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Nanodiamond applications in skin preparations

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The biocompatibility and nontoxicity of nanodiamonds (NDs) in combination with their excellent physical performance have rendered them attractive candidates for biomedical applications. NDs have great potential in drug nanoformulations because of their small size compared with other carbon nanomaterials. They are nontoxic with excellent adsorption properties and can be formulated into skin care products. Even though NDs have shown encouraging potential in skin preparations, only a few studies have reviewed their application in topical drug delivery systems. Therefore, here we focus on the application of NDs in skin care preparations, skin cancer medication, and wound healing. We also highlight the development of topical drug delivery by NDs and their cytotoxicity.

Introduction

Nanomaterials are particles <100 nm in at least one or more external dimension. They exhibit other physical and chemical activities that result from their increased surface area, as well as quantum effects. Owing to their superior properties, nanoparticles (NPs) have emerged as alternative promising materials for biomedical applications [1].

Transdermal drug delivery, the non-invasive delivery of drugs through the skin into the bloodstream, could revolutionize the treatment of several skin diseases [2]. Such delivery methods are an attractive alternative to other drug administration routes, such as oral delivery, intravenous administration, and hypodermic injection [3–6]. Utilizing nanocarriers, such as nanospheres and NPs, can improve transdermal drug delivery. Microparticles and NPs can not only enhance drug absorption into the skin, but also release drugs in a controlled manner for a prolonged period of time. They also increase drug permanence [7–12], ensuring direct contact with stratum corneum [13], penetrating skin appendages [14,15], and protecting drugs against chemical or physical instability [16–18]. Recent advances in nanoscience and nanotechnology have made carbon-based nanomaterials attractive research targets. Many researchers are focusing on the utility of fullerenes, nanotubes, and diamond NPs for various industrial applications and drug delivery applications [19–22]. Based on their size, common commercial diamond NPs can be categorized into three groups: nanocrystalline diamond (NCD) particles (10–100 nm), diamondoids (1–2 nm), and ultrananocrystalline diamond particles (UNCD) (2–10 nm). Detonation NDs (characteristic size of primary particles ~4–5 nm) are UNCDs that have been commercialized for biomedical applications [23]. NDs show important properties, such as biocompatibility, low toxicity, and a tailorable surface chemistry, which can be easily modified to facilitate bioconjugation and accessibility. Their unique optical, mechanical, and thermal properties with stable photoluminescence characteristics have made them good candidates for a range of applications in drug delivery [24,25].

NDs were recently introduced as suitable carriers for improving transdermal drug delivery for the treatment of skin diseases. They have also been developed for treating superficial tumors, such as skin cancers. NDs can deliver drugs transdermally to tumors or inflammation sites and are able to reduce drug toxicity on normal tissues [26,27]. They can also provide regenerative therapies for wound healing by accelerating the healing process while avoiding damage to healthy cells or tissues [28–30]. NDs are widely used in cosmetic and healthcare products because of their large surface area, which allows a large amount of the therapeutic agent to be

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loaded onto the particles; NDs also improve drug durability and robustness and disperse insoluble drugs in water [31]. However, the toxicology and environmental safety of NDs are of legitimate concern and need to be examined by specific toxicological studies before the wider application of this new technology.

Here, we review the application of NDs in skin care products, their effectiveness in wound healing, and *in vitro* and *in vivo* experiments on skin cells using NDs.

Applications of NDs in skin care products

The Environmental Working Group lists 139 cosmetic products containing NDs that are currently on the market (www.cosmeticdatabase.com). NDs are nontoxic and are listed as materials with low hazard score (1 out of 10) [32]. A significant area of ND application is for ND-impregnated cosmetics [33]. Given the excellent absorption attributes of NDs, they can be formulated into various skin care products, such as facial lotions, deodorants, skin cleansers, dermal strips, soaps, and exfoliates [34].

NDs can improve the penetration of active ingredients into skin and enhance their therapeutic activity [35,36]. The hydrophilic nature of diamond powder is related to the high concentrations of OH groups on its surface. NDs are able to bind to the skin surface, reduce transepidermal water loss (TEWL), affect the function of the hydrolipid skin surface, and protect skin from aging [37]. Protection against ultraviolet rays (UVR) is another potential application of NDs in healthcare products [38] as a result of unique optical properties that enable them to protect against harmful UV radiation [39]. A sunscreen containing functionalized NDs for protection against some types of skin cancer was patented by Sung in 2011 [40,41]. Thus, the biocompatibility and nontoxicity of NDs combined with their excellent physical performance render them attractive candidates for reflecting and scattering UV radiation [39,42].

In vitro and *in vivo* studies of NDs

In vitro and *in vivo* studies have been performed to explore the mechanistic and physiological behaviors of NDs for use in applications from drugs to medical implants.

For example, Lim *et al*. evaluated different properties of NDs for topical drug delivery applications. To increase the dispersal ability of NDs, detonated NDs were functionalized via their carboxyl group. Z-average sizes of carboxylated NDs (ND–COOH) at pH 5.0, 6.0, and >7.0 were reported as 1049.9, 177.9, and ~50 nm, respectively. Increasing the pH (from 5.0 to 9.0) significantly reduced the sizes of ND–COOH agglutinates and increased their zeta potential values (14.90, 22.50, and 31.58 mV, respectively). The skin permeation profiles of eugenol and ND–COOH/eugenol complexes were assessed at 32 °C by a Franz diffusion cell system using excised hairless mouse skin. The amount of eugenol released increased to1195.28 $\mu g/cm^2$ with the ND–COOH/eugenol complexes compared with 780 $\mu g/cm^2$ with the eugenol solution after 24 h. Thus, NDs improved the *in vitro* skin permeation by >50% and adsorbed a drug amount equivalent to 80% of their own weight. ND–COOH and ND–COOH/eugenol complexes did not have any significant toxic effects on murine macrophage cells. The reactive oxygen species (ROS) scavenging effects of eugenol and ND–COOH/eugenol complexes were studied using 2′,7′-dichlorodihydrofluorescein diacetate (DCF), cupric reducing antioxidant capacity (CUPRAC), and 1,1-diphenyl-2-picryl-hydrazil (DPPH). A remarkable reduction in ROS was observed with the ND–COOH and ND–COOH/eugenol complexes compared with the negative control (Table 1). Therefore, ND–COOH was introduced as an excellent topical drug delivery system with improved permeability, higher stability, and minimal safety issues [43].

Different medical implants have been covered by the chemical vapor deposition (CVD) of NCDs. Diamond powder particles (DPPs), which are known for their outstanding performance and reproducibility, are an extension of NCDs. Skin toxicity examinations revealed that, unlike the allergic reaction of patients to nickel and chromium, no allergic reaction was observed against DPPs. *In vivo* and *in vitro* skin studies showed that NCD coatings do not result in any toxic and allergic reactions of the systems studied so far [44] (Table 1).

Horie *et al*. examined the cellular effects of three kinds of ND: ND-A, ND-B, and ND-C. The average secondary particle size, ND concentration, and zeta potential were 16 nm, 71 mg/ml (7.1 wt. %), and −51.68 mV for ND-A; 8.4 nm, 78 mg/ml (7.8 wt.%), and +42.39 mV for ND-B; and 3.1 nm, 69 mg/ml (6.9 wt.%), and +45.58 mV for ND-C. Different dispersal concentrations (1.0, 0.1, and 0.01 mg/Ml) were tested on human skin keratinocytes (HaCaT) and A549 cells for 6 and 24 h. Mitochondrial activity, intracellular ROS level, apoptosis, lipid peroxidation, colony formation, and cellular uptake were examined. Exposure to ND-A (100 and 0.1 mg/ml) for 24 h decreased mitochondrial activity in HaCaT and A549 cells. By contrast, the cell viabilities of ND-B and ND-C exposed A549 cells decreased significantly. Neither apoptosis nor necrosis was observed in A549 cells exposed to any concentration of ND. By contrast, an increase in apoptosis was observed in HaCaT cells following ND exposure at a concentration of 1.0 mg/ml. In A549 cells, there was no inhibition of colony formation following exposure to ND at any concentration. Compared with A549 cells, colony formation of ND-A-exposed HaCaT cells was inhibited in a concentration-dependent manner. The number of cells included in a colony was smaller than in unexposed cells in both ND-exposed A549 and HaCaT cells. ND-B and ND-C did not result in any colony formation. Cellular uptake of ND particles was observed in both A549 and HaCaT cells. Compared with unexposed cells, there was no change in either the intracellular ROS or lipid peroxidation level in ND-A-exposed A549 and HaCaT cells. In addition, ND-A did not induce oxidative stress in the cultured cells, whereas ND-B and ND-C increased the intracellular ROS level. There was no increase in lipid peroxidation in cells exposed to either ND-B or ND-C [45] (Table 1).

Schrand *et al*. assessed the effect of the surface chemistry of NDs (2–10 nm) on cellular cytotoxicity. NDs with acidic or basic functional groups (–COOH, –COONa, and $–SO_3Na$) were prepared and applied to three different cell types (macrophages, keratinocytes, and PC-12 cells). NDs without acidic or basic functional groups (2–10 nm) were used as positive controls. NDs with different functional groups did not cause any significant cytotoxic or genotoxic effects on any of the cell types included in the study (Table 1) [46].

Stanishevsky *et al*. studied the surface modification of NCD films, DNPs, and carbon nanospheres (average size ~5 nm). NCD films were deposited onto polished Ti–6Al–4 V discs using a gas $H_2/CH_4/N_2$/He mixture and a chamber pressure of 65 Torr. The surface temperature was maintained at 700 °C during the

TABLE 1

In vitro and in vivo studies of nanodiamonds in skin cells

Type of nanodiamond	Biological model	Parameter evaluated	Results	Refs
ND–COOH/eugenol (1049.9 nm at pH 5, 177.9 nm at pH 6), ~50 nm at pH >7.0); zeta potentials: 14.90 mV at pH 5.0; 22.50 mV, at pH 7.0; 31.58 mV, at pH 9.0	*In vitro:* porcine cadaver skin, RAW 264-7 cells	Cytotoxicity, skin permeation, ROS	No toxicity ND–COOH/eugenol complex showed ~50% higher released amount compared with eugenol solution Cumulative released amount of eugenol after 24 h ($\mu g/cm^2$): ND–COOH/eugenol complex; 1195.28; eugenol solution; 780 Decrease in ROS	[43]
Diamond powders (0.3 ÷ 1.7, 0.2 ÷ 1.8 μm)	*In vivo:* guinea pigs, human back skin	Irritation allergic reaction	No allergic reaction	[44]
ND-A (16 nm), ND-B (8.4 nm), ND-C (3.1 nm); zeta potentials: ND-A, − 51.66 mV; ND-B, +42.39 mV; ND-C, +45.58 mV; concentration: 1.0, 0.1, 0.01 mg/ml (6, 24 h)	*In vitro:* human keratinocyte HaCaT cells	Cell viability, ROS, apoptosis	Negative zeta potential of ND-A showed stronger cellular influences Positive zeta potential of ND-B and ND-C decreased cell viability and increased intracellular ROS level Slight apoptosis, inhibition of colony formation in HaCaT cells at 1.0 mg/ml	[45]
NDs (2–10 nm): unfunctionalized, acid-functionalized, base-functionalized	*In vitro:* neuroblastoma, macrophage, keratinocyte, PC-12 cells	Cytotoxicity	No toxicity	[46]
ND (5 nm) O-, H-, and F-terminated surfaces	*In vitro:* human mesenchymal stem cells (MSCs), PAM 212 (healthy mouse epidermal keratinocytes), CH 72 (cancerous mouse epidermal mouse keratinocytes), RAW 264-7 (mouse macrophages), Au565 (human breast cancer cells), NIH3T3 (mouse fibroblasts), mouse bone marrow-derived dendritic cells (BMDC)	Cellular response, cytotoxicity	Attachment, proliferation of various cells affected by surface functionalization No cytotoxicity	[47]
Microcrystalline (100–300 nm) and UNCD (5–10 nm)	*In vitro:* normal human dermal fibroblast (NHDF) cells, pheochromocytoma (PC12) cells	Adhesion properties of diamond surface on NHDF	UNCD exhibited better cell adhesion than microcrystalline diamond Cell adhesion forces strongest on UV-treated UNCD	[48]
UNCD (150 nm thick): coated and uncoated with microporous silicon nitride	*In vitro:* human epidermal keratinocytes	Comparison of human epidermal keratinocyte growth	UNCD coating did not alter viability of human epidermal keratinocytes	[49]
NDs (5 and 100 nm)	*In vivo:* C57BL/6J mouse *In vitro: HaCaT keratinocytes, MEFs*	Comparison of UVB protection of NDs, nanosized TiO_2 and ZnO	100-nm NDs exhibited superior UVB attenuation in HaCaT keratinocyte model 5- and100-nm NDs exhibited superior UVB attenuation compared with nanosized ZnO in MEF model	[50]
Titanium alloy microneedle coated with N-UNCD (350 nm thick)	*In vitro and in vivo:* cadaveric porcine skin	Skin penetration	Capability for skin penetration	[51]
NDs (4–5 nm): ND R1 and ND R2	*In vitro:* human cervical cancer (HeLa), murine melanoma (B16F10) cells	Antiproliferative effect, ROS, cytotoxicity, apoptosis	Improved antiproliferative effects Reduced pro-oxidant, cytotoxic and pro-apoptotic activities	[52]
NDs (4–5 nm)	*In vitro*: murine melanoma (B16F10) cell	Mitotic processes, cell growth	Antimitotic properties Inhibited cell growth	[53]

process, followed by gradual cooling step in pure hydrogen plasma. Resulting H-terminated films had an average grain size of 5 nm according to X-ray diffraction spectroscopy. An O-terminated hydrophilic surface was produced by treating with H_2/O_2 plasma after 1 h. Processing of H-terminated films in F_2 gas in a closed chamber at 100 °C for 48 h produced F-terminated hydrophobic NCD films. The flexibility of NDs and NCD films for specific applications was adjusted by surface modifications. The attachment and proliferation of various cells were strongly influenced by the type of surface functionalization. Cells readily adhered to, and proliferated on, H-terminated NCD, but not on F- or O-terminated NCD films. H-terminated NCD surfaces promoted cell adhesion, and proliferation (Table 1). Given the nature of NCD, it is not obvious to what degree the available surface sites on NCD films or carbon NPs can be occupied by functional groups. None of the modified NDs were cytotoxic. Although there was clear selectivity of the cellular response to H, O, and F surface-terminated NCD films, the performance and role of specific functional groups on carbon NPs has yet to be investigated [47].

Adhesion properties of microcrystalline diamonds and UNCDs with various functional groups (H-terminated, undecylenic acid-functionalized, and UV-treated) on normal human dermal fibroblast (NHDF) cells indicated a direct correlation between initial cell adhesion forces and subsequent cell growth. Cell adhesion forces were observed to be strongest on UV-treated UNCDs (Table 1). UV spectroscopy of cell growth showed that UNCDs were intrinsically more biocompatible than microcrystalline diamonds. UV irradiation of the diamond surface in air caused oxygenation of the surface. Hydrophilic oxygenated surfaces provided a better platform for initial cell adhesion and subsequent cell growth compared with H-terminated surfaces [48].

Skoog *et al*. deposited a UNCD film (~150 nm thick) on a microporous silicon nitride membrane via a microwave plasma chemical vapor deposition method. Comparison of the growth of human epidermal keratinocytes on UNCD-coated microporous silicon nitride membranes with those on uncoated microporous silicon nitride membranes by MTT assay indicated that the UNCD-coated membranes did not significantly alter the viability of these cells (Table 1). These results revealed that UNCD-coated membranes could be used for improving skin sealing around percutaneous implants [49].

The attenuation efficiencies of NDs (5 and 100 nm) in attenuating UV radiation were studied in C57BL/6J mice, mouse embryonic fibroblasts (MEFs), and HaCaT cells. Results were compared with nano-sized TiO_2 and ZnO. The 100-nm NDs displayed superior UVB attenuation compared with the other NPs in the HaCaT keratinocyte model; both 5- and 100-nm NDs showed superior UVB attenuation compared with nano-sized ZnO in the MEF model (Table 1). A positive effect of the protective efficiency of 100-nm NDs was demonstrated over nano-sized TiO_2 in mouse skin, with 2 mg/cm^2 of NDs efficiently reducing over 95% of UVB radiation. Thus, NDs were found to be feasible and safe substances for preventing UVB-induced skin damage [50].

Skoog *et al*. fabricated titanium alloy microneedles with a coating of nitrogen-incorporated UNCDs (N-UNCDs). Microneedles were micromachined from a widely used medical titanium alloy, ASTM F136 ELI Ti-6Al- 4 V. N-UNCDs (~350 nm thick) were deposited on the microneedles using microwave plasma chemical vapor deposition to develop their biocompatibility, increase their hardness and mechanical strength, and create an electrochemically stable surface. Cadaveric porcine skin was used to evaluate the skin penetration capabilities of the device. Diamond-coated titanium microneedle arrays were able to permeate full-thickness cadaveric porcine skin without microneedle fracture or diamond film delamination. The N-UNCD coating demonstrated an acceptable level of adhesion to the titanium alloy substrate. *In vitro* electrochemical detection of dopamine and uric acid was performed using unmodified N-UNCD electrodes at physiologically relevant analyte concentrations. The results demonstrated the potential application of the N-UNCD coating of titanium alloy microneedles for enhancing skin penetration and transdermal electrochemical biosensing [51] (Table 1).

The biomedical use of plant drugs loaded into NDs (4–5 nm crystallite primary size) in cancer therapy was investigated by Gismondi *et al*. NDs were synthesized by detonation and purified via an oxidation to remove metals and sp^2 carbons from the outer shell of the NDs. NDs were opportunely modified by two types of reducing process: chemical reduction (ND R1) and plasma reduction (ND R2). Each type of ND (ND, ND R1, and ND R2) was then functionalized with ciproten (C) or quercetin (Q) at different concentrations (5, 10, 20, 50, 80, 100, 150, and 200 mg/ml) for 30 min, and 2, 4, 6, 8, 24, 48, and 72 h (h). The amount of intracellular ROS, cytotoxicity levels, apoptosis, and antiproliferative effects of pure ND, ND R1, and ND R2 or funtionalized NDs (+C and +Q) on human cervical cancer (HeLa) and murine melanoma (B16F10) cells were studied. The results showed that ND +C, NDR2 +C, NDR1 +Q, and NDR2 +Q caused a reduction in cell proliferation better than did treatment with their respective pure secondary metabolites (C and Q). In addition, all MTT data clearly demonstrated that the NDs amplified the cell growth inhibitory properties of ciproten and quercetin on HeLa and B16F10 cells. The authors also observed that, in both cell lines, ciproten bioactivity was increased just after conjugation with ND and ND R2, whereas ND R1 and ND R2 were able to enhance the antiproliferative effects of quercetin on the cells. No sample showed any significant alteration in cell proliferation after 24 h of treatment. For each sample, cytotoxicity was absent, or minimal, after 24 h of treatment. Therefore, structural and chemical modifications of ND surfaces can affect the bioactivity of transported drugs [52].

Gismondi *et al*. coupled reduced detonation NDs (4–5 nm crystallite primary size) with a plant secondary metabolite, citropten (5,7-dimethoxycoumarin), and showed that the complex reduced B16F10 tumor cell growth more effectively than did treatment with pure citropten. The identification of metaphasic nuclei and irregular disposition of β-actin in the cell cytoplasm supported the hypothesis that citropten coupled with NDs demonstrated antimitotic characteristics in B16F10 cells (Table 1). Thus, ND-citropten is considered an effective treatment for skin cancer [53].

Wound-healing applications of NDs

Skin comprises several layers that are essential to its function and response to injury: epidermis, dermis, and hypodermis. When an injury occurs, damaged skin tissue naturally begins a repair process, named wound healing [54]. Wound healing is a spontaneous and dynamic repair process in the injured tissue and includes hemostasis, inflammation, proliferation, and remodeling (or scar

tissue formation) [55]. The early phase after skin trauma is hemostasis, and hemorrhage is a main cause of early death. Wound healing attempts to repair the damaged tissue by re-establishing the integrity of the injured tissue and replacing lost tissue [56]. The absence of adequate repair leads to prolonged healing time for damaged skin, major disability, and even death. Thus, it is necessary to design proper wound dressings to protect wounds from further damage and to improve wound healing [57].

ND-based gene delivery platforms for wound healing are attractive because NDs have a high surface chemistry that is amenable to various modifications to help carry genes and effect cell entry [58]. In wound healing, overcoming the limited distribution of growth factors to the wound site is being investigated by means of gene transfer. For instance, bovine collagen as a biocompatible matrix is a supporting gene therapy vector that works as a growth factor for tissue repair.

Adamantane (1–2 nm) is the smallest species of H-terminated cubic diamond and contains only ten carbon atoms. Bellocq *et al*. prepared a synthetic biocompatible material comprising a linear β-cyclodextrin-polymer and an adamantane-based cross-linked polymer (CD-ADA), to deliver bovine collagen to cultured cells by the use of an adenoviral delivery vector. The combined polymers produced an extended network by chemical reactions between cyclodextrins and adamantanes. The network properties relied on the molecular weight and number of adamantanes on the cross-linking polymer. Alternative factors, such as replacement of β-cyclodextrin (host) and adamantane (guest) with other cyclodextrins (such as α, γ, and substituted members) and inclusion molecules (guests), offer a logical step for designing network features. These concepts illustrated increased rates and migration of collagen. According to the results obtained with the CD-ADA construct, this can function as a tunable and biocompatible matrix for recombinant adenovirus-mediated gene delivery to local wound sites [59] (Table 2).

ND-based drug platforms have also been examined for the elution of a range of therapeutically active compounds. Insulin is a potential wound-healing compound and a vascularization-promoting agent for severe burns and other conditions. Shimkunas *et al*. conjugated bovine insulin noncovalently to detonated NDs (approximately 5–10 nm thick) by physical adsorption in an aqueous solution. pH-dependent desorption was observed in a sodium hydroxide alkaline environment. In neutral and alkaline solutions, combination of NDs with insulin at a 4:1 ratio showed $79.8 \pm 4.3\%$ adsorption and $31.3 \pm 1.6\%$ desorption, respectively. In addition, a 5-day desorption assay in NaOH (pH 11) and a neutral solution showed $46 \pm 4\%$ and $2.2 \pm 1.5\%$ desorption, respectively [60] (Table 2).

Mytych *et al*. indicated the potential hormetic effects of low concentrations of NDs and SiO_2-NPs using normal human facial skin fibroblasts (FSF1) undergoing serial passaging in culture. Some well-documented effects of hormetins in human cells were proven to slow aging, extend the replicative lifespan, accelerate wound healing, increase differentiation and angiogenesis, and improve tolerance to other stresses. Optimized wound-healing effects were obtained at low ND and SiO_2-NP concentrations (up to 0.5 mg/ml) [61] (Table 2).

Pacelli *et al*. investigated the potential of NDs as carriers for the controlled release of therapeutic molecules for regenerative and wound-healing applications. The authors formulated a thermosensitive hydrogel using gelatin, chitosan, and NDs that provided the sustained release of exogenous human vascular endothelial growth factor (VEGF). The addition of NDs resulted in improved mechanical characteristics of the injectable hydrogels without affecting their thermosensitive gelation properties. Biocompatibility of the generated hydrogel was investigated by *in vitro* assess-

TABLE 2

Published data on wound-healing applications of nanodiamonds

Type of nanodiamond	Biological model	Parameter evaluated	Results	Refs
CD-ADA construct (cyclodextrin- and adamantane-containing polymers)	*In vitro:* human skin fibroblasts (CCD-1074sk cell)	Cutaneous wound repair by gene delivery	CD-ADA construct can serve as a highly tunable, biocompatible matrix for recombinant adenovirus-mediated gene delivery to local wounds	[59]
ND (5–10 nm thick)–insulin complexes	*In vitro:* RAW 264.7 macrophages, 3T3-L1 fibroblasts	Wound healing	ND–insulin might accelerate healing process, decrease incidence of infection by releasing insulin in alkaline wound areas	[60]
NDs ($<$ 10 nm particle size) and silica NPs (SiO_2-NP) (12-nm primary particle size)	*In vitro:* normal diploid human facial skin fibroblast cells (FSF1)	Wound healing	ND and SiO_2-NP at low concentration (up to 0.5 mg/ml) enhanced wound healing ability and slowed aging	[61]
ND (220 nm); zeta potential: -9.4 ± 0.9 mV	*In vitro*: immortalized human umbilical vein endothelial cells (HUVECs)	Release study, wound healing	Provided sustained release of VEGF for wound-healing applications	[62]
Carboxylated NDs/cellulose nanocomposite membranes (CNM-CND): Size: 86.59 ± 5.50 nm; zeta potential: -40.50 ± 4.00 mV	*In vitro*: HeLa cells	Cytotoxicity, release study, wound dressing	No toxicity Greater release of DOX at pH 5.5 than at pH 7.4. Potentially suitable for wound dressings	[63]
Chitosan/bacterial cellulose composite films containing NDs (5 nm)	*In vitro*: fibroblast L929 cells	Cytotoxicity, wound dressing	Reasonable cytocompatibility Flexible platform for wound dressing	[64]
Uniform fibers of CS/medical grade/NDs (3 nm)/bacteria	*In vitro:* mouse skin fibroblast cells (L929)	Wound dressing	Potentially suitable for wound healing applications	[65]

ment of apoptotic gene expression and anti-inflammatory interleukin production. The inclusion of the ND-VEGF complex in the hydrogel network resulted in the sustained release of this angiogenic growth factor. These results demonstrate the potential application of NDs for formulating a biocompatible, thermosensitive, and multifunctional hydrogel platform that can function as a filling agent to modulate hydrogel properties, as well as a delivery platform for the controlled release of bioactive molecules and growth factors [62] (Table 2).

Luo *et al*. developed a carboxylated ND-cellulose nanocomposite membrane (CND-CNM) for the controlled release of doxorubicin (DOX) for potential application as a wound dressing. Drug loading efficiency and release were examined using doxorubicin hydrochloride as a model drug. Comparing the drug release profile from the membranes at pH 7.4 and 5.5 demonstrated greater DOX release at higher pH. An *in vitro* cytotoxicity assay of the membranes confirmed that DOX-loaded CND-CNMs exhibited lower cytotoxicity compared with free DOX (Table 2). Thus, the authors provided proof of concept for using these membranes for the loading and release of bioactive compounds for wound dressing [63].

Ostadhossein *et al*. suggested the potential application of chitosan/bacterial cellulose composite films including DNPs as a flexible platform for wound dressing. The authors examined the effect of NDs on mechanical, physiochemical, and biological properties of the films. Microstructural studies indicated uniform ND dispersion in the matrix and slight agglomeration at concentrations above 2 wt%. Hydrogen bonds between NDs and the polymer matrix were approved by FTIR. X-ray diffraction analysis showed decreased crystallinity of the polymer matrix in the presence of NDs. An approximately 3.5-fold increase in the elastic modulus of the composite film was obtained by the addition of 2 wt% NDs. Results of colorimetric analysis demonstrated that the composite films were transparent but turned gray and semitransparent at high ND concentrations. A reduction in the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) gap was observed, which resulted in a red shift and higher absorption intensity towards the visible region. Mitochondrial activity determined in L929 fibroblast cells demonstrated the biocompatibility of this nanocomposite film (>90%) after 24 h incubation [64] (Table 2).

Mahdavi *et al*. prepared chitosan-based biopolymers containing medical grade NDs (MND) (3 nm, up to 3 wt%) and bacterial cellulose (33 wt%) by electrospinning. Scanning electron microscopy revealed the formation of uniform fibers with diameters in the range of 80–170 nm. Results revealed that the ND-modified mats are potentially suitable for wound healing [65] (Table 2).

Concluding remarks

Owing to their excellent biocompatibility, NDs can provide a good foundation to improve the application of drug delivery vehicles to treat skin cancer. As a result of their high adsorption rate, the addition of NDs to skin care products can also enable active ingredients to work at a maximum potential. NDs are able to carry more of the active ingredients than are traditional formulations and can penetrate deeper into the skin layers. As well as being fully and rapidly absorbed by skin, the high water absorption capacity of NDs keeps the skin hydrated for longer. NDs might also enhance wound healing and could be used for wound dressing. ND-insulin clusters with a high surface area could hasten the healing process and reduce the incidence of infection by releasing insulin in alkaline wound areas. Cytotoxicity and allergic reaction studies of NDs have indicated that they do not induce any significant cytotoxic or genotoxic effects, whereas UVB attenuation abilities in a HaCaT keratinocyte model and MEFs demonstrated that NDs significantly reduced the UVB intensity to a safe range, highlighting the potential protective effects of NDs against the development of skin cancer.

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