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Nanodiamond-containing composites for tissue scaffolds and surgical implants: A review

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ABSTRACT

Due to promising properties such as low toxicity against different cell lines, being highly stable fluorescent without showing photobleaching, and good surface properties, nanodiamonds have gained ever-increasing attention for various biomedical applications including bioimaging and therapeutic applications. Various methods are used for the fabrication of nanostructured diamond, the commonly used of which is the denotation technique. Newer approaches are being practiced for the modification and functionalization of their surfaces by different biomolecules suitable for interaction with considered targets. In this review, the scope and recent advancement in the field of nanodiamonds for biomedical applications particularly their application for nanocomposite scaffold and implants are discussed.

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1. Introduction

Nanocarbons involve a diverse structural family, one of which is nanodiamonds (NDs). NDs can be found in the form of nano-sized diamondoids, fullerenes, amorphous carbon, foam, platelets, whiskers, bells, peapods, cones, rods, horns, onions, and tubes [1-4]. A detailed investigation of NDs was initiated in Russia in the 1960s. Since then, these nanoparticles have attracted significant attention because they can be produced on large scale by cost-effective processes based on the detonation process of carbon-containing explosives. Moreover, nanodi-

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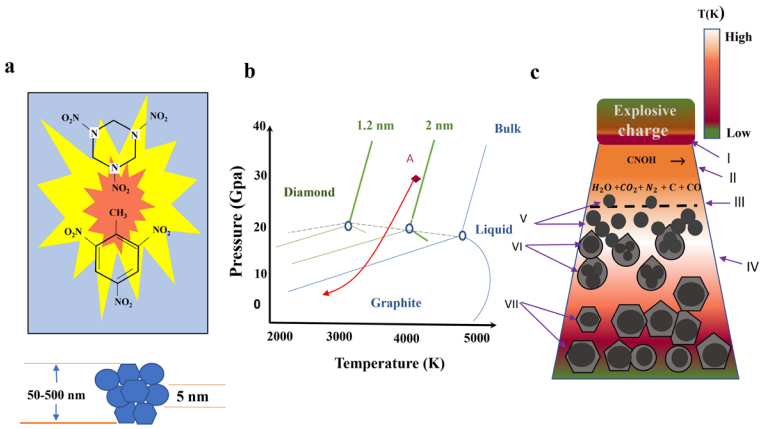

Fig. 1. Fabrication of nanodiamonds through detonation.

amonds are highly biocompatible, they can be easily functionalized like bioconjugation, and they have a small particle size of about 5 nm. A few products based on carbon nanomaterial such as derivatized fullerenes [5-8] or adamantane derivatives (e.g. memantine, amantadine, and rimantadine) have found ways to medicinal practice; however, ND suspensions have offered promising outcomes when used in animals and human patients suffering from cancer [9-12].

Similar to other nanoparticles such as metallic nanoparticles, carbon nanoparticles, and quantum dots, NDs can be used for the production of therapeutic agents for tissue scaffolds, anti-bacterial treatments, anti-viral treatments, gene therapy, delivery vehicles, diagnostic probes, as well as the preparation of new medical devices like nanorobots [13-19]. Moreover, the prospective exploitation of nanodiamonds can be applied for bioanalytical purposes including purification of proteins or biolabeling by the application of NDs with high fluorescence properties [20, 21]. The limitless potential of bio nanotechnology is demonstrated by the interaction of engineered nanostructured materials (e.g. ceramic, metallic, polymeric, and composite materials) at the molecular level which acts highly specific [22, 23] Nevertheless, the establishment of novel analytical methods, development of diverse nanofabrication approaches, and miniaturization of devices, e. g. BioMEMS is required for taking advantage of the advances of NDs in clinical trials [24-29].

This paper presents an overview of the processing and purification of nanodiamond particles, their properties, as well as their biomedical applications. In this review, the focus will be on the biological applications of NDs, especially in scaffolds and implants. Lastly, existing challenges and prospective directions in the development of NDs in biotechnology, engineering, and medicine will be discussed.

2. Nanodiamonds

At ambient pressure and temperature, the most stable allotrope of carbon is known to be graphite and diamond has a metastable state [30-32]. Besides the subtle difference in the energy state of graphite and diamond (0.02 eV), the energy barrier existing between the two phases is high (~0.4 eV per atom). Therefore, the transformation of the phases requires high pressure and temperature and/or catalyst [33, 34]. Due to the dependence of the Gibbs free energy on the surface energy, the cluster size should be included in the nanoscale carbon phase diagram in addition to temperature and pressure[35, 36]. Nanoscale diamonds consist of a core with sp^3 carbon structures together with disorder/defect and sp^2 carbon on the surface, which is available with single-digit nanoscale individual particles in colloidal suspension [37-39].

Diamond nanostructures in the size range of 1 to 100 nm are in the forms of pure-phase particles, 2-D diamond nanoplatelets, 1-D diamond nanorods, and diamond films. Ultra-nanocrystalline diamond (UNCD) is a special group of nanodiamonds with a size of a few nanometers, and their characteristics can be distinguished from other nanostructured diamonds with sizes larger than 10 nm [40-42]. In the 1960s, "detonation nanodiamond" (DND) or ultradispersed diamond" (UDD) with characteristic sizes of 4 to 5 nanometer were fabricated in the former USSR using detonation of carbon-containing explosives. At the end of the 1990s, Argon National Laboratory in the U. S. developed pure-phase UNCD films via chemical vapor deposition (CVD). Their characteristic grain sizes were between 2 to 5 nm [42-44].

Today, baffling nanodiamond arrays are available for investigations. Various methods have been developed for the fabrication of these nanomaterials including the detonation method (Fig. 1), high-energy ball milling of diamond microcrystals produced at high temperature and high pressure (HPHT) [45, 46], laser ablation [47], ultrasound cavitation [48], electron irradiation of carbon 'onions' [49], ion irradiation of graphite [50], chlorination of carbides [51], autoclave preparation from supercritical fluids [52], and chemical vapor deposition (CVD) with plasma assistance [53]. Among these techniques, the first three methods are employed commercially.

According to astronomical observations, the presence of NDs in the protoplanetary disks of some star types has been demonstrated [54, 55]. However, scientists are still investigating the origins of these cosmic sources. For using NDs for research and industrial applications, the large-scale production of nanodiamonds is needed. In this paper, we will review the production, modification, and applications of NDs, while concentrating on their application for tissue engineering.

3. Synthesis and purification of nanodiamonds

Carbon-containing explosives can provide a source of energy for the transformation of carbon to nanodiamonds (Fig. 1a) [34, 56, 57]. This method is eco-friendly and by using this technique, we can dispose of old munitions, like Composition B, while using other explosives might be possible. The detonation of explosives occurs in a closed chamber that is filled by water/ice coolant (wet synthesis) or an inert gas coolant (dry synthesis). Detonation soot that is used for naming the resultant products is composed of diamond particles in the range of 4 to 5 nm (~75 wt%) accompanied by other allotropes of carbon and impurities. Based on cooling media, the carbon yield is about 4 to 10 wt% of the explosive [56-59].

The mechanism of ND formation by detonation was proposed by Danilenko [34, 56]. The Jouguet point temperature and pressure (point

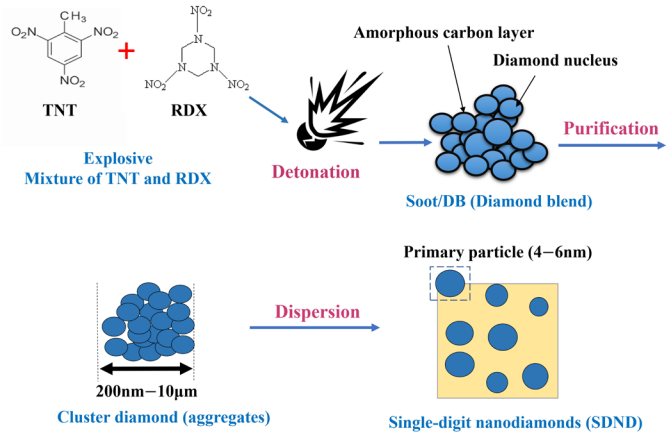

Fig. 2. Processing stages of NDs.

A in Fig. 1b) do not reach the point to form liquid bulk carbon, however, they are enough for the production of liquid carbon at the nanoscale (Fig. 1b). For nanocarbon, the liquid carbon region is shifted to lower temperatures, and there is a slight shift in the nanodiamond stability region to higher pressures (Fig. 1b). Therefore, the nucleation of NDs occurs by liquid carbon condensation and crystallization in the supersaturated carbon vapor (Fig. 1c). Other explosive-based methods, such as using waves for the production of NDs from graphite, fabricate NDs with crystallite sizes greater than 10 nanometers.

As mentioned, about 25–85 wt% of the detonation soot is graphitic carbon, and about 1–8 wt% of that is composed of incombustible impurities such as metals and oxides. Therefore, for most applications, it is required to be purified [57, 60]. The sources of the metallic impurities are the steel walls of the chamber (iron and other metals) where detonation occurs and the igniter that initiates detonation (typical azides of copper, lead, or silver). These impurities can be found on the outer surface of ND aggregates or inside them; hence, the aggregates must be disintegrated for the removal of the trapped impurities [61, 62].

For the removal of non-diamond carbon on an industrial scale, liquid oxidants such as HNO_3, $HClO_4$, H_2O_2/HNO_3 under pressure, Na_2O_2, KOH/KNO_3, $K_2Cr_2O_7$ in H_2SO_4, or a mixture of HNO_3 and H_2SO_4, are used for the purification of detonation soot [56, 57]. To reduce the concentration of non-carbon impurities to less than 0.5 wt%, further exposure to HCl and other treatments are carried out. Using a liquid phase for purification is hazardous and expensive so that 40% of the product cost is contributed to this process. An alternative environmentally-friendly approach for the removal of non-diamond carbon is its oxidation by air or ozone-enriched air at a high temperature [60, 63]. Oxidizing in the air is an eco-friendly, robust, and cost-effective purification method, which can change the content of diamond from ~25 wt% to more than 95 wt%. Because of oxidation, different presented functional groups are removed from the surface of NDs, and oxygen-containing surface functional groups, mainly carboxylic acids and anhydrides are produced. Consequently, different nanodiamond grades can be converted to materials that contain a high diamond content with unified surface chemistry [3, 60].

In comparison with the acid-purified NDs, the aggregate size of ozone-purified ones in aqueous dispersions is about 160-180 nm and a higher amount of faceted particles in the range of 3 to 5 nm is produced [63, 64]. Moreover, due to highly acidic surface groups in the hydrosols purified by ozone, their pH is very low; for instance, the pH value for 10 wt% hydrosol is between 1.6 and 2. Additionally, the electrokinetic potential (ζ-potential) varies from -50 mV for polydispersed specimens to -100 mV for the fraction with the size of 20-30 nm and the potential remains constant between the pH values of 2 to 12. Thus, the most promising technique for the purification of NDs is gas oxidation. The reduction of surface in a hydrogen atmosphere has been practiced, however, the removal of non-diamond carbon was not complete [65].

4. Surface modification and de-agglomeration of nanodiamonds

Commercial NDs are often required to undergo further processing and modification. This is due to their high content of non-diamond carbon and incombustible impurities, the large average size of aggregates, and unsuitable surface chemistry for proposed applications [19, 66, 67].

Even though the diameter of NDs is between 4 to 5 nm, there is a tendency to aggregation in the particles and the size of the aggregates is larger in common commercial ND suspensions, which are sometimes resistant to ultrasonic treatment. Even though the presence of the aggregates could be beneficial for some application such as drug delivery [68] or chromatography [69], de-aggregation of the particles into individual primary ones are often required for exploiting the advantages of NDs [70].

Osawa et al. [71] developed a microbead (SiO_2 or ZrO_2)-assisted ultrasonic de-aggregation process using the suspension of NDs, which was reported to be able to yield individual ND colloidal solutions with diameters of 4-5 nm. Using microbeads bring about some complications, mainly contamination with bead material and the graphite layer that forms on the surfaces of nanodiamonds [72]. It is also required to remove metal contaminants and amorphous carbon that was released from the aggregates during milling. On the other hand, the re-aggregation of the particles occurs during the purification of milled diamonds with the help of liquid oxidizers [72]. According to recent studies, sufficiently purified and oxidized particles in the air are allowed for subsequent isolation of stable hydrosol nanoparticles with a diameter of 4-5 nm by centrifugation [73].

Recently, the de-aggregation of particles from microscale aggregates to stable nanoparticles with a diameter in the range of 5-20 nm has been practiced by dry milling in cheap and abundant media like sugar and water-soluble salts. These media do not produce contaminants [74]. The reduction of NDs in borane with the help of ultrasonic treatment was reported to yield finer aggregates [75]. Aggregates with a diameter of ~20 nm were also obtained with surface graphitization and subsequent functionalization [76]. Nanodiamond aqueous colloids containing stable single particles were also obtained by hydrogen treatment at high temperatures and the nanoparticles with the size of 2-4 nm were isolated using a centrifugation process at above 10,000 rpm [77, 78].

Re-aggregation of NDs after additional surface functionalization is a concern regarding the processing of nanodiamonds. Because of capil-

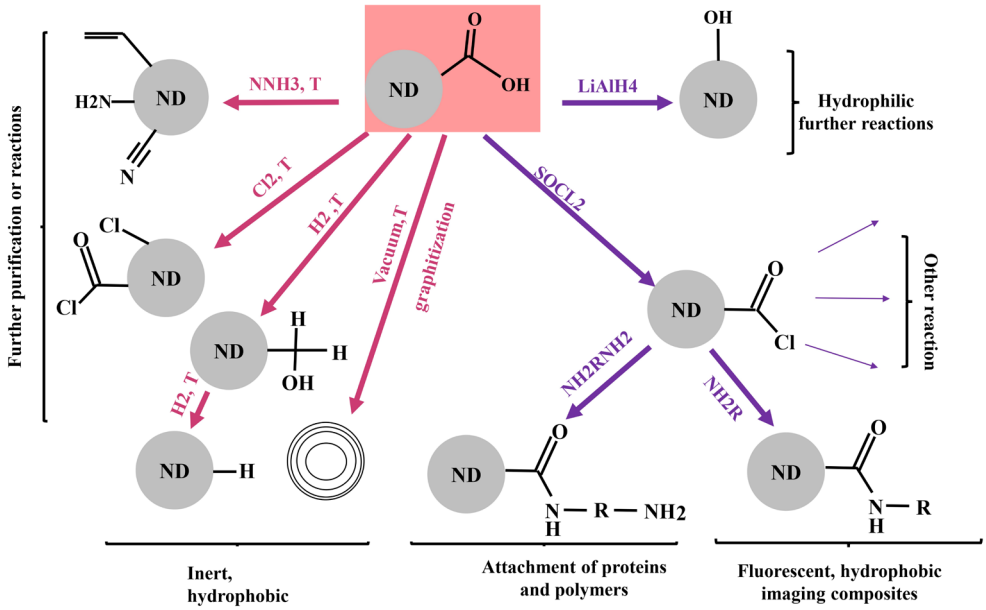

Fig. 3. Surface modification of NDs.

lary forces that attract the particles together, re-aggregation may also be promoted by drying (for storage). It could be caused by attractive van der Waals forces, which make the functionalization an arduous task. An approach for the prevention of re-aggregation after drying is the treatment of NDs in NaCl solution with the assistance of ultrasound [61, 79]. The prevention results from the attachment of Na^+ ions to the surfaces of NDs. Halting re-aggregation of NDs fabricated via NaCl-assisted milling can also be explained by this reason [74].

NDs can be separated into fractions by their size and weight using centrifugation [80, 81]. Unlike methods that introduce contamination (e.g. bead milling), centrifugation is contamination-free and ND fractions (different sizes) are possible to be isolated suitable for various applications. For instance, only small particles can be used for drug delivery, while NDs that can form photonic structures for light diffraction are required to have aggregate sizes above 100 nm [82]. Obtaining small NDs by centrifugation is economically feasible when NDs purified by air or ozone are used rather than acid-purified NDs. This is because of a higher fraction of fine aggregates achieved by the former approaches. Although single-digit NDs can be extracted by ultracentrifugation, the yield is low. Therefore, the dispersion of NDs into individual particles requires the development of facile, scalable, and non-contaminating techniques. The processing of NDs from synthesis to de-aggregation is illustrated in Fig. 2.

NDs are superior to carbon nanotubes (CNTs) and other graphite-based nanoparticles in terms of the possibility to attach various functional groups to their surfaces [44]. This capability not only provides complex surface functionalizations but also preserves the promising characteristics of the diamond core [83]. Nevertheless, understanding their interaction mechanism with their surrounding materials and the alleviation of adverse effects such as aggregation is essential [84, 85]. The different functional groups that exist on the surface of commercial NDs can be utilized for covalent functionalization; however, starting with carboxylated NDs prepared by ozone and air purification methods, and then making use of the COOH groups' rich chemistry is more convenient. As a result of the hydrogen microwave chemical vapor deposition plasma treatment at temperatures higher than 700 °C, COOH groups are completely removed and hydrogenated NDs are produced by the complete removal of oxygen from the surface [86].

In comparison with gas treatments in the temperature range of 400-850 °C, milder conditions are required for wet chemistry. An enhanced selectivity is provided by this approach through the conversion of plenty of functional groups known in organic chemistry (Fig. 3). In several wet chemical reactions, reactive C–Cl and C–F bonds on the surface generated by photochemical chlorination and halogen annealing have also been used [87, 88]. Long alkyl chain terminations were produced on the surface of NDs by esterification of O-H terminations with acylchlorides [89]. O-H terminates are also involved in silanization/de-aggregation [90]. Also, the modification of NDs by taking advantage of the graphitic carbon chemistry can be performed. Graphite carbon can be either intrinsically present or formed by surface graphitization. Strong bonds of C–C can be formed between the surface group and the graphitic shell, while C–X bonds (where X is S, O, N, etc.) are created by methods that work based on the chemistry of ND functional groups [91, 92].

Diazonium chemistry and Diels–Alder reactions have been used for the functionalization of nanodiamond graphitic shells. Diazonium chemistry has been utilized with hydroxylated nanodiamond for the production of C–O–C bonds between the diamond core and the attached moiety, and also with hydrogenated NDs for the C–C bond formation [93]. Even though there are different options for surface functionalization of NDs, the uniformity and purity of the starting materials surface chemistry strongly affect the outcome [94, 95]. The development of quantitative analysis for the evaluation of different groups present on the surface of NDs is a challenge in this regard.

5. Properties of nanodiamond

5.1. Fluorescence

The promising fluorescence properties of NDs results from nitrogen atoms next to a vacancy called nitrogen-vacancy (NV) centers in NDs. To create NV centers, NDs are irradiated with high-energy particles such as protons, electrons, and helium ions, and then vacuum annealed at 600-800 °C [96, 97]. During irradiation, vacancies are formed in the diamond structure, and the annealing treatment leads to the migration of the vacancies and their entrapment by N atoms present in NDs [98]. Different spectra are emitted based on the NV center types that would be either negatively charged (NV^-) or neutral (NV^0). The spin ground state of $S = 1$ of NV^- centers provide the possibility of spin polarization by

optical pumping and manipulation through electron paramagnetic resonance. Additionally, NV^- centers possess long spin coherence times. In isotopically clean diamonds, fluorescent NV centers have been favored for quantum computing [99], while in NDs, NV centers are being studied for biomedical imaging [96], high-resolution magnetic sensing [100-102], and fluorescence resonance energy transfer applications [103].

Diamond synthesized at high temperatures and high pressures contain about hundreds of ppm of native substitutional nitrogen and thereby they are the candidate for developing bright photoluminescent [45, 96]. These materials are then ground to nanoparticles containing single-digit NDs [45]. The concentration of electron-irradiation-generated fluorescent NV defects is not significantly affected by the nanocrystal size; however, the produced fraction of NV-defects is reduced with the decrease in the nanodiamond size due to electron traps at the surface [104].

With the help of medical imaging, it is possible to detect and diagnose a variety of diseases, and thereby the healthcare industry is seeking innovations in the imaging field [105-108]. Up to now, NDs fabricated by explosives have not been considered as promising materials for NV-center-based imaging applications. In recent years, individual pristine NDs with a diameter of 5 nm synthesized by the detonation of TNT and hexogen precursors have shown NV-center-based intermittent luminescence [102]. Additionally, larger NDs (above 20 nm) with trapped NV centers fabricated by graphite and hexogen [109] and TNT and hexogen [110] precursors have exhibited stable luminescence. Doping of nitrogen into the ND core and the *in situ* creation of NV centers are influenced by numerous factors including the cooling conditions and the nitrogen amount in the precursors. The reasons for the low intensity of fluorescence in NDs synthesized from explosives are the existence of internal defects and the proximity of surface defects dependent on their size [102, 109].

Adsorbing [111] or linking [66, 112] of different fluorophores onto NDs can also produce fluorescent particles. NDs with fluorophore linkage can move through cellular sections with varying pH values without changing cell viability or causing degradation of fluorophore linked to the surface over a long period [113]. Octadecylamine was linked to carboxylic groups on the surfaces of NDs and thus, bright blue fluorescent NDs were formed [114]. Not only do fluorescent NDs benefit from the promising properties of semiconductor quantum dots, namely, high photostability, small size, and bright multicolor fluorescence, but also they show rich surface chemistry, biocompatibility, and non-toxicity. These properties would probably revolutionize *in vivo* imaging [96, 115, 116].

Zhang et al. [117] prepared the multimodal nanodiamonds by attaching monoclonal antibodies and drug-oligonucleotide conjugates labeled by fluorescent onto the surface of ND. They reported that by these linkers, it is possible to quantify the ND conjugates and observe intercellular regions. Dong et al. [118] prepared fluorescent nanodiamond-based composites by a simple and novel method. The hydrophilic polymer/ functionalized ND composite was reported to be suitable for different biomedical applications because of their good potential and physicochemical properties. Also, they reported that these samples have strong fluorescence intensity, low toxicity, and high water dispersibility. According to the result of cell uptakes, the cells could internalize the fluorescent nanodiamond-based composites. Sarkar et al. [119] used a new background-free imaging method and reported that this technique enhances the ratio of signal-to-background up to 100 times. Also, an improvement was observed in the fluorescent nanodiamond imaging capabilities on diverse fluorescence imaging platforms.

5.2. Biocompatibility

Although glassy carbon and diamond are not toxic, carbon nanoparticles cannot be assumed also non-toxic. Because of a variety of processes for purification and different options for surface modification used by various manufacturers, the toxicity caused by NDs is a concern [120-122]. *In vitro* and *in vivo* properties such as gene program activity, cell viability, and *in vivo* physiological and mechanistic behavior in the presence of NDs have been investigated [66, 120, 123-127].

Researchers demonstrated that instilled NDs within the trachea had a low level of pulmonary toxicity. The ND amount decreased with time in the alveolar region. Moreover, after 28 days after exposure, ND-burdened macrophages were observed in the bronchia [9, 125, 128, 129]. Systemic toxicity and serum indicators of the liver were not affected by using high dosages of intravenously administered ND complexes [127].

Mohan et al [126] studied worm reproduction, cytotoxicity, and stress response activity of fluorescent NDs with an average hydrodynamic size of about 120 nanometers in *Caenorhabditis elegans* worm. They reported that bare ND particles remained in the lumen of the worm, while NDs coated with bovine serum albumin or dextran were adsorbed into its intestinal cells. Transferring NDs injected into worm gonads to the larvae and offspring was observed, however, this did not affect the survival or reproductivity of the worms. It was also indicated that fluorescent NDs are non-toxic and do not cause stress in the worm, thus, they are suitable for *in vivo* imaging. Overall, the biocompatibility and toxicity of newly developed NDs should be carefully investigated.

6. General applications

ND additives have been utilized for metal plating (electrolytic/electroless) for a long time [130, 131]. In recent years, these particles have found ways to other applications. In this section, different applications of NDs are addressed.

Tribology and lubrication: Adding detonation soot containing diamond to lubricants causes about 5% reduction in fuel consumption and prolongs the engine life [130]. Two reasons contribute to this effect: 1) the existing graphite has a lubrication effect: 2) friction is reduced on sliding surfaces by NDs through polishing away asperities. The enhancement in tribological performance is provided when purified NDs are dispersed alone or together with metal nanoparticles or polytetrafluoroethylene (PTFE) in oils or greases [132]. The initial assumption about the lubrication mechanism was acting as 'ball bearings', however, recent studies suggested different contributing mechanisms. For instance, the wear mechanism for Al alloys is significantly affected by the viscosity of the ND suspension, while a decrease in wear and friction of carbon steel is the result of the embedment of NDs from the lubricant into the surface of the carbon steel [114]. It is possible to tailor the surface chemistry of NDs so that they can be dispersed in various systems such as water and oil [133].

Because of the microscopic ball-bearing effect, carbon onions can offer an efficient lubrication effect. Generally, in contrast to expectations, the lubrication mechanism is more complex, but it can be accepted that both carbon onions and NDs embed into the surfaces of metals and thereby leading to the separation of sliding surfaces and prevention of wear resulting from metal-metal adhesion [134, 135].

Nanocomposites: Nanocomposites have shown promising properties suitable for a variety of applications [136-139]. NDs have been suggested as excellent fillers for composites owing to their rich surface chemistry, and good thermal and mechanical characteristics. Also, the fillers can tailor these composites for biomedical applications due to the diamond core's chemical stability and biocompatibility. It has been reported that the ND addition to polymers caused the enhancement of their thermal conductivity [140, 141], electromagnetic shielding [142], adhesion [143], wear resistance [144], and mechanical strength [47, 141, 145-148]. On the other hand, in the case of using aggregated or non-purified NDs, degradation in properties has been observed, which confirms the necessity of proper functionalization and well dispersion of these

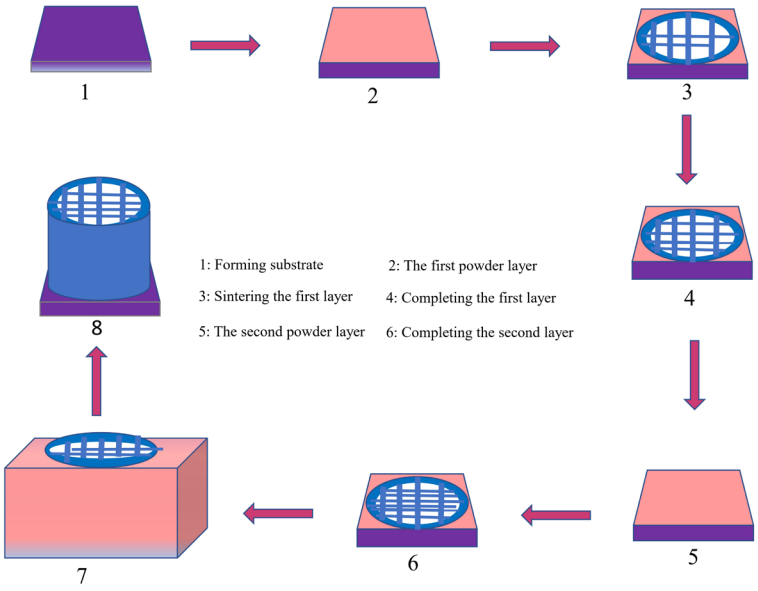

Fig. 4. Schematic illustration of the selective laser sintering method.

particles.

The addition of small amounts of NDs to transparent poly(vinyl alcohol) showed improved mechanical properties [149]. Surface chemistry can control the interfacial interaction between the matrix and NDs as well as their dispersion in the matrix. If NDs are modified, they can covalently bond with metal, ceramic, polymeric matrices, and further enhancement can be provided for composites [146, 150, 151].

Drug delivery: A drug delivery system is required to have some properties including scalability, dispersibility in water, the capability of carrying various therapeutics, and biocompatibility [152-156]. Another important property is the targeted therapy potential combined with imaging possibility. Most of these requirements are met by NDs [157-161].

Delivery of doxorubicin (Dox) by ND-based systems has been shown to be safe and effective [162-164]. ND-Dox complexes were utilized for the treatment of liver cancer (LT2-M) and breast cancer (4T1) models. The application of ND-Dox complexes increased the circulation half-time to 10 times of unmodified doxorubicin, and the doxorubicin expelling capacity of tumors decreased. Other benefits of drug delivery systems based on ND-Dox are reported to be a noticeable decrease in the tumor size, the absence of myelosuppression, and the absence of mortality in the case of high delivery doses [123, 165, 166]. Besides, delivering small molecules polyethyleneimine 800 (PEI800)-coated NDs were investigated for the delivery of nucleic acids. Different loadings have been delivered by NDs such as siRNA for specific cancers [158], small molecules in acidic environment [3], proteins [3, 157], and covalently bonded drugs [167, 168].

The functional groups are present on the surface of ND, especially oxygenated moieties including hydroxyl, ether, ketone, lactone, carboxylic acid. ND is a widely applicable nanocrystalline due to its structural stability, natural biocompatibility, and non-toxic nature. According to previous works, ND has a role as a drug carrier of dexamethasone, 4-hydroxytamoxifen, purvalanol A, and doxorubicin for blood cancer, breast cancer, liver cancer, and colon cancer therapies, respectively [169].

Protein mimics: Owing to promising properties such as low cytotoxicity, ability to self-assemble, rich surface chemistry, stable core, and small size, NDs are being used for mimicking globular proteins [170, 171]. Besides the ability to deliver drugs, other molecules such as genetic material can be delivered across cellular membranes. NDs are also able to mimic other functions of proteins. For instance, proteins that are involved in the folding/unfolding of DNA are highly alkaline histones. During this process, a nucleosome that contains histones in its core, spool the DNA strand around itself. NDs not only can be increased close to the size of histones by selective air oxidation [172] but also by employing covalent bonding of amino groups, their surface alkalization is possible [146]. The alkaline NDs can then be folded with DNA to form artificial nucleosomes. Functionalized NDs can also mimic some proteins' enzymatic functions due to their catalytic properties [173].

Tissue scaffolds and surgical implants: Due to the restoring potential for damaged tissue, regenerative medicine, and tissue engineering are of great interest [174-177]. Like protein-coated materials, ND monolayers have been indicated to act as a suitable platform for the growth of neuronal cells [178]. Reinforcement of biodegradable polymers with NDs provides prospective advantages for the synthesis of multifunctional tissue engineering scaffolds due to their biocompatibility, superior mechanical properties, delivering biologically active molecules and drugs, and tunable surface chemistry [179]. One of the ND-containing polymer composites studied for biomedical applications is ND octadecylamine (ODA)-poly (l-lactic acid) (PLLA). PLLA is a biodegradable and biocompatible polymer, however, its mechanical properties cannot satisfy the requirements for load-bearing implants. It has been reported that the incorporation of ND-ODA and its good dispersion enhanced Young's modulus and hardness of the composites close to those obtained for human cortical bone. Also, no changes in proliferation and morphology of murine 7F2 osteoblast cells cultured on the ND-ODA-PLLA scaffold were observed. Therefore, clinically relevant properties are obtained by these composites while offering high scalability and non-toxicity [123, 179-181]. The remarkable enhancement of properties caused by the addition of NDs could suggest these materials to be used in a wider range of biomaterials.

7. Scaffolds based on nanodiamond composites

7.1. Fabrication methods

Selective laser sintering (SLS): In this method, polymer/ND powders first are poured on a workbench, and then the powders are sintered selectively by laser-based layer-by-layer sintering following a pre-designed scaffold model. At the final step, the sintered scaffold is achieved by removing the residual powder [67]. This process is schematically illustrated in Fig. 4.

Feng et al. [182] synthesized poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV/ND, PHBV/MoS_2, and PHBV/ND/MoS_2 composite

powders via a solution mixing technique. Selective laser sintering with laser power of 2 W, scanning speed of 200 mm/s, and a spot diameter of 50 μm was used for the fabrication of cylindrical scaffolds with a height of 10 mm and a diameter of 8 mm. PLLA/ND composite scaffolds were also prepared by Shuai et al. [67] using the selective laser sintering method.

Electrospinning: This technique is a facile, efficient, fast, and cost-effective route for the production of nanofibers from a polymeric melt or solution via applying electrostatic forces [183, 184]**.** This method is used for the fabrication of fibrous polymeric scaffolds [185, 186]. In this technique, ND particles are dispersed in a polymer solution, and then the polymeric solutions are loaded into a syringe equipped with a metallic needle. The electric potential is applied to the metallic needle and aluminum foil is used to collect the as-spun nanofibers. The parameters that contribute to the electrospinning method are temperature, feed rate, polymer concentration, voltage, and distance between the collector and needle [179, 187, 188]. The synthesis of poly (lactic-co-glycolic acid)/nanodiamond (PLGA-ND) composite was reported using the electrospinning of a dimethylformamide and methylene chloride solution. In comparison with pure PLGA fibers, PLGA-ND fibers were noticeably thicker. The results demonstrated that cell spreading of human mesenchymal stem cells (hMSCs) improved compared to pure PLGA. This is due to the presence of oxygen termination of NDs that provides hydrophilicity in ND-containing scaffolds [189, 190]. Houshyar et al. [187] performed wound healing dresses based on polycaprolactone (PCL) and ND using electrospinning. Enhanced moisture and wicking management in NDs results from different hydrophilic groups on their surfaces. Furthermore, excellent cellular activities and no cytotoxicity were exhibited by the composites**.**

In-situ polymerization: ND nanocomposite scaffolds can be prepared by in-situ polymerization. ND particles are usually functionalized and the nanodispersion is added to a monomer-containing solution. Finally, self-assembly and polymerization of monomers occur in the presence of functionalized ND [179, 191]. Alishiri et al. [192] polymerized acrylate-terminated polyurethane-acrylate diluents (APUA) in the presence of 2- hydroxyethyl methacrylate (HEMA)-grafted ND. This method led NDs to well dispersion in APUA resulting in the enhancement of mechanical properties. Ultra-high molecular weight polyethylene (UHMWPE)/ND nanocomposites were also successfully synthesized by in-situ polymerization based on bi-supported Ziegler-Natta catalyst. The results showed that the mechanical properties of both silane-modified and unmodified NDs were improved [193].

Solvent casting: Due to the solubility of biopolymers in various solvents, solvent casting has been considered as a facile and commonly used technique for biopolymer film synthesis. Solubilization, casting, and drying are the steps that are involved in this approach [167]. For the preparation of ND-containing nanocomposites by solvent casting, nanodiamonds are first ultrasonically dispersed in a solution. The polymer then is added to the prepared solution; afterward, the mixture is poured on a glass substrate or in a mold followed by the complete evaporation of the solvent [194].

Maitra et al. [195] incorporated acid purified ND in a PVA film to improve the mechanical properties of the polymer for applications in broader biomedical areas [196]. Fox et al. [148] also used the solvent casting method to reinforce the polycaprolactone (PCL) film with fluorescent ND. In this regard, a mixture of PCL and fluorescent ND in methanol was prepared followed by casting for the production of free-standing films. Sun et al. [144] synthesized chitosan and ND-COOH composite films by using a solvent of acetic acid. The results showed the improvement of mechanical properties owing to a strong interaction between chitosan polymer chains and carboxyl groups of ND. Zhang et al. [123] added ODA-grafted ND to a mixture of chloroform and PLLA and showed that the addition of nanodiamonds enhanced Young's modulus and hardness of PLLA.

The preparation of nanocomposites by solvent casting is a suitable method; however, selecting an appropriate solvent that can dissolve both ND particles and polymer would be a critical issue affecting ND aggregation. Thus, the functionalization of ND is necessary in most cases to achieve uniform distribution in polymeric films [197, 198].

7.2. Nanocomposite scaffolds and implants

ND-containing composite scaffolds are gaining increasing attention for biomedical research and applications. Shuai et al. [67] modified ND by phospholipid and incorporated the modified particles in PLLA scaffolds using selective laser sintering. The hydrophilic head (–OH) of phospholipid was adsorbed on ND surfaces (–COOH), while its hydrophobic tails were arranged toward the polymeric PLLA matrix. Therefore, a layer of phospholipid covered ND particles. Because of the repulsive force between the hydrophobic tails, phospholipids are forced away from each other leading to better dispersion of NDs in the PLLA matrix. Compared to unmodified scaffolds, phospholipid-modified composite scaffolds showed an increase in Vickers hardness, the compressive modulus, and compressive strength by 88.2%, 163.2%, and 162.8%, respectively. It was reported that the prepared scaffolds acted as a proper platform for cell adhesion, growth, and migration, indicating their potential for bone tissue engineering.

Houshyar et al. [187] used the electrospinning technique for the fabrication of PCL/ND nanofibrous scaffolds. It was proposed that the scaffolds possessed the requirements for wound healing including the restriction of microbial activities and the promotion of epithelial cell proliferation. The outcomes of adding ND to PCL were a delay in the scaffolds' thermal degradation, better moisture management, and higher thermal stability. The proliferation of Chinese hamster ovarian (CHO) cells for PCL-5%ND after 1, 3, and 7 incubation days exhibited 43%, 38%, and 22% enhancement. Also, microbial activity decreased with the increase in the ND content. Fox et al. [177] also reported the synthesis of PCL/ND composites by the solvent casting method. The results demonstrated that hydrophilicity and surface roughness of the ND-PCL composite films were higher than those of PCL alone. Moreover, their degradation was slightly enhanced and the tensile strength decreased. Osteoblast adhesion increased with an increase in the ND loading. Finally, a 3D composites scaffold was produced by extrusion revealing the promising potential for tissue regeneration.

Apicella et al. [199] fabricated bio-mechanical scaffolds based on ND and poly (hydroxy-ethyl-methacrylate) hydrophilic matrix for tissue engineering. The hybrid material was reported to be potent for biomimetic, osteoinductive, and osteoconductive applications as biomechanical bones. Owing to the enhanced mechanical strength, these hybrid materials can be a replacement for traditional hydrogels with lower mechanical properties for bone regeneration; they can also be used as coatings onto metal trabecular scaffolds. Recreation of micro-and macro-distribution of bone deformations and stresses occur in osteoinductive ceramic/polymer-coated micro-trabecular metal scaffolds.

Nunes-Pereira et al. [143] used solvent casting to add different types of ND into Poly (vinylidene fluoride) (PVDF). According to the results, the thermodynamic stability as well as the optical properties of the samples could be tailored by the addition of ND nanofillers. Also, the dielectric losses of the nanocomposites remained constant, and the dielectric constant increased while was independent of the filler concentration. Moreover, ND nanoparticles were found to be non-toxic. It was concluded that the prepared nanocomposites were promising material for biomedical applications owing to cell culture properties of the polymer and nanodiamond potential for drug delivery and protein functionalization.

Feng et al. [182] embedded ND particles into MoS_2 nanosheets and

Table 1.
ND-containing composites for scaffolds and implants

Matrix	ND size (nm)	Functionalization	Fabrication method	Cell type	Conclusion	Ref.
PCL	45	-	Solvent Casting	Human osteoblasts	Incorporation of ND resulted in increased hydrophilicity and tailored degradation of the composites compared to base PCL No cytotoxicity	[177]
PCL	-	Acid treatment+octadecylamine	Electrospinning	Human lens epithelial (HLE)	Tensile Strength of FND/PCL composites increased compared to pure PCL No cytotoxicity	[151]
PVDF	<10	-	Solvent casting	MC3T3-E1 pre-osteoblast cells	No toxicity	[143]
PLGA	-	-	Electrospinning	Human mesenchymal stromal cells (hMSCs)	PLGA-ND membranes exhibited higher hardness and Young's modulus. No cytotoxicity	[189]
PVDF/bioglass scaffold	5–10	-	Selective laser sintering	MG 63 cells	Tensile strength of the scaffolds increased by 23.01% Bioactivity of the samples increased Improved osteoinductive properties	[201]
Chitosan	5	Acid treatment	Solvent casting	Cell wall of fungi	Addition of 1 wt % nanodiamond improved the young's modulus and hardness of the composites by 239% and 69% respectively.	[144]
PCL	<10	-	Electrospinning	CHO	The surface energy of the PCL-ND composite increased by the addition of ND which resulted in better moisture management, proliferation, and cell attachments. No cytotoxicity More thermal stability Increased crystallization temperature	[187]
Polydiallyldimethylammonium chloride (PDDA)	4	-	Coating of NDs using Polyelectrolyte multilayers (PEMs)	Human fetal osteoblasts (hFOBs)	Cell Viability increased by 40% Increased cell adhesion Feasibility of NDs as a coating material for biomedical applications and drug delivery vehicles.	[202]
Magnesium	<10	-	Powder metallurgy	Fibroblast (L-929)	The corrosion resistance of the MG-5ND composites increased by 4.5% compared to pure Mg Biocompatible and No Cytotoxicity	[203]
PLLA	5	-	Solution casting followed by compression molding	-	10 wt% of ND-ODA led to an increase in the strain to failure by 280% and an increase in fracture energy by 310% in comparison to pure PLLA. Bonelike apatite is formed on the ND-ODA/PLLA scaffolds when tested in SBF Solution, which may increase the osteoinductive properties.	[204]
PCL	-		Electrospinning	NIH/3 T3 cells	The addition of 1 wt% of ND resulted in increased young's modulus, Tensile Strength, and percentage elongation to break. High cell proliferation rate for 1 wt% ND/PCL No cytotoxicity	[205]
Poly(lactic acid) (PLA)	<10	-	Electrospinning	-	Incorporation of 1 wt % nanodiamond in PLA improved the tensile strength and young's modulus of the composites by 239% and 161% respectively.	[161]
Poly (L-lactide-co-e-caprolactone) (poly(LLA-co-CL))		-	Anionic polymerization	stem cell line UE7T-13	Young's modulus of 10 wt% composites increased by 6 times. Biocompatible and no cytotoxicity for all contents (i.e. 1, 5, 10, 50 wt%)	[206]
APU	-	Quaternary ammonium	In-situ polymerization	MC3T3-E1 cells	Improved mechanical properties Hydrophilicity increased by incorporation of NDs Crystallinity improved which resulted in tailored degradation rates No cytotoxicity	[200]
Gelatin	-	-	Electrospinning	Human adipose-derived stem cells (hASCs)	Enhanced cell viability and proliferation Increased scaffold stiffness No cytotoxicity	[207]

then the co-dispersion nanostructure was added to poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) by selective laser sintering to produce scaffolds for bone regeneration. The restacking of the molybdenum oxide nanosheets was restrained by the placement of NDs between adjacent nanosheets. Additionally, the aggregation of NDs was prevented by the steric hindrance effect of the MoS_2 nanosheets. Consequently, the compressive and tensile strengths of scaffolds containing ND and MoS_2 were increased by 52% and 94%, respectively. The mechanisms that contribute to the strengthening of the scaffolds include crack pinning, crack bridging, crack deflection, as well as pulling out of ND particles and MoS_2 nanosheets. Moreover, the scaffolds showed good cell viability.

Wang et al. [200] added polycation-modified ND loaded with Ag to acrylate-terminated polyurethanes (APU) for cartilage tissue implants using in-situ polymerization. The results indicated that the crystallinity of the nanocomposites increased compared to pure APU, showing a strong interaction between APU and nanodiamonds. Release-killing of the Ag nanoparticles and contact-killing of cationic polymers resulted in excellent antibacterial activity of the nanocomposites. Additionally, the addition of polyethylene glycol to APU increased its degradability rates significantly. Moreover, the synthesized scaffolds showed low toxicity. Overall, the combined effects of hydrophilicity and crystallinity provided proper degradation rates for APU, which was reported to be adaptable to the cartilage tissue-healing rate. Some research studies focusing on the development of ND-containing composites for scaffolds and implants have been summarized in Table 1.

Table 1. ND-containing composites for scaffolds and implants

8. Conclusions and Future insights

NDs have shown all the ideal properties needed for biomedical applications. Many research activities in various biomedical applications have focused on the application of nanodiamonds. However, some challenges should be addressed including re-aggregation prevention, cost reduction, poor cell affinity, controlling by-product degradation, and controlling surface chemistry. As a result, scientists continuously study NDs to shed light on the surface structure and chemistry to develop functional materials with improved properties. Composites containing biopolymers and NDs are also attracting the attention of scientists leading to the introduction of novel materials and methods into this area. In the near future, the approval of ND application in implants is expected. Therefore, a new era for the application of nanodiamonds in the biomedical field will be opened. The prospective applications of nanodiamonds will be in various multifunctional devices for simultaneous cell targeting, drug delivery, and image reactions.

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Conflict of interest

The authors declare that there is no conflict of interest.

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