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Scanning Electron Microscopy in modern dentistry research

Microscopia Eletrônica de Varredura na pesquisa odontológica moderna

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Abstract

The purpose of this article was to review the usage of Scanning Electron Microscopy (SEM) in dentistry research nowadays, through a careful and updated literature review. By using the key-words Scanning Electron Microscopy and one of the following areas of research in dentistry (Endodontics, Periodontics and Implant), in international database (PubMed), in the year of 2012 (from January to September), a total of 112 articles were found. This data was tabled and the articles were classified according to the usage of SEM (magnification and type of detector) and if this information was obtained either in the summary or only in the full-text article. A critical review was also performed, with new guidelines regarding the usage of SEM in modern dentistry research.

Keywords

Scanning electron microscopy; endodontics; periodontics; implant.

Introduction

Scanning Electron Microscopy (SEM) has been a useful tool in research for quite some time, with articles being published using SEM in dentistry since 1962 [1]. Nowadays, most research laboratories in universities have their own scanning electron microscope and the technique has significantly evolved.

SEM allows the visualization of images at high magnification (50x – 10.000x and above). In this technique, an electron beam scans the surface of the sample to produce a variety of signals, the characteristics of which depend on many factors, including the energy of an electron beam and the nature of the sample, since a beam of electrons hit the sample and the response is collected by a detector, as described by Saghiri et al. [2]. There is no usage of light and the color of the sample does not influence on the image, which is something very important in dentistry, where dental tissues and dental materials tend to be white or have light colors, which makes the usage of optical microscopes hard.

The most common detectors used in SEM are secondary electron detectors (ETD - Everhart-Thornley Detector or SE1/SE2 in high-vacuum or LFD - Large-Field Detector, in low-vacuum) and back-scattered electron detector (BSED). According to Tarrant [3], the difference between them is that secondary electrons have been ejected from the outer electron shell of an atom as a result of impact from a high energy electron, having relatively low energies (up to about 50 eV, compared to the 1 - 30 keV of the beam electrons) and highlight the surface features (topography) of the sample. On the other hand, back-

scattered electrons are beam electrons that have undergone sufficient elastic 'collisions' with atomic nuclei and consequent changes in direction to exit the surface of the sample. BSED will provide an image with difference of phases, based on the difference of the atomic number (Z) of the surfaces. Regions of lower atomic number (such as Al, Si, C) in a sample will appear darker than areas of higher atomic number (such as Fe, Cu, W), which will appear brighter (phenomenon known as Z contrast).

High-vacuum images are mostly common obtained, since teeth surfaces can be fixed and dried-out. High-vacuum provides images with higher magnification, but samples need to be conductive. Since neither teeth nor dental materials (composites, ceramics, cements, for instance) are conductive, sputtering of the samples is necessary, with the usage of Au or Au-Pd target allows. Carbon coating is also used, depending on the research.

The purpose of this article was to review the usage of SEM in research nowadays, through a careful and updated literature review, by using the key-words Scanning Electron Microscopy in the following areas of research in dentistry (Endodontics, Periodontics, Implant), in international database (PubMed), in the current year (January to September). Data was tabled according to the information given either in the summary or in the article (type of detector and magnification).

Critical review

Each abstract of all articles was read and their corresponding full-text article, if available online, was examined searching for the information regarding SEM: type of detector (secondary electron or back-scattered) and magnification. This information should be written in all images regarding SEM, since it is extremely important and all SEM images come with a legend where this information is. However, sometimes authors tend to cut the original legend of SEM images and do not reproduce that information throughout the text. If that was the case, although it is easily possible for a well-trained observator to identify if a SEM image was taken with a secondary electron or a back-scattered electron detector, this information should be reproduced by the authors in any study when using SEM images. If that information was not written in the article, it was considered not available (N/A). The results are described in Table 1.

In Endodontics, SEM is used mainly to evaluate bacterial leakage within the root canal, bacterial biofilm formation [4] and also to evaluate fracture patterns regarding root posts and filling cements. Topographic analysis of the dentin surface after different rotary instruments and techniques is also a common purpose of study [5, 6].

SEM is particularly important in Endodontics when the gap formed between the filling material and the dentin wall is analyzed or measured. According to Souza et al.[7], replicas should be made and evaluated before samples are prepared for SEM examination in order to differentiate genuine gaps from artifactual gaps created after vacuum desiccation in conventional scanning electron microscopes. The usage of SEM technology in Endodontics allows visualization of root/dentin structures, with different heights, without altering the focus. In addition, since SEM figures are in gray scale, the color of dentin does not influence in obtaining a correct focus, limitation which is found in optical stereomicroscopes.

In Implants, morphologic evaluation of titanium surface is currently researched, including after nanohydroxyapatite-coating [8, 9], as well as biofilm formation on implant surface [10]. Atomic force microscopy, EDS and X-ray photoelectron spectroscopy is usually associated with SEM topographic analysis before and after several treatments [8, 11-13]. Implants are naturally conductive, therefore if only the visualization of the implant surface is aimed, charging is not necessary if low-vacuum SEM is available. However, if either biofilm formation or other sources of film coating on the implant surface is the goal, then sputtering of the samples becomes essential.

In our literature review, BSED was used in a minority of articles (10.9% in Implant and 0.2% in Endodontics). A recent article [14] stated that BSED-SEM is an invaluable method for studying the histology of the hard, mineralised components of poly-methyl methacrylate (PMMA) or other resin embedded dental tissues. However, the author suggested the use of triiodide ion in Lugol's iodine solution to stain the block surface prior to the application of any conductive coating – and the latter can be omitted if charging is suppressed by use of poor vacuum conditions in the SEM sample chamber. The method permits the use of archival tissue, and it is valuable in studies of both normal growth and development and pathological changes in bones and joints, including osteoporosis and osteoarthritis, and tissue adaptation to implants.

According to Saghiri et al.[2], cell observation under SEM requires prior use of a fixative like osmium

tetroxide and glutaraldehyde. This process of fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutaraldehyde, sometimes in combination with formaldehyde and other fixatives and optionally followed by post-fixation with osmium tetroxide. Cell adhesion to surface and other biological interactions may occur differently, according to the process of fixation, since different methodologies are found in literature [15-17]. No studies regarding comparison of different fixation process for specimens to be analyzed by SEM have been performed to this date.

Authors tend to briefly describe how the samples were prepared for SEM. Unfortunately, not all authors understand that the type of sputtering used in studies merits discussion. For conventional imaging in SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Metal objects require little special preparation for SEM except for cleaning and mounting on a specimen stub [2].

Nonconductive specimens, as described before (teeth, composites and ceramics) tend to charge when scanned by the electron beam, and especially in secondary electron imaging mode, this may cause scanning faults and other image artifacts, which is why samples are usually coated with an ultra-thin coating of electrically-conducting material, commonly gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation. Coating prevents the accumulation of static electric charge on the specimen during electron irradiation. According to Saguiri et al. [2], there are two reasons for coating, even when there is enough specimen conductivity to prevent charging: (a) to increase signal and surface resolution, especially with samples of low atomic number (Z); and (b) improvement in resolution arises because backscattering and secondary electron emission near the surface are enhanced and thus a higher-quality image of the surface is formed.

Poor coating can lead to charging, as shown in Figure 1 in low-vacuum, when is possible to observe non-conductive samples at lower magnifications. When this occurs, the image becomes difficult to focus, even when astigmatism is corrected. When coating is improved, the image becomes easily focused. It is important to mention that in low-vacuum, very high magnifications are difficult. Therefore, in Figure 2 it is possible to observe the same sample as in Figure 1, after coating was improved and the magnification lowered.

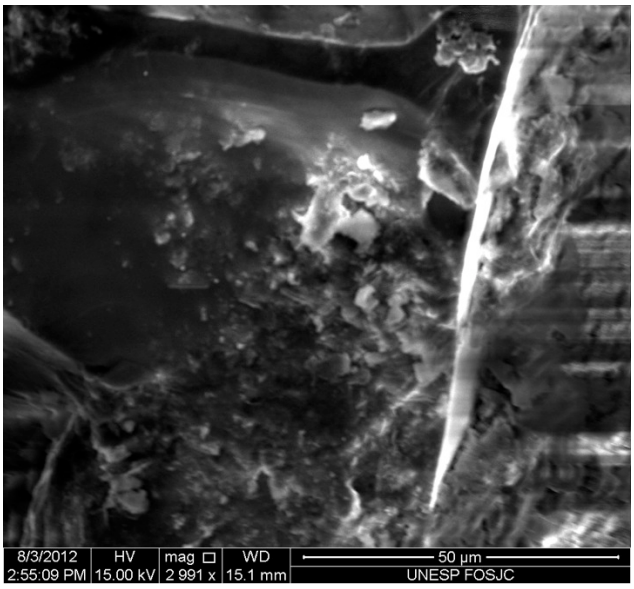

Figure 1 – Specimen with poor coating, at low-vacuum, at high magnification. It is possible to observe the lack of focus and the charging on the surface in secondary electron mode.

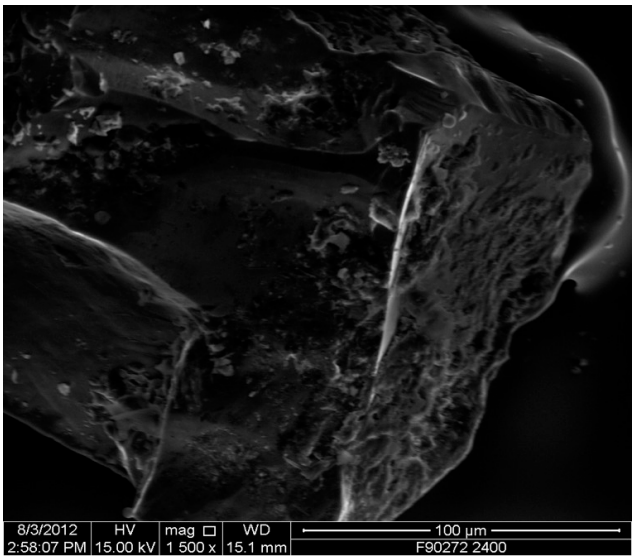

Figure 2 – Specimen with improved coating, at low-vacuum, with lower magnification. It is possible to observe a better focus and absence of charging on the surface in secondary electron mode.

In our analysis, of all the 112 studies, only few mentioned how the specimens were prepared and coated [8, 15, 16, 18, 19-21]. Most articles just mentioned that specimens were prepared for analysis under SEM, not describing the process of increasing the conductivity of the specimens. Different coating procedures for the same samples could generate image artifacts. Also, the voltage (HV) used for generating the image, as well as the spot are most of the times not mentioned in the text.

In our experience, the mean HV for analyzing samples in dentistry varies from 12.5 to 20 and the spot from 4-7. However, this data is just from our experience and has not yet been compared to any other studies, since unfortunately articles hardly mention these values.

When high magnification is necessary, usually Field Emission Gun (FEG) SEM is used. The difference between FEG-SEM and thermionic W or LaB6 emitters is that the former uses electrical current to heat up a filament. When the heat is enough to overcome the work function of the filament material, the electrons can escape from the material, forming the electron beam, which will go through electromagnetic lenses and then hit the surface of the sample. The way electrons interact with the sample is captured by detectors, which will transmit the image to the computer. Thermionic sources have relative low brightness, evaporation of cathode material and thermal drift during operation. Field Emission is one way of generating electrons that avoids these problems. A Field Emission Source (FES); also called a cold cathode field emitter, does not heat the filament. The emission is reached by placing the filament in a huge electrical potential gradient. The FES is usually a wire of Tungsten (W) fashioned into a sharp point. The significance of the small tip radius (~ 100 nm) is that an electric field can be concentrated to an extreme level, becoming so big that the work function of the material is lowered and electrons can leave the cathode. FEG-SEM uses Field Emission Source producing a cleaner image, less electrostatic distortions and spatial resolution < 2nm.

When FEG-SEM is used, different types of detectors can be used, such as TLD (Through Lenses Detector), which allows higher definition images, at greater magnification. In our research, this type of detector was only mentioned in few articles [9, 22]. This inf ormation is very important since higher magnifications (such as 50.000x and above) are mainly achieved in FEG scanning electron microscopes, not in thermionic tungsten (W) or LaB6 beam microscopes.

Regarding the articles which presented the information throughout the text or as picture legends, few described the magnification or the detector type in the abstract (14%, 17% and 23%, in periodontics, endodontics and implant, respectively). It is understandable that since summaries tend to present maximum number of words, many authors tend to minimize information. Thus, it is difficult to understand why many authors tend to cut the original SEM figures, eliminating the technical information which is presented in the legend of every SEM figure and not describing that information in the text. For researchers to evaluate the morphology of their specimens, it is essential that correct parameters are used and for purpose of comparison, it would be appreciated if similar magnification is used, which is something that was not observed in our analysis.

In other areas of interest, such as Medicine, using different analytical SEM approaches, applying a wide spectrum of accelerating voltages (1–30 kV) and various BSED and SE detectors in combination with FEG-SEM, it is possible to obtain precise 3D distribution of chromatin patterns in centromeres [23]. Such techniques are currently being described, focusing on the technical aspects, for which advantages and limitations are discussed. Taking limitations into consideration, combined SEM techniques still provide novel and—until now—elusive information for structural elements. Research in Dentistry should also follow this scientific pattern, discussing and evaluating current SEM techniques.

Researchers should be aware of these guidelines when thinking about SEM. Standardization of imaging principles is as important as the experiment itself. When correct principles are observed and followed, it becomes easier for students and readers of scientific articles to understand how SEM figures were obtained. Also, if there is interest researchers can easily based their innovating studies on previous one, comparing their morphological results, using the same protocols.

In conclusion, although SEM is an extremely important tool for research in dentistry, researchers should provide full information when using SEM figures, since the comparison of results is only possible when similar magnifications is used. In addition, how the sample was processed, regarding conductivity, what type of microscope was used (tungsten, LaB6 beam microscopes or FEG-SEM) is crucial information that should also be in the article. Lack of information makes the understanding of results, as well as the comparison, difficult for any researcher when using SEM technology.

Table 1 – Description of the data

Area of knowledge	Number of articles	Mean Mag	Variation (Mag)	Detector	Information
Endodontics	41	5000x	30x – 80.000x	51.8% SE 0.2 % BSED 48% N/A	17% Summary 83% Full-text
Periodontics	7	1168x	17x – 5.000x	28.5% SE 71.5% N/A	14% Summary 86% Full-text
Implant	64	8809x	20x-100.000x	18.75% SE 10.9% BSED 64% N/A 6.35% TLD (FEG)	23% Summary 77% Full-text

Legend: Mag – Magnification; SE – Secondary Electron; BSED – Back-Scattered Electron Detector; N/A – Not Available; TLD – Through Lenses Detector; FEG – Field Emission Gun

Resumo

O objetivo deste artigo foi revisar a utilização de Microscopia Eletrônica de Varredura (MEV) na pesquisa odontológica atual, por meio de uma cuidadosa e atualizada revisão de literatura. Utilizando as palavras-chave Microscopia Eletrônica de Varredura e uma das seguintes áreas de pesquisa odontológica (Endodontia, Periodontia e Implante), em uma base de dados internacional (PubMed), no ano de 2012 (de Janeiro a Setembro), um total de 112 artigos foram encontrados. Este dado foi tabelado e os artigos foram classificados conforme a utilização de MEV (aumento e tipo de detector) e se esta informação foi obtida no resumo ou no artigo completo. Uma revisão crítica também foi realizada, com novos direcionamentos relacionados ao uso de MEV na pesquisa odontológica moderna.

Palavras-chave:

Microscopia eletrônica de varredura; endodontia; periodontia; implante.

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