COMPARISON OF DENTINE SURFACE MICRO-MORPHOLOGY OF FIVE RESIN BONDING CONDITIONERS USING SCANNING ELECTRON MICROSCOPY

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CONTENTS

ABSTRACT

Dentine bonding with resin systems has continued to challenge researchers and clinicians. Current resin systems available are based on the concept of micro-mechanical retention which involves three steps. The dentine is conditioned which removes the smear layer as well as demineralises the dentinal matrix. A priming agent is used which optimizes the matrix (mineral removed and an expanded organic substrate is left behind). This is followed by monomer penetration (into this matrix) and its polymerization to form the hybrid layer.

Recently introduced products attempt to simplify the bonding sequence by reducing the number of steps and thereby hoping to eliminate errors, save time and perhaps deliver a better clinical result.

The aim of this study was to compare the conditioned surface, derived using five resin systems. The systems tested were 3M, SDI, Clearfil SE Bond (Kuraray), Prime & Bond ^{NT} (Dentsply) and Prompt L-Pop (ESPE). The hypothesis being tested was that there was no difference between conditioned dentine surfaces derived using these systems.

Middle dentine disks of were obtained from twenty five extracted caries free, unrestored human molars. A smear layer was created on the disk, which were then divided into five groups of five specimens. The five products being tested were applied according to

manufacturer's instructions onto the dentinal disks. Thereafter the disks were washed in either water, (3M etch, SDI etch, and Prompt L-pop) alcohol (Clearfil SE bond primer) or in acetone (Prime & Bond ^{NT}). They were then fixed, washed and dehydrated in ethanol. After the final ethanol step the specimens were dried using hexamethyldisilazane (HMDS) and viewed under a field emission scanning electron microscope. The dentinal disks were scored for presence of debris and the quality of their collagen matrix.

The results showed the SDI etch and Prime and Bond ^{NT} had significant debris present so were ineffective in conditioning the dentine. The other three, whilst successful in removing debris, did not produce an optimally expanded dentinal substrate for monomer penetration.

In conclusion there were differences between the conditioned surfaces. None of the five conditioning agents produced an ideal surface, with debris removed and an expanded demineralised dentinal matrix.

STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

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INTRODUCTION

Restorative dentistry continues to look for successful aesthetic materials, which require minimal tooth preparation. Bonding of the restorative material to tooth is the first step in the process.

Resin bonding to enamel has a proven clinical record. Buonocore [1] showed that using phosphoric acid on the enamel surface increased the adhesion of acrylic filling materials considerably. Scanning Electron Microscope (SEM) studies [2,3,4], later showed how phosphoric acid was able to create a preferential loss of prism material from the enamel and as a result create micro-porosities (Figure 1.1). The adhesive resins could then flow into these and create resin tags. It was these tags that gave the resin-enamel bond its strength.

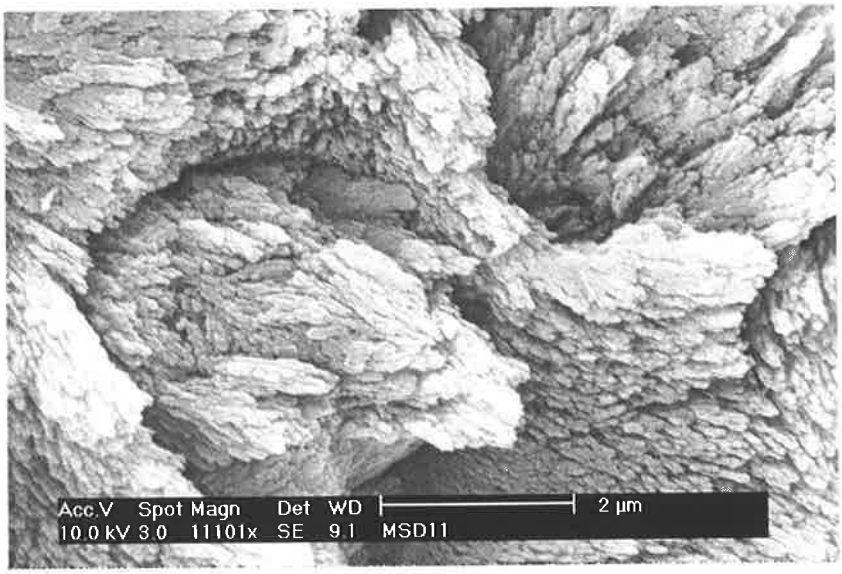


Figure 1-1: Phosphoric acid etched enamel

Silverstone et al[5] concluded that exposure of human dental enamel to acid solutions in-vitro produced three basic etching patterns.

Type 1: prism core material preferentially removed leaving the prism peripheries relatively intact.

Type 2: peripheral regions of prisms removed preferentially.

Type 3: random pattern with both type 1 and 2 observed along with a more random pattern in which the pattern could not be related to prism morphology.

While enamel bonding has been successful, dentine bonding has proved much more difficult to achieve. As a substrate dentine is different to enamel. Dentine is a hydrated biological composite structure[6]. By volume it is 50% mineral, rich in carbonate and deficient in calcium, 30% organic matrix which is largely type I collagen and about 20% fluid. This fluid is similar to plasma but poorly characterized [7].

Evolution of dentine bonding agents

Initial attempts to acid etch dentine proved unsuccessful. Etched dentine responded very differently to etched enamel due to its different structure.

Bowen [8, 9, 10, 11] in his series of articles developed the concepts of:

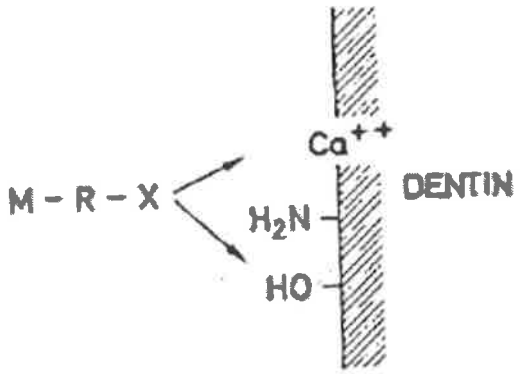
- Surface coupling i.e. a molecule, which is capable of bonding chemically with dentine as well as a resin substrate.

- The NPG-GMA agent (addition reaction product of N-Phenylglycine and Glycidyl methacrylate)
- Suggested the optimum concentration of NPG-GMA in 10% acetone resulted in best bond strengths.

This research was significant because until then attempts to bond to dentine after acid etching were proving unsuccessful. Hydrophobic, unfilled resins did not adhere well to dentine because of the dentine's fluid content. For the first time the bond strength between resin- dentine was not being completely destroyed by water. It was suspected that the NPG-GMA was bonding to the mineral phase of dentine because in-vitro bond strengths seemed significantly higher to fluoro-apatite surfaces, (which contained no organic material) compared to bovine tendon (which was predominantly collagen). NPG-GMA was commercially available in a restorative material as Cervident (SS White Co., Lakewood, NJ). Clinical trials however of this material proved unsuccessful. Harris et al[12] found that 6 months after placement only 55% of restorations were still in place.

Cervident became the first generation dentine bonding agent. Although Cervident was clinically unsuccessful, it did introduce the idea of chemical interactions between adhesive and dentine groups and stimulated the development of second generation products.

Second generation products were introduced in the early 1980's and were based on chemical interactions between adhesive and dentine groups. The products introduced aimed to bond with the inorganic and organic phase of dentine, (Figure 1-2).



Dentin adhesive M-R-X with a potential for reaction with either Ca^{++} ions of the inorganic part, or with NH_2 or OH groups of the organic part of the dentin

Figure 1-2: Potential chemical dentine bonding sites [50]

By this stage, the idea of chemical interaction between dentine substrate and resin systems was firmly entrenched. Clinical trails however were not promising, (Table 1-1).

Table1-1: Clinical observations of loss of Class V restorations placed with dentine bonding agents

Report	System	Enamel	Restorations lost by number of months 1.5	3	6	12
Tyas[13]	Scotchbond + Silux	No	6	18		
	Scotchbond + Silar	No	20	44		
	Dentine Adhesit + Silux	No	52	74		
Doering & Jensen[14]	Scotchbond + Silux	Yes		1		
Ziemiecki et al[15]	Scotchbond + Silar	No		13	24	36.5
Dennison et al[16]	Scotchbond + Silux	No		13	17	30
	Scotchbond + Silux	Yes		0	8	12.2
Ziemiecki et al[17]	Creationbond + Resin				73.4	
	Creationbond + Resin	Yes			51.5	
Doering et al[18]	Scotchbond + Silux	No		24	26	
	Dentine bond + Silux	No		91	97	
	Creationbond + Marathon	No		100	100	

The newer dentine bonding agents continued to perform poorly. When enamel was etched and used then better results were achieved[14,16] compared with dentine alone.

Researchers continued to look at new ways to bond to dentine. Dentine as a substrate was receiving close attention and SEM played an important role in this. Garberlogio[19] et al showed that there are about 40,000 tubules per mm^2 in dentine making it a very porous structure. The area of space occupied by tubules varied from less than 1%, just beneath the enamel to more than 22% near the pulp[20]. This was due to the convergence of tubules as they approached the pulp chamber. As a result one would have expected the bonding

mechanisms in deep versus superficial dentine to be different.

The smear layer had been isolated under the light microscope in the 1950's, but it was not well understood. SEM, with its improved resolution and the large depth of field, made detailed analysis of surface morphology and composition possible.
SEM analysis of the dental debris which formed during cutting of dental tissues, demonstrated both organic and inorganic components. Furthermore the quality and quantity of the layering was directly influenced by operating conditions. For example a course diamond abrasive, used dry, produced the thickest deposits[21].

The smear layer became a central focus for researchers. It seemed logical that ultimate strength of any dentinal bonding would be only possible with successful treatment of the smear layer which formed each time teeth were cut. Researchers were not however entirely sure how to deal with the smear layer. If the smear layer was retained then there was a risk of decreased bonding strengths, but if removed then, it was argued that the dentinal substrate would be left permeable and sensitive.

Third generation systems were developed with view to manage the smear layer and can be broadly classified based on their treatment of the smear layer.

A: Dissolve and remove the smear layer

This category included Gluma (Columbus Dental) and Scotchbond 2 (3M Dental

Products, St Paul, MN). They used an acidic primer to remove the smear layer and thereafter attempted to form a chemical bond between resin and dentine (organic or inorganic phase).

B: Modify the smear layer

This category included systems such as Tenure (Den-Mat Corp) and Mirage Bond (Chameleon Dental products, Inc, Kansas City, KS). They used oxalate to form an insoluble precipitate that sealed the dentinal tubules to protect the pulp.

Hansen[22] reported three-year cumulative survival rates of 96% with Gluma and 66% with Scotchbond 2 for cervical erosions restored with dentine bonding agents.

As data on dentine bonding accumulated previously held ideas were questioned. Etching of dentine (with strong acids) had been previously discouraged on two accounts. Firstly acid etching would remove mineral from dentine and as a result leave dentine devoid of the mineral phase. Systems reliant of the mineral phase to bond with would be compromised. Secondly as a substrate, dentine was seen to be porous, with the dentinal tubules linking it directly to the pulp. Acid etching dentine would be therefore like indirectly etching the pulp. Previous studies had shown that if dentine was etched prior to the placement of composite resins almost invariably a massive bacterial invasion in the dentinal tubules occurred. As a result severe pulpal inflammation occurred in these teeth[23, 24, 25, and 26].

In Japan in the meantime something different was happening. Practitioners were routinely taught to acid etch dentine prior to resin placement. This worked well and it wasn't increasing dentinal sensitivity as might have been expected with open dentinal tubules [27a]. Brannstrom[27] showed that the acid etchants did not produce appreciable damage or inflammation to the pulp even if directly applied to the pulp exposures. Later Hume et al[28] suggested that the most important variable that determines whether or not a pulpal reaction will occur following acid etching of dentine is the adequacy of the subsequently placed restorative materials to seal the cavity margins, prevent micro-leakage, and block bacterial substances from penetrating through dentinal tubules to the pulp. Acid etching dentine which led to pulpal reactions in the earlier studies were due to bacteria and their products leaking into the depths of the cavity rather than as a direct effect of the acids[29,30].

As a result of these studies acid etching of dentine was no longer seen as a deleterious step. The idea of chemical bonding dentine with resin systems lost its significance and gave way to the current understanding of dentine bonding.

Current concepts

The formation of the hybrid layer is thought of as the standard bonding mechanism of the current adhesive systems. It relies on micro-mechanical entanglement, between resin and dentine substrate as the basis of bond strength. Nakabayashi et al [31] was the first to

describe the intermingled layer of collagen and resin referred to as the hybrid layer. They simultaneously etched dentine and enamel using 10% citric acid - 3% ferric chloride and then primed surfaces with 4-methacryloxyethyl trimellitate anhydride (4-META). SEM analysis showed that the monomers penetrated the tissues deep and good adhesion was provided by the intertubular lock. The paper was published in 1982 and at the time most researchers were interested in chemical bonding using coupling agents. It was not until Van Meerbeek et al [32] showed the morphology of the hybrid layer using SEM and TEM, that the significance of original finding were realised. A micromechanical entanglement of exposed collagen fibrils occurred at the decalcified dentine surface layer. Above this decalcified zone was the adhesive/low viscosity resin whilst below it was unaltered inter and intra-tubular dentine.

The formation of the hybrid layer involves three steps;

Step 1: Etching (conditioning)

This step etches dentine using a phosphoric acid. The smear layer is removed, the intertubular dentine is demineralized (the peritubular dentine is also demineralized but to a lesser extent). The removal of the intertubular dentine leaves behind a collagen meshwork, (Figure 1-3).

Step 2: Priming

After the etching agent is rinsed off, a primer consisting of a solvent (alcohol, acetone, or water) with one or more hydrophilic monomers is applied. Primer

molecules contain two functional groups – a hydrophilic group which has an affinity for the dentine surface and the hydrophobic (methacrylate) group with affinity for resin. The primer wets and penetrates the collagen meshwork, raising it almost to its original level. The primer also increases the surface energy, and hence wettability of the dentine.

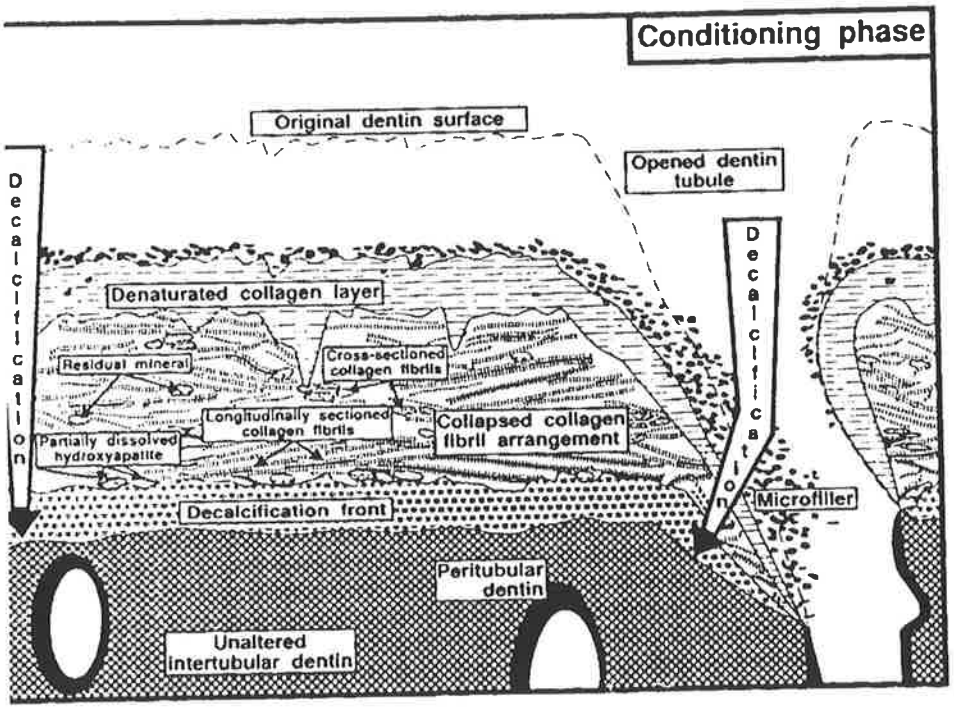


Figure 1-3: Schematic representation of the ultrastructure of the resin-dentine interdiffusion zone at the conditioning phase[32].

Step 3: Bond

Unfilled resin is applied and penetrates into the primed dentine, co-polymerizing with the primer to form an intermingled layer of collagen and resin known as the 'hybrid layer', (Figure 1-4)

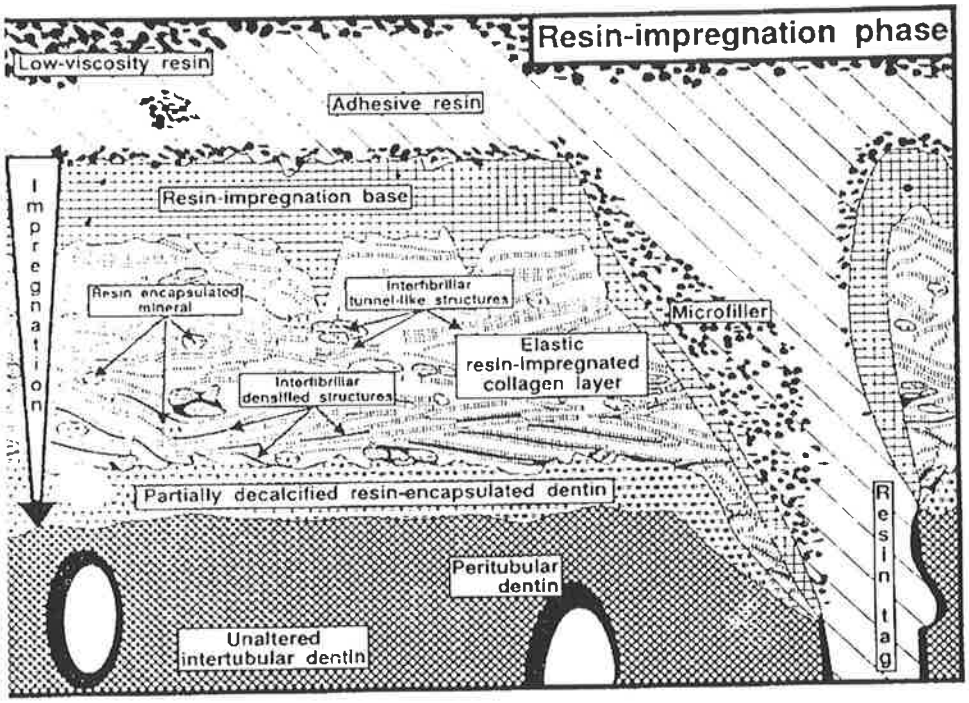


Figure 1-4: Schematic representation of the resin-dentine inter-diffusion zone at the resin impregnation phase[32].

The current systems were originally introduced as three step procedures. Since then further developments that have taken place. Systems claiming to be fourth, fifth and beyond-generational products have been released onto the market. While their clinical

delivery has changed, (Table 1-2) they are still reliant on the micromechanical bond between resin and dentine.

The three step bonding systems were regarded by some as being complicated. The multiple steps were perceived to be time consuming and there was always the possibility of incorporating errors into the steps. The two and one step systems attempt to simplify the bonding procedures, without compromising the quality of the hybrid layer formed.

Table 1-2: Adhesive systems available

System	Step 1	Step 2	Step 3	Example
3-step	Etch	Prime	Bond	All Bond 2, ScotchbondMP+, Amalgambond, Prime & Bond 2.1.
2-step	Etch + Prime Etch	Bond Prime + Bond		Etch & Prime 3.0, Resculin Aqua-prime, Clearfil Liner Bond 2
1-step	Etch + Prime + Bond			Prompt L-Pop.

Dentine conditioning is an important prerequisite in dentine bonding. A good conditioner must firstly dissolve the smear layer formed during cavity preparation. This allows the resin system access to the dentinal matrix. The dissolved smear layer is either rinsed away with water, (3-step systems) or incorporated into the resin-dentine entanglement, (2-step and 1-step systems).

The second function of the conditioner is to remove the mineral from the dentine matrix, and leave behind an organic matrix composed predominantly of collagen fibres. This exposed collagen must be kept in an expanded state (Figure 1-5) to allow for monomer penetration. Exposed collagen is very sensitive to collapsing on itself (Figure 1-6). This Would be detrimental to bonding because there would be no longer any spaces available for monomer to penetrate, and it would result in poor quality bond formation.

The current systems available employ different conditioners. The 3-step system has a phosphoric acid etch. This is usually thickened with silica or polymers to allow easier handling, and to enable it to stay where required. The 2-step system has an acidic monomer which allows conditioning and priming in one step. The 1-step system which has been only recently introduced combines etch, prime and bond into one step. Prompt L-Pop (ESPE) is one of the first commercially available 1-step systems. It consists of a two-component adhesive consisting of a methacrylated phosphoric acid derivate and water. The two fluids are kept in separate receptacles and are mixed with one another simply by squeezing out.

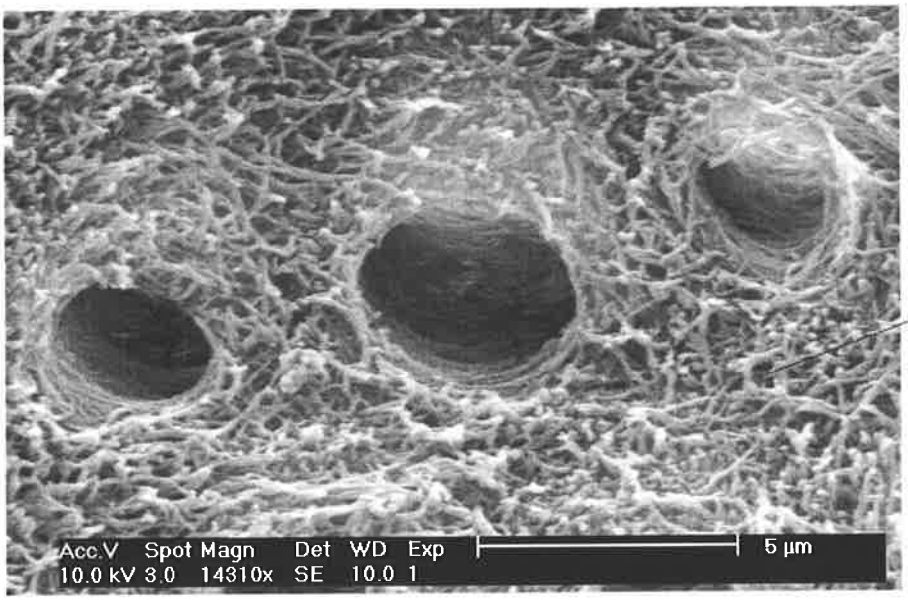


Figure 1-5: Expanded collagen matrix

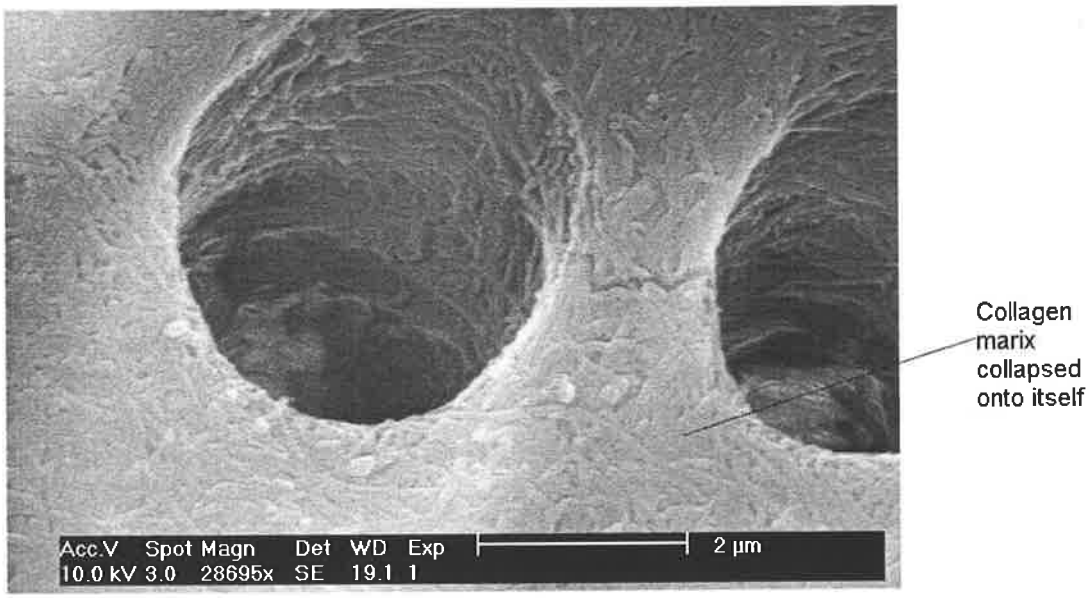


Figure 1-6: Collapse collagen matrix

Aims of the study

The aim of this study was to compare the conditioned dentine surface derived using each of five currently available resin systems. The systems used were

1. Phosphoric etch; 3M
2. Phosphoric etch; SDI
3. Clearfil SE Bond; Kuraray
4. Prime and Bond^{NT}; Dentsply
5. Prompt L-Pop; ESPE

The hypothesis being tested was that there was no significant difference between conditioned dentinal surface derived using these systems.

MATERIALS AND METHODS

Dentine disk preparation

Extracted caries-free, unrestored human molars, which had been stored, for up to six months, in an aqueous solution of 0.5% chloramine at 4°C were used for this study. Dentine disks of approximately 1000 μm thick were obtained using an Isomet slow-speed saw (Buehler, Lake Bluff, Illinois, USA). A blade, (Unicorn,Van Moppes), removed the roots at the cemento-enamel junction with the first cut and then enamel was removed with a parallel cut leaving behind the dentinal disk. Twenty five dentinal disks were obtained in this way.

A smear layer was then created on top of the disk by sanding with 600-grit silicon carbide sandpaper (Carborundum Abrasive, Australia) under running tap water for 60 seconds[33]. The disks were then divided into five groups, A-E (Table 2-1), of five specimens. Dentine conditioner was applied from the five resin systems being tested following the manufacturer's instructions. They were then rinsed for 15 seconds in water (Groups A, B, E), ethanol rinse (Group 3) or acetone rinse (Group D). The disks were then immediately immersed in 4% paraformaldehyde with 2.5% glutaraldehyde in phosphate buffered with saline (PBS) buffer at pH 7.4 for 24 hours. After fixation the disks were rinsed with 20 mls of PBS washing buffer for one hour with 2 changes, followed by distilled water for one minute. They were then dehydrated in ascending grades of ethanol shown below.

25% ethanol for 20 minutes,

50% ethanol for 20 minutes,

75% ethanol for 20 minutes,

95% ethanol for 30 minutes and

100% ethanol for 60 minutes.

Table 2-1: Product information

Group	Name/ Batch no.	Type	Manufacturer
A	Phosphoric etchant 9ml: 7423	Three step system	3M Scotchbond (USA)
B	Acid etch gel Lot 990549	Three step system	Southern Dental Industries Ltd (Aus)
C	Clearfil SE Bond Lot 00049A	Two step system	Kuraray Co Ltd (Japan)
D	Prime & Bond ^{NT} Lot 000710	Two step system	Dentsply Caulk (USA)
E	Pompt L-Pop Lot 62152	One step system	ESPE (Germany)

After the final ethanol step the specimens were immersed in hexamethyldisilazane $[(CH_3)_3SiNHSi(CH_3)_3]$, HMDS, for 10 minutes, placed on a filter paper inside a covered

glass vial, and air dried at room temperature[34].

After drying, the disks were mounted on aluminium stubs (ProScitech, Qld, Australia) and a carbon dag applied. The disks were then coated using a carbon and gold coating in an evaporative coater (Denton Vacuum, DV-502).

The composition of the products used is listed below. (composition of SDI etch gel not available)

Table 2.2: Composition of Scotchbond Etching Gel 7423 (3M) [51].

Ingredient Name	**Percentage**
Water	54 – 62
Phosphoric Acid	34 – 68
Silica	3 – 7

Table 2-3: Composition Prime & Bond [NT, 37].

Di- and trimethacrylate resin
Functional amorphous silica
PENTA (dipentaerythritol penta acrylate monophosphate)
Photoinitiators
Stabilizers
Cetylamine hydrofluoride
Acetone

Table 2-4: Composition of Clearfil SE Bond (Kuraray) [38].

PRIMER:	BOND:
10-Methacryloyloxdecyl dihydrogen phosphate (MPD)	10-Methacryloyloxdecyl dihydrogen phosphate (MPD)
2-Hydroxyethyl methacrylate (HEMA)	Bis-phenol A diglycidylmethacrylate (Bis-GMA)
Hydrophylic dimethacrylate	2-Hydroxyethyl methacrylate (HEMA)
dl-Camphorquinone	dl-Camphorquinone
N,N-Diethanol-p-toluidine	N,N-Diethanol-p-toluidine
Water	Silanated colloidal silica

Table 2-5: Composition of Prompt L-Pop (ESPE) [39].

Liquid 1 (red blister)	Liquid 2 (yellow blister)
Methacrylated phosphoric esters	Water
Initiators	Fluoride complex
Stabilizers	Stabilizers

Dentine disk analysis

The disks were observed under a Philips XL-30 Field Emission Scanning Electron Microscope (FE SEM), at an accelerating voltage of 10 KV, at a working distance of approximately 10 mm.

Each disk was viewed under increasing magnification from 50 X, up to 32,000 X. Five random locations were chosen on the disk and electron micrographs were recorded at magnifications of 4000 X, 8000 X, and 16,000 X. Any other areas of interest besides these points were also noted.

The dentinal disks were then evaluated for the presence of debris and the state of its collagen matrix.

Debris

The dentinal disks were examined for presence of debris. Five random sites were selected for each disk. At 4000× magnification a 10×10 grid was dropped onto the image and each of the 100 cells was examined for debris (Figure 2-1).

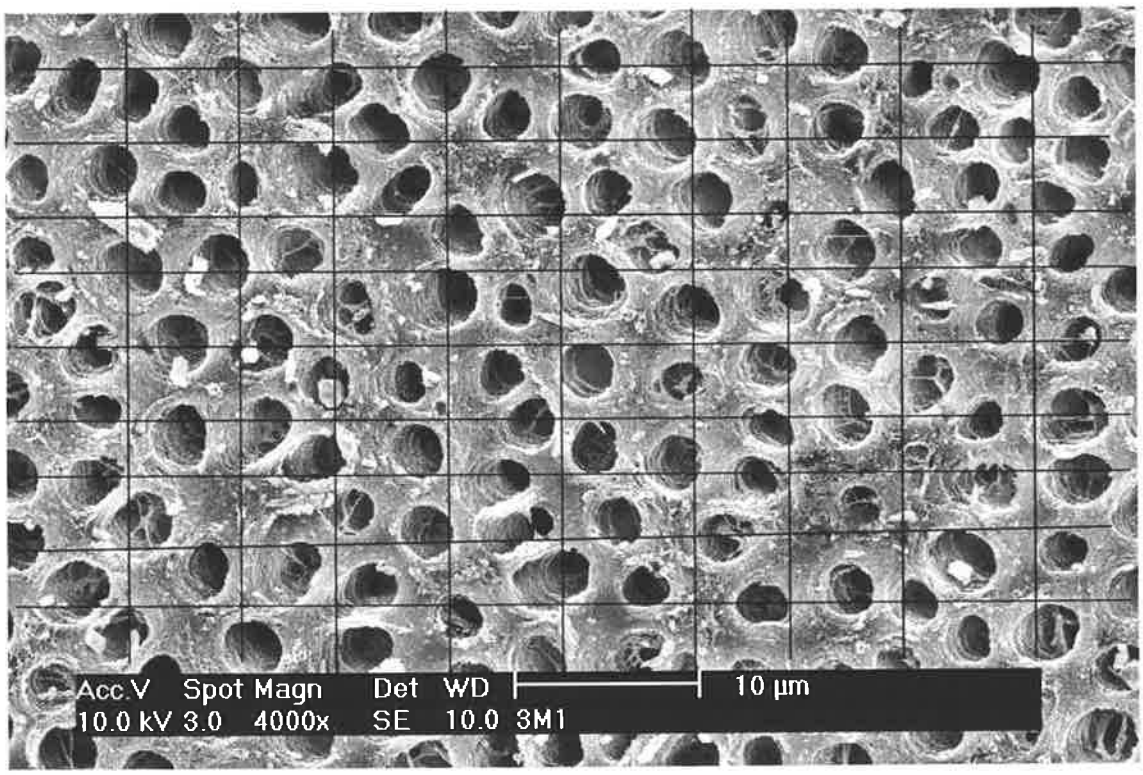


Figure 2-1: Dentine etched with Scotchbond Etching gel 7423 (3M), 34 -38% phosphoric acid @ 4000x magnification.

Each cell was scored. If no debris was present in the cell then it was scored as 1, while debris present meant the cell was scored as 0. In this way five sites on each of the five

disks, for each of the five materials were scored for debris.

Collagen matrix

Electron micrographs at 4000x magnification were examined for the quality of their collagen matrix. The same 10x10 grid (as used for debris analysis) was dropped onto the image and each of the 100 cells was scored for the level of collagen collapse.

Each cell was given a score based on collagen matrix morphology. A score of 0 was given when the cell was covered by debris. It meant that a qualitative assessment of the collagen could not be made, (Figure 2-2, 2-3). A collagen score of 1 was given to a cell which was free of debris, but the collagen was completely collapsed (Figure 2-4, 2-5). A cell was scored as 2 when collagen matrix was visible, but had partially collapsed, (Figure 2-6).

The scoring was based on a qualitative assessment of the collagen present. Where collagen strands could be seen individually it was scored as 2. If they were clumped together or totally collapsed then the cell was scored as 1. In a cell where both 1 and 2 score collagen was present the score given fitted the predominant category as seen in Fig 2-6. A score of 3 was given when collagen appeared in a fully expanded form, (Figure 2-7).

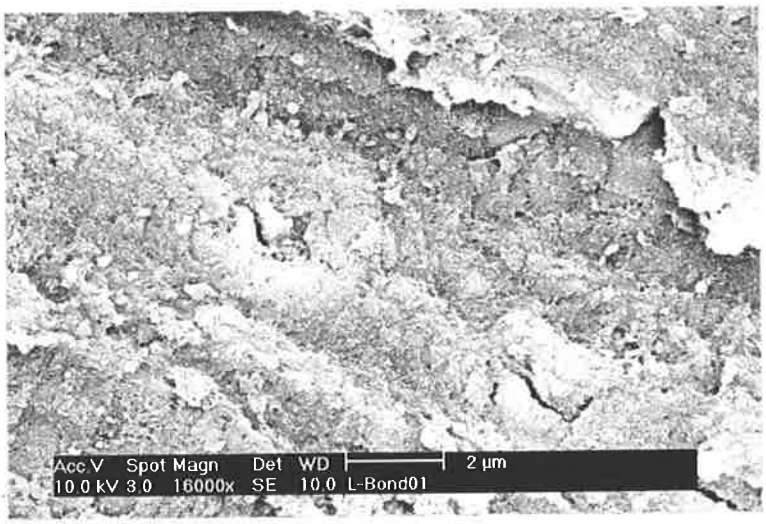


Figure 2-2: A sample where all cells have scored 0.

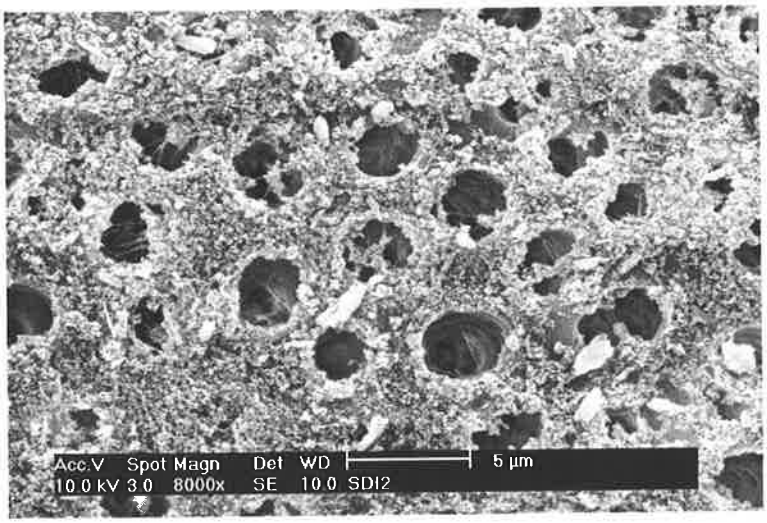


Figure 2-3: Sample with cell score of predominantly 0 scoring cells even though dentinal tubules are visible

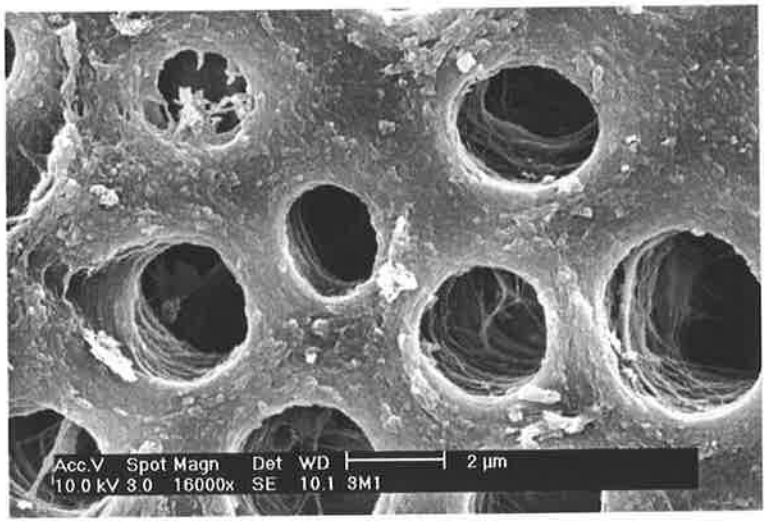


Figure 2-4: Sample with cells predominantly scoring 1; collagen matrix is present but collapsed.

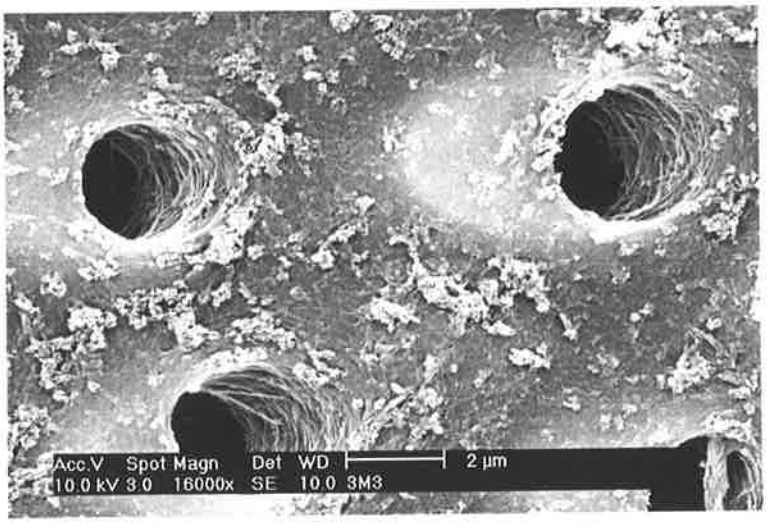


Figure 2-5: Collagen clumped together, with cell scores of 1.

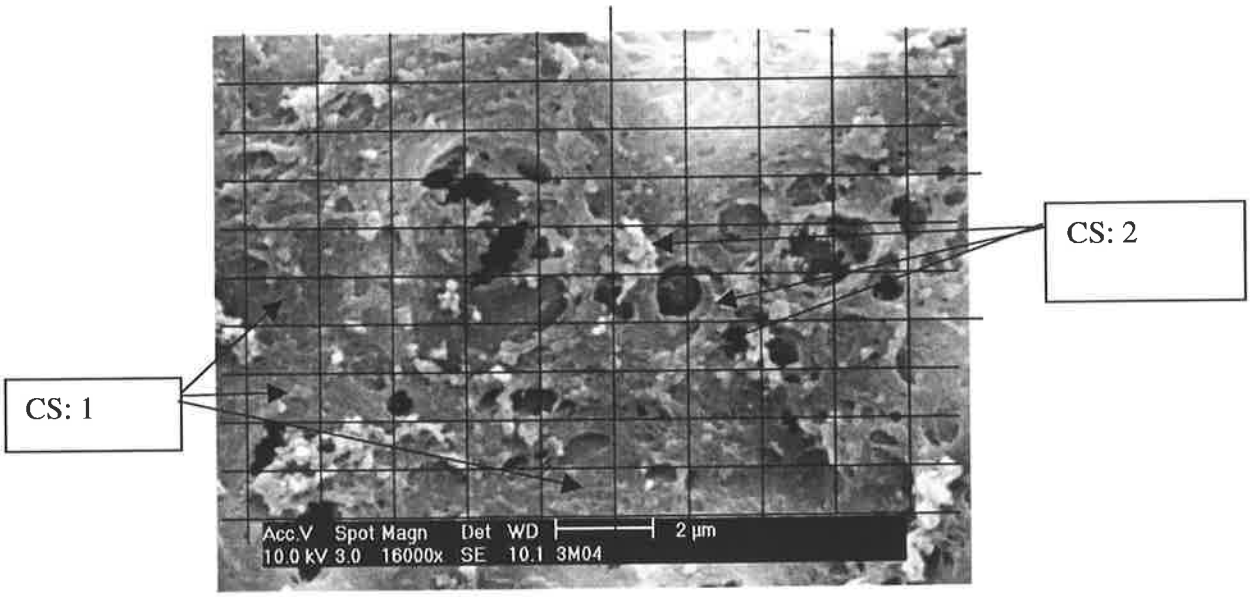


Figure 2-6: Sample with cells scoring scores of 1 and 2.

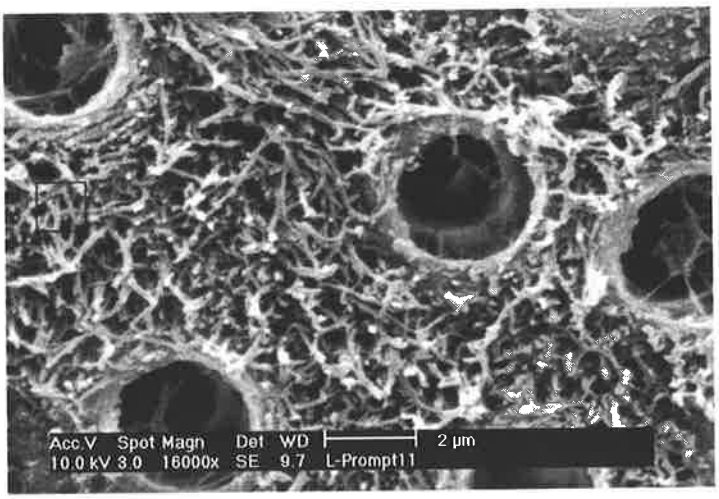


Figure 2-7: Sample with expanded collagen matrix, and cell scores 3.

STATISTICAL ANALYSIS

The data obtained from the scoring of prevalence of collagen exposure was subjected to two way analysis of variance (ANOVA). The statistical analysis was carried out with the software system (SPSS-X).

In these analyses collagen scores were re-scaled and unscorable, debris covered surfaces were excluded and scores in the range 1 to 3 were scaled to the range 0-2 to indicate total matrix collapse (0), partially expanded (1), and expanded (2).

RESULTS

Debris

The distribution of surface debris scores are summarised in Table 3-1 where a score of 0 indicates that no cells in the examined areas were free from debris and a score of 100 suggests that no cells contained debris.

Table 3-1: Distribution of debris scores

	Sites*	Mean score	Standard deviation
3M etch	25	94.4	4.6
SDI etch	25	0.2	0.8
Clearfil SE Bond	25	91.1	19.3
Prime and Bond ^{NT}	25	0.0	0.0
Prompt L-Pop	25	94.0	19.8

* 5 sites for each of 5 specimens for each material, except for Clearfil SE Bond for which we utilised 4 specimens and averaged for the 5th.

In general, specimens conditioned with 3M, Clearfil SE Bond and Prompt L-Pop tended to be free from debris (mean scores greater than 90) while the surfaces conditioned with SDI etch and Prime and Bond ^{NT} generally retained debris (mean scores less than 1).

This overall pattern was confirmed by the analysis of variance comparing the variation in Scores between specimens treated with the same material and between materials (Table 3-2).

Table 3-2: ANOVA comparing debris scores between specimens, and between materials.

	SS	Df	MS	F	p
Materials	260116.9	4	65029.2	447.1	<0.0001
Specimens	916.6	4	229.1	1.5	0.19
Interaction	3352.6	16	209.5	1.4	0.13
Within	14545.6	100	145.5		
Total	278931.7	124			

The ANOVA revealed significant differences in surface debris scores between materials but no significant differences in scores between specimens with the groups treated with each of the conditioners.

Collagen

The distribution of collagen surface micro-morphology scores are summarised in Table 3-3, where a score of 0 indicates that collagen matrix was collapsed in all 100 surface cells for the area considered and a score of 200 indicated that the matrix was fully expanded in all 100 scored cells. No data for the SDI etch or Prime and Bond ^{NT} treated samples are included in this analysis as the surfaces where consistently obscured by debris.

Table 3-3: Distribution of debris scores.

	Sites*	Mean score	Standard deviation
3M etch	25	2.2	6.5
Clearfil SE Bond	25	0.0	0.0
Prompt L-Pop	25	54.4	41.8

* 5 sites for each of 5 specimens for each material

These data indicated that the collagen matrix was generally collapsed in the specimens treated with 3M etch and Clearfil SE Bond (mean scores<2.2) but was more often expanded in specimens treated with Prompt L-Pop. The score of 54.4 would be obtained if this percentage of scores cells showed partly expanded matrix (scaled score of 1) or if 27.2 percent of cells showed fully expanded matrix or there was some intermediate distribution of partly and fully expanded areas.

The analysis of variance confirmed the significance of the observed differences between the materials (Table 3-4).

Table 3-4: ANOVA comparing collagen scores between specimens and between materials.

	SS	Df	MS	F	p
Materials	47479.8	2	23739.9	107.0	<0.0001
Specimens	11067.9	4	2767.0	12.5	<0.0001
Interaction	18554.2	8	2319.2	10.5	<0.0001
Within	133.1.0	60	221.8		
Total	90411.92	74			

In addition to indicating that collagen micro-morphology scores differed significantly between treatment groups, the ANOVA also indicated significant variation between specimens treated with the same conditioner and that this pattern of variation differed between the treatment groups (indicated by the significant interaction term). This indicates that the resultant collagen micro-morphology is highly variable both within and between groups.

DISCUSSION

Debris

Permeability of dentine to adhesive agents is of crucial importance to obtain good dentinal bonding[35]. On conditioning, the dentine is demineralized and resin infiltration is expected to fill the space. As the mineral is solubilized during conditioning, channels are created around the collagen fibres. Monomer particles then can diffuse into this matrix.

Monomer diffusion is influenced by

- Steric restrictions based on their size (monomer).
- Intrinsic diffusion coefficients.
- Presence of a clear pathway.

Size and intrinsic diffusion coefficient depend upon the monomer properties. Collagen fibrils devoid of mineral are thought to have 20nm spaces between them, (Figure 4-1).

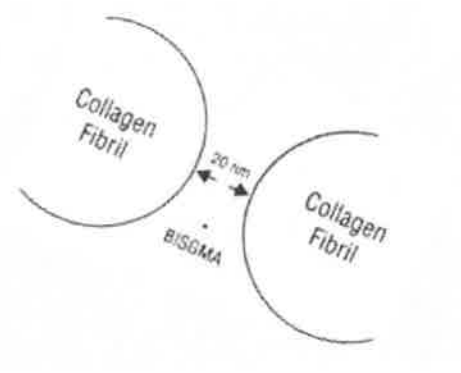


Figure 4-1: Adjacent collagen fibres at the demineralized dentinal surface[35].

Provided monomer particles are below this diameter they can pass through the network. Intrinsic diffusion coefficients will determine the level of penetration of the demineralized matrix that can occur, (Figure 4-2).

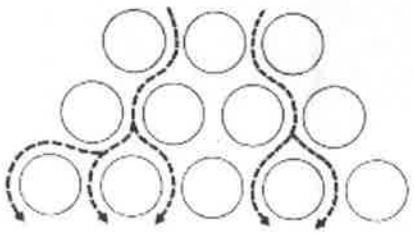
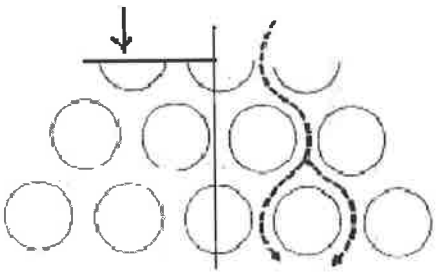


Figure 4-2: Schematic drawing of the increased diffusion path of monomers brought about by the tortuous nature of the diffusion channels[35].

Presence of surface debris would limit monomer flow, (Figure 4-3).



Presence of debris (left side), would obstruct monomer diffusion through the collagen matrix. The right side free from obstruction provides a clear monomer pathway.

Fig 4-3: Limitations with debris present.

GROUPS A & B – 3 STEP SYSTEMS (SDI and 3M etch)

Etching agents are often marketed as gel in order to facilitate handling. The clinician can control the spread of the acid over the tooth surface and visually identify the presence of the acid.

The acid gels comprise of phosphoric acids thickened with either silica or polymer. Perdigao et al [36] have previously raised the possibility of silica micro-particles leaving a particulate residue on the dentine surface. In their study silica gel etchants were grouped into one category. The results of this study have shown significant difference exists in debris present even between silica thickened etchants. As a result it cannot be assumed that all silica thickened conditioners will produce the same conditioned dentine morphology.

One possible explanation of the difference may be related to compositional differences between the commercial products tested. The percentage of silica particles in Scotchbond etching gel 7423 (3M) ranges between 3-7.(Table 2.2). Direct communication with SDI research facilities, indicated their etching gel also incorporated silica as a thickener. The exact composition of the gel product was not forthcoming.

The current study did not carry out a chemical analysis of the debris on the occlusal surface. Perdigao et al [36] using X-ray micro-analysis, (based on a single sample), confirmed the particles to be silica. One would expect the debris to reflect the composition of individual products and x-ray micro-analysis would be required to confirm this. The debris on the SDI sample seemed to adhere strongly to the dentinal disk because rinsing with 10, 20 or even 30 seconds triplex syringe did not remove it (Figure 4-4, 4-5, and 4-6).

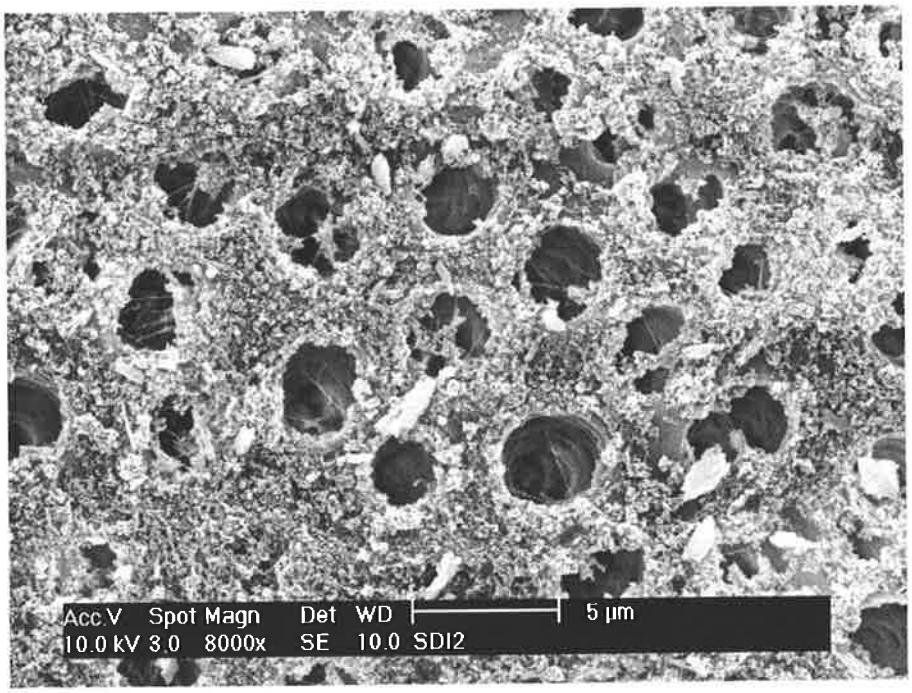


Figure 4-4: Application SDI etch 15 seconds; 10 seconds triplex syringe.

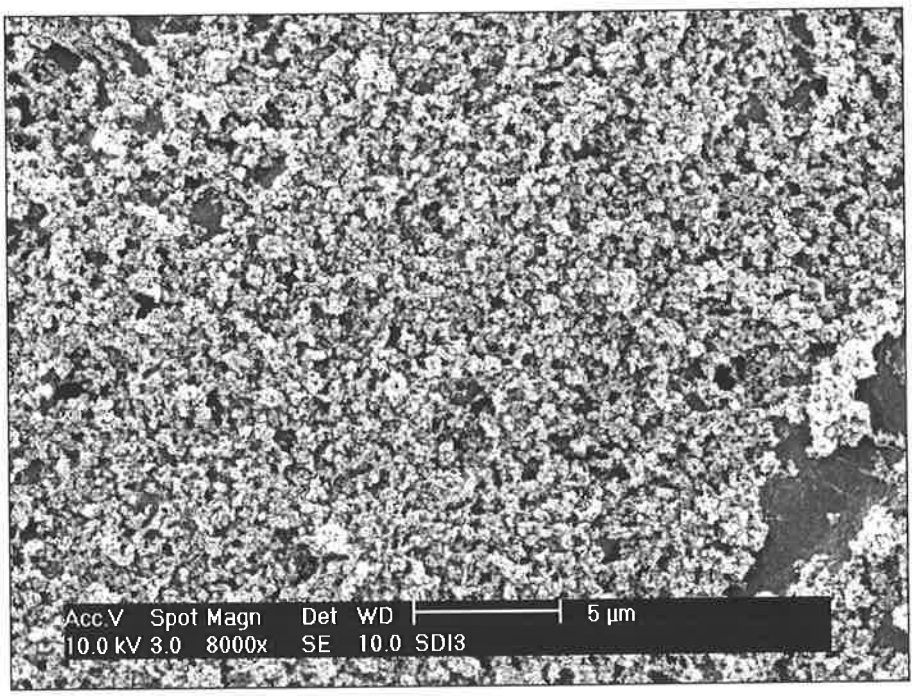


Figure 4-5: Application SDI etch 15 seconds; 20 seconds triplex syringe.

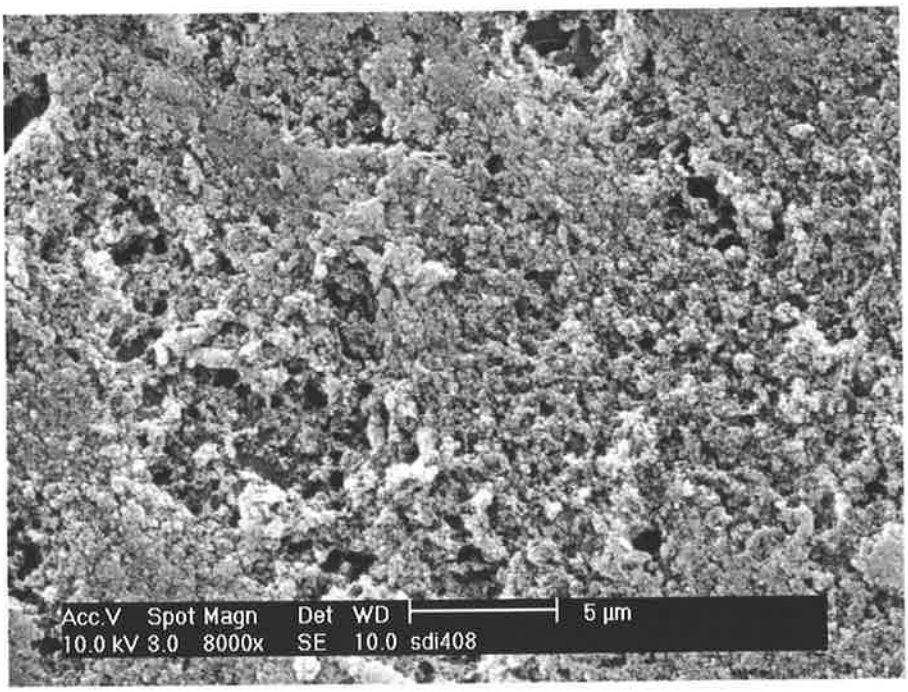
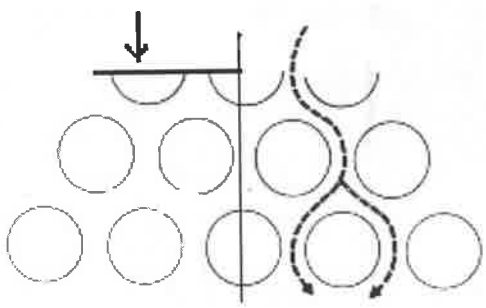


Figure 4-6: Application SDI etch 15 seconds; 30 seconds triplex syringe.

In a clinical situation, the debris would continue to adhere to the dentine surface even after an extended rinse with the triplex syringe, and dentinal bonding would incorporate this debris.

The surface debris would be expected to restrict the flow of monomer molecules into the matrix (Figure 4-7).



Left side of the picture with SDI etch, surface debris would obstruct monomer diffusion through the collagen matrix, shown on the right by the dotted lines.

Figure 4-7: Debris obstructing monomer flow.

The 3M gel's etchant morphology did not appear to leave debris on the occlusal surface. The smear layer was washed clean and the dentinal tubules were clearly visible (Figure 4-8).

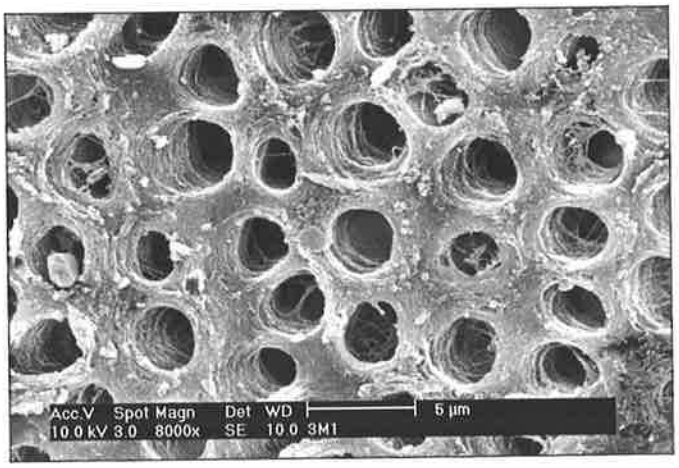


Figure 4-8: 3M acid etch surface, minimal debris on surface.

GROUP D -2 STEP SYSTEM (Prime and Bond ^{NT})

On its original release into the market (Prime & Bond ^{NT}, Table 2-3), it was advertised as being able to be used without any conditioning when working on dentine[37].

Results of this study indicate significant debris is left on the dentinal surface when conditioned with Prime and Bond ^{NT} (Figure 4-9).

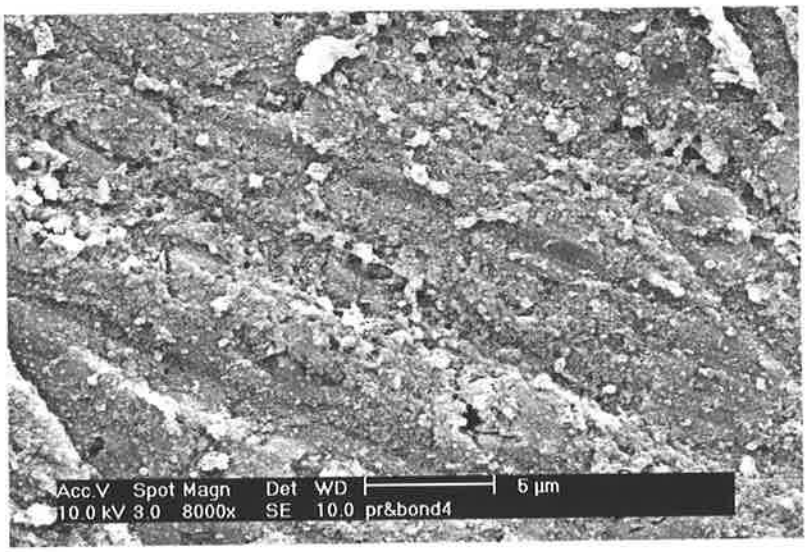


Figure 4-9: Prime and Bond ^{NT}, debris on surface.

Surface analysis would be required to determine the composition of the debris, but one would expect that it would interfere with monomer penetration.

GROUPS C & E – 2 STEP AND SINGLE STEP SYSTEM (Clearfil SE Bond and Prompt L-Pop)

Clearfil SE Bond (Kuraray) and Prompt L-Pop (ESPE) did not leave significant debris (Figure 4-10, and Figure 4-11).

The conditioning agents used in both Clearfil SE Bond and Prompt L-Pop have been listed previously on Table 2-4, and Table 2-5 respectively.

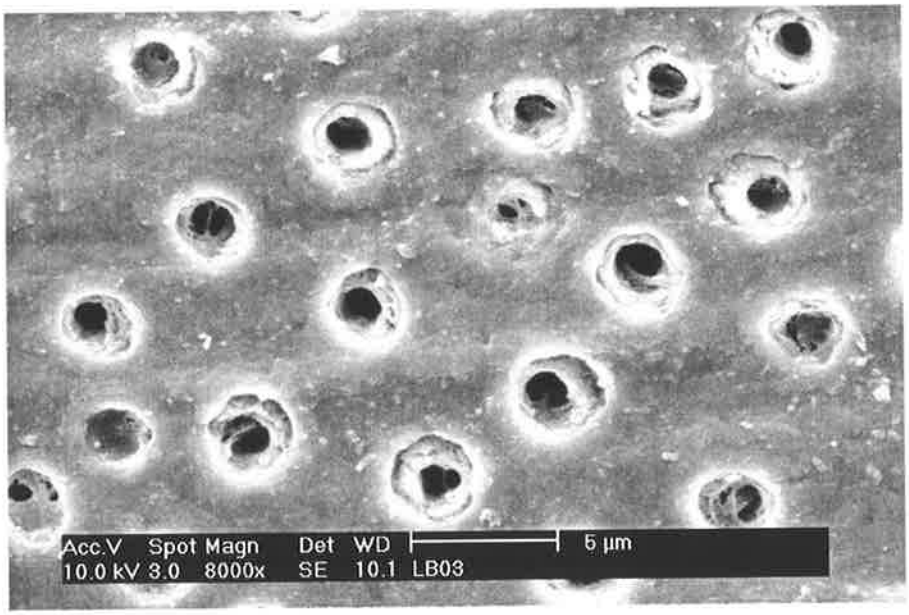


Figure 4-10: Clearfil SE Bond, no debris on surface.

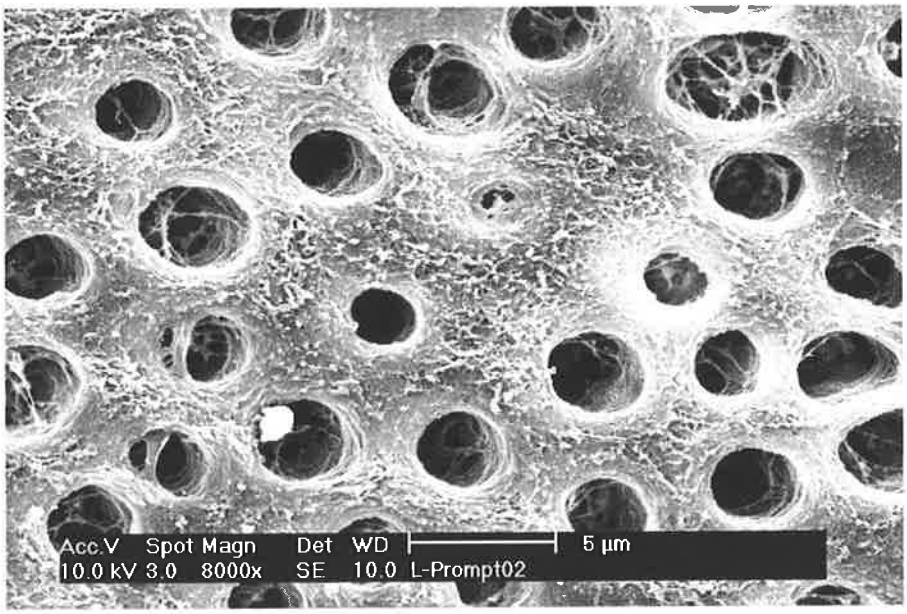


Fig4-11: Prompt L-Pop, no debris on surface.

IMPLICATIONS

Effective treatment of debris is an important prerequisite in the formation of a successful hybrid layer. The results of this study show that it cannot be assumed that the

conditioning agents are equally effective in removal of debris from the dentinal surface. One would expect the ability of debris removal to have a direct impact on the resin-dentine bond strengths.

Perdigao et al [36], in a previous study evaluated the shear bond strengths of composite resins to dentine etched with different types of gels and found no significant statistical difference. However, they classified silica gel etchants into one category and, based on the results of this study, we know that is not necessarily always the case. A study which tested a variety of gel etchants with known composition would be valuable to determine if there was a specific composition which resulted in maximal bond strengths. This is difficult because quite often the product composition changes over time. Clinical success is the ultimate parameter but again due to the rapid change in products, by the time a longitudinal study is completed more than likely the product is no longer used. It is of note that no clinical data, even anecdotal, suggests that the SDI gel is adversely affecting dentine bonding. This indicates that many factors are likely to be affecting bond strengths and that dentine bonding is still not fully understood.

In another study Ferrari et al [40] compared in-vitro formation of the resin tags, and adhesive lateral branches on conditioned and unconditioned human dental surfaces using Prime and Bond [NT]. They found regular tag and branch formation existed on the conditioned specimens but not on the unconditioned dentine. Regular tag and branch

formation would be expected to contribute positively to bond formations.

Prime and Bond ^{NT} is the successor to Prime & Bond 2.1. It has incorporated nanofillers, which are reputed to be only about 7 nm in diameter. The average dentinal tubule is about 800nm and the interfibrillar spaces of 20 nm. Theoretically the nanofillers could penetrate into dentinal tubules and inter-fibrillar matrix and as a result provide extra retention, known as nano-retention. Perdigao et al [41] have shown under SEM that nanofillers were penetrating into the dentinal tubule and the microspaces between the collagen fibres. Whether this will mean increased bond strengths is not entirely clear. It is fair to assume at least comparable bond strengths to traditional systems would be expected provided the dentine is conditioned. Only longitudinal clinical data will indicate if this translates into a proven clinical advantage.

Clearfil SE Bond (Kuraray) and Prompt L-Pop (ESPE) were effective in removing debris from the dentinal surface. This study was designed to allow a direct view of dentinal morphology after conditioning. As a result the conditioning agents were washed off after application, Clearfil SE Bond sample with alcohol, Prompt L-Pop samples with water, (Prime and Bond ^{NT} with acetone) because the products were alcohol, water (and acetone) respectively. In a clinical situation the conditioners would not be rinsed off as they have been designed to be incorporated into the final hybrid layer. Similarly the smear layer whilst dissolved would also be incorporated in the final hybrid layer. Whether this

would be detrimental to final bond strengths is not entirely clear. It is suggested that no differences exist in in-vitro bond strengths with or without smear layer removal for Prompt L-Pop (Direct communication, Roland Richter, ESPE). In-vitro bond strengths of Clearfil Liner Bond 2V have been investigated. Nakajima et al [42] used micro-tensile bond strengths to compare Clearfil Liner Bond 2V, with Scotchbond Multi-Purpose and All Bond 2 on normal and caries affected dentine. Clearfil Liner Bond 2V outperformed the other systems in both environments. Wilder et al [43] showed that shear bond strengths of conventional three step and simplified two step systems (including Clearfil Liner Bond 2V) were not significantly different.

These results suggest that the incorporation of the smear layer should make little difference, and two or one step techniques with an effective conditioning agent would provide a clinical advantage, due to ease of application and time efficiency.

Collagen Matrix

Once the dentine has been conditioned, the dentine matrix, with its mineral content removed, is suspect to collapse. Changes in the size of the spaces between collagen fibrils in mineralized, de-mineralized, air-dried, and re-expanded states are shown below, (Figure 4-12).

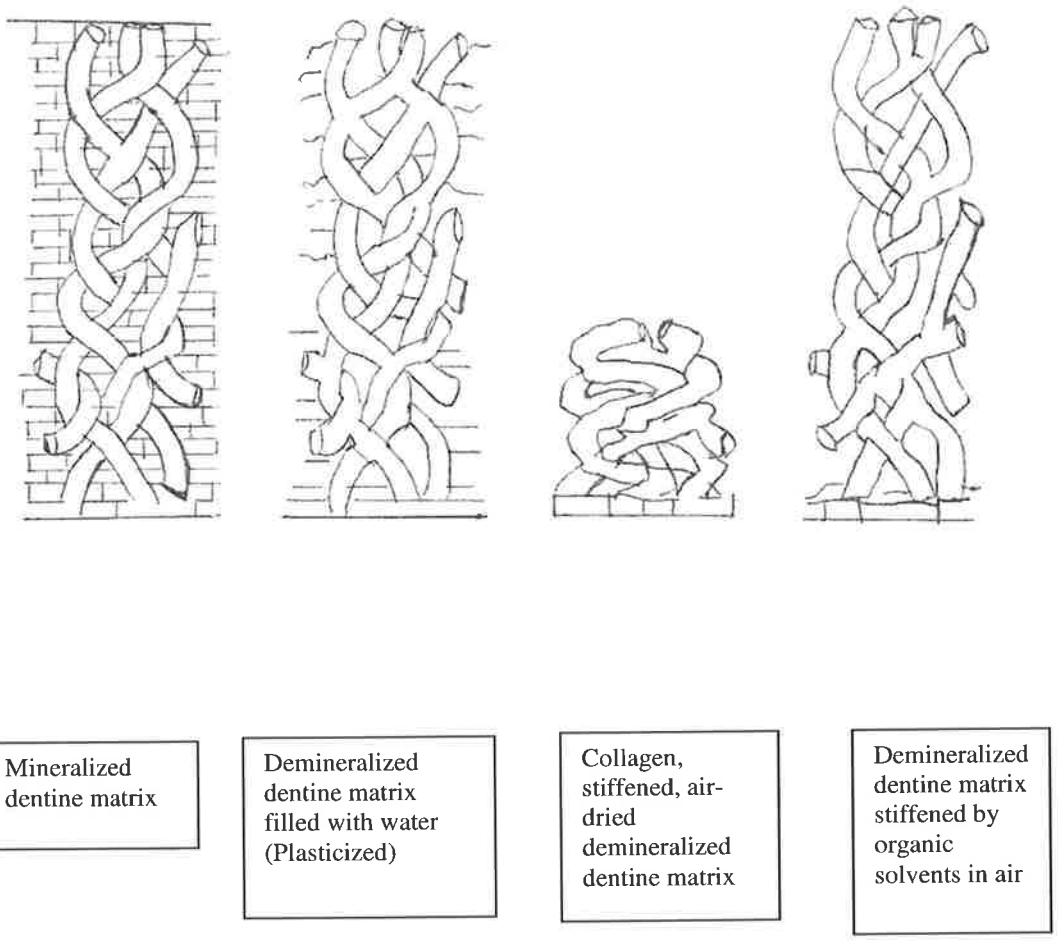


Figure 4-12: Dentinal matrix possible states [44].

Two plausible explanations have been suggested for possible collagen collapse.

- The de-mineralized network is floating or suspended in water. Each fibril is separated from the other by a water-filled space, which occupies the space that

was previously occupied by apatite crystallites. As the water-supported collagen network is air-dried, the amount of water separating the fibrils disappears as the water evaporates, and the collagen fibrils come closer together in all three dimensions. This would result in the loss of space between the fibrils; the space needed for monomer infiltration[44].

- Carvalho et al [45] suggested an alternative explanation. As water is evaporated from the collagen network the collagen fibrils shorten slightly. However because they are interconnected, this shortening of surface fibrils summates rapidly, causing the underlying soft, compliant network to be pulled down.

A collapsed dentinal matrix would result in a thinner hybrid layer. One may suspect that this may lead to poorer quality of dentine bonding.

The results of this study showed that in the three groups where debris had been effectively removed, there was significant collapse of the collagen matrix.

IMPLICATIONS

SEM is a widely used tool in dental materials science. There are diverse protocols for preservation of dental tissues [48]. The main aim is to preserve tissue structure in near life-like condition as possible. Whilst previous studies [34] have compared different fixation

and post fixation protocols, for preservation of dentinal tissue, there is little research that has quantitatively analyzed the results obtained. Almost all of the data obtained from microscopic investigations has been used as an adjunct to describe what is thought to be happening across the whole sample. No mention has been made as to whether the photomicrographs shown were the norm or the exception. The results of this study show that within a given sample large variations can occur. Whether these are related to microscopic techniques or an accurate reflection of what may be happening in-vivo is not entirely clear. More controlled studies are required which determine whether the photomicrographs used are in fact an accurate representation of the entire sample or are an exception in an otherwise ambiguous sample, presented to support the author's opinion.

This study also suggests that collagen collapse is widespread, and occurred in almost every conditioned specimen. This raises two possible scenarios. Either the results obtained may in fact be due to inappropriate microscopic techniques or that the micro-morphology of conditioned dentine surface is very variable and rarely involves the expanded collagen matrix, which is the required substrate for micro-mechanical resin-dentine bonding.

Microscopic techniques

This study is a microscopic investigation, and as a result it is very technique sensitive. It is feasible that the results are artifacts and have resulted due to technique variations that have occurred during sample preparation.

Sodium cacodylate buffer as suggested by Perdigao et al [47] with glutaraldehyde has been replaced in this study by using a phosphate buffer with 4 % paraformaldehyde, 2.5% glutaraldehyde. The main reason for this change is because sodium cacodylate preparation contains arsenic. This is a health hazard if inhaled or on contact with the skin, it can cause dermatitis, liver and kidney inflammation.
It is unlikely that the use of a phosphate rather than cacodylate buffer would have any adverse affects on the results, because Prompt L-Pop samples did show a preserved matrix (discussed below).

SEM examination requires the sample to be dried prior to examination. Conventionally this has involved air drying the specimen. The liquid component is extracted from the specimen. Dentine is a biological substrate with 20% fluid (by volume). If the dentine specimen is air dried then as the fluid evaporates the remaining tissue structures clumps together. While this may be advantageous if we were investigating cell junctions of the specimen, in the demineralized dentinal the matrix devoid of its mineral content would simply collapse. As a result alternative techniques have been developed aimed at

preserving the integrity of the biological tissues [46].

Critical Point drying (CPD) had been the technique of choice for tissue fixation by most researchers, to prepare biological samples with a fluid component.

The water content of the specimen is successively replaced by ethanol, amyl acetate and liquid carbon dioxide, and the later is then heated to a little above its critical point in an enclosed space. Above the critical point the liquid becomes a gas which can be released from the specimen. The result is that artifacts caused by the crystals and phase boundaries in frozen -dried preparations are eliminated. Dentinal specimens, would display a collagen matrix not distorted by collapse onto underlying dentine.

In a study such as this one which was designed to evaluate the effects of conditioners on dentinal surface, preservation of surface detail was a minimal requirement.
CPD however does have some disadvantages. The process requires special equipment and it is time consuming. It takes about 1.5 hours and requires constant monitoring during this time.

A previous study by Perdigao et al [34] had compared four post fixation techniques for human dentine. These included Critical Point Drying (CPD), Hexamethyldisilizane (HMDS) drying, Peldri II drying, and air drying. The dentinal specimens were observed in cross-sectional and longitudinal planes, using a field emission scanning electron

microscope. They found that the HMDS drying preserved the collagen network and the micro-porosity of the demineralized dentine surface, better than the other techniques. As a result HMDS drying [47] was adapted as the method of fixation for this study. It had shown to be successful in preserving dentinal tissue, and it involved 10 minutes of immersion time compared with 1.5 hours as was needed with CPD.

The collapse may have been caused due to errors in my post fixative technique. Perdigao et al [47] suggest after final ethanol step, the specimens are to be immersed in HMDS for 10 minutes. In this study I used the HMDS liquid sparingly. Instead of immersing the sample in HMDS I covered the sample with HMDS using a pipette. This may have contributed partially to the collapse seen post fixation in the samples.

However, this is unlikely to be the major cause of the collapse because there were samples in the Prompt L-Pop series which showed optimal expansion of the collagen matrix. These were produced with similar techniques and interestingly within each sample there were variations with no collapse to areas of maximal collapse, Figure 4-13.

The collagen matrix is very susceptible to collapse. Once mineral content has been removed the matrix has a tendency to fall onto itself. This quality was highlighted when two samples were conditioned with 3M then one sample was ultasonicated for 30 minutes prior to being fixed, and the other was not. The ultrasonicated sample showed optimal

expansion of the dentinal matrix, Figure 4-14, whilst the other showed matrix collapse, Figure 4-15.

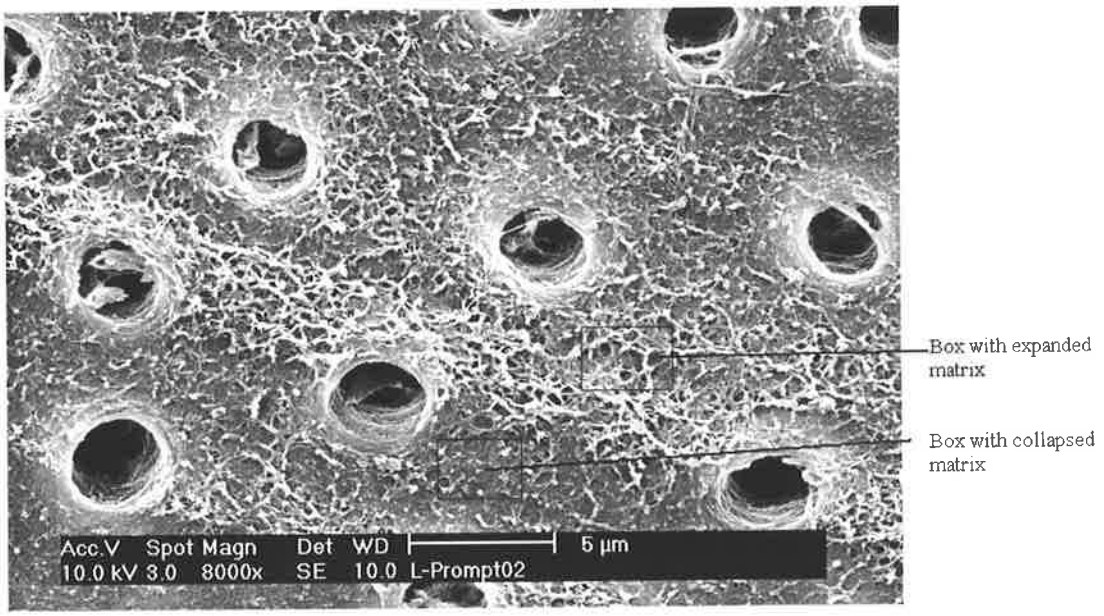


Figure 4-13: Variations in collagen matrix within a same sample.

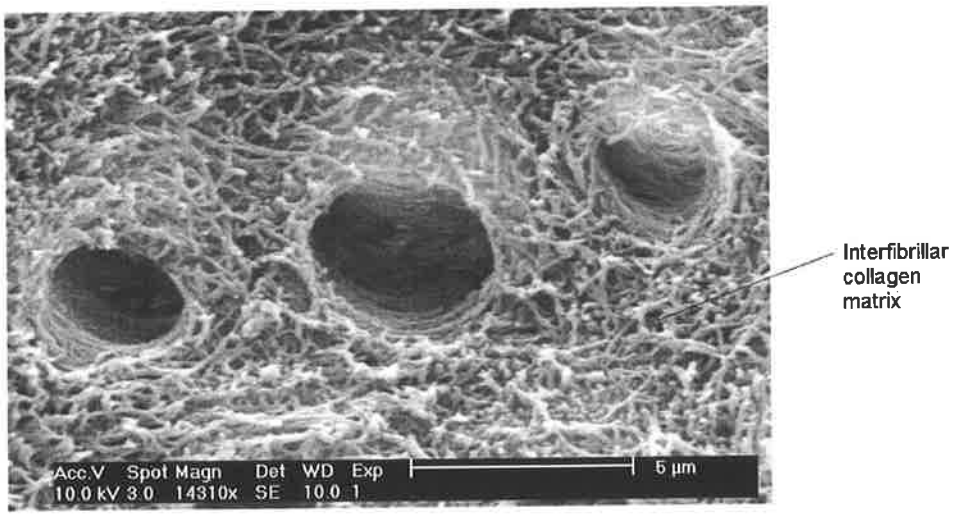


Figure 4-14: Dentine matrix conditioned with phosphoric acid; rinsed; and ultrasonicated for 30 minutes prior to fixing for SEM viewing.

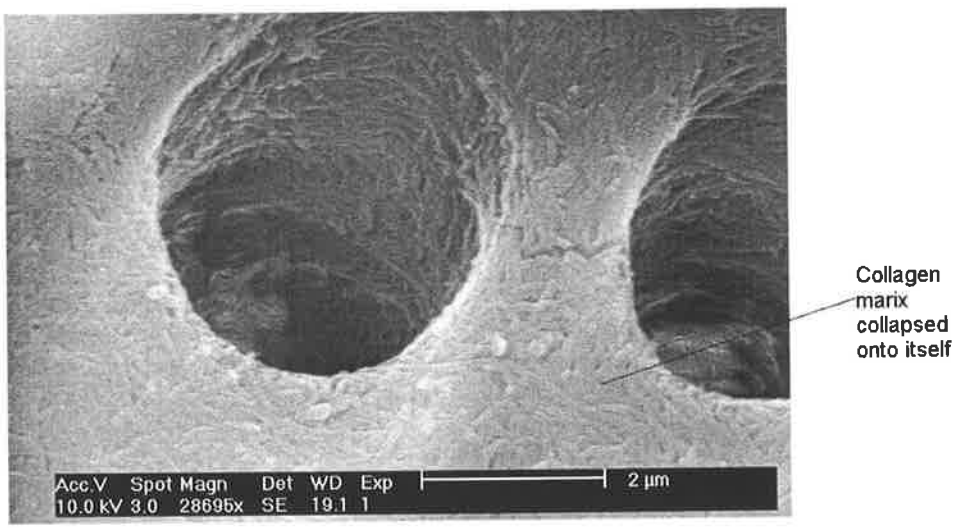


Figure 4-15: Dentine matrix conditioned with phosphoric acid, rinsed and fixed for SEM viewing.

In-vivo

The hybrid layer formation relies on penetration of monomer molecules, into the labyrinth network of collagen formed by dissolution of hydroxyapatite crystals between collagen fibres. The expanded collagen matrix, is widely accepted as the optimal substrate for monomer penetration.

The results of this study show this may not necessarily be true. Even with our best efforts, the collagen matrix was susceptible to collapse. It was only in an ultrasonicated sample where optimal expanded matrix was visible. This highlights the potential problems faced by clinicians. It would be difficult to imagine in a clinical situation that we were producing optimally expanded dentinal matrices each time we conditioned the tooth. One step and two step techniques may be advantageous because there is lesser chance of tooth desiccation after application, which may minimise matrix collapse.

More research is required to determine the relationship between the hybrid layer and quality of final dentine bond in an in-vivo environment.

CONCLUSIONS

Of the five conditioning agents tested none produced an ideal dentinal surface, with debris removed and an expanded collagen matrix. SDI etch and Prime and Bond ^{NT} had significant debris present, so were ineffective in conditioning. The other three whilst successful in debris removal did not produce an optimally expanded dentinal substrate for monomer penetration. One step and two step techniques may be advantageous because they simplify the steps required and also because they may have a better chance of preventing collagen collapse.

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APPENDICES

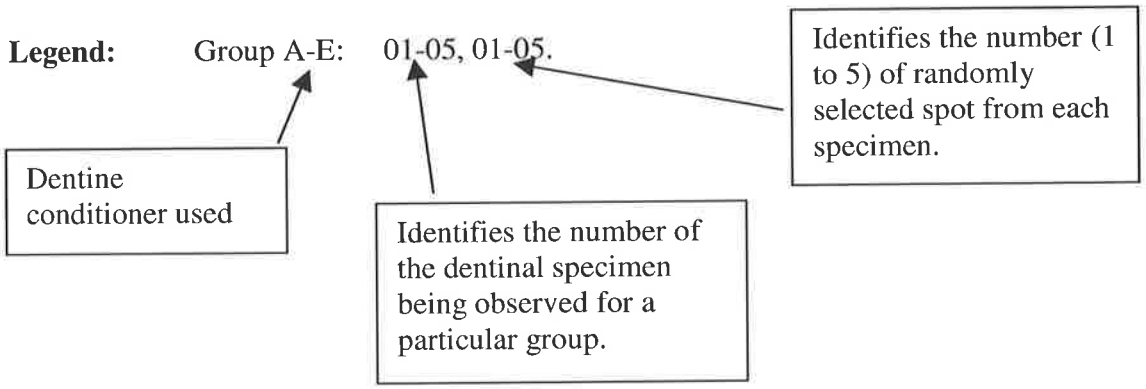
Group A: 3M Scotchbond; Phosphoric etchant

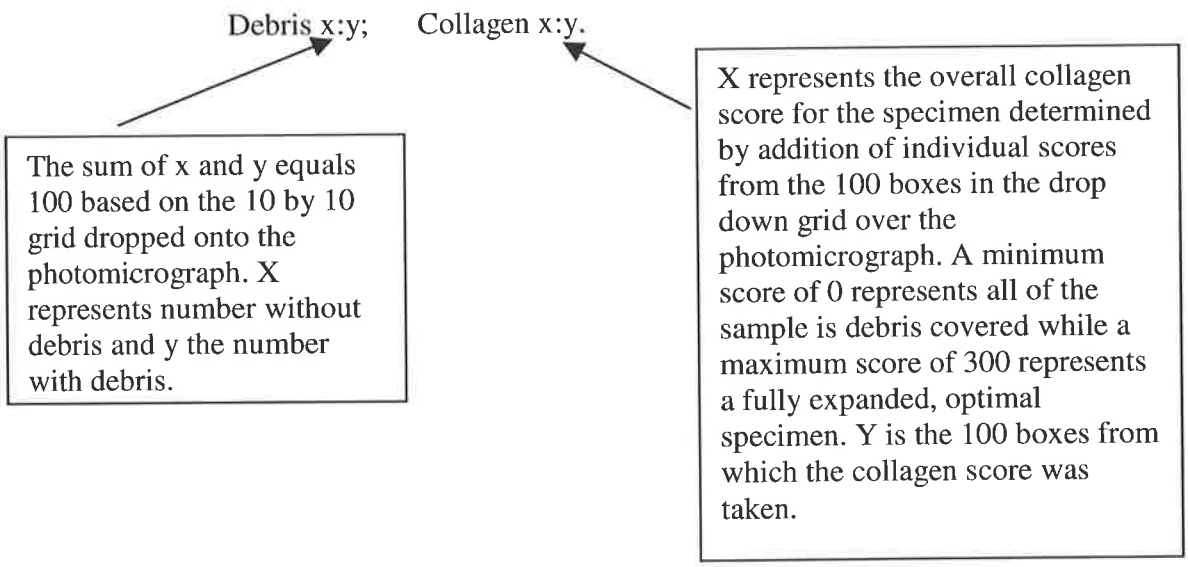
Group B: Southern Dental Industries; Acid etch gel

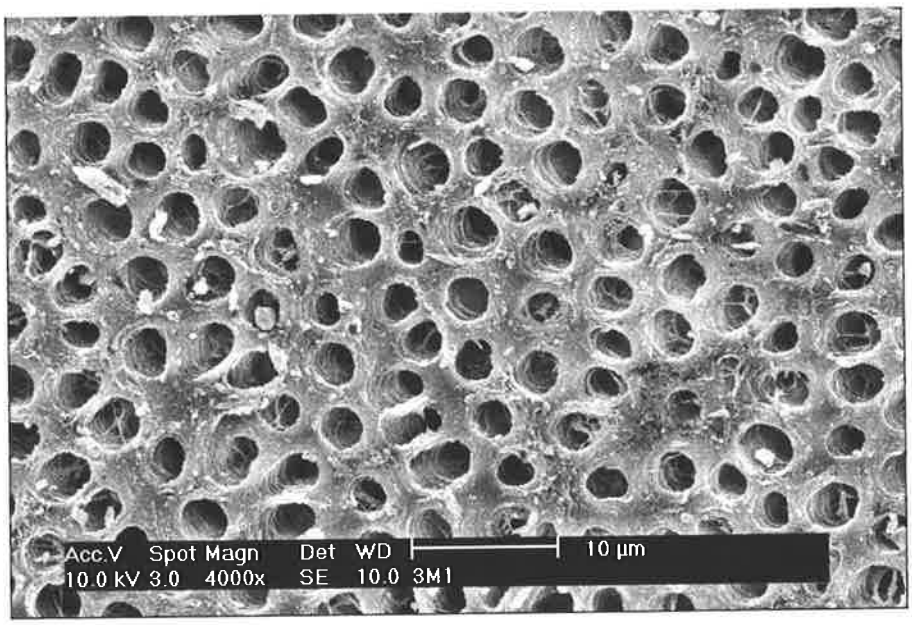
Group C: Kuraray Co Ltd; Clearfil SE Bond

Group D: Dentsply; Prime & Bond ^{NT}

Group E: ESPE; Prompt L-Pop







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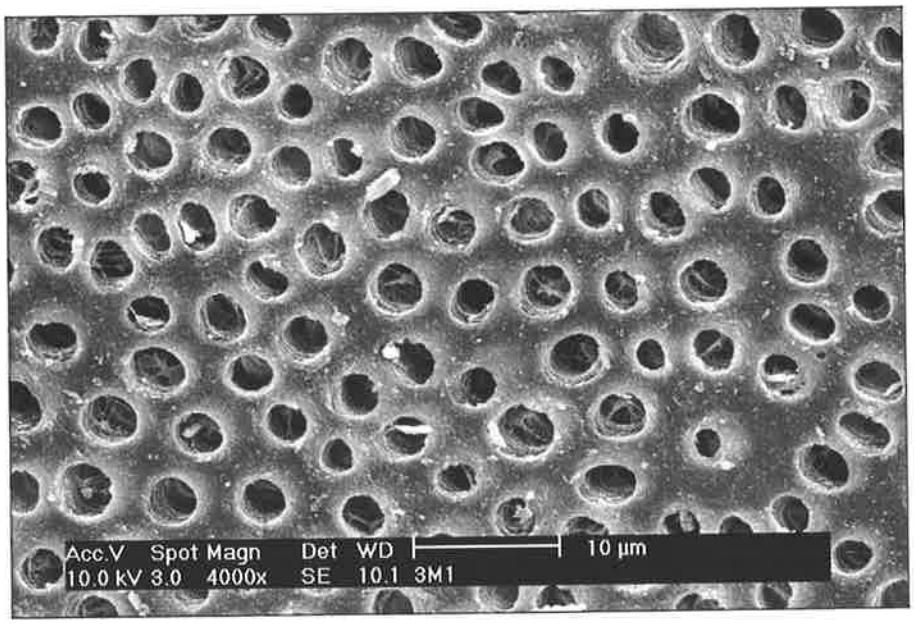
Debris 89:11 Collagen 100:100.



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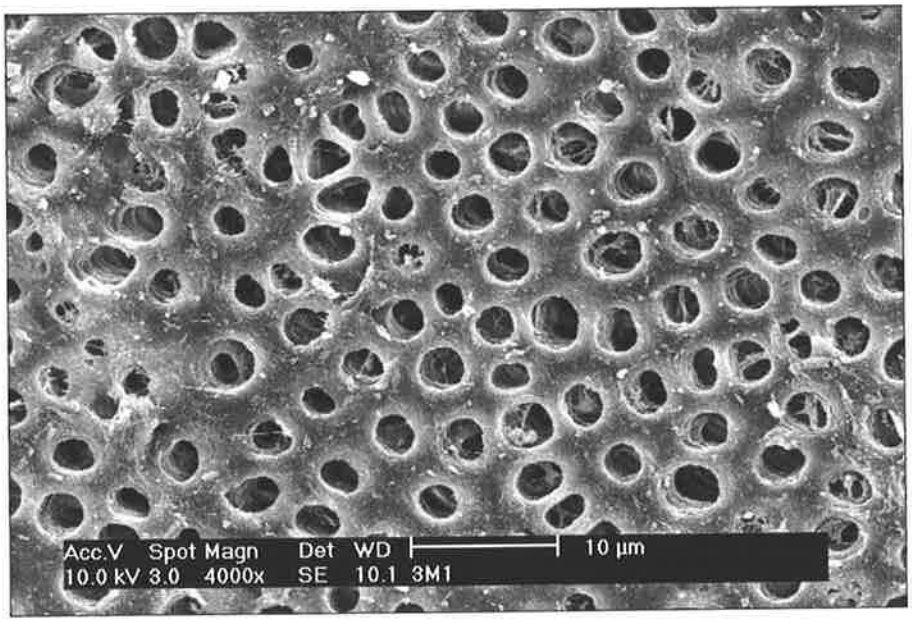

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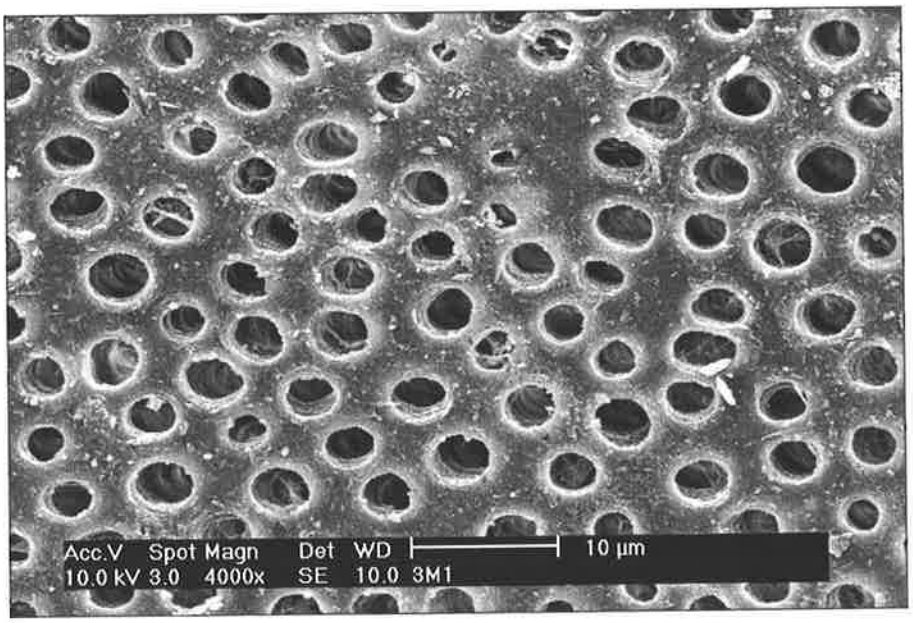

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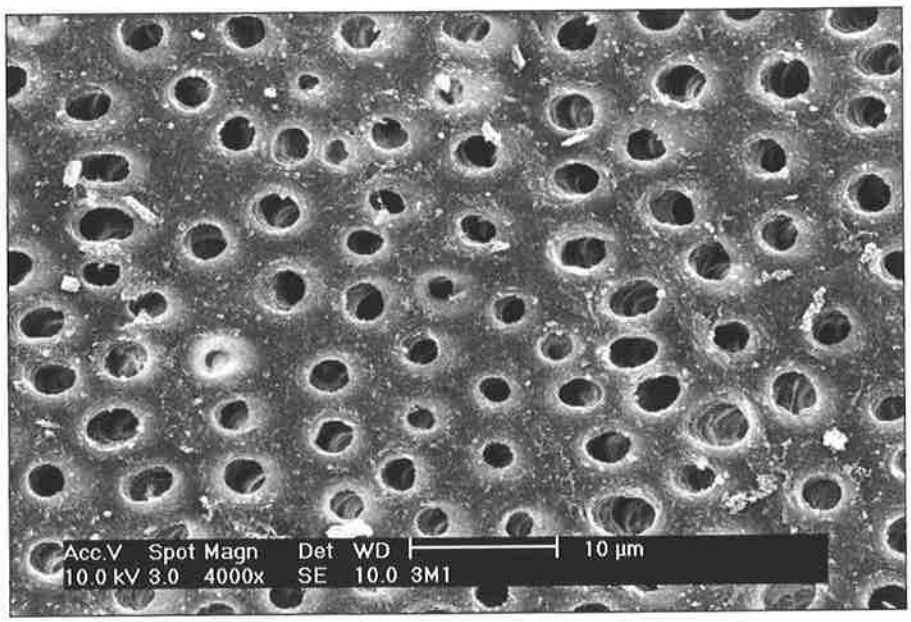
Debris 86:14; Collagen 100:100



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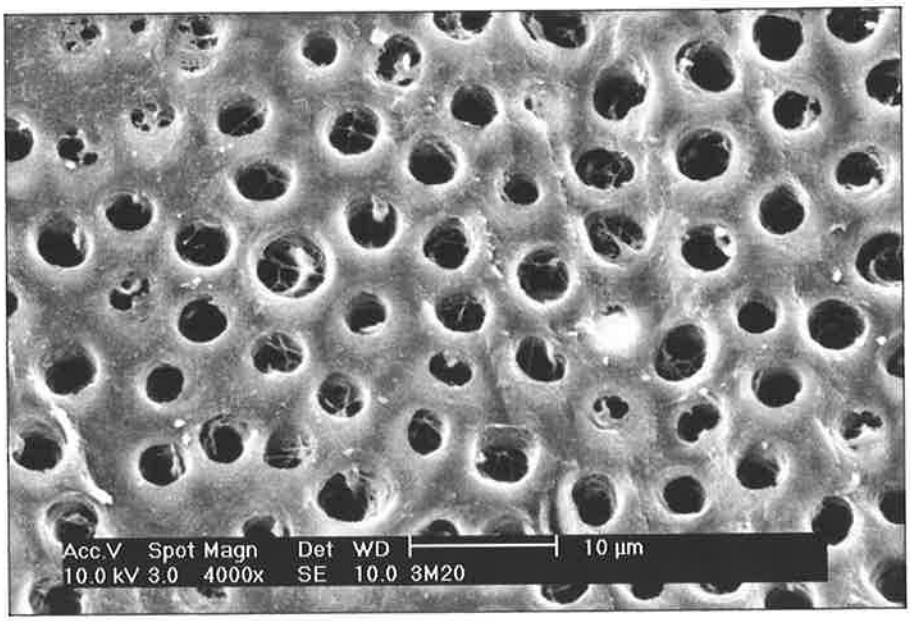

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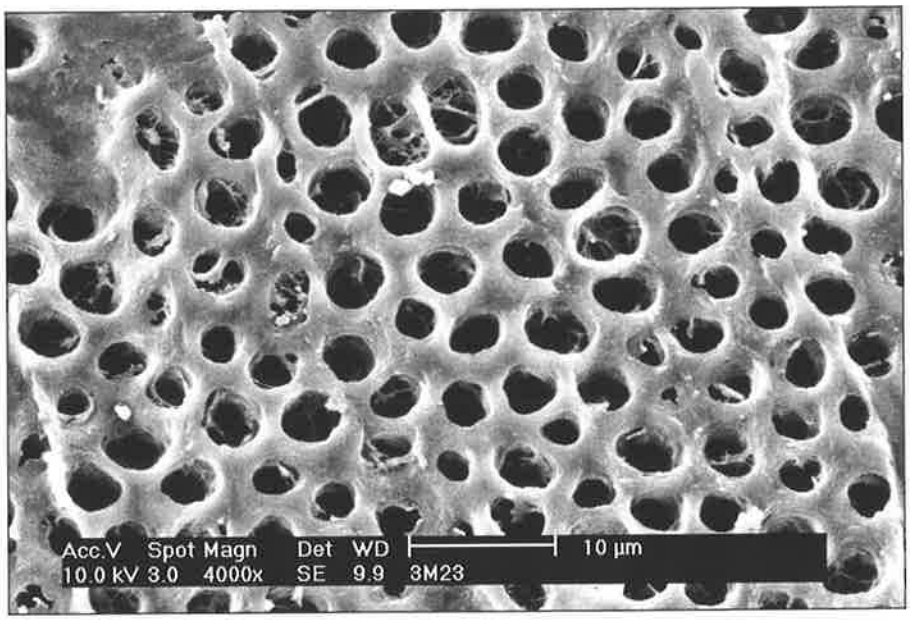

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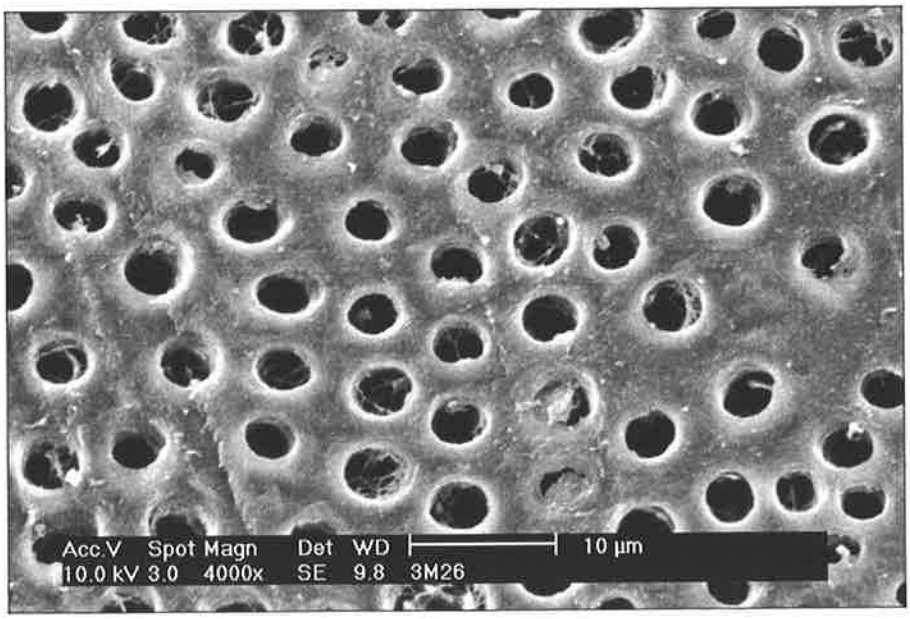

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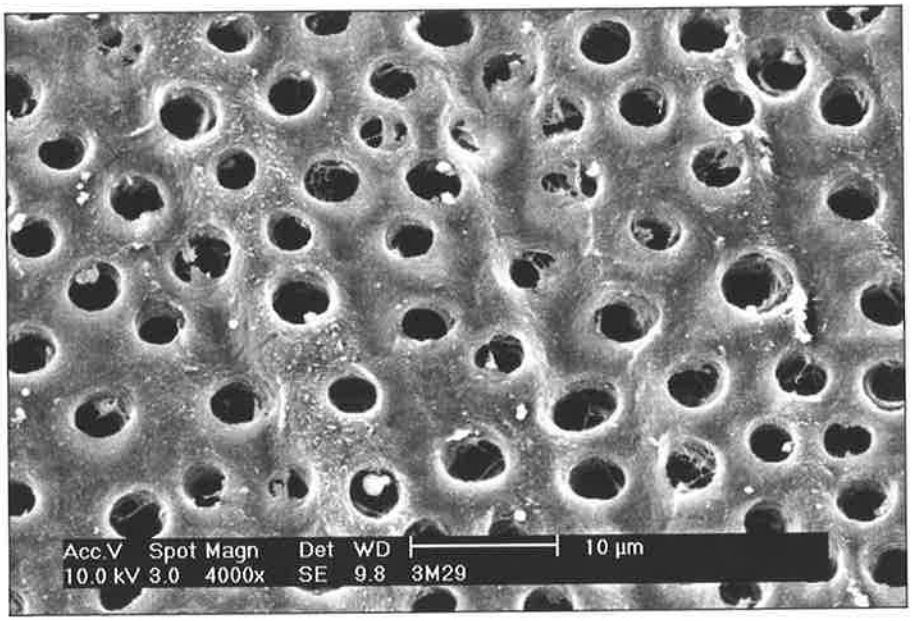

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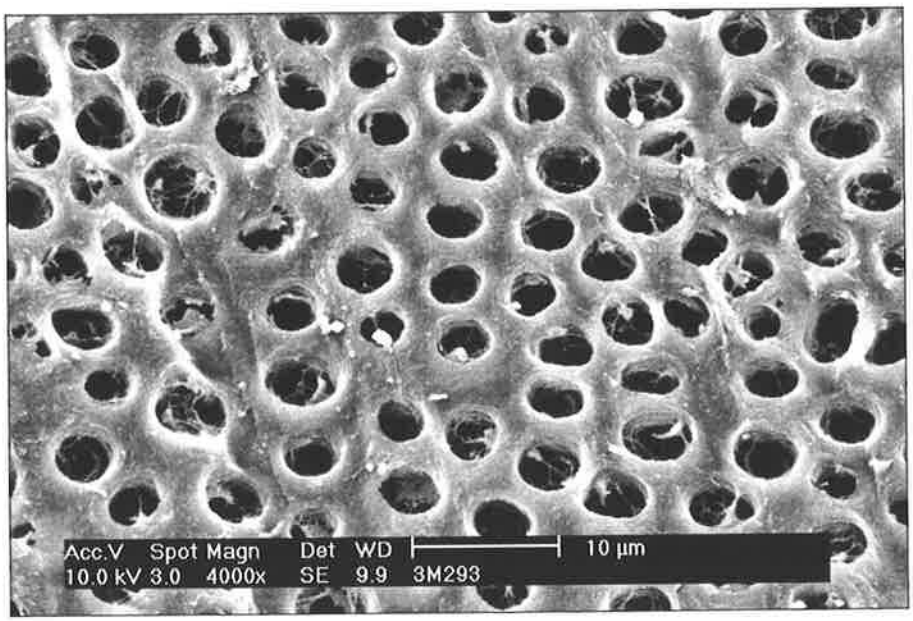

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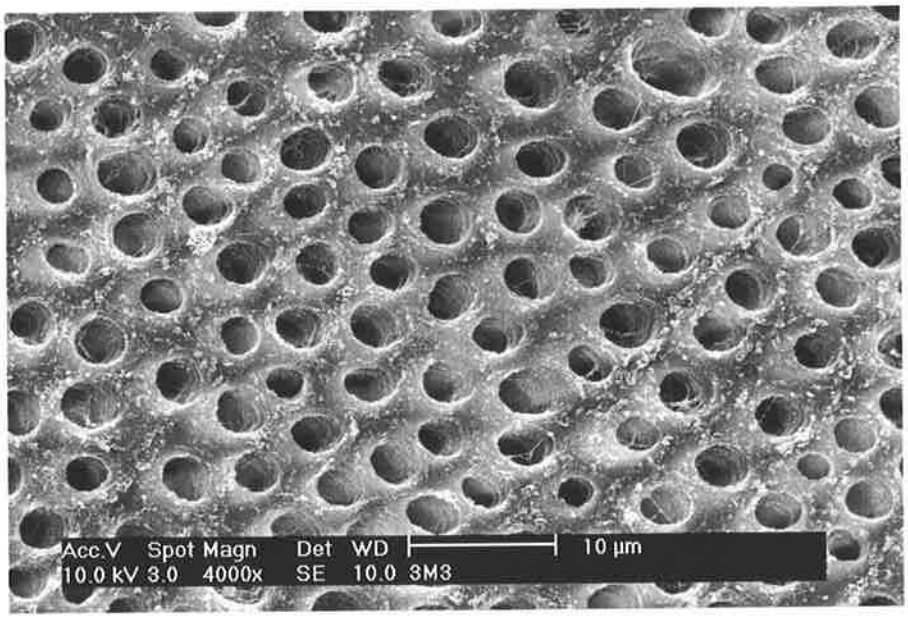

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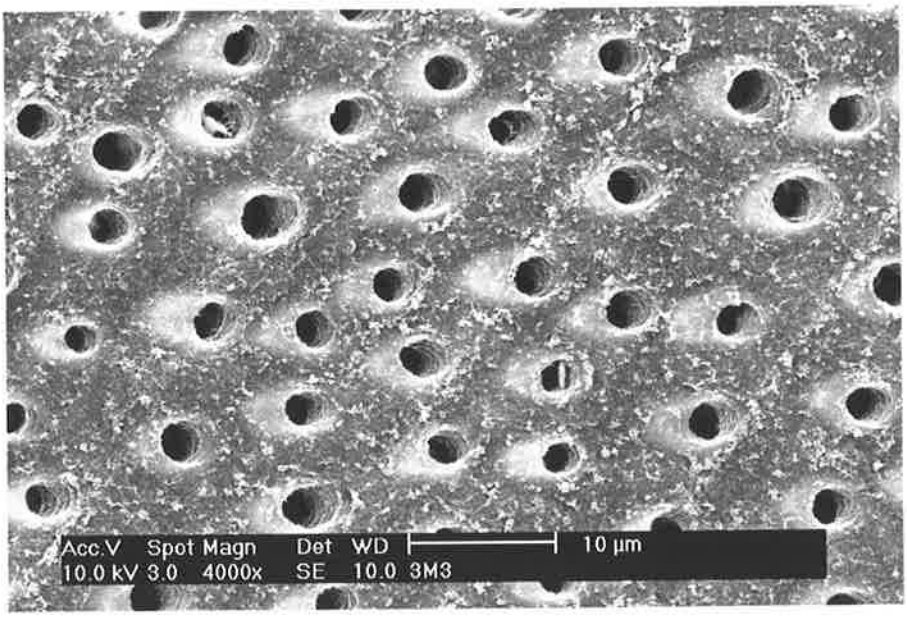

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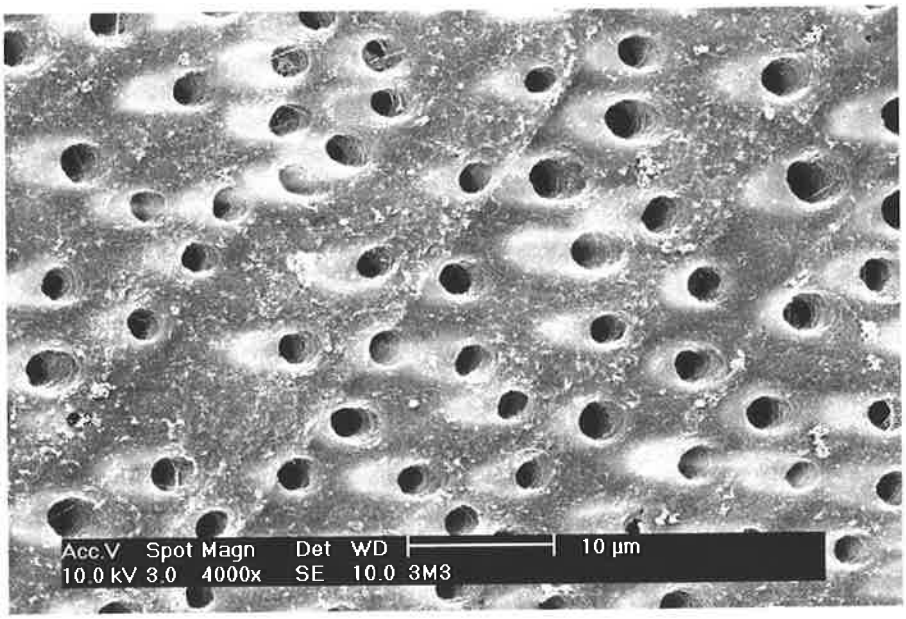
Debris 100:0; Collagen 100:100.



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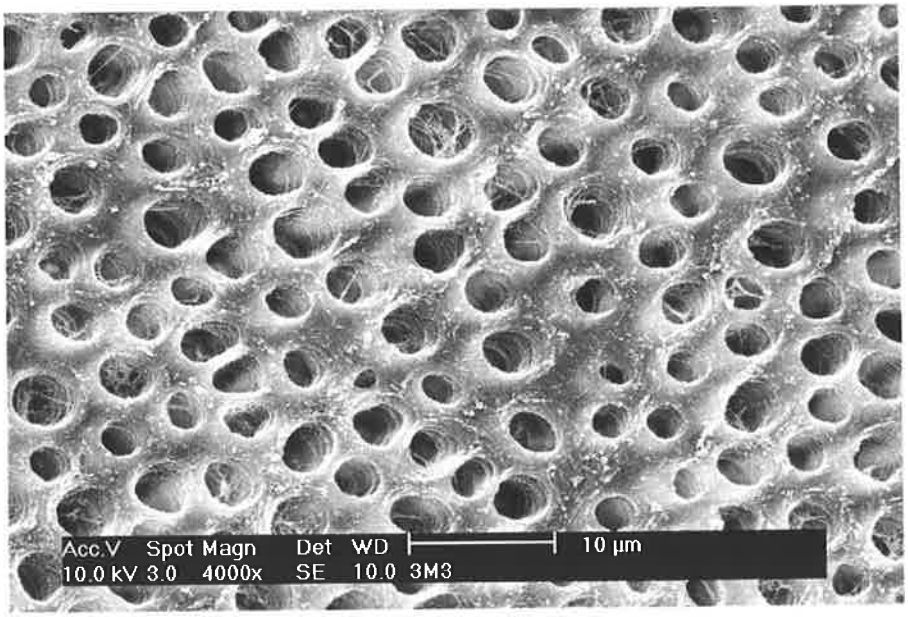

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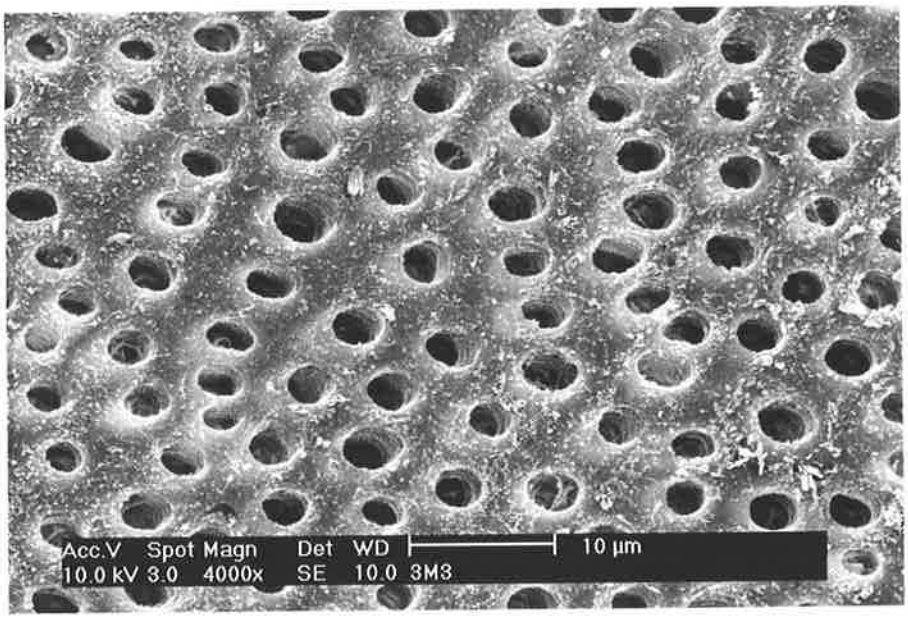

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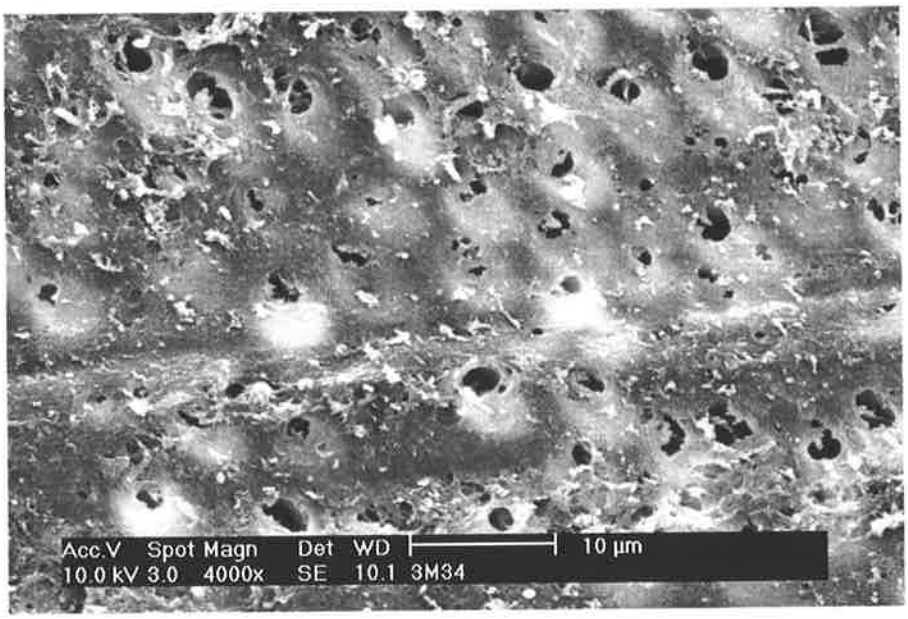

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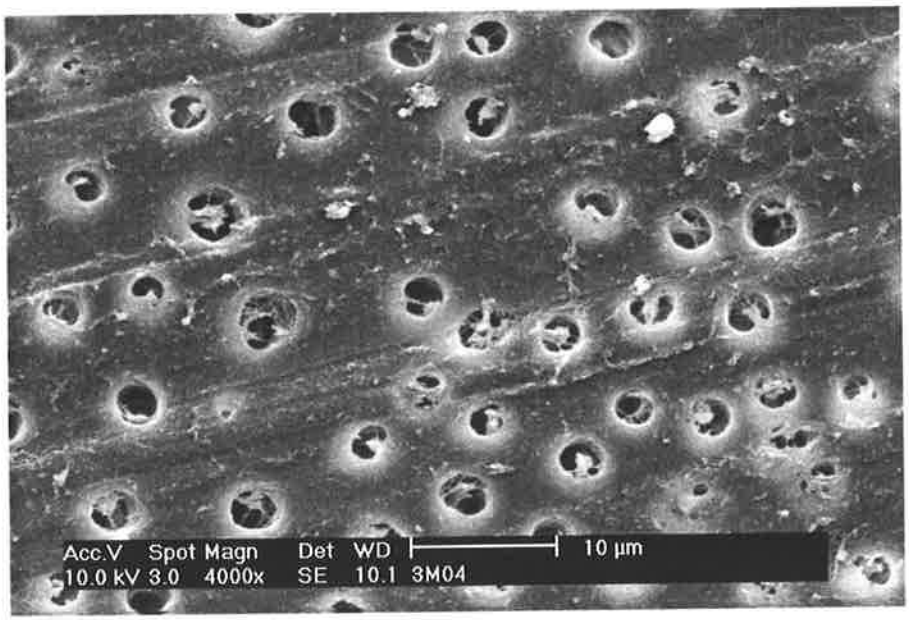

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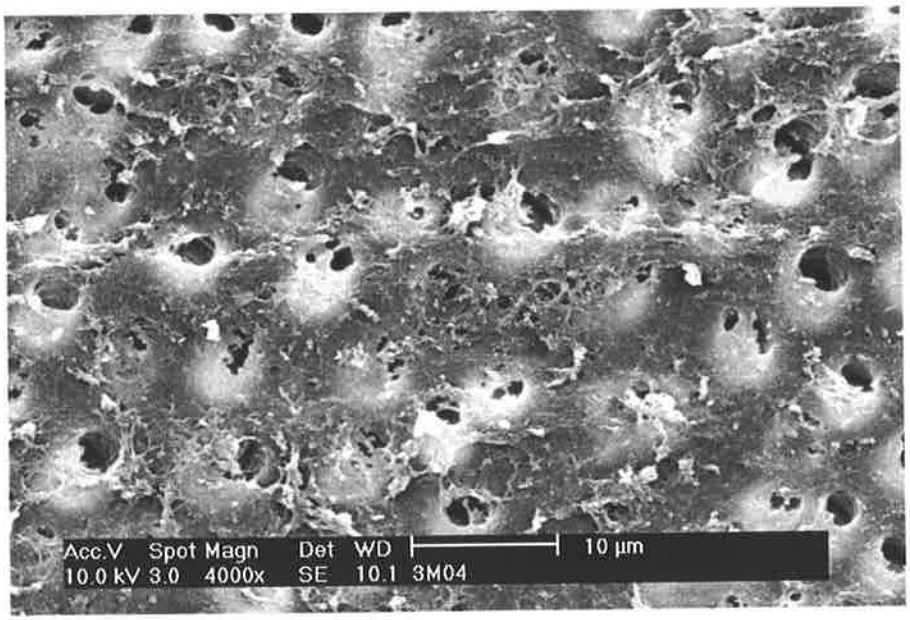

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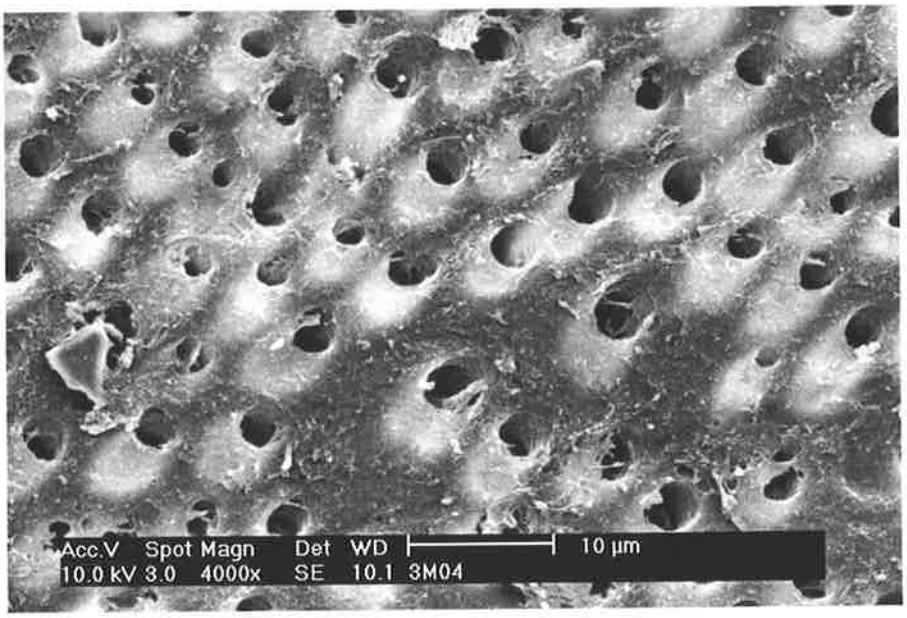

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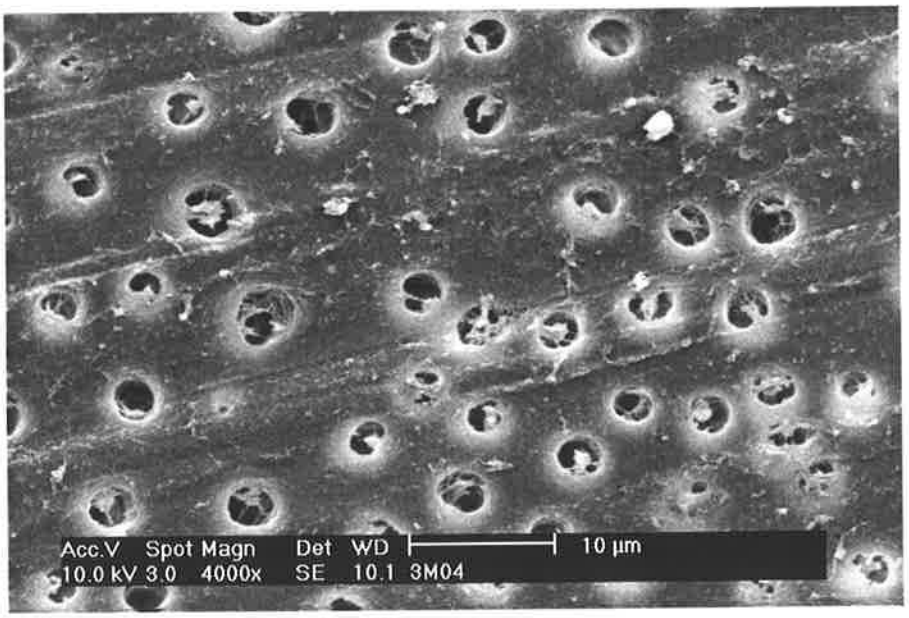

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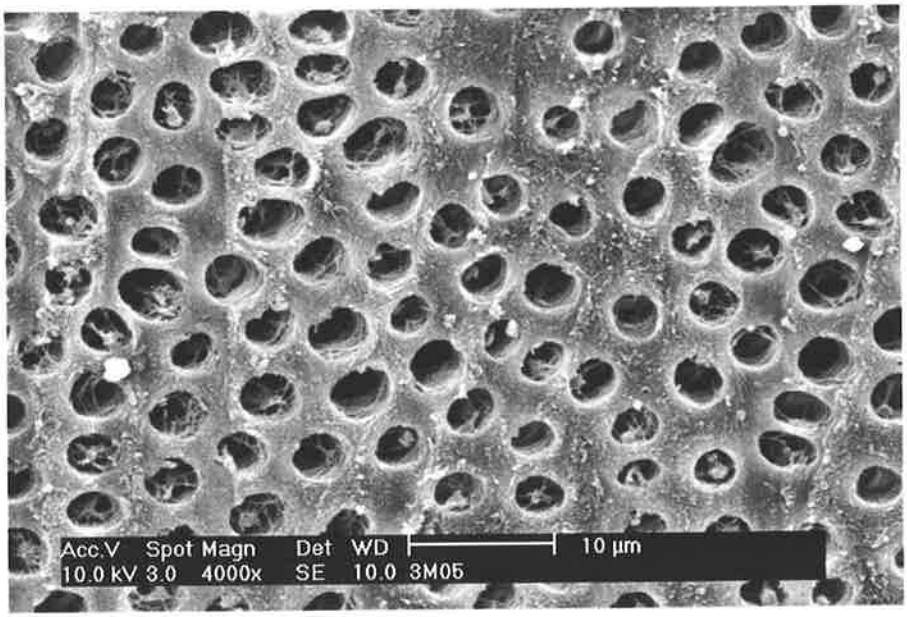

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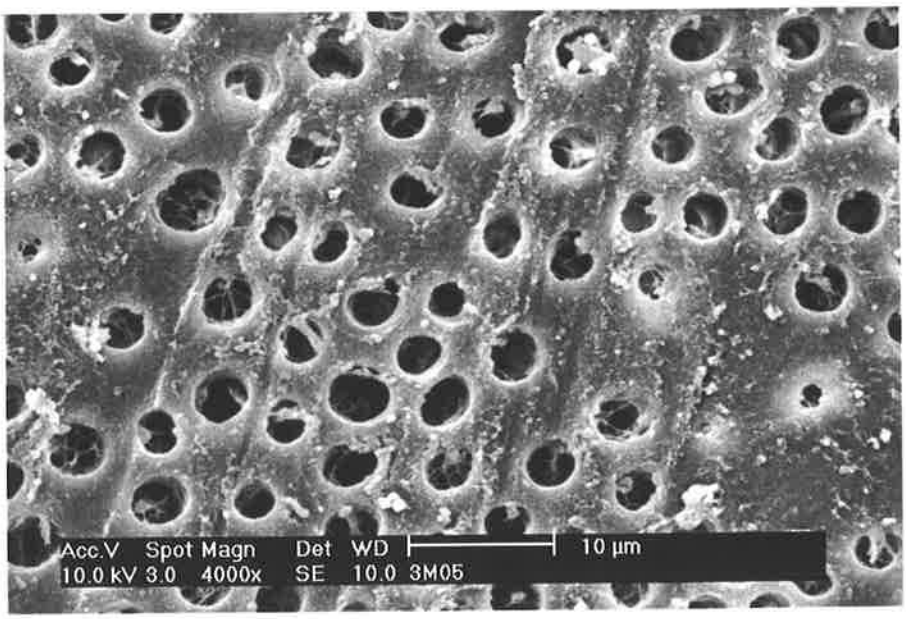

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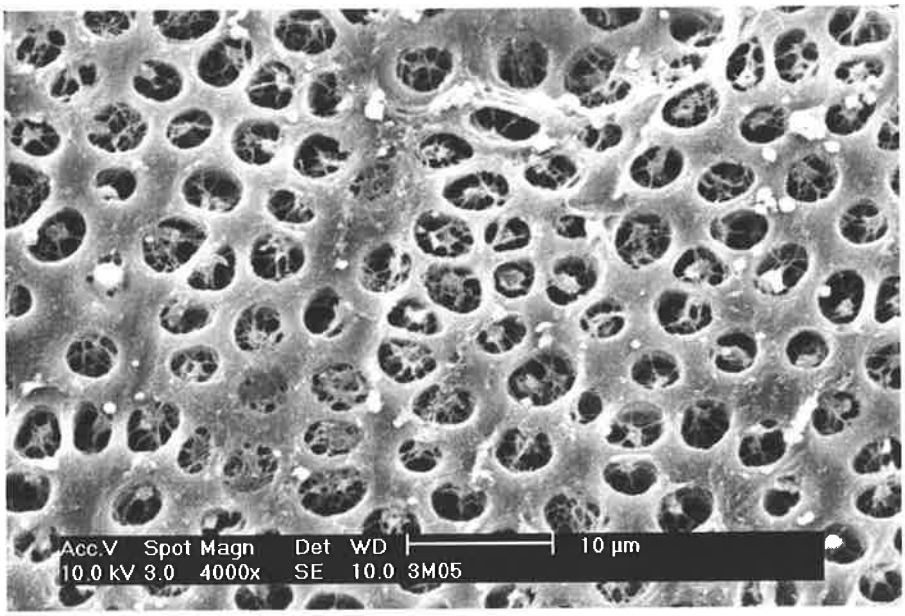

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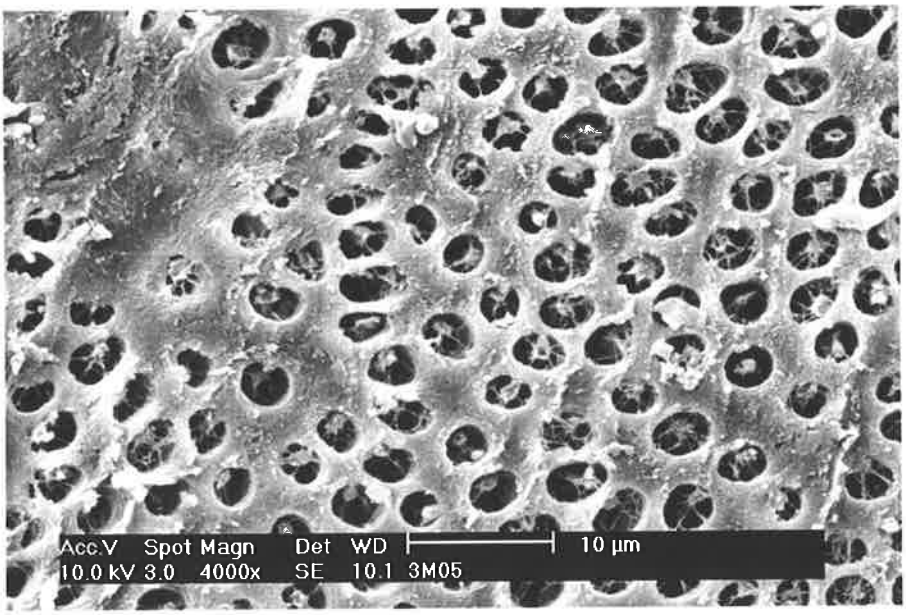

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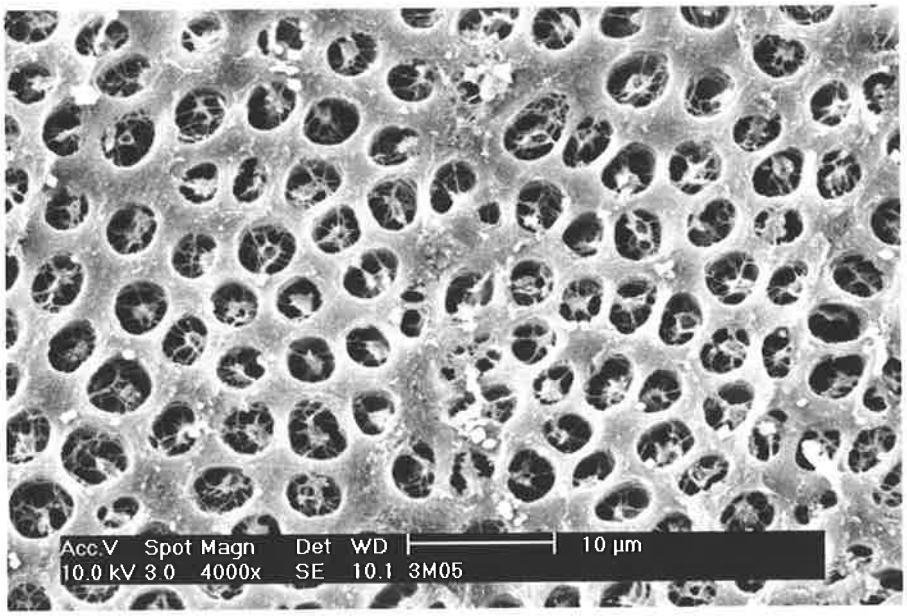

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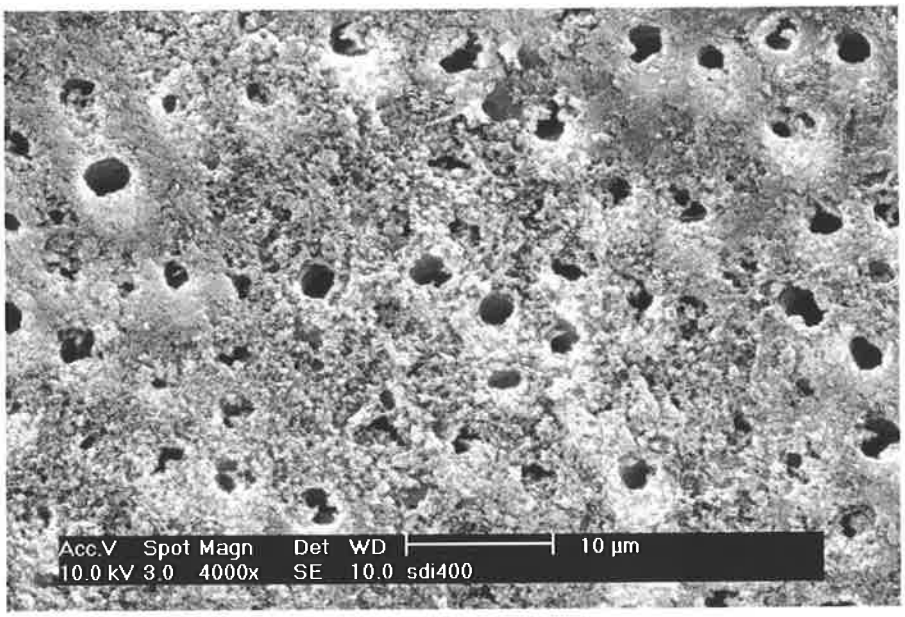

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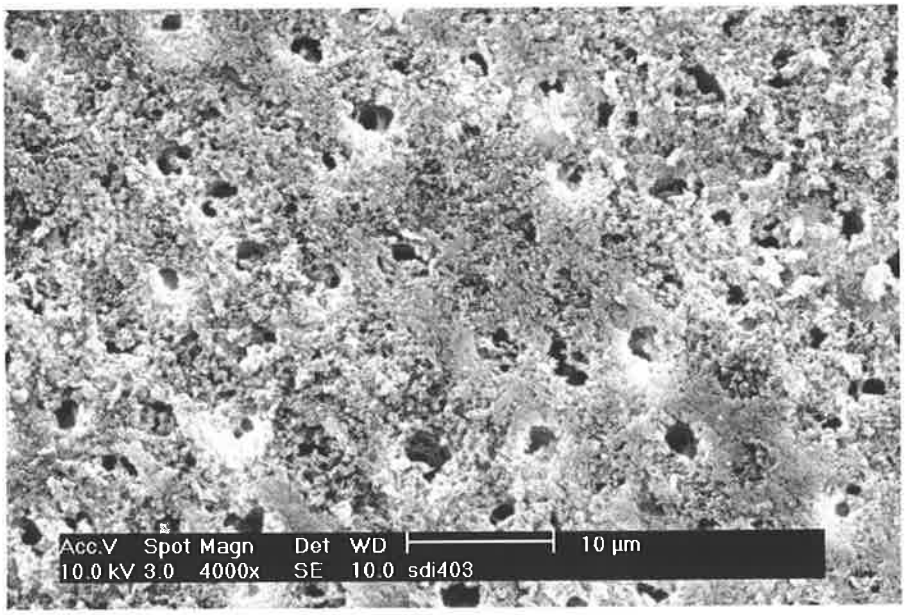

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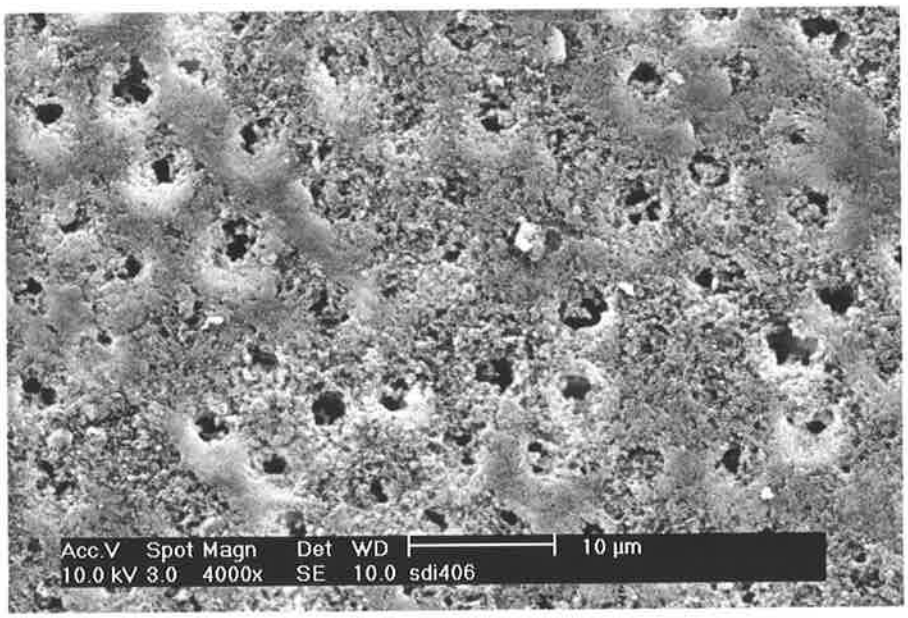

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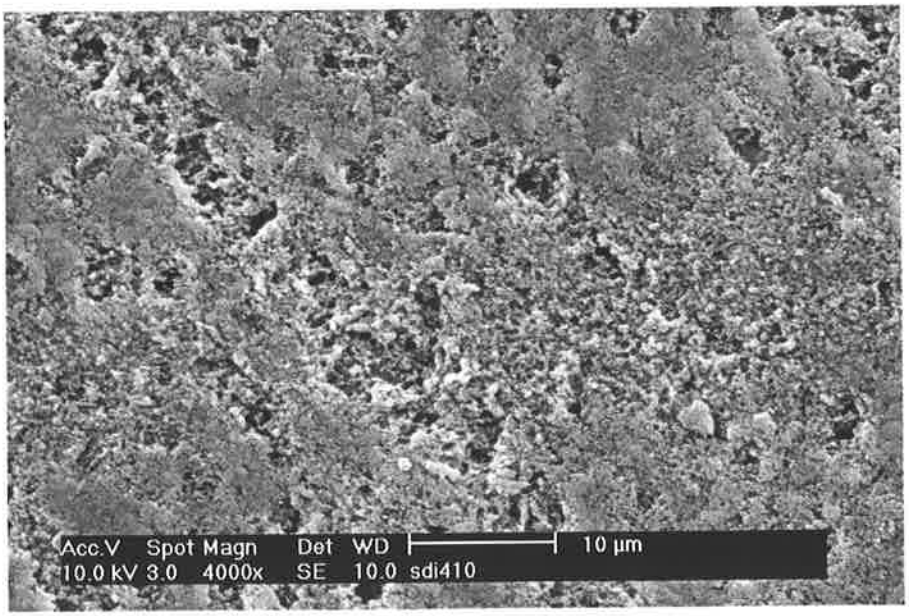

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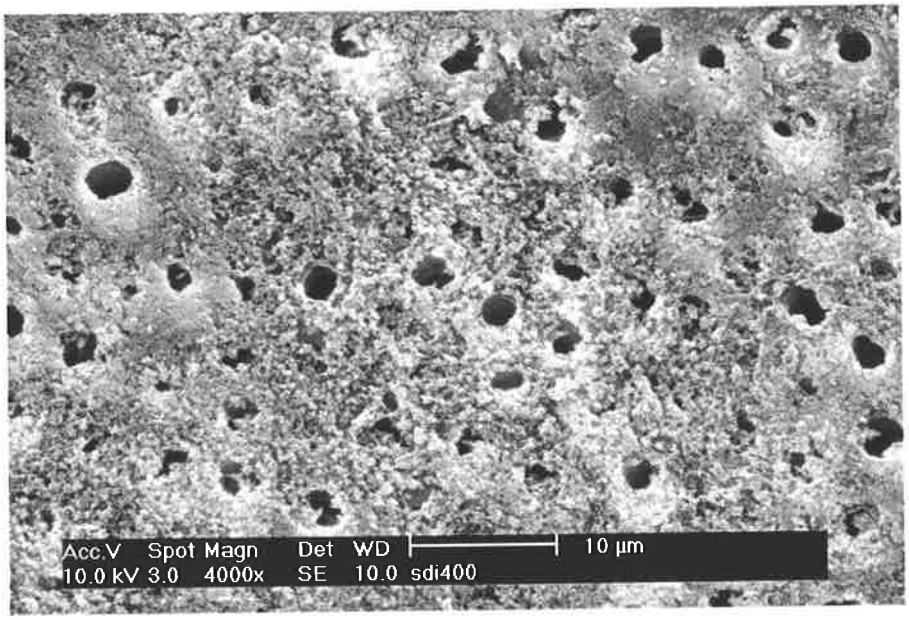

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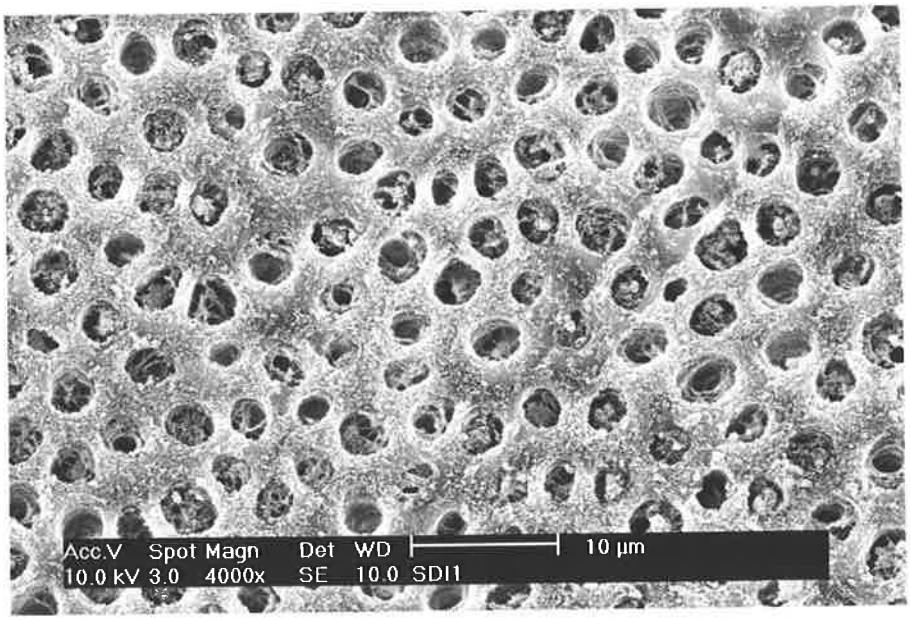

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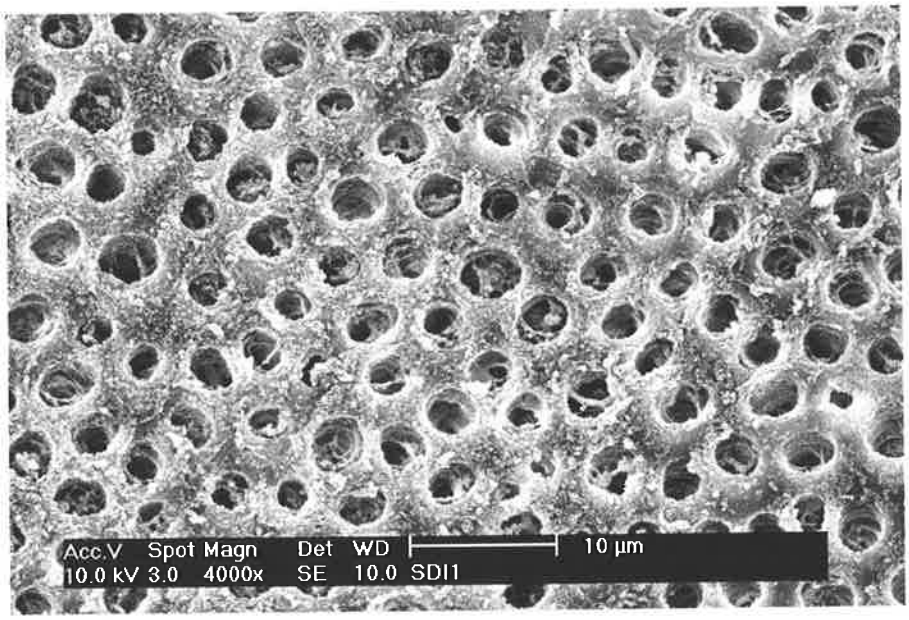

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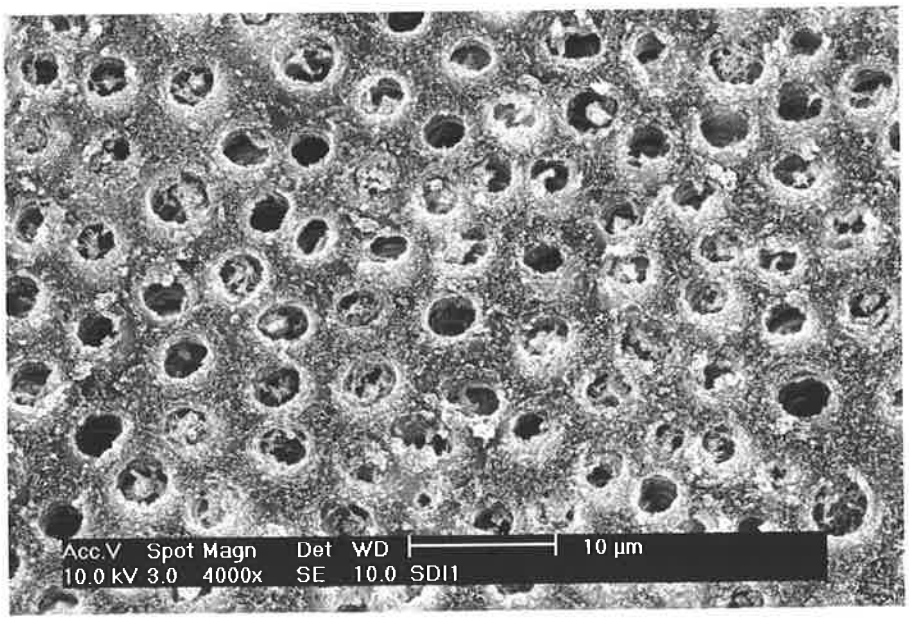

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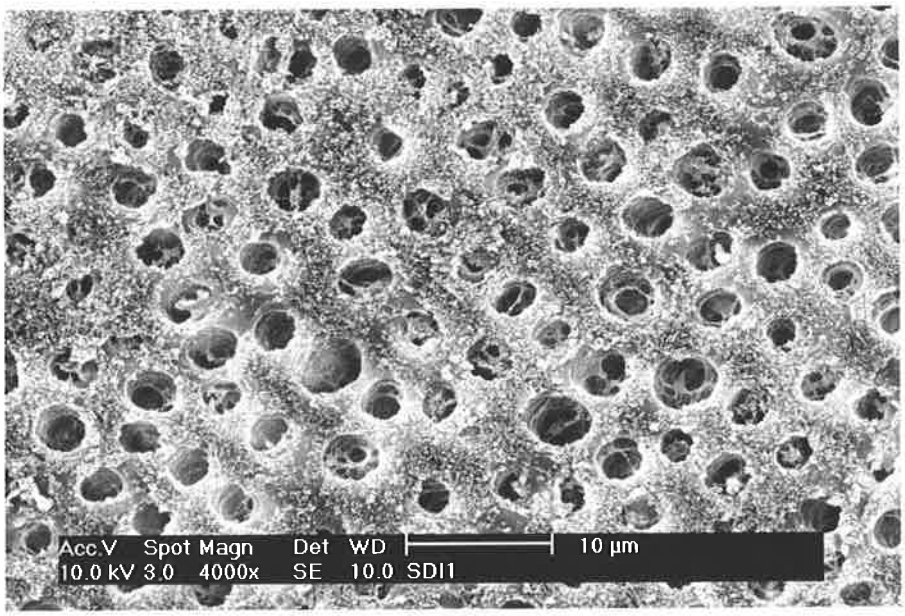

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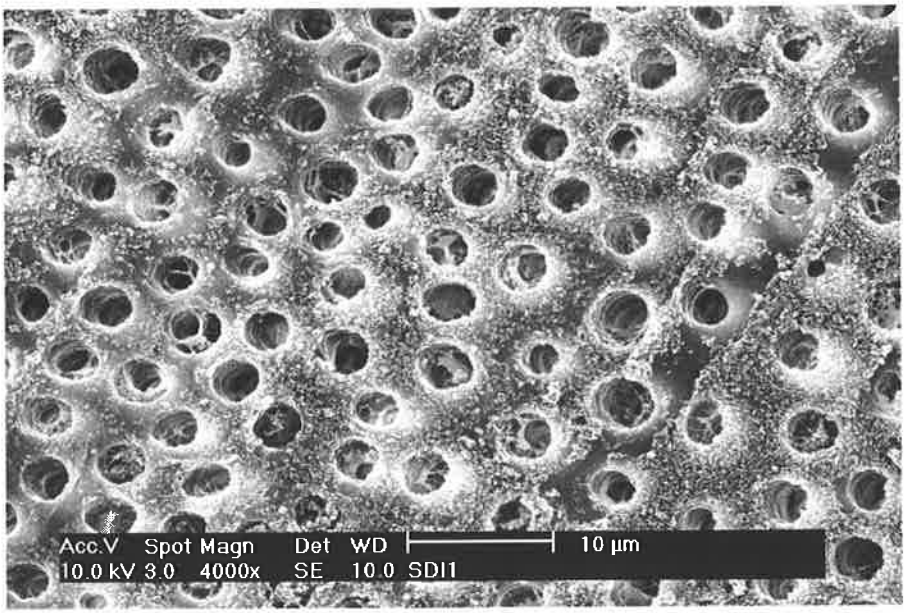

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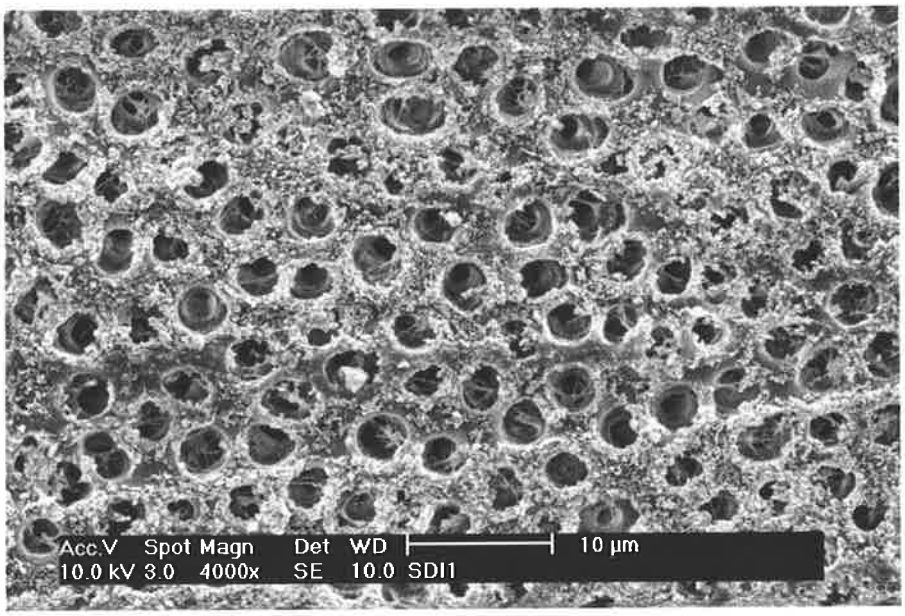

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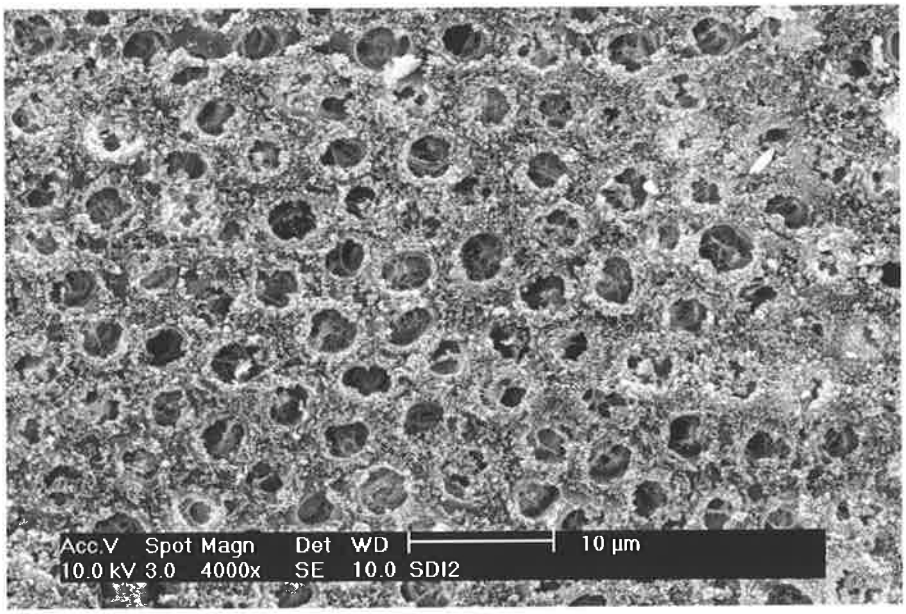

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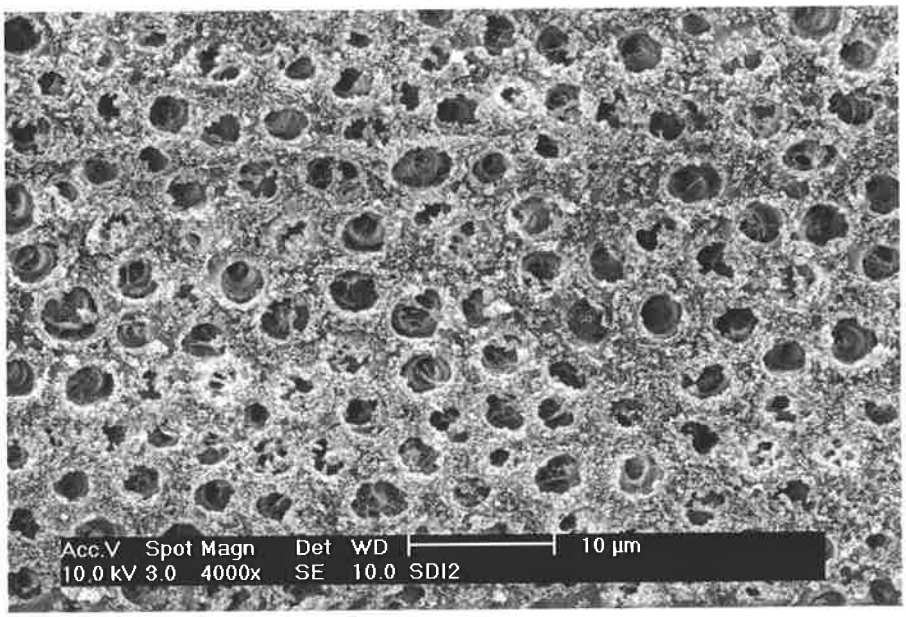

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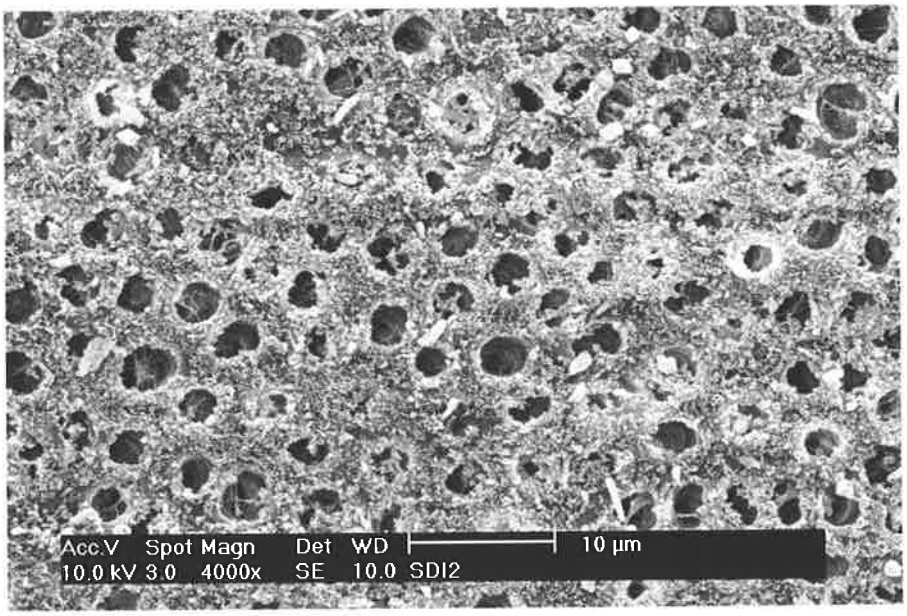

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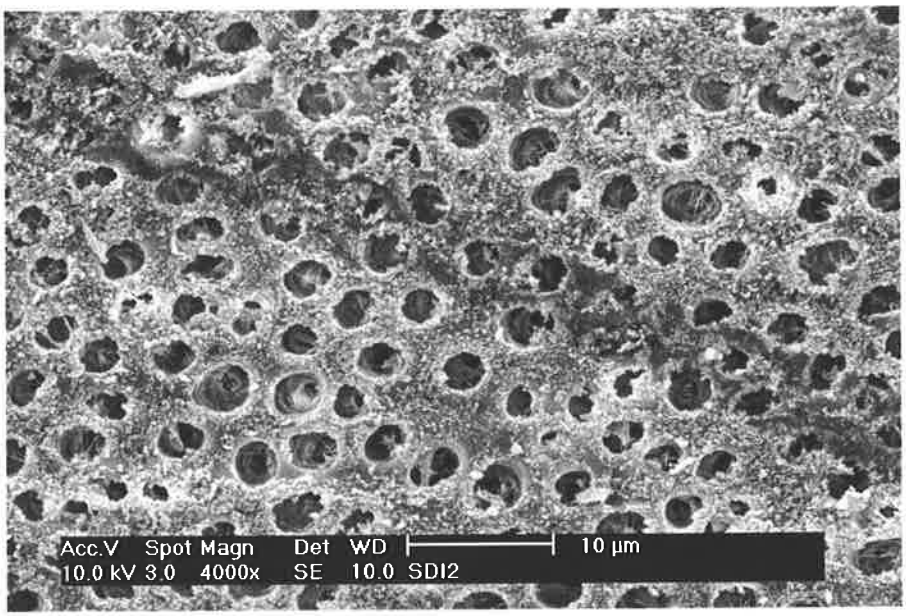

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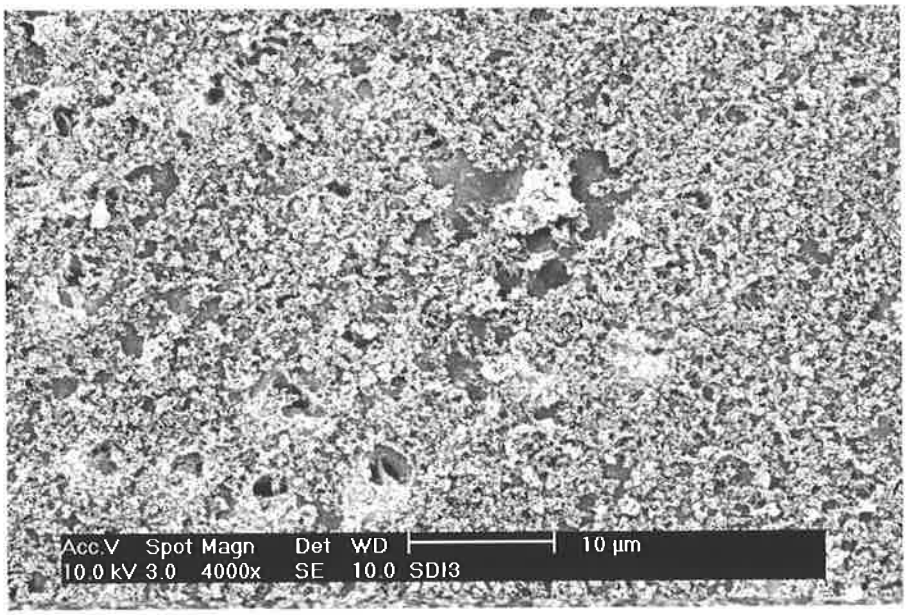

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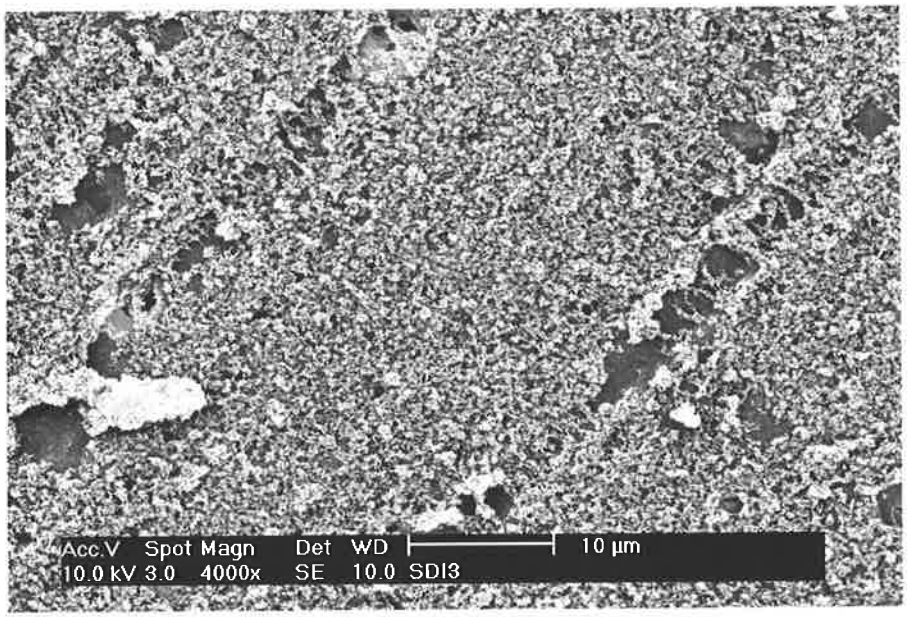
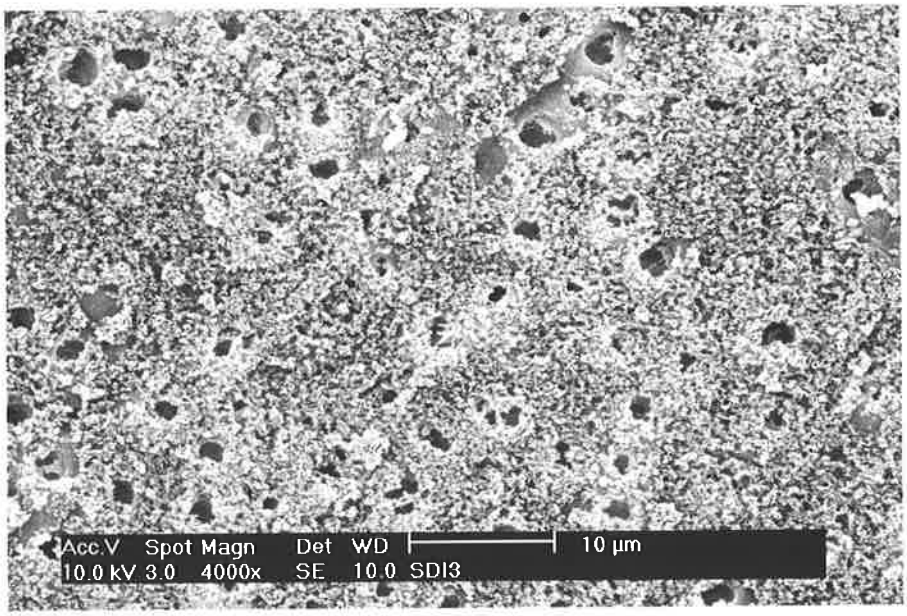


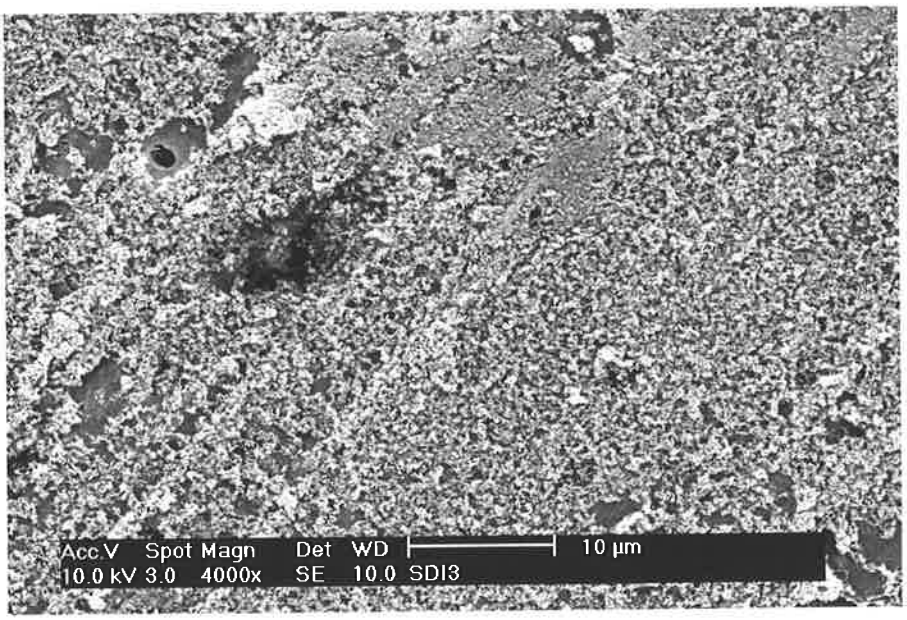
Group B: 04,02.

Debris 0:100; Collagen 0:100.



Group B: 04,03.

Debris 0:100; Collagen 0:100.



Group B: 04,04.

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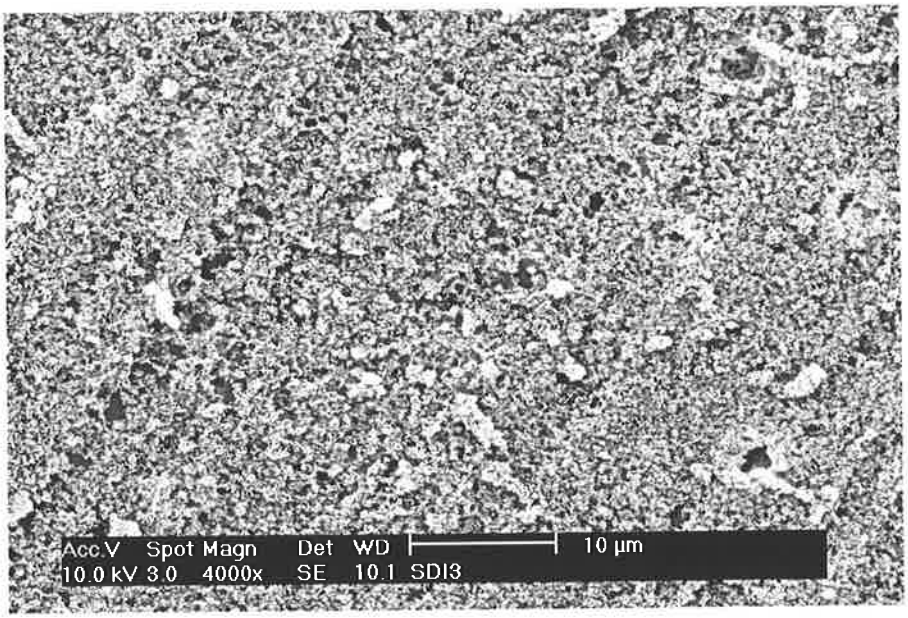
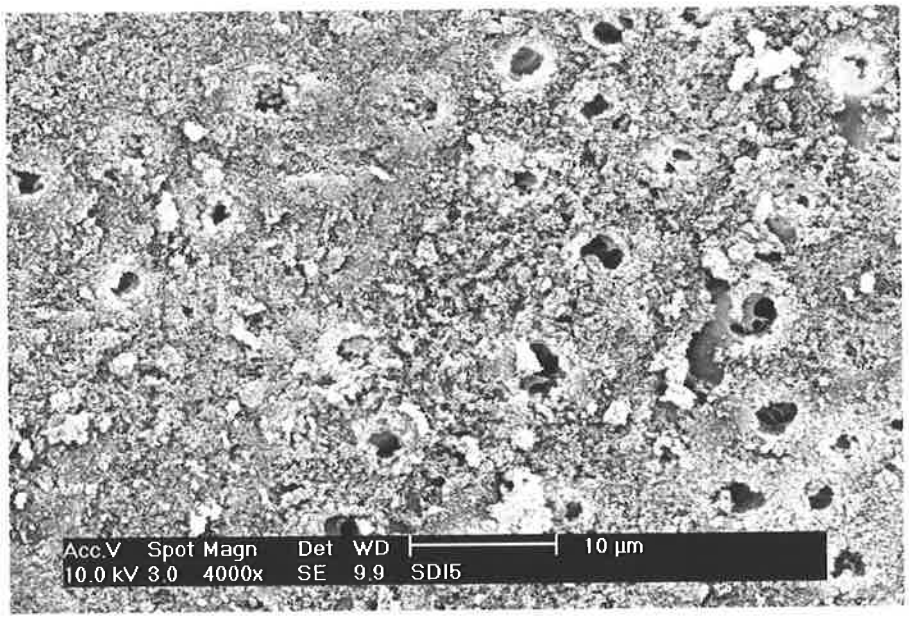


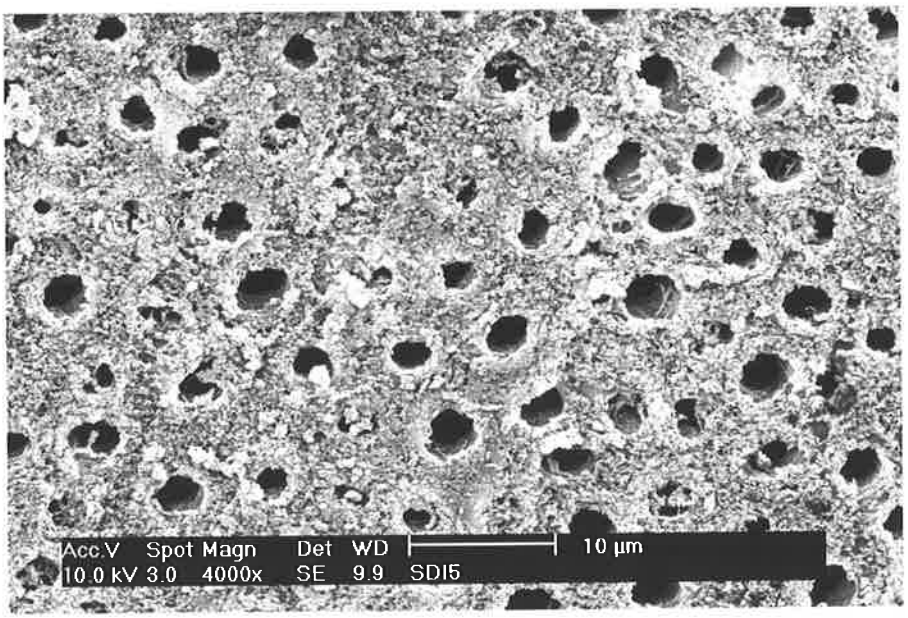
Group B: 04,05.

Debris 0:100; Collagen 0:100.



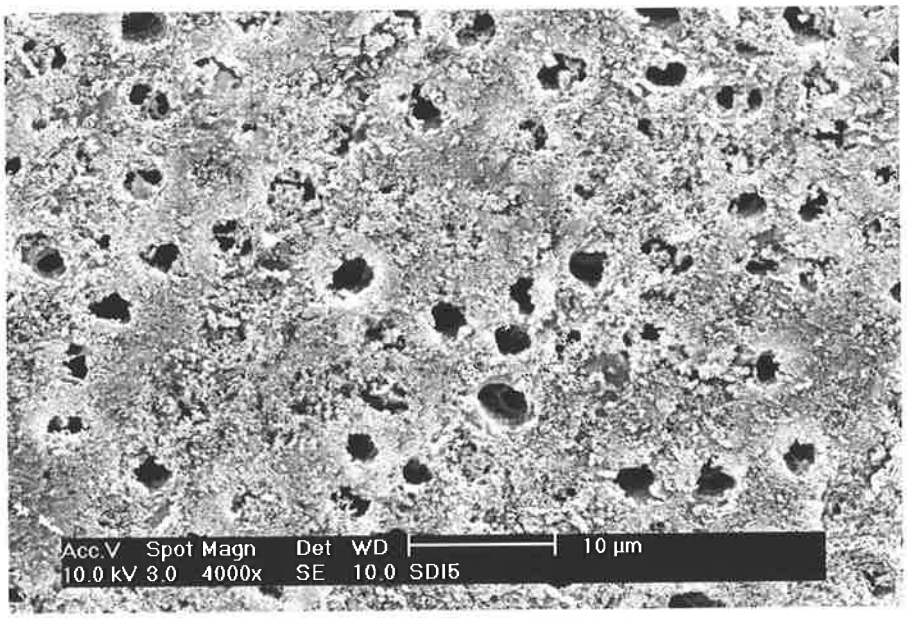
Group B: 05,01.

Debris 0:100; Collagen 0:100.



Group B: 05,02.

Debris 0:100; Collagen 0:100.



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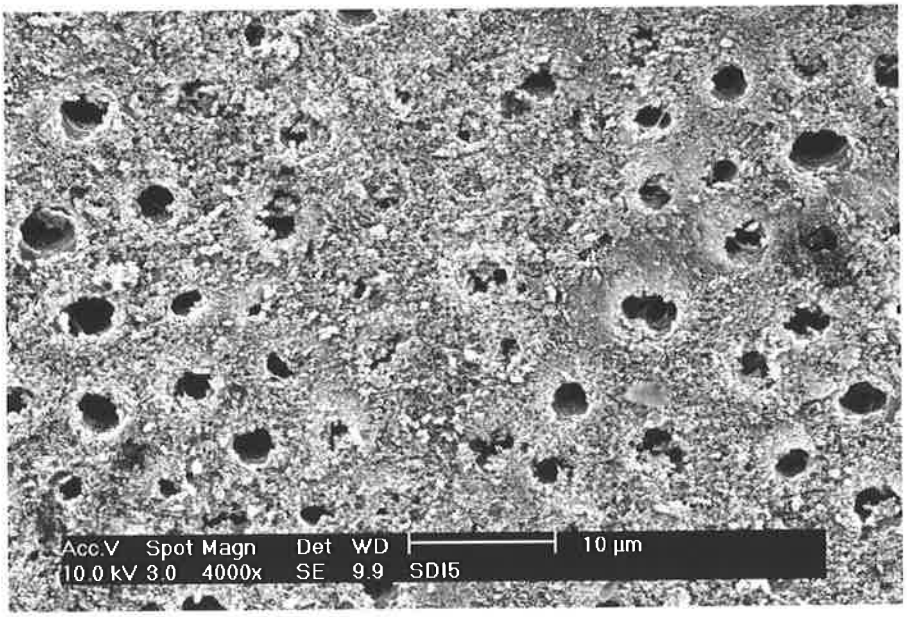

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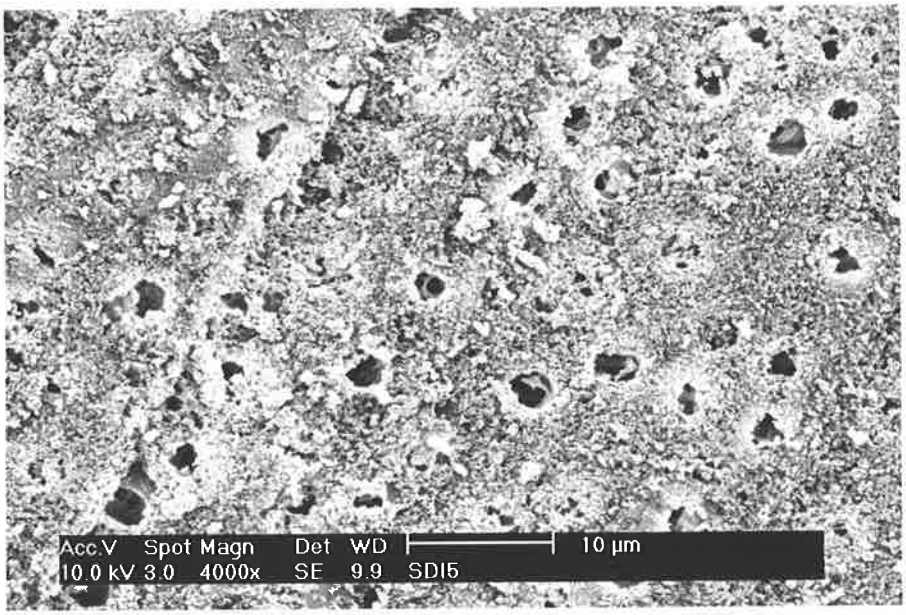

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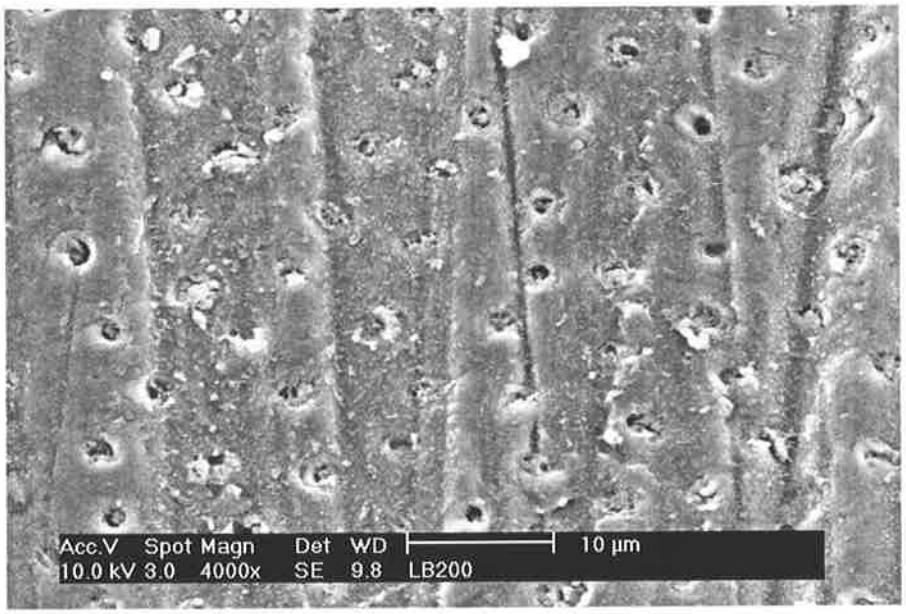
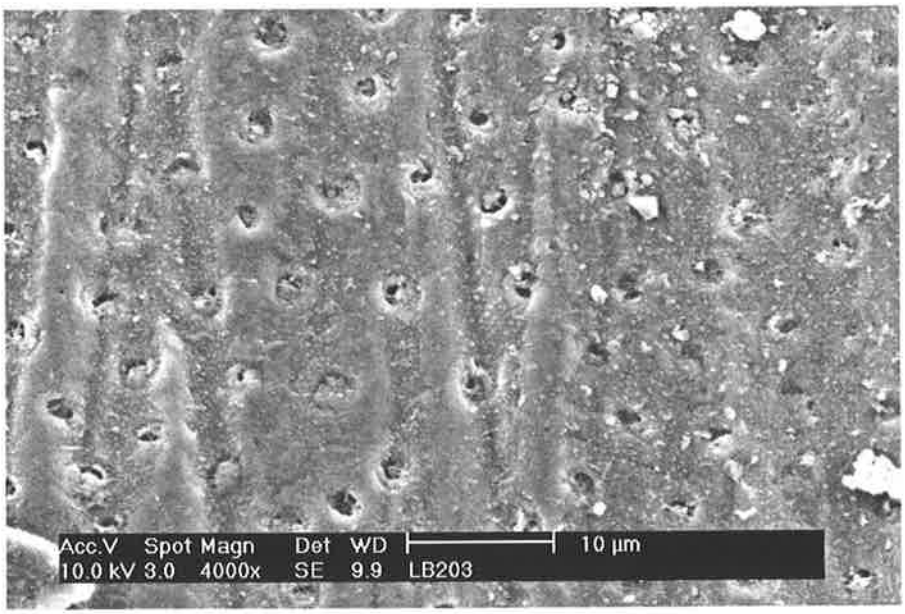


Group C: 01,01.

Debris 98:02; Collagen 100:100.



Group C: 01,02.

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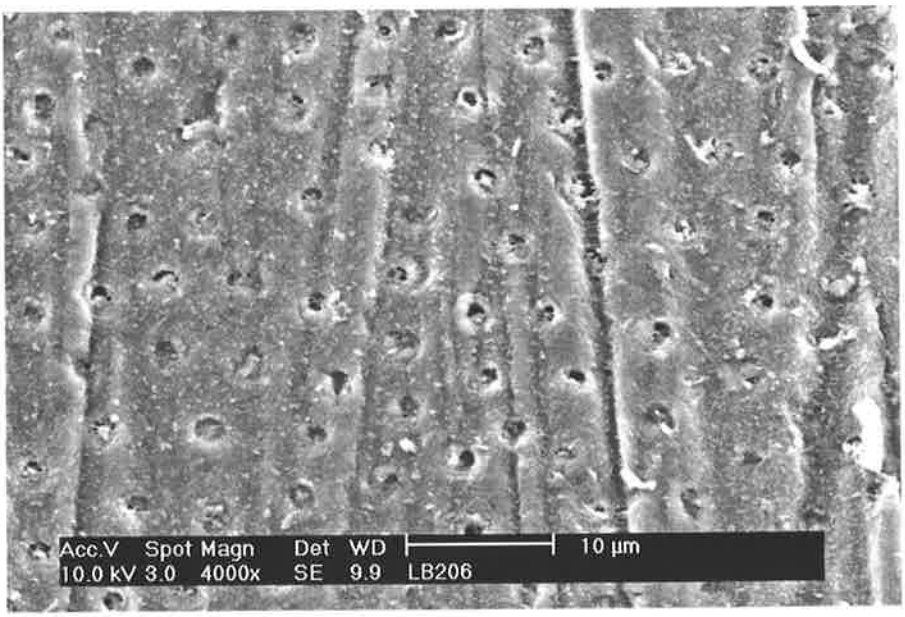

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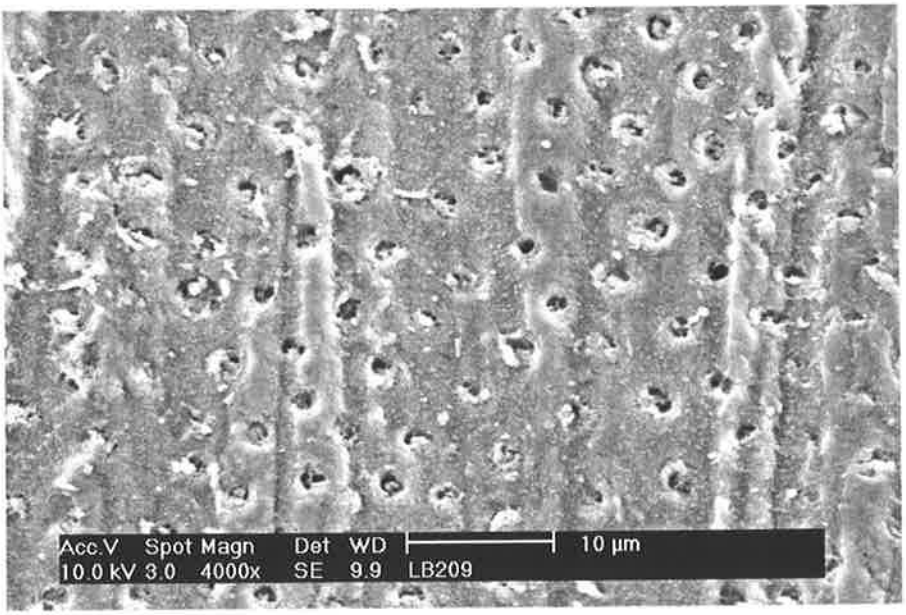

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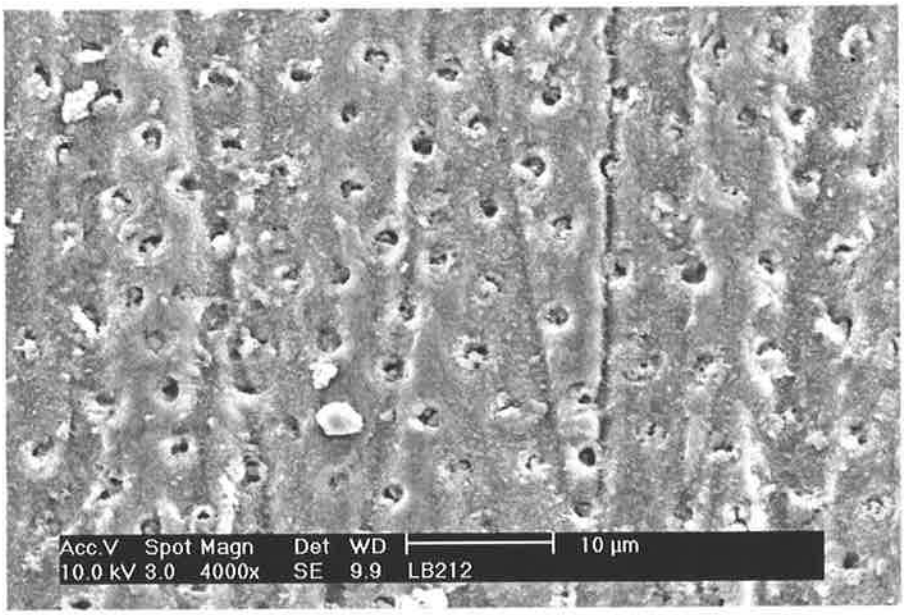

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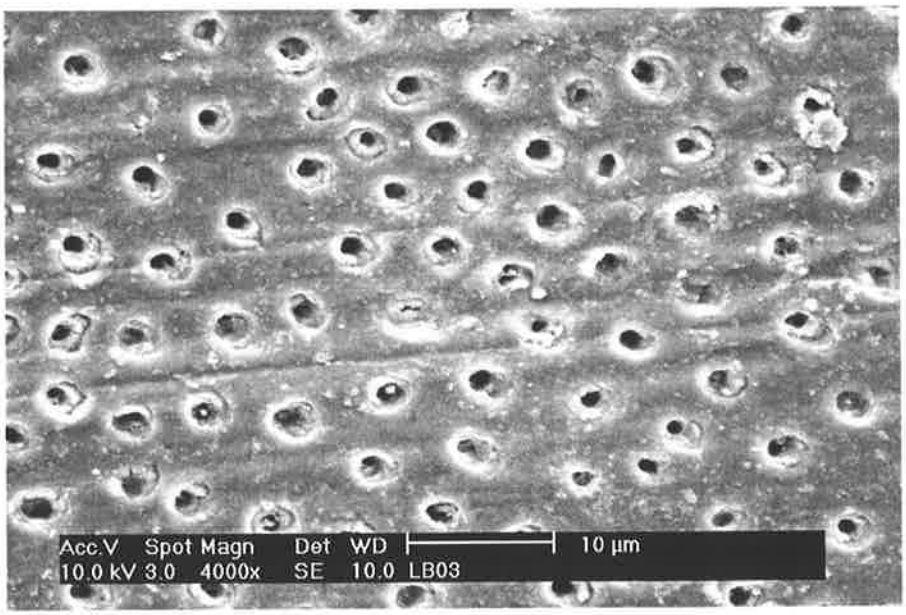

Group C: 02,01.

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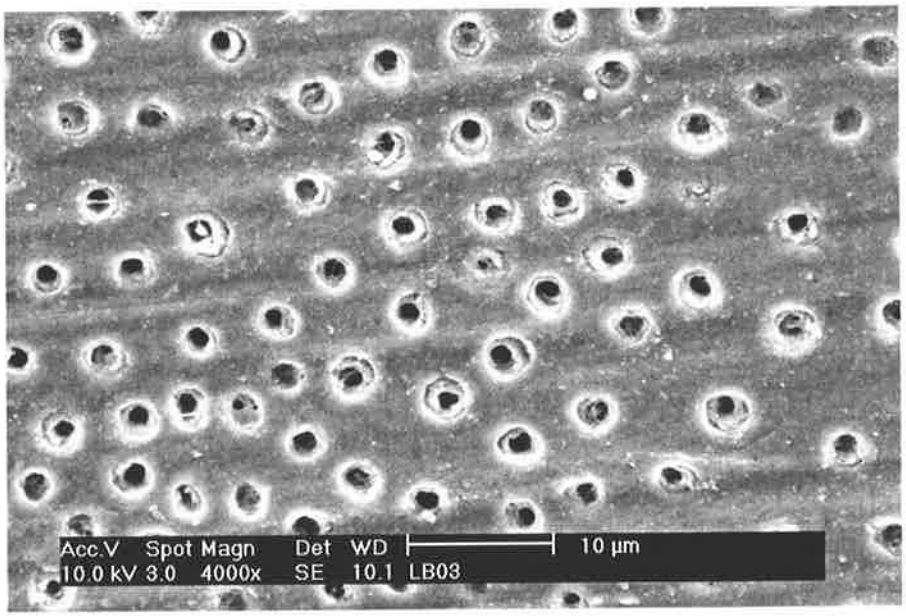

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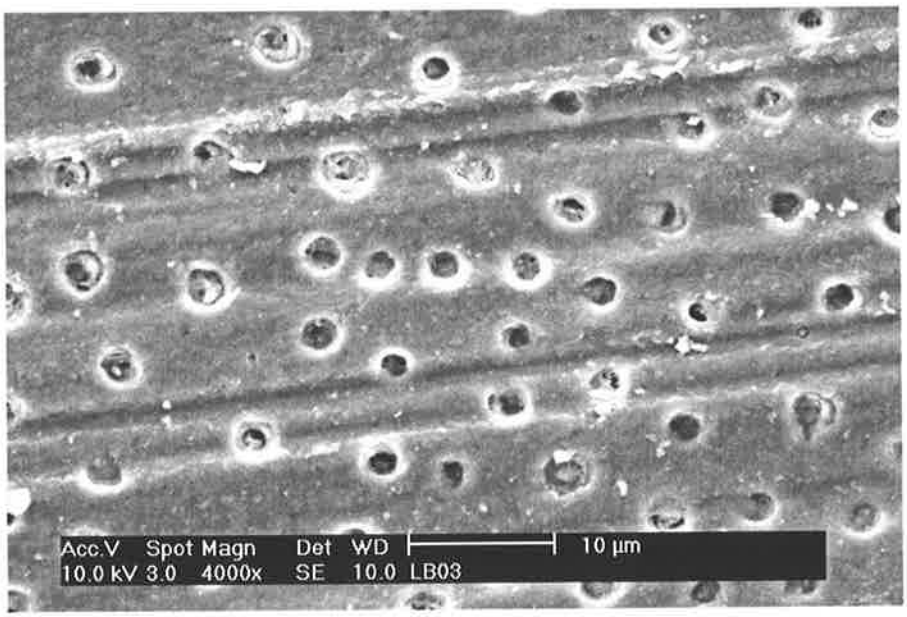

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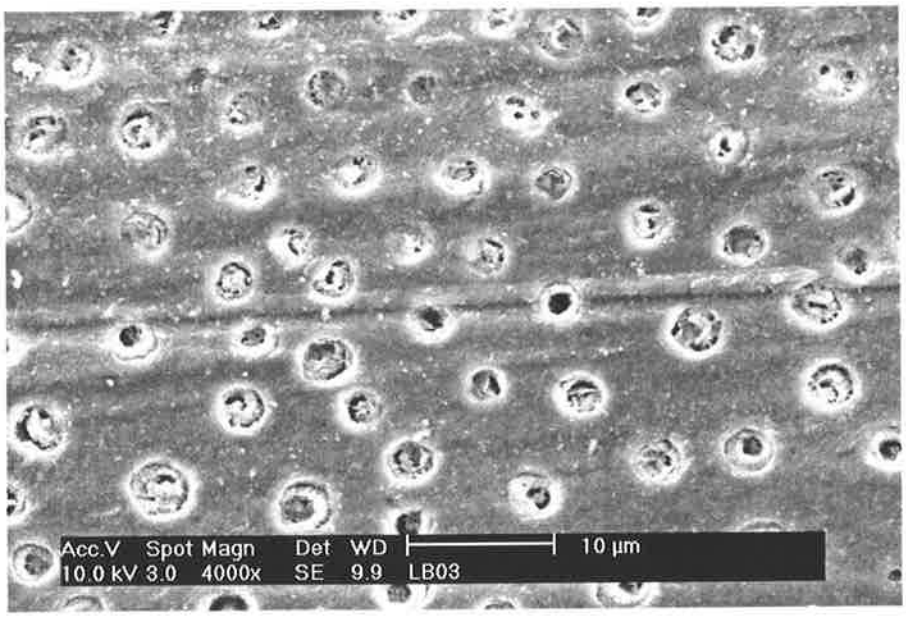
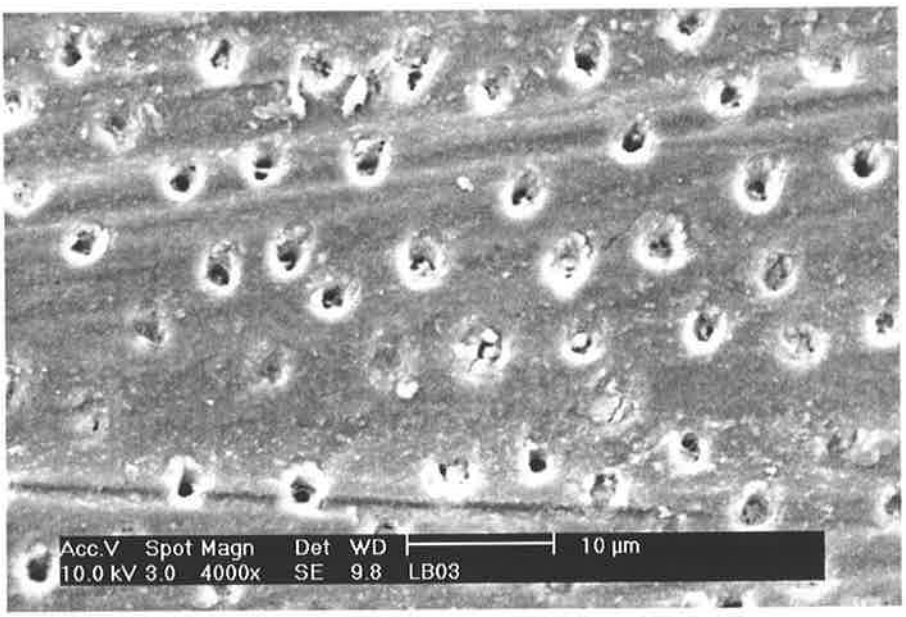


Group C: 02,04.

Debris 98:02; Collagen 100:100.



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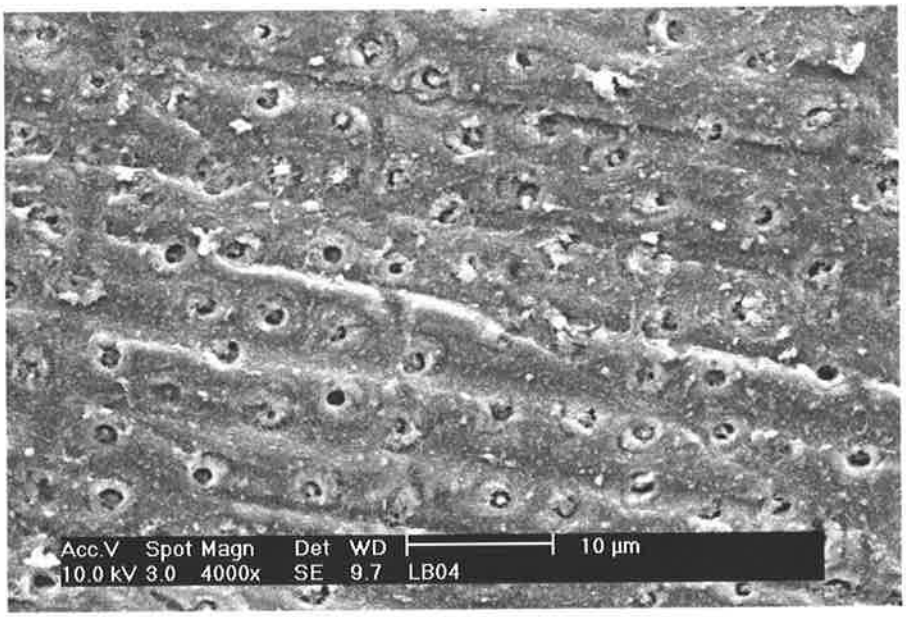
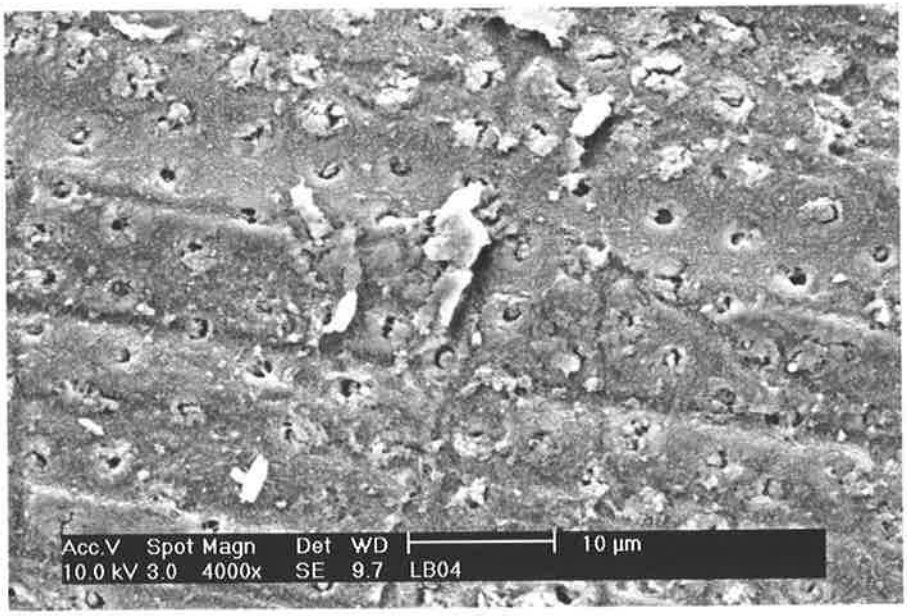


Group C: 03,01.

Debris 89:11; Collagen 100:100.



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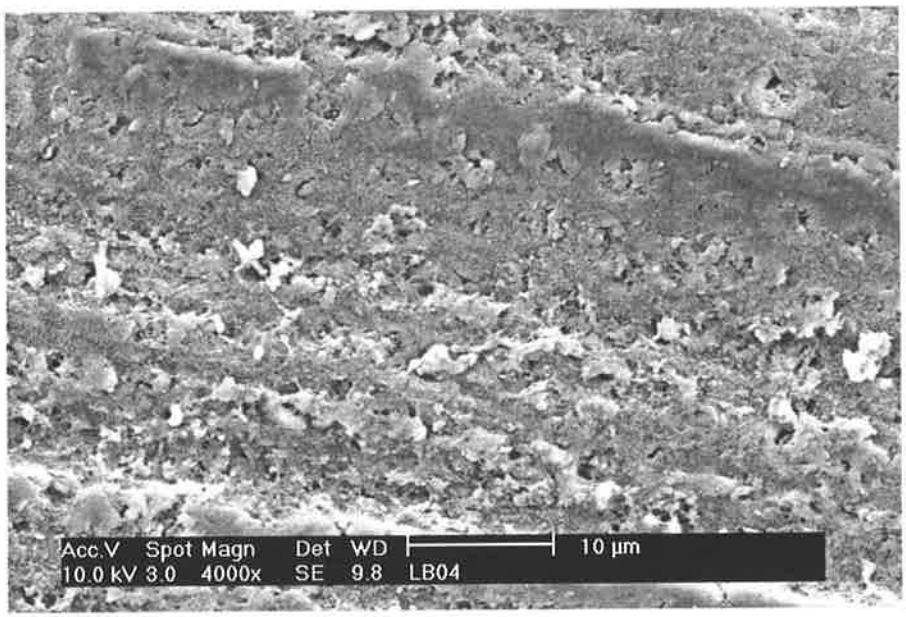

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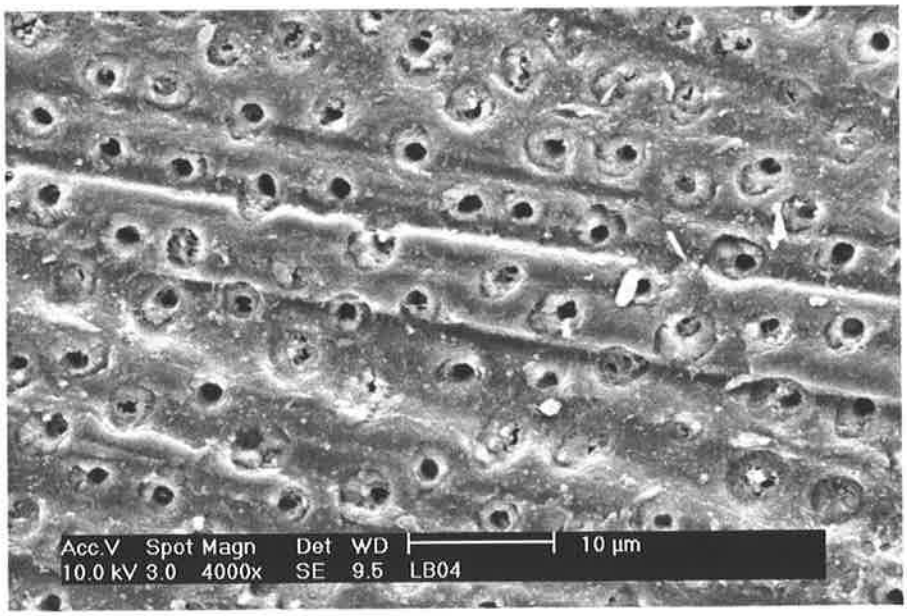

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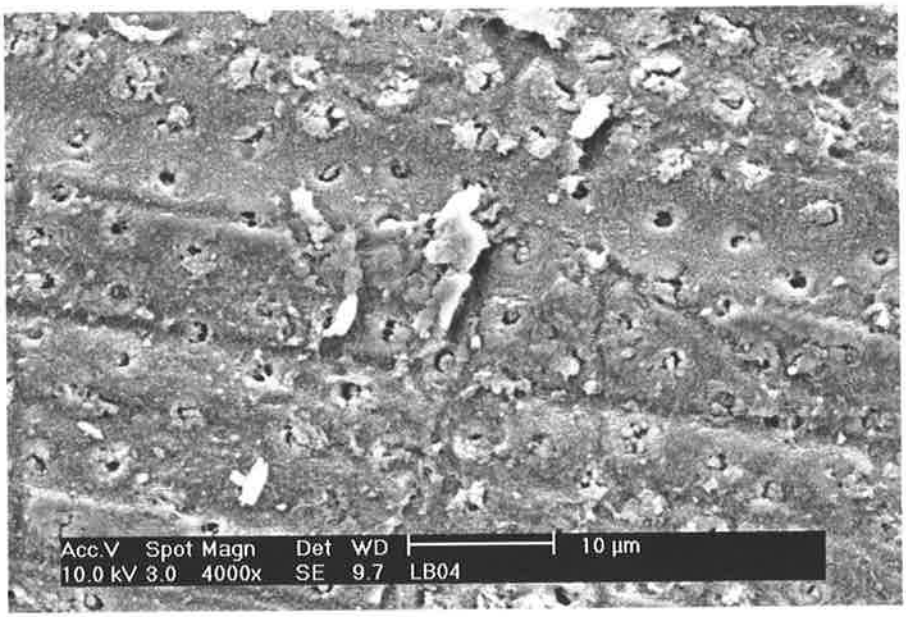

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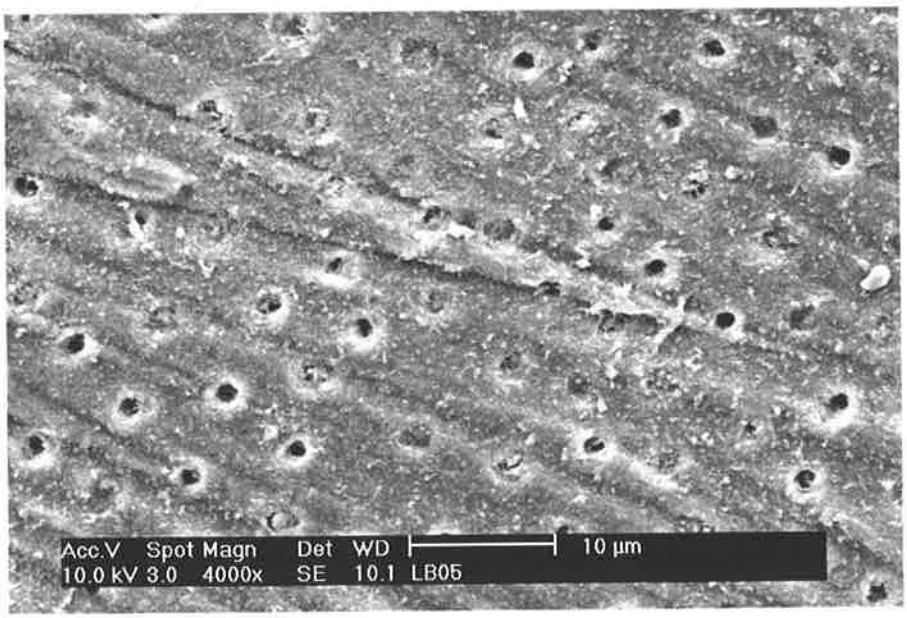

Group C: 04,01.

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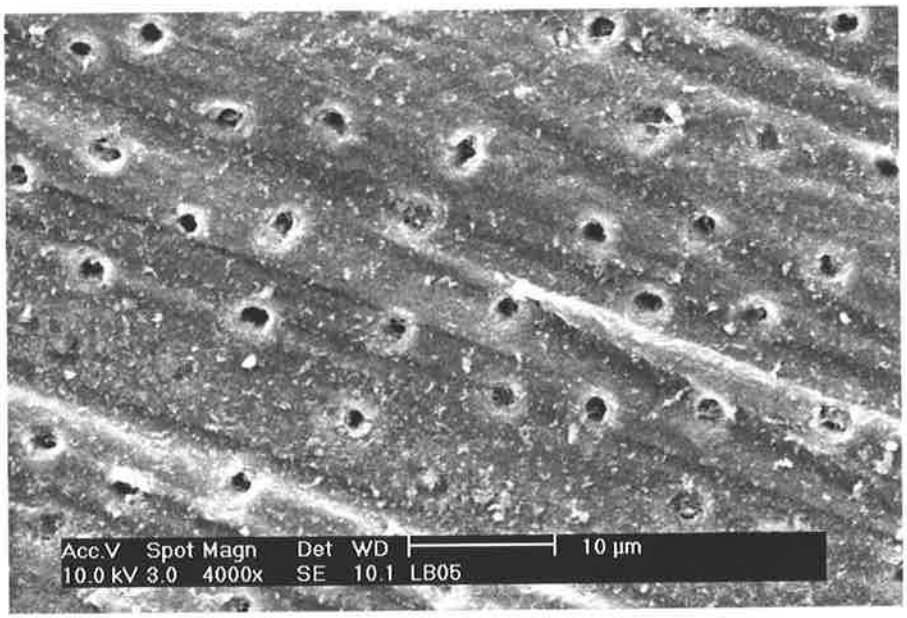

Group C: 04,02.

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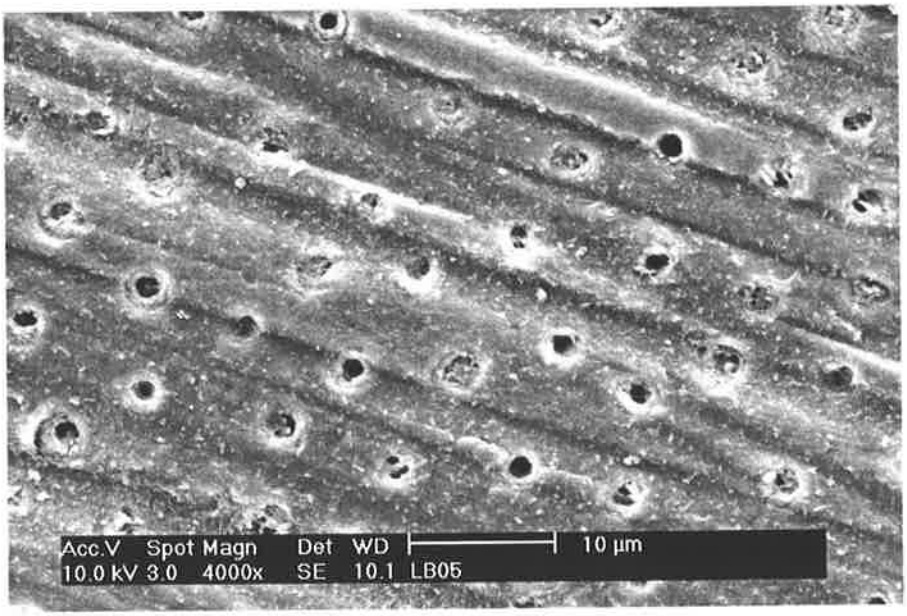

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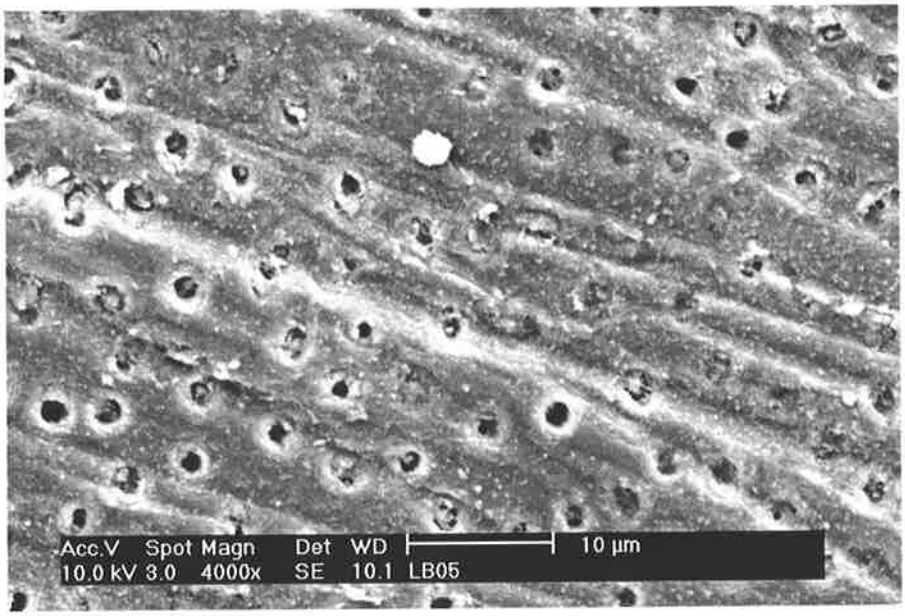

Group C: 04,04.

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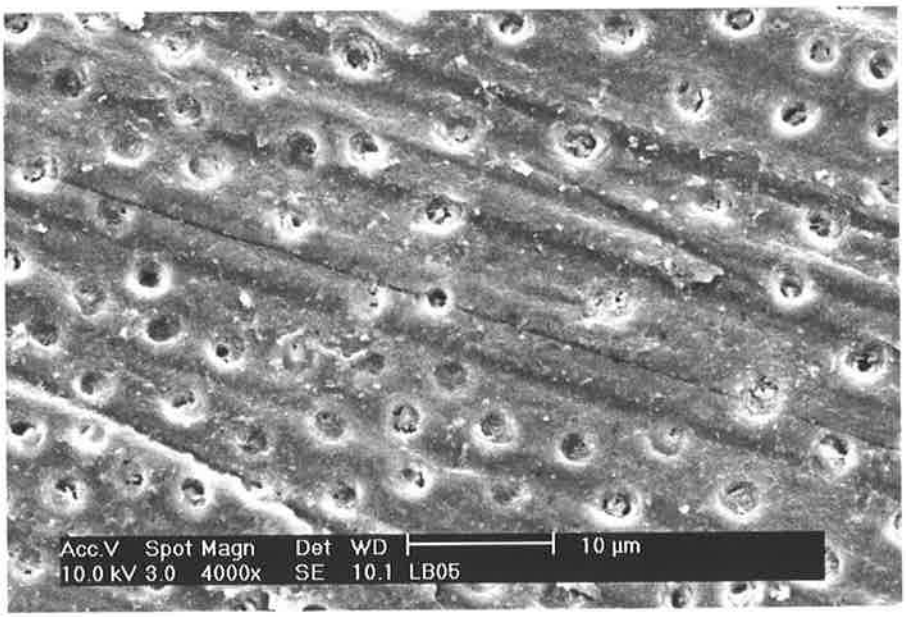

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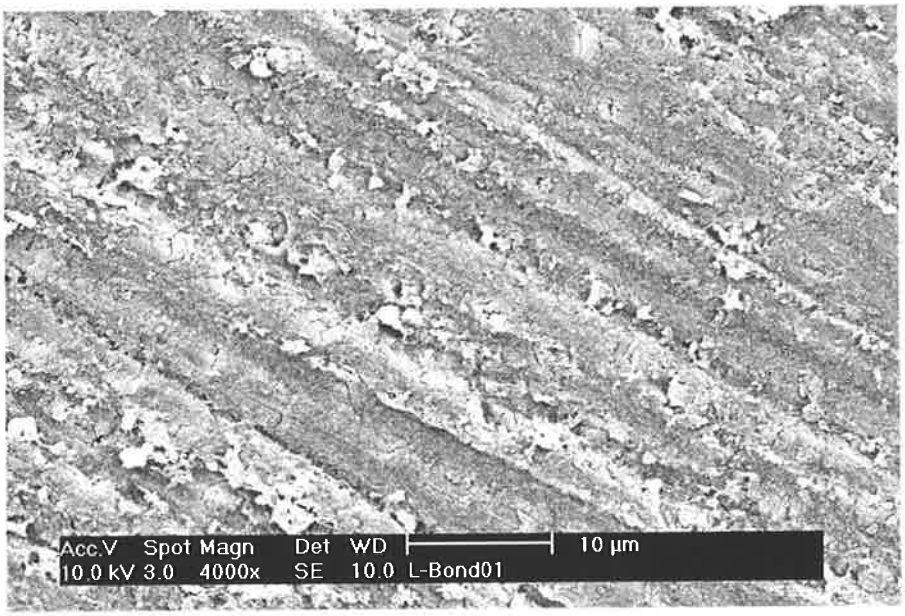
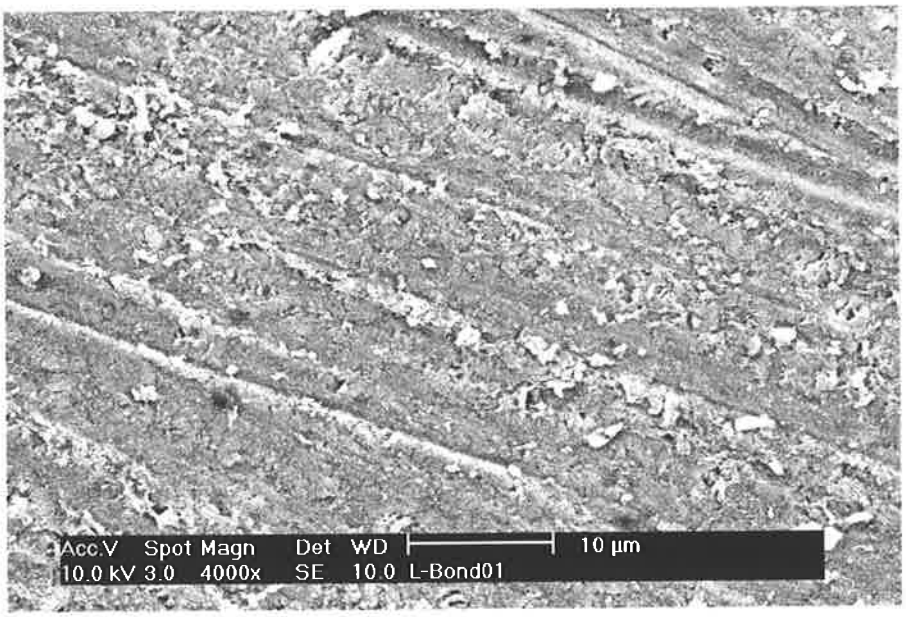
Debris 96:04; Collagen 100:100.



Group D: 01,01.

Debris 0:100; Collagen 0:100.



Group D: 01,02.

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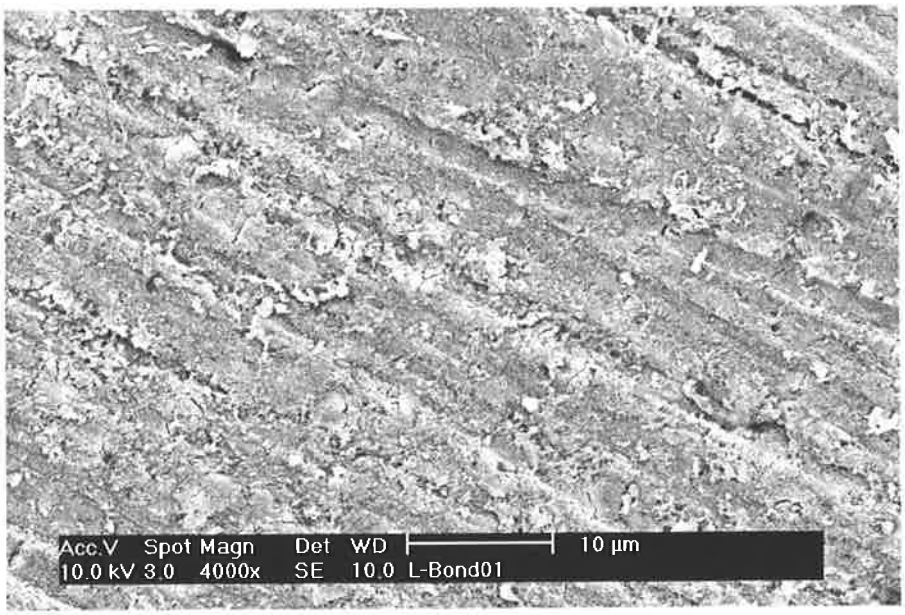
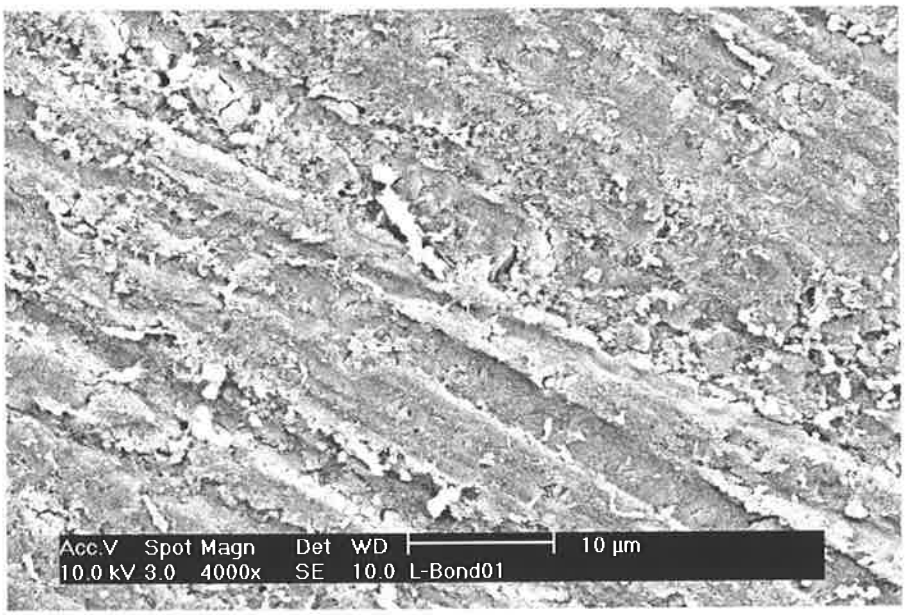


Group D: 01,03.

Debris 0:100; Collagen 0:100.



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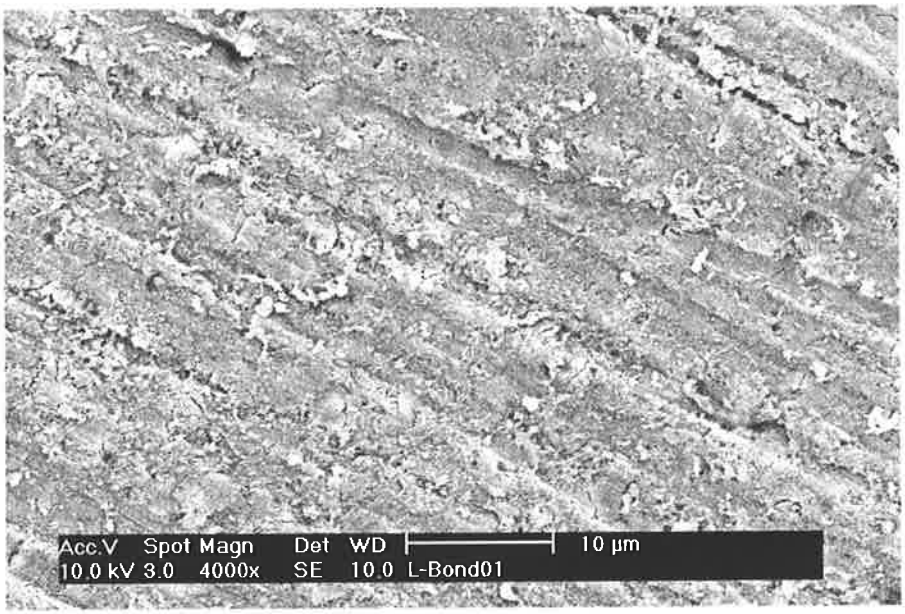
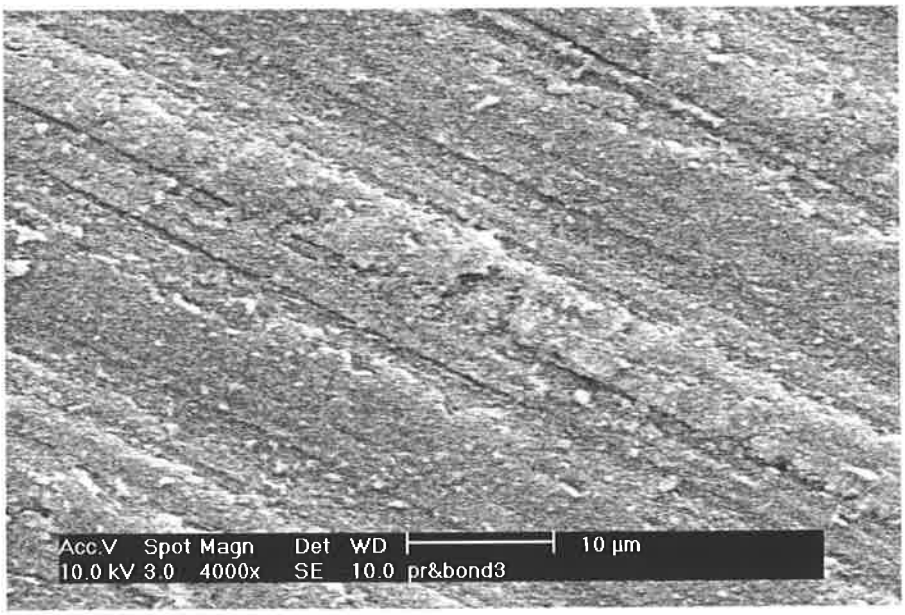


Group D: 01,05.

Debris 0:100; Collagen 0:100.



Group D: 02,01.

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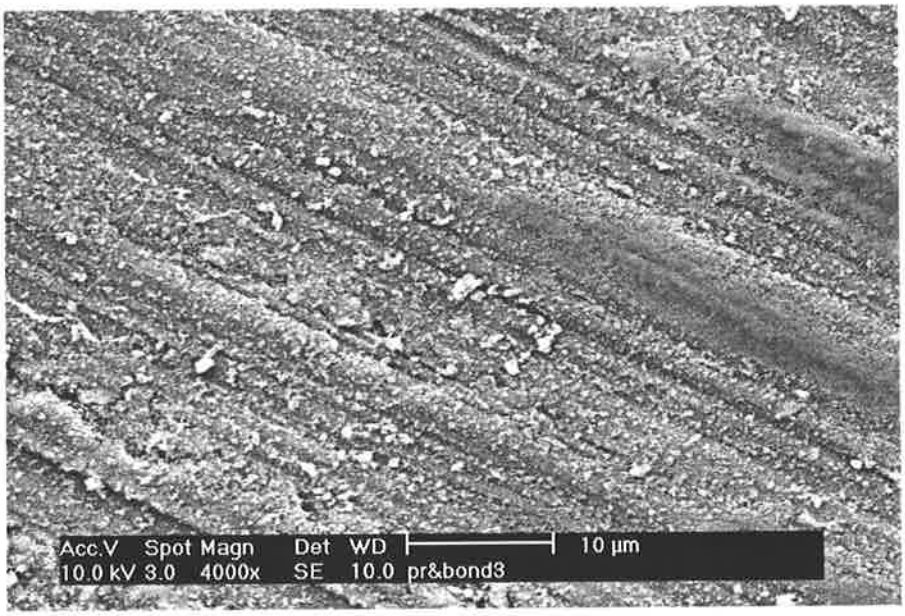

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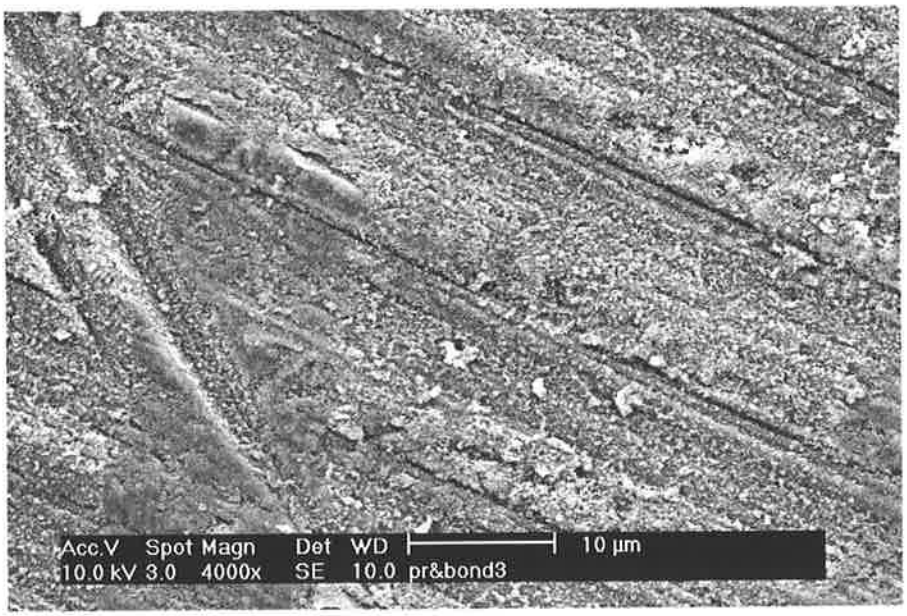

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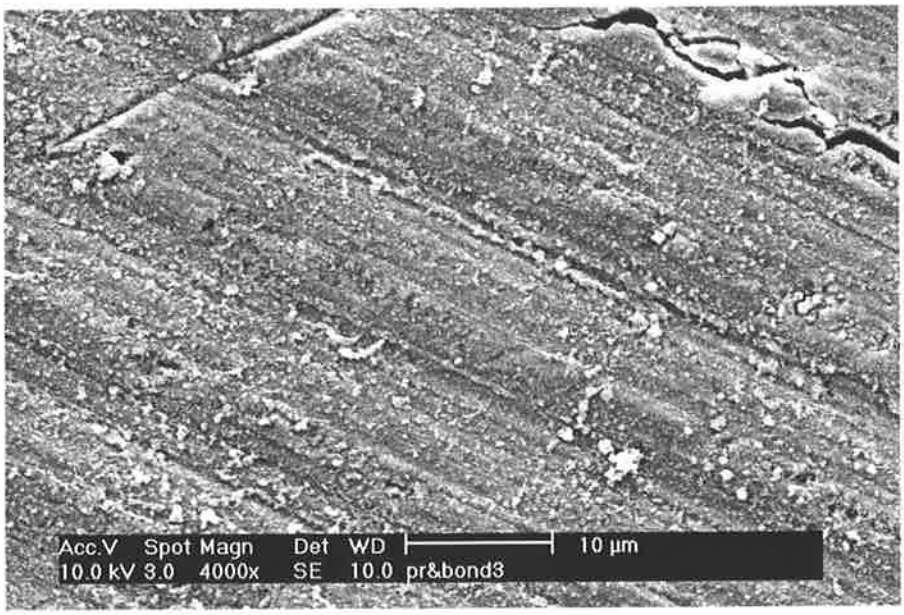

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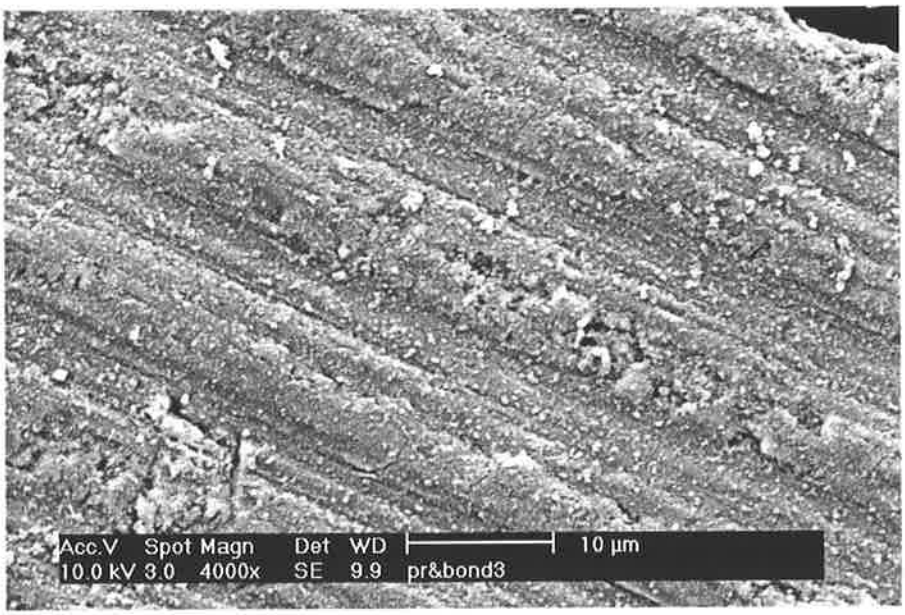

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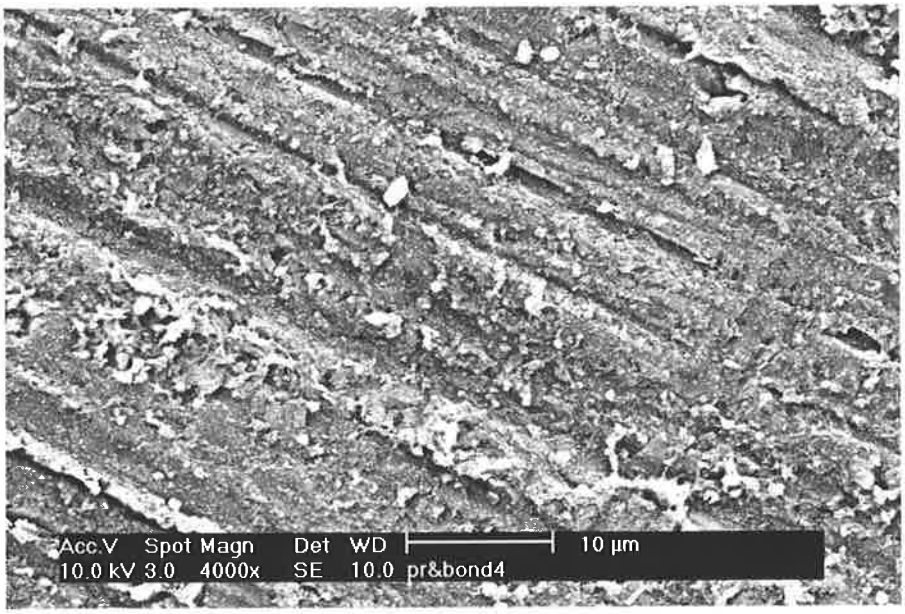

Group D: 03,01.

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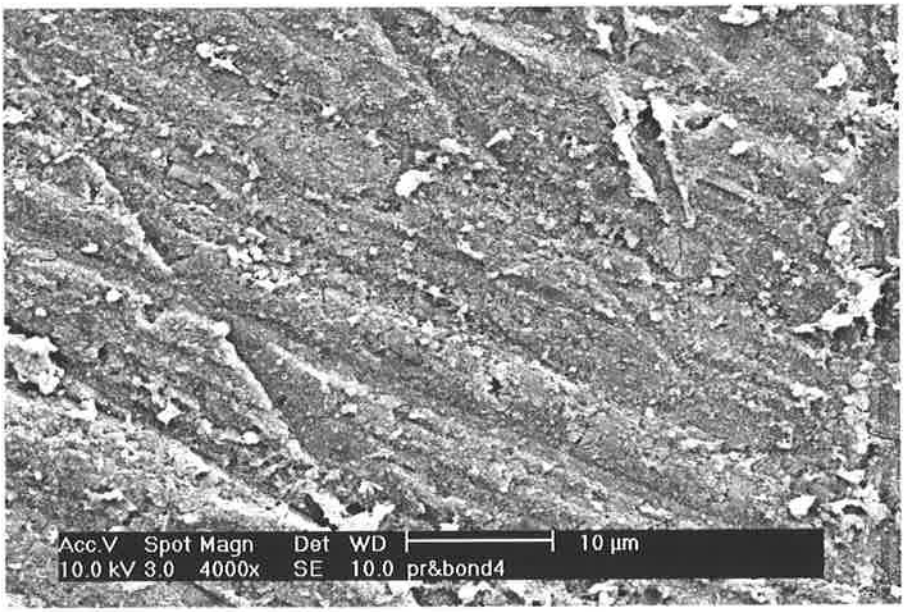

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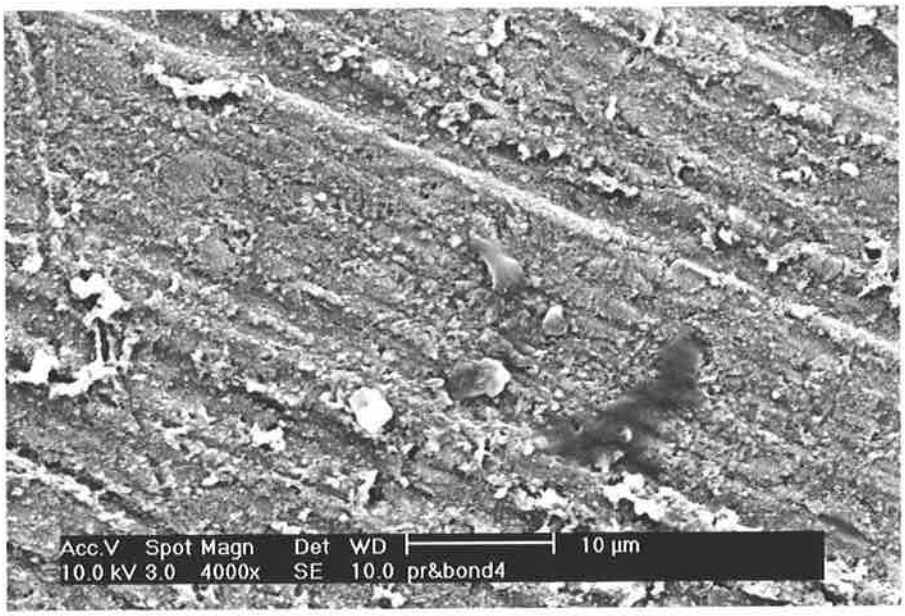

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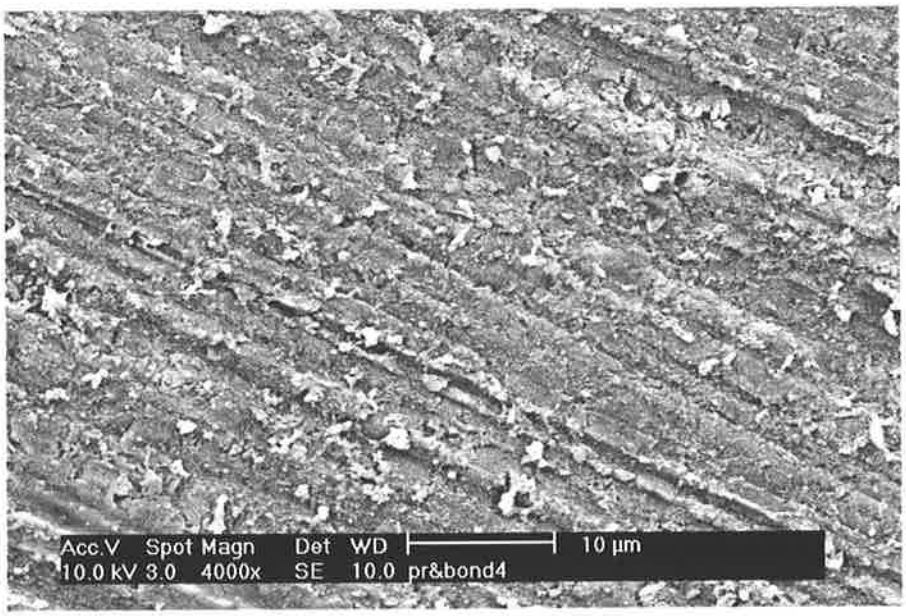

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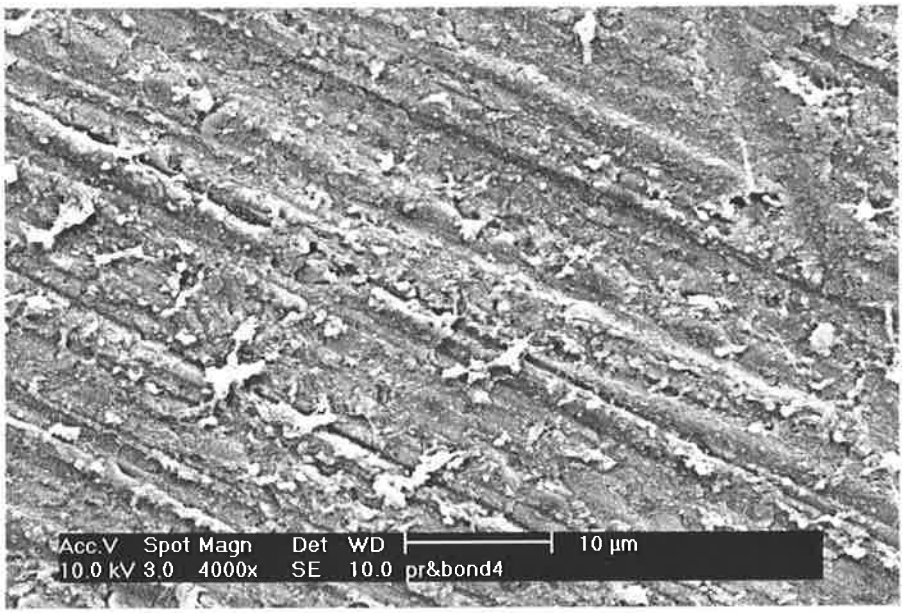

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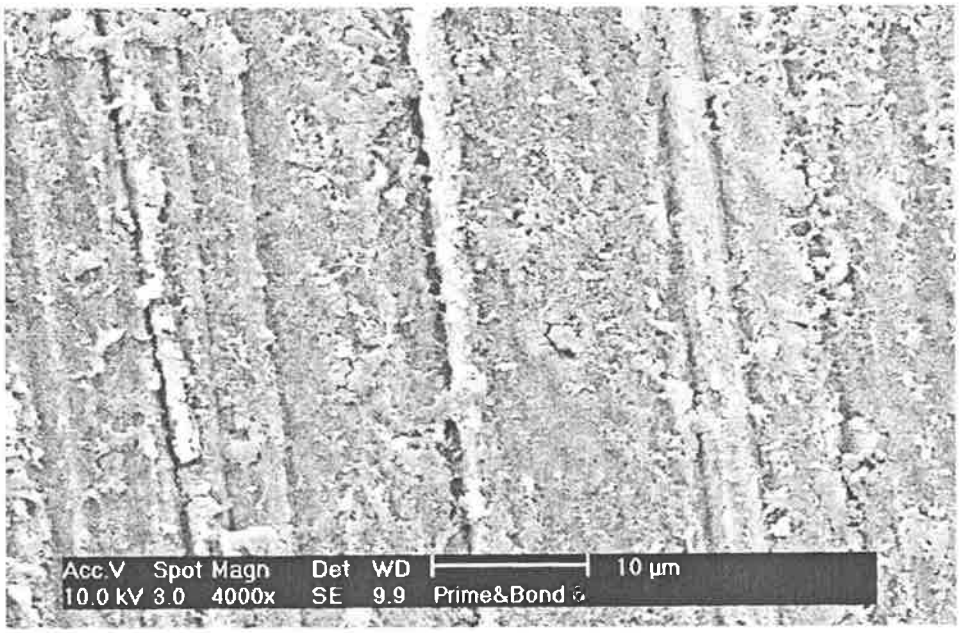

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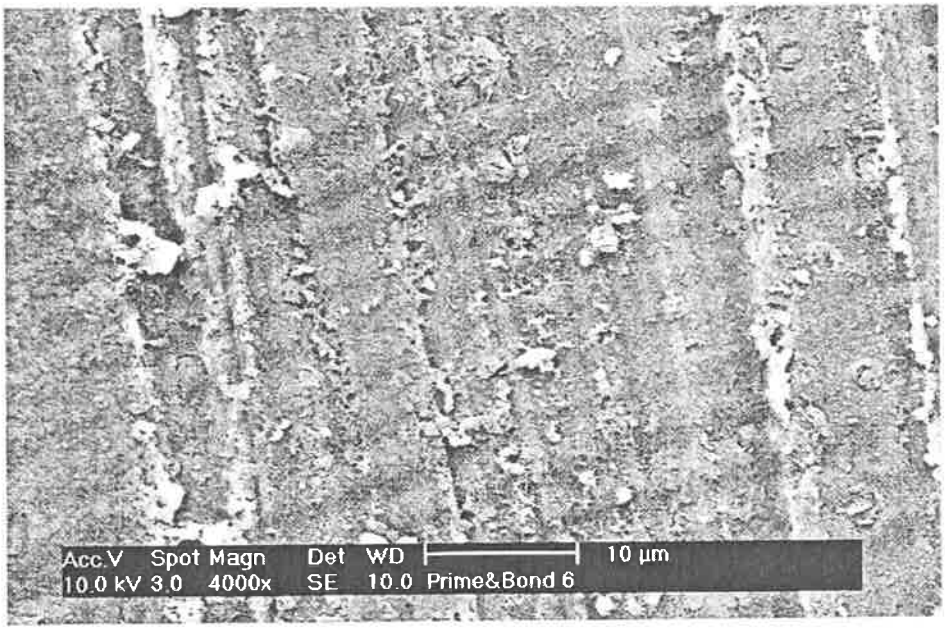

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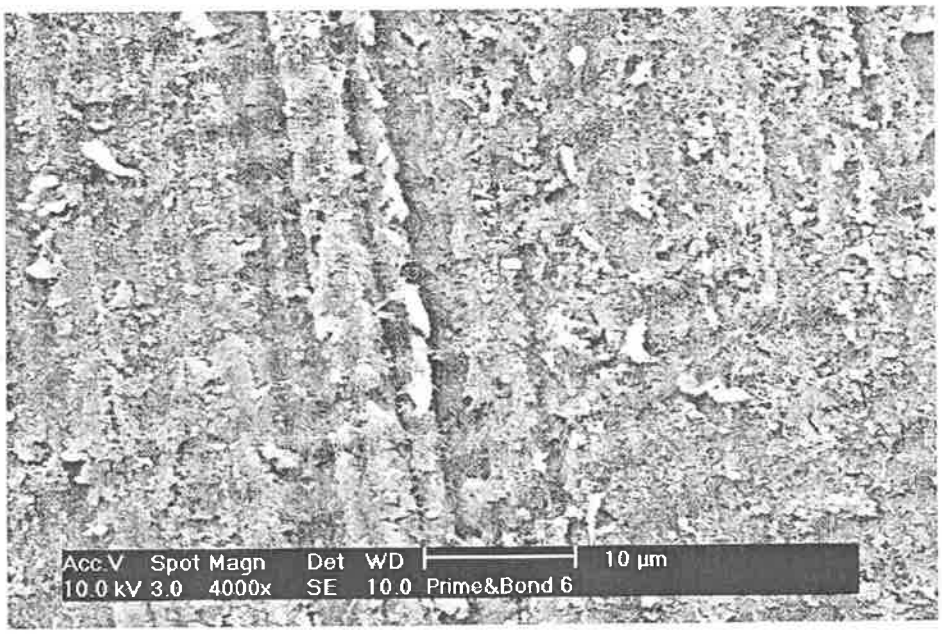

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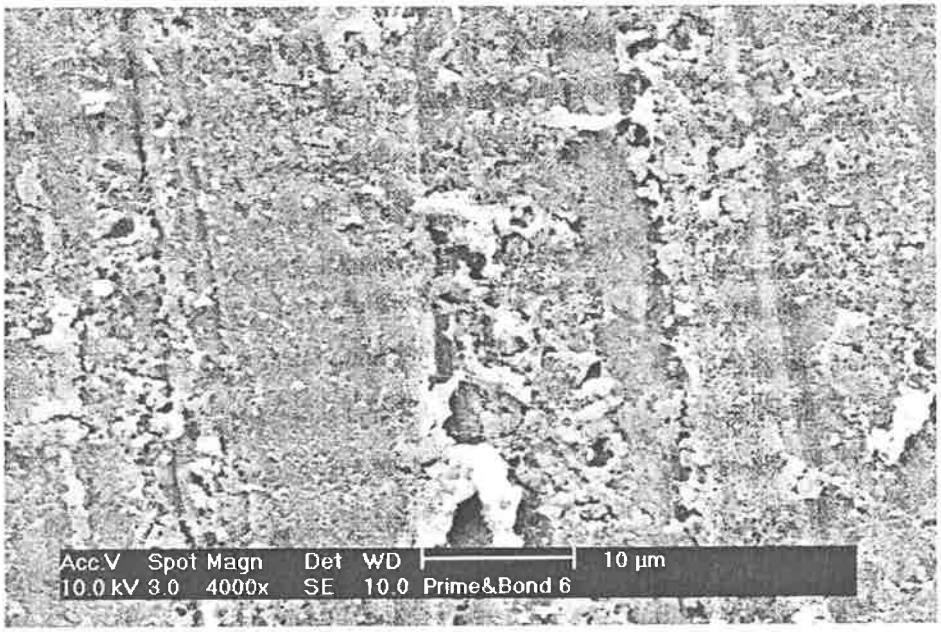

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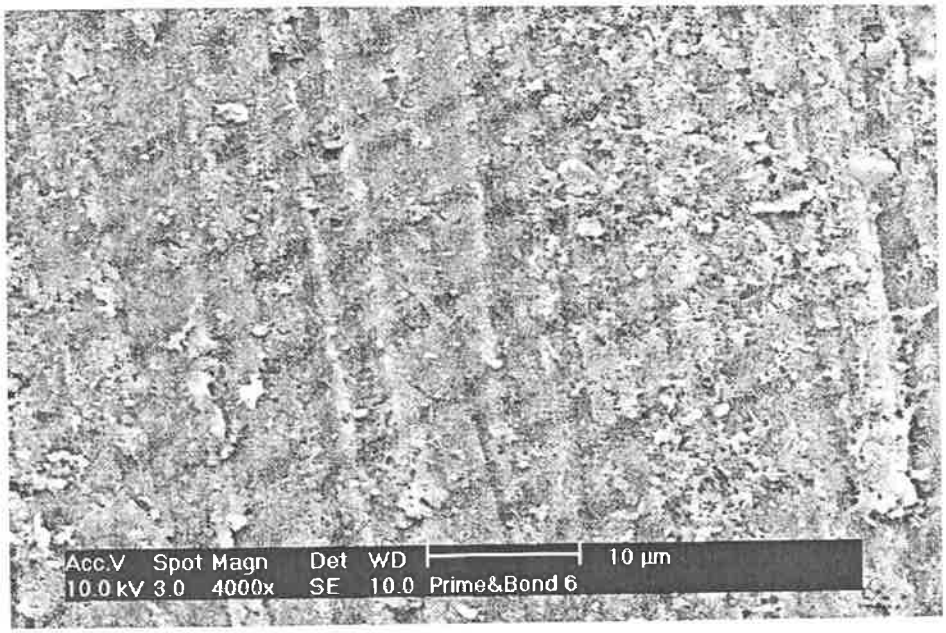

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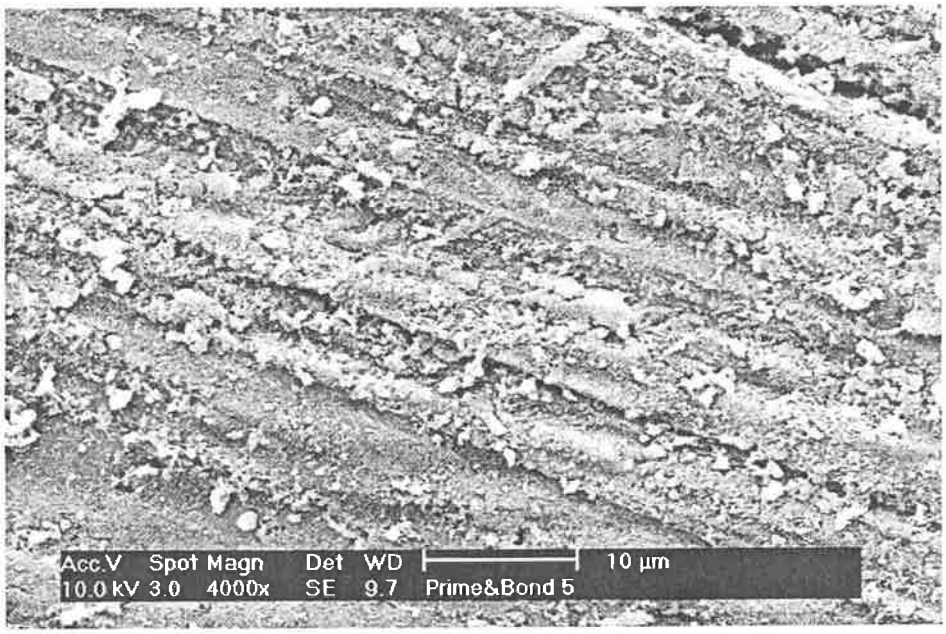

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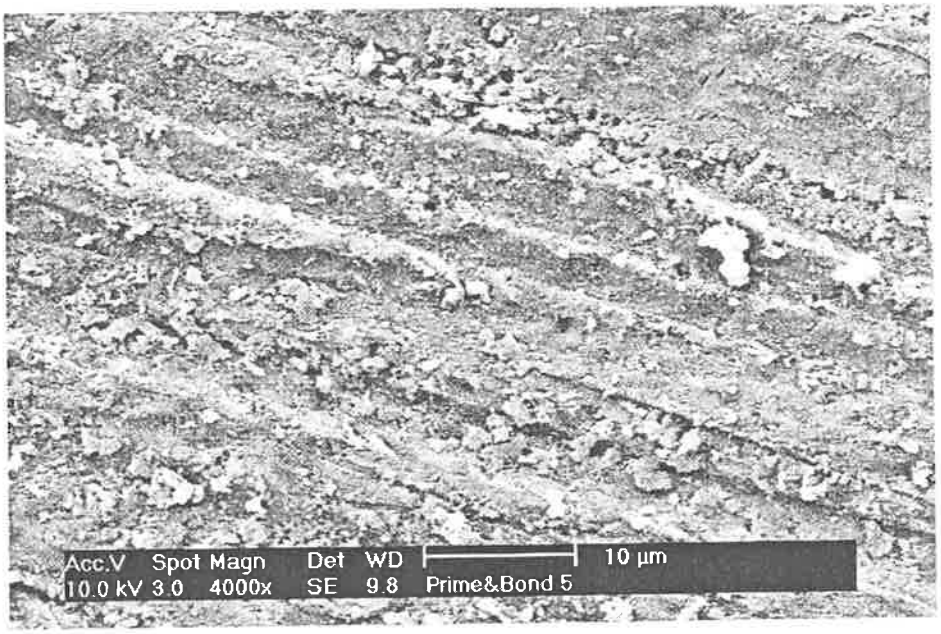

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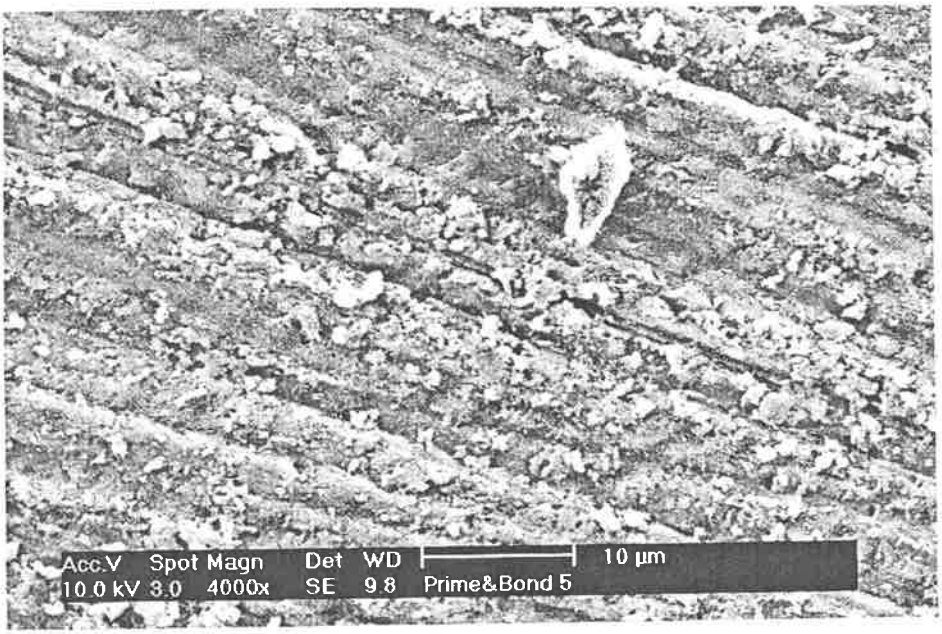

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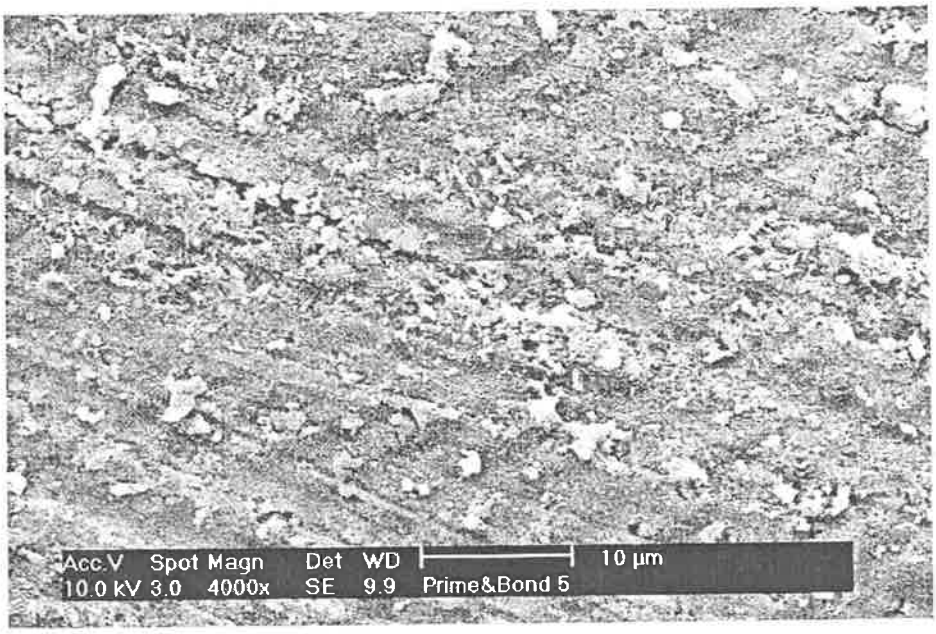

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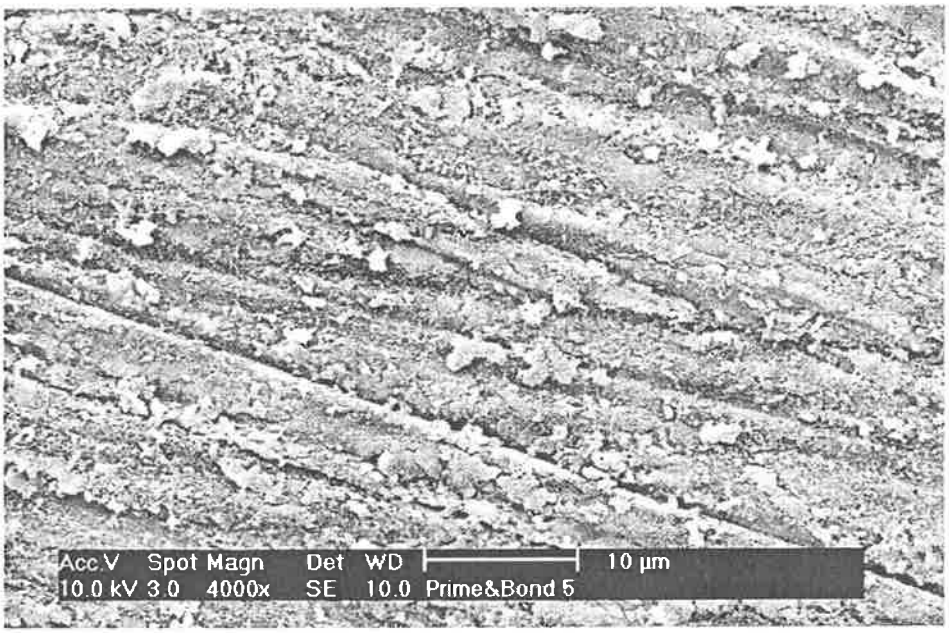
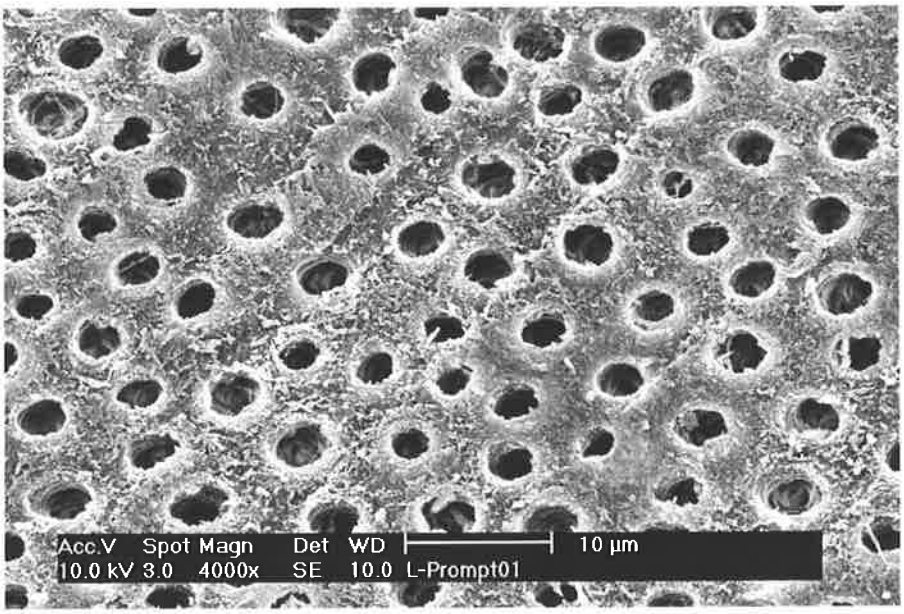
Debris 0:100; Collagen 0:100.



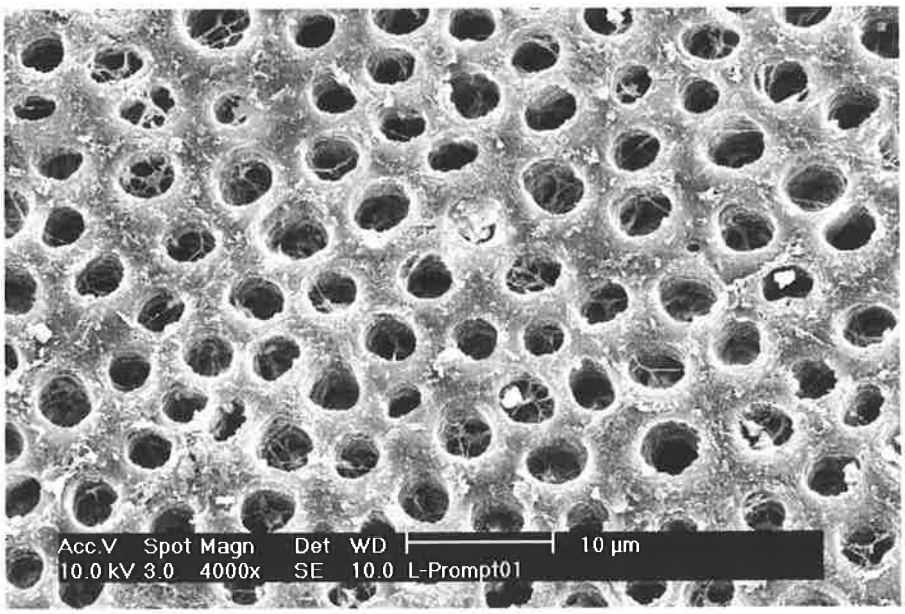
Group D: 05,05.

Debris 0:100; Collagen 0:100.



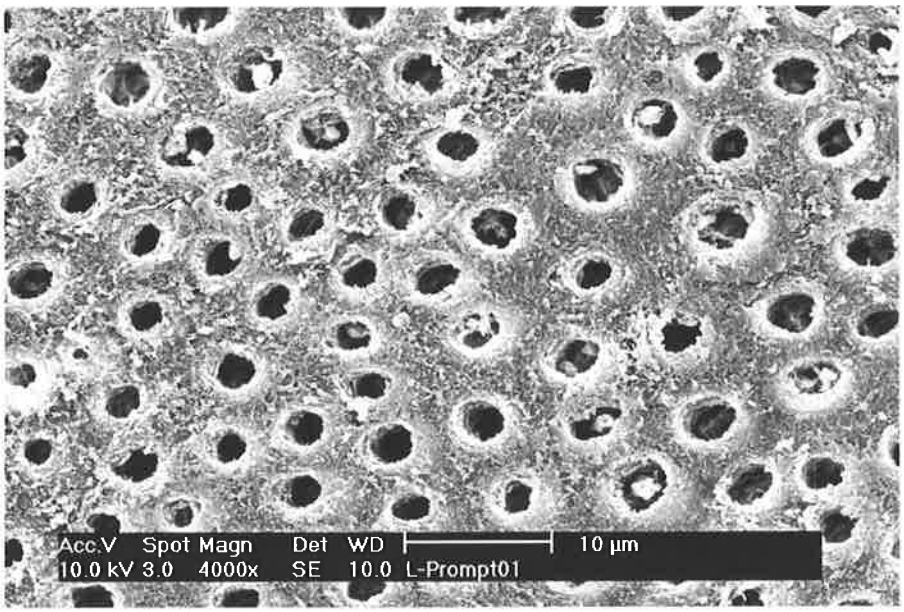
Group E: 01,01.

Debris 100:0; Collagen 124:100.



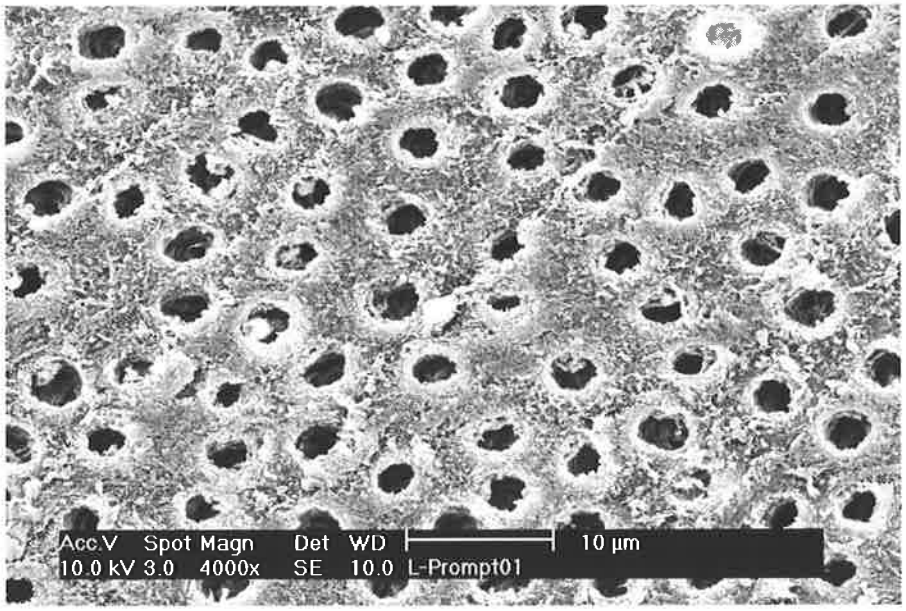
Group E: 01,02.

Debris 97:03; Collagen 100:100.



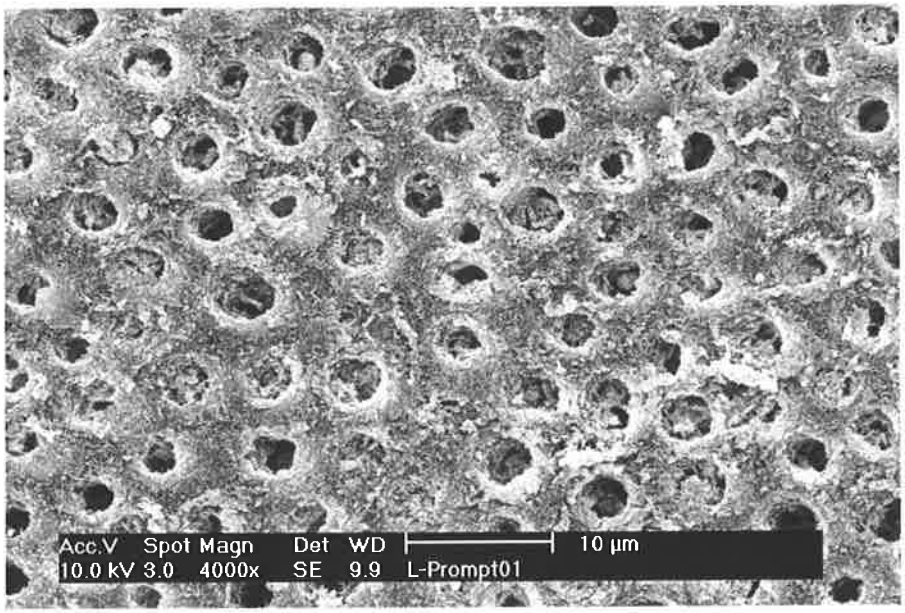
Group E: 01,03.

Debris 99:01; Collagen 141:100.



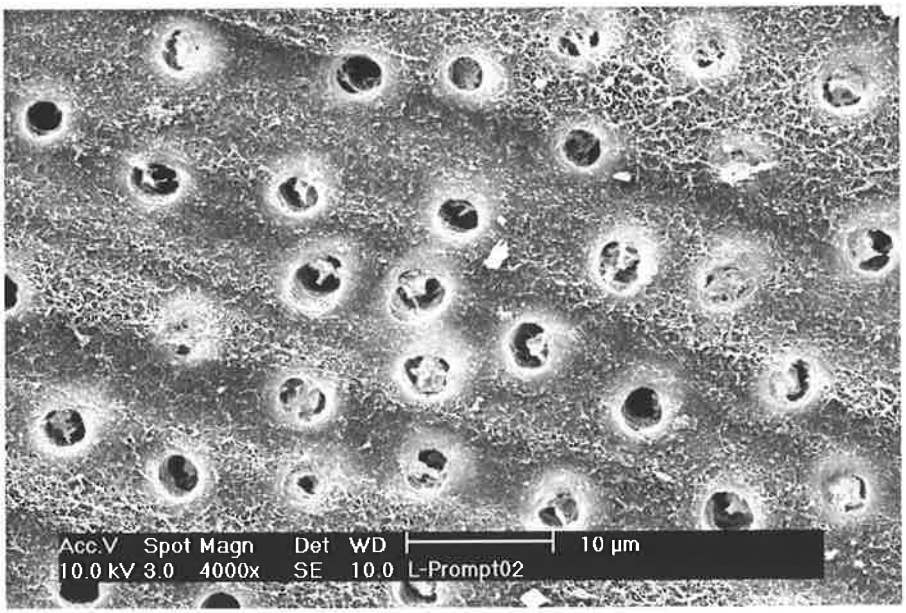
Group E: 01,04.

Debris 98:02; Collagen 139:100.



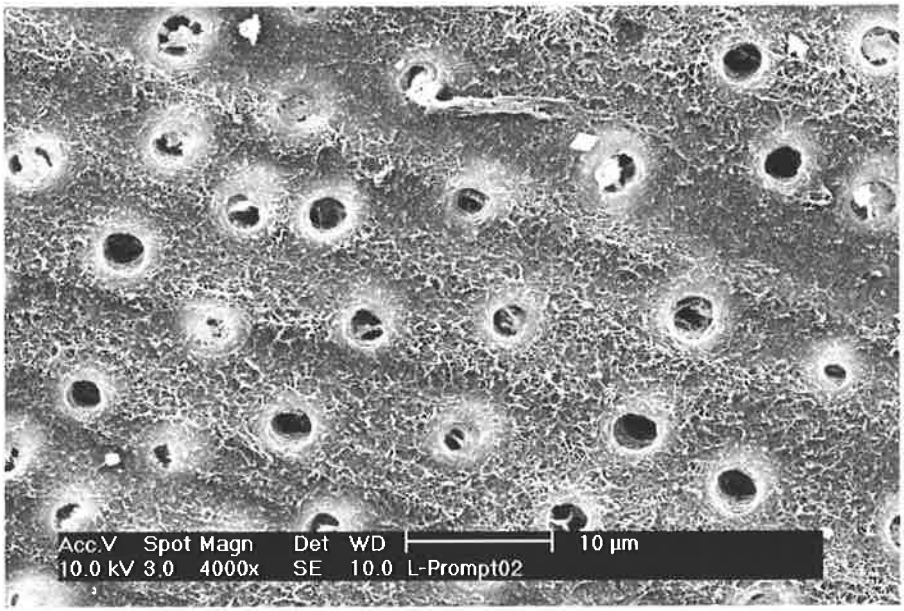
Group E: 01,05.

Debris 98:02; Collagen 150:100.



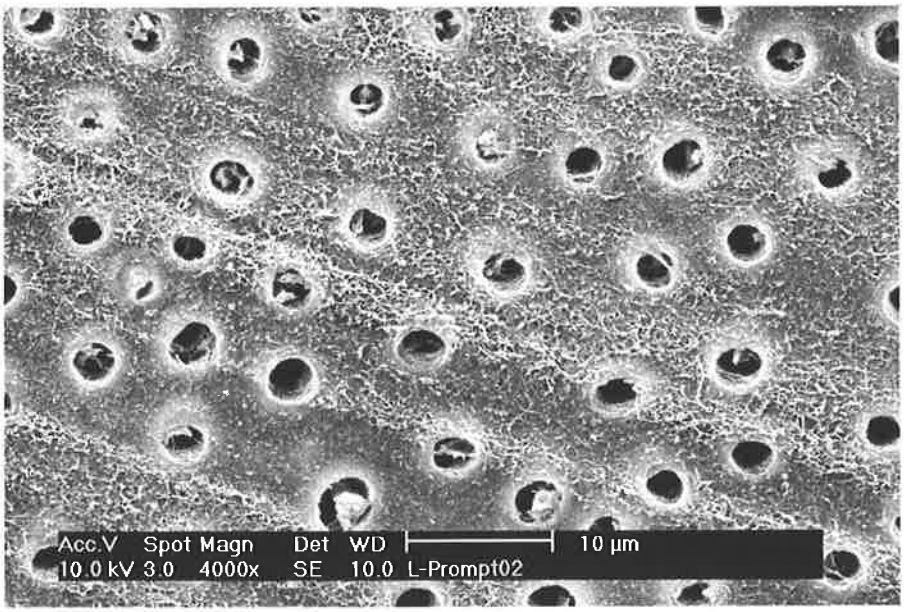
Group E: 02,01.

Debris 98:02; Collagen 194:100.



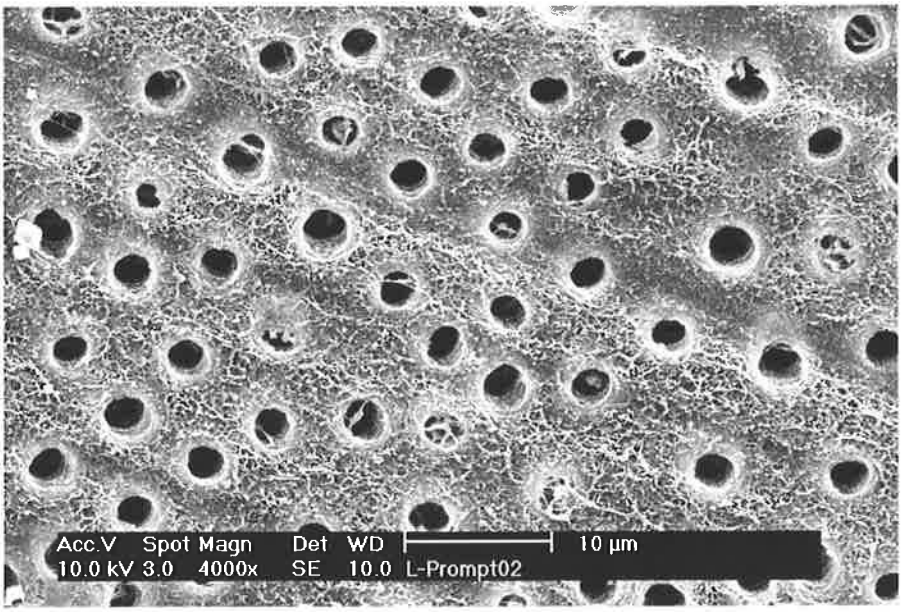
Group E: 02,02.

Debris 94:06; Collagen 225:100.



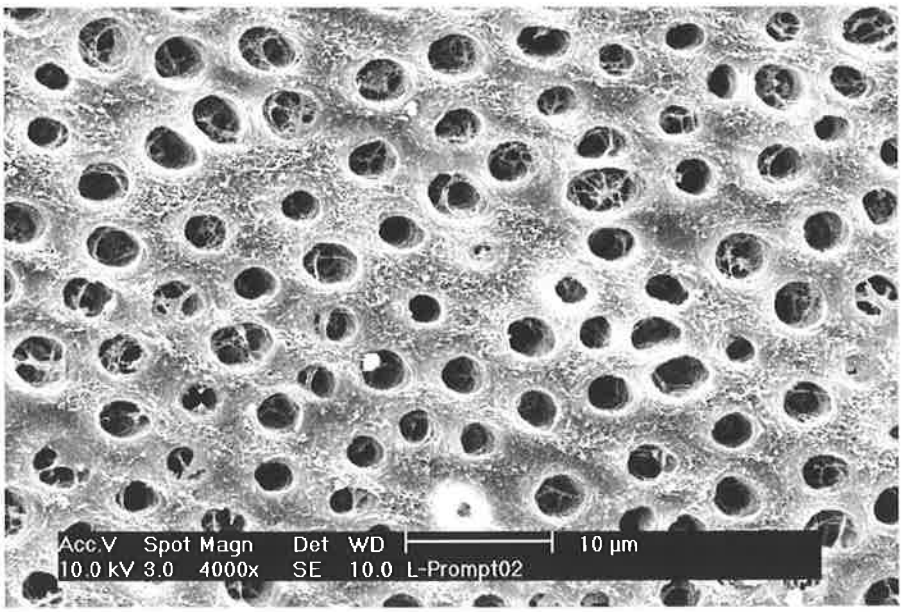
Group E: 02,03.

Debris 100:0; Collagen 222:100.



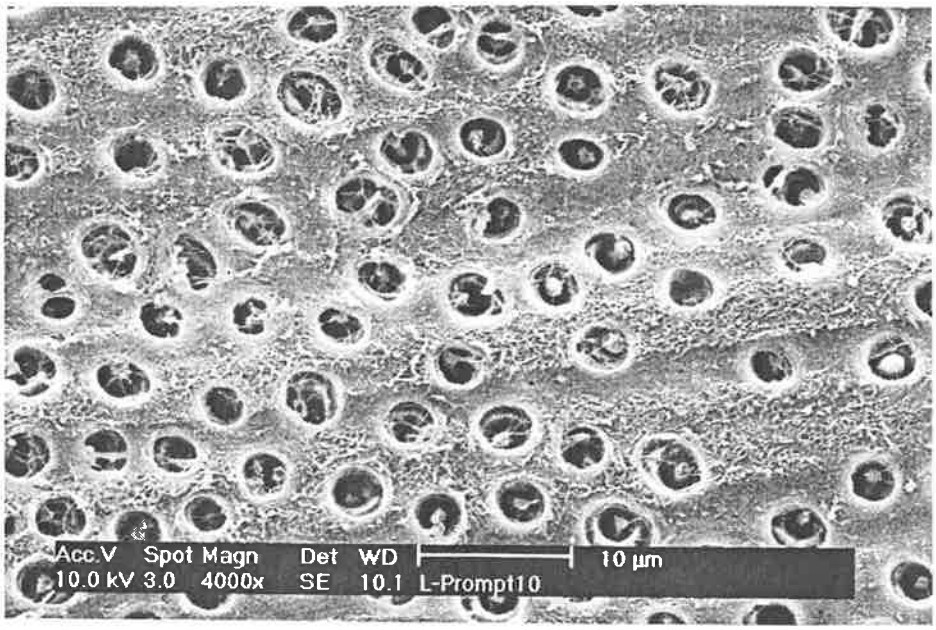
Group E: 02,04.

Debris 99:01; Collagen 224: 100.



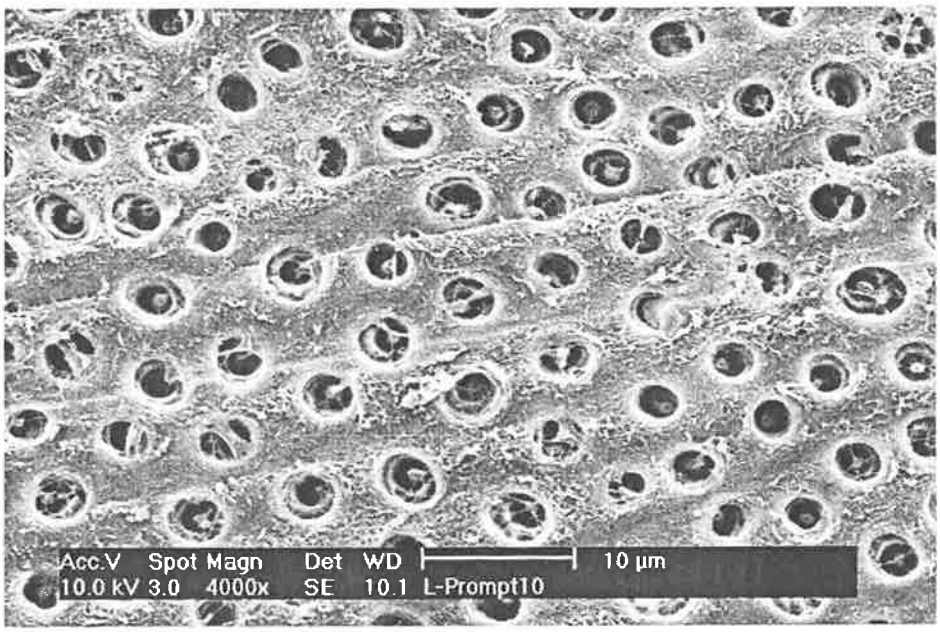
Group E: 02,05.

Debris 100:0; Collagen 133:100.



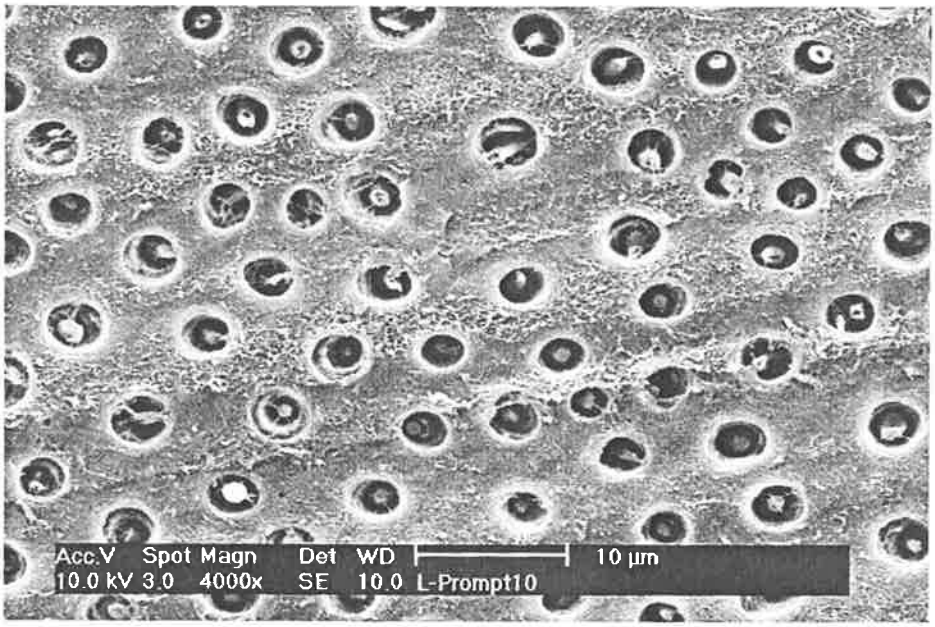
Group E: 03,01.

Debris 97:03; Collagen 162:100.



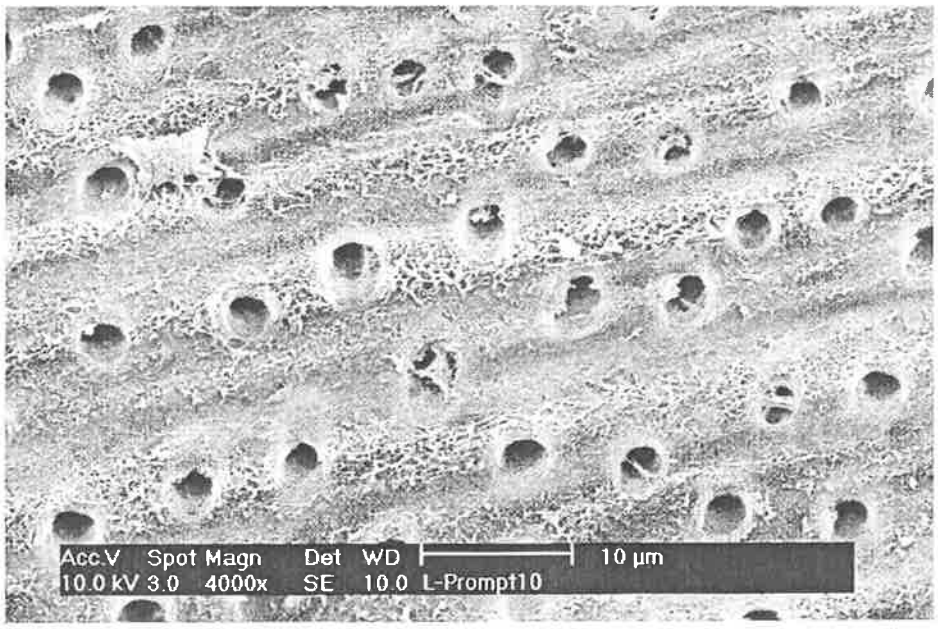
Group E: 03,02.

Debris 95:05; Collagen 135:100.



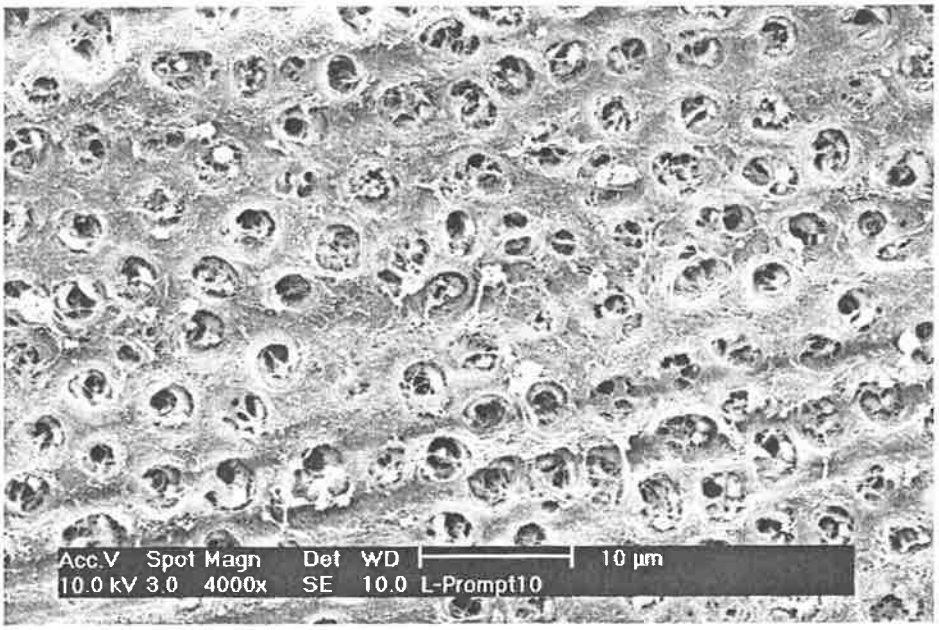
Group E: 03,03.

Debris 100:0; Collagen 155:100.



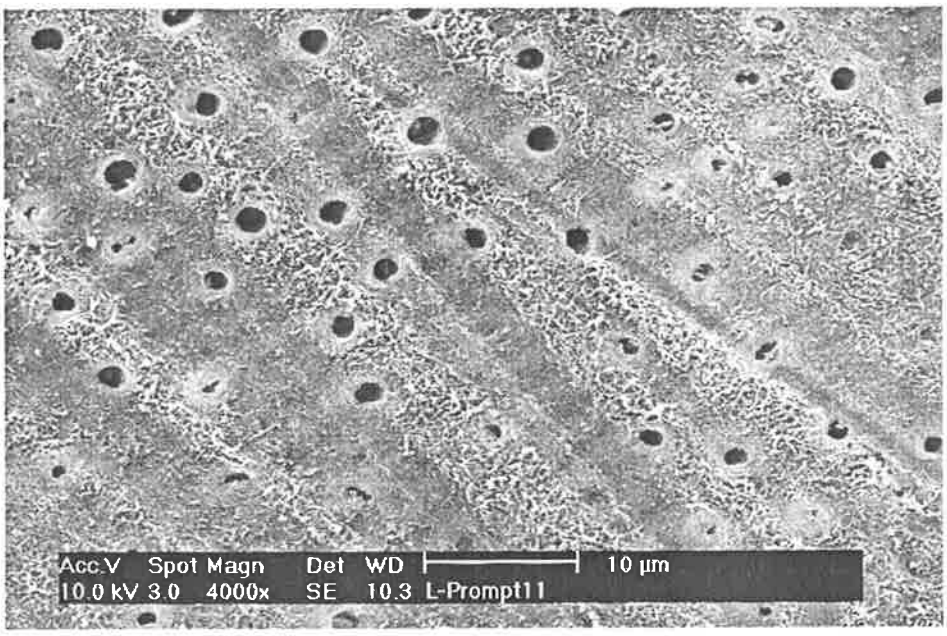
Group E: 03,04.

Debris 100:0; Collagen 173:100.



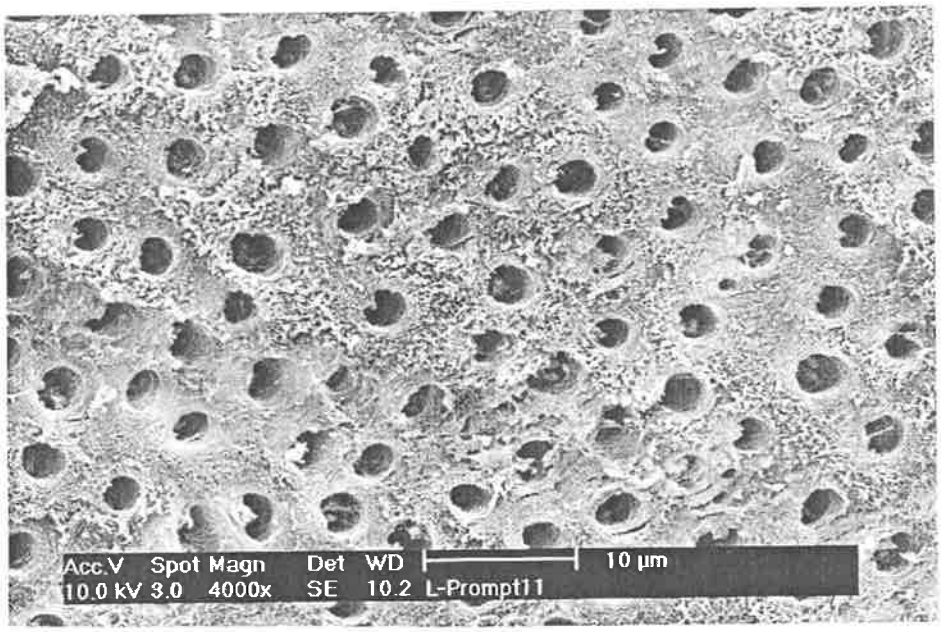
Group E: 03,05.

Debris 88:12; Collagen 106:100.



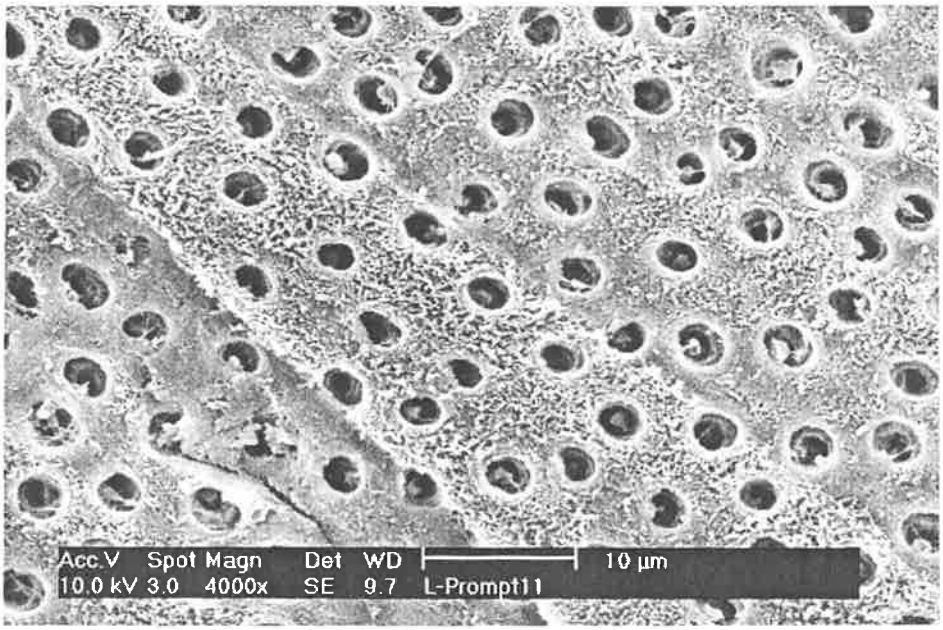
Group E: 04,01.

Debris 99:01; Collagen 194:100.



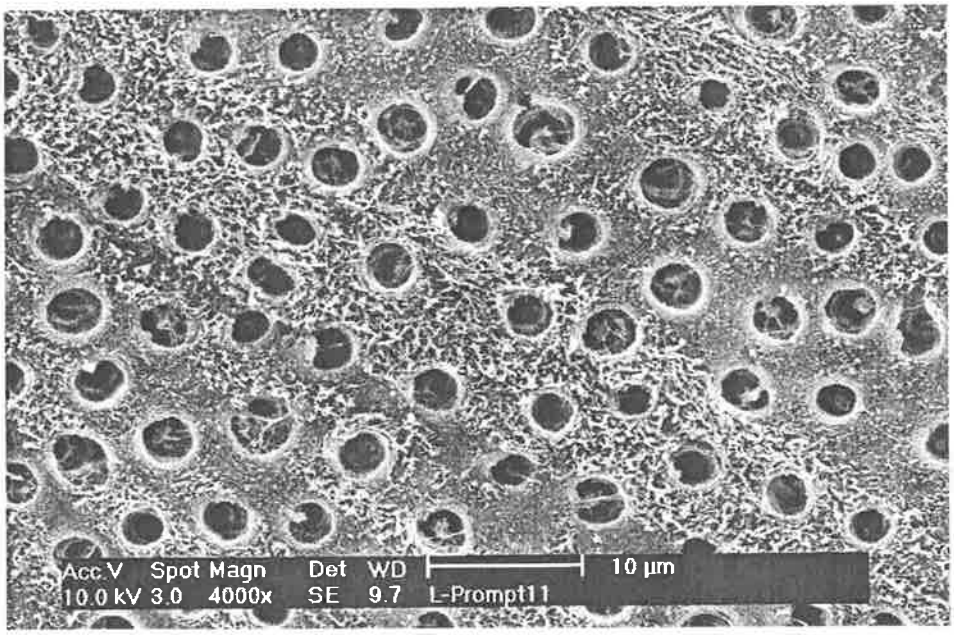
Group E: 04,02.

Debris 96:04; Collagen 153:100.



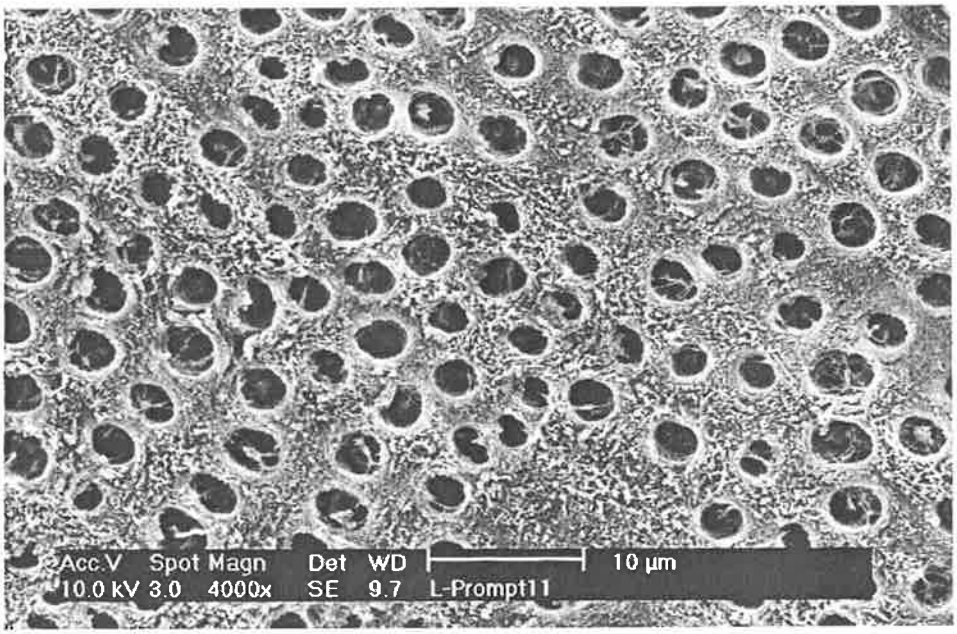
Group E: 04,03.

Debris 100:0; Collagen 192:100.



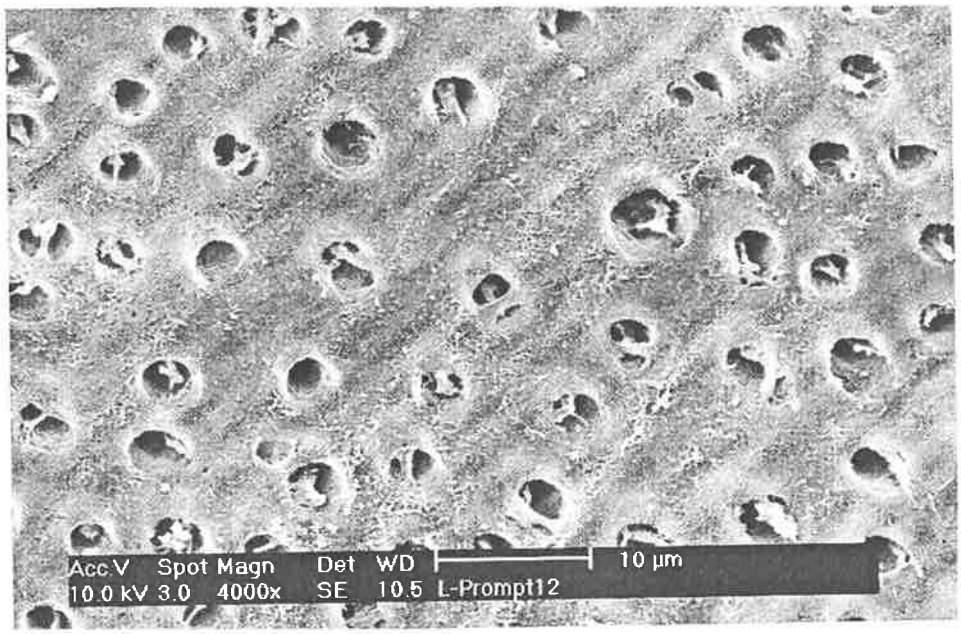
Group E: 04,04.

Debris 100:0; Collagen 213:100.



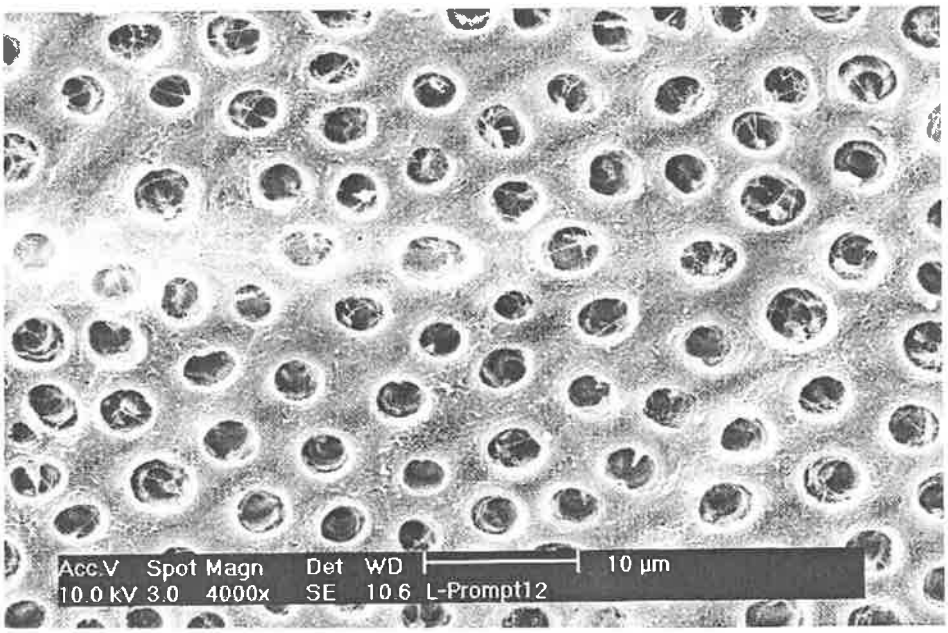
Group E: 04,05.

Debris 100:0; Collagen 183:100.



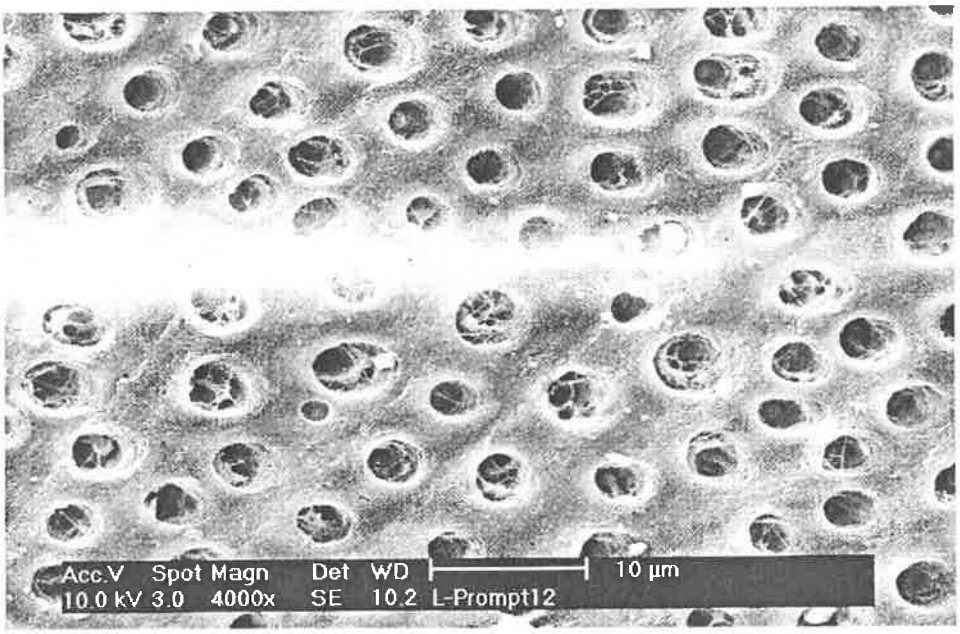
Group E: 05,01.

Debris 98:02; Collagen 113:100.



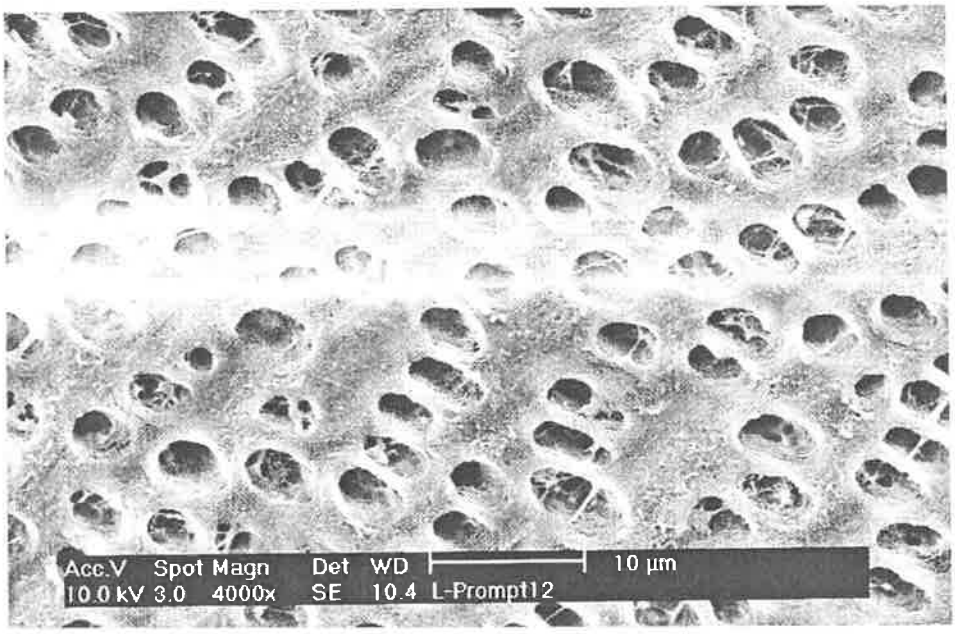
Group E: 05,02.

Debris 100:0; Collagen 109:100.



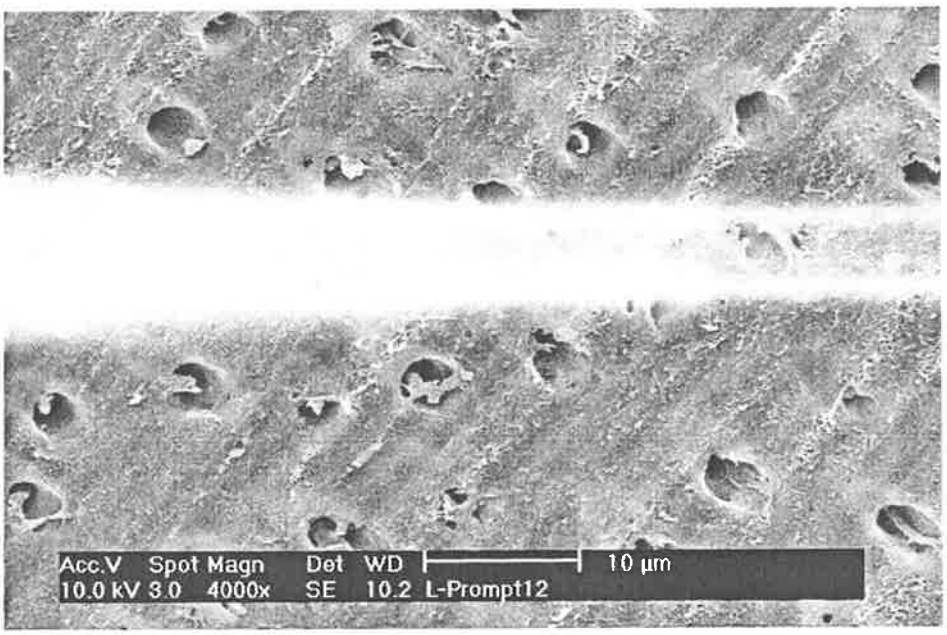
Group E: 05,03.

Debris 94:06; Collagen 100:100.



Group E: 05,04.

Debris 100:0; Collagen 100:100.



Group E: 05,05.

Debris 100:0; Collagen 121:100.