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TOOTH COLOR AND STRUCTURE CHANGES INDUCED BY TETRACYCLINE IN RAT.

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국문초록

테트라싸이클린에 의한 백서 치아의 색상 및 구조 변화

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테트라싸이클린에 의한 치아 변색은 심미성을 고려한 임상 치료시 커다란 난제로 남아 있다. 본 연구는 백서 치아에 테트라싸이클린을 주입하여 야기되는 색상과 구조적인 변화도를 측정하여 향후 연구에 기초자료를 제공하기 위해 시행되었다. 24 마리의 백서를 각기 8 마리씩 3 군으로 분류하여 대조군은 정상적인 사육을, 식염수군은 TC군과 동량의 식염수를 주사하였으며 TC군은 12 ml에 용해시킨 60 mg의 테트라싸이클린을 복강내로 2 주간 주입하였다. 최종 주입후 1 주간 정상 사육한 다음 cervical dislocation법에 의해 희생시켜 시편을 채득한 후 각 치아를 횡단하여 실물현미경과 FlexCam을 이용하여 컴퓨터에 영상을 저장한 다음 색변화량을 Adobe Photoshop으로 측정하였으며 구조변화는 주사전자 현미경을 이용하였다. 치아색 변화량의 유의성 검정은 ANOVA와 Scheffe test를 이용하였으며 본 실험 결과는 다음과 같다.

1. 각 군의 a* 수치는 대조군 -6.12 (1.18), 식염수군 -8.00 (1.33), TC군 -18.56 (2.52)로 나타났으며 b* 수치는 대조군 1.12 (2.13), 식염수군 1.62 (1.45), TC군 28.67 (5.18)을 보였다. 즉 테트라싸이클린에 의해 a*와 b*의 수치가 유의성있게 ($p<0.01$) 초록색과 노란색 방향으로 변화하였다.
2. 테트라싸이클린에 의해 치경부 법랑질 외면이 검게 변색되었으며 울퉁불퉁한 양태로 변화되었다. 그러나 법랑소주의 형태, 방향등은 차이를 보이지 않았다.
3. 각 군간 상아세관의 수, 방향, 크기등이 차이가 없었다. 치수측에서의 관찰결과 대조군과 식염수군에서는 상아세관들의 입구가 크고 명확하게 보인 반면 TC군에서는 입구가 좁고 부분적으로 막힌 양태도 관찰되었다.

I. Introduction

There are basically 2 types of teeth discoloration (caused by extrinsic factors and by intrinsic congenital or systemic factors). Discolored teeth not only made people unesthetic and also psychologically-deficient state. Various approaches including veneering and placement of esthetic crowns were done to overcome this problem, but these techniques need to cut down teeth and are apt to encroach the periodontal tissue. So bleaching techniques are becoming a more commonly used treatments. With proper case selection, careful diagnosis, treatment planning, and appropriate procedures, these bleaching techniques will provide esthetic results without harming teeth.

Tetracycline antibiotics discovered in 1948 had been used primarily for upper respiratory conditions. It was safe, effective, and less expensive than the penicillins but the deleterious side effect of teeth discoloration was incurred when ingested at a young age or by the patient's mother during pregnancy. This discoloration is shown predominantly in dentin and cementum and only slightly in enamel and the affected dentin exhibits incremental lines, which are visible in ground section under incandescent light[1].

H_2O_2 is a material of choice for bleaching due to the ability of oxidation of pigments[2]. Discoloration by tetracycline has been treated in 2 ways. One is Vital bleaching and the other is intentional nonvital approach. Several researchers[3-6] reported the results of vital bleaching of tetracycline discoloration, but the effects might be temporary and perhaps only a surface phenomenon. Another treatment of intentional internal bleaching was done and it was reported that the whitened color didn't regress after 4 years[7]. But with this method, the occurrence of the external resorption should be considered[8].

The purpose of this study is to assess the amount of change in color and structure by tetracycline and to offer a basis of further developed and more safe, new technique which we have to discover. Because of too small specimens to use current techniques, new trial was done to evaluate the color, that is, images of rat teeth were captured into a computer via stereomicroscope through FlexCam (VIDEOLABS$_{TM}$, Japan). Color spaces were determined with an image analysis system, Photoshop 3.0 (Adobe Systems Inc., USA).

II. Materials and methods

24 Albino male rats were used (wt. 350–400 gm). After obtaining rats, they were fed routinly for a week for their adaptation to new environment. They were divided into 3 groups of 8 rats each. The first group was served as a control with normal feeding and the second group was injected with 12 ml of saline. In TC group, 60 mg TC [Chlortetracycline (Teracyclin$_{cap.}$, ChongKeunDang, Korea)] dispersed in 12 ml of saline solution was peritoneally injected daily for 2 weeks. One week after the final injection, rats were sacrificed by cervical dislocation and teeth were extracted and coded in random two digit numbers. Eighteen rats were used in a test of color change and six rats were used in the evaluation of structural change.

Color change

After taking both maxilla and mandible, 4 anterior teeth (2 teeth in each jaw) were pull out. 2 samples were obtained in each tooth by horizontal sectioning in middle and gingival portion and were ground on 600 grit sandpaper to obtain a flat base. Samples were cleaned

in a ultrasonic bath and were storaged in a light-proof case to prevent color change by light. Sectioned images were examined with a stereomicroscope (x15) under uniform illumination and they were storaged into a computer (Pentium, P54C133) via image delivering unit, FlexCam.

Using an image analysis system, Photoshop 3.0 software, color space (L*, a*, b*) was investigated at 12 different sites nearby the pulp space and its mean value was taken as a representative one for each sample. An analysis of variance followed by a Tukey post-hoc evaluation and t-test were used to determine whether there were any differences among groups.

Change in tooth structure

Teeth were horizontally sectioned and ground flat for the examination of outer surface morphology. To evaluate the structural change in enamel and dentin, teeth were fractured longitudinally by tapping a pre-formed lingual groove. Samples were gold plated for observation in the scanning electron microscope (JSM-5200, JEOL, Japan).

III. Results

Color change

Representative photomicrograph is shown in Fig 1. Fig 1a shows the image of control group and Fig 1b corresponds to saline group. Teeth in these groups appeared to be white, but Fig 1c representing TC group looks more yellowish than Fig 1a and 1b.

Table 1 shows color space values of each group. The results in color space a* in Mean (S.D.) were : -6.12 (1.18) for control, -8.00 (1.33) for saline, and -18.56 (2.52) for TC. The color space b* were : 1.12 (2.13) for control, 1.62 (1.45) for saline, and 28.67 (5.18) for TC. The changes in color spaces for both a* and b* after the TC injection were statistically significant ($p<0.01$) (Table 2, 3, 4, 5). TC caused color changes for the direction of green and yellow hue. But the change of L* showed different pattern, namely, saline injection lightened teeth but teeth became dark by tetracycline injection. (Table 6, 7).

Comparing middle area with gingival area, there was no difference except only in color space a* of mandibular teeth in control group (Table 8, 9) and only in color space b* of

Table 1. Color space values(mean± S.D.)(n=12 per group)

Groups	Area		L*	a*	b*
Control	Maxilla	Middle	76.90± 2.34	-6.62± 0.87	0.08± 1.69
		Gingival	76.48± 3.23	-6.65± 0.65	-0.49± 0.84
	Maxilla	Middle	72.69± 2.28	-4.90± 1.00	2.32± 2.58
		Gingival	74.93± 4.03	-6.32± 1.27	2.55± 1.19
Saline	Maxilla	Middle	82.19± 3.45	-8.55± 1.12	2.05± 1.71
		Gingival	81.75± 1.97	-8.31± 1.39	0.92± 1.50
	Maxilla	Middle	79.91± 0.90	-7.53± 1.36	2.08± 1.03
		Gingival	80.96± 2.30	-7.60± 1.28	1.44± 1.37
TC	Maxilla	Middle	78.34± 2.87	-15.54± 2.14	25.58± 3.77
		Gingival	79.24± 2.66	-19.61± 1.24	28.59± 1.86
	Maxilla	Middle	78.53± 3.10	18.50± 1.91	28.99± 5.92
		Gingival	78.92± 2.67	20.59± 1.35	31.85± 6.17

Table 2. ANOVA test of a* among groups

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	4317.1789	2158.5895	6798.3087	0.0000
Within Groups	141	448.0453	3.1776		
Total	143	4765.2243			

Table 3. Tukey-HSD test for a*

Groups	TC	Saline	Control
TC			
Saline	*		
Control	*	*	

* : significance at 95% level

Table 4. ANOVA test of b* among groups

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	23860.5621	11930.2811	1069.5712	0.0000
Within Groups	141	1572.7514	11.1543		
Total	143	25433.3135			

Table 5. Tukey-HSD test for b*

Groups	Control	Saline	TC
Control			
Saline			
TC	*	*	

* : significance at 95% level

Table 6. ANOVA test of L* among groups

Source	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	2	860.2592	430.1296	48.1296	0.0000
Within Groups	141	1238.8131	8.7859		
Total	143	2099.0723			

Table 7. Tukey-HSD test for L*

Groups	Control	TC	Saline
Control			
TC	*		
Saline	*	*	

* : significance at 95% level

Table 8. t-tests for paired samples of maxillary middle and gingival sections in Control group

Variable	Paired Differences			t-value	df	2-tail Sig.
	Mean	SD	SE of Mean			
L*	0.4097	4.266	1.231	0.33	11	0.746
a*	0.0278	1.072	0.309	0.09	11	0.930
b*	0.5694	1.603	0.463	1.23	11	0.244

Table 9. t-tests for paired samples of maxillary middle and gingival sections in Control group

Variable	Paired Differences			t-value	df	2-tail Sig.
	Mean	SD	SE of Mean			
L*	-2.2431	3.584	1.035	-2.17	11	0.053
a*	1.4167	1.895	0.547	2.59	11	0.025*
b*	-0.2292	2.637	0.4761	-0.30	11	0.769

* : significance at 95% level

Table 10. t-tests for paired samples of maxillary middle and gingival sections in Saline group

Variable	Paired Differences			t-value	df	2-tail Sig.
	Mean	SD	SE of Mean			
L*	0.4375	3.778	1.091	0.40	11	0.696
a*	-0.2361	2.316	0.669	-0.35	11	0.731
b*	1.1319	1.437	0.415	2.73	11	0.020*

Table 11. t-tests for paired samples of maxillary middle and gingival sections in Saline group

Variable	Paired Differences			t-value	df	2-tail Sig.
	Mean	SD	SE of Mean			
L*	-1.0486	2.228	0.643	-1.63	11	0.131
a*	0.0625	1.220	0.352	0.18	11	0.862
b*	0.6320	1.620	0.468	1.35	11	0.204

* : significance at 95% level

Table 12. t-tests for paired samples of maxillary middle and gingival sections in TC group

Variable	Paired Differences			t-value	df	2-tail Sig.
	Mean	SD	SE of Mean			
L*	-9.9028	4.479	1.293	-0.70	11	0.500
a*	4.0694	2.654	0.766	5.31	11	0.000*
b*	-3.3125	4.776	1.379	-2.40	11	0.035*

* : significance at 95% level

Table 13. t-tests for paired samples of maxillary middle and gingival sections in TC group

Variable	Paired Differences			t-	df	2-tail
	Mean	SD	SE of Mean	value		Sig.
L*	-0.3889	4.462	1.288	-0.30	11	0.768
a*	2.0903	2.809	0.811	2.58	11	0.026*
b*	-0.8472	9.873	2.850	-1.00	11	0.339

* : significance at 95% level

maxillary teeth in saline group (Table 10, 11). But with TC injection, greater difference was observed between middle and gingival portions : gingival areas appeared to be more greenish and yellowish than middle areas except color space b* of mandibular teeth, but similar pattern could be seen in that comparison, even if there was no significant difference (Table 12, 13).

Change in tooth structure

In a preliminary stereomicroscopic view, it was known that the gingival portion of labial enamel surface in TC group (Fig. 2c) was dark and different from the other samples (Fig. 2a, 2b). From SEM images, it was revealed that the outer gingival surfaces of control and saline groups (Fig. 3a, 3b) were relatively smooth, but those in TC group were uneven with an impression of melting/fusion (Fig. 3c). But similar less irregular pattern was seen in middle portion regardless of groups.

Fractured specimens were used for examining the inner enamel and dentin structure. Enamel was composed of outermost amorphous, aprismatic layer and inner prismatic layer (Fig. 4). All groups showed a regular pattern of rod orientation with no specific difference in enamel area (Fig 5a, 5b, 5c).

Dentinal area was evaluated in two ways, one was taken from pulpal side and the other was from fractured side. No specific differences were noted in number, direction, and dimension of the dentinal tubules among groups from fractured side-view (Fig. 6a, 6b, 6c). In pulpal-side view, control and saline groups showed clear, definite tubule openings (Fig. 7a, 7b) but in TC group, less openings and a portion of amorphous area were seen(Fig. 8a, 8b).

Discussion

In modern society, physical appearance influences one's impression of and reaction to others and one may fall into a psychotoxic state due to one's smile and figure. So there are increasing numbers of patients who recognize the importance of an attractive smile and are interested in procedures that can improve their looks.

There are basically 2 types of teeth discoloration. One is attacked by extrinsic factors such as coffee, tea, smoking etc. and the other is brought about by intrinsic congenital or systemic influences. Various approaches have been done to overcome this problem such as veneering and cosmetic crowns, but these techniques need to cut down teeth and are apt to encroach the periodontal tissue. So more conservative bleaching techniques are becoming a more commonly used treatments in the dental office. Bleaching techniques are far superior to any prosthetic treatments.

But all of the teeth discoloration couldn't be corrected with only these bleaching techniques. One of the most distressing and treat-

ment-ineffective discoloration is that from tetracycline. These antibiotics discovered in 1948 have long been used primarily for upper respiratory conditions, but it was noted that the deleterious side effect of teeth discoloration was incurred when ingested at a young age or by the patient's mother during pregnancy. Schwachman[9] first reported this phenomenon and the FDA issued a warning about the dental staining properties of TC given during tooth formation, usually before the age of eight years.

Because of dentin's greater absorbance of TC due to larger surface area of dentin apatite crystals than that of enamel apatite crystals[10], this discoloration is seen predominantly in dentin and cementum and only slightly in enamel[1]. It is believed that the tetracycline fluorophore is deposited in calcifying teeth and bone in a calcium complex of the inorganic portion of tissues and Mello[11] found that TC chelates with calcium at the hydroxylapatite surface of mineralizing dentin to form tetracycline-orthophosphate which results in tooth discoloration. Although the TC absorbed in bone can be released through normal bone remodeling, but that deposited in enamel or dentin is permanently positioned. It is believed this released TC from bone would be free to reenter the circulation and be incorporated in other dentin[12].

The color and severity of stain depend on several factors, including the type of drug[13], dosage, time of administration, duration, and route of administration. The resultant color varies from yellow to brown to gray to blue-black. From the results of this study, color space a* value changed from -6.12 ± 1.18 to -8.00 ± 1.33 by saline injection and to -18.56 ± 2.52 by TC injection. In addition, color space b* value also changed from 1.12 ± 2.13 to 1.62 ± 1.45 by saline and to 28.67 ± 5.18 by tetracycline. This corresponds to a fact that the color of rat teeth changed into the direction of green and yellow hue by TC. Feinman[14] established a classification system for varying degrees of TC discoloration and the success potential by bleaching. Because the samples in this study were cross-sectioned ones, it is impossible to classify them according to this system, if we assort the discoloration of rat teeth by TC, it may belong to the 1st degree. If much more dose could be injected, the result would be different, but in a preliminary study, it was known that unfortunately rat couldn't endure that dose.

Characteristically, yellow teeth by TC fluoresce when exposed to UV light and this yellow color changes gradually darken to brown or gray-brown. As the color changes, tooth loses its fluorescence. This is thought to be due to the degradation of TC, which is accelerated by exposure to sunlight[15]. So the anterior teeth are usually the first to darken, while the more protected posterior teeth retain their yellow color for longer periods. Others have reported that exposure to sunlight can cause continued darkening of some TC-stained teeth, depending on the type of drug[16] or its photooxidation by-product[17].

H_2O_2 is a material of choice for bleaching due to the oxidation potential of pigments[2,18] and it flows freely through enamel and dentin [19,20] because of the relatively low molecular weight (30g/mol)[21]. These discolored teeth by TC has been treated in 2 ways. One is vital bleaching and the other is intentional nonvital approach. Many scientists[3-6] studied vital bleaching technique, but the results might be temporary and perhaps only a surface phenomenon. Another test of intentional internal bleaching("walking bleaching") was done[7] and it was reported that the bleaching effect persisted after 4 years. But with this method,

the cases of external resorption were noted by Harrington et al[22] and Madison et al[8]. Although the occurrence of external resorption is not common and can be prevented with the use of calcium hydroxide, it'll be better if we can solve this type of discoloration with an vital bleaching technique.

In this study, the amount of change in color space and tooth structure was evaluated to offer a basis for further research about discoloration from TC. Several methods, such as using naked eyes[23], Colorimeter[24], and spectrophotometer[25] are being used for color evaluation. In general, instrumental measurements are more objective procedures that eliminate the potential for human bias and variability. But in this study, the sample of rat teeth is too small and can't be evaluated easily by naked eyes and Colorimeter. If the photograph was taken and estimated by the spectrophotometer, a lot of errors would be incurred by much more experimental steps, so its image was magnified under a stereomicroscope (x 15) and was input directly into a computer via FlexCam and the color space L*, a*, b* was checked by the image analysis system, Photoshop 3.0.

L*, a*, b* color space was established by the Commission Internationale de L'Eclairage in 1978 and this CIELAB system is related to human color perception in all three dimensions or directions of color space. Equal distances in the color space represent approximately equally perceived steps. L* represents the degree of gray and corresponds to value or brightness and a* ia a hue-chroma parameter in the red-green direction and b* is in the blue-yellow axis. High L* value means a bright or white specimen. Positive a* values are red while negative values are green and positive b* values are yellow while negative values are blue. Color space influences each other, for example, a change in the a* or b* direction can also result in a shift in the L* direction. In this study, the color space L* values increased with saline injection (from 75.25 ± 3.58 to 81.20 ± 2.43), but lower values were noted by TC injection (78.40 ± 3.83). It may be the result of discoloration by TC, that is, more green and yellow hue may influence the lowering of the L* values.

Structural changes associated with TC were reported[26] and it was noted that matrix formation and initial enamel mineralization would be hindered too[27]. In this study, dark, tortuous, fusion-like pattern was seen in the outer gingival surface of TC group (Fig. 2c, 3c), which is different from those of other control (Fig. 2a, 3a) and saline groups (Fig. 2b, 3b). It was considered that this might be the result of disturbance from TC in the process of tooth formation. Fractured specimens were used for examining the inner enamel and dentin structure. Rat enamel was composed of outermost amorphous, aprismatic layer and inner prismatic layer like human (Fig. 4). There was no specific difference in enamel area among groups (Fig 5a, 5b, 5c). In dentinal area, no specific differences were noted in number, direction, and dimension of the dentinal tubules among groups from fractured side-view (Fig. 6a, 6b, 6c). In pulpal-side view, less openings and a portion of amorphous area were seen in TC group (Fig. 8a, 8b) as compared with clear, definite openings in control and saline groups (Fig. 7a, 7b). It's not clear whether these aspects are correlated with bleaching effect now, this should be revealed from further studies. These enamel and dentinal figures were not consistent with the other report [28]. This disagreement might be made from a different type of drug and dosage.

As previously stated, new technique was used in this study because of the difficulties

in using current techniques, but it is thought that this method also has some disadvantages which are to be corrected. At first, it is impossible to use the standardized light source. Secondly, spoid tool in Photoshop could catch only 5 x 5 pixels area, mean color space value of each sample had to be calculated from the data obtained at several sites. Finally, the data couldn't be input into a computer automatically, it needs lots of time and labor. But it might be deduced this technique is another helping mean to the study about discoloration. In addition, a great deal of efforts should be done to develop new, ideal bleaching agents and techniques that could overcome the flaws of the current methods.

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EXPLANATION OF FIGURES

Fig. 1. Representative photographs. Tooth appeared to be white in 1a (control group) and 1b (saline group), but 1c (TC group) looks more yellowish than 1a and 1b.

Fig. 2. Photograph representing gingival area of outer enamel surface. 2c (TC group) is darker than 2a (control group) and 2b (saline group).

Fig. 3. SEM images showing Fig. 2. Surfaces of 3a (control group) and 3b (saline group) are relatively smooth, but uneven figure is seen in 3c (TC group) with an impression of melting/fusion.

Fig. 4. Enamel structure of rat tooth. Outermost amorphous, aprismatic layer (b) and inner prismatic layer (a).

Fig. 5. Photograph representing inner prismatic layer. All groups show a regular pattern of rod orientation with no specific difference. (a) : control group, (b) : saline group, (c) : TC group.

Fig. 6. Dentin area (fractured-side view). No differences in number, direction, and dimension of the dentinal tubules among groups. (a) : control group, (b) : saline group, (c) : TC group.

Fig. 7. Dentin area (pulpal-side view). Clear, definite openings are seen in 7a (control group) and 7b (saline group).

Fig. 8. In TC group, less openings (8a) and a portion of amorphous roof area (8b) can be seen.

Fig. 1

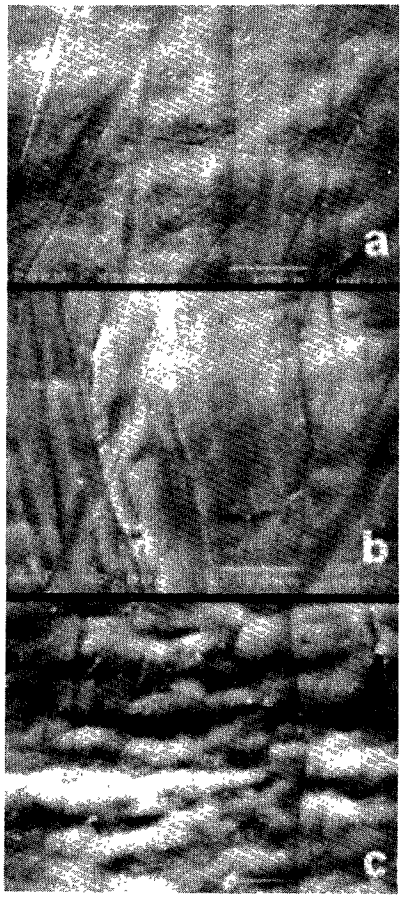

Fig. 3

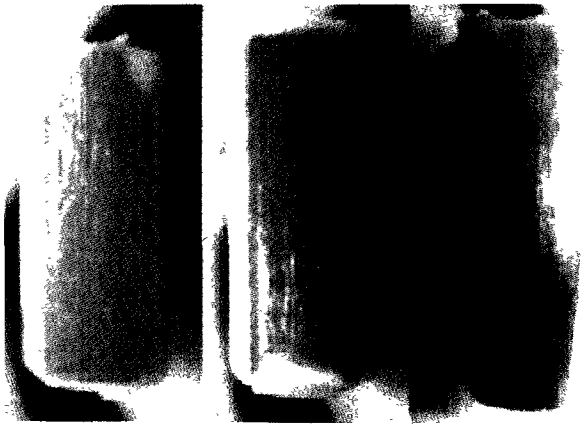

Fig. 2

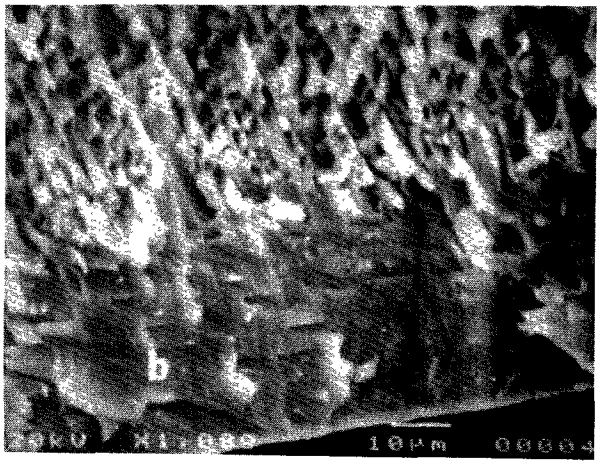

Fig. 4

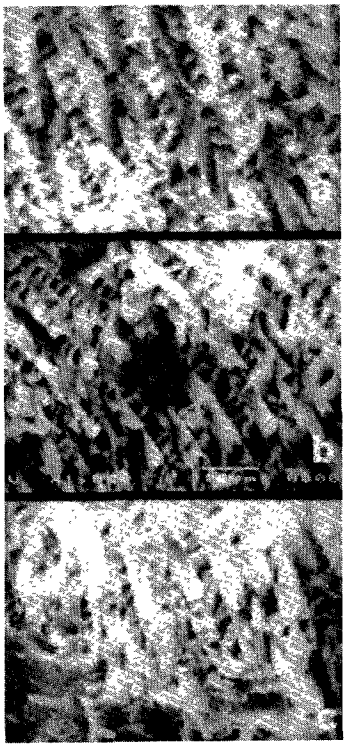

Fig. 5

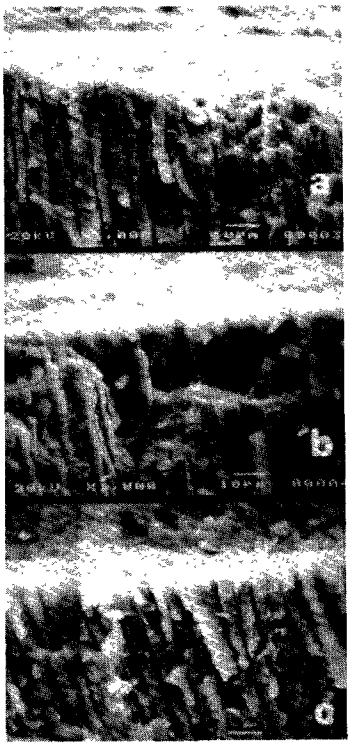

Fig. 6

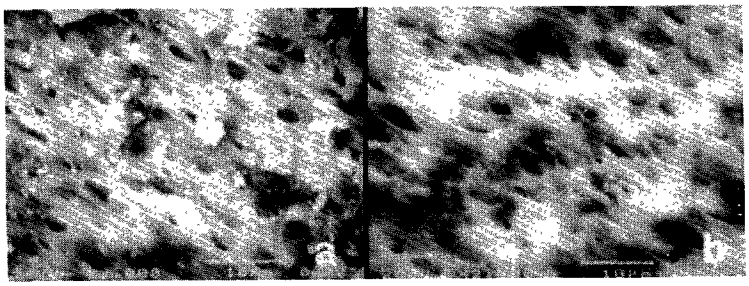

Fig. 7

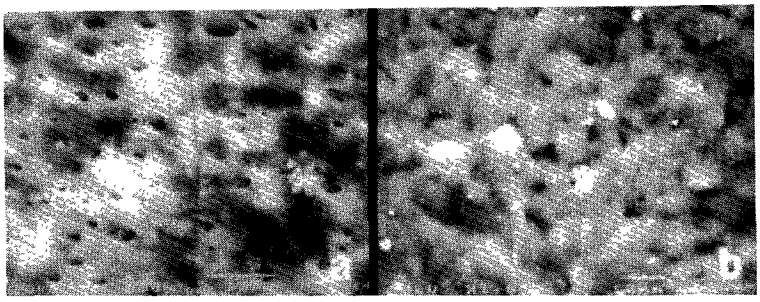

Fig. 8