Editorial

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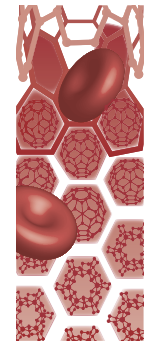

Landing a lethal blow on bacterial infections: an emerging advance of nanodots for wound healing acceleration

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Wound infection is a localized pathological defect in which microorganisms invade skin lesions and decelerate the healing process by triggering inflammation and preventing reepithelization via rapid colonization or biofilm formation [1]. Studies have demonstrated that microorganisms can be seen to accumulate in 6% of acute wounds and over 90% of chronic ones, denoting a therapeutic challenge due to their resistance to conventional antibiotics [2]. This demonstrates that bacterial infections in wounds can become more severe over time and increase healthcare costs when left untreated. To circumvent the above-mentioned challenge, various nanotechnological advancements are under investigation to develop economically viable, multifunctional, potent and ecofriendly therapeutics with novel mechanisms of action as new sources of antibacterial agent [3]. Cutaneous wound healing usually involves topical delivery, which makes nanotherapeutics relatively easy to formulate, generally as wound dressings.

Nanomaterials employed for this purpose either exhibit intrinsic properties beneficial for wound treatment and/or can be used as delivery vehicles for therapeutic agents [3]. In this editorial, we aim to provide a balanced discussion on the fundamental aspects of antimicrobial nanodots for wound repair. We focus on the potential of organic and inorganic nanodots/quantum dots (QDs), tiny zero-dimensional particles of small size (usually smaller than 50 nm), for the promotion of wound healing through inhibition of bacterial growth in the skin lesion or overcoming developed antibiotic resistance in the local infections. Current challenges and possible future research directions are also presented.

Organic nanodots for infected wound repair

Antibiotics have been used imprudently over the last few decades, leading to a dramatic increase in the prevalence of antibiotic resistance and subsequent elevation of life-threatening infections through open wounds. Therefore, synthesis of advanced antibacterial substances, such as organic nanodots, has recently gained increased attention because of its potential to be game-changing in the near future.

Organic carbon and graphene nanodots can be prepared by simple, affordable and tailorable approaches and stimulate proper healing process without scar formation. This is mainly through the maintenance of the moisture in wound area and killing bacteria through free radical formation or mediating photothermal inhibition of bacterial growth [4–7]. These nanomaterials are currently under investigation for combined therapy of wound infection to ensure proper killing effect on bacteria and inhibition of bacterial regrowth due to the possible resistance. A wound bandage of cotton fabrics containing H_2O_2 and graphene QDs was reported in a pioneering work for synergistic and rapid wound closure [4] through peroxidase-like activity of graphene QDs, which can catalyze the decomposition of H_2O_2 and generate free radicals of •OH with higher antibacterial activity and lower toxicity as compared with the or H_2O_2. Hybrids of graphene QDs–silver (Ag) nanoparticles have also been reported for combined H_2O_2 decomposition and near infrared-irradiated phototherapy of wound infection [7]. Nevertheless, more studies

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describing the potential of nanodot-mediated near infared phototherapy of infected wounds are yet to be conducted, making a full assessment of this modality and its plausible mechanism currently problematic. Therefore, more investigation in this area is needed; otherwise, phototherapy will remain on the fringes of mainstream infected wound management.

One of the ground-breaking examples in this field is the development of an injectable hydrogel composed of folic acid, polydopamine (PDA), Zn^{2+} and carbon QD-decorated ZnO (C/ZnO) nanoparticles [5]. Since wounds are often irregular in shape, injectable hydrogels are suitable platforms to match the wound shapes and allow complete coverage of damaged skin by a 3D structure that resembles its soft extracellular matrix (ECM). In this example, carboxyl groups of folic acid and catechol in PDA could rapidly chelate Zn^{2+} and form a hydrogel through metal–ligand coordination. In addition, PDA could coat the surface of C/ZnO and contribute to the uniform distribution of nanodots within the hydrogel network. C/ZnO nanoparticles were able to produce reactive oxygen species (ROS) upon excitation with visible or infrared light, and subsequently kill bacteria in a very short time by oxidative effect on the phospholipids, proteins and nucleic acids of the pathogens [6]. In addition to excellent photothermal performance of carbon QDs and PDA for short-time antibacterial effect, sustained release of zinc ions resulted in long-lasting prevention of bacterial infection, as well as fibroblast regrowth. This study suggested that preparing ECM-mimicking multifunctional 3D networks composed of organic nanodots can bring a new perspective on the reconstruction of bacteria-infected wounds.

Multidrug-resistant (MDR) is currently a major challenge in infected bacterial wounds. Therefore, applying nanodots to fight against wounds infected with MDR bacteria can have a substantial impact. As an example, spermidine modified carbon QDs (size $\approx$ 4.6 nm) were synthesized and tested against MDR bacteria, *methicillin*-resistant *S. aureus* (MRSA) [8]. The minimal inhibitory concentration value of spermidine–carbon QDs was 25,000-fold lower than that of spermidine alone. In fact, spermidine–carbon QDs can damage bacterial membrane through multivalent hydrogen bonding and electrostatic interaction of spermidine–carbon QDs to the proteins, porins and/or peptidoglycans on cell membranes, resulting in the inhibition of membrane synthesis through the disruption of the permeability and respiratory function of the cell membrane. In addition, MRSA-infected wounds demonstrated faster healing through efficient epithelialization and collagen formation when spermidine modified carbon QDs were used as a dressing material. Similarly, nitrogen-doped carbon QDs with the size range of 2–5 nm have demonstrated the same therapeutic effect as vancomycin on wounds infected with MRSA [9]. These nanodots have the features of negligible toxicity *in vivo*, easy synthesis and low production cost.

Inorganic nanodots for infected wound repair

Inorganic materials arise as effective antimicrobial substitute, especially when formulated as ultra-small particles, due to high surface-to-volume ratio that allows a large and efficient contact area with bacteria. This leads to destructive effect on the normal function of bacterial membrane, infliction of DNA damage and generation of ROS, all contributing to reduce or break resistance mechanisms of bacteria [10]. Furthermore, association or functionalization of inorganic nanodots with different therapeutic moieties minimizes side effects and increases therapeutic effect through a synergistic antimicrobial effect in the wound area [11].

Ag nanoparticles possess versatile antibacterial properties, particularly in the form of nanodots, owing to abundant Ag^{+} ions on their surface and high capability to generate ROS [12]. As a wound dressing agent, Acticoat® is the most successful example of commercially available Ag nanoparticle-based products in the market. Nevertheless, further attempts on bench to bedside movement of advanced formulations are proceeding to overcome associated toxicity and increase therapeutic efficacy by Ag nanodots. In one recent example of a novel wound therapy using Ag nanodots, the Ag nanoparticles (21–70 nm) embedded in a matrix of plant-based cellulose nanocrystals were prepared both in ointments and strips [13]. Topical application of the wound dressing ointment exerted early neovascularization, enhanced collagen deposition and faster reepithelialization, ultimately leading to better and faster healing of acute wounds as compared with the strips, owing possibly to higher gaseous and fluid exchange in ointment treated wounds. These therapeutic actions are due to the synergistic action of the cellulose nanocrystal in high-absorption capacity of wound exudates with the antimicrobial function of Ag nanodots through enhanced adherence and penetration to the bacterial membrane and plausible interaction with intracellular proteins [13].

Gold (Au) nanostructures have also been emerging as promising candidates for wound healing owing to their intrinsic biocompatibility, excellent multifunctionality, as well as antioxidant and optical properties [14,15]. A study on 3 nm-Au nanodots, functionalized with the antimicrobial surfactin peptide showed potent antimicrobial activity with 80-fold lower minimal inhibitory concentration values compared with surfactin alone. High-surface

area, and thus, the large amount of surfactin ligands on the surface of Au nanoparticles could increase the efficiency of interaction with the bacterial walls and their consequent disruption, resulting in faster healing and better epithelialization of wounds infected with MRSA [16]. In another study, 5 nm Au nanoparticles coated with egg-white hydrolysate and 2-mercapto-1-methylimidazole were developed, presenting prominent antibacterial activity and enhanced healing in infected wounds through the same mechanism explained for surfactin coated Au nanodots [17]. Photobiomodulation therapy is another strategy applied in wound healing through Au QDs, using low-level light to stimulate cellular and metabolic functions. Spherical 22 nm Au nanoparticles have been reported for photobiomodulation therapy of wounds through the shortening of the inflammatory phase, induction of angiogenesis and increasing collagen production in a rat model [18].

Copper (Cu) nanodots are another promising platform proposed for the treatment of nonhealing infected wounds because of the therapeutic activities of Cu ions, their catalytic activity on the upregulation of growth factors' genes and proteins (e.g., angiogenic VEGFs), and stabilizing effect on hypoxia-inducible factors [19]. Cu can also stabilize ECM proteins, while promoting fibrinogen and collagen formation during the healing process [20]. In addition, Cu based nanodots demonstrate remotely controllable photothermal killing effect on bacteria by converting absorbed light energy into heat [21]. Recently, albumin-stabilized 6 nm copper sulfide (CuS) nanodots demonstrated excellent antibacterial effect and accelerated wound healing compared with bigger CuS nanoparticles (20 nm) as a result of the photothermal effects of CuS nanodots, release of Cu^{2+}, and photodynamic generation of ROS. CuS nanodots, under laser irradiation, not only promoted the healing process but also imparted antimicrobial activity against MDR bacterial infection without damaging healthy dermis of infected wound model in diabetic mice [22].

Conclusion & future perspective

In addition to the given examples, other types of inorganic nanodots, such as bismuth, MXene, black phosphorous, titanium dioxide, terbium hydroxide, silicon, iron oxide and zinc oxide have emerged as promising candidates for the healing of infected wounds [20]. Nevertheless, in our view, it is still quite early to fully evaluate the potential of nanodots for real-life therapy of infected wounds based on the present research findings. This is mainly due to the lack of established standards and protocols, which may pave the way to speed-up the clinical transition of developed nanodots for wound healing. For example, the way researchers grow their bacteria is often vague and the 'silent' power of possible cross-contamination of infected wounds in animal models is commonly ignored. Furthermore, it is also worth emphasizing that due to the novelty of the topic, the role of biotic factors, such as species/strains tested and physiological variations, are poorly understood. Therefore, we recommend stricter standards are urgently needed to achieve more reliable and comparable data in various studies.

Since the safety investigations of nanodots started earlier than their antimicrobial and wound healing effect, there is currently a plethora of findings on the toxicity evaluation of both organic and inorganic nanodots. Despite present controversies, there is an agreement on high biocompatibility in therapeutic concentrations for most of nanodots and the dependency of toxicity on the size and chemical nature of nanodots. Moreover, there is not yet any comprehensive attempt dealing with the surface functionalization of novel nanodots with targeting ligands for efficient attack to bacteria in the wound, combining conventional antibiotics with emerging potential of nanodots in phototherapy, or developing dual therapy of infected wounds by phototherapeutic nanodots incorporated within angiogenic or immunoregenerative hydrogels. All these would be promising research directions for the future.

Overall, we hope the roadmap provided here will encourage scientists to tackle current bottlenecks for the exploitation and commercialization of antimicrobial nanodots in actual clinical practice of wound healing and cure or prevent tens of thousands of wound infections each year.

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