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# Carbon nanotubes reinforced with natural/synthetic polymers to mimic the extracellular matrices of bone – a review

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| **Corresponding Author:** | Sivaranjani Arumugam, Ph.D<br>Nagoya University<br>Nagoya, Aichi-Ken JAPAN |
| **First Author:** | Sivaranjani Arumugam, Ph.D |
| **Order of Authors:** | Sivaranjani Arumugam, Ph.D |
| | Yang Ju, Ph.D |
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| **Suggested Reviewers:** | Kaliyappan T, Ph.D<br>Professor, Pondicherry Engineering College<br>tkaliyappan@pec.edu |
| | Stalin T, Ph.D<br>Professor, Alagappa University Faculty of Science<br>drstalin76@gmail.com |
| **Opposed Reviewers:** | |
| **Response to Reviewers:** | |

# Carbon nanotubes reinforced with natural/synthetic polymers to mimic the extracellular matrices of bone – a review

**Sivaranjani Arumugam*, Yang Ju**

Department of Micro-Nano Mechanical Science & Engineering, Graduate School of Engineering, Nagoya University, Nagoya 464-8603, Japan

## Abstract

The continuous failure of conventional materials applied for the orthopedic implant was due to the deficiency or poor integrations of implant materials to the juxtaposed bone and stress-strain imbalances between the interfaces of tissues and implant materials. Therefore, the fabrication of a suitable bioactive scaffold for bone tissue engineering is considered a vital requisite to mimic the extracellular bone matrix. Numerous researches were reported to fabricate a suitable bioactive scaffold to improve cell adhesion, proliferation, and differentiation so far. However, for the past two decades, the research on carbon nanotubes (CNTs)-reinforced composites employed for the biomedical field is increasing day-by-day by its outstanding properties. Moreover, it is essential to choose a biocompatible polymer with greater affinity to act as an extracellular matrix as well as to attract CNTs and to facilitating the homogeneous distribution of CNTs in aqueous and organic solvents. The development of CNTs-based composites in bone tissue engineering is presented in this review based on the last ten years of research. The detailed information about the structural-functions and defects of bone, and the importance of CNTs-functionalized natural and synthetic polymers, and their potential activity in bone regenerations and bone replacements have been reviewed.

## Keywords

Carbon nanotubes; natural polymers; synthetic polymers; composite materials; bone tissue engineering.

Corresponding author email: sivaranjaniarumugam88@gmail.com (Sivaranjani Arumugam),

## 1. Introduction

Nanotechnology is considered as an emerging field of science that utilizes the outstanding physical and chemical properties of nano-sized materials which are not available in bulk solids (Fig. 1), hence, it received tremendous interest among worldwide research communities [1]. The combination of nanotechnology and tissue engineering (Fig. 2) provides a wide platform for nanostructured materials in biomedical applications [2]. Particularly, research in bone tissue engineering has generated essential demands and interests in the biomedical field due to the wide variety of bone disorders in day-to-day life. Specifically, every year almost 20 million people are affected by various accidents and diseases worldwide [3]. Also, in recent days bone disorders such as fracture, back pain, infection, osteoporosis, tumors, rheumatic diseases, and scoliosis are the major concern due to the increasing growth of population, scarceness of regular physical exercise, and obesity [4]. Therefore, the fabrication of appropriate bioactive material is an important requisite for bone tissue engineering to mimic natural bone matrices. More specifically, the development of nanostructured materials in bone tissue engineering applications is one of the recent prominent research interests. Compare to bulk solids nanostructured materials have excellent physical and chemical properties due to the nanoscale proportions.

Since the discovery in 1991 carbon nanotubes (CNTs) received major concern among other nanoscale materials [5]. CNTs are allotropes of carbon, the carbon atoms in CNTs covalently bonded with one another through $sp^2$ hybridizations like graphene (Fig. 3). Each carbon atom attached by a C=C bond with another carbon atom and every carbon has three σ-bond and one π-bond. As shown in Fig. 3a, the electronic configuration of the ground state has only two unpaired electrons in 2p orbital. In the excited state, one electron from 2s orbital

promoted to 2p orbital, and then one electron from 2s orbital and two electrons from 2p orbital mixed to form $sp^2$ hybridization. The energy levels of hybridized orbitals are greater than s-orbital and lesser than 2p orbital. These three electrons from $sp^2$ hybridized orbitals can form σ-bond with another carbon atom in CNTs and the remaining one electron from 2p orbital form π-bond with another carbon atom as shown in Fig. 3b. The $sp^2$ bonds in CNTs are stronger than the $sp^3$ bonds in a diamond. The $sp^2$ bonds provide strength to CNTs and the π bonds are the reason for the electrical properties of CNTs [6].

Based on the geometrical alignment of carbon bonds, CNTs are classified into armchair, zigzag and chiral nanotubes (Fig. 4) [7]. Moreover, the sheets of graphite rolled up into cylindrical carbon nanotubes. Based on the number of cylindrical tubes, CNTs are classified into single-walled (single cylinder), double-walled (two concentric nanotube cylinders) and multi-walled (multiple concentric nanotube cylinders) carbon nanotubes, as shown in Fig. 5 [8]. Usually, the diameters of the CNTs are in nanometers and the length in several micrometers. The theoretical surface area of single-walled carbon nanotubes (SWCNTs) is measured as 1315 $m^2/g$ which makes it an ideal candidate for nano-robotics and biomedical applications. The theoretical surface area of multi-walled carbon nanotubes (MWCNTs) is 100 $m^2/g$ and the inter-distance between each layer of MWCNTs is approximately 0.36 nm [8,9]. The unique structure and aspect ratio (length and diameter ratio) of CNTs influences their properties. CNTs showed better electrical conductivity (2.6 micro-ohm-centimeters) than copper (1.6 micro-ohm-centimeters) and the mobility of CNTs (100,000 $cm^2/Vs$) are higher than other semiconductor materials (InSb: 77,000 $cm^2/Vs$,) at optimum temperature [10]. CNTs have high tensile strength (63 GPa) and elastic modulus (1 TPa) and also thermally stable up to 2800 °C under vacuum [11, 12].

The outstanding electrical and thermal conductivity, elasticity, optical, biocompatibility, different aspect ratio, and shape of the tube structure make CNTs a suitable candidate to enhance cell growth and differentiation to establish particular tissue. However, CNTs have poor solubility/dispersibility in organic and aqueous solvents. As a remedial measure, organic/ biomolecules have covalently or non-covalently functionalized with CNTs to improve the solubility/dispersibility, biocompatibility, and biodegradability of CNTs [13, 3]. The functionalization of biological molecules onto CNTs can enormously increase the dispersibility/solubility of CNTs in both an aqueous and organic medium, which facilitates the development of novel products for the applications of biotechnology, biomedicine, and bioengineering discipline. Hence, CNTs are suggested as a smart material for biomedical applications over conventional materials.

Numerous inorganic biominerals, ceramics, bioactive glass, and biopolymers have already used for bone tissue engineering, but it was not suitable for load-bearing applications like natural bone, which is due to their inferior mechanical properties viz., brittle nature, low fracture toughness and fragile in tension [14]. Under this circumstance, CNTs reinforced composites have been introduced for the application of bone tissue engineering. The unique structural, mechanical, chemical, electrical, and thermal properties of CNTs make their composites versatile candidates in an appropriate field. Therefore, the importance of CNTs functionalized with natural/synthetic polymers composites and their developments in the field of bone tissue engineering are presented in the comprehensive review. Moreover, the basic structure and functions of bone, various bone defects, and the need for bone tissue engineering to treat, regenerate, or replace the diseased/ injured bone are also highlighted in this review.

## 2. Structure and functions of bone

Bone is a rigid organ that creates the skeletal structure of the body and the skeleton is organized by long (such as arm, leg, and back), flat (skull, jaw, ribs, and sternum), short (little bones in the hand), sesamoid (found in joints throughout the body) and irregular bones (hipbones and the vertebrae). Usually, bone serves as an internal framework to support the body and protects important organs, and also helps to move the body. The skeleton of the human body is composed of nearly 300 bones during birth, after which few bones were fused and the total count was decreased to 206 in adulthood. Two major types of bones in our body are thick (i) cortical bone and spongy like (ii) trabecular bone. Trabecular bone provides strong support to the weight-bearing bones and cortical bone on the outside forms the shaft of the long bone. Generally, bone is composed of 40% of organic and 60% of inorganic components. The organic component is composed of the primary matrix known as collagen which is the richest protein (about 90%) in the body that forms bone, cartilage, skin, and tendons. The major inorganic components of bone are calcium and phosphorus. Moreover, bone is a mineral reservoir of the body, which can store 99% of calcium and 85% phosphorus in the body. Bone also consists of small amounts of other inorganic components viz., magnesium, sodium, and bicarbonate. The increased or decreased level of calcium in the blood can stop the functions of muscles and nerves, but bone plays a vital part to maintain the calcium level of blood. The two major categories of bone cells are (i) osteoclasts and (ii) osteoblast (which consisting of osteoblasts, osteocytes, and lining cells) (Fig. 6). The first category viz., osteoclasts can resorb the bone, and the second category viz., osteoblasts form the bone, osteocytes support to maintain the bone and the lining cells are helps to cover the bone surface.

## 3. Bone defects and bone tissue engineering

Bone fractures are classified into (i) greenstick (partial fracture which occurs in one side of the long axis of bone), (ii) transverse (occurs straight across the bone), (iii) oblique (complete fracture occurs at a plane oblique to the long axis of the bone), (iv) spiral (twisted), (v) avulsed (pulling away of tendon or ligament from the main mass of the bone), (vi) segmental (two distinct fracture lines that completely isolates a segment of bone), (vii) comminuted (broken into several pieces), etc. (Fig. 7) [15]. As discussed in the previous section, the excellent structure and functions of bone cells and tissues can self-heal small-scale bone fractures or defects, but critical bone defects occurred by trauma, congenital, surgical resections, severe injuries, damage in soft-tissues, aging, and comorbidities (such as diabetes) cannot heal by themselves [4, 16]. The repair of the bone defect through tissue engineering is observed as the best approach because bone defects are treated by the patient's tissue during the time of regeneration [17]. At the beginning of the year 2001, research and development in tissue engineering were pursued by 3300 scientists with an annual expenditure of 600 million USD, moreover, every year 3.5 million bone grafts are performed worldwide [18]. The term bone tissue engineering is defined as a complete understanding of the structure and mechanics of bone and tissue formation as well as the knowledge in the successful development of bone regeneration. Bone tissue engineering is one of the emerging fields in regenerative medicines. Initially, bone defects due to surgeries, diseases, fractures, etc., were treated by (i) isografts, (ii) autografts, (iii) allografts, and (iv) xenografts (Fig. 8). Isografts are performed between identical twins, autografts are done by using the tissues of the same person, allografts are carried out from the donor of a similar species, and xenografts are achieved from the donor of a different species. But these natural grafts exhibited plenty of demerits which include lack of donor species, the possibility of disease transference, lack of availability, or reproducibility. In this situation, wide varieties of research have focused to

synthesize biocompatible/biodegradable polymers for bone tissue engineering and regenerative medicines [19].

Also, compared with conventional bone grafts, engineered bone tissues have been considered as an efficient alternate due to their continuous supply along with no disease transmission [17]. The major intention of bone tissue engineering is to induce bone regeneration through effective biomaterials. There are three major components have reported for effective tissue engineering and which includes (i) well-structured scaffolds and their vital substrates to induce tissue growth and development, (ii) source of cells to expedite respective tissue formation, and (iii) growth factors to direct the cell growth and differentiation within the scaffold. Many researchers have widely reported numerous scaffolds so far to promote (1) osteoconduction (i.e. deposition of inorganic bone minerals and collagen) and (2) osteoinduction (osteogenic differentiation). Also, biocompatibility and biodegradability are the two major concerns to design favorable scaffold materials. Biocompatible denoted as the capability of a scaffold to support cell growth and tissue regeneration without causing inflammation. Biodegradation allows the scaffolds to disappear in togetherness with tissue formation. Therefore, biodegradation can deny the need for a second surgery to remove implant materials. Particularly, the selection of good scaffold materials can enhance biocompatibility and biodegradability. Based on these criteria, three vital scaffold materials have been used by researchers so far in bone tissue engineering which include (i) ceramic materials, (ii) natural, and (iii) synthetic polymers [20]. The studies related to the combination of these vital materials are discussed in this review.

## 4. Similarities and differences of CNTs-scaffolds with natural extracellular matrix

In general, the natural extracellular matrix is composed of collagen, proteoglycans, adhesion proteins, and signaling molecules [21]. The extracellular environment is generated by a complex network of biochemical and biophysical signals to cells by a wide range of mechanisms and factors [22]. The characteristics of the synthetic extracellular matrix should be as much as similar to the natural extracellular matrix to replace damaged tissue or to activate tissue regeneration by providing ideal conditions. Nanomaterials are of interest in the area of bioengineering for the fabrication of scaffolds due to their similarities with the extracellular matrix, i.e., their physical and chemical properties, configurations, and dimensions influence the cellular interactions which lead to tissue regeneration. Among the other class of nanomaterials, CNT has potential biomedical application due to its unique properties like high surface area, low weight, high thermal and electrical conductivity, good chemical stability, and high mechanical strength [23, 24]. For that purpose, CNTs-based scaffolds are great alternatives for tissue engineering.

However, it has some differences with natural extracellular matrixes. For example, the challenges that researchers have to overcome are manipulating and integrating CNTs with biocompatible organic molecules because it is chemically inert and has poor dispersion in solvents [25]. An appropriate functionalization can induce the dispersion of bundled CNTs in an aqueous environment; as a result, it can reduce the hydrophobicity of CNTs [26]. For bone regeneration and bone tissue engineering, CNTs-based composites are considered biomimetic of collagen fiber at the cell hierarchical level [27]. The CNTs-based scaffolds have special features that are common to the natural extracellular matrix, i.e., the high degree of flexibility/elasticity of CNT is similar to the natural extracellular matrix [28, 29]. The high degree of flexibility is

directly corresponding to the cohesion of the solid [30]. The lower hardness of the matrix enhances the speed of the cells to move to tissue which facilitates the cell reconstitution [31].

The porosity of a similar diameter is a common factor between CNT and extracellular matrix [30]. Since the natural extracellular matrix is highly porous, the high degree of porosity in CNTs makes them an effective scaffold, which has served as a key factor in tissue integration [32]. Moreover, CNTs have a large surface area which is another important characteristic found in the natural extracellular matrix [33]. CNTs can be functionalized with different functional groups that facilitate the solubility of the nanotubes in aqueous and organic solvents. To obtain a more integrated network, desirable molecules are functionalized onto the tube surface, which can promote the interconnection between the numerous pores of the support [34]. Another important characteristic of CNT is that can form 3D architecture like natural extracellular matrix [35, 36]. The significant property of CNTs is that they can interact with proteins and DNA [34]. Therefore, these features of CNTs are analogous with the natural extracellular matrix that makes CNTs the most efficient candidate for tissue engineering applications.

**5. The advantage and the biological effect of CNTs in biomedical application**

The highly beneficial electrical, mechanical and chemical properties of CNTs, and the ability to functionalize with a wide variety of organic and inorganic molecules make CNTs an ideal candidate for several biomedical applications which include drug delivery, biosensing, and tissue engineering [37-39]. For example, CNTs are widely employed as nanocarriers for drug and gene delivery due to their favorable size and biocompatibility. Since CNT exhibits exceptional cell transfection capabilities, the use of CNTs as nanocarriers has shown great benefits [40]. Taking advantage of the photothermal properties of CNTs with NIR laser stimulation is considered to be an excellent way to treat cancer [41]. In biosensing applications,

the presence of CNTs on the surface of the electrode enhances the sensitivity of electrochemical detection by accelerating the faster electron transfer [42]. Correa-Duarte et al. reported that the ability of CNT to form 3D architecture is interesting for cell proliferation enhancement and tissue engineering [43]. However, the issue of toxicity of CNTs in the living biological system is a major concern so far. Researchers are constantly trying to answer the question related to CNTs toxicity. Usually, CNTs can be entered into an organism via three different ways such as inhalation, ingestion, and injection [40].

Inside the organism, it spreads throughout the body by reaching the bloodstream and then leaves the body by excreting urine or it can be accumulated into the secondary organs (if the immune system fails to eliminate it) [44-46]. The most careful way to use CNT is to prevent them from entering the organism freely. The metal impurities in CNTs can have the possibility to enhance toxicity which leads the cell death via mitochondrial destruction and oxidative stress [47]. However, this issue can be solved by functionalizing some organic molecules onto CNTs. Functionalization can significantly enhance the dispersibility and biocompatibility of CNTs [48]. However, the biocompatible nature of CNT is connected to some parameters viz., functionalization, dispersion, and length. For example, Ali-Boucetta et al. reported that covalently functionalized CNTs have a tendency to excrete via urine, but pristine or non-covalently functionalized CNTs are accumulated in the liver and spleen [44]. The functionalization of small hydrophilic organic molecules onto CNTs can significantly enhance the dispersibility and biocompatibility. Also, the functionalization method needs prior to purification/activation through oxidation. Since metal impurities are the source of cell damage and oxidative stress, purification is considered an important step. Moreover, the purification

process reduces the length of the CNTs length and improves further functionalization and dispersion [40].

## 6. Ceramic materials functionalized-CNTs composites

Ceramic materials viz., hydroxyapatite, calcium, and tricalcium phosphate were frequently employed for bone tissue engineering, which is corresponding to their osteoconductivity, biocompatibility, high crystallinity, and stiffness. More specifically, hydroxyapatite with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ belongs to the calcium phosphate family, which is analogous to inorganic minerals of natural bone. Hence, the biocompatibility of HAp has been used to enhance osteoblast adhesion and proliferation in the field of fracture treatment, spinal fusion, craniomaxillofacial reconstruction, total joint replacement, and revision surgery [49]. However, the poor mechanical properties, brittle and slow degradation nature of ceramic materials cannot meet the mechanical properties of natural bone. To overcome these demerits, CNTs have been used to facilitate the mechanical properties of inorganic bioactive minerals. Our previous studies reported CNTs-nanohybrids for bone tissue engineering through covalent functionalization of biocompatible polymers/dendrimers with CNTs followed by the stabilizations of nanostructured hydroxyapatite [13, 3, 50]. As we discussed in the previous section, critical bone defects cannot heal by themselves, but it can be supported by biomaterial supports [4]. The combination of synthetic polymers provides mechanical strength and processability, whereas biopolymers provide favorable circumstances for cell proliferation and bone growths [16]. Hence, the combinations of polymeric materials with CNTs and their biological functions in bone tissue engineering were discussed in this review.

## 7. Importance of polymer functionalized CNTs composites

Generally, ideal scaffold materials require outstanding mechanical properties, biocompatibility, well-established architecture to colonize and organize the cells, and to ensure the integration of scaffold with bone tissue [51]. Recently, the chemical functionalization of polymer molecules with CNTs has been used in various biomedical and industrial applications. Further, the functionalization of polymer molecules with CNTs can enhance the dispersion of CNTs in organic and aqueous medium as well as enables the separation of CNTs from carbonaceous and metal impurities. In this situation, the selection of the right polymer molecules for effective functionalization is a challenging factor to improve CNTs' solubility without affecting their remarkable properties as well as to enrich the biocompatible and biodegradable efficiency of the materials. Mostly, natural polymers viz., collagens, chitosan, gelatin, hyaluronic acid, silk, glycosaminoglycan, fibrin, elastin, and alginate possess exceptional chemical similarities with the extracellular matrix of various tissues as well as with outstanding structural, biocompatible, and biodegradable properties. These biopolymers can have an ability to degrade into $H_2O$ and $CO_2$ in the body, hence it is non-toxic to health even at high concentrations [52].

Also researchers have substantial interest in synthetic polymers viz., poly(lactic acid) (PLA), polyvinyl alcohol (PVA), poly(glycolic acid) (PGA), polycaprolactone (PCL), poly(ethylene glycol) (PEG) and poly(lactide-co-glycolide) (PLGA) due to their essential biodegradable/biocompatible property. The well-established structures of the polymers act as a template to control the structure of composite materials [53, 54]. However, they have some limitations like slow degradation during drug release. Although the research interest in bone tissue engineering has started nearly three decades ago, still it's showing tremendous improvements every year. This review provides a detailed survey of research and improvements on biocompatible polymers functionalized-CNTs nanocomposites in bone tissue engineering.

Moreover, for the past ten years, the studies published in science indexed journals by using biocompatible polymers functionalized-CNTs nanocomposites for bone tissue engineering was provided in this review.

## 8. Natural polymers-functionalized CNTs composites

### 8.1. *CNTs-collagen composites*

As discussed in the previous section, collagen is the most important component of the extracellular matrix of bone which provides structural support, an important role in bone formation, and also facilitates the bone healing process. During bone formation, the fibrous protein of collagen impregnated with calcium phosphate. These excellent parts of collagen fibers in bone formation and their matrix structure, reinforcing and composite-like nature are attracted by researchers [55]. Moreover, the combination of CNTs and collagen offers composite materials with enhanced elasticity, tensile strength, and strain than pure collagen matrix. Specifically, Hirata et al. developed a CNTs-coated 3D collagen scaffold and the rat primary osteoblasts (ROBs) differentiation was observed earlier than uncoated collagen sponge [56]. Besides, more quanta of bone formation were noted on the day of 28 and 56 around CNT-coated collagen sponge and new bone was also attached directly to CNTs.

A three-dimensional biocomposite scaffold was prepared by the combinations of mineral trioxide aggregate (MTA), type I collagen, and CNTs [57]. The in vitro osteoblastic response such as mineralization capabilities, viability, cell morphology, and migration of the scaffold was investigated using MC3T3-E1 cells. Collagen sheet compounded with CNTs served as a template to carry recombinant human bone morphogenetic protein-2 (rhBMP-2), also composite showed the earlier establishment of bone tissue in the mouse back muscle, and the efficiency is relatively higher when employing the collagen sheet alone. After this inference, many

researchers have reported the in vitro as well as in vivo bone regeneration efficiency of CNTs in bone tissue engineering applications. For example, Tanaka et al. reported 3D CNTs-blocks containing rhBMP-2, which has implanted in muscles of mouse and produced ectopic bone, and comparable bone marrow densities were observed when PET-collagen reinforcements were employed with rhBMP-2 [58].

Moreover, the addition of MC3T3-E1 preosteoblasts with MWCNT blocks exhibited more compression strength on cortical bone. Hence, MWCNTs-blocks served as excellent fillers for bone defects and good scaffold material for the regeneration of bone. The report demonstrates that CNTs act as osteoconductive scaffolds to allow osteoblasts to proliferate onto their surface and act as a functional scaffold to promote bone tissue regeneration through interacting with a living body, and collagen obtainable as a carrier for bone morphogenetic protein (BMP). Similarly, a silicon-based composite was fabricated by coating collagen onto a silicon substrate and followed the attachment of CNTs by cross-linking collagen with a carboxylic group of acid-functionalized-CNTs [59]. Here, collagen has chosen to increase cell attachment, and CNTs to enhance the electrical conductivity of the composite film. The osteogenic differentiation of mesenchymal stem cells (MSCs) has enhanced while applying electric current in the presence of electrically conductive CNTs in a silicon-collagen composite.

A porous scaffold was developed by integrating 0.5 % CNTs with collagen and hydroxyapatite composites [60]. The obtained scaffold was found as the best one to promote the cell proliferation and spreading of bone marrow MSCs, mRNA, and protein expressions of bone sialoprotein and osteocalcin, respectively. Moreover, the in vivo experiment was employed to treat the defect of rat calvarial (8mm) and the result indicates the development of new bone using 3D reconstructions of micro-computed tomography (CT), hematoxylin/eosin (HE), and

Masson staining. Composites of nano-hydroxyapatite, collagen I, and carbon nanotubes (nHA/ColI/MWCNT) scaffold were prepared via blending and freeze-drying method, later, it was incorporated with bone morphogenetic protein (BMP-9) [61]. This scaffold material showed a high degree of porosity (between 88 and 153 $\mu$m) which facilitates the scaffold to interact with bone tissue. Moreover, the compressive strength and compressive modulus of the scaffolds are increased by increasing the amount of CNTs. The *in vitro* result suggests that scaffold derived from 1% loading of CNTs improved cell differentiation of bone marrow mesenchymal stem cells (BMSCs) into osteoblasts and the *in vivo* result of the scaffold was also induced more bone formation.

8.2. *CNTs-chitosan composites*

Chitosan is the best example of a natural polymer that is highly biocompatible and biodegradable hence it is considered a promising candidate for biomedical applications. The previous studies on chitosan proved that the biocompatible and biodegradable nature of chitosan facilitates the proliferation of mesenchymal and osteoblasts [62, 63]. The functionalization of chitosan with CNTs can be achieved by covalent or non-covalent functionalization. In 2010, Zhao et al. developed biocompatible CNTs/chitosan composites by using phosphotungstic acid to modify CNTs. Since the modification was mild and effective, the intrinsic structure of CNTs was retained. The cytotoxicity of the composite was examined on Mouse fibroblasts (L-929) cells and the result indicates that the obtained composite was biocompatible for bone tissue engineering application [64]. Liao et al. demonstrated the synthesis of MWCNTs combined with polyvinyl alcohol and chitosan Nanofibrous scaffolds by electrospinning method and subsequently cross-linked with glutaraldehyde vapor [65]. The scaffold with high porosity and smaller diameter (160 nm) improved the in vitro cell proliferation of mouse fibroblasts (L929).

Venkatesan et al. prepared MWCNT grafted with chitosan and hydroxyapatite composites through the freeze-drying method i.e., -COOH group of MWCNT-COOH was bonded with $–NH_2$ group of chitosan and followed the coordination interaction of hydroxyapatite with MWCNT-chitosan matrix (Fig. 9) [49]. The hydrophilic nature of chitosan and hydroxyapatite acts as an extracellular matrix which improved the homogeneous distribution of MWCNT. The efficiency of the material was examined against human osteoblast-like MG-63 cells and the results showed enhancement in cell proliferation, alkaline phosphatase activity, protein concentration, and mineralization [49, 66]. Three-dimensional porous chitosan composite has been reported by Zhang et al. by the integration of SWCNT (by magnetically synthesized), nanocrystalline hydroxyapatite (treated hydrothermally), and chitosan [67]. The composite obtained from 20 wt% of hydroxyapatite exhibits enhanced tensile strength and compressive moduli than that of pure chitosan (control). Also, the study demonstrates that nanocrystalline hydroxyapatite and SWCNT obtained after magnetic synthesize offers a favorable cellular environment to improve the cell proliferation and adhesion of Human fetal osteoblasts (CRL-11372).

Chen et al. prepared chitosan/CNTs/HAp composites with high compressive strength (33.2 to 105.5 MPa) and elastic modulus (509.9 to 1089.1 MPa) [68]. The in vitro cell line studies are carried out on preosteoblast MC3T3-E1 cells and the results considerably improved osteoblast proliferation onto the surface of CS-MWNTs/HA, hence the report claimed that the obtained composites are favorable for bone tissue engineering. Fonseca-García et al. employed an ice segregation-induced self-assembly process to develop a biomimetic scaffold by the integration of MWCNTs, chitosan, and nanostructured hydroxyapatite (CTS/MWCNT/nHAp). The efficiency of the scaffold was investigated by using MSCs derived from the periosteum.

Based on the structural arrangement, surface properties and cell viability (99 %) of the CTS/MWCNT/nHAp scaffolds can be applied as a favorable material for bone tissue engineering application [69]. Mineral-substituted hydroxyapatite combined with carboxymethyl chitosan and CNTs to improve the biological properties of hydroxyapatite. The obtained composite coated onto Ti-6Al-4V alloy to improve the biological and mechanical properties of orthopedic material [70]. Chitosan-grafted CNTs (MWCNTs and SWCNTs) and chitosan-grafted hydroxyapatite complex are prepared by cross-linking through glycerol phosphate [71]. Mechanical properties of the obtained material were improved by the addition of hydrogel nanofillers with faster sol/gel transition. The cross-linked CNTs and hydroxyapatite in thermosensitive gels offered injectable composites with potential properties such as good bioactivity, prolonged drug release, enhanced mechanical properties, and reduced gelation time.

In another study, polyethyleneimine (PEI)-grafted CNTs and chitosan substrate was employed as a nanocarrier for gene delivery system and to examine the transfection efficiency of BMSCs (Fig. 10). The results indicate >82% of cellular uptake and enhanced delivery of pDNA into BMSCs. This report emphasized the significant role of chitosan to improve the transfection efficiency of BMSCs [72]. To improve the biocompatibility of bone regeneration implant, nanostructured CNTs-chitosan hybrid solution was prepared and coated onto a titanium plate by electrophoretic deposition process [73]. Osteoblastic cell responses of CNTs-chitosan hybrid were examined on osteoblastic MC3T3-E1 cell line and the results exhibit stimulations on cell adhesion. Further, the nanotopographical feature of the hybrid coating improved the large quantity of protein adsorption and sustainably released them. Cancian et al. reported thermosensitive chitosan hydrogels consisting of N-octyl-O-sulfate chitosan stabilized CNTs. Biocompatibility of the scaffold materials was examined by calcification studies i.e. deposition

of calcium salts onto the surface and interface structure of scaffold was evaluated and which can play a crucial role in bone fracture treatment [74].

Further, CNTs-chitosan composites scaffolds were prepared from the composites of MWCNTs, chitosan, and β-Glycerophosphate by Gholizadeh et al [75]. The water uptake characteristics, porosity, and electrical conductivity of the composites were increased by increasing f-MWCNT up to 1 w/v%. Further, the compressive and tensile strength of the scaffold was increased by adding the CNTs up to 0.5%. The cell line results indicate that the composite obtained between 0.1 - 0.5 w/v% was appropriate for bone tissue engineering application and which because the functionalized MWCNTs makes the nanotubes more biocompatible and soluble in solvents and also the polymeric structure of the composites has not exhibited significant cytotoxicity, unlike other structured composites. In another report, the chitosan-MWCNTs scaffold was applied for bone repair and regeneration studies. Water-soluble tetrazolium salt assay and double staining methods were performed to examine the cytotoxic, apoptotic, and necrotic effects of MWCNTs-chitosan scaffold on chondrocyte cell lines. The results suggest that cell viability of chondrocyte cell lines was not significantly affected by the scaffold material, hence, CNTs-chitosan composites were not exhibited toxic effects on cells, and also double staining method can identify the necrotic and apoptotic effects of chitosan-MWCNT nanocomposite on respective cell lines. The stress-strain curve indicates the exceptional mechanical properties of the scaffold and the addition of CNTs enhanced the elongation strength [76]. Chitosan nanoparticle revealed the proliferation of canine MSCs without exhibiting intense toxicity [77].

Highly porous collagen functionalized with CNTs, chitosan, and HAp (Col/f-MWCNT/CS/HA) composites scaffold was prepared by the freeze-drying method [78]. The Ca/P

ratio of the reported Col/f-MWCNT/CS/HA composite was 1.52 which is analogous to the Ca/P ratio of natural bone (1.6). The groups viz., -OH, -$NH_2$, and –C=O presented in collagen have formed a hydrogen bond with -$NH_2$ and –OH groups of chitosan and –C=O and –OH groups of f-MWCNT (Fig. 11). The biomineralization of the composites was employed by the biomimetic method using simulated body fluid. The elastic modulus and compressive stress of the composite were measured before (523 and 37 kPa) and after mineralization (523 and 1112 and 57 kPa) which helps to uphold the structural unity throughout the bone regeneration. The swelling (513.9 - 481.05 %), porosity (98 - 95.7 %), and contact angle (87.8 - 76.7°) of Col/f-MWCNT/CS/HA were investigated before and after biomineralization and compared with collagen, chitosan, and Col/f-MWCNT. Sharmeen et al. reported the research on polyethylene glycol functionalized with carbon nanotubes/gelatin-chitosan composites with greater thermal, mechanical, and water swelling properties [79]. The functionalization of CNTs with porous gelatin/chitosan matrix increased the stiffness, dampness, and microfibrillar within pore walls can be used in bone tissue engineering.

Recently, biocompatible CNTs-nanohybrids were synthesized by our research group through covalent functionalization of CNTs with biocompatible polymers viz., chitosan, polyacrylamide (PAM), PLA, and PEG, and followed by the immobilizations of nanostructured-hydroxyapatite. The obtained results indicate that the nanohybrids were not inducing any significant toxicity on MG-63 cells. Besides, the biocompatible nature of chitosan, nanostructured hydroxyapatite and coordination bond between chitosan and hydroxyapatite played a crucial role in the cell membrane and which helps to attain effective cell attachment and proliferation of MG-63 cells. Therefore, the reported nanohybrids are expected as potential candidates for bone tissue engineering [13].

8.3. *CNTs-gelatin composites*

Gelatin is a low-cost denatured collagen peptide with a molecular weight of 100 kDa, and also it is a biocompatible, biodegradable, and non-immunogenic high molecular weight polypeptide derived from controlled partial hydrolysis of collagen. The gel or film-forming properties of gelatin are mainly applied in pharmaceutical applications. Even though it is widely used for wound healing and drug delivery application, weak mechanical and fast degradable properties make them a poor candidate for bone graft materials [80]. The extensive hydrophilic characteristic of gelatin is the major factor for its low barrier and mechanical properties. To overcome these demerits, a wide variety of methods viz., crosslinking, composites with biopolymers, hydroxyapatite, bioactive glasses, or reinforcing with CNTs have reported [81, 82]. To mimic the properties of collagen fibrils, acid group functionalized CNTs were covalently functionalized with the terminal amine groups of gelatin molecule through an amide linkage, and then HAp crystals are further accumulated onto gelatin-grafted *f*-CNTs (Fig. 12) [83]. The obtained multilayered core-shell structure significantly improved the tensile strength, elastic modulus, elongation, and biocompatible properties compared to pure gelatin.

Three-dimensional gelatin/MWNTs/HAp nanofibrous scaffold has been prepared by the electrospinning technique [84]. Here, gelatin molecules attached with MWNTs/HAp via hydrogen bonding, and the incorporation of MWNTs/HAp molecules enhanced the porosity and mechanical strength of the scaffold material. Also, HAp nanoparticles exhibit the chelating effects to promote osteogenesis and mineralization of human fetal osteoblastic cells and MWNTs showed a synergetic effect in apatite formation. Acid-functionalized MWCNTs used to strengthen freeze-dried gelatin/chitosan scaffold, and which showed porous structure with a pore size of 80-300 μm, the compressive strength of 411 kPa, and modulus of 18.7 MPa [85]. Moreover, the COOH-

functionalized MWCNTs reinforced gelatin/chitosan composites showed improved *in vitro* bone-like apatite formation in simulated body fluid.

8.4. *CNTs-hyaluronic acid composites*

Hyaluronic acid or sodium hyaluronate (HY) is a high molecular-weight polysaccharide and composed of the repeating disaccharide units of *D*-Glucuronic acid and *N*-acetyl-D-glucosamine [86]. It is widely found in the extracellular matrix of mammalian tissues [87]. This HY can improve the proliferation, differentiation, and migration of osteoprogenitor cells by binding with CD44 cell surface receptors [88-90]. Even though, Sodium hyaluronate (HY) speeds up the bone repair process, the poor stability of HY in the aqueous environment has slowed down its utility in bone tissue engineering. Moreover, to enhance stability, CNTs have been reinforced with HY. Mendes et al. reported the synthesis of HY–CNTs composites with improved dynamic mechanical properties than pure HY [86]. The trabecular bone formation was evaluated after seven days of surgery of Wistar rats and the results showed an increased % of bone formation and collagen type I expression.

It was demonstrated that CNTs combined with sodium hyaluronate (HY-CNTs) accelerates bone repair in the tooth socket of rats [91]. The result obtained after 7 days indicates that the direct uptake of HY-CNTs with low concentration has not exposed any significant changes in the cardiovascular function of the rats. In another report, titanium surfaces coated with sodium hyaluronate (HY)-functionalized CNTs biocomposites exhibits the deposition of mineralized bone nodules and increased mRNA expression of type I and III collagen, bone morphogenetic proteins 2 and 4, and osteocalcin [92, 93]. CNTs associated with hyaluronate (HY) biocomposites were employed for the bone restoration of rat tibiae. The bone defect has made by a 1.6 mm diameter drill and then the histological and morphometric analyses

were performed on the 7$^{th}$ and 14$^{th}$ days. The obtained histomorphometric exhibited improved percentage with highly ordered and denser bone trabeculae. Also, the Tibiae sample of an animal performed with HY-CNTs showed greater expression of vascular endothelial growth factor (VEGF), collagen I (Col I), bone morphogenetic protein-2 (BMP-2), and osteocalcin (OCN) [94].

## 9. Synthetic polymers-functionalized CNTs composites

### 9.1. *CNTs-poly(lactic acid) composites*

Poly(lactic acid) is the most important biodegradable polymer due to its outstanding biological and mechanical properties. However, in tissue engineering, it is difficult to mimic the extracellular matrix of bone by using a single component. Under this situation, poly(lactic acid) reinforced with CNTs composites have been reported to improve the mechanical, electrical, thermal, and chemical properties along with cell proliferation and osteoblastic differentiation. For example, carbon nanotubes-calcium phosphate (CP) hybrid nano-powders were prepared by adding 0.1 % of ionically modified CNTs (mCNTs) and 0.25 % of CP, the mCNTs-CP hybrid was mixed with 50 % of PLA and thus produced mCNTs-CP-PLA nanocomposites [95]. The obtained nanocomposites showed excellent biological responses viz., proliferation and differentiation, and protein expressions. In some other reports, 3D PLA/MWCNTs nanocomposites scaffolds were prepared by a pressure-activated microsyringe microfabrication method that allows the direct fabrication of preferred microstructures [96]. The intrinsic mechanical property of the scaffolds varies between 60 and 170 MPa by changing the ratio of CNTs and PLA. The fabricated scaffold showed higher stiffness, porosity (60-75 %), and cell viability (>75 %) on human fetal osteoblasts (hFOB) compared with pure 3D microfabricated PLA scaffold.

Poly(D, L-lactic acid) has been applied for bone regeneration, however, the smooth surface and hydrophobic nature restricts cell adhesion. This issue has rectified later by introducing poly(D, L-lactic acid) with vertically aligned-CNTs and hydroxyapatite. The *in vivo* study exhibited that the obtained scaffold mimics immature bone and also induced bone remodeling [97]. Highly porous and super-hydrophilic poly(D, L-lactic acid)/vertically aligned CNTs/hydroxyapatite nanocomposite scaffold has prepared by adopting two methods viz., electrodeposition, and immersion in simulated body fluid. The fabricated material supports human chondrocyte attachment and reduces type I Collagen mRNA expression [98]. 3D-printed scaffold reinforced with polylactic acid/CNT filaments is prepared by the melt extrusion technique [99]. Comparably improved mechanical properties and porosity were observed than pure polymer scaffolds and biocompatibility was noticed on human MSCs for 24h incubation of the newly fabricated composite filaments.

9.2. *CNTs-poly(ethylene glycol) composites*

The biocompatibility of poly(ethylene glycol) received greater attention among various polymers. Specifically, the functionalization of CNTs with a water-soluble polymer like polyethylene glycol showed high dispersibility in an aqueous medium. The covalent approach of CNTs with PEG provides water-soluble composites with controlled composition and reproducible properties and thereby offers significant importance in biomedical applications. Xiao et al. synthesized two types of CNTs composites viz., acid-functionalized MWCNTs (AO-M), and PEG covalently-functionalized MWCNTs (PEG-M) [100]. Afterward, the biomineralization technique has applied to deposit hydroxyapatite onto AO-M and PEG-M, and thus produced HA-AO-M and HA-PEG-M, respectively (Fig. 13). The cytotoxicity of the samples viz., Raw-M, AO-M, PEG-M, HA-AO-M, and HA-PEG-M were examined by using the

cells of neonatal rat's mandibular osteoblasts. The cell morphologies observed on HA-PEG-M were analogous to that of control. Moreover, the cell growth on HA-PEG-M was much higher than the other four samples (Fig. 14). The results strongly indicate that the covalent PEGylation and followed in situ deposition of HAp significantly improved the biocompatibility of the scaffold material, on the other hand, the rounded and non-adherent cells found in raw MWCNTs and AO-M medium indicates a substantial % of cell death. Therefore, the biocompatibility and dispersion ability of PEG, outstanding biocompatibility and osteoconductivity of HAp, and the exceptional mechanical properties of MWNTs in HA-PEG-M collectively offer the potential application in bone tissue engineering.

9.3. *CNTs-poly(lactic-co-glycolic acid) composites*

The uses of synthetic biodegradable polymers provide rapid advancement in the field of tissue engineering. Moreover, synthetic polymers with high biocompatibility, biodegradability, and enhanced cell proliferation and differentiation have already been reported extensively on the formation of bone graft substitutes [101, 102]. Poly(lactic-co-glycolic acid) (PLGA) is an FDA (Food and Drug Administration) approved and frequently used biodegradable, cost-effective, and highly processable synthetic polymer. This PLGA degrades into lactic and glycolic acid, which are harmless to cells and tissues, and also it can be excreted by the normal metabolic pathway [103-105]. However, the poor mechanical properties of PLGA have been resolved by nanostructured materials like CNTs. Lin et al. reported the formation of poly(lactic-co-glycolic acid)/MWCNTs-COOH nanocomposites [106]. The in vitro biocompatibility of the nanocomposites was examined on MSCs. The in vitro degradation was tested for seven weeks and the result indicates that the hydrolytic degradation of PLGA accelerated by carboxyl-functionalized MWCNTs. Further, the PLGA/c-MWCNT nanocomposites showed good cell-

adhesion and viability along with high production of alkaline phosphate. Hence, it is reported that the nanocomposites can induce MSCs and to differentiate MSCs into osteoblast.

Sitharaman et al. reported the differentiation of bone marrow-derived marrow stromal cells (MSCs) to osteoblasts through photoacoustic (PA) effect using SWCNT-PLGA films [107]. The cell differentiation was examined by quantitative (alkaline phosphatase, calcium, and osteopontin) and qualitative (alizarin red stain) assay. The report demonstrates that the osteo-differentiation of MSCs was improved by PA stimulation and SWCNTs in PLGA films. Similarly, Gupta et al. developed SWCNT and PLGA composites to examine the interaction of human BMSCs and MC3T3-E1 cells through cell proliferation, growth, mineralization, gene expression, and extracellular matrix formation [108]. The results confirmed that the uniform distribution of SWCNTs onto the PLGA matrix was not disturbed the degradation rate. Besides, the biocompatibility of the composites was revealed from the normal and non-stressed morphologies of the MC3T3-E1 and hBMSCs cells. In 2015, Gupta et al. demonstrated in vivo biocompatibility of SWCNT/PLGA composite in a subcutaneous implant rat model [109]. The composite mimics the properties of human trabecular bone. The potential of the composites was observed for 12 weeks and the results indicate that the slow degradation rate of PLGA. The slow degradation rate of the composite material may be favorable for prolonged duration studies because the lesser percentage of SWCNTs in the composite can highly reduce the toxicity of the implants. Recently, PLGA-based carbonaceous composites are reported by strengthening 1 wt% of CNTs, graphene, and activated carbon for bone tissue engineering [110]. These composite materials exposed noble mechanical properties viz., tensile strength, swelling ratio, and degradation percentages. The improved hydrophilicity and protein adsorption of the composites

enhanced cell proliferation and cell differentiation. Therefore, the report demonstrates the efficiency of PLGA-based carbonaceous composites in bone regeneration.

9.4. *CNTs-polyvinyl alcohol composites*

Natural and synthetic polymers are widely used as scaffold materials for biomedical applications. However, biodegradable/biocompatible synthetic polymers have greater consideration due to their tunable properties. Polyvinyl alcohol (PVA) has considerable interest in tissue engineering, drug delivery, and wound dressing due to its favorable biocompatibility and physicochemical property. The physicochemical characteristics and viscoelastic properties of PVA were analogous to articular cartilage and which received special attention than other polymeric hydrogels. Usually, hydrogels are applied for cartilage tissue engineering to offer a 3D structure to exchange articular cartilage and to absorb an enormous quantity of biological fluids and water. The remarkable mechanical properties of CNTs provide structural reinforcement to the hydrogels. In 2012, PVA and PVA-reinforced with CNTs nanoparticles were employed to treat osteochondral defects [111]. The cytotoxicity of the scaffolds on Vero fibroblast-type cells was evaluated by studying the metabolic activity and morphology of the cells. The osteogenic differentiation of MSCs was observed from the nodules of the mineralized organic matrix by alkaline phosphatase assay and alizarin red-S staining. Similarly, PVA-CNTs nanocomposites scaffolds were developed and their physicochemical and mechanical properties were analyzed [112]. The in vitro cell adhesion, proliferation, and cell differentiation of MG-63 cells are examined by MTT, alkaline phosphatase, and alizarin red staining assay along with collagen quantification.

9.5. *CNTs-polycaprolactone composites*

Polycaprolactone is a hydrophobic and semi-crystalline polymer that possesses favorable solubility and melting point with significant blend compatibility. The superior viscoelastic and rheological properties of PCL makes it a versatile candidate for bone tissue engineering. Pan et al. reported a solvent evaporation technique for the synthesis of MWNTs-PCL scaffolds [113]. This MWCNT/PCL composite showed improved cell proliferation and differentiation of bone-marrow-derived stromal cells (BMSCs) and also the composites obtained by 0.5 wt% of MWCNTs showed higher cell proliferation and differentiation than composite derived from higher wt % of MWCNTs. In the same year, PCL integrated with hydroxyapatite and ionically modified CNTs-nanocomposites scaffold with 3D pore has prepared via robocasting (Fig. 15) [114]. The PCL-HA-CNT scaffold has implanted on rat subcutaneous tissue for four weeks and thus produced soft fibrous tissues with neo-blood vessels on the 3D pores of the scaffold without causing any inflammation.

Later, 3D printed PCL-HA filled with CNT scaffolds have prepared to stimulate the growth of MG-63 cells [115]. The scaffold derived from 2 wt% of CNTs offers excellent mechanical property and electrical conductivity. Also, the compressive strength (~ 4 MPa) of the scaffold was compatible with trabecular bone. Flores-Cedillo et al. reported three different MWCNTs/PCL composites such as randomly distributed MWCNTs/PCL, aligned-MWCNTs/PCL, and β-glycerol phosphate (BGP)-modified with aligned-MWCNTs/PCL for bone regeneration [116]. These composites showed interesting mechanical and physicochemical properties, for example, BGP-modified MWCNTs/PCL showed a similar tensile strength of cancellous bone substitute (10–20 MPa). This study demonstrates that MWCNTs (0.3 wt%) were not exposed to toxicity on human dental pulp stem cells (HDPSCs). Further, BGP modified MWCNTs/PCL composites showed a greater proliferation of HDPSCs for up to 21 days.

Also, insulin-like growth factor-1 (IGF) coated PCL-PLA scaffolds such as (i) IGF-PCL-PLA, (ii) CNT-PCL-PLA and (iii) CNT-IGF-PCL-PLA were prepared via surface coating of CNTs and IGF using photoimmobilization method (Fig. 16) [117]. The in vitro results of CNT-IGF-PCL-PLA showed a high proliferation rate of MSCs. Moreover, scaffold (ii) and (iii) exhibited a strong ability to prevent cellular senescence. The in vivo studies of the same scaffolds accelerated the healing rate of bone without toxicity and hence it is reported that the obtained materials can increase the life span of cells (Fig. 17). Three-phased MWCNTs/nano-hydroxyapatite/polycaprolactone composite scaffold (mwCNT/nHA/PCL) was fabricated by solvent evaporation technique [118]. The obtained three phases of MWCNT/nHA/PCL exhibited high miscibility and strong interfacial force in 1/15/84 (wt%), and also the scaffold possesses small porosity and slow degradation along with massive crystallized hydroxyapatite in SBF solution. Moreover, the scaffold exhibits improved cell proliferation and cell differentiation of MG63 cells.

## 10. Conclusions and perspectives

The fabrication of a promising bioactive scaffold to mimic the extracellular matrix of natural bone is a major concern so far. The research and development of nanomaterials have received considerable attention and demands in the applications of the medical arena. Particularly, carbon nanotubes-based composite materials provided a versatile platform in the biomedical field by their superior mechanical, electrical, and chemical properties than conventional biomaterials. The development of CNTs-based composites gained popularity in tissue engineering. The superior physicochemical properties of CNTs have enhanced cellular interactions, adhesion, proliferation, and osteogenesis differentiation which provide improvements in bone replacement and growth. The large surface area, biocompatibility, and

stimulation of CNTs have extended their activity as nanocarriers for drug delivery and cellular transportation for bone diseases and defects. Although CNTs have made significant advances and growths in the application of bone regeneration and repair, there are still some issues that need to be addressed through the concerted efforts of researchers around the world, from the experimental stage to clinical application.

The first major challenge that researchers have to overcome is hydrophobicity, i.e., the poor solubility/dispersibility of CNTs in aqueous and organic solvents. Constant efforts should be made to functionalize appropriate biomolecules with CNTs to enhance the aqueous solubility and biocompatibility of CNTs in biomedical applications. The issue of toxicity of CNTs in the living system is the second major challenge to be solved. The safety evaluation and the assessment of potential risk are obligatory to confirm its specific biomedical uses. The metal impurities in CNTs can enhance toxicity, resulting in cell death through mitochondrial damage and oxidative stress. However, it should be rectified by functionalizing appropriate biocompatible polymers onto CNTs. To enhance the dispersibility and to minimize toxicity, CNTs are functionalized with suitable organic molecules through covalent and non-covalent functionalization. The biocompatibility of CNT is interconnected with functionalization, dispersion, and length. The functionalization can significantly induce the dispersion of bundled CNTs and biocompatibility in an aqueous environment. Therefore, innovative technologies and strategies to modify the tubular structure of CNTs need to be further studied and developed.

For bone tissue engineering, CNTs are mainly employed as reinforcement filler in the scaffold to reinforce mechanical and biological properties. It is highly necessary to fabricate suitable biocompatible and biodegradable scaffold materials for bone tissue engineering. Therefore, the development of CNTs functionalized with biocompatible natural or synthetic

polymer composites gained popularity in bone tissue engineering. The superior physicochemical properties of CNTs-based polymer composites have enhanced cell adhesion, proliferation, and differentiation which provided improvements in bone replacement and growth. Overall, scaffolds made up of CNTs-polymer composites are emerging as excellent materials with suitable structural and biological features, innovative strategies, and the ability to explore future prospects for bone tissue regeneration and engineering.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Figures

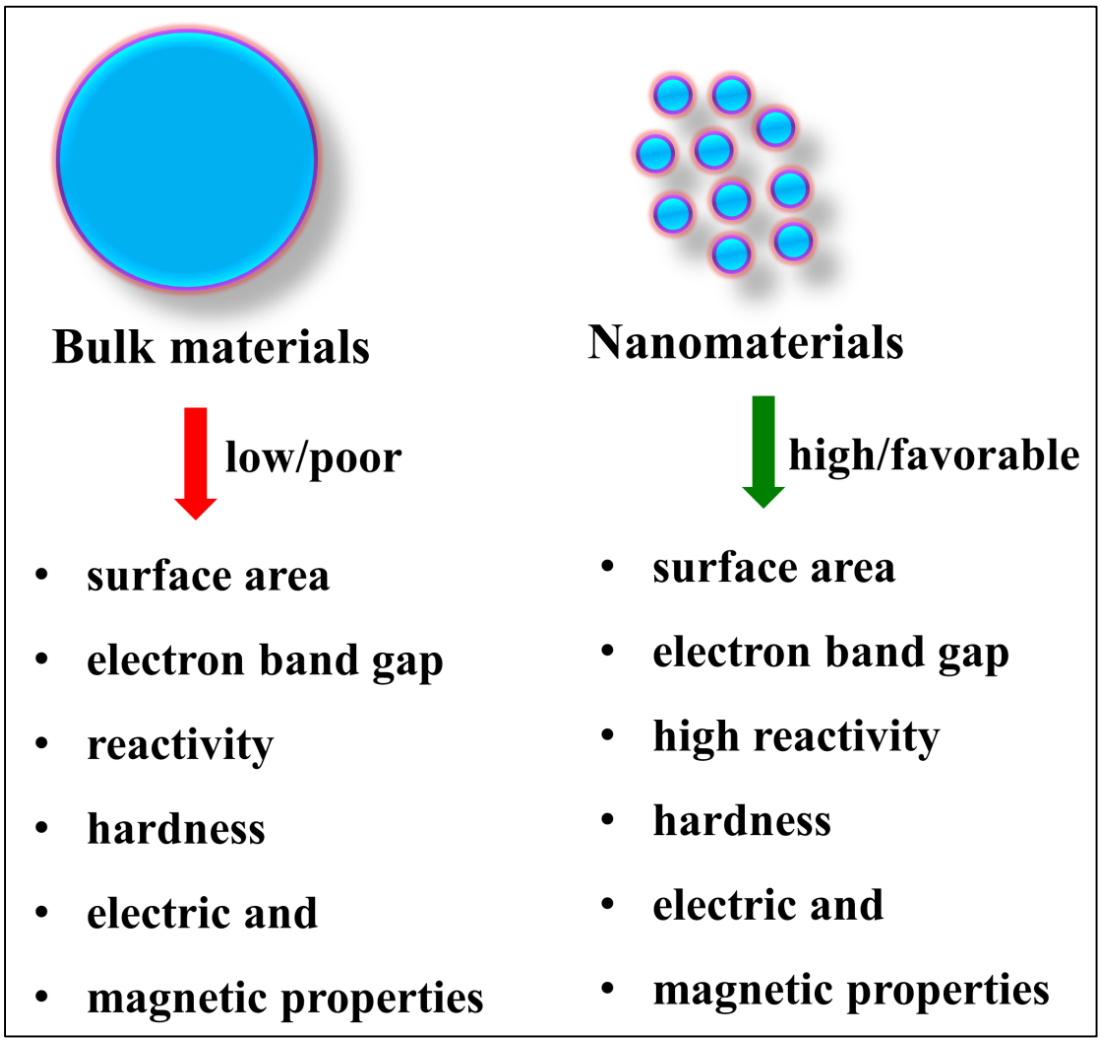


**Fig. 1. The schematic illustration represents the outstanding properties between nano and bulk materials.**

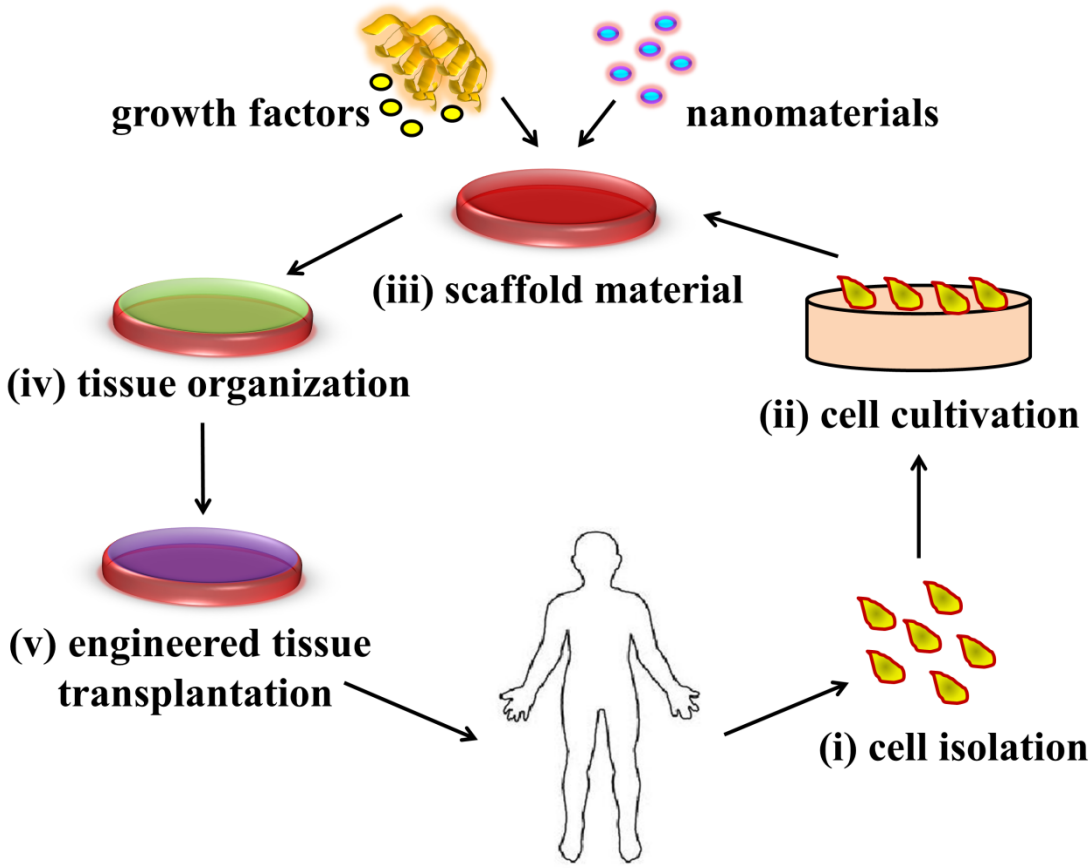


**Fig. 2. Schematic illustration of nanomaterials involved in biomedical applications.**

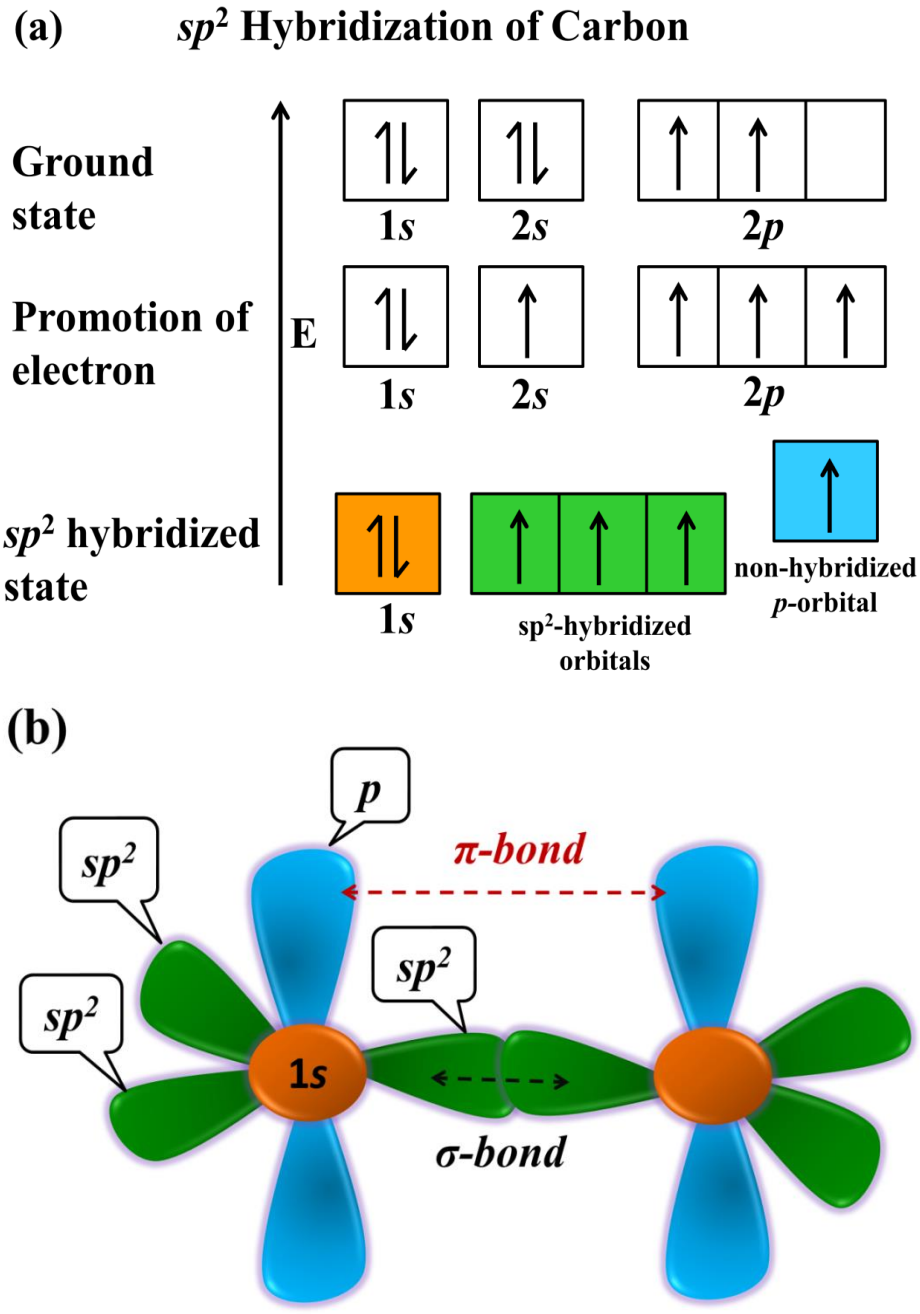


**Fig. 3. Schematic representation of (a) $sp^2$ hybridization of carbon and (b) carbon atoms in CNTs covalently bonded with one another through $sp^2$ hybridizations.**

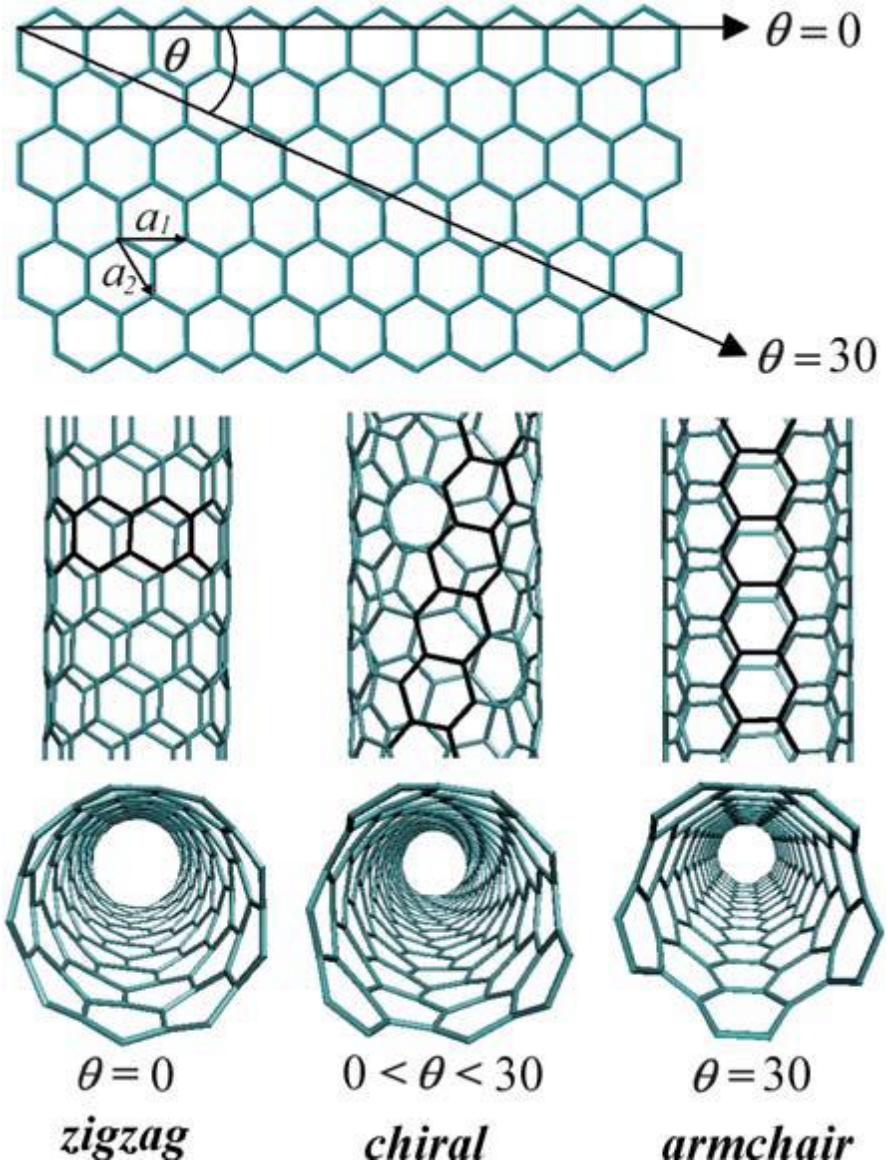


**Fig. 4. Types of CNTs obtained from geometrical alignment. Reproduced from Ref. [7] with permission from the Royal Society of Chemistry.**

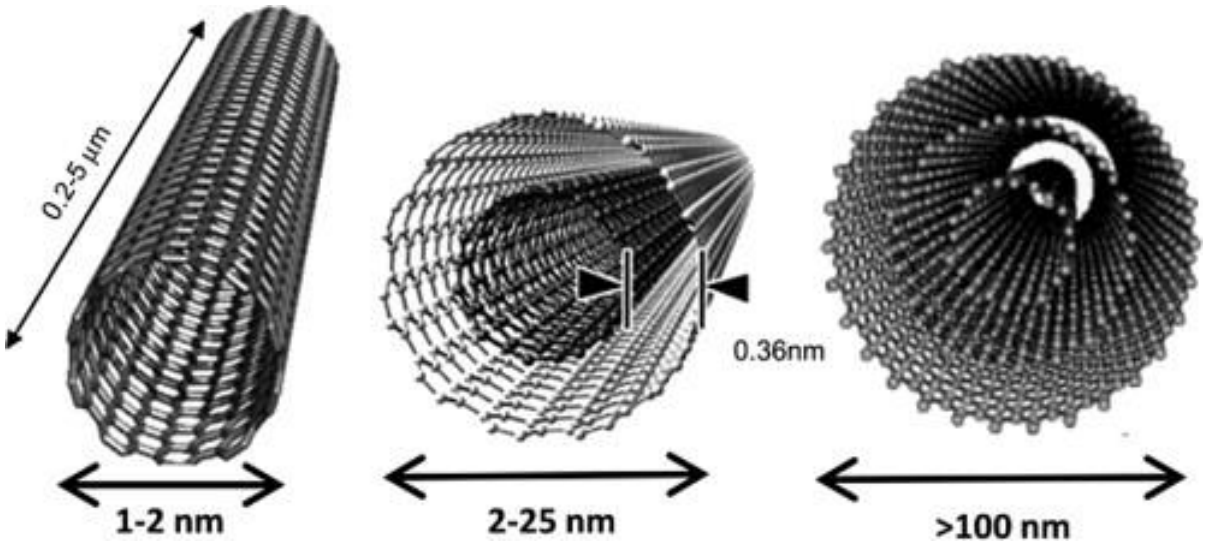


**Fig. 5. Schematic representations of single-, double- and multi-walled CNTs. Reproduced from Ref. [8] with permission from Elsevier.**

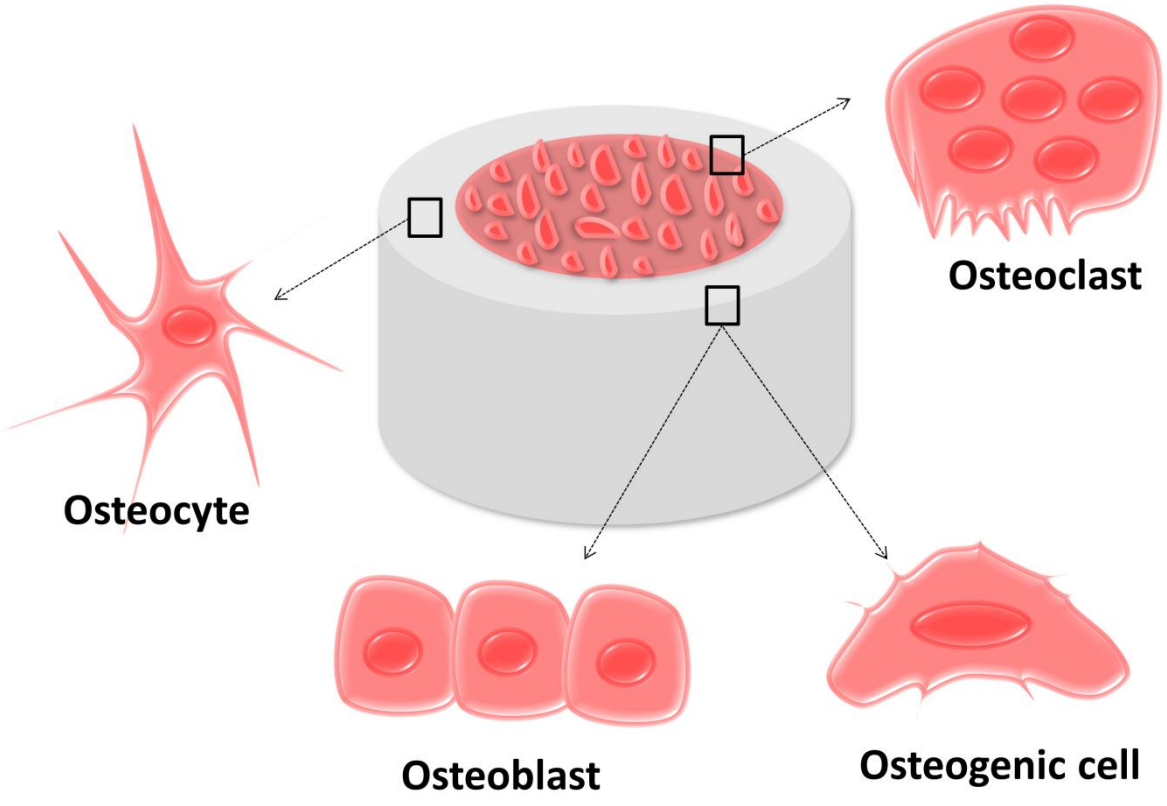


**Fig. 6. Classifications of bone cells**

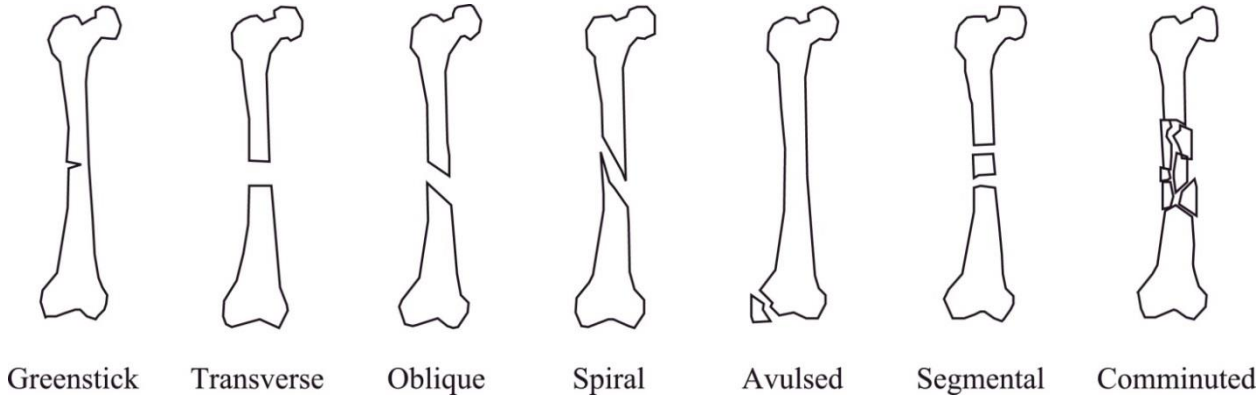


**Fig. 7. Different types of bone fractures. Reproduced from Ref. [15] with permission from Elsevier.**

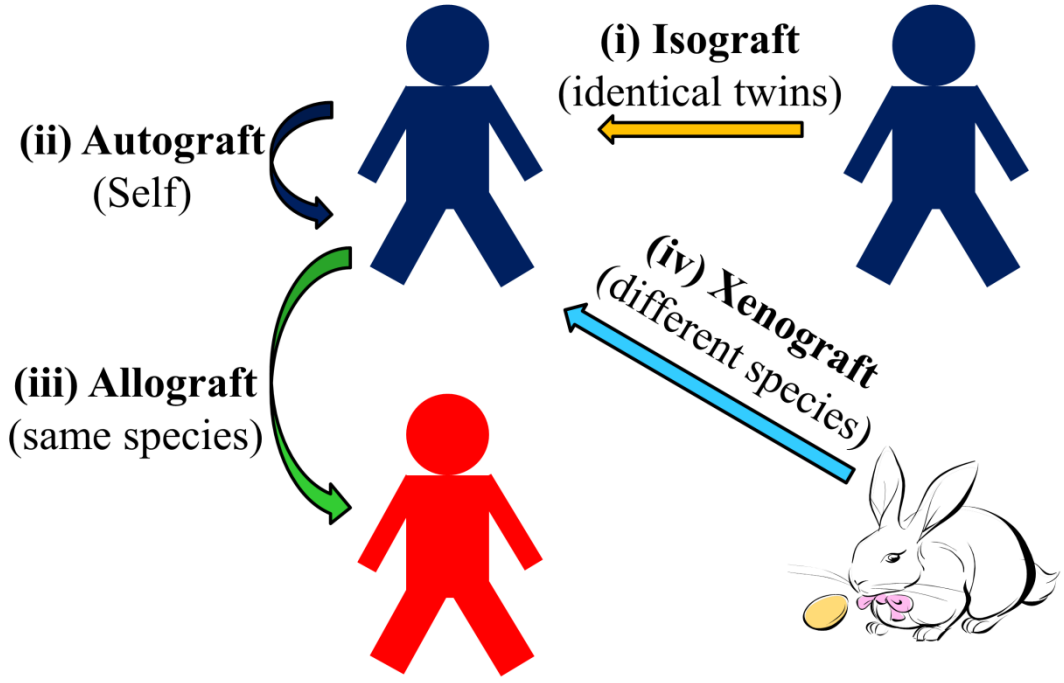


**Fig. 8. Types of transplants**

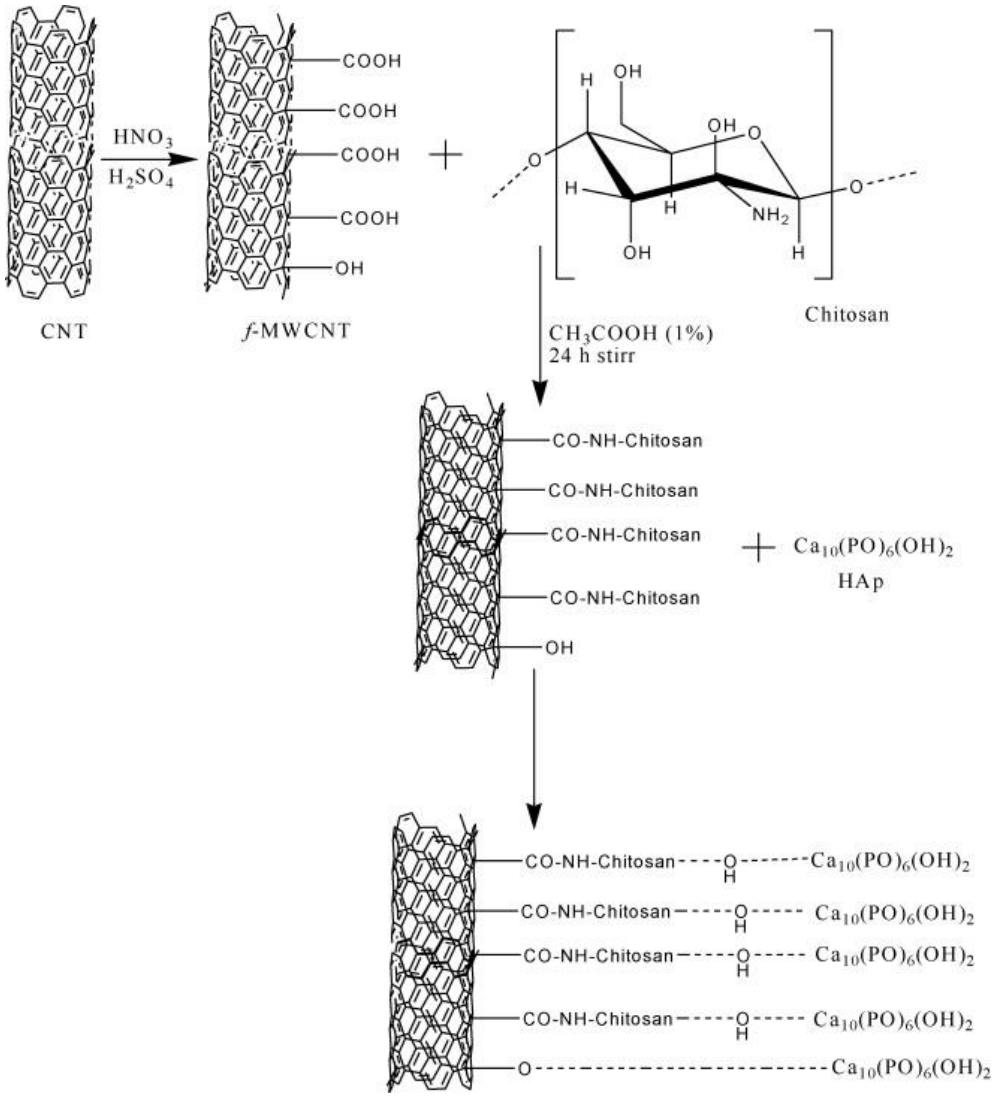


**Fig. 9. Scheme for the formation of MWCNT-grafted chitosan-HAp composite. Reproduced from Ref. [49] with permission from Elsevier.**

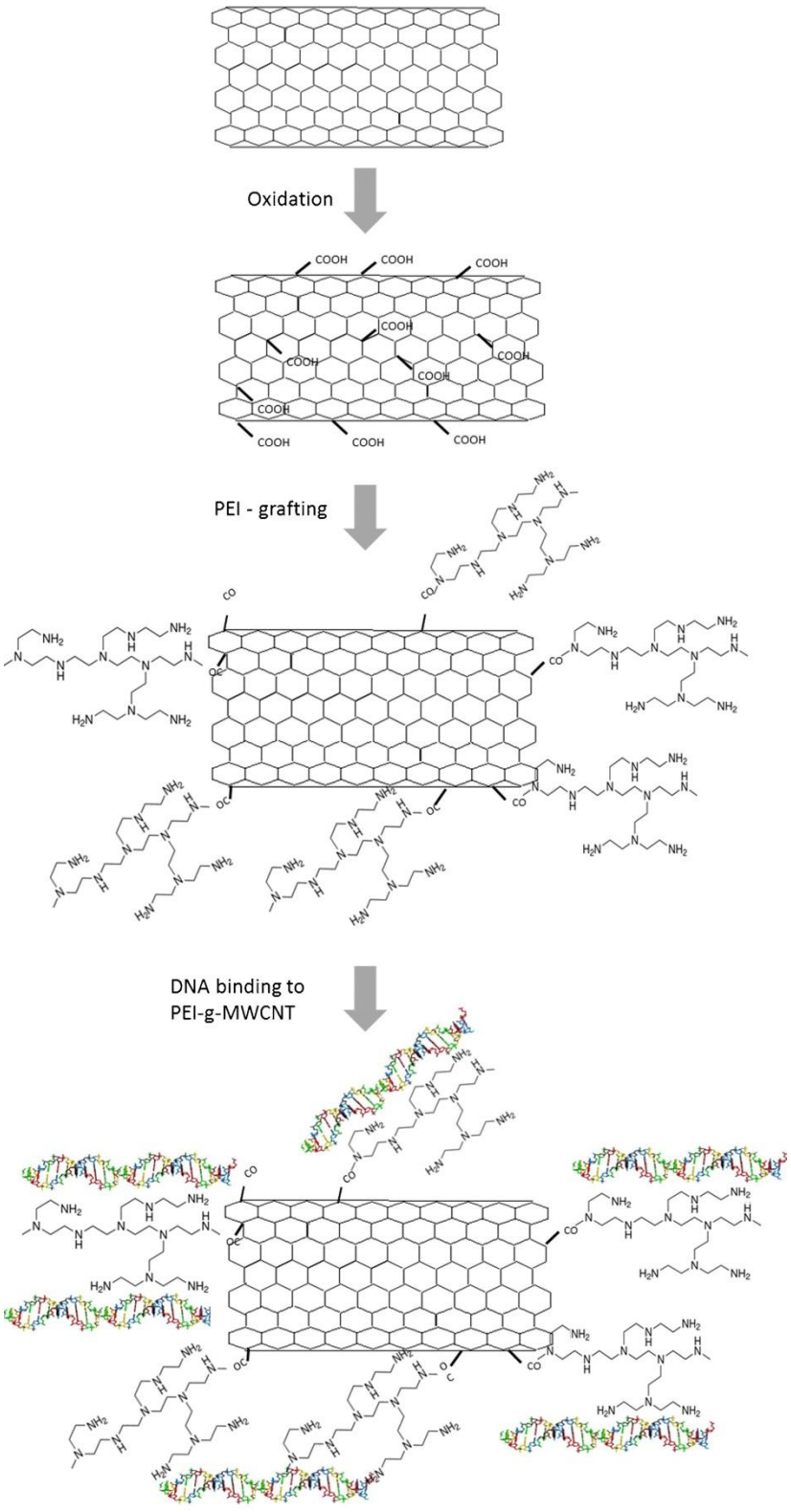


**Fig. 10. Schematic representation of PEI-grafted CNTs-chitosan and their interaction with DNA. Reproduced from Ref. [72] with permission from Elsevier.**

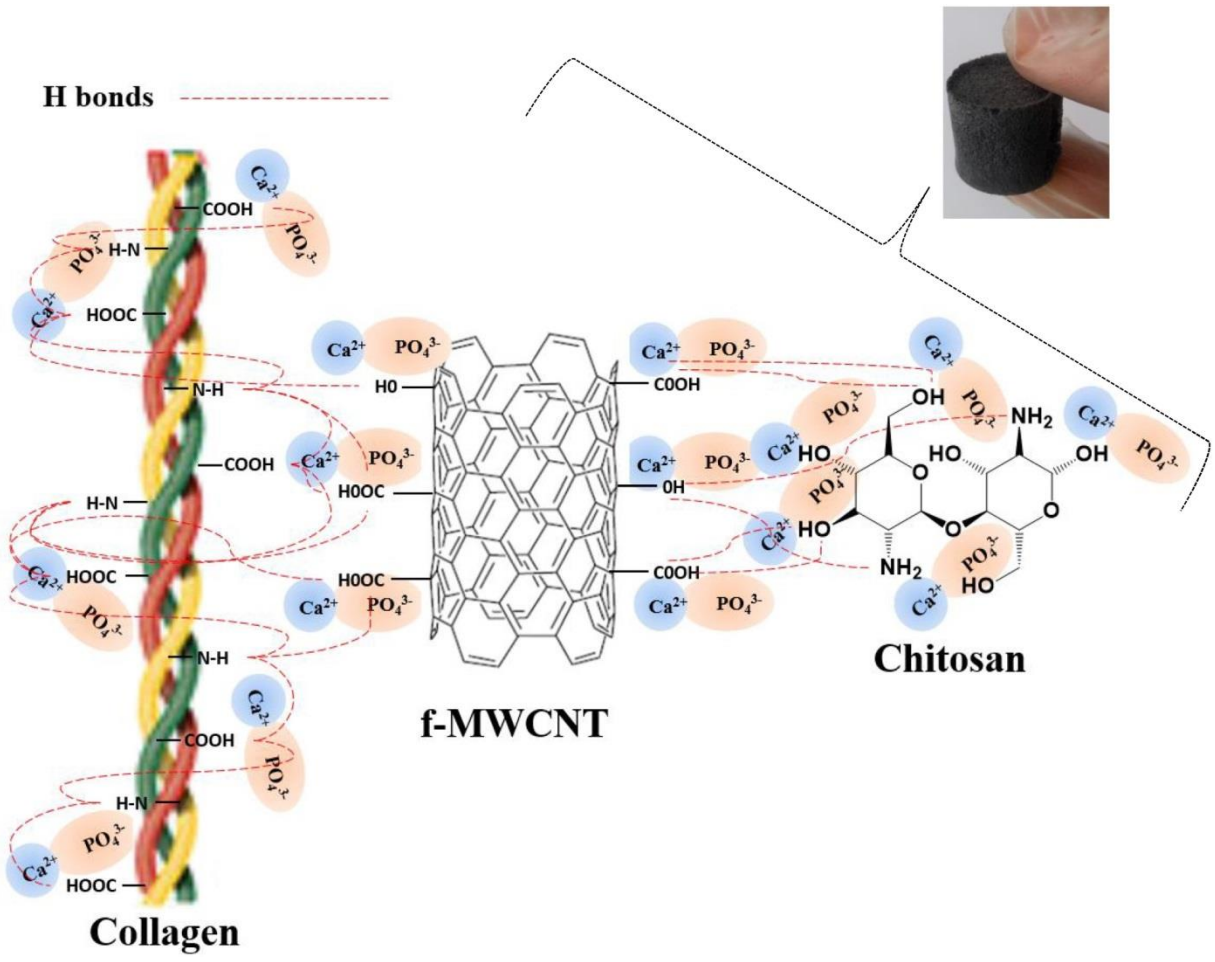


**Fig. 11. Biomineralization of collagen functionalized with CNTs, chitosan, and HAp composites using simulated body fluid. Reproduced from Ref. [78] with permission from Elsevier.**

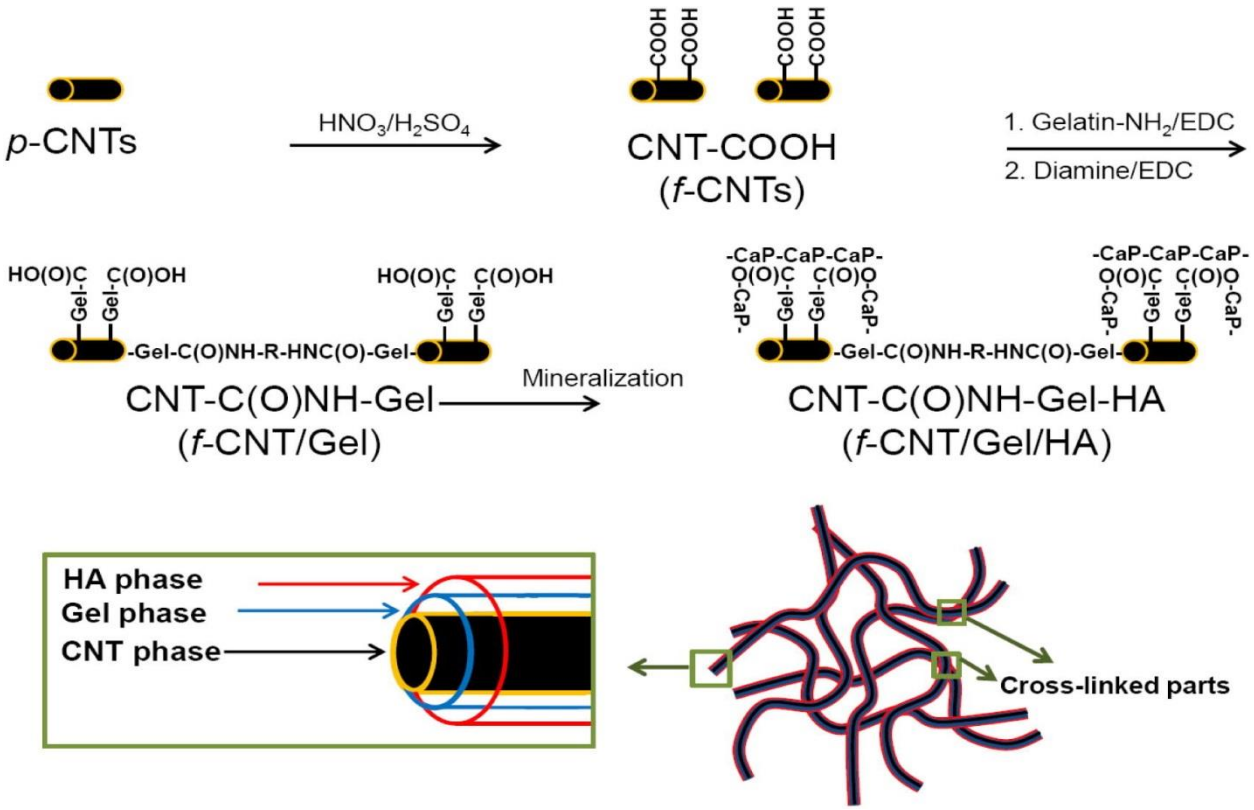


**Fig. 12. Scheme for the preparation of f-CNT/Gel/HA multilayered core-shell structure. Reproduced from Ref. [83] with permission from Elsevier.**

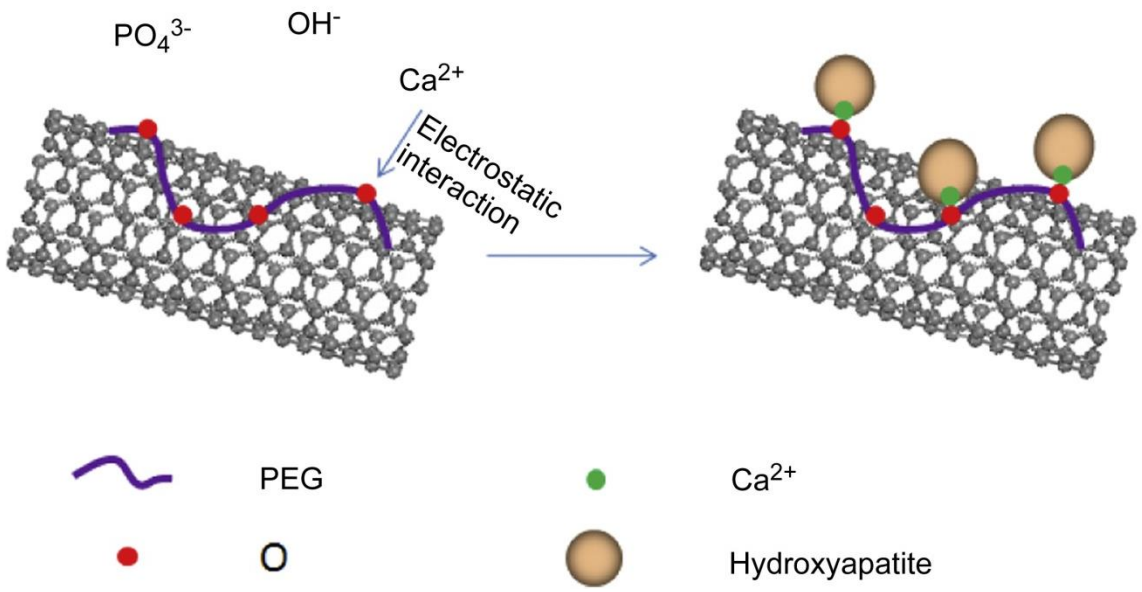


**Fig. 13. Scheme for the biomineralization of HA onto PEG-M. Reproduced from Ref. [100] with permission from Elsevier.**

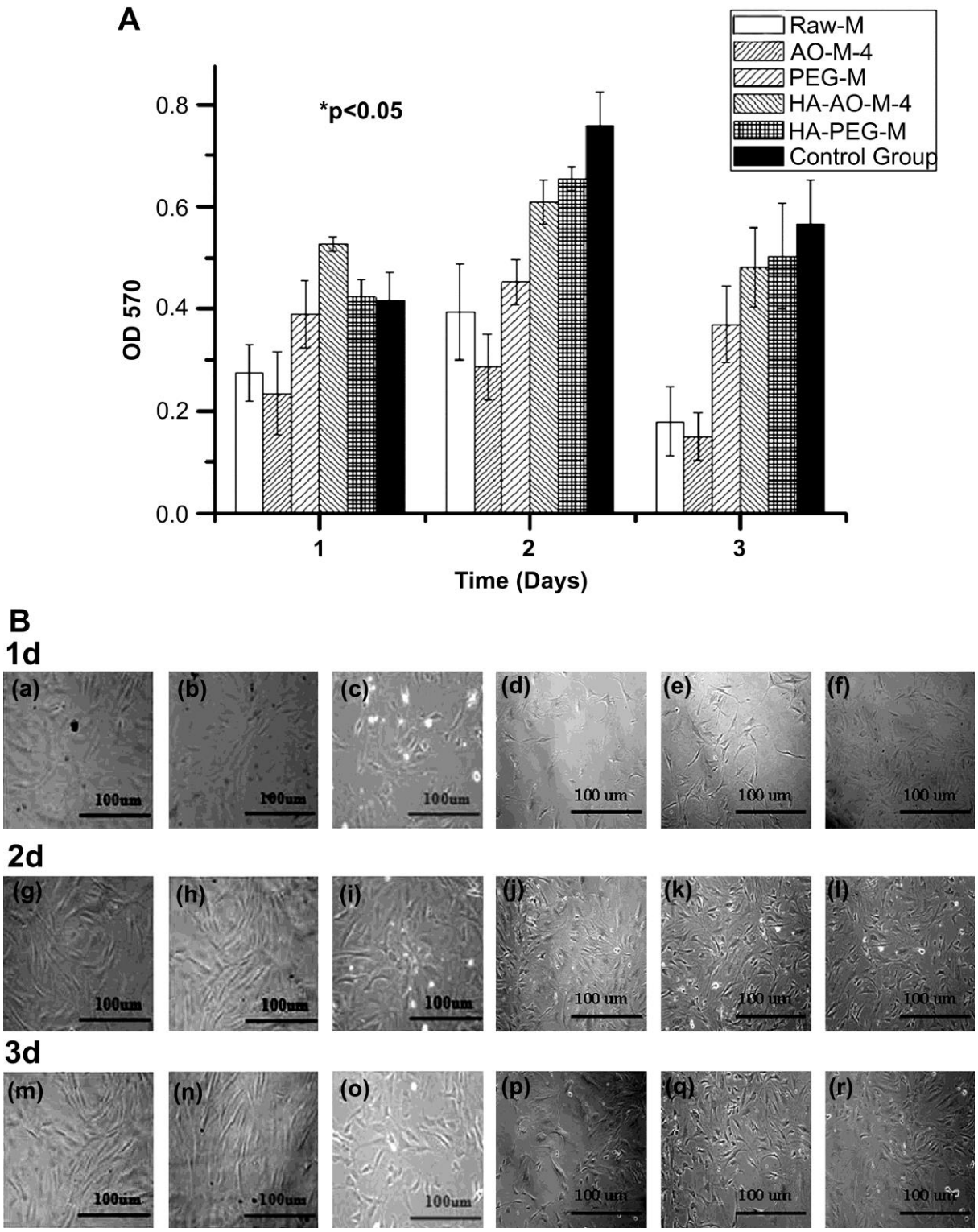


**Fig. 14. (A) MTT and (B) optical microscope results of the growth of osteoblasts on Raw-M (a, g, m), AO-M-4 (b, h, n), PEG-M (c, i, o), HA-AO-M-4 (d, j, p), HA-PEG-M (e, k, q) and control group (f, l, r) for day 1, 2 and 3, respectively. Reproduced from Ref. [100] with permission from Elsevier.**

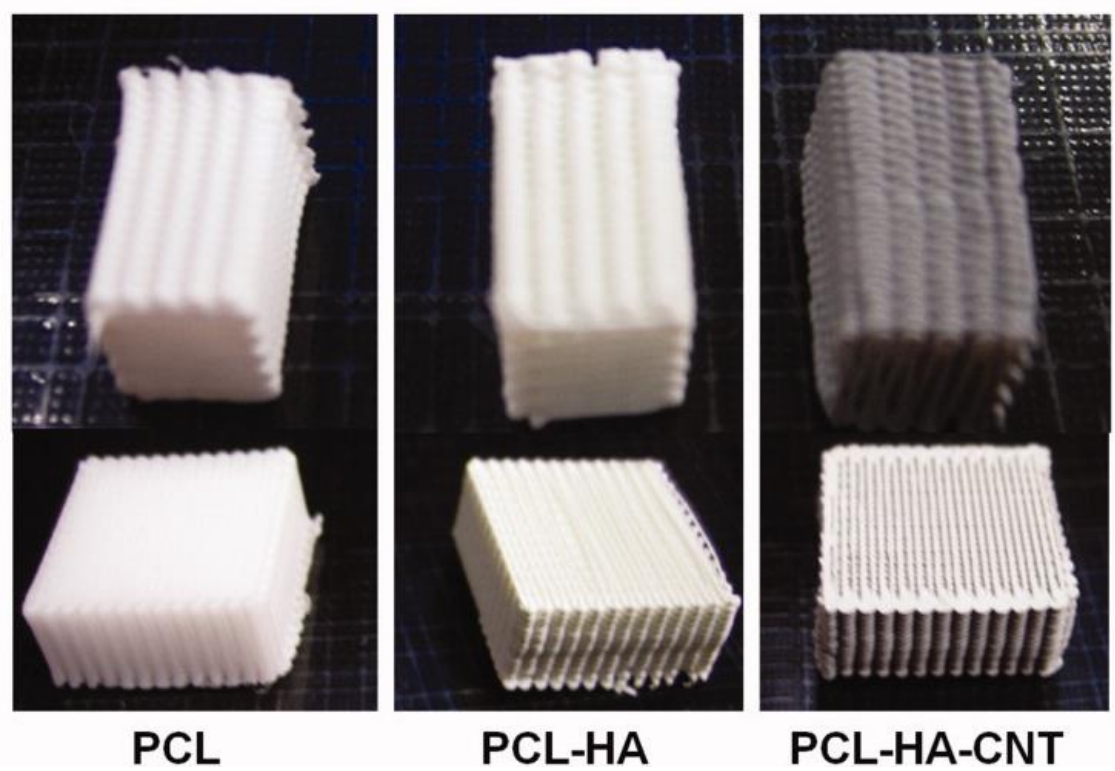


**Fig. 15. Optical images of PCL, PCL-HA and PCL-HA-CNT with different compositions. Reproduced from Ref. [114] with permission from John Wiley & Sons, Inc.**

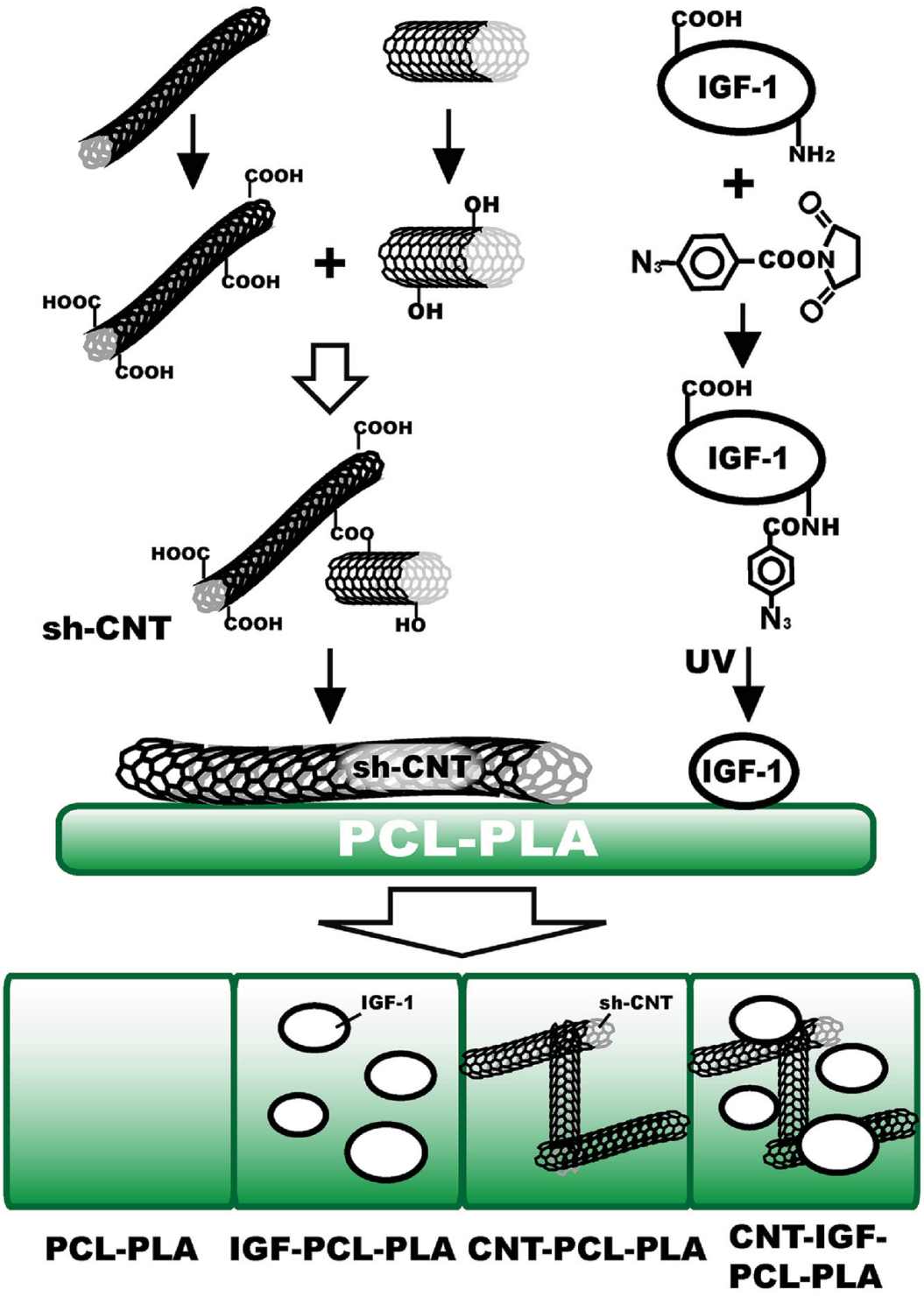


**Fig. 16. Schematic representation of self-assembled CNTs and IGF onto the PCL-PLA scaffold. Reproduced from Ref. [117] with permission from John Wiley and Sons.**

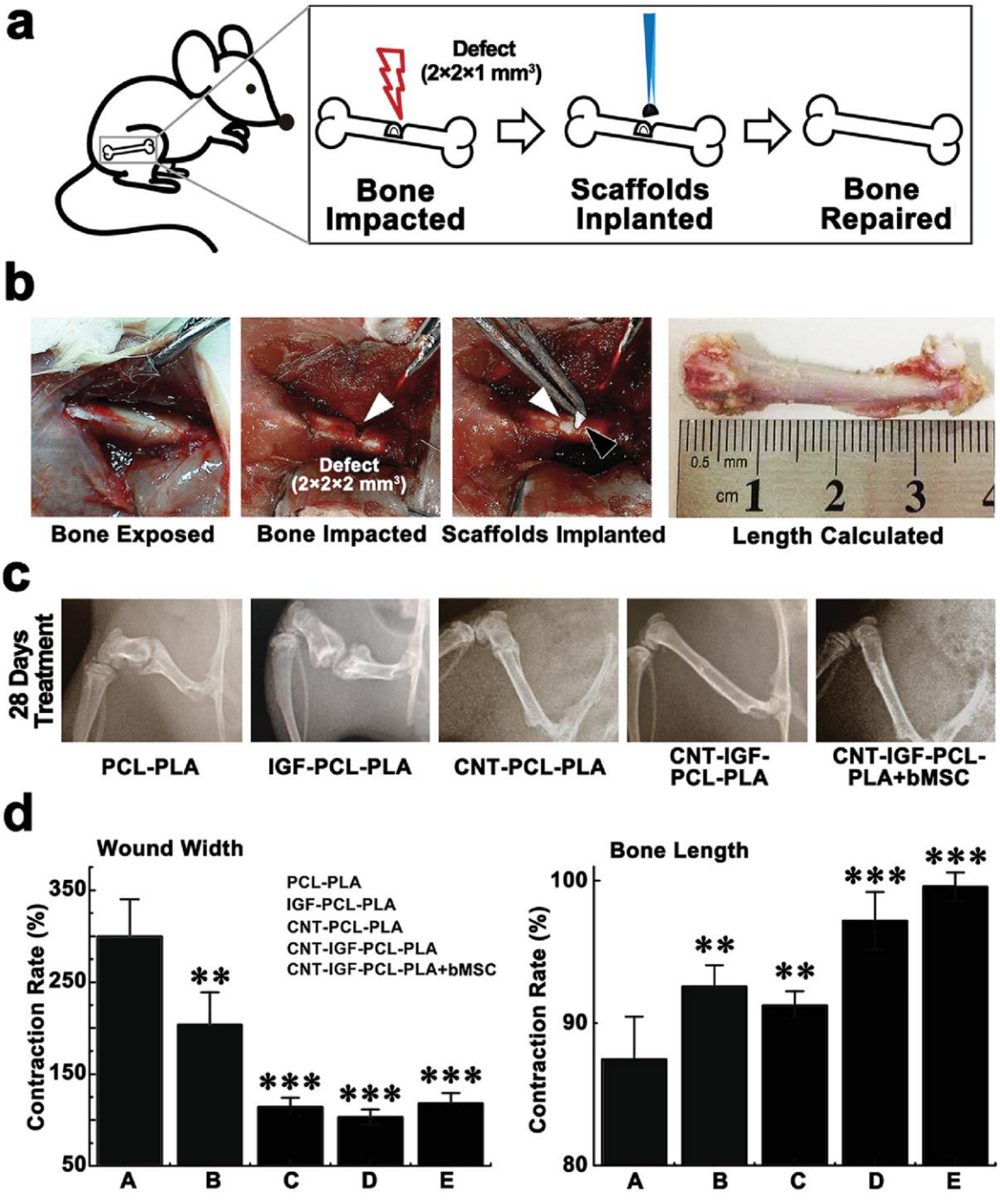


**Fig. 17. a) Schematic illustration of the SD rate's operation. b) Images of scaffold implantation (white arrow: exposing bones, black arrow: implanted scaffolds, white bar: defect area. c) X-ray analysis indicates the curing of bone (28 days after surgery). d) Bar graphs represents the wound width and bone length ($p$<0.05 : *, 0.001<$p$<0.01: **, and $p$<0.001: ***) compared with PCL-PLA. Reproduced from Ref. [117] with permission from John Wiley and Sons.**

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: