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Material-specific contrast in the ESEM and its application in dentistry

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Abstract The Environmental Scanning Electron Microscope (ESEM) equipped with a Gaseous Secondary Electron Detector (GSED) was used to image and analyze materials of different density, composition and structure applied in dentistry. Under ESEM conditions (at a H_2O vapor pressure of 1–10 Torr) the hydrated surfaces of native teeth, which were coated with different polymers, generated a topographic and also a material specific contrast. The backscattered (BSE) and the secondary (SE) electrons involved into the imaging process produced a cascade-dependent mixed signal at the GSED. The material-specific contrast, generated by the BSE cascade, depends mainly on the atomic number z of the investigated material. The topographic contrast is based principally on the SE cascade. For the exact differentiation of the specific signal components inside of the ESEM, we additionally used a backscattered electron detector (BSED), the application of which allowed us to detect pure BSEs and no signals from cascade-dependent electrons. Conventional scanning electron microscopy (CSEM) used to investigate and image the structures of teeth and applied dental materials needs time-consuming and often artifact-inducing preparation steps before the partially hydrated specimen can be investigated, whereas the ESEM technology permits the imaging of hydrated organic structures with no prior specimen preparation. In the ESEM the interfaces between the hydrated organically structured tooth surfaces and the artificially applied polymer materials with its specific bond characteristics can be analyzed very fast and repeatedly (e.g. after etching series) at a reproducible high quality level.

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Introduction

ESEM-specific aspects: ESEM signal components

The Environmental Scanning Electron Microscope (ESEM) is an electron optical instrument which enables the examination of the surfaces of soft, hydrated, unfixed, uncoated and electrically insulating specimens with depth of field and magnifications to that typically afforded by CSEM. The ability of the ESEM to yield three-dimensional information from surfaces of bulk biological material in its ''natural'' state and to vary the environmental conditions inside of the specimen chamber (gas pressure and specimen temperature) has opened new application fields in the medically oriented material sciences [1]. ESEM technology neither needs high-vacuum conditions nor time-consuming and artifact-producing preparation steps like chemical fixation, drying processes with organic solvents or critical point drying with CO_2. Surrounded by a 100% vapor atmosphere, the biological structures are able to bind and preserve structural bonds or associated water at their surfaces even at low working pressures in the specimen chamber. Moreover, specimen cooling and manipulation of water vapor pressure is possible, allowing the specific variation of charging effects and image quality [2–4]. But as a rule, a high hydration degree of the specimen surface (wet mode) is not essential because the electron signals are amplified by a cascade-ionization process occurring in the water vapor surrounding the specimen, which means that

the gas is not inert, it is just actively involved in the imaging process [5].

The efficiency of gas amplification depends on the pressure of the gas, the detector bias and the working distance [6]. The factors and nature of this amplification process have been analyzed experimentally. It was shown by Fletcher et al. [7] that mainly backscattered (BSE) as well as secondary electrons (SE) contribute to the signal cascade and that the two electron signals vary according to the pressure of the amplifying gas. Fletcher et al. [7] reported that BSE signals become more significant at higher pressures of the amplifying gas. The SE signal is a result of the inelastic collisions of primary electrons within a few nanometers of the sample surface. These ''original'' SE give rise to a useful high-resolution signal and their emission depends on δ the SE emission coefficient. The BSE arise from elastic collisions within the interaction volume of the sample and their emission efficiency, η, is a function of the average atomic number [7, 8].

Specific considerations on the different sources of the amplified ESEM signal e.g., primary electron beam scattered electrons, background signaling electrons or other unspecific signaling effects are described and discussed elsewhere [2, 9]. The utilization of specific charge contrasts for ESEM imaging applying a GSE-Detector is described by Watt et al. [10].

An overview of the development and principles of the ESEM technology is given by Danilatos [11].

Medical aspects: the composite–adhesive–tooth interface

The achievement of a constant and reliable gap-free bond between dentin/enamel and adhesive/composite interfaces is sought after in dentistry but is difficult to realize. One reason is that the adhesives, responsible for the mechanical contact between composites and the hydrated dentin/enamel surfaces, are often insufficiently polymerized. This is mostly due to the presence of spittle (an enzyme–water mixture), oxygen and dentin liquid at the tooth surfaces, where the bonding of the adhesive/primer combinations shall take place. Another reason for insufficient polymerization is the restricted accessibility for UV-light and heat, initiating the polymerization process.

A further problem is the formation of gaps between the different hydrated tooth materials dentine and enamel and the composites during the polymerization process, due to a shrinkage process [12]. Inside these gaps inflammations caused by bacteria can occur. The primer, as a bonding agent, will prevent the appearance of such gaps by the formation of ''resin-tags''. These tags are produced from a deep resin penetration into the dentin-tubuli, a process that will seal the tooth surface after resin polymerization [13].

The purpose of this contribution is to show that the ESEM technology is particularly suitable to image and analyze the interfaces between enamel and dentin structures and the artificially applied polymer materials. More precisely, ESEM investigations are time-saving, reproducible and avoids the artifacts of CSEM techniques allowing a routinely and reproducible quality control of dental applications and moreover, the realization of specific experiments in sequential steps (e.g., etching-series or the application of multiple composite layers).

Methodical background and experimental setup

The interaction of composites with adhesive materials and of adhesives with natural dentin and enamel was investigated and analyzed using a model system. In this system the surfaces of extracted human teeth were cut under defined conditions, etched and coated with composites and adhesives (for details see section ''Analysis of composite–adhesive–tooth interfaces''), or the compound quality of different composites alone was investigated in the EM (for details see section ''Analysis of composite–composite interfaces'').

EM investigations were carried out with a XL30-ESEM (FEI Philips Company, Netherlands) using a LaB_6-Cathode and a solid state GSE- or BSE detector and a specimen cooling stage. Images were produced with the Digital Image Scanning System Diss 5, developed by Point-Electronic, Halle, Germany.

Analysis of composite–adhesive–tooth interfaces

Material

To analyze the bonding strength and characteristics of composites with the dental matrix, human molar teeth were prepared. For the cutting and grinding processes we applied a system provided by EXACT Apparatebau (Norderstedt, Germany). Etch series were carried out using a 37.5% phosphoric acid-gel, Ecusit®-Etch. Ecusit®-Composit was used as composite material and as adhesive the Ecusit®-Primer/Mono was applied. All Ecusit® products were produced by DMG (Chemisch-Pharmazeutische Fabrik GmbH, Hamburg, Germany).

Preparation methods

The investigated teeth were, as schematically shown in Fig. 1F, sectioned in the sagittal plane (A–A) and subsequently grinded.

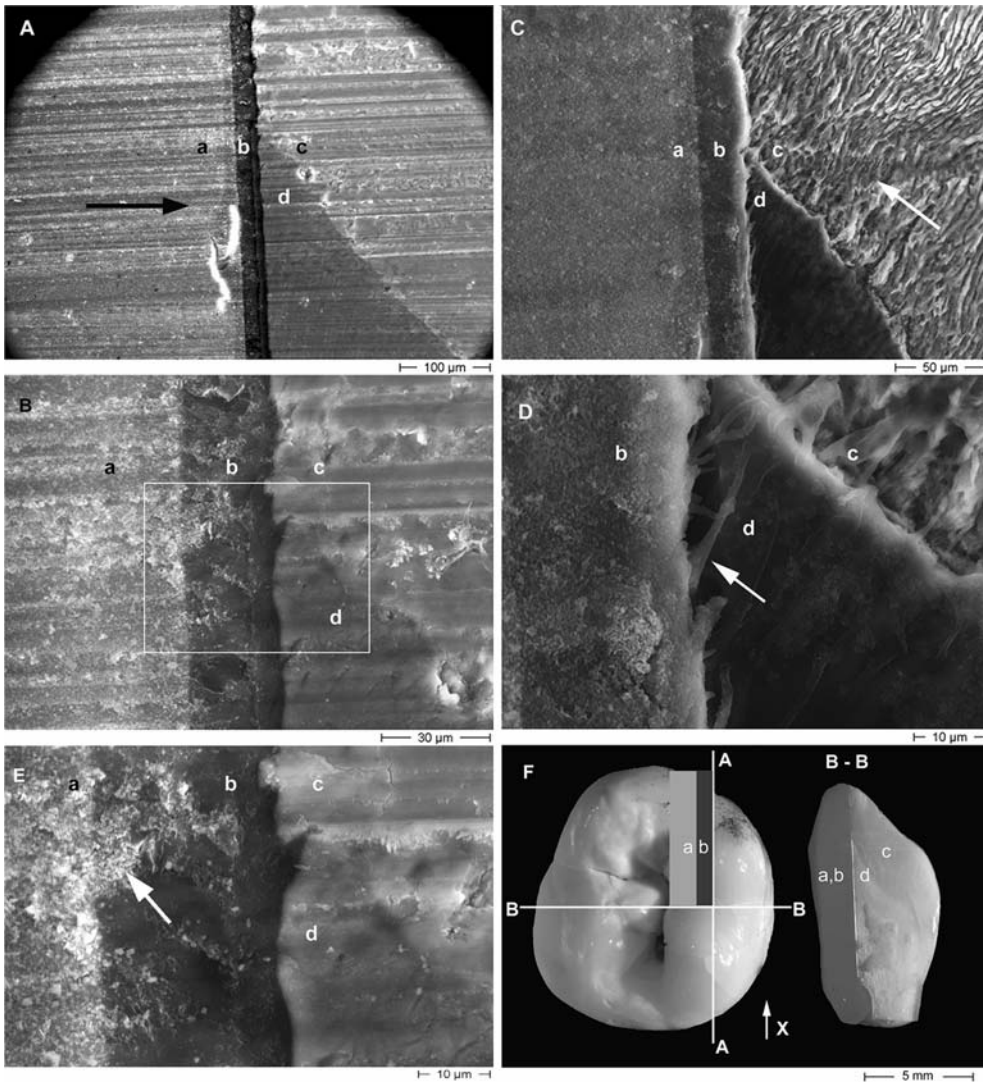

Fig. 1 **A–E** ESEM micrographs of a human molar tooth using a GSE-detector (25 kV, spot size 4, 4 Torr). The tooth was sectioned as shown in **F** (overview), etched and coated with composites and adhesives. Figs. **A**, **B**, **E**; Interfaces of a: composite, b: adhesive, c: enamel, d: dentin, at different magnifications, preparation details see section ''Analysis of composite–adhesive–tooth interfaces''). Arrow in **A**: sectioning direction, in **E**: displacement of composite components. Figs. 1**C** and **D**: Identical area as shown in **A**, after etching with HCl. Arrow in **C**: structural lesions in the enamel, in **D**: resin-tags in the etched dentin layer

The uncovered dentine and enamel surface was etched for 15 s or 30 s respectively with the phosphoric acid-gel described above, washed thoroughly for 15 s with water and dried with compressed air leaving residual moisture on the dentine and enamel surface in order to simulate a wet-bonding process [14].

In the next step a 1:1 mix of the Ecusit-Primer (Component A with B) was applied on the dentine and enamel surface using a brush and rubbed in for approx. 20 s. Excessive primer was removed with compressed air followed by the application of the third primer component, Ecusit-Mono, onto the dentine and enamel surface which was pretreated with the Ecusit-Primer. Ecusit-Mono was rubbed in with a dry brush for again 20 s. This layer was polymerized with UV light for 20 s. The completing application and polymerization of Ecusit-Composit for 40 s under UV light was carried out successively in thin layers.

For the ESEM documentation such a pretreated tooth was sectioned again in the sagittal plane (see Fig. 1F, plane B–B) and investigated in the area where the composite has been applied (Fig. 1A, B, E). After documentation the cutting area was etched with 1% HCl for 60 s [15]. The etching process removed preferentially the organic tooth material and less adhesive and composite material (see Fig. 1C, D). After the etching process, the surface of the tooth was washed thoroughly with distilled water for 20 s.

ESEM investigation

The tooth was investigated in the ESEM at room temperature at a working distance of 5–8 mm, an angle of 0°and at a H_2O vapor pressure of 3–4 Torr inside the chamber. A special device, not described in detail here, allowed us to relocate selected specimen areas in the µm-range.

Analysis of composite–composite interfaces

Material

The composites used are heterogeneous and consist of ultra-fine glass particles, embedded into a UV- or temperature-sensitive resin matrix. In order to investigate the interfaces between the two different composite materials (both produced by DMG), a fineglass-hybridcomposite (Ecusit®: Barium-Glas, SiO_2, see Figs. 2A, 3A) and a micro-filled composite (EcuSphere®-Shine: SiO_2, see Figs. 2B, 3B) were applied. After polymerization of the first composite layer, a second one was applied onto its surface and also polymerized.

Preparation methods

Methods for fractured unpolished specimen: In order to analyze the specific material and topographic contrast generated from the surfaces of composite–composite interfaces, the material was carved at both sides and fractured, resulting in a fracture pattern that was not superposed by artificial traces of a cutting process (see Fig. 2A, B).

Methods for fractured and polished specimen: In order to minimize the topographic part of the GSE signal the fractured area, shown in Fig. 2A, B was polished and reinvestigated, see Fig. 3A, B.

ESEM investigation

The fractured and unpolished composites, (Figs. 2A, B), were investigated in the ESEM at 4 °C, a working distance of 9 mm, a tilting angle of 45°(A) and 0°(B) and at a H_2O vapor pressure of 5 Torr with an GSE detector.

*The fractured and polished composite*s were first investigated with the GSED at a tilting angle of 0° (Fig. 3A) with the above-described parameter. Subsequently, the same material was investigated with the BSED (Fig. 3B) The H_2O vapor pressure was 2.6 Torr and the temperature was 1.1 °C.

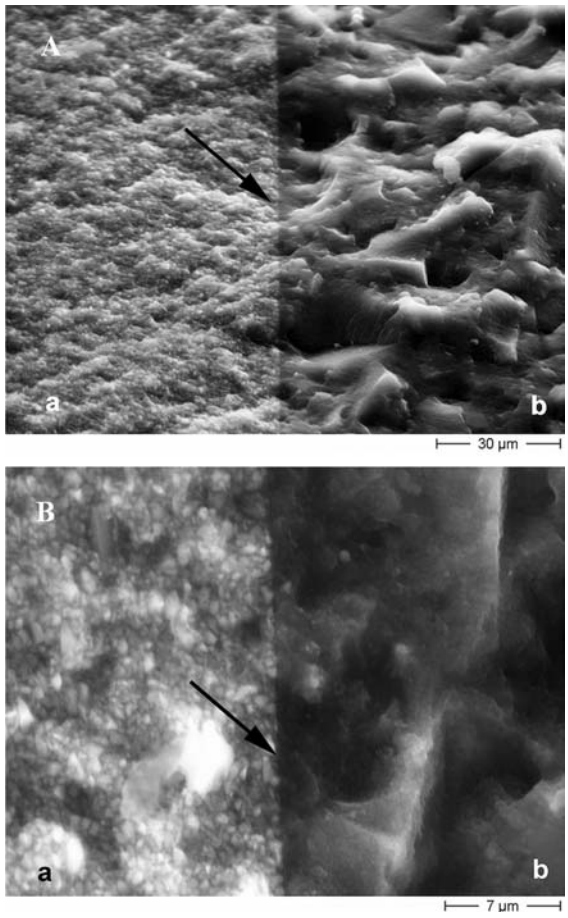

Fig. 2 **A**, **B** ESEM-GSED micrographs, showing the surfaces of different composites (preparation details see section ''Analysis of composite–composite interfaces'') after fracturing. Arrows in **A** and **B**: borderline between the two composites a and b. Tilting angle in **A** is 45° and 0°in **B**, a higher magnification out of **A**

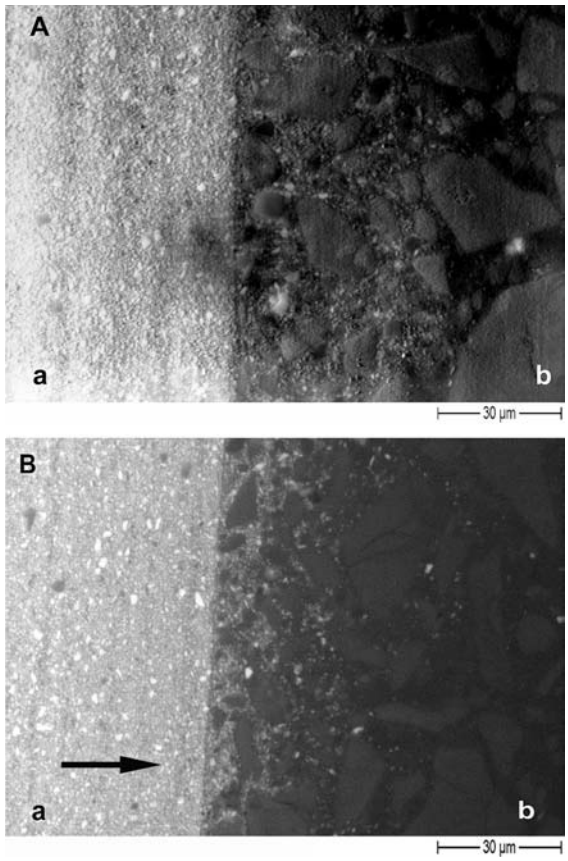

Fig. 3 **A**, **B**: ESEM micrographs, showing the polished surfaces of the same fractured composites depicted in Figs. 2**A** and **B**, tilting angle in both figures is 0°. The GSE-detector was used for **A** and the BSE-detector for **B**, arrow: displacement of composite components

Results and discussion

Conventional SEM of tooth surfaces and possible preparation artifacts

It is often argued that dental material does not need complex dehydration protocols, because of its high percentage of inorganic compounds. But dentin is a vital structure and its environment is moist. In vitro evaluation of dentin adhesives does not generally take into account the presence of fluids. And resin–dentin bonding agents have been shown in vitro to be adversely affected by the presence of moisture [16]. Moreover, if teeth treated with adhesives under moist conditions (wet-bonding procedures, see section ''Preparation methods'') are exposed thereafter direct to high-vacuum conditions without any prior dehydration steps (controlled alcohol series and CP-drying), the still hydrated material is dried very fast until reaching high-vacuum conditions. This harsh drying process often results in a collapse of fine organic surface structures by air-drying and the appearance of strong drying tensions inside the different hydrated zones of the tooth. As a consequence, cracks and fissures can appear which possibly makes it difficult to analyze the interconnection zones or interfaces of the different applied materials. Moreover, insufficient polymerized resins can evaporate during the evacuation procedure resulting in shrinkage processes or changes in the surface structure of the applied components. These possible artifacts complicate the exact analysis of the interaction processes between the tooth surface and adhesives or composites.

ESEM investigations are ''biocompatible''

The ESEM in contrast to CSEM is ''biocompatible'' because it tolerates water and tooth structures contain, bind and are surrounded by water, which is an integrated component of their bio-matrix and environment. ESEM technology thereby avoids the dehydration artifacts of CSEM, described above, and it allows the investigation of the partly hydrated and controlled laminated tooth surfaces at low pressure and in a hydrated environment. Moreover, the absence of an artificial conductive surface layer allows multiple etching series or repeated surface coating with adhesives or composites. Ten minutes after a specific pretreatment the specimen can be reinvestigated and analyzed in the ESEM. This significantly shortened time period between the medical-induced preparation and EM analysis opens not only new application fields, but also saves time and money within the context of clinical-diagnostic work routines.

Charge contrast imaging in the ESEM

The absence of a conductive metal layer on the specimen surface has another advantage: the material-specific contrast of the different polymer materials (see Fig. 2) is directly visible with the GSE detector. Moreover, Watt et al. [4] could show that it is possible to implant a limited amount of charge in an uncoated sample. These implanted electrons modify secondary electron emission from the sample surface and produce contrast between areas of different conductivity, allowing the direct imaging of subtle compositional variations and micro-structural features, see also [17]. The imaging data of our study indicate that a mixture of this charging contrast and the material-specific contrast enabled us to differentiate diverse elements of the composites, e.g., fineglass elements in a micro-filled composite (see Fig. 2A, B), subsequently discussed in detail.

Material-specific and topographic contrast in the ESEM

Based on our data we can demonstrate that an ESEM, equipped with a GSE detector, provides many possibilities to reproducibly analyze and image materials of different density, composition and structure. Under ESEM conditions (at a H_2O vapor pressure of 1–10 Torr) the hydrated surfaces of native teeth, which were coated with different polymers, generated a material-specific BSE-contrast and also a topographic SE-contrast. And both, BSE and SE produced a constant cascade-dependent mixed signal at the GSED.

The images based on this mixed signal made it possible to visualize precisely (a) the distinct interfaces of composites, adhesives and tooth material and (b) the different elements of the composites by material-specific signals and moreover their specific topography by a clear definable topographic contrast as demonstrable by stereo images. This precise optical differentiation was even possible at surfaces where mechanically induced surface artifacts superimposed the original matrix (Fig. 1A, B).

Etched (Fig. 1C, D), fractured (Fig. 2A, B) and polished surfaces (Fig. 3A, B) and their different components and topography could be electron optically analyzed with ease, one after the other and immediately after the specific dental applications.

Analysis of composite-adhesive-tooth interfaces (Fig. 1A–D)

The cutting process of the tooth itself induces specific cutting pattern (see Fig. 1A, overview), the arrow indicates the cutting direction. As a result the specific contrast and roughness of the different materials is superimposed by this

artificial pattern (Fig. 1A, B) and its topographic contrast (see Fig. 1A, B, E). But despite this superposition, the different applied components (Fig. 1A, a: composite, b: primer) and the tooth structures (Fig. 1A, c: enamel, d: dentin) are directly visible and distinguishable with the GSE detector. These cutting structures nearly disappear if the surface is etched. Merely very deep structural lesions in the enamel are still detectable (Fig. 1C, arrow) although the etching process has nearly reduced all cutting traces, as shown in Fig. 1C and D. As clearly demonstrated in Fig. 1C, new surface pattern are formed, but the specific material contrast remains. The interfaces between composite (Fig. 1A: a) and primer (Fig. 1A: b) can be exactly distinguished by a distinctive difference in contrast. The interfaces between the primer (1A: b) and the surface structures of the tooth (1A: c, enamel) and (1A: d, dentin) are much clearer to detect due to the typical etch pattern and its specific topographical contrast.

The first section of the tooth in the sagittal plane (Fig. 1F: A–A) opened up some of the dentin channels. The penetration of the primer into these channels can be clearly shown only after the etching process with HCl (compare Fig. 1A, C: plane d). The etching process removed preferentially most of the dentin (Fig. 1C: d) and left the polymerized primer that had filled these channels. These structures built in the resin–dentin inter-diffusion zone (Fig. 1D, arrows) are described as resin-tags and are of major importance in bond strength between resin and dentin [15]. This example shows clearly that by using ESEM technology, comparative investigations—on the basis of identical specimens—can be carried out with ease.

Moreover, the cascade-dependent mixed signal at the GSED could clearly detect the displacement of composite components from the zone ‘a’ in Fig. 1B and E into the primer zone ‘b’ (arrow). This relocation of material induced by the cutting process itself often complicates the exact detection of the interaction zones and borderlines between two different materials.

Analysis of fractured composite/composite interfaces (Figs. 2, 3)

The 45°-image of the fractured composite interaction zones allows a direct analysis of the different composite components, its interaction and boundary by specific material contrast. The clear and sharp delineation by contrast is additionally supported by the fracture pattern topography of the different composites and their specific topographic signals (see Fig. 2A: a and b). On the left side (a) the fine structures of the ‘‘light-cured fine-glass hybrids’’ are clearly visible and on the right side (b) the coarser structures of the ‘‘light-cured microfiller hybrid composite’’ are detectable. At higher magnification at an angle of 0° the fine-glass particles and their distribution can be analyzed. We think that these particles are presentable by a specific charge contrast, which allows the direct imaging of subtle compositional variations and micro-structural features as described by Griffin [17, 1998]. Stereo pictures indicated that this specific charge contrast can be detected in deeper regions up to 3 μm and it should be pointed out that even after longer irradiation no unspecific charging effects were detectable.

In order to compare directly (a) the cascade-dependent fraction of the BSE signals at the GSE detector and (b) the direct BSE signals, we used a BSE detector and investigated the surface of an identical specimen.

The above described displacement of composite components, in this case induced by polishing the fracture faces of the two composites, from the zone ‘a’ in Fig. 3B into the zone ‘b’ (arrow), can be easily detected by the BSE signal. Whereas the displaced fine-glass structures are hardly detectable in the GSE picture (see Fig. 3A) but are superposed by the topographical signal of the coarser structures of the ‘‘light-cured microfiller hybrid composite’’. The topography of these coarse structures is still present in the GSE signal, beside the polishing process, whereas the BSE signal does not detect this hybrid composite.

Conclusion

Based on the results presented here we suggest that one of the most exciting future applications of the ESEM technology is the direct and fast analytical imaging of biocompatible organic and inorganic materials, including the 3D analysis of the interaction zones between mixtures of inorganic nano-sized materials with biological surfaces or matrices.

Moreover, using the ESEM chamber as a mini-laboratory, correlative investigations of identical specimen areas after specific treatment like etching, in situ polymerization, cooling and water condensation experiments or in situ laser-treatment are possible. This again permits the sequential analysis of all different steps necessary for the production of stable composites between biological surfaces and artificial but biocompatible materials.

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