ATOMIC FORCE MICROSCOPY CHARACTERIZATION OF A THREE-DIMENSIONAL PORCINE COLLAGEN MATRIX FOR PERIODONTAL APPLICATIONS UNDER HYDRATED CONDITIONS

C. Cojocaru[1], D. I. Virvescu[1*], Oana-Maria Butnaru[1], Daniela Argatu[1], F. C. Bida[1], St. L. Toma[2], V. Constantin[1], I. Luchian[1]
1. Grigore T. Popa University of Medicine and Pharmacy Iasi, Romania
2. Gheorghe Asachi Technical University of Iasi, Romania
[*]Corresponding author: E-mail: dragos.virvescu@umfiasi.ro

ATOMIC FORCE MICROSCOPY CHARACTERIZATION OF A THREE-DIMENSIONAL PORCINE COLLAGEN MATRIX FOR PERIODONTAL APPLICATIONS UNDER HYDRATED CONDITIONS (Abstract): The **aim** of this study was to characterize the nanoscale surface topography of a three-dimensional porcine collagen matrix under hydrated conditions, simulating a physiologically relevant environment. **Materials and methods:** Atomic force microscopy (AFM) was performed in hydrated conditions to evaluate surface topography, deflection signal, three-dimensional reconstruction, surface profile, and quantitative roughness parameters. **Results:** The collagen matrix exhibited a continuous, moderately undulating surface without detectable structural defects. Topographic analysis revealed a homogeneous distribution of elevations and depressions, consistent with an isotropic collagen network. Deflection imaging enhanced the visualization of local slope variations, indicating microstructural heterogeneity. Three-dimensional reconstruction confirmed uniform spatial organization with gradual height transitions. Quantitative analysis demonstrated moderate roughness (Sa = 0.1179 µm; Sq = 0.1765 µm) and limited maximum height (Sz = 1.541 µm), suggesting the absence of pronounced irregularities. Height distribution showed a centered dispersion around the mean plane. **Conclusions:** The hydrated collagen matrix presents a stable, continuous, and moderately rough surface morphology, which may support favorable biological interactions and its potential application in soft tissue and periodontal regeneration. **Keywords:** ATOMIC FORCE MICROSCOPY, COLLAGEN MATRIX, SURFACE TOPOGRAPHY, HYDRATED CONDITIONS, ROUGHNESS, BIOMATERIALS, PERIODONTAL REGENERATION, NANOSCALE MORPHOLOGY.

INTRODUCTION

Collagen-based biomaterials are widely used in regenerative dentistry and periodontology due to their biocompatibility, biodegradability, and structural similarity to the native extracellular matrix (1, 2). Porcine-derived collagen matrices, in particular, have been increasingly employed as substitutes for autogenous grafts in soft tissue augmentation procedures, offering advantages such as reduced morbidity and simplified surgical protocols (3, 4). The surface morphology of these biomaterials plays a critical role in their biological performance, influencing protein adsorption, cell adhesion, proliferation, and tissue integration (5, 6). At the micro- and nanoscale levels, surface topography has been shown to modulate cellular behavior and affect the interaction between biomaterials and the surrounding biological environment (7).

Atomic force microscopy (AFM) is a powerful technique for nanoscale surface characterization, providing high-resolution topographical data and information regarding surface roughness and local mechanical interactions (8). Unlike other imaging methods, AFM allows the investigation of soft biomaterials under hydrated conditions, which more closely resemble physiological environments (9).

Hydration significantly affects the structural and mechanical properties of collagen-based materials, leading to changes in surface morphology, compliance, and topographical features (10). In hydrated conditions, collagen matrices may exhibit a more relaxed structural configuration, potentially reducing apparent surface roughness and altering the visualization of microstructural details (11). Despite the widespread clinical use of collagen matrices, limited data is available regarding their nanoscale surface characteristics under physiologically relevant conditions (12). Most existing studies focus on dry-state analysis, which may not accurately reflect vivo behavior.

Therefore, the aim of the present study was to characterize, using atomic force microscopy, the surface topography of a three-dimensional porcine collagen matrix under hydrated conditions, with particular emphasis on morphological features and rough surface parameters.

MATERIALS AND METHODS

Materials. A commercially available three-dimensional porcine collagen matrix, Mucoderm® (Botiss biomaterials GmbH Zossen-Germany), was used in this study. The material consists of a collagen-based structure designed for soft tissue applications. Prior to analysis, 30 samples were prepared according to standard handling protocols and maintained for 20 minutes in hydrated conditions (sterile NaCl 0.9% solution) according to the manufacturer recommendations. Hydration is an essential step in order to obtain an optimal oral integration in a clinical setting.

AFM images were acquired on synthetic tissue samples in a hydrated state to evaluate surface topography under conditions closely resembling the physiological environment. Hydration affects both the mechanical behavior and the morphological configuration of the surface, inducing structural relaxation of the collagen network and facilitating a more accurate representation of surface irregularities, depressions, and local topographical variations.

Sample Preparation. The specimens of appropriate dimensions (10 mm diameter) placed on a stable support suitable for AFM analysis. Measurements were performed in wet conditions, ensuring the presence of a hydration layer throughout the scanning process. This approach was selected to preserve the native structural configuration of the collagen matrix and to minimize artifacts associated with dehydration.

Atomic Force Microscopy Analysis. Atomic Force Microscopy (AFM) investigations were carried out using an EasyScan 2 system (Nanosurf AG, Liestal, Switzerland), equipped with a highly sensitive PPP-NCHAuD cantilever (NanoAndMore, USA), characterized by a resonance frequency of approximately 330 kHz, a spring constant of 44 N/m, and a length of 125 μm. Image acquisition was performed in Dynamic Force mode (Non-Contact Mode), while data processing was conducted using the dedicated Nanosurf EasyScan 2 analysis software. To ensure stable immobilization of the synthetic tissue and enhance measurement reproducibility, the samples were mounted on commercially available glass slides coated with poly-L-lysine (PLL), which promotes electrostatic adhesion of biological specimens. AFM scans were carried out under hydrated conditions, and prior to imaging, the substrates were allowed to reach thermal equilibrium with the scanning cell environment in order to minimize experimental variability.

The scans were performed over an area of 5 × 5 μm, allowing detailed visualization of surface morphology at the micro- and nanoscale. Several imaging channels were recorded, including: height (topography) images, deflection images, three-dimensional surface reconstructions

Surface Analysis. Topographic images were analyzed to evaluate the overall morphology and spatial distribution of surface features. Deflection images were used to enhance the visualization of local slope variations and microstructural boundaries.Three-dimensional reconstructions were generated to assess the spatial organization of the surface. Surface profiles were extracted along selected cross-sections to evaluate height variations.

Roughness Parameters. Surface roughness parameters were calculated from AFM data, including following parameters:Sa (arithmetical mean height),Sq (root mean square roughness), Sz (maximum height), Ssk (skewness), Sku (kurtosis).These parameters were used to quantify the amplitude and distribution of surface irregularities.

Statistical Representation. Height distribution histograms were generated to evaluate the distribution of surface elevations relative to the mean plane. This allowed the assessment of symmetry, dispersion, and the presence of extreme values within the dataset.

RESULTS

AFM Topography Analysis. AFM topography images acquired on the synthetic tissue in a hydrated state revealed a surface characterized by a non-uniform relief, dominated by heterogeneously distributed morphological variations. An alternation between slightly elevated regions and localized depressions was observed, suggesting a discontinuous spatial organization at the micro- and nanoscale levels (fig. 1).

The surface exhibited a relatively homogeneous appearance in terms of material continuity, with no evidence of abrupt discontinuities or major structural defects. The relief was predominantly defined by rounded features, while transitions between areas of maximum and minimum height were gradual.

The topographical configuration indicated the presence of randomly distributed morphological units, without a clearly identifiable preferential alignment. Furthermore, no filamentous formations or dominantly oriented structures were observed, suggesting a predominantly isotropic character of the analyzed surface.

Overall, the topographic image reflected a continuous surface structure, with moderate variations in relief and without extreme features such as sharp peaks or pronounced depressions.

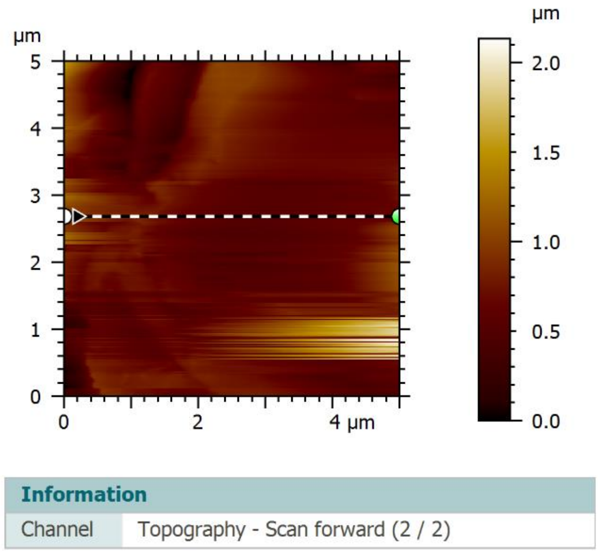


Fig. 1. Surface topography of the synthetic tissue under hydrated conditions as revealed by AFM.

AFM Deflection Image Analysis. The AFM deflection image revealed a pronounced distribution of local contrast, reflecting significant variations in the interaction between the probe tip and the surface of the synthetic tissue. Regions of differing intensity were observed, corresponding to local slope variations and fine morphological discontinuities (fig. 2).

The deflection signal highlighted structural details that were not as clearly visible in the topographic image, particularly at the boundaries between morphological units. Well-defined contours of surface features were identified, along with transition zones where surface inclination changes were more pronounced.

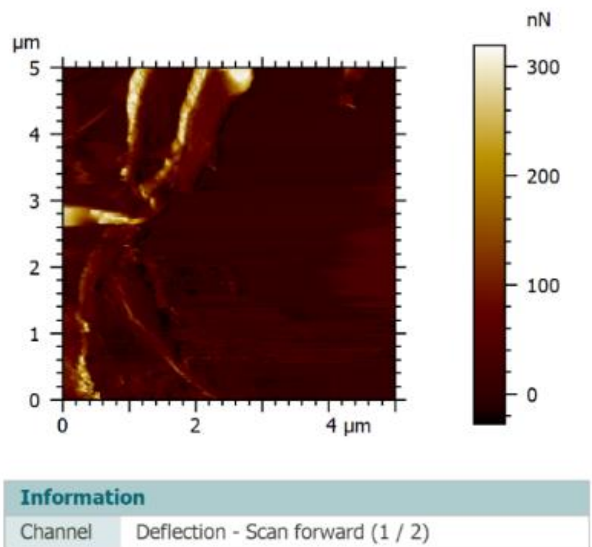


Fig. 2. Representative AFM deflection image of the synthetic tissue under hydrated conditions (5 × 5 µm scan area)

The image exhibited a granular contrast pattern, with an alternation between bright and dark regions, suggesting the presence of micro-scale heterogeneities at the surface level. No major discontinuities or evident artifacts were observed, indicating good scanning stability under hydrated conditions. Furthermore, the distribution of the deflection signal did not indicate a clear preferential orientation, supporting the predominantly isotropic character of the surface structure.

Three-Dimensional Surface Reconstruction Analysis. The three-dimensional reconstruction of the synthetic tissue surface obtained by AFM revealed a relief characterized by continuous altimetric variations, non-uniformly distributed across the analyzed area. The surface exhibited an undulating configuration, with an alternation between elevated regions and depressions, without the presence of abrupt discontinuities or well-defined dominant structures (fig. 3).

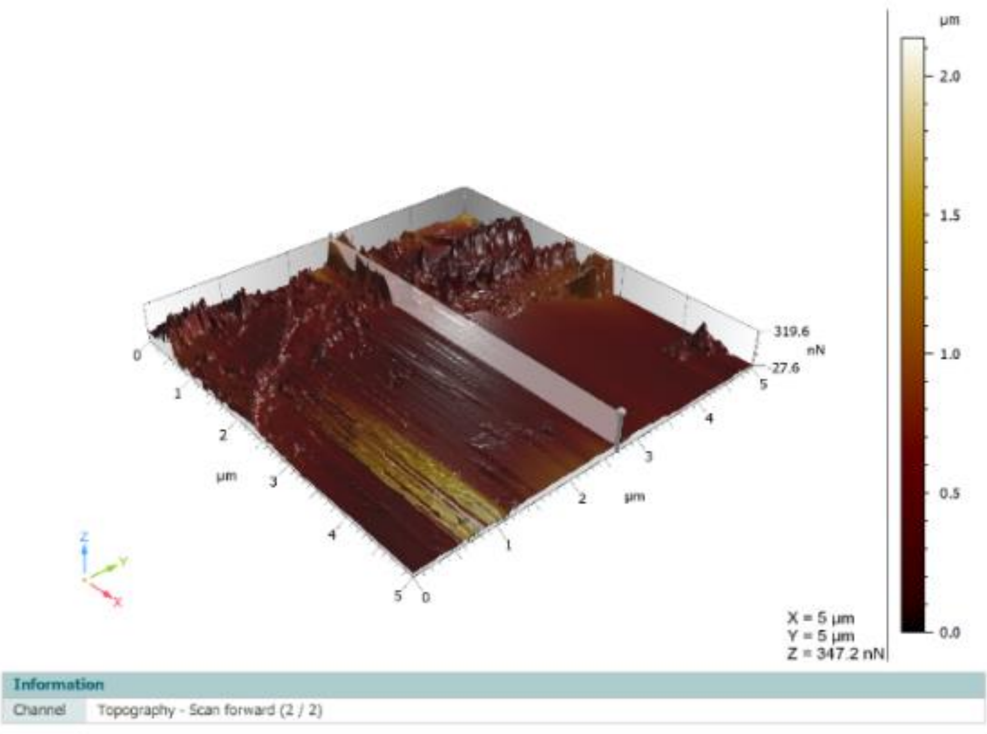


Fig. 3. Three-dimensional reconstruction of the synthetic tissue under hydrated conditions.

The morphological elements appeared to be relatively randomly distributed, without a clearly identifiable preferential orientation, indicating an isotropic spatial organization. The height of the structures varied gradually, and transitions between regions were smooth, suggesting a continuous distribution of the material at the surface level.

The three-dimensional aspect confirmed

the presence of morphological units with rounded shapes, without sharp peaks or deep depressions. The overall relief was characterized by moderate height variations, uniformly distributed, providing a coherent representation of the spatial configuration of the surface. Overall, the 3D reconstruction highlighted a continuous surface with moderate relief and without extreme heterogeneities.

Surface Profile Analysis. The surface profile obtained from a cross-sectional analysis revealed continuous altimetric variations along the analyzed distance, reflecting the undulating configuration of the synthetic tissue surface under hydrated conditions. The profile trace indicated an alternation between regions of maximum and minimum height across the entire length of the section (fig. 4).

Height differences were expressed at the nanometer scale, and the amplitude of the variations was moderate, with no evidence of sharp peaks or abrupt depressions. Transitions between maxima and minima were gradual, suggesting a continuous distribution of the material at the surface level.

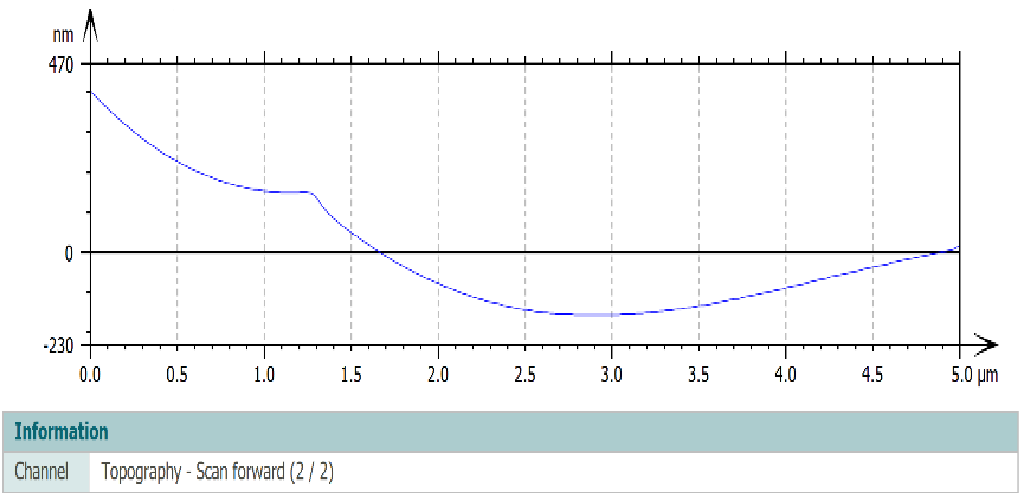


Fig. 4. Representative AFM cross-sectional surface profile of the synthetic tissue under hydrated conditions.

Along the length of the profile (corresponding to the scanned dimension, in the micrometer range), no areas with abrupt discontinuities or localized material accumulations were identified. The profile exhibited a relatively uniform character, with regular height fluctuations, indicating a balanced spatial organization. Overall, the cross-sectional profile confirmed the presence of a moderately rough surface, with uniformly distributed variations and no extreme

Surface Roughness Analysis. Surface roughness parameters derived from AFM analysis revealed a moderate level of altimetric variation of the synthetic tissue surface under hydrated conditions. The amplitude parameters (Sa = 0.1179 µm and Sq = 0.1765 µm) indicated a relatively uniform distribution of surface heights relative to the mean plane, supporting the continuous nature of the surface relief (tab. I).

The maximum height parameter (Sz = 1.541 µm) reflected the difference between the highest peak and the deepest valley, indicating the absence of pronounced peaks or deep depressions. This observation is consistent with the topographical and cross-sectional profile analyses, where no major discontinuities were identified.

TABLE I.

Surface roughness parameters of the hydrated synthetic tissue

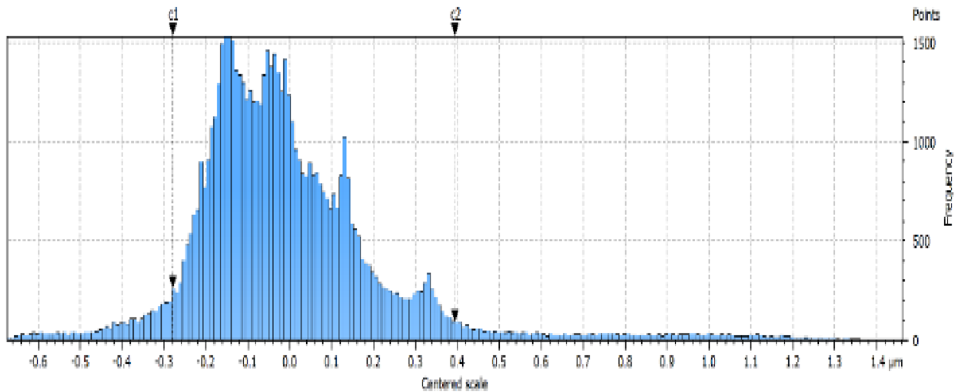
ISO 25178-2 - Roughness (S-L)			
S-filter (λs): None			
F-operation: None			
L-filter (λc): Gaussian, 0.0025 mm			
Height parameters			
Sq	0.1765	µm	
Ssk	1.334		
Sku	7.873		
Sp	1.069	µm	
Sv	0.4719	µm	
Sz	1.541	µm	
Sa	0.1179	µm	

The dimensionless shape parameters of the height distribution (Ssk = 1.334 and Sku = 7.873) suggested a slightly skewed distribution with a tendency toward asymmetry, depending on the obtained values. This indicates a relatively balanced distribution of peaks and valleys, with a possible predominance of either protruding or recessed features. Overall, the roughness analysis confirmed the presence of a moderately developed surface relief, uniformly distributed and without extreme heterogeneities at the level of the analyzed surface.

Height Distribution Histogram Analysis. The height distribution histogram revealed a concentration of values around the central region, indicating that the majority of surface points were located close to the mean plane. The range of height values extended from negative to positive values (expressed in nanometers), reflecting the overall amplitude of altimetric variations (fig. 5).

The highest frequency was observed in the central region of the distribution, where a pronounced peak was identified, suggesting the predominance of height values close to the mean. The distribution exhibited a moderate width, indicating a relatively controlled dispersion of height values.

The shape of the histogram was relatively symmetrical or slightly skewed, depending on the exact distribution of the data, suggesting a balanced or mildly inclined distribution of peaks and valleys. No extended tails or dominant extreme values were observed, indicating the absence of major surface irregularities. Overall, the histogram confirmed the moderate nature of height variations and their relatively uniform distribution across the analyzed surface.



Information	
Channel	Topography - Scan forward (2 / 2)

Individual cursors	Unit	c1	c2
Height	µm	-0.279	0.3938

Cursor sets	c2-c1	Unit
Height difference	0.6727	µm
% of points between cursors	90.00	%

Fig. 5. Height distribution histogram of the synthetic tissue surface as obtained by AFM.

DISCUSSION

The surface topography observed under hydrated conditions reflects the influence of hydration on the morphological configuration of the synthetic tissue. The presence of water has been associated with a redistribution of the material at the surface level, leading to a more uniform appearance and attenuation of topographical contrast. Such behavior is consistent with previous studies demonstrating that hydration can induce structural relaxation and increased compliance in collagen-based materials (10, 13).

The rounded morphology of surface features and the gradual transitions between regions can be correlated with the compliant nature of hydrated soft biomaterials, in which water acts as a plasticizing agent, reducing stiffness and smoothing surface irregularities (14). Consequently, the interaction between the AFM probe tip and the surface is influenced by this hydrated state, resulting in a relatively "smoothed" representation of the surface relief compared to measurements performed under unhydrated conditions (15).

The random distribution of morphological units suggests the absence of a clearly defined directional organization or, alternatively, the masking of an underlying structural arrangement due to hydration effects.

This observation supports the interpretation of an isotropic structural response of the material under the experimental conditions employed, a characteristic commonly reported for collagen matrices lacking preferential fiber orientation (16).

The overall topographical appearance is consistent with a continuous and flexible surface structure, which may have direct implications for interactions with the surrounding biological environment, particularly in the presence of fluids. Surface properties such as roughness and compliance are known to influence protein adsorption and subsequent cellular behavior, including adhesion and proliferation (7,17).

At the same time, potential measurement-related effects specific to wet conditions must be considered. Capillary interactions, variations in contact forces, and hydration-induced changes in tip-sample interaction dynamics may subtly influence the recorded topographical configuration (8). These factors are well-documented in AFM studies performed in liquid environments and should be considered when interpreting nanoscale surface features (18).

The AFM deflection image provided complementary information to topographical data, being particularly sensitive to local slope variations and rapid changes in surface relief. The high contrast observed can be associated with height gradients across the surface, allowing for a clearer delineation of boundaries between morphological structures.

Under hydrated conditions, the deflection response was influenced by the complex interaction between the AFM probe tip and the hydrated surface layer. The presence of water contributes to modifications in contact forces and local mechanical response, leading to an enhancement of contrast in certain regions and attenuation in others (18,19). The well-defined contours observed in the deflection image suggest that, although the overall topography appears relatively smoothed due to hydration effects, local slope variations remain detectable and may even be amplified in the deflection signal.

This observation highlights the increased sensitivity of deflection imaging to fine morphological details, particularly at the interfaces between structural units. The granular appearance of the image can be interpreted as the result of microstructural heterogeneity of the synthetic tissue, combined with tip-surface interaction effects in a liquid environment (18, 20).

The absence of a preferential orientation is consistent with the topographical findings, supporting a predominantly isotropic structural organization. At the same time, it is important to consider that AFM measurements performed under hydrated conditions may be affected by additional factors, such as capillary forces, variations in local adhesion, and changes in tip-sample interaction dynamics (21, 22). These effects can influence the deflection signal and contribute to the observed contrast without exclusively reflecting the actual surface geometry (9).

The three-dimensional reconstruction enabled a more intuitive evaluation of the spatial distribution of height variations, confirming the continuous and relatively uniform nature of the surface observed in the topographic images. The undulating configuration can be associated with the material response under hydrated conditions, where the presence of water influences local material redistribution and reduces topographical contrast. Rounded surface features and the absence of abrupt discontinuities are consistent with the compliant behavior of hydrated collagen-based structures, in which water induces structural relaxation and attenuates sharp height variations (20, 24).

The relatively random distribution of three-dimensional features further supports the absence of a preferential directional organization, in agreement with the observations from both topography and deflection imaging. Such behavior is characteristic of collagen matrices exhibiting isotropic structural organization under hydrated conditions, as reported in recent biomaterials studies (25).

The 3D reconstruction also facilitated the identification of spatial relationships between elevated and depressed regions, demonstrating that height variations were gradually distributed without the accumulation of critical regions. This configuration is consistent with a continuous material structure exhibiting increased surface flexibility, which may play a role in modulating interactions with the surrounding biological environment (26).

The cross-sectional profile analysis provided a quantitative perspective on surface height variations, supporting the qualitative observations derived from topography and three-dimensional reconstruction. The regular alternation between maxima and minima is indicative of an undulating surface architecture typical of hydrated soft biomaterials (27). The moderate amplitude of altimetric variations suggests a stable surface configuration, without the presence of major local defects. The absence of abrupt transitions further supports a homogeneous material distribution and highlights the influence of hydration in smoothing surface irregularities.

The smooth and continuous shape of the profile can be correlated with the compliant behavior of the hydrated surface layer, where water reduces local stiffness and contributes to the homogenization of the surface relief (28). The relatively uniform distribution of variations לאורך the entire profile length supports the hypothesis of an isotropic surface organization, consistent with the findings obtained from topographic and deflection analyses. Additionally, the absence of localized accumulations of height variations suggests a stable structural response, which may have favorable implications for the functional behavior of the material in wet environments (29).

The roughness parameters obtained were consistent with the observed topographical configuration and reflected the direct influence of hydration on surface behavior. The presence of water was associated with a reduction in altimetric contrast and a homogenization of the surface, leading to moderate values of Sa and Sq. Similar trends have been reported in recent AFM studies of collagen-based and soft biomaterials analyzed under hydrated conditions (30).

The moderate values of the Sz parameter can be interpreted as a consequence of the absence of major structural discontinuities and a stable material response in wet conditions, which agrees with the continuous surface observed in the 3D reconstruction. The shape parameters of the height distribution (Ssk and Sku) provided additional insight into the nature of the surface relief. A near-symmetrical distribution suggests a balanced presence of peaks and valleys, while slight deviations may indicate a modest predominance of either protruding or recessed features. These findings are consistent with the rounded morphology and gradual transitions observed in AFM images (31).

Overall, the results indicate that under hydrated conditions, the synthetic tissue surface exhibits a more uniform and morphologically stable behavior compared to typical dry-state analyses. This surface homogenization may have direct implications for surface interactions, including adhesion phenomena and local mechanical response, which are critical for biomaterial performance in physiological environments (32).

The height distribution histogram further supported the topographical and cross-sectional findings, confirming that the surface was characterized by moderate variations and a relatively uniform organization. The concentration of values around the mean plane indicates the predominance of low-relief regions, typical of hydrated soft materials (33).

The moderate width of the distribution reflects controlled surface variability and suggests the absence of extreme heterogeneities. This behavior can be attributed to the effect of hydration, which contributes to surface smoothing and reduction of altimetric contrast (34). The approximately symmetrical shape of the histogram indicates a balanced distribution of peaks and valleys, consistent with a stable structural configuration.

The absence of extended tails or dominant extreme values suggests the lack of major defects or structural discontinuities, further supporting the continuous nature of the material. Additionally, the observed distribution is influenced not only by intrinsic surface characteristics but also by experimental conditions, including tip-surface interactions and the presence of water, which may contribute to the attenuation of height variations and the clustering of values around the mean (35).

Hydration was associated with an attenuation of topographical contrast and a homogenization of the surface relief, leading to increased compliance of the superficial layer. This aspect is clinically relevant, as hydrated conditions closely mimic the physiological environment in which these biomaterials are typically applied.

The predominantly isotropic character of the surface, together with the uniform distribution of height variations, suggests a predictable material behavior when in contact with soft tissues. Such an organization may promote a more uniform interaction with the biological environment and reduce the risk of localized stress concentrations or uneven tissue integration.

The moderate values of surface roughness parameters indicate a surface that is sufficiently active from a topographical perspective to support biological interactions, without exhibiting extreme irregularities that could compromise mechanical stability or local surface behavior. This balance may be considered favorable for processes such as cell adhesion and the initial stabilization of the biomaterial.

The height distribution, characterized by a concentration of values around the mean plane and the absence of extreme values, further supports the presence of a morphologically stable and homogeneous surface. From a clinical perspective, this uniformity may contribute to more controlled integration and predictable interactions with biological fluids and tissue components.

CONCLUSIONS

The results of the atomic force microscopy analysis demonstrated that, under hydrated conditions, the surface of the porcine-derived collagen matrix exhibits a moderately developed, continuous, and relatively uniform relief, characterized by gradual altimetric variations and the absence of major structural discontinuities.

This morphological configuration reflects a stable structural behavior and a balanced distribution of surface features.

Overall, the identified topographical characteristics suggest that the analyzed collagen matrix exhibits surface properties highly compatible with use in hydrated environments, providing a stable and uniform structural support. These features may have favorable implications for the clinical performance of the biomaterial, particularly in terms of soft tissue adaptation and interaction with the biological environment.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflicts of interest and this research received no external funding.

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