Carbon Nanotube for Bone Repair

20

Jayachandran Venkatesan and Se Kwon Kim

Contents

Abstract

In the recent years, significant development has been achieved in tissue engineering for the artificial bone preparation. Metals, polymers, and ceramics are widely used biomaterials for bone implant. Apart from this, the infant material carbon nanotube (CNT) is an emerging biomaterial in the recent days, which are being checked for bone tissue engineering. CNT has unique properties such as electrical, mechanical, and thermal properties. Thus, addition of CNT in the polymer, ceramic, and metal matrix will be enhancing the function of the CNT.

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In this chapter, CNT–polymers, CNT–hydroxyapatite, and CNT/Bioglass composite biomaterials have been discussed and explored for bone tissue engineering application.

Keywords
Polymers • Bioglass • Chitosan • Toxicity • Hydroxyapatite

1 Introduction

In the recent years, significant development has been achieved in artificial biomaterials to treat the loss, defects, or failure of the bone. Autograft and allograft are promising materials to treat the bone defect or replacements; however, both methods are having disadvantages in donor sites and dangerous diseases transmissible. Thus, researchers have been paid an attention in the preparation of artificial bone materials using polymers, ceramics, and metals. Presently, metals are widely used for the treatments of bone defects and replacement due to its mechanical properties. Several synthetic and natural biopolymers, bioceramics, are being currently used to make the artificial bone; those biomaterials are in the laboratory stage to clinical level today. But still the problem exists in mechanical strength; to address this issue, carbon nanotube (CNT) might be a promising biomaterial to solve the problem. The common tissue engineering culture, replacement, and implant procedure have been shown in Fig. 20.1.

2 Bone Tissue Engineering

The bone is a hierarchical structure and it is made up of bioceramics (hydroxyapatite) and complicated biopolymer (collagen) as a major portion. The bone is not only giving the structural arrangement of the body; it keeps the internal organs such as the heart, brain, and lungs safe. Collagen and non-collagenous proteins are playing major role in the bone metabolisms. The loss, fracture, and diseases of the bone are the painful ones and also affect the human normal life. Several biomaterials are being used for the purpose of bone tissue engineering to solve the issues. Poly(methyl methacrylate), polyglycolic acid, polyvinylpyrrolidone, poly(propylene fumarate), polydopamine, polyvinyl alcohol, polycaprolactone, collagen, chitin, chitosan, and alginate [1, 2] are some of them. Synthetic and natural polymers have advantages and disadvantages. Synthetic polymers are in the problem of degradation and unwanted byproducts produced while degradation. Natural polymers degraded quickly and are also inexpensive. Apart from the polymers, ceramics are widely used biomaterials in the bone tissue engineered materials. Hydroxyapatite (HAp) materials are widely used in the bone tissue engineered materials due to its excellent biocompatibility with bone tissue. The schematic procedure of bone graft substitute has been shown in the Fig. 20.2.

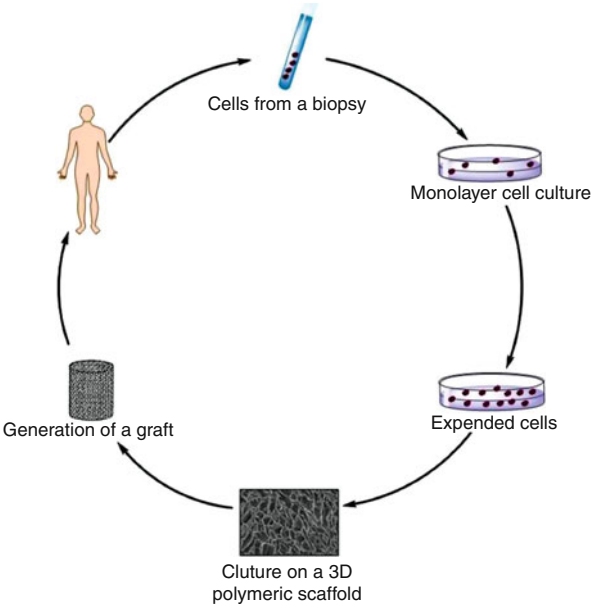

Fig. 20.1 Basic tissue engineering

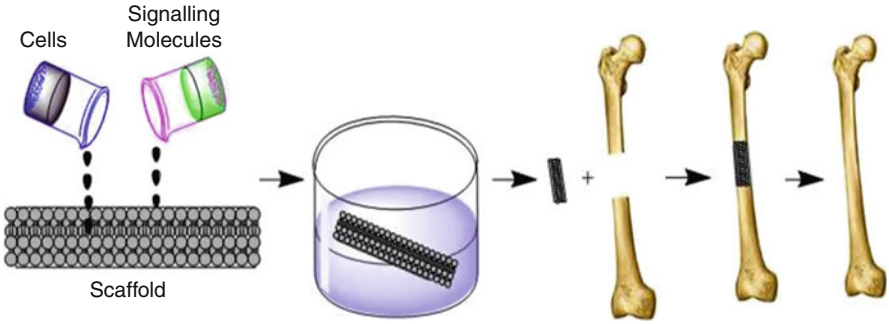

Fig. 20.2 Schematic procedure for tissue engineering scaffold transplantation

3 Carbon Nanotube

Carbon has different kinds of allotropes and it can be available in different forms such as diamond, fullerene, graphite, carbon nanotubes, and more recently graphene. Among the carbon nanotubes, single-walled carbon nanotube (SWNT) and multiwalled carbon nanotube (MWNT) are the most extensively studied biomaterials for various applications, as shown in Fig. 20.3.

CNT has unique properties such as electrical, mechanical, and thermal properties; thus, researchers are trying to use CNTs in the preparation of artificial bone

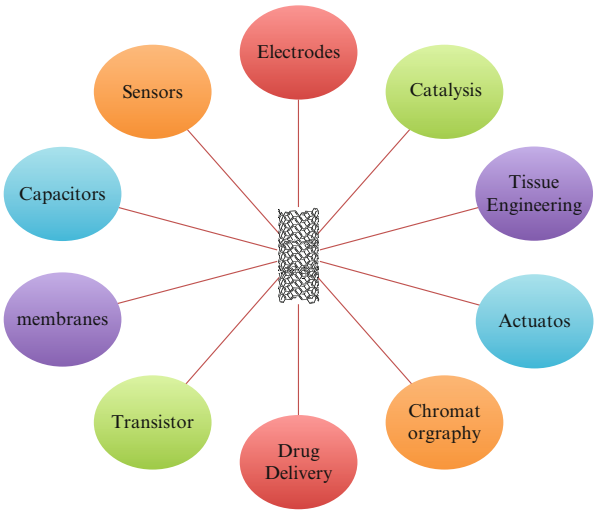

Fig. 20.3 Application of carbon nanotube

materials. But, the toxicity of CNTs is an important concern, whether it can be used for biometrics or not. It is a burning question in the recent research. In addition, the toxicity of CNTs is still obscure. Several controversy reports have been published in online for decades.

Horrison et al. suggest that CNTs can be used for improved tracking of cells, sensing the microenvironments, distribution of transfection agents, and scaffold incorporation with the host's body [3]. In another study, the comparison of MWNT and SWNT toxicities was performed at in vivo condition; CNTs' toxicity is dependent on size, shape, length, chemical surface, and aspect ratio [4] (Fig. 20.4). CNT alone was implanted in animal femur model without any binder, as a result of no severe inflammatory response and no toxicity, and it may be useful for bone regeneration [5]. The orientation of CNT is also important regarding toxicity concern; Namgung et al. suggested that aligned CNT networks exhibited enhanced proliferation and osteogenic differentiation compared to those on randomly oriented CNT networks [6].

Fullerene, graphene, and diamond are allotropes of carbon. To find out the toxicity of carbon allotropes, fullerenes C60 were deposited on microscopic glass coverslips with different heights of 128 ± 8 nm, 238 ± 3 nm, 326 ± 5 nm, and 1043 ± 57 nm. Until 326 ± 5 nm of fullerene layer, the adhesion and proliferation of human osteoblast-like MG 63 cells was similar as in control cells on polystyrene dishes. By increasing the layer content, 1043 ± 57 nm in height, the cells grew preferentially in grooves among the prominences. In another case, nanodiamond was deposited on silicon substrates and provided an excellent substrate for the adhesion, growth, and osteogenic differentiation of MG 63 cells [7, 8]. The gene transfection efficiency of cells grown on the CNT and graphene-coated substrates was improved up to 250 % that of cells grown on a cover glass [9].

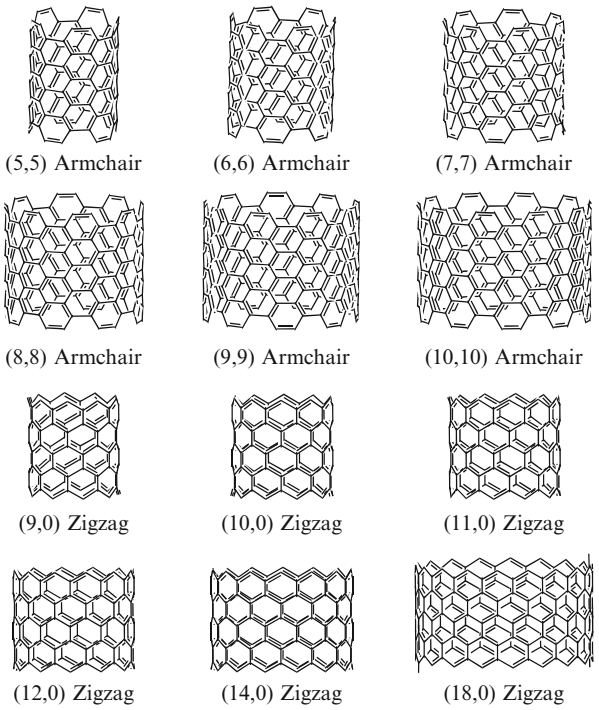

Fig. 20.4 Different type of CNTs

In the case of MWNT, adjoining bones induce little local inflammatory reaction, show high bone tissue compatibility, permit bone repair, become integrated into new bone, and accelerate bone formation stimulated by rhBMP-2 [10]. In addition, few reports suggest that functionalization of CNT as carboxylated SWNTs and carboxylated MWNT inhibited the proliferation, osteogenic differentiation, adipogenic differentiation, and mineralization of MSCs [11].

The addition of CNTs in the composite materials is expected to be promising for high load-bearing orthopedic implants and does not only decrease the toxicity of the CNT but is also expected to mimic the natural function of the bone. The extensive review has been written for CNT, which can act as biomaterials for tissue regeneration [12–16].

The in vivo (mice nasal, oral, intratracheal, and intraperitoneal) study has been performed with pure MWNTs and N-doped MWNT. Extremely high concentrations of N-doped MWNT nanotubes administrated directly into the mice's trachea only induced granulomatous inflammatory responses. Importantly, all other routes of administration did not induce signs of distress or tissue changes on any treated mouse. The functionalizations of N-doped MWNT nanotubes are less harmful than MWNTs or SWNTs and might be more advantageous for bioapplication [17].

Functionalization of CNT significantly reduced its toxicity and is also used for several biomedical applications such as bone regeneration, neural regeneration,

drug delivery, and gene delivery [18]. The toxicity of SWNT has been reduced with the functionalization of poly-L-Lysine and used for cell adhesion [19, 20]. The metabolic activity of 3T3 cells was also dependent on SWNT preparation and concentration [21].

4 Carbon Nanotube Composites Biomaterials for Bone Tissue Engineering

4.1 Carbon Nanotube–Polymer Nanocomposite

The electrical and mechanical properties of CNTs are important key properties, which can be used for bone tissue engineering. Electricity properties of CNT might be used to stimulate the cell towards osteogenic differentiation, and mechanical properties can be used to mimic the mechanical strength of the bone. Several polymers have been widely used in tissue engineering due to their multifunctional nature, such as biocompatibility, biodegradability, favorable mechanical properties, being good for cell adhesion, direct contact with body fluids in vivo, and also being useful for cell adhesion, proliferation, and differentiation [22]. Poly(lactic acid), poly(glycolic acid), poly(ε-caprolactone), chitosan, and collagen have emerged as a class of biomaterials of growing interest for application in surgery, drug delivery, and bone tissue engineering.

4.2 Carbon Nanotube–Collagen Nanocomposite

Collagen is the promising biomaterial in bone tissue engineering in the recent years; the addition of collagen in MWNT could improve the surface properties for the cell growth and other osteogenic differentiation and increases the DNA content on the MWNT-coated sponge after 1 week, which is higher than on an uncoated collagen sponge. There was no significant difference between the estimated ALP activity normalized by DNA quantity on the MWNT-coated sponge and that on the uncoated collagen [24]. The fibril structure of collagen and nanotube structure of carbon has been shown in the Fig. 20.5.

In another study, it was proven that MWNT could be used for bone tissue engineering. Significant bone formation, earlier differentiation, alkaline phosphatase, and osteopontin contents have been observed in MWNT-coated collagen sponge scaffolds with rat primary osteoblast cell line, compared to uncoated sponges. Significantly more bone formation in vivo was observed around the MWNT-coated sponges than around the uncoated sponges [23, 24]. In another research, collagen–CNT composite materials were checked for bone tissue engineering [25]. The scanning electron microscopy (SEM) images of cells on collagen sponge and MWNT-coated sponge have been shown in Fig. 20.6.

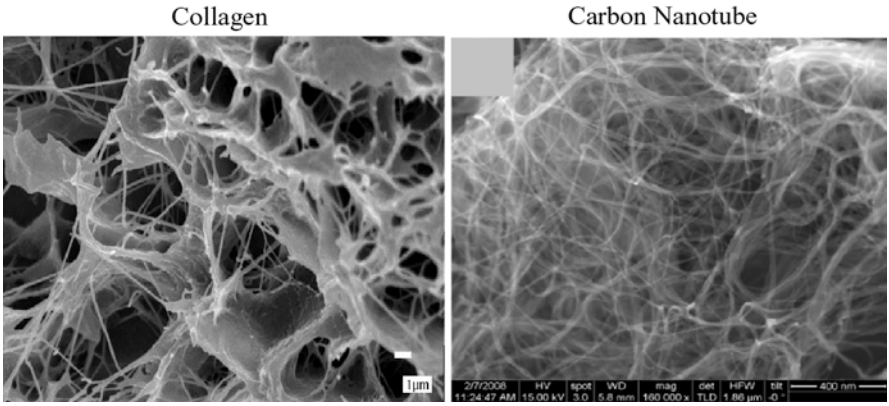

Fig. 20.5 Scanning electron microscope images of Collagen vs. CNT

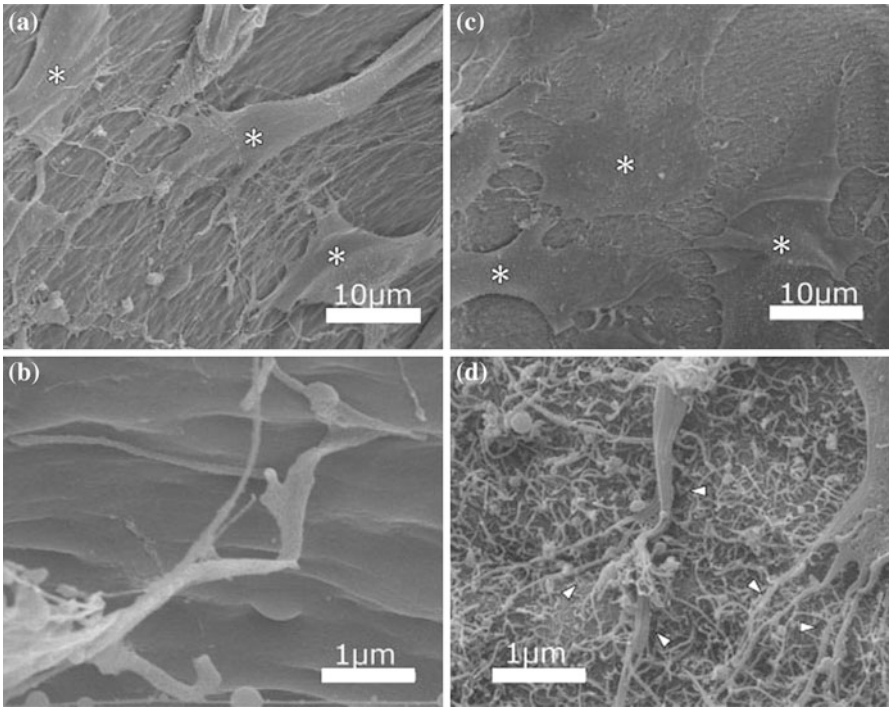

Fig. 20.6 SEM observation after 1-week incubation: cells (*asterisk*) grown on the (**a**) collagen sponge and (**c**) MWNT-coated sponge. SEM images at higher magnification: (**b**) collagen sponge, and (**d**) cytoplasmic elongations (*arrowhead*) intertwined with MWCNTs on the surface of the MWCNT-coated sponge [24]

4.3 Carbon Nanotube–Polylactic Acid Nanocomposite

Polyhydroxy acids are widely used biomaterials in therapeutic devices. Poly(lactic-co-glycolic acid) MWNT composite materials have been prepared using electrospinning and colloidal approach. As proven, incorporation of MWNTs in PLGA scaffolds was prepared to significantly promote fibroblast attachment, spreading, and proliferation when compared with PLGA fibrous mats and macroporous PLGA films without MWNTs [26]. In another study, different kinds of method (solvent casting technique) have been used to prepare the biodegradable PLGA/MWNT. The presence of MWNTs increased the mechanical properties of the nanocomposite. A seven-week period in vitro degradation test showed the addition of c-MWNTs accelerated the hydrolytic degradation of PLGA. Compared with control groups, MSCs cultured onto PLGA/c-MWNT nanocomposite exhibited better adhesion and viability and also displayed significantly higher production levels of ALP over 21 days of culture [27]. Electrically conductive nanofibers of polylactic acid with MWNT have been prepared using electrospinning methods. They found that cellular elongation and proliferation were mainly dependent on the electrical stimulation whereas the topographical features played a minor role [28].

The mechanical strength has been increased by the introduction of the CNT in the poly(propylene fumarate) matrix [29]. In addition, good cell viability, osteoconductivity, and marrow stromal cells demonstrated equally good cell attachment and proliferation on all scaffolds made up of different materials at each porosity [30, 31].

4.4 Carbon Nanotube–Chitosan Nanocomposite

Chitosan is a biopolymer and has considerably been employed as a scaffold in orthopedic and other biomedical applications due to its biocompatibility, biodegradability, pore formation behavior, suitability for cell ingrowth, and intrinsic antibacterial nature [1, 32, 33]. However, chitosan-based composite biomaterials have optimum mechanical strength and low interconnected porosity for cell attachment, which needs to be improved further. The addition of CNT in the chitosan matrix can solve the mechanical issues. For this, several reports have been published in the recent years; pristine SWNT, acid-functionalized SWNT, and glucosamine-functionalized SWNT (0.001–1.0 % wt/vol) were checked in vitro for bone tissue engineering, increasing concentrations of SWNT and resulting in a decrease of cell viability, which was dependent on SWNT preparation.

Venkatesan et al. [1] explained about chitosan–carbon nanotube composite scaffold preparation, mechanical strength, in vitro biological activity, and chemical interaction between chitosan and carbon nanotube [1] (Fig. 20.7).

Abarrategi et al. performed experiment with MWNT–chitosan composites for bone tissue engineering and interestingly found that implantation of MWNT–chitosan scaffolds adsorbed with rhBMP-2 in muscle tissue and ectopic formation of bone tissue and in vivo [34].

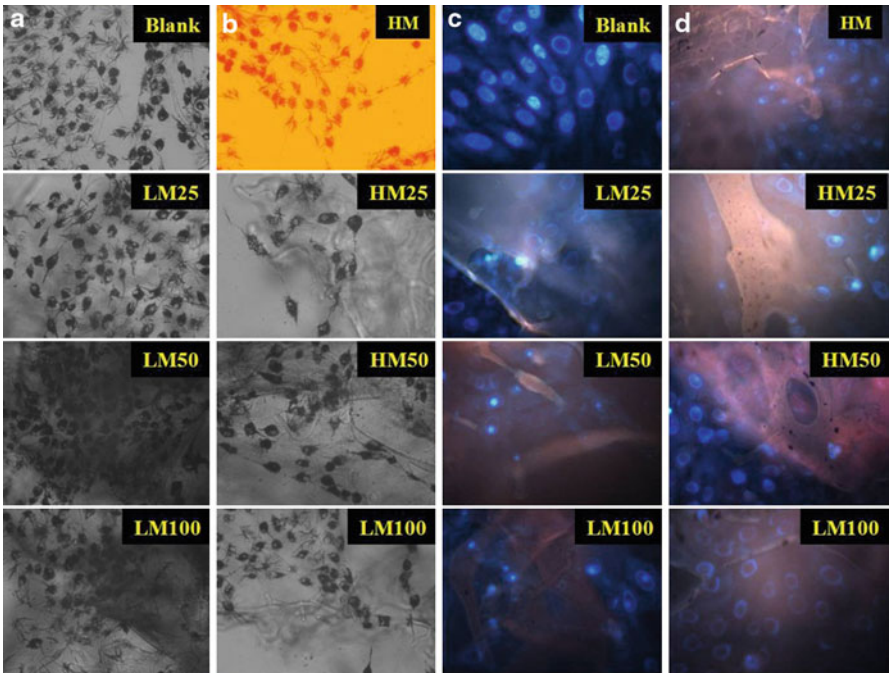

Fig. 20.7 Optical microscopy images of chitosan and their composite scaffolds (magnification = 20×) after the addition of MTT solution. (**a** and **b**) Blank, Low and high molecular weight chitosan scaffolds and their composite scaffolds, (**c** and **d**) Fluorescence microscopy images of chitosan and chitosan/f-MWCNT composite scaffolds (magnification = 40×) after Hoechst stain

4.5 Carbon Nanotube–Polycaprolactone Nanocomposites

Polycaprolactone (PCL) is a degradable polymer; it can degrade by hydrolysis of ester linkage in the physiological conditions. Thus, it has gained great importance in the preparation of artificial implantable biomaterials. Coaxial electrospun PCL, MWNT, and a hydrogel consisting of polyvinyl alcohol and polyacrylic acid have been prepared for skeletal muscle tissue replacement. Incorporation of MWCNT in the polymer matrix increased the conductivity and biocompatible was observed. MWCNT-containing scaffolds had higher strength than the rat and pig skeletal muscle. Although the mechanical properties were higher than the muscle, the PCL-containing MWCNT scaffold shows promise as a potential bioartificial nanoactuator for the skeletal muscle [35].

Micro fabricated CNT–polycaprolactone composites, by changing the ratio of CNT to polydopamine, the elastic modules of the nanocomposite, can vary between 10 and 75 MPa. In addition, PCL–CNT nanocomposite was able to sustain osteoblast proliferation and modulate cell morphology [36]. Pan et al. prepared the MWNTs/PCL composite scaffolds via solution evaporation technique.

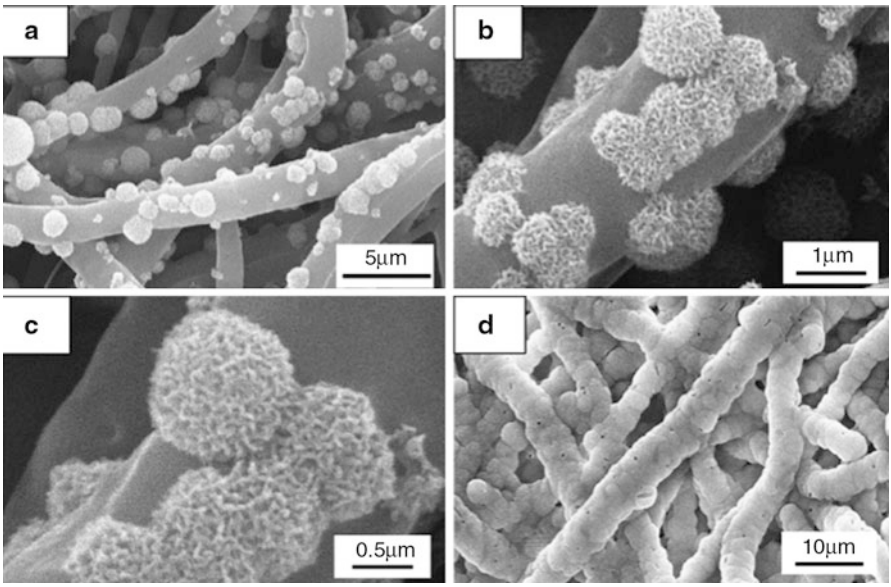

Fig. 20.8 SEM images of the PLGA/MWNTs scaffolds after immersion in 1.5 × SBF for (**a**), (**b**), (**c**) 7 d and (**d**) 14 d

The scaffolds with low concentration (0.5 wt%) of MWNTs can enhance the proliferation and differentiation of the BMSCs more than that with higher concentration of MWNTs. It is concluded that MWNTs/PCL composite scaffolds have the potential for bone tissue engineering, and the relatively low concentration of MWNTs (0.5 wt%) is preferred [37].

4.6 Carbon Nanotube–Hydroxyapatite Nanocomposite

Hydroxyapatite (HAp) is one of the widely checked biomaterials for bone tissue engineering in the last two decades [38]. HAp is a bioceramics material used as bone implants because of its chemical composition that is similar to the inorganic portion of the bone and teeth [39, 40]. HAp has been used in clinical bone graft procedures for more than 25 years. But its poor tensile strength and fracture toughness compared with the bone make it unsuitable for major load-bearing devices. CNTs with their high aspect ratio and excellent mechanical properties have the potential to strengthen and toughen HAp without offsetting its bioactivity [41].

Composite scaffolds composed of PLGA with MWNTs were prepared by electrospinning, and scaffolds were immersed in a simulated body fluid (1.5 × SBF) at 37 °C for 7, 14, and 21 days for biomimetic mineralization. After mineralization, apatite crystals were deposited on the PLGA/MWNTs composite scaffolds [42] (Fig. 20.8).

SDS has been used to carry out the biomimetic mineralization on MWNT with Ca/P solution [43]. BMP-2 has been used with the PLLA, CNT, and HAp scaffolds. Three-dimensional porous PLLA scaffolds have been mixed with SWNT, HAp, and BMP2; the role of the different biomimetic components added to the PLLA matrix was deciphered, with BMP2-added scaffolds showing the highest biomimetic activity on cells differentiating to mature osteoblasts [44].

Electrospinning a suspension consisting of PLLA, MWNT, and HAp membrane has been reported, enhanced the adhesion and proliferation of periodontal ligament cells (PDLCs) by 30 %, and inhibited the adhesion and proliferation of gingival epithelial cells by 30 %, compared with the control group [45]. Self-assembled nHAp/MWNT and collagen/MWNT composite were prepared. Spindle-shaped units that are detached from the MWNT template are able to maintain the ordered parallel structure of the nHAp polycrystalline fibril [46]. The HAp/MWNT composites were prepared by solution blending. The fracture toughness and flexural strength were improved by 50 % and 28 % separately when the volume percentage of MWNTs reached 7 % [47]. Some of the researchers said that CNTs with micro HAp containing composite materials are not recommended as a bone restorative material [48].

4.7 Carbon Nanotube–Bioglass Nanocomposite

Bioglass is composed of SiO_2, Na_2O, CaO, and P_2O_5 in specific proportions; it is well proven that high amount of calcium and phosphorous can be used for apatite formation. Important advanced material can be produced by the addition of CNT in Bioglass for bone tissue engineering. Highly porous 45S5 Bioglass-based foam scaffolds were coated with MWNT by electrophoretic deposition technique. Increased electrical conductivity was reported by the addition of MWNT coasting [49] (Fig. 20.9).

Poly(3-hydroxybutyrate) composites with bioactive glass particles and MWNTs have been reported. The presence of MWNTs (2–7 wt%) increased the surface roughness, and small amount of MWNT in the composite materials enhanced MG-63 osteoblast-like cell attachment and proliferation compared to composites with higher concentration of MWNTs [50]. 45S5 Bioglass-ceramic scaffolds were fabricated by the foam replication method and coated with CNT using EPD. In vitro cell culture using MSCs was carried out on both scaffold systems (with and without CNT coating) over a 4-week period. No cytotoxic effects of the CNT were observed under the conditions of the present experiments. Although a lower initial cell viability on the CNT-coated scaffolds were observed, no significant differences were found after 4 weeks of culture compared with the uncoated scaffolds. This work therefore shows that there is in principle no significant improvement of cellular responses by creating a CNT coating on this type of highly bioactive scaffolds. However, the electrical conductivity introduced by the coating might have the potential to increase cell viability and differentiation when cell culture is carried out under the effect of electrical stimulation [51].

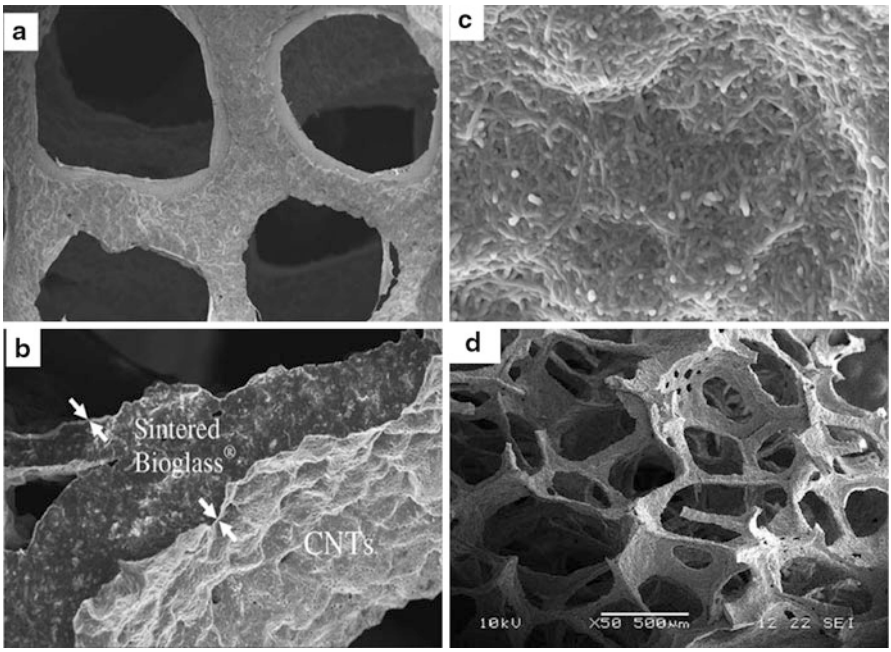

Fig. 20.9 SEM images showing the typical microstructure of a CNT coated scaffold, obtained by EPD (2.8 V, 10 min) at (**a**) low, (**b**) medium and (**c**) high magnifications. The CNT coating is indicated by the *arrows* in (**b**), (**d**) SEM micrograph showing the 3D microstructure of the highly porous glass–ceramic scaffold developed from Bioglass Ò powder by the foam replica technique [49]

4.8 Carbon Nanotube Coating on the Polymeric Surface

Surface chemistries TiO_2 nanotubes with carbon-coated TiO_2 nanotubes were compared for cell behaviour. The roles played by the material surface chemistry of the nanotubes did not have effects on the adhesion, growth, or morphology, but had a major influence on the ALP activity of osteoblast cells, with the original TiO_2 chemistry having higher ALP levels. Different chemistries caused different levels of osteogenic differentiation in MSCs; however, it was the carbon-coated TiO_2 nanotubes that had the greater advantage, with higher levels of osteo differentiation [52].

Conductive and nontoxic composites of CNF with agarose have been reported and demonstrated that these CNFs can be used for cell attachment and response both in vitro and in vivo [53]. Bhattacharya et al. reported the effects of layer by layered CNT composite on osteoblasts were compared against the effects by commercially available pure titanium. Cell proliferation on the CNT composite and Titanium were similar. When implanted in critical-sized rat calvarial defect, the CNT composite permitted bone formation and bone repair without signs of rejection or inflammation [54].

5 Challenges and Future Directions

Until now, CNT is a promising biomaterial and is used for different fields such as tissue engineering, drug delivery, and biosensors:

1. The toxicity and biocompatibility are important parameters for biomedical application. To find out the exact toxicity of CNT is a challenge for the current researchers; several kinds of research tools and experiments are available, but the toxicity of CNT is varied with production process, availability of toxic metals, size, functionalization, etc.
2. Surrounding tissues will come into surface contact with CNT composites, and compatibility between CNT and host cells must be addressed.
3. Amount of carbon nanotube in the polymeric matrix can also play a major role in osteogenic differentiation.

6 Conclusions

As a conclusion, with the initial stage of CNT in biomedical application, we are not concluding anything in this point. It will take several years, whether CNT can be used as implant material or not. However, it is difficult to use CNT alone in bone-related implant, due to formation of abacas sheet and aggregation. This will be avoided by using functionalization of CNT. Functionalization of CNT is an open way to use CNT as a potential material for further research. Thus, CNT–polymer with ceramics composites will be promising materials for the repair of bone defects.

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