Surface functionalized carbon nanopipettes for real-time intracellular signaling sensing

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Statement of Purpose: The objective of this work is to advance the study of cell signaling by creating novel tools for intracellular sensing. Herein we describe unique nanoscale functionalized carbon nanopipettes (CNP) and the processing methods we elaborated for their reproducible manufacturing. The full potential of CNPs to measure, sense or capture specific signaling molecules inside the cell is not realized, lest the CNP is functionalized to react with molecules of interest. Thus, in this paper, we also describe the design and the validation protocols used to create reactive groups on the surface of the probe and utilize these groups to attach particles or bioactive molecules on to the surface of CNP for using it as analytical probes [1]. This paper additionally focuses on the evaluation of successful attachment of particles and biomolecules which can be detected using well established instrumental methods. Thus, gold nanoparticles, and biotin and fluorescent streptavidin molecules which can be detected and quantified easily under the fluorescent optical microscope were attached.

Methods:

The carbon nanopipettes (CNPs) are formed by depositing carbon inside pulled quartz micropipettes to form a continuous, conductive film along the quartz capillary's interior surface. Subsequently, the tip of the quartz capillary is etched to expose a desired length of carbon pipe.

Functionalization of CNPs with gold nanoparticles: The covalent attachment of gold nanoparticles to the surface of CNPs consists of three steps: carboxylation, thiolation and nanoparticles attachment.

Carboxylation: In order to create the carboxyl groups, CNPs were heat treated at 400^{o}C in air for 30 minutes. We have found this is the optimal temperature in order to keep the CNPs structurally unaffected.

Thiolation & nanoparticles attachment: Carboxylated CNPs were reacted with cysteamine hydrochloride in the presence crosslinking molecule EDAC in PBS solution at pH 7.4 for 16 hours. This reaction leaves the CNPs surface terminated with thiol groups. These thiolated CNPs were then dipped into the gold nanoparticles solution for 24 hrs in order to facilitate the covalent attachment of nanoparticles to the surface via thiol groups.

Biotinylation of functionalized CNPs & detection of streptavidin: In order to covalently attached the biotin molecules, the surface of gold nanoparticles on the CNPs were aminated by using the linker molecule cysteamine hydrochloride. Each CNP was immobilized with a fixed concentration 1mg/ml solution of biotin in PBS at pH 7.4. These CNPs were then dipped into three different concentration solutions of fluorescent labeled streptavidin, 0.1 µg/ml, 40 µg/ml, 200 µg/ml respectively. Reaction time for each pipettes were kept at 30 minutes. These CNPs were observed under the fluorescent microscope in order to detect and quantify the captured streptavidin. Gold functionalized CNPs without biotin were used as controlled probes. Average fluorescent intensity was calculated with the help of 'Image J' software.

Results: Electron microscopy results showed the uniform coating of CNP with gold nanoparticles. Fluorescent microscopy (fig. 1(a)) revealed the observable differences in the fluorescent intensity of biotinylated probes with different concentration of immobilized streptavidin. On the contrary the probes without biotin didn't show any fluorescence (fig. 1(b)). The average fluorescent intensity calculated over the exposed carbon tips reveals the linear variation with the different concentration of immobilized streptavidin (fig. 1(c)).

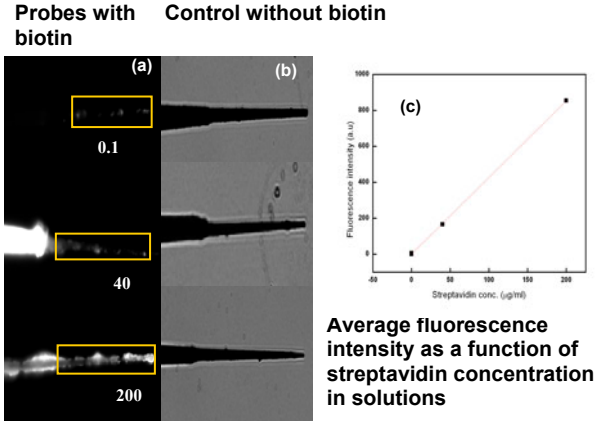

Figure 1.

Conclusions: The above results suggest that these functionalized CNPs can selectively detect and quantify the streptavidin molecules in solution. It can be concluded that there is no measurable non-specific interaction of streptavidin with the probes. These functionalized CNPs can be used as affinity based biosensors. They also are being used to detect and quantify intracellular proteins, as these CNPs can penetrate the cell membrane without damaging it.

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References:

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