

Original Article

Enzymatic dissolution of calcium oxalate calculi using oxalate decarboxylase: an in vitro pilot study

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Abstract: Introduction: Chemical dissolution of kidney stones has been studied, yet clinical application remains elusive. Previous research highlights the potential of biological systems to prevent or dissolve kidney stones, including the oxalate decarboxylase (ODC) enzyme, which catalyzes the conversion of oxalate into formate and carbon dioxide. This study examines the capability of ODC in promoting calcium-oxalate (Ca-Ox) stone breakdown in preclinical models. Methods: After IRB approval and patient consent, patient-derived Ca-Ox stones (equal distribution of monohydrate and dihydrate) were categorized into five size groups (1-5 mm) and exposed to increasing ODC amounts (50-800 µg, Creative Enzymes®) in artificial urine at pH 4 and 37 °C. Dissolution was evaluated over 2 hours to 7 days by measuring changes in stone weight with a precision analytical balance and diameter with a high-resolution digital microscope. Controls were included for each size group. Final analyses incorporated infrared spectroscopy to confirm dissolution products. Results: Control stones showed minimal variability in weight and size, with median changes under 2%. Across all ODC amounts and stone sizes, no consistent trends in weight or diameter reduction were observed, and apparent reductions were attributed to measurement variability rather than true dissolution. For the 50 µg ODC amount, the relative weight change ranged from -14% to +0.5%. For the 800 µg ODC amount, the relative weight change ranged from -7% to +16%. Biochemical analysis of reaction precipitates failed to detect stone breakdown products. Conclusion: ODC did not achieve measurable Ca-Ox dissolution under the tested conditions. This supports the notion that established Ca-Ox stones contain bonds highly resistant to enzymatic breakdown. Further investigations under more extreme experimental settings are warranted.

Keywords: Oxalate decarboxylase, calculi, calcium, oxalate, dissolution

Introduction

The dissolution of urolithiasis using chemical agents has been documented for decades. Despite this, the clinical goal of achieving rapid and effective dissolution via chemolysis remains unmet. Over the past 25 years, significant research efforts have been dedicated to exploring innovative approaches, particularly leveraging biological systems [1]. Among these, plant and microbial enzymes, known for their ability to catabolize salts, have emerged as promising candidates for addressing this challenge [2, 3]. Most of the investigated agents have primarily targeted radiolucent stones, particularly those composed of uric acid [1, 4, 5]. While the dissolution of uric acid stones through

chemical urinary alkalization is well established in clinical practice and can successfully dissolve even large calculi [5], the breakdown and dissolution of calcium-based stones have yet to be translated into routine clinical care. Consequently, current research efforts are increasingly focused on oxalate-degrading microorganisms and enzymes.

Calcium-oxalate (Ca-Ox) calculi is a prevalent condition, with hyperoxaluria being a primary risk factor. Hyperoxaluria is a condition characterized by excessive urinary excretion of oxalate, resulting from either increased endogenous oxalate production (primary hyperoxaluria), increased intestinal absorption (enteric hyperoxaluria), or dietary factors. Elevated uri-

nary oxalate promotes the formation of Ca-Ox crystals and is one of the strongest risk factors for recurrent kidney stone disease. In severe or untreated cases, persistent crystal deposition can lead to chronic kidney disease, kidney failure, and systemic oxalosis [6, 7].

Recent advances in the understanding of oxalate metabolism have highlighted the potential of oxalate-degrading microorganisms and enzymes as novel strategies for the prevention and treatment Ca-Ox stones. Among these, *Oxalobacter formigenes* has received particular attention because of its ability to metabolize intestinal oxalate, thereby reducing urinary oxalate excretion and mitigating hyperoxaluria. Epidemiological studies have demonstrated an association between loss of *O. formigenes* colonization (often following antibiotic exposure) and increased stone recurrence, while animal models have shown its presence may offer protection by reducing diet-induced hyperoxaluria, in some cases up to 70% [3, 8-10]. However, translation into clinical practice has been limited by difficulties achieving stable intestinal colonization, susceptibility to antibiotics, and inconsistent efficacy across human studies. Free oxalate-degrading enzymes have emerged as an alternative approach, however, significant barriers remain before these therapies can be clinically implemented. Calcium-containing stones are highly stable and poorly soluble under physiologic conditions, and most dissolution agents that show efficacy in-vitro require extreme pH conditions, high concentrations, or prolonged exposure times that may be impractical or unsafe in vivo. Furthermore, effective dissolution is hindered by limited penetration into the stone core, stone heterogeneity, inadequate contact between the agent and the entire stone surface, and concerns regarding local toxicity and systemic absorption. Additional challenges specific to enzyme-based therapies include inhibition by reaction products and the need for improved enzyme engineering and delivery systems. Consequently, despite encouraging proof-of-concept studies, no safe and effective chemolytic therapy for calcium-containing urinary stones has yet been established for routine clinical practice, and further advances are required to achieve clinically meaningful dissolution rates and treatment effect sizes [11].

Oxalate decarboxylase (ODC), a member of the cupin superfamily, catalyzes the cleavage

of oxalate into carbon dioxide and formate (Oxalate $\rightarrow$ Formate + CO₂). Unlike Ca-Ox, calcium-formate is significantly more water-soluble, potentially reducing the risk of stone formation. The rationale for this study emerges from experimental evidence highlighting the promise of enzyme-based therapies. Thalji et al. (2011) incubated Ca-Ox crystals with ODC, observing 15-fold increased calcium levels within 3-5 days, indicating stone dissolution, while controls showed no change after 15 days [12]. Building on this foundation, our preclinical study integrates laboratory-based experiments to evaluate the efficacy of ODC in facilitating the breakdown of genuine Ca-Ox kidney stones.

Experimental protocol

This in-vitro study investigates the effectiveness of varying amounts of ODC (the enzyme) in dissolving Ca-Ox stones of different sizes (the substrate), retrieved from real patients, under simulated physiological conditions. To reflect potential clinical relevance, we assessed macroscopic outcomes across different exposure durations. The study was conducted in the state-of-the-art Urology research laboratories at the Icahn School of Medicine at Mount Sinai, which support advanced research in molecular biology, chemistry, and translational science through access to specialized equipment, core facilities, and expert faculty.

Stone samples (substrate)

Following institutional review board approval (STUDY-24-01118, 1/30/2025, Ichan School of Medicine at Mount Sinai) Ca-Ox stones were collected from patients undergoing surgical stone removal. After compositional analysis using Fourier-transform infrared (FTIR) spectroscopy, only stones composed of 100% calcium oxalate were included for the experiment, with an equal distribution of 50% calcium oxalate monohydrate and 50% calcium oxalate dihydrate. Using sieves the selected stones and fragments were categorized by size into 5 diameter groups: 1 mm, 2 mm, 3 mm, 4 mm, and 5 mm (**Figure 1A**). These sizes were chosen to replicate small stones and residual fragments commonly encountered in clinical practice, which are considered clinically relevant, as they have been associated with less favorable outcomes compared with completely stone-free patients [13, 14]. Overall, 30 stone fragments were included in the study, with 6 fragments allocated to each size category.

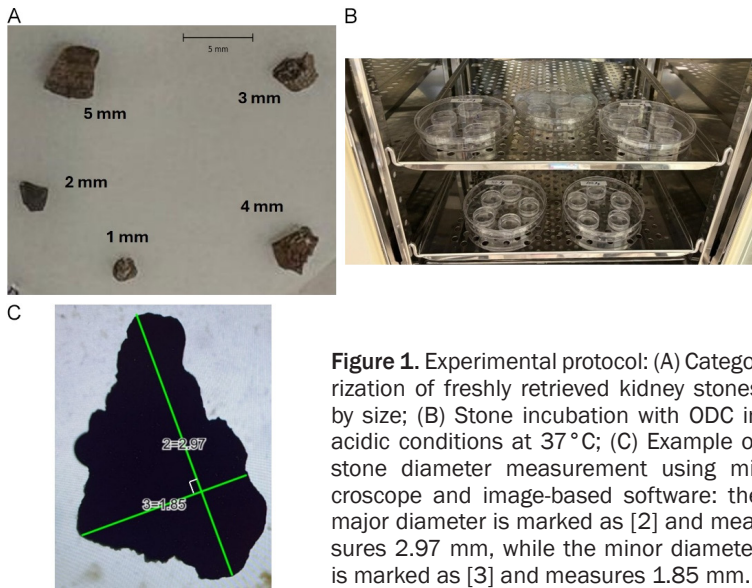


Figure 1. Experimental protocol: (A) Categorization of freshly retrieved kidney stones by size; (B) Stone incubation with ODC in acidic conditions at 37°C; (C) Example of stone diameter measurement using microscope and image-based software: the major diameter is marked as [2] and measures 2.97 mm, while the minor diameter is marked as [3] and measures 1.85 mm.

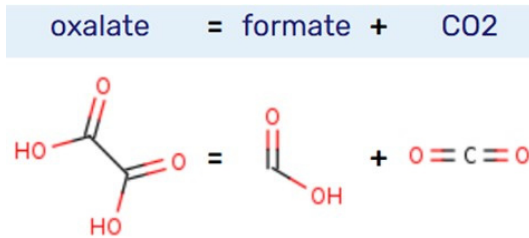


Figure 2. Oxalate decarboxylase catalytic reaction.

ODC enzyme

ODC from *Bacillus subtilis* (cat. no. EXWM-4764; Creative Enzymes®; USA), was used in this study for its specific catalytic ability to convert oxalate into formate and carbon dioxide (**Figure 2**). This recombinant enzyme, supplied as a lyophilized powder, has a molecular weight of approximately 43 kDa, stored at +4°C for short term and -20°C~-80°C for long term, and its catalytic activity is optimized in acidic environment. It is considered non-hazardous research-grade enzyme, requiring standard laboratory precautions. According to the manufacturer's assay protocol, one unit of enzyme activity is defined as the amount of enzyme that converts 1 µmol of oxalate to formate and CO₂ per minute at pH 3.0 and 37°C.

The reaction protocol, outcomes and measurements

The reaction settings were based on previous studies and the enzyme manufacturer's in-

structions [12]. Ten milligrams of lyophilized ODC powder were reconstituted in 10 mL of 0.1% sodium azide (NaN₃; CAS no. 26628-22-8; cat. no. 08591; Sigma-Aldrich; USA) solution to enhance enzyme stability during the reaction. Five stones from each diameter group were included in the experiment, totaling 25 reactions. For each diameter group, different amounts of ODC were tested: 50 µg, 100 µg, 200 µg, 400 µg, and 800 µg (5 enzyme amounts × 5 size groups). Stones and ODC were placed together in 5 mL of artificial urine (cat. no. SAE0074; Sigma-Aldrich; USA), adjusted

to pH 4 to provide an acidic environment favorable for ODC activity while approximating the lower end of urinary pH which is safe to the urothelium [15], and incubated at 37°C to simulate physiological conditions. Each stone was incubated in a separate container (**Figure 1B**). Control samples without enzyme were included for each stone size (5 stones × 1 control group). No biological replicates were included in either the enzyme-treated or control groups. Stone dissolution and structural breakdown were assessed at multiple time points: 2 hours, 24 hours, 2 days, 4 days, and 7 days.

Clinically relevant outcomes included stone weight and diameter. At each time point, the transparent containers were placed under a high-resolution digital microscope with up to ×100 magnification, and images of the stones were captured and uploaded to the research computer. Image-based measurements were conducted using *Photo Measure*, an open-access, browser-based software tool (<https://eleif.net/photomeasure>). The software enables scale calibration based on a known reference and allows for accurate measurement of distances within the same plane. For each stone, the two largest perpendicular diameters were measured to determine the major and minor axes (**Figure 1C**). For weight measurements, stones were removed from their containers, dried under a heating lamp for 30 minutes, and weighed using an Ohaus Discovery precision analytical balance (Model DV215CD; OHAUS

Oxalate decarboxylase and Ca-Ox stone dissolution

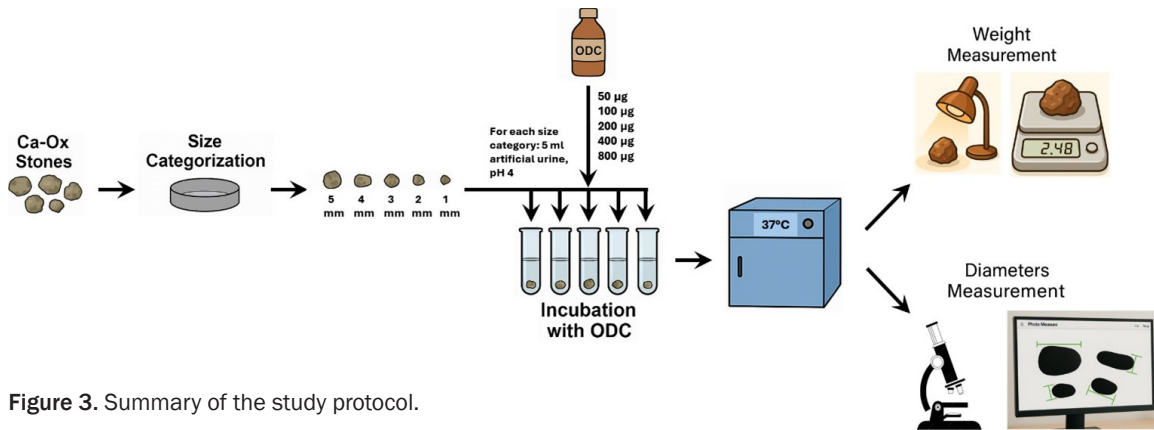


Figure 3. Summary of the study protocol.

corp., Parsippany, NJ; USA) with 0.1 mg readability. Following documentation of both diameters (mm) and weights (grams), the stones were returned to their respective containers to continue the reaction and measurements were compared to baseline dimensions recorded at time zero. To biochemically confirm stone dissolution, at the final time point (day 7), the stones were removed, the remaining solution was dried, and the resulting precipitate was analyzed for dissolution products (minerals or crystalline constituents) via infrared spectroscopy. The full study protocol is illustrated in **Figure 3**.

Statistical analysis

Results are presented descriptively as absolute values and percentage changes in stone weight relative to baseline. Data management and descriptive calculations were performed using Microsoft Excel v.16.0 (Microsoft Corporation, Redmond, WA, USA).

Results

Controls

Supplementary Table 1 elaborates the changes in weight, major diameter, and minor diameter across time points for the control group, stratified by stone size (1-5 mm). Across all control group size categories (1-5 mm) and evaluated time points, the median relative change in weight was 1.8%, in major diameter 1.6%, and in minor diameter 0.6%. Weight changes ranged from 5.8% (2 hours) to 18.1% (4 days) for 1 mm stone, -10.2% (2 hours) to 17.7% (4 days) for 2 mm stone, -2.7% (24 hours) to 1.8% (1

week) for 3 mm stone, -3.3% (2 days) to 15.7% (4 days) for 4 mm stone, and 0% (24 hours) to 1.7% (1 week) for 5 mm stone. Major diameter changes ranged from -6.22% (4 days) to 5.1% (1 week) for 1 mm stone, 0% (24 hours) to 9.5% (4 days) for 2 mm stone, -3.1% (2 hours) to 11.6% (1 week) for 3 mm stone, -3.7% (24 hours) to 11.2% (4 days) for 4 mm stone, and -3.9% (2 hours) to 16.4% (4 days) for 5 mm stone. Minor diameter changes ranged from -5.2% (4 days) to 5.3% (2 hours) for 1 mm stone, -15.7% (24 hours) to 6.9% (1 week) for 2 mm stone, -9.3% (24 hours) to 0.6% (1 week) for 3 mm stone, -7.7% (2 hours) to 14.2% (1 week) for 4 mm stone, and -3.4% (2 hours) to 13.3% (1 week) for 5 mm stone.

ODC did not reduce stone weight

No apparent changes, trends or patterns in stone weight were observed across different stone sizes or ODC amounts throughout the study period, compared to controls (**Figure 4**). More deviations were observed in the smaller stones (1 mm and 2 mm), however, with no obvious pattern (**Figure 4A** and **4B**). No consistent pattern indicated that higher enzyme concentrations were associated with greater stone weight reduction for any stone size.

For the lowest ODC amount (50 µg), after 7 days the relative weight change was -8%, -14%, -3%, -2% and 0.5% for 1 mm, 2 mm, 3 mm, 4 mm and 5 mm, respectively. For the highest ODC amount (800 µg), after 7 days the relative weight change was -6%, 16%, -7%, -4% and -2% for 1 mm, 2 mm, 3 mm, 4 mm and 5 mm, respectively. There is no clear evidence from

Oxalate decarboxylase and Ca-Ox stone dissolution

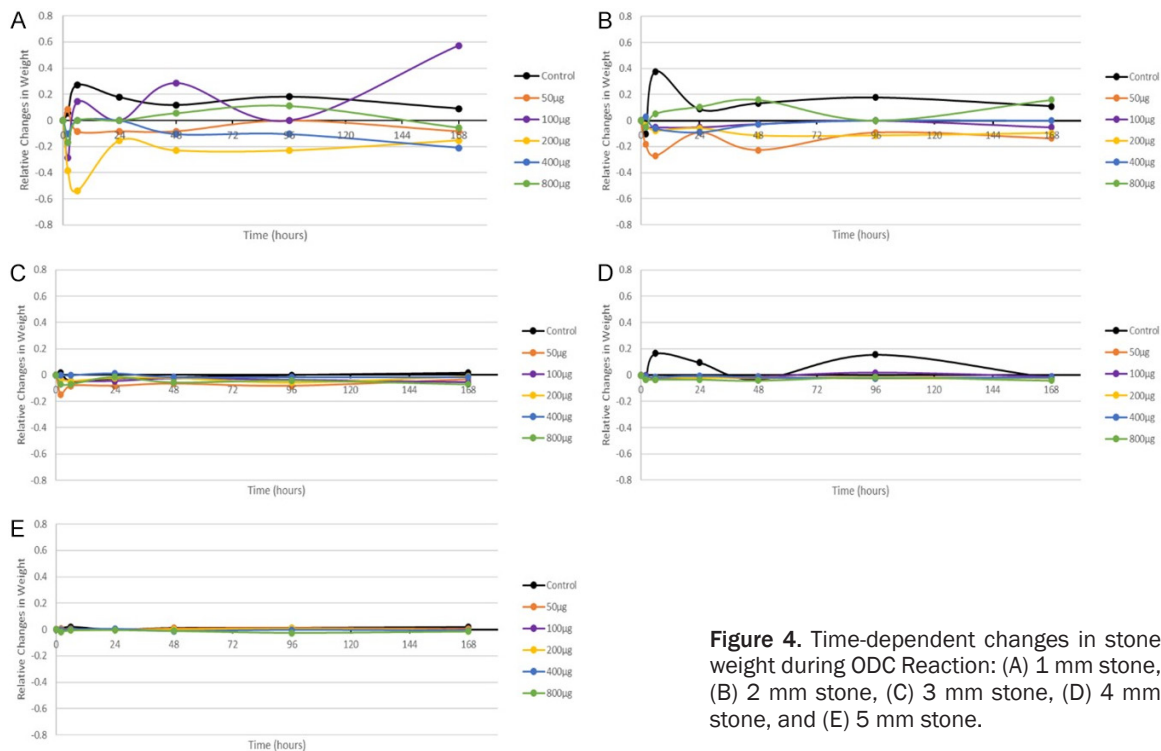


Figure 4. Time-dependent changes in stone weight during ODC Reaction: (A) 1 mm stone, (B) 2 mm stone, (C) 3 mm stone, (D) 4 mm stone, and (E) 5 mm stone.

these results to indicate reliable stone dissolution. The greatest observed weight reduction in the experiment was -27% and occurred with 50 µg of ODC after a 2-day reaction with a 2 mm stone. However, subsequent measurements showed a decrease to -10%, suggesting this was likely due to measurement variability rather than true stone dissolution.

ODC did not reduce stone diameter

No apparent changes, trends or patterns in the major (**Figure 5**) or minor (**Figure 6**) diameter of the stones were observed across different stone sizes or ODC amounts throughout the study period, compared to controls. No consistent pattern indicated that higher enzyme concentrations were associated with greater stone diameter reduction for any stone size.

Lack of biochemical evidence of stone dissolution

The precipitates from all 25 containers were analyzed for stone breakdown products. However, no detectable levels of dissolution products were identified in any of the samples (**Supplementary Table 2**). The analysis predominantly revealed an unidentified organic compound, potentially representing accumulation of the ODC enzyme.

Discussion

Ca-Ox kidney stone formation is a multifaceted process that involves supersaturation, crystallization, Randall's plaques, lipid metabolism, cell death modulation of renal tubular damage, and macrophage-renal cell inflammation interactions [16]. The concept of chemical dissolution for kidney stones has long been pursued as a means to avoid the morbidity and risks of surgical intervention, with some success demonstrated for uric acid stones through urinary alkalinization. These strategies exploit the inherent solubility of uric acid under higher pH conditions, making medical dissolution feasible and clinically applied therapy in select patients. In contrast, calcium-based stones (particularly Ca-Ox monohydrate) remain highly resistant to chemolysis, with no currently available agents capable of achieving clinically meaningful dissolution. As such, while dissolution therapy represents a reality for uric acid stones, it continues to be an unmet challenge for Ca-Ox stones. The goal of our study was to evaluate the feasibility of a similar approach using the ODC enzyme and real patient stones at varying real world fragment sizes commonly encountered in clinical practice.

Although prior in-vitro study demonstrated that ODC can catalyze oxalate breakdown and pro-

Oxalate decarboxylase and Ca-Ox stone dissolution

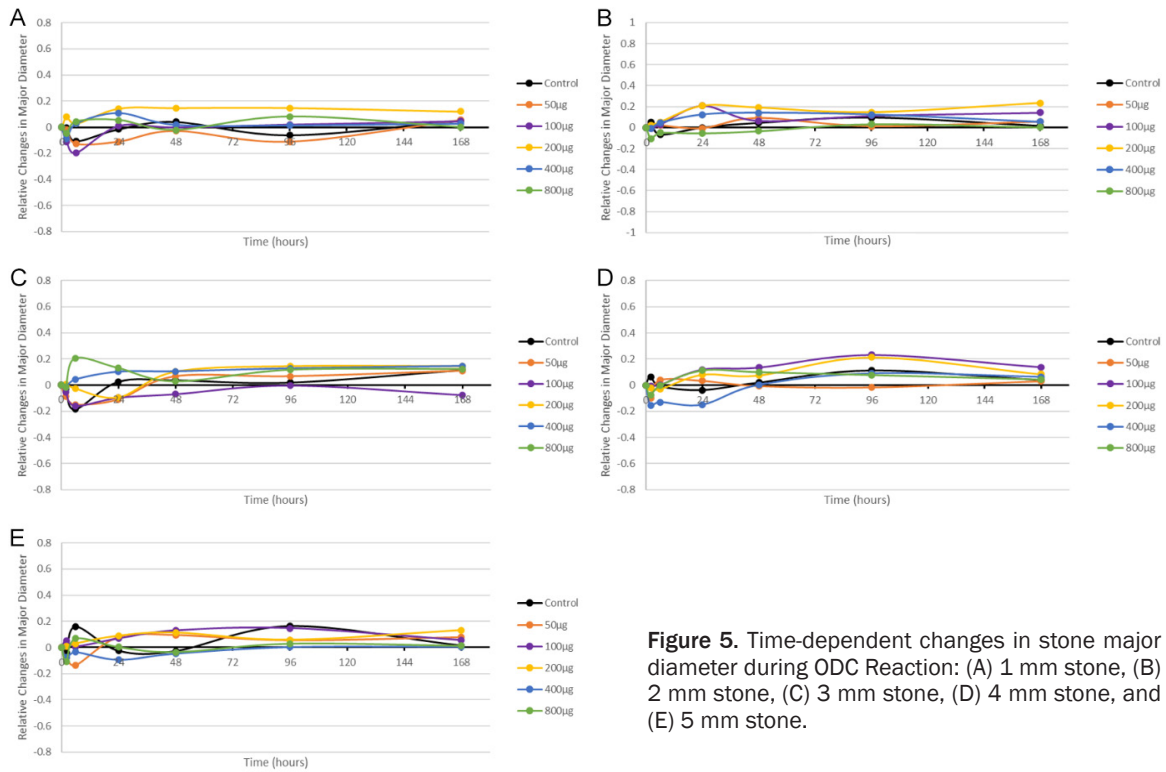


Figure 5. Time-dependent changes in stone major diameter during ODC Reaction: (A) 1 mm stone, (B) 2 mm stone, (C) 3 mm stone, (D) 4 mm stone, and (E) 5 mm stone.

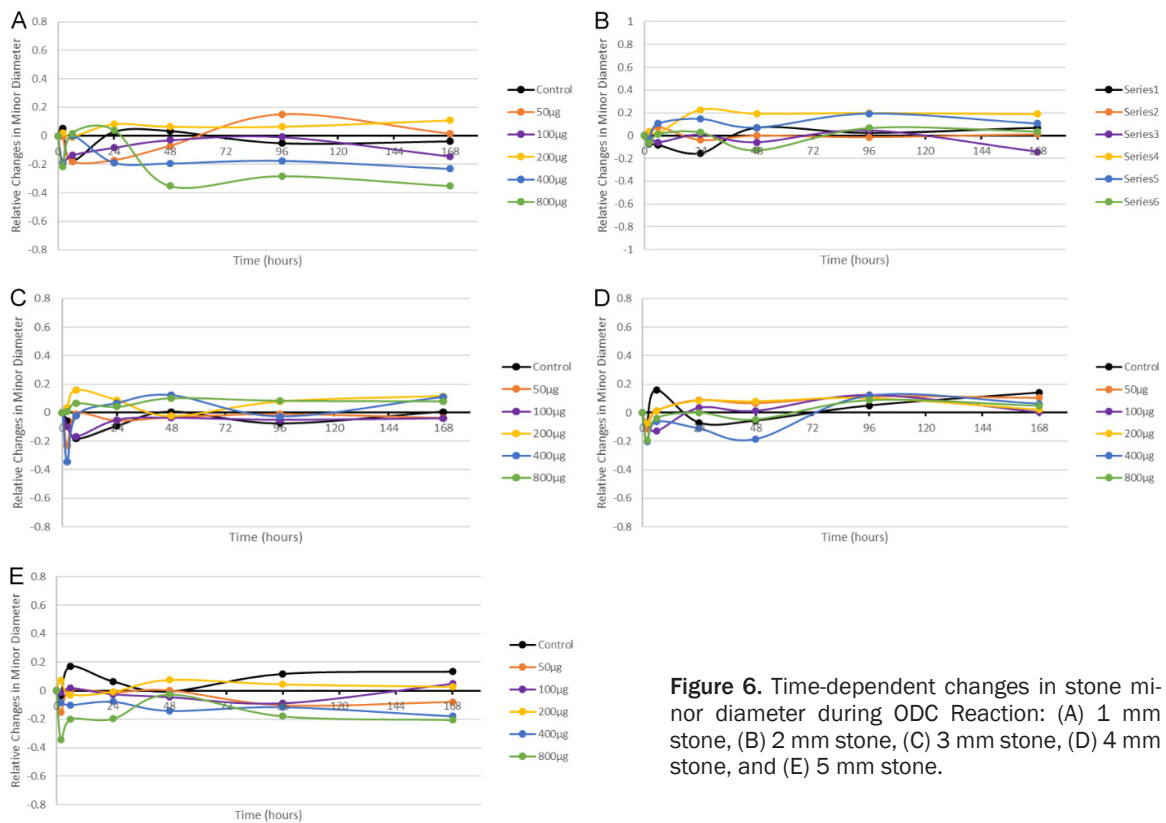


Figure 6. Time-dependent changes in stone minor diameter during ODC Reaction: (A) 1 mm stone, (B) 2 mm stone, (C) 3 mm stone, (D) 4 mm stone, and (E) 5 mm stone.

mote artificial Ca-Ox crystal dissolution [12], our study on established kidney stones did not

reproduce these findings. No consistent, dose-dependent reductions in stone weight or diam-

eter were observed across stone sizes, and residue analysis after seven days revealed no evidence of dissolution products. These null findings may reflect differences in substrate or reaction environment, or simply the limited ability of ODC to act with clinical relevance on the bonds of well-established Ca-Ox kidney stones.

We initially hypothesized that real kidney stones, containing urinary proteins within their crystal matrix, would enhance dissolution by increasing crystal strain, as suggested by prior cell culture models [17]. However, this approach might eventually introduced several challenges. The inherent insolubility of Ca-Ox monohydrate under physiological conditions likely hindered chemolytic dissolution from the outset and limited enzymatic conversion by restricting oxalate release [18]. This may explain why ODC was effective on artificial crystals but failed to act on real, clinically relevant heterogeneous kidney stones.

Moreover, although we attempted to replicate optimal physiological conditions for ODC activity, the reaction microenvironment may require alternative optimization to achieve more effective dissolution. The dissolution kinetics of calcium oxalate have been shown to be influenced by complexing agents such as citrate, where it can bind calcium and promote dissolution as shown in previous studies [19]. Hippuric acid has also been shown to increase dissolution rates in artificial urine, which further shows the complex interplay between stone crystals, ODC, and these agents whose absence may explain the null results [20]. Another potential mechanistic consideration pertains to urine pH, which was adjusted to optimize enzymatic activity yet may nevertheless remain suboptimal for sustained catalysis. Additionally, static incubation of the samples may have limited diffusion of enzyme and subsequent turnover of the stone surface for further oxalate enzymatic conversion. The weighted importance of all these factors is unknown but certainly could have played a significant role in the negative results of the study. We believe that the strong association between calcium and oxalate is the key factor limiting the effectiveness of ODC, as it hinders oxalate liberation from the stone matrix and consequently reduces its accessibility to enzymatic degradation.

This study is the first to utilize real patients' stones with an equal distribution of both Ca-Ox monohydrate and dihydrate stone composition for enzymatic chemolysis. Our findings suggest that ODC alone is unlikely to serve a practical chemolytic role for pre-formed Ca-Ox stones, at least under standard physiological conditions. Nevertheless, further work is needed to examine its potential role in urinary oxalate handling with the aim of kidney stone prevention rather than dissolution. This study also demonstrates that any chemolysis agent for Ca-Ox stones, including ODC, will likely benefit from a multimodal approach, such as considering the use of other chelating agents, varying pH micro-environments, and protein-crystal matrix disruption to influence the dissolution of authentic patient stones.

The limitations of this study are mainly related to its methodology. First, limited sample size at the dose per path level. Second, macroscopic metrics such as mass and diameter may not detect subtle or early dissolution and do not employ serial solution chemistry to examine kinetics. Third, it is unclear if further exposure beyond seven days would have made a meaningful difference. Finally, our investigation focused on dose-dependent effects, while the reaction microenvironment was held constant. Ultimately, despite methodological constraints, our pioneering use of enzymatic catalysis on real-world kidney stones provides a roadmap for future studies, laying the groundwork for a clinically viable approach to dissolving the otherwise 'unbreakable' Ca-Ox stones.

Conclusion

This pioneering in-vitro study demonstrated that Ca-Ox kidney stones are resistant to enzymatic degradation by ODC under physiologic conditions. Although modifications in the reaction environment or enzyme concentration might yield more favorable results, it is also plausible that the Ca-Ox bonds in established kidney stones are inherently resistant to ODC-mediated catalysis. Nevertheless, this study establishes key concepts and methodologies to guide future investigations.

Disclosure of conflict of interest

None.

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Oxalate decarboxylase and Ca-Ox stone dissolution

Supplementary Table 1. Control group results: temporal changes of stone weight and dimensions across size categories

Stone Size	Variable	2 hours	24 hours	48 hours	96 hours	168 hours
1 mm	ΔWeight	5.80%	17.60%	11.70%	18.10%	9.00%
	ΔMajor Diameter	-0.52%	-1.55%	4.12%	-6.22%	5.15%
	ΔMinor Diameter	5.30%	2.65%	3.31%	-5.26%	-3.97%
2 mm	ΔWeight	-10.20%	8.80%	13.30%	17.70%	11.10%
	ΔMajor Diameter	4.90%	0.00%	4.40%	9.50%	1.60%
	ΔMinor Diameter	-6.90%	-15.70%	6.90%	2.30%	6.90%
3 mm	ΔWeight	1.70%	-2.70%	-1.80%	-	1.80%
	ΔMajor Diameter	-3.10%	2.50%	3.70%	-	11.60%
	ΔMinor Diameter	-5.80%	-9.30%	0.60%	-	0.60%
4 mm	ΔWeight	-2.90%	9.70%	-3.30%	15.70%	-2.10%
	ΔMajor Diameter	6.50%	-3.70%	1.90%	11.20%	3.80%
	ΔMinor Diameter	-7.70%	-6.90%	-5.50%	5.10%	14.20%
5 mm	ΔWeight	0.71%	0.00%	1.10%	1.19%	1.75%
	ΔMajor Diameter	-3.98%	-2.42%	-2.59%	16.39%	1.55%
	ΔMinor Diameter	-3.40%	6.55%	-0.49%	11.78%	13.35%

Supplementary Table 2. Precipitate analysis report

SOURCE-1	Comment
Color	Tan
Size	mm
Weight	mg
Calcium Oxalate Monohydrate	CANCELED*
Calcium Oxalate Dihydrate	CANCELED*
Calcium Phosphate (Hydroxyl)	CANCELED*
Calcium Phosphate (Carbonate)	CANCELED*
CaHPO ₄ (Brushite)	CANCELED*
Calcium Phosphate	CANCELED*
Calcium Carbonate	CANCELED*
Mg NH ₄ PO ₄ (Struvite)	CANCELED*
MgHPO ₄ (Newberyite)	CANCELED*
Uric Acid	CANCELED*
Uric Acid Dihydrate	CANCELED*
Ammonium Acid Urate	CANCELED*
Sodium Acid Urate	CANCELED*
2,8 Dihydroxyadenine	CANCELED*
Xanthine	CANCELED*
Cystine	CANCELED*
Cholesterol	CANCELED*
Calcium Bilirubinate	CANCELED*
Bilirubin	CANCELED*
Calcium Palmitate	CANCELED*
Calcium Stearate	CANCELED*
Triamterene	CANCELED*
Drug or Metabolite	CANCELED*

*No detectable results.