The Effect of Hydroxyapatite on the Remineralization of Dental Fissure Sealant.

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감사의 글

논문이 완성되기까지 자상하게 지도해주시고 격려해주신 최형준 지도 교수님께 감사를 드리며, 실험설계를 처음부터 끝까지 도와주신 이용근 교수님과 애정어린 관심과 조언을 아끼지 않으신 최병재 교수님께 감사드립니다. 아울러 관심있게 지켜봐 주신 이종갑 교수님, 손흥규 교수님, 이제호 교수님, 김성오 교수님께 감사드리며, 실험의 기술적인 부분을 세심하게 도와주신 치과재료학교실 여러분들께도 깊은 감사를 드립니다.

계속되는 실험 속에 용기를 준 소아치과 의국원 모두에게 감사드리며, 특히 실험실을 그림자처럼 함께한 윤상일 선생님, 논문의 완성에 좋은 의견을 제시해준 문성환 선생님, 홍은경 선생님, 고동현 선생님, 류정아 선생님 그리고, 삼성병원의 김학수 선생님께 감사드립니다.

끝으로 한결같은 사랑으로 돌보아 주시며 늘 저의 빈자리를 채워주시는 부모님께 깊은 감사를 드립니다. 소중한 동생 희성과 10년을 넘게 항상 옆에서 든든한 후원자가 되어주었던 친구들 자옥, 찬민, 준홍, 우진, 세훈에게도 깊은 감사의 뜻을 전합니다.

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Abstract

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The purpose of this study was to investigate the remineralization of enamel in the human tooth by fissure sealant containing various amount of hydroxyapatite. Prior to remineralization experiments, the necessary requirements of the dental fissure sealant, the curing depth and the curing time, were measured with the content of the hydroxyapatite according to the standard of ISO 6874(international organization for standardization,1988). Various amount of hydroxyapatite was mixed uniformly using sonicator up to 20wt% to the fissure sealant. In spite both the curing time and the curing depth were decreased with increasing the content of hydroxyapatite, all samples were satisfied the ISO requirements. Experimental remineralization samples were produced by bonding fissure sealant containing various amount of hydroxyapatite to human tooth enamel using manufacturer's information. After immersion to the simulated body fluid(SBF) at 36.5^{o}C for 4 weeks, the bonding strength and the

surface morphology were examined using Instron and scanning electronic microscope, respectively. The bonding strength between the fissure sealant and the human teeth was drastically enhanced with the amount of hydroxyapatite. The remineralization zone could be observed along with the boundary of hydroxyapatite and fissure sealant using a scanning electronic microscope.

According to the result, we can predict the adhesion between the fissure sealant and tooth was enhanced.

In conclusion,

1. The curing time of the fissure sealant was decreased with increasing HA content. More than 10wt% of HA showed significant difference ($P<0.05$). The curing time was in the range of ISO standard which did not affect the physical characteristics.

2. The curing depth of the fissure sealant was decreased slightly with increasing HA content, however, there was no significant difference ($P>0.05$). The curing depth was in the range of ISO standard which did not affect the physical characteristics.

3. As the content of HA increases, the bonding strength between the sealant and the tooth surface tends to show an increase. More than 5wt% of HA showed significant difference ($P<0.05$).

4. Under SBF, HA in the sealant showed a remineralization effect in the interface with the calcium phosphate layer.

If the new hydroxyapatite composite sealant was treated in adequate time, the longevity and caries prevention would be improved.

In addition to the bonding strength of the sealant containing the hydroxyapatite, longterm observation and an assessment of the microleakage would support this study.

Keywords: hydroxyapatite, remineralization, dental fissure sealant, bonding strength

The Effect of Hydroxyapatite on the Remineralization of Dental Fissure Sealant.

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(Directed by professor Hyung Jun Choi, DDS, PhD)

I. Introduction

The main component of fissure sealant is Bis-GMA resin. Fissure sealant, a dental esthetic material, is used for caries prevention. However, in many cases, fissure sealant is fallen out because of the microleakage between the fissure sealant and tooth. Bacterial invasion is produced through the microleakage and then the secondary dental caries is produced(Hembree, 1986). Many clinicians try to minimize the microleakage in dental esthetic restoration in order to prevent the dental caries.

Hydroxyapatite(HA) is a major inorganic component of hard tissue in the human body. Synthetic HA finds many applications, especially as

a biomaterials. Among them, the most important ones are as adsorbents for proteins and enzymes and for artificial teeth and bones(H. Tanaka, 1999). Several polymer-hydroxyapatite composites have been developed as bone cements, dental implants or bone substitute material (N. Ignjatovic, 1999).

The term “enamel remineralization” has been used such as enamel repair, rehardening, mineral deposition, an improved acid resistance and a decreased brushing abrasion (K. Collys , 1993).

HA is known to remineralize the tooth when applied to the enamel surface. On the basis of this, HA was added to the fissure sealant to induce the remineralization effect between the enamel interface, thereby eliminating the microleakage and enhancing the bonding strength.

To provide an environment similar to intraoral, test specimens were immersed at 36.5˚C similar to body temperature in simulated body fluid(SBF) which has a composition as saliva for 4 weeks. The Ca ions and the P ions in the saliva have a great solubility and therefore mineralization and remineralization take place easily. The previous studies implicated that the action of Ca or P ions in the saliva, assist the HA particles to undergo remineralization and become one body with

the surrounding enamel.

For this study, the remineralization effect can be expected to improve the adhesion between dental material and human tooth. The purpose of this study is to investigate the remineralization of enamel in the human tooth by the fissure sealant mixed with various amount of HA.

II. Materials and Methods

1. Materials

Commercially available dental fissure sealant and HA were purchased to prepare the composite of fissure sealant and HA. Concise™ (3M/ESPE, USA) was selected in this study with a light curing source (XL 3000, 3M / ESPE, USA).

Calcium phosphate tribasic(Sigma-Aldrich Inc., USA) was selected in this study as HA. It's Molecular fomula is $Ca_5(OH)(PO4)_3$ and Molecular weight is 502.3.

To provide an environment similar to intraoral, test specimens were maintained at 36.5˚C similar to body temperature in simulated body fluid(SBF) which has a composition as saliva.

Fig. 1. Pit & fissure sealant (Concise^{TM}) used in this study.

Fig. 2. Light curing XL 3000 device used in this study.

Fig. 3. Calcium phosphate tribasic used in this study.

Table 1. The components of SBF in this study.

List	Material	X1 (1L)	X1 (500ml)	X1.5 (1L)	X2 (1L)
1	NaCl	7.996	3.998	11.994	15.992
2	$NaHCO_3$	0.35	0.175	0.525	0.7
3	KCl	0.224	0.112	0.336	0.448
4	$K_2HPO_4.3H_2O$	0.174	0.087	0.261	0.348
5	$MgCl_2.6H_2O$	0.305	0.1525	0.4575	0.61
6	1M-HCl	40ml	20	40	40
7	$CaCl_2$	0.278	0.139	0.417	0.556
8	Na_2SO_4	0.071	0.0355	0.1065	0.142
9	$NH_2C(CH_2OH)_3$	6.057	3.0285	9.0855	12.114

2. Methods

1) Producing the sealant containing the hydroxyapatite

Various amount of HA was mixed uniformly using sonicator up to 20 wt% to the fissure sealant. For equal distribution of the mixed HA, Sonicator (SH-2100, Saehan, Korea) was used for sonication. Air bubble was completely removed in a vacuum oven below 50˚C. The pure Concise™ was used as the control.

Table 2. Sample identification of sealants in this study.

Sample I.D	Sealant	wt% of HA
Control	Concise™	0
HA-1	Concise™	1
HA-5	Concise™	5
HA-10	Concise™	10
HA-15	Concise™	15
HA-20	Concise™	20

2) Curing time

The curing time was determined as a necessary requirement of the dental fissure sealant in accordance with ISO 6874.

Place the sealant, prepared in accordance with the manufacturer's instructions, in the mould and maintain the apparatus at $(37 \pm 1)^{o}C$. Take care to exude air bubbles and slightly overfill the mould. Press the mould and a strip of the film under a microscope slide to exude excess material. Remove the microscope slide, leaving the film in place, and gently place the exit window of the energy source against the film. Irradiate the sealant for 20 seconds that recommended by the manufacturer. Record the period from the time when the energy source is turned on to the time when the peak temperature occurs. Repeat this procedure four more times and calculate the mean of the five determinations as the curing time.

The curing time shall not exceed that stated by the manufacturer or 60s, whichever is the lesser.

3) Curing depth

The curing depth was determined as a necessary requirement of the dental fissure sealant in accordance with ISO 6874.

After completing the procedure of the curing time, remove the sealant from the mould. Remove the uncured surface film from the top and bottom of each specimen by wiping with a tissue. Using the micrometer, measure the height of the test specimen. Record this height as the curing depth. Carry out determinations on five test specimens.

The curing depth shall be not less than 1.5mm.

4) Bonding strength

Total 80 specimens were prepared for the measurement of the bonding strength between fissure sealant and the human teeth. Experimental specimens were produced by bonding fissure sealant containing various amount of HA to human tooth enamel using manufacturer's information. The polyethylene tube (diameter - 4mm) was used to bond the fissure sealant to the enamel. All specimens were immersed into the simulated body fluid at 36.5^{o}C for 4 weeks. The bonding strength was determined using universal testing machine (Instron, UK).

Fig. 4. Samples for the investigation of bonding strength.

5) Surface observation

The surface and cross-section of the specimens were observed using SEM (S 2000, Hitachi, Japan).

6) Statistical evaluation

The statistical significant differences were analyzed by Mann-Whitney U test with the level of significance at $P<0.05$.

Data were summarized as median and range.

III. Result

1. Curing time

The curing time of the fissure sealant was decreased with increasing HA content (Table 3, Fig. 5).

More than 10% of HA showed significant difference ($P<0.05$).

All the samples containing any amount of HA were satisfied the requirement of ISO 6874 curing time.

Table 3. Curing time of sealants incorporated with different amount of hydroxyapatite.

Run	Curing time(s)					
	Control	HA-1	HA-5	HA-10	HA-15	HA-20
1	19.45	19.2	19.12	18.56	18.74	19.24
2	18.94	18.95	19.1	18.24	18.97	17.37
3	19.32	18.43	18.77	17.45	18.42	18.32
4	19.4	19.62	19.21	18.56	17.41	18.47
5	18.72	18.51	19.1	18.67	17.67	18.24
Average	19.166	18.942	19.06	18.296	18.242	18.328

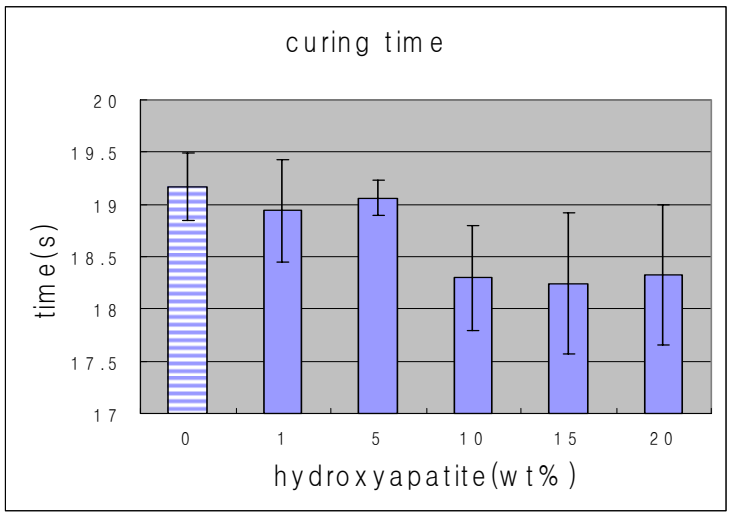


Fig. 5. Curing time of sealants incorporated with different amount of hydroxyapatite.

2. Curing depth

The curing depth of the fissure sealant was decreased slightly with increasing HA content, however, there was no significant difference ($P>0.05$) (Table 4, Fig. 6).

All the samples containing any amount of HA satisfied the requirement of ISO 6874 curing depth.

Table 4. Curing depth of sealants incorporated with different amount of hydroxyapatite.

Run	Curing depth(mm)					
	Control	HA-1	HA-5	HA-10	HA-15	HA-20
1	7.32	7.1	6.85	6.52	6.12	5.78
2	7.02	6.92	7.01	6.43	6.14	5.47
3	7.13	7.01	6.97	6.57	6.02	5.97
4	7.34	6.98	6.78	6.74	5.97	5.64
5	7.27	6.89	6.71	6.34	6.21	5.63
Average	7.216	6.98	6.864	6.52	6.092	5.698

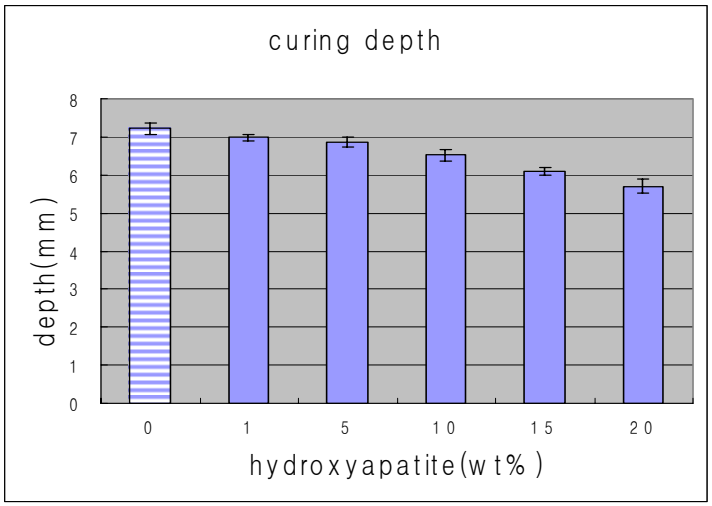


Fig. 6. Curing depth of sealants incorporated with different amount of hydroxyapatite.

3. Bonding strength

The bonding strength was drastically increased with increasing HA content (Table 5, Fig. 7).

More than 5% of HA showed significant difference ($P<0.05$).

The maximum strength reached to 10.08 MPa when 20% of HA, which is 17% higher than the control.

Table 5. Bonding strength of the enamel and sealants incorporated with different amount of hydroxyapatite.

Run	Bonding strength (MPa)					
	Control	HA-1	HA-5	HA-10	HA-15	HA-20
1	8.62	8.55	9.00	9.50	9.75	10.30
2	8.42	8.38	9.18	9.84	9.80	10.00
3	8.79	8.62	9.29	9.43	9.59	9.85
4	8.18	8.71	9.23	9.19	9.46	9.98
5	9.01	8.79	8.98	9.47	9.80	10.30
Average	8.60	8.61	9.14	9.49	9.68	10.08

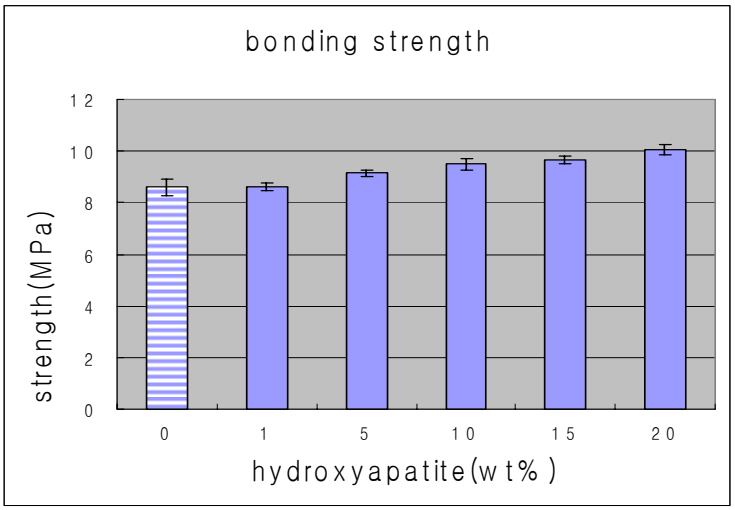
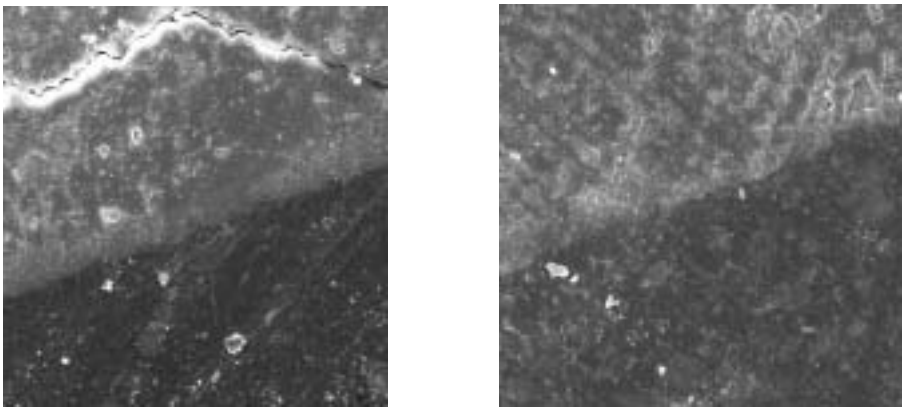


Fig. 7. Bonding strength of the enamel and sealants incorporated with different amount of hydroxyapatite.

4. Microstructure

According to the observation by SEM, the remineralized zone could be observed along with the interface between HA and fissure sealant, while there was no sign of remineralization in the control group(Fig. 8). This remineralized zone could be seen more clearly as the amount of HA increased. Under SEM, an amorphous white zone was seen in the interface between the tooth and the sealant. This interface was not distinct but it seemed to be the remineralization zone.

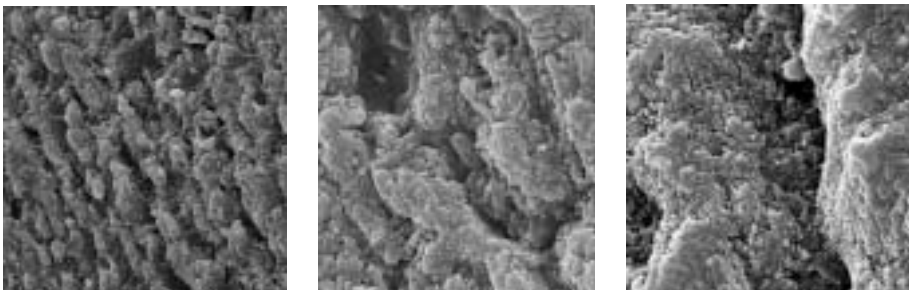


(a) **(b)**

Fig. 8. Interface between the tooth and the sealant by SEM (×1000).

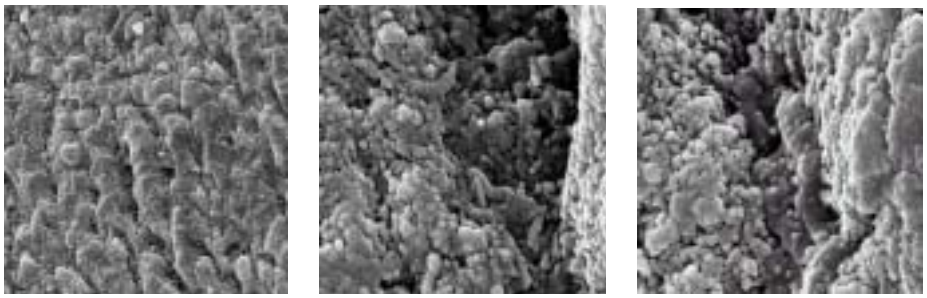
(a) experimental group – HA 20 wt% (b) control group

Under SEM, it seemed to be more recrystalization of HA in enamel surface of experimental group compared with that of the control(Fig 9, 10).



(a)(×2000) **(b)(×10000)** **(c)(×20000)**

Fig. 9. Enamel surface of control group by SEM.



(a) (×2000) **(b) (×10000)** **(c) (×20000)**

Fig. 10. Enamel surface of experimental group–HA 20wt% by SEM.

IV. Discussion

Many clinicians have used fissure sealant to prevent dental caries, however, in many cases the microleakage occurred between tooth enamel and fissure sealant because of the low physical property and weak bonding strength of fissure sealant.

Recently the filled sealant was developed to improve the physical property and bonding strength. But increase in filler content led to decrease in flowability, inconvenience of clinical application, and there were several studies reporting that filler content doesn't even make much difference in the bonding strength. (Marcushamer, 1997)

The filler content of filled sealant on market ranged from 45 to 55 wt%. In this study, HA was added up to 20 wt%.

In previous study (Atwan; Sullivan 1987), the bonding strength of unfilled sealant ranged from 4.74 to 6.074 MPa.

In this study, the bonding strength of the control group was 8.60 MPa, while that of HA-20wt% group was 10.07 MPa which is 17% higher than the former. That means, in this study, increased bonding strength was obtained with less content of filler than the filled sealant on market.

HA is well-known bioactive material and the component of the bone

and the tooth. It is neutral, and its crystal structure is stable in the body due to its stability in alkaline solution. It has high compressive strength and no toxicity(M. Saito, 1994).

The previous studies showed that the HA particles become remineralized by the Ca or P ions in the saliva, and become one body with tooth enamel. HA has the optimal condition to be used as filler material(G. Willems, 1993).

The use of HA in restorative dentistry offers several promising advantages, including intrinsic radio-opaque response, enhanced polishability, and improved wear performance, mechanical property (Labella R, 1994).

As a filler component in polymer-based composites, amorphous calcium phosphate has been shown to be an effective agent for remineralizing in vitro caries-like enamel lesions artificially induced in extraced bovine incisors(Antonucci et al.,1995).

Amorphous calcium phosphate was proposed as the most suitable filler for use as a remineralizing agent in sealant, adhesive, base and lining materials where protection against mineral loss and restoration of mineral in dental tissues would be desirable(Skrtic et al.,1995).

Bioactivity is defined as the ability of a material to generate a surface

apatite layer that provides the bonding interface with tissues.

It is well knowen that, in vitro, ionic exhange at the interface between calcium phosphates and SBF leads to the formation of surface apatite(L.L.Hench, 1996).

In vitro study, Bis-GMA containing composite exhibited formulated silanized HA filler revealed that composite forms a calcium phosphate layer on its surface in SBF(C.Santos, 2001).

In this study, HA was added to a commercial fissure sealant Concise™ up to 20 wt%. To find out the physical characteristics of this newly made sealant, the curing time, curing depth and the bonding strength was compared with the control. And the remineralization effect of HA on the enamel surface was observed under SEM.

According to the result, we can predict that the adhesion between the fissure sealant and tooth was enhanced.

The new composite sealant has a remineralization effect against microleakage and bacterial invasion. The new composite sealant can challenge the concept of dental caries prevention by enhancing the adhesion of sealant and tooth.

If the new HA composite sealant was treated in adequate time, that of the longevity and caries prevention would be improved.

In addition to the bonding strength of the sealant containing the HA, longterm observation, an assessment of the microleakage with a dye and assessment of the mineral density profile with Microradiographs would support this study.

V. Conclusion

In order to investigate the remineralization of enamel in the human tooth by the fissure sealant mixed with various amount of hydroxyapatite up to 20 wt%, it's curing depth, curing time, bonding strength with enamel and the surface of enamel were evaluated. Test specimens were maintained at 36.5˚C in SBF for 4 weeks. The following conclusions could be drawn from this investigation.

1. The curing time of the fissure sealant was decreased with increasing HA content. More than 10wt% of HA showed significant difference ($P<0.05$). It was in the range of ISO standard which did not affect the physical characteristics.

2. The curing depth of the fissure sealant was decreased slightly with increasing HA content, however, there was no significant difference ($P>0.05$). It was in the range of ISO standard which did not affect the physical characteristics.

3. As the content of HA increases, the bonding strength between the sealant and the tooth surface tends to show an increase. More than 5wt% of HA showed significant difference ($P<0.05$).

4. Under SBF, HA in the sealant showed a remineralization effect in the interface with the calcium phosphate layer.

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국문 요약

치면열구전색재의 재석회화에 대한 하이드록시아파타이트의 효과

연세대학교 대학원 치의학과

박 상 욱

지도교수 : 최 형 준

이 연구의 목적은 다양한 정도의 하이드록시아파타이트를 함유한 치면 열구전색재에 의해 법랑질에서 재광화 효과를 알아보는 것이다. 본 연구에서는 기존의 필러를 포함하지 않은 치면열구전색재 제품인 Concise^{TM}(3M Co., St. Paul., MN, U.S.A.)에 하이드록시아파타이트를 1, 5, 10, 15, 20wt%로 첨가하여 새로운 수복용 치면열구전색재를 제조하였다. 이에 대한 물리적 성질로서 ISO 6874 기준에 맞는 치면열구전색재의 요건을 충족시키기 위해 하이드록시아파타이트의 함유량에 따라 중합 시간과 중합 깊이를 측정하였다. 기준에 맞는 하이드록시아파타이트를 함유한 치면열구전색재를 가지고 치아의 법랑질에 접착하여 SBF 에서 36.5°C 수조에 4주동안 보관하여 결합강도를 측정하고 표면형태를 알아보기 위해 전자현미경 사진을 관찰하였다.

기존의 제품인 Concise™ 대조군과 비교 시험하여 다음과 같은 결과를 얻었다.

1. 치면열구전색재의 중합시간은 하이드록시아파타이트 양이 증가함에 따라 감소하였다. 10wt% 이상에서 군간의 유의차가 있었다($P<0.05$). 이것은 ISO 6874의 기준을 만족하였고 치면열구전색재의 물리적 성질에는 영향을 미치지 못했다.

2. 치면열구전색재의 중합깊이는 하이드록시아파타이트 양이 증가함에 따라 약간 감소하는 경향을 보였다. 그러나 군간의 유의차는 없었다($P>0.05$). 이것은 ISO 6874의 기준을 만족하였고 치면열구전색재의 물리적 성질에는 영향을 미치지 못했다.

3. 하이드록시아파타이트 양이 증가함에 따라 치아표면과 치면열구전색재 사이의 결합강도는 증가하는 경향을 보였다. 5wt% 이상에서 유의차가 있었다($P<0.05$).

4. SBF에 보관한 결과 치면열구전색재 안의 하이드록시아파타이트에 의해 법랑질과 치면열구 전색제의 경계면에서 재석회화 효과로 칼슘-인 층이 관찰되었다.

이상의 실험결과로부터 하이드록시아파타이트를 첨가한 것은 기존의 치면열구전색재의 물성을 약화시키지 않고, 결합 강도는 하이드록시아파타이트의 함량이 증가함에 따라 증가하는 것으로 판명되었다. 이는 하이드록시아파타이트의 재광화 효과를 통해 미세누출을 억제하는 효과를 기대하여 이차우식을 방지하는 치면열구전색재의 기능을 더욱 강화 시킬 것이라 판단된다. 앞으로 미세누출억제의 효과를 더 밝혀내고 하이드록시아파타이트의 실란 처리를 통해 하이드록시아파타이트와 치면열구전색재간의 결합력을 향상시키는 연구가 더 필요할 것으로 사료된다.

핵심되는 말: 하이드록시아파타이트, 재광화, 치면열구전색재, 결합강도