

# MECHANICS МЕХАНИКА



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## Electric-Field-Assisted Formation of a Biomimetic Organomineral Coating on Natural Human Tooth Enamel: Morphology and Surface Mechanical Properties



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### Abstract

**Introduction.** The development of coatings capable of reproducing the structural and functional properties of dental enamel is of considerable interest for dental materials science and biomimetic surface engineering. Despite the progress achieved in biomimetic calcium-phosphate systems, the most common approaches still rely on multistep protocols that are highly sensitive to interfacial-layer formation conditions and do not always ensure simultaneous reduction of deposition time, control of coating morphology, and reproducible surface mechanical response. The objective of this study was to experimentally evaluate the feasibility of one-step formation of a biomimetic hybrid nHAp/PDA coating in an electric field using isolated electrodes, and to determine the effect of the deposition mode on surface morphology and the surface microhardness of the “coating–substrate” system.

**Materials and Methods.** Segments of native human permanent tooth enamel were used as a model of a natural apatite-containing substrate. Four surface conditions were compared: native enamel, an nHAp/AA layer formed after acid conditioning, a PDA/nHAp coating obtained by sequential deposition, and a hybrid coating formed via simultaneous electric-field-assisted mineralization and accelerated dopamine polymerization. Deposition was performed in a potentiostatic cell with isolated copper electrodes. Surface morphology was evaluated using scanning electron microscopy and atomic force microscopy (AFM). Surface mechanical response was assessed by Vickers microhardness testing at a 50 g load, AFM mapping of indentation imprints, and local nanoindentation.

**Results.** The one-step electric-field-assisted mode was found to produce the densest and most uniform surface layer, approximately 1  $\mu\text{m}$  thick, with a minimum roughness of about 20 nm. Sample D demonstrated the highest surface microhardness values, reaching approximately 310 VHN, whereas native enamel showed values of approximately 280 VHN, sample B — about 120 VHN, and sample C — about 190 VHN. One-way ANOVA confirmed a statistically significant effect of sample type on microhardness ( $p < 0.001$ ). AFM mapping of the indentation imprints confirmed the accuracy of optical diagonal measurements on the textured surface.

**Discussion.** The increased surface microhardness of the electric-field-assisted sample appears to be associated with more organized interfacial interactions involving polydopamine and a denser packing of the mineral component of the coating. At the same time, Vickers microhardness testing and AFM nanoindentation characterize different scale levels of the mechanical response and should therefore be interpreted as complementary methods.

**Conclusion.** It is shown that one-step formation of a hybrid nHAp/PDA coating in an electric field using isolated electrodes makes it possible to obtain a morphologically organized layer with a surface mechanical response comparable to that of intact enamel. The proposed approach appears promising for the accelerated formation of functional organomineral coatings on apatite-containing substrates.

**Keywords:** electric field, organomineral coating, native enamel, microhardness atomic force microscopy

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Оригинальное эмпирическое исследование

## Электрополевое формирование биомиметического органоминерального покрытия на природной эмали человеческого зуба: морфология и поверхностные механические свойства

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### Аннотация

**Введение.** Разработка покрытий, способных воспроизводить структурно-функциональные свойства зубной эмали, представляет значительный интерес для стоматологического материаловедения и биомиметической инженерии поверхностей. Несмотря на развитие биомиметических кальций-фосфатных систем, наиболее распространённые подходы по-прежнему основаны на многостадийных протоколах, чувствительных к условиям формирования межфазного слоя, и не обеспечивают в полной мере одновременного сокращения времени осаждения, контроля морфологии покрытия и воспроизводимого механического отклика поверхности. Цель данной работы состояла в экспериментальной оценке возможности одностадийного формирования биомиметического гибридного nHAp/PDA-покрытия в электрическом поле с использованием изолированных электродов, а также в установлении влияния режима осаждения на морфологию поверхности и микротвёрдость системы «покрытие–подложка».

**Материалы и методы.** В качестве модели природной апатитовой подложки использовали сегменты нативной эмали постоянных зубов человека. Сравнивали четыре состояния поверхности: нативную эмаль; слой nHAp/AA, сформированный после кислотного кондиционирования; покрытие PDA/nHAp, полученное в последовательном режиме; гибридное покрытие, сформированное при одновременной электрополевой минерализации и ускоренной полимеризации дофамина. Осаждение выполняли в потенциостатической ячейке с изолированными медными электродами. Морфологию поверхности оценивали методами сканирующей электронной и атомно-силовой микроскопии. Поверхностный механический отклик исследовали по микротвёрдости Виккерса при нагрузке 50 г, AFM-картированию отпечатков и локальной наноиндентации.

**Результаты исследования.** Установлено, что одностадийный электрополевой режим обеспечивает формирование наиболее плотного и равномерного поверхностного слоя толщиной порядка 1 мкм с минимальной шероховатостью около 20 нм. Для образца D зарегистрированы наибольшие значения поверхностной микротвёрдости — около 310 VHN; для нативной эмали они составили около 280 VHN, для образца В — около 120 VHN, для образца С — около 190 VHN. Однофакторный дисперсионный анализ подтвердил статистически значимое влияние типа образца на микротвёрдость ( $p < 0,001$ ). AFM-картирование отпечатков подтвердило корректность оптической оценки диагоналей на текстурированной поверхности.

**Обсуждение.** Повышение поверхностной микротвёрдости образца, сформированного в электрополевом режиме, связано, по-видимому, с более организованным межфазным взаимодействием при участии полидофамина и более плотной упаковкой минеральной составляющей покрытия. При этом микротвёрдость Виккерса и AFM-наноиндентация характеризуют различные масштабные уровни механического отклика и должны интерпретироваться как взаимодополняющие методы.

**Заключение.** Показано, что одностадийное формирование гибридного nHAp/PDA-покрытия в электрическом поле с использованием изолированных электродов позволяет получить морфологически организованный слой с поверхностным механическим откликом, сопоставимым с интактной эмалью. Предложенный подход представляет интерес для ускоренного формирования функциональных органоминеральных покрытий на апатитсодержащих подложках.

**Ключевые слова:** электрическое поле, органоминеральное покрытие, природная эмаль, микротвёрдость, атомно-силовая микроскопия

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**Introduction.** Enamel is a highly mineralized, hierarchically organized tissue, whose mechanical characteristics are determined by the orientation of apatite crystallites, compositional gradient, and structural anisotropy [1]. The scientific and applied significance of developing coatings capable of partially reproducing or restoring the specified characteristics is associated not only with the tasks of local regeneration of hard dental tissues, but also with the creation of controlled biomimetic systems in which the interphase organization and mechanical response are regulated by the composition and mode of formation of the coating [2].

Modern approaches to remineralization and the production of enamel-like coatings include systems based on functional inorganic materials, organic matrices, and polymer carriers [3]. A separate direction is represented by hydrogel systems that make it possible to simulate a gel-like mineralization environment and control the local delivery of ions [4]. Hydroxyapatite (nHAp) is of significant interest as a functional material with high bioactivity and structural similarity to the mineral phase of hard tissues [5]. Clinical and materials science reviews confirm the high potential of hydroxyapatite-containing systems for the prevention of damage and restoration of enamel [6]. However, the use of predominantly mineral systems does not always provide stable adhesion of the coating to the enamel surface and controlled interphase organization at the coating – substrate interface, which limits the reproducibility of the morphology and mechanical properties of the formed layer.

At the same time, polydopamine (PDA) is considered as one of the most universal interfacial components due to its pronounced adhesive properties and the ability to initiate the binding of the inorganic phase to the substrate [7]. Modern reviews devoted to the chemistry of polydopamine show that such films not only stabilize the surface, but also form a functional interphase capable of directing mineral formation [8]. Although accelerated deposition schemes involving the  $\text{CuSO}_4/\text{H}_2\text{O}_2$  system can significantly reduce the time to obtain a more uniform layer [9], the problem of reproducible control of the morphology and interphase organization of hybrid coatings remains challenging. For dental applications, it is important that PDA is able to initiate mineral formation on demineralized enamel [10] and enhance the remineralization effect in one-step coatings combining polydopamine and fluoride ion [11]. It is also shown that polydopamine coatings affect the nature of nucleation of the calcium phosphate phase on the surface of mineralized substrates [12].

At the same time, alternative biomimetic matrices, including amelogenin-like systems, are capable of directing the oriented formation of an enamel-like mineral structure, but, as a rule, require more complex multistage protocols [13]. Electrokinetic approaches, on the contrary, improve the transport of ions into the thickness of the enamel, but by themselves do not solve the problem of controlled interfacial organization of a hybrid coating [14]. Previous work by the authors shows the possibility of rapid deposition of hybrid hydroxyapatite-polydopamine layers on natural enamel [15]. However, the question of whether one-step coating formation in an electric field with isolated electrodes can simultaneously ensure reduced deposition time, controlled morphology, and a reproducible surface mechanical response, remains insufficiently studied. The possibility of more localized control of deposition processes and interphase interaction under conditions of spatial separation of electrode processes, which potentially makes it possible to increase the homogeneity and structural organization of the formed hybrid layer, is of particular interest.

The objective of this work is to experimentally evaluate the possibility of one-step formation of a hybrid nHAp/PDA coating in an electric field using isolated electrodes, as well as to establish the effect of the deposition mode on the surface morphology and surface microhardness of the coating – substrate system.

This study answers the following questions.

1. Does a one-step electric-field-assisted mode provide a more uniform and structurally organized coating compared to sequential deposition schemes?
2. Are morphological changes in the surface accompanied by an increase in surface microhardness?
3. Are the results of Vickers microhardness and local AFM nanoindentation consistent in assessing the mechanical response of the formed hybrid layer?

**Materials and Methods.** The study included the following main phases.

**Phase 1:** Preparation of dental enamel samples. Enamel segments from permanent human teeth without visible carious lesions, cracks, or restorations were used as substrates. The enamel samples were prepared, processed, and the test series were formed according to a protocol previously described in detail for a related deposition system [15]. At the selection phase, the teeth were subjected to a cursory clinical examination to confirm the absence of carious lesions, defects, including erosions and wedge-shaped lesions, as well as visually detectable changes in the enamel structure.

Dental enamel segments measuring 5x5 mm<sup>2</sup> and approximately 2 mm thick were obtained using a low-speed diamond saw with water cooling. After segmentation, the sections were placed in sealed containers with constant humidity, where they were stored until the start of experimental studies.

**Phase 2:** Formation of series A and B. Sample A represented native enamel and served as a control group. To obtain Sample B, the native enamel surface was conditioned with 37% phosphoric acid for 30 seconds, followed by deposition of an nHAp/AA layer.

**Phase 3:** Formation of series C. After surface conditioning with 37% orthophosphoric acid for 30 s and subsequent alkaline activation, a hybrid layer was sequentially formed: first, dopamine was polymerized for 2 h, then nHAp was deposited. This resulted in a PDA/nHAp coating, which allowed evaluating the contribution of polydopamine to the sequential organization of the interfacial layer and mineral phase.

**Phase 4:** Formation of series D. Sample D was obtained in one step by combined electric field mineralization and dopamine polymerization. The coating was formed in a potentiostatic cell with insulated copper electrodes that were not in contact with the working solution. The basic one-step deposition mode included a TRIS buffer with pH = 8.5, dopamine hydrochloride — 2 mg/ml, 5 mM CuSO<sub>4</sub> 6H<sub>2</sub>O, 20 mmol H<sub>2</sub>O<sub>2</sub>, suspension with a mean particle size of 20–30 nm and a final concentration of 1 mg/ml. Aspartic acid was used as the amino acid component at a concentration of 0.1 mg/ml. The voltage was approximately 45 V, the distance between the electrodes was approximately 4 mm, and the process duration was 4 h. The use of the Cu<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> system was consistent with published data on the accelerated deposition of more homogeneous PDA films [8]. This design allowed for a sequential evaluation of the contributions of pretreatment, mineral phase, polydopamine, and the electric field. A separate control series without an electric field or without an accelerating system was not included in this study, which was taken into account when interpreting the results obtained.

**Phase 5:** Morphological analysis of the samples obtained. The surface morphology was examined using scanning electron microscopy (SEM) on a JEOL JSM-6700F instrument (JEOL, Japan) and atomic force microscopy using the IMBUA-nano ultramicroscopy setup at the Brazilian Synchrotron Radiation Laboratory (LNLS), which combined scanning near-field optical microscopy (s-SNOM) with infrared radiation from the synchrotron. SEM was used to assess layer continuity, substrate overlap, and determine coating thickness on transverse cleavages. IMBUA-nano equipment was applied to analyze nanorelief, surface profiles, and indentation geometry. Roughness was quantified using parameter Ra on 10 × 10 μm scans. The selection of these techniques and their application parameters followed the previously used protocol for studying hybrid coatings on enamel [15, 16].

**Phase 6:** Assessment of mechanical properties and statistical processing. Surface mechanical properties were assessed by Vickers microhardness using an HVS-1000 device (TIME Group Inc., China) at a load of 50 g and a holding time of 15 s; 10 measurements were performed for each sample. Since the thickness of the coating was finite, the measured value was interpreted as the surface microhardness of the composite coating-enamel system, and not as the intrinsic hardness of the isolated layer [16]. For sample D, AFM mapping of Vickers fingerprints and local analysis of DvZ/DFL curves were additionally performed.

This sequence of phases made it possible to compare the effect of pretreatment, the method of forming the hybrid layer, and the deposition mode on the morphological and mechanical characteristics of the coating-enamel system.

Statistical processing of the results was performed using one-way ANOVA analysis of variance followed by multiple pairwise comparison of groups using the Tukey test; differences were considered statistically significant at  $p < 0.05$ .

**Research Results.** In accordance with the objective of the work, the morphology of coatings formed in different modes was first analyzed, and then their surface mechanical response was assessed. A comparison of the coating formation modes showed that the type of interphase interaction affects significantly the final surface morphology (Table 1). For sample B, in which an nHAp/AA layer was deposited after pretreatment, a developed, but thin and poorly integrated layer with a thickness of about  $0.4 \mu\text{m}$  was formed. According to morphometric analysis, the surface roughness in this series reached  $47.8 \pm 5.6 \text{ nm}$ , while for native enamel, it was  $22.1 \pm 3.4 \text{ nm}$ . With the sequential introduction of polydopamine in sample C, the coating thickness increased to approximately  $0.9 \mu\text{m}$ , and parameter Ra decreased to  $25.4 \pm 3.2 \text{ nm}$ . The most pronounced effect was recorded for sample D: the one-step electric-field-assisted mode provided a more uniform substrate coverage and the formation of a dense layer  $1.0\text{--}1.2 \mu\text{m}$  thick with a minimum roughness of  $18.3 \pm 2.6 \text{ nm}$ , which may indicate a higher degree of morphological ordering of the coating.

According to AFM data, sample D is characterized by the presence of ordered nanoaggregates of approximately  $50\text{--}80 \text{ nm}$  in size, forming a distinct relief and denser surface packing. The presence of denser packing and ordered nanoaggregates is consistent with the assumption of a more organized interfacial structure of the coating [13, 18].

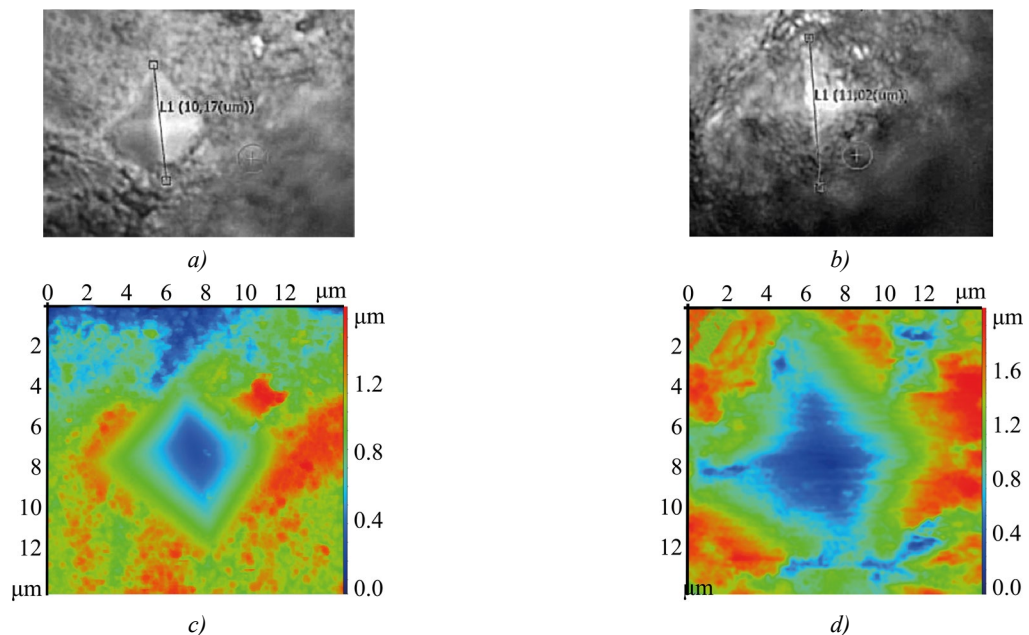
Table 1

Vickers Surface Microhardness at a Load of 50 g

Indicator	A, native enamel	B, nHAp/AA	C, PDA/nHAp	D, (electric-field-assisted)
VHN, M $\pm$ SD	280 $\pm$ 20	120 $\pm$ 10	190 $\pm$ 13	310 $\pm$ 22

Microhardness measurements showed that the minimum value was found for sample B — approximately 120 VHN. For sample C, an increase in hardness to approximately 190 VHN was recorded. The maximum values were obtained for sample D — approximately 310 VHN, which is slightly higher than the average value for native enamel — approximately 280 VHN. One-way analysis of variance showed a statistically significant effect of sample type on microhardness ( $p < 0.001$ ). According to post-hoc analysis using Tukey test, significant differences were found between all pairs of samples, with the exception of pair A–D, which indicated the absence of statistically significant differences between sample D and intact enamel under the conditions of the experiment.

To verify the mechanical properties of sample D, optical images of the Vickers indenter marks on native enamel and in the electric field series were first analyzed (Fig. 1 *a, b*). To more accurately determine the geometry of the marks on the textured surface, they were then further examined using AFM mapping (Fig. 1 *c, d*). According to this analysis, for sample D, values of about 320 VHN were obtained at a load of 50 g and about 290 VHN at a load of 10 g. The obtained values are consistent with the results of the optical microhardness tester and confirm that the high level of surface microhardness is not an artifact of measuring the diagonals of the indentation on the textured surface (Fig. 1 *a–d*).



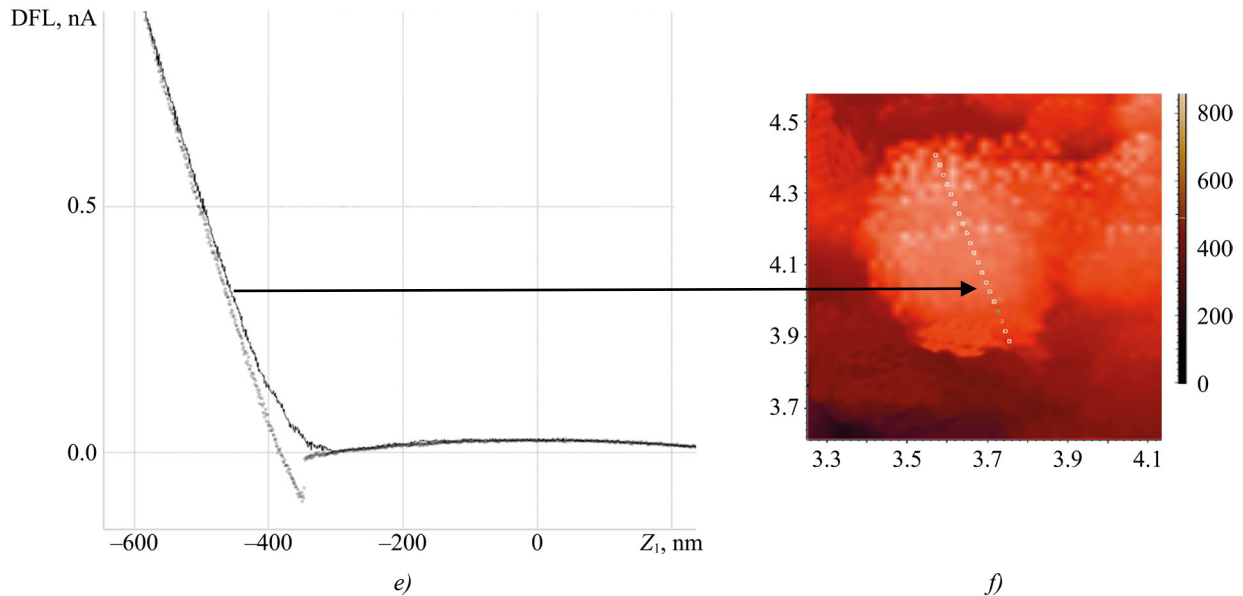


Fig. 1. Mechanical characterization of native enamel and sample D: *a, b* — optical images of Vickers indenter prints; *c, d* — AFM topography of prints; *e* — typical force curve of local AFM nanoindentation; *f* — AFM image of the surface area where the force response was recorded

To further evaluate the local mechanical response of the near-surface layer, AFM nanoindentation was performed.

During local AFM nanoindentation at depths less than 50 nm, the response of the near-surface PDA-rich shell with a Young's modulus of approximately 1 GPa was recorded. A typical force curve is shown in Figure 1 *d*, and the surface region where the force response was recorded is shown in Figure 1 *f*.

**Discussion.** The results obtained show that the higher surface microhardness of sample D is associated not only with the presence of the mineral component, but also with the nature of the interfacial organization of the hybrid layer. This is consistent with existing data indicating that polydopamine can act as an adhesive interphase and simultaneously affect the early stages of mineral formation [8, 10, 12].

Compared to sample B, which formed a thin and poorly integrated layer after acid conditioning, and sample C, where the morphology improvement was achieved mainly due to the sequential introduction of PDA, the electric-field-assisted mode provided a denser coating packing and a more uniform substrate overlap. Probably, a more organized interphase structure and reduced localized coating heterogeneity contributed to a stable distribution of mechanical load under indentation. However, the presented data do not allow us to definitively link the increase in hardness solely to the effect of the electric field, as chemical acceleration of polymerization could also have made a significant contribution.

Crucially, the recorded microhardness characterizes not the isolated coating, but the composite coating – substrate system. Comparison of the layer thickness of approximately 1.0–1.2  $\mu\text{m}$  with the strain scale under a 50 g load shows that the substrate contribution to the measured mechanical response remains significant. Therefore, the obtained values are more accurately interpreted as the surface microhardness of the system as a whole. In this sense, Vickers indentation and AFM nanoindentation data are complementary: the former reflects the integrated response of the hybrid layer and the substrate, while the latter characterizes the local properties of the near-surface PDA-rich shell [16].

The obtained data confirm that the combination of an organic interphase and a mineral component can play an important role in the formation of mechanically stable enamel-like coatings. The results are consistent with modern approaches, according to which the most promising biomimetic materials for hard tissues combine a controlled organic interphase and a mineral component [2, 17, 18]. In this context, a one-step scheme with isolated electrodes is of interest not only as a remineralization option, but also as an engineering strategy for the accelerated formation of an organized surface layer. A limitation of the study remains the lack of a dedicated control series that would allow for a complete separation of the contributions of the electric field and chemical polymerization accelerator. This makes it impossible to fully separate the contributions of the electric field and chemical accelerator to the observed changes in the morphology and mechanical properties of the coating. Further research should include dedicated control modes and an assessment of the long-term stability of the resulting coatings, including the use of a combination of machine learning methods and algorithms [19].

**Conclusion.** A method has been developed for the one-step formation of a hybrid nHAp/PDA coating in an electric field using insulated electrodes.

It is shown that the electric field mode provides the formation of a denser and morphologically organized layer compared to sequential deposition schemes and is accompanied by the highest values of surface microhardness — up to 310 VHN at a load of 50 g.

The results of AFM analysis of the indentations and local nanomechanical testing confirm the consistency of the mechanical response assessment at different scale levels.

The data obtained allow us to consider the proposed approach as a promising method for accelerating the formation of functional organomineral coatings on apatite-containing substrates. Further research should be focused on varying the electric field parameters, controlling the layer thickness, and assessing the long-term stability of the coating under combined chemical-mechanical effects.

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