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Mesoporous nanotubes as biomaterials

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Abstract: This review provides an overview of selected recent research efforts that employ the use of mesoporous nanotubes in a biomaterial context, e.g. principally as a therapeutic or biosensing platform. We focus on the compositions of alumina, boron nitride, silica, silicon, titania, and zinc oxide, along with selected accounts involving single-walled carbon nanotubes. Where known, attention is directed toward the biodegradability and biocompatibility of a given nanotube type, its tunability of size and surface chemistry, and relevance of these parameters to its function as a biomaterial.

Keywords: nanotube, drug delivery, biosensor, alumina, boron nitride, silica, silicon, titania

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1 Introduction

While a diverse range of mesoporous morphologies are available for investigation, the appealing simplicity of a one-dimensional hollow nanotube construct, with associated high surface area along with interior/exterior curved interfaces, provides unique opportunities in the observation of new physical properties and chemical reactivity relevant to a range of disciplines. While tubular crystals of naturally-occurring minerals have been known for some time [1], single walled carbon nanotubes (SWCTs) have garnered a lion's share of attention in the last decade [2]. The range of commonly-investigated nanotube materials has subsequently been extended to a rather lengthy list, as outlined in Table 1.

Much of the initial focus of investigations of these nanotubes has centered on their relevance to energy-related areas such as battery technologies and photovoltaics. Given the charge of the journal *Mesoporous Biomaterials*, this specific review entails highlighting known recent studies (within five years or less, where possible) of selected nanotubes that retain the desired porous dimension in the mesoporous range relevant specifically to either biosensing or therapeutic (e.g. principally drug delivery) applications. The discussion presented herein is organized according to composition, sub-classified within each by fabrication, fundamental properties (biocompatibility/biodegradability), and application. Highlights of a given material's desirable properties for a particular biorelevant application are identified where possible, along with remaining challenges for clinical implementation.

2 Alumina Nanotubes

We begin with a brief but focused discussion on nanotubes of aluminum oxide (alumina, Al_2O_3). It is appropriate to begin with this composition, given the fact that nanoporous alumina membranes are used in a widespread manner as templates for the attempted formation of other nanotube types (titania, silicon) via infiltration, annealing, and etching.

Interestingly, prior investigations have established the utility of nanoporous alumina membranes to possess improved osteoblast adhesion and proliferation (relative to amorphous alumina) for orthopedic-relevant applications [3] and also support viability and functionality of encapsulated beta cells for the ultimate use in immunoisolated devices [4]. Nevertheless, in order to legitimately probe size dependent effects, methods must be employed to separate membrane assemblies into individual nanotubes. These are highlighted below.

2.1 Alumina Nanotube Fabrication

The base alumina nanoporous membranes are prepared by anodization in dilute H_3PO_4/H_2SO_4 [3, 4]. Some procedures add a second anodization step under pulsed galvanostatic conditions to improve pore structure. Any remaining aluminum substrate can be removed by wet chemical etching in a mixture of dilute $CuCl_2$ and HCl. Free-standing alumina nanotubes are obtained by immersion into the same acid solution followed by ultrasonic

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Table 1: Known mesoporous nanotubes investigated for biomaterial applications

Nanotube Type	Focus	(+) Advantages / (–) disadvantages	*References*
Al_2O_3	Drug delivery	(+) Ease of preparation / (–) brittleness	[3–6]
BN	Drug delivery, tissue engineering, radiation therapies	(+/–) Chemical inertness (both a positive & negative effect, depending on application) / (–) length control	[7–22]
SiO_2	Drug delivery, tissue engineering, biosensing	(+) Diverse range of preparative routes / (–) Agglomeration (depending on surface chemistry)	[23–36]
Si	Drug delivery, tissue engineering	(+) Bioresorbability; semiconductor / (–) multistep fabrication & low yield	[37–46]
SWCNT	Drug delivery, tissue engineering, biosensing	(+) Ease of large scale preparation / (–) toxicity in the absence of surface modification	[47–75]
TiO_2	Drug delivery, tissue engineering, biosensing	(+) Large scale fabrication and ease of osteointegration (in orthopedics)	[76–86]
ZnO	Drug delivery, biosensing	(+) Cost, ease of fabrication / (–) challenges with drug loading	[87–92]

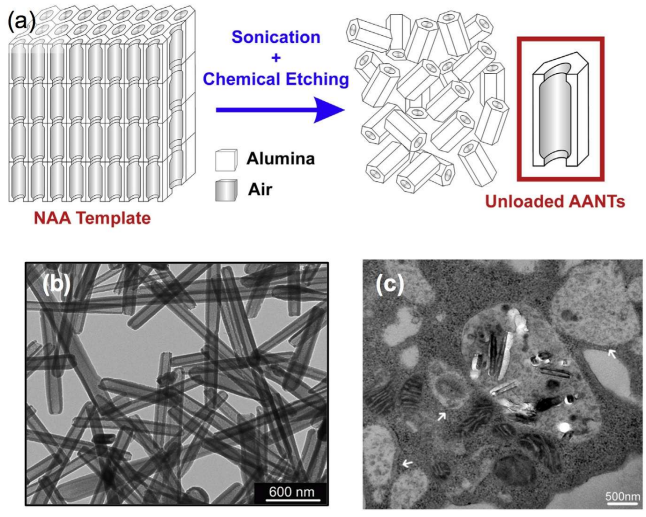

Figure 1: Alumina nanotubes: (a) generation of discrete alumina NTs from alumina nanoporous membranes [6]; (b) TEM image of isolated alumina NTs with widths on the order of 100 nm and lengths ~700 nm [6]; (c) TEM image of alumina NTs internalized by RAW 264.7 macrophage cells [6].

treatment [5]. Typical inner diameters for the nanotubes prepared by this route are on the order of 30 nm, falling within the mesoporous regime [5].

2.2 Alumina Nanotube Biocompatibility

Mesoporous alumina nanotubes have not enjoyed the widespread investigation as witnessed for the case of silica and titania, for example. Alumina is typically considered as a bioinert material; nevertheless, its ability to be carefully constructed in nanotube form under controlled fabrication conditions does make it an excellent candidate for an evaluation of the toxicity of high aspect ratio nanomaterials in general. A detailed study by Wang and coworkers evaluated alumina nanotubes with aspect ratios ranging from 7.8 to 63.3, and multiple cytotoxicity assays (beyond simple cell viability and morphology) were conducted with RAW 264.7 mouse macrophage cells and MDA-MB 231-TXSA human breast cancer cells [5]. Not surprisingly, the resultant toxicity patterns were cell-type dependent and strongly related with nanotube dose, length of time, and very importantly, nanotube aspect ratio. Long ratio nanotubes triggered enhanced cell death, morphological changes, tumor necrosis factor α (TNF-α) release, etc. than short nanotubes. The toxic aspect ratio 'window' of these nanotubes was determined to be 7.8, reported to be relatively shorter than that of other high aspect ratio nanomaterials [5].

2.3 Alumina nanotubes - therapeutic relevance

Drug Delivery

Given the above results, other research groups have evaluated the ability of a non-toxic alumina nanotube material to host and release a tumor necrosis factor-relevant

apoptosis-inducing ligand, Apo2L/TRAIL [6]. Experiments with these nanotubes using a combination of transmission electron microscopy (TEM) and fluorescence microscopy demonstrated significant uptake of alumina nanotubes by the same RAW 264.7 mouse macrophage cells and MDA-MB 231-TXSA human breast cancer cells noted earlier. These alumina nanotubes could load more than 100 micrograms of the Apo2L/TRAIL ligand per mg of nanotube, and in studies with MDA-MB 231-TXSA human breast cancer cells, an associated significant reduction in cell viability is observed due to the induction of apoptosis (as monitored by changes in Caspase-3 activity). Importantly, and encouragingly, these high loading capacities facilitated cancer cell death in relatively short times [6].

Given cost considerations and ease of fabrication, these results for this relatively under-explored composition of nanotubes will likely stimulate further work as a consequence.

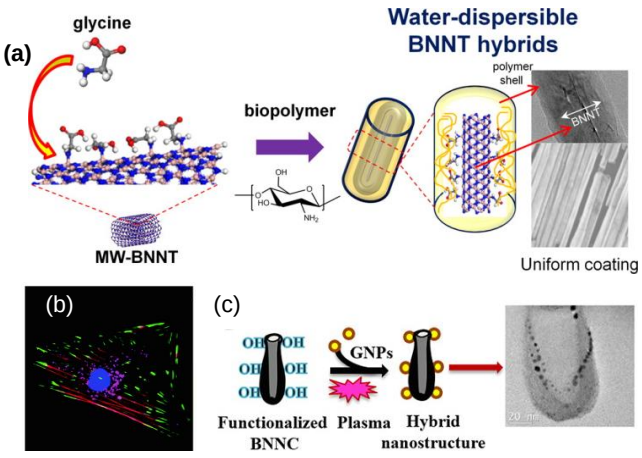

Figure 2: Boron Nitride (BN) nanotubes: (a) schematic illustration of glycinetemplated biopolymer coating process for these nanoubes [15]; (b) Internalization of functionalized BN NTs by a mesenchymal stem cell [17]; (c) Plasma-assisted functionalization of BN NTs with Au nanoparticles [16].

3 Boron Nitride Nanotubes

In nanotube form, boron nitride forms a unique contrast to its isoelectronic analog, carbon (CNTs). Its chemical inertness, specifically resistance to oxidation, along with mechanical strength and intrinsic radiation adsorption properties, suggests novel utility in selected biomedical applications [7].

3.1 Boron Nitride Nanotube Fabrication

Typical fabrication techniques for boron nitride (BN NTs) nanotubes have been inspired by methods established for the growth of CNTs, mainly via arc-discharge and chemical vapor deposition (CVD) routes [8, 9]. Additional methods under refinement have focused on the use of rather extreme conditions (laser heating or ball milling at high pressures) [10–12] or more sophisticated refinement of the above-noted CVD techniques [13]. These methods are effectively summarized in a 2010 review by Goldberg et al. [7]. In spite of extensive efforts, the large scale synthesis of boron nitride nanotubes with suitable diameters and purity for biomedical applications (requiring less complex reaction conditions) remains a challenge, likely the strongest challenge to its widespread implementation for such purposes. It is also clear that the preparative method employed has a very sensitive influence on nanotube width (pore size); for example, laser ablation methods can produce BN NTs with inner diameters of 3–15 nm [14], while ball milling can provide meso-scale inner widths in the range of 20–40 nm [15].

3.2 Boron Nitride Nanotube Biocompatiblity and surface chemistry dependence

Given its chemical inertness, one of the most common formulations to evaluating the cytotoxicity of boron nitride nanotubes has been their coating with different biocompatible polymer species. One of the most noteworthy is a recent (2014) study involving noncovalent functionalization of boron nanotubes with four surfactants: pluronic (P123), polyethyleneimine (PEI), pluronic (F127) and ammonium oleate [16]. Cytotoxicity levels in four different cell lines (Vero, Chang liver, MCF7 and A549) were evaluated by MTT assays, with the pluronic-coated boron nanotubes showing the most promising biocompatibility versus the cell lines tested.

Other biocompatible coatings onto the surface of BNNTs have also been investigated, including the polycationic polymer chitosan, polyanions such as hyaluronan (HA), and polyzwitterionic polymers such as chitosan-phosphorylcholine (CH-PC) [17]. However, for such coatings to adsorb onto the nanotube surface, glycine must be present onto the BN, achieved with relatively mild conditions. The role of glycine in this process is 2-fold: the amine terminus presumably binds to the B-sites of BNNTs, while the carboxylic acid function provides ionic anchoring sites for interactions with polyelectrolytes [17]. Such functional-

ized nanotubes have not been yet been evaluated for their biocompatibility, however.

3.3 Boron Nitride nanotubes - therapeutic relevance

3.3.1 Drug Delivery

It should be pointed out that selected surfactant-coated BN nanotubes noted above have also been evaluated for their antibacterial activity. Specifically, quantitative turbidity assays indicate that PEI-coated BN nanotubes exhibit strong bacterial activity against *Escherichia coli* and *Staphylococcus aureus* [16]. In addition, chemotherapeutics such as tamoxifen and paclitaxel loaded into pluronic-coated BNNTs were tested in MCF7 and A549 cells. It is found that DNA fragmentation and fluorescence-based assays are consistent with an apoptotic pathway of cell death for these particular cancer cells, inferring the possible further exploration of such nanovector constructs for targeted drug delivery [16].

One rather unique approach to tacking the nanotube length issue by Ponraj et al has been addressed by the generation of a pulsed plasma in liquid at atmospheric pressure by a nanosecond pulsed voltage, and its effect on suspended boron nitride nanotubes [18]. Such an approach was used to significantly reduce the length of boron nitride nanotubes; furthermore, with the presence of gold nanoparticles in the solution, hybrid boron nitride/gold nanoparticle solutions were created. These nanotubes were loaded with the established anticancer drug doxorubicin and an average of 97% of prostate cancer cells (DU145) were killed. Such effects presumably occur for plasma-treated BNNTs because of their shorter length, facilitated cellular uptake, and drug loading capacity [18].

3.3.2 Tissue engineering

The approach of coating BN nanotubes with specific biocompatible species has also been found to impart useful therapeutic properties to such hybrid materials relevant to tissue engineering. A range of coatings, with different areas of emphasis in the realm of tissue engineering, has been investigated. One example evaluated the influence of BN NTs functionalized with gum arabic on its cytocompatibility and subsequent differentiation of mesenchymal stem cells [19]. *In vitro* assays were performed on mesenchymal stem cells to evaluate the cytocompatibility of the functionalized BN NTs in terms of cell viability and metabolic activity. The differentiation of the mesenchymal stem cells into adipocytes and osteocytes after treatments with non-toxic concentrations of BN NTs was assessed at both the gene and phenotype levels [19]. Unfortunately, it was found that the differentiation of the MSCs into adipocytes was enhanced by the presence of the nanotubes, with no measureable elevation of osteogenesis.

In another application, the piezoelectric properties of BN NTs were exploited (using low-frequency ultrasound) for purposes of exploring its relevance to cardiovascular tissue engineering [20]. Three-dimensional constructs, in microfiber form, were obtained via ultrasonic irradiation; it was found that C2C12 myoblasts differentiate into viable myotubes and internalize the BN NTs. Both 2D and 3D models were further evaluated by investigating the expression of connexin 43 (C × 43, involved in cell crosstalk and mechano-transduction) and myosin, a myogenic differentiation marker. Maximum expressions of C × 43 and myosin were detected in the 3D model relative to all controls, a marked contrast to standard 2D cultures where BN NTs (with ultrasound) both deplete myosin synthesis and reduce C × 43 mRNA levels [20]. Such results for authentic three-dimensional scaffolds are encouraging, but certainly reinforce the necessity of drawing conclusions from proper *in vitro* models.

3.3.3 Therapies taking advantage of radiation sensitivity

Finally, it should be pointed out that some limited investigations highlight the known neutron absorbing ability of boron in such nanotubes to act as a therapeutic vehicle relevant to Boron Neutron Capture Therapy (BNCT). Boron neutron capture therapy (BNCT) is a form of cancer therapy which exploits the preferential accumulation of boron-containing compounds in tumor sites. Irradiation by a neutron beam subsequently causes the ^{10}B to split into an α particle and a lithium nucleus, both of which cause damage localized to the cells in which they are contained.

One study has evaluated dispersions of BN NTs coated with poly-L-lysine solutions along with functionalization with a fluorescent probe (quantum dots) for tracking and folic acid for targeting purposes [21]. *In vitro* studies of this material with glioblastoma multiforme cells indicate some selectivity in nanotube uptake, suggesting a possible use in clinical BNCT for cerebral tumors.

A recent patent application also describes a formulation that combines BN NTs functionalized with a IgG antibody species via a suitable linker for covalent attach-

ment and housed in a polymeric chitosan carrier.[22] The intended focus of such a composite is also in neutron-based therapies such as BNCT.

4 Silica Nanotubes

4.1 Silica Nanotube Fabrication

Silica nanotubes have been prepared by a diverse number of routes, all of which employ active templating – by either organic, inorganic, or biomolecular species – to achieve the desired morphology. For example, use of single-walled carbon nanotubes as templates provide silica nanotubes with diameters ranging from 5–23 nm [23]. A recent review highlights a multiple synthetic methodologies that have been successfully engaged for this purpose [24]. Other strategies have emerged recently, such as work by Gao et al. highlighting use of a nickel hydrazine complex – nanorod precursor species that permits sensitive tailoring of length of the given silica nanotube product length via hydrazine concentration employed [25]. The range of lengths reported span from 37 to 340 nm; associated inner diameters are also tunable (from 10–20 nm) based on choice of surfactant in this case.

4.2 Silica Nanotubes: Bioresorption Studies

One of the most detailed studies of the dissolution behavior or silica nanotubes comes from studies by Hu and coworkers, who evaluated both the pH dependence as well as the thickness dependence of such phenomena [26]. Similar to mesoporous silicon [27], the degradation product of these silica nanotubes is monomeric silicic acid, $Si(OH)_4$.

In terms of pH dependence, not surprisingly, negligible dissolution is observed at pH = 1; however, at neutral pH (7), approximately 35% is degraded in 5 h, and shifting to a pH = 8 results in over 80% dissolution in the same time period [26]. Nanotube wall thickness demonstrates a similar sensitivity to dissolution. At this slightly elevated pH value of 8, silica nanotubes with average wall thicknesses of 14 nm dissolve less than 20% in the 5 h time window, while diminishing this value to ~9 nm dramatically increases the percent silica dissolved to ~80%. The authors then exploit the above sensitivity to demonstrate pH dependent drug release of the anticancer drug camptothecin from this system [26].

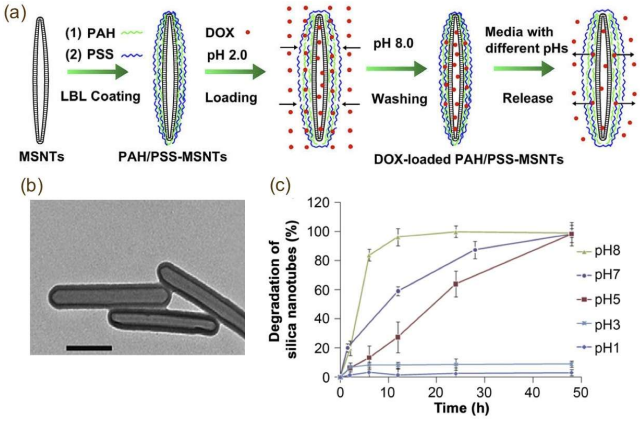

Figure 3: Silica Nanotubes: (a) Basics of coating silica nanotubes with polyelectrolyte layer-by-layer assembly, with concomitant drug loading (such as with doxorubicin (DOX) [31]; (b) TEM image of silica nanotubes of 14 nm length [26]; (c) Dissolution of silica nanotubes 9 nm length as a function of pH (at 37°C) [26].

4.3 Silica nanotubes – therapeutic relevance

Gene therapy

Along with the above example with camptothecin, a diverse range of drug delivery options has been explored using this type of nanomaterial. One of the early noteworthy examples include attempted gene therapy, whereby silica nanotubes were functionalized with primary amine moieties (via silicon alkoxide precursors), then electrostatically loaded with a gene encoding green fluorescent protein protein (GFP), exposed to monkey kidney cells, and the cells subsequently found to express GFP [28]. Transfection efficiencies of 10–20% were reported for this system. A second example of a gene therapy delivery system based on silica nanotubes entails a far more complex platform with the use of polyethylene imine (PEI) that not only binds the nucleotide of interest, but also entangles multiple silica nanotubes in a single construct [29]. To diversify their potential biomedical relevance, specifically in area of imaging, these investigators also loaded superparamagnetic iron oxide nanocrystals along with visibly emissive quantum dots into the nanotube interior, making these quite complex compositions. Transfection efficiencies on the order of 60–70% percent have been reported with this system [29]. The possible issue of cytotoxicity due to the presence of the cadmium-containing quantum dots was not addressed, however.

Drug Delivery – Other Complex Combinations

This theme of more complex designs in terms of nanotube surface functionalization and impact on delivery has been addressed for other therapeutics, including more complex platforms addressed by Chen and coworkers [30]. Using amine-functionalized silica nanotube surfaces, these workers created a mutlistage platform that contained ibuprofen (for eventual release), immobilized enzyme (vide infra), and CdS quantum dots. The presence of the amine functionality is found to reduce the ibuprofen release rate from the matrix [30]. While the quantum dots are presumably present for imaging reasons also in this case, once again the ultimate toxicity of the CdS quantum dot in an *in vivo* context was not discussed, however.

Layering of polyelectrolytes is a proven strategy in nanomaterial design, and has been applied to silica nanotubes as well for pH-controlled release of the established chemotherapeutic doxorubicin. In a recent study, as-prepared silica nanotubes were exposed to a layer by layer assembly of poly(allylamine hydrochloride) and sodium poly(styrene sulfonate) to load and release the positively charged drug doxorubicin [31]. A second related design was prepared by alternately coating sodium alginate and chitosan onto amine-functionalized mesoporous silica nanotubes, which were used as vehicles for the loading and release of the negatively charged model drug sodium fluorescein. Controlled release of the drug molecules from these delivery systems was achieved by changing the pH value of the release medium. An evalution of the cell-killing efficacy of the loaded doxorubicin against human fibrosarcoma (HT-1080) and human breast adenocarcinoma (MCF-7) cells was found to be pH dependent, suggesting a relevance to pH-controlled drug delivery systems [31].

A more recent study has exploited the reduced nanoscale pore channel width of silica nanotubes to in principle decrease drug particle size, increase drug surface area, and thereby increase drug dissolution rate for drugs with intrinsically poor aqueous solublity [32]. This is exemplified in this study by the incorporation of the relatively insoluble drug cilostazol into mesoporous silica nanotubes. Gelatin coatings of different thicknesses were employed to further tune drug delivery release rates by barrier coatings.

Finally, it should be pointed out that non-standard release triggering mechanisms have been applied to drug-loaded silica nanotube platforms, such as the work published by Kapoor and Bhattacharyya regarding the use of ultrasound to release ibuprofen from silica nanotubes [33]. Not surprisingly, drug release profile is strongly dependent on ultrasonic exposure protocol, with shorter pulses (ca. 30 sec) and shorter time intervals between successive ultrasonic pulses producing higher amounts of released ibuprofen. Additional investigations on less stable drugs or specific clinical candidates with challenging release conditions remain to be explored at this stage, however.

4.4 Mesoporous Silica Nanotubes for Biosensing

In multiple examples, enzymes have been immobilized on the surface of mesoporous silica nanotubes. The perceived rationale for this strategy is to improve the stability of a given enzyme with regard to temperature, pH, and long-term storage. This is exemplified with studies of the attachment of penicillin G acylase [34] and lysoszyme [35] to porous hollow silica nanotubes. The system most relevant to authentic biosensing entails the immobilization of glucose oxidase to silica nanotubes, as it is sensitive probe of glucose concentrations in biological fluids [36]. In this regard, enzyme activities were found to increase with increasing surface coverage of adsorbed protein (up to a relative activity scale of 98.6%), then effectively diminish slightly and saturate at values approaching 90%. This slight reduction at higher enzymatic loadings is attributed to aggregation of adsorbed glucose oxidase and associated restriction of mass transfer of substrate to/the nanotube surface [36]. A more quantitative evaluation of glucose oxidase sensing platforms have been carried out for other nanotube compositions, however (*vide infra*).

5 Silicon Nanotubes

5.1 Silicon Nanotube Fabrication

Nanotube materials based on elemental silicon form a complementary system to the silica materials described above, as mesoporous silicon particles and films demonstrate a well-established dissolution chemistry exploited for drug delivery [37, 38], sustained release, and enhancement during dissolution phenomena in tissue engineering applications such as calcification for bone regrowth [39–41]. This is in addition to the key advantage of silicon as a semiconductor, to be exploited in the long term for ‘smart’ applications involving the exploitation of Si in a monolithic structure that has both sensing and therapeutic functions on a single platform. Ideally in such a platform there

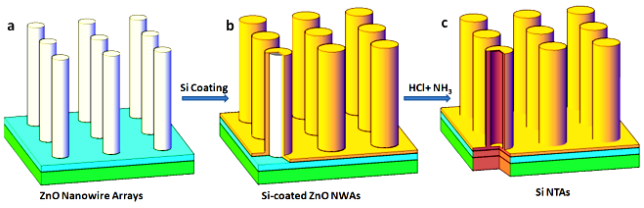

Figure 4: Si nanotube array (Si NTA) fabrication by sacrificial templating. Si is deposited (CVD with silane) onto ZnO NWs at 500°C, followed by removal of the ZnO core by etching with HCl and NH_3.

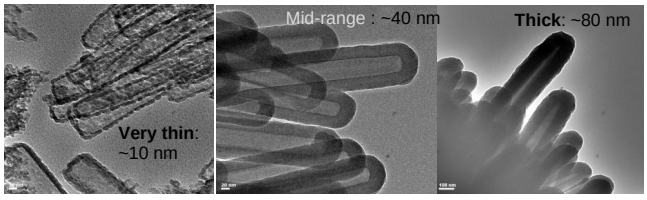

Figure 5: Representative cases of silicon nanotubes prepared by a sacrificial templating method, showing (a) porous sidewalls; (b) medium thick walls approx. 20 nm thick, and (c) extremely thick Si nanotube sidewalls ca. 80 nm thick.

will be electronic communication between the two functions.

In terms of nanotube synthesis, multiple entries to the formation of nanotubes elemental silicon have been investigated, including routes that involve incomplete filing of a porous alumina template [42]. This process is very difficult to control and achieve uniformity in nanotube morphology (e.g. it is easy to overfill a given pore and produce solid nanowire structures). Another published route employs gas phase condensation, a process that yields a large amount of amorphous, ill-defined particles and only few nanotubes on a given surface [43].

The process currently employed in our laboratories relies on the initial formation of a zinc oxide (ZnO) vertical nanowire array template, followed by the deposition of a surface silicon layer of a desired thickness (using silane as a Si precursor), and finally, use of a gas phase etching process involving He-diluted HCl / NH_3 gas mixtures at a modest temperature (450°C) to remove the ZnO core and produce the resultant hollow nanotubes [44].

We have established the ability of such a process to create SiNTs of a sensitively tunable shell thickness, ranging from 10 nm to ~100 nm. The former nanotube possesses a rather unique porous morphology with small mesopores in the 2–5 nm regime (Fig. 5a), providing opportunities for facilitated infiltration and release. We have also demonstrated the ability to tune inner diameter of the nanotube, from ~30 nm to hundreds of nm, with implications for the size/dimensions of the resultant loaded species (therapeutic or sensing probe molecule) that can be confined within the nanotube cavity.

5.2 Silicon Nanotubes: Bioresorption Studies

The observation of a porous morphology in the very thin walled SiNT variant poses the question of possible dissolution of these nanotubes in aqueous media over time. Knowledge of such behavior is critical to use of these SiNTs to *in vivo* functions such as sustained drug delivery and/or tissue engineering.

In this regard, silicon dissolution assays have been carried out with the use of molybdate-based spectrophotometric-based methods. For the case of the thin porous sidewall (10 nm) SiNT exposed to aqueous buffer at physiological temperature (37°C), 80% of a $\sim$ 60 µg sample will resorb in a 48 h period, and complete dissolution within 72 h [44]. This dissolution behavior is similar to that of high porosity bioactive nanostructured mesoporous silicon prepared by the anodization of crystalline silicon [27]. Use of thicker Si side walls, lower temperatures, and an absence of salts in the water greatly reduce these dissolution kinetics.

5.3 Silicon nanotubes – therapeutic relevance

Drug Delivery

There are ample opportunities to demonstrate useful drug delivery with these semiconducting nanotubes. One of the more unique examples published thus far lies with the case of the loading of superparamagnetic iron oxide nanocrystals into these nanotubes, with the long term goal of magnetic-field assisted drug delivery of a relatively high density of these nanocrystals, and functionalized with a targeting/therapeutic moiety onto the outer SiNT surface [45].

To date, there are two methods for loading nanocrystals such as magnetite (Fe_3O_4) into SiNTs, depending on the magnetite crystal size and the absence/presence of pores in the nanotube wall. If the silicon walls are thin and porous (< 12 nm), and the magnetite size small enough (~4 nm), then simple diffusion is sufficient for satisfactorily complete loading of a given nanotube. If the SiNT wall thickness is greater, non-porous, and/or the magnetite nanocrystal diameter is larger, then the silicon nan-

otube array must be 'opened' at one end by physical removal from its underlying substrate.

Measurements of the fundamental magnetic properties of these loaded SiNTs have found that in all cases the blocking temperature (T_B) remains significantly below room temperature, ensuring their suitability for use in biological environments.

Gene therapy

The relative large internal surface areas of SiNTs, capable of housing a loaded therapeutic, coupled with the presence of targeted genes to the outer nanotube surface has given impetus for its investigation in gene therapy. For such experiments, aminopropyltriethoxysilane (APTES) is utilized as a linker, whereby the siloxy species can covalently bind to surface silicon atoms of the nanotube, and the amine terminus exposed (and available for coupling with a polynucleotide) [46].

Initial evaluation of cytocompatibility with HEK 293 cells and these SiNTs confirm that SiNTs have no adverse effects on this human cell line. In preliminary transfection experiments, plasmid DNA (pDNA) expressing the enhanced green fluorescence protein gene under control of an inducible interferon-beta promoter (IFNβ-eGFP) was selected. Optimal ratios of the binding of APTES-SiNTs to pDNA, evaluated by agarose gel electrophoresis, demonstrated that the suitable ratio of silicon to pDNA was about 35:1; indicating the positive charge density on the surface of SiNTs is not very high. The eGFP expression from HEK 293 cells was evaluated after 24 hrs, 48 hrs, and 72 hrs transfection. eGFP expression with these plasmid-bound Si NTs required 72 hours transfection. Experiments with additional surface functionalization strategies are currently underway to reduce this incubation time.

6 Single-Wall Carbon Nanotubes (SWCT)

Carbon nanotubes are of significant interest because of their elemental simplicity and structural uniqueness (i.e. they can be isolated in single atom thick wall form). The number of reported studies for SWCNTs is large enough such that informative review articles summarizing both therapeutic [47, 48] and biosensing [49] relevance have been compiled recently. Thus, we restrict our discussion here to the relevant topics (fabrication, biocompatibility / biodegradability (if the latter property has been evaluated), therapeutics, biosensing) that are especially noteworthy and/or has appeared after a given review has been published.

6.1 Single Wall Carbon Nanotube Fabrication

Original accounts of carbon nanotube synthesis are well known for the heterogeneity of reaction products produced (multiwalled species, and for the single walled tubes, mixtures subsequently identified as semiconducting, metallic, and insulating structures) [50]. In terms of inner width, SWCNTs possess a range of 2–30 nm. The evolution and refinement of chemical vapor deposition techniques applied to SWCNT formation have subsequently been refined to overcome some of these initial hurdles in fabrication [51, 52]. High volume production with fluid bed reactors capable of reactant diffusion and thermal transfer to the requisite metal catalyst have resulted in significant reduction in production costs for carbon nanotubes in general [53]; however, isolation of SWCNTs with the desired purity and chirality requires a combination of density gradient centrifugation with surfactants encompassing the nanotubes, or alternatively, gel chromatography [54, 55]. Yet as pointed out by DeVolder et al., in spite of such significant improvements, the cost of SWCNT production is still too expensive to be exploited in commercial products on a large scale [56].

One of the key steps in assessing feasibility of carbon nanotubes for biological applications lies in altering chemistry of the nanotube surface. Multiple comprehensive reviews have recently summarized the progress in this area (including one published late last year) [48], so for the sake of brevity we distill the highlights of the key adopted strategies in this section.

The earliest, and one of the most extensively utilized methods entails an oxidative treatment of the carbon centers to produce carboxylic acid moieties on the sidewalls, ends, or defect sites of the SWCNT [47]. This of course has yielded additional synthetic openings to produce nanotubes with more than one or more additional type of surface species, often put into place by the use of well-known cycloaddition reactions. The range of available functionalities is extremely diverse, in addition to the carboxylic acid species, including acylation, amidation, esterification, and polyethylene glycol (PEG)-ylation [48]. Tabular compilations of the known range of functional groups attached to SWNCTs are readily found in references 47 and 48.

A second major success in SWCNT functionalization lies in the general approach of non-covalent wrapping by

polymers, biopolymers, surfactants and other amphiphilic molecules. Some of the most popular choices in this regard include 'proton-rich' polymers such as polyethyleneimine (PEI), polyacrylic acid (PAA), and dendrimeric polyamidoamine species (PAMAM) [57]. This has resulted in the dispersion of relatively large amounts of nanotube species in aqueous via straightforward processes that are readily scalable.

6.2 Single Walled Carbon Nanotube Biocompatibility / Bio-resorption Studies

Two parameters of SWCNTSs play a role in their biological response: length and surface chemistry. Early studies with non-functionalized multiwalled CNTs highlighted concerns with the possibility of mesothelioma in the case of nanotube bundles beyond a certain length (20 μm) [58]. Subsequent investigations with functionalized MWCNTs less than 1 μm in length did not pose a toxicological risk with regard to intrapleural administration and retention [59]. The hypothesis that carboxylic acid-functionalized SWCNTs, in the presence of strongly oxidative enzymes such as horseradish peroxidase (HRP) or myeloperoxidase (MPO), should undergo significant degradation and ultimate resorption, was a significant advance to this field [60]. This approach has been evaluated significantly and expanded to include the use of a simulated phagolysosome environment (PSF, phagolysosomal stimulating fluid; simulates the acidic oxidizing environment present in endosomes and phagolysosomes of macrophages) for a range of COOH-functionalized nanotubes [61]. To facilitate this degradation, a phosphatidylserine coating on COOH-functionalized SWCNTs apparently initiates a digestion signal to macrophages, monocytes, etc to begin the process [62]. An informative review of the efforts to date regarding this topic has been published by Bianco and co-workers [63]. A very recent report by Donkor et al. has tackled the length dependence issue on cellular uptake of SWCNTs. In this work, very short (< 80 nm) SWCNT functionalized with PEG were exposed to different cell lines, with an analysis in terms of cell-type dependent cellular uptake, intracellular localization, excretion, and in some cases partitioning at cell division [64]. Confocal fluorescence imaging and flow cytometry analysis of three cell types (HeLa, human hepatoma, and HUVEC) indicate that PEGylated SWCNT shorter than 35 nm might not be suitable for active targeting but may find merit in gene transfection (due to its ability to spontaneously traverse the nuclear membrane) [64]. SWCNTs

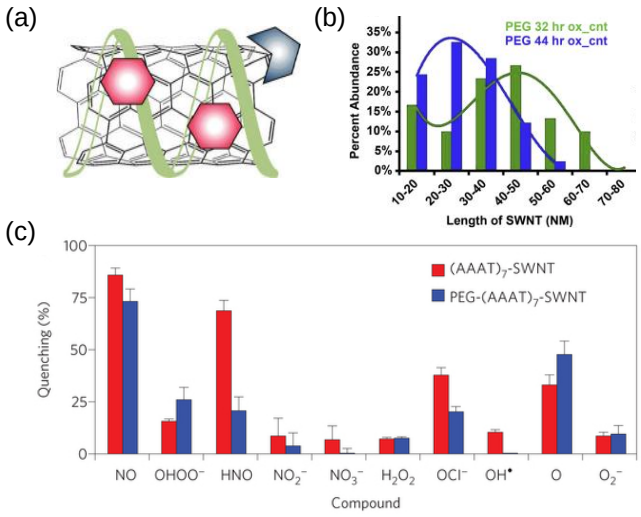

Figure 6: Single Wall Carbon Nanotubes: (a) typical SWNCT construct functionalized with charged polymer (ribbon) and drug/prodrug (hexagon- outer surface), and targeting moiety (arrowhead) [45]; (b) control of SWCNT length by acid oxidative treatment [64]; (c) Sensitivity of SWCNT fluorescence to NO, NO-containing species, and various O species [75].

with an average length of 30 nm were rapidly excreted by HeLa and hepatoma cells, but for the case of HUVEC, lysosomal retention is observed. Such results clearly demonstrate not only the sensitivity of nanotube length to cellular response, but identity of cell type as well.

6.3 Single Wall Carbon Nanotubes – Therapeutic Relevance

Drug Delivery with SWCNTs

As mentioned earlier, this is a well-investigated area of nanotube research, with the dominant disease model addressed with this platform being cancer. A recent review by Fabbro et al. outlines the key themes investigated thus far, involving the effects of covalent attachment of selected important anticancer drugs to the SWCNT surface, including doxorubicin, methotrexate, taxanes, platinates, camptothecine and gemcitabine [47]. As with any nanoscale drug delivery vehicle, targeting is a key issue of course, and multi-group functionalization of the nanotube surface is required; commonly employed moieties used in the reported studies include the well-known RGD peptide [65], folic acid [66], biotin [67], and selected antibodies [68]. Overall a number of very promising results have been reported, with advantages touted in terms of the capability of SWCNTs to act as a needle and deliver biologically-active

molecules directly to the cytoplasm [47]. The large surface area functionalized with targeting ligands and (in principle) internal cavity that can be loaded with additional useful species (e.g. magnetic nanoparticles) are clear advantages of the use of SWCNTs. However, due to the diverse experimental conditions employed across these multiple studies, precise comparisons as to the efficacy of these designs are often difficult.

Gene therapy

The ability of these low dimensional SWCNTs to act in a needle-like fashion is also relevant to exploitation in the field of gene delivery. With the nanotube in a sense acting like a spear, the target cell nucleus can ideally be penetrated and thus transfected with the nucleotide of interest from the nucleotide carrier [69]. Multiple additional criteria must be satisfied by the vector to ensure feasibility, however, including sufficient loading of the gene of interest, cellular translocation, intracellular delivery, controllable gene expression and minimal immunogenicity / toxicity [57].

In a rather brief period of time, a significant diverse range of 'genetic cargos' have been evaluated, including small interfering RNA (siRNA) [70], microRNA (miRNA) [71], plasmid DNA (pDNA) [72], oligonucleotides (ODNs) [73], and DNA/RNA aptamers [74]. These studies, both *in vitro* as well as *in vivo*, are effectively summarized in tabular form in a recently-published review [57].

Again, while multiple significant advances have been realized, serious challenges remain [57]. These include sensitive evaluation of the dose of nucleotide cargo to SWCNT carrier, determining optimal means of delivery *in vivo*, and maintaining control of gene expression for sustained periods.

6.4 Single Wall Carbon Nanotubes – Biosensing

As with other aspects of SWCNTs applied to biological systems, biosensing platforms utilizing these nanotubes as an active element have attracted immense interest over the past decade. A comprehensive review has appeared earlier this year [49]; hence as with other well-studied nanotube systems, we focus in this section on unique aspects associated with this topic, including selected results appearing since the release of the above article.

These sensing platforms have been classified into the following categories: electrochemical (mainly amperometric-based); physical (optical, calorimetric, piezoelectric), and SWCNT field effect transistors [49]. Prior surface functionalization of the nanotube is usually required for optimal signal response upon its interaction with the analyte, usually enzyme or nucleotide in origin. The identity of analytes that have been analyzed by SWCNT biosensors to date is rather diverse and extensive, including proteins / enzymes, nucleotides, viruses, bacteria, and metabolites [49].

Of those very recent reports, we wish to briefly highlight here a recent publication by Strano and co-workers involving a near IR fluorescent SWCNT structure capable of detecting NO in tissue [75]. Interestingly, this construct entails fabrication of luminescent nanotubes on a flexible hydrogel substrate detection with a detection limit of about 1 μM; it is claimed that such a structure is the first reversible, direct optical sensor for NO capable of *in vivo* operation. The stability of such sensors *in vivo* for over a year (at least 400 days, with a negligible change of activity observed) is indeed impressive and has the potential for even longer time periods of performance, given an absence of photobleaching in nanotube emission. Two modes of operation have been demonstrated with this sensor: injection followed by localization within the liver, as well as direct implantation within tissue. Further development of this platform, thereby providing more fundamental knowledge associated with tissue inflammation, cancer and cell signaling applications, is eagerly anticipated.

Not surprisingly, major challenges to widespread implementation of the above structures remain: (1) cost (given the steps associated with SWCNT isolation and purification) and (2) legitimate thermal stability and operating lifetime of these SWCNT-based biosensors. The above NO sensor based on a stable luminescent SWCNT species certainly addresses the latter concern, but focused efforts from investigators working on these challenges continue.

7 Titanium oxide (Titania) nanotubes

Titania remains an extensively explored category of nanotubes. While of significant interest to the fields of dye-sensitized photovoltaics and battery-related electrochemistry, titania nanotubes have been widely investigated for biomaterial properties including tissue engineering and drug delivery.

7.1 Titania Nanotube Fabrication

Standard fabrication of titania NTs often employ the anodization of titanium foil or related metal shape in a weak acid electrolyte. However, in a study of some practical significance published earlier this year, Gulati and coworkers have explored several factors systematically that strongly influence nanotube fabrication [76]. These workers specifically analyzed the role of electrolyte aging, water content, voltage / time of anodization, and the substrate dimensions for optimization of the fabrication of nanotubes on curved surfaces such as Ti wires. As a consequence, an overall optimal fabrication procedure, including anodization parameters, was presented that yield stable, well adherent titania nanotubes of high-quality [76]. In terms of nanotube inner diameter, a rather broad selection of possible sizes can be achieved experimentally, most commonly in the range of 30–100 nm [77]; the latter value is formally beyond the standard IUPAC classification for a mesoporous material.

Another relatively recent study involving more sophisticated fabrication of titania NTs include the use of an oscillatory voltage during the anodization process to produce periodically modulated internal structures in array form [78]. These periodic modulated structures were also subsequently fractured by ultrasonication into liberated capsule form. The intended goal of producing such periodic structures is the formation of nanotubes capable of enhanced drug loading and extended duration of release (relative to conventional titania nanotubes).

7.2 Titania Nanotube Biocompatability

Several key studies have interrogated complementary compatibility aspects of titania nanotube properties relevant to its use *in vitro* and / or *in vivo*.

One early study investigated the adsorption of key blood proteins (albumin, fibrinogen, and immunoglobulin-g) on titania nanotube arrays using a combination of micro-BCA assays and X-ray photoelectron spectroscopy (XPS) [79]. The adhesion and activation of platelets was investigated using live-cell staining, viability assays (MTT), and SEM. Whole-blood clotting kinetics was evaluated by measuring the free hemoglobin concentration, with SEM used to image clot formation. Overall, these results indicate increased blood serum protein adsorption, platelet adhesion and activation, and whole blood clotting kinetics on titania nanotube arrays, results that have clear implications for their use in a clinical context.

A follow up investigation interrogated the *in vitro* immune response of titania nanotubes compared to titanium surface controls. Immune cell functionality (of leukocytes, thrombocytes and trace amounts of erythrocytes) was evaluated by cellular viability, adhesion, proliferation, morphology, and cytokine/chemokine expression [80]. Results indicated a decrease in short- and long-term monocyte, macrophage and neutrophil functionality on titania nanotube arrays as compared to the control medical-grade titanium substrate. Thus a reduced stimulated immune response induced by these titania nanotube arrays is certainly desirable and worthy of further assessment.

Finally, one rather pragmatic study to point out here involves the explicit evaluation of the role of sterilization method on titania nanotube cytocompatibility. Zhao et al. investigated autoclaving, ultraviolet irradiation and ethanol immersion, on the cytocompatibility (primary rat calvarial osteoblasts) in the presence of titania nanotubes (with an average diameter of 80 nm) as well as titania nanostructured ‘nets’ with an average pore diameter of 25 nm [81]. UV and ethanol sterilization apparently induce higher initial cell adhesion and proliferation compared to autoclaving, whereas UV irradiation produces optimal adhesion, proliferation, as well as differentiation (as represented in terms of gene expression) in the presence of these nanotubes [81]. These authors suggest that differences between sterilization protocols may be responsible for published differences in the *in vitro* response of titania nanotubes.

7.3 Titania nanotubes – Therapeutic Relevance

Drug Delivery

The literature regarding drug delivery from mesoporous titania nanotubes is rather vast and has been reviewed in a very recent article from earlier this year by Dusic and co-workers [82]. It should be emphasized that many of the drug delivery studies reported involve delivery of antibacterial or anti-inflammatory compounds to suppress possible bacterial infections / immune responses associated with a titanium-containing implant. Thus in this section we mention only rather relatively newly reported studies involving drug delivery from titania nanotubes of a rather unique nature.

One of the most recent and innovative of these reports involves the use of a titanium wire containing a titania nanotube layer loaded with indomethacin (an anti-

inflammatory drug) and micelle-based carrier (tocopheryl PEG succinate) [83]. For this platform, Au nanoparticles were included for the purpose of including a transducer, as RF signals were generated from a customized RF generator to trigger an *in vitro* release. In their reported experiments over a 2.5 hr time window, 92% of loaded drug and 68% of loaded carriers were released using a short RF exposure of 5 min, in contrast to control values of 31% of drug and 11% of carriers lacking a RF trigger [83]. Thus it would appear that RF triggered release in this type of titania nanotube is indeed possible and warrants further exploration.

Tissue engineering

Given the prevalence of titanium metal alloy–based orthopedic implant materials, one of the great intrinsic appeals of the use of titania nanotubes in this type of application is to exploit both the titanium oxygen surface chemistry, along with the high surface area of mesoporous nanotubes, to enhance the osteointegration of a given implant. Hence technically the formation of new bone in a tissue engineering context is of a rather limited region between implant and existing bone.

One of most influential of these studies involves early (2007) evaluation by Desai and co-workers of titania nanotubes (formed by an anodization method) to promote osteoblast differentiation of marrow stromal cells and extracellular matrix production, and enhance short- and long-term osseointegration *in vitro*; *in vivo* biocompatibility of these nanotube-containing interfaces was also assessed histologically after four weeks by implanting surfaces in a male Lewis rat model [84]. It was found that cells cultured on nanotubular surfaces showed higher adhesion, proliferation, alkaline phosphatase (ALP) activity (a marker for osteogenic differentiation), and bone matrix deposition compared to flat titanium surface controls; furthermore, *in vivo* biocompatibility results suggested that nanotubular titania does not cause chronic inflammation [84]. This well-cited report stimulated an extensive number of additional investigations (for example, see ref. [85]) of titania nanotubular structures as non-planar orthopedic implants.

A very recent account by Wan et al. describes the use of titania nanotubes to act as a possible scaffold for large area bone tissue engineering [86]. These TiO_2 nanotubes, with an average outer diameter of 100 nm and (aggregated) wall thickness of ca. 7 nm, were synthesized using the template-assisted sol-gel method followed by calcination. Bacterial-based cellulose was used as the template for nanotube formation in this case. The large surface area of such tubular scaffolds (1629 m^2 g) result in an enhancement of cell growth and proliferation, along with improved alkaline phosphatase (ALP) activity and mineralization, when compared to ordinary culture plate controls [86]. It is noted here that the ALP activity of the scaffold is as high as commonly-employed hydroxyapatite-coated nanofibrous scaffolds, a very encouraging result for further development of this nanotube-based material.

8 Zinc Oxide Nanotubes

While extensively explored in nanowire form, ZnO nanotubes remain under-investigated for biomaterial purposes. The lack of toxicity [87] and ease of fabrication for ZnO nanostructures do suggest ample opportunities in this regard. Nevertheless, a few studies are reported for nanotube platforms with zinc oxide as a key component, and these are brought to the reader's attention to give a proper perspective on the diversity of nanotube compositions available.

8.1 Zinc oxide Nanotube Fabrication

The ease of ZnO synthesis in nanoscale dimensions is one of its most appealing properties. Most approaches involve initial formation of a ZnO nanowire-type structure, followed by transformation of these NWs into NTs using judicious control of etching of the ZnO (with either dilute HCl or KOH) to form a hollow interior with tubular shape [88]. One account describes the use of silk as a templating agent for ZnO multiwall nanotube formation, but the tubes lack consistent cylindrical shape and are heavily aggregated [89]. The mesopores associated with these type of nanotube fall within the 20–50 nm range [89].

8.2 Zinc oxide nanotubes – therapeutic relevance

Studies of the release of active therapeutics from NT designs based on ZnO are quite limited; in fact, a scrutiny of the recent literature finds only one report along these lines. Specifically, Yuan et al. utilize a ZnO nanowire array, in conjunction with layer-by-layer alternating templating assembly of the polyelectrolytes PBI and PAA to form nanotube architectures that house and subsequently release model drug compounds [90]. While in principle a system

of great opportunity, the incorporation of authentic therapeutics remain to be evaluated for such designs.

8.3 Zinc oxide nanotubes for Biosensing

Far more work has been carried out for the case of modified ZnO nanotubes and applications relevant to biosensing. For example, in contrast to the semi-quantitative measurements performed on a silica nanotube platform described above, Yang and co-workers have fabricated ZnO NT arrays on ITO substrates (and Nafion coating) with immobilized glucose oxidase, with a sensitivity of 30.85 μA cm^{-2} mM^{-1}, a relatively low limit of glucose detection of 10 μM, and Michaelis-Menten constant K_M^{app} of 2.59 mM [91].

More recent investigations have continued to refine explore glucose sensing with such nanotubes. Kong and co-workers [92] utilize a well-defined hexagonal ZnO nanotube array (again with immobilized glucose oxidase) to produce a biosensor with a 3 sec response time and a sensitivity of 21.7 μA cm^{-2} mM^{-1}. An impressive detection limit of 1 mM is reported, but with a surprisingly far larger Michaelis-Menten constant of 19 mM (relative to the initially-reported ZnO NT structure noted above) [92].

Utilizing a multiwalled ZnO nanotube structure fabricated from silk templates, Zhao et al have fabricated a chitosan/ZnO NT/Au structure with immobilized glucose oxidase with detailed measurements of a relatively fast (< 2 sec) and sensitive response (47.2 μA mM^{-1} cm^{-2}) [90]. Interestingly, their associated Michaelis-Menten binding constant of 1.09 mM is improved relative to that of the initial ZnO NWA platform of Yang et al. [92].

Given the competion between groups on this platform, further refinement of these ZnO NT-based biosensors specifically for glucose monitoring is anticipated.

9 Overall Summary

This review has attempted to cover some of the key issues in the evaluation of selected inorganic mesoporous nanotubes for therapeutic and diagnostic use. The amount of preclinical research carried out thus far on silica, titania, SWCNTs, along with BN NTs has indeed been impressive, and interesting opportunities in the further evaluation of silicon, alumina, and zinc oxide nanotubes for their possible applications in biotechnology clearly exist. While challenges for each material have been identified, it is hoped that dedicated research on all of the above compositions will continue at an enthusiastic pace.

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