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Dental Applications of Nanodiamonds

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Abstract: Nanodiamonds (NDs) have been used in various fields of medicine such as drug delivery, tissue regeneration and gene therapy. Although there has been research carried out investigated the potential of these remarkable materials in dentistry, to date no review has been published to summarize the studies conducted. Due to their target cell specificity, small size and fluorescence they have also been found to be usefulness in dentistry. Main applications of NDs in dentistry and medicine include guided tissue regeneration, reinforcement of polymers and drug delivery to treat infections and cancers. Recent research also suggests that NDs can also be used as bioactive or antibacterial dental implant coatings. However, to date, the research conducted on their biocompatibility is limited and inconclusive. Hence, substantially more *in vitro* and *in vivo* studies are required to envisage the future of NDs in dentistry. It is hoped that in the next decade these promising materials will find a variety of uses in routine dentistry.

Keywords: nanodiamond, nanotechnology, dental materials, drug delivery, implants, guided tissue regeneration, resin composites, acrylics

1. Introduction

Nanometer-sized particles have been used in various fields of medicine. Drug-delivery, protein-delivery and imaging are few of many uses of nanoparticles in biomedicine. Additionally, nano-particles can also be used to improve the mechanical properties and bioactivity of surgical devices such as implants and graft materials.[1-4]

In recent years, significant amount of research has been conducted to harness the excellent mechanical and biological properties of carbon-based nano-particles. Graphene oxide[5-7], carbon nanotubes[8-10] and nanodiamonds[11-14] have been studied for possible applications in dentistry as well as medicine. Nanodiamonds (NDs) were first developed by scientists in the USSR during the 1960s and revealed to the public during the mid '80s. Initially, the particles were used as protective water-resistant coatings and as additives in motor oil but now have found expediency in medicine.[15] NDs can be synthesized via detonation, chemical vapors deposition, ultrasonic cavitation, irradiation with pulsed lasers and application of high temperature / high pressure (HTHP).[12,15-17] As shown in Figure 1, apart from the route of synthesis, NDs are also classified according to their size: nanocrystalline particles (1 to150 nm), ultra-nanocrystalline particles (2 to 10 nm), and diamondoids (1 to 2 nm).[15,18] A schematic diagram of a nanodiamond crystal is shown in Figure 2. A 'surface shell' composed of graphite surrounds an 'inert core' which is primarily made of diamond.[12] Unlike the inert core which is stable, the surface layer of ND is unstable and reactive. The surface layer can be stabilized if it reacts with other function groups or the conversion of sp^3 hybridization to sp^2 bond configuration of carbon. Surface modification initially involves treatment of ozone which introduces – COOH (carboxyl) groups on the surface of the ND. The carboxyl groups are then converted to different groups by heat treatment in specific gases. For instance, heat treatment in F_2 introduces fluoride groups and Cl_2 may give rise to formation of acylchlorides on the surface. The mechanical properties of NDs are similar to

those of traditional, full sized diamonds making them extremely hard (Mohs scale of hardness: 10, absolute hardness: 1600) and non-biodegradable[12]. However, because of their small size, they are easily excreted out of the body [12].

There are various uses of NDs in medicine. Due to their target specificity, anti-bacterial properties and small size, they are primarily used as carriers for: chemotherapeutic anti-cancerous agents and anti-bacterial drugs.[14,19-21], growth factors such as bone morphogenic protein 2 (BMP 2) and fibroblast growth factor (FGF)[22], and DNA for gene therapy[23]. Additionally, because they can be made fluorescent and are small enough to be taken up by cells, NDs are also used in imaging[24-26] and cell studies.[27,28] Furthermore, they can also be used as nano-sized fillers to improve the mechanical properties of composite polymers.[29]

Lately, recent advances in dental materials have involved the incorporation of nano-sized particles and surface modifications to improve their mechanical and biological properties of dental implants, directive restorative materials and tissue regenerative materials.[1-4,30] Some examples of nano-sized particles that have been used in dentistry are: bioceramics such as hydroxyapatite (HA)[31-36] fluorohydroxyapatite[37-39] (FHA) and bioglass[40,41] for implant-modification and guided tissue regeneration and nano-sized fillers such as silica and alumina to improve the mechanical and optical properties of restorative materials.[42,43]. Hence, as shown in Figure 3, it is not surprising that nano-sized crystals such as NDs have also forayed into the field of dentistry and related fields. Recently, gutta-percha (GP) reinforced with anti-bacterial NDs has been evaluated as a possible endodontic material.[44]. Because of their bio-fluorescent properties and ability to function as nano-sized fillers, NDs have been incorporated in scaffolds for guided tissue regeneration of periodontal bone to make cellular-level imaging easier and to improve the mechanical properties of scaffolds.[22]. Moreover, they have also been used to

reinforced resin composites and denture base polymers.[45,46] The aim of this review is to provide an overview of the research that has been conducted on NDs as dental biomaterials. In addition, the potential of NDs for dental applications and toxicological aspects have been discussed.

2. Applications of NDs in Dentistry

2.1 Guided tissue regeneration

Uncontrolled periodontal disease can lead to bone loss and eventual tooth loss.[47] Over the last two decades, resorbable guided tissue regeneration (GTR) membranes have been used to stimulate the regeneration of periodontal bone by acting as a barrier between the bone and the gingival epithelium.[48] However, few of the biggest limitations of using biodegradable GTR materials are their limited mechanical properties and possibility of adverse tissue reactions.[48]

Poly-L-lactide (PLLA) fibrous and membranous scaffolds containing octadecylamine-functionalized NDs have superior mechanical properties when compared to those not containing any NDs.[49,50] Human osteoblast-like MG 63 cells can be cultured on NDs more effectively than on polystyrene culture dish which indicates their potential to be used in GTR scaffolds.[51] Indeed, growth factors such as BMP-2 and FGF carried by NDs simultaneously in injectable form, have been observed to stimulate osteoblast differentiation *in vitr.*[22] A stronger scaffold capable of delivering bioactive drugs and growth factors to the periodontal tissues could potentially overcome many limitations of the currently available GTR membranes.

In addition to PLLA, nanodiamond-loaded elecrospun poly(lactide-co-glycolide) (PLGA)[52,53] and poly(L-lactide)-co-(ε-caprolactone) (poly(LLA-co-CL) scaffolds[54] have been seen to have favourable effects on the proliferation and growth of human osteoblast-like MG-63 and mesenchymal cells. Furthermore, the nanodioamond-loaded poly(LLA-co-CL) scaffolds have been used to deliver BMP-2 to periodontal tissues *in vivo* and can induce bone growth

significantly morecompared to unmodified scaffolds.[54,55]. A summary of studies conducted on nanodiamond-loaded scaffolds is presented in Table 1. As shown in Figure 2, research has focused on developing ND composite scaffolds either in form of injectable hydrogels or membranes to deliver various therapeutic drugs or regenerative growth factors to dental tissues. However, more *in vivo* and *in vitro* studies are required to ascertain the future of these scaffolds in regenerative periodontics.

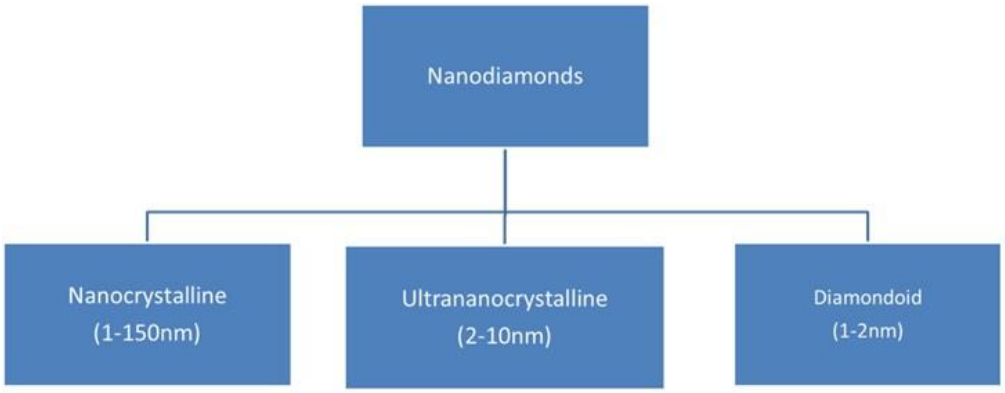

Fig. 1: Classification of nanodiamonds according to crystal size

Table 1.A summary of studies conducted on nanodiamond-loaded scaffolds. NDs, nanodiamonds.

Study	Type of study	Composition of tested scaffold		Mean size and characteristics of ND particles in scaffold	Results
		ND composition	Polymer and type of scaffold		

Zhang et al 2010[50]	*In vitro*	1, 3, 10% 5 nm Octadecylamine-functionalized NDs	Poly-L-Lactide electrospun nanofibers	28 – 32 nm oligomers	Stimulation of MG63 proliferation and differentiation. 200% improvement in Young's modulus and 800% increase in hardness with 10% NDs.
Parizek et al 2012[52]	*In vitro*	0.7% 5 nm NDs	Poly(lactide-co-glycolide) electrospun nanofibers	~ 200 nm clusters	Higher stimulation of MG63 proliferation and differentiation than control. Negligible inflammation observed.
Zhang et al 2012[49]	*In vitro*	1, 3, 10% 5 nm Octadecylamine-functionalized NDs	Poly-L-Lactide electrospun nanofibers	~ 30 nm spheroid	More apatite formed on tested scaffold. 280% and 301% improvement in strain and fracture respectively when 10% nanodioamonds used.

Moore et al 2013[22]	*In vitro*	Injectable 5 nm NDs loaded with BMP-2 and FGF simultaneously in solution	None	100 nm clusters	Dual release of BMP-2 and FGF stimulated osteobloast differentiation and proliferation.
Xing et al 2013[54]	*In vitro* and *in vivo*	10^{17} crystals/mL, 5 nm	Poly(L-lactide)-co-(ε-caprolactone) electrospun nanofibers	Not stated	Significantly more bone formation seen in scaffold + NDs after 12 and 24 weeks.
Suliman et al 2013[55]	*In vitro* and *in vivo*	2 w/v% 5 nm NDs loaded with BMP-2	Poly(L-lactide)-co-(ε-caprolactone)electropsun nanofibers	Not stated	Significantly more bone formation and BMP-2 release seen in scaffold + NDs.
Brady et al 2015[53]	*In vitro*	0.7 wt% 5 nm NDs	Poly(lactide-co-glycolide) electrospun nanofibers	Not stated	Higher stimulation of hMSCs proliferation and differentiation than control. Negligible inflammation observed.

2.2 Reinforcement of dental polymers

In addition to their excellent biological properties and small size, NDs can also function as fillers of various polymers used in dentistry. NDs can be incorporated to epoxy resins, polymers generally used to construct dies, to improve their mechanical properties. Aminated, i.e. combination with amine groups, NDs can be covalently bonded to epoxy to produce composites which have 50% higher Young's modulus and significantly lesser creep when compared to unmodified epoxy.[56] Similarly, a 25% vol.% loading of NDs to epoxy can improve the Young's modulus by up to 470% and hardness by up to 300%.[29]

One of the major requirements of denture materials is adequate strength to withstand the occlusal forces in the oral cavity.[57,58] Acrylic dentures are known to fracture where the denture base is thin [63]. There are several methods of strengthening dentures. Plasticizing agents, nano-fillers and polymer or glass fibers.[58-60] can be used to reinforce denture base polymers. Recent research by Protopapa*et al.* studied the mechanical properties of polymethylmethacrylate (PMMA) denture polymer reinforced by addition of 20nm nanoclusters of NDs.[46] They observed that the PMMA resin containing 0.83 wt% NDs, at 2084 MPa, had a much higher modulus of elasticity (Young's modulus) when compared to unmodified resin which had a modulus of 1184 MPa in addition to improved fracture resistance and impact strength.

Resin composites, being the material of choice for direct esthetic restorations, have a disadvantage of having lower mechanical properties when compared to materials such as dental amalgam, porcelain and alloys.[57,58] Although significant improvements have been achievement as far as the mechanical properties of composites are concerned, they still have not matched stress-bearing attributes of dental amalgam and alloys.[43] It has been observed that resin composites loaded with 220 nm nanodiamond particles at 0.15 wt% concentration can increase the flexural strength from 74.52 MPa to 112.67 MPa and cause the elastic modulus to rise from

2.56 GPa to 5.67 GPa.[45] Although nanodiamond-filled resin composites do not have significantly higher surface hardness when compared to conventional resins, it has been suggested that composites containing a NDs have a significantly lesser cytotoxicity when mouse fibroblasts are seeded on them.[61] However, properties such as bond strength of nanodiamond-composites to tooth structure and microleakage have not been studied yet. Hence, more *in vitro* testing is required before the composites can be applied in the clinical practice.

Incorporation of NDs to direct restorative materials not only is promising from the mechanical point of view, these composites could give rise to a new class of bioactive composites which could be used to deliver medicaments to the pulp and root canal. Some Young's moduli of nanodiamond-modified polymers are presented in Table 2.

2.3 Endodontic treatment and regenerative endodontics

Root canal treatment (RCT) or endodontic treatment involves the extirpation of the coronal and radicular pulp followed by removal of the infected dentine via instrumentation and antimicrobial irrigants – a step known as 'cleaning and shaping'.[62] After the cleaning and shaping of the root canal, a restorative material is place inside the canal to seal (or obturate) the canal. Conventionally, the rubber-based guttapurcha (GP) has been used as the endodontic material of choice due ease of removal and radiopacity.[63] However, due to their limited mechanical properties and leakage, conventional endodontic materials are not sufficient in many cases to completely disinfect the canal and infection might prevail in spite of obturation.[64-66].

Table 2. Young's moduli of nanodiamond-reinforced dental polymers compared with those of unmodified resins, enamel and dentine. PMMA, polymethylmethacrylate; ND-PMMA,

nanodiamond-reinforced polymethylmethacrylate; ND-Epoxy, nanodiamond-reinforced epoxy; ND-Resin composite, nanodiamond-reinforced resin composite.

Material	**Young's modulus (GPa)**	**Reference**
Dentine	15	67-70
Enamel	40-83	67,68,70,71
Epoxy	3.4	29
ND-Epoxy (25 vol%)	12	29
PMMA	1.184	46,72,73
ND-PMMA (0.83 wt%)	2.084	46
Resin composite	2.56	45
ND-Resin composite (0.15 wt%)	5.67	45

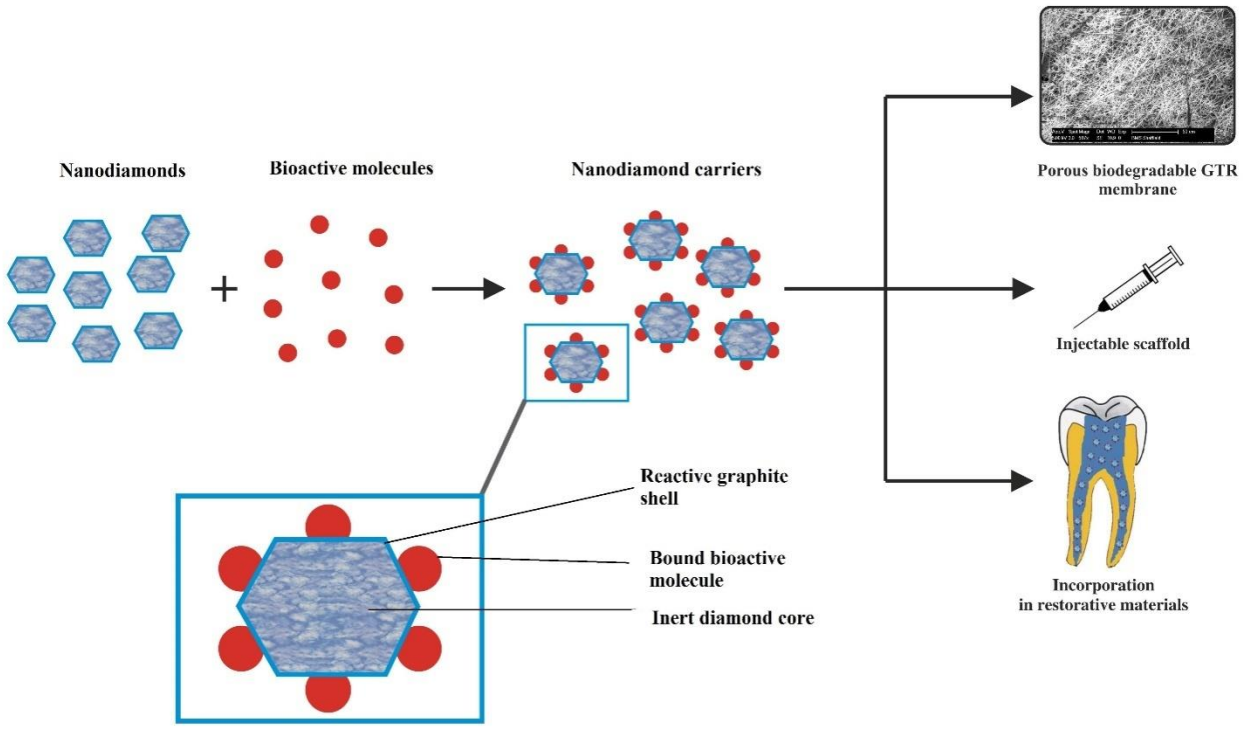

Fig. 2: Schematic diagram of a nanodiamond (ND) and the various ways NDs can be used in tissue regeneration and drug delivery. A 'surface shell' composed of graphite surrounds an 'inert core' which is primarily made of diamond. Unlike the inert

core, the surface layer is reactive and can be bound with various chemical groups such as ketones, hydroxyls and carboxylswhichcan in turn bind to bioactive molecules such as drugs and growth factors and delivered to dental tissues via scaffolds and restorations.

Incorporation of NDs in GP has made it made possible to deliver power antibiotics to the root canal. In a recent study, Lee *et al.*[44]successfully incorporated NDs bound to amoxicillin (ND-amoxicillin) into GP and observed that not only the composite ND-GP exhibited enhanced bactericidal properties against *Streptococci* via prolonged drug release but increased Young's modulus, tensile strength and yield strength were also observed.[44] This study maybe the first of many which could lead to development of endodontic materials that not only can deliver antibiotics to the canal but also carry regenerative growth factors to enhance root and pulpo-dentine regeneration. Indeed, human dental pulp cells cultured on oxygen-terminated NDs have demonstrated enhanced growth and differentiation in addition to formation of mineralized tooth tissue indicated the tooth-regenerative potential of nano-diamonds but until now, the *in vivo* of these NDs has not been witnessed.[74]

2.4 Modification of dental implants

Prior to being used as dental implant, the surface of the implant material has to be modified in order to improve its biocompatibility. Imparting osseoconductive properties and increasing the hydrophilicity of implants enhances osseointegration.[30] However, even with the recent advancements in dental implant modification, failures of implants are still observed.[75-77] Many of these failures have been linked to the failure of the bioactive surface coating and per-implant infections.[75,76]

Recently, titanium dental implants have been coated with NDs.[78] Due to their ability to deliver antimicrobial drugs and osseogenerative growth factors, ND coatings could be used to enhance osseointegration and overcome the shortcomings of current implant surface modifications. Additionally, anti-bacterial coatings such as Ag/ND could be used as anti-contaminant implant co atings owing to their antimicrobial properties against *E. coli*[79]. The anti-microbial properties of NDs have been attributed due to the presence of oxygen-containing groups and charges on their surface [56]. However, to date no studies have attempted to determine the bond strength or mechanical properties of the ND layer coated on dental implants. Mechanically undermined coatings are known to chip off during screwing of the implant or may laminate prematurely leading to failure of osseointegration [30]. Therefore, the mechanical properties and the bond strength of ND-coatings must be determined they are used in the clinical environment.

2.5 Drug delivery and cancer treatment

Various forms of drug-delivery systems that can be used to deliver therapeutic agents to site of oral pathology have been studied.[80,81]As illustrated in Figure 4, Ability of NDs to carry drugs and release them in acidic cancer tissues makes them ideal carriers of anti-cancer agents.[12,15] Effective administration against breast cancer tissues indicate that NDs could be used in hydrogels to deliver doxorubicin to oral cancer lesions.[82,83] An adjunct administration of ND-delivered drugs to conventional anti-cancer treatment could have a synergistic effect on the outcome. Indeed, recent research has shown that it could be possible to safely deliver multiple drugs at the same time using NDs. [84] One potent effect of using NDs is that they can induce an increased leakiness of cancer drugs through vascular barriers to improve drug deliver to target cells.[13] Furthermore, small interfering RNA (siRNA) can be delivered into cancer cells via NDs to stop their proliferation by impeding the expression of oncogenes.[85] Recent delivery of plant-

derived anti-cancer drugs, ciproten and quercetin into cancer cells suggest a promising natural treatment of cancer.[11]

Although research shows great promise in using NDs to deliver drugs, robust *in vitro* and *in vivo* studies have to be conducted to study the effect of ND-delivered anti-cancer drugs on cancer cells before they can be deemed safe clinically.

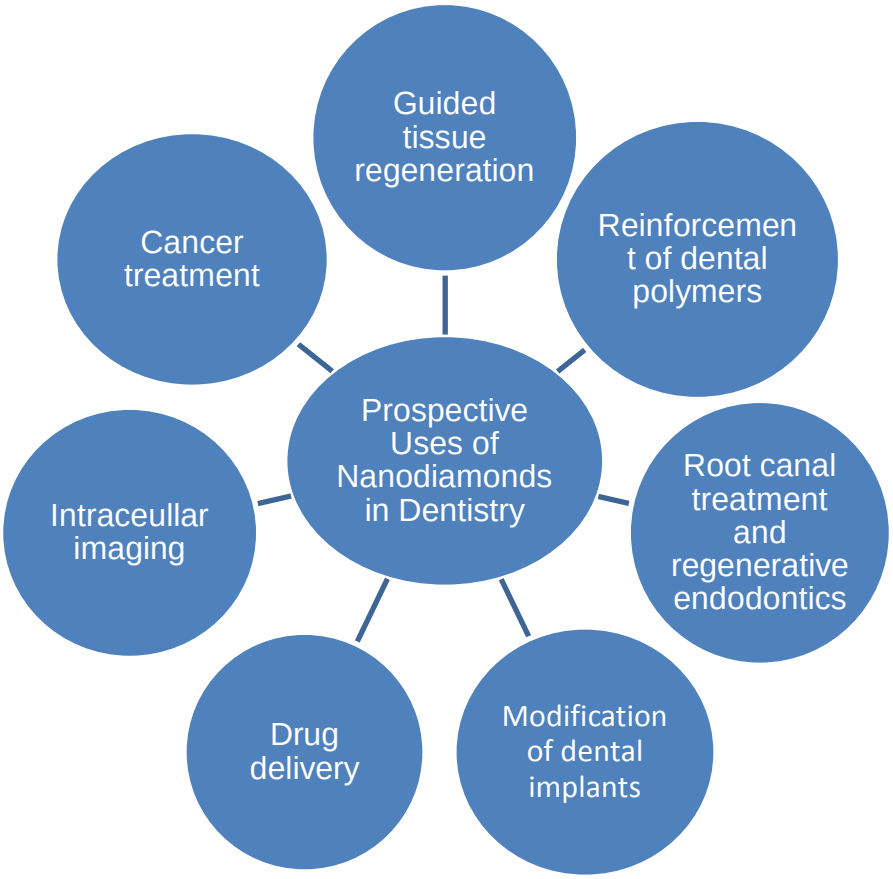

Fig. 3: Major applications of nanodiamonds in dentistry and medicine.

3. Drawbacks of nanodiamonds

The major concerns of using NDs are their potential toxicity and biocompatibility. To date, research conducted on the biocompatibility of NDs has not concluded to what extent the particles are biocompatible. Impure NDs can contain metals such as iron (Fe) which can be potentially non-biocompatible.[12]Hence, for clinical use, highly pure NDs are recommended. Studies indicate that NDs at concentrations higher than 50μg/ml could cause evident toxicity in human lymphocytes and concentrations lower than that can still cause DNA damage.[86]Conversely, i*n vivo* studies conducted on zebrafish suggest that the cytotoxicity of NDs is not dose-dependent but is rather linked to the functional groups bound to them. Cationic attachments on NDs exhibit a higher toxic potential than anionic attachments.[87]Nevertheless, assay studies on the production of reactive oxygen species (ROS) when NDs are exposed to neuroblastomas, macrophages, keratinocytes, and PC-12 cells has observed no significant toxic effects of NDs once they are inside the cells.[88] It is safe to assume that the cytotoxicity of NDs still has to be studied further before they can be used clinically. More *in vitro* and *in vivo* studies are required to study the effect of NDs on dental tissues such as pulp, periodontium, oral mucosa.

Another disadvantage of NDs is that, too date, no large-scale clinical trials have been conducted to determine their safety and efficacy in the aforementioned applications. As stated above, it is unknown how the NDs would affect the human tissues in the clinical setting. Indeed, if NDs are used in GTR scaffolds, their mechanical properties and physical properties must withstand the different occlusal forces in the oral cavity. Meticulous mechanical as well biological testing is required before ND-containing composites are used in routine dental practice. Additionally, phase separation of matrix and ND fillers may lead to diminished mechanical properties of ND-polymer composites. Indeed, this phase separation is observed with other polymers too [2] and may cause premature failure of ND-incorporated restorations and polymers. One way of

overcoming this may be via incorporation of coupling agents [89] but, because of lack of further studies, it is known how they would function in the oral cavity under occlusal and masticatory stresses.

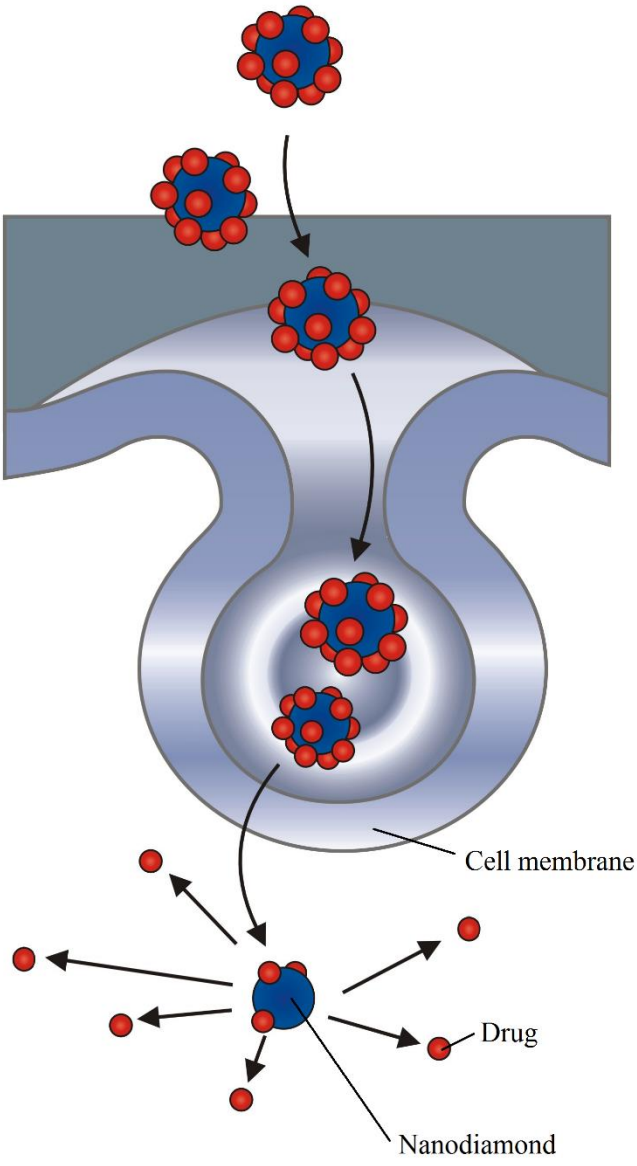

Figure 4. The extremely small size of nanodiamonds makes it possible for them to be taken up into cells by endocytosis. Due to the low pH in the inflammatory and cancer cells, the drug is released into the intracellular matrix.

4. Future outlook and conclusion

NDs could be used in a variety of areas of dentistry. Research indicates that they not only can improve the mechanically properties of GTR materials but also can carry growth factors and drugs to the oral tissues to provide a more personalized, targeted form of therapeutics [89]. Furthermore, because of their extremely small size, they can be used to reinforce restorative and prosthodontic polymers such as acrylics, resin composites and epoxy. Moreover, they can also be used asosseoconductive and/or antibacterial coatings on dental implants to minimize peri-implant infections and enhance osseointegration. Recent research also indicates that NDs could also be used to deliver intracanal antibiotics and improve the mechanical of endodontic materials. However, to date, the research conducted on the biocompatibility of ND-composites is limited and inconclusive. Hence, extensive *in vitro* and *in vivo* studiesarerequired to envisage the future of NDs in dentistry. It is hoped that in the next decade these promising materials will find a variety of uses in routine dentistry.

Conflicts of Interest

The authors declare no conflict of interest

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