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Ultrastructural studies on implants failure with immediate or late loading

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ABSTRACT

Objectives. The purpose of the present study was to evaluate by scanning electron microscope (SEM) and energy dispersive spectrophotometry (EDS), the degree of bone mineralization of the tissues on the surface of failure dental implants with immediate or late loading.

Materials and method. In the study, 8 dental explants from 8 clinically healthy, non-smoking patients were taken. All implants were inserted by the same dental surgeon, 4 of them were immediate loaded and 4 were late prosthetically loaded by the same dentist. The ablation of the implants was performed as atraumatically as possible and they were collected in sterile containers and sent to the BIOMAT Research Center, where they were subjected to SEM and EDS analyses. The ratios between the chemical elements calcium and nitrogen (Ca/N), phosphorus and nitrogen (P/N), respectively calcium and phosphorus (Ca/P) were calculated for each of the 8 samples and were statistically analyzed.

Results. If we compare the degree of coverage of the implants with bone tissue in different phases of mineralization, we find that the bone structures occupy a larger surface area of the implants in the cases with immediate loading compared to the cases with late loading. Thus, out of 4 immediately loaded implants, 2 were completely covered, one showed very little exposed areas, and the fourth showed alternating covered and uncovered areas. Regarding the late loaded implants, three showed alternation between covered and uncovered areas and only one was completely covered with bone.

Conclusions. The degree of coverage of the explants with bone tissue was better represented for the immediate loading cases. The degree of mineralization of the bone tissue covering the explants was higher for cases with immediate loading. To confirm the obtained results, it is necessary to expand the study on larger batches of samples.

Keywords: SEM, EDS, failure of implants, immediate loading, late loading

INTRODUCTION

The bone tissue is in a continuous evolution and structural change, dictated by the mechanical stresses to which it is subjected and reflected by the compositional variations that denote the health status of the bone [1]. Scanning electron microscope (SEM) examination of explants is an investigation that can be performed repeatedly, at several points, allows the morphological study of the mineralized bone tissue without damaging the sample [2] and generates ample results that allow the investigation of the bone-implant interface. In addition, the energy dispersive spectrophotometry (EDS) analysis allows studying the chemical composition of the tissues present on the surface of the implants, makes it pos-

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sible to assess the degree of bone mineralization by calculating the ratios between some chemical elements and allows the detection of elements that could have migrated into the bone from the inserted implant. Recent studies have used the ratio between calcium and phosphorus (Ca/P), calcium and nitrogen (Ca/N), phosphorus and nitrogen (P/N) to evaluate the degree of bone mineralization [2-4], but until now the degree of mineralization at the bone-implant interface has only been analyzed in a few studies [5-7]. The purpose of the present study was to evaluate comparatively, by means of current SEM and EDS techniques, the degree of bone mineralization of the tissues on the surface of dental explants with immediate or late loading, based on the quantification of the mass percentages of some chemical elements in the bone composition.

MATERIAL AND METHOD

The study was carried out according to a protocol approved by the Ethics Committee of the Faculty of Dental Medicine of the Titu Maiorescu University in Bucharest (no. 5/12.01.2017), respecting the Helsinki declaration and human rights, without harming the patients or the environment. In the study, 8 dental explants were taken from 8 clinically healthy and non-smoking patients, without bruxism. All cylindrical Straumann implants, with SLActive© surface, were inserted by the same surgeon in the postextraction healed bone, 4 of them were immediately loaded and 4 were late prosthetically loaded by the same dentist. Loading was achieved with screw-attached fixed prosthetic restorations, and cases with anterior edentulous benefited from bone augmentation with autologous bone and provisional prosthesis. The immediate loading was carried out 2 days after the insertion of the implants, and the late one after more than 2 months. After the insertion of the implants, the patients were advised to perform a rigorous oral hygiene, they were instructed on the brushing technique to apply, in association with the water flosser and interdental brushes. They were also informed about the need to schedule every 6 months for the application of professional sanitizing in the dental office (scaling, professional brushing, perio flow). However, the 8 patients were not consistent with the recommendations, so that after variable periods of time, the occurrence of biological failure of the implants was found (low secondary stability compared to the primary stability, the presence of local sensitivity and changes in the periimplant gingiva). After the failure of the implant-prosthetic treatment was found, the ablation of the implants was performed as atraumatically as possible, by manual unscrewingand and they were collected in sterile containers and sent to the BIOMAT Research Center within the Politehnica University of Bucharest, where they were subjected to SEM analyses with a Phenom microscope ProX equipped with energy dispersive spectrophotometer. For those samples, histological analysis was not performed because it could not have brought information related to the mass percentages in which the chemical elements that we followed in the study were found and the ratios between them. The samples did not require special preparation for the examination, they were handled with sterile tweezers in order not to be contaminated by touch and in turn not to contaminate the person who performed the SEM and EDS analyses. SEM examination was performed from coronal to apical level of each explant at different magnifications, depending on the areas of interest. Representative images were taken for each explant. EDS analysis was performed for each sample at one single point, in mass and atomic percentages. The ratios between the chemical elements calcium and nitrogen (Ca/N), phosphorus and nitrogen (P/N), respectively calcium and phosphorus (Ca/P) were calculated for each of the 8 samples. The ratio values between the three chemical elements were statistically analyzed with Excel (Office 365) © Microsoft.

RESULTS

Based on the percentages of reports obtained between the three chemical elements of interest, the analyzed areas can be classified into four categories: 1. Category 1, represented by bone with very low mineralization, characterized by very low mass percentages of Ca, N and P, but also very low Ca/N and P/N ratios. 2. Category 2, represented by bone with reduced mineralization, characterized by very low mass percentages of Ca, N and P, but also low Ca/N and P/N ratios. 3. Category 3, represented by bone with average mineralization, characterized by average mass percentages of Ca, N and P, but also by ratios with average values between Ca/N and P/N. 4. Category 4, represented by bone with increased mineralization, characterized by increased mass percentages of Ca, N and P, but also by ratios with high values between Ca/N and P/N. Table 1 shows the 4 categories of weight percentages for each of the tracked chemical elements, respectively Ca, P and N at the level of the analyzed samples. The Ca/N, P/N and Ca/P ratios of each sample are summarized in Table 2. The retrieved images show that the mineralized tissues are not uniformly distributed at the implant interface. Sample 1 (Figure 1a) is represented by an implant that was immediately loaded, from a 50-year-old female patient with partial maxillary edentulous. The Straumann implant inserted into a bone of 7.5 mm thickness (obtained after augmentation with autologous bone) and D2 density according to Misch, had a diameter of 3.3 mm and a length of 12 mm. At the time of insertion, the implant had a primary stability of 41 ISQ (implant stability quotient), which had increased two days after implantation to 43 ISQ, at the time of immediate provisional loading. The patient did not return to regular check-ups, nor she performed a proper hygiene, so that 14 months after loading, she presented with local pain sensitivity and a secondary stability of 32 ISQ, which necessitated the ablation of the implant. The removed implant was sent for SEM and EDS analysis. On the entire examined surface, the intimate adhesion of the mineralized tissue to the implant is found, which demonstrates the presence of the ossification process under the influence of functional pressures. Regarding the chemical composition of the tissues established by EDS analysis, we find the presence of N in very low weight percentages, Ca in low weight percentages and P in moderate weight percentages in this sample. At the same time, the Ca/N ratio is increased, the P/N is moderate, while the Ca/P is very low, which signifies the presence of a bone in the process of maturation. Sample 2 (Figure 1b) is an implant that was late loaded and failed after 18 months. The sample comes from a 60-year-old male patient with maxillary partial edentulous. The Straumann implant was inserted into a bone of 8.5 thickness and D3 density according to Misch and had a diameter of 4.1 mm and a length of 14 mm. At the time of insertion, the implant had a primary stability of 38 ISQ, which two months after implantation, at the time of late immediate loading, had increased to 49 ISQ. The patient did not return to regular check-ups and did not performed proper hygiene, so that 18 months after loading he presented with local pain sensitivity and a secondary stability of 33 ISQ, which required the ablation of the implant. In this case, we find the alternation between implant areas intimately covered with bone tissue and exposed areas. N is present in high weight concentration, Ca is moderate, and P is low. The Ca/N ratio is moderate, the P/N ratio is very low, and the Ca/P ratio is low, suggesting the presence of medullary bone undergoing mineralization. Sample 3 (Figure 1c) is an implant that was late loaded and failed after 16 months. The sample comes from a 56-year-old male patient, with mandibular partial edentulous. The Straumann implant inserted into a bone region of 7.3 thickness and D3 density according to Misch had a diameter of 3.3 mm and a length of 10 mm. At the time of insertion, the implant had a primary stability of 41 ISQ, which two days after implantation, at the time of immediate provisional loading, had increased to 42 ISQ. The patient did not return to regular check-ups nor did he performed proper hygiene, so that 16 months after loading he presented with local pain sensitivity and a secondary stability of 33 ISQ, which necessitated the ablation of the implant. As with sample 2, the bone tissue does not uniformly cover the surface of the implant. The weight percentages of Ca and P are very low, and those of N are moderate. Calculating the Ca/N ratio for sample 3, it falls into category 3, the P/N and Ca/P ratios fall into category 2, suggesting overall the presence of bone undergoing mineralization. Sample 4 (Figure 1d) is an implant that was immediately loaded from a 58-year-old female patient with partial mandibular edentulous. The Straumann implant inserted in a bone with a thickness of 7.3 mm (obtained after augmentation with autologous bone) and D3 density according to Misch had a diameter of 3.3 mm and a length of 10 mm.

At the time of insertion, the implant had a primary stability of 39 ISQ, which two days after implantation, at the time of immediate provisional loading, had increased to 42 ISQ. The patient did not return to regular check-ups, nor she performed proper hygiene, so that 9 months after loading, she presented in dental office with local pain sensitivity and a secondary stability of 31 ISQ, which necessitated the ablation of the implant. On the surface of the implant, there are areas covered by adherent bone tis-

TABLE 1. The weight percentages of Ca, P and N at the level of the studied samples

Types of bone	Ca	P	N
Category 1 (medullary bone)	Very low (0.21-0.52)	Very low (0.44-0.76)	Very low (1.46-5.49)
Category 2 (mineralizing bone)	Low (0.53-0.84)	Low (0.77-1.07)	Low (5.5-9.5)
Category 3 (mature bone)	Moderate (0.85-1.16)	Moderate (1.08-1.39)	Moderate (9.6-13.6)
Category 4 (cortical bone)	High (1.17-1.47)	High (1.4-1.71)	High (13.7-17.8)

TABLE 2. Ca/N, P/N and Ca/P ratios for the 8 studied samples

No. Sample	Ca/N ratio	P/N ratio	Ca/P ratio
Sample 1	0.25	0.28	0.66
Sample 2	0.16	0.05	1.31
Sample 3	0.17	0.17	1
Sample 4	0.12	0.054	2.25
Sample 5	0.22	0.13	1.73
Sample 6	0.03	0.1	0.38
Sample 7	0.24	0.17	1.39
Sample 8	0.19	0.38	0.51

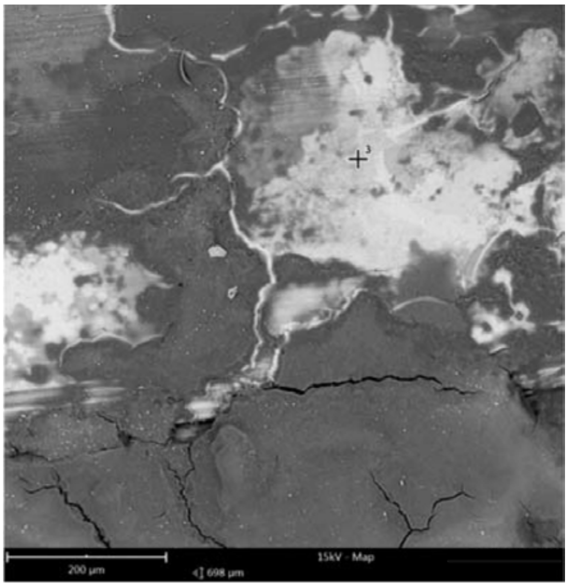
TABLE 3. Correlation between the 3 reports (Ca/N, Ca/P and P/N)

	Correlation between the reports		
Type of Loading	Ca/N→Ca/P	Ca/N→P/N	Ca/P→P/N
Immediate	0.118547228	0.764014618	-0.51091793
Late	0.376038324	0.237120445	-0.81098054

sue, alternating with relatively small areas where the metal is exposed. In the case of this sample, N is found in moderate weight percents, Ca is found in high weight percents, and P is found in very low weight percents. The Ca/N ratio is low, the P/N ratio is very low, and the Ca/P ratio is increased, suggesting the presence of cortical bone. Sample 5 (Figure 1e) is an implant that was late loaded and failed after 13 months. The sample comes from a 52-year-old patient with mandibular partial edentulous. The Straumann implant inserted into a bone of 7.3 thickness and D3 density according to Misch had a diameter of 3.3 mm and a length of 14 mm. At the time of insertion, the implant had a primary stability of 42 ISQ, which two days after implantation, at the time of immediate provisional loading, had increased to 43 ISQ. The patient did not return to regular checkups, nor he performed proper hygiene, so that 13 months after loading, he presented with local pain sensitivity and a secondary stability of 33 ISQ, which necessitated the ablation of the implant. This implant has areas covered by adherent bone tissue on the surface, alternating with areas where the metal is exposed. In the case of this sample, N and P are found in very low weight percentages and Ca is in low weight percentages. The Ca/N ratio is increased, P/N is low, and Ca/P is moderate, suggesting the presence of category 2 bone. Sample 6 (Figure 1f) is an implant that was immediately loaded from a 58-year-old female patient with partial mandibular edentulous. The Straumann implant inserted into an 8.5 mm thick bone region (obtained after augmentation with autologous bone) and D1 density according to Misch had a diameter of 4.1 mm and a length of 12 mm. At the time of insertion, the implant had a primary stability of 42 ISQ, which two days after implantation, at the time of immediate provisional loading, had increased to 43 ISQ. The patient did not observe proper hygiene, so that 6 months after loading, she presented with local pain sensitivity and a secondary stability of 32 ISQ, which necessitated the ablation of the implant. On the surface of the implant, the presence of a dense bone tissue is visible, which completely covers the surface of the implant. Regarding the weight percentages of Ca, P and N quantified by EDS, we find that Ca is found in low quantity, P is in very low weight percentages and N is in increased weight percentages. Analysing the ratios between the chemical elements mentioned above we note that all 3 are very low, suggesting the local presence of medullary bone. Sample 7 (Figure 1g) is an implant that was immediately loaded from a 51-year-old female patient with maxillary partial edentulous. The Straumann implant inserted in a bone region with a thickness of 7.7 mm (obtained after augmentation with autologous bone) and D1 density according to Misch had a diameter of 3.3 mm and a length of 12 mm. At the time of insertion, the implant had a primary stability of 43 ISQ, which two days after implantation, at the time of immediate provisional loading, had increased to 44 ISQ. The patient did not perform proper hygiene, so that 18 months after loading she presented with local pain sensitivity and a secondary stability of 32 ISQ, which required ablation of the implant. Examining the surface of the implant, we note the presence of well-represented bone tissue, with very few areas where the uncovered implant is visible. Regarding the weight percentages of Ca, P and N quantified by EDS, we find: Ca is found in moderate percentages, P is in low percentages, N is in very low percentages. Analyzing the ratios between the chemical elements mentioned above, we note the presence in sample 7 of mature bone, given that the Ca/N ratio is moderate, the P/N ratio is high, and the Ca/P ratio is very low. Sample 8 (Figure 1h) is an implant that was late loaded from a 53-year-old female patient with maxillary partial edentulous. The Straumann implant inserted in a bone region with a thickness of 7.7 mm (obtained after augmentation with autologous bone) and D1 density according to Misch had a diameter of 3.3 mm and a length of 12 mm. At the time of insertion, the implant had a primary stability of 42 ISQ, which two months after implantation, at the time of immediate provisional loading, had increased to 48 ISQ. The patient did not perform proper hygiene, so that 18 months after loading, she presented with local pain sensitivity and a secondary stability of 35 ISQ. Examining the implant, we note the presence of well-represented bone tissue, which uniformly covers the surface. Regarding the weight percentages of Ca, P and N quantified by EDS, we find that all 3 elements are found in very low percentages. Analysing the ratios between the chemical elements mentioned above, we note the presence at the level of sample 8 of bone undergoing mineralization, considering that the Ca/N ratio is increased, the P/N ratio is low and the Ca/P ratio is moderate. The values of Ca/N, P/N and Ca/P ratios were entered into Excel (Office 365) © Microsoft, with which statistical analysis of the data was also performed. Table 3 shows the correlation between the three reports, calculated with the CORREL function in Excel. The correlation coefficients that can be considered significant after applying the CORREL function are only those between Ca/N → P/N for immediate loading (0.764) and respectively Ca/P → P/N for late loading (-0.81). Also with the Excel software, we performed the descriptive statistical analysis of the data, respectively: mean (chart 1), variance (data scatter) (chart 2), sum (chart 3) and mean square deviation (standard deviation) (chart 4).

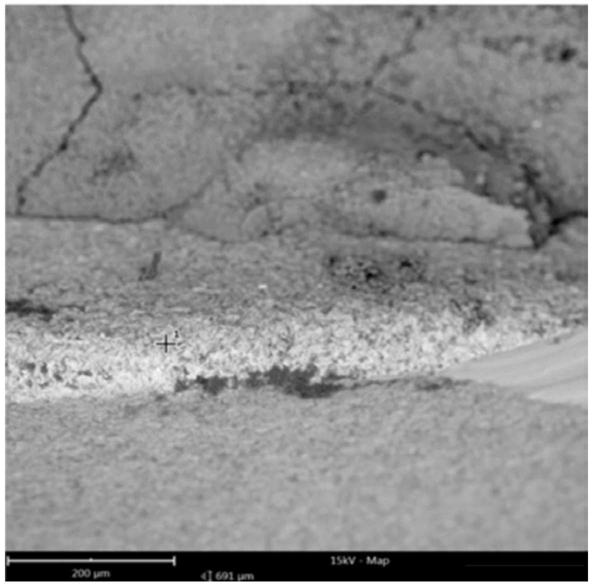
DISCUSSIONS

In 2008, at the ITI Consensus Conference, the different loading protocols were defined: a- conventional or delayed loading: loading of implants more than two months after their placement; b- early loading: loading carried out between 1 week and 2 months after implant insertion; and c- immediate loading: loading carried out in the first week after implant placement. The failure of the implant-prosthetic treatment implies the presence of painful sensitivity, change in color of the gingiva and low secondary stability compared to primary stability. Currently, there are very few studies on animal [2,8-10] or human models [4,5] in which an attempt was made to obtain information on the chemical composition of peri-implant tissues through ultrastructure



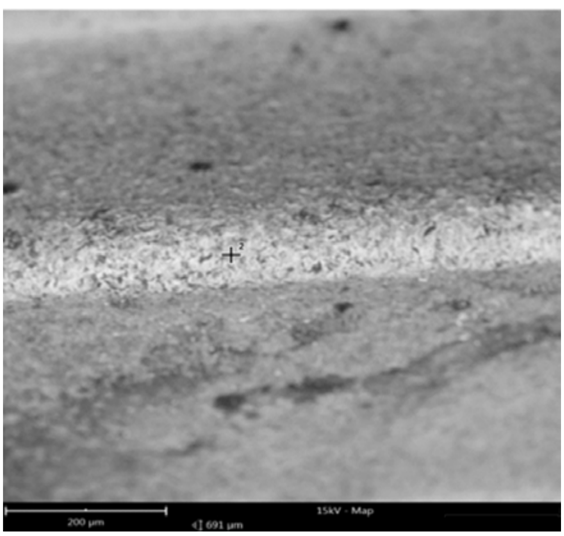
a

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	5.30	3.00
15	P	Phosphorus	0.91	1.14
20	Ca	Calcium	0.46	0.75



b

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	22.45	15.58
20	Ca	Calcium	0.56	1.10
15	P	Phosphorus	0.55	0.84

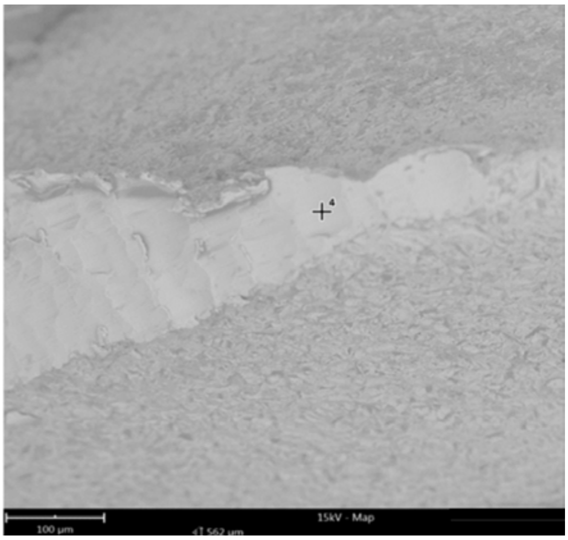


c

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	17.50	12.55
15	P	Phosphorus	0.34	0.44
20	Ca	Calcium	0.10	0.21

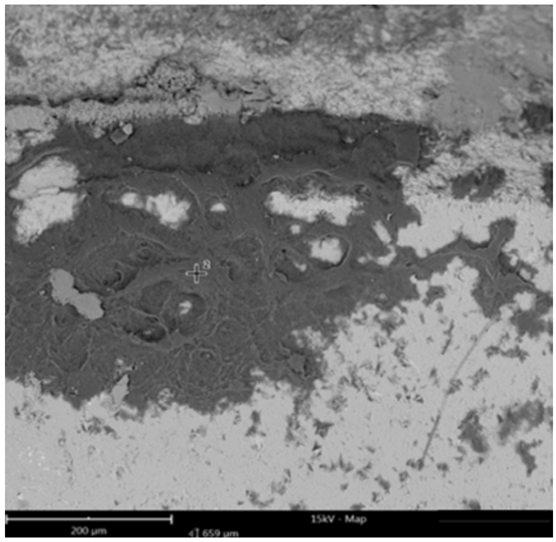
d

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	18.80	11.96
20	Ca	Calcium	0.79	1.44
15	P	Phosphorus	0.45	0.64



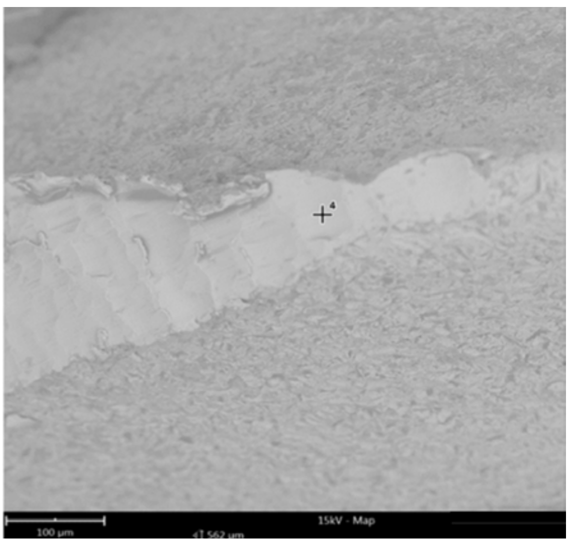
e

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	7.86	3.48
20	Ca	Calcium	0.60	0.76
15	P	Phosphorus	0.45	0.44



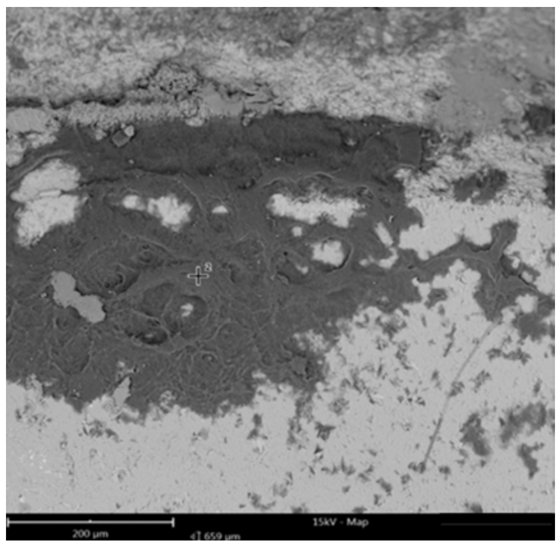
f

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	20.96	17.75
15	P	Phosphorus	0.91	1.71
20	Ca	Calcium	0.27	0.65



g

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	7.90	4.60
20	Ca	Calcium	0.66	1.11
15	P	Phosphorus	0.62	0.80



h

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
7	N	Nitrogen	4.17	1.46
15	P	Phosphorus	0.71	0.55
20	Ca	Calcium	0.28	0.28

FIGURE 1. SEM images and EDS analyses of samples areas
a - sample 1, b - sample 2, c – sample 3, d – sample 4, e – sample 5, f – sample 6, g – sample 7, h – sample 8

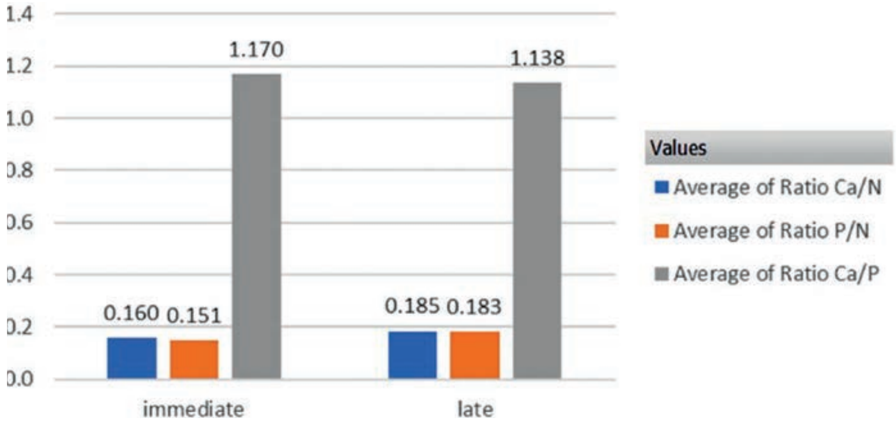


CHART 1. Graphic representation of the average of the Ca/N, Ca/P and P/N reports for the two types of loading (immediate and late)

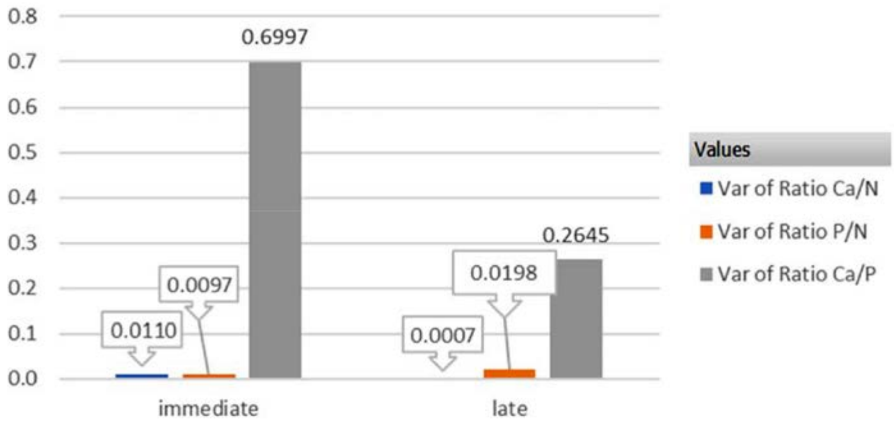


CHART 2. Graphic representation of the variance (dispersion of data) for the two types of loading (immediate and late)

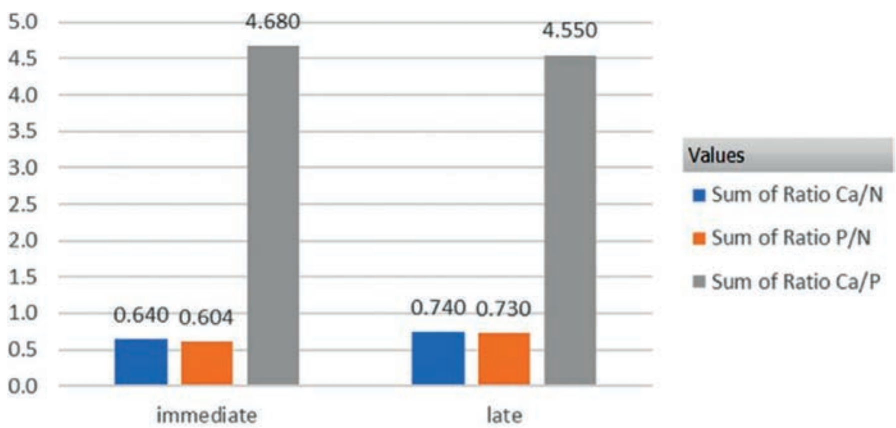


CHART 3. Graphic representation of the sum of reports for the two types of loading (immediate and late)

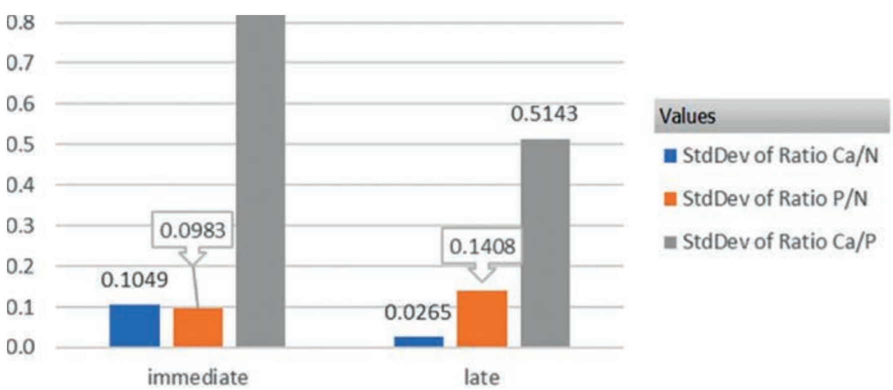


CHART 4. Graphic representation of the mean square deviation (standard deviation) of the ratios calculated for the two types of loading (immediate and late)

techniques. Unlike optical microscopy, SEM associated with EDS analysis in the present study, as in another similar one performed on mini-implants [5], the detection of the presence and quantification in weight and atomic percentages of some chemical elements such as Ca, P and N, to identify the different types of bone structures present at the implant interface. Ca and P are part of the inorganic component of bone, while N is part of the organic components of the bone structure. Depending on the percentages in which the respective chemical elements were found and the ratios between them, 4 types of bone were detected in the present study, with different degrees of mineralization: medullary bone, bone undergoing mineralization, mature bone and cortical bone. The presence of medullary bone on the surface of dental implants has also been identified in other studies performed on animal models [7,9,11]. Bone undergoing mineralization is characterized by the presence of osteocytes and osteons, which indicate an intense remodeling activity [12,13]. After certain periods of time, if the implant is integrated, the mineralizing bone will turn into mature bone, which evolves over years into cortical bone [5]. Instead, the medullary bone area will remain relatively stable as a reservoir of young bone cells, on which bone apposition will continue. Like other recent similar studies [1,5,7], our study shows that the bone-implant interface is an area characterized by dynamism and heterogeneity, which can be poorly mineralized, partially mineralized, or mineralized. The presence of poorly mineralized bone at the bone-implant interface was also detected in explants extracted at longer periods of time after insertion [2], but this area is narrower the longer the implant has been in operation. Gandolfi et al [2] performed a study on 9 explants that were loaded for periods of time greater than 10 years and detected the presence of homogeneous, richly mineralized bone with very reduced areas of poorly mineralized bone at the implant interface. If we compare the degree of coverage of the implants with bone tissue in different phases of mineralization, we find that the bone structures occupy a larger surface area of the implants in the cases with immediate loading compared to the cases with late loading. Thus, out of 4 implants loaded immediately, 2 were completely covered (samples 1 and 6), one showed very little exposed areas (sample 7), and the fourth showed alternating covered and uncovered areas (sample 4). Regarding the late-loaded implants, three showed alternation between covered and uncovered areas (samples 2, 3 and 5) and only one was completely covered (sample 8). From the statistical analysis performed, it appears that, for the analyzed cases, the degree of ossification (Ca/P ratio) was better represented for the immediate loading, compared to the late loading. Currently, immediate loading [14-17] is increasingly used with good long-term results, but also for the fastest possible reintegration of patients into society.

CONCLUSIONS

1. The study of the explants under the electron microscope associated with the EDS analysis allowed making assessments on the bone-implant interface from a structural and compositional point of view, providing information on the mineral and organic content of the bone, compared for cases of immediate or late loading. 2. The degree of coverage of the explants with bone tissue was better represented for the immediate loading cases. 3. The degree of mineralization of the bone tissue covering the explants was higher for cases with immediate loading. 4. The limits of the present study are given by the small number of samples examined. To confirm the obtained results, it is necessary to expand the study on larger batches of samples.

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