



ISSN: 2231-3656

International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR | Vol. 16 | Issue 4 | Oct-Dec -2026

www.ijpir.com

DOI: <https://doi.org/10.61096/ijpir.v16.iss4.2026.962.970>

Mitochondrial Reactive Oxygen Species in Neutrophil Biology: Mechanisms, Netosis, and Therapeutic Perspectives

S. Mishra^{1*}, P. Kumar¹, A. Mishra¹

Shree Krishna College of Pharmacy, Sitapur, Uttar Pradesh, India

Correspondence: S. Mishra

E-mail: sakshammishra0322@gmail.com



Published on:
04.10.2026
Published by:
Futuristic
Publications
2026| All rights
reserved.



Creative
Commons
Attribution 4.0
International
License.

Abstract: Neutrophils have traditionally been viewed as highly glycolytic, short-lived effector cells in which mitochondria primarily govern apoptosis. This interpretation is now incomplete. Although mitochondrial oxidative phosphorylation contributes little to the acute energetic requirements of mature neutrophils, mitochondria function as spatially organized signaling platforms that regulate calcium handling, migration, degranulation, survival, hypoxic adaptation, antimicrobial activity, and neutrophil extracellular trap (NET) formation. Mitochondrial reactive oxygen species (mROS), generated mainly through electron leakage at respiratory-chain complexes I and III, operate at low-to-moderate levels as second messengers but become injurious when production exceeds antioxidant control. In neutrophils, mROS interact with NADPH oxidase 2 (NOX2), mitogen-activated protein kinases, phosphoinositide 3-kinase, protein kinase C, mitochondrial calcium uptake, and peptidylarginine deiminase 4 (PAD4). These interactions create stimulus-specific routes to NET formation. Protein kinase C activation by phorbol esters generally favors NOX2-dependent, lytic NETosis, whereas calcium ionophores, sterile injury, and selected inflammatory signals can induce NOX-independent NET formation that depends strongly on mitochondrial calcium loading and mROS. Oxidized mitochondrial DNA released within NETs is not merely structural material; it acts as a damage-associated molecular pattern capable of activating Toll-like receptor 9 and cyclic GMP-AMP synthase-stimulator of interferon genes signaling, thereby linking mitochondrial redox stress to type I interferon responses, thrombosis, endothelial injury, autoimmunity, and cancer progression. Therapeutic approaches include mitochondria-targeted antioxidants, mitochondrial calcium and dynamics modulators, PAD4 inhibition, DNase-based NET dismantling, blockade of oxidized mitochondrial DNA sensing, and disease-specific upstream anti-inflammatory strategies. However, broad suppression of mROS or NETosis may compromise antimicrobial defense. Future translation therefore requires stimulus-resolved biomarkers, rigorous NET detection, cell-selective delivery, and treatments that normalize pathological mitochondrial signaling without eliminating protective neutrophil functions.

Keywords: Mitochondrial reactive oxygen species; neutrophils; NETosis; neutrophil extracellular traps; mitochondrial DNA; PAD4; NOX2; inflammation

1. Introduction

Neutrophils are the most abundant circulating leukocytes in humans and are rapidly recruited to sites of infection, sterile injury, and tissue stress. Their classic antimicrobial repertoire includes phagocytosis, granule exocytosis, production of reactive oxygen species (ROS), and release of neutrophil extracellular

traps (NETs). These responses are effective because they concentrate proteases, oxidants, antimicrobial peptides, and chromatin around invading microorganisms. The same potency also creates a biological hazard: when neutrophil activation is excessive, prolonged, or spatially misdirected, oxidants and NET-associated proteins damage endothelium, activate coagulation, expose autoantigens, and amplify inflammatory signalling.^[1,2]

For decades, mitochondrial function in mature neutrophils was considered limited because these cells generate most of their ATP through glycolysis and contain fewer mitochondria than many other leukocytes. This metabolic profile remains correct, but the conclusion drawn from it has changed. Neutrophil mitochondria are now understood as signaling organelles rather than major power plants. They shape intracellular calcium, produce localized ATP for migration, regulate apoptosis, support hypoxic responses, control redox-sensitive signaling, and provide mitochondrial ROS (mROS) that can either prime host defense or promote inflammatory injury.^[3,4]

The distinction between NOX2-derived ROS and mROS is central to contemporary neutrophil biology. NOX2 generates the high-amplitude oxidative burst required for efficient killing of many microbes, as illustrated by the severe infections observed in chronic granulomatous disease. By contrast, mROS are frequently produced at lower intensity and in restricted subcellular domains. These signals can regulate NOX2 assembly, degranulation, integrin activation, chemotaxis, HIF-1 α stabilization, and NET formation. Under calcium overload, hypoxia, mitochondrial dysfunction, or sterile stress, mROS may become a dominant oxidative source and can drive NETosis independently of NOX2.^[5,6]

This narrative review synthesizes current evidence on the generation and regulation of mROS in neutrophils, their contributions to core neutrophil functions, their mechanistic role in distinct NETosis pathways, and their translational relevance. Particular emphasis is placed on the calcium-mitochondria-PAD4 axis, crosstalk between mitochondria and NOX2, oxidized mitochondrial DNA as an inflammatory mediator, methodological limitations in mROS and NET measurement, and therapeutic strategies that seek to reduce pathological neutrophil activation while preserving antimicrobial competence.

2. Neutrophil Mitochondria: Signaling Organelles in a Glycolytic Cell

Mature neutrophils possess an extensive but functionally specialized mitochondrial network. Glycolysis supplies most ATP needed for the respiratory burst and phagocytosis, yet mitochondrial membrane potential and electron transport remain biologically meaningful. Early experiments showed that disruption of mitochondrial function impaired chemotaxis even when bulk cellular ATP was not immediately depleted, suggesting that mitochondria provide localized signals required for polarization and movement. Subsequent work linked mitochondrial dynamics, calcium uptake, and organelle positioning to cytoskeletal rearrangement and directional migration.^[3,7]

Mitochondrial function also changes across the neutrophil life cycle. During granulopoiesis, mitochondrial metabolism and autophagy contribute to differentiation and maturation. In circulating mature cells, mitochondria participate in apoptosis through BCL-2 family proteins, mitochondrial outer-membrane permeabilization, and caspase activation. In inflamed tissues, survival signals such as GM-CSF, hypoxia, and microbial products can delay apoptosis, extending the period during which neutrophils release oxidants and proteases. Thus, the same organelle contributes to maturation, acute activation, and inflammatory resolution.

Mitochondrial organization is dynamic. Mitofusin 2 supports mitochondrial fusion and interactions with the endoplasmic reticulum, while dynamin-related protein 1 promotes fission. These processes influence calcium transfer, membrane potential, and mROS generation. In neutrophils, mitochondrial calcium uptake can facilitate polarization and migration, whereas excessive calcium entry increases electron leak and mROS. The mitochondrial calcium uniporter (MCU) therefore occupies an important regulatory position between receptor-driven calcium flux and downstream effector responses.

A useful conceptual distinction is that mitochondrial bioenergetics and mitochondrial signaling are not equivalent. A neutrophil may rely minimally on oxidative phosphorylation for total ATP yet depend strongly on mitochondrial membrane potential, local ATP release, calcium buffering, and redox signaling. This explains why mitochondrial inhibitors can alter migration, degranulation, survival, or NET formation without producing the global energetic collapse expected in an oxidative cell.

3. Generation and Regulation of Mitochondrial ROS

mROS arise primarily when electrons escape the respiratory chain and reduce molecular oxygen to superoxide. Complexes I and III are commonly regarded as major sites, although the dominant source

depends on substrate supply, membrane potential, oxygen tension, and reverse electron transport. Superoxide is converted by mitochondrial superoxide dismutase to hydrogen peroxide, which can diffuse or be transported across membranes and modify redox-sensitive proteins. At controlled concentrations, this chemistry supports signaling. At excessive concentrations, it oxidizes lipids, proteins, and mitochondrial DNA.

Several events increase mROS in activated neutrophils. First, cytosolic calcium elevations promote mitochondrial calcium uptake. Moderate calcium can coordinate metabolism and signaling, whereas overload increases membrane potential instability and electron leak. Second, inflammatory receptor signaling activates phosphoinositide 3-kinase, protein kinase C, Rac GTPases, and mitogen-activated protein kinases, creating bidirectional communication between NOX2 and mitochondria. Third, hypoxia modifies electron transport and favors mROS-dependent stabilization of HIF-1 α , which prolongs neutrophil survival and supports inflammatory adaptation ^[6]. Fourth, mitochondrial damage, defective mitophagy, and altered fission-fusion balance can increase oxidant production and mitochondrial DNA release.

Neutrophils possess antioxidant systems including superoxide dismutases, glutathione-dependent enzymes, peroxiredoxins, and catalase-related pathways. The biological result depends on the ratio between oxidant generation and antioxidant capacity, the subcellular site of production, and the duration of exposure. This point is important because the term “ROS” is often used as if all species were interchangeable. Superoxide, hydrogen peroxide, hypochlorous acid, hydroxyl radicals, and peroxynitrite differ in lifetime, diffusion, reactivity, and targets. Rigorous interpretation therefore requires chemical specificity and orthogonal validation rather than reliance on a single fluorescent probe ^[8].

Experimental antioxidants also require caution. Diphenyleneiodonium is frequently used as a NOX inhibitor but can inhibit flavoproteins outside NOX2, including components of mitochondrial electron transport. MitoTEMPO and related triphenylphosphonium-linked compounds accumulate in polarized mitochondria and are useful tools, yet their effects depend on membrane potential and concentration. SkQ1 similarly targets mitochondrial oxidative processes and has been shown to reduce fMLP-induced oxidative burst and granule exocytosis, supporting the idea that mROS participate upstream of major neutrophil responses ^[9]. Mechanistic conclusions are strongest when pharmacological experiments are combined with genetic, biochemical, and imaging approaches.

4. mROS in Core Neutrophil Functions

4.1 Chemotaxis and adhesion

Neutrophil recruitment requires front-rear polarization, actin remodeling, integrin activation, and coordinated calcium flux. Mitochondria accumulate in functionally relevant cellular regions and help sustain local signaling. mROS can modulate ERK and other redox-sensitive pathways that influence β 2-integrin activation and adhesion. Mitochondrial calcium uptake and fission also support directional migration. Excessive mROS, however, may impair motility, promote aberrant adhesion, and trap neutrophils in tissues, thereby converting an adaptive recruitment response into persistent inflammation. ^[23]

4.2 Oxidative burst and degranulation

NOX2 is the principal source of the high-output respiratory burst, but mROS can amplify or facilitate NOX2 activation. In human neutrophils stimulated with the chemoattractant fMLP, scavenging mitochondrial oxidants with SkQ1 reduced oxidative burst and exocytosis of azurophilic and specific granules ^[9]. These findings support a feed-forward model in which receptor signaling increases mROS, mROS promotes assembly or activity of NOX2-related pathways, and NOX2-derived oxidants further alter mitochondrial redox status. This crosstalk allows a small mitochondrial signal to control a much larger antimicrobial response.

4.3 Antimicrobial defense

mROS can contribute directly to microbial killing in selected infections. During *Streptococcus pneumoniae* infection, mitochondrial oxidants accounted for a substantial proportion of intracellular neutrophil ROS, and mitochondria-targeted scavenging impaired bacterial killing. Adenosine A2B receptor signaling acted as a negative regulator of this pathway, indicating that mROS production is integrated with extracellular danger and metabolic signals ^[10]. Such evidence warns against assuming that mitochondria-targeted antioxidants are universally anti-inflammatory without antimicrobial cost.

4.4 Survival and hypoxic adaptation

Inflamed tissues are frequently hypoxic. Neutrophils remain functional in these environments partly because glycolysis limits their dependence on oxygen-consuming ATP generation. Nevertheless, mitochondria are needed for hypoxic signaling. mROS promote stabilization of HIF-1 α , a transcription factor that delays apoptosis and sustains antimicrobial functions.^[6] demonstrated that neutrophil mROS increase under hypoxia and support high-level HIF-1 α stabilization. This response can be protective during infection, but prolonged HIF-1 α activity may delay resolution and intensify tissue injury.

4.5 Cell death decisions

Neutrophils can undergo apoptosis, necrosis, pyroptosis, or NET-associated death. The intensity and location of ROS influence this choice. Moderate mitochondrial signaling may delay apoptosis or prime effector functions, while severe mitochondrial damage can trigger apoptosis or shift cells toward NETosis. UV-induced sterile injury provides an instructive model in which increasing mROS and oxidative DNA damage move neutrophils from apoptosis toward NET release^[11]. Thus, mROS are not simply toxic by-products; they help determine how neutrophils terminate their response and what inflammatory material remains.

5. NETosis: Stimulus-Specific Pathways and the Role of mROS

NETs are extracellular chromatin structures decorated with histones, myeloperoxidase, neutrophil elastase, and other antimicrobial proteins. The term NETosis is often used broadly, but NET release can occur through mechanistically distinct routes. Lytic NETosis involves chromatin decondensation, nuclear-envelope breakdown, mixing of nuclear and granule contents, plasma-membrane rupture, and cell death. Non-lytic or “vital” NET release can occur rapidly through vesicular export while neutrophils retain selected functions. NET material may derive predominantly from nuclear DNA or, under specific conditions, mitochondrial DNA.^[12,2,13,14,15]

5.1 NOX2-dependent NETosis

Phorbol 12-myristate 13-acetate (PMA) is the prototypical laboratory stimulus for NOX2-dependent NETosis. PMA activates protein kinase C, the Raf-MEK-ERK pathway, and NOX2 assembly. Superoxide and downstream oxidants promote release of neutrophil elastase and myeloperoxidase from granules, chromatin remodeling, and nuclear-envelope disintegration. This process develops over hours and is markedly reduced by NOX2 deficiency or inhibition. ROS-induced oxidative DNA lesions and the recruitment of base-excision repair proteins may create strand breaks and chromatin relaxation that facilitate NET release.^[16,14]

mROS are not necessarily absent from this pathway. They may prime NOX2, influence granule mobilization, or amplify redox signaling. However, PMA-induced NETosis is generally dominated by NOX2-derived oxidants, and its sensitivity to mitochondrial scavengers varies with experimental conditions.

5.2 Calcium-driven, NOX-independent NETosis

Calcium ionophores such as ionomycin and A23187 induce a distinct pathway. Rapid cytosolic calcium elevation activates calcium-dependent potassium channels, promotes mitochondrial calcium uptake, increases mROS, and activates PAD4. PAD4 converts arginine residues in histones to citrulline, weakening histone-DNA interactions and supporting chromatin decondensation. Douda et al. (2015) identified a small-conductance calcium-activated potassium channel 3-mROS axis in ionomycin-induced NET formation. In this context, mitochondrial uncouplers, channel inhibitors, and mROS scavengers suppress NET release, whereas NOX2 inhibition has limited effect.^[5,17]

This distinction is clinically relevant because calcium-driven NETosis may be prominent during sterile injury, crystal-induced inflammation, thrombosis, and exposure to damage-associated molecules. It also explains why neutrophils from patients with defective NOX2 can still form NET-like structures under selected conditions. The route is not simply a weaker substitute for NOX2-dependent NETosis; it produces different kinetics, signaling intermediates, DNA composition, and inflammatory consequences.

5.3 Oxidative DNA damage and chromatin remodeling

ROS can oxidize guanine to 8-oxoguanine and activate base-excision repair. Neutrophils contain repair proteins such as OGG1, PARP, PCNA, and DNA polymerase β . During NETosis, repair-associated nicking

and polymerase activity may loosen chromatin and promote genome-wide decondensation. Azzouz et al. (2021) showed that ROS-induced DNA oxidation and repair are mechanistically linked to NET formation. More recent work extends this concept to mROS-driven sterile injury, where oxidative DNA damage can direct the transition from apoptosis to NETosis.^[11,18,16]

5.4 Mitochondrial DNA-containing NETs

Yousefi et al.^[12] demonstrated that viable neutrophils primed with GM-CSF and stimulated through C5a or TLR4 could release NETs containing mitochondrial rather than nuclear DNA. This process was ROS dependent and did not require immediate cell death. Mitochondrial DNA is particularly inflammatory because it retains bacterial-like CpG motifs and is susceptible to oxidation near sites of mROS production. Oxidized mitochondrial DNA can activate TLR9 and cytosolic DNA-sensing pathways, converting NETs from passive antimicrobial scaffolds into potent immunostimulatory complexes.

In systemic lupus erythematosus, low-density granulocytes show enhanced spontaneous NETosis and release oxidized mitochondrial DNA.^[19] demonstrated that mitochondrial ROS were required for this process and that oxidized mitochondrial DNA promoted type I interferon signaling through STING. These findings provide a direct molecular bridge among mitochondrial stress, NETosis, and interferon-driven autoimmunity.

6. Pathophysiological Consequences

6.1 Autoimmunity

NETs expose DNA, histones, granule enzymes, and post-translationally modified proteins to the immune system. Oxidized mitochondrial DNA is especially interferogenic and can sustain plasmacytoid dendritic-cell activation and type I interferon production. In lupus, this pathway may create a self-amplifying loop: inflammatory cytokines prime neutrophils, mROS oxidize mitochondrial DNA, NETs release oxidized DNA, innate DNA sensors increase interferon, and interferon further primes pathogenic neutrophil subsets. PAD4-dependent histone citrullination may add additional neopeptides relevant to rheumatoid arthritis and other autoimmune conditions.^[19,15]

6.2 Thrombosis and endothelial injury

NETs provide a scaffold for platelets, red blood cells, von Willebrand factor, and coagulation proteins. Histones damage endothelium, while neutrophil elastase and other proteases alter anticoagulant pathways. mROS-driven NETosis may therefore contribute to immunothrombosis during sepsis, acute lung injury, ischemia-reperfusion, and autoimmune vasculopathy. Mitochondrial DNA and oxidized phospholipids further amplify endothelial and platelet activation. The therapeutic challenge is to reduce occlusive NET accumulation without eliminating localized NET-mediated pathogen containment.^[13,2]

6.3 Infection and sepsis

During infection, mROS can improve bacterial killing and support NOX2 activity. Yet excessive mitochondrial oxidants may promote endothelial injury, delayed neutrophil apoptosis, and diffuse NET release. Sepsis therefore illustrates the context dependence of mROS: insufficient production may impair clearance, while uncontrolled production may intensify organ dysfunction. Timing is likely decisive. Early enhancement of antimicrobial function and later promotion of resolution may require different therapeutic approaches.^[10]

6.4 Cancer

Tumor-derived cytokines and metabolic signals recruit and reprogram neutrophils. NETs can capture circulating tumor cells, facilitate adhesion to endothelium, awaken dormant cells, establish premetastatic niches, and contribute to thrombosis. Mitochondrial dysfunction and mROS may favor NET formation in tumor-associated neutrophils, while oxidized mitochondrial material activates inflammatory pathways in the tumor microenvironment. Nevertheless, neutrophils can also exert antitumor activity. Broad depletion or pan-NET inhibition may therefore have unpredictable effects unless directed toward a defined pathological pathway.^[2]

6.5 Hypoxia and inflammatory tissue damage

Hypoxic organs, including inflamed lung, ischemic myocardium, and poorly perfused tumors, favor mROS-HIF-1 α signaling. This preserves neutrophil survival and activity but can prolong exposure to

proteases and NETs. The same adaptation that helps neutrophils function in oxygen-poor infection sites can delay resolution in sterile disease. Therapeutic strategies that modify this axis must distinguish host-protective hypoxic adaptation from chronic inflammatory persistence.^[6]

7. Therapeutic Perspectives

7.1 Mitochondria-targeted antioxidants

MitoTEMPO, MitoQ, SkQ1, and related compounds concentrate antioxidant activity within mitochondria. In experimental systems, MitoTEMPO reduces calcium ionophore-induced mROS and NET formation, while SkQ1 suppresses chemoattractant-induced oxidative burst and degranulation. In lupus-prone models, reducing mitochondrial oxidation can decrease oxidized mitochondrial DNA release and interferon-associated pathology. These results establish proof of concept, but translation remains difficult. Triphenylphosphonium-linked molecules depend on mitochondrial membrane potential, can accumulate differently across tissues, and may affect protective antimicrobial responses. Dose, timing, and cell-selective delivery are therefore essential.^[9,19]

7.2 Modulation of mitochondrial calcium and dynamics

The MCU, calcium-activated potassium channels, Drp1, and mitofusins regulate the calcium-mROS interface. Inhibiting excessive mitochondrial calcium uptake or pathological fission could reduce mROS and NETosis. However, these proteins are widely expressed and contribute to essential functions in many organs. Systemic inhibition may create cardiac, neurological, or metabolic toxicity. Neutrophil-targeted nanoparticles, short treatment windows, or disease-localized delivery may improve the therapeutic index.

7.3 PAD4 inhibition

PAD4 is a major effector of calcium-dependent chromatin decondensation. Small-molecule PAD4 inhibitors reduce NET formation in numerous preclinical models and may be particularly relevant when mROS acts upstream through calcium signaling. PAD4 inhibition is not equivalent to mROS inhibition: it blocks a downstream chromatin step while leaving mitochondrial signaling and antimicrobial oxidants partly intact. Yet PAD4-independent NET pathways exist, and complete blockade may interfere with host defense. Biomarkers of histone citrullination and stimulus-specific NET signatures may help identify responsive patients.^[17]

7.4 NET dismantling with DNase

Recombinant DNase I degrades extracellular DNA and can reduce NET viscosity and microvascular obstruction. Its established use in cystic fibrosis supports clinical feasibility. Nevertheless, DNase dismantles the DNA scaffold without necessarily neutralizing histones, proteases, or oxidized mitochondrial fragments. In some settings, fragmentation may increase dissemination of inflammatory NET components. Combination approaches that include histone neutralization, protease inhibition, or enhanced clearance may be more effective than DNase alone.

7.5 Blocking mitochondrial DNA sensing

Oxidized mitochondrial DNA activates TLR9 and the cGAS-STING pathway. In interferon-driven autoimmunity, inhibiting these sensors may interrupt downstream inflammation without fully suppressing neutrophil antimicrobial ROS. STING and TLR9 inhibitors are therefore attractive, especially when biomarkers demonstrate oxidized mitochondrial DNA-rich NETs. The limitation is that DNA sensing is important for antiviral and antitumor immunity. Patient selection and infection surveillance are mandatory.

7.6 Upstream inflammatory and receptor pathways

Cytokines such as GM-CSF, IL-8, IL-18, and type I interferons can prime mitochondrial redox responses and NETosis. Adenosine A2B receptor signaling can restrain mROS during pneumococcal infection, while microRNA-223 has been reported to reduce calcium influx, mROS, and IL-18-associated NET formation. Blocking upstream cytokines or receptors may provide disease specificity, but the same pathway may have opposite effects in infection and sterile inflammation. Mechanism-based treatment should therefore begin with the dominant disease context rather than the assumption that all mROS is harmful.^[10,20]

7.7 Improving resolution rather than suppressing activation

A promising alternative is to restore the normal termination of neutrophil responses. Promoting apoptosis and macrophage efferocytosis, enhancing mitophagy, accelerating NET clearance, and correcting metabolic stress may reduce inflammatory persistence while retaining early antimicrobial activity. This strategy fits the temporal biology of neutrophils: activation is useful initially, but failure to resolve becomes pathogenic.

8. Methodological and Translational Challenges

Several technical issues complicate the field. First, many probes described as mROS indicators are not fully specific for a single reactive species. MitoSOX is sensitive to oxidation and mitochondrial accumulation but can produce misleading signals if membrane potential changes or if oxidation products are not resolved. Strong studies combine imaging with flow cytometry, electron paramagnetic resonance, mass spectrometry, genetically encoded sensors, or direct measurement of oxidative products^[8].^[21]

Second, NET quantification requires more than extracellular DNA staining. Sytox Green detects membrane-impermeable DNA but cannot by itself distinguish NETosis from necrosis. Recommended approaches demonstrate co-localization of DNA with neutrophil proteins such as myeloperoxidase or elastase, assess citrullinated histone H3 where appropriate, use live-cell imaging to define kinetics, and exclude other forms of cell death. Plasma cell-free DNA is not a NET-specific biomarker. MPO-DNA or elastase-DNA complexes improve specificity but still require careful assay validation.^[21]

Third, PMA and ionomycin are valuable mechanistic tools but do not represent all physiological stimuli. PMA directly activates protein kinase C, whereas ionomycin creates a large calcium influx. Conclusions based only on these agents may not generalize to bacteria, immune complexes, crystals, activated platelets, complement, or cytokines. Comparative experiments should include disease-relevant stimuli and neutrophils from patients.

Fourth, human and murine neutrophils differ in receptor expression, granule composition, metabolism, and NET responses. Preclinical efficacy of mitochondrial inhibitors may therefore overestimate clinical benefit. Finally, neutrophil heterogeneity matters. Mature high-density neutrophils, immature populations, low-density granulocytes, and tumor-associated neutrophils may use mitochondrial pathways differently. Single-cell transcriptomics, metabolic profiling, and spatial imaging should be integrated with functional assays to identify the populations that produce pathogenic mROS-rich NETs.^[22]

9. Future Research Priorities

Future work should move from broad descriptions of “oxidative stress” toward stimulus-, species-, and compartment-specific redox biology. Important priorities include defining how mitochondrial complex I versus complex III contributes to particular neutrophil responses; mapping the sequence linking calcium entry, MCU activity, mitochondrial dynamics, mROS, PAD4, and DNA repair; and determining when mitochondrial DNA-containing NETs arise in vivo.

Clinical translation requires validated biomarkers that distinguish NOX2-dominant NETosis from mROS-dominant NETosis. Candidate panels may combine oxidized mitochondrial DNA, mitochondrial DNA-protein complexes, citrullinated histone H3, MPO-DNA complexes, and disease-specific cytokines. Longitudinal studies should establish whether these markers predict flare, thrombosis, organ injury, or treatment response.

Therapeutic development should prioritize neutrophil-selective or lesion-targeted delivery. Short-lived nanoparticles, antibody-guided carriers, prodrugs activated in inflamed tissue, or inhaled formulations for pulmonary disease may reduce systemic toxicity. Trials should monitor infection, wound healing, vaccine responses, and tumor surveillance rather than focusing only on inflammatory endpoints.

A final priority is temporal treatment design. Early infection may benefit from intact or even enhanced mROS-mediated killing, whereas later sterile inflammation may require suppression of mROS, NET clearance, and promotion of apoptosis. The most effective intervention may therefore change across the course of the same illness.

10. Conclusion

Mitochondria have emerged as central regulators of neutrophil behavior despite their limited contribution to bulk ATP production. mROS connect calcium signaling, mitochondrial dynamics, NOX2 activation, degranulation, migration, hypoxic adaptation, survival, and NET formation. Their role is especially

prominent in NOX-independent NETosis and in the generation of oxidized mitochondrial DNA-rich NETs that activate interferon and thrombo-inflammatory pathways.

This biology presents both opportunity and risk. Mitochondria-targeted antioxidants, PAD4 inhibitors, DNase, mitochondrial calcium modulators, and DNA-sensing antagonists can reduce pathological NET-associated inflammation in experimental models. Yet mROS also support microbial killing and adaptive neutrophil responses. Successful therapy will therefore require precision rather than indiscriminate antioxidant suppression. Mechanistically defined patient selection, robust mROS and NET assays, targeted delivery, and attention to disease stage are necessary to transform mitochondrial neutrophil biology into safe and effective treatment.

Declarations

Funding: No external funding was received for preparation of this review.

Conflict of interest: The author(s) declare no conflict of interest.

Ethics approval: Not applicable. This article is a narrative review and did not involve human participants or experimental animals.

Data availability: No new dataset was generated or analyzed for this article.

References

1. Papayannopoulos, V. (2018). Neutrophil extracellular traps in immunity and disease. *Nature Reviews Immunology*, 18(2), 134–147. <https://doi.org/10.1038/nri.2017.105>
2. Wang, H., et al. (2024). Neutrophil extracellular traps in homeostasis and disease. *Signal Transduction and Targeted Therapy*, 9, 235. <https://doi.org/10.1038/s41392-024-01933-x>
3. Cao, Z., et al. (2022). Roles of mitochondria in neutrophils. *Frontiers in Immunology*, 13, 934444. <https://doi.org/10.3389/fimmu.2022.934444>
4. Vorobjeva, N. V., et al. (2023). Role of mitochondria in the regulation of effector functions of neutrophils. *International Journal of Molecular Sciences*, 24(18), 14150. <https://doi.org/10.3390/ijms241814150>
5. Douda, D. N., Khan, M. A., Grasemann, H., & Palaniyar, N. (2015). SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. *Proceedings of the National Academy of Sciences of the United States of America*, 112(9), 2817–2822. <https://doi.org/10.1073/pnas.1414055112>
6. Willson, J. A., et al. (2022). Neutrophil HIF-1 α stabilization is augmented by mitochondrial ROS produced under hypoxia. *Blood*, 139(2), 281–286. <https://doi.org/10.1182/blood.2021011010>
7. Nathan, C. (2006). Neutrophils and immunity: Challenges and opportunities. *Nature Reviews Immunology*, 6(3), 173–182. <https://doi.org/10.1038/nri1785>
8. Murphy, M. P., Bayir, H., Belousov, V., et al. (2022). Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nature Metabolism*, 4, 651–662. <https://doi.org/10.1038/s42255-022-00591-z>
9. Vorobjeva, N., Galkin, I., Pletjushkina, O., Golyshev, S., Zinovkin, R., Prikhodko, A., Pinegin, V., Kondratenko, I., & Pinegin, B. (2017). Mitochondrial reactive oxygen species are involved in chemoattractant-induced oxidative burst and degranulation of human neutrophils in vitro. *European Journal of Cell Biology*, 96(3), 254–265. <https://doi.org/10.1016/j.ejcb.2017.03.003>
10. Herring, S. E., et al. (2022). Mitochondrial reactive oxygen species regulate neutrophil antimicrobial activity against *Streptococcus pneumoniae*. *Infection and Immunity*, 90(4), e00531–21.
11. Azzouz, D., et al. (2023). Mitochondrial ROS and base excision repair steps leading to DNA damage-dependent NETosis. *Frontiers in Immunology*, 14, 1175793. <https://doi.org/10.3389/fimmu.2023.1175793>
12. Yousefi, S., Mihalache, C., Kozłowski, E., Schmid, I., & Simon, H. U. (2009). Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death & Differentiation*, 16(11), 1438–1444. <https://doi.org/10.1038/cdd.2009.96>
13. Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., Weinrauch, Y., & Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. *Science*, 303(5663), 1532–1535. <https://doi.org/10.1126/science.1092385>

14. Fuchs, T. A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., Weinrauch, Y., Brinkmann, V., & Zychlinsky, A. (2007). Novel cell death program leads to neutrophil extracellular traps. *Journal of Cell Biology*, 176(2), 231–241. <https://doi.org/10.1083/jcb.200606027>
15. Kaplan, M. J., & Radic, M. (2012). Neutrophil extracellular traps: Double-edged swords of innate immunity. *Journal of Immunology*, 189(6), 2689–2695. <https://doi.org/10.4049/jimmunol.1201719>
16. Azzouz, D., Khan, M. A., Palaniyar, N., & colleagues. (2021). ROS induces NETosis by oxidizing DNA and initiating DNA repair. *Cell Death Discovery*, 7, 113. <https://doi.org/10.1038/s41420-021-00491-3>
17. Vorobjeva, N. V., & Chernyak, B. V. (2020). NETosis: Molecular mechanisms, role in physiology and pathology. *Biochemistry (Moscow)*, 85(10), 1178–1190. <https://doi.org/10.1134/S0006297920100065>
18. Azzouz, D., et al. (2024). How do ROS induce NETosis? Oxidative DNA damage, repair, and neutrophil extracellular trap formation. *Antioxidants*, 13(10), 1245.
19. Lood, C., Blanco, L. P., Purmalek, M. M., Carmona-Rivera, C., De Ravin, S. S., Smith, C. K., Malech, H. L., Ledbetter, J. A., Elkon, K. B., & Kaplan, M. J. (2016). Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. *Nature Medicine*, 22(2), 146–153. <https://doi.org/10.1038/nm.4027>
20. Liao, T. L., et al. (2021). MicroRNA-223 inhibits neutrophil extracellular traps formation through regulating calcium influx and mitochondrial reactive oxygen species. *Scientific Reports*, 11, 15676. <https://doi.org/10.1038/s41598-021-95028-0>
21. Hoppenbrouwers, T., Autar, A. S. A., Sultan, A. R., Abraham, T. E., van Cappellen, W. A., Houtsmuller, A. B., van Wamel, W. J. B., van Beusekom, H. M. M., van Neck, J. W., & de Maat, M. P. M. (2017). In vitro induction of NETosis: Comprehensive live imaging comparison and systematic review. *PLOS ONE*, 12(5), e0176472. <https://doi.org/10.1371/journal.pone.0176472>
22. Rosales, C. (2018). Neutrophil: A cell with many roles in inflammation or several cell types? *Frontiers in Physiology*, 9, 113. <https://doi.org/10.3389/fphys.2018.00113>
23. Kolaczowska, E., & Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. *Nature Reviews Immunology*, 13(3), 159–175. <https://doi.org/10.1038/nri3399>