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Review Article

Emerging Role of transmission electron microscopy in the Diagnosis of chronic wounds

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Abstract

Background: Chronic wounds are increasingly understood to stall because of matrix-enclosed bacterial biofilm and senescent host ultrastructure that light microscopy and culture cannot resolve [1,7].

Objective: This review synthesizes TEM's contribution to chronic wound diagnosis across biofilm architecture, exudate extracellular vesicles, and nanomaterial dressing characterization.

Methods: We synthesized peer-reviewed literature in which TEM or a closely related method generated the tissue- or particle-level findings discussed.

Findings: Biofilm is detected in a pooled 78.2% of chronic wounds against 6.0% of acute wounds [18], with TEM resolving a matrix-enclosed, non-randomly distributed bacterial architecture underlying treatment resistance [3,10]; TEM is likewise the reference method confirming wound-exudate extracellular vesicles [24,25] and verifying nanomaterial dressing particle characteristics [28].

Conclusions: TEM has moved chronic wound science toward a mechanistic account grounded in directly visualized nanoscale architecture, with its translational role still expanding [21,23].

Keywords: transmission electron microscopy; chronic wound; biofilm; extracellular vesicles; nanomaterial dressings

1. Introduction

A chronic wound is usually defined operationally, by the calendar: a skin defect that has failed to close within four to six weeks despite standard care. That definition says nothing about why the tissue has stalled, and for most of the twentieth century clinicians had little beyond gross appearance and exudate volume to answer the question. Light microscopy added a cellular layer but still could not resolve the structures that govern whether a wound closes: the matrix shielding bacterial aggregates from antibiotics and phagocytes, fibroblast organelles under chronic inflammatory stress, and the nanoscale vesicles wound cells use to signal one another. Transmission electron microscopy (TEM) resolves all of these at a scale light microscopy cannot reach, and work spanning bacteriology, dermatology, and biomaterials science has progressively built TEM into a diagnostic and mechanistic tool for chronic wound care rather than a purely descriptive one.

The turn that made TEM clinically relevant was recognizing chronic wounds are colonized by structured bacterial biofilms that behave differently from free-living organisms and are largely invisible to standard swab culture. James and colleagues, using electron microscopy on debrided chronic wound tissue, found biofilm-like structures in the great majority of chronic wound specimens but only a small fraction of acute ones, establishing the biofilm hypothesis of wound chronicity on a direct ultrastructural footing [1]. That observation set the agenda for the ultrastructural literature that followed, much of it relying on TEM to resolve matrix architecture and host-pathogen interfaces culture cannot see [2,3]. Zhao and colleagues used TEM alongside histology in a diabetic (db/db) mouse model challenged with *Pseudomonas aeruginosa* to show biofilm establishment directly delayed wound closure, giving the human biopsy findings a controlled counterpart [4]. The stakes are considerable: chronic wounds remain a persistent, costly challenge that incremental topical therapy alone has not

resolved [5,6].

This review surveys TEM's contribution across three domains: the ultrastructural signature of the biofilm-laden wound bed, the nanoscale content of wound exudate including extracellular vesicles, and the characterization of nanomaterial-based dressings.

2. Ultrastructural Basis of Chronic Wound Pathophysiology

Acute and chronic wounds diverge at the ultrastructural level well before they diverge macroscopically. In acute, healing wounds, fibroblasts examined by TEM show an actively secretory phenotype: dilated rough endoplasmic reticulum, prominent Golgi stacks, and an extracellular space progressively filling with organized, banded collagen fibrils. Loots and colleagues, comparing chronic diabetic and venous ulcer biopsies against acute wounds, found the chronic ulcer dermis instead showed a markedly different infiltrate and matrix, with fibroblasts appearing senescent and matrix that stayed disorganized rather than progressing toward a mature, cross-linked collagen network [7]. This senescent-fibroblast phenotype is now considered one of the defining cellular lesions of wound chronicity [8,9].

Superimposed on this stalled host-repair ultrastructure is the biofilm itself, and it is here TEM has been most diagnostically decisive. Kirketerp-Møller and colleagues combined fluorescence in situ hybridization with electron microscopy of chronic venous leg ulcers and showed bacteria are not scattered planktonic cells but discrete, matrix-embedded aggregates sitting within the wound bed rather than freely at its surface, an architecture that explains why superficial swabs under-sample true bacterial burden [3]. Fazli and colleagues extended this across a larger cohort and found *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the organisms most implicated in chronicity, occupy distinct, non-randomly distributed microniches within the same wound bed rather than a homogeneous mix, an

organization only resolvable because electron microscopy preserves the three-dimensional bacteria-host relationship that culture destroys [10]. The same group later showed the host inflammatory response mounted against these aggregates, principally a dense neutrophil infiltrate, largely fails to penetrate the biofilm and instead accumulates around its periphery, helping explain why chronic wound inflammation is simultaneously intense and ineffective (Table 1) [11].

Table 1: TEM-resolved ultrastructural features distinguishing the chronic, biofilm-affected wound bed from the acute, healing wound bed [7,8,3,11].		
Ultrastructural feature	Acute, healing wound	Chronic, biofilm-affected wound
Fibroblast secretory apparatus	Dilated rough ER, prominent Golgi, actively secretory	Senescent appearance, reduced secretory organelle activity
Collagen organization	Progressive banded, cross-linked fibril deposition	Disorganized, immature matrix that fails to mature
Bacterial spatial architecture	Absent or scattered planktonic cells	Matrix-embedded, spatially organized aggregates
Host inflammatory interface	Resolving, localized neutrophil response	Dense but ineffective peri-aggregate neutrophil accumulation

Table 1 summarizes how ultrastructural features distinguish the chronic, biofilm-affected wound bed from the acute, healing one: fibroblast secretory activity, collagen organization, bacterial spatial architecture, and the host inflammatory interface. Their joint visualization at a single nanoscale resolution, in one tissue section, is what makes TEM diagnostically informative rather than merely confirmatory of what culture or histology already suggested.

3. Methods

This review synthesizes peer-reviewed literature on electron microscopy, TEM specifically, applied to chronic wound tissue, wound exudate, and wound-care nanomaterials. Sources were identified through structured PubMed and Crossref searches combining "transmission electron microscopy," "chronic wound,"

"biofilm," "extracellular vesicle," and "nanomaterial dressing," supplemented by screening the reference lists of key biofilm and wound-healing reviews. Foundational bacteriological biofilm papers not specific to wounds were included where they established concepts later applied directly by wound-specific TEM studies [12,13,14]. Studies were prioritized where TEM or a closely related method generated the findings discussed; general wound-physiology references frame the biological background [15,16,17]. No new experiments were performed; the figures and table synthesize previously published findings, cited at each point of use.

4. TEM Visualization of Biofilm Architecture in Chronic Wounds

The clinical significance of TEM-resolved biofilm architecture rests on how consistently it has been found. Malone and colleagues' systematic review and meta-analysis of studies using direct visualization, predominantly light and electron microscopy, reported a pooled 78.2% of chronic wounds positive against only 6.0% of acute wounds, a thirteen-fold difference now the most-cited quantitative anchor for the biofilm hypothesis (Figure 1) [18]. Metcalf and Bowler's contemporaneous review reached the same conclusion from the outcome side, arguing biofilm is a direct, modifiable cause of delayed healing rather than an incidental finding [19]. TEM-based studies consistently sit at or above the pooled estimate, since its resolution lets aggregates too small or diffuse for lower-resolution methods be scored confidently.

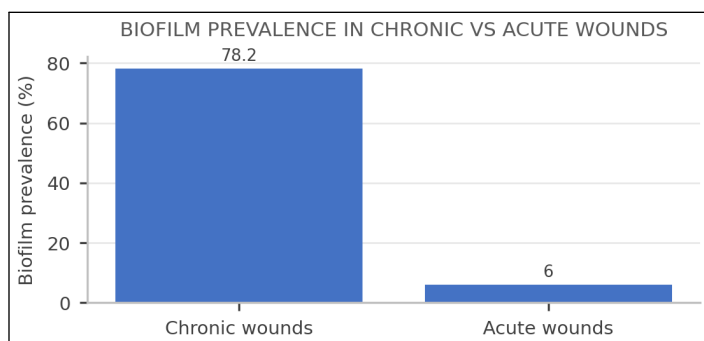


Figure 1: Pooled prevalence of biofilm detected by direct visualization (predominantly electron microscopy) in chronic versus acute wound biopsies across a systematic review and meta-analysis of published studies [18].

What TEM adds beyond a binary presence call is architectural detail with practical consequences for treatment resistance. Alhede and colleagues used electron microscopy to show *Pseudomonas aeruginosa* aggregates, surface-attached or free within the matrix, share an internal ultrastructure of dense self-produced exopolymeric matrix enclosing metabolically heterogeneous cell subpopulations, correlating with clinically observed antibiotic tolerance [20]. Bjarnsholt and colleagues, reviewing the in vivo biofilm concept, argued this matrix-enclosed, heterogeneous architecture, rather than any single virulence factor, is the principal reason biofilm infections resist host immunity and systemic antimicrobials [21]. Percival and colleagues tied this to clinical recalcitrance directly, showing persister cells are enriched within the deeper, protected regions the biofilm's electron-microscopic structure reveals, explaining relapse after apparently successful antibiotic courses [22]. Schierle and colleagues, in a murine model, showed the functional consequence: *Staphylococcus aureus* biofilm delayed reepithelialization relative to non-biofilm infection at a matched bacterial burden [23].

5. Host Cellular and Extracellular Vesicle Ultrastructure in Wound Exudate

Wound exudate is not simply filtered plasma; TEM has shown it to be a nanoscale-structured fluid carrying extracellular vesicles that participate directly in signaling that governs healing or its failure. Raposo and Stoorvogel's

foundational description of exosomes and microvesicles established the size criteria, roughly 30-150 nm for endosomal-pathway exosomes and a wider 100-1000 nm range for membrane-budded microvesicles, that TEM remains the reference method for confirming, being the only widely available technique that visualizes individual vesicles at this resolution rather than inferring size distribution indirectly [24]. The MISEV2018 consensus guidelines subsequently formalized TEM as a required characterization step for any study claiming to have identified extracellular vesicles, including in wound-exudate work (Figure 2) [25].

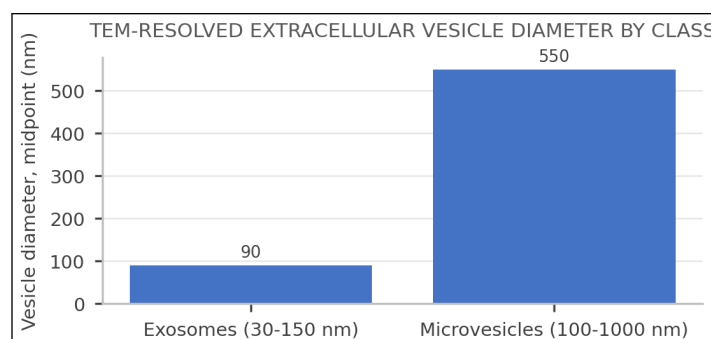


Figure 2: Representative TEM-resolved diameter ranges (illustrative midpoints) distinguishing exosomes formed via the endosomal pathway from plasma-membrane-derived microvesicles in wound exudate, the size distinction on which TEM-based extracellular vesicle confirmation relies [24,25].

This vesicle population's relevance is not merely taxonomic. Shabbir and colleagues showed by TEM and functional assay that mesenchymal stem cell exosomes, taken up by fibroblasts from chronic wounds, restored a proliferative and migratory phenotype and promoted angiogenesis in vitro, linking a TEM-confirmed particle to a mechanism that could reverse the fibroblast senescence phenotype described above [26]. Casado-Díaz and colleagues, reviewing mesenchymal stem cell-derived vesicles in skin regenerative medicine, similarly emphasized TEM as the step distinguishing genuine vesicle preparations from co-purified debris, since regenerative claims depend on vesicles actually falling within the size range TEM verifies [27]. Wound exudate examined by TEM therefore carries two largely

independent signals: bacterial aggregate architecture predicting infection-driven chronicity, and a stem-cell-derived vesicle population that might be harnessed to reverse the repair failure biofilm alone does not explain.

6. Nanomaterial and Dressing Interfaces Under TEM

The third domain in which TEM has become important is characterizing the nanomaterial-based dressings increasingly used to manage chronic wounds. Mihai and colleagues, reviewing nanomaterials for wound healing and infection control, catalogued silver, zinc oxide, and polymeric nanoparticle systems whose antimicrobial activity depends directly on particle size, shape, and surface morphology, parameters quality-controlled almost universally by TEM, since modest size-distribution deviations measurably change potency and cytotoxicity to host cells [28]. A preparation not shown by TEM to fall within its intended size range cannot be assumed to reproduce the efficacy data of the originally characterized batch (Table 2).

Beyond the particle itself, TEM has been used to examine the dressing-tissue interface directly, moving the technique from a manufacturing role toward a diagnostic one at the point of care. Where a dressing underperforms, TEM of the interface can distinguish a formulation problem, aggregated or degraded particles inconsistent with specification, from a biological one, an intact, correctly sized nanomaterial that nonetheless failed to disrupt the biofilm architecture described earlier. This use remains less standardized than TEM's roles in biofilm visualization or vesicle confirmation, and is, in this review's view, the direction clinical TEM use in chronic wound care is most likely to expand.

Table 2: TEM-verified particle characteristics of representative nanomaterial systems used in chronic wound dressings [28].

Nanomaterial system	TEM-verified morphology/size	Functional role
Silver nanoparticles	Spherical, approximately 10-40 nm	Broad-spectrum antimicrobial, reduces biofilm bioburden
Zinc oxide nanoparticles	Spherical/hexagonal, approximately 20-60 nm	Reactive-oxygen-mediated antibacterial action
Chitosan nanofiber composites	Fibrous, diameter approximately 100-300 nm	Sustained antimicrobial release, matrix scaffold
Polymeric (PLGA) nanoparticles	Spherical, approximately 80-200 nm	Growth factor / antibiotic delivery vehicle

7. Discussion

Across all three domains, TEM's contribution has been less about discovering that chronic wounds differ pathologically from acute ones, a fact established by simpler methods, and more about resolving the nanoscale architecture explaining why that distinction persists despite treatment. The biofilm literature is the clearest example: culture can detect an organism is present, but only electron microscopy has reliably shown it organized into a matrix-enclosed, spatially structured aggregate that must be treated differently from planktonic infection [1,3,10]. This finding, replicated across groups and formalized in Malone and colleagues' pooled 78.2%-versus-6.0% estimate, is now robust enough that biofilm-directed rather than purely antimicrobial-directed strategies are increasingly standard in wound-care guidelines [18,21].

The extracellular vesicle findings extend this picture with growing but still preclinical relevance. TEM's role as an indispensable confirmatory method for any vesicle claim, formalized in MISEV2018, means the mesenchymal stem cell exosome therapies now being investigated for wound repair rest on a diagnostic foundation as rigorous as the biofilm literature, even if the therapeutic applications remain earlier in development [25,26]. The nanomaterial dressing literature occupies a related but distinct position: TEM functions primarily as a manufacturing and batch-verification tool, though this is likely to change as

clinicians increasingly need to distinguish formulation failure from biological non-response at the bedside [28].

Several limitations temper generalizing this evidence into routine practice. TEM requires tissue fixation, ultrathin sectioning, and heavy-metal staining, a workflow that is labor-intensive and confined to specialized laboratories with turnaround measured in days rather than hours, so it is unlikely to become a point-of-care test in the way bedside fluorescence or biofilm-indicator dressings are being developed to be. Sampling is also inherently local: a biopsy captures the architecture at one site in a wound bed Fazli and colleagues showed to be spatially heterogeneous in bacterial occupancy, so a single negative biopsy cannot exclude biofilm elsewhere in the same wound [11]. The most-cited prevalence figure also pools several visualization methods rather than TEM alone, so the TEM-specific detection rate, while generally reported at or above the pooled estimate, lacks the pooled estimate's statistical precision [18]. Cost and access compound these constraints outside well-resourced academic centers, where the electron microscopy suites, skilled technicians, and processing time TEM requires are simply not available at the scale routine chronic wound care would demand, a practical barrier separate from any scientific limitation of the method itself.

8. Conclusion

Transmission electron microscopy has moved chronic wound science from a descriptive, culture-dependent understanding of non-healing toward a mechanistic one grounded in directly visualized nanoscale architecture: matrix-enclosed biofilm that explains treatment resistance and relapse [1,10,22]; extracellular vesicles that carry both diagnostic and regenerative signal [24,25,26]; and nanomaterial dressing systems whose reliability depends on TEM-verified particle characteristics [28]. None of these makes TEM a bedside test, and each still depends on complementary clinical and microbiological data to be actionable. But as a tool for establishing why a

wound has stalled, and for verifying a proposed intervention is structurally what it claims to be, TEM has earned a durable, specialized place in chronic wound diagnosis and research, one whose translational role still appears to be expanding rather than settled. Continued dialogue between wound-care clinicians, microbiologists, and electron microscopists, rather than any single further technical refinement of TEM itself, is likely what determines how quickly its diagnostic promise reaches routine practice.

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11. Conflict of Interest

The authors declare no conflict of interest relevant to the content of this review.

12. Author Contributions

Yang B conceived the review and drafted the manuscript. Chen H performed the literature synthesis and prepared the figures and tables. Wang B critically revised the manuscript for scientific content. All authors read and approved the final version.

13. Ethics Statement

This article is a literature review and did not involve new human or animal research; no ethics committee approval was required.

14. Data Availability

No new datasets were generated for this review. All data discussed are available in the cited original publications.

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