Expert Opinion

Potential applications of boron nitride nanotubes as drug delivery systems

Gianni Ciofani

Center of MicroBioRobotics c/o Scuola Superiore Sant'Anna, Italian Institute of Technology, Smart Materials Lab, Viale Rinaldo Piaggio, Pontedera (Pisa), Italy

In recent years, there has been an explosion of research in the 'bio-nano' field, with the discovery and introduction of ever more fascinating materials for applications as drug delivery systems, sensors, transducers, and so on. The author's group, for the first time in the literature, proposed boron nitride nanotubes as a valid alternative to carbon nanotubes and other kinds of inorganic materials, because of their improved chemical properties that theoretically guarantee better stability and compatibility in a biological context. In this paper, the bio-applications of boron nitride nanotubes that have emerged in the literature are summarized, with special attention given to their exploitation as safe drug delivery and targeting carriers. Finally, the possibility of combining their physical and chemical properties is approached, highlighting the features that render these innovative nanovectors unique and exceptional candidates for many bio-applications.

Keywords: biocompatibility, boron neutron capture therapy, boron nitride nanotubes, drug delivery, drug targeting, nanocarriers, nanomedicine

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1. Introduction

The explosive growth of nanotechnology in the last few years has produced dramatic innovations in pharmacology, and it is revolutionizing the delivery of biologically active compounds. The long-term objective of drug delivery systems is the ability to target selected cells and/or receptors within the body. At present, the development of new drug delivery techniques is driven by the need, on one hand, to target drugs more effectively to the site of disease and, on the other hand, to identify new ways to deliver new classes of pharmaceuticals that cannot be effectively delivered by conventional means. Nanotechnology is critical in reaching these goals. Nanoparticle formulations make use of the fact that an enlarged surface/volume ratio results in enhanced activity. Nanoparticles are also useful as drug carriers for the effective transport of poorly soluble therapeutics. When a drug is suitably encapsulated in nanoparticulate form, it can be delivered to the appropriate site, released in a controlled way and protected from undergoing premature degradation. This results in higher efficacy and dramatically minimizes undesirable side effects. Such nanoparticulate delivery systems can be used to treat more effectively cancer and a wide range of other diseases, which require high-potency drugs [1].

Among the huge variety of inorganic vectors that nanotechnology offers, ceramic nanotubes have attracted considerable interest in pharmacology research. The properties and characteristics of carbon nanotubes (CNTs), for example, are being heavily researched, and scientists have just begun to investigate the potential of these structures. However, in some cases CNTs have already proved to serve as safer and

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more effective alternatives to traditional drug delivery methods. They can serve as vehicles carrying therapeutic drugs, vaccines and nucleic acids deeply into the cell, to desired targets, responding to static and dynamic energetic fields [2]. The discovery of nanoparticles that can combine chemical and physical properties during the drug delivery process has the potential to revolutionize biomedical research, as they can show superior performance over other traditional vectors. The advantage lies in a unique, unprecedented combination of electrical, magnetic, optical and chemical properties, which is promising for the development of new classes of drug complexes and therapies.

Notwithstanding, the concerns about cytotoxicity of a new class of nanomaterials such as CNTs are not yet fully addressed in the literature, which often offers contrasting conclusions [3,4], hence the necessity to find alternative suitable nanomaterials. In this article, it is shown how boron nitride nanotubes (BNNTs) could represent a valid vector for several applications in drug delivery research and, more generally, in nanomedicine. The main findings and other applications that have just started to emerge, very recently, among the scientific community are summarized. Future applications and realistic scenarios are approached and discussed.

2. Boron nitride nanotubes as drug delivery systems

Boron nitride nanotubes [5] are of significant interest for the scientific community. In fact, similarly to carbon nanotubes, they have attracted attention because of potentially unique and important properties for structural and electronic applications [6]. A boron nitride nanotube is a structural analogue of a carbon nanotube in nature: alternating B and N atoms entirely substitute for C atoms in a graphitic-like sheet with almost no change in atomic spacing; despite this similarity, carbon and boron nitride nanotubes have many different properties. It has been found that BNNTs have excellent mechanical properties, with a measured Young's modulus of 1.22 ± 0.24 TPa. Moreover, whereas CNTs vary from semiconducting to conducting behavior depending on chirality and diameter of the product, BNNTs have a constant band gap of ~ 5.5 eV [7].

Recent investigations have confirmed that BNNTs also have excellent piezoelectric properties. *Ab initio* calculations of the spontaneous polarization and piezoelectric properties of BNNTs have demonstrated that they function as excellent piezoelectric systems with response values larger than those of piezoelectric polymers, and comparable to those of wurtzite semiconductors [8]. In addition, BNNT bending forces have been measured directly by high-resolution transmission electron microscopy (TEM), and real-time video-recording of their elastic kinking deformation have confirmed their marked flexibility [9]. Recently, Bai *et al.* have experimentally verified deformation-driven electrical transport and the first evidence of piezoelectric behavior in multi-walled BNNTs [10]. The insulating character of an individual BNNT can be modified with tube squeezing between two gold contacts inside a transmission electron microscope. A notable current of several tens of nanoamperes is then able to flow through the tube. Such transport has been confirmed to be reversible, and disappears almost completely after tube reloading. These observations underpin the very high potential of BNNTs as efficient new nanoscale transducers.

Albeit all these impressive properties, whereas in the last few years applications of CNTs in the field of biotechnology had been largely proposed (e.g., biosensors, DNA chip, and nanovectors for drug, protein and gene delivery [2]), biomedical applications of BNNTs have just begun to emerge [11]. In **Table 1**, a summary of the main examples of BNNT bioapplications and investigations during very recent years is reported: before the experiments started in the author's laboratories, only Zhi and co-workers had investigated the interactions between BNNTs and various protein species [12] and DNA [13].

If the high chemical stability of BNNTs is an index of better biocompatibility, on the other hand it results in the problem of their poor dispersibility in the solvents commonly used for biological applications. The first issue to be addressed for biomedical applications is therefore the preparation of stable and biocompatible BNNT dispersions in aqueous media. For the first time, in the author's laboratories, aqueous BNNT dispersions based on non-covalent wrapping with polyethyleneimine and poly-L-lysine have been prepared and tested *in vitro*. Studies were reported on the cytocompatibility of BNNTs on human neuroblastoma cells and it was demonstrated, for the first time, that they do not have adverse effects on viability, metabolism and cellular replication of this cell line. The fluorescent labeling of BNNTs with quantum dots enabled their tracking in the cellular uptake and the investigation of the uptake mechanism [14-16]. These assays confirmed significant energy-dependent internalization of BNNTs by different types of mammalian cell (PC12 neuronal-like cells, human neuroblastoma, C2C12 myoblasts, etc.).

Of course, the intrinsic toxicity of these first tested wrapping agents did not allow the investigation of high BNNT concentrations. Very recently, glycol-chitosan was found to be an effective and absolutely compatible polymer that enables highly concentrated and stable BNNT dispersions to be obtained and tested (**Figure 1**), thus enabling the *in vitro* safety of these nanotubes, checked with many different, independent and complimentary toxicity assays (MTT, WST-1, live/dead assay, DNA quantification, apoptosis detection and oxidative stress investigation [17]), to be confirmed further.

After these preliminary but most interesting results, attention began to be given to applications of BNNTs. Chen and collaborators, for example, explored their use as cell delivery agents using single-stranded DNA as cargo [18]. A fluorescein isothiocyanate (FITC)-labeled DNA oligomer was loaded onto the surface of BNNTs by passive adsorption, and Chinese hamster ovary (CHO) cells were then incubated

Table 1. Summary of the main examples of BNNT bioapplications and investigations.

Investigation/application	Functionalization approach	Cargo type	Ref.
Investigation of interactions between BNNT and protein	Direct complexation; non-covalent wrapping with 1-pyrenebutyric acid *N*-hydroxysuccinimide ester	Proteins	[11]
Investigation of interactions between BNNT and DNA	Direct complexation	DNA	[12]
Preparation of BNNT aqueous dispersions	Non-covalent wrapping with PEI	N/A	[13]
BNNT cytocompatibility evaluation on human cells; uptake investigation	Non-covalent wrapping with PEI; covalent modification of the coating	Quantum dots	[14]
Differentiation of myoblasts in the presence of BNNT	Non-covalent wrapping with PLL; covalent modification of the coating	Quantum dots	[15]
Innovative polymeric coating: extension of biocompatibility study	Non-covalent wrapping with glycol-chitosan	N/A	[16]
BNNT as carriers for DNA and proteins	Non-covalent wrapping with glycodendrimers; ligand–receptor interactions with the coating; direct complexation	DNA, proteins	[17]
BNNT-polymer composite for tissue engineering applications	BNNT-polymer composites	N/A	[18]
Exploitation of the BNNT magnetic properties for drug targeting	Non-covalent wrapping with PEI; covalent modification of the coating	Quantum dots	[19]
Exploitation of the BNNT electric properties for cell electroporation	Non-covalent wrapping with PLL	N/A	[20]
Preliminary study of application of BNNTs in the boron neutron capture therapy	Non-covalent wrapping with PLL; covalent modification of the coating	Quantum dots, folic acid	[21]

BNNT: Boron nitride nanotube; PEI: Polyethyleneimine; PLL: Poly-L-lysine.

with the FITC-DNA-BNNTs for 12 h. Fluorescence microscopy revealed that FITC-DNA-BNNTs were internalized by the cells in a manner dependent on the carrier. The cytocompatibility and safety of BNNTs was confirmed further after wrapping them with a glycodendrimer.

Recently, Lahiri *et al.* proposed an intriguing application in the field of tissue engineering [19]. Biodegradable polylactide/polycaprolactone copolymer (PLC) was reinforced with BNNTs, resulting in composite films with improved mechanical properties and biocompatibility, for applications in orthopedic scaffolds. These constructs showed impressive improvements of the mechanical properties and, most interestingly, osteoblast cell viability study on polymer films revealed a 30% increase in live-to-dead cells ratio with BNNT addition in PLC. Gene expression study of osteoblast cells, grown on the composite films, showed a dramatic increase in levels of expression of the transcription factor Runx2, indicating accelerated osteoblast differentiation and growth in the presence of BNNTs.

All these results confirm the high potential of BNNTs as nanovectors for plenty of applications in biomedicine. The challenge, however, is the exploitation not only of the chemical properties of BNNTs, but also of the physical features outlined in the Introduction. The possible exploitation of the magnetic properties of BNNTs has been reported for the first time in [20]. SQUID (superconducting quantum interference device) analysis shows the typical trend of superparamagnetic materials, mainly due to the presence of small Fe particles, catalytic residuals of the production process. *In vitro* tests performed on human neuroblastoma SH-SY5Y cells demonstrated that cellular uptake of fluorescent labeled BNNTs can be easily modulated with an external magnetic field, thus enabling active drug targeting: magnetic properties of BNNTs can be used for this aim, the drug being easily chemically bound to the external coating.

Dielectric properties of BNNTs can be exploited to obtain local enhancement of an electric field, thus improving the electroporation efficiency in eukaryotic cells. Experimental evidence demonstrated that, performing electroporation after cells are allowed to interact with BNNTs, small molecules could be translocated across the cell membrane of

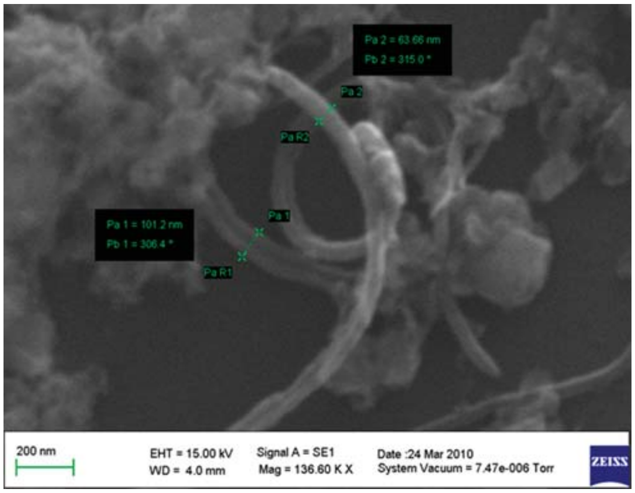

Figure 1. Scanning electrical microscope imaging of glycol-chitosan-coated boron nitride nanotubes (purchased from the Nano and Ceramic Materials Research Center, Wuhan Institute of Technology, China). Diameter size measurements are provided.

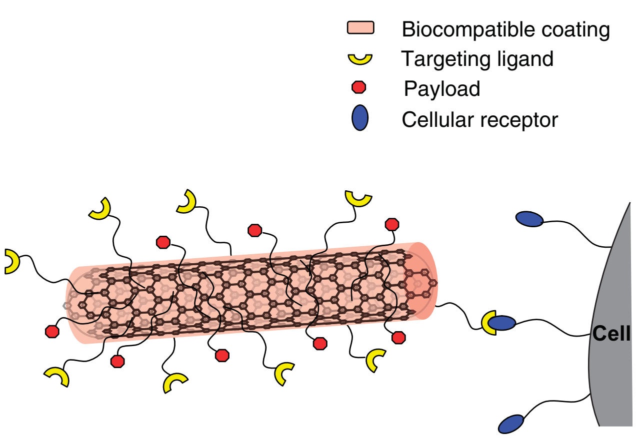

Figure 2. Schematization of a boron nitride nanotube as a drug delivery and targeting system.

human neuroblastoma cells by using a very low electrical field, maintaining a high level of viability, metabolism and proliferation, as demonstrated by specific assays. The use of controlled electric fields to aid cell permeabilization for enhanced cellular uptake of molecules has distinct potential for clinical application in drug delivery, gene therapy, vaccination and electrochemotherapy for solid cancers [21].

Finally, interesting perspectives in the field of boron neutron capture therapy (BNCT) are also under consideration [22]. BNCT is a binary modality therapy that has the potential for effective treatment of many forms of cancers, in particular cerebral glioblastoma multiforme. It is based on the neutron capture reaction $^{10}B(n, \alpha)^{7}Li$, where a ^{10}B atom captures a low-energy thermal neutron and spontaneously decays to produce the linear recoiling particles ^{4}He (α particle) and ^{7}Li. Highly energetic α particles are potentially capable of killing individual cancer cells (where a boron-based compound accumulates) while sparing the healthy normal parenchyma. In this case, BNNTs are proposed as a suitable boron-containing compound, and therefore as a drug *per se,* rather than a drug delivery system, after that, their selective uptake by cancer cells occurs.

3. Conclusion

The introduction of new inorganic materials in biomedical research is often followed by a huge debate about their biosafety and their interactions with living matter. Let us consider, for example, the case of carbon nanotubes: even many years after they were proposed as bio-nanovectors and as drug delivery systems, many contrasting results and opinions could be still found in the literature about their cytocompatibility.

In this report, data have been summarized suggesting that boron nitride nanotubes, innovative ceramic nanostructures, are not cytotoxic in the concentrations tested and in the cell models considered, thus showing that their use in therapeutic or diagnostic applications is feasible and realistic. Their functionalization with appropriate coatings and targeting ligands enables their exploitation as drug delivery systems, biomarkers and biosensors (**Figure 2**). Their unique physical properties, joined with their compatibility, make BNNTs an optimal candidate for an impressive variety of applications in the nanodomain.

4. Expert opinion

The necessity for much more biological testing on BNNTs, eventually *in vivo,* before a final statement on their safety is evident and mandatory for an actual exploitation of these nanovectors in the biomedical field. The classical questions about their final fate inside the cells, about their bioaccumulation, excretion, and so on, should be absolutely approached in the near future.

If the use of such an innovative vector raises all these concerns, are there any relevant advantages, with respect to more traditional nanovectors (polymeric-based nanoparticles, liposomes, etc.), that justify an extensive, time-consuming and cost-demanding research? In the author's opinion the answer is obviously affirmative. Nanoscale structures and materials (e.g., nanoparticles, nanowires, nanofibers and nanotubes), thanks to a unique combination of chemical and physical features, open many new and, until a few years ago, unbelievable possibilities for biomedicine. It has already been shown and discussed how the physics of BNNTs could dramatically improve the efficiency and the targeting ability of a drug delivery system. Let us now stress other unexplored features, such as the peculiar optical and piezoelectric properties, which could be exploited in the near future to achieve an actual nanotransducer system, able to interact with cells and tissues sensitive to electrical stimuli.

The increasing interest in BNNTs, demonstrated by an ever increasing number of publications, as indicated by the ISI Web of Science database, could soon lead to an explosion of their applications in nanopharmacology and, more generally, in all the sectors of nanomedicine, with the big challenge of obtaining an active nanodevice able to foster different functions inside a living system.

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Declaration of interest

The author states no conflict of interest and has received no payment in preparation of this manuscript.

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Affiliation

Gianni Ciofani PhD
Center of MicroBioRobotics
c/o Scuola Superiore Sant'Anna,
Italian Institute of Technology,
Smart Materials Lab,
Viale Rinaldo Piaggio, 34-56025
Pontedera (Pisa), Italy
Tel: +39050883019; Fax: +39050883497;
E-mail: g.ciofani@sssup.it