

Review Article

3D Nanostructures for Tissue Engineering, Cancer Therapy, and Gene Delivery

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The self-assembling is a spontaneous progression through which objects of nanophase/molecules materialize into prepared collections. Several biomolecules can interact and assemble into highly structured supramolecular structures, for instance, proteins and peptides, with fibrous scaffolds, helical ribbons, and many other functionalities. Various self-assembly systems have been established, from copolymers in blocks to three-dimensional (3D) cell culture scaffolds. Another advantage of self-assembly is its ability to manage a large variety of materials, including metals, oxides, inorganic salts, polymers, semiconductors, and various organic semiconductors. The most basic self-assembly of 3D nanomaterials is three primary forms of nanostructured carbon-based materials that perform a critical role in the progress of modern nanotechnologies, such as carbon nanotubes (CNTs), graphene, and fullerene. This review summarized important information on the 3D self-assembly nanostructure, such as peptide hydrogel, graphene, carbon nanotubes (CNTs), and fullerene for application in gene delivery, cancer therapy, and tissue engineering.

1. Introduction

Nanostructure materials are those materials which have their dimensionality in the range of nanometers [1, 2]. Nanomaterials are synthesized using two major approaches: top-down and bottom-up techniques. Self-assembly is spontaneous assembly of constituents to form a complex nanostructure in the absence of significant external intervention. There are two types of self-assembly intermolecular and

intramolecular self-assembly. Self-assembly is highly useful because it provides the path for the aggregation of structures, which are very small, to modify individually into the organized patterns that often give various functions to materials. Self-assembly is the result of a combination of weak forces such as noncovalent interactions, hydrogen bonds, electrostatic interactions, pi-pi stacking, hydrophobic forces, van der Waals forces, and chiral dipole-dipole interactions. Self-assembly of nanomaterials is affected by interparticle

interactions, particle size, and particle shape [3]. Self-assembled nanomaterials represent a classic of induced non-covalent interactions [4]. The cooperative association of disordered nanomaterial building blocks contributes to the spontaneous creation of more ordered (or organized) nanostructured structures [5]. A highly structural nanoparticle assembly is assessed for application in wiring, superlattice creation, and rings. Several self-assembly systems were created, from copolymers in blocks to three-dimensional (3D) cell culture scaffolds. Because of its applications in optical materials, nanoelectronics, computing, photonics, medical imaging, and diagnostics, the functional material assembly draws attention. Interactions between the materials, particle size, and particle composition affect nanomaterial self-assembly [4–6].

There are several standard features of the various types of self-assembly developments in nanoscience that allow for the adoption of a conceptual framework in the context of the following three phases (Figure 1).

Spontaneous self-assembly of spherical and nonspherical nanoparticles is distinguished from one another. Polydispersed spherical nanoparticles, 5% self-assembled 2D or 3D compact structures, and nonspherical nanoparticles exhibit different ways of self-assembly [8].

Significant instances of the self-assembly mechanism are originated in biomaterials, where the combination of different macromolecular components and the coordination of their activities allow for extremely complex functions of biological interest [9–11]. For instance, folding polypeptide chains within different functional types of proteins or conformation nucleic acids are essential instances of procedures of self-assembly in numerous biological functions [12, 13]. Proteins are an excellent instrument for modern nanotechnology and serve as building blocks for quaternary frameworks and functional self-assembled nanostructures [14]. These nanoassemblies were used to generate hierarchical protein nanostructures, counting 1D (tubules/strings/nanowires), 2D (nanorings/networks), and 3D (hydrogels and crystalline frames) [15]. There are also three main forms of carbon-based nanostructured materials amongst the utmost common organic nanomaterials, which play an important role in the development of modern nanotechnologies, specifically graphene, carbon nanotubes (CNTs), and fullerene (Figure 2). Such nanomaterials are significant nanoplatforms in the creation of emerging nanotechnologies, with good mechanical properties, high aspect ratio, outstanding optical activity, and multifunctional surface properties [7, 16, 17]. Of particular interest is the use of self-assembling CNTs in biomedical applications. Single-layer carbon nanotubes (SL/CNTs) are capable of transferring imaging agents (radioisotopes or fluorophores) or medicines to certain tumors and provide major recompenses over other methods based on nanoplatforms [18, 19]. A fullerene-based nanocarrier tool for doxorubicin drugs has recently been designed to treat potential lung cancers [20]. Fullerene derivatives with well-established functional properties are also capable of nanostructures for bioactive macromolecules that are optimally distributed [21]. Gra-

phene is used as a matrix that interferes with various cells and biomolecules [2, 22]. The peculiar characteristics of graphene, such as excellent physicochemical, electrical, large surface area, and biocompatibility, have been revealed in the past decade, leading to ongoing research into the use of graphene nanomaterial in various clinical applications and regenerative medicine [22]. The graphene-based materials can appear as nanomaterials of the next decade [23]. Graphene promotes interest in biosensing and bioimaging as two-dimensional, three-dimensional, and hybrid. The researchers should find the use of graphene nanomaterials in different tissue scaffolds as a significant field of interest shortly [2, 24–26].

The mechanisms of self-assembly of biomolecules to different nanostructures have been extensively studied, and some reviews of the synthesis, design, and applications of self-assembled biomolecular nanomaterials have been recorded [27–29]. For example, Yang and colleagues presented a summary of the self-assembly of proteins into different supramolecular products, in which the design techniques for the self-assembly of proteins were applied and discussed in detail [30]. Willner and Willner reviewed the applications of nanostructures and nanomaterials constructed on biomolecules for sensing and nanodevice manufacturing [31]. After studying these reports, we realized that contributing a review on the self-assembled 3D poly functionalized nanostructures is still valuable for us. Therefore, in this study, application of 3D self-assembly (such as hydrogels, CNTs, graphene oxide, nanodiamonds, and buckminsterfullerene) was explained in gene delivery, small molecule drug delivery, cancer therapy, and tissue engineering.

2. 3D Self-Assembled Nanostructures for Tissue Engineering

Tissue engineering celebrates the ability to reconstruct and remove damaged sections of the body by developments in the medical industry. The significant growing need for organ and tissue transplants has sparked ongoing research into the cell's rejuvenating properties. It promoted the development of a new tissue engineering approach, as the primary response to tissue and organ destruction. The key factors to be discussed in cell regeneration include the structure and origin of the cells, the scaffolding materials used, scaffolding design, the cell, and the outer tissue forming environment [32, 33]. Because of the nanoscale structure of human tissues (Figure 3), advances in nanotechnology have led to advances in regenerative medicine with the potential to replicate nanoscale composition and function of human tissues and organs [34].

Peptide amphiphiles, carbon nanotubes (CNTs), self-assembled peptides, electrospun fibers, and layer structures are among the most widely used nanomaterials [21]. The development of new nanostructures composed of bioactive molecules capable of directly and reproductively interacting with cell receptors and proteins to control procedures such as cell proliferation, cell differentiation, cell production, and tissue and organ regeneration dedifferentiation has called natural attention to this. Various studies using

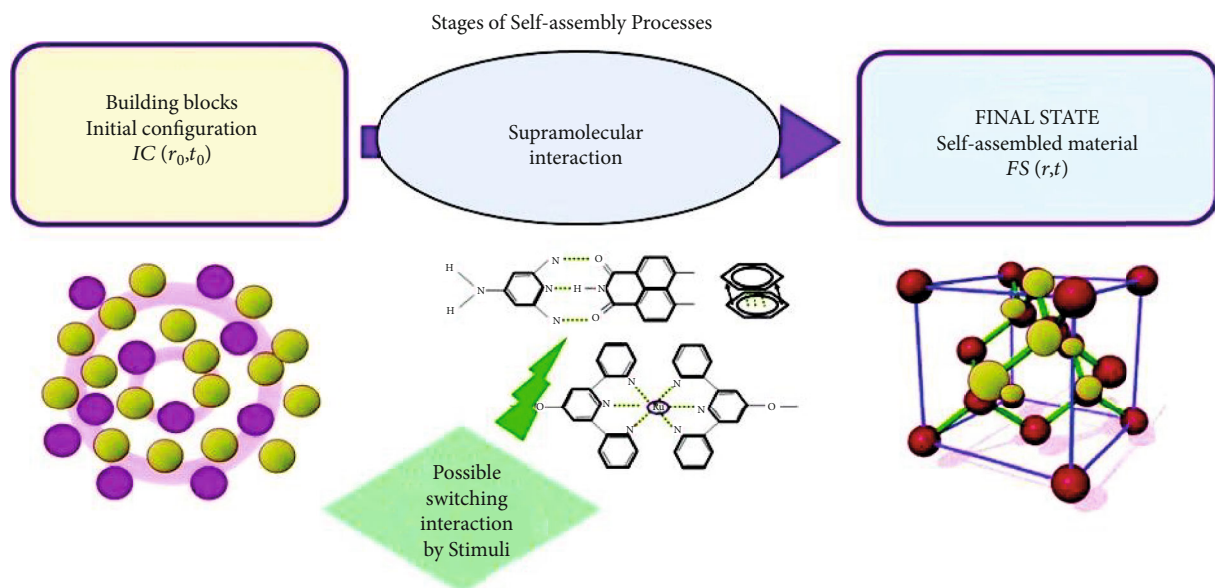


FIGURE 1: Conceptual structure showing the significant phases of the self-assembly procedure in nanoscience. IC: initial configuration; FS: final state; r_0 : initial radius; t_0 : initial time; r : radius; t : time [7].

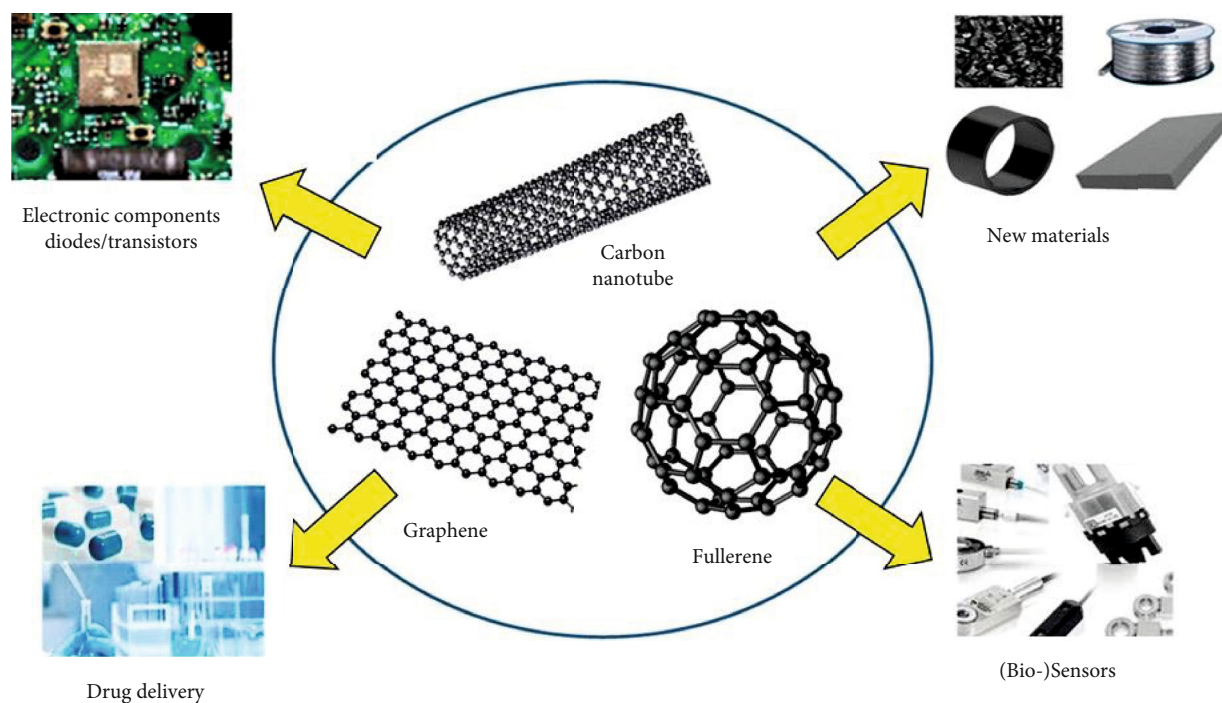


FIGURE 2: Morphology and critical uses of certain carbon-dependent nanoplateforms for nanotechnology development (carbon nanotubes, graphene, and fullerene) [7].

nanostructured materials have shown the feasibility of this method and its use in the regeneration of various tissues (such as the heart, bone, nerve, cartilage, lung, skin, and vascular) by improving the biological properties of cells such as cell adhesion, proliferation, and cell differentiation [3, 37–40], respectively. In the *in vivo* framework, the cells are located in three-dimensional (3D) microenvironments,

encompassed by certain cells and the extracellular matrix (ECM) whose elements, such as elastin, laminin, and collagen, are organized into nanostructures (i.e., triple helices, fibers) with various bioactive reasons for cell homeostasis regulation in three-dimensional (3D) microenvironments; the cells are *in vivo* surrounded by other cells and an extracellular matrix (ECM) whose components, such as

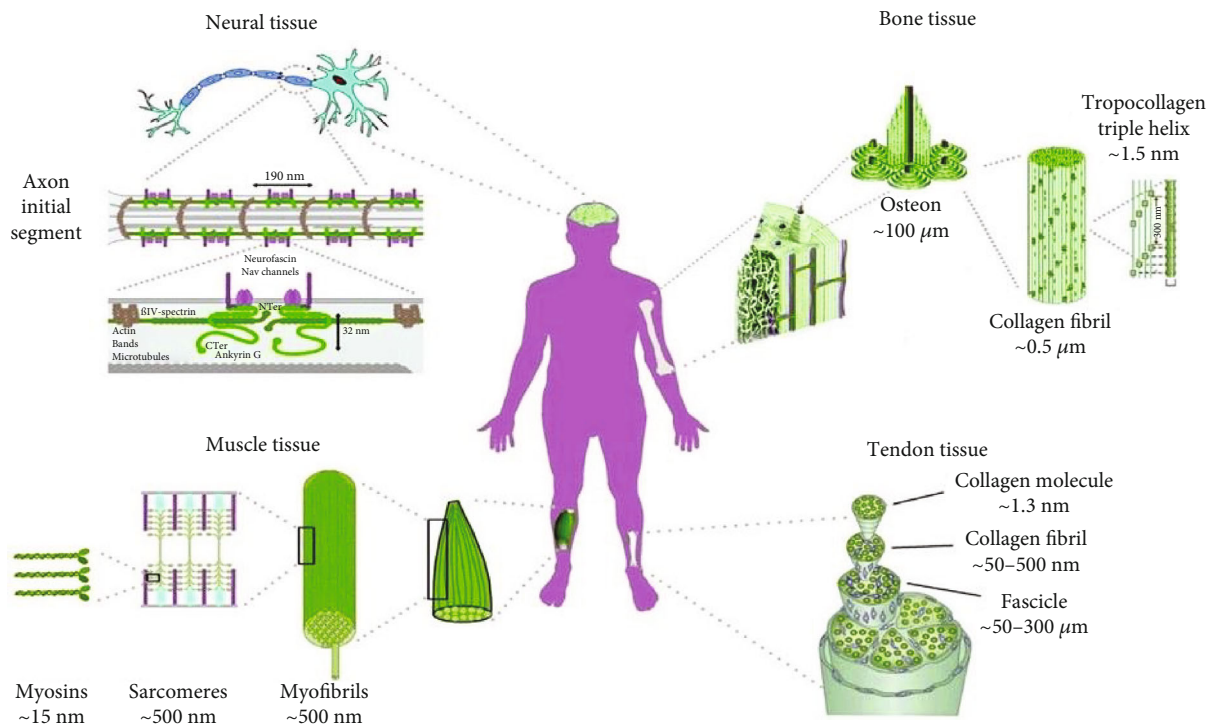


FIGURE 3: Characteristics of nanoscale human tissue. Within neural tissue, the axon's initial portion is responsible for producing future neuronal behavior and is organized on a nanoscale. This system is arranged regularly in a longitudinal direction and is placed radially. That architecture is essential for neuronal activity on a nanoscale [35]. The compact bone tissue comprises of osteons (100 mm), which are strengthened fibers shaped by aggregated type I collagen and calcium phosphate nanoparticles [34]. Myofibrils are produced in parallel, creating sarcomeres in the muscle tissue. The sarcomeres are made up of actin-consistent protein filaments and organized in conjunction with dense filaments made up of myosin aggregates. Nanoscale may be the dimensions of myofibrils, sarcomeres, and myosin. A tendon fiber comprises of a complex network of tendon fibers in the tendon tissue (50–500 nm), composed moreover of molecules of collagen (w1.3 nm) [36].

collagen, elastin, and laminin, are organized into nanostructures (i.e., fibers, triple helices) with different bioactive motives regulating the homeostasis cell [41, 42].

2.1. 3D Self-Assembled Peptide Hydrogels in Tissue Engineering. Bone tissue is a particularly complex example of such a composite because it contains multiple levels of hierarchical organization. Various nanoscale protein filaments (e.g., peptides) can be inserted into high-aspect nanofibers, which can replicate the in vivo cells' internal microenvironment. Such nanofibers wrap round body cells that stretch long distances across their surfaces and act as cables that mechanically link and sustain adjacent cells through the creation of three-dimensional networks (Figure 4(a)) [43]. For example, one form of peptide amphiphiles has been investigated to produce nanofibers for bone tissue engineering via a pH-induced self-assembly process [44]. These peptide amphiphiles contain several main structural features, including long hydrophobic alkyl tails that accumulate in aqueous solution to drive self-assembly, four consecutive cysteine residues forming disulfide bonds to polymerize self-assembled structures, a bonding region of three glycine residues to provide flexibility for the hydrophilic head group, a single phosphorylated serine residue that strongly interacts with calcium

ions to improve mineralization, and an Arg-Gly-Asp peptide ligand to enhance cell adhesion [37] (Figure 4(b)). Dithiothreitol-treated peptide amphiphiles at pH 8 are soluble in aqueous solution and begin self-assembled at pH 4. Fibers with an average diameter of 7 nm and a duration of up to many micrometers are produced and can be analyzed using electron microscopy with cryotransmission (Figure 4(c)).

In addition, a family of peptide amphiphiles has been shown to self-assemble into nanofiber networks by modifying both the concentration of pH and salt ion (e.g., sodium and potassium) in aqueous solutions. Due to their amphiphilic nature, separate model oligopeptides can also be self-assembled into nanofibers [38]. The first of the oligopeptides creators, EAK 16-II, a 16-amino acid peptide, was contained in zuotin, a yeast protein that was initially described as binding to left-handed Z-DNA [39]. This natural peptide had an AEAEAKA amino acid sequence—KAEAEAKAK, which can form a stable β -sheet structure and self-assemble into hydrogels in various shapes when the sodium/potassium concentration in aqueous solution is balanced. These peptide scaffolds can boost the adhesion, proliferation, and differentiation of mammalian cells [40]. Holmes [41] researched neuronal cell adhesion and differentiation using Ac-RADARADARADA-NH₂ (RAD16-I).

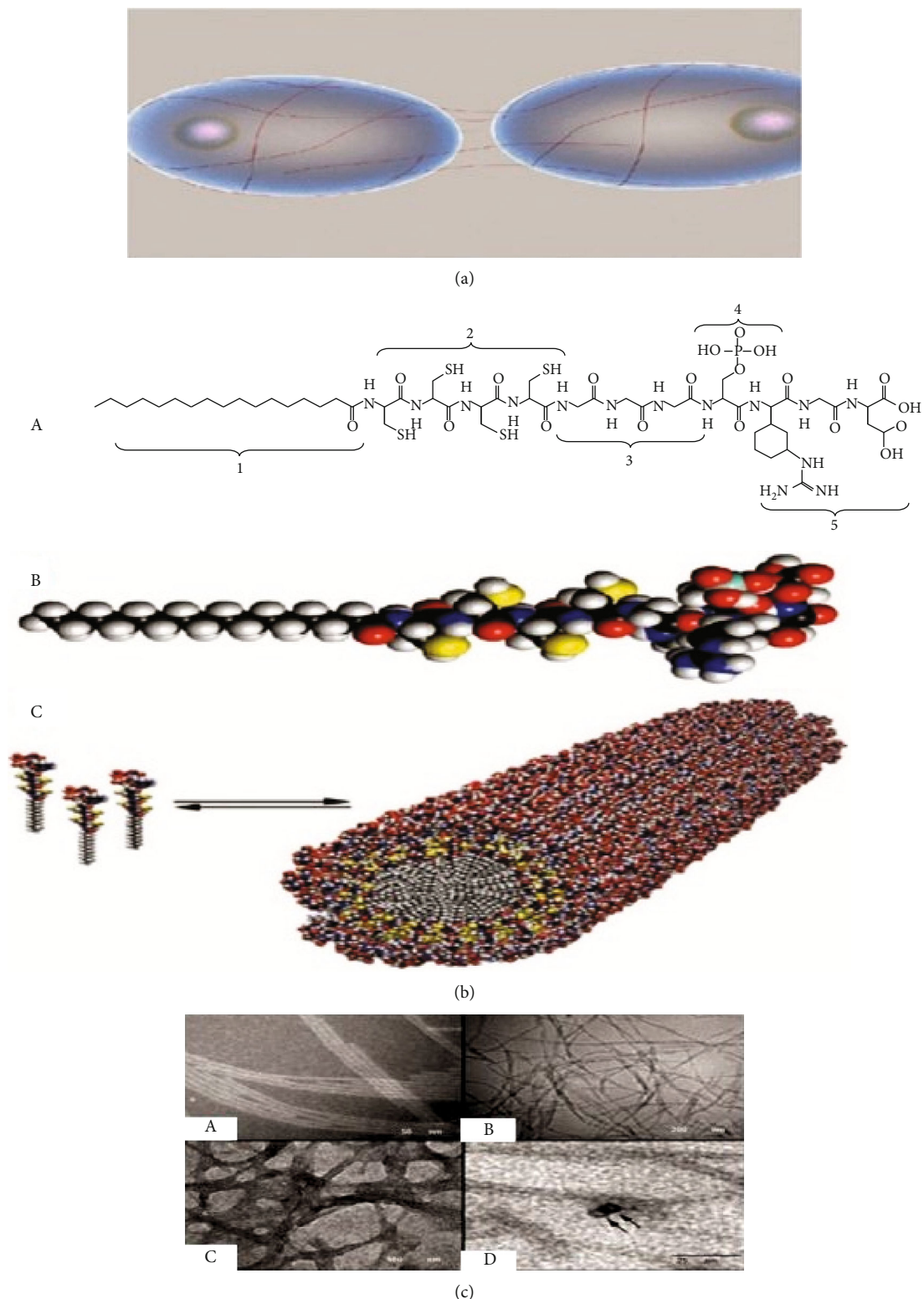


FIGURE 4: Self-assembly process used in developing 3D scaffolds. (a) An overview of the filamentous receptor cell nanostructures [43]. (b) Chemical composition of (A) amphiphilic peptides and (B) molecular sequence. Collection of colours: C, black; H, white; O, red; N, blue; P, cyan; and S, yellow. (C) Amphiphilic peptide self-assembly schemes into a cylindrical micelle. (c) Microscopic images of (A) negative stain (phosphotungstic acid) TEM of the self-assembled nanofibers before covalent capture, (B) vitreous ice cryo-TEM of the fibers reveals the diameter of the fibers, (C) positive stain (uranyl acetate) TEM of the self-assembled nanofibers after oxidative crosslinking, and (D) thin section TEM of positively stained (uranyl acetate) nanofibers after oxidative crosslinking and embedding in epoxy resin [37].

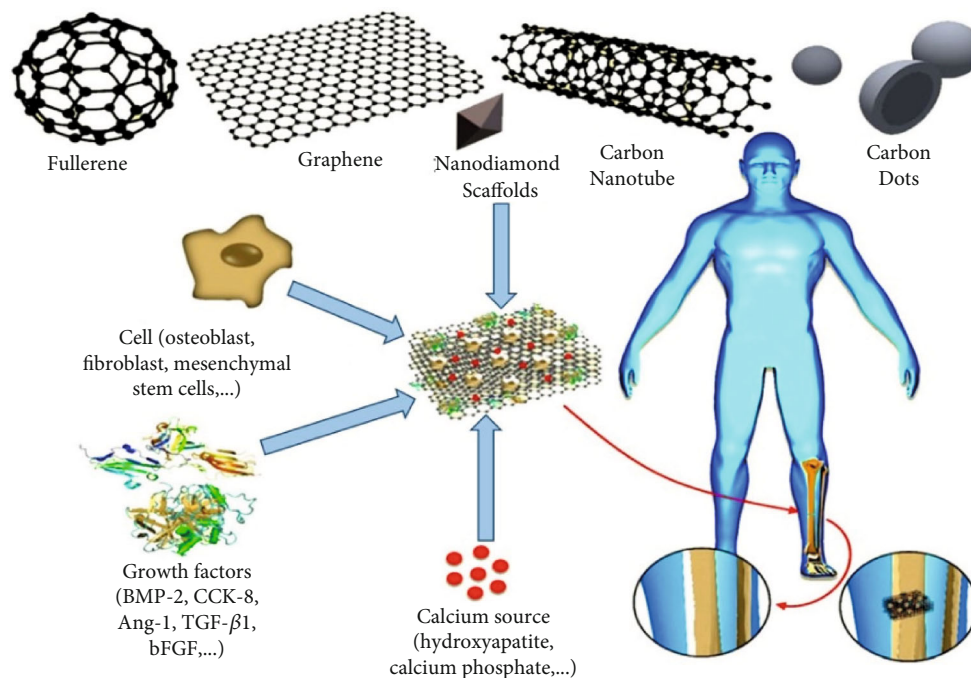


FIGURE 5: Use of carbon-dependent nanomaterials as scaffolds in the engineering of bone tissues [45].

Two small self-assembling peptide hydrogel scaffolds, and Ac-RARADARADADADA-NH₂ (RAD16-II), showed that mouse neurons can develop active dendrites on these scaffolds.

2.2. Carbon-Based Nanomaterials in Tissue Engineering. All the carbon nanomaterials on one or more ends are bioactive. Most exhibit high bone tissue engineering capabilities with acceptable mechanical possessions, no osteoblast cytotoxicity, and endogenous antibacterial activity (deprived of the use of exogenous antibiotics) [42] (Figure 5).

2.2.1. Tissue Engineering by Graphene-Based Nanomaterials. GO-based systems provide a wide variety of applications for engineering bone and tissue regeneration. GO nanomaterials' remarkable advantages are the wide surface area, adequate wettability, excellent mechanical properties, strong adhesion power, and quick start of stimulation performance. Besides, these materials may solve the weak interaction between bioceramics and biopolymers by incorporating strong electrostatic and p-p stacking interactions [46, 47]. GO would also definitely start to draw experts from prospective bone regeneration fields and other tissue engineering programs. Below are three items related to the usage of GO for scaffolding bone tissue. First, the existence of GO in natural biopolymer-based scaffolds has more potent stimulating effects on the bone tissue mineralization cycle than synthetic polymers. Second, the presence of GO in the matrix of polymer scaffolds will stimulate the growth and spread of bone cells on both natural and synthetic polymer scaffolding surfaces. However, on the GO synthetic polymer scaffold, the fraction of dead cells was higher than that of the natural biopolymer scaffold from the GO. Third, while the proportion of dead cells on the GO synthetic polymer scaffold was higher

than that of the GO natural biopolymer, GO natural biopolymer scaffolds can produce better mechanically resistant bone tissue. Table 1 summarizes the results of GO nanomaterials and their application in bone tissue engineering.

Omidi et al. made a carbon dot/chitosan hydrogel and found it to be biocompatible with *Staphylococcus aureus* and have antibacterial efficacy [56]. It has been shown that chitosan hydrogel composites filled with carbon dots composed of citrate-conjugate ammonium hydrogen have enhanced mechanical properties. These nanomaterials were extremely pH-reactive and found to be highly successful for wound cure. Consequently, carbon dots/chitosan nanocomposite both have pH responsiveness and antibacterial properties. In tissue engineering, this study has the potential to improve wound healing. GQDs have been used in recent research for regenerative, and stem cell-based uses in tissue engineering. Many researchers have used stem cells to organize them across a variety of categories, utilizing several techniques. GQDs can be used to promote stem cell differentiation in the right conditions. Qiu et al. looked at how GQDs perform an important osteogenic differentiation function [57]. In particular, GQDs have been shown to affect the early activation of osteogenesis. This nanomaterial increases the abundance of calcium, too. Due to their low toxicity, differentiation, and excellent mechanical properties, these particles are highly valuable in the regenerative medicine field. Tissue engineering's future depends on the three-dimensional (3D) scaffolds created by exciting new biomaterials. Recent advances in tissue engineering for applications in 3D graphene scaffolds are shown in Figure 6 [58].

2.2.2. Carbon Nanotubes in Tissue Engineering. Due to its exceptional biocompatibility, carbon nanotubes/nanofibers (CNTs/CNFs) are seen as potential candidates for

TABLE 1: GO-based nanoparticle application in the engineering of bone tissue.

| Method of NP synthesis | Cell type | Mechanical strength (MPa) | Application | Ref. |
|---|---|---------------------------|----------------------|------|
| Modified Hummers' method | mMSCs | 53 | In vivo | [48] |
| Modified "Hummers' and Offeman's" method | Osteogenesis of MC3T3-E1 preosteoblasts | — | In vivo | [49] |
| Modified Hummers' method | MC3T3 cells | 10.0 | In vitro | [50] |
| Modified Hummers' method | Rat mesenchymal stem cell line | 14 ± 0.7 | In vivo and in vitro | [51] |
| Modified Hummers' method | Mesenchymal stem cells | 134.4 ± 26.5 | N/A | [51] |
| Electrostatic LBL assembly followed by electrochemical deposition | MC3T3-E1 osteoblast | 85.94 ± 10.76 | N/A | [52] |
| Prepared by chemical oxidation of graphite flakes following a modified Hummers' process | Human osteoblast cell line Saos-2 | 12.69 ± 0.86 | N/A | [53] |
| Modified Hummers' and Offeman's method | Osteoblastic MC3T3 E1 cell | 0.125 | In vitro and in vivo | [53] |
| Modified Hummers' method | Human periodontal ligament stem cells | — | In vitro | [54] |
| Biomimneralization of GO/C scaffolds | Bone marrow stromal cells | 0.65 | In vivo and in vitro | [55] |

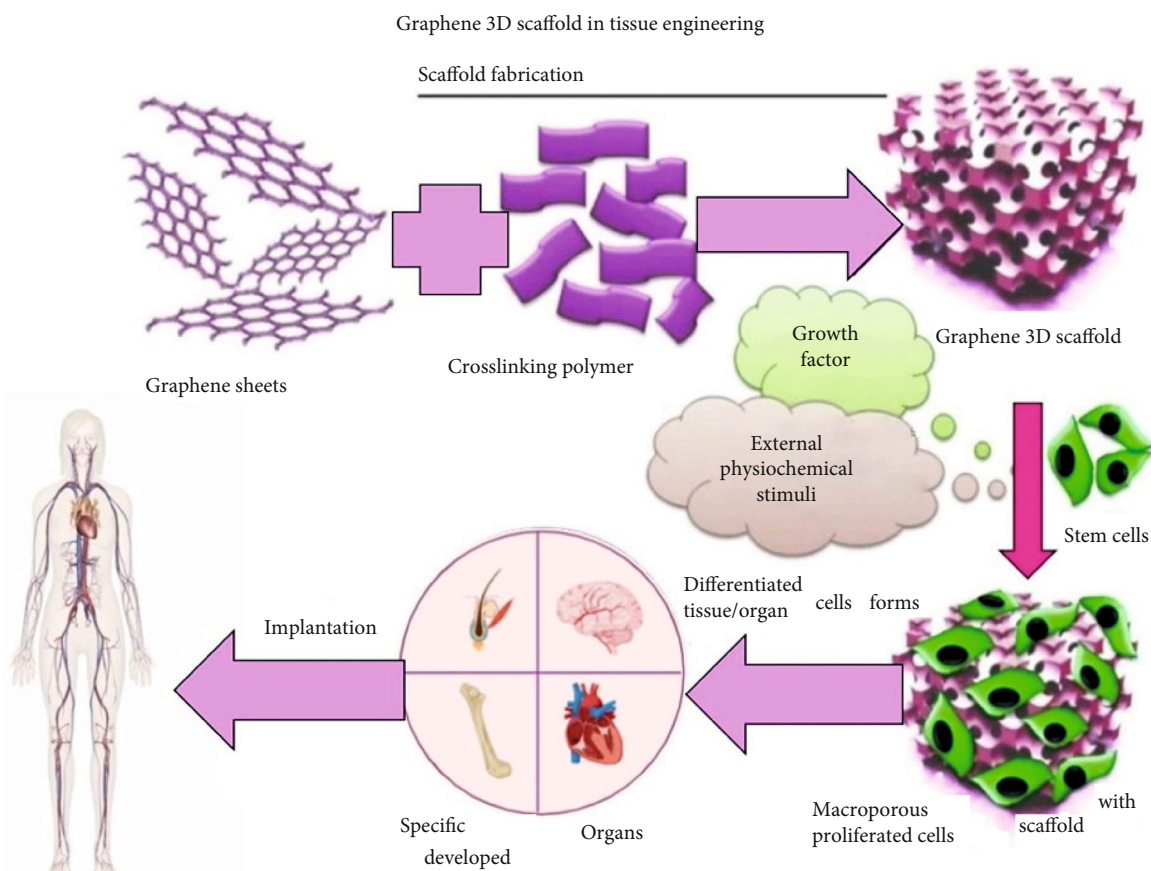


FIGURE 6: Schematic diagram of 3D graphene scaffolding in tissue engineering [58].

applications in bone tissue engineering, electrical and mechanical possessions [59]. In a new study by Price et al., osteoblast adherence by a diameter of 60 nm CNF significantly improved and, at the same time, diminished competi-

tive cells (smooth muscle cells and fibroblasts) in order to induce sufficient osseointegration [60].

Sitharaman et al. recently published an in vivo study of the ultrashort SWCNT polymer nanocomposites (single-

walled carbon nanotubes), following up to 12 weeks of implantation in rabbit femoral condyles and subcutaneous pockets. For 4 to 12 weeks, nanocomposites had intense hard and soft tissue reactions [61]. Hirata et al. studied MWCNT-coated 3D-C scaffolds and checked the adhesion of the cells to MWCNT-coated C sponges. Their study of actin stress fibers showed that the tension in Saos2 cells, which developed on materials covered with CNT, became more apparent after seven days of growth. MWCNT coating gives the cell culture a 3D scaffold, which is more fitting than SWCNT [62].

The structural and molecular dynamics of the scaffold microenvironments can be discussed in the interaction between different types of CNTs and their effect on bone cell growth and attachment. Because of its wide specific surface area, SWCNTs can give more space for efficient cell adhesion to the scaffold. At the same time, MWCNTs will manage the positive contact between the cells and the scaffold surface due to the more aggregated condition of the MWCNTs. While the cytotoxicity of CNTs continues to be a problem in bone tissue engineering because of the complicated interactions between CNTs and cellular processes, the inclusion of CNTs in the scaffold matrix may enhance cell interactions. Due to the smaller number of oxygen atoms in functional groups of functionalized CNT, the spreading and aggregation of cells within the scaffold microenvironments is less successful than GO-based scaffolds. Table 2 summarizes some of the observations regarding the use of CNT-based materials in bone tissue engineering.

2.2.3. Fullerenes in Bone Tissue Engineering. Fullerenes are closed-cage structures consisting of roughly spherical carbon atoms and hybridized by sp^2 . The fullerenes C60 and C70 are more widespread than the whole of other types. A natural application of the fullerene compounds provides alternative types of development in bone tissue. The spherical molecular structure of the fullerenes allows their use in biomedicine as free radical scavenger agents [70]. For instance, because neuroprotective agents are HIV particle inhibitors, fullerene materials incorporate new behavior.

As a consequence of findings of enhanced cell adhesion to fullerene biomaterials, advances in bone tissue engineering have drawn significant attention in recent years [71]. Bacakova et al. developed fullerene-coated carbon nanofibers capable of increasing the adhesion of osteoblastic MG 63 cells and increasing cell proliferation by up to 4.5 times in 7 days [72]. This work recorded fullerene-based microarrays which were prepared using a metallic “nanomask” to improve the growth and adhesion of MG 63 osteoblast bone cells. Hierarchical surface morphology has played a crucial role in the formation of cells because the cells are almost entirely located between the prominences of the grooves. However, fullerene-based biomaterial did not allow the cells to extend by more than 1 mm in height. That is explained by the hydrophobic nature of the materials fullerene [73]. In another research, this group proposed that complements and other carbon nanoparticles could be therapeutic agents for arthritic bone diseases. Their consequences showed that fullerene materials were stable and did not cause DNA damage or alter

osteoblastic MG 63 and U-2 OS cells morphology. However, they could increase the biological function of bone cells [74].

2.3. 3D Self-Assembled Nanostructures for Use in Scaffolds. Scaffolds can be called the tissue engineering field’s “beating heart.” Without proper scaffolding, the cells cannot expand in an artificial setting. Bone cells are perhaps the essential forms among all the separate cells in the human body, providing a well-designed scaffold for constructed living bones. Research has shown that nanostructured materials through desirable cell surface possessions can enable more protein interactions than conventional materials to support more effective new bone development [75, 76].

2.3.1. Graphene Family Materials as Scaffold or a Reinforcement Material in Scaffold. In bone tissue engineering, the most common technique is to reproduce the bone remodeling and regeneration processes as natural. Biocompatible scaffold, biodegradable, and osteoinductive or osteoconductive techniques must reach three dimensions [77]. This form of scaffold would provide an ideal microenvironment for imitating the extracellular matrix (ECM) for osteogenic cell binding, division, differentiation and proliferation, and growth factor carriers [78]. Graphene should make the full surface area of the substratum suitable as a flexible biocompatible scaffold for cell differentiation and osteogenic differentiation [79]. For example, 3D graphene foams used as substrates for human mesenchymal stem cells (hMSC) have shown their capacity to preserve stem cell viability and facilitate osteogenic differentiation [80]. In addition, 3D graphene (3DGp) scaffolding and 2D graphene (2DGp) coating have been shown to induce the differentiation of periodontal ligament stem cells (PDLSC) into mature osteoblasts by higher rates of mineralization and graphene-related upregulated bone-related genes and proteins with or without chemical inductors [81]. GO was placed by Han et al. on Ti scaffolds, modified with polydopamine (PDA). After that, a separate form of gelatin microspheres (GelMS) was encapsulated in BMP-2 and vancomycin. The drug-containing GelMS were subsequently placed on GO/Ti scaffolds and stabilized by usable GO groups (Figure 7). The novel scaffolds play an essential role in bone recovery and tolerance for bacterial infections [82]. Substance P (SP) is a neuropeptide containing 11 strictly retained amino acids [83]. It includes, for example, inflammatory wound healing, control, and angiogenesis in many procedures, and is necessary to facilitate the recruitment of MSC implants [84]. So apart from the BMP-2, this peptide, SP, was applied to the GO-coated Ti surface by La et al. The dual delivery mechanism of GO-coated Ti has demonstrated the continuous release of BMP-2 and SP, and the ability of SP to activate MSC mi-integration. In vivo, the Ti/SP/GO/BMP-2 group showed more magnificent new bone formation in the mouse calvary relative to implant recruitment by SP for the Ti/GO/BMP-2 population [85].

A significant factor in the design of tissue engineering scaffolds is the mechanical strength and longevity of the

TABLE 2: Carbon nanotubes used in the manufacture of bone tissues.

| Method of NP synthesis | Type of NPs | Cell type | Mechanical strength (MPa) | Application | Ref. |
|---|---|--|---------------------------|-------------|------|
| In situ hybrid CNTs with bacterial cellulose (BC) | BC | Osteogenic cells | 0.474 | In situ | [63] |
| Chemical vapor deposition (CVD) | Hydroxyapatite | Osteoblastic and fibroblast (L-929) cells | 89 | In situ | [64] |
| Thermal | Hydroxyapatite | Human osteoblast sarcoma cell lines | — | In vitro | [65] |
| CVD | Graphene (G) nanosheets and HAp-polyether ether ketone (PEEK) | MG-63 cells and human bone marrow stromal cells (hBMSCs) | 78.65 | In vitro | [66] |
| Freeze-drying method | Polyvinyl alcohol (PVA) | Osteoblast cells | 215.00 ± 9.20 | In vitro | [67] |
| Freeze-drying method | Polysaccharide HAP | MG 63 cell line | 0.222 | In vitro | [68] |
| Thermal | Poly (butylene adipate-co-terephthalate) (PBAT) | MG63 osteoblast-like cells | 3.5 | In vitro | [69] |

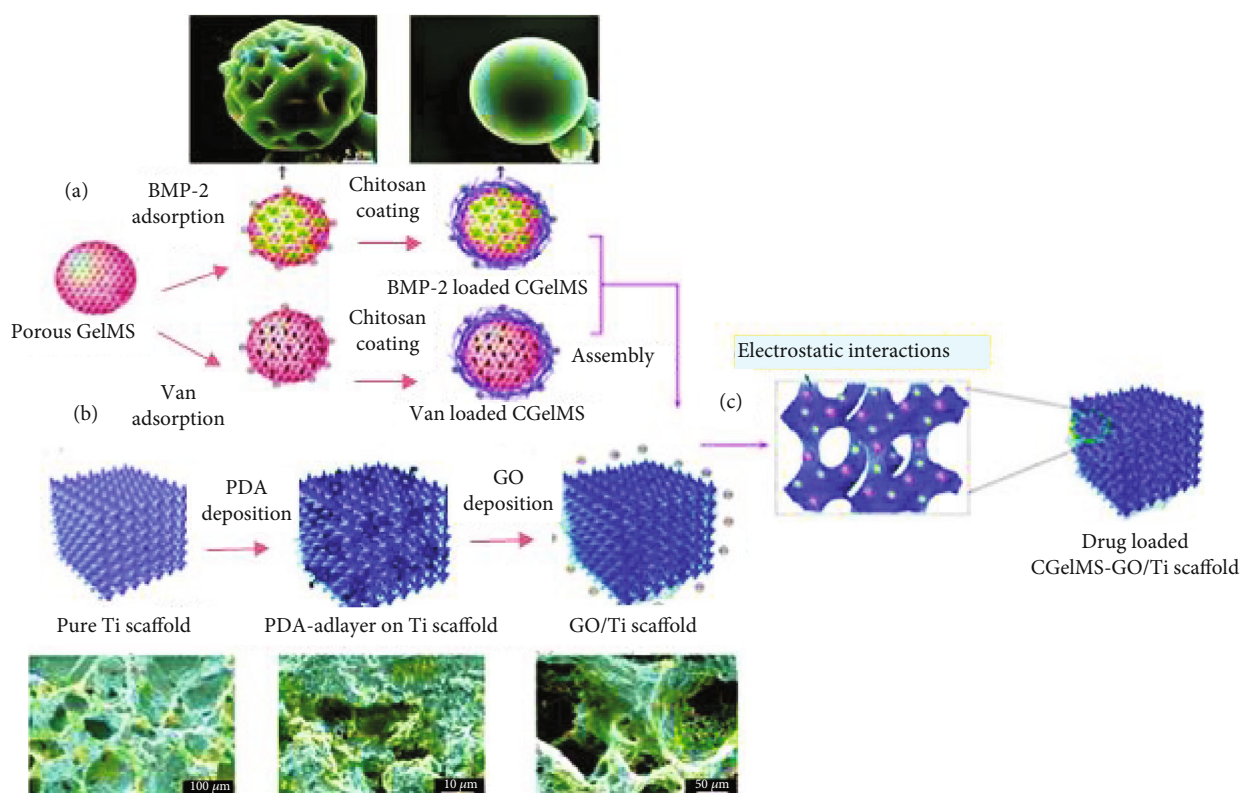


FIGURE 7: Schematics and electron micrograph examination of the preparation of the new GO/Ti scaffold: BMP2- and Van-loaded CGelMS are immobilized by electrostatic interactions on the GO/Ti scaffold between functional GO and CGelMS groups [82].

material. GO-based composites have very porous structures and high mechanical strength, which give strong prospects for regeneration to Liang et al. scaffolds. It is reported that composite scaffolds HAp/collagen (C)/poly(lactic-co-glycolic acid)/GO (nHAp/C/PLGA/GO) facilitate the proliferation of MC3T3-E1 cells (Figure 8), [86]. For scaffold preparation, they formulated nHAp/C/PLGA/GO nanomaterials with a particular percentage of GO weight and measured the mechanical properties of the scaffold. The findings showed

that the dynamics would raise the mechanical strength of the scaffold by 1.5 wt. percent of GO and provide a good cell adhesion and propagation substratum.

One of the critical factors influencing the mechanical possessions of the bone-shaped in tissue engineering is the adhesion of bone cells to the substrate at the center [88]. A variety of work has focused on this subject over the last few years—for example, Mahmoudi and Simchi. A nanofibrous matrix was developed using electrospun material to improve

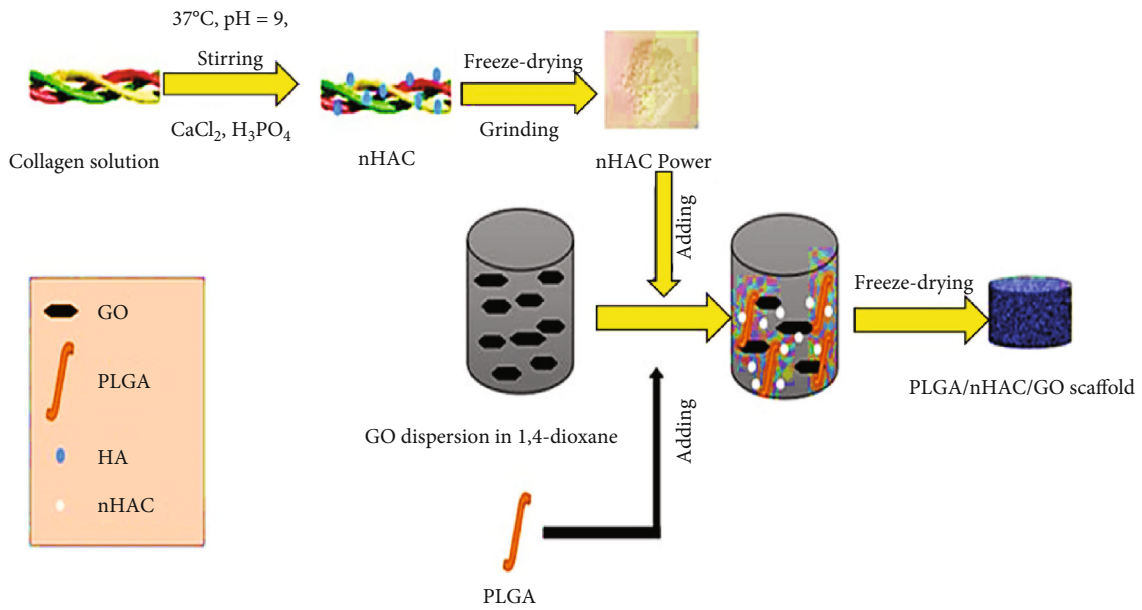


FIGURE 8: Experimental schematic techniques for the preparation of nHAp/C/PLGA/GO scaffold [87].

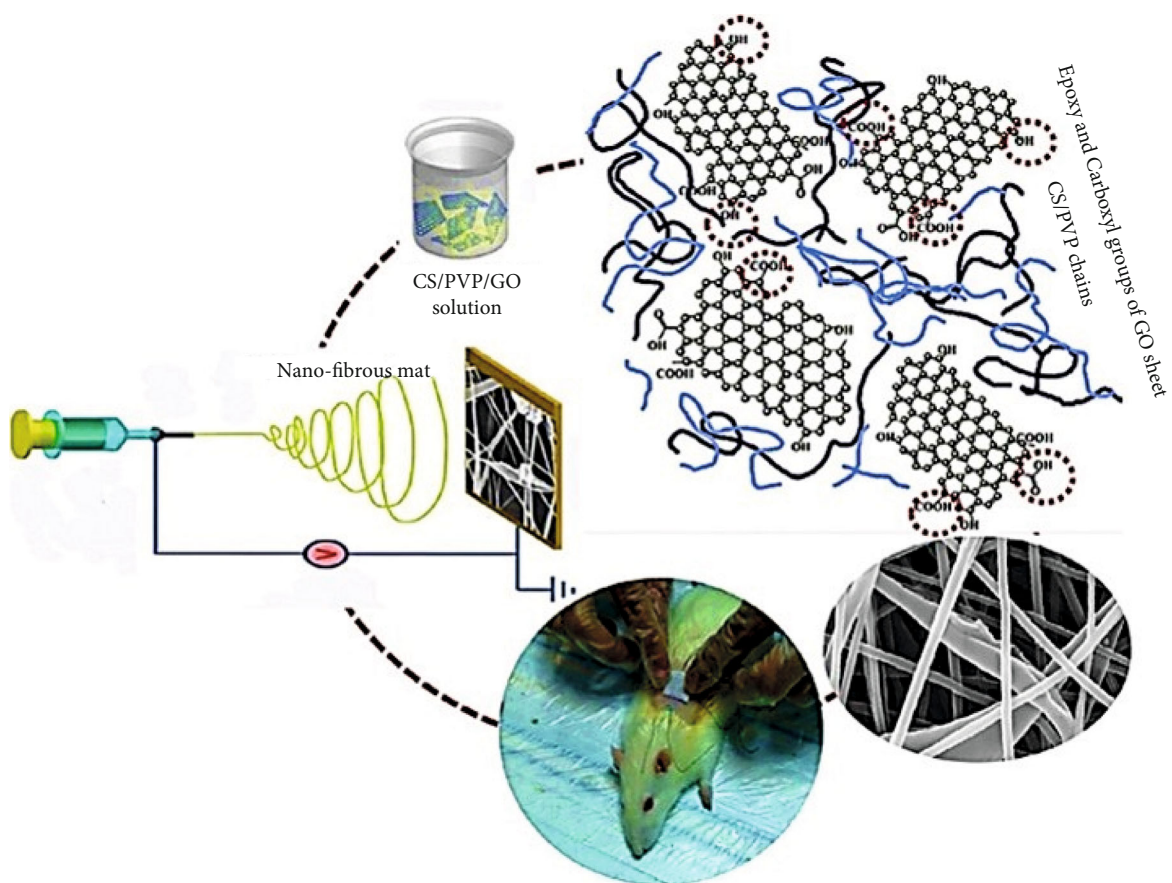


FIGURE 9: Biopolymer-GO composites are made using chitosan (CS), poly (vinyl pyrrolidone) (PVP), and GO using a process of electrospinning [89].

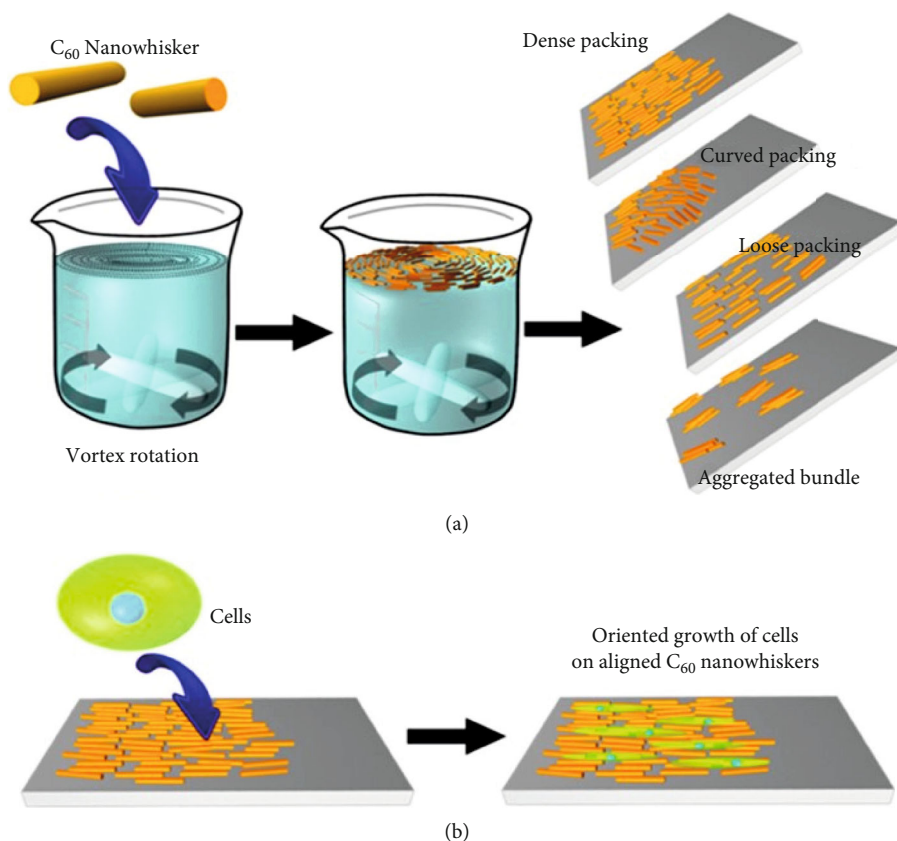


FIGURE 10: Schemes for (a) the alignment of C₆₀NWs at an air–water interface by vortex flow and (b) aligned C₆₀NWs as scaffold for directing cell growth [74].

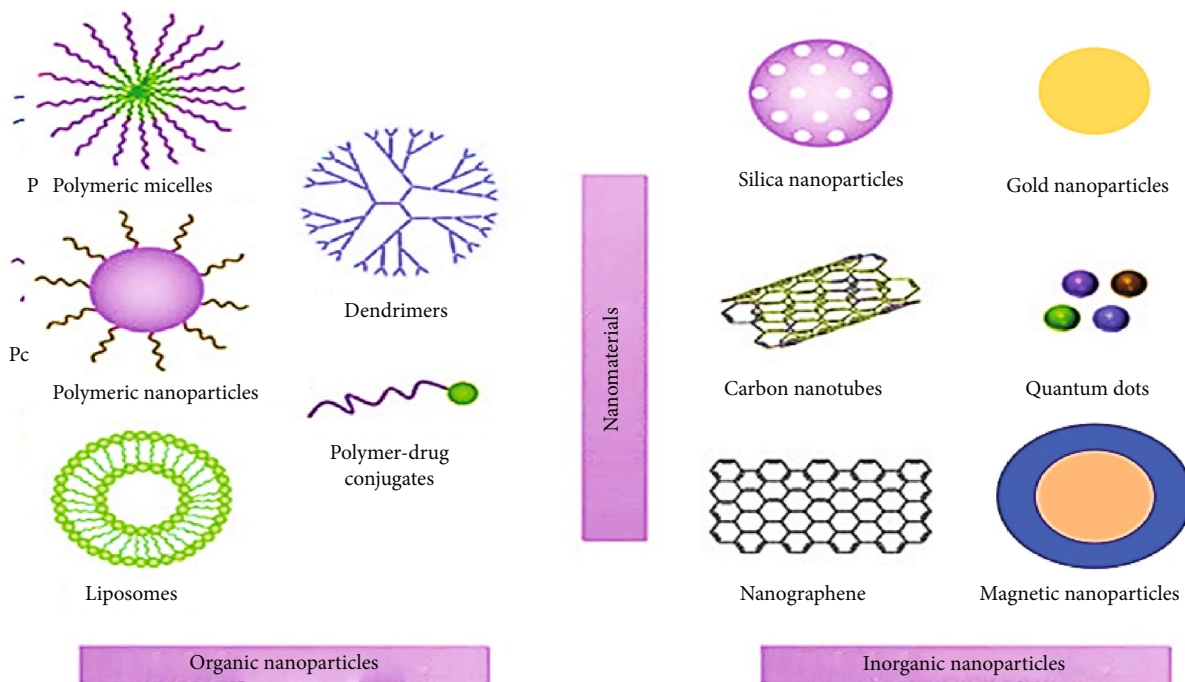


FIGURE 11: Representation of nanomaterials used for cancer therapy, including organic and inorganic nanoparticles [91].

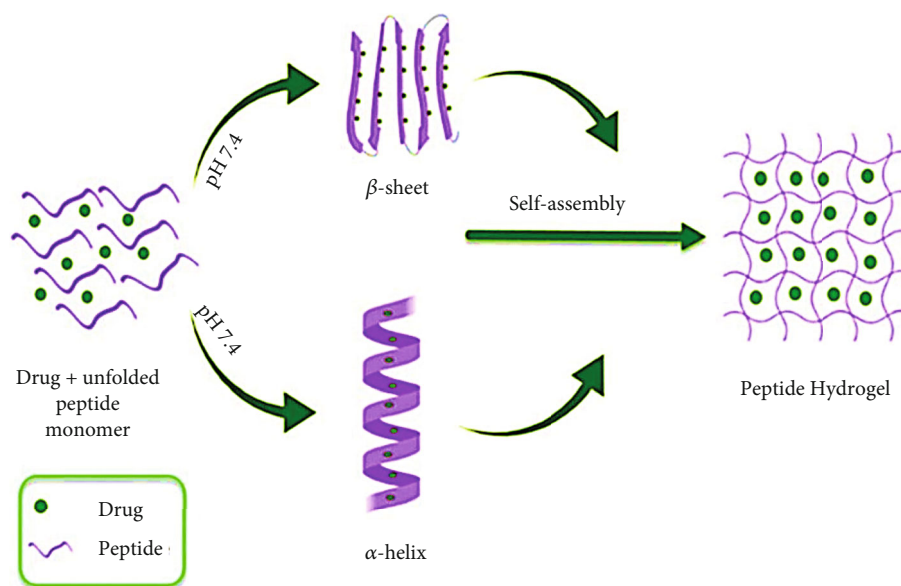


FIGURE 12: Peptides are self-assembled into hydrogels for drug delivery [93].

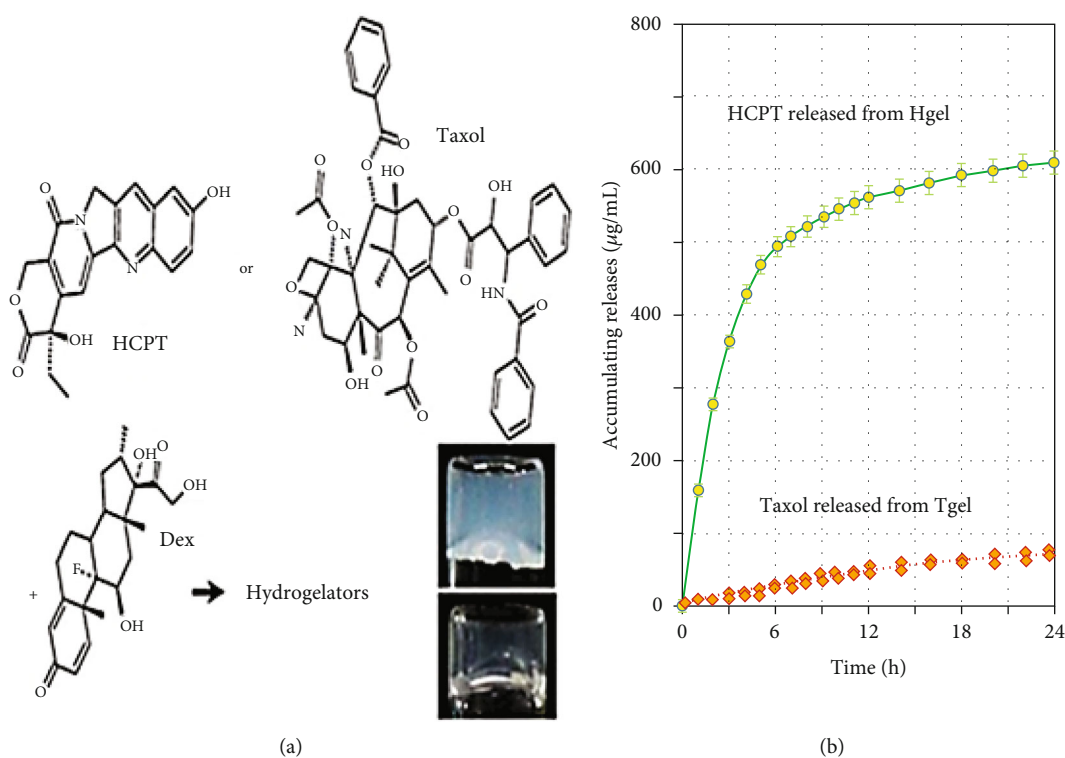


FIGURE 13: Codelivery of two anticancer drugs via a peptide-based hydrogel system [93].

the bonding forces within the bone cells. Because of this, they used high mechanical resistance and biocompatibility biopolymers and GO hybrids, and then a natural closure rate for wounds. The experimental design approach to this material are shown in Figure 9 [89].

2.3.2. Scaffolds of Fullerene Materials. Scaffolds of fullerene materials have specific situations in various and broad ranges of scaffolds in bone tissue engineering. These have strong

calming effects on bone cell proliferation, with minimal cytotoxicity. Scaffolds dependent on fullerenes have more roughness and hydrophobicity. It would improve the potential for controlling the cell connection and strengthen the bone tissue thickness, and then the mineralization phase, which is an entirely remarkable feature of carbon nanomaterials. The fullerene molecule structure enables the anatomy of the final bone tissue to be organized and regulated [45]. In order to stimulate the growth of osteoblastic MG 63 bone cells,

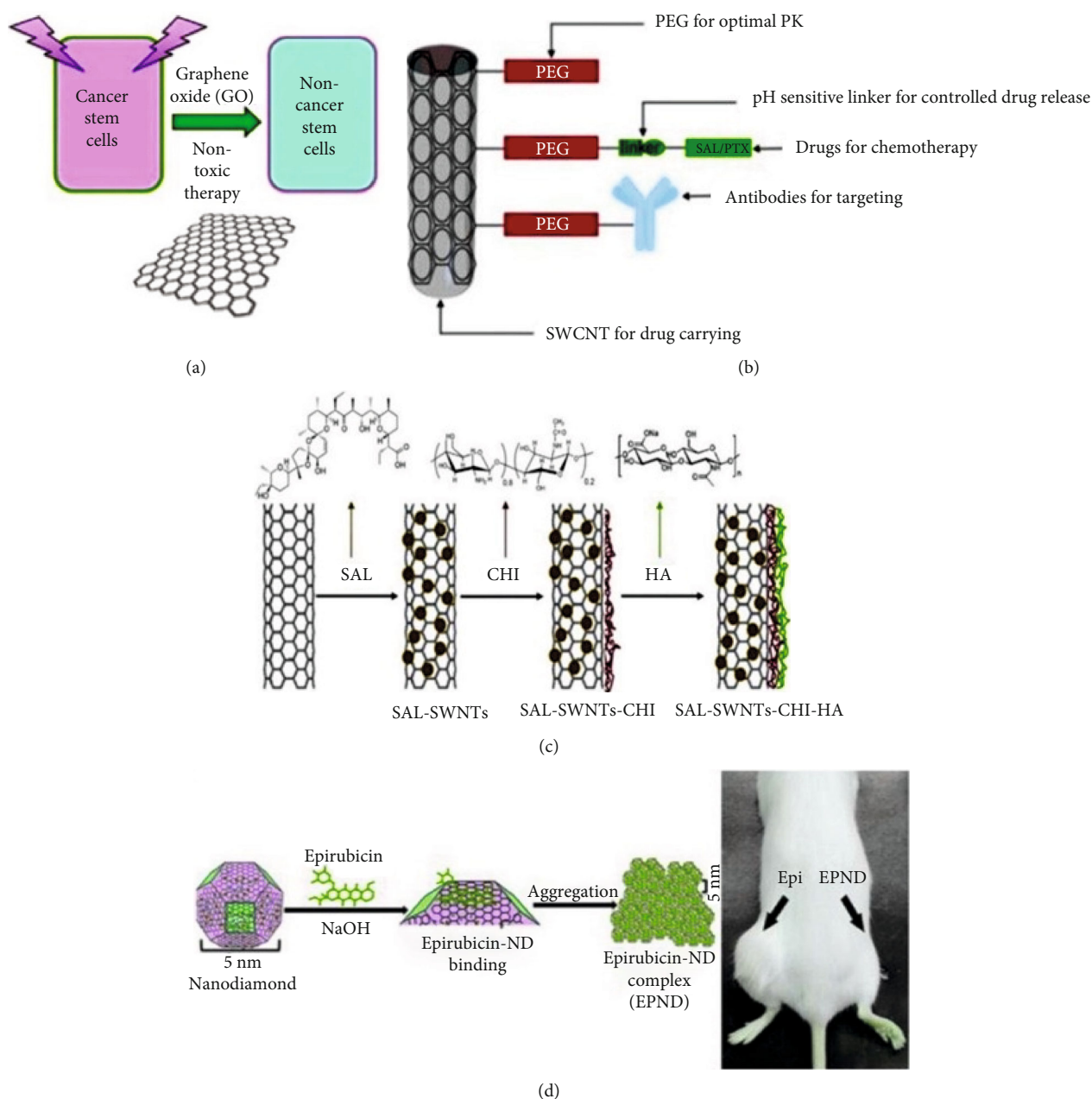


FIGURE 14: (a) Graphene oxide (GO): cancer stem cells targeted at the difference with nanotherapy [71]. (b) Schematic illustration of a processing method for SAL-SWNTs-Chi-HA (chitosan- (CHI-) coated single-wall carbon nanotubes (SWCNTs) treated with hyaluronic acid (HA) salinomycin (SAL)) [102]. (c) Schematic diagram showing the concept of drug carriers as operational SWCNTs [103]. (d) Left: a graphical model demonstrating the surface and chemical composition of (ND) and epirubicin (Epi). Epirubicin-nanodiamond (EPND) complex synthesis and aggregation. [104].

Krishnan et al. described a new method for making and depositing fullerene nanowhiskers on scaffolds. This was achieved by rotational flow of solutions that comprise fullerene nanowhiskers, allowing normal arranged arrays to be deposited on a glass substrate. They observed that fullerene nanowhiskers' normal, coordinated deposition had better biological activity than a random deposition. The distance from the vortex core of the glass substratum played a vital role in morphology of the formation. Samples produced at the edge of the vortex fluid had a more normal morphology

than samples from core vortex sections. A processing schematic for this sample is shown in Figure 10 [74].

Cancer accounts for millions of deaths per year, and the number of new confirmed cases is increasingly growing due to the rise and aging of the world's population. While several advancements in early detection and novel therapy procedures have now been established in clinical practice, several important issues still need to be resolved in order to treat cancer efficiently and to reduce many drawbacks created by traditional therapies. Nanomedicine appeared as an up-

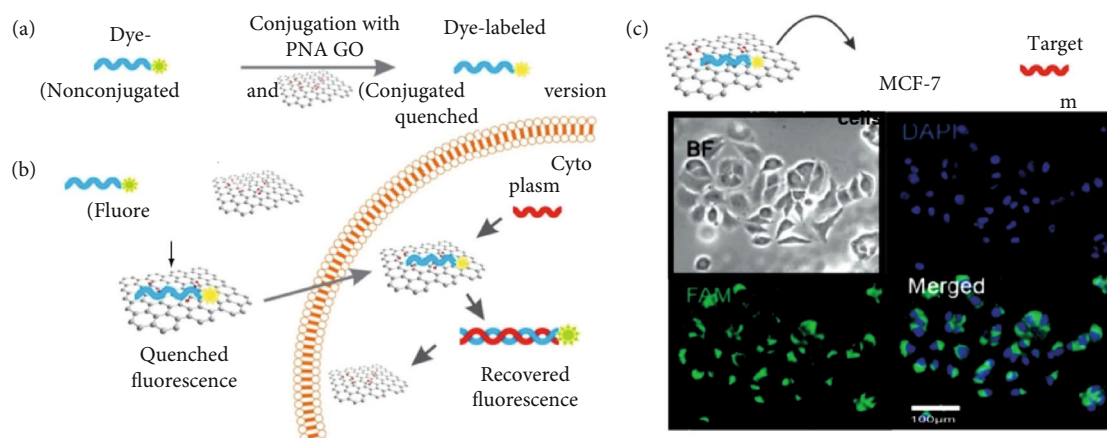


FIGURE 15: Nanotechnology focused on graphene used to identify miRNAs. (a) The dye-labeled PNA becomes fluorescent when nonconjugated; however, fluorescence becomes quenched after contact with GO. (b) Nanoparticle graphene oxide technique for intracellular miRNA identification has been used as a fluorescence quencher for the identification and tracking of dye-labeled nucleic acid probes in living cells by miRNA multiplex in vitro. Dye-labeled PNA fluorescence is quenched when interacting with GO, and further hybridization of the PNA with the target miRNA has helped activate the GO surface PNA sample and recovery of fluorescence. The ability of the PNA-GO probe to enter cells and the minimization of nonspecific fluorescence signal allows sensitive monitoring of multiple miRNA targets even in living cells with low basal fluorescence. (c) GO-sample fluorescent microscopy of MCF-7 cancer cells for quantitative miR-21 detection. MCF-7 cells had substantial fluorescence due to the strong expression of miR-21. GO: graphene oxide; PNA: peptide nucleic acid [118].

and-coming method to promote both early detection and successful tumor therapy. A plethora of various inorganic and organic multifunctional nanomaterials was ad hoc developed to satisfy the increasing need for new cancer treatment solutions [90]. As shown in Figure 11, a wide variety of nanomaterials were produced using organic, inorganic, lipid, and protein compounds usually within 1–100 nm varieties and delivering various antitumor drugs by fine-tuning the chemical structure, size, and form (morphology) capable of regulating nanomaterial functionality.

2.4. 3D Self-Assembled Peptide Hydrogels in Cancer Therapy. Peptide hydrogels are leading carriers in many medical applications because of their exceptional structural and behavioral properties [92]. Numerous self-assembled peptides have been developed which have the potential to be an antitumor drug delivery nanocarriers. Figure 12 shows the peptides that are self-assembled into hydrogels.

By self-assembling its molecules, the peptide produces structures identical to nanotubes. With adequate mechanical power, stability, and biocompatibility, the microscale length of these nanotubes is calculated. It was also found that its thermal and chemical properties were within the appropriate range [94]. Self-assembled hydrogels based on peptides have significant effects in stabilizing and regulating the release of anticancer drugs. Some examples of self-assembled peptide-based hydrogels in tumor cells were receptive to microenvironmental conditions [95]. Mao et al. first advanced a drug delivery device focused on a self-assembled peptide hydrogel. The study group integrated two chemotherapeutic drugs and reported a significant increase in medication safety, as seen in Figure 13 [96]. The device showed a controlled release of medicinal products through hydrolysis of the ester bond, thus dem-

onstrating the potential for targeted antitumor delivery. Due to their lower cost and tunable properties, small peptide hydrogels are reported to be more beneficial for the delivery of drugs [97].

2.5. Graphene Oxide for Cancer Therapy. Previous research has shown that GO can be used to monitor targeted cancer, to stop tumor growth, and to prevent tumor cell movement [2, 98, 99]. Phototherapy focused on transdermal nanographene oxide-hyaluronic acid (NGO-HA) conjugates recorded for skin cancer melanoma using a near-infrared (NIR) laser in 2014; however, studies which used GO in CSC therapy for cancer treatment are uncommon. Fiorillo et al. have shown that GO prevents tumors from growing in six separate lines of cancer cells (prostate, pancreatic, breast, vaginal, lung, and brain cancer) through different types of tumors. They used the tumorsphere method to evaluate GO-targeted therapy and clinically measured the production and extension of tumorspheres from individual CSCs under conditions independent of anchorage. The results suggested that GO specifically targets a phenotypic worldwide property of CSCs, which may decrease the amount of bona fide CSCs by splitting which inhibiting them (Figure 14(a)) [100, 101]. The author here, in a nutshell, presents evidence that GO-based therapy may be successful in reducing CSCs by inhibiting several main signal pathways and then splitting CSCs.

2.6. Carbon Nanotubes in Cancer Therapy. Carbon nanotubes are nanostructures of cylindrical graphene with advanced nanotechnologies and clinical research, for example, water solubility, cell membrane penetration, strong drug load performance, photothermal, low toxicity, tumor selectivity, photoacoustic, and radiant properties [105, 106].

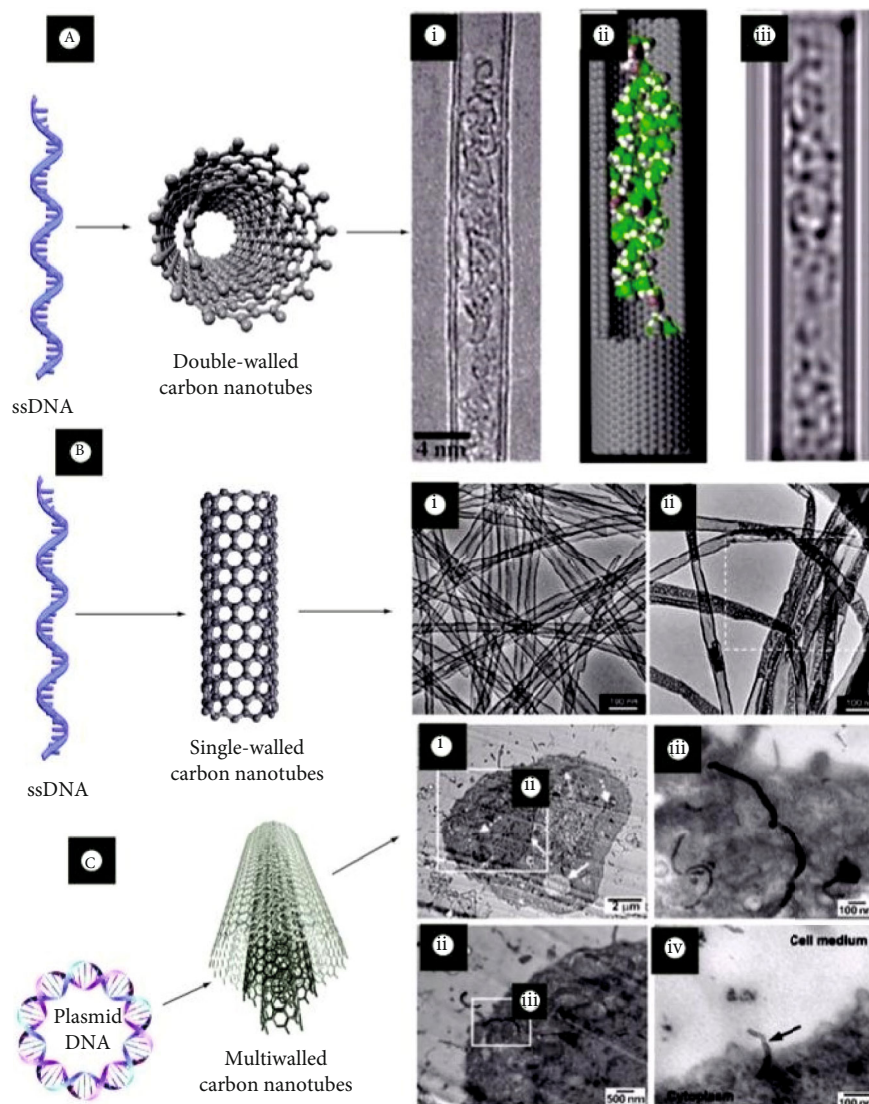


FIGURE 16: DNA encapsulation and gene transition. (a) Double-walled nanotubes of carbon, which encapsulate ssDNA. (i) A transmission image of ssDNA molecules composed of 30-base cytosine homopolymers (defined as C30) encapsulated double-walled carbon nanotube by an electron microscope (TEM). (ii) Layout TEM image structure and (iii) simulation of TEM image. (b) A single-walled carbon nanotube encapsulated with ssDNA. TEM photographs, after soaking, of a solution ssDNA (i) without CaCl_2 and (ii) CaCl_2 (DNA-CaCl_2). The ssDNA internalization happened at (iii). (c) Multiwalled carbon nanotubes which are used for strong plasmid DNA encapsulation. The cells in HeLa were treated with DNA-multiwalled carbon plasmid nanotubes during the encapsulation process. Box magnifications for each cell are given (ii and iii). (iv) A multiwalled carbon nanotube which separates the cell membrane. White dotted arrow: chromatin; white dotted arrow: mitochondria; white dotted arrow: atomic membrane; white arrow: vacuole. To: ssDNA [118].

Burke et al., in 2012, mentioned that breast cancer stem cells (BCSCs) were found to be immune to thermal carbon nanotube therapy and loss of proliferative capacity after thermal nanotube therapy [107]. Thus, nanotube-assisted thermal therapy will destroy all the isolated cells that form the bulk of a tumor simultaneously. In 2014, Yao et al. developed a gastric CSC-specific drug delivery system (SAL-SWNT-CHI-HA complexes) centered on single-wall chitosan-coated carbon nanotubes (SWChNTs) packed with the hyaluronic acid (HA) and salinomycin (SAL) structure. The designed system can extract gastric CSCs selectively (Figure 14(b)) [108]. Al Faraj et al. suggested a technique utilizing biocompatible multimodal SWCNTs that were func-

tionized with CD44 antibodies and enhanced direct anti-CD44 targeting, resulting in promising breast targeting findings for CSCs and potential for further clinical trials [103]. Shortly afterward, the same community combined paclitaxel and salinomycin drugs in the murine xenograft model combined SWCNTs (Figure 14(c)) similarly to fight breast cancer and CSCs simultaneously, and the results revealed an increased therapeutic effect of combination therapy compared to care for independent nanocarriers or free suspension of medication. Consequently, the optimized drug delivery mechanism for conjugated SWCNTs has enormous potential to effectively treat breast cancer by attacking both CSCs and cancer cells [103].

TABLE 3: Graphene-based nanocarriers used for gene delivery.

| Graphene-based nanomaterial | Gene | Target cell in the study | Ref. |
|--|-----------------------------------|-----------------------------|-------|
| Graphene oxide low-molecular-weight branched polyethyleneimine | Luciferase reporter gene | HeLa and PC-3 cell lines | [116] |
| Graphene-polyethyleneimine (25 kDa) | EGFP | HeLa cells | [123] |
| Graphene oxide-chitosan | Luciferase reporter gene | HeLa cells | [129] |
| Grafted ultrasmall graphene oxide-polyethyleneimine | EGFP | H293T and U2Os cell lines | [124] |
| Graphene-polyethyleneimine (25 kDa) | Luciferase reporter gene | HeLa cells | [123] |
| Graphene oxide-gold nanorods-polyethyleneimine | EGFP and luciferase reporter gene | HeLa cells | [125] |
| Graphene oxide-gold nanoparticles-polyethyleneimine | EGFP and luciferase reporter gene | HeLa cells | [125] |
| Reduced graphene oxide PEG low-molecular-weight branched polyethyleneimine | Luciferase reporter gene | PC-3 and NIH/3T3 cell lines | [128] |

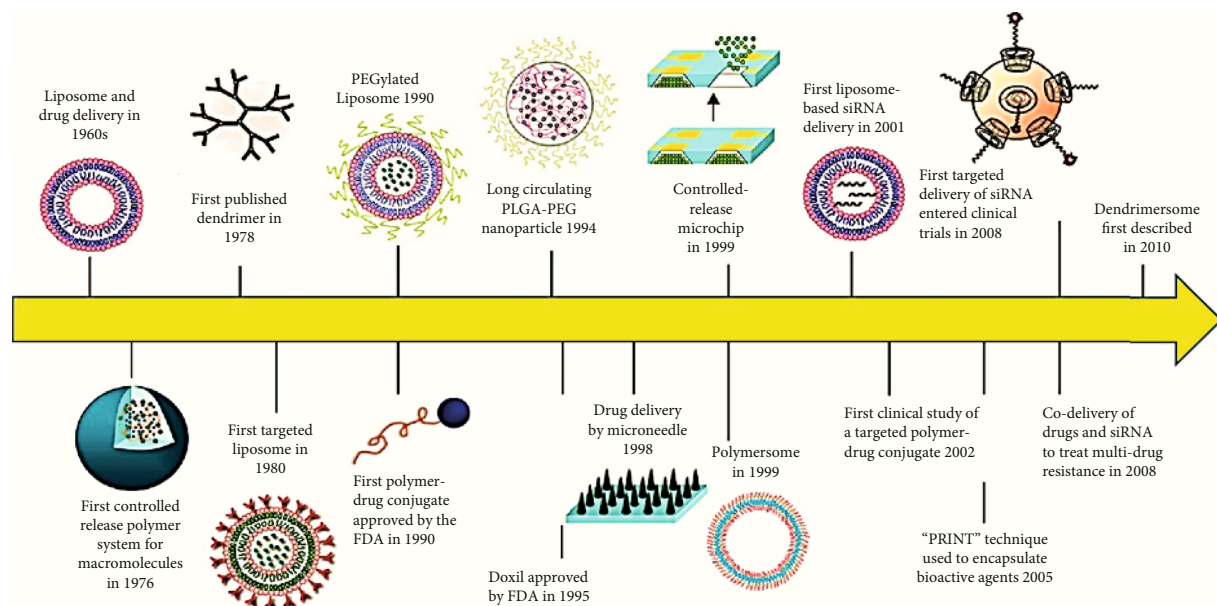


FIGURE 17: Delivery of nanotechnology-based drugs in the timeline. Here, we highlight those delivery systems which serve as essential milestones in drug delivery history [131].

2.7. Nanodiamonds in Cancer Therapy. Nanodiamonds are carbon semi-octahedral systems with a wide variety of biological and chemical elements, as well as small molecules, genetic content, biomolecules, and imaging agents [109]. Nanodiamonds (NDs) have shown excellent delivery capacity and excellent biocompatibility among a wide range of vehicles based on nanomaterials [110]. Zhao et al. showed that detonation of nanodiamond with hyperbranched polyglycerol (dND-PG) coating charged with an anticancer drug and conducted by an active targeting moiety might lead to tumor cells becoming highly preferential toxic via different absorption mechanisms while minimizing macrophage absorption and toxicity [111]. The nanodiamond drug complex, also developed by physical adsorption of epirubicin on nanodiamonds, has proven to be a highly successful nanomedicine-based solution for overcoming chemoresis-

tance in hepatic CSC. As shown in Figure 14(d), the resultant EPND complex, Epirubicin@nanodiamonds, has improved care over unmodified epirubicin [104]. The probability of binding different bioactive molecules to carbon molecules such as cell-specific ligands makes carbon-dependent nanomaterials an important option for cancer therapy through targeting CSCs.

2.7.1. 3D Self-Assembled Nanostructures for Gene Delivery.

Despite recent advancements in multiple nucleotide-based therapies, the efficacy of gene therapy in clinical procedures remains limited due to less efficient delivery routes to the targeted tissue or cells [112]. Nanotechnology has rendered a significant advance in the production of healthy and efficient gene carriers in recent decades [113]. Nonviral gene delivery carriers can be made from several materials, including

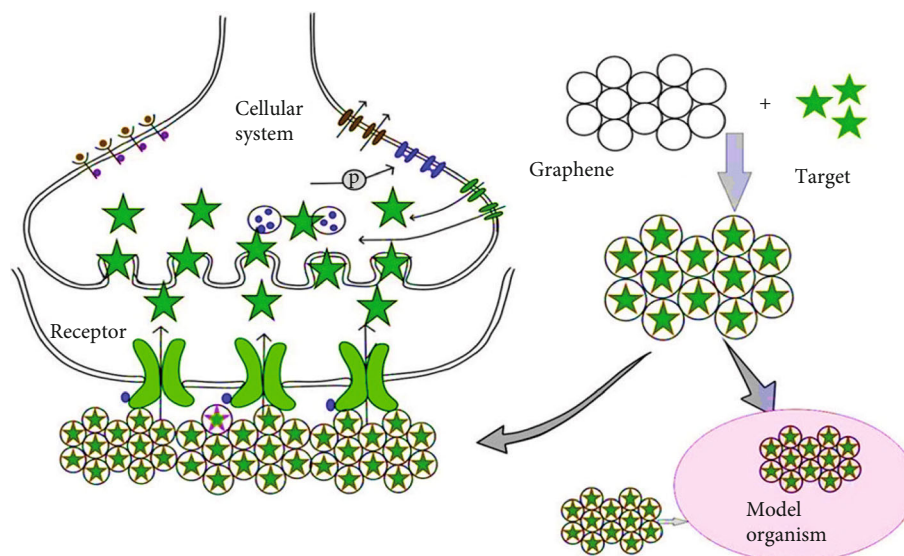


FIGURE 18: Graphene as target carrier (gene or small molecular drug) [134].

inorganic nanoparticles, carbon nanotubes, liposomes, protein-based nanoparticles, and peptides, as well as nanoscale polymeric materials, and have gained popularity in recent years due to their protection, versatility in nucleic acid packaging, and ease of processing. Significant attempts were made to increase the efficiency of nonviral gene transmission by fair and semirational design as an ideal gene carrier that should have many functions to resolve the obstacles in the gene transfection process at different levels.

2.7.2. Gene Delivery with Graphene-Based Nanomaterials. Graphene and its derivatives have been increasingly used in many biomedical fields as sheet-like carbonic nanomaterials [114, 115]. Graphene-based platform applications currently apply to the distribution of genes as a nanocarrier. Provided the unique structure and chemistry of graphite nanoparticles, they have a high potential for gene processing. Biostability, cellular uptake, and improved efficiency of gene processing, graphene surfaces, and their derivatives have been adjusted with various polymers or ligands to boost biocompatibility [114, 116]. Graphene-based nanosheets with a vast hybridized sp² carbon region may interact with further molecules, including DNA and RNA nucleic acids, as well as with drugs. They may also be used as moving genes or as protectors and carriers of specimens involved in miRNA detection (Figures 15 and 16) [117].

DNA can also interact with nanomaterials based on graphenes [119]. Low pH and strong ionic resistance DNA adsorption can be built with tiny fragments [120], in which they are covered sterically from nuclease (DNase) attacks [121]. One of the advantages of preserving DNA is that a robust gene transfer vector will thus be proficient in successful cellular uptake. Graphene is, therefore, an exciting option for plasmid transmission, uniquely when it is functionalized with cationic polyethyleneimine that interacts well with DNA phosphate groupings [119]. Kim et al. combined low-molecular-weight polyethyleneimine branched to GO to provide the cytomegalovirus promoter

with a plasmid that controls luciferase gene expression [116]. Feng and Liu initially delivered a plasmid to HeLa cells carrying the enhanced green fluorescent protein (EGFP) encoding gene, using a different nanocarrier [122]. Chen et al. used a higher-molecular-weight polyethyleneimine [123] in which cytotoxicity to polyethyleneimine decreased with GO presence. In the presence of 10% fetal bovine serum (FBS), they also transmitted the luciferase reporter gene to HeLa cells using this gene delivery mechanism. They demonstrated that the transfection efficiency of serum proteins did not decrease [108]. Zhou and his colleagues recently transferred ultrasmall GO plasmid DNA (pEGFP) to mammalian cell lines and zebrafish embryos [124]. By contrast, Xu et al. encapsulated inorganic nanoparticles and gold nanorods in GO nanosheets that not only decreased cytotoxicity dramatically, allowing polyethyleneimine to work better, yet also achieved high transfection efficiency and improved viability of HeLa cells [125]. Although polyethyleneimine is not the only gene transmission component that can be added to the nanomaterials based on graphene, in this experiment, Bao et al. used a chitosan-like GO (a nanosystem frequently administered to HeLa cells for a plasmid containing the luciferase gene) and demonstrated that this complex could condense DNA for rapid cell absorption through agarose gel electrophoresis [126]. Additionally, the use of PEG and polyethyleneimine, for example, to improve the efficiency of plasmid DNA transfection, can also be performed in GO. This gene carrier was also found to be light-responsive, as described by Feng et al. [127]. Another example of an environmentally sensitive nanosystem is Kim et al.'s photothermally regulated gene delivery carrier, which integrates branched low-molecular-weight polyethyleneimine and reduced GO through PEG. The increased efficacy of the gene transfection of this nanocarrier was due to a rapid endosomal escape through locally mediated heat [128]. Table 3 reviews several of the graphene-based nanocarriers used for the gene delivery.

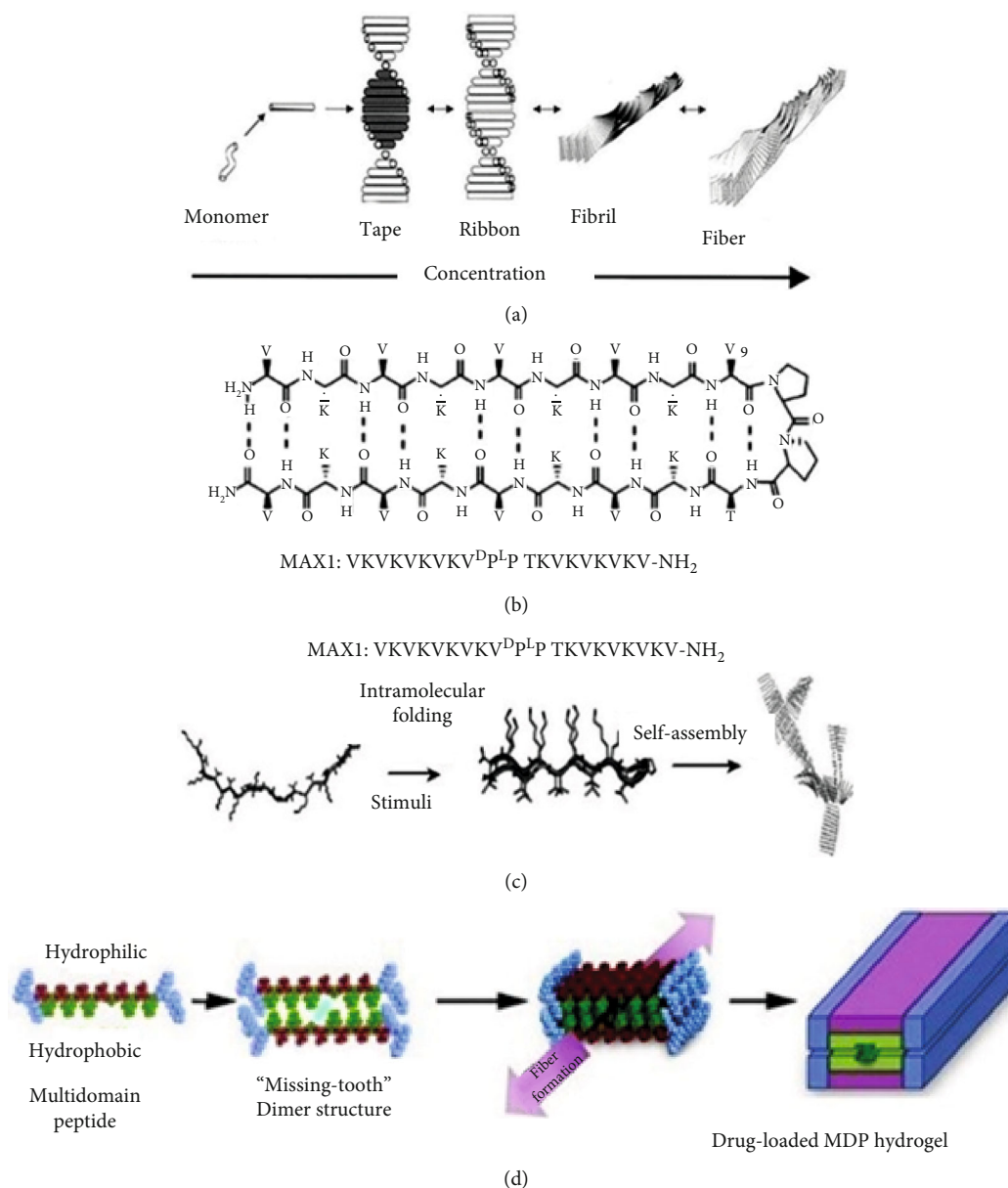


FIGURE 19: (a) Hierarchical model of self-assembly, from monomer to fiber. (b) MAX1 and (c) β -hairpin sequence intramolecular random-coil folding of the MAX1 peptide followed by self-assembly. (d) The MDP assembly named "broken tooth" exposes the vacuum produced during fiber packaging. MDP: multidomain peptides [143].

3. 3D Self-Assembled Nanostructures for Small Molecule Drug Delivery

Small molecules are amongst the most vital biological function molecules found in most medicines to date. Many organic molecules exhibit low solubility in aqueous media and insufficient delivery of products as the primary cause of around 40 percent of all medication failures. While high-profile strategies continue to improve a variety of chemical agents for the treatment of complicated disease processes, there is an increasing need for suitable methods of molecular delivery to be established, which are useful and practical. Precise spatiotemporal regulation of a large variety of hydrophobic and neutral small molecules is therefore essential. Recent developments in nanotechnology have introduced smart and

new therapeutic nanomaterials using various targeted approaches [130]. The application of nanotechnology in medicine and, more specifically, the drug market is expected to expand even more than it has over the last two decades. As delivery vehicles, a range of organic/inorganic nanomaterials and technologies were used to develop effective therapeutic methods (Figure 17) [131].

3.1. *Graphene and Graphene Oxide as Nanomaterials for Small Molecule Drug Delivery.* Mechanisms of drug delivery utilizing graphene-dependent nanosources have been researched since 2008 [132, 133]. Proteolytic enzyme development within the cytoplasm often interferes with the drug delivery process. GO is used to provide the carrier genes and medicines with efficacy (Figure 18). The GO biological

community (COOH and OH) allows for the mixing of numerous polymers and biomolecules (DNA, ligand, and protein) [134]. Methods contain its cationic polymer functionality, like PEI [24]. It is known as a nonviral gene vector, as it intensively communicates with negatively charged DNA and RNA phosphate ions [24]. Its varieties of transfection are simple and effective boost cell selectivity and decrease cell toxicity. The use of PEI-functional GO transmission of the Bcl-2-target antiapoptotic family protein siRNA and anticancer drug DOX exhibited a synergistic impact providing enhanced transfection ability with reduced PEI cytotoxicity and enhanced anticancer effectiveness [135]. Also established was a photochemically regulated gene delivery carrier in which low-molecular-weight PEI and rGO were combined with hydrophilic polyethylene glycol (PEG) and plasmid DNA and physiochemical assays were found to be stable [136]. FeO nanoparticles offer multifunctional and multimodal GO for broad organic and medicinal applications [128]. The delivery of anti-inflammatory ibuprofen drugs utilizing chitosan-containing GOs has been reported [137].

Graphene's unmodified basal plane sites with free surface π electrons are hydrophobic and can create interactions for charging drugs and covalent modifications [123]. Part of drug delivery is due to differences in the concentration of temperature, pH, light, and salt. Polymers sense the fundamental changes, and the medication is released. GO biopolymers are pH sensitive and are, therefore, often used as smart transporters for the delivery of drugs [138]. In this respect, the use of folic acid containing of nano-GO (NGO) called FA-NGO for the treatment of tumors has conclusively demonstrated pH-sensitive delivery in the case of DOX and camptothecin [139]. Ibuprofen and 5-fluorouracil anti-inflammatory drugs with distinctive hydrophilicity were also administered utilizing a pH-dependent CS-GO complex [140]. GO is being modified to carry a carrier of water-soluble cancer drugs. The functionalized PEG NGO can allow more soluble physiological and aqueous solutions [141].

3.2. Self-Assembled Peptide and Protein Nanofibers for Small Molecule Drug Delivery. Hydrogels offer a commonly employed and efficient tool for the distribution of small molecule medications and biological therapies self-assembly as shown in Figure 19 (e.g., proteins and DNA), as their physical and chemical properties may be adjusted to suit the release profile of the encapsulated cargo and are treated under moderate conditions conducive to cargo survival [142].

Soil-based hydrogels can be used for the delivery of small medicinal molecules. For instance, hydrogels that consist either of a high-molecular-mass silk protein (SPH) or an SPH composite and a low-molecular-mass silk protein (SPL) release the drug buprenorphine at concentration-related SPH levels [144]. This concentration of SPH was hypothesized as leading to a denser network of nanofiber and, in turn, a more tortuous route through which buprenorphine moves to avoid the hydrogel. This was also confirmed by SPH/SPL composite hydrogels, where 10% SPH with 6% SPL had a higher diffusion rate than 10% SPH with 2%

SPL, respectively. RADA16 gels, for example, published small-molecule encapsulated colors such as phenol red, bromophenol blue, 3-PSA, 4-PSA, and Coomassie Brilliant Brown G-250 (CBBG) at levels associated with chemical coloring [145]. In particular, after seven days, bromophenol blue and CBBG did not elute out of the gels, indicating that they had not adsorbed nanofibers directly into RADA16. Besides, 3-PSA has eluted the more electrostatically charged 4-PSA at a faster pace, indicating the net charge of the drugs that affect their release kinetics. The diffusivity of red, 3-PSA, and 4-PSA phenols decreased as the RADA16 concentration increased similarly to the SPH/SPL gels mentioned above.

4. Conclusion and Future Perspectives

This research summarized the application of 3D self-assembled polyfunctional nanostructures such as carbon nanotubes (CNTs), graphene fullerene, and peptide hydrogels that are used successfully in tissue engineering, gene delivery, and cancer therapy. Some nanocarbon allotropes, such as GO, CNTs, fullerenes, CDs, NDs, and their derivatives, have high potential as bone cell proliferation scaffolds and can be used to rebuild bones. Also, nanotube-assisted thermal therapy can kill all of the specialized cells that form the bulk of a tumor simultaneously. Because of their nanoscale size, photoluminescence properties, large specific surface area, and antibacterial activity, graphene family materials possess significant potential for bone tissue engineering, drug/gene delivery, and cancer treatment.

Self-assembly of nanostructural materials is theoretically valuable and has produced new resources to revolutionize the biological and biomedical sciences. In this study, application of 3D self-assembled nanostructures such as carbon nanotubes (CNTs), graphene and fullerene, peptide hydrogels for use in tissue engineering, gene delivery, and cancer therapy has been summarized. Besides, they have applications in drug delivery, vaccine delivery, and photothermal therapy. Therefore, their application can be examined in these cases as well. Also, there are other categories of 3D self-assembled nanostructures that have exciting applications in medicine, which are essential and practical to study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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