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Scanning electron microscopy and energy-dispersive X-ray spectroscopy on the degree of bone mineralization at the bone-implant interface

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ABSTRACT

Background. The purpose of this research was to evaluate the degree of mineralization as well of the chemical composition at the interface of failed dental implants with surrounding bone.

Material and methods. The analysis was performed on human histological samples by the aid of a scanning electron microscope and an energy-dispersive x-ray spectroscopy (ESEM-EDX).

Results. The middle and the apical region of the implant surfaces had higher quantity of bone tissue deposition. A higher degree of mineralization was observed in these two regions as well.

Conclusion. From the analysis performed by the aid of scanning electron microscopy, it appears that the bone tissue deposition is better represented in the middle and apical region of the implants.

Keywords: dental implants, mineralization, prosthetic restorations

INTRODUCTION

Dental implantology evolved very fast from the first implants proposed by Branemark to a variety of implant shapes with different combination of macro- and microstructure, different types of grafting materials, prosthetic components and materials, all of these with the purpose to fulfill patient's needs [1]. As the branch increased in numbers of doctors performing the augmentations (hard and soft tissue) and implant placement, the number of surgeries increased as well and become available for lots of patients [1]. As a consequence, the complications associated with this kind of procedures are more often and range from intraoperative or postoperative related complications. Depending on the moment when they take place, surgical complications are intraoperative, early or late postoperative [2].

One of the most common induced complications associated with dental implants is the periimplantitis which has many possible causes. Overloading, incorrect management of peri-implant soft and hard tissue (lack of fixed, keratinized gingival), systemic diseases (diabetes mellitus, immunosuppression, cardiovascular disease) and drug therapies, faults in prosthetic design, poor oral hygiene (including missing checkups), history of periodontitis, Iatrogenic causes (e.g. peri-cementitis), smoking [2-5].

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Peri-implant bone loss should be prevented from early stage of treatment planning to latest stage of prosthetic restoration conception [6]. The pathology generally comprises a destructive inflammatory process around dental implants, association peri-implant bone loss, bleeding on probing and peri-implant pockets.

There are multiple ways to analyze the bone-implant interface from clinical, intraoral evaluation, to radiological examination, histologic analysis, percussion test, reverse torque test, periotest, resonance frequency analysis (RFA) device cutting torque resistance analysis, or, environmental scanning electron microscopy (ESEM) [7,8].

Electron microscopy makes is possible to obtain magnifications of 3,000,000x, so that nowadays the technique is frequently used in the study of different types of materials. In the electron microscopy, the illumination source is a beam of high-energy electrons. In scanning electron microscopy, a focused beam of electrons is formed with increased energy, which scans the specimen targeted for analysis.

MATERIALS AND METHODS

The study was carried out according to a protocol approved by the Ethics Committee of the clinic Dental Institute, located in Bucharest Romania, without harming the environment or the patients, respecting human rights and the Declaration of Helsinki.

We studied 9 dental implants that belonged to a number of 9 patients of the dental clinic. All the implants were inserted by the same surgeon in native bone. The prosthetic restorations were made by the same prosthodontist.

The ablation of the implants after the therapeutic failure was done by manual unscrewing, as atraumatic as possible for the patients. After implant removal, the implants were harvested in a dry environment, in individual sterile containers, and transported to BIOMAT, Research Center of the Faculty of Materials Science and Engineering of the National University of Science and Technology POLITEHNICA Bucharest.

In the BIOMAT Research Center, the collected implants were analyzed with the aid of the scanning electron microscope (SEM) Phenom ProX. The samples were not subjected to any special preparation in order to be examined with the electron microscope and to perform a spectrophotometric analysis (EDX). The handling of the samples was carried out with the help of sterile tweezers to avoid contamination of the samples.

The SEM examination was performed by scanning the samples from the apical region to the coronal region. Images with a magnification of 500x were taken from three areas of interest: apical, the middle region and coronal. The EDX analysis was performed for each sample in three points (apical, coronal and in the middle area of the implant) as well. For each area of interest were quantified (in atomic and mass percentages) 10 chemical elements: titanium (Ti), nitrogen (N), oxygen (O), carbon (C), calcium (Ca), boron (B), aluminum (Al), vanadium (V), fluorine (F) and phosphorus (P).

The information on the amounts of Ca, N and P found on the surfaces of the implants was taken from the tables with the percentage values of the elements provided by the EDX analyzer. The ratios between the elements were calculated (respectively Ca/N, Ca/ P and P/N) and were statistically analyzed with Office 365 – Microsoft Excel to appreciate the degree of bone mineralization of each region.

No histological analysis was needed; this would not have brought additional information of interest for the present study.

For the classification of the examined bone areas, we used the same quantifications used by Prati et al [9] for the atomic percentages of the chemical elements Ca, N and P (Table 1), but also for the Ca/N, P/N and Ca/P ratios (Table 2).

TABLE 1. Association between the 4 bone types and (procentual) atomic values of C, P and N in the analysed regions

Mineralization regions	Ca	P	N
Bone region 1 – Bone marrow-poorly mineralised region	Very low (<1.2)	Very low (<1.1)	High (>13)
Bone region 2 – Bone remodeling process - medium mineralized region	Moderate (1.21-1.75)	Moderate (1.11-1.5)	Moderate (11-12)
Bone region 3 – Mature bone-high mineralized bone	High (1.76-3)	High (1.51-2)	Low (<11)
Bone region 4 – Cortical bone-high mineralized bone similar to cortical bone	Very high (>3.1)	Very high (>2.1)	Moderate (11-12)

The data on the atomic percentages of Ca, N and P, as well as the atomic ratios Ca/N, Ca/P and P/N, obtained from the 9 implants were introduced into a database and analyzed statistically using the IBM SPSS program. Statistics, version 20.

The aim of the statistical analysis was to investigate the degree of mineralization of the bone located along the surface of some human dental implants (from the coronal, middle and apical region), by comparing the atomic percentages of Ca, N and P, as well as the atomic ratios of Ca/N, Ca/P and P/N.

The study was carried out on failed implants, which were recovered 3 to 9 months after implantation.

TABLE 2. EDX atomic ratio of Ca/N, P/N and Ca/P of the analysed regions

Mineralization regions	Ca/N	P/N	Ca/P
Bone region 1 – Bone marrow-poorly mineralised region	Very low (<0.08)	Very low (<0.08)	Very low (<1.2)
Bone region 2 – Bone remodeling process - medium mineralized region	Moderate (0.081-0.16)	Moderate (0.081-0.2)	Moderate (1.21-1.5)
Bone region 3 – mature bone-high mineralized bone	High (0.17-0.25)	High (0.21-0.25)	High (1.51-1.8)
Bone region 4 – Cortical bone-high mineralized bone similar to cortical bone	Very high (0.25)	Very high (>0.26)	Very high (>1.81)

RESULTS

All 9 implants were analyzed in the apical, coronal and middle region. For each point of interest images were taken with the scanning electron microscope, an EDX analysis was performed and the atomic percentage of the chemical elements was listed in tables.

Sample 6 (Figures 1 and 2) is represented by an implant that came out from 57 years old, non-smoker patient, with dyslipidemia treated with atorvastatin. The patient additionally presented hypertension being treated with selective beta-blockers. The implant was inserted in native bone (edentulous site 12), with D3 bone density after Misch classification. The Dentix Milenium implant had a diameter of 3.75 mm and a length of 13 mm.

From the analysis of the acquired images, it appears that the bone tissue deposition is better quantitatively represented in the middle and apical region of the implant.

From a qualitative point of view, nitrogen is found in high atomic percentages in the coronal zone, and Ca and P are present in very low atomic percentages. The Ca/P ratio is medium and the P/N and Ca/N ratios are very low.

TABLE 3. Atomic percentage of the chemical elements in the coronal area of the sample 6

Element number	Element symbol	Element name	Atomic conc.	Weight conc.
7	N	Nitrogen	33.83	32.06
8	O	Oxygen	24.93	26.98
6	C	Carbon	23.94	19.45
5	B	Boron	13.13	9.61
22	Ti	Titanium	2.80	9.08
13	Al	Aluminium	0.85	1.56
23	V	Vanadium	0.14	0.47
20	Ca	Calcium	0.14	0.38
15	P	Phosphorus	0.10	0.22
9	F	Fluorine	0.13	0.20

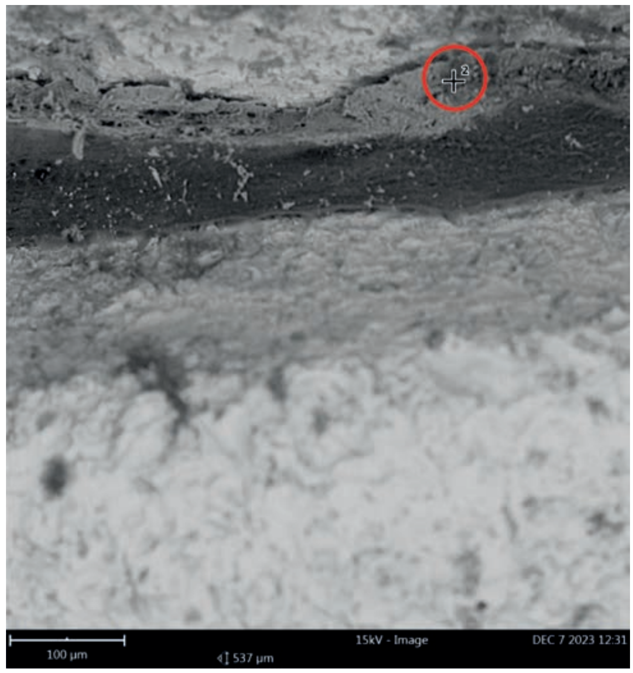

FIGURE 1. Scanning electron microscopy image of sample 6 (coronal region of the implant)

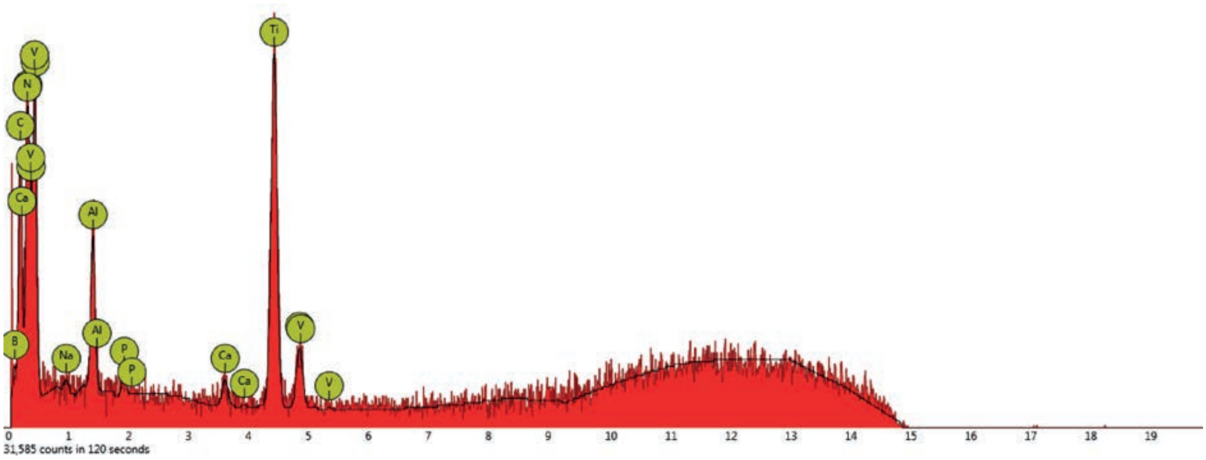

FIGURE 2. EDX analysis performed at a coronal point of sample 6

In the middle zone nitrogen is in low quantity, but phosphorus and calcium are in very high quantities. The ratios between the 3 elements are very high.

In the apical zone, nitrogen and phosphorus are present in increased amounts, and calcium in very increased amounts. The P/N ratio is moderate, and the Ca/N and Ca/P ratios are very high. Both in the middle and apical areas, we observe high mineralized bone tissue.

The different ratio of Ca/N, Ca/P and P/N between the three regions taken into analysis (coronal, middle and apical) is listed in Tables 4 and 5.

Analyzing the 9 implants, in the coronal area, the average of the atomic percentages indicates a weakly mineralized bone; thus, the average atomic percentages of Ca is 1.19 (<1.2), P is 0.69 (<1.1), and N is 21.02 (>13). In the same sense, the mean of Ca/N atomic ratios is 0.78, which, moreover, is also representative of this investigated area, indicating a highly mineralized bone (>0.25); also, the average Ca/P atomic ratio is 3.29 (>1.81). The mean of P/N ratios is 0.06, indicating medium mineralized bone (reference range being 0.081-0.2).

Instead, in the median area, the average of the atomic percentages of Ca and P indicates highly mineralized bone (similar to cortical bone): for Ca it is 8.76 (>3.1) and for P it is 4.55 (>2, 1); again, the exception appears in the average N atomic percent of 13.11, which corresponds to poorly mineralized bone (>13). A similar interpretation can be made based on the average of Ca/N ratios, which is 0.77 (>0.25) and Ca/P, which is 1.87 (>1.81). Contrary to the results obtained from the analysis of atomic percentages, the average of the P/N ratios indicates a highly mineralized bone (similar to cortical bone), being 0.41 (>0.26).

Similar to the midzone, the average of the atomic percentages of Ca and P in the apical zone indicates a highly mineralized (brain-like) bone: for Ca it is 5.17 (>3.1) and for P it is 2.25 (>2.1); the exception appears at the average atomic percentage of N, of 19.15, which corresponds to a poorly mineralized bone (>13). Likewise, if we refer to the average Ca/N atomic ratios, which is 0.29 (>0.25), Ca/P, which is 2.28 (>1.81). In contrast, the mean P/N ratios of 0.13 indicate medium mineralized bone (reference range being 0.081-0.2).

DISCUSSION

In scanning electron microscopy, a focused beam of electrons with increased energy is formed, which scans the specimen to be analyzed. Different effects may result in the moment of interaction between

TABLE 4. Atomic percentage for Ca, N and P measured by the aid EDX for each sample in coronal middle and apical region of the implant

	Atomic percentage Ca			Atomic percentage P			Atomic percentage N		
	C	M	A	C	M	A	C	M	A
Sample 1	0.12	5.39	1.48	0.9	2.41	0.94	19.6	19.74	13.3
Sample 2	5.45	10.19	14.03	3.86	6.63	6.47	12.42	9.42	21.95
Sample 3	0.06	7.5	0.78	0.22	4.95	0.57	3.96	8.12	24.67
Sample 4	0.83	7.71	2.78	0.47	4.24	1.77	24.18	8.79	7.82
Sample 5	0.16	2.32	0.35	0.17	1.67	0.15	28.13	18.85	28.87
Sample 6	0.14	15.7	12.28	0.1	6.41	1.97	33.83	10.72	16.12
Sample 7	3.39	6.58	4.22	0.17	3.4	2.42	19.67	17.15	17.98
Sample 8	0.23	16.73	5.15	0.13	7.47	3.27	20.46	13.37	24.57
Sample 9	0.38	6.72	5.46	0.19	3.84	2.73	26.92	11.87	17.05

TABLE 5. Ca/N, P/N, Ca/P ratio for each sample in the three areas of interest

	Ca/N ratio			P/N ratio			Ca/P ratio		
	C	M	A	C	M	A	C	M	A
Sample1	0.01	0.27	0.11	0.05	0.12	0.07	0.13	2.24	1.57
Sample3	0.44	1.08	0.64	0.31	0.7	0.29	1.41	1.54	2.17
Sample 3	0.02	0.92	0.03	0.06	0.61	0.02	0.27	1.51	1.37
Sample 4	0.03	0.88	0.36	0.02	0.48	0.23	1.77	1.82	1.57
Sample 5	0.01	0.12	0.01	0.11	0.1	0.01	0.94	1.39	2.33
Sample 6	0.004	1.46	0.76	0.003	0.6	0.12	1.4	2.45	6.23
Sample 7	0.17	0.38	0.23	0.01	0.2	0.13	19.94	1.94	1.74
Sample 8	0.01	1.25	0.21	0.01	0.56	0.13	1.77	2.24	1.57
Sample 9	0.01	0.57	0.32	0.01	0.32	0.16	2	1.75	2

the electron beam and the electrons in the sample [10]. Most of the electron beam interacts with the sample and undergoes elastic and inelastic scattering, but some of the electron beam will not be scattered. In the last case, the direction of the primary electron's changes, preserving their energy. Electrons that have been inelastically scattered change their direction of travel and partially lose their energy [10]. Although the energy of the electron beam mostly reaches the specimen as heat, other secondary events occur outside the specimen. Secondary events are represented by the emission of secondary electrons, backscattered electrons and characteristic X-rays [10]. If an incident electron changes direction and transfers some of its energy to an atom in the sample, secondary electrons are generated.

Each incident electron can produce several secondary electrons following the interaction with the sample surface, these being more abundant and at the same time representing the most used imaging signal in scanning electron microscopy. Backscattered electrons are formed following the collision of an incident electron with an atom in the specimen, at which point the electron partially loses its energy and is scattered 180°, "backwards". At the exit from the sample to be examined, part of the backscattered electrons can generate several secondary electrons [10]. The number of backscattered electrons is directly proportional to the atomic number of the chemical elements present in the specimen: the higher the atomic number, the brighter that region appears. X-ray emission occurs as a result of the voltage drop of an atom in the sample after a secondary electron is produced [10]. A key factor in scanning electron microscopy is the interaction volume, represented by the region where the incident electron beam penetrates the specimen [10]. Scanning electron microscopy provides a lot of information about the morphology, topography, composition and crystallographic nature of the analyzed specimens, overcoming most of the limitations of optical microscopy [10].

The X-ray spectrum emitted by the specimen provides both qualitative and quantitative information, allowing the identification of the elements present in the sample and the amount of each element, in mass and atomic percentages [10]. In addition to the emitted X-rays, a small number of secondary X-rays can also be generated when the primary X-rays pass through the specimen and interact with the sample atoms [11,12].

The examination of each sample with the scanning electron microscope SEM was carried out by analyzing their entire surface from apical to coronal. Images with a magnification of 500x were taken from three areas of interest: implant apex, the middle region of the implant and the coronal region. The degree of mineralization was firstly validated and recognized by Gandolfi et al both in experiments on animals [13] and on human subjects [9,14]. This study allowed the determination in atomic and mass percentages of the organic (nitrogen) and inorganic (calcium and potassium) components, as well as the ratios between them. Based on the ratios between the inorganic and organic components, the bone-implant "interface" was divided into 4 areas, from the least mineralized or "medullary bone" to the one with a very high mineral content, respectively "bone cortical" [9,15,16]. The peri-implant region is constantly subjected to bone remodeling processes. Intricate regions with different degrees of mineralization can be observed on the surface of the implant [9,15]. Area no.1 was defined as a region with a low degree of mineralization, as "medullary bone", which is in close contact with the surface of the dental implant [9]. According to studies, neoplastic bone tissue with a lamellar structure is formed in the bone area placed in immediate proximity to the implant [16]. The area no.2 is in the course of mineralization, having a complex structure, in terms of vascularization, cell migration and proliferation. The active remodeling process was characterized by areas of bone resorption and the presence of osteons and osteocytes [9,17]. According to other studies, area no. 3 is characterized by the presence of mature bone, with a high degree of mineralization, without any resorption process. This was mainly highlighted at a distance of 200-300 microns from the implant surface [9]. Area no.4 is characterized by a bone tissue with a higher degree of mineralization than area no.3, a tissue similar to the cortical bone [9]. This type of tissue was identified at a distance of 1-1.5 mm from the implant surface. Area no.1, the "medullary bone" will remain a reservoir of cells involved in bone remodeling processes. With the passage of time through a continuous process of bone mineralization, area no.2 will transform into area no.3, to later become a highly mineralized tissue, comparable to the cortical bone [9].

Other authors performed studies on osseo integrated implants, having as result higher Ca/N and Ca/P values especially in the coronal region in comparison to the apical area of the implants [10]. Most cases from our research showed a high mineralized bone in the apical and middle region, supporting the idea of periimplantitis affecting firstly the crestal bone.

CONCLUSION

From the analysis performed by the aid of scanning electron microscopy, it appears that the bone tissue deposition is better represented in the middle and apical region of the implants. Besides the bone quantity, the quality in terms of mineralization is higher in the same areas.

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