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Research review paper

Carbon nanotubes leading the way forward in new generation 3D tissue engineering

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ABSTRACT

Statistics from the NHS Blood and Transplant Annual Review show that total organ transplants have increased to 4213 in 2012, while the number of people waiting to receive an organ rose to 7613 that same year. Human donors as the origin of transplanted organs no longer meet the ever-increasing demand, and so interest has shifted to synthetic organ genesis as a form of supply. This focus has given rise to new generation tissue and organ engineering, in the hope of one day designing 3D organs *in vitro*. While research in this field has been conducted for several decades, leading to the first synthetic trachea transplant in 2011, scaffold design for optimising complex tissue growth is still underexplored and underdeveloped. This is mostly the result of the complexity required in scaffolds, as they need to mimic the cells' native extracellular matrix. This is an intricate nanostructured environment that provides cells with physical and chemical stimuli for optimum cell attachment, proliferation and differentiation. Carbon nanotubes are a popular addition to synthetic scaffolds and have already begun to revolutionise regenerative medicine. Discovered in 1991, these are traditionally used in various areas of engineering and technology; however, due to their excellent mechanical, chemical and electrical properties their potential is now being explored in areas of drug delivery, *in vivo* biosensor application and tissue engineering. The incorporation of CNTs into polymer scaffolds displays a variety of structural and chemical enhancements, some of which include: increased scaffold strength and flexibility, improved biocompatibility, reduction in cancerous cell division, induction of angiogenesis, reduced thrombosis, and manipulation of gene expression in developing cells. Moreover CNTs' tensile properties open doors for dynamic scaffold design, while their thermal and electrical properties provide opportunities for the development of neural, bone and cardiac tissue constructs.
This review will provide an update on the use of CNTs in 3D organ generation.

Contents

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Introduction

The Annual Report published by the National Health Service (NHS) in 2011/2012 estimated over 7000 patients on the transplant waiting list, in the United Kingdom alone (NHS, 2013). Despite this demand, less than 4000 patients actually received a transplant by the following year (NHS, 2013). This model, where demand critically outstrips supply, is not confined to the UK but is a worldwide problem (Langone and Helderman, 2003; Molzahn et al., 2003). Adding to this problem is the risk of losing an organ because of immune rejection. While, this can be prevented in most cases, with life-long immunosuppressive therapy, this approach is not always reliable. Moreover, chronic immune suppression leaves patients vulnerable to opportunistic infections and certain types of cancer, with non-melanoma skin cancer being the most common in solid organ transplant recipients (Fisherman, 2013; Ramsay, 2013; Specchio et al., 2014). Organ shortage and post-operative immune suppression has thus seen focus shift from traditional transplant methods to tissue engineering. This approach could not only provide additional organ supply, but bypasses the complications of immune suppression and organ rejection. Recent advances in this field have taken the concept of developing 3D organs from our laboratory into patients, with the world's first synthetic trachea transplant performed in 2011 (Jungebluth et al., 2011). This has established a platform for developing more complex organs such as the liver, bladder and heart (Atala et al., 2012).

The 3D construction of an organ or tissue is achieved by creating an environment similar to the extracellular matrix (ECM), a parameter that has repeatedly proved problematic (Chan and Leong, 2008; Geckil et al., 2010). In order to achieve this, scaffolds need to demonstrate optimum mechanical strength and chemical stability, while also being highly porous, for the diffusion of oxygen, metabolites, and cell-cell interactions. They also need to support and encourage cell and protein adhesion, proliferation and differentiation. While the physical and mechanical properties of scaffolds are constantly researched and altered, recent studies have praised techniques such as electrospinning for scaffold development, as this can create a nanofibrous network optimal for cell attachment (Bidez et al., 2006; Li et al., 2006). Such scaffolds, similar to ECM, define a local biochemical and mechanical niche with the physical regulation that cells need in order to develop (Dalby et al., 2007). More recent studies have examined scaffold support and emphasised its requirement to mimic native ECM for stem cell growth and regulation (Discher et al., 2009; Voog and Jones, 2010).

CNTs, discovered in 1991 (Iijima, 1991), present a novel option to modify and improve the chemical and mechanical properties of biomaterials and polymers. These nanomaterials have already sparked enthusiasm in regenerative medicine in terms of targeted ultrasound imaging (Wu et al., 2014), drug delivery, thermal ablation (Fabbro et al., 2012; Madani et al., 2013b) and *in vivo* biosensor applications (Vashist et al., 2011), with further interest lying in their ability to transform scaffolds for tissue generation. This interest is attributed to numerous properties of CNTs, which include tensile strengths ranging from 11–200 GPa (Yu et al., 2000), a Young's modulus of 0.27–1.34 TPa (Demczyk et al., 2002; Iijima et al., 1996), electrical conductivities as high as 10^4 S/cm^2 and thermal conductivities as high as 5000 Wm^{-1} K (Nardecchia et al., 2012; Orlita et al., 2008). Other valuable properties of CNTs include microwave radiation absorbance (Leelaviwat et al., 2012; Sharon et al., 2005) and near infrared (NIR) optical fluorescence of 900–1600 nm (Leeuw et al., 2007; O'Connell et al., 2002). In this paper we will explore the role of CNTs in polymer nanocomposite scaffolds. These are defined by a combination of a matrix or filler where at least one dimension of the system, is on the nanoscale (less than or equal to 100 nm) (Byrne and Gun'ko, 2010; Mozafari et al., 2012). Finally, we will present case studies in which we review the chemical, electrical, and mechanical properties of CNTs for the purpose of creating biochemically optimised scaffolds to be used in 3D constructs for neuronal, bone and cardiac tissue engineering.

Understanding native extracellular matrix function

Understanding the native structure and function of ECM will allow the analysis of CNT properties to be put in context, in terms of tissue engineering. The ECM makes up the surrounding cellular environment and consists of both chemical and physical components, which will be explain in this section. There are 300 different structural proteins embedded in this matrix, arranged as insoluble fibres (Hynes and Naba, 2012). Reticular and elastic fibres, collagen and proteoglycans will influence mechanical tension transmitted to the tissue and these can tighten tissue structure, promote cell compaction and determine cell morphology. Importantly the ECM, with embedded nanosized collagen fibrils, provides an intricate 3D structure for cell and protein attachment and support. These are 20–400 nm in diameter and can expand up to hundreds of microns (Lu et al., 2008; Toroian et al., 2007). Cell and protein adhesion is optimised by a large surface area, created by the dispersion of these fibrils within the ECM.

In terms of cell signalling, the ECM provides a medium allowing for intricate communication within a cellular network. This communication is mediated by numerous growth factors, cytokines, chemokines and proteoglycans. Proteoglycans can bind sulphate groups and define biochemical boundaries for cell interaction (Hynes and Naba, 2012). The ECM is also able to provide and bind extracellular proteins to support these activities. Physical and chemical components need to co-operate and support each other's functions for optimum tissue development, and so despite mechanical strength the ECM is still highly porous for cell signalling and oxygen diffusion. Through these components the ECM regulates a complex environment that interacts closely with cells to regulate migration, adhesion, growth, differentiation, gene expression, phenotype, apoptosis and homeostasis.

Carbon nanotube properties

CNTs are sheets of graphite rolled into seamless cylindrical tubes that, depending on their geometry, can be divided into two categories: single-walled carbon nanotubes (SWCNTs) (Fig. 1A) and multi-walled carbon nanotubes (MWCNTs) (Fig. 1B). MWCNT diameters range from 2–100 nm, while SWCNTs show diameters between 0.8–2 nm (De Volder et al., 2013), with some reporting diameters less than 0.7 nm when synthesised with iron nitrate (Arjmandi et al., 2009).

Both SWCNTs and MWCNTs have excellent mechanical properties, where their flexible hexagonal network of carbon atoms gives them a Young's modulus of 0.27–1.34 TPa (Demczyk et al., 2002; Iijima et al., 1996) and a tensile strength ranging from 11–200 GPa (Yu et al., 2000). The disciplined arrangement of their carbon atoms linked together via strong sp^2 bonds explains how CNTs exhibit superior electrical conductivities as high as 10^4 S/cm^2 and thermal conductivities as high as 5000 Wm^{-1} K (Nardecchia et al., 2012; Orlita et al., 2008).

Despite mechanical, conductive and thermal properties, CNTs are highly porous and lightweight with SWCNTs pores generally less than 1 nm in diameter, and MWCNTs pores varying between 4 and 30 nm

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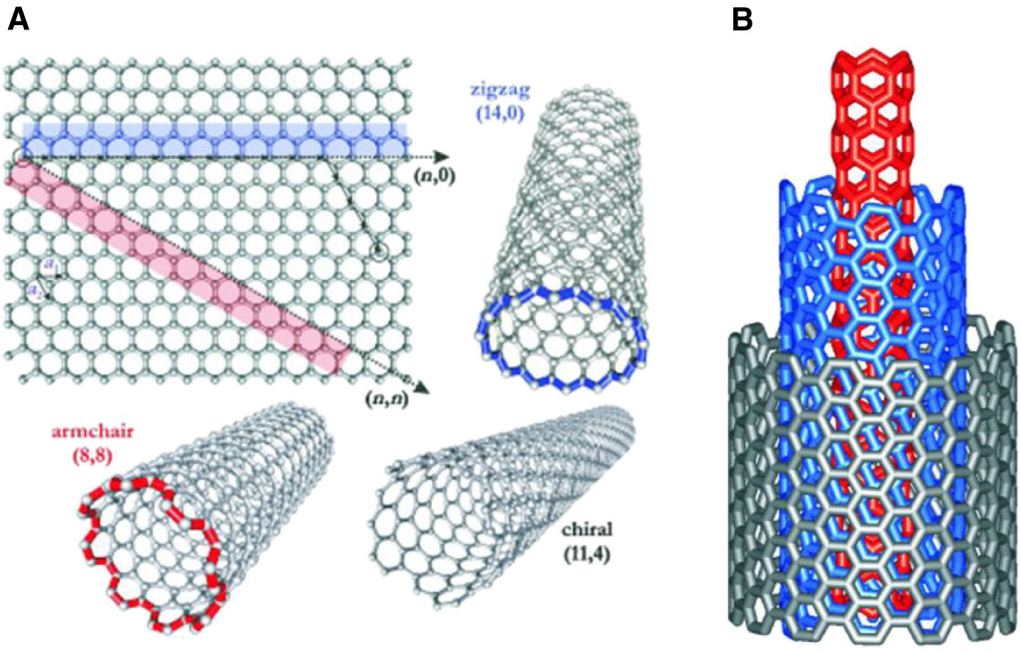

Fig. 1. Depiction of (A) SWCNT with zigzag, armchair and chiral structures, and (B) MWCNT with multiple concentric tubes of grapheme (Balasubramanian and Burghard, 2005).

(Jagtoyen et al., 2000). These nano-dimensions provide CNTs with significantly large surface areas and similar surface roughness to the naturally occurring collagen fibres of the ECM (Lu et al., 2008; Voge and Stegemann, 2011; Zhang et al., 2010). Through CNTs' ability to structurally mimic collagen fibres, they have been documented to greatly influence cell adhesion, proliferation and differentiation (Lu et al., 2008; Ross et al., 2012; Zhang et al., 2008).

Carbon nanotube incorporation into polymer scaffolds and hydrogels for tissue generation

Mechanical and structural properties

The mechanical and structural properties of CNTs can be used to enhance current scaffold design, and CNTs have already been incorporated into numerous constructs including collagen, poly-L-lactide (PLLA), polycarbonate-urethane (PCU), polycaprolactone (PCL) and polystyrene (PS). Previous studies have shown that while scaffolds made solely from these polymers are porous, they do not possess the necessary mechanical properties for *in vivo* implantation (Meng et al., 2009; Paratala and Sitharaman, 2011). Studies on the incorporation of CNTs into polymeric scaffolds have generated promising results with most reporting a substantial increase in the tensile and compressive moduli of the composite scaffolds (Fig. 2) (Martínez-Pérez et al., 2011; Olivas-Armendáriz et al., 2010; Pan et al., 2012). CNT incorporated PS polymers show a 36–42% and 25% increase in elastic modulus and tensile strength, respectively (Qian et al., 2000). SWCNT-PLLA composites show a 5% decreased in polymer crystallinity, 12% increase in tensile strength and a reduced rate of degradation (Mackle et al., 2011). These structural changes can prove very useful for the purpose of developing structural organs (such as bone) *in vitro* and aiding regeneration *in vivo* (Cheng et al., 2013), as well as offering support for other tissues. The latter will be explored in the case studies that follow.

Finally, CNTs have been shown to influence the rate of scaffold degradation. 3-dimentional, highly porous polyurethane containing CNTs scaffolds, have been shown to display significantly improved mechanical stability at a CNT concentration of up to 5%, without affecting the polymer's degradation rate (Jell et al., 2008). It was also shown that CNTs prevent accelerated degradation in PLLA scaffolds (Mackle et al., 2011). This is vital, as a scaffold needs to degrade at a rate that will

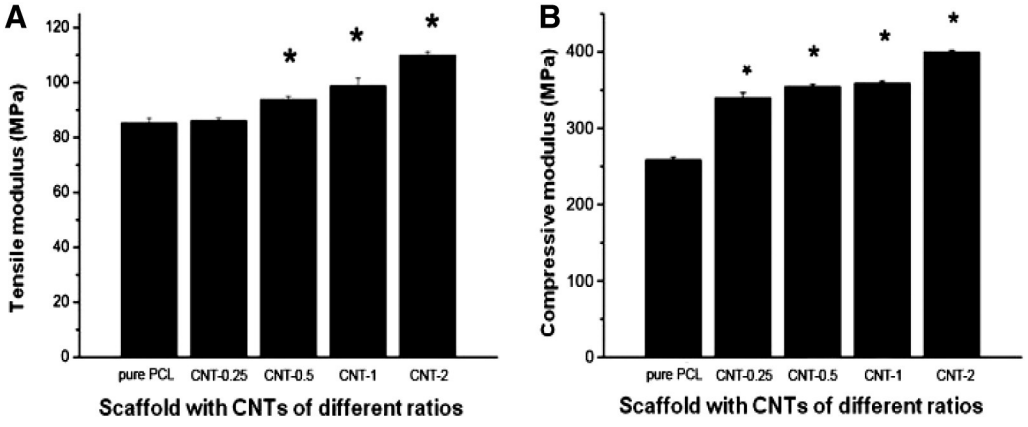

Fig. 2. Experimental and computational effective moduli for tensile strength for polycaprolactone (PCL) scaffolds of varying CNT concentrations. Data represents mean ± standard deviation for n = 6, *P < 0.05 (compared to pure PCL scaffolds) (Pan et al., 2012).

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still support cell growth, but eventually allow blood vessel support and cytokine interaction with cells, and the formation of tissue that is structurally and chemically independent of the original scaffold. Useful reviews on this topic are presented by Coleman et al. (2006) and Armentano et al. (2010).

Cell and protein interaction and support

In vitro studies with CNTs have demonstrated that they can adsorb extracellular and serum proteins, improving their level of interaction with cell cultures (Li et al., 2009) as this adsorption makes scaffolds more bioactive and biocompatible (Lizundia et al., 2012; Zhao et al., 2005). This phenomenon is exploited by functionalisation of CNTs, where peptides and serum proteins can be placed on CNT walls, either by simple adsorption or chemical functionalisation and will be further discussed in Carbon Nanotube Functionalisation.

Biocompatibility is only useful, however, if CNTs can spatially and temporally arrange the adhered cells and proteins. It is important to understand that the arrangements and size of CNTs will affect cell adhesion, growth and differentiation. Mwenifumbo et al. (2007) showed that cell adhesion and growth is indirectly proportional to CNT size, with osteosarcoma cells growing best at 14 nm CNT diameters as smaller diameter CNTs have relatively greater surface areas and hence a greater potential to bind with growth promoting and adhesive proteins. It was also shown by Oh et al. (2009) that stem cell differentiation could be dictated solely by CNT size, through still unclear mechanisms (Oh et al., 2009). Moreover, the orientation of CNTs within the polymer has also been shown to dictate cell growth and direction. SWCNTs aligned on glass substrates, by centrifuge forces, show 120% increase in mesenchymal stem cells (MSCs) proliferation and increased differentiation down osteocyte linages, when compared to randomly orientated SWCNTs (Namgung et al., 2011). The orientation of SWCNTs is thought to affect polymer tension, which in turn mechanically activates the synthesis of cell adhesion mediators. Furthermore, in a study by Meng et al. (2010) cell adhesion mediators RhoA, ROCK2 and FAK were up regulated in cells grown on aligned MWCNTs, and this was shown to support vascular endothelial cell proliferation. These were also shown to enhance extracellular collagen type IV secretion (which is the most common component of the basal lamina, used for cell attachment and communication).

These findings have led to novel methods for aligned CNT incorporation into polymer scaffolds, with electrospinning emerging as a powerful technique to disperse and align functionalised CNTs (Meng et al., 2010; Shao et al., 2011). Electrospinning is a relatively recent method to synthesise CNT nanocomposites. This process involves drawing out CNTs from the tip of a sharp conical meniscus, using an applied electric charge. The focussing of the meniscus into a sharp conical shape controls the orientation of CNTs dispersed in solution both along the streamlines and also the fibre axis. There are many advantages of using electrospinning over other fabrication methods as electrospinning allows for simultaneous alignment of CNTs along a single axis while maintaining the structural integrity of them individually. It is also a one-step, low cost, safe and relatively uncomplicated technique compared to complicated, multi-step contemporary manufacturing methods (Yeo and Friend, 2006). Other techniques to create these scaffolds, such as nanopatterning, self-assembly, and CNT sheet stretching are explored in papers by Dvir et al. (2011) and Kim et al. (2013).

Electrical and thermal properties

CNT incorporated hydrogels show exciting potential for cell culturing and cell sheet preparation. Chen et al. (2013) investigated Poly(N-isopropylacrylamide) (PNIPAAM)-based hydrogels with interpenetrating MWCNTs for cell sheet engineering and demonstrated that the PNIPAAM hydrogels with MWCNTs exhibit increased elastic moduli and hydrophobicities, compared to those scaffolds without MWCNTs. They conclude that higher elastic moduli and hydrophobicity are crucial for epithelial Madine-Darby canine kidney (MDCK) cells to attach to the hydrogel. Hydrogel cell sheet harvesting was achieved by temperature manipulation and was only possible in MWCNT gels, due to their thermal stability and the influence of MWCNTs enhanced cell attachment (Fig. 3). CNTs thermal properties can be further exploited in microwave based thermal drug or cell signalling factor release and infrared fluorescence microscopy imaging (Veetil and Ye, 2009).

In terms of electrical stimulation, the excellent conductive properties of CNTs can be exploited in the design of scaffolds used in neural, bone and cardiac tissue engineering. Such scaffolds should, ideally, mimic the electrical properties of nerves and myocytes and have the ability to electrically stimulate developing tissues (Bosi et al., 2012). A summary of various properties of CNTs, interesting for multiple applications in tissue engineering, is provided in (Table 1).

Carbon nanotube functionalisation

The incorporation of CNTs into polymers has not been without complication as CNTs are naturally chemically inert and disperse poorly in numerous solvents. CNT functionalisation is thus required and involves the addition of functional groups such as carboxyl or alcohol groups to the walls and ends of the nanotubes. This should prevent CNT aggregation and allow for their incorporation into polymer scaffolds (Fig. 4) (Balasubramanian and Burghard, 2005; Harrison and Atala, 2007). Biofunctionalisation of CNTs surface with bioactive molecules, such as carbohydrates or peptides can help improve the biocompatibility and bioactivity of the scaffold. Once again their large surface area makes CNTs useful for tissue engineering purposes as large amounts of biomolecules can be placed onto the nanotubes. This is explored by Bianco et al. (2011).

It is important to note that the bonds formed between CNTs and functional groups are weak enough to allow for a wide range of reactions to take place within the biochemical environment, but not too weak for rapid chemical decay to proceed (Balasubramanian and Burghard, 2005; Newman et al., 2013). This is vital for scaffold integrity, chemical stability, degradation and cell interaction.

As seen with size and orientation, the functional groups added onto CNTs can affect cell growth and differentiation. The effect of pristine SWCNTs studied against carboxyl functionalised SWCNTs found that these carboxyl groups inhibited cell proliferation (Mu et al., 2009). The same results were observed with MSCs, where carboxylated CNTs show increased Smad and BMP inhibition, indicating reduced cell growth and communication (Liu et al., 2010). On the other hand, the charged nature of the carboxyl groups has been shown to promote chondrocyte growth and ECM protein expression (Chahine et al., 2014). While these different results could be explained by cell type, it is more likely to be a case of the duration and nature of functionalisation. Results from our laboratory showed that when functionalising CNTs with nitric and sulphuric acids, functionalisation periods of 5 h, compared to 2 h and 0.5 h, increase cell viability (Madani, 2013). We have also explored the nature of the bonds formed during carboxyl functionalisation and conclude that covalent bonding in both MWCNTs and SWCNTs lowers cell death and toxicity.

Carbon nanotube toxicity

CNTs hold enormous potential for tissue engineering, which will only come to fruition once toxicology is handled. *In vivo* cytotoxicity of CNT based scaffolds should be assessed carefully to ensure there is no negative effect on the engineered organ, surrounding organs or the health of the patient in general. Nanoparticles can damage and cause cell death by creating forms of reactive oxygen species (ROS) (Pulskamp et al., 2007), rupturing cell membranes (Kang et al., 2008), and by evoking immune responses and chronic inflammation. These negative interactions can cause significant disruption and damage due

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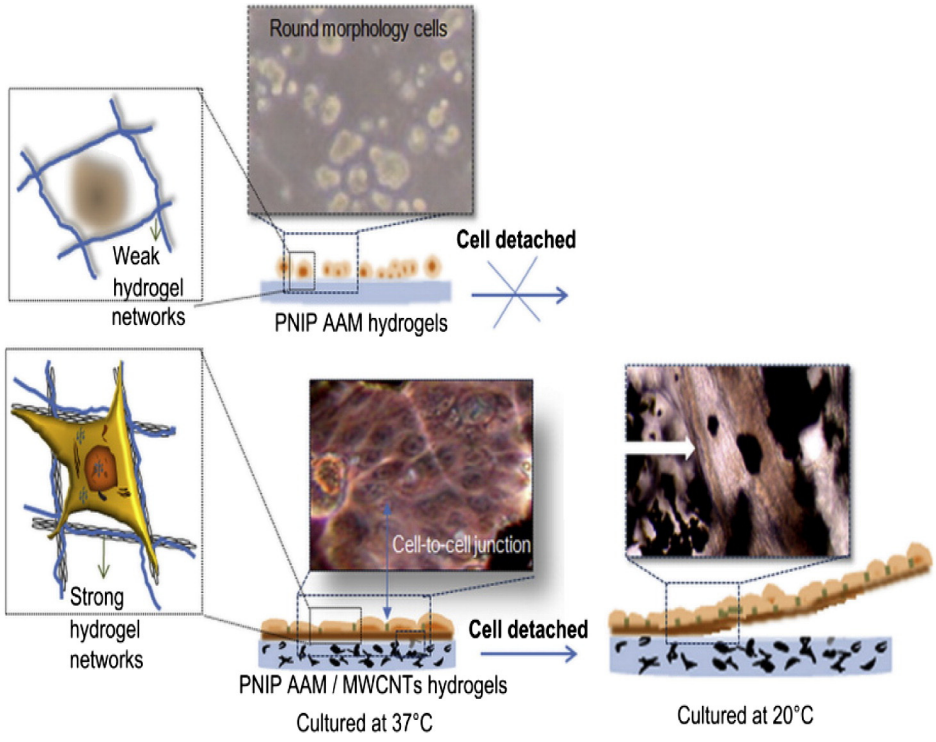

Fig. 3. PNIPAAM/MWCNT hydrogels, showing how MWCNTs, increase cell adhesion and spreading. MWCNT cell sheet harvesting is conducted by lowering the temperature to 20 °C (Chen et al., 2013).

to their small size and large surface area. These implications are examined in the following papers (Buzea et al., 2007; Magrez et al., 2006; Nel et al., 2006).

While studies have shown conflicting results, four aspects of CNT toxicity are uniformly presented, summarised in (Fig. 5). 1) Increased CNT concentrations free in solution or in polymer scaffolds correlates to reduced cell growth and increased apoptosis (Madani et al., 2013a). 2) CNT size and shape influences toxicity. While smaller CNT show enhanced cell and protein adherence, the small diameter means that they are more likely to interfere with cell membranes and evoke an immune response (Nygaard et al., 2009). On the other hand, CNT rods longer than macrophages, 20 μm, will not be phagocytosed and degraded but will result in 'frustrated' phagocytosis and inflammation (Madani et al., 2012). 3) Synthesis, post-fabrication treatments and functionalisation influence CNT purity and toxicity (Lanone et al., 2013). 4) In each case cytotoxicity will also depend on the cell type and so CNT functionalisation and size needs be to be matched accordingly. This final point will be difficult to control and put into practice as CNTs can migrate from desired implant sites (Usui et al., 2008).

Table 1
Summary of the physical and chemical properties of CNTs.

Structural family	Fullerene
Types	2 types: SWCNTs and MWCNTs
Atomic bonding	Van der Waals forces (specifically, pi-stacking)
Chemical bonding	sp^2 bonds (CNTs are stronger than diamonds as the latter has chemical sp^3 bonding)
Solid density	1.3–1.4 g/cm^3
Specific strength	Up to 48,000 kn.m.kg^{-1} (Tensile strength 11–200 GPa)
Young's modulus	0.27–1.34 TPa
Size of the nano diameter	0.8–2 nm and 2–100 nm for SWCNTs and MWCNTs respectively
Electrical conductivity	Superconductive (10^4 S/cm^2 at any one point)
Thermal	Excellent 5000 Wm^{-1} K and microwave radiation absorbance
Optical properties	Near infrared fluorescence (900–1600 nm)

Each of these attributes are reviewed by Lanone et al. (2013) and in most cases they propose that the potential cytotoxic effects of nanoparticles can be avoided by changing the synthesis and/or purification methods as well as by correctly functionalising the surface of CNTs. Methods and functionalisation options for CNT adaption are presented in recent publication (Madani et al., 2012). In this paper various methods for CNT nanocomposite synthesis are also discussed as a means to minimise toxicity induced by CNT migration. Enhancing overall CNT biocompatibility and biodegradability can further reduce migrated CNT toxicity, which is discussed by Bianco et al. (2011).

It is clear that further research and clarification is needed in terms of CNT cytotoxicity. For each cell type optimum lengths and guidelines for CNT size and functionalisation need to be defined, standardised and reliable methods for evaluating CNT toxicity *in vivo* are required. Yamashita et al. (2010) have attempted this and in their study they define CNTs dimensions (length: 1–2 μm, diameter between 5–10 nm) to minimise toxicity and cell interference in alveolar epithelial cells (carcinoma cell lines used) (Yamashita et al., 2010). Liu et al. (2014) have also taken a step in this direction by proposing Drosophila embryos as a tool to assess the mechanisms behind cellular and developmental MWCNT cytotoxicity in living organisms.

Due to the numerous physical and chemical parameters that need to be considered when discussing toxicity, as well as different CNT toxicity with differ cell types, we have chosen to present relevant studies, which explore toxicity in the cells and scaffolds discussed, in the case studies that follow.

CNT and organ engineering/regeneration case studies

Neural tissue engineering

Nerve loss can be the result of external trauma, anoxia, hypoglycemia, diabetes and viral infection. Nerve axons can show some regeneration, however this is limited to 5 mm and often coaptation is required (Sedaghati et al., 2011) in which the damaged nerve ends are surgically joined. Other treatments include nerve grafts, both

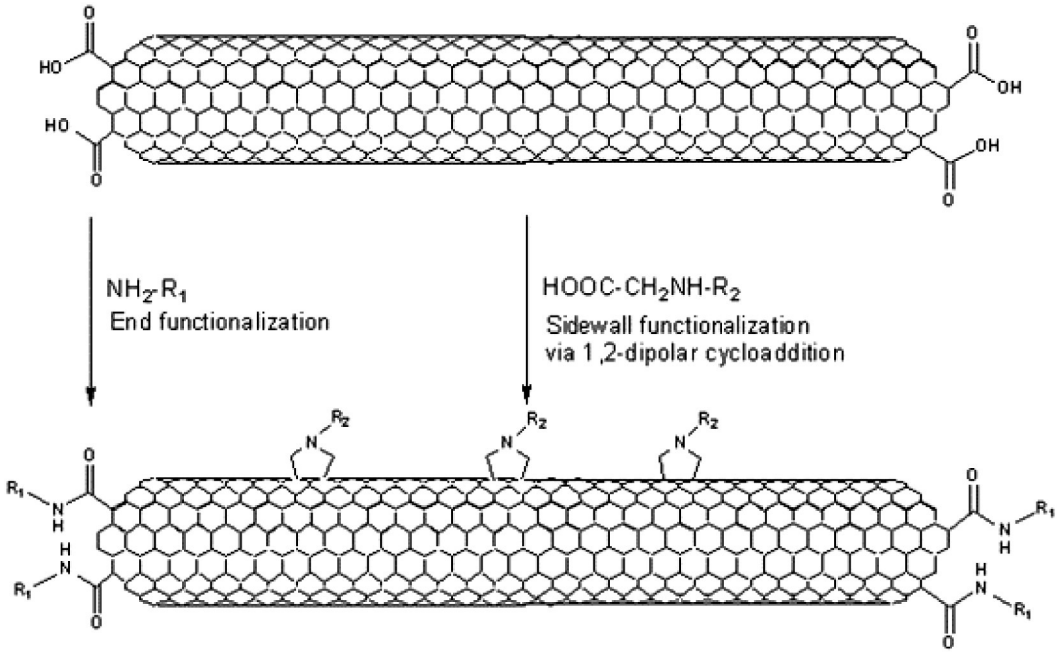

Fig. 4. Heterogeneous functionalisation of CNTs. This shows both tip and side wall adaption, shown to reduce CNT toxicity and increase CNT biocompatibility (Harrison and Atala, 2007).

autologous and bioprosthetic. These are explained, along with other nerve conduits, in a review by Sedaghati et al. (2011). In terms of nerve regeneration and engineering, scaffolds need to have a larger diameter than the nerve, to avoid nerve damage and compression and to allow neurotrophic factor release and diffusion by the proximal site of axon injury (Mackinnon and Dellon, 1990). Flexibility is crucial to aid growing axons *in vitro,* and to allow for surgical manipulation *in vivo* (Hudson et al., 2000). Furthermore, scaffolds should be stable but without sharp boarders as these sites have been shown to cause a granulomatous inflammatory response (Sedaghati et al., 2011). Scaffolds should also mimic the ultimate tensile strength (UTS) and elastic modulus of nerve tissue. These have been determined as 2.7 and 0.57 MPa, respectively with decellularised nerve tissue showing a UTS of 1.4 and elastic modulus of 0.58 MPa (Arslantunali et al., 2014; Borschel et al., 2003). Finally, scaffold nanotopography is essential, as neural processes such as dendrites vary from the nano to microscale (Arslantunali et al., 2014), and so require an appropriately sized environment for neural interaction and maturation.

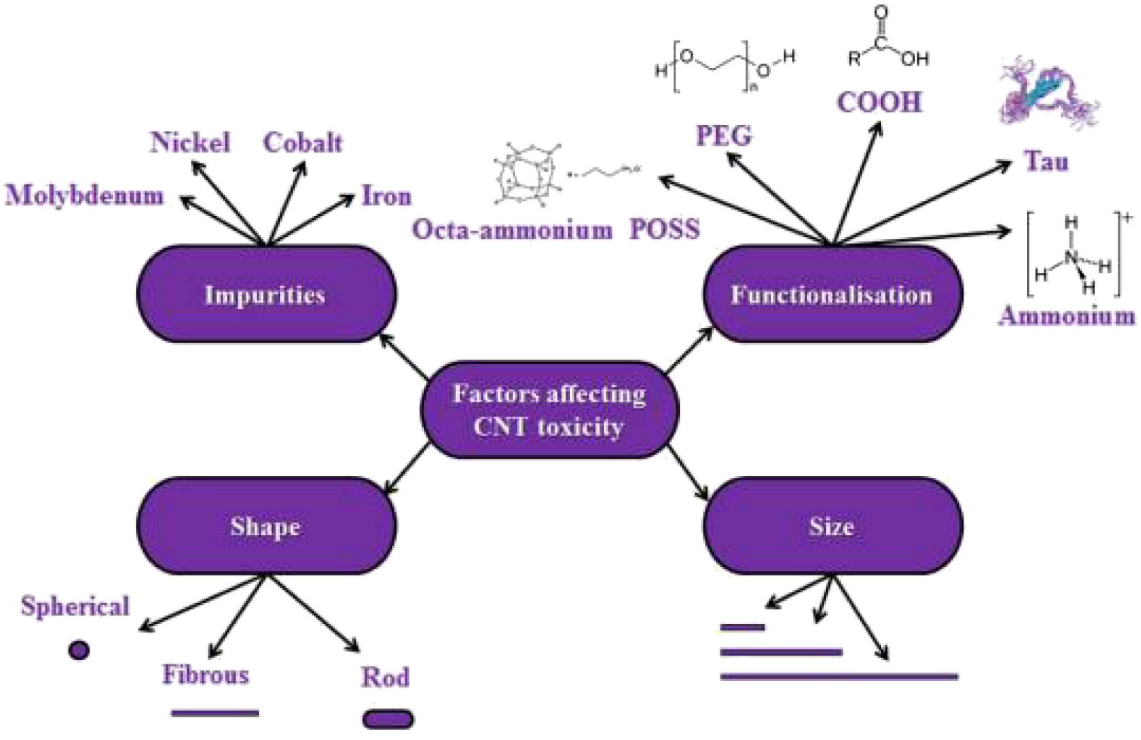

Fig. 5. Summary of the factors affecting CNT toxicity.

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Polymer scaffolds like PLLA and PCU, and biomaterials like collagen fit some of these criteria, however they lack the essential properties of appropriate tensile strength and electrical conductivity. CNTs can provide conductive properties (with conductivity densities of 10^4 S/cm); along with mechanical and tensile scaffold enhancements.

Conductive nerve guides show improved peripheral nerve regeneration (Sisken et al., 1989) and axon remyelintation (George et al., 2009). Gheith et al. (2006) reported that neuronal cells grown on SWCNT multilayer films showed stimulated nerve repair. 4-hydroxynonenal-MWCNTs have shown improved embryonic rat neuronal growth and long-term viability, with double axon length and three times more neurite branching, compared to those without MWCNTs (Mattson et al., 2000). Arslantunali et al. (2014) reported SHSY5Y neuroblastoma cell clustering and communication when cultured on 1% MWCNT-pHEMA hydrogels and that pristine pHEMA showed monolayer coverage. This clustering, through the use of axon like processes indicated cell communication and progress towards an organised 3D neuronal network. In terms of MWCNT conductivity, these were shown to preserve cell viability when 1 V and 2 V potentials were applied. These voltages, however, did not improve cell alignment or differentiation (Arslantunali et al., 2014). Furthermore, pHEMA composite conduits with MWCNTs, 6–13 nm in diameter and 12–20 μm in length, were presented as ideal for nerve engineering due to their superior neural process interaction. In the same study, pHEMA compisites at 3% MWCNT concentration showed optimal elastic moduli of 0.41 MPa and a UTS of 2 MPa. This is ideal for the purpose of decellularised neural tissue.

In terms of collagen composites, pheochromocytoma rat cells grown on electrically conductive CNT-collagen scaffolds show that CNTs, even at low concentrations of 5%, significantly improve neuronal growth and differentiation (Cho and Borgens, 2010). Furthermore, it was demonstrated that, if electrically stimulated, these cells could increase their neurite spreading on the surface of the composite (Fig. 6).

Excitingly, in 2012, conductive CNT ropes (diameter of 1 mm and length of 1.5 cm) were designed and these showed superior differentiation, maturity and neurite outgrowth (Huang et al., 2012). The spiral arrangement of the rope is thought to direct neurite extensions. Assessment after 3 weeks shows long-term superior support offered by CNT ropes and electrical stimulation of 5 mV (intermittent stimulation (25 ms off, 25 ms on) for 15 min) showed neurite outgrowths of up to 146 μm, 60 μm more than the non-stimulated group (Fig. 7). Gamma enolase (ENO2) and MECP2 are genes expressed in mature neurons, and at 3 weeks gene expression levels were highest in neural stem cells cultured on the CNT rope stimulated at 5 mV. Syn 1 gene expression, coding for synapsin found on synaptic vesicles, was down regulated at week 1 on the non-stimulated CNT rope, indicating that CNTs aid growth and neurite development, but does not support complex development. Electrical stimulation however increased Syn 1 expression during week 1, showing that it is required to stimulate synaptic network formation. This activity could be explained by the fact that applied electrical stimulation mimics the endogenous electric field created at the start of nerve repair (Li and Jiang, 2011; Huang et al., 2012) and that this signal is aided and dispersed by the 3D structural network formed by CNTs (Jin et al., 2013).

Cellot et al. (2009) present a more detailed mechanism behind improved synaptic formation and nerve stimulation in neural tissue grown on conductive CNTs. Here it is proposed that CNTs favour reverse action potential propagation back up the axon and into the dendrite. This potential is thought to generate increased dendritic calcium concentration in the process called calcium electrogenesis. This process is dependent on dendrite interactions with surrounding cell stroma and is closely linked to a phenomenon called ADP or after-depolarization potential (Cellot et al., 2009). This prolonged potential was shown to enhance synaptic maturation and network formation and can increase the frequency of neuronal firing. These observations were also

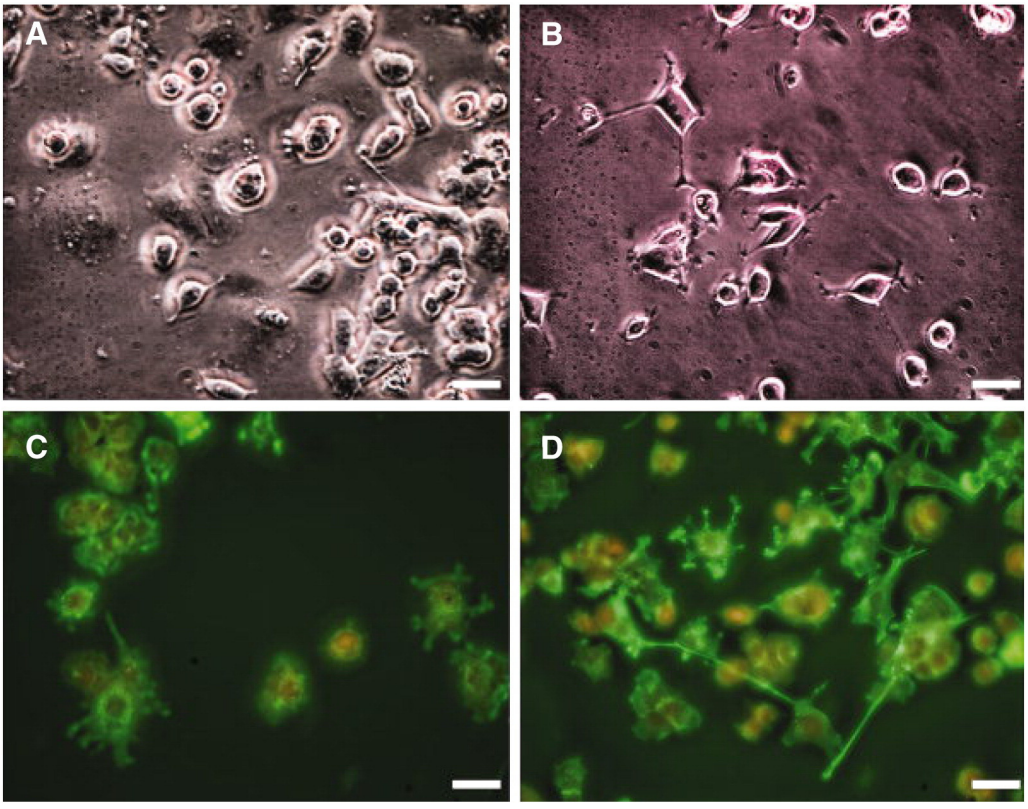

Fig. 6. Phase contrast and representative fluorescent images of pheochromocytoma cell neurite extension after 2 days in culture. A and C: CNT-collagen composite with 5% MWCNT loading. B and D: Electrically stimulated CNT-collagen composite with 5% MWCNT loading. Electrically conductive surfaces increase cell adhesion, proliferation, and neurite extension. Scale bar represents 20 μm (Cho and Borgens, 2010).

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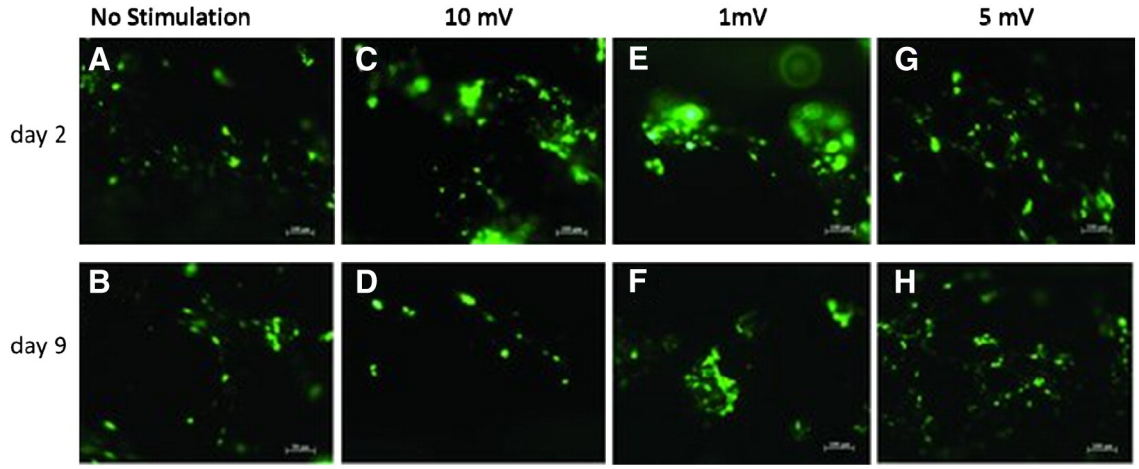

Fig. 7. Fluorescence microscopy of neural stem cells at days 2 and 9. Electrical stimulation intermittent (25 ms on, 25 ms off for 15 min) for 10Mv, 1 mV and 5 mV. Showing enhanced neurite growth, axonal elongation and possible network formation. (Huang et al., 2012).

documented by Lovat et al. (2005) who cultured hippocampal neurons on MWCNT-glass plates. The conductive properties of CNTs enhanced natural neural action potentials, stimulating the network as a whole. MWCNT-glass cultures displayed, on average, a 6-fold increase in the frequency of spontaneous postsynaptic currents to 6.67Hz. The spontaneous action potential frequency of these neurons changed from 0.22 Hz in cultures without MWCNTs, to 1.34 Hz in MWCNT-glass cultures (Lovat et al., 2005).

These observations show that the relationship of CNTs with neurons can affect neuronal information processing and establish and support network formation. It is important to note that this interaction is a combination of nanotopography, CNT-cell interaction and electrical stimulation. These results were not seen with other conductive materials such as indiumtin oxide (ITO), which lack nanotopography (Cellot et al., 2009).

Studies by Arslantunali et al. (2014) and Huang et al. (2012) assessed CNT toxicity and reported that both neuroblastoma and neural stem cells were not affected by the presence of MWCNT or CNT ropes, and presented these as viable electrical conducts for *in vitro* nerve genesis and *in vivo* nerve regeneration.

Bone tissue engineering

Bone tissue engineering is one of the main areas where CNTs are being widely investigated, with over 50 scientific papers published on their application. A comprehensive review on this topic was recently published by Newman et al. (2013).

CNTs were used by Ogihara et al. (2012) as reinforcement in alumina composites. Mechanical testing revealed a 120% increase in the fracture toughness of the material and the study demonstrated that osteoblasts, chondrocytes, smooth muscle cells and fibroblasts have superior proliferative ability, compared to those seeded on non-composite alumina. They also investigated composite alumina substrates *in vivo,* using 15-week-old Japanese white rabbits, in which CNT composites showed direct integration into bone tissue and showed reduced inflammatory responses compared to the control substrates. Other studies have also reported an increase in alkaline phosphate and calcium deposition, as well as osteoblast cell adhesion, in cases where CNT-PU composite scaffolds were used (Price et al., 2003; Sato and Webster, 2004). This indicates natural bone deposition encouraged by these scaffolds.

Apart from the physical properties, the electrical properties of CNTs have also been used in the field of bone tissue engineering with the hopes of developing an electrically active biomaterial, similar to natural bone. Applying alternating currents (10 μA at 10 Hz) to MWCNT-PLLA scaffolds seeded with osteoblasts have shown a 46% increase in cell proliferation and a 307% increase in extracellular calcium deposition, compared to non-stimulated controls (Supronowicz et al., 2002). Shao et al. (2011) also examined electrospun osteoblast seeded MWCNT-PLLA composite substrates, with aligned MWCNTs, under the presence or absence of electric stimulation. In the presence of electrical stimulation (direct current (DC) of 100 μA) improved proliferation and cell alignment was seen, with cells growing and showing elongation in the direction of the applied current.

The effects of CNTs on osteoblast cell membrane function and collagen secretion were studied by Zanello et al. (2006) in the hope of determining how CNTs affect bone matrix formation. They concluded that in the presence of CNTs the electrical function of cell membranes was maintained. This study supports the hypothesis that CNTs can enhance L-type calcium channel formation and electrical propagation. Enhanced channel function showed improved calcium deposition. Another interesting finding in this study was that the formation of hydroxyapatite crystals (the largest mineral component of the natural bone) on SWCNTs and MWCNTs mirrored that of osteoblasts cultured on woven bone. Moreover, cells grown on 1.5 nm diameter SWCNTs showed diameters of 40 μm, which resembles natural osteoblast size. This phenotype could result from the fact that these SWCNTs are similar in size to the triple helix collagen fibres found in natural bone (Zanello et al., 2006).

Major efforts have been put into investigating the toxicological effects of CNTs for bone tissue engineering applications, most of which report that CNTs, either within a composite or added to substrates, improve cell proliferation and bone tissue formation. These studies are discussed in a review by Newman et al. (2013). A conflicting study in 2009 should be mentioned, as it reported that carboxylated SWCNTs suppress bone morphogenic protein signalling (Mu et al., 2009). Newman et al. (2013) who reviewed various studies on CNTs and their potential application for bone engineering also presented a discussion to explain conflicting results. They conclude that CNTs affect osteocyte proliferation in a time dependant manner. This cycle starts with an initial period of cell death, followed by the removal of the toxic potential of CNTs through biodegradation and macrophage and neutrophil activity. This removal happens in a time dependant manner and is affected by CNT functionalisation, size and biocompatibility. The removal or biodegradation of CNTs consequently results in an increase in osteoblast proliferation (Newman et al., 2013).

Cardiac and vascular tissue engineering

CNTs have been studied as fillers to improve mechanical and chemical properties of various cardiovascular tissue engineered constructs (Meng et al., 2013). CNT-incorporated photo-cross-linkable gelatin methacrylate

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(GelMA) hydrogels seeded with neonatal rat cardiomyocytes were able to create functional cardiac patches and demonstrated that, by adding CNTs, the mechanical integrity and electrophysiological functions of the construct improves significantly. In the case of myocardial tissues cultured on 50 μm thick CNT-GelMA, these patches showed a 3 fold increase in spontaneous synchronous beating rates and an 85% lower excitation threshold, compared to those cultured on pristine GelMA hydrogels (Fig. 8) (Shin et al., 2013).

In another study, CNT scaffolds promoted cell division and maturation in cardiomyocytes with the idea that the CNT scaffolds can improve cardiomyocyte growth and maturation by gene expression manipulation (Martinelli et al., 2013). CNT substrates increase the α-myosin heavy chain and up-regulate sarcoplasmic reticulum Ca^{+2} by 2 fold. Cardiomyocytes growth on CNT substrates were also documented to show no signs of pathological hypertrophy, seen by reduced levels of β-myosin heavy chain and skeletal α-actin. They also demonstrated that cardiomyocytes on CNTs had a more mature electrophysiological phenotype of syncytia and intracellular calcium signalling. This was seconded by a study by Zhang et al. (2008) who showed that SWCNTs and MWCNTs can increase gene expression of focal adhesion kinase (FAK). This is a broadly expressed cytoplasmic tyrosine kinase, which is a downstream component of an integrin regulated signaling pathway, and is vital for the regulation of migration, proliferation and survival of cardiomyocytes during development and maturation.

CNTs ability to conduct electrical impulses holds potential for *in vivo* cardiomyogenesis. Initial studies on cardiomyogenesis utilised the demethylating agent 5-azacytidine in MSCs, however, the use of this was limited to *in vitro* work as it lead to cell apoptosis *in vivo* (Bulut et al., 1999). In an attempt to overcome this problem, MSCs cultured in media containing varying concentrations of CNTs and MSCs seeded onto CNT-PLLA scaffolds were examined. These were placed in a bioreactor providing a controlled electrical stimulus of 0.15 V/cm at a frequency of 1Hz for 14 days. The electrical current conducted through the CNTs was shown to cause MSCs morphology to elongate and encouraged reorientation of cells perpendicular to applied current flow (Fig. 9) (Mooney et al., 2012). Additionally, the electrically stimulated cells in CNT medium showed a 40-fold increase in the cardiac marker for myosin heavy chain (CMHC) and an increase in cell number. In the same study, electrically stimulated CNT-PLLA seeded scaffolds showed increased CMHC and cardiac troponin T (CTT), indicating increased interaction between actin and myosin chains. An increase in cardiac marker C43, which is a gap junction protein found in myocardium, was also seen on electrically stimulated CNT-PLLA scaffolds, indicating increased communication between cells. These findings open doors for engineering cardiac patches and organs *in vitro*. The use of CNTs' conductivity could not only aid this generation, but also presents an opportunity to implant these cardiac patches or seeded scaffolds and electrically stimulate them to differentiate *in situ* using pacemaker technology (Mooney et al., 2012).

In terms of 3D organ engineering, thrombosis, coagulation and blood vessel formation should be considered. Blood clotting is a leading cause of morbidity and mortality following operative treatment, especially in cardiovascular and pancreatic implant surgeries (Cashion et al., 2010; Meng et al., 2010; Tu et al., 2010). CNTs are increasingly being studied for anticoagulation purposes. This has resulted in shifting attention from traditional anticoagulant drugs, to endothelial cells, as these are considered more effective for their long-term anticoagulant effect. This is due to the cells' ability to regulate the secretion of two factors:

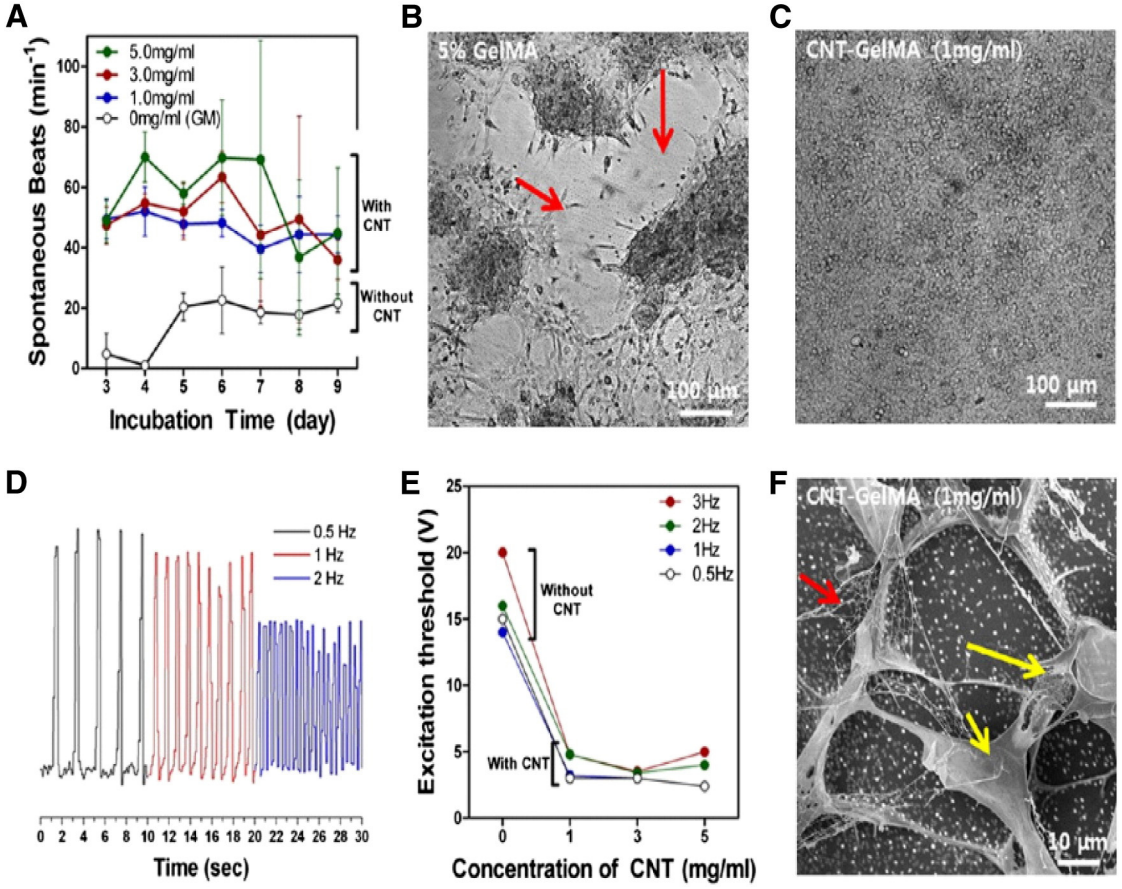

Fig. 8. Improved mechanical integrity and advanced electrophysiological functions of cardiac tissues on CNT-GelMA. (A) Spontaneous beating rates of cardiac tissues recorded daily from days 3–9 with and without CNTs. Phase contrast images showed cardiac tissues ruptured on (B) pristine GelMA but remain intact on (C) CNT-GelMA on day 5. (D) Recording of synchronous beating signal of a tissue sample in response to applied external electric fields. (E) Excitation threshold of cardiac tissues on CNT-GelMA was 85% lower than that on pristine GelMA. (F) SEM image shows morphology of cardiac cells on CNT-GelMA. Red arrow: cytoplasmic prolongations adhered to CNTs. Yellow arrows: flat cell bodies (Shin et al., 2013).

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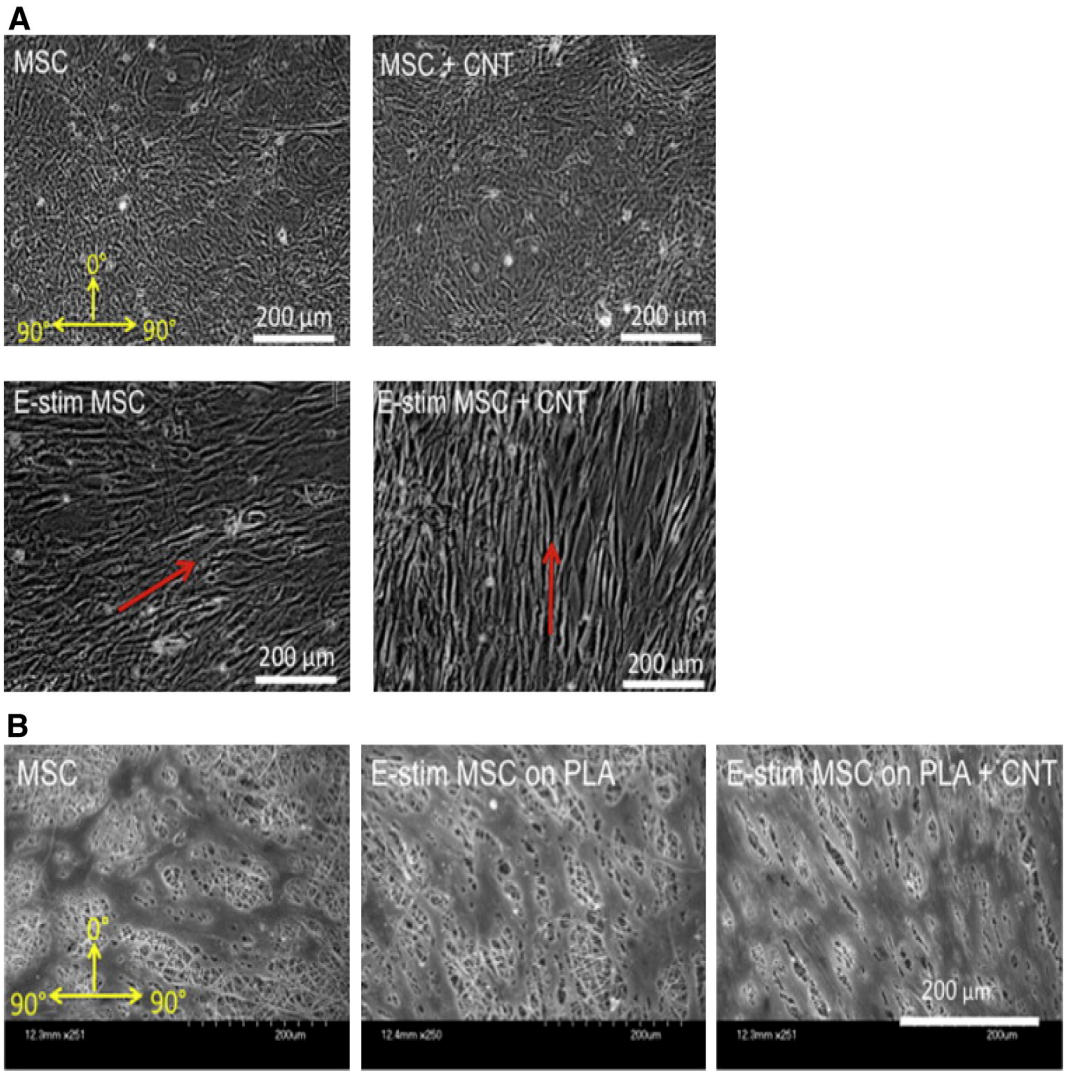

Fig. 9. Overview of the effect of CNT and/or electrical stimulation on MSC morphology in (A) MSC culture containing CNT after 14 days electrical stimulation (Magnification 10×) elongated in shape compared to the fibroblastic morphology of the unstimulated control cultures and (B) MSC seeded on CNT scaffolds after 10 days electrical stimulation appeared to have an elongated morphology (Mooney et al., 2012).

Tissue factor (TF), and Plasminogen activator inhibitor-1 (PAI-1). These are usually released when endothelial cells are damaged or disrupted (Meng et al., 2010). In a recent study it was shown that the physical nanofibrous structure plays a crucial role to inhibit TF release by human umbilical vein endothelial cells (HUVEC), and scaffolds with incorporated MWCNTs showed further ability to inhibit the release of PAI-1, when compared to nanofibrous scaffolds without MWCNTs (Meng et al., 2010). MWCNTs were also speculated to play a role in endothelial cell arrangement and orientation. This was shown through the activation of GTPases Rac, Rho and the GTPase activating protein Cdc42. Signals from these proteins are relayed to the nucleus via the MAP kinase pathway and are thought responsible for increased cell proliferation, cytoskeletal changes and ultimately cell alignment.

Blood vessel development is an important consideration when discussing complex organ (cardiac) synthesis. Chaudhuri et al. (2010) while assessing the ability of CNTs to deliver antiangiogenic drugs to tumor blood vessels, showed that drug free CNTs actually promote angiogenesis *in vitro* and *in vivo*. Excitingly, CNTs promoted endothelial tubulogenesis, which indicates advanced angiogenesis. The mechanism proposed suggests that CNTs increase integrin clustering, which results in the phosphoryation of FAK and subsequent activation of phosphinositide-3-kinase cell signaling (Fig. 10). This finding is important as the development of blood vessels in engineered organs, as discussed above, is a vital step towards tissue survival, support, and independent growth from the original scaffold.

The above-mentioned studies do not report on CNT toxicity, however a study by Kroustalli et al. (2013) who grew endothelial and myofibroblast cells at 5% and 10% MWCNT concentrations, on MWCNT-chitosan scaffolds, reports that MWCNTs did not show cell toxicity and did not cause cell apoptosis.

In another study, the 3D printing process, coaxial bioprinting, was used to incorporate MWCNTs into alginate vascular conduits, to enhance the mechanical properties of these constructs (Dolati et al., 2014). The conduits synthesised showed superior physical properties with seamless cylindrical inner and outer diameters of 461 µm and 930 µm, respectively, and lengths just over 1 m long. Tensile strength increased significantly from 110 kPa in 3% alginate conduits without MWCNTs to 238 kPa at 1% MWCNT concentrations. Elastic moduli also increased from 105 kPa to 305 kPa at 1% MWCNT concentrations. In terms of perfusion, tests conducted in a perfusion chamber with cell media, showed that MWCNT alginate conduits demonstrate successful perfusion capability. Conduits up to 1 m showed increased and directed

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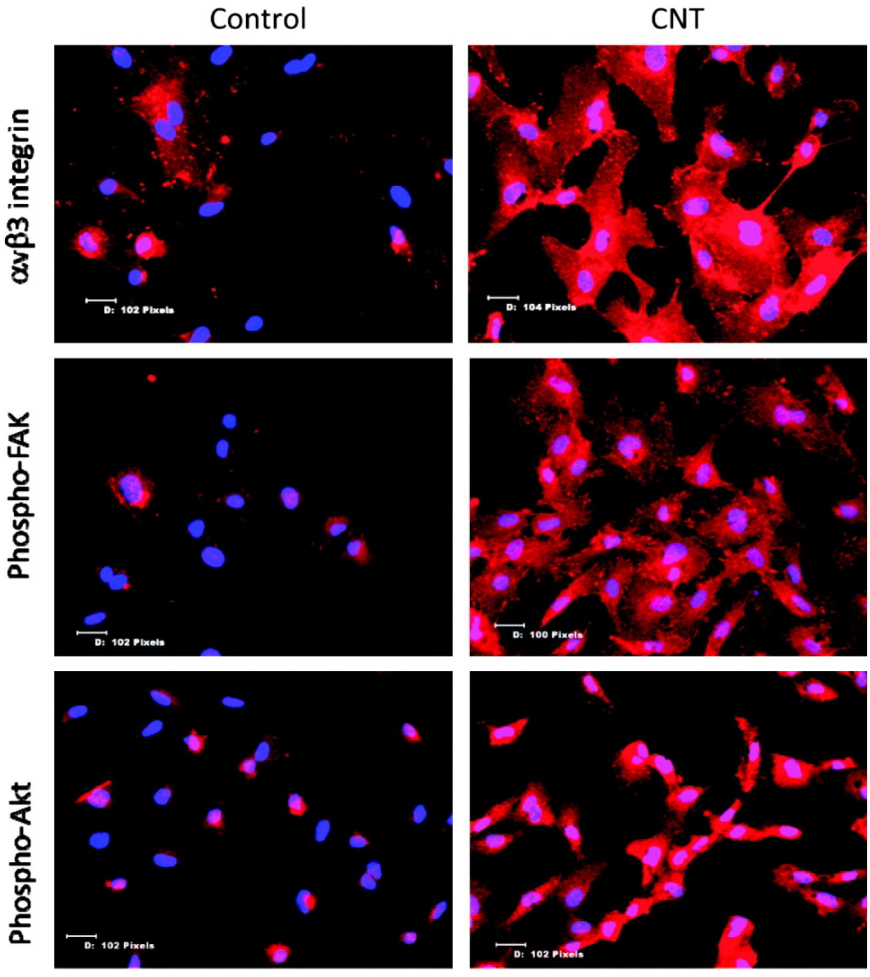

Fig. 10. HUVEC cells incubated with and without CNTs. Antibodies against αvβ3 integrin, phospho-FAK, and phospho-Akt and probed with Alexa 594-conjugated secondary antibody. Magnification x 20. Scale bar is shown in pixels. One pixel = 0.22 micrometers (Chaudhuri et al., 2010). The clustering of integrins on CNTs results in phosphorylation of FAK, which can then activate PI3kinase that phosphorylates Akt, which has been implicated in angiogenesis.

diffusion of cell media in a radial manner, similar to the flow seen in natural blood vessels, with the highest diffusion rates seen in the 1% MWCNT concentration group.

This printing process with MWCNTs was reported to initially damage human coronary artery smooth muscle cells. However, following printing, cell viability and proliferation increased upon introduction into *in vitro* culture. Long-term analysis, however, showed reduced ECM and damaged cell nuclei as a result of MWCNT incorporation. This study concluded that CNT-induced toxicity reduced the biological performance of the conduits, however, they did not report on the type of CNTs used nor the functionalisation methods adopted. This study highlights significant enhancements of CNTs in alginate conduits for blood vessel engineering and these properties should not be dismissed because of toxicology. Instead coaxial bioprinting should be conducted with differently functionalised and processed CNTs, as the results from this study in terms of tensile and elastic improvements as well as enhanced permeability should not be overlooked.

Prospective studies

In this section we present prospective areas of investigation, which are centred around the CNT properties discussed in this paper.

Dynamic scaffolds

CNTs' elastic and tensile strength could aid dynamic scaffold design. In terms of cardiac, bladder and nerve engineering, this may be very useful in simulating a dynamic, *in vivo* environment. Tian et al. (2010) have documented the importance of dynamic growth using human bone marrow MSCs cultured on PLLA scaffolds in dynamic and static environments. The results propose a significant role for dynamic cultures in inducing MSCs to express smooth muscle- specific genes and proteins. The effect of dynamic culture was also more influential to cell growth than the presence or absence of growth factors PDGF-BB and TGF-b1. At Day 6, dynamic growth factor rich culture has produced $5x10^3$ smooth muscle cells, while its static counterpart had produced $3x10^3$ cells (Tian et al., 2010)

Controlled cell division

Nanotopographical properties of CNT have been shown to increase cell adhesion and communication, but what about controlling cell proliferation? Keeping cell division in check will become highly important when working with stem cells. It is also important to consider controlled cell division in the implanted organ, especially when an organ transplant is required due to cancer. Carbon nanofibres (CNFs) incorporated into polyurethane show reduced transitional and squamous urothelial carcinoma cell proliferation (Tsang et al., 2011). Results demonstrated that both transitional and squamous cell proliferation rates decreased over time on substrates with increased CNF concentration in PU. While no *in vivo* experiments have been conducted for CNT scaffolds, this research provides opportunities for scaffold design in terms of keeping cell proliferation under control.

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Table 2
Summary of the most recent and significant studies on incorporating CNTs into polymers for tissue and organ regeneration and generation.

Author, Year of Publication	CNT Property Tested and Application	Scaffold Used	Cell Used	Outcome
Supronowicz et al. (2002) & Shao et al. (2011)	Structure and Conductivity	MWCNT-PLLA	Osteoblasts	Increased proliferation and calcium production, stimulated by MWCNTs and electrical current.
Lovat et al. (2005)	Structure and Conductivity	MWCNT-glass plates	Hippocampal neurons	The conductive property of CNT enhanced natural neural action potentials, stimulating the network as a whole. MWCNT-glass cultures displayed, on average, a 6-fold increase in the frequency of spontaneous postsynaptic currents.
Gheith et al. (2006)	Structure and Conductivity	SWCNT multilayer films	Neural cells	Both electrically stimulated and unstimulated multilayers showed nerve repair. Enhanced on electrically stimulated films.
Zanello et al. (2006)	Structure and Conductivity	CNT	Osteoblasts	CNTs and electrical stimulation improved cell membrane electrical function, and calcium ion channel function. Hydroxyapatite layer made by osteoblasts was similar to that of woven bone. CNTs can enhance L-type calcium channel formation and electrical propagation. Enhanced channel function showed improved calcium deposition.
Zhang et al. (2008)	Structure	SWCNTs and MWCNTs	Cardiomyocytes	SWCNTs demonstrated more ability than MWCNTs to affect gene expression, cell elongation and viability.
Cellot et al. (2009)	Structure and Conductivity	CNT	Neural Cells	CNTs favour reverse action potential propagation back up the axon and into the dendrite. This potential is thought to generate increased dendritic calcium concentration in the process called calcium electrogenesis.
Armentano et al. (2010)	Structure (Cell proliferation)	MWCNTs in PU scaffolds	Osteoblasts	Increased osteoblast proliferation and calcium production in scaffolds with MWCNTs.
Chaudhuri et al. (2010)	Structure and composition	CNT	Endothelial cells	CNTs promoted endothelial tubulogenesis and thus advanced angiogenesis. Method proposed is that CNT promote integrin clustering which results in FAK phosphorylation and activation of kinase cell signaling.
Cho and Borgens (2010)	Structure and Conductivity	CNT-Collagen	PC12 pheochromocytoma cells	5% concentrations of CNTs showed optimum cell growth. Electrical stimulation showed increased neurite spreading.
Meng et al. (2010)	Structure (Cell adhesion and vascularization)	PU scaffold with MWCNT incorporation	Human umbilical vein endothelial cells (HUVECs)	Aligned nanofibrous structures and MWCNTs can act as extracellular signals to control cell behavior such as growth, proliferation and differentiation. Endothelial cells aligned along CNT defined axis. MWCNT also promoted increased collagen type IV secretion.
Mackle et al. (2011)	Structure and Composition (Scaffold strength and degradation)	SWCNTs incorporated into PLA scaffolds, concentration 2%	Human MSC	5% decrease in polymer crystallinity, a 12% increase in tensile strength and physical stability. Reduced rate of degradation No cytotoxic effect on the cells.
Mooney et al. (2012)	Conductivity (Cell differentiation)	CNT culture and CNT-PLLA scaffolds	MSCs	Electrical current through the CNTs was shown to cause MSCs elongation and encouraged reorientation of cells. Also showed increased cardiac marker and protein expression in CNT scaffolds. Importantly, showed the presence of gap junction protein C43 possibly highlighting CNTs role in encouraging cell communication.
Huang et al. (2012)	Structure and Conductivity	CNT rope	Neural stem cells	Neurite outgrowth increased in CNT ropes and spiral structure seems to guide extensions. Upregulation of ENO2 and MECP2 genes, showed nerve maturity. Electrical stimulation of 5 mV increased Syn 1 expression, showed complex nerve development. Voltages above 5 mV were damaging to cells.
Li et al. (2012)	Structure and Composition (Increased cell and protein attachment)	MWCNT- coated collagen sponges	Osteoblasts	Increased cell adhesion. DNA content in the cells attached to the MWCNTs was higher while an increased ability to adsorb proteins was observed.
Ogihara et al. (2012)	Structure, Tensile strength (Cell attachment and development)	CNT reinforced alumina composites	Osteoblasts, chondrocytes, smooth muscle cells and fibroblasts	120% increase of fracture toughness of material. Enhanced cell proliferation. *In vivo*, constructs showed direct integration into bone and improved inflammatory response.
Arslantunali et al. (2014)	Structure and Conductivity	MWCNT-pHEMA	SHSY5Y neuroblastoma cells	MWCNTs seemed to promote cell clustering and communication. Cells seemed to form 3D like structure. Electrical stimulation did not cause cell alignment and MWCNTs showed cell protection against electric stress.
Chen et al. (2013)	Structure and Thermal properties	PNIPAAM-MWCNT hydrogels	MDCK cells	MWCNTs increased cell adhesion and thermal properties allowed for cell sheet harvesting through temperature manipulation.
Kroustalli et al. (2013)	Structure and Composition (Scaffold stability, non-toxicity)	Chitosan composites with incorporated MWCNTs	Endothelial cells and vascular myofibroblast cells	Mechanical structure of scaffold was optimal with a 5% MWCNT concentration. Additionally, showed that MWCNT scaffolds were not toxic to both cell types.
Martinelli et al. (2013)	Structure and Conductivity (Influenced gene expression and cell development)	MWCNTs of 20–30 nm on glass	Cardiomyocytes	Improved cardiomyocyte growth through CNTs ability to influence gene expression. Cells growth on CNTs also demonstrated electrophysiological phenotype of syncytia and intracellular calcium signaling.
Shin et al. (2013)	Structure and Conductivity (Scaffold stability, elasticity and cell development)	CNT-incorporated GelMA hydrogels	Neonatal rat cardiomyocytes	CNTs improved mechanical integrity, electrophysiological functions, elasticity and hydrophobicity. Myocytes showed increased differentiation towards a functioning cardiac system.
Dolati et al. (2014)	Structure, tensile strength and porosity	MWCNT into alginate vascular conduits, 3D printed	Human coronary artery smooth muscle cells.	The conduits synthesised showed superior physical properties with seamless cylindrical inner and outer diameters. Tensile strength and elastic moduli increased significantly. MWCNTs were reported to damage human coronary artery smooth muscle cells.

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Monitoring tissue development and viability

In terms of conductive and optical properties, CNTs can be used for application in biosensors. SWCNTs show fluorescence in the near infrared optical range. These properties minimise optical imaging interferences (tissue absorbing, light scattering, autofluorescence and photobleachng) and SWCNTs have also been shown to detect real-time nitric oxide in living cells. This is important as nitric oxide is produced in tissue inflammation and cell apoptosis (Iverson et al., 2013). CNTs' combined optical abilities could allow for the monitoring of the bio-distribution and cell viability in developing tissues and organs *in vivo*. This could be achieved through developing CNTs for the analysis and monitoring of ion transport, enzyme/cofactors interactions and protein as well as metabolite secretion (Harrison and Atala, 2007).

Conclusion

In summary, increasing demand for organs and shortage of suitable transplants has led to great advances in tissue engineering and regenerative medicine, specifically in the field of scaffold design. Studies have shown that the mechanical properties and nanotopography of scaffolds are highly important in their functionality and application in tissue and organ engineering; with successful scaffolds mimicking native cell ECM structure and function. CNTs bring fascinating advantages to the field of tissue engineering and scaffold design for the following reasons: 1) their superior mechanical properties with elastic moduli and tensile strength, make them suitable for composite scaffolds and future dynamic scaffold design. 2) CNT size, shape, surface roughness and surface area structurally mimics that of collagen fibres embedded in cell ECM, providing a 3D network to support and guide cell proliferation, differentiation and communication. 3) CNTs ability to interact with and adsorb extracellular proteins allows enhanced cell interaction and scaffold biocompatibility. 4) CNTs offer increased cell support, which is important for angiogenesis and vascularisation, while still allowing scaffold degradation. 5) Unique thermal and conductive properties enhance scaffold design and improve cell differentiation in a multitude of engineered tissues (Table 2).

Despite many obstacles on the road towards developing fully functional organs, recent progress discussed in this review shows that this goal is not far from realisation, and suggests that CNTs could lead the way forward into a new generation of 3D organ genesis.

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