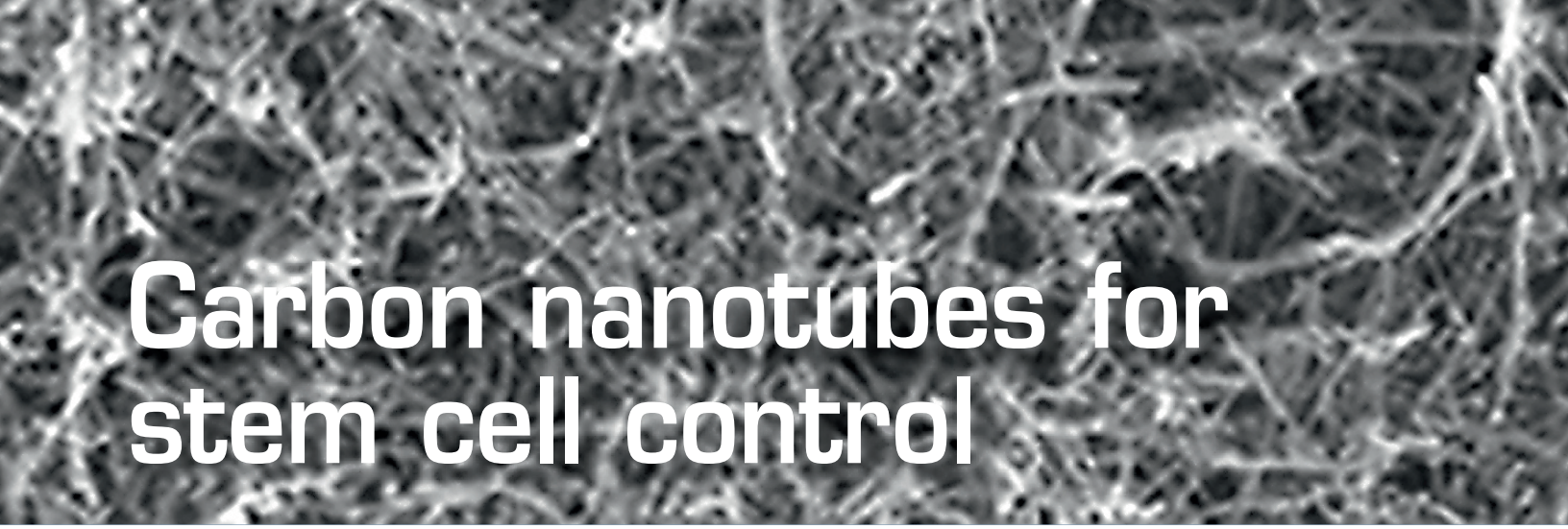

Carbon nanotubes for stem cell control

In the past decade, two major advancements have transformed the world of tissue engineering and regenerative medicine—stem cells and carbon nano-dimensional materials. In the past, stem cell therapy seemed like it may present a cure for all medical ailments, but problems arose (i.e., immune system clearance, control of differentiation in the body, etc.) that have hindered progress. But, with the synergy of carbon nano-dimensional materials, researchers have been able to overcome these tissue engineering and regenerative medicine obstacles and have begun developing treatments for strokes, bone failure, cardiovascular disease, and many other conditions. Here, we briefly review research involving carbon nanotubes which are relevant to the tissue engineering and regenerative medicine field with a special emphasis on carbon nanotube applications for stem cell delivery, drug delivery applications, and their use as improved medical devices.

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One of the main goals of tissue engineering and regenerative medicine is to replace diseased or damaged tissue with biological substitutes that can reestablish or maintain normal tissue function. Major advancements in stem cell research, tissue transplantation, and material science and engineering have supported the ever changing development of tissue engineering and regenerative medicine. In the past decade, two major advancements have transformed the world of tissue engineering and regenerative medicine—stem cells and nanotechnology (the study and control of materials at length scales ≤ 100 nm[1]).

In biology it is known that all cells arise from other cells, but stem cells are defined as undifferentiated (unspecialized) cells that can renew themselves and can also give rise to one or more specialized cell types that have specific functions in the body[2] (Fig. 1). For example, tumor initiation starts as a single mutation in just one cell. As the tumor cell grows, it divides exponentially, which gives rise to several different cell types that can be traced back to the single tumor 'stem' cell. This can also be seen in extreme medical examples called teratomas (an encapsulated tumor with many components resembling normal derivatives of all three germ layers)

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or teratocarcinomas (a germ cell tumor that is a mixture of teratoma with embryonal carcinoma). Biological research has been able to locate stem cells in the bone marrow[3], adipose tissue[4], myocardium[5,6], skin[7], embryos[8], and many other organs[9,10] (Fig. 2), but it wasn't until researchers found a way to grow them in the lab[11-13] that the tissue engineering and regenerative medicine fields took off. Through this breakthrough, stem cell therapy seemed like it could provide the cure for all medical problems, as significant advancements in cardiac disease[14], neurological complications[15], cancer treatments[16], and Parkinson's disease[17] were made.

However, major pitfalls arose that basic stem cell therapy could not overcome by itself. For example, when alone stem cells are more likely to develop into tumor cells[18], and how one controls this is still being widely debated[19-21]. Also, the issue of how to deliver stem cells still needs to be solved. Stem cells can be injected intravenously[14,22] into their designated areas, but keeping them in their desired location (without migration) and controlling their differentiation are problems that have still not been fully resolved[23,24].

These concerns have resulted in a synergy between stem cell research and the use of nanotechnology. Like regenerative tissue engineering, which involves directing the growth of cells to form higher order structures, nanotechnology is using the same bottom-up ideology to focus on building simple elements from multifaceted structures and materials. Nanotechnology revolves around the use of materials which possess at least one physical dimension between 1 – 100 nm to create structures and materials that have novel properties (known as nanomaterials). This is very important, as most biological components (i.e., DNA) involve some aspect of nano-dimensionality. Knowing this, it is logical to use nanomaterials for tissue engineering and regenerative medicine. For example, iron oxide superparamagnetic nanoparticles and quantum dots have been used to track the bio-distribution of stem cells and other types of cell *in vivo*[25,26]. Also, numerous nanomaterials can have multifunctional capabilities that both target and image in a biological environment[27]. One category of nanomaterials that has transformed tissue engineering and has strong potential for improving stem cell technologies are carbon nanotubes.

Following the discovery of carbon nanotubes (CNTs) and other carbon cylinders[28,29], carbon-based nanotechnology has rapidly become a platform technology for a variety of uses, including biomedical applications. CNTs have attracted increasing attention due to their mechanical, electrical, thermal, optical, and structural properties, and the ability to produce them in many different shapes and sizes (Fig. 3)[30]. CNTs are ordered, hollow nanostructures consisting of carbon atoms bonded to each other via sp^2 bonds, which are stronger than sp and sp^3 bonds and are the key ingredient in providing CNTs with their excellent mechanical strength as well as their high electrical and thermal conductivity. CNTs can be imagined as forming a graphene sheet rolled into a cylinder. One graphene sheet rolled up will form a single-walled carbon nanotube (SWCNT), while more than one concentric graphene sheet creates a multi-walled carbon nanotube (MWCNT). Single wall carbon nanotubes usually have diameters that typically range from 0.5 to 1.5 nm and a length that ranges from 100 nm to up to several micrometers. MWCNTs have larger diameters (can be more than 100 nm) due to multilayer structures.

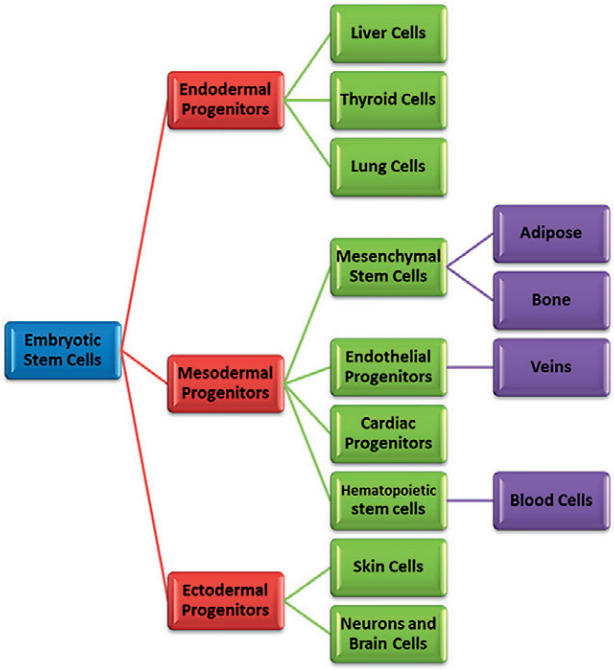

Fig. 1 Flow chart of the stem cell lineage originating from embryotic stem cells (this begins when a sperm fertilizes an egg). Stem cells possess various abilities when it comes to differentiating into different cell-types. One type of stem cell can produce any other cell-type of a given organism. Other stem cells can only produce cells of a given tissue type or a few cell-types in a given tissue.

Due to the superb mechanical, electrical, and surface properties of CNTs, they are ideal candidates for a wide range of applications such as structural materials[31], sensors[32], field emission displays[33], hydrogen storage materials[34], tips for scanning probe microscopy[35], and nanometer-sized semiconductor devices[36]. For example, metallic SWCNTs can carry currents over 50 fold greater than normal metals[37], but more importantly, by having a high aspect ratio (and consequently high surface areas with many dangling bonds on their side walls) CNTs have strong potential for biomedical applications, including neural regeneration[38], cardiomyocyte growth[39], muscle regeneration[40], orthopedic tissue growth[41], and induced pluripotent stem cell applications[42]. More importantly, CNTs have the potential to bridge the gap between stem cell therapy and nanotechnology for numerous biomedical applications. This review focuses on research involving CNTs relevant to tissue engineering and regenerative medicine, with a special emphasis on CNT applications for stem cells, drug delivery applications, and their use as improved medical devices.

CNTs and stem cells

As stated above, over the past few decades there have been significant advances in stem cell therapy and tissue engineering for the repair and replacement of damaged tissue and organs. In parallel, nanotechnology and nanomaterials, in particular CNTs, have emerged showing a pronounced potential for creating the next generation of biomaterials. By combining the two, a series of publications have shown how material-based approaches can be used to control the fate of mesenchymal stem cells (MSCs)—a type of stem cell that can be isolated from adults and

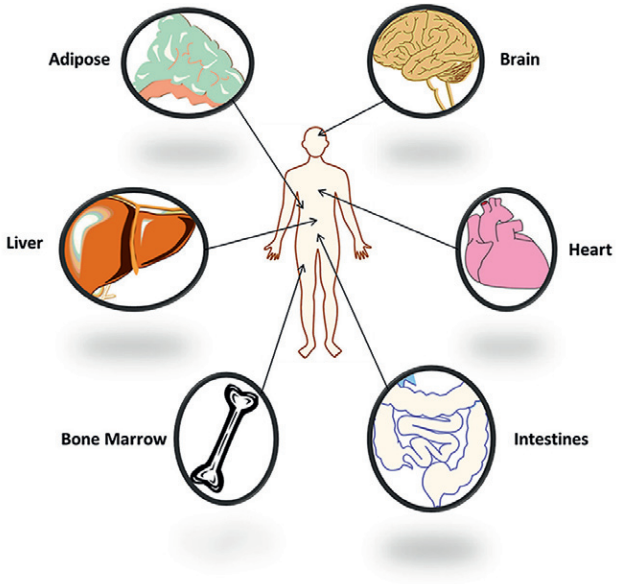

Fig. 2 Some locations where research has found adult stem cells and their niches in the body. Adult stem cells are typically called multipotent cells and can generate all the cell types of the organ from which they originate. This is different than embryonic stem cells, which are pluripotent (can differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm). Adult stem cell research is not considered to be controversial, since they are derived from adult tissue samples rather than destroyed human embryos. In the past decade, adult stem cells have gained attention from the research community since they can be genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells (able to differentiate into all derivatives of the three primary germ layers), known as induced pluripotent stem cells.

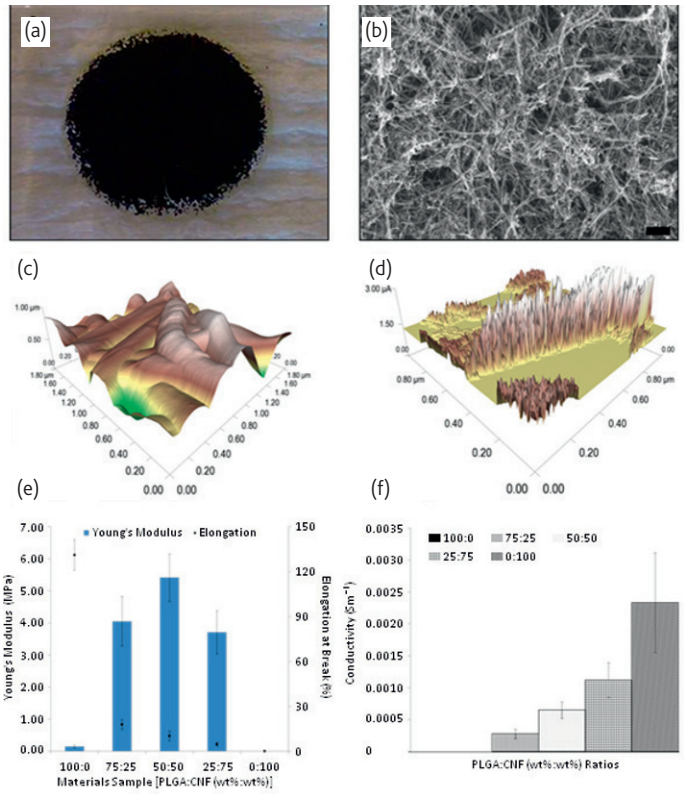

Fig. 3 The many ways CNTs can change material characteristics, particularly, by adding 100 nm diameter CNTs to biodegradable poly(lactic-co-glycolic-acid) (PLGA) polymers to create a conductive biodegradable composite for cardiovascular applications. CNTs were added to a PLGA solution at various weight percent ratios using the nomenclature PLGA:CNF (wt%:wt%). (a) shows a 25:75 composite on a glass substrate. (b) Scanning electron microscope image of a 25:75 composite at ×10 000 magnification (scale bar = 1 μm). An Asylum MFP-3D atomic force microscope (scan rate at 1.0 Hz, AC mode with a 256 × 256 scan points and lines) image showing that CNTs can increase surface nanometer features (c) and conductivity (d) of a 27:75 [PLGA:CNF (wt%:wt%)] sample. (e) CNTs are able to increase the Young's modulus of a PLGA matrix while decreasing its elongation at failure (experiments were conducted using a Rheometric Scientific minimat 2000 at a speed of 50 mm/min as stated by the ASTM international standard test method for tensile properties of plastics destination D638). (f) Conductivity can also be altered by the addition of CNTs to the PLGA matrix (measurements were completed with the use of a 4-point measurement apparatus).

cultured in the laboratory to expand for transplantation to restore tissue. One study showed that these cells sense and respond to the stiffness of the substrate they are cultured on demonstrating that MSCs differentiated into bone cells when cultured on stiff substrates (8 – 17 kPa), but gave rise to neuronal cells under identical conditions when soft substrates (0.1 – 1 kPa) were used[43]. Another study used substrates that were patterned (pits and linear features) to show that cells that were allowed to spread, differentiated into bone cells, whereas those in a rounded shape became adipocytes (or fat cells)[44]. Using patterned substrates, it was also determined that MSCs responded to the geometry of their shape: on a star-shaped island, the cells favored a bone-cell fate, whereas those on flower-shaped (1000, 2500, and 5000 μm^2) islands of the same size differentiated into adipocytes[45]. Following a strategy of engineering a material to dictate the fate of a stem cell, researchers created a material with nanofeatures (square-shaped pits 120 nm in size, separated by 180 nm in between pits) and showed that MSCs cultured on a nanostructured substrate maintained their multipotency—the ability of a cell to differentiate into a limited number of cell types or into a closely related family of cells—for up to eight weeks[46]. This demonstrated that stem cell organization and signaling properties of the cytoskeleton can therefore be engineered with nanopatterned substrates to define the position, shape, and size of the cell membrane focal adhesions which dictate cellular function[47].

Using CNTs and MSCs, Mooney and colleagues[48] showed that CNTs could be beneficial for tissue engineering and regenerative medicine. Using a COOH-functionalized SWCNT, Mooney *et al*.[48] were able to differentiate MCSs along adipogenesis, osteogenesis, or chondrogenesis lines while not adversely affecting cell viability, proliferation, or increasing cell death—thus indicating minimal material toxicity. More importantly, they revealed that SWCNTs migrated through the cell wall to a nuclear location after 24 hours, but did not change the cellular ultrastructure or adversely affect the cell[48]. These findings could be used to design CNTs for emerging technologies in drug delivery and stem cell organelle specific engineering applications.

Another study assembled layer-by-layer SWCNT-polyelectrolyte multilayer thin films for neural stem cell applications[49]. Using mouse embryonic neural stem cells (NSCs), it was shown that the nanostructure of the layer-by-layer SWCNT-polyelectrolyte thin film had no adverse

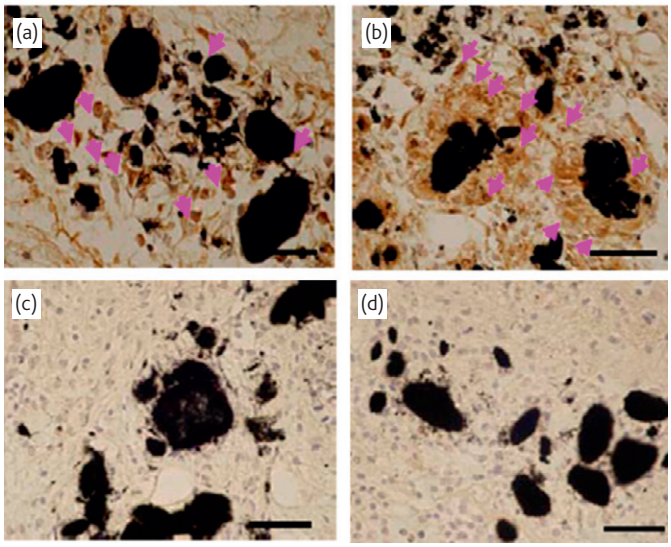

Fig. 4 Increased stem cell differentiation into neurons after three weeks of implantation of hydrophobic carbon nanotubes and stem cells into stroke damaged neural tissue of rats. Immunostaining of stroke damaged brain sections where stem cells and carbon nanotubes (black) were implanted. (a) Nestin = marker for stem cells; (b) MAP2 = marker for neurons; (c) GFAP = marker for astrocytes; and (d) CD11b = marker for meningeal cells. All double stained a priori *with BrdU. Dark brown (arrows) show positively double stained neurons with no scar tissue forming cells (astrocytes and meningeal cells) surrounding carbon nanotubes. Bars = 50 μm. Similar results shown up to eight weeks.*

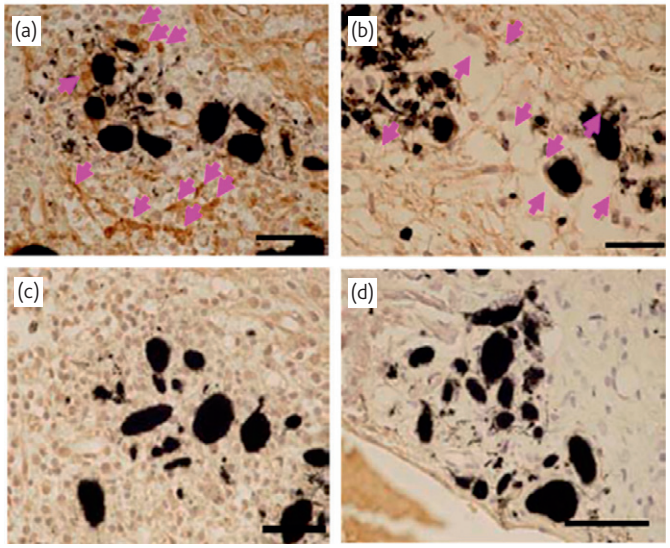

Fig. 5 Increased stem cell differentiation into neurons after three weeks of implantation with hydrophilic carbon nanotubes (CNTs) into stroke damaged neural tissue of rats. Immunostaining of stroke damage brain sections where stem cells and carbon nanotubes (black) were implanted. (a) Nestin = marker for stem cells; (b) MAP2 = marker for neurons; (c) GFAP = marker for astrocytes; and (d) CD11b = marker for meningeal cells. All double stained a priori *with BrdU. Dark brown (and arrows) show positively double stained neurons with no scar tissue forming cells surrounding carbon nanotubes. Scale bars = 50 μm. Similar results shown up to eight weeks. Such stem cell and CNT implantation returned motor function faster than stem cells alone.*

effect on the differentiation of NSCs. In fact, the NSCs behaved similarly to those cultured on standard poly-L-ornithine (PLO)—one of the most widely used growth substrates for neural stem cells—in terms of cell viability, the development of neural processes, and the appearance and progression of neural markers, and may even induce preferential differentiation into specific lineages[49].

In a preliminary study, the authors imbedded neural stem cells (Wistar rats, isolated for later implantation from one to three day old neonates) into carbon nanofibers to see if neural capabilities in rats could be reestablished after an induced stroke[50]. *In vivo* preliminary results demonstrated that unfunctionalized carbon nanofibers/nanotubes can: 1) conduct electricity when implanted into damaged, non-conductive, regions of the brain; 2) mimic the nanometer features of key proteins found in neural tissue (such as laminin) to promote neuron functions and decrease glial scar tissue formation; 3) anchor stem cells to promote their differentiation into neurons when implanted into damaged regions of the brain; 4) remain non-toxic and non-migratory when used at low concentrations (less than 25:1 [solution (μl): CNTs (μg)] ratio) in the brain; and 5) most importantly, return motor skills to stroke-induced rats at least three times faster than injecting stem cells alone[50].

Furthermore, it was shown that only the neural stem cells impregnated with carbon nanofibers differentiated into neurons (Figs. 4 & 5). This may be due to: 1) the high electrical conductivity of carbon nanofibers; 2) the high surface reactivity of carbon nanofibers which according to *in vitro* studies[51] increased the adsorption of laminin from serum; and/or 3) size similarities to laminin (a 70 nm cruciform protein easily mimicked through carbon nanomaterials). Moreover, this could be because the carbon nanofibers allowed for a favorable substrate for the neural stem cells to adhere to, thus, keeping them in the local environment (i.e., anchoring them) so that they could be stimulated to differentiate into neurons by the conductive carbon nanofibers.

Even with these advancements, whenever discussing CNTs, toxicity questions arise. Using mouse embryonic stem cells (ESCs) and MWCNTs, researchers found that the MWCNTs can accumulate and induce apoptosis in mouse ECSs and activate the tumor suppressor protein p53 within two hours of exposure[52]. Furthermore, genotoxicity (toxicity at the molecular level) was observed by the increased expression of two isoforms of the base excision repair protein 8-oxoguanine-DNA glycosylase 1 (OGG1), a double strand break repair protein Rad 51, phosphorylation of H2AX histone at serine 139, and SUMO modification of XRCC4 with an increased mutation frequency by 2-fold compared with the spontaneous mutation frequency in normal mouse ESCs[52]. Furthermore, using MSCs and carboxylated SW- and MWCNTs, it was demonstrated that CNTs inhibited the proliferation, osteogenic differentiation, adipogenic differentiation, and mineralization of MSCs[53]. Investigations like this may sound alarming, which they should, but it is important to note how the researchers delivered and investigated CNT toxicity[54]. Reports of toxicity of CNTs are usually presented when the mode of delivery is free floating in media[52,53], not for a substrate with CNTs embedded, whereas CNTs embedded into a substrate or made as a substrate promote cellular growth[48,49]. Depending on the mode of exposure and/or material presentation, CNTs may lead to toxicity or promoted tissue growth

(although significantly more research is required). Furthermore, it is important to emphasize that not all CNTs are the same. Some impurities may be left over from unreacted catalysis known to be toxic to cells, while others have fully reacted catalysts presenting relatively pure CNTs to cells. Such differences in CNTs can clearly have profound influences on CNT toxicity.

CNTs for transportation of pharmaceuticals and therapeutics

New strategies for the delivery of drugs and molecular probes into cells are in high demand due to the poor cellular penetration of many small molecules and an increasing number of macromolecules, including proteins and nucleic acids within cells[55]. These approaches, in which a poorly permeating drug or probe molecule is covalently attached to a transporter to produce a cell-penetrating conjugate, offer a partial solution to this problem through the use of lipids, polyethylene glycols (PEGs), and peptides with relative levels of success[56-58]. In the advent of the nanotechnology revolution, CNTs may provide an alternative solution to this problem (Fig. 6). For example, in recent CNT research, it was reported that SWCNTs are capable of penetrating inside mammalian cells for bioactive agent transportation and therapeutic purposes[59-61]. The nanoscale dimensions of SWCNTs, combined with their high aspect ratios, as well as their structural and electronic properties, make them amiable to high cargo loading; thus SWCNTs show great promise as candidates for a new class of transport system for the detection and treatment of cancer and diseases[62-64].

For example, the structural change in deoxyribonucleic acid (DNA) upon shifting from the B to Z conformation, sufficiently perturbs the electronic structure of SWCNTs such that the change can be detected optically from living cells that have taken up DNA-SWCNT complexes[65]. This and other research has demonstrated how CNTs can be used as sensors within living cells[66,67]. Furthermore, exposing cells containing SWCNTs to near infrared radiation kills the cells due to the efficient optical-to-thermal energy conversion of SWCNTs, demonstrating that they can potentially be used in targeted cancer therapies to eliminate cancer cells[68]. There are also a number of reports that CNTs facilitate the transport of bound oligonucleotides, peptides, and proteins across the plasma membrane[60,69-71].

Despite these and other intracellular applications not listed here[72-74], there remain significant technical challenges to the realization of the potential benefits of CNTs in medicine. Namely, CNTs are extremely hydrophobic, bundle together, and are insoluble in water. Also as previously mentioned, current research suggests that CNTs used for transportation and therapeutics can be toxic to cells[75,76]. On the contrary, it has been shown that the manner in which carbon based nanomaterials are introduced into the host systems will greatly affect the toxicity of the materials. The assay being used to assess cytotoxicity is also critical since different assays can produce different results and readings—a false positive toxicity result can show up when one looks at 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) results after adding CNTs to cell culture media whereas CNT toxicity is negligible when using other tetrazolium salt assays[54]. Even though one can assess that carbon based nanomaterials are cytotoxic at some concentrations (since everything is toxic at some concentration), the larger problem lies with the presence of impurities that are left behind during the synthesis of the material. If one could remove these impurities, one could reduce the appearance of toxicity and promote cell growth[77]. Clearly, such issues have plagued the investigation of CNTs for bioactive agent transport.

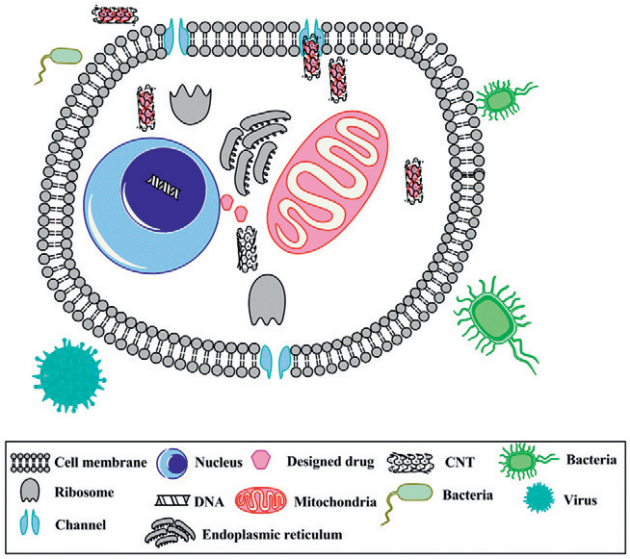

Fig. 6 The ability of SWCNTs to penetrate inside the cellular walls provides innovative ways for transporting and administering drugs and molecular probes into cells as demanded by the poor cellular penetration of many small molecules and an increasing number of macromolecules. A drawing is presented of drug encapsulated SWCNTs penetrating the cellular wall through an ion channel within the cellular membrane and administering therapeutics that would not otherwise be allowed into the cell. The cellular wall is not damaged through this process as indicated by the protection of its organelles from outside bacteria.

CNTs as improved biomaterials

The development of nanotechnology and the synthesis of new CNTs as improved biomaterials have already had a great impact on existing macro- and micro-technologies and provide the ability to better mimic the native microenvironment for biomedical applications[78,79]. Due to their exceptional electrical, mechanical, chemical properties as well as their high aspect ratio, CNTs have been used in biomedical applications since 2004[79]. For tissue engineering purposes, CNTs have been important materials in four main areas including improved cell tracking and labeling, sensing cellular behavior and micro/nano-environments, delivery of transfection agents and growth factors, and as tissue scaffolding[79]. Numerous papers, articles, books, and research have been devoted to the utilization, development, analysis, implication, and understanding of nanotechnology and more specifically CNTs for tissue engineering and regenerative medicine applications as described below[79-81].

For example, the extracellular matrix (ECM) is a dynamic and highly-organized nanocomposite which defines the space that native tissue occupies (Table 1). It is an essential component for tissue engineering as it regulates essential cellular functions such as cellular differentiation, proliferation, adhesion and migration[82]. Nanomaterials have been exploited for the creation of ECM-like structures and compensate

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Table 1: Extracellular matrix (ECM) complexities and how CNTs are creating biomaterials that mimic the native ECM

Organ example	Natural tissue structure	Engineered CNT scaffold
Heart	The ECM of muscle tissue, like the heart, facilitates cardiomyocytes (heart cells) to couple mechanically to each other and to form elongated and aligned cell bundles that create an anisotropic syncytium.	Nanogrooved surfaces and aligned CNTs embedded into a matrix are suitable for cardiac tissue engineering applications because they force cardiomyocytes to align.
Bone	Bone is a nanocomposite material consisting primarily of a collagen rich organic matrix and inorganic hydroxyapatite nanocrystallites which serve as a chelating agent for ECM mineralization by osteoblasts.	Integrating CNTs and hydroxyapatite to create a stiff and porous scaffold.
Liver	Cells composing epithelial tissue are polarized and contact three types of surfaces for efficient mass transfer.	Scattered and non-aligned CNTs modified with surface molecules embedded in a matrix can promote cell adhesion and tissue polarity.

for some limitations that some popular synthetic polymers (such as poly(lactic acid) (PLA) and poly(lactic-co glycolic acid) (PLGA)) lack[78]. These limitations include weak mechanical properties, lack of electrical conductivity, the absence of adhesive micro and nanoenvironment-defining moieties, and the inability of cells to self-assemble into three-dimensional (3D) tissues[78]. In addition, CNTs can be easily functionalized for different biomedical applications. CNTs provide structural support due to their extraordinary mechanical strength and hold a great promise as scaffolds for tissue engineering applications. In addition, the viscoelastic behavior of CNTs is similar to that observed in soft-tissue membranes thus making them ideal for tissue engineering applications[83].

Many studies have shown that mechanical properties are significantly improved when CNTs are dispersed in conventional polymer solutions. For example, MWCNTs blended with a chitosan polymer showed significant improvements in mechanical properties (increasing tensile modulus from 1.08 GPa to 2.15 GPa and from 37.7 MPa to 74.3 MPa for tensile strength) compared with those of chitosan[84]. Chitosan is a natural biopolymer and has been widely used in many tissue engineering applications as it is biocompatible and biodegradable, has multiple functional groups, and is highly soluble in aqueous media[84-87]. On the other hand, chitosan has low to moderate mechanical strength which limits its use in load-bearing biomedical applications. To overcome this limitation, chitosan/MWCNTs composites were prepared by a simple solution-evaporation method. MWCNTs were homogeneously dispersed within a chitosan polymer matrix. By blending only 0.8 wt% MWCNTs, the tensile modulus and strength of the chitosan/MWCNT composites were greatly improved by about 93 % and 99 % (2.15 GPa for tensile modulus and 74.3 MPa for tensile strength), respectively, when compared to neat chitosan[84].

CNTs can also improve the mechanical properties of hydrogels, creating porous hydrogels with tunable mechanical properties. Highly crosslinked, 3D hydrogel microenvironments have high stiffness, but they might limit cellular proliferation, migration, and morphogenesis[88-90]. To create 3D hydrogels with controlled mechanical properties, Shin *et al.*[90] coated CNTs with a thin layer of photo-crosslinkable gelatin methacrylate (GelMA), which is known to allow for cell encapsulation and proliferation. The addition of CNTs significantly improved the mechanical properties of GelMA hydrogels without decreasing their porosity. The elastic modulus of the GelMA hydrogels increased from 15 kPa to 60 kPa when only 0.5 mg/mL of CNTs were coated. In addition, the CNT-GelMA hybrid hydrogels had high toughness and strong tensile strengths and they maintained their ability to elongate under an applied force. NIH-3T3 cells (a standard fibroblast cell line) and human mesenchymal stem cells were further encapsulated in CNT-GelMa hybrid microgels at varying concentrations of CNTs from 0 to 0.5 mg/mL. Both cell types were spread and proliferated after encapsulation in CNT-GelMA hybrid microgels. 3T3 cells had greater than 90 % cellular viability after 48 hours for all CNT concentrations[90]. Apart from polymer and hydrogel enhancement, CNTs can also be used to reinforce ceramic matrices. For example, CNTs/barium titanate ($BaTiO_3$) composites were fabricated by adding 1 wt% of CNTs[91]. Moreover, CNTs were homogenously distributed into a hydroxyapatite (HA) coating using plasma spraying[92]. Incorporating CNTs into a brittle bioceramic coating improved its fracture toughness (from 0.39 MPa $m^{1/2}$ to 0.61 MPa $m^{1/2}$) by 56 %[92]. Hence, CNTs can be used to enhance the mechanical properties of hydrogels, polymers, and ceramics to create better scaffolds to mimic native tissues.

Conclusions

In this review, some of the recent progress using CNTs for stem cell research and tissue engineering and regenerative medicine was presented. Research in this area is still in its early developmental stage and much more research is needed to fully appreciate the potential and limitations of CNTs across all of medicine. The verdict is still out concerning whether properly synthesized CNTs present any significant toxicity to its host and if longer term CNT exposure will have negative health outcomes. Even if studies on completely pure CNTs showed significant adverse health consequences, many researchers are confident that CNTs could be functionalized to avoid such problems. It is now known that CNTs do play a positive role in stem cell differentiation in short term animal studies on healing damaged tissue, but the long term effects remain to be addressed. It is also already clear that CNTs have transformed the medical research world, resulting in the development of novel bio-transporters and drug delivery systems to significantly improve medical device performance. Researchers are continually improving their knowledge on how CNTs affect and control their biological surroundings by designing new and innovative ways to apply CNTs to stem cells and tissue engineering technologies. The future looks bright for the synergy of CNTs and stem

cell therapies while enhancing biomaterial performance and developing innovative tactics for cellular transport and therapeutics. mt

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