Review

# Carbon based nanomaterials for tissue engineering of bone: Building new bone on small black scaffolds: A review

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## HIGHLIGHTS

- Bone tissue engineering allows stem cells to form mechanically adequate new bone.
- Nanomaterial scaffolds allow cell adhesion, growth, and differentiation.
- Carbon nanomaterials have good properties as scaffolds for bone tissue engineering.
- Includes graphene oxide, carbon nanotubes, fullerenes, carbon dots, and nanodiamond.
- Biocompatibility, low toxicity, and a nano-patterned surface form ideal scaffold.

## GRAPHICAL ABSTRACT
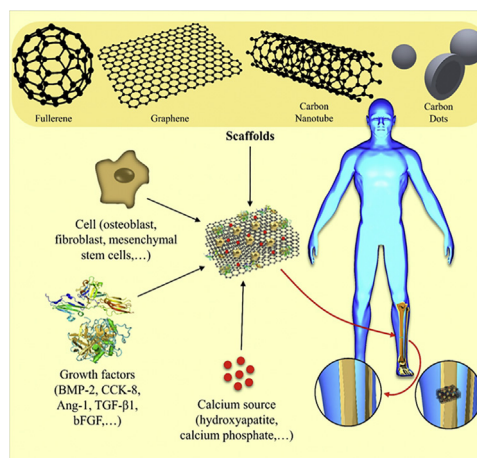


## ARTICLE INFO

## ABSTRACT

Tissue engineering is a rapidly-growing approach to replace and repair damaged and defective tissues in the human body. Every year, a large number of people require bone replacements for skeletal defects caused by accident or disease that cannot heal on their own. In the last decades, tissue engineering of bone has attracted much attention from biomedical scientists in academic and commercial laboratories. A vast range of biocompatible advanced materials has been used to form scaffolds upon which new bone

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can form. Carbon nanomaterial-based scaffolds are a key example, with the advantages of being biologically compatible, mechanically stable, and commercially available. They show remarkable ability to affect bone tissue regeneration, efficient cell proliferation and osteogenic differentiation. Basically, scaffolds are templates for growth, proliferation, regeneration, adhesion, and differentiation processes of bone stem cells that play a truly critical role in bone tissue engineering. The appropriate scaffold should supply a microenvironment for bone cells that is most similar to natural bone in the human body. A variety of carbon nanomaterials, such as graphene oxide (GO), carbon nanotubes (CNTs), fullerenes, carbon dots (CDs), nanodiamonds and their derivatives that are able to act as scaffolds for bone tissue engineering, are covered in this review. Broadly, the ability of the family of carbon nanomaterial-based scaffolds and their critical role in bone tissue engineering research are discussed. The significant stimulating effects on cell growth, low cytotoxicity, efficient nutrient delivery in the scaffold microenvironment, suitable functionalized chemical structures to facilitate cell-cell communication, and improvement in cell spreading are the main advantages of carbon nanomaterial-based scaffolds for bone tissue engineering.
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## Introduction

Scaffolds can be called "the beating heart" of the tissue engineering field. Without the appropriate scaffold, the growth of cells in an artificial environment is not possible. Among all the various cells of the human body, bone cells are one of the most critical types that require a well-designed scaffold to allow engineered living bone. There is a growing need to repair damaged tissues such as bones or replace them with new healthy ones. Research into new approaches to create such scaffolds has been intensified in recent years, and tissue engineering combined with nanotechnology is now looked upon as a promising alternative to the existing conventional repair strategies [1,2]. This multidisciplinary science is a novel approach to the restoration and reconstruction of damaged tissues. It aims to grow specific and functional tissue that can behave as well (or even better) than natural tissue [3]. Basic science (chemistry, physics and engineering) is combined with life sciences (biology and medicine) in order to enhance the function of damaged tissue [4]. Kidney was the first organ to be transplanted between identical twin brothers. Ronald Herrick conducted this transplant in 1954. In this procedure the donor and recipient were genetically identical which avoided adverse immune response (rejection) [5]. According to recent statistics from the US Department of Health and Human Services, 22 people die each day while waiting for a transplant [6]. The aim of tissue engineering is to overcome existing transplant bottlenecks by modeling biological structures with the eventual aim to construct artificial organs. Engineers working in the field of tissue engineering utilize natural or synthetic materials to fabricate scaffolds. Scaffolds should be biocompatible without any stimulation of excessive inflammation, or response by the immune system. Furthermore, scaffolds should be compatible with tissue-specific cell types and with the environments found in the body of the individual who will receive the tissue [7,8]. Bone is unique amongst tissue engineering targets, since mechanical strength becomes of paramount importance, in addition to good biocompatibility and satisfactory biological function. Some studies have been undertaken to investigate the use of carbon-based nanomaterials for bone tissue engineering *in vivo*. For instance, Sitharaman et al. utilized CNT/biodegradable polymer nanocomposites for bone tissue engineering in a rabbit model. They utilized single-walled carbon nanotubes (SWCNTs), especially ultra-short SWCNTs (US-SWCNTs) to fabricate polymeric scaffold materials. Their results showed the significant effects of the scaffold composition on the cell behavior and the growth rate in the microenvironment of the scaffold surface. In their report, the CNT scaffolds that did not possess the appropriate surface chemical composition did not perform well for cell growth. Their results indicated that a suitable chemical composition played a critical role in bone cell proliferation and growth [9]. Therefore, the exact influence of the scaffold surface chemical composition requires further broad studies. Nanomaterials such as carbon-based, metallic and metalloid nanoparticles play a pivotal role in tissue engineering [10–16]. Nowadays, nanocarbon materials have been used extensively in energy transfer and energy storage applications. Fullerenes, graphene and CNTs are some of the most widely studied nanocarbon structures [17,18]. These nanomaterials have diameters ranging from tens of nanometers to hundreds of nanometers [19]. They possess unique structures and properties which make them promising candidate materials for use in biomedical applications, such as tissue engineering and regenerative medicine. Moreover, carbon nanomaterials have been used as secondary structural reinforcing agents to enhance the mechanical properties of two- and three-dimensional cell culture scaffolds such as hydrogels and alginate gels [20].

Graphene (G) materials may be superior to other carbon nanomaterials such as CNTs due to their lower levels of metallic impurities and the need for less time consuming purification processes to remove the entrapped nanoparticles [21]. However, on the other hand, CNTs possess some unique properties like a cylindrical shape with nanometer scale diameters, longer lengths (4100 nm) and very large aspect ratios. Moreover other physical and mechanical properties of CNTs are important such as high tensile strength $\geq 50$ GPa, Youngs modulus $\geq 1$ TPa, conductivity $\sigma_{in} \geq 10^7$ S/m, maximum current transmittance $J_{in} \geq 100$ MA/cm$^2$, and density $\rho \leq 1600$ kg/m$^3$ [17].

All carbon nanomaterials have been shown to be bioactive for one or more purposes. Many show a high capability for bone tissue engineering, with good mechanical properties, no cytotoxicity toward osteoblasts, and display an intrinsic antibacterial activity (without the use of any exogenous antibiotics) [22]. Due to these advantageous properties they have been widely investigated for bone tissue engineering applications, either as a matrix material or as an additional reinforcing material in numerous polymeric nano-composites [20]. In this review, the applications of carbon-based scaffolds including GO, CNTs, CDs, fullerenes, nanodiamonds (NDs) and their derivatives and compositions in bone tissue engineering have been covered (Fig. 1).

For broad and comprehensive coverage of the application of carbon nanomaterials in bone tissue engineering, the following keywords were employed: scaffold, GO, CNTs, fullerenes, CDs, nanodiamonds, bone tissue engineering, cell proliferation, osteogenic differentiation, cell spreading, biocompatibility, cytotoxicity and mechanical strength. The focus of this review is on reports that have been published in the last 3–4 years and have been cited in Google scholar and Scopus websites.
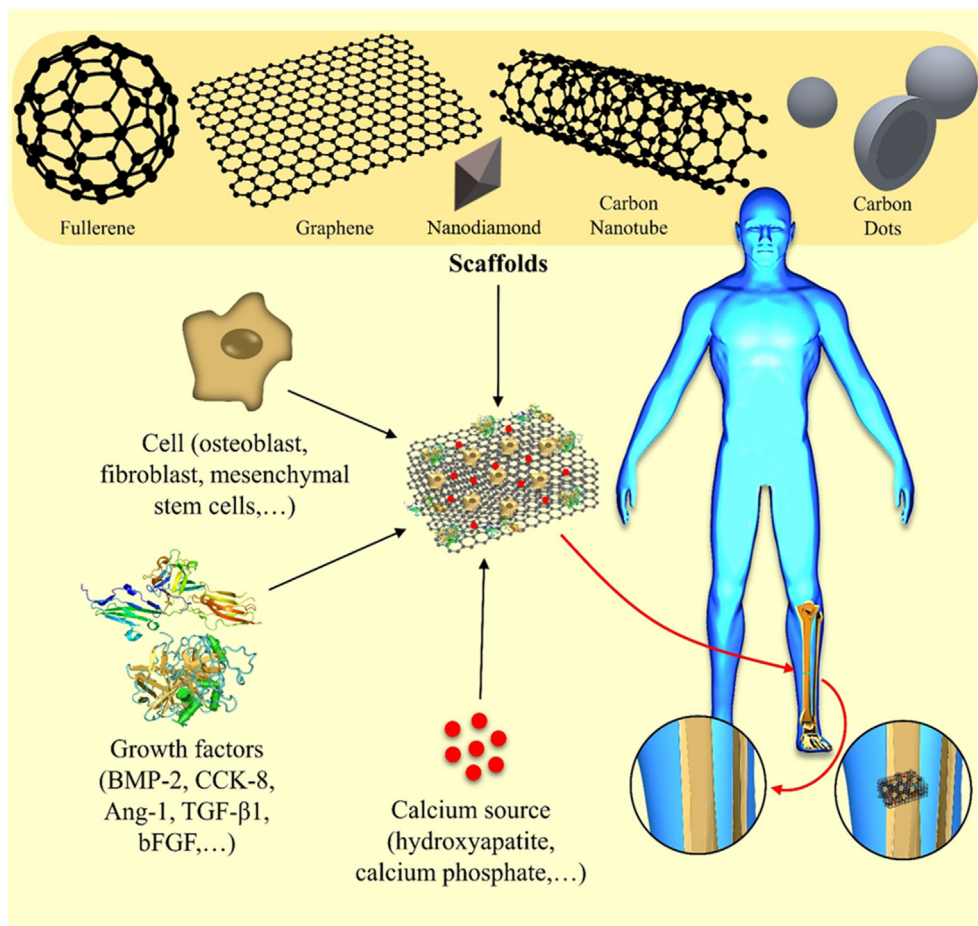
**Fig. 1.** Application of carbon-based nanomaterials as scaffolds in bone tissue engineering. Different carbon-based nanoparticles such as CNTs, G, fullerenes and CDs and NDs could act as scaffolds or matrices for various bone forming cells, growth factors and sources of calcium.

## Graphene oxide in bone tissue engineering

G is one allotrope of the crystalline forms of carbon, taking the form of a single monolayer of $sp^2$-hybridized carbon atoms arranged in a hexagonal lattice. It is the basic structural element of many other allotropes of carbon, such as graphite, charcoal, CNTs and fullerenes. Each carbon atom has two σ-bonds and one out-of-plane π-bond linked to neighboring carbon atoms. This molecular structure is responsible for the high thermal and electrical conductivity, unique optical behaviors, excellent mechanical properties, extreme chemical stability, and a large surface area per unit mass. Additionally, by chemical and physical manipulation, G sheets can be restructured into single and multi-layered G or GO. GO is a compound of carbon, oxygen, and hydrogen in variable molecular ratios, achieved by treating graphite with strong oxidizing agents. Because of the presence of oxygen, GO is more hydrophilic than pure G, and can more easily disperse in organic solvents, water, and different matrices [23,24]. Recently, basic studies on the physicochemical properties GO, have shown that the hydrophilicity [25], mechanical strength [26], high surface area [27] and adhesive forces [28] are related to how the G sheets interact with each other. This interaction can occur by π-π stacking of [29], electrostatic or ionic interactions, and van der Waals forces depending on the exact structure of the functionalized sheets. These various interactions make possible specifically tailored applications of GO-based materials for tissue engineering in different organs, biosensor technology, and medical therapeutics [30,31]. Different "Gum-metal" titanium-based alloys like Ti(31.7)-

Nb(6.21)-Zr(1.4)-Fe(0.16)-O can be admixed with GO-based materials to enhance their mechanical and electrical properties. Depending on the proposed application, GO can be functionalized in a number of ways. For instance, one way to ensure that the chemically-modified G disperses easily in organic solvents is to attach amine groups through organic covalent functionalization. This makes the material better suited to function in biodevices and for drug delivery [32]. Reports have shown the beneficial effects of kaolin-based materials on the toxicity of G-based materials [33,34]. Nowadays, the non-toxicity of G-based nanomaterials that are in the form of 2D-substrates or 3D-foams is the one of the most interesting issues in designing bioactive scaffolds for different human and animal stem cells differentiation processes [18,21,35]. G-nanoparticle composites have also shown good potential in tissue engineering because of the appropriate ability for surface modification, acceptable cytotoxicity and biodegradability [36]. In 2015, Xie et al. reported a facile and versatile method that can be used to synthesize these structures based on colloidal chemistry. In their study, they started with aqueous suspensions of both GO nano-sheets and citrate-stabilized hydroxyapatite (HAp) nanoparticles. Hydrothermal treatment of the blends of suspensions increased the G to GO ratio, and entrapped colloidal HAp nanoparticles into the 3D-G network owing to formation of a self-assembled graphite-like shell around them. Dialysis of this shell preparation led to deposition of uniform NPs onto the G walls. The results showed that G/HAp gels were extremely porous, mechanically strong, electrically conductive and biocompatible, thus promising as scaffolds for bone tissue engineering.

This study has great importance because it studies the effects of G and GO sheet morphology on the artificial bone tissue quality. In 2015, Lee et al. investigated whether nanocomposites of reduced graphene oxide (rGO) and HAp could promote the osteogenic differentiation of MC3T3-E1 preosteoblasts and stimulate new bone cell growth. rGO/HAp nanocomposites significantly promoted spontaneous osteo-differentiation of MC3T3-E1 cells without any inhibition of their proliferation. This improved osteogenesis was verified by measurement of alkaline phosphatase (ALP) activity as a marker of the early stage of osteo-differentiation and mineralization of calcium and phosphate as the late stage. Moreover, rGO/HAp nanocomposites meaningfully increased the expression process of osteopontin and osteocalcin. Likewise, rGO/HAp nanocomposite grafts were found to increase new bone cells formation in animal models without any inflammatory response. rGO/HAp nanocomposites could be suitable for the design of a new class of dental and orthopedic bone grafts to facilitate bone regeneration due to their ability to stimulate osteogenesis. Fig. 2 displays field emission scanning electron microscopy (FESEM) images of the rGO/HAp nanocomposites reported in the study [37].

Acrylic polymers or polymethylmethacrylate (PMMA) based materials have been applied in biomedical applications since the 1930s. They were first utilized for odontology and subsequently in orthopedic applications. Many attempts have been made to improve their mechanical properties due to their initial comparative weakness. One of the ways to accomplish this, is the addition of a reinforcing filler or fibers into the polymer matrix. Carbon based nanomaterials, including CNT powders, G and GO have been investigated due to their ability to improve the mechanical properties, thermal and electrical conductivity. For example, in 2017, Paz et al. studied G and GO nano-sized powders, with a loading ranging from 0.1 to 1.0 *w/w* % as reinforcement agents for PMMA bone cement. They examined the mechanical properties of the resulting PMMA/G and PMMA/GO nanocomposites such as: bending strength, bending modulus, compression strength, fracture toughness and fatigue performance. They found that the mechanical strength of PMMA/G and PMMA/GO bone cements was enhanced at low loading ratios (≤0.25 wt%), especially the fracture toughness

and fatigue performance. This was attributed to the G and GO inducing deviations in the crack fronts and hampering crack propagation. It was also observed that a high functionalization ratio of GO (as compared with G) resulted in better improvements due to the creation of stronger interfacial adhesion between GO and PMMA. The use of a loading ratio ≥0.25 wt% led to a decrease in the mechanical properties as a consequence of the formation of agglomerates as well as to an improvement in the porosity [38].

Moreover, the formation of highly porous 3D nanostructure networks and with a favorable microenvironment makes it possible to use GO in bone tissue engineering [39]. In 2016, Kumar et al. prepared PEI (polyethyleneimine)/GO composites for application in bone tissue engineering as scaffolds. They claimed that the PEI/GO could encourage proliferation and formation of focal adhesion complexes in human mesenchymal stem cells cultured on poly (ε-caprolactone) (PCL). The PEI/GO composite induced stem cell osteogenic differentiation causing near doubling of ALP expression and more mineralization compared to unmodified PCL with 5% filler content, and was about 50% better than GO alone. 5% PEI/GO was as effective as addition of soluble osteoinductive factors. They attributed this phenomenon to the enhanced absorption of osteogenic factors due to the amino and oxygen-containing functional groups on the PEI/GO leading to boosting of the stem cell differentiation process. Moreover, they reported that PEI/GO exhibited a better intrinsic bactericidal activity compared to neat PCL with 5% filler ingredients and GO alone. They concluded that PEI/GO-based polymer composites could function as resorbable bioactive biomaterials, as an alternative to using less stable biomolecules in the engineering orthopedic devices for fracture stabilization and tissue engineering. The polymer and GO nanocomposites not only have superior morphological properties for scaffolds, but their high bioactivity makes it possible to allow repair of bone defects [40]. The mechanical strength and stability of the material is an important factor in the design of scaffolds for tissue engineering. GO-based composites possess highly porous structures and great mechanical strength that gives them good potential for bone regeneration scaffolds. Liang et al. reported that HAp/collagen (C)/poly(lactic-co-glycolic acid)/GO (nHAp/C/PLGA/GO) composite scaffolds could stimulate proliferation of MC3T3-E1 cells (Fig. 3) [41]. They prepared nHAp/C/PLGA/GO nanomaterials with various GO weight percentage for preparation of scaffolds, measured the mechanical properties of the scaffold.

The results showed that 1.5 wt% GO could increase the mechanical strength of the scaffold and provided a good substrate for adhesion and proliferation of the cells. In addition to these advantages, the presence of GO in (nHAp/C/PLGA/GO) improved the hydrophilic properties of the scaffolds, which can facilitate the adhesion of cells. Changes in contact angle with different percentages of GO increased the wettability of the scaffold surface due to the presence of more hydroxyl functional groups in the GO. The nHAp/C/PLGA/GO scaffolds showed different pore diameters (0–200 nm) and the sample with 1.5% GO had the best mechanical strength. Increasing the weight percentage of GO also increased the MC3T3-E1 osteoblast cell proliferation rate. There were more cells measured at 1, 3, 5 and 7 days with the nHAp/C/PLGA/GO scaffold with 1.5%wt GO compared to lower GO weight percentage. SEM images of the cell proliferation illustrated the GO effect (Fig. 4). According to SEM images, the cell numbers (white areas) after 3, 5 and 7 days for 1.5% GO were higher than those with 0%, 0.5% and 1% GO [41].

Recently, Natarajan et al. described composites of galactitol-polyesters that had different percentages of GO and a high modulus and low toxicity. The mechanical strength decreased when the weight percentage of GO increased from 0.5 to 1.0%. A further increase of GO up to 2% wt gave an even worse influence on the mechanical stability. Therefore the GO weight percentage seems
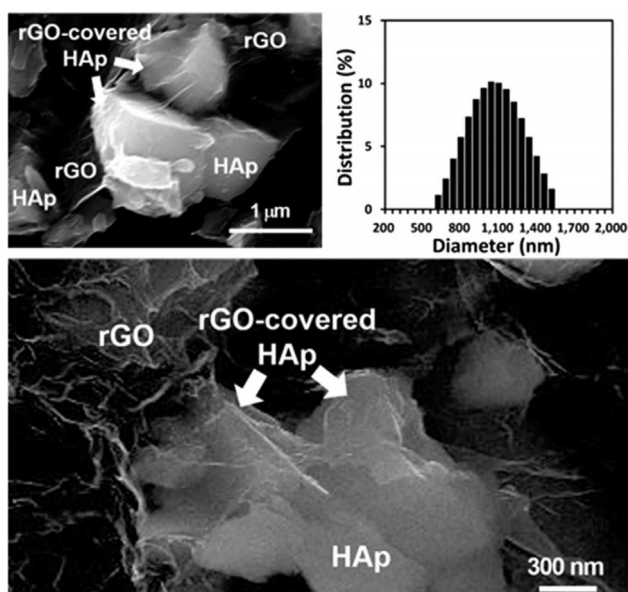


**Fig. 2.** FESEM images of rGO/HAp nanocomposites. The morphology of the HAp was irregular-shaped granules with a mean particle size of 960 ± 300 nm, with the HAp particles partly covered and interconnected by a network of rGO [37]. Open access article with no copyright permission.
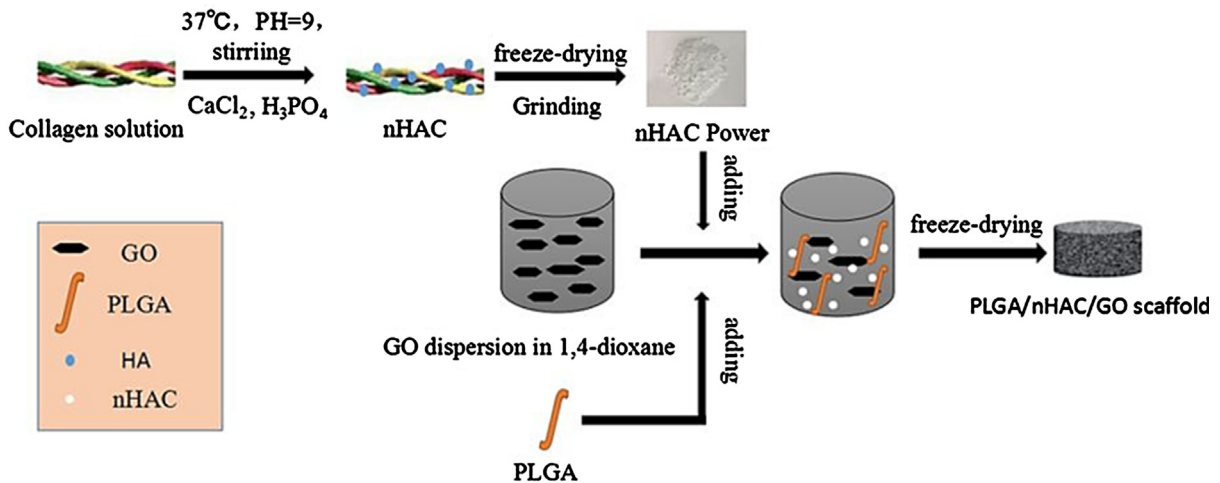
**Fig. 3.** Experimental schematic procedures for nHAp/C/PLGA/GO scaffold preparation [41]. Open access article with no copyright permission.
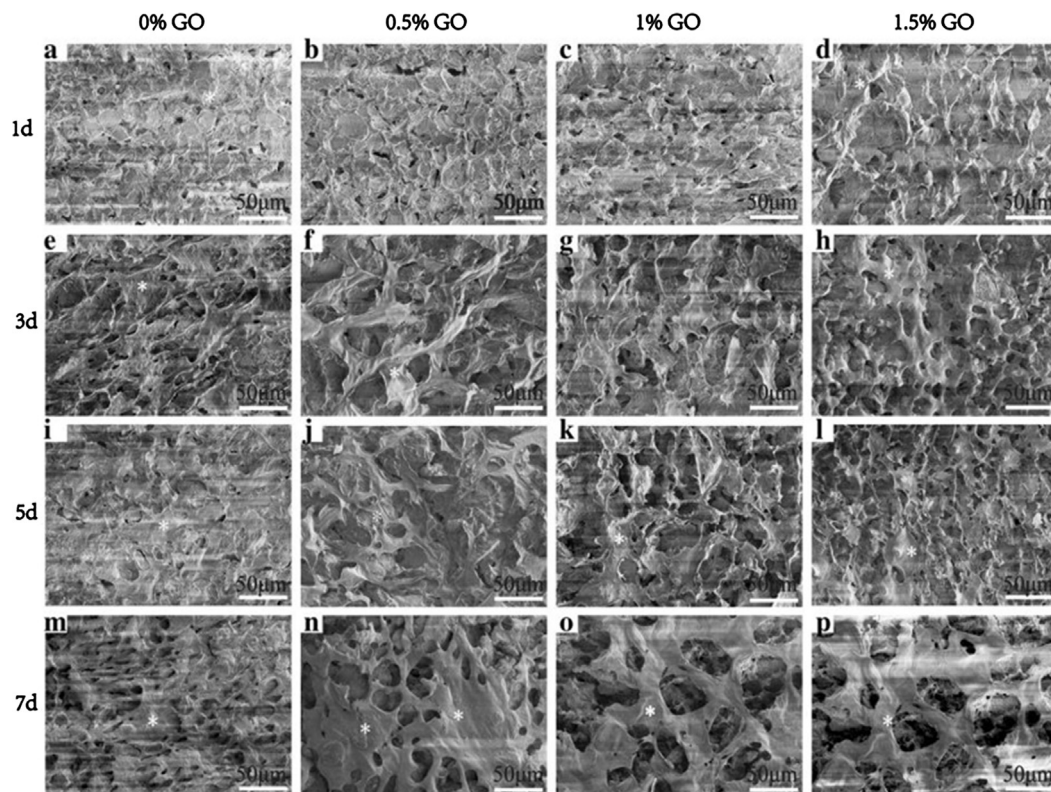


**Fig. 4.** SEM images of MC3T3-E1 osteoblast cell proliferation with different amounts of GO in the nHAp/C/PLGA/GO scaffolds. (NB the white areas shows the cells) [41]. Open access article with no copyright permission.

to be an important factor in scaffolds for bone regeneration [42]. Recently, Zhou and coworkers developed composite fibrous scaffolds for bone regeneration produced from poly(3-hydroxybuty rate-co-4-hydroxybutyrate) and GO by an electrospinning fabrication technique. The obtained materials showed high porosity, hydrophilic surface, mechanical stability and could stimulate osteogenic differentiation [43]. In another study, Luo et al. described the fabrication of PLGA-GO fibrous biomaterial scaffolds for bone regeneration with good cell adhesion that stimulated proliferation and osteogenic differentiation of human mesenchymal stem cells. Composite scaffolds with GO and PLGA can stimulate expression of osteogenesis-related genes, which control the

production and release osteocalcin and non-C proteins [44]. GO composite scaffolds could also be candidates as sensitizing agents for photothermal therapy or magnetic hyperthermia of tumors. Zhang et al. described paramagnetic nanocomposite ($Fe_3O_4$/GO) scaffolds based on GO and $Fe_3O_4$ for hyperthermia of bone tumor cells for the first time. The tumor cells could proliferate on the scaffold substrate, and when an adjustable external magnetic field was applied there was a controllable increase in temperature. Three-dimensional β-tricalcium phosphate-based scaffolds with surfaces modified by $Fe_3O_4$/GO (named β-TCP–Fe–GO) could also be employed in bone regeneration. The external magnetic field could increase the tumor cell temperature up to 50–80 °C, for a 1% $Fe_3O_4$/

GO composite. 75% of the target cells were destroyed, and moreover the results for osteogenic differentiation and proliferation of rabbit bone marrow stromal cells (rBMSCs) were better than without β-TCP–Fe–GO [45].

Recent studies have suggested that the presence of certain metal ions at precise concentrations in scaffold materials could accelerate bone cell proliferation. In this regard, Kumar et al. investigated strontium ion release from hybrid rGO(rGO-Sr) nanoparticles and its effect on osteoblast proliferation and differentiation. They used a PCL matrix with rGO-Sr composite for the scaffold with a strontium weight percentage in rGO of 22% [46]. The advantages of GO in tissue engineering can be summarized as mechanical strength and hydrophilicity to enhance the scaffolds, increasing the adhesion, and accelerating the proliferation of cells. One example is a poly(propylenefumarate)/polyethyleneglycol/GO-nanocomposite-based scaffold (PPF/PEG-GO) reported by Díez-Pascual et al. Their studies showed that the PPF/PEG-GO nanocomposite was the best candidate for bone tissue engineering and medical applications. Along with different amounts of PEG in the PPF polymer, the addition of GO enhanced the physiochemical properties of the PPF/PEG based scaffold. The increase in mechanical strength, biodegradability, a high rate of cell growth and osteogenic differentiation of bone cells on this scaffold were better than the PPF/PEG-based polymer alone. The SEM images and schematic representation of the composite are shown in Fig. 5 [47].

In continue, Song et al. developed a composite foam with 3D-rGO and polypyrrole on nickel as a mechanically stable bone regeneration scaffold. This demonstrated good ability to stimulate MC3T3-E1 osteoblastic cell proliferation (6.6 times). This new class of scaffold were fabricated using a layer-by-layer (LBL) method and an electrochemical deposition technique, proposed to be a low-cost and simple strategy for scaffold fabrication [48]. However, one of unsolved challenges in bone tissue engineering is the weak attachment between biopolymers and bioceramics at the molecular scale. However, Peng et al. reported the application of GO as a potential solution for this problem. They reported that electrostatic and π-π interactions have a key role in the formation of strong interactions between polyether-etherketone (PEEK) biopolymer and HAp bioceramic [49]. Scaffolds are highly porous biomaterials which can be used as drug loading vehicles to reduce pain and inflammation in surgical sites in the bone. Ji et al. introduced an aspirin-loaded C-GO-HAp-based scaffold, fabricated by LBL biomineralization technique. The loading and controlled release of aspirin from the porous scaffold substrate (300 nm pore size) significantly reduced pain and

inflammation in the bone surgical site. Wu et al. prepared a GO-based β-tricalcium phosphate bioactive ceramic as a bone regeneration scaffold with high osteogenic ability both *in vivo* and *in vitro*. They found that the addition of GO to β-tricalcium phosphate improved osteogenic proliferation and activated signaling pathways within human bone cells compared to β-tricalcium phosphate alone [50]. The adhesion of bone cells to the underlying substrate is one of the important factors that can influence the mechanical properties of the bone produced in tissue engineering. In recent years, many studies have concentrated on this issue. For instance, Mahmoudi et al. developed a nanofibrous matrix for enhancement of adhesive forces between bone cells, using electrospun material. They used biopolymers and GO hybrids for this purpose with good mechanical strength and biocompatibility, and subsequently an efficient wound closure rate. The experimental design process of this material is illustrated in Fig. 6 [51].

In summary, GO based materials have a broad range of applications in bone regeneration and tissue engineering. The high surface area, suitable wettability, remarkable mechanical properties, high adhesion ability, and rapid onset of stimulation effects are impressive advantages of GO nanomaterials. Moreover, these materials can solve the weak interaction between bioceramics and biopolymers by introducing strong electrostatic and π-π stacking interactions. Therefore, GO will likely continue to attract the attention of scientists for bone regeneration and other fields of tissue engineering in the future. Three points concerning the use of GO in bone tissue engineering scaffolds are as follows. Firstly, the presence of GO in the natural biopolymer-based scaffolds has better stimulant effects on the mineralization process of bone tissue in comparison to synthetic polymers. Secondly, the presence of GO in the polymeric scaffold matrix can facilitate the growth of bone cells and their spreading process on the scaffold surface for both the natural and synthetic polymers, but the fraction of dead cells on the GO synthetic polymer scaffold was higher than GO natural biopolymer scaffold. Thirdly, although the fraction of dead cells on the GO synthetic polymer scaffold was higher than GO natural biopolymer, GO natural biopolymer scaffolds can produce bone tissues with better mechanical strength.

A summary of reports about GO nanomaterials and their application in bone tissue engineering is shown in Table 1. The contents of this Table cover physiochemical properties of GO nanomaterials, synthesis methods, clinical trials and the type of scaffolds that have been used. Also, the various stem cells, different growth factors and nanomaterials that have been applied.
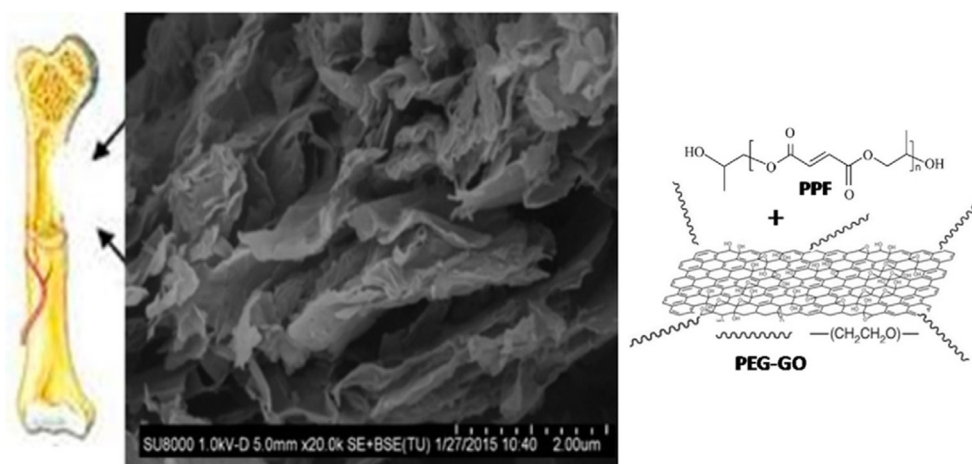


**Fig. 5.** SEM image of PPF/PEG-GO composite and molecular representation of PPF matrix with PEG-GO that have been applied as a scaffold for bone tissue engineering [47]. Copyright ACS reprinted with permission.
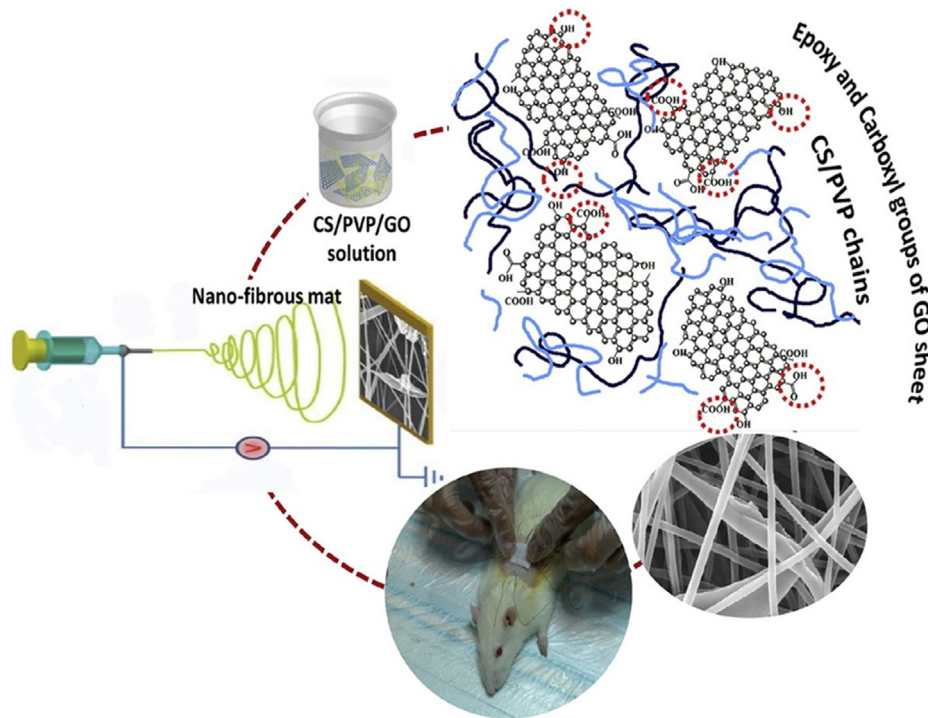
**Fig. 6.** The fabrication process of the biopolymer-GO composite involves chitosan (CS), poly(vinyl pyrrolidone) (PVP) and GO using an electrospinning method [51]. Copyright Elsevier reprinted with permission.

**Table 1**
Applications of GO-based nanoparticles in bone tissue engineering.

| Method of NP synthesis | Type of NPs | Growth factor | Cell type | Mechanical strength (MPa) | Application | Ref. |
|---|---|---|---|---|---|---|
| Electrostatic LBL assembly followed by electrochemical deposition | HAp and polypyrrole | N/A | MC3T3-E1 osteoblast | 185.94 ± 10.76 | N/A | [48] |
| Biomineralization of GO/C scaffolds | C | BMP-2 | Bone marrow stromal cells | 0.65 | *In vivo* and *in vitro* | [52] |
| Modified "Hummers and Offeman" method | HAp | N/A | Osteogenesis of MC3T3-E1 preosteoblasts | – | *In vivo* | [53] |
| LBL technique with biomimetic mineralization | C-HAp nanocomposite film | N/A | mMSCs | – | *In vitro* | [54] |
| Modified Hummer's method | C/Hyaluronic acid (HA) containing an osteogenesis-inducing drug simvastatin (SV) | N/A | MC3T3 cells | 10.0 | *In vitro* | [55] |
| Modified Hummer's method | PLGA, tussah silk fibroin (SF) | N/A | mMSCs | 53 | *In vivo* | [56] |
| Modified Hummers method | C/PVP nanocomposite | N/A | Rat mesenchymal stem cell line | 14 ± 0.7 | *In vivo* and *in vitro* | [51] |
| Modified Hummers method | PLGA nanofiber scaffolds | N/A | Mesenchymal stem cells | 134.4 ± 26.5 | N/A | [57] |
| Modified Hummers method | Sodium titanate | N/A | Human periodontal ligament stem cells | – | *In vitro* | [58] |
| Prepared by chemical oxidation of graphite flakes following a modified Hummers process | HAp rods with good biocompatibility incorporated into PLA | N/A | Human osteoblast cell line Saos-2 | 12.69 ± 0.86 | N/A | [59] |
| Modified Hummers and Offeman method | C sponge | N/A | Osteoblastic MC3T3-E1 cell | 0.125 | *In vitro* and *in vivo* | [60] |
| Modified Hummers and Offeman method | HAp | N/A | Human mesenchymal stem cells | – | *In vitro* | [61] |
| N/A | PMMA/PLC fluorapatite (FA) | N/A | MG63 osteoblast cells | 66.5 ± 4.4 | *In vitro* | [62] |

## Carbon nanotubes in bone tissue engineering

CNTs are allotropes of carbon with a long thin cylindrical morphology. They have unique properties that make them useful materials in different fields such as electronics, nanotechnology, optics, and particularly in the human-machine interface at a cellular level. SWCNT and multi wall CNT (MWCNT) are of considerable interest for a variety of biomedical purposes based on their impressive physical properties. They have a tensile strength $\geq 50$ GPa, Young's modulus $\geq 1$ TPa, conductivity $\sigma_{in} \geq 10^7$ S/m, maximum

current transmittance $J_{in} \geq 100\,MA/cm^2$, density $\rho \leq 1600\,kg/m^3$, all of which are important in these advanced biocompatible composite materials [17,63,64]. The SWCNTs have a diameter about 0.8–2 nm. The length of CNTs varies from less than 100 nm to as long as several cm. Nanobiomaterials like CNTs with protein/peptide attachments have been widely studied and optimized using material engineering methods. However pristine CNTs need to be functionalized in order to be used effectively. The biocompatibility of CNTs is still uncertain, due to their toxic nature and insolubility, and their similarity to asbestos fibers. Additional investigations are required to assure their biocompatibility. In spite of these reservations, there is no doubt that the CNTs could be extremely promising because of their exceptional mechanical strength, ultrahigh specific surface area, excellent electrical and thermal conductivity. The two categories of CNTs, SWCNTs and MWCNTs can both be used in tissue engineering. In SWCNTs, a cylindrical tube-like structure is formed by rolling up a single G sheet; while MWCNTs are made of multi-layered G cylinders with higher diameter (∼5 nm; depending on the number of layers) that are concentrically nested like rings in a tree trunk. Both SWCNT and MWCNT show high tensile strength, ultra-lightweight and high chemical and thermal stability. Moreover, it has been proved that CNTs can buckle and reversibly collapse as determined by the stiffness and resilience. CNTs have an axial Young's modulus of about 1 TPa and a tensile strength of 150 GPa caused by the hexagonal molecular network having high stiffness of the C—C bonds. Consequently, CNTs function as stiff materials, which have the capacity to deform either electrically or under compression. The modification of the CNT surface or functionalization of their surface can be an efficient method for enhancement of cell-scaffold interactions and subsequently the cell spreading on the scaffold surface microenvironment. For functionalization of the CNT surface, many strategies have been reported. Covalent functionalization is divided into three major approaches: (i) Cationic, anionic and radical polymerization; (ii) Click chemistry (biomolecules, metal hybrids nanomaterials and macromolecules); and (iii) Electrochemical polymerization. In order to compare and contrast covalent and non-covalent functionalization methods for CNTs, their general features are summarized below [65].

*Non-Covalent Functionalization:*
- van der Waals interaction
- Structural network is retained
- No loss of electronic properties
- Wrapping of molecules around the CNT surface
- Uses adsorption of polymers, surfactants, biomolecules, nanoparticles, etc.

*Covalent Functionalization:*
- Formation of stable chemical bonds
- Destruction of some $\pi$-bonds
- Loss of electronic properties
- Uses side-wall attachment and end-cap attachment
- Reactions include oxidation, halogenation, amidation, thiolation, hydrogenation, etc.

The broad polymeric materials have been used for functionalization of CNTs for scaffold designing aims. The synthetic and natural biopolymers are their general categories. Biodegradable polymers such as PVA (polyvinyl alcohol), PEG [66], PLGA, PLA, and PU (polyurethane) [67] which are all synthetic polymers, and C, gelatin, CS, and SF (natural polymers) [68–70], as well as biodegradable ceramics, such as bioactive glass [71,72] have all been reported to serve as scaffolds for tissue engineering. The use of some kinds of materials is limited in bone tissue, because of specific disadvantages. These include polymers (because of their poor mechanical strength and Young's modulus,) and ceramics (because of their brittleness).

Shokri et al. presented a new approach to fabrication of a nanocomposite scaffold for bone tissue engineering by using a composite of bioactive glass (BG), CNTs, and CS in different ratios. They found that a specific combination of these three materials had the best mechanical, chemical, and cell-stimulating properties, and was the most appropriate for repairing trabecular bone tissue [73]. In 2016, Li et al. successfully fabricated CNT-HAp composites by a double *in situ* synthesis, combining the first *in situ* synthesis of CNTs in HAp powder by chemical vapor deposition (CVD), with a second encapsulation of CNTs into HAp by a sol-gel method. The flexural strength of the composite was up to 1.6 times higher than that of pure HAp, and higher than that of conventionally prepared CNT/HAp composites. These CNT/HAp composites increased the proliferation of fibroblast cells in comparison to those fabricated by traditional methods. (Fig. 7) [74].

In 2016, Zhang et al. fabricated nanoHAp/polyamide-66 (nHAp/PA66) porous scaffolds by a phase inversion method. In their study, CNTs and SF were used to modify the surface of the nHAp/PA66 scaffolds by freeze-drying and cross-linking. The nHAp/PA66 scaffolds with CNTs and SF performed well as bone tissue engineering scaffolds. Furthermore, a dexamethasone (DEX)-loaded CNT/SF-nHAp/PA66 composite scaffold could promote osteogenic differentiation of bone mesenchymal stem cells, and drug-loaded scaffolds were proposed to function as effective bone tissue engineering scaffolds. Many studies have been reported concerning the effect of CNTs coated on scaffold surfaces on cell growth and proliferation [75–79]. Hirata et al. studied 3D-C scaffolds coated with MWCNTs and investigated cell adhesion to MWCNT-coated C sponges. Their analysis of the actin stress fibers revealed that after seven days of culture, stress was more evident in Saos2 cells growing on CNT-coated materials. MWCNT-coating creates a more suitable 3D scaffold for cell culture compared to SWCNTs [77]. Studying the impacts of LBL assembled CNT-composite on osteoblasts *in vitro* and on *in vivo* rat bone tissue, Bhattacharya et al. found that CNT-coated materials could increase cell differentiation as measured by ALP activity. These studies suggested that CNTs might have some interesting biofunctionalities [78,80,81]. Zanello et al. studied the use of CNTs for osteoblast proliferation and bone formation, concluding that CNTs carrying a neutral electric charge produced the highest rate of cell growth, and observed the production of plate-like crystals correlating with a change in the cell attachment in osteoblasts cultured on MWNTs [80]. Cellular senescence in biological organs frequently occurs through an ontogenetic process, and occurs naturally to a great extent in embryogenesis. It is a natural and necessary process in the development of individual organisms and in organs. Chen et al. synthesized surface-modified PCL-PLA acid scaffolds using a combination of self-assembled heterojunction CNTs and insulin-like growth factor-1 (IGF1). They investigated cellular senescence and the possible underlying mechanism by characterizing the functionality and cell biology features of these scaffolds and demonstrated the anti-senescence functionality of the self-assembled heterojunction CNT-modified scaffolds in bone tissue engineering, being able to accelerate bone healing with extremely low *in vivo* toxicity [82]. Park et al. suggested a new method for the biosynthesis of a CNT-based 3D scaffold by *in situ* hybridizing CNTs with bacterial cellulose (BC). As there are some difficulties in the fabrication of 3D-microporous structures using CNTs [83–87], the *in vivo* applications of CNTs are still very limited. In order to have enough surface and space for cell adhesion, migration, growth, and tissue formation in tissue engineering scaffolds, it is necessary to construct the 3D-microporous structure. Because of the structural features of MWCNTs, 3D-MWCNT-based morphologies are considered a good choice for scaffolds/matrices in tissue engineering [88]. To obtain effective bone grafts, the use of nano-scale fibers was
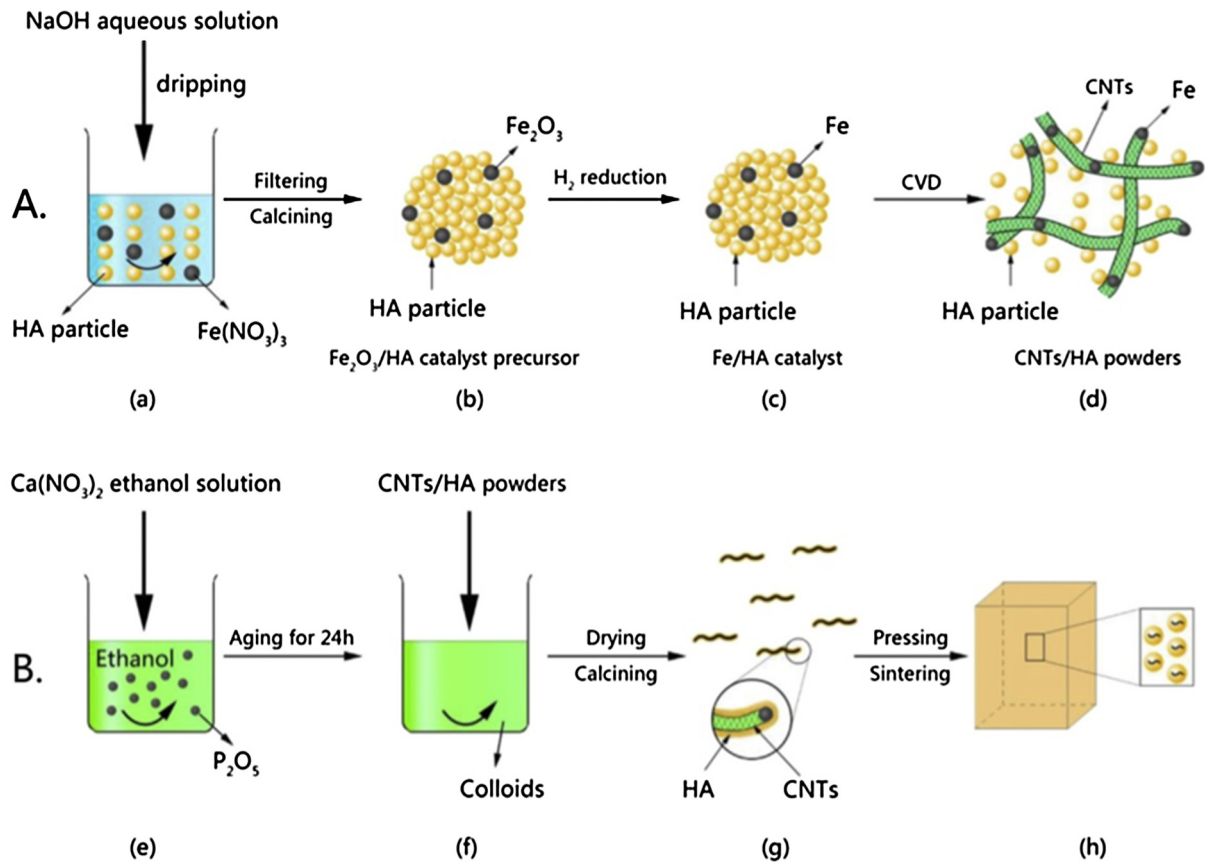
**Fig. 7.** Fabrication procedure of CNT/HAp composites: (A) *In situ* synthesis steps of CNT/HAp composite powders by CVD: (a) preparation process of the catalyst precursor by a deposition-precipitation route, (b) formation of the $Fe_2O_3$/HAp catalyst precursor (first calcination process), (c) homogeneous spread of the active Fe nanoparticles on the surface of HAp powder, (d) *in situ* synthesis of CNTs on HAp particles by CVD; (B) *in situ* modification of the CNTs with HAp by a sol-gel method: (e) preparation of the colloids, (f) formation of the colloids by aging for 24 h, (g) formation of the CNTs at HAp powder (twice calcination), (h) fabrication of the bulk composite by pressing and sintering steps [74]. Copyright Elsevier reprinted with permission.

reported [89]. The appropriate mechanical properties allowed better cell attachment to these fibers. DeVolder et al. developed a PLGA-C hydrogel system which can be used to enhance the performance of osteoconductive matrices [90]. Henriksson and Berglund studied the structure, as well as the physical and mechanical properties of nanocomposite films constructed from microfibrillated cellulose (MFC) and from MFC in combination with melamine formaldehyde (MF), and confirmed that the BC had a 3D-microporous structure. Other studies have shown that some structural aspects of BC are favorable for tissue engineering scaffold applications, including large pores and the presence of nano-scale fibers in the 3D-structure [91–94]. As a result, Park et al. proposed the hybridization of CNTs with BC to provide an environment suitable for bone regeneration *in vivo,* combining the osteogenic effects of CNTs and the good scaffold properties of BC. C is a natural polymer suitable for construction of biocompatible cell scaffolds. The structural properties and cellular interactions of C with a wide range of other biomaterials used in tissue engineering have been studied [95–97]. Among the different types of C, Type I C is the major organic component of bone tissue. In this regard, having analyzed a 3D-biocomposite scaffold produced using a combination of type I C, mineral trioxide aggregate (MTA) and MWCNTs, Valverde et al. showed that combinations of type I C, MTA and MWCNT are biocompatible, and therefore may be useful as bone tissue mimetics. The 3D-scaffold fabrication and experimental design are depicted in Fig. 8. As a brief explanation, the MTAs are calcium silicate materials that have been used for stimulating the biomineralization process in bone tissue engineering [98].

Because of the tunable properties of synthetic polymers, they have attracted great interest in the tissue engineering field. PVA has appropriate physicochemical properties and a biocompatible nature so it has been used in tissue engineering, wound dressings and drug delivery [99,100]. On the other hand, PVA has poor mechanical strength. This disadvantage of PVA has limited its applications in bone tissue engineering. Hence, many researchers have tried to improve the mechanical and biological performance of PVA as a biomaterial. One way is to add an appropriate and biocompatible reinforcement material into the PVA matrix in order to improve the mechanical features. The reinforcement of the polymer matrix by CNT may result in improved mechanical and viability [101]. Kaur and Thirugnanam demonstrated the utility of PVA–CNT nanocomposite scaffolds for accelerating bone tissue regeneration, especially when the concentration of CNT was relatively low. They also showed that the dispersion of CNT in PVA matrix was homogeneous because of the interactions between carboxylic acid functionalized CNT with PVA, and this combination resulted in improvement in the surface morphology, biological activity, protein adsorption, and mechanical properties of the nanocomposite scaffolds [102]. Although it is widely accepted that CNTs have unique properties, there is one drawback that may limit the application of CNTs in the field of biomechanics. The outer walls of pristine CNTs are relatively inert and do not undergo many chemical reactions. As a result, in order to provide biocompatibility and solubility [103,104] special functionalization methods are required. In this regard, there are two approaches, which are noncovalent and covalent functionalization [103,104]. In the noncovalent approach, long polymer chains (e.g. polystyrene sulfonate) are wrapped
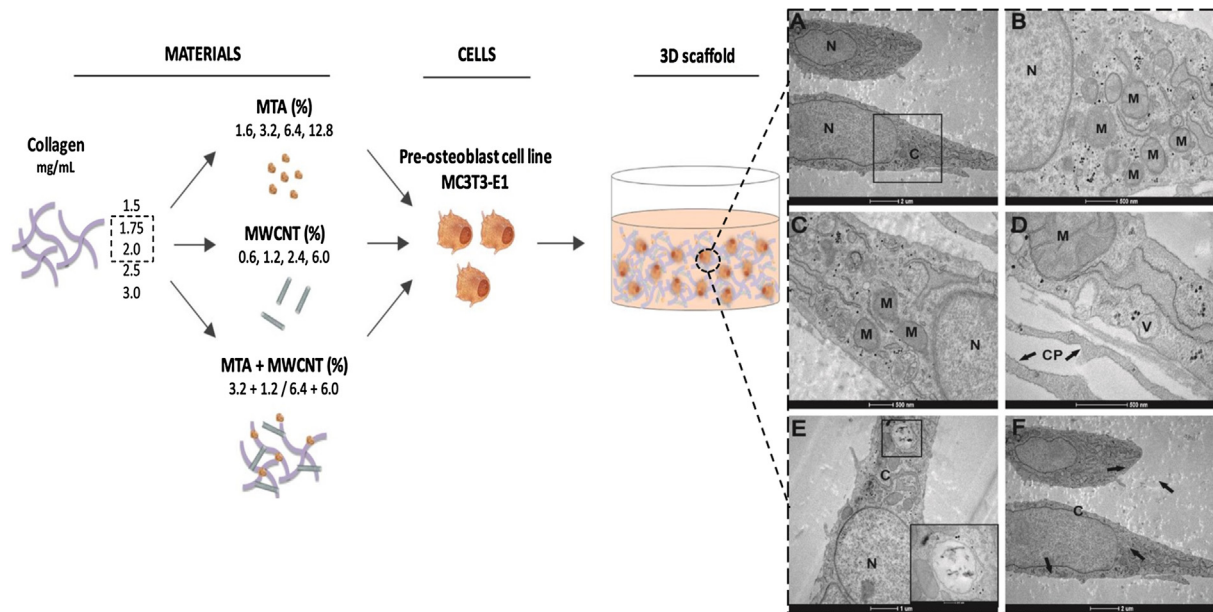
**Fig. 8.** Schematic of 3D scaffold fabrication and parameters varied in experimental design and TEM image of cell proliferation on scaffolds in Ref. [98]. Copyright Elsevier reprinted with permission.

around the CNTs and the CNTs are dispersed in the polymer matrix, while in the covalent or chemical approach, direct covalent bonds are formed with the carbon atoms [105]. Noncovalent modification involves relatively mild conditions (sonication, room temperature, etc.) and does not affect the basic CNT structure [106] nor their optical and electrical properties [103–107]. The covalent approach is used in most of the current functionalization methods and also ensures a strong bond between the CNTs and the coupling agent. However covalent modification may result in partially loss of the mechanical strength of CNTs (depending on the severity of the oxidation conditions) and also takes a longer time than noncovalent modification [108].

In the comparison between different types of CNTs, and their influence on bone cell growth and attachment, the structural and molecular interactions within the scaffold microenvironments can be discussed. SWCNTs with their high specific surface area can supply more sites for efficient adhesion of cells on the scaffolds, while for MWCNTs, it is possible that the more aggregated state of MWCNTs will disrupt the efficient connection between the cells and the scaffold surface. Although the cytotoxicity of CNTs in bone tissue engineering is still a challenge because of the complicated interactions between CNTs and cellular processes, the presence of CNTs in the scaffold matrix could enhance cell-scaffold interactions. Because of the smaller number of oxygen atoms contained in the functional groups of functionalized CNTs, the cell spreading and aggregation in the scaffold microenvironments are less efficient than GO-based scaffolds. Some reports that discussed the application of CNT-based materials in bone tissue engineering have been summarized in Table 2.

## Carbon dots in bone tissue engineering

The term CDs refers to the zero dimensional carbon nanomaterials about 10 nm in size [118]. CDs can be spherical [119], crystalline or amorphous containing $sp^2$ [120] or $sp^3$ hybridized carbon atoms that have been synthesized with laser irradiation on carbon sources [121]. The interesting physical and optical properties of CDs have encouraged their use for biological application [122]. CDs have a broad band of wavelength absorption ranging

from 260 to 320 nm [123,124] and size-dependent optical emission, a high quantum yield for photoluminescence [125], low toxicity [126,127] a tunable surface that have been explained broadly by the Wang group [128,129] and suitable electron transfer properties [130,131]. These properties make CDs a good option for applications in biomedicine [132], biosensors [133–135], solar cells [136,137], supercapacitors [138] and photocatalysts [139,140]. Recently, the potential of CDs and other carbon nanomaterials has been tested in bioimaging applications [123,141], drug delivery [142] and in bone tissue engineering fields [143]. Other applications have involved optoelectronics [144], biosensing [145], bioimaging [146], medicinal [147] and catalysis [148]. CDs-based biological scaffolds have been suggested as materials for bone regeneration, and to repair bone defects. Gogoi et al. developed CD-peptide composites embedded in a tannic acid and PU matrix for *in vivo* bone regeneration. Their results indicated that a mixture of 10% wt gelatin in polymeric CD-peptide exhibited the best biological activity, in terms of osteoblastic adhesion, osteogenic differentiation, and cell proliferation [149]. According to this work, four different peptides (viz. SVVYGLR [150], PRGDSGYRGDS [151], IPP [152], CGGKVGKACCVPTKLSPISVLYK [153]) could be used as bioactive properties in scaffolds. These four peptides can stimulate angiogenesis, adhesion, osteoblast differentiation, and osteogenesis, respectively. In another report, they found that a CD@HAp composite in a PU matrix as a scaffold (CD@HAp/PU) showed good biological activity. This new CD-based scaffold exhibited good potential for bone tissue engineering and used cheap and disposable materials for the hydrothermal synthesis of HAp. The best Ca/P (calcium/phosphorus) ratio that was obtained (1.69) compared well with that of natural bone sample Ca/P ratio (1.67). Study in MG 63 osteoblastic cells revealed that these CD-based nanocomposites had excellent mechanical properties and good osteogenic activity. According to the results, the uniform distribution of the CDs in HAp, and cross-link formation between CDs and PU were the reasons for the high mechanical strength of the scaffolds. Some studies have indicated that the effect of surface functionalization on cross-link formation improves intermolecular interactions and mechanical properties in scaffolds. Cell proliferation results showed that CD-based scaffolds were superior to CD-free scaffolds after 7 days. The CDs help the HAp to distribute

**Table 2**
Application of carbon nanotubes in bone tissue engineering.

| Size | Method of NP synthesis | Type of NPs | Growth factor | Cell type | Mechanical strength (MPa) | Application | Ref. |
|---|---|---|---|---|---|---|---|
| 10–20 nm | *In situ* hybrids CNTs with bacterial cellulose | BC | Col-BMP-2 | Osteogenic cells | 0.474 | *In situ* | [83] |
| 15 nm | Thermal | HAp | N/A | Human osteoblast sarcoma cell lines. | – | *In vitro* | [109] |
| 30 to 70–175 nm | CVD | HAp | CCK-8(Cell Counting Kit-8) | Osteoblastic and fibroblast (L-929) cells | 89 | *In situ* | [74] |
| 9.5 nm | Freeze drying method | PVA | N/A | Osteoblast cells | 215.00 ± 9.20 | *In vitro* | [102] |
| N/A | CVD | G/SWCNT | N/A | rMSCs | – | N/A | [110] |
| 30 nm and a length of 10–30 μm | CVD | G nanosheets and HAp-PEEK | N/A | MG-63 cells and hBMSCs | 78.65 | *In vitro* | [111] |
| N/A | N/A | C/ COOH-SWCNTs nanocomposite films | TGF-Beta 1 | rBMSCs | – | *In vitro* | [112] |
| <8 nm | Freeze drying method | Polysaccharide HAp | N/A | MG 63 cell line | 0.222 | *In vitro* | [113] |
| 20–25 nm | N/A | HAp | N/A | Human fibroblast cells (CCD-18 Co) | 37.43 | *In vitro* | [114] |
| S-CNTs, 10–20 nm L-CNTs, 40–60 nm in length | N/A | Surface-modified PCL/PLA scaffolds | Insulin-like growth factor-1 | rBMSCs | – | *In vitro* | [82] |
| <8 nm | Freeze drying method | Tricomponent scaffold with an oxidized fMWCNT alginate HAp | N/A | MG-63 cell line | 0.072 | *In vitro* | [115] |
| 10–20 nm | Thermal | Poly (butylene adipate-co-terephthalate) (PBAT) | N/A | MG63 osteoblast-like cells | 3.5 | *In vitro* | [116] |
| 200–1200 nm | N/A | BG and C | N/A | MG63 osteoblast cell line | 4.6 ± 0.5 | *In vitro* | [73] |
| N/A | Arc discharge method | Type I C, MTA | N/A | osteoblastic MC3T3-E1 cells | – | *In vitro* | [98] |
| 9.5 nm | N/A | 3D C scaffold and β-tricalcium phosphate (β TCP) nanoparticles | N/A | Fibroblast cells | – | N/A | [117] |

uniformly on the polymer matrix for efficient bone regeneration. The efficient proliferation of mesenchymal stem cells plays a key role in replacing damaged or defective organs such as bone tissue. One of the main challenges in the bone tissue engineering field is the efficient mineralization throughout the entire body of the scaffold. CDs with appropriate shape and size can help to solve this problem. Shao et al. introduced CDs as novel materials for efficient osteogenic differentiation of rBMSCs and improvement of the mineralization process. In addition to these advantages, the biocompatibility, nontoxicity and facilitation of osteoblastic gene expression are other benefits of CDs [154]. Sarkar et al. developed CD-carboxymethylcellulose-HAp as a material for osteogenic bone regeneration scaffolds. They suggested that the simple one-pot fabrication method with good biocompatibility, excellent ability for drug loading and specific bone regeneration properties was highly economical [155].

In summary, the unique optical, structural and electron transfer properties of CDs open the way to novel medical, catalytic, bioimaging, biosensing, optoelectronic and especially biological applications. One of the best and critical biological applications of CDs concerns bone tissue engineering. Briefly, the efficient cell interactions and cross-link formation of CDs provided excellent mechanical properties of bone regeneration scaffolds. The uniform and regular distribution of CDs in the matrix can help improve the biological activity of cells. The simple fabrication methods and low toxicity are the most prominent properties of CDs in bone tissue engineering scaffolds. Basically, CDs have been used as an agent that can stimulate osteogenic activity. Therefore, in comparison to the other types of carbon nanomaterials, this ability of CDs is rare. Additionally, the significant effect of CDs on the mechanical strength of the formed bone tissue is related to the density of cells on the scaffold; this issue is more significant for CDs in comparison to other carbon nanomaterials.

## Fullerenes in bone tissue engineering

Fullerenes are closed cage structures composed of sp$^2$ hybridized carbon atoms, with a roughly spherical shape. The C60 and C70 fullerenes are more common than other types of fullerenes. Fullerene materials, have attractive physiochemical properties, which have applications in medicinal chemistry [156], to perturb biological membranes and exert antibacterial activity [157], and in pharmacology [158]. The biological application of fullerene materials opens new avenues in bone tissue engineering. The spherical molecular structure of fullerenes make it possible to use them as free radical scavenger agents in biomedicine [159,160]. For example, fullerene materials show interesting behavior as HIV inhibitors [161] and as neuroprotective agents [162]. Applications in bone tissue engineering have attracted attention of many researchers in recent years, due to observations of an increase in adhesion of cells onto fullerene biomaterials. Bacakova et al. developed carbon nanofibers coated with fullerene layers, which could enhance the adhesion of osteoblastic MG 63 cells, and also increase the cell proliferation up to 4.5 time over 7 days [163]. This group also described fullerene-based microarrays prepared using a metallic "nano-mask" to improve the growth and adhesion of MG 63 osteoblastic bone cells. The hierarchical surface morphology played a key role in cell growth, the cells localized almost exclusively in the grooves between the prominences. In addition, the fullerene-based biomaterial did not allow the cells to grow more than 1 μm in height. The hydrophobic surface of fullerene materials could be the reason for this observation [164]. In another report, this group suggested that fullerenes and other carbon nanoparticle could be therapeutic agents for arthritic bone diseases. Their results indicated that fullerene materials were safe and did not cause DNA damage or alter the morphology of MG 63 and U-2 OS osteoblastic cells, but could increase the biological activity

of bone cells [165]. Krishnan et al. described a new method for the fabrication and deposition of fullerene nanowhiskers onto scaffolds to stimulate growth of osteoblastic MG 63 bone cells. This was accomplished by rotational flow of solutions containing fullerene nanowhiskers allowing the deposition of regular aligned arrays on a glass substrate. They found that the regular aligned deposition of fullerene nanowhiskers had better biological activity than a random deposition. The distance of the glass substrate from the vortex center played a key role in the alignment morphology. Samples fabricated at the edge part of the vortex solution had more regular morphology than samples from central parts of the vortex. A schematic of this sample fabrication is shown in Fig. 9 [166].

Scaffolds of fullerene materials have specific situations in various and broad range of scaffolds in bone tissue engineering. They have good stimulant effects on bone cell proliferation and only low cytotoxicity. Fullerene-based scaffolds have more hydrophobicity and more roughness. This may increase the ability to controlling the cell attachment, improving the bone tissue thickness, and subsequently the mineralization process that is a truly unique property of carbon nanomaterials. The shape of the fullerene molecule makes it possible to organize and control the morphology of the final bone tissue.

## Nanodiamond particles (NDs) in bone tissue engineering

ND particles are a new type of semiconductor quantum dots with advanced applications in medicine and biotechnology [167]. The first nano-scale diamond particles were introduced in 1960s, but they remained relatively unknown until the late 1990s. After that they attracted attention since they exhibited various useful properties [168]. They have high surface areas and tunable surface structures, with useful mechanical and optical properties [169]. These particles are very rigid, biocompatible, conductive, electrically resistant and chemically stable [170]. Multifunctional bone scaffolds can be designed from different polymers and nanoparticles. However, an important future role of NDs particles in tissue engineering cannot be neglected [171]. Zhang et al. fabricated a special bone scaffold using PLLA polymer and octadecylamine-functionalized NDs (ND-ODA) (Fig. 10). These scaffolds were fluorescent and stable as a result of the NDs component. Moreover, they had not adverse effects on cell proliferation and were considered to be safe. In this study, the combination of ND-ODA/PLLA with murine osteoblast (7F2) cells for more than one week showed less harmful consequences. Moreover, NDs and ND-ODA were non-
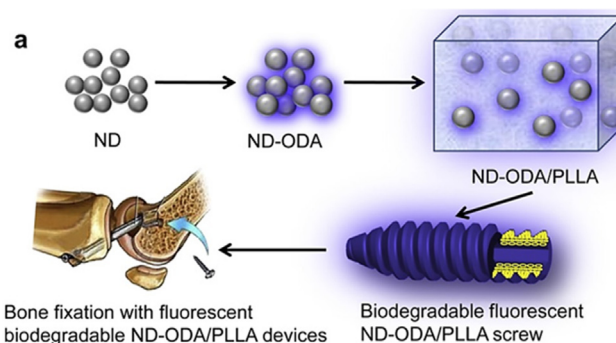


**Fig. 10.** Schematic image of the synthesis of PLLA and ND-ODA and its function in bone tissue engineering [172].
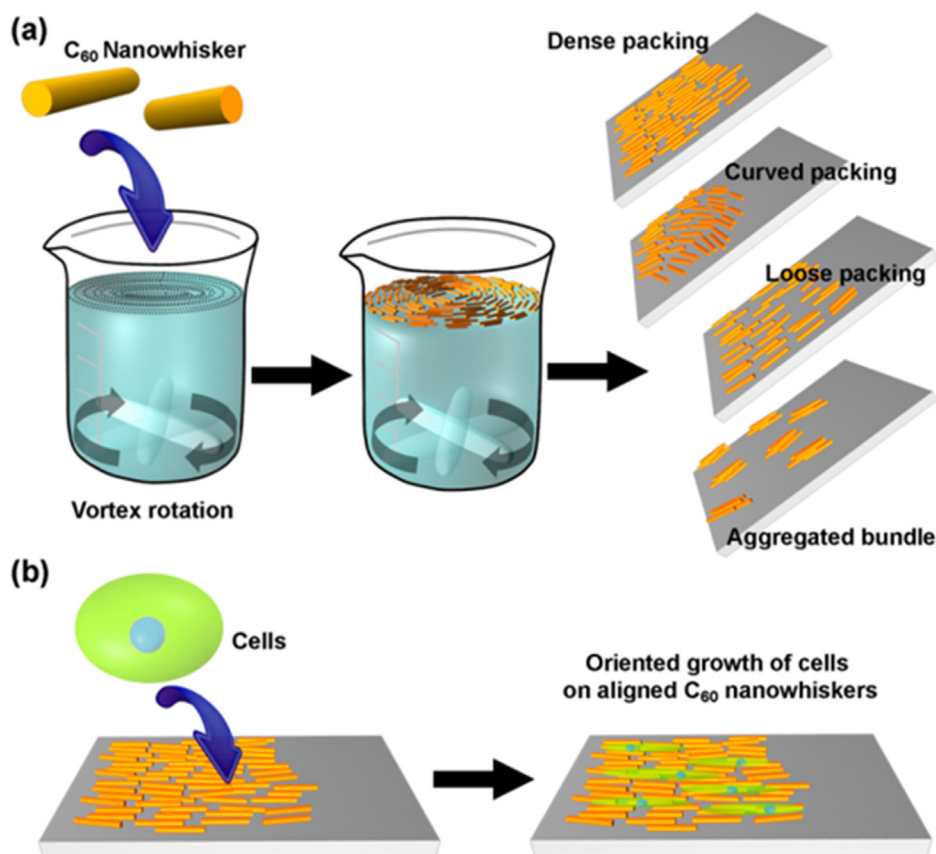


**Fig. 9.** Schematic representation of vortex solution method for fullerene nanowhiskers deposition on glass substrates and different alignments of nanowhiskers [166]. Copyright ACS reprinted with permission.

toxic and biodegradable and could be a good alternative in bone tissue engineering [172].

Following this investigation, Zhang et al. achieved a significant enhancement in the properties of ND-ODA/PLLA scaffolds in bone tissue engineering. They added 10% wt of ND-ODA to pure PLLA which resulted in 280% enhancement of the strain at failure and a 310% improvement of the fracture force in tensile strength [173]. In another study conducted by Parizek et al. polymers coated with NDs, were fabricated for bone tissue engineering. They utilized the electrospinning method for this purpose. Nanofibrous membranes included a PLGA copolymer and NDs gave nanoparticles with 270 ± 9 nm diameter. However, pure PLGA nanoparticles were 218 ± 4 nm diameter. Moreover, the areas of the fiber pores were $0.46 \pm 0.02\ \mu m^2$ and $1.28 \pm 0.09\ \mu m^2$ in pure PLGA samples. Therefore, PLGA-ND exhibited thicker and smaller fibers in comparison to pure PLGA. They found that PLGA-ND had high mechanical resistance and could bind to human osteoblast-like MG-63 cells and enable them to proliferate. The advancements of this technique are being safe and non-toxic, as well as being non-inflammatory [174]. Various significant properties of ND particles makes them reliable and suitable for a broad range of biomaterial and medical applications, including those in bone tissue engineering [175,176]. As a final note, NDs are known to be the only non-toxic carbon nanomaterial, that recently has been used for bone tissue engineering. In comparison to the other carbon nanomaterials, the acceptable mechanical strength, efficient osteogenic activity, good stimulatory effects on the mineralization, together with anti-inflammatory properties are the main advantage of these nanomaterials.

## Conclusions and future perspectives

In this review, the application of carbon nanomaterials as biologically compatible, mechanically stable and commercially viable candidates for use in bone tissue engineering successfully has been summarized. Many nanocarbon allotropes such as GO, CNTs, CDs, fullerenes, NDs and their derivatives have high potential for use as scaffolds for bone cell proliferation and could be used for bone repair applications. The large surface area, good biocompatibility, appropriate biodegradability and excellent stimulation effects on gene expression and proliferation in bone cells are the main advantages compared to other materials. Nevertheless, further study about the low cytotoxicity and possible adverse environmental effects will be necessary before these materials can be clinically tested. Studies into these materials are remarkably expanding, and the next few years will tell us whether their promise will be fulfilled. The availability of carbon nanomaterials on a commercial scale, and their relatively simple synthetic methods can facilitate the usage of these nanomaterials in clinical applications. The cytotoxicity of these materials is mostly acceptable for artificial bone tissue production, but for some of them the risk of unwanted cell death and disruption of cell growth is higher. Although there are limited reports about controlling the cytotoxicity of scaffolds, their cytotoxic effects or disruption of pathways are still unknown. The ability of carbon nanomaterials to undergo surface modification with different chemical compositions or functional groups, are another advantage of these materials, that make it possible to control cell-scaffold interactions. The cell spreading on the scaffold surface directly depends on the chemical composition, and the presence of oxygen containing functional groups that can facilitate cell movement and cell-cell communication. With regard to mechanical properties, carbon nanomaterials can overcome the challenges of the poor mechanical strength of some scaffolds that has caused problems for many years. The large specific surface area, high porosity and effective biological interactions suggests

these nanomaterials may be the most appropriate scaffolds for bone tissue engineering. Finally, evidence shows the good biodegradability of carbon nanomaterials in bone tissue engineering process. The cellular behavior, nutrient exchange efficiency, and the cellular microenvironment on the scaffold surface can be affected by the biodegradation process emphasizing the need for preparation with the highest possible quality.

## Conflict of interest

*The authors have declared no conflict of interest.*

## Compliance with Ethics Requirements

*This article does not contain any studies with human or animal subjects.*

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## References

[1] Khademhosseini A, Langer R. A decade of progress in tissue engineering. Nat Protocol 2016;11(10):1775–81.

[2] Van Vlierberghe S, Dubruel P, Schacht E. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. Biomacromolecules 2011;12(5):1387–408.

[3] Czernuszka J, Sachlos E, Derby B, Reis N, Ainsley C. Tissue engineering scaffolds. WO2003022319A1; 2002.

[4] Krishnamoorthy N, Tseng YT, Gajendrarao P, Sarathchandra P, McCormack A, carubelli I, et al. A novel strategy to enhance secretion of ECM components by stem cells: relevance to tissue engineering. Tissue Eng 2017;24(1-2):145–56.

[5] Merrill JP, Harrison JH, Murray J, Guild WR. Successful homotransplantation of the kidney in an identical twin. Trans Am Clin Climatol Assoc 1956;67 (166):6.

[6] Shafiee A, Atala A. Tissue engineering: toward a new era of medicine. Ann Rev Med 2017;68:29–40.

[7] Gomes ME, Rodrigues MT, Domingues RMA, Reis RL. Tissue engineering and regenerative medicine: new trends and directions-a year in review. Tissue Eng Part B Rev 2017;23(3):211–24.

[8] Murdock MH, Badylak SF. Biomaterials-based in situ tissue engineering. Curr Opin Biomed Eng 2017;1:4–7.

[9] Sitharaman B, Shi X, Walboomers XF, Liao H, Cuijpers V, Wilson LJ, et al. *In vivo* biocompatibility of ultra-short single-walled carbon nanotube/ biodegradable polymer nanocomposites for bone tissue engineering. Bone 2008;43(2):362–70.

[10] Pashazadeh P, Mokhtarzadeh A, Hasanzadeh M, Hejazi M, Hashemi M, de la Guardia M. Nano-materials for use in sensing of salmonella infections: recent advances. Biosens Bioelectron 2017;87:1050–64.

[11] Mokhtarzadeh A, Eivazzadeh-Keihan R, Pashazadeh P, Hejazi M, Gharaatifar N, Hasanzadeh M, et al. Nanomaterial-based biosensors for detection of pathogenic virus. Trends Analyt Chem 2017.

[12] Eivazzadeh-Keihan R, Pashazadeh P, Hejazi M, de la Guardia M, Mokhtarzadeh A. Recent advances in Nanomaterial-mediated Bio and immune sensors for detection of aflatoxin in food products. Trends Analyt Chem 2016;87:112–28.

[13] Berglund L, Forsberg F, Jonoobi M, Oksman K. Promoted hydrogel formation of lignin-containing arabinoxylan aerogel using cellulose nanofibers as a functional biomaterial. RSC Adv 2018;8(67):38219–28.

[14] Villaça JC, da Silva LCRP, Adilis K, de Almeida GS, Locatelli FR, Maia LC, et al. Development and characterization of clay-polymer nanocomposite membranes containing sodium alendronate with osteogenic activity. Appl Clay Sci 2017;146:475–86.

[15] Fakhrullin RF, Lvov YM. Halloysite clay nanotubes for tissue engineering. Future Medicine; 2016.

[16] Komiyama M, Mori T, Ariga K. Molecular imprinting: materials nanoarchitectonics with molecular information. Bull Chem Soc Jpn 2018;91 (7):1075–111.

[17] Gerasimenko AY, Ichkitidze LP, Podgaetsky VM, Selishchev SV. Biomedical applications of promising nanomaterials with carbon nanotubes. Biomed Eng 2015;48(6):310.

[18] Shin SR, Li YC, Jang HL, Khoshakhlagh P, Akbari M, Nasajpour A, et al. Graphene-based materials for tissue engineering. Adv Drug Deliv Rev 2016;5:255–74.

[19] Wang J, Hu Z, Xu J, Zhao Y. Therapeutic applications of low-toxicity spherical nanocarbon materials. NPG Asia Mater 2014;6(2):84.

[20] Yu X, Tang X, Gohil SV, Laurencin CT. Biomaterials for bone regenerative engineering. Adv Health Mater 2015;4(9):1268–85.

[21] Shadjou N, Hasanzadeh M. Graphene and its nanostructure derivatives for use in bone tissue engineering: recent advances. J Biomed Mat Res A 2016;104(5):1250–75.

[22] Umeyama T, Imahori H. Photofunctional hybrid nanocarbon materials. J Phys Chem C 2012;117(7):3195–209.

[23] Hasanzadeh M, Shadjou N, Mokhtarzadeh A, Ramezani M. Two dimension (2-D) graphene-based nanomaterials as signal amplification elements in electrochemical microfluidic immune-devices: recent advances. Mat Sci Eng C 2016;68:482–93.

[24] Yousefi M, Dadashpour M, Hejazi M, Hasanzadeh M, Behnam B, de la Guardia M, et al. Anti-bacterial activity of graphene oxide as a new weapon nanomaterial to combat multidrug-resistance bacteria. Mat Sci Eng: C 2017;74:568–81.

[25] Marcano DC, Kosynkin DV, Berlin JM, Sinitskii A, Sun Z, Slesarev A, et al. Improved synthesis of graphene oxide. ACS Nano 2010;4(8):4806–14.

[26] Soler-Crespo RA, Gao W, Xiao P, Wei X, Paci JT, Henkelman G, et al. Engineering the mechanical properties of monolayer graphene oxide at the atomic level. J Phys Chem Lett 2016;7(14):2702–7.

[27] Chang WC, Cheng SC, Chiang WH, Liao JL, Ho RM, Hsiao TC, et al. Quantifying surface area of nanosheet graphene oxide colloid using a gas-phase electrostatic approach. Anal Chem 2017;89(22):12217–22.

[28] Gao Y, Liu LQ, Zu SZ, Peng K, Zhou D, Han BH, et al. The effect of interlayer adhesion on the mechanical behaviors of macroscopic graphene oxide papers. ACS Nano 2011;5(3):2134–41.

[29] Qu S, Li M, Xie L, Huang X, Yang J, Wang N, et al. Noncovalent functionalization of graphene attaching [6, 6]-phenyl-C61-butyric acid methyl ester (PCBM) and application as electron extraction layer of polymer solar cells. ACS Nano 2013;7(5):4070–81.

[30] Georgakilas V, Tiwari JN, Kemp KC, Perman JA, Bourlinos AB, Kim KS, et al. Noncovalent functionalization of graphene and graphene oxide for energy materials, biosensing, catalytic, and biomedical applications. Chem Rev 2016;116(9):5464–519.

[31] Li MT, Liu M, Yu YH, Li AW, Sun HB. Laser-structured graphene/reduced graphene oxide films towards bio-inspired superhydrophobic surfaces. Bull Chem Soc Jpn 2018;92(2):283–9.

[32] Mitran V, Dinca V, Ion R, Cojocaru VD, Neacsu P, Dinu CZ, et al. Graphene nanoplatelets-sericin surface-modified Gum alloy for improved biological response. RSC Adv 2018;8(33):18492–501.

[33] Kryuchkova M, Fakhrullin R. Kaolin alleviates graphene oxide toxicity. Environ Sci Technol Lett 2018;5(5):295–300.

[34] Michaela F, Zhe Teo W, Pumera M. Environmental impact and potential health risks of 2D nanomaterials. Environ Sci Nano 2017;4(8):1617–33.

[35] Menaa F, Abdelghani A, Menaa B. Graphene nanomaterials as biocompatible and conductive scaffolds for stem cells: impact for tissue engineering and regenerative medicine. J Tissue Eng Regen Med 2015;9(12):1321–38.

[36] Lee WC, Lim CH, Kenry SuC, Loh KP, Lim CT. Cell-assembled graphene biocomposite for enhanced chondrogenic differentiation. Small 2015;11(8):963–9.

[37] Lee JH, Shin YC, Lee SM, Jin OS, Kang SH, Hong SW, et al. Enhanced osteogenesis by reduced graphene oxide/hydroxyapatite nanocomposites. Sci Rep 2015;5.

[38] Paz E, Forriol F, Del Real JC, Dunne N. Graphene oxide versus graphene for optimisation of PMMA bone cement for orthopaedic applications. Mater Sci Eng C 2017;77:1003–11.

[39] Xie X, Hu K, Fang D, Shang L, Tran SD, Cerruti M. Graphene and hydroxyapatite self-assemble into homogeneous, free standing nanocomposite hydrogels for bone tissue engineering. Nanoscale 2015;7(17):7992–8002.

[40] Kumar S, Raj S, Sarkar K, Chatterjee K. Engineering a multi-biofunctional composite using poly (ethylenimine) decorated graphene oxide for bone tissue regeneration. Nanoscale 2016;8(12):6820–36.

[41] Liang C, Luo Y, Yang G, Xia D, Liu L, Zhang X, et al. Graphene oxide hybridized nHAC/PLGA scaffolds facilitate the proliferation of MC3T3-E1 Cells. Nanoscale Res Lett 2018;13(1):15.

[42] Natarajan J, Madras G, Chatterjee K. Development of graphene oxide-/galactitol polyester-based biodegradable composites for biomedical applications. ACS Omega 2017;2(9):5545–56.

[43] Zhou T, Li G, Lin S, Tian T, Ma Q, Zhang Q, et al. Electrospun poly(3-hydroxybutyrate-co-4-hydroxybutyrate)/graphene oxide scaffold: enhanced properties and promoted in vivo bone repair in rats. ACS Appl Mater Interface 2017;9(49):42589–600.

[44] Luo Y, Shen H, Fang Y, Cao Y, Huang J, Zhang M, et al. Enhanced proliferation and osteogenic differentiation of mesenchymal stem cells on graphene oxide-incorporated electrospun poly(lactic-co-glycolic acid) nanofibrous mats. ACS Appl Mater Interface 2015;7(11):6331–9.

[45] Zhang Y, Zhai D, Xu M, Yao Q, Chang J, Wu C. 3D-printed bioceramic scaffolds with a Fe3O4/graphene oxide nanocomposite interface for hyperthermia therapy of bone tumor cells. J Mater Chem B 2016;4(17):2874–86.

[46] Kumar S, Chatterjee K. Strontium eluting graphene hybrid nanoparticles augment osteogenesis in a 3D tissue scaffold. Nanoscale 2015;7(5):2023–33.

[47] Díez-Pascual AM, Díez-Vicente AL. Poly(propylene fumarate)/polyethylene glycol-modified graphene oxide nanocomposites for tissue engineering. ACS Appl Mater Interface 2016;8(28):17902–14.

[48] Song F, Jie W, Zhang T, Li W, Jiang Y, Wan L, et al. Room-temperature fabrication of a three-dimensional reduced-graphene oxide/polypyrrole/hydroxyapatite composite scaffold for bone tissue engineering. RSC Adv 2016;6(95):92804–12.

[49] Peng S, Feng P, Wu P, Huang W, Yang Y, Guo W, et al. Graphene oxide as an interface phase between polyetheretherketone and hydroxyapatite for tissue engineering scaffolds. Sci Rep 2017;7:46604.

[50] Wu C, Xia L, Han P, Xu M, Fang B, Wang J, et al. Graphene-oxide-modified β-tricalcium phosphate bioceramics stimulate in vitro and in vivo osteogenesis. Carbon 2015;93:116–29.

[51] Mahmoudi N, Simchi A. On the biological performance of graphene oxide-modified chitosan/polyvinyl pyrrolidone nanocomposite membranes: In vitro and in vivo effects of graphene oxide. Mater Sci Eng C 2017;70:121–31.

[52] Xie C, Lu X, Han L, Xu J, Wang Z, Jiang L, et al. Biomimetic mineralized hierarchical graphene oxide/chitosan scaffolds with adsorbability for immobilization of nanoparticles for biomedical applications. ACS Appl Mater Interface 2016;8(3):1707–17.

[53] Lee JH, Shin YC, Lee SM, Jin OS, Kang SH, Hong SW, et al. Enhanced osteogenesis by reduced graphene oxide/hydroxyapatite nanocomposites. Sci Rep 2015;5:18833.

[54] Žáková P, Kasálková NS, Kolská Z, Leitner J, Karpíšková J, Stibor I, et al. Cytocompatibility of amine functionalized carbon nanoparticles grafted on polyethylene. Mater Sci Eng C 2016;60:394–401.

[55] Unnithan AR, Sasikala ARK, Park CH, Kim CS. A unique scaffold for bone tissue engineering: an osteogenic combination of graphene oxide–hyaluronic acid–chitosan with simvastatin. J Ind Eng Chem 2017;46:182–91.

[56] Shao W, He J, Sang F, Wang Q, Chen L, Cui S, et al. Enhanced bone formation in electrospun poly (l-lactic-co-glycolic acid)–tussah silk fibroin ultrafine nanofiber scaffolds incorporated with graphene oxide. Mater Sci Eng C 2016;62:823–34.

[57] Luo Y, Shen H, Fang Y, Cao Y, Huang J, Zhang M, et al. Enhanced proliferation and osteogenic differentiation of mesenchymal stem cells on graphene oxide-incorporated electrospun poly (lactic-co-glycolic acid) nanofibrous mats. ACS Appl Mater interface 2015;7(11):6331–9.

[58] Zhou Q, Yang P, Li X, Liu H, Ge S. Bioactivity of periodontal ligament stem cells on sodium titanate coated with graphene oxide. Sci Rep 2016;6:19343.

[59] Liu C, Wong HM, Yeung KWK, Tjong SC. Novel electrospun polylactic acid nanocomposite fiber mats with hybrid graphene oxide and nanohydroxyapatite reinforcements having enhanced biocompatibility. Polymers 2016;8(8):287.

[60] Nishida E, Miyaji H, Kato A, Takita H, Iwanaga T, Momose T, et al. Graphene oxide scaffold accelerates cellular proliferative response and alveolar bone healing of tooth extraction socket. Int J Nanomed 2016;11:2265.

[61] Lee JH, Shin YC, Jin OS, Kang SH, Hwang YS, Park JC, et al. Reduced graphene oxide-coated hydroxyapatite composites stimulate spontaneous osteogenic differentiation of human mesenchymal stem cells. Nanoscale 2015;7(27):11642–51.

[62] Pahlevanzadeh F, Bakhsheshi-Rad H, Hamzah E. In vitro biocompatibility, bioactivity, and mechanical strength of PMMA-PCL polymer containing fluorapatite and graphene oxide bone cements. J Mech Behav Biomed Mater 2018;82:257–67.

[63] Revathi S, Vuyyuru M, Dhanaraju MD. Carbon nanotube: a flexible approach for nanomedicine and drug delivery. Carbon 2015;8(1).

[64] Ahmadi H, Ramezani M, Yazdian-Robati R, Behnam B, Azarkhiavi KR, Nia AH, et al. Acute toxicity of functionalized single wall carbon nanotubes: a biochemical, histopathologic and proteomics approach. Chem-Biol Interact 2017;275:196–209.

[65] Kumar S, Rani R, Dilbaghi N, Tankeshwar K, Kim KH. Carbon nanotubes: a novel material for multifaceted applications in human healthcare. Chem Soc Rev 2017;46(1):158–96.

[66] Chen J, Du Y, Que W, Xing Y, Chen X, Lei B. Crack-free polydimethylsiloxane–bioactive glass–poly (ethylene glycol) hybrid monoliths with controlled biomineralization activity and mechanical property for bone tissue regeneration. Colloids Surf B Biointerfaces 2015;136:126–33.

[67] Dong Z, Li Y, Zou Q. Degradation and biocompatibility of porous nano-hydroxyapatite/polyurethane composite scaffold for bone tissue engineering. Appl Surf Sci 2009;255(12):6087–91.

[68] Marelli B, Ghezzi CE, Mohn D, Stark WJ, Barralet JE, Boccaccini AR, et al. Accelerated mineralization of dense collagen-nano bioactive glass hybrid gels increases scaffold stiffness and regulates osteoblastic function. Biomaterials 2011;32(34):8915–26.

[69] Lei B, Shin KH, Koh YH, Kim HE. Porous gelatin–siloxane hybrid scaffolds with biomimetic structure and properties for bone tissue regeneration. J Biomed Mater Res B 2014;102(7):1528–36.

[70] Lei B, Wang L, Chen X, Chae SK. Biomimetic and molecular level-based silicate bioactive glass–gelatin hybrid implants for loading-bearing bone fixation and repair. J Mater Chem B 2013;1(38):5153–62.

[71] Jones JR, Ehrenfried LM, Hench LL. Optimising bioactive glass scaffolds for bone tissue engineering. Biomaterials 2006;27(7):964–73.

[72] Brown RF, Day DE, Day TE, Jung S, Rahaman MN, Fu Q. Growth and differentiation of osteoblastic cells on 13–93 bioactive glass fibers and scaffolds. Acta Biomater 2008;4(2):387–96.

[73] Shokri S, Movahedi B, Rafieinia M, Salehi HA. new approach to fabrication of Cs/BG/CNT nanocomposite scaffold towards bone tissue engineering and evaluation of its properties. Appl Sur Sci 2015;357:1758–64.

[74] Li H, Zhao Q, Li B, Kang J, Yu Z, Li Y, et al. Fabrication and properties of carbon nanotube-reinforced hydroxyapatite composites by a double in situ synthesis process. Carbon 2016;101:159–67.

[75] Mehra NK, Verma AK, Mishra PR, Jain NK. The cancer targeting potential of D-α-tocopheryl polyethylene glycol 1000 succinate tethered multi walled carbon nanotubes. Biomaterials 2014;35(15):4573–88.

[76] Mundra RV, Mehta KK, Wu X, Paskaleva EE, Kane RS, Dordick JS. Enzyme-driven bacillus spore coat degradation leading to spore killing. Biotechnol Bioeng 2014;111(4):654–63.

[77] Hirata E, Uo M, Nodasaka Y, Takita H, Ushijima N, Akasaka T, et al. 3D collagen scaffolds coated with multiwalled carbon nanotubes: initial cell attachment to internal surface. J Biomed Mater Res B 2010;93(2):544–50.

[78] Bhattacharya M, Wutticharoenmongkol Thitiwongsawet P, Hamamoto DT, Lee D, Cui T, Prasad HS, et al. Bone formation on carbon nanotube composite. J Biomed Mater Res A 2011;96(1):75–82.

[79] Bajaj P, Khang D, Webster TJ. Control of spatial cell attachment on carbon nanofiber patterns on polycarbonate urethane. Int J Nanomed 2006;1(3):361.

[80] Zanello LP, Zhao B, Hu H, Haddon RC. Bone cell proliferation on carbon nanotubes. Nano Lett 2006;6(3):562–7.

[81] Mooney E, Mackle JN, Blond DJP, O'Cearbhaill E, Shaw G, Blau WJ, et al. The electrical stimulation of carbon nanotubes to provide a cardiomimetic cue to MSCs. Biomaterials 2012;33(26):6132–9.

[82] Chen WY, Yang RC, Wang HM, Zhang L, Hu K, Li CH, et al. Self-assembled heterojunction carbon nanotubes synergizing with photoimmobilized IGF-1 inhibit cellular senescence. Adv Healthc Mater 2016;5(18):2413–26.

[83] Park S, Park J, Jo I, Cho SP, Sung D, Ryu S, et al. In situ hybridization of carbon nanotubes with bacterial cellulose for three-dimensional hybrid bioscaffolds. Biomaterials 2015;58:93–102.

[84] Ryu S, Lee C, Park J, Lee JS, Kang S, Seo YD, et al. Three-dimensional scaffolds of carbonized polyacrylonitrile for bone tissue regeneration. Angew Chem 2014;126(35):9367–71.

[85] Abarrategi A, Gutierrez MC, Moreno-Vicente C, Hortigüela MJ, Ramos V, Lopez-Lacomba JL, et al. Multiwall carbon nanotube scaffolds for tissue engineering purposes. Biomaterials 2008;29(1):94–102.

[86] Serrano MC, Gutiérrez MC, del Monte F. Role of polymers in the design of 3D carbon nanotube-based scaffolds for biomedical applications. Prog Polym Sci 2014;39(7):1448–71.

[87] Nardecchia S, Carriazo D, Ferrer ML, Gutiérrez MC, del Monte F. Three dimensional macroporous architectures and aerogels built of carbon nanotubes and/or graphene: synthesis and applications. Chem Soc Rev 2013;42(2):794–830.

[88] Correa-Duarte MA, Wagner N, Rojas-Chapana J, Morsczeck C, Thie M, Giersig M. Fabrication and biocompatibility of carbon nanotube-based 3D networks as scaffolds for cell seeding and growth. Nano Lett 2004;4(11):2233–6.

[89] Fujihara K, Kotaki M, Ramakrishna S. Guided bone regeneration membrane made of polycaprolactone/calcium carbonate composite nano-fibers. Biomaterials 2005;26(19):4139–47.

[90] DeVolder RJ, Kim IW, Kim ES, Kong H. Modulating the rigidity and mineralization of collagen gels using poly (lactic-co-glycolic acid) microparticles. Tissue Eng A 2012;18(15–16):1642–51.

[91] Vandamme EJ, De Baets S, Vanbaelen A, Joris K, De Wulf P. Improved production of bacterial cellulose and its application potential. Polym Degrad Stabil 1998;59(1–3):93–9.

[92] Yeo M, Lee H, Kim G. Three-dimensional hierarchical composite scaffolds consisting of polycaprolactone, β-tricalcium phosphate, and collagen nanofibers: fabrication, physical properties, and in vitro cell activity for bone tissue regeneration. Biomacromolecules 2010;12(2):502–10.

[93] Venugopal JL, Sharon C, Aw T, Ramakrishna S. Interaction of cells and nanofiber scaffolds in tissue engineering. J Biomed Mater Res B 2008;84(1):34–48.

[94] Henriksson M, Berglund LA. Structure and properties of cellulose nanocomposite films containing melamine formaldehyde. J Appl Polym Sci 2007;106(4):2817–24.

[95] Kiran S, Nune K, Misra R. The significance of grafting collagen on polycaprolactone composite scaffolds: processing–structure–functional property relationship. J Biomed Mater Res A 2015;103(9):2919–31.

[96] Rodrigues CVM, Serricella P, Linhares ABR, Guerdes RM, Borojevic R, Rossi MA, et al. Characterization of a bovine collagen–hydroxyapatite composite scaffold for bone tissue engineering. Biomaterials 2003;24(27):4987–97.

[97] Wang Y, Zhang L, Hu M, Liu H, Wen W, Xiao H, et al. Synthesis and characterization of collagen-chitosan-hydroxyapatite artificial bone matrix. J Biomed Mater Res A 2008;86(1):244–52.

[98] Valverde TM, Castro EG, Cardoso MHS, Martins-Junior PA, Souza LMO, Silva PP, et al. A novel 3D bone-mimetic scaffold composed of collagen/MTA/MWCNT modulates cell migration and osteogenesis. Life Sci 2016;162:115–24.

[99] Paradossi G, Cavalieri F, Chiessi E, Spagnoli C, Cowman MK. Poly (vinyl alcohol) as versatile biomaterial for potential biomedical applications. J Mater Sci Mater Med 2003;14(8):687–91.

[100] Baker MI, Walsh SP, Schwartz Z, Boyan BDA. review of polyvinyl alcohol and its uses in cartilage and orthopedic applications. J Biomed Mater Res B 2012;100(5):1451–7.

[101] Zhao X, Zhang Q, Chen D, Lu P. Enhanced mechanical properties of graphene-based poly (vinyl alcohol) composites. Macromolecules 2010;43(5):2357–63.

[102] Kaur T, Thirugnanam A. Tailoring in vitro biological and mechanical properties of polyvinyl alcohol reinforced with threshold carbon nanotube

[103] Liu R, Chen Y, Ma Q, Luo J, Wei W, Liu X. Noncovalent functionalization of carbon nanotube using poly (vinylcarbazole)-based compatibilizer for reinforcement and conductivity improvement in epoxy composite. J Appl Polym Sci 2017;134(26).

[104] Stevens JL, Huang AY, Peng H, Chiang IW, Khabashesku VN, Margrave JL. Sidewall amino-functionalization of single-walled carbon nanotubes through fluorination and subsequent reactions with terminal diamines. Nano Lett 2003;3(3):331–6.

[105] Habibizadeh M, Rostamizadeh K, Dalali N, Ramazani A. Preparation and characterization of PEGylated multiwall carbon nanotubes as covalently conjugated and non-covalent drug carrier: a comparative study. Mater Sci Eng C 2017;74:1–9.

[106] Punetha VD, Rana S, Yoo HJ, Chaurasia A, McLeskey Jr JT, Ramasamy MS, et al. Functionalization of carbon nanomaterials for advanced polymer nanocomposites: a comparison study between CNT and graphene. Prog Polym Sci 2017;67:1–47.

[107] Kim SW, Kim T, Kim YS, Choi HS, Lim HJ, Yang SJ, et al. Surface modifications for the effective dispersion of carbon nanotubes in solvents and polymers. Carbon 2012;50(1):3–33.

[108] Fu Q, Lu C, Liu J. Selective coating of single wall carbon nanotubes with thin SiO2 layer. Nano Lett 2002;2(4):329–32.

[109] Khalid P, Hussain MA, Rekha PD, Arun AB. Carbon nanotube-reinforced hydroxyapatite composite and their interaction with human osteoblast in vitro. Hum Exp Toxicol 2015;34(5):548–56.

[110] Yan X, Yang W, Shao Z, Yang S, Liu X. Graphene/single-walled carbon nanotube hybrids promoting osteogenic differentiation of mesenchymal stem cells by activating p38 signaling pathway. Int J Nanomed 2016;11:5473.

[111] Feng P, Peng S, Wu P, Gao C, Huang W, Deng Y, et al. A nano-sandwich construct built with graphene nanosheets and carbon nanotubes enhances mechanical properties of hydroxyapatite–polyetheretherketone scaffolds. Int J Nanomed 2016;11:3487.

[112] Wang J, He C, Cheng N, Yang Q, Chen M, You L, et al. Bone marrow stem cells response to collagen/single-wall carbon nanotubes-COOHs nanocomposite films with transforming growth factor beta 1. J Nanosci Nanotechnol 2015;15(7):4844–50.

[113] Rajesh R, Ravichandran YD, Reddy MJK, Ryu SH, Shanmugharaj AM. Development of functionalized multi-walled carbon nanotube-based polysaccharide–hydroxyapatite scaffolds for bone tissue engineering. RSC Adv 2016;6(85):82385–93.

[114] Gholami F, Ismail S, Noor AFM. Development of carboxylated multi-walled carbon nanotubes and bovine serum albumin reinforced hydroxyapatite for bone substitute applications. J Aust Ceram Soc 2017;53(1):117–27.

[115] Rajesh R, Ravichandran YD. Development of a new carbon nanotube–alginate–hydroxyapatite tricomponent composite scaffold for application in bone tissue engineering. Int J Nanomed 2015;10(Suppl. 1):7.

[116] Rodrigues BVM, Silva AS, Melo GFS, Vasconscellos LMR, Marciano FR, Lobo AO. Influence of low contents of superhydrophilic MWCNT on the properties and cell viability of electrospun poly (butylene adipate-co-terephthalate) fibers. Mater Sci Eng C 2016;59:782–91.

[117] Miyaji H, Murakami S, Nishida E, Akasaka T, Fugetsu B, Umeda J, et al. Evaluation of tissue behavior on three-dimensional collagen scaffold coated with carbon nanotubes and β-tricalcium phosphate nanoparticles. J Oral Tiss Eng 2018;15(3):123–30.

[118] Sun YP, Zhou B, Lin Y, Wang W, Fernando KAS, Pathak P, et al. Quantum-sized carbon dots for bright and colorful photoluminescence. J Am Chem Soc 2006;128(24):7756–7.

[119] Fang Y, Guo S, Li D, Zhu C, Ren W, Dong S, et al. Easy synthesis and imaging applications of cross-linked green fluorescent hollow carbon nanoparticles. ACS Nano 2011;6(1):400–9.

[120] Ray SC, Saha A, Jana NR, Sarkar R. Fluorescent carbon nanoparticles: synthesis, characterization, and bioimaging application. J Phys Chem C 2009;113(43):18546–51.

[121] Hu SL, Niu KY, Sun J, Yang J, Zhao NQ, Du XW. One-step synthesis of fluorescent carbon nanoparticles by laser irradiation. J Mater Chem 2009;19(4):484–8.

[122] Zheng XT, Ananthanarayanan A, Luo KQ, Chen P. Glowing graphene quantum dots and carbon dots: properties, syntheses, and biological applications. Small 2015;11(14):1620–36.

[123] Li L, Wu G, Yang G, Peng J, Zhao J, Zhu JJ. Focusing on luminescent graphene quantum dots: current status and future perspectives. Nanoscale 2013;5(10):4015–39.

[124] Baker SN, Baker GA. Luminescent carbon nanodots: emergent nanolights. Angew Chem Int Ed 2010;49(38):6726–44.

[125] Li X, Wang H, Shimizu Y, Pyatenko A, Kawaguchi K, Koshizaki N. Preparation of carbon quantum dots with tunable photoluminescence by rapid laser passivation in ordinary organic solvents. Chem Commun 2010;47(3):932–4.

[126] Lin L, Rong M, Luo F, Chen D, Wang Y, Chen X. Luminescent graphene quantum dots as new fluorescent materials for environmental and biological applications. TrAC Trends Anal Chem 2014;54:83–102.

[127] Luo PG, Sahu S, Yang ST, Sonkar SK, Wang J, Wang H, et al. Carbon "quantum" dots for optical bioimaging. J Mater Chem B 2013;1(16):2116–27.

[128] Jelinek R. Bioimaging applications of carbon-dots. In: Carbon quantum dots. Springer; 2017. p. 61–70.

[129] Wang Y, Hu A. Carbon quantum dots: synthesis, properties and applications. J Mater Chem C 2014;2(34):6921–39.

[130] Li H, Kang Z, Liu Y, Lee ST. Carbon nanodots: synthesis, properties and applications. J Mater Chem 2012;22(46):24230–53.

[131] Lim SY, Shen W, Gao Z. Carbon quantum dots and their applications. Chem Soc Rev 2015;44(1):362–81.

[132] Chen B, Li F, Li S, Weng W, Guo H, Guo T, et al. Large scale synthesis of photoluminescent carbon nanodots and their application for bioimaging. Nanoscale 2013;5(5):1967–71.

[133] Li H, Zhang Y, Wang L, Tian J, Sun X. Nucleic acid detection using carbon nanoparticles as a fluorescent sensing platform. Chem Commun 2011;47(3):961–3.

[134] Wei W, Xu C, Ren J, Xu B, Qu X. Sensing metal ions with ion selectivity of a crown ether and fluorescence resonance energy transfer between carbon dots and graphene. Chem Commun 2012;48(9):1284–6.

[135] Shi W, Li X, Ma H. A tunable ratiometric pH sensor based on carbon nanodots for the quantitative measurement of the intracellular pH of whole cells. Angew Chem 2012;124(26):6538–41.

[136] Huang JJ, Zhong ZF, Rong MZ, Zhou X, Chen XD, Zhang MQ. An easy approach of preparing strongly luminescent carbon dots and their polymer based composites for enhancing solar cell efficiency. Carbon 2014;70:190–8.

[137] Mirtchev P, Henderson EJ, Soheilnia N, Yip CM, Ozin GA. Solution phase synthesis of carbon quantum dots as sensitizers for nanocrystalline TiO2 solar cells. J Mater Chem 2012;22(4):1265–9.

[138] Wei Y, Liu H, Jin Y, Cai K, Li H, Liu Y, et al. Carbon nanoparticle ionic liquid functionalized activated carbon hybrid electrode for efficiency enhancement in supercapacitors. New J Chem 2013;37(4):886–9.

[139] Yu H, Zhao Y, Zhou C, Shang L, Peng Y, Cao Y, et al. Carbon quantum dots/TiO2 composites for efficient photocatalytic hydrogen evolution. J Mater Chem A 2014;2(10):3344–51.

[140] Gao S, Chen Y, Fan H, Wei X, Hu C, Wang L, et al. A green one-arrow-two-hawks strategy for nitrogen-doped carbon dots as fluorescent ink and oxygen reduction electrocatalysts. J Mater Chem A 2014;2(18):6320–5.

[141] Pan D, Guo L, Zhang J, Xi C, Xue Q, Huang H, et al. Cutting sp2 clusters in graphene sheets into colloidal graphene quantum dots with strong green fluorescence. J Mater Chem 2012;22(8):3314–8.

[142] Wang X, Su X, Lao J, He H, Cheng T, Wang M, et al. Multifunctional graphene quantum dots for simultaneous targeted cellular imaging and drug delivery. Colloids Surf B Biointerfaces 2014;122:638–44.

[143] Gogoi S, Kumar M, Mandal BB, Karak N. Nano-bio engineered carbon dot-peptide functionalized water dispersible hyperbranched polyurethane for bone tissue regeneration. Macromol Biosci 2017;17(3).

[144] Yuan F, Li S, Fan Z, Meng X, Fan L, Yang S. Shining carbon dots: synthesis and biomedical and optoelectronic applications. Nano Today 2016;11(5):565–86.

[145] Sun X, Lei Y. Fluorescent carbon dots and their sensing applications. TrAC Trends Anal Chem 2017;89:163–80.

[146] Zhang J, Yu SH. Carbon dots: large-scale synthesis, sensing and bioimaging. Mater Today 2016;19(7):382–93.

[147] Gaddam RR, Mukherjee S, Punugupati N, Vasudevan D, Patra CR, Narayan R, et al. Facile synthesis of carbon dot and residual carbon nanobeads: implications for ion sensing, medicinal and biological applications. Mater Sci Eng C 2017;73:643–52.

[148] Shen LM, Liu J. New development in carbon quantum dots technical applications. Talanta 2016;156:245–56.

[149] Gogoi S, Kumar M, Mandal BB, Karak N. A renewable resource based carbon dot decorated hydroxyapatite nanohybrid and its fabrication with waterborne hyperbranched polyurethane for bone tissue engineering. RSC Adv 2016;6(31):26066–76.

[150] Egusa H, Kaneda Y, Akashi Y, Hamada Y, Matsumoto T, Saeki M, et al. Enhanced bone regeneration via multimodal actions of synthetic peptide SVVYGLR on osteoprogenitors and osteoclasts. Biomaterials 2009;30(27):4676–86.

[151] Zhang SFG, Zhao X. Designer self-assembling peptide nanofiber scaffolds for 3D tissue cell cultures. Seminars in cancer biology. Elsevier; 2005.

[152] Huttunen MM, Pekkinen M, Ahlström MEB, Lamberg-Allardt CJE. Effects of bioactive peptides isoleucine-proline-proline (IPP), valine-proline-proline (VPP) and leucine-lysine-proline (LKP) on gene expression of osteoblasts differentiated from human mesenchymal stem cells. Br J Nutr 2007;98(4):780–8.

[153] Bergeron E, Senta H, Mailloux A, Park H, Lord E, Faucheux N. Murine preosteoblast differentiation induced by a peptide derived from bone morphogenetic proteins-9. Tiss Eng A 2009;15(11):3341–9.

[154] Shao D, Lu M, Xu D, Zheng X, Pan Y, Song Y, et al. Carbon dots for tracking and promoting the osteogenic differentiation of mesenchymal stem cells. Biomater Sci 2017;5(9):1820–7.

[155] Sarkar C, Chowdhuri AR, Kumar A, Laha D, Garai S, Chakraborty J, et al. One pot synthesis of carbon dots decorated carboxymethyl cellulose-hydroxyapatite nanocomposite for drug delivery, tissue engineering and Fe 3+ ion sensing. Carbohydr Polym 2018;181:710–8.

[156] Sivaraman N, Srinivasan TG, Vasudeva RPR. Natarajan R.QSPR modeling for solubility of fullerene (C60) in organic solvents. J Chem Inf Comput Sci 2001;41(4):1067–74.

[157] Bosi S, Da Ros T, Castellano S, Banfi E, Prato M. Antimycobacterial activity of ionic fullerene derivatives. Bioorg Med Chem Lett 2000;10(10):1043–5.

[158] Sidorov LN, Yurovskaya MA, Borschevskii AY, Trushkov IV, Ioffe I. Fullerenes. Moscow: Ekzamen; 2005. p. 688.

[159] Grebowski J, Kazmierska P, Krokosz A. Fullerenols as a new therapeutic approach in nanomedicine. BioMed Res Int 2013.

[160] Chaudhuri P, Paraskar A, Soni S, Mashelkar RA, Sengupta S. Fullerenol–cytotoxic conjugates for cancer chemotherapy. Acs Nano 2009;3(9):2505–14.

[161] Sijbesma R, Srdanov G, Wudl F, Castoro JA, Wilkins C, Friedman SH, et al. Synthesis of a fullerene derivative for the inhibition of HIV enzymes. J Am Chem Soc 1993;115(15):6510–2.

[162] Dugan LL, Turetsky DM, Du C, Lobner D, Wheeler M, Almli CR, et al. Carboxyfullerenes as neuroprotective agents. Proc Natl Acad Sci 1997;94(17):9434–9.

[163] Bacakova L, Grausova L, Vacik J, Fraczek A, Blazewicz S, Kromka A, et al. Improved adhesion and growth of human osteoblast-like MG 63 cells on biomaterials modified with carbon nanoparticles. Diam Relat Mater 2007;16(12):2133–40.

[164] Bacakova M, Lisa V, Bacakova L. Regionally-selective adhesion and growth of human osteoblast-like MG 63 cells on micropatterned fullerene C60 layers. J Optoelectron Adv Mater 2008;10(8):2071–6.

[165] Kopova I, Bacakova L, Lavrentiev V, Vacik J. Growth and potential damage of human bone-derived cells on fresh and aged fullerene C60 films. Int J Mol Sci 2013;14(5):9182–204.

[166] Krishnan V, Kasuya Y, Ji Q, Sathish M, Shrestha LK, Ishihara S, et al. Vortex-aligned fullerene nanowhiskers as a scaffold for orienting cell growth. ACS Appl Mater Interface 2015;7(28):15667–73.

[167] Raty JY, Galli, Giulia BC, Van Buuren TW, Terminello LJ. Quantum confinement and fullerenelike surface reconstructions in nanodiamonds. Phys Rev Lett 2003;90(3):037401.

[168] Mochalin VN, Shenderova O, Ho D, Gogotsi Y. The properties and applications of nanodiamonds. Nat Nanotechnol 2012;7(1):11.

[169] Chang YR, Lee HY, Chen K, Ch Chang C, Tsai DS, Fu CC, et al. Mass production and dynamic imaging of fluorescent nanodiamonds. Nat Nanotechnol 2008;3(5):284.

[170] Ho DN. Applications in biology and nanoscale medicine 2010;vol. 10:978–81.

[171] Grausova L, Bacakova L, Kromka A, Potocky S, Vanecek M, Nesladek M, et al. Nanodiamond as promising material for bone tissue engineering. J Nanosci Nanotechnol 2009;9(6):3524–34.

[172] Zhang Q, Mochalin VN, Neitzel I, Knoke IY, Han J, Klug CA, et al. Fluorescent PLLA-nanodiamond composites for bone tissue engineering. Biomaterials 2011;32(1):87–94.

[173] Zhang Q, Mochalin VN, Neitzel I, Hazeli K, Niu J, Kontsos A, et al. Mechanical properties and biomineralization of multifunctional nanodiamond-PLLA composites for bone tissue engineering. Biomaterials 2012;33(20):5067–75.

[174] Parizek M, Douglas TEL, Novotna K, Kromka A, Brady MA, Renzing A, et al. Nanofibrous poly (lactide-co-glycolide) membranes loaded with diamond nanoparticles as promising substrates for bone tissue engineering. Int J Nanomed 2012;7:1931.

[175] Pfaff A, Beltz J, Ercal N. Nanodiamonds as antioxidant carriers: applications for drug delivery. Free Radic Biol Med 2018;128:S48.

[176] Woodhams B, Ansel-Bollepalli L, Surmacki J, Knowles H, Maggini L, De Volder MF, et al. Research data supporting "Graphitic and oxidised high pressure high temperature (HPHT) nanodiamonds induce differential biological responses in breast cancer cell lines" 2018.

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