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SEM and EDS analysis of tooth root surfaces sectioned with bur or Er:YAG laser and irradiated with Nd:YAG laser

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Abstract

This study evaluated the dentin surface of 20 bovine mandibular incisors whose roots were sectioned using a high-speed bur or Er:YAG laser and irradiated with Nd:YAG laser. Specimens were divided into two groups: in the first, teeth were sectioned using a high-speed bur under constant cooling; in the second, teeth were sectioned using Er:YAG laser (pulse mode, 10 Hz, 500 mJ [5W]) applied at a distance of about 3 mm from the tooth under constant air and water spray cooling. Both groups had two surfaces; one treated with Nd:YAG laser, and the other with no surface treatment. Scanning electron microscopy (SEM) was used to obtain images of the surfaces, and energy dispersion spectroscopy (EDS) produced data about their molecular structure. The nonparametric Kruskal-Wallis test was used for comparisons between the four groups. The level of significance was set at 5%. SEM analyses revealed that surfaces sectioned using a high-speed bur had a smoother surface and fewer open dentinal tubules than the surfaces sectioned using Er:YAG laser. Resection with a bur may reduce dentin permeability in comparison with Er:YAG laser alone or in combination with Nd:YAG laser used for surface treatment.

Key words: Apicoectomy; Er-YAG Lasers; Nd-YAG Lasers; Microscopy, Electron, Scanning; Spectroscopy, Energy dispersion.

1 INTRODUCTION

Apical resection, also called apicectomy, is a surgical procedure in which the root apex is removed. Several methods have been developed, and most use high- or low-speed burs. High power lasers have also been tested as a technological resource for apicectomies[1].

According to Oliveira et al.[2] (2004), apical surgery is an alternative treatment for teeth when root canals are calcified or perforated, canals are contaminated or have a complex anatomy, posts cannot be removed, or root canals have bacterial infections that are refractory to conventional treatment.

Laser apicectomy has some advantages, because it sterilizes dentinal tubules by vaporizing bacteria, melts and recrystallizes irradiated dentin and seals dentinal tubules, which does not occur when high- or low-speed burs are used.

In this study, scanning electron microscopy (SEM) was used to evaluate the effect of sectioning roots using a bur or Er:YAG laser and of applying Nd:YAG laser on the structure of the root surface, and energy dispersion spectroscopy (EDS) was used to describe the chemical composition of these surfaces.

2 MATERIAL AND METHODS

2.1 Sample

Twenty bovine mandibular central incisors were obtained from Frigorífico Silva in Santa Maria, Brazil. After extraction from the bovine mandibles, the teeth were washed and scraped to remove periodontal ligament remnants, and then stored in deionized water at 4^{o} C, which was changed weekly.

2.2 Groups

The teeth were randomly divided into two groups of 10 specimens according to the type of sectioning to be performed. After sectioning, one of the dentin surfaces was kept intact (subgroups 1A and 2A) and the other was irradiated with Nd:YAG laser (subgroups 1B and 2B) (Table I).

2.3 Tooth sectioning

In group 1, the 10 teeth were sectioned in the middle third of the root using a high-speed 4138 cylindrical diamond bur (KG Sorensen) at 280,000 rpm under constant irrigation with 0.9% saline solution applied with the handpiece tip at 90 degrees to the long axis of the tooth.

In group 2, the teeth were sectioned in the middle third of the root using Er:YAG laser (pulse mode, 10 Hz, 500 mJ [5W] under constant air water spray cooling applied at a distance of about 3 mm from the tooth.

2.4 Treatment of dentin surface

Each specimen had two surfaces sectioned with the same type of instrument. One surface was kept intact, while the other two subgroups (1B and 2B) were irradiated with Nd:YAG laser (pulse mode, 20 Hz, 200 mJ [4 W]) under no cooling for 60 s. The optical fiber was placed at about 2 mm from the dentin surface, according to the protocol described by Pozza (2005) [4,5,6]. Laser was applied following a zigzag line in the buccolingual and mesiodistal directions so that the whole surface exposed by sectioning was treated. After each use, the optical fiber tip was cut with a special cutting device.

The two sections of all teeth were marked with water-resistant black ink according to group and subgroup to which they belonged. After that, all specimens were stored in deionized water at 4^{o} C; one of their ends was embedded in utility wax to keep the sectioned surface free and constantly immersed in water.

A Twinlight Laser Dental® unit (Fotona, Slovenia), previously calibrated by a brand certified company, was used for both lasers.

2.5 Scanning Electron Microscopy and Energy Dispersion Spectroscopy

The samples were sent for analysis in the Center for Microscopy and Microanalyses of Pontifícia Universidade do Rio Grande do Sul, Porto Alegre, Brazil, An XL-30 Phillips scanning electron microscope was used for analyses. This microscope also had an EDS system that was used to detect the chemical composition of the specimens and to map contents all over the sectioned areas.

The specimens were kept in a desiccator for two weeks. After that, they underwent gold sputter coating using an ion deposition technique because the biological specimens lacked electrical conduction.

The specimens were position on stubs to provide good visualization of the sectioned surface. Ethyl cyanoacrylate was used for fixation and to keep specimens in the correct position.

SEM images of the 40 sectioned surfaces were obtained at 35x, 1000x and 3000x magnifications. The electron beam energy was 20 keV, and beam diameter was 4.9 nm. The morphology of the specimen surfaces was evaluated using the secondary electron detector at a 70x magnification.

SEM images were also obtained using the backscattered electron (BSE) detector because it provides better contrast between materials that have important differences in atomic weights. EDS analyses to detect chemical elements of each surface were performed using a Si(Li) solid state detector.

2.6 Statistical analysis

Tables, graphs and descriptive statistics (means and standard deviations) were used for analyses together with the tests described below.

First, the Kolmogorov-Smirnov test was used to test data normalcy. Results indicated that some of the variables under analysis were not normally distributed; therefore, nonparametric tests were selected for comparisons. The nonparametric Kruskal-Wallis test was used for the comparisons between the 4 groups.

The level of significance was set at 5% ($p \leq 0.05$). The SPSS 10.0 software was used for data analysis and statistic calculations.

3 RESULTS

Scanning Electron Microscopy (SEM)

SEM revealed that specimens in group 1A, which were sectioned using a bur and did not receive surface treatment with Nd:YAG laser, had a smooth surface, smear layer and, except for one specimen, fully obliterated dentinal tubules (Figure 1).

Specimens in group 1B, which were sectioned using a high-speed bur and treated with Nd:YAG laser, also had a smooth surface and little exposure of open dentinal tubules, and fusion of the smear layer (Figure 2).

All specimens in group 2A, which were sectioned using Er:YAG laser and did not receive surface treatment with Nd:YAG laser, had an irregular surface and, except for 2 specimens, and their dentinal tubules were partially open (Figure 3).

All specimens in group 2B, which were sectioned using Er:YAG laser and received surface treatment with Nd:YAG laser, had an irregular surface, and half of the specimens had open dentinal tubules (Figure 4).

The results of EDS analysis are described in Table II.

The results of the nonparametric Kruskal-Wallis test revealed a significant difference between the groups for the following chemical elements:

- C (carbon): Group 1A had significantly greater values than groups 1B and 2A, which were not different from each other. Group 2B did not show any significant difference ($p=0.05$).

- P (phosphorus) Group 1A had significantly lower values than all the other groups, followed by groups 2A and 2B, which were not different from each other. Group 1B had significantly greater values than all the other groups ($p=0.008$).

The contents of other elements (O, Mg and Ca) were not statistically different between groups.

4 DISCUSSION

This study was conducted with bovine teeth (permanent mandibular incisors) because they are easy to obtain, their dentin is similar to human dentin, and they provide a standardized root morphology as there is little variation in their forms and sizes [7,8].

The analysis under SEM revealed that the specimens that were sectioned using a bur (groups 1A and 1B) had smoother surfaces than the specimens sectioned with Er:YAG laser, as described in the study conducted by Dutra Corrêa et al. [9] This may probably be assigned to the fact that Er:YAG laser was used in pulse mode and did not section dentin smoothly [1,9,10,11].

According to Kessler et al.[12] and Gown-Soares et al.[13], surfaces sectioned with a bur are smoother and, because of that have greater dentin permeability than those sectioned using Er:YAG laser. In this study, the opposite was seen, as the surfaces sectioned using Er:YAG laser had a greater concentration of open dentinal tubules than the remaining surfaces after sectioning with a bur. This may be explained by the fact that the remaining smear layer on the surface that was sectioned using a bur obliterated the dentinal tubules, as seen under SEM, whereas the Er:YAG laser ablated the smear layer.

Smooth surfaces as a result of apicectomies favor tissue repair in the periodontal ligament and make it easier to place retrofilling material; they also make it more difficult for microorganisms to proliferate [12].

SEM showed that smear layer fusion was found on the surface of specimens in the groups in which Nd:YAG laser was used (1B and 2B), something not seen in the other groups (1A and 2A). Smear layer fusion and recrystallization was apparently responsible for the smaller number of open dentinal tubules, at least in group 2B.

Practically all the specimens used in this study had cracks or fractures visualized under electron microscopy. This is a frequent finding in this type of analysis because of drying and the vacuum in the internal system of the microscope. When there is a minimal amount of humidity in the specimen, the decrease in atmospheric pressure to obtain vacuum produces an expansion of this liquid inside the tubules, which causes dentin fractures or cracks.

The chemical substances analyzed in this study under EDS were carbon, oxygen, magnesium, phosphorus and calcium. Dentin is composed of water (about 12%), organic material (18%), and inorganic or mineral material (70%), which consists of hydroxyapatite molecular units, whose formula is Ca10(PO4)6(OH)2, as well as some calcium phosphates, carbonates, sulfates and other substances, such as fluoride, copper, zinc and iron [14].In bovine teeth, the presence of fluoride is practically null, because the contact of these animals with fluoridated water is rare, and toothpastes and mouth washes are not used.

In the four types of treatment used for dentin surfaces in this study, the variation in the percentage of magnesium, oxygen and calcium was not statistically significant.

Carbon was found in significantly greater amounts in group 1A (teeth sectioned using a bur and not treated with Nd:YAG laser) than in groups 1B (teeth sectioned with a bur and treated with Nd:YAG laser) and 2A (teeth sectioned with Er:YAG laser and with no surface treatment), but the values were not statistically different between the last two groups. Group 2B (sectioned using Er:YAG laser and surface was treated with Nd:YAG laser) did not have statistically different results from any of the other groups. This is contrary to what was found by Lustosa [15], who adopted a different method and found that the group in which a high-speed bur was used had the lowest carbon values under spectroscopy when compared with teeth that undergo apicectomy using CO2 or Er:YAG laser.

Tanji[16] suggested that, without cooling, surfaces irradiated with Er:YAG laser had cracks and carbonization areas generated by a large increase in temperature on the irradiated surface.

In this study, cooling was used in all groups in which specimens were sectioned with a bur or laser. Group 1A, in which the surface was the result of the use of a bur under cooling with no smear layer fusion, had the highest percentage of carbonates, which suggests that excessive heat was generated due to the use of the bur despite the use of spray cooling, and that such heat was even greater than that generated by the use of Er:YAG laser.

Further studies should be conducted using the same parameters to evaluate human teeth or other types of laser, such as excimer or CO2 lasers, to clarify the consequences and advantages of using these techniques.

5 CONCLUSION

Our findings suggest that:

a) the surfaces resulting from sectioning teeth with a high-speed bur were smoother under SEM than the surfaces obtained when teeth were sectioned using Er:YAG laser.

b) The dentin surfaces that were sectioned using a bur and were not treated with Nd:YAG laser had greater carbon content values than surfaces sectioned using laser, but SEM did not reveal any signs of carbonization in any of the groups. The surfaces sectioned using a bur had a lower percentage of open dentinal tubules, which suggests that this method to section dentin may produce dentin with lower permeability than the dentin sectioned with Er:YAG laser only, or also treated with Nd:YAG laser.

REFERENCES

1. Paghdiwala AF. Root resection of endodontically treated teeth by Er:YAG laser radiation. J endodont 1993;19(2):91-94.

2. Oliveira RG et al. Scanning eletron microscopy (SEM) and optical microscopy: effects of Er:YAG and Nd:YAG lasers on apical seals after apicoectomy and retrofill. Photomed Laser Surg 2004;22(6):533-536.

3. Arens DL, Levy GC. A comparison of dentin permeability after bur and laser apicoectomies. Compendium of Continuing Education in Dentistry 1993;14(10):1290-1298.

4. Pozza, DH. Avaliação, por infiltração de corante, de tecnologias *Laser* em cirurgias paraendodônticas (TESE). Universidade Federal da Bahia, 2005.

5. Baraldi CE e Puricelli E. Estudo in vitro das alterações morfológicas da superfície de raízes submetitas à apicetomia e irradiadas com *laser* de Nd:YAG. Ver fac odont ufrgs 2000;40(2):29-35.

6. Çelik EU et al. Effect of different laser devices on the composition and microhardness of dentin. Operative Dentistry 2008;33(5): 496-501

7. Mello JB e Mello GPS. *Laser* em odontologia. São Paulo: Editora Santos, 2001, 174p

8. Anido A. Dentina humana e bovina: estudo da profundidade de desmineralização e da espessura da hibridização empregando-se um sistema adesivo convencional ou autocondicionante: análise em MEV

(TESE). São José dos Campos, Universidade Estadual Paulista – Faculdade de Odontologia, 2005.

9. Dutra Corrêa M et al. Estudo comparativo ao microscópio de luz da morfologia das dentinas bovina e humana. Rev. ABO nac 2005;13(3):179-83.

10. Sasaki KM et al. Compositional analysis of root cementum and dentin after Er:YAG laser irradiation compared with CO_2 lased and intact roots using Fourier transformed infrared spectroscopy. J Period Res 2002a;37(1):50-59.

11. Sasaki KM et al. Morphological analysis of cementum and root dentin after Er:YAG laser irradiation. Lasers Surg Med 2002b;31(2):79-85.

12. Kessler G, Koren R, Kessler A. Long-term clinical evaluation of endodontically treated teeth by 15F CO_2 microprobe, three years clinical follow-up of 1512 root canals: *in vivo* study. In: Conf in laser Dent, 5. Society of Photo-Optical Instrumentation Engineers 1999;3593:27-29.

13. Gouw-Soares S, Lage Marques JL, Eduardo CP. Apicoectomy by Er:YAG laser: permeability and morphological study of dentine cut surface. In: Int Las Cong. Athens, Greece. Proceedings 1996;365-370.

14. Oda M, Matos AB, Liberti EA. Morfologia da dentina tratada com substâncias dessensibilizantes: avaliação através de microscopia eletrônica de varredura. Rev Odont Univ São Paulo 1999;13(4):337-342.

15. Lustosa-Pereira AC, Pozza DH, Cunha A, Dedavid BA, Duarte-de Moraes JF, Gerhardt-de Oliveira M. Analysis of the morphology and composition of tooth apicesapicectomized using three different ablation techniques. Med Oral Patol Oral Cir Bucal. 2011 March 1;16(2):e225-e230.

16. Tanji EY, Eduardo CP. Estudo in vitro da variação de temperatura em dentina irradiada com o *laser* de Er:YAG - análise termográfica.(TESE). FOUSP, São Paulo;2002.

Table I – Groups according to sectioning and treatment of dentin surface

Table II – Comparison of Wt % values between study groups

Figure 1 – SEM image of a specimen in group 1A under 35x, 1000x and 3000x magnifications. This specimen has concentric cracks (arrow), as most study specimens, due to drying and removal of air from inside the unit. Surface is very irregular, has a few grooves and no exposed or open dentinal tubules.

Figure 2 – SEM image of a specimen in group 1B under 35x, 1000x and 3000x magnifications. Smooth appearance and smear layer fusion. Arrow points to open dentin tubule.

Figure 3 – SEM image of a specimen in group 2A under 35x, 1000x and 3000x magnifications. Rough and irregular surface with extensive exposure of open dentinal tubules.

Figure 4 – SEM image of a specimen in group 2B under 35x, 1000x and 3000x magnifications. Irregular surface, although application of Nd:YAG laser resulted in smear layer fusion and gave it a smoother appearance that in group 2A. Despite that, open dentinal tubules (arrows) are seen.

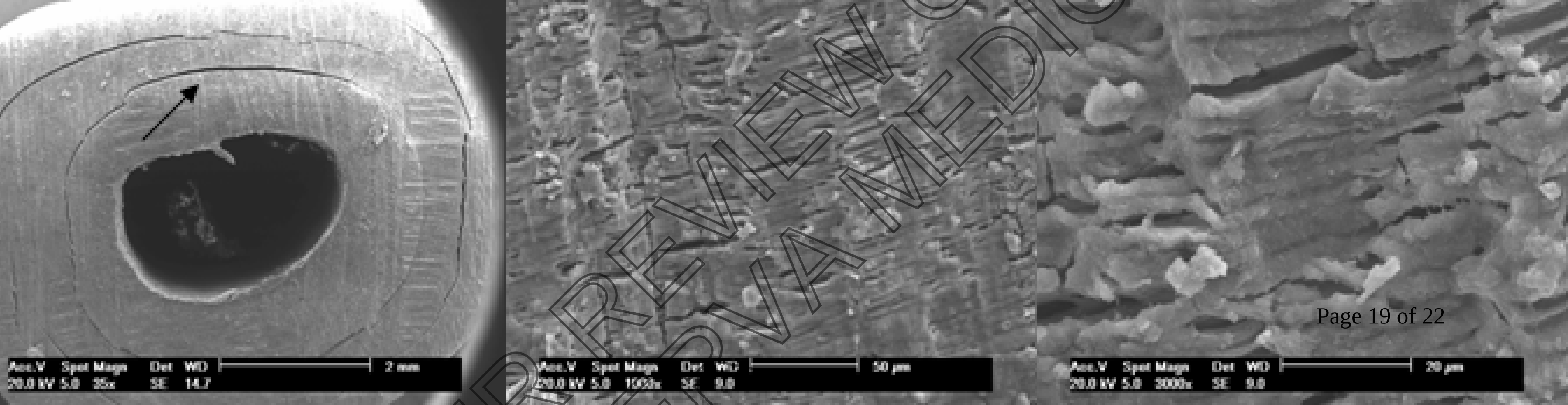
Table I – Groups according to sectioning and treatment of dentin surface

GROUP	N	SECTIONING	SUBGROUP	SURFACE TREATMENT
1	10	Diamond bur	1A	no
			1B	Nd:YAG laser
2	10	Er:YAG laser	2A	no
			2B	Nd:*YAG Laser*

Table II – Comparison of Wt % values between study groups

Element	*Group*	*N*	*Minimum*	*Maximum*	*mean*	*Standard deviation*	*p*
C	Group 1A	10	5.46	16.67	9.54^{A}	3.34	0.050
	Group 1B	10	4.23	15.28	6.80^{B}	3.79	
	Group 2A	10	5.55	9.99	6.92^{B}	1.26	
	Group 2B	10	4.40	11.08	7.66^{AB}	2.13	
O	Group 1A	10	23.28	33.65	28.66	2.89	0.226
	Group 1B	10	23.57	29.36	26.59	1.71	
	Group 2A	10	25.02	31.21	27.95	2.05	
	Group 2B	10	25.07	30.27	28.00	1.89	
Mg	Group 1A	10	0.00	1.64	0.81	0.57	0.230
	Group 1B	10	0.66	1.78	1.18	0.37	
	Group 2A	10	0.69	1.81	1.27	0.29	
	Group 2B	10	0.58	2.64	1.35	0.65	
P	Group 1A	10	19.98	26.51	22.26^{A}	1.69	0.008
	Group 1B	10	21.40	26.51	24.78^{B}	1.53	
	Group 2A	10	19.27	25.25	23.57^{C}	1.82	
	Group 2B	10	21.54	25.25	23.53^{C}	1.32	
Ca	Group 1A	10	33.97	42.74	37.72	2.86	0.186
	Group 1B	10	32.81	43.54	40.43	3.22	
	Group 2A	10	36.39	44.59	39.70	2.51	
	Group 2B	10	36.23	44.58	39.45	2.89	

* Means followed by the same letter do not differ from each other.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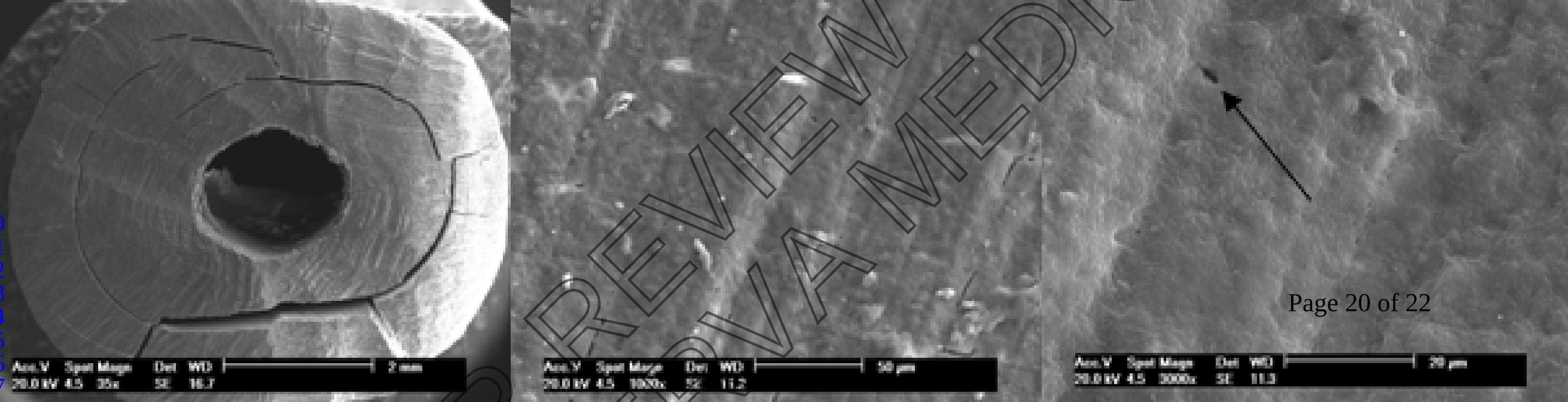
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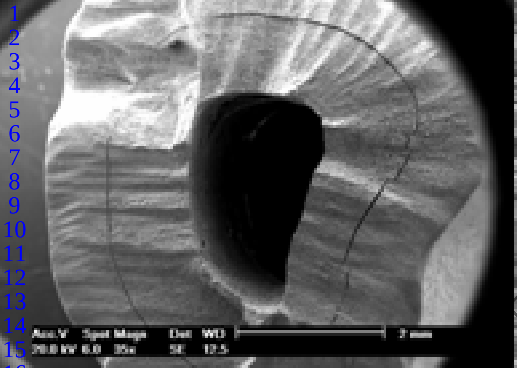

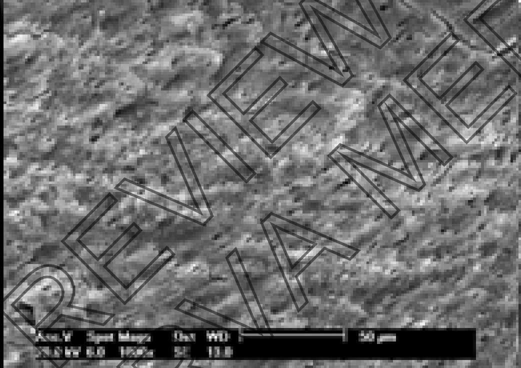

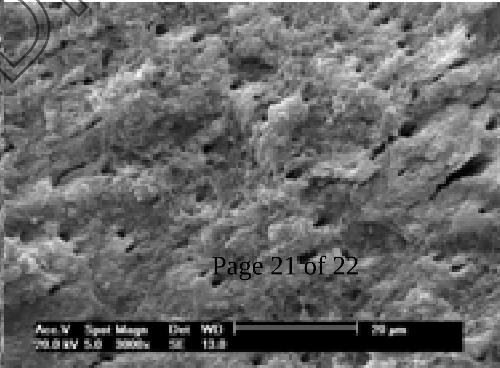

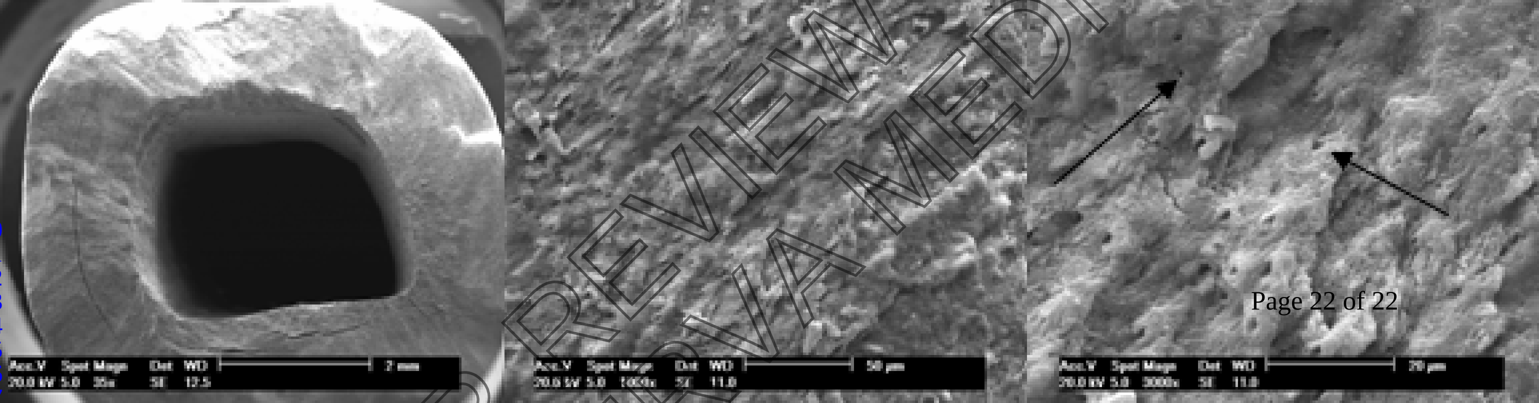
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