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Binding and purification of plasmid DNA using multi-layered carbon nanotubes

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ABSTRACT

We propose a new method for the separation of nucleic acids using multi-layered carbon nanotubes (CNTs) as an adsorbent. According to agarose gel electrophoresis, oxidized water-stable CNTs adsorb certain forms of nucleic acids, such as high molecular weight RNA, chromosomal DNA, linear and denatured forms of plasmid DNA. However, CNTs do not adsorb supercoiled form of plasmid DNA. Nucleic acids bound to CNTs can be readily removed by centrifugation whereas supercoiled plasmid DNA remains in solution. Upon the addition of divalent metal ions supercoiled plasmid DNA forms relatively stable complexes with CNTs due to chelation. Thus, new details about association of nucleic acids with CNTs were revealed and stoichiometry of the complexes was estimated. Our results can be used for fine purification of supercoiled plasmid DNA for gene therapy applications as well as manipulation of nucleic acids for biosensor design.

1. Introduction

Development of materials and devices for manipulation of biological objects is one of the main problems of biotechnology. The use of nanomaterials for such purpose is reasonable as their dimensions are comparable to that of biomolecules in addition to broad range of physicochemical and biological characteristics exhibited by nanomaterials (Kane and Stroock, 2007). In this respect carbon nanotubes (CNTs) with organized graphene-like structure are one of the most promising nanomaterials which are capable to controllably affect biological systems at different levels (Yang et al., 2008).

CNTs are a candidate for developing materials for tissue engineering applications. In particular, CNTs and their composites with proteins are used for fabricating cell culture substrates. These substrates were shown to be non-toxic and biocompatible towards mammalian cells including stem cells in vitro (Harrison and Atala, 2007; Mooney et al., 2008). CNTs can provide a good support for the adhesion and proliferation of mammalian cells as well as for modulation of their differentiation (Abarrategi et al., 2008).

The mechanisms by which CNTs affect cell behaviour may involve the adsorption of cellular factors and extracellular matrix proteins (Abarrategi et al., 2008; Kam and Dai, 2005), direct effect on mechanical properties (Wang et al., 2005) and electrical conductivity (Malarkey et al., 2009) of matrixes. Doping of polymeric coating with CNTs allowed direct electrical stimulation of osteoblasts to assist their proliferation and production of bone components (Supronowicz et al., 2002).

Owing to high surface area and capability for chemical modification CNTs are a promising carrier for biomacromolecules. Proteins can be readily attached to the surface of water dispersed CNTs through physical adsorption (Munge et al., 2005) and chemical linking (Prashanth et al., 2006). CNT–enzyme conjugates exhibit high catalytic activity and stability (Prashanth et al., 2006) and can be used as ultra-sensitive labels for DNA detection (Munge et al., 2005).

While proteins are non-specifically adsorbed on CNTs by means of hydrophobic interactions (Kam and Dai, 2005), the binding of nucleic acids to CNTs seems to occur in a more complicated manner depending on molecular weight and conformation of nucleic acids. Single-stranded oligonucleotides were shown to wrap around CNTs due to adsorption of nitrogen bases onto nanotube wall (Zheng et al., 2003). It was shown that single-layered CNTs bind to major groove of DNA in preference to GC sequence resulting in destabilization of DNA duplex (Li et al., 2006).

One of the promising applications of interactions between CNTs and nucleic acids is the separation of nucleic acids based on their conformations. This biotechnological problem particularly covers large-scale isolation of plasmid DNA for gene therapy and vaccination (Ferreira, 2005). Plasmid DNA produced by bacteria and some

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