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Review article

Multifunctional nanodiamonds in regenerative medicine: Recent advances and future directions

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A B S T R A C T

With recent advances in the field of nanomedicine, many new strategies have emerged for diagnosing and treating diseases. At the forefront of this multidisciplinary research, carbon nanomaterials have demonstrated unprecedented potential for a variety of regenerative medicine applications including novel drug delivery platforms that facilitate the localized and sustained release of therapeutics. Nanodiamonds (NDs) are a unique class of carbon nanoparticles that are gaining increasing attention for their biocompatibility, highly functional surfaces, optical properties, and robust physical properties. Their remarkable features have established NDs as an invaluable regenerative medicine platform, with a broad range of clinically relevant applications ranging from targeted delivery systems for insoluble drugs, bioactive substrates for stem cells, and fluorescent probes for long-term tracking of cells and biomolecules *in vitro* and *in vivo*. This review introduces the synthesis techniques and the various routes of surface functionalization that allow for precise control over the properties of NDs. It also provides an in-depth overview of the current progress made toward the use of NDs in the fields of drug delivery, tissue engineering, and bioimaging. Their future outlook in regenerative medicine including the current clinical significance of NDs, as well as the challenges that must be overcome to successfully translate the reviewed technologies from research platforms to clinical therapies will also be discussed.

1. Introduction

Nanodiamonds (NDs) are an emerging class of carbon nanomaterials which possess a unique set of chemical, physical, and biological properties essential for the design of innovative therapies in the fields of drug delivery, tissue engineering, and bioimaging. Over the last two decades, several synthesis techniques have been optimized to enable the fabrication of NDs with a defined size, morphology, and homogeneous surface chemistry. The discovery of improved purification methods and surface modification strategies has also facilitated the production of NDs with precise and customizable features [1]. The inherent physical and chemical properties of NDs make them suitable candidates as delivery agents for many therapeutic molecules. For instance, their high surface area to volume ratio and tunable surface chemistry enables the high loading capacity of small molecules containing amine groups or other polar moieties by physical adsorption. Following this reversible loading technique, poorly soluble drugs such as anthracyclines can be non-covalently linked to NDs that possess hydroxyl or carboxyl groups on their surfaces [2]. This facile loading process is efficient and does not require any chemical modification. Moreover, NDs have demonstrated to improve the therapeutic efficacy of many chemotherapeutic agents by increasing their dispersivity in water, facilitating their sustained release, shielding the drug from inactivation, and bypassing the mechanisms of chemoresistance [3,4]. These significant improvements in the delivery of chemotherapeutic agents have inspired researchers to study NDs for the sustained release of other therapeutic molecules, such as growth factors, peptides, and genes [5]. NDs are rapidly internalized by cells but do not readily undergo exocytosis, which localizes the release of bioactive molecules within the cell for an enhanced therapeutic effect [6]. Proteins such as insulin and bone morphogenetic protein-2 (BMP-2) have been adsorbed onto carboxylated NDs and administered both *in vitro* and *in vivo* to achieve a pH-dependent sustained release [7,8]. Additionally, the presence of polar groups on the surface of NDs enables the nanoparticle to adsorb positively charged polymers such as polyethyleneimine or polylysine which serve as intermediate cationic layers to promote the adsorption of DNA and RNA [9].

Meanwhile, NDs have also been evaluated as a nanofiller for reinforcing the mechanical properties of composite scaffolds to rival that of human tissue [10,11]. By establishing covalent or ionic bonds with

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the polymeric chains during the scaffold preparation, NDs can be used to modulate the mechanical properties of polymeric networks to mimic the structure of both soft and hard tissues of the human body [12]. NDs have also found applications as bioactive coatings to improve the tribological properties and reduce the mechanical wear of orthopedic implants [13]. The high biocompatibility of NDs in comparison with other carbon nanomaterials such as graphene oxide or single and multi-walled carbon nanotubes represents a significant advantage for NDs and suggests the high probability for the clinical translation of ND-based treatments [14].

Finally, the optical properties of fluorescent NDs (FNDs) have sparked a great interest among researchers for the use of these nanoparticles as imaging probes. NDs can be modified to introduce nitrogen vacancies in their inner diamond core that emit a highly stable fluorescence. These nitrogen vacancies, which emit a bright fluorescence in the far-red spectrum, are located within the sp^3 carbon lattice structure allowing for surface modification without disrupting the vacancy centers or reducing the fluorescence intensity. FNDs possess high photostability, high quantum efficiency and longer fluorescent lifetimes when compared to other organic fluorophores used for cellular imaging [15].

In this review, we will highlight the strategies available for the synthesis and the chemical modification of NDs' surface with particular attention to how they may affect their biocompatibility. This section will be followed by an overview of the possible applications of NDs in the field of drug delivery, tissue engineering and bioimaging describing the current challenges yet to overcome (Fig. 1A). Lastly, particular emphasis will be given to the design of multidisciplinary approaches in which NDs can be employed as a nanocarrier for drugs or genes while functioning as fluorescent probes or as nanofilling agents in bone tissue scaffolds. The ability of NDs to present multimodal functionality is what makes them truly unique from other nanomaterials, and thus, NDs have a very bright future as both a research tool and as a clinical theranostic platform.

2. Synthesis and functionalization of NDs

NDs were first discovered in 1963 by researchers in the USSR who were performing detonation tests with carbon-based explosives. Upon detonating a mixture of 2,4,6-trinitrotoluene (TNT) and 1,3,5-trinitroperhydro-1,3,5-triazine (RDX) in a blast chamber, the researchers found that the soot contained 4–5 nm diamond particles accompanied by graphite and other non-diamond carbon particles [16]. Despite their early discovery, the properties of these nanoparticles were not researched for biomedical applications until the beginning of the 21st century. NDs can be produced in different sizes such as nanocrystalline particles (1–150 nm) or ultra-nanocrystalline particles (2–10 nm). The core of the nanoparticle is a sp^3 hybridized carbon lattice that is surrounded by sp^2 hybridized carbon and various oxygenated functional groups [17].

The size of the diamond core, the distribution of the sp^2 regions in the outer shell, as well as the diversity of reactive functional groups on the NDs' surface, are substantially influenced by the route of synthesis and the composition of the reactants. In addition, the physicochemical properties can also be affected by the purification steps, which are necessary for removing undesired impurities that are introduced during synthesis. An overview of these fundamental aspects will be examined with an emphasis on strategies aimed to homogenize the NDs' surface chemistry, which is an essential requirement for their potential application in biomedical research.

2.1. Synthesis techniques

Several methods are available for the fabrication of NDs including detonation synthesis, chemical vapor deposition (CVD), high-pressure high-temperature (HPHT), and laser techniques. Each one of these technologies has a profound impact on the size and surface properties of the obtained NDs. The choice of a particular strategy can also influence the degree of agglomeration and level of purity of the nanoparticles, which are both aspects that need to be accurately tested prior to further use of NDs in drug delivery, tissue engineering, or bioimaging applications. For this reason, signficant efforts have been made to fabricate NDs with controllable size and homogeneous surface features to reduce the level of variability associated with their fabrication. Both the advantages and limitations of each synthesis approach will be discussed in the following sections with particular attention to the emerging solutions designed to achieve a precise control over NDs' properties.

2.1.1. Detonation synthesis

Detonation nanodiamonds (DNDs) are formed by high temperature and pressure reactions resulting from the detonation of mixtures of carbon explosives such as TNT and RDX or 2,4,6-triamino-1,3,5- trinitrobenzene (TATB) in oxygen-deficient reaction chambers. The combustion reaction produces extreme operating conditions at which sp^3 diamond becomes the thermodynamically favored bulk phase of carbon; shortly after detonation (< 100 ns) the diffusive coagulation of clustered carbon atoms results in the formation of this bulk sp^3 phase [18]. As the soot of detonation is cooled at a controlled rate in either a gas or liquid phase medium, sp^2 hybridized carbon and other impurities are deposited in layers surrounding the diamond lattice. These partially graphitized diamond crystallites, known as DNDs, have an average diameter of 4–5 nm and possess a truncated octahedral morphology (Fig. 2A) [19]. Overall, the detonation synthesis is a process associated with a poor degree of control over the ND purity. This issue is due to the presence of non-carbon species in the explosive reactants, which introduces in the lattice and the outer shell of DNDs a wide diversity of impurities such as nitrogen, metals, and carbides [20].

For this reason, several purification steps are necessary, including magnetic separation, filtration, and oxidation with sulfuric acid and nitric acid. Removal of the sp^2 carbon shell can also be achieved by oxidizing NDs in air with ozone at high temperatures without the utilization of a corrosive acid [21]. Though more environmentally friendly, these steps are expensive and increase the final cost of fabrication significantly. Another drawback of the detonation approach is the limited control over the surface chemistry along with the considerable variability in surface chemistry from batch to batch. DNDs typically present oxygen functional groups on their surfaces in the form of carbonyl, hydroxyl, epoxy or carboxyl groups. Additionally, the composition of functional groups on the surface may also vary according to the process of cooling after the reaction which can be obtained in a dry chamber with an inert gas or in an aqueous environment using water.

Finally, another limitation of this technique is that the resulting DNDs have a strong affinity to form aggregates in aqueous suspension, and these clustered networks can form up to 200 nm in diameter [22]. DNDs tend to aggregate due to the high density of reactive groups on the surface and the small size. The process of aggregation can be caused by the formation of covalent bonds among the particles or other types of weak interactions including hydrogen bonds, van der Waals forces and π-π stacking due to the presence of graphitic regions. Therefore, in addition to removing metallic impurities, DNDs require a further step of purification after their synthesis to increase their colloidal stability. These strategies will be further analyzed in the following section.

2.1.2. Deagglomeration strategies of DNDs

To effectively overcome the problem of agglomeration and create stable suspensions after detonation synthesis, several factors can be modulated such as the surface charge, the steric hindrance, and the size distribution of DNDs. Each of these factors can impact the stability of DNDs once suspended in an aqueous medium and ultimately determines their suitability for biomedical applications. For instance, it is well established that highly charged nanoparticles do not aggregate due to electrostatic repulsion. Based on this concept, a straightforward