Enhanced Capture of Cancer cells on Aptamer-Modified Topographical Surfaces

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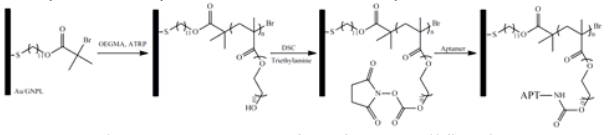
Statement of Purpose: Circulating tumor cells (CTCs) are considered to be crucial in the progression of cancer, and the detection of CTCs may be a useful approach in diagnosis, treatment and prognosis.[1, 2] However, CTCs are present in very small numbers in blood: one CTC cell per 10^5 to 10^7 normal blood cells. Therefore CTC enrichment is a prerequisite for the development of CTC analysis as a cancer diagnostic tool. However in a realistic setting, e.g. human blood, many other cells will compete with target cells. Moreover, proteins may adsorb non-specifically and thus interfere with the interactions of specific receptors on the cell surface and surface immobilized aptamers. Many studies gave data only for single cell or binary cell systems. In this work, we prepared gold nanoparticle layers (GNPLs) with controlled surface morphology by a simple and convenient method; we also investigated POEGMA as an antifouling spacer for the aptamer. The B leukemia CTC cell, Ramos cell, was selected as a target to study the selective capture capability of cell specific aptamer-modified GNPLs of varying surface roughness in serum-free and serum-containing cell culture conditions.

Methods: Modification of Gold/GNPLs with aptamers. 2.5 OD TD05 APT (for single-stranded DNA, 1 OD ≈ 33 μg) (The sequence of the TD05 APT is: 5′-CAC CGG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CTC CCG GTG TTT TT-$(CH_2)_6$-HS-3′) was dissolved in 2 mL phosphate buffered saline (PBS) (2.25 nmol/L). Smooth gold and nanoparticle surfaces were treated with the aptamer solution at room temperature for 3 h and then washed with PBS to remove excess aptamer. The samples (Au-APT and GNPL-APT) were then dried under N_2.

Modification of Gold/GNPLs with aptamers via POEGMA spacer. The procedures for the preparation of POEGMA-modified smooth gold and nanoparticle surfaces have been reported previously.[3] POEGMA-modified Au surfaces and GNPLs (Au-POEGMA and GNPL-POEGMA) were treated with N,N'-disuccinimidyl carbonate (DSC) in dry acetonitrile at room temperature for about 5 h. The DSC modified surfaces (Au-POEGMA-NHS and GNPL-POEGMA-NHS) were then washed with acetonitrile and dried under nitrogen. The samples were immersed in aptamer solution (The sequence of the TD05 APT is: 5′-CAC CGG GAG GAT AGT TCG GTG GCT GTT CAG GGT CTC CTC CCG GTG TTT TT-$(CH_2)_6$-NH_2-3′) for 3 h at 37 °C to give the APT-modified Au-POEGMA (Au-POEGMA-APT) and APT-modified GNPL-POEGMA (GNPL-POEGMA-APT) surfaces (as shown in Scheme 1).



Scheme 1. Process of surface modification.

Results: In serum-free binary cell mixtures containing Ramos cancer cells and CEM cells, the density of Ramos cells adherent to highly rough GNPL-APT (GNPL3-APT) was 19 times that of CEM cells. However, in serum-containing conditions, the specificity of GNPL-APT for Ramos cells was much reduced (Figure 1a). In order to improve the Ramos selectivity of the APT modified surfaces in serum conditions, POEGMA was introduced as a protein resistant element. The densities of Ramos cells on the four POEGMA surfaces modified with aptamer were, respectively, 0.9, 1.5, 3.5, and 6.6 times those of CEM (Figure 1b), indicating that the selectivity of GNPL-POEGMA-APT for Ramos cells was significantly enhanced with increase in surface roughness. This effect may be attributed to the protein resistance of the POEGMA, excluding the surface proteins that might "shield" the immobilized aptamer.

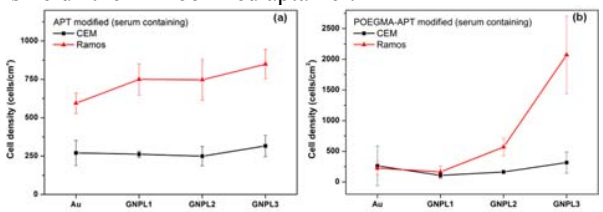


Figure 1. Selective capture of Ramos cells on APT modified smooth gold and GNPL(1 to 3) surfaces without (a) and with (b) the POEGMA spacer in serum-containing conditions.

Conclusions: Micro/nano-structured gold nanoparticle layers with varying surface roughness were modified with TD05 aptamer. In mixtures of Ramos and CEM cells under serum-free conditions, the density of Ramos cells on the roughest GNPL surface was 19 times that of CEM. However, the selectivity of GNPL-APT surfaces was much less in serum-containing conditions. GNPL-POEGMA-APT surfaces with POEGMA as an antifouling spacer, showed good selectivity for Ramos cells in serum-containing medium, and selectivity increased with increasing surface roughness. The density of Ramos cells was 6.6 times that of CEM cells on the roughest (GNPL3-POEGMA-APT) surface. The data generated in this study suggest that surfaces combining appropriate chemical composition and micro/nano topographic structure may be useful for cell separation, including the isolation of cancer cells for diagnosis.

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References:

[1] Braun, S., et al. *N Engl J Med*. 2000, 342: 525-533.
[2] Alunni-Fabbroni, M., et al. *Methods* 2010, 50: 289-297.
[3] Zhou, F., et al. *Macromol Biosci* 2012, 12: 1391-1400.