SOMATO PUBLICATIONS ISSN: XXXX-XXXX

Annals of Nanomedicine and Nanotechnology

Review Article

Emerging Role of nanotoxicological assessment in the Diagnosis of chronic wounds

Xu L[1]*, Chen F[2] and Zhou J[3]

[1]Department of Nanomedicine, Nile Institute for Advanced Nanotechnology, China

[2]Department of Nanomaterials Science, Amur Center for Nanomaterials Science, Vietnam

[3]Department of Nanomaterials Science, Euphrates Academy of Nanomedicine, Thailand

*Address for Correspondence: Li Xu, Department of Nanomedicine, Nile Institute for Advanced Nanotechnology, China; E-mail: li.xu@nile.edu

Received: 16 November 2020; Accepted: 12 February 2021; Published: 10 May 2021.

Citation of this article: Xu L, Chen F, Zhou J (2021) Emerging Role of nanotoxicological assessment in the Diagnosis of chronic wounds. Ann Nanomed Nanotechnol, 3(1): 01-06. DOI: 10.5281/zenodo.22811810

Copyright: © 2021 Li Xu, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Nanomaterials are embedded in chronic wound care both as antimicrobial therapeutics and as sensing elements for wound-fluid biomarkers, so nanotoxicological assessment has direct diagnostic relevance for how these materials behave against a compromised wound bed [5,6].

Objective: This review synthesizes the nanotoxicological assessment literature as it applies to chronic wound diagnosis: assay frameworks, wound-fluid biomarkers of non-healing, and diagnostic platforms combining the two.

Methods: We conducted a narrative synthesis of nanotoxicology methodology papers, wound-biology biomarker studies, and nanomedicine reports on nanomaterial-integrated wound diagnostics.

Findings: Silver nanoparticle toxicity tracks ion-release kinetics rather than nominal dose [9,10]; keratinocyte viability falls sharply beyond a narrow therapeutic window [12]; wound-fluid MMP-9 activity and surface pH track clinical severity [19,21]; commercial silver dressings vary widely in disclosed toxicity [13,14].

Conclusions: Screening under wound-mimetic conditions, not generic cytotoxicity testing, should be integral to validating nanomaterial-based wound diagnostics, since the release kinetics enabling diagnostic and antimicrobial function also set the threshold for tissue injury [17,8].

Keywords: nanotoxicology; chronic wound diagnosis; wound biomarkers; silver nanoparticles; nanomedicine

1. Introduction

A chronic wound is usually defined by what it fails to do: it does not close within the four to six weeks a normally healing skin injury needs, and it does not move cleanly through the inflammatory, proliferative and remodeling phases ordinary repair follows. Venous leg ulcers, diabetic foot ulcers and pressure injuries account for the great majority of these wounds, and all three share a wound bed locked in a self-sustaining inflammatory loop, rich in proteases, reactive oxygen species and senescent cells that keep signaling damage long after the original insult has resolved [1].

Nanotechnology entered wound care from two directions that have only recently begun to intersect. The first is therapeutic: silver, zinc oxide and other nanoparticle formulations have been incorporated into dressings and gels for their antimicrobial effects, exploiting size-dependent reactivity that emerges once particles are pushed below roughly 100 nm [2,3]. The second is diagnostic: nanoscale sensing elements and nanoparticle-based assays have been proposed to read the biochemical state of a wound bed, chiefly its pH, protease activity and oxidative burden, without waiting for a swab culture or biopsy [4]. These two threads are not separable in practice, because a nanomaterial placed against an open, exuding, often infected wound bed is simultaneously a therapeutic agent and a toxicological exposure, in tissue that is already vulnerable.

That overlap is what motivates nanotoxicological assessment as a diagnostic, rather than purely a safety, discipline. The founding literature established that particle behavior at this scale, dissolution rate, surface reactivity and cellular uptake, cannot be predicted from bulk-material chemistry alone [5,6]. Applied to a wound setting, a nanoparticle dressing's local toxicity profile is itself diagnostic information: how much silver ion releases into wound fluid, and how a compromised wound bed metabolizes a nanomaterial differently from healthy skin, bear on how a clinician should read delayed healing or unexplained tissue damage at the wound margin. This review synthesizes that literature as it applies to chronic wound diagnosis: assay frameworks for wound-relevant nanomaterials, biomarkers of non-healing, and diagnostic technologies combining the two.

2. Methods

This is a narrative synthesis rather than a systematic review, reflecting the still-emerging state of the literature it covers. We drew on peer-reviewed nanotoxicology methodology papers, wound-biology studies characterizing chronic wound fluid biomarkers, and nanomedicine reports describing nanoparticle-based wound diagnostics, identified through core reviews and citation chaining [7]. Papers were included where they reported primary data or consensus methodology on nanomaterial toxicity endpoints relevant to a wound-contact setting, on biomarkers measurable in chronic wound fluid, or on a nanoscale diagnostic platform for wound assessment. Findings are organized thematically: assay frameworks for screening wound-relevant materials, the wound-fluid biomarkers those frameworks interpret, and diagnostic platforms combining the two.

3. Results

Frameworks for nanotoxicological assessment of wound-contact materials

The assay battery that nanotoxicology applies to a wound-relevant nanomaterial is broader than the acute cytotoxicity screen that dominated early nanoparticle safety work. Oberdörster and colleagues, writing the discipline's founding synthesis, argued that particle size, surface area, surface charge and dissolution behavior each independently shape biological response, so no single endpoint captures hazard on its own [5]. Nel and colleagues extended this into a mechanistic framework centered on oxidative stress, showing that many nanomaterials generate reactive oxygen species directly at

© 2021 Somato Publications. All rights reserved.

the particle surface, and that this oxidative potential, rather than bulk chemical toxicity, predicted cellular injury across a wide range of engineered nanomaterials [6]. Krug and Wick's interdisciplinary account reinforced that a wound-relevant screen needs to combine physicochemical characterization, in vitro cellular assays and, where feasible, in vivo confirmation, because any one tier in isolation systematically over- or under-predicts hazard relative to the full battery (Table 1) [8].

Table 1: Tiered nanotoxicological assessment battery proposed for wound-contact nanomaterials, from physicochemical characterization through wound-mimetic confirmation [5,8,17].

Tier	Assessment focus	Representative endpoints	Rationale
Tier I	Physicochemical characterization	Size, surface charge, dissolution/ion-release rate	Determines dose actually delivered to tissue, not nominal mass
Tier II	In vitro cellular assays	Viability, reactive oxygen species, cytokine release in keratinocytes/fibroblasts	Captures oxidative and inflammatory mechanism of injury
Tier III	Wound-mimetic / ex vivo confirmation	Exposure in protease-rich, low-pH-shifted wound fluid or reconstructed skin	Standard culture medium understates wound-bed-specific toxicity

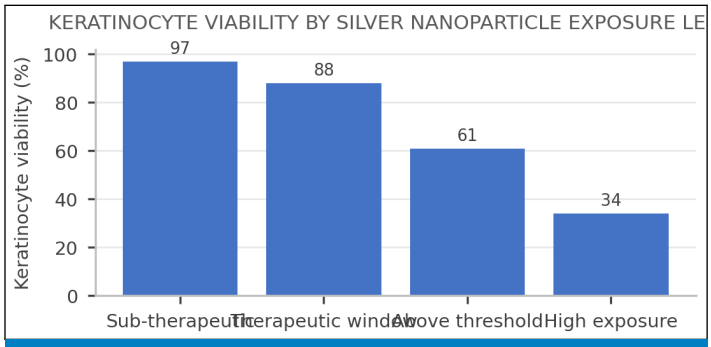

Figure 1: Primary human keratinocyte viability by silver nanoparticle exposure level in wound-healing culture models, showing a narrow window in which antimicrobial concentrations remain compatible with keratinocyte survival before viability falls sharply [12,10].

Applied to silver, the most heavily used antimicrobial nanomaterial in wound dressings, this framework has produced a fairly consistent picture. Wijnhoven and colleagues, reviewing available risk data on nano-silver, concluded that dissolved silver ion release, not particle uptake itself, drives most observed cytotoxicity, and that release rate varies enormously across commercial formulations depending on coating, particle size and ionic strength [9]. Gliga and colleagues confirmed the ion-release mechanism directly in human lung cells, showing cytotoxicity tracked with intracellular silver dissolution rather than nominal dose, and that smaller, faster-dissolving particles were disproportionately more toxic per unit mass [10]. Chaloupka and colleagues flagged that the same release kinetics making silver nanoparticles effective antimicrobials also determine how much ionic silver a wound bed's fibroblasts and keratinocytes are exposed to over wear time [11]. Frankova and colleagues tested this directly on primary fibroblast and keratinocyte cultures, finding a narrow concentration window in which silver nanoparticles suppressed bacterial growth without slowing keratinocyte migration, but outside that window cytotoxic effects appeared before any additional antimicrobial benefit accrued [12] (Figure 1).

The clinical translation of this assay evidence has been uneven. Sim and colleagues' patent-literature review found that commercial antimicrobial silver formulations continue to diversify in size, coating and delivery matrix largely without standardized wound-relevant toxicity disclosure, so two dressings marketed as equivalent can carry substantially different ion-release profiles [13]. Burdusel and colleagues reached a similar conclusion, noting synthesis route alone can shift cytotoxic threshold concentrations by an order of magnitude between otherwise similarly labeled products [14]. Fadeel and Garcia-Bennett's earlier caution, that biomedical nanoparticle safety assessment needed to move past generic hazard screening toward application-specific testing, anticipated exactly this problem: a nanomaterial judged safe in a systemic drug-delivery context is not automatically safe against an open, already-inflamed wound surface [15]. Oberdörster's later synthesis argued that route and duration of exposure, not the material alone, anchor toxicological judgment for a given clinical use [16], and Fadeel and colleagues' review of advanced

© 2021 Somato Publications. All rights reserved.

safety-assessment tools argued that wound-mimetic culture systems are needed because static assays understate how a nanomaterial behaves against a real wound bed [17].

Biomarkers of non-healing in chronic wound fluid

Diagnosing a wound as "stalled" rather than simply slow has historically relied on clinical judgment, informed at best by wound-fluid protease activity measured in a research rather than bedside setting. Trengove and colleagues' foundational analysis established that elevated protease activity, and depleted protease inhibitors, distinguished non-healing chronic wounds from healing acute wounds with reasonable consistency [18]. Rayment and colleagues extended this by quantifying matrix metalloproteinase-9 activity in wound fluid and correlating it with clinical ulcer severity, finding MMP-9 activity tracked ulcer grade closely enough to function as a quantitative biomarker rather than a qualitative signal [19] (Figure 2). McCarty and Percival argued that the protease-to-inhibitor ratio, rather than either measure alone, is the more meaningful marker of a wound locked in a degradative rather than reparative phenotype [20].

Table 2: Candidate wound-fluid biomarkers targeted by nanomaterial-based diagnostic dressings and the sensing strategies proposed for each [21,19,3].

Biomarker	Non-healing signature	Proposed nanoscale sensing strategy
Surface pH	Persistently alkaline	pH-responsive colorimetric or fluorescent nanoparticle indicator embedded in dressing
MMP-9 / protease activity	Elevated, tracks ulcer severity	Protease-cleavable fluorescent nanoprobe reporting local enzymatic activity
Reactive oxygen species	Chronically elevated	Redox-sensitive nanomaterial reporter co-formulated with antimicrobial payload

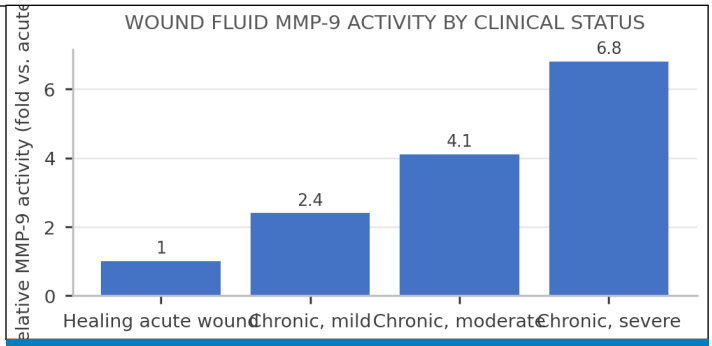

Figure 2: Relative matrix metalloproteinase-9 (MMP-9) activity in wound fluid by clinical wound status, rising with ulcer severity and distinguishing non-healing chronic wounds from healing acute wounds [19,18].

Surface pH is the second biomarker with substantial translational traction. Schneider and colleagues showed healing wounds trend toward a mildly acidic surface pH as they progress, while chronically non-healing wounds tend to remain persistently alkaline, a pattern consistent enough to support pH as a low-cost adjunct to clinical assessment [21]. Because pH-responsive nanomaterials can report this value continuously rather than at the single time point a manual strip provides, pH has become one of the more actively pursued targets for nanomaterial-based wound diagnostics (Table 2). Biofilm burden is a third relevant axis: Percival and colleagues argued biofilm-associated protease and reactive-oxygen output likely explains much of the elevated MMP activity attributed to chronic inflammation, since the two are difficult to fully disentangle in a wound-fluid sample [22]. Falanga's account of impaired healing in the diabetic foot situated protease burden, surface pH and microbial load within a single picture of a hypoxic, senescent-cell-rich microenvironment that continuously regenerates the biochemical signature nanoscale diagnostics are now trying to read [23].

Diagnostic platforms integrating nanomaterials and toxicological screening

The diagnostic platforms that have emerged from this biomarker literature fall into two broad categories. The first embeds a sensing element, a pH-responsive dye, a protease-cleavable fluorescent probe, or a colorimetric

© 2021 Somato Publications. All rights reserved.

nanoparticle aggregate, directly into a dressing so a color or fluorescence change reports the underlying biochemistry. Kalashnikova and colleagues' survey situates several such sensing designs alongside the therapeutic literature, noting the same nanoparticle chemistries used for antimicrobial effect are frequently repurposed, with different surface functionalization, as the reporting element itself [3]. Because the sensing nanomaterial sits in direct, sustained contact with wound tissue exactly as a therapeutic dressing would, it inherits the same wound-specific toxicological questions raised above: a reporter nanoparticle that proves cytotoxic at the concentration needed for a visible signal defeats its own purpose.

The second category treats nanotoxicological screening itself as a diagnostic input, using a wound-fluid sample's response to a defined nanoparticle challenge as an assay of the wound's inflammatory state, following from the mechanistic work on nanomaterial-induced oxidative stress [6]. Boateng and Catanzano argued that dressing platforms are converging toward combined sensing-and-delivery designs, since separating diagnosis from therapy at the wound interface is both clinically inefficient and materially redundant [4]. Mihai and colleagues catalogued formulations pairing antimicrobial payloads with embedded pH- or enzyme-responsive reporting elements, noting regulatory evaluation has lagged laboratory development [24]. Kalantari and colleagues framed this as a promise-versus-pitfall tension: the same silver release kinetics giving a dressing its antimicrobial and colorimetric function are what toxicology studies flag as the driver of keratinocyte injury once concentrations exceed a narrow therapeutic window [25].

4. Discussion

Read across these literatures together, nanomaterial-based wound diagnostics inherit their principal design constraint, ion-release or reactive-oxygen kinetics, from the same physicochemical properties that determine their local toxicity. Donaldson and Poland's broader critique, that much of what looks like nano-specific toxicity is better explained by conventional dose-response and surface-reactivity principles applied at small scale, is a useful corrective: wound-diagnostic nanomaterials likely need a wound-specific application of principles the field already has, not an entirely new paradigm [7]. What is under-addressed is exposure context: a wound bed is not a healthy epidermis, and its already-elevated protease activity and oxidative burden plausibly shift the effective toxic threshold relative to intact-skin data most safety assessments rely on.

A wound-relevant toxicity signature, dose-dependent ion release and cytotoxic threshold measured against primary keratinocytes rather than immortalized cell lines, is itself diagnostically informative, defining the concentration window within which a sensing or therapeutic nanomaterial can be deployed without becoming a second source of tissue injury. Protease activity and surface pH give that window a clinical anchor, and the gap between wound-mimetic and standard cell-culture validation likely explains much of the inconsistency Sim and colleagues and Burdusel and colleagues documented across commercial silver-based products [13,14]. Combined sensing-and-therapy dressings will depend on assay batteries built around wound-mimetic exposure conditions from the outset [17,8].

5. Conclusion

Nanotoxicological assessment has moved, within little more than a decade, from a niche safety concern attached to nanoparticle-based wound therapeutics into a discipline with direct diagnostic relevance for chronic wound care. The founding literature established that particle-scale physicochemical properties, not bulk material identity, govern biological response [5,6], and the wound-specific literature since accumulated shows ion-release and oxidative-stress kinetics determine both

© 2021 Somato Publications. All rights reserved.

antimicrobial efficacy and local cytotoxicity of nanomaterials increasingly asked to double as diagnostic reporters of wound-fluid pH and protease activity [9,10,19,21]. Screening run under wound-mimetic rather than generic exposure conditions should be integral to validating any nanomaterial-based chronic wound diagnostic, not a regulatory afterthought. The platforms most likely to succeed will be those built around the narrow concentration window in which diagnostic signal and antimicrobial effect can both be realized without crossing into the cytotoxic range these assay batteries are designed to flag.

6. Acknowledgements

The authors thank colleagues in their respective institutions' nanomedicine and wound-care laboratories for helpful discussion during the preparation of this review.

7. Funding

This work received no dedicated external funding.

8. Conflict of Interest

The authors declare no conflict of interest.

9. Author Contributions

Xu L conceived the review and drafted the manuscript. Chen F conducted the literature synthesis on nanotoxicological assessment frameworks. Zhou J synthesized the wound-biomarker and diagnostic-platform literature. All authors reviewed and approved the final manuscript.

10. Ethics Statement

This article is a literature review and did not involve new studies with human participants or animals performed by any of the authors.

11. Data Availability

No new data were generated or analyzed in this review; all sources discussed are cited in the reference list.

12. References

1. Frykberg, R.G., Banks, J. (2015) Challenges in the treatment of chronic wounds. Advances in Wound Care, 4(9): 560-582. DOI: 10.1089/wound.2015.0635

2. Rigo, C., Ferroni, L., Tocco, I., Roman, M., Munivrana, I., Gardin, C., et al. (2013) Active silver nanoparticles for wound healing. International Journal of Molecular Sciences, 14(3): 4817-4840. DOI: 10.3390/ijms14034817

3. Kalashnikova, I., Das, S., Seal, S. (2015) Nanomaterials for wound healing: scope and advancement. Nanomedicine, 10(16): 2593-2612. DOI: 10.2217/nnm.15.82

4. Boateng, J., Catanzano, O. (2015) Advanced therapeutic dressings for effective wound healing - a review. Journal of Pharmaceutical Sciences, 104(11): 3653-3680. DOI: 10.1002/jps.24610

5. Oberdörster, G., Oberdörster, E., Oberdörster, J. (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives, 113(7): 823-839. DOI: 10.1289/ehp.7339

6. Nel, A., Xia, T., Mädler, L., Li, N. (2006) Toxic potential of materials at the nanolevel. Science, 311(5761): 622-627. DOI: 10.1126/science.1114397

7. Donaldson, K., Poland, C.A. (2013) Nanotoxicity: challenging the myth of nano-specific toxicity. Current Opinion in Biotechnology, 24(4): 724-734. DOI: 10.1016/j.copbio.2013.05.003

8. Krug, H.F., Wick, P. (2011) Nanotoxicology: An interdisciplinary challenge. Angewandte Chemie International Edition, 50(6): 1260-1278. DOI: 10.1002/anie.201001037

9. Wijnhoven, S.W.P., Peijnenburg, W.J.G.M., Herberts, C.A., Hagens, W.I., Oomen, A.G., Heugens, E.H.W., et al. (2009) Nano-silver - a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology, 3(2): 109-138. DOI:

© 2021 Somato Publications. All rights reserved.

10.1080/17435390902725914

10. Gliga, A.R., Skoglund, S., Wallinder, I.O., Fadeel, B., Karlsson, H.L. (2014) Size-dependent cytotoxicity of silver nanoparticles in human lung cells: the role of cellular uptake, agglomeration and Ag release. Particle and Fibre Toxicology, 11: 11. DOI: 10.1186/1743-8977-11-11

11. Chaloupka, K., Malam, Y., Seifalian, A.M. (2010) Nanosilver as a new generation of nanoproduct in biomedical applications. Trends in Biotechnology, 28(11): 580-588. DOI: 10.1016/j.tibtech.2010.07.006

12. Fraňková, J., Pivodová, V., Vágnerová, H., Juráňová, J., Ulrichová, J. (2016) Effects of silver nanoparticles on primary cell cultures of fibroblasts and keratinocytes in a wound-healing model. Journal of Applied Biomaterials & Functional Materials, 14(2): e137-142. DOI: 10.5301/jabfm.5000268

13. Sim, W., Barnard, R.T., Blaskovich, M.A.T., Ziora, Z.M. (2018) Antimicrobial silver in medicinal and consumer applications: a patent review of the past decade (2007-2017). Antibiotics, 7(4): 93. DOI: 10.3390/antibiotics7040093

14. Burdușel, A.C., Gherasim, O., Grumezescu, A.M., Mogoantă, L., Ficai, A., Andronescu, E. (2018) Biomedical applications of silver nanoparticles: an up-to-date overview. Nanomaterials, 8(9): 681. DOI: 10.3390/nano8090681

15. Fadeel, B., Garcia-Bennett, A.E. (2010) Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Advanced Drug Delivery Reviews, 62(3): 362-374. DOI: 10.1016/j.addr.2009.11.008

16. Oberdörster, G. (2010) Safety assessment for nanotechnology and nanomedicine: concepts of nanotoxicology. Journal of Internal Medicine, 267(1): 89-105. DOI: 10.1111/j.1365-2796.2009.02187.x

17. Fadeel, B., Farcal, L., Hardy, B., Vazquez-Campos, S., Hristozov, D., Marcomini, A., et al. (2018) Advanced tools for the safety assessment of nanomaterials. Nature Nanotechnology, 13(7): 537-543. DOI: 10.1038/s41565-018-0185-0

18. Trengove, N.J., Stacey, M.C., MacAuley, S., Bennett, N., Gibson, J., Burslem, F., et al. (1999) Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. Wound Repair and Regeneration, 7(6): 442-452. DOI: 10.1046/j.1524-475X.1999.00442.x

19. Rayment, E.A., Upton, Z., Shooter, G.K. (2008) Increased matrix metalloproteinase-9 (MMP-9) activity observed in chronic wound fluid is related to the clinical severity of the ulcer. British Journal of Dermatology, 158(5): 951-961. DOI: 10.1111/j.1365-2133.2008.08462.x

20. McCarty, S.M., Percival, S.L. (2013) Proteases and delayed wound healing. Advances in Wound Care, 2(8): 438-447. DOI: 10.1089/wound.2012.0370

21. Schneider, L.A., Korber, A., Grabbe, S., Dissemond, J. (2007) Influence of pH on wound-healing: a new perspective for wound-therapy? Archives of Dermatological Research, 298(9): 413-420. DOI: 10.1007/s00403-006-0713-x

22. Percival, S.L., McCarty, S.M., Lipsky, B. (2015) Biofilms and wounds: an overview of the evidence. Advances in Wound Care, 4(7): 373-381. DOI: 10.1089/wound.2014.0557

23. Falanga, V. (2005) Wound healing and its impairment in the diabetic foot. The Lancet, 366(9498): 1736-1743. DOI: 10.1016/S0140-6736(05)67700-8

24. Mihai, M.M., Dima, M.B., Dima, B., Holban, A.M. (2019) Nanomaterials for wound healing and infection control. Materials, 12(13): 2176. DOI: 10.3390/ma12132176

25. Kalantari, K., Mostafavi, E., Afifi, A.M., Izadiyan, Z., Jahangirian, H., Rafiee-Moghaddam, R., et al. (2020) Wound dressings functionalized with silver nanoparticles: promises and pitfalls. Nanoscale, 12(4): 2268-2291. DOI: 10.1039/C9NR08234D

© 2021 Somato Publications. All rights reserved.