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Vaccine Delivery by Carbon Nanotubes

Novel nanomaterials, such as carbon nanotubes, are under active investigation for potential use in biomedical applications. In this issue of *Chemistry & Biology*, researchers describe antigen-antibody interactions and immune responses using peptide-carbon nanotube conjugates.

The potential of novel nanomaterials, such as carbon nanotubes, is enormous. These tubular arrangements of sp2 hybridized carbon atoms are under active investigation due to their phenomenal physical properties [1]. For example, they can function as semiconductors in nanoscale devices, be spun into the toughest material (man-made or natural [2]), or act as actuators with a force generation 100 times larger than that of mammalian muscles.

Carbon nanotubes (CNT) exist in two types, single wall (SWNT) and multi wall (MWNT). SWNT have lengths of up to 10 μm and diameter of up to 2 nm, depending on the production process. MWNT are thicker and longer. CNT are not easily processed due to their lack of solubility in many solvents. However, the CNT carbon atoms present an excellent platform for chemical functionalization. Noncovalent and covalent functionalization has been utilized to overcome the problem of processability (see [3–6] and references therein).

In the last five years, inorganic nanomaterials such as nanocrystals, nanowires, and nanotubes have been receiving an increasing amount of attention for potential biomedical applications. Nature has spent billions of years assembling nanoscale building blocks (such as lipids, peptides, and nucleic acids) into complex and functional structures. For example, selectivity and recognition at the molecular scale, such as antibody-antigen interactions, is a critical feature of living systems. However, nature has not had the opportunity to produce biomolecular interactions with the desired nanoscale materials [7]. It has been shown that selective design of peptides can be used to control interactions between the biological and nonbiological world. Peptide sequences have been used to bind to metal particles [8] and carbon nanotubes [4, 9, 10].

The interactions between carbon nanotubes and biological materials are mainly being investigated for biosensing (see [11, 12] and references therein). The basic concept for utilizing carbon nanotubes as transducers in biosensing applications is the ability to enable specific interactions with the analyte through functionalization of the nanotube surface and characterization of specific interactions (i.e., sensing) and reducing nonspecific interactions.

In an important new development, the work by Bianco, Prato, and collaborators [13] published in this issue demonstrates the potential use of carbon nanotubes in vaccine delivery. The basic concept for utilizing carbon nanotubes in vaccine delivery is to link the antigen to carbon nanotubes while retaining its conformation and thereby inducing antibody response with the right specificity. In addition, carbon nanotubes should not trigger a response by the immune system, i.e., they should not possess intrinsic immunogenicity.

In previous work, the Bianco and Prato research group's carbon nanotubes were covalently functionalized with a pyrrolidine ring through the 1,3-dipolar cycloaddition

of azemethine ylides [14]. In a subsequent publication, amino-derivatized nanotubes were covalently linked to a peptide sequence derived from the foot-and-mouth disease virus (FMDV), generating monoconjugated peptide-CNT [4]. Now this functionalization method has extended to enable the linkage of two FMDV peptide sequences to amino-derivatized carbon nanotubes (bis-conjugated peptide-CNT) [13]. Standard characterization techniques such as HPLC, NMR, and microscopy were used to confirm the formation of the peptide-nanotube covalent bonds.

To establish that nanotube-linked peptides cover the same conformational space as free peptides, antigen-antibody interactions were measured in vitro using surface plasmon resonance measurements. It was found that the antibody (anti-FMDV peptide mAb with anti-mouse Fcγ antibody) did interact with free peptides and peptide-conjugated nanotubes but not with pristine carbon nanotubes. Moreover, a qualitative analysis showed no difference in response between free peptide and peptide-conjugated nanotubes, thus establishing that nanotube-linked peptides cover the same conformational space as free peptides.

Immune responses to FMDV peptide were measured in vivo using BALC/c mice. It is well known that the FMDV peptide needs to be coupled to either a carrier protein or a T-helper epitope to render it immunogenic. Now it has been established that the peptide coupling to carbon nanotubes produces the same result.

The anti-peptide antibody responses (with ovalbumin bystander help) were measured using ELISA and were most significant for bis-conjugated peptide CNT. More importantly, the responses were directed to just the peptides and not the molecular link between peptides and carbon nanotubes. As a result, no anti-carbon nanotube antibodies could be detected. This could suggest that carbon nanotubes do not trigger an immune response.

Understanding the interaction of nonbiological materials (such as carbon nanotubes) with biological systems (such as peptides) is essential for the realization of biological applications with novel nanomaterials such as carbon nanotubes.

The research described by Bianco et al. in this issue [13] advances the potential application of carbon nanotubes as drug delivery systems. One can imagine that in the (distant) future, instead of receiving a vaccine shot by syringe, a patient may lick a lollypop coated with functionalized carbon nanotubes acting as vaccine delivery systems.

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New Structural Insights into the Inhibition of Serine Proteases by Cyclic Peptides from Bacteria

The inhibition of enzymes employing a nucleophilic serine residue by natural products has been studied for many years. More recently, high-resolution structural analyses have begun to augment kinetic analyses. In this issue of *Chemistry & Biology*, Schulz and colleagues describe the crystal structure of scyptolin A, a cyclic peptide produced by cyanobacteria, complexed with elastase. Together with structures for a related inhibitor bound to trypsin, the work may assist in the design of reversible serine protease inhibitors.

Classic enzymology studies combined with pioneering structural biology have led to the serine proteases being among the best characterized of all enzyme families.