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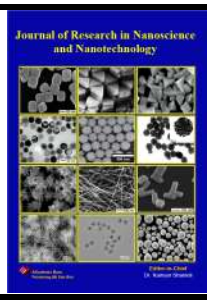

Exploring Red Blood Cell Fragility in Diabetes: A Nanoscale View of Calcium Dysregulation and Oxidative Stress

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ABSTRACT

Oxidative stress and disrupted calcium homeostasis are key contributors to vascular dysfunction in diabetes mellitus. This study explores their combined effects on red blood cell (RBC) membrane integrity, emphasizing nanoscale alterations associated with membrane fragility. RBCs isolated from healthy individuals and patients with poorly controlled diabetes (HbA1c >15%) were exposed to oxidative stress (250 nM H_2O_2), elevated extracellular calcium (1.25 mM $CaCl_2$), their combination, and a recovery condition using the calcium chelator EDTA. Hemolysis was monitored spectrophotometrically over a period of 12–20 days to assess changes in membrane stability. Exposure to elevated calcium significantly increased osmotic fragility in both groups, with diabetic RBCs demonstrating heightened susceptibility. While oxidative stress alone induced minimal hemolysis, it markedly amplified calcium-mediated membrane damage under combined conditions. The addition of EDTA effectively restored membrane stability, highlighting the pivotal role of calcium dysregulation in membrane destabilization. These findings suggest that diabetic RBCs exhibit intrinsic nanoscale membrane vulnerability, which is further intensified by the synergistic interaction between calcium overload and oxidative stress. This model provides mechanistic insight into membrane destabilization processes relevant to diabetic vascular complications and underscores calcium modulation as a potential therapeutic target.

Keywords:
Red Blood Cells, Oxidative Stress, Calcium Dysregulation, Diabetes Mellitus, Membrane Fragility, Nanoscale Analysis

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1. Introduction

Diabetes mellitus is a major risk factor for vascular complications, particularly those involving microvascular dysfunction and impaired endothelial integrity (1). Among the key pathological drivers of these complications are elevated levels of reactive oxygen species (ROS) and disrupted intracellular calcium (Ca^{2+}) homeostasis, both of which are consistently observed in poorly controlled diabetes (2). These alterations are strongly associated with vascular abnormalities such as endothelial dysfunction, reduced angiogenic capacity, and increased microvascular fragility (3,4).

At the cellular level, oxidative stress and Ca^{2+} overload exert profound effects on membrane stability. They disrupt lipid organization, alter cytoskeletal architecture, and impair intercellular communication, ultimately compromising membrane integrity (5,6). From a nanoscale perspective, these changes manifest as alterations in membrane fluidity, permeability, and structural organization, which are critical determinants of cellular resilience under stress conditions (7).

Red blood cells (RBCs) provide a practical and physiologically relevant model for studying membrane destabilization. Despite lacking organelles, RBC membranes share key structural and biochemical characteristics with vascular cell membranes, including lipid composition and susceptibility to oxidative and Ca^{2+}-mediated damage (8,9). Hemolysis, as a measurable outcome of membrane disruption, serves as a sensitive indicator of membrane fragility (10). Importantly, variations in hemolytic behavior reflect underlying changes in membrane phospholipid composition, cytoskeletal integrity, and intracellular levels of Ca^{2+} and ROS, making RBCs a valuable surrogate system for investigating vascular stress mechanisms (11,12).

Under physiological conditions, ROS and Ca^{2+} function as essential signaling mediators, regulating cellular metabolism and homeostasis (13). However, in pathological states such as chronic hyperglycemia, their roles shift toward promoting cellular injury (14). Their interaction is highly interconnected: ROS modulate intracellular Ca^{2+} levels through effects on membrane transport systems, while elevated Ca^{2+} further enhances ROS production via activation of enzymatic pathways (15,16). This bidirectional relationship creates a self-amplifying cycle that accelerates membrane destabilization and cellular damage (17).

Experimentally, hydrogen peroxide (H_2O_2) is widely used to model oxidative stress, while calcium chloride ($CaCl_2$) serves as a reliable inducer of Ca^{2+} overload (18). The combined application of these factors provides a controlled framework for examining the synergistic effects of oxidative and calcium stress on membrane integrity. Additionally, calcium chelators such as EDTA offer a means to probe the specific contribution of Ca^{2+} to membrane destabilization and to evaluate potential protective strategies.

In this study, we investigate RBC membrane fragility under conditions of oxidative stress and Ca^{2+} dysregulation, with particular emphasis on their combined effects at the nanoscale level. Using spectrophotometric analysis of hemolysis, we compare responses between healthy individuals and patients with poorly controlled diabetes. Furthermore, the protective role of Ca^{2+} chelation is assessed to better understand the mechanistic contribution of calcium in membrane destabilization. This approach provides insight into fundamental processes underlying diabetic vascular complications and highlights potential avenues for therapeutic intervention.

2. Methods

2.1 Study Population

Blood samples were collected from two groups: (i) eight healthy volunteers aged 18–25 years with no known metabolic disorders, and (ii) eight diabetic patients aged 45–65 years with glycated

hemoglobin (HbA1c) levels greater than 15%, indicating poor glycemic control. The A1c level exceeding 15 represents the maximum degree of diabetes-induced cellular stress, and our objective was to maintain the cell at its external limit (externe) under these extreme conditions. Informed consent was obtained from all participants prior to sample collection in accordance with institutional ethical guidelines.

2.2 Sample Preparation

Heparinized venous blood samples (5 mL each) were collected from participants and processed to isolate RBCs. The whole blood was first centrifuged at 1,500 × g for 10 minutes to separate the plasma. The resulting RBC fraction was then washed three times with an isotonic buffered saline solution (155 mM NaCl, 10 mM phosphate buffer, pH 7.4, 300 mOsm) to remove residual plasma and buffy coat.

For the osmotic fragility assay, the washed RBCs were resuspended in a hypotonic buffered saline (BS; 125 mM NaCl, 10 mM phosphate buffer, pH 7.4, 250 mOsm) to a final hematocrit of 8%. The reduced osmolarity of 250 mOsm was intentionally selected to create a consistent, mildly hypoosmotic stress on the RBC membrane. Adjusting the hematocrit to 8% served a dual purpose: it minimized the background level of spontaneous hemolysis while ensuring that subsequent spectrophotometric measurements of hemoglobin release would fall within the instrument's optimal linear detection range.

2.3 Experimental Conditions

The osmotic fragility test was designed to systematically evaluate the effects of oxidative stress and Ca^{2+} imbalance on red blood cell (RBC) membrane integrity. The assay utilized five experimental groups, each containing a 3 mL aliquot of RBC suspension in a specific test medium as described in Table 1. This design allowed for the isolation and combination of key stressors: H_2O_2 to induce oxidative damage, $CaCl_2$ to disrupt intracellular Ca^{2+} homeostasis, and EDTA as a Ca^{2+} chelator to inhibit Ca^{2+}-mediated effects. The groups progress logically from a control baseline to individual stressors, their combination, and finally a rescue condition to confirm the role of Ca^{2+}.

Table 1. Experimental groups and media composition of the osmotic fragility test.

Tube	Composition
1	Control (BS only)
2	BS + 250 nM H_2O_2
3	BS + 1.25 mM $CaCl_2$
4	BS + 1.25 mM $CaCl_2$ + 250 nM H_2O_2
5	BS + 1.25 mM $CaCl_2$ + 250 nM H_2O_2 + 10 mM EDTA

2.4 Rationale for Concentrations

The basal solution (BS) consisted of 125 mM NaCl and 10 mM mono-/di-potassium phosphate, adjusted to pH 7.4, with a final osmolality of 250 mOsm. This buffer was used as the standard incubation medium for all experimental conditions. Oxidative stress was induced using 250 nM H_2O_2, a concentration that reflects levels reported during severe inflammatory states (19). This condition was designed to model inflammation-associated oxidative damage. The Ca^{2+}-related effects were evaluated by supplementing the medium with 1.25 mM $CaCl_2$, corresponding to the physiological

concentration of free Ca^{2+} in human plasma. To assess Ca^{2+} chelation, 10 mM EDTA was added, representing a therapeutically relevant concentration of this Ca^{2+}-binding agent.

The experimental design included five conditions. Tube 1 served as the control group and provided baseline hemolysis data. Tube 2 simulated oxidative stress typical of severe inflammation. Tube 3 examined the effects of physiological Ca^{2+} alone. Tube 4 evaluated the combined impact of oxidative stress and Ca^{2+} overload, while Tube 5 assessed the protective effect of Ca^{2+} chelation under simultaneous oxidative and Ca^{2+} stress conditions.

2.5 Incubation and Measurement

Samples were stored at +4°C under continuous gentle rotation to prevent sedimentation. At 48-hour intervals, tubes were removed, centrifuged at 1,500 × g for 10 minutes, and the optical density of the supernatant was measured at 540 nm using a Pharmacia spectrophotometer to quantify hemoglobin release as a marker of hemolysis. This procedure was repeated until approximately 50% hemolysis was observed in the control samples. Primary hemolysis was defined as the initial 1–3% hemolysis observed during the first measurement for each sample, while 50% hemolysis corresponded to an optical density indicating 45–50% RBC lysis. Across both groups, primary hemolysis typically occurred within 2–4 days, and 50% hemolysis within 12–20 days. Complete (100%) hemolysis was defined using 8% hematocrit RBC suspensions in distilled water as the reference standard.

Note on methodological considerations: The hemolytic effect of H_2O_2 is attributed to its oxidative impact on cytoskeletal proteins, membrane phospholipids, and enzymes involved in energy production and Ca^{2+} homeostasis. Although H_2O_2 promotes oxidation of hemoglobin to methemoglobin (which absorbs less at 540 nm and may lead to slight underestimation of hemolysis), this limitation is acceptable within the context of this comparative study between healthy and diabetic groups.

3. Results

Figure 1 shows the time-dependent progression of hemolysis in red blood cells (RBCs) from healthy and diabetic individuals under different experimental stress conditions. The graph illustrates that under baseline control conditions, both groups exhibit minimal and statistically similar hemolysis over time. However, a clear divergence occurs under oxidative stress induced by H_2O_2, where diabetic RBCs demonstrate a notable resistance, showing only 0.24% hemolysis compared to 0.89% in healthy cells. This suggests an adaptive response to chronic oxidative stress in diabetes. In contrast, elevated Ca^{2+} alone causes a moderate and comparable increase in hemolysis for both groups. The most critical finding is observed under combined Ca^{2+} and peroxide stress, where the protective advantage of diabetic RBCs is completely abolished, and hemolysis rates converge sharply between the groups, indicating a synergistic destabilizing effect. The inclusion of the Ca^{2+} chelator EDTA in the combined stress medium markedly reduces hemolysis, confirming the central role of Ca^{2+} in mediating membrane damage. While the conversion of hemoglobin to methemoglobin by H_2O_2 may lead to a slight underestimation of absolute hemolysis, this technical factor affects all samples equally and does not alter the comparative outcome. Collectively, Figure 1, visually demonstrates that diabetic RBCs, while resistant to isolated oxidative stress, possess a specific membrane vulnerability that is unmasked by the synergistic action of concurrent Ca^{2+} overload and oxidative stress.

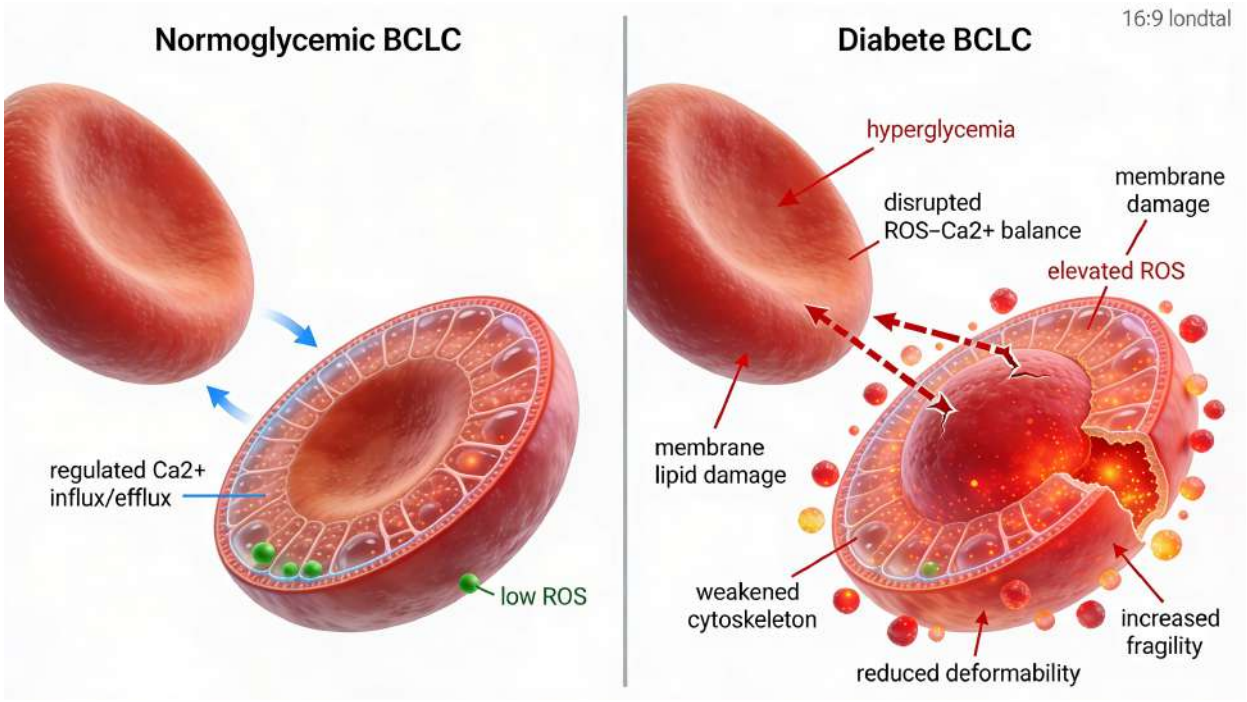

Figure 1. Schematic overview of membrane stability in healthy and diabetic RBCs. The left panel shows normoglycemic RBCs with intact cytoskeleton and regulated Ca^{2+} homeostasis, while the right panel illustrates diabetic RBCs, where hyperglycemia-induced oxidative stress disrupts ROS–Ca^{2+} balance, leading to membrane damage, reduced deformability, and increased fragility.

A comparative analysis was conducted to evaluate the effects of Ca^{2+} and H_2O_2 on RBC osmotic fragility in healthy and diabetic subjects. Five experimental conditions (Tubes 1–5) were tested, and hemolysis parameters, including primary and 50% hemolysis, were assessed both within each group and between groups. Table 2 presents the effects of various extracellular medium compositions on erythrocyte fragility in both control and diabetic blood samples. Table 2 compares five different medium conditions, measuring both initial hemolysis (IH) and 50% hemolysis (CH50) values across control and diabetic groups. The experimental conditions tested include:

(1) Baseline medium containing 125 mM NaCl in PBS
(2) Baseline medium with 250 nM H_2O_2
(3) Baseline medium supplemented with 1.25 mM $CaCl_2$
(4) Baseline medium with both 250 nM H_2O_2 and 1.25 mM $CaCl_2$
(5) Baseline medium containing 250 nM H_2O_2, 1.25 mM $CaCl_2$, and 10 mM EDTA

Results indicate that diabetic erythrocytes demonstrate significantly increased fragility compared to controls under most conditions.

It is noteworthy that condition 2, when H_2O_2 was used, caused significant hemolysis only in the diabetic group ($P \leq 0.001$ for CH50). In condition 3, when $CaCl_2$ was used, it produced a significant increase in IH and CH50 values for both groups, with the effects being more pronounced in diabetic samples ($P \leq 0.001$). Combined treatment of H_2O_2 and $CaCl_2$ (condition 4) resulted in the most sustained and significant increase in all parameters in both groups ($P \leq 0.001$ to $P \leq 0.004$). The addition of EDTA as a Ca^{2+} chelator and antioxidant (condition 5) appeared to exert protective effects, with only diabetic CH50 showing a significant change ($P \leq 0.028$). These findings suggest that diabetic

erythrocytes exhibit enhanced susceptibility to oxidative stress and Ca^{2+}-mediated membrane destabilization.

Table 2. Effect of extracellular milieu on erythrocyte fragility in control and diabetic blood.

No.	Medium Composition (Final Con.)	Control Group		Diabetic Group		Comment
		IH	CH50	IH	CH50	
1	125 mM NaCl, PBS	—	—	—	—	—
2	125 mM NaCl, PBS, 250 nM H_2O_2	—	—	—	$P \leq 0.001$ ↑	Increase 50% hemolysis was observed only in the diabetic group due to the difference in antioxidant capacity between the two groups.
3	125 mM NaCl, PBS, 1.25 mM $CaCl_2$	$P \leq 0.003$ ↑	$P \leq 0.027$ ↑	$P \leq 0.001$ ↑	$P \leq 0.001$ ↑	More significant increase in diabetic 50% hemolysis vs healthy due to differences in Ca^{2+} hemostasis.
4	125 mM NaCl, PBS, 250 nM H_2O_2, 1.25 mM $CaCl_2$	$P \leq 0.001$ ↑	$P \leq 0.002$ ↑	$P \leq 0.004$ ↑	$P < 0.001$ ↑	—
5	125 mM NaCl, PBS, 250 nM H_2O_2, 1.25 mM $CaCl_2$, 10 mM EDTA	—	—	—	$P \leq 0.028$	Ca^{2+} and free radical scavengers improve membrane strength. This effect is more evident in the diabetic group.

Footnotes: Initial hemolysis (IH) and 50% hemolysis (CH50) were used as indicators of membrane stability. PBS consisted of potassium phosphate buffer (K_3PO_4/KH_2PO_4, 0.01 M each). $CaCl_2$ (1.25 mM) represents physiological free plasma Ca^{2+} levels in diabetes. H_2O_2 at 250 nM reflects concentrations observed during severe inflammation, compared with normal physiological levels (~2×10^{-10} M) (20). EDTA was used as a Ca^{2+} chelator with antioxidant properties.

Figure 2 presents a detailed comparative analysis of RBC stability between healthy and diabetic individuals by measuring their breakdown, or hemolysis, over time under various stress conditions. The vertical Y-axis represents the percentage of hemolysis, which quantifies the extent of RBC rupture, while the horizontal X-axis represents the incubation time in days, illustrating how hemolysis progresses over a 12 to 20-day period.

The experiment systematically tested RBC fragility by exposing cells to different media: a control solution, a solution containing H_2O_2 to induce oxidative stress, and media with Ca^{2+} alone or in combination with peroxide. Under baseline control conditions, RBCs from both healthy and diabetic donors showed minimal and statistically identical hemolysis, indicating comparable initial membrane integrity.

A clear and notable divergence emerged under oxidative stress induced by H_2O_2. In this condition, RBCs from diabetic individuals demonstrated significantly greater resistance, exhibiting only 0.24% hemolysis compared to 0.89% for healthy RBCs. This finding suggests an adaptive cellular response to chronic oxidative stress, a well-known feature of diabetes. In contrast, the introduction of Ca^{2+} into the medium resulted in a moderate and similar increase in hemolysis for both groups, with no significant difference between them.

The most critical observation came from the combined stress condition, where RBCs were exposed to both Ca^{2+} and H_2O_2 simultaneously. In this environment, the protective advantage observed in

diabetic RBCs against peroxide alone was completely abolished. The hemolysis rate for diabetic RBCs increased sharply, nearly matching that of healthy cells, with the difference between groups becoming borderline significant.

This result reveals a specific vulnerability in the diabetic RBC membrane, indicating that while these cells may adapt to individual stressors, their defense mechanisms are overwhelmed when faced with multiple concurrent challenges. This insight helps explain part of the hematological complications associated with diabetes, where RBCs must endure complex physiological stresses in vivo.

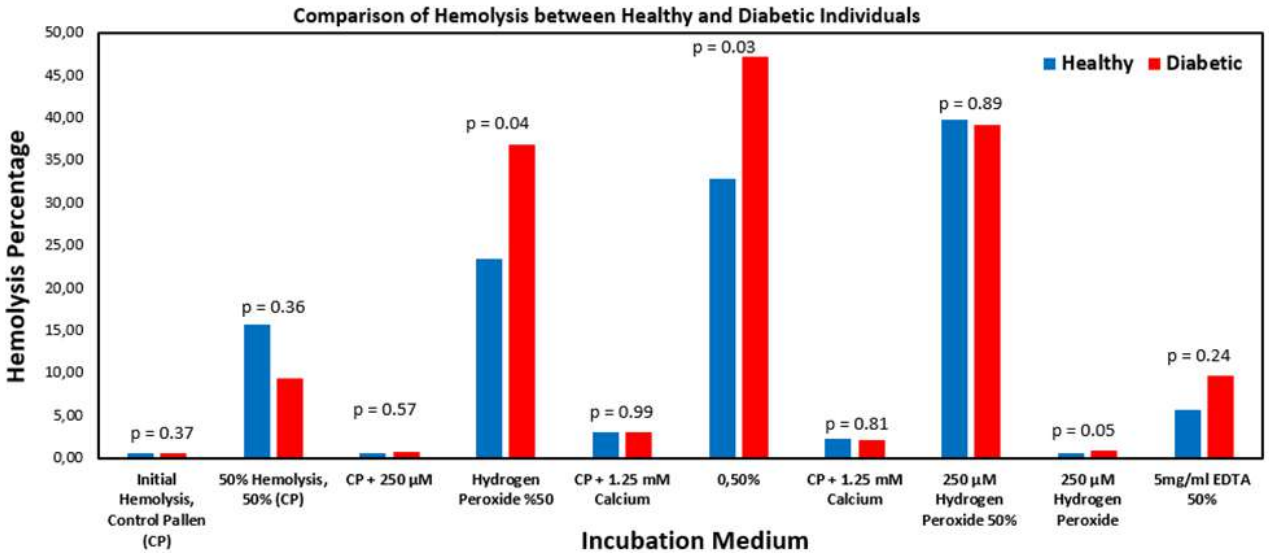

Figure 2. Comparative analysis of osmotic fragility in healthy versus diabetic RBCs under controlled stress conditions.

3.1 Synergistic Oxidative and Calcium Stress Underlies Diabetic RBCs Fragility

The experimental findings reveal distinct patterns of membrane fragility in diabetic RBCs under controlled stresses. Under baseline conditions in an additive-free medium, diabetic RBCs exhibited no significant difference in initial hemolysis but demonstrated a slight reduction in osmotic fragility at the 50% hemolysis point. This paradoxical resilience can be attributed to mild oxidation of the cytoskeletal protein spectrin, which alters its biomechanical properties (21). This modification stems from a chronic state of oxidative stress in diabetes, characterized by a systemic reduction in antioxidant enzyme capacity (22) that elevates free radical levels. Under laboratory conditions, this pre-existing oxidation can subtly modify membrane mechanics, thereby influencing spectrin-mediated membrane adhesion and cohesion (23).

When exposed to exogenous oxidative stress via 250 nM H_2O_2, hemolysis increased significantly in both groups, with diabetic RBCs showing markedly greater susceptibility. This aligns with their compromised endogenous antioxidant defenses, rendering them less capable of neutralizing additional oxidative insult. Elevating extracellular Ca^{2+} to 1.25 mM further induced substantial membrane destabilization, an effect more pronounced in diabetic cells. This highlights Ca^{2+} role as a primary mediator of membrane damage and points to an underlying impairment in Ca^{2+} homeostasis within diabetic erythrocytes.

Simultaneous exposure to elevated Ca^{2+} and oxidative stress produced the most severe membrane disruption, indicating a synergistic interaction that led to the highest recorded hemolysis levels. A

technical note is that H_2O_2 can convert hemoglobin to methemoglobin, which has a lower optical absorbance, potentially causing a slight underestimation of true hemolysis. However, as this effect occurs equally across samples, it does not affect the comparative results. The data robustly confirm that Ca^{2+} overload and oxidative stress jointly destabilize the RBC membrane, with diabetic cells displaying heightened vulnerability.

The central mechanistic role of ionic Ca^{2+} was confirmed by adding the chelator EDTA to the combined stress medium. By sequestering free Ca^{2+}, EDTA substantially restored membrane integrity and reduced hemolysis in both groups to near-control levels, though a higher residual fragility persisted in diabetic samples. This indicates an intrinsic membrane weakness in diabetes beyond acute Ca^{2+} toxicity. While EDTA's protective effect is mechanistically informative, its pharmacodynamic profile limits its clinical applicability.

Temporally, diabetic RBCs showed consistently elevated fragility across all stressors. The hemolysis progression curves visually underscore their inherent susceptibility, which is profoundly exacerbated by combined oxidative and Ca^{2+} stress. All differences between healthy and diabetic cells were statistically significant ($p \leq 0.001$), robustly highlighting this synergistic pathway as a critical contributor to erythrocyte pathology and a potential mechanism underlying vascular complications in diabetes.

4. Discussion

This study reveals key differences in Ca^{2+} homeostasis and antioxidant capacity between diabetic and healthy RBCs, with implications for vascular complications in diabetes. Diabetic RBCs were consistently more susceptible to hemolysis under all stress conditions ($p \leq 0.001$), reflecting intrinsic membrane fragility due to chronic metabolic dysregulation. The Ca^{2+} emerged as the primary mediator of membrane destabilization, inducing significant hemolysis at physiological concentrations (1.25 mM), with diabetic RBCs showing greater vulnerability (47.25% vs. 32.85% at 50% hemolysis). The H_2O_2 (250 nM) alone had modest effects but enhanced Ca^{2+}-induced damage, highlighting the interplay between Ca^{2+} overload and oxidative stress through pathways including ATPase inhibition, mechanosensitive channel activation, NADPH oxidase stimulation (24), and cytoskeletal protein degradation. EDTA chelation substantially reduced Ca^{2+}-induced hemolysis (5.66% in healthy vs. 9.70% in diabetic RBCs), confirming Ca^{2+} central role, though residual fragility in diabetic cells indicates additional pathological mechanisms. Prolonged exposure under energy-depleted, mildly hypoosmotic conditions allowed progressive destabilization of cytoskeletal proteins such as actin and spectrin, modeling chronic vascular stress in diabetes. Given structural parallels between RBC and endothelial membranes, these findings provide mechanistic insight into diabetic microangiopathy and support the investigation of antioxidant and Ca^{2+}-modulating therapies and RBC fragility as predictive biomarkers for vascular complications.

Schematically contrasts the membrane architecture of a healthy RBC with that of a diabetic RBC exposed to sustained oxidative stress. In the healthy state (left), the lipid bilayer is smooth, and the underlying actin–spectrin cytoskeleton remains intact; physiological H_2O_2 is efficiently detoxified by antioxidant enzymes and cytosolic Ca^{2+} is kept low by active Ca-ATPase pumps. Under diabetic conditions (right), hyperglycaemia drives overproduction of ROS (25) and actin polymerization in the rat retinal vasculature (26) and vascular smooth muscle (27), elevating intracellular H_2O_2 that oxidises membrane lipids and proteins. The resulting oxidative inhibition of Ca-ATPases, together with Ca^{2+} influx through mechanosensitive channels, raises cytosolic Ca^{2+} to levels that activate the protease calpain (Cas), which promotes the aggregation of cytoskeleton proteins (28). This progression leads to membrane blebbing, loss of deformability, and eventual microvesiculation (29).

These alterations shorten RBC lifespan and provide a mechanistic link between oxidative Ca^{2+} stress and the osmotic fragility commonly observed in diabetes.

In biological systems, free radicals are highly unstable and cannot directly cross cell membranes. Upon generation, they are rapidly converted into H_2O_2, which is membrane-permeable (30). Under physiological conditions, intracellular antioxidant enzymes immediately neutralize H_2O_2. However, during severe inflammation or metabolic stress, free radical production exceeds intracellular antioxidant capacity, leading to elevated ROS levels, compromised membrane integrity, and increased osmotic fragility.

The significant difference in time to 50% hemolysis observed in H_2O_2-containing media between healthy and diabetic groups can be attributed to differences in antioxidant capacity. Numerous studies have documented reduced antioxidant enzyme activity in diabetes (31,32), including decreased levels of superoxide dismutase, catalase, and glutathione peroxidase. Similarly, the pronounced difference in hemolysis progression in Ca^{2+}-containing media reflects disparities in Ca^{2+} homeostasis between the groups. The differences in 50% hemolysis between healthy and diabetic groups reflect fundamental differences in their Ca^{2+} homeostatic mechanisms. The role of elevated intracellular Ca^{2+} in increasing cellular fragility and reducing membrane stability has been well-established in multiple experimental systems (33).

The protective effect of EDTA on osmotic fragility highlights its dual role as both a Ca^{2+} chelator and an indirect antioxidant (34). By sequestering extracellular Ca^{2+}, EDTA prevents Ca^{2+}-mediated activation of oxidative pathways (35) and membrane-associated proteases (36). The residual differences in hemolysis observed between healthy and diabetic RBCs, even in the presence of EDTA, may reflect variations in intrinsic cellular redox state, membrane lipid composition, and Ca^{2+} buffering capacity. Although clinical use of EDTA is limited due to its non-selective chelation properties and potential side effects, this RBC osmotic fragility model provides a valuable platform for studying the effects of antioxidant and Ca^{2+}-regulating interventions on membrane stability.

The extended time course required to achieve 50% hemolysis (12–20 days) allowed sustained interaction between intracellular Ca^{2+} and H_2O_2 with cytoskeletal proteins, particularly actin, which is highly sensitive to both elevated Ca^{2+} and oxidative modifications. Previous studies have demonstrated spatial alterations in actin organization under conditions of increased intracellular Ca^{2+} and H_2O_2 exposure. Our laboratory's previous work showed that prolonged exposure of isolated RBC membranes to supraphysiological Ca^{2+} concentrations at 4°C induces actin aggregation and progressive cytoskeletal protein degradation proportional to both Ca^{2+} concentration and exposure duration (37).

Furthermore, intracellular Ca^{2+} and free radicals exhibit a bidirectional amplification relationship: increases in one mediator promote increases in the other through multiple mechanisms, including mitochondrial dysfunction, Ca^{2+}-dependent activation of NADPH oxidase (38), and ROS-mediated impairment of (Na/K) ATPases (39). The heightened sensitivity of actin to Ca^{2+} is particularly relevant to vascular complications because actin plays a central structural role in maintaining the integrity of endothelial cell membranes and the secretory organelles responsible for basement membrane component synthesis and deposition. Disruption of the actin cytoskeleton in endothelial cells compromises barrier function, increases vascular permeability (40, 41) and impairs angiogenic responses, all hallmark features of diabetic microangiopathy.

The biochemical and structural parallels between RBC membranes and vascular endothelial cell membranes, including shared mechanisms of Ca^{2+} and redox homeostasis, support the utility of RBCs as an accessible experimental model. This model can facilitate investigation of membrane-protective interventions and provide mechanistic insights translatable to endothelial dysfunction in diabetic vascular disease.

5. Conclusion

This study confirms that RBCs possess an intrinsic membrane fragility, demonstrating consistently greater susceptibility to hemolysis under stress. The Ca^{2+} overload, even at physiological concentrations, emerged as the primary destabilizing mediator by promoting cytoskeletal protein aggregation, particularly actin. Furthermore, oxidative stress, modeled by H_2O_2, significantly amplified this Ca^{2+}-induced membrane damage synergistically. These combined effects, which are pronounced in the diabetic cohort due to compromised antioxidant and homeostatic mechanisms, underscore the role of Ca^{2+} and redox imbalance in cellular injury. Importantly, Ca^{2+} chelation with EDTA effectively restored membrane stability, highlighting Ca^{2+}-modulating pathways as potential therapeutic targets for diabetic vascular complications. Given the structural similarities between RBCs and vascular endothelial cells, this in vitro osmotic fragility model provides a sensitive and accessible platform for investigating microangiopathy mechanisms and evaluating protective interventions before clinical trials.

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Conflicts of Interest

The authors declare no conflicts of interest.

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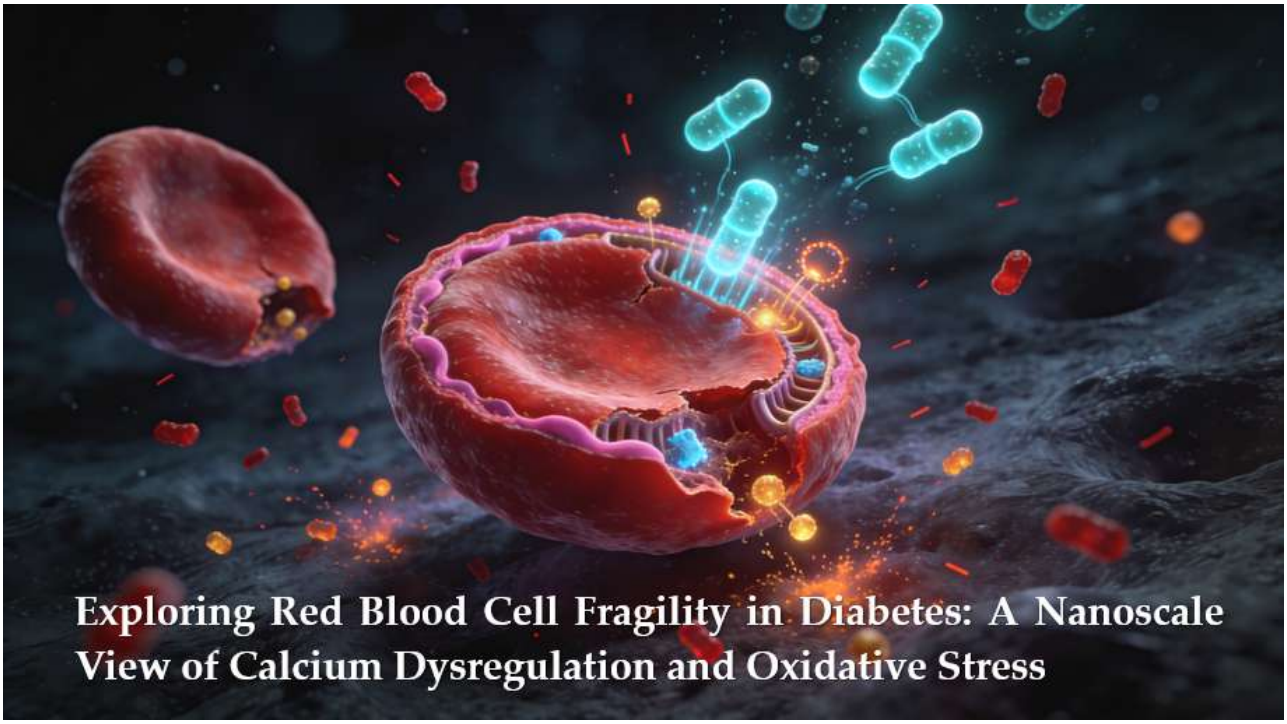

Graphical Abstract