Opportunities and challenges for antimicrobial nanostructured materials in the management of skin infection

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Abstract
Worldwide the management of skin and soft tissue infections represents a burden for health care systems that demands additional scientific efforts. Despite the combined advances in modern medicine from different disciplines, chronic non-healing topical wounds, still represent an unresolved clinical challenge. Nanotechnology has contributed significantly to the development of advanced therapeutic and diagnostic approaches in wound care. In this perspective, recommendations on the design of nano-based approaches for the management of infected non-healing chronic wounds are suggested. Preclinical results have demonstrated that nanostructured antimicrobial-loaded dressings and hydrogels can reduce pathogenic bioburden and restore the wound physiologic balance. Future clinical trials that ensure meaningful results are recommended.

Perspective
Skin microbiota is composed of commensal bacteria, fungi and viruses. Some of those skin microorganisms prevent their host from pathogen colonization, thanks to the secretion of different antimicrobial peptides such as cathelicidins, histatins, and β-defensins [1]. They also boost the host immune system, regulate uncontrolled overgrowth of opportunistic commensal microorganisms, modulate the skin inflammatory response, and participate in the degradation of skin proteins and triglycerides in sebum, being some of the degradation byproducts beneficial or protective for the skin itself (i.e., providing moisturization, free radical protection, lowering the pH, etc.) [2].
Metagenomic sequencing has overcome the limited identification that culture based methods provide for some commensal bacteria present in our skin and, nowadays, is commonly accepted that four phyla (*Actinobacteria, Proteobacteria, Bacteriodetes and Firmicutes*) and specifically three genera (*Staphylococcus, Corynebacterium and Propionibacterium*) comprise ~60% of the species identified [3]. Despite this general identification, the bacteria present show inter- and intrapersonal variations depending on the anatomical area they colonize, which is mainly dependent on the moist, dry or sebaceous local environments, the ethnicity, sex, age, hygiene habits, the geographical location of the host, etc. [4,5].

This varied commensal skin microbiota and also exogenous pathogenic species can compromise the skin when a laceration, puncture or cut occurs generating an infected wound. As mentioned before, commensal bacteria byproducts induce the host´s immune system response but also, when colonizing, they are also able to attenuate this response to promote their own growth. Wound bacterial contamination is usually resolved by debridement, cleansing and by using topical 2antiseptics, but if bacterial colonization occurs, wound healing is delayed. Different pre-existing conditions in the host such as immunodeficiency disorders, smoking, drugs or alcohol use, diabetes, poor nutrition, poor circulation, uncontrolled edema, etc. could further delay healing and the infection can become chronic [6]. Chronic non-healing wounds normally present a polymicrobial pathogenic microbiota, high levels of proteases, unresolved inflammation, hypoxia, reduced cell proliferation, slough and even necrotic tissue [7,8]. Not only chronicity but also biofilm formation further delays wound healing and hinders treatment. A recent systematic review and meta-analysis revealed the presence of bacterial biofilm in almost 80% of the human chronic non-healing wounds [9]. Bacterial biofilms are polymicrobial in nature and show increased tolerance to antibiotic treatments [10]. Chronic non-healings wounds (e.g., diabetic foot ulcers, pressure ulcers, venous and arterial leg ulcers, etc.) remain as a serious burden for healthcare systems along with the additional concerns of the global prevalence of obesity and diabetes (i.e., pathologies highly susceptible to develop chronic wounds) and increased life expectancy. According to UN, by 2050, the elderly are expected to account for 35 % of the total population in Europe [11] and in the US, government estimates predict 77 million elderly in the US by 2060 [12]. These numbers raise serious concerns considering that this population group the one with the highest risk of developing topical chronic wounds due to other potential underlying chronic conditions as well as due to age-associated changes in their skin such as reduced inflammatory response, lower levels of extracellular matrix and growth factors, delayed epithelialization, deficient cellular recruitment and reduced angiogenic activity [13].

The physiological healing process after wounding starts with blood coagulation and hemostasis to stop bleeding, then the activation of the immune system produces an acute inflammatory response, afterwards, cell proliferation, migration and regeneration to the wound bed take place while connective tissue remodeling restores the damaged tissue. But no always the physiological process is efficient and delayed wound closure or even chronicity occur.

Acute and chronic wounds are managed by personnel from different clinical disciplines depending on the wound type (burns, diabetic foot ulcers, osteomyelitis, surgical site wounds, etc.) being always recommended to treat also potential underlying conditions which might interfere with the physiological healing process (i.e., hemostasis, inflammation, granulation, and maturation). However, despite the combined advances in modern medicine from different disciplines, chronic non-healing wounds still represent an unresolved clinical challenge.

Nanotechnology has contributed significantly to the development of therapeutic and diagnostic systems used in the management of acute and chronic infected wounds. Nanostructured advanced wound dressings loaded with active principles such as antiseptics, antibiotics, growth factors, essential oils, anti-inflammatory drugs, local anesthetics, reporter molecules, etc. have been fabricated (mainly as electrospun dressings or as hydrogels) showing demonstrated benefits [14]. Most of the dressings

are fabricated using synthetic polymers (e.g., cellulose derivatives, rayon, polyesters, etc.) but also biopolymers such as alginate, bacterial cellulose [15], chitosan [16] or bio-aerogels [17] are used taking advantage of their superior biocompatibility. Some of those dressings are able to identify the presence of pathogenic infection. For instance, reporter wound dressings composed of lipidic nanovesicles that release a fluorescent dye when in contact with pathogenic wound biofilms have been reported using an *ex vivo* porcine skin model of infected burn wounds [18]. Some other reporter nanostructured dressings are also able to monitor temperature changes, associated to the presence of inflammation or infection, having simultaneously selective antifouling and biocidal activity [19].

Other nanostructured dressings provide with a local release of active principles to not only speed up the regenerative process but also to offer mechanical support, homeostasis, vascularization, wound exudates absorption, pain relief, and gas exchange while avoiding wound maceration. Advanced antibiotic-loaded nanostructured wound dressings have shown successful results in preclinical models even when benchmarked against commercial dressings [20, 21]. In their colloidal form, and as hydrogels, nanoparticulate systems, used as carriers of active principles, have also proven beneficial *in vitro* and in preclinical models of acute and chronic infected wounds including (Figure 1): i) superior antimicrobial effect compared to the equivalent dose of the free antimicrobial compound [22]; ii) diminished chances of developing bacterial resistance by promoting the use of antibiotic-free treatments including the use of metal nanoparticles [23] or by using photodynamic therapy based on nanoparticulated photosensitizers [24], by their combinations [25] or by using photothermally activated nanoparticles [26]. In that regard, it is important to point out that metal nanoparticles display multiple mechanisms of antimicrobial action and consequently the chances of genetic adaptation of the bacteria against multiple arrests diminish compared to antibiotics which normally are target specific; however, bacterial resistance to metal nanoparticles has also been demonstrated [27] and warrants further studies; iii) metal nanoparticles can potentiate the effect of conventional antibiotics [28] or combined synergetic effects of different antimicrobials (i.e., antimicrobial peptides and metal nanoparticles) are possible within the same nanoparticulated carrier [29]; iv) a prolonged duration of the antimicrobial action using sustained or controlled delivery systems are also well documented [30]; v) induced angiogenesis, collagen deposition, and re-epithelialization using metal nanoparticles embedded in hydrogels have been reported [31]. In this case, copper metal organic framework nanoparticles embedded within an antioxidant citrate-based hydrogel showed reduced copper ion toxicity while wound closure rates were accelerated in an splinted excisional dermal wound diabetic mouse model; vi) antibiotic-loaded nanoparticles have also shown the ability to change the bacterial phenotype from resistant to susceptible [32]; vii) nanoparticles have shown improved solubility for highly hydrophobic poorly available antimicrobial drugs [33]; viii) simultaneous loading of different drugs and procollagen components within the same nanoparticulated carrier is also possible [34]; ix) improved antimicrobial selectivity by using antibody-functionalized nanoparticles is also feasible [35]; x) improved biofilm permeability and eradication compared to standard antibiotic treatments is also demonstrated [36]; xi) infection reporting ability by using fluorescent imaging of infected wounds is documented, for instance, in response to the presence of specific bacteria using toxins and enzymes secreted by bacteria to trigger the lysis of

nanoparticles containing fluorescent dyes [37], xii) nanoparticles can increase the stability of enzymatically sensitive antimicrobial peptides [38], xiii) multifunctionality is also demonstrated when using polydopamine coated carbon nanotubes combined with gelatin-grafted-dopamine to render antibacterial, adhesive, antioxidant and conductive action [39], etc.

Several other advanced nanostructured surfaces fabricated using bio-based materials such as those composed of genetically engineered protein polymers (recombinant protein polymers) [40], or using bio-inspired surfaces [41] or incorporating antimicrobial biologics (i.e., cationic proteins and peptides) [42] represent an opportunity to push forward the development of advanced antimicrobial dressings. A summary of representative effective nano-based approaches used in the *in vivo* management of acute and chronic infected wounds is shown in Table 1.

Besides all those benefits of nanostructured wound dressings and nanoparticulated colloidal systems have to offer, still, infected non-healing wounds remain as an unresolved clinical issue. This perspective is intended to provide with a rationale understanding on the clinical problem to help when proposing a new nano-based antimicrobial solution in the management of chronic non-healing wounds. First, it is important to highlight that there is limited evidence of the demonstrated benefits of advanced wound dressings in the management chronic wounds. Probably, the polymicrobial and dynamic nature of bacterial biofilms present on those chronic non-healing wounds as well as the intra e interpersonal variations observed in the skin microbiome are responsible for the difficulties in the standardization of protocols. Clinical trials that ensure meaningful results are recommended considering the stratification of specific non-healing infected wounds sharing common characteristics and with the final goal of understanding the mechanisms responsible for the chronicity. Recommendations in the development of nano-based approaches for the management of infected non-healing chronic wounds are directed towards:

i) The understanding of the dynamic and complex aerobic and anaerobic polymicrobial skin microbiome and its cross talk with the host immune system. More fundamental research is needed in this field.

ii) The development of different strategies to reverse the antibiotic resistant bacterial phenotype into a sensible one to be able to re-use already existing antimicrobials.

iii) The clear differentiation between commensal and pathogenic bacteria to be able to decide the antimicrobial treatment and restore the balance of the skin microbiome.

iv) The development of faster diagnostic systems to identify the pathogenic microorganism or microorganisms responsible for the delayed healing. Clinical readouts of efficacy rely on indirect or anatomic measurements, which occur over prolonged time scales. Those should be sped up considering the dynamic microbiota present on the wound bed.

v) The development of strategies to revert the bacterial sessile phenotype to an antibiotic sensitive planktonic state.

vi) The development of different strategies to treat chronic non healing wounds while simultaneously boost the potency of a robust immune response.

vii) The development of nanoparticulate systems containing active principles should demonstrate exponential benefits over the administration of the

equivalent dose of the free active principles to make a difference. The benefit should be synergetic instead of merely additive.

viii) The development of reporter therapeutic nanosystems which in real time send back information about the outcome of the proposed antimicrobial regime could revolutionize the field in the future. Such technology can potentially advance precision medicine by helping identify the antibiotic resistant and non-antibiotic resistant bacteria early on while minimizing adverse effects and facilitate a rapid change in the antimicrobial regime.

ix) The studies should include necessary controls, such as the comparative evaluation of the antimicrobial alone (i.e., without the corresponding nanostructured carrier). Most of the studies do not show a complete bacterial eradication in the life span analyzed which, would result in a potential bacterial re-growth; therefore, long term studies are needed. Some others, which use pathological animal models (e.g., diabetic murine models), do not previously corroborate the intended chronicity of the wound despite of the fact that it is known that those animal models show wound healing impairment. The lack of standardization in the protocols followed also hinders the comparison between independent results.

All in all, nanotechnology have demonstrated that combination wound dressings containing active principles and the combination of different therapeutic approaches (e.g., photothermal therapy and antimicrobials) are, in fact, successful therapeutic methods for the management of acute and chronic non-healing wounds. Most of the scientific papers include an *in vitro* validation of the proposed nanostructured dressings/hydrogels but they *in vivo* validation in preclinical models is not widely reported and, more importantly, in most of the studies a comparative analysis with commercially available conventional or advanced dressings is not included. Currently, effective results in preclinical models have demonstrated how combination dressings and hydrogels can reduce pathogenic bioburden and restore the wound physiologic balance. Therefore, a large body of scientific evidence has been presented to support the use of those nanostructured dressings and hydrogels to grant future clinical trials. Reporter nanostructured wound dressings and hydrogels can also help to identify the pathogenic bacterial phenotype present in the wound bed and, consequently, select the most appropriate antimicrobial regime reducing the chances to develop drug resistance.

Conflict of interest
The author declares no conflicts of interest to disclose.

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Figure 1. Reported strategies and derived benefits of nanostructured systems in the management of infected topical wounds.
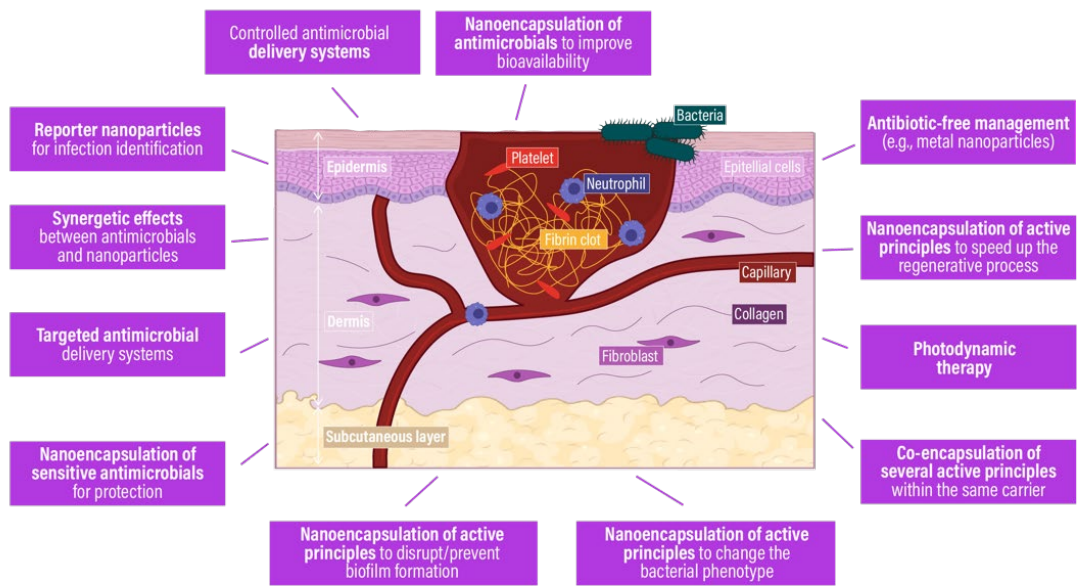
Table 1. Representative nano-based approaches used in the *in vivo* management of acute and chronic infected wounds showing a superior antimicrobial effect compared to the equivalent dose of the free antimicrobial compound or to placebos or untreated controls

Nanosystem	Antimicrobial compound	Pathological model	Targeted bacteria	Control	Antimicrobial loaded in the nanosystem	Observations	Reference
Colloid based on Ag nanoparticles polydopamine (PDP) and Prussian blue (PB)	Ag + photothermal therapy	Infected full-thickness cutaneous wound model in diabetic mice	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Only PB, PB/PDP, and untreated control	Single mode therapy produced only a limited effect compared to untreated controls. At day 12 < 1log (CFU/mL) reduction was observed for the controls.	The combination of photothermal therapy and ionic silver release. At day 12 a 2 log (CFU/mL) reduction was observed for the combined nanocomposite	[25]
Colloid based on ciprofloxacin (CIP)-loaded ceria decorated block copolymer poly(ε-caprolactone)-block-poly (glutamic acid) vesicles	Ciprofloxacin	Infected full-thickness cutaneous wound model in diabetic mice	*S. Aureus*	PBS, free ciprofloxacin, ciprofloxacin loaded vesicles as controls	At day 5 free ciprofloxacin and ciprofloxacin-loaded vesicles reduced the bacterial burden in 4 and 6 log (CFU/mL), respectively	Ciprofloxacin-loaded vesicles containing ceria almost no bacterial colonies were found and the wound section was completely closed and new epidermis was formed	[28]
Colloid based on surfacting-functionalized gold nanoclusters	Surfactin (an antimicrobial peptide)	Infected full-thickness wounds on Sprague Dawley rats	MRSA	Free surfactin and untreated control	At day 6 post-infection free surfactin treated group showed a higher number of bacterial cell colonies than the colloid based on surfacting functionalized gold nanoclusters	At day 12, the colloid treated group showed a complete re-epithelialization, thicker granulation tissue and more extensive development of hair follicles when compared with that of the two control groups	[29]
Hydrogel glass nanocomposite	Curcumin	Infected murine burn model	MRSA and *Pseudomonas aeruginosa*	Coconut oil, empty nanocomposite, free curcumin	≈2 log reduction (CFUs/gram of tissue)	Only 1 log reduction (CFUs/gram of tissue) for free curcumin	[33]
Hydrogel based on a pH-responsive antimicrobial peptide and a NIR-activatable cyanine dye containing proline to promote extracellular matrix formation	pH-responsive antimicrobial peptide + photothermal therapy	Infected full-thickness skin defect wound on type II diabetic mice	*Escherichia coli* or MRSA	Non loaded hydrogel, cyanine dye, proline w/o. NIR irradiation	Photothermal damage alone and the peptide alone were unable to completely eliminate the experimental bacterial biofilm	Wound healing in the group treated with the hydrogel containing the cyanine dye and the proline activated with light was found to be much faster than the other control groups	[34]
NB-201 nanoemulsion	Benzalkonium chloride	Infected skin split-thickness injury porcine model and murine skin abrasion model	MRSA	Placebo and PBS	5 log reduction in colony-forming units (CFUs) per gram of tissue	Reduced necrosis, inflammation and proinflammatory cytokines in treated infected wounds	[43]
Hydrogel based on gelatin-grafted-dopamine, chitosan and polydopamine-coated carbon nanotubes	Doxycycline	Infected full-thickness mouse skin defect wound	*S. aureus* and *E. coli*	PBS and wounds dressed with Tegaderm™, and hydrogel w/o. antibiotic	Hydrogels w. and w/o the antibiotic showed faster wound closure than control	Significantly better wound closure occurred in the antibiotic-loaded hydrogel than in the controls	[44]

Electrospun polycaprolactone (PCL) dressing containing APA-coated gold nanoparticles	Aminopenicillanic acid, APA	Infected full-thickness wounds on Wistar rats	*E. coli*, MDR *E. coli*, *P. aeruginosa* and MDR *P. aeruginosa*	PCL/gelatin nanofibrous scaffolds, and gauze	MDR *E. coli* and MDR *P. aeruginosa* infected wounds: At day 14 gauze and PCL gelatin reduced in 2 and 1 log, respectively the CFUs per wound	MDR *E. coli* and MDR *P. aeruginosa* infected wounds: At day 14, APA-containing electrospun dressing reduced 5 log the CFUs per wound in both cases	[45]
Hydrogel based on CIP-loaded polydopamine (PDA) nanoparticles (NPs) and glycol chitosan	Ciprofloxacin + photothermal therapy	Infected full-thickness mouse skin defect wound	*S. Aureus*	Free ciprofloxacin (CIP), untreated, PDA treated, NIR light, CIP-free hydrogel used as controls	No bacterial load reduction was observed for any of the controls.	A 2 log reduction (CFUs/gram of tissue) was observed in the combined effect of CIP and the NIR-activated photothermal action of the PDA light absorbing nanoparticles	[46]
Poly(ethylene oxide) and poly(D,L-lactide) (50:50) blend electrospun nanofibers	Nisin	Infected full-thickness excisional wounds in mice	Bioluminescent *S. aureus* Xen 36	Non-loaded nanofibrous wound dressings	At day 7 from the initial inoculum (10^8 CFU/mL) only 1 log reduction was observed for the non-loaded nanofibrous wound dressings	A 7 log reduction was observed for the nisin-loaded nanofibrous wound dressings	[47]
pH/redox dual-responsive hydrogel based on ε-polylysine-coated MnO_2 nanosheets and insulin-loaded self-assembled aldehyde Pluronic F127 micelles	ε-Polylysine	Infected full-thickness cutaneous wound model in diabetic mice	MRSA	Ampicillin, hydrogel with and w/o. insulin and self-assembled aldehyde Pluronic F127 micelles	At day 14, <1 log reduction (CFU/mL) for the controls including ampicillin (990 µl ampicillin solution, 2 µg/ml)	MnO_2 nanosheets are scavengers of ROS (H_2O_2) catalyzing tits decomposition to O_2. At day 14, both hydrogel (with and w/o. insulin) groups showed the most effective bactericidal action (almost 7 log reduction CFU/mL) compared to other control groups	[48]
Thymol-loaded PCL electrospun dressings	Thymol	Infected full-thickness excisional wounds in mice	*S. Aureus*	PCL dressings, free thymol, chlorhexidine and untreated control	One day post-infection, a few colonies were detected in wounds treated thymol-loaded PCL dressings mats while a high number of colonies appeared in wounds treated with the equivalent dose of free thymol	Thymol-loaded PCL dressings and chlorhexidine groups showed at least 2 log reduction (copies/mL) in the number of bacterial strain copies	[49]
Colloid based on NO-releasing poly(lactic-co-glycolic acid)-polyethylenimine NPs	Nitric oxide	Infected full-thickness cutaneous wound model in mice	MRSA	Untreated mice were used as control.	No bacterial cell counts were reported in the in vivo study	A faster wound healing observed with the nanobased treatment was attributed to the bactericidal effect as well as wound healing activity of the NO	[50]
Colloid based on mesoporous silica nanoparticles containing amoxicillin and decorated with lysozyme and hyaluronic acid	Amoxicillin and lysozyme	An infected mouse wound model	*S. Aureus*	Untreated and the combination of amoxicillin and lysozyme	The quantitative culture of excised tissues revealed only 1 log (CFU/mL) reduction for the group treated with the combination of amoxicillin and lysozyme compared to the untreated control	The quantitative culture of excised tissues revealed 3 log (CFU/mL) reduction for the group treated with the nanobased treatment compared to the untreated control	[51]
Colloid based on copper containing MOFs	Copper + photothermal therapy	Infected excisional cut wounds in rats	*S. Aureus*	Untreated and conventionally treated with a commercial dressing	No bacterial cell counts were reported in the in vivo study	The photothermal and photocatalytic activity of the Cu-doped MOF produced that 14 days after treatment the wounds in the treated group were significantly healed, while those in the control group and in the	[52]

						group treated with a commercial dressing remained	
A thermoresponsive in-situ forming sprayable hydrogel containing Ag-nanoparticles decorating reduced graphene oxide nanosheets	Ag	Two full-thickness round skin wounds in rats	MRSA	Untreated control	No bacterial cell counts were reported in the in vivo study	99.85% of the infected wound areas were healed after 12 days for the treated group whereas only 54% of wound areas were closed in the untreated group at the same time	[53]
Niacin MOFs encapsulated microcapsules with alginate shells and copper-/zinc-niacin framework cores	Niacin-copper-zinc	Infected full-thickness skin defect model in mice	E. Coli	PBS, alginate microcapsules (ALGM), Cu-MOFs, niacin Cu-MOF-laden microcapsules (M-Cu-MOFs), and niacin Cu-MOF- and Zn-MOF-laden microcapsules (M-Cu&Zn-MOFs)	No bacterial cell counts were reported in the in vivo study	Wounds treated with M-Cu&Zn-MOFs experienced a significantly shorter healing times and better physical condition than other groups	[54]
Hydrogel based on sodium hyaluronate containing gold-silver nanoshells (AgAuNSs) for a theragnostic approach	Ag + photothermal therapy	Infected full-thickness cutaneous wound model in mice	MRSA	Untreated control Ag + laser, AuAgNSs, and AuAgNSs + laser	No bacterial cell counts were reported in the in vivo study. Qualitatively Gram staining on histological sections revealed much fewer cocci-shaped MRSA in the treatment group (AuAgNSs + laser)	Combined effect of ionic silver release and photothermal antimicrobial therapy. Compared to the controls the wound area shrank by 50% 6 days after AuAgNSs + laser treatment, and almost fully healed at day 8	[55]