Biological Uses of Chemically Dervatized Nanodiamonds

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All major forms of carbon at the nanoscale - fullerenes, nanotubes and nanodiamond (ND), the later in the forms of both particulate and films, appear to be the valuable materials for biomedical applications. The focus of our research group is applications of nanodiamond particles as biological tracers, scavengers, and delivery vehicles of biologically active molecules. Particulate nanodiamond possesses a number of distinct properties that make it an attractive biotechnological material. These distinct properties include very low cytotoxicity, fluorescence with little photobleaching and blinking characteristics, straightforward functionalization with chemical groups and biomolecules, large surface area ($400 m^2/g$), ability to be sterilized under high temperature and pressure conditions, and a variety of other unique characteristics.

One of the reported bioapplications of ND will include application of detonation nanodiamonds as probes for protein capture and electrophoretic collection of the analyte. This application is based on high electrophoretic mobility and controlled surface charges of nanodiamonds. To demonstrate this approach, a biotinylated nanodiamond bioconjugate was synthesized for the specific capture of streptavidin protein. The ND was also chemically conjugated to the fluorescent dye derivative of rhodamine (TAMRA) to facilitate microscopic detection. First, NDs were chemically modified to produce amine surface groups (ND-NH_2). The N-hydroxysuccinimide conjugates of TAMRA and biotin were coupled to ND-NH_2 to produce the singly conjugated particle TAMRA-ND (T-ND) and the doubly conjugated particle TAMRA-ND-Biotin (T-ND-B). These products form stable aqueous colloidal suspensions with a zeta potential of +40 mV for an average particle size of 150 nm. Streptavidin was captured by the T-ND-B bioconjugate probe and this nanoparticle-protein complex was collected from solution by electrophoresis on field tip arrays.

Use of NDs for biolistic gene and small molecule delivery will be also discussed. Detonation nanodiamond particles were coated with either DNA, synthetic ethylene precursor, ethylene antagonist or labeled with fluorescent dyes and delivered into bacteria, yeast, insect cells and plant tissues using a Bio-Rad particle delivery system PDS-1000/He [1]. We found that biolistic delivery of mechanically and chemically stable detonation nanodiamonds is a very effective means for the transformation of cells using the particles as a means of delivery of DNA and biologically active molecules. Alternatively, onion-like carbon (OLC) obtained by high temperature vacuum annealing of nanodiamonds, can be also derivatized with biomolecules and biolistically delivered to cells and tissue. Benefits of using this type of nanocarbon particles due to their very high absorbance of electromagnetic radiation in visible and infrared regions will be also discussed.

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[1] V. Grichko, V. Grishko, O. Shenderova, *Nanobiotechnology* **2**, 37 (2007).