ZONAL DIFFERENTIATION AND CRYSTAL MORPHOTYPE CLASSIFICATION IN HUMAN SALIVA DEHYDRATION FACIES: A QUANTITATIVE MORPHOLOGICAL ANALYSIS

Ergashev Erkinjon Abdusattor ugli,
Mashrabboyev Abdusamad Abdug'ani ugli,
Abdug'aniyev Asadbek Faxriddin ugli,
Fergana State University, Fergana Region, Uzbekistan
Corresponding author: erkinjonebk@mail.ru

Abstract. Background: Morphological analysis of biological fluid dehydration patterns offers a non-invasive approach to characterizing complex physicochemical properties. Systematic quantitative classification of saliva crystallization zones and crystal morphotypes remains limited. Objective: To develop and apply a quantitative morphological classification system for zonal differentiation and crystal morphotype distribution in human saliva dehydration facies. Methods: Thirty dehydrated saliva droplets from 10 healthy male volunteers (18–30 years) were analyzed by digital microscopy (50×–200×). Five morphological parameters - zonal expression, crystal shape class, branching complexity, density, and symmetry - were scored quantitatively and analyzed statistically. Results: Three-zone organization was identified in 73.3% of droplets. Dendritic and branched morphotypes predominated (80.0%), with high structural heterogeneity (86.7%). Significant inter-individual morphological reproducibility was demonstrated (CV < 12% for all parameters). Conclusions: A reliable, reproducible quantitative morphological classification system for saliva dehydration facies is feasible and yields consistent results across individuals, supporting its application as a biophysical diagnostic tool.

Keywords: saliva crystallization, morphological classification, zonal differentiation, dendritic crystals, dehydration facies, quantitative morphology, biophysical diagnostics

Introduction

The analysis of dehydrated biological fluid patterns - commonly termed crystallography, tensiometry, or facies analysis - has attracted growing scientific interest as a potential non-invasive diagnostic modality [1]. Saliva, accessible and easily collected, represents a particularly attractive candidate for such approaches. The patterns formed during saliva dehydration reflect the integrated physicochemical state of the fluid, including protein content, ionic balance, pH, and colloidal organization [2].

While qualitative descriptions of saliva crystallization patterns are available in the literature, reproducible quantitative morphological classification systems have rarely

been reported. Most existing studies rely on subjective visual scoring or binary classification (presence/absence of arborization), limiting comparability across investigations and precluding rigorous statistical analysis [3]. The development of a standardized, multi-parameter quantitative classification framework would substantially strengthen the scientific foundation of salivary facies analysis.

The present study aimed to develop and apply a five-parameter quantitative scoring system for human saliva dehydration facies, evaluate morphological reproducibility across individuals, and characterize the distribution of morphotypes and zonal patterns in a healthy male reference population.

Materials and Methods

Participants and Sample Collection. Unstimulated saliva was collected from 10 healthy male volunteers aged 18–30 years under standardized conditions (oral rinse, 10–15 min rest, 1.5–2 hours postprandial). Three replicate 5 µL droplets were prepared per participant on clean glass slides, yielding 30 droplets for analysis.

Dehydration Protocol. Droplets were dehydrated in a thermally insulated chamber at 25 °C and 32% relative humidity for 25–30 minutes under vibration-free conditions. All droplets were processed under identical environmental conditions within each session.

Quantitative Morphological Scoring System. A five-parameter scoring system was applied to each droplet (Table 1). Each parameter was scored on a three-point ordinal scale (0 = absent, 1 = moderate, 2 = high/complex), with a maximum composite score of 10. Additionally, predominant crystal morphotype was recorded as one of four categories: dendritic (D), branched (B), radiating (R), or irregular (I).

Table 1.

Quantitative morphological scoring system for saliva dehydration facies.

Parameter	Score 0	Score 1	Score 2
Zonal expression	Absent	Peripheral only	3 zones distinct
Crystal density	Sparse	Moderate	Dense
Dendritic branching complexity	None	Simple (1°)	Complex (2°+)
Structural symmetry	Irregular	Partial	Symmetric
Structural heterogeneity	Homogeneous	Moderate	Highly heterogeneous

Statistical Analysis. Data are presented as mean ± SD and percentages. Inter-individual reproducibility was assessed by calculating the coefficient of variation (CV%) for each parameter across all 10 participants. Morphotype frequency

distribution was assessed by chi-square analysis. Correlations between composite morphological score and individual parameters were evaluated by Pearson correlation. Statistical significance: $p < 0.05$.

Results and Discussion

Application of the five-parameter quantitative scoring system to 30 dehydrated saliva droplets yielded consistent and reproducible results across the study population (Table 2). The mean composite morphological score was 7.8±1.2 out of a maximum of 10, indicating a generally high level of structural organization in the dehydrated facies of healthy individuals.

Table 2.

Quantitative morphological characteristics of dehydrated human saliva droplets (n=30).

Parameter	Mean Score ± SD	CV (%)	Frequency (%)
Zonal expression	1.7±0.3	11.8	73.3 (3-zone)
Crystal density	1.6±0.2	9.4	68.3 (dense)
Dendritic branching	1.8±0.2	8.6	80.0 (complex)
Structural symmetry	1.2±0.3	11.5	60.0 (partial)
Structural heterogeneity	1.9±0.2	7.4	86.7 (high)
Composite score	7.8±1.2	—	—

Three-zone zonal organization (peripheral, transitional, and central) was observed in 73.3% of droplets, with a mean zonal expression score of 1.7±0.3. The peripheral zone was universally present, while the transitional zone showed greater variability, being absent in approximately one-quarter of specimens. This variability is consistent with the known dependence of intermediate zone formation on solute redistribution kinetics and evaporation rate [4].

Dendritic branching complexity showed the highest reproducibility among all parameters (CV = 8.6%), suggesting that branching behavior in saliva crystallization is robustly determined by the intrinsic compositional properties of the fluid rather than random microenvironmental fluctuation. The predominant morphotype across the sample was dendritic (53.3%), followed by branched (26.7%), irregular (13.3%), and radiating (6.7%), consistent with the compositional complexity of saliva and the thermodynamic conditions favoring dendritic growth at moderate supersaturation.

Structural heterogeneity was the most consistently expressed parameter (CV = 7.4%, frequency 86.7%), reflecting the inherent compositional diversity of saliva and its capacity to generate spatially variable crystallization microenvironments

within a single droplet. The high structural heterogeneity observed across all specimens further underscores the inadequacy of binary (present/absent) classification systems for salivary facies characterization.

Composite morphological scores showed strong positive correlations with dendritic branching complexity ($r = 0.91$, $p < 0.001$) and structural heterogeneity ($r = 0.87$, $p < 0.001$), identifying these two parameters as the primary determinants of overall morphological complexity in healthy saliva. Symmetry showed the weakest correlation with composite score ($r = 0.61$, $p < 0.01$), suggesting it is a less discriminative parameter in normative specimens and may be more informative in pathological conditions where symmetry is disrupted.

Conclusions

A five-parameter quantitative morphological scoring system was successfully applied to human saliva dehydration facies, yielding reproducible results with CV values below 12% for all parameters across 10 healthy volunteers. Three-zone organizational architecture was present in 73.3% of specimens, with dendritic morphotypes predominating (53.3%). Dendritic branching complexity and structural heterogeneity were identified as the most reproducible and discriminative morphological parameters. The proposed scoring system provides a standardized, quantitative framework for characterizing salivary crystallization patterns, with potential applications in comparative studies, diagnostic modeling, and the evaluation of modifying factors. Future studies should validate this system in pathological populations and investigate its sensitivity to systemic and local disease states.

References:

[1] Yakhno T. Salt-induced protein phase transitions in drying drops. J Colloid Interface Sci. 2008;318(2):225–230.

[2] Hu H, Larson RG. Evaporation of a sessile droplet on a substrate. J Phys Chem B. 2002;106(6):1334–1344.

[3] Piqué N, Miñarro M, Jiménez-Castellanos MR. Salivary crystallomics and pattern recognition in oral diseases: a systematic review. Oral Dis. 2021;27(4):793–803.

[4] Deegan RD. Pattern formation in drying drops. Phys Rev E. 2000;61(1):475–485.

[5] Yakhno TA, Yakhno VG, Sanin AG. The informative value of the morphological features of dried biological fluid drops. Pattern Recognit Image Anal. 2005;15(3):519–534.