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A study of the porosity of some samples of dentinal substitute material from extracted human teeth

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Abstract

The aim of the present study was to investigate the level of which the endodontic pathology and/ or the preparation and application procedures of dentinal substitute influenced the quality of the restorative material (Biodentine, Septodont, France). It is well known the fact that Biodentine, Septodont offers a bioactive substitute to dentine, it is highly biocompatible, presenting a short setting time of aproximatively 10 minutes, which is beneficial in endodontic procedures. Nevertheless, at the same time, it is highly dependent on the conditions under which it is mixed and applied on the dentinal surface and also on the various endodontical clinical pathology, thus eventually becoming a potential issue regarding the quality of the restorative dentinal substitute.

In the current study we analyzed microscopically the incidence of pores and cracks and we estimated the total porosity values of four samples of Biodentine, Septodont that were separated from extracted teeth with different endodontic pathology (iatrogenic furcation perforation, internal resorbtion with perforation, external radicular resorbtion and deep coronal lesion restored by sandwich technique). We analyzed two fragments from each sample, out of which two thin sections were cut and from the obtained areas we estimated the total porosity value for each Biodentine sample, using a Leica DM EP polarizing microscope (Switzerland).

Locally, in the mass of samples 1 and 3, microcracks and intercommunications between them and certain pores were detected. After the microscopic examination of pores and estimation of total porosity for all four samples of Biodentine separated from extracted teeth, very low incidence of pores and no microcracks were identified in sample number 4, probably due to judicious preparation and applying of the material, under the presumptions of the fact that the instructions of setting and preparation were in accordance to the manufacture and the application in the dental cavity was done under no humidity conditions. This fact is consistent with other authors' findings. In sample 2 and 4 there were no cracks identified, whereas the incidence of pores in sample number 1, 2 was lower and comparable, while in sample number 3 was higher. A possible explanation for this fact would be a vicious procedure regarding the preparation and application methods under the conditions of a higher local humidity potential.

Keywords: porosity, dentinal substituent biomaterial, microcracks

1. Introduction

Biodentine (Septodont, France) is a bioactive and biocompatible material acting like a dentinal substitute, which can be successfully recommended in endodontics, restorative dentistry and pediatric dentistry, for a large variety of clinical situations and due to its efficacy, compatibility and the conservation of tooth structure. [1] Biodentine is a two part component material, the powder containing Tricalcium silicate, Dicalcium silicate, Calcium Carbonate and Oxide as filler and Zirconium Oxide as a radio- opacifier.; the liquid contains calcium chloride as a setting accelerator and hydrosoluble polymer, as a water reducing agent. The method of applying Biodentine does not require any supplementary conditioning of the dentinal surface, since the sealing of the material is provided by biomechanical retention, the material penetrates the dentinal tubules. Biodentine can be shaped like natural dentine and can be bonded with adhesives before final restoration with composite resin. [4, 3]

However, the presence of pores in dental materials has implications for the mechanical strength and for the possibility of infiltration of these materials by biological fluids or various microorganisms.

Porosity is defined as the pore volume (Vp) of a material relative to the total volume of the material (Vt) multiplied by 100. The porosity is expressed as a percentage. [6]

$O_T [\%] = Vp / Vt \times 100$

It can be said of a total porosity in which all the pores present are taken into account and an effective porosity where only pores communicating with each other are taken into account and which also communicate with the surface of the fragment or sample of the material considered.

The value of total porosity would be significant in terms of reducing the mechanical strength of the material and the value of effective porosity would be important in terms of the speed at which the material could be infiltrated.

In order to estimate the porosity of endodontic materials and to appreciate the characteristics of the pores in these materials, microscopic imaging methods can be applied on samples of the material. For micron and submicron pores is suitable electron microscopy investigation, whereas for pores having dimensions of the order of more than 10-20 μm can be used successfully optical microscopy. Stereomicroscopy could allow the pores to be seen from the surface of the sample of material or fragments drawn from it, and transmitted light microscopy on thin sections allows for the observation of the pores inside the material at the depths at which the sections are chosen. Transmitted light microscopy offers the advantage of operating at 1000x magnification orders that are well above those achieved by conventional laboratory stereomicroscopes (about 90x).

Working with microscopy in transmitted light on thin sections, a fully tridimensional pores and porosity cannot be evaluated. Thus, it is operated on one or more sections made from the material sample. The more section areas for the same sample are evaluated, the closer we can get closer to the actual true porosity value in three- dimensionality.

Although sometimes used in current language, the wording that a dental material "has porosities" is not considered appropriate. Since porosity is a percentage value, it is not correct for several samples to be spoken for a given sample except when two values have been estimated: one of total porosity and one of effective porosity. This makes sense when the pores of the material are at least partially communicating and, as has been shown before, the communicating pores also communicate with the surface of the material sample.

As a rule, those who speak of "porosities" refer not to percentages of porosity, but to domains of the reference material in which pores are preferentially concentrated, which in fact means a non-uniform distribution of pores in three-dimensional space. We believe that invoking the presence of such areas where pores are concentrated in dental materials should be justified by the application of investigative methods for those cases. [5]

2.Material and Methods

In the present paper we examined microscopically the pores and we estimated the total porosity values for three samples of Biodentine (Septodont, France) that were separated from four the extracted teeth that presented various pathologies (1 molar with iatrogenic perforations at the furcation, a tooth with internal radicular resorption with perforation, a tooth with external radicular resorption and the last one with restoration of deep coronal lesion, through sandwich technique) after this material has been applied previously in the endodontic space. The time elapsed between application and tooth extraction is between 2-4.5 years. The teeth were provided from three clinicians under different preparation and obturations conditions.

The previously applied Biodentine masses were separated by cutting under the endodontic stereomicroscope (Zeiss, Germany) of dental hard tissues around them and not by fragmenting them in order not to alter the microstructure of the material.

From each sample of recovered material, two thin sections were made: each recovered Biodentine sample was cut into two relatively equal parts, which were then ground to obtain two new planar surfaces at some distance of the initial section. Of the two fragments thus obtained were carried out microscopic thin sections and fuse them to the original with special epoxy resin prepared by microscopic glass slides and then thinning them by polishing with carborundum powder to a thickness of 10 μ m.

The characteristics of the pores and the areas occupied by them in the section plane were determined using a Leica DM EP polarizing microscope. Digital microscopic images were performed with a Leica DMC 4500 microscope camera (Switzerland).

In order to estimate the total porosity value for each Biodentine sample, pores and even other voids (such as those corresponding to the open micropores) were determined in several sets of microscopic micropores, these being reported on the total surface area of microdomains. In order to facilitate the evaluation of the surfaces corresponding to the pores and openings of the microcracks, in digital photos, each such microdomain has been superimposed since shooting a 1600 mesh grid operating on a 100x magnification order. The reference surface unit was chosen to be the one corresponding to an eye of the grid. Thus, for each microdomain, the estimated porosity value is given by the number of meshes (cells) of the grid corresponding to the pores, relative to the total mesh size of the grid (1600). Because a pore does not occupy an exact number of mesh grids, subdivisions of the mesh surface have been taken into account, with an accuracy of 0.1. The surfaces of the pores were marked on the digital images to ensure that they were not made in any microbe omissions (Figure 1). Each image was recertified by several members of the team.

What we considered the porosity / opening space of the microcircuits corresponds to the very transparent areas where there is practically no Biodentine material, but only the crosslinking resin of the section.

The open space of the microfissure was taken into account by estimating the fraction occupied by it in each grid eye through which the crack passes (Figure 1 - lower left). In the case of grid loops where it would be clear that the crack passes, if free space could not be detected through an appropriate transparency area, it was not taken into account.

For each Biodentine sample porosity was determined by 6 sets of 8 adjacent microdomains (located next to each other), respectively 3 sets for each section. Finally, average values were determined for each set and for each sample. Microscopic images corresponding to a set of microdomains for the first Biodentine sample are shown in Fig. a- h.

It is important to specify the fact that in the case of tooth number 4, the one presenting deep and large coronal lesion restored by the sandwich technique with Biodentine, in all samples analyzed, the incidence of pores and cracks was extremely low, this fact being in accordance to most of the findings described in the literature. [1, 2, 7, 8, 9] (Table no. 4, Figure no. 7)

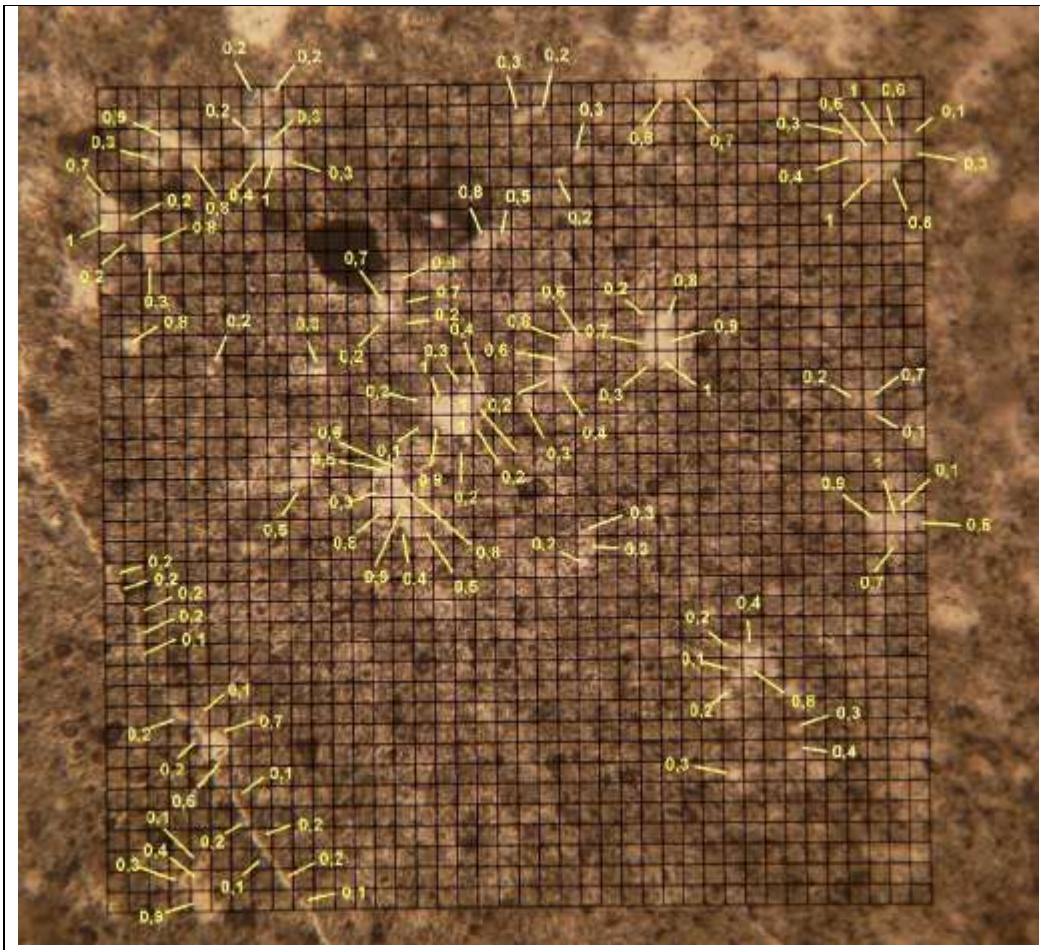
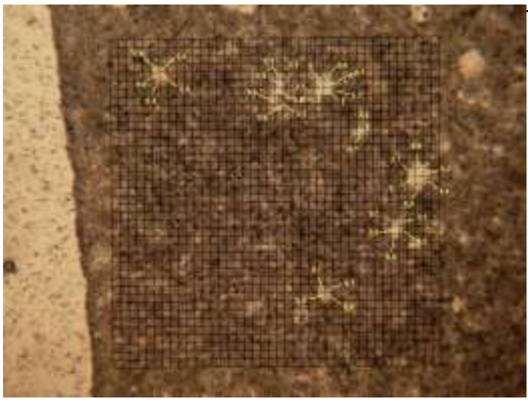


Fig. 1 Marking the areas corresponding to the pores, as well as cracks, in the digital images of the microscopic microdomains analyzed, corresponding to the first tooth analyzed- iatrogenic furcation perforation (the microscopic magnification order is 100x)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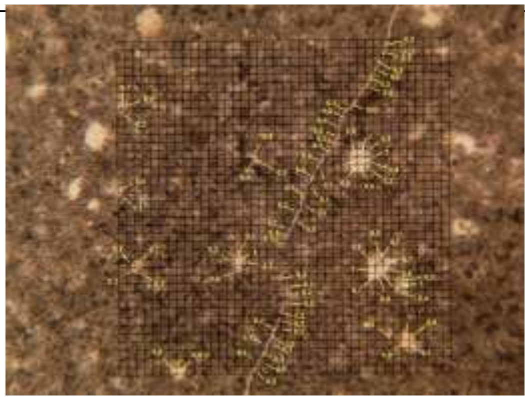
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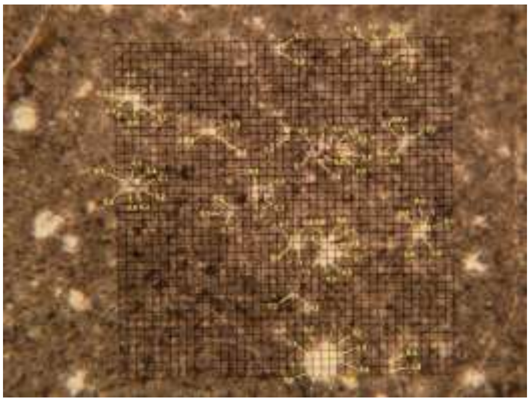
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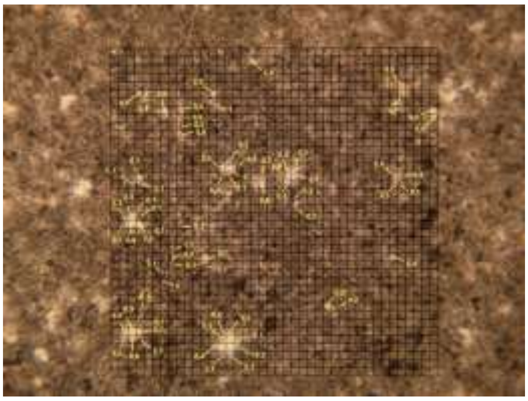
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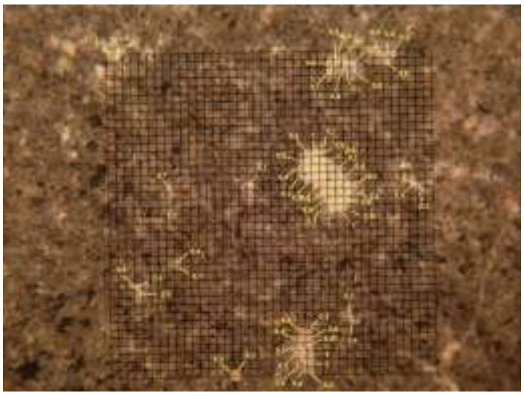
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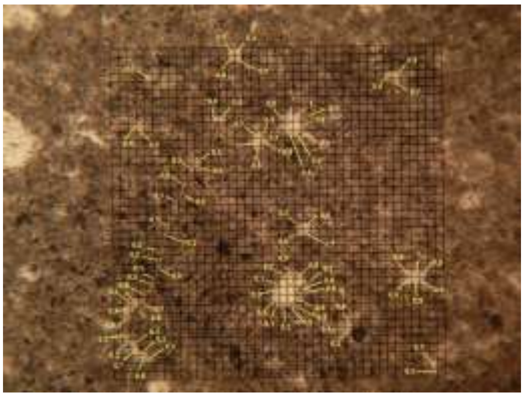
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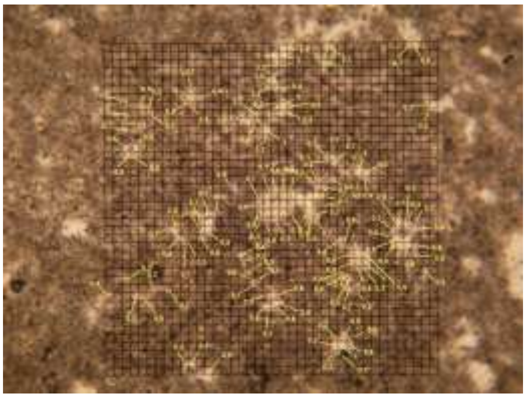
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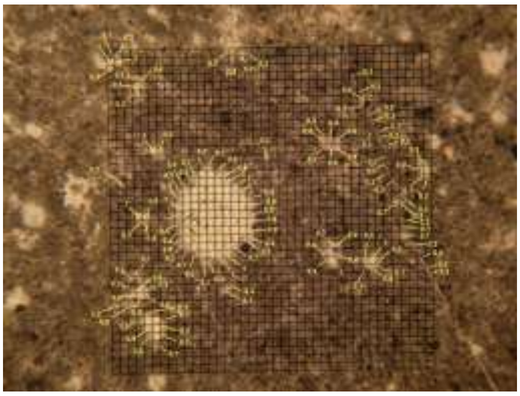

h.

Fig. 2 a-h Microscopic photos corresponding to a first set of microscopic fields adjacent to analyzes for the first Biodentine sample. The magnification order of all images is 100x. The surfaces corresponding to the pores and cracks are marked

2.Results and Discussions

The presence of the accelerator allows the setting of the material in 12 minutes and the presence of water, as a reducing agent avoids the formation of cracks within the material. The literature offers information about the fact that the presence of cracks was induced after setting of cements containing high percentage of water [3, 4].

From a microscopic point of view, the material presents itself as cryptocrystalline mass, with unevenness in terms of transparency, with a pronounced optical isotropic character and containing two types of voids: pores and cracks.

The pores can be divided into two categories according to morphology: pore with anisometric (irregular) and quasi-spherical pores (Fig. 3.4). Those in the first category are predominant.

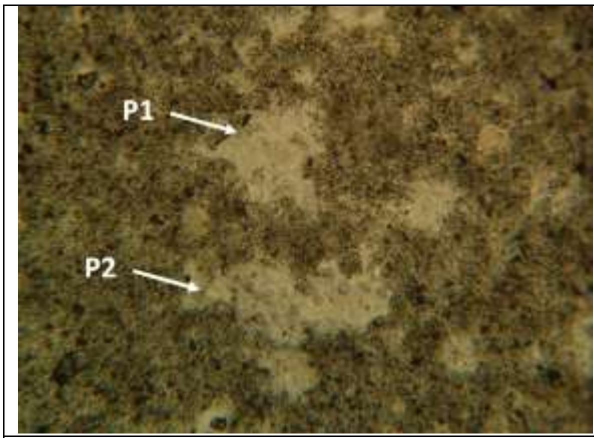


Fig. 3. Pores with irregular shapes (anisometric). The maximum dimensions are apparent: 140 μ m 176 μ m P1 and P2 (250X).

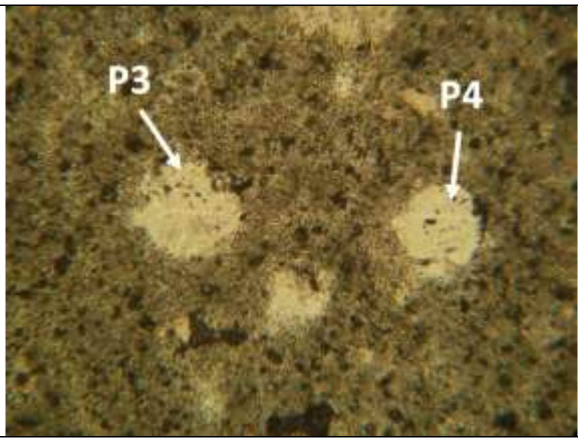
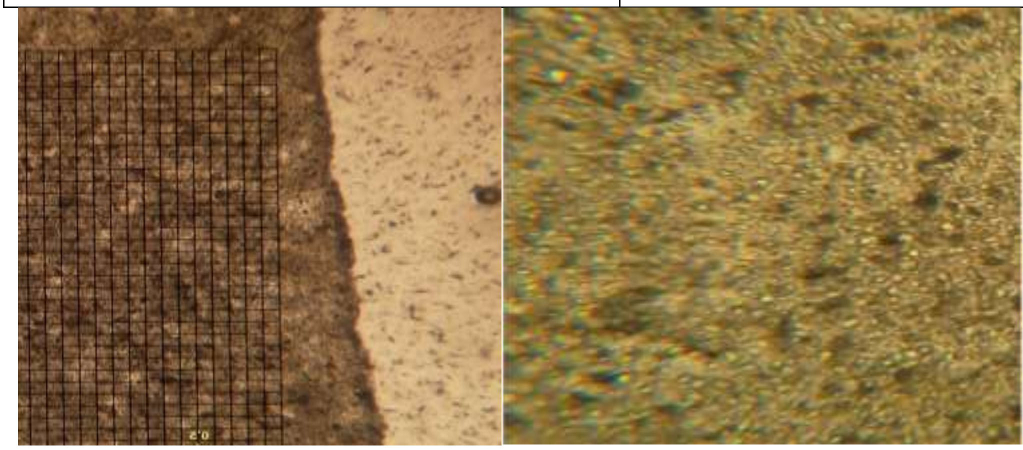


Fig. 4. Pores quasi-spherical forms. The maximum dimensions are apparent: 100 μ m 80 μ m for P3 and P4 (250X).



a) b)

Fig. 7 a, b- Microscopic photos corresponding to a first set of microscopic fields adjacent to analyzes for the fourth Biodentine sample. The magnification order of all images is 140x. The surfaces corresponding to a very low, almost neglected number of pores and no cracks are observed.

It is important to specify the fact that in the case of tooth number 4 (presenting deep and large coronal lesion restored by the sandwich technique with Biodentine), all samples analyzed presented a very low incidence of pores and absence of cracks (Table no. 4, Figure no. 7), under the presumptions of the fact that the instructions of setting and preparation were in accordance to the manufacture and the application in the dental cavity was done under no humidity conditions. This fact is consistent with other authors' findings [1, 2, 7, 8, 9]

The dimensions of these pores in the section plane are apparent dimensions. Obviously, the section plane is unlikely to capture all pores after their maximum diameters / dimensions. Still, we evaluated all the sections to identify the largest apparent sizes found for pores. We believe it provides a picture of the largest absolute dimensions in our Biodentine samples. Thus, we found that the maximum apparent size for the 0.29 mm spherical pores and for those with irregular shapes 0.22 mm.

Locally, in the mass of material at samples 1 and 3 were also encountered microcracks. The maximum openings of these microfissures observed in the section plane are approx.5-6 μ m. We found that these cracks are not continuous, meaning that there can be shoulder to plan our sections to the ends of the sample at both ends. These microcracks are lost in the section plane at a time (Figure 5). It suggests that they practically do not completely separate the samples of Biodentine into smaller fragments and that they are primary, so formed when the semi-consolidated material was placed by the clinician in the endodontic space. In both samples where the microcracks were encountered, there were also interconnections between them and certain pores (fig. 6).

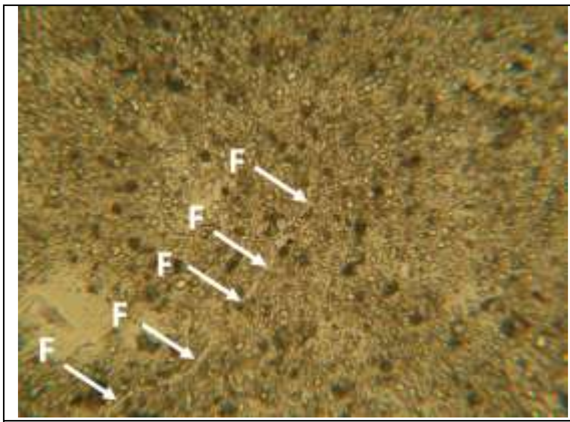


Fig. 5. Detail of the terminal area of a crack; In the upper part of the image the crack does not continue in the section plane (400x).

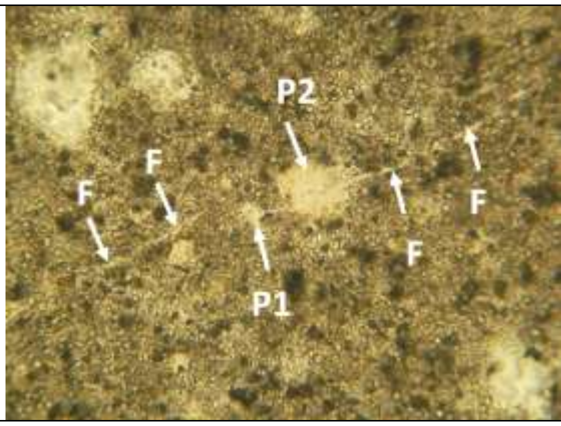


Fig. 6. Detail of the interconnection of some pores (P1 and P2) with a microfissure (F) (375x)

The frequency of microsamples in samples was as follows:

- Sample 1 of Biodentine - with rare microfissures - 0.35 microcracks per microdomain analyzed (17 to 48 microdomains cracks);
- Sample 2 of Biodentine – no microfissures;
- Sample 3 of Biodentine – with more frequent microcracks of 0.48 microfissures per microdomain analyzed (21 cracks on 48 microdomains);
- Sample 4 of Biodentine- no microfissures.

Because we were looking to estimate total porosities for microdomains analyzed and on Biodentine samples, where we encountered the microfissures, their corresponding gaps were taken into account and added to the pore size value. Thus, the following porosity values are presented in Tables 1-3.

Table 1. Porosity values for Biodentine sample 3

	Set of microdomains analyzed	1	2	3	4	5	6	7	8	Average values
Surface section 1	**1**	**1.39**	**1.96**	**3.44**	**2**	**4.14**	**2.78**	**5.64**	**7.54**	**3.61**
	2	**2.47**	**2.89**	**1.85**	**3.54**	**4.03**	**2.13**	**4.94**	**3.98**	**3.23**
	3	**3.18**	**2.24**	**2.83**	**2.71**	**5.06**	**3.22**	**3.85**	**4.29**	**3.42**
Surface section 2	**1**	**2.93**	**7.79**	**1.77**	**3.54**	**3.82**	**1.73**	**7.82**	**3.31**	**4.09**
	2	**3.21**	**3.77**	**1.28**	**2.07**	**3.52**	**2.29**	**6.15**	**4.92**	**3.40**
	3	**2.96**	**4.21**	**2.06**	**3.89**	**3.77**	**7.62**	**3.28**	**4.57**	**4.05**
									Average values/sample	**3.63**

Table 2. Porosity values for Biodentine sample 2

	Set of microdomains analyzed	1	2	3	4	5	6	7	8	Average values
Surface section 1	**1**	**1.85**	**4.19**	**2.23**	**4.2**	**3.75**	**1.67**	**3.68**	**3.03**	**3.08**
	2	**2.37**	**2.04**	**3.45**	**1.74**	**2.3**	**4.25**	**1.87**	**1.64**	**2.46**
	3	**1.93**	**2.41**	**1.56**	**6.28**	**3.62**	**2.41**	**4.54**	**3.25**	**3.25**
Surface section 2	**1**	**4.08**	**2.79**	**3.14**	**2.65**	**2.24**	**1.76**	**5.02**	**2.48**	**3.02**
	2	**2.61**	**6.32**	**1.65**	**2.78**	**2.17**	**2.72**	**4.51**	**2.15**	**3.11**
	3	**3.98**	**2.51**	**2.15**	**2.18**	**5.29**	**2.86**	**2.37**	**2.06**	**2.93**
									Average value/sample	**2.97**

Table 3. Porosity values for Biodentine sample 3

	Set of microdomains analyzed	1	2	3	4	5	6	7	8	Average values
Section surface 1	**1**	**4.1**	**5.15**	**4.21**	**3.64**	**4.24**	**7.71**	**5.27**	**4.26**	**4.82**
	2	**2.31**	**5.26**	**7.04**	**4.47**	**3.18**	**2.05**	**4.34**	**5.46**	**4.26**
	3	**4.28**	**3.24**	**6.69**	**4.76**	**4.24**	**6.84**	**5.27**	**5.91**	**5.15**
Section surface 2	**1**	**5.12**	**4.52**	**4.62**	**4.99**	**2.68**	**5.79**	**5.61**	**4.86**	**4.77**
	2	**4.2**	**8.36**	**3.82**	**2.47**	**4.57**	**6.22**	**5.84**	**6.24**	**5.22**
	3	**3.62**	**4.17**	**4.66**	**4.95**	**4.29**	**8.17**	**7.85**	**5.88**	**5.45**
									Average value/sample	**4.95**

Table 4. Porosity values for Biodentine sample 4

	Set of microdomains analyzed	1	2	3	4	5	6	7	8	Average values
Surface section 1	**1**	**1.09**	**0.96**	**0.44**	**1**	**0.14**	**1.78**	**0.64**	**0.54**	**0.82**
	2	**1.47**	**0.89**	**0.85**	**0.54**	**0.08**	**1.03**	**1.04**	**0.99**	**0.86**
	3	**1.08**	**1.04**	**0.83**	**0.76**	**0.26**	**1.02**	**0.83**	**0.39**	**0.77**
Surface section 2	**1**	**0.39**	**1.09**	**1.77**	**0.54**	**0.85**	**0.75**	**0.89**	**1.13**	**0.92**
	2	**0.21**	**1.17**	**1.08**	**0.07**	**0.52**	**0.29**	**0.15**	**0.90**	**0.54**
	3	**0.95**	**0.21**	**1.06**	**0.87**	**0.67**	**0.63**	**0.28**	**0.52**	**0.64**
									Average values/sample	**0.75**

4. Conclusions

1. It is important to specify the fact that in the case of tooth number 4, presenting deep and large coronal lesion restored by the sandwich technique with Biodentine, in all domains analyzed, the incidence of pores and cracks was extremely low, this fact being in accordance to most of the findings described in the literature.

2. Although the microscopic analysis of the other three sections revealed the fact that most pores are non-communicating, however, locally we noticed that some pores (regardless of form) are interconnected by microcracks. Although it does not completely separate the material samples, we have found that local microcracks starting from several marginal areas of the Biodentine samples interconnect themselves, thus opening up the possibility that the material placed in the endodontic space could be subjected to microinfiltration.

3. Microfissures were only microscopically identified in samples 1 and 3. In sample 2 and 4 no cracks were found on the microscopic scale. This suggests some aspects: microcracks have not occurred in the preparation of sections that we have applied and that the material can be applied in the endodontic space without such discontinuities occurring.

4. Microfissures through their apertures do not contribute significantly to the total void volume and thus to the total estimated porosity per sample. The occurrence of the microfissures is due to the fact that when applying the material in the endodontic space robably it lost its plasticity, the working time being probably cleared, so that during the scarring these microdisintentions appeared.

5. In the case of those with more pores, it highlights the fact that there are no communications, however, locally we noticed that some of them are interconnected through cracks, which opens the way for a discussion about the eventual possibility of infiltration of this material, in cases where instructions for use, mixing instructions and dispensing and condensation conditions have not been accurately followed.

6. A potential issue is the fact that Biodentine highly depends on how it is mixed and the conditions under which it is applied and prepared.The proportions between powder and liquid should be respected and at the same time, the preparation and application of the material should be in accordance to the manufacturer's instructions, but also the degree of moisture regarding the topical application in the specific endodontics pathology (furcation perforations) might influence the setting and the mechanical properties of the material.

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