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DETERMINATION OF THE SYNTHETIC HYDROXYAPATITE LIFE CIRCLE USED IN DENTAL PRACTICE

Dental materials are developed as general materials for specific aplication in oral environment. To determine the functional properties of these materials, nonstandard approach and specific methods need to be utilized. In this study, two methodologies of material testing were used - artificial aging and quantification of visual informations for life circle assesment of hydroxyapatite (Hap) based materials. Hap was chemically synthetized, resulting in high purity and crystallinity of the material. Artificially produced Hap is used in stomatology for repair of bone tissue, as a filling for periodontal defects, and as a preservative augmentation for alveolar ridges. These materials are also used for definitive root canal obturation in endodontic therapy procedure as an apical plug or as complete filling material. This research study was focused on the analysis of the bonding properties of Hap-based materiales to the root canal walls. The methodology of artificial aging was used together with the quantification of visual informations for the purpose of quantifying the Hap bonding properties and bonding quality. Experiments were done in vitro, with the artificial saliva as the agressive agent. The experimental teeth were analysed by a high resolution optical microscope for the morphological characterisation of the bonding layer. A model for the bond life circle assesment was developed. Hap-based materials showed favorable properties for dental use. The presented results proved that the combination of the two methodologies (artificial aging and quantification of visual informations) could be used as a tool for analysing the material-dentine interaction.

Keywords: hydroxilapatite; material; modeling; life circle; bonding; aging; dentine.

Synthetic hydroxyapatite (Hap) belongs to the group of ceramic biomaterials with a wide range of use in medicine and dentistry [1]. It is produced in two forms, non-porous and porous, and it is also available in granules and cubes. The final morphology and stoichiometry of Hap depend on how it was synthesized. In addition to the ceramic form of Hap, the cement form of Hap has been studied since 1991. It is produced by direct crystallization in vivo without heating to high temperatures in oder to obtain a structurally stable implant.

Hap was synthetised chemically [2] and obtained wih has high purity and crystallinity. Artificially synthetised Hap proved to be a successful substitute for natural Hap [4]. This material is used in stomatology for repair of bone tissue, as a filling for periodontal defects, and as a preservative augmentation for alveolar ridges [3,4]. It is also used for definitive root canal obturation in endodontic therapy procedure as an apical plug or as complete filling material.

Numerous studies showed that all forms of hydroxyapatite are biocompatible. Hap does not induce a giant cell reaction, considerable inflammation or elevated serum Ca and P [5,6]. In addition to being biocompatible, synthetic Ca-phosphates have an effect on the formation of the dentine tissue bridge [7]. These synthetic materials possess both osteointe-

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grative and osteoconductive properties and improve mechanical properties over other bone substitutes.

Clinical confirmation of osteoconductive and stimulative properties on bone reparation is observed on endodonticaly treated teeth after an appropriate observation period (12 to 24 months).

An ideal material for sealing tooth cavities, root or bone should prevent leakage, and also be non-toxic, non-carcinogenic, and biocompatible with the tissue fluids and dimensionally stable. The presence of moisture should not affect its sealing ability. For practical purposes, it should be easy to use and be radioopaque in order to be recognized on the radiograph. This research study was focused on the analysis of the bonding properties of Hap in the root canal.

Numerous different experimental setups were developed in order to evaluate the effect of the oral environment and its ingredients (saliva, bacteria and microorganisms) on the stability of dental materials. Each one of them was proposed in order to simulate different factors of the oral environment [7]. Most of these models comprised a solution which was considered: a) to simulate as close as possible the conditions in the oral cavity or b) to accelerate the degradation in order to meet a time effective answer whether the tested materials are resistant to degradation. Some of them intended to simulate the effect of the environmental conditions, different pH levels found in the oral cavity, others the impact of thermal fluctuations occurring in the oral environment and some the solubility action of food elements. It has been often attempted to incorporate all these factors in a solution, which could simulate saliva. Therefore, numerous artificial saliva models were developed. It is clear that a solution, which simulates all chemical, thermal and biochemical elements, would be ideal to reproduce the oral conditions and eventually predict certain properties of dental materials behaviour *in vivo*. In the presented study, the so-called Ira and Shannon prescription [12] was used with ingredients presented in Table 1.

Duplicating exactly the properties of human saliva is impossible due to the inconsistent and unstable nature of natural saliva. This instability also makes natural saliva itself inappropriate for use in standardized *in vitro* studies. Although there is a long history of artificial saliva development, it is characterized by generally *ad hoc* formulations. A large assortment of many different artificial saliva compounds is available. Very few of them were found to contain all major ionic components with concentrations in the physiological range.

Saliva should be considered as a potential corrosive factor for materials. Water is able to enter the polymer matrix. The outcome of this entrance is thought to be a swelling of the matrix and the hydrolitical degradation of the silane bond between filler and matrix. This process is proposed to be long lasting in the oral cavity, but much longer in *in vitro* set-ups with either distilled water or artificial saliva. This study was undertaken with the aim to determine the life circle of synthetic Hap used as a material for definitive tooth root canal obturation.

Table 1. Ira and Shannon prescription [12]

Component	Content, mass%
KCl	0.06
NaCl	0.08
$MgCl_2$	0.005
$CaCl_2$	0.001
K_2HPO_4	0.08
KH_2PO_4	0.03
Methyl-p-hydroxybenzoate	0.2
Corigens	0.4
Sorbitol	2.275
Na-carboxyethylcellulose	1.00
NaF	0.0004
Water	95.8436

Mathematical description of correlations (models) for materials properties changes is a great challenge for both theoretical and applied investigations [8]. Researchers are always in a dilemma about the level of interactions for considering change of chosen parameter with time. From a practical point of view, investigating the interactions that lead to evident changes is adequate. This problem can be solved by using working models that correlate one of the morphological parameters with a certain characteristic of the produced material.

The basis of the model is defining a function (model) of the investigated property change with time:

$$I = f(x_1, x_2, \dots, x_n) I_0 \tag{1}$$

In relation to the initial value at time $\tau = 0$, $f(x_i)$ can be a constant or a function. According to the basic postulates of stereology [9-11], the surface and volume distribution of a measured/determined morphological parameter are mutually equal in the first approximation. This provides working models for correlation of the investigated property change with the corresponding changes of the appropriate parameter.

Selection of morphological parameter for describing and quantifying changes is of substantial significance for analysis and modelling. In this case, the measuring of bond degradation during time was done by measuring the length of bond destruction in the prepared samples. In order to quantify the bonding properties, the index of bonding destruction was calculated [7,10]. The definition is simple - the index of bonding destruction, *IOB*, presents the percent of the destructed bond line as a function of time:

$$IOB = \frac{L_{\mathrm{tot}} - L_{\mathrm{dest}}}{L_{\mathrm{tot}}} \qquad (2)$$

where *IOB* is the index of bonding destruction, L_{tot} is total bonding length and L_{dest} is the length of the destructed bond.

The aim of this research was to analyse the Hap-dentine bond properties, in order to predict the life circle assessment of this material in endodontic practice. The other goal was to analyse the possibilities of simultaneous use of artificial aging and quantification of visual information as tools for materials behaviour analysis in practice.

MATERIAL AND METHODS

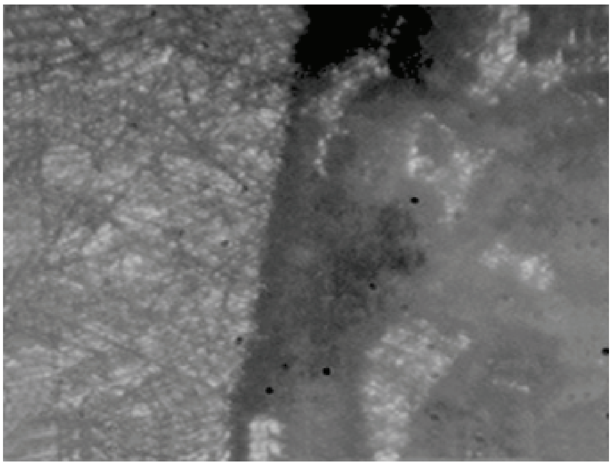
The thirty human anterior single rooted teeth, freshly extracted due to severe periodontal destruction, were chosen for this study. The teeth were instrumented using the Crown down technique and nickel-titanium rotary instruments, irrigated with 2.5% NaOCl, and lubricated with chelating agent. Root canals were obturated with Hap-paste (Hap-composite material - a mixture of 65% Hap Pat. No. 285/91 with 35% of calcium-sulphate and sterile saline) as an apical sealer-plug, and Sankin Type I (Sankin Industry, Japan) the hydroxyapatite-based root canal sealer and gutta-percha cones were used to complete definitive root canal filling.

The teeth were stored at 37 °C and 100% humidity for one week to allow the sealers to set. Some root samples were grooved longitudinally on the bucal and lingual surfaces, and were split into 2 halves. Other samples were cross sectioned at the coronal, middle and apical level. Stability, porosity and bonding properties of the Hap material-root dentine interface, and the changes induced by the aging methodology were investigated by analysing the adhesive properties using quantification of visual informations methodology [9].

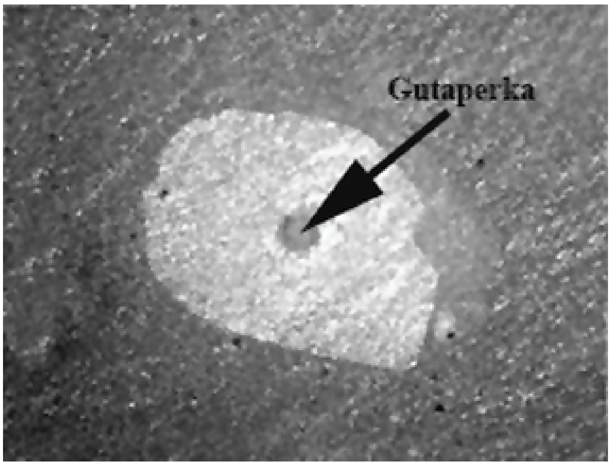
Sets of five experimental tooth samples were immediately analysed as the zero samples, which gave the results for the untreated samples (0 days of experiment). Other sets of five tooth root samples were treated with artificial saliva solution and analysed after 7, 14, 21, 28 and 35 days of the treatment.

RESULTS AND DISCUSION

Qualitative exsminstion of samples proved that there were changing in bonding during time. The optical microscopy images of the starting samples (at $\tau = 0$ days) are presented in Figure 1. The transversal and longitudinal images show the same quality bond between dentine and the Hap. The bond layer is stable and does not have any discontinuites. In the transversal section (Figure 1B), the position of gutta-percha is visible.



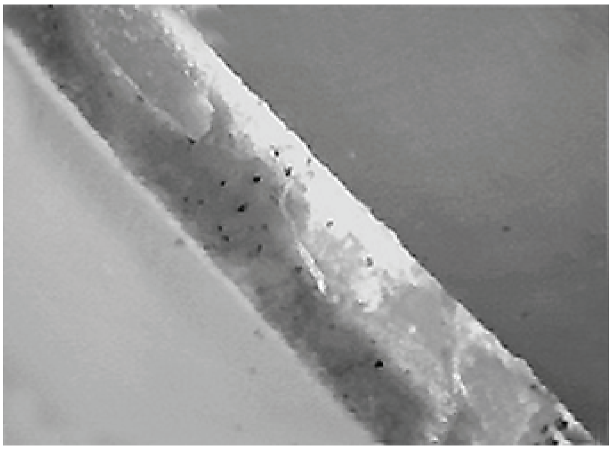
(A)



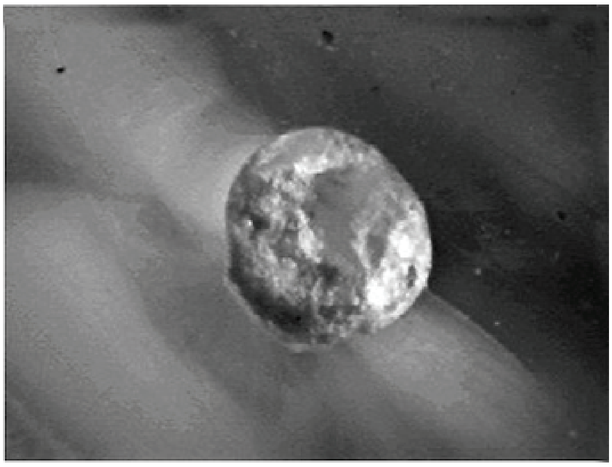
(B)

Figure 1. Starting sample: A - longitudinal section and B - transversal section.

Figure 2 presents the images of samples after $\tau = 35$ days of treatment in the artificial saliva. The destruction of the bonding layer is clearly visible on both sections. However, the remains of Hap material still exist on the dentine surface. We have the situation that after the end of experimental cycle the bond damage was such that there was no significant functional amount of Hap.



(A)



(B)

Figure 2. Samples after 35 days of treatment in artificial saliva; A - longitudinal section and B - transversal section.

Quantitative mesurements of bond destruction, measuring the length of damaged interface between the Hap and dentine for all the samples is presented in Table 2.

Table 2. Results of IOB evaluation

Time, days	*IOB* / %	Standard deviation, %
0	0.1	0
7	1.9	0.6
14	3.05	0.9
21	5.2	2
28	20.9	5.2
35	45.91	12.4

The folowing Figure 3 presents graphicaly the Index of bond destruction during the experiments.

The obtained data present the differences in the bond destruction gradient during experimental time of 35 days. The gradient of *IOB* changes is small during the first 20 days. After that, large changes in bond destruction rate is observed, together with greater dissipation of results in that period (*SD* values). This suggests that there were possible changes in the mechanism of bond destruction after a period of 20 days. This can be explained by the bond resistance potential, in this case Hap-dentine, which was lowered by the destruction agent over time. This potential reaches a critical value in the period of 17 to 20 days, after which the material, in this case bond Hap-dentine, does not have the capacity to resist further bond damages. Modeling of *IOB* changes during time was done by the exponential model:

$$Y = Ae^{bx} \tag{3}$$

where Y is *IOB*, and x is the time of exposure to the artificial saliva.

This model is frequently used as the basic model of chemical reaction kinetics and system decay. Experimental and model values are presented in Figure 4.

Values for the model parameters were A = = 0.0013 and b = -3.36, with the correlation coefficient of R^2 = 0.996. The differences between observed values and values predicted by the model suggest that there must be some changes in the mechanisms of bond destruction over time. To analyse this, measured values of *IOB* were split into two *IOB* classes – longitudinal and transfersal direction (Figure 5). Both IOB values were stable during the first 20 days. The visible bond destruction starts after that time but with different gradients in longitudinal and transfersal directions. This can be explained with the assumption that transversal IOB can be accepted as the local, spot damage of bond, while the longitudinal is line destruction. This is analogous to crack developement in materials, which starts as a local material irregularity and then with time becomes the dominant entity, which leads to material damage.

The same reasoning can be applied to *IOB*. The first bond irregularities happen as local spot damages. Then, over time, local isolated irregularities start forming clusters and spread over longitudinal directions. This means that in the transversal sections the layer still exists, which could be an indication of the existence of some different mechanism of adhesion.

Modeling of *IOB* changes with time for both measurement directions was done using the exponential model. Obtained model coefficients are presented in Table 3.

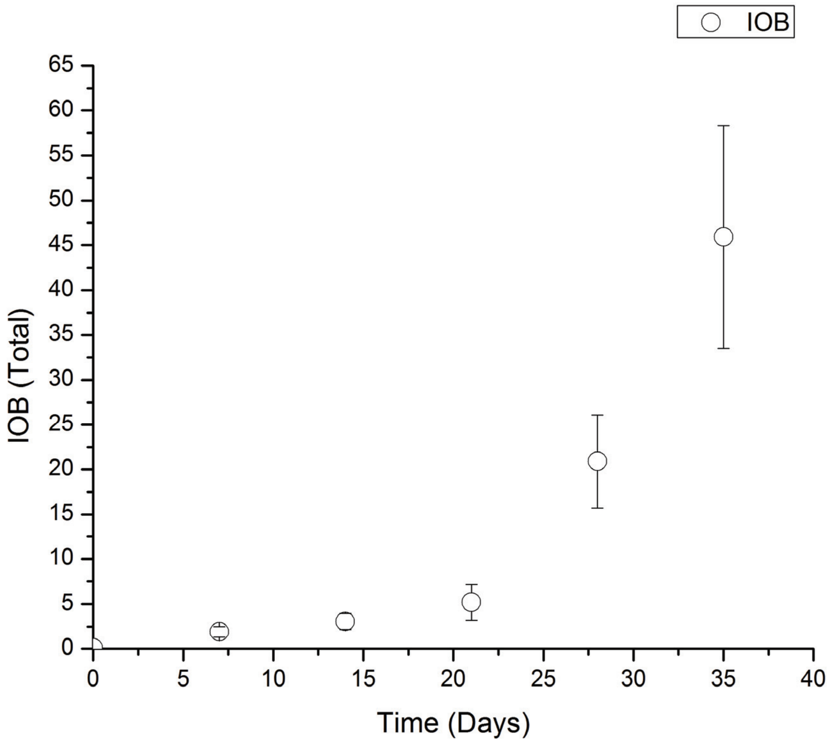


Figure 3. IOB time dependence.

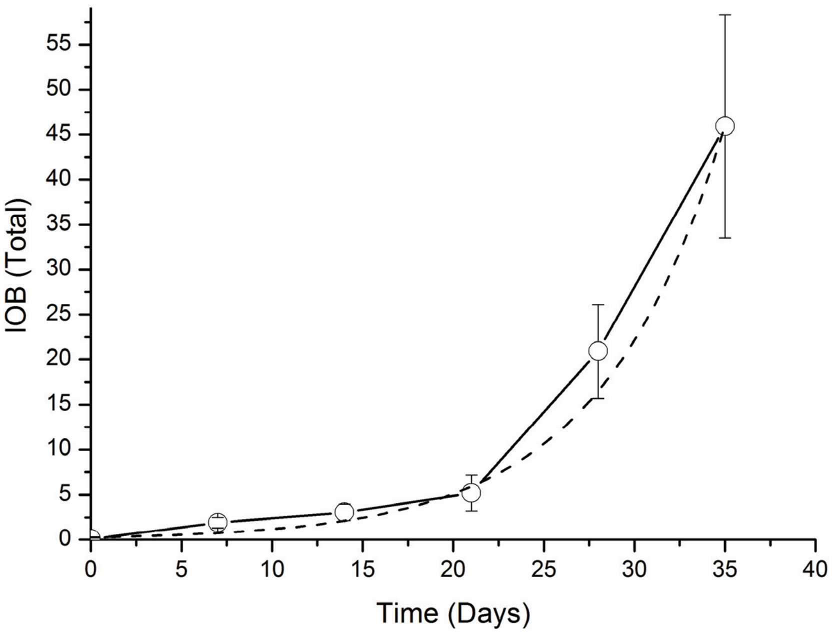


Figure 4. Measured IOB and model values.

The values of *A* in the longitudinal direction are smaller then the value of the same coefficient in the transversal direction. This fact suggests that bond destruction does not start exclusively from local damage on the Hap-dentine inteface.

The value of coefficient *b* is larger in the longitudinal direction. This coefficient could be assumed as the gradient of IOB changes during time. The value points out that the gradient of bond destruction is higher in the transversal direction. This is proven by the experiments and indicates the diferences in bond destruction in the examined directions.

These results can be used to improve the suggested qualitative model of Hap behavior in the rooth

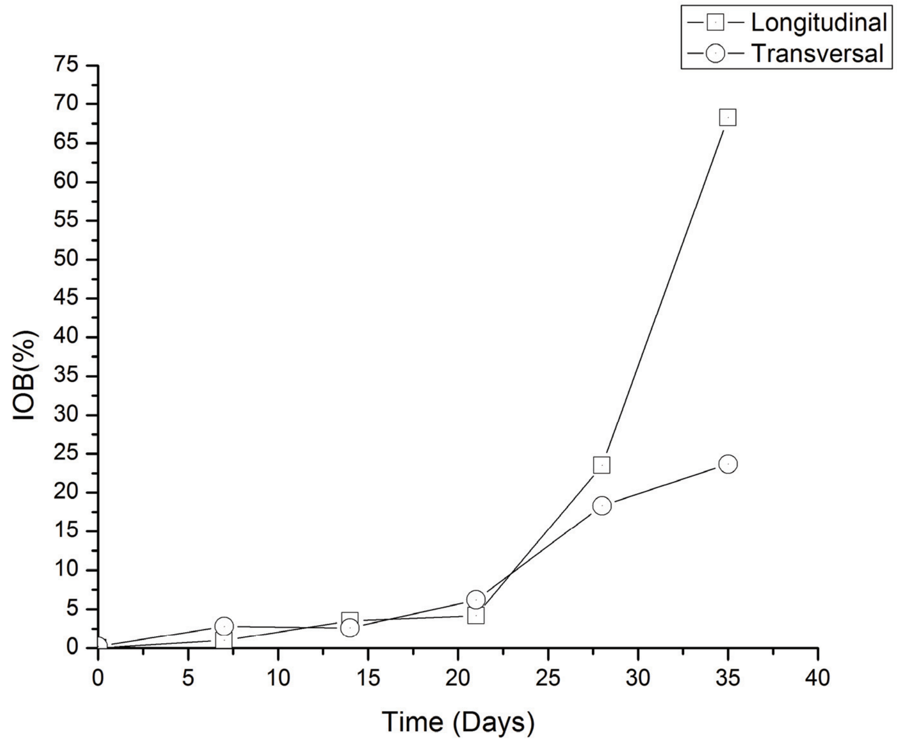


Figure 5. Change of IOB with time for longitudinal and transversal directions.

Table 3. Values of model coefficients

Parameter	Longitudinal (R^2 = 0.994)	Transversal (R^2 = 0.920)
A	0.238	1.418
b	-6.17	-12.18

canal. The methodology of canal obturation is based on the use of two materials - sealant and the gutta-percha [15]. The material destruction by the aging agent starts from the center of the canal, not from the material/dentine interface. In that case *IOB* changes are favored in the direction with higher gradient, *i.e.*, the longitudinal one, which can be seen on the macro scale.

The properties of Hap material create conditions for developing bonding mechanisms other than adhesion, such as chemical bonding or diffusion. This means that Hap could establish a more stable bond to the dentine than adhesion. This is evident on the microscale, after 35 days, on all of the transversal sections examined. Small amounts of the Hap material still remain connected to the dentine, besides the fact that all bonding layers are damaged.

CONCLUSION

Hap proved to have favorable properties for use as a material in dental parctice. It has the ability to induce secondary bonding mechanisms, which can improve the material-dentine bond. To find the exact mechanism of Hap ahdesion, this research must be expanded with microchemical analysis of the bond layer.

Modeling can be used for developing the so--called resistance potential of materials as a starting point for prediction of life circle assesment in practical use. These results showed that the gradients of IOB could be one of the potential means of quantification of resistance potentials.

The presented results proved that the combination of two methodologies - artificial aging and the quantification of visual informations - could be used as a tool for analysing material-dentine interactions in dental practice.

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NAUČNI RAD

ODREĐIVANJE ŽIVOTNOG CIKLUSA SINTETIČKOG HIDRIKSILAPATITA U STOMATOLOŠKOJ PRAKSI

Materijali koji se koriste u stomatološkoj praksi su specifični materijali, koji su razvijani prema specifičnoj nameni u oralnom okruženju. Za određivanje funkcionalnih svojstava ovih materijala razvijen je nestandardni pristup koji je obuhvatio kombinovanje dve metodologije: testiranje materijala primenom veštačkog starenja i kvantifikacije vizuelnih informacija za određivanje veka upotreba materijala na bazi hidriksilapaptita (Hap). Korišćeni Hap materijal je hemijski sintetizovan i posedovao je veliku čistoću i kristaličnost. Ovaj materijal se koristi u stomatološkoj praksi za reparaciju koštanih tkiva, kao i za isunjavanje peridentalnih defekata, a u poslednje vreme i kao definitivna ispuna kanala korena zuba. Istraživanja bila su usmerena na analizu vezivnih svojstava Hap materijala i zidova kanala zuba. Pomenute metodologije veštačkog starenja i kvantifikacije vizuelnih informacija korišćene su u cilju određivanja kvantifikovanja adhezivnih svojstava Hap kao i samog kvačliteta veze. Eksperimenti su uradjeni in vitro sa veštačkom pljuvačkom kao agensom starenja. Eksperimentalni zubi su analizirani optičkim mikroskopom u cilju morfološke karakterizacije vezivnih slojeva. Na osnovu tih rezultata razvijen je model za predviđanje veka upotrebe Hap materijala. Sam materijal je pokazao dobra svojstva za korišćenje u stomatološkoj praksi. Dobijeni rezultati pokazali su da kombinacija primenjenih metodologija može da se koristi kao alat za karakterizaciju interakcija na dodiru materijala i dentina.

Ključne reči: hidroksilapatit; materijal; modelovanje; vek upotrebe; vezivanje; starenje; dentin.