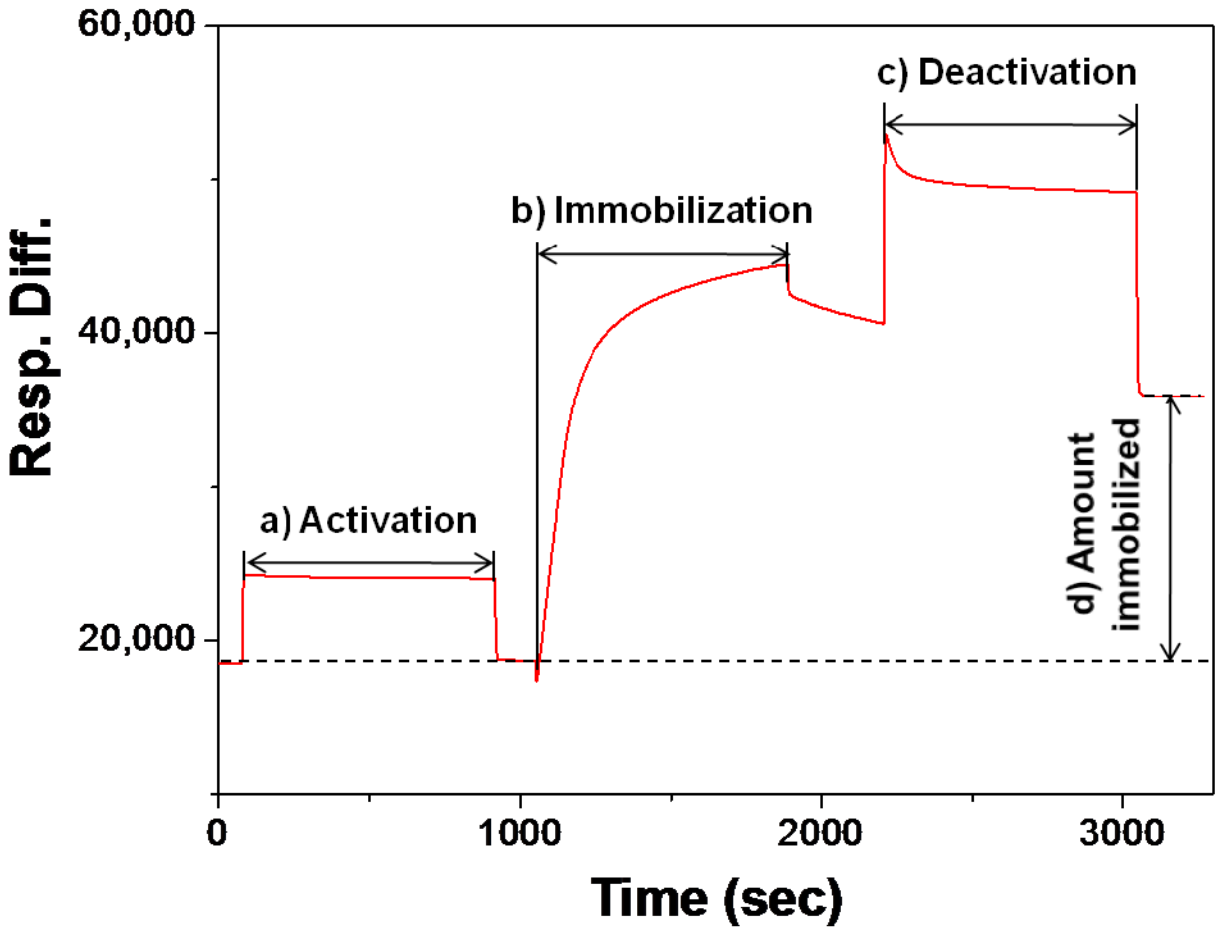
The coating of CD24 on microspheres (10 μm in diameter) was also confirmed using FACS following the same method described above, as shown in Supplementary Figure 4. The streptavidin-coated microspheres (negative control) showed the lowest fluorescence intensity, and the isotype control showed a slightly higher intensity than the negative control. A significantly higher intensity was observed from the CD24-coated microspheres, indicating that CD24 was successfully immobilized on the microsphere surfaces.



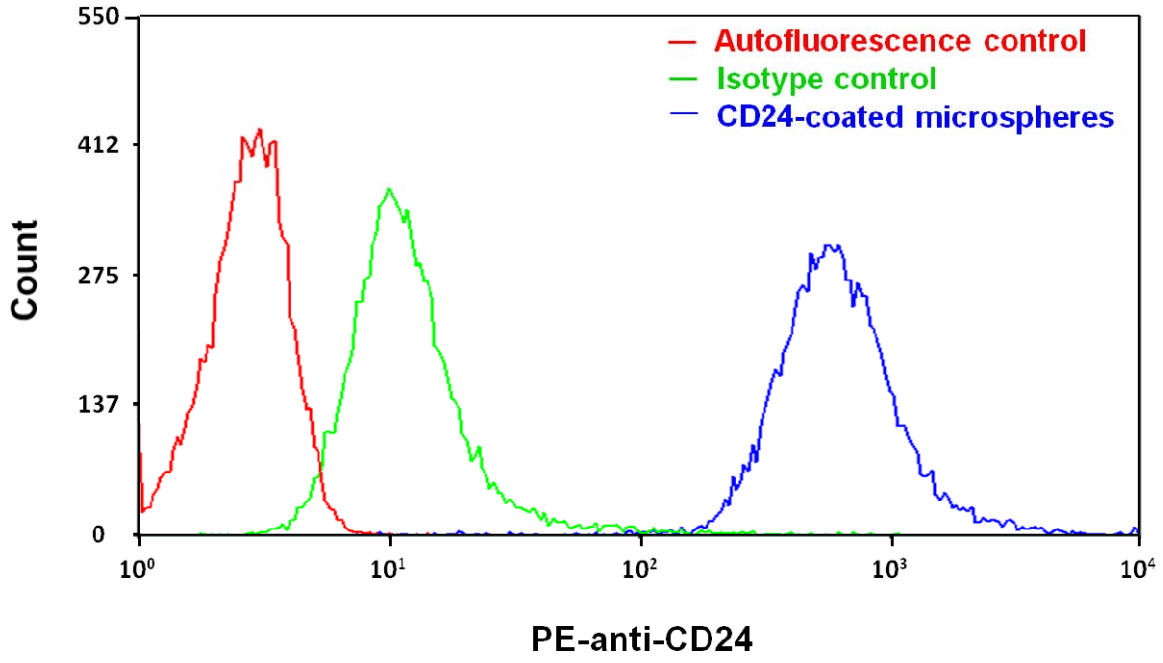
Supplementary Figure 1. FACS histograms of PE fluorescence intensities from MCF-7 cells. The cells were treated with PE-conjugated anti-CD24 (blue), PE-conjugated anti-$IgG_{2a,\,k}$ for an isotype control (red), or were untreated (black). Based on the fluorescence intensity of each group, it was confirmed that MCF-7 cells have two subpopulations, CD24 negative and CD24 positive.



Supplementary Figure 2. Time-course images of (a and b) nonfunctionalized microspheres and (c and d) CD24-coated microspheres without Ca^{2+} in buffer, both under shear stress of 0.08 dyn/cm^2 on E-selectin-immobilized surfaces. No interactions between the microspheres and the surfaces were observed in the either condition, indicating that non-specific interaction of the microspheres is minimal and the CD24/E-selectin interaction is Ca^{2+} dependent.



Supplementary Figure 3. An SPR sensorgram of E-selectin immobilization on a CM5 sensor chip. The amount of the immobilized protein on the sensor chip was determined by the difference in resonance unit before and after immobilization of E-selectin (d). The sensor chips with the immobilized shift of more than 8000 RU were used for subsequent binding analyses. The immobilization process was performed by three steps: (a) activation of the CM5 sensor chip using a mixture of EDC and NHS; (b) injection of E-selectin; and (c) termination of the remaining reactive ester residues on the sensor chip using ethanolamine.



Supplementary Figure 4. FACS histograms of PE fluorescence intensities from the various microspheres. The microspheres were treated with PE-conjugated anti-CD24 (blue), PE-conjugated anti-$IgG_{2a,\,k}$ for an isotype control (green), or were untreated (red). The significantly increased fluorescence intensity from the CD24-coated microspheres confirms that CD24 coating was performed successfully.