In Vivo Study on the Biocompatibility of New Resin-based Root Canal Sealers

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국문초록

신개발 레진 계열 봉함제의 생체친화성에 관한 연구

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목 적

근관 치료의 최종 목적인 근관계의 영구적인 충전을 위해 사용되는 근관 봉함제는 많은 연구와 개선을 거쳐서 현재는 다양한 성분의 봉함제가 시판되고 있다. 이중에서 레진이 주성분인 봉함제는 조작이 편리하고 흐름성이 좋으며 근관의 벽에 높은 밀폐성을 보이고 충분한 작업시간과 높은 방사선 불투과성을 가지는 장점을 가짐에도 불구하고 높은 초기 생체 독성을 나타내는 단점을 가지고 있다.

본 실험에서는 기존의 상용화된 제품 중 레진이 주성분인 두 종류의 봉함제(AH 26, AH plus)와 산화 아연이 주성분인 봉함제(Pulp Canal Sealer EWT)와 국내에서 새로이 개발한 제품으로서 레진이 주성분인 두 종류의 봉함제(Adseal-1,2)를 생체조직에 매식하여 국소적인 반응을 비교하여 생체친화성을 알아보고자 하였다.

방 법

수종 봉함제의 생체 친화성을 알아보기 위하여 64마리의 Sprague-Dawley rat을 사용하였다. 봉함제의 피하조직 매식을 위해 길이와 직경이 각각 5와 1.5mm인 폴리에틸렌 테프론 관을 사용하였으며 이를 에탄올과 증류수로 세척한 후 고압증기멸균을 시행하였다. Rat에 대하여 케타민으로 복강내 마취를 시행한 후 배부를 면도하고 iodine으로 소독한 다음 네 곳에 절개를 시행하였으며 blunt dissection을 통해 깊이 10mm 이상의 피하조직 pocket을 형성하였다. 각각의 봉함제를 제조사의 지시에 따라 혼합 후 즉시 멸균된 테프론 관에 주사기를 이용하여 담은 다음에 봉함제가 흐르지 않게 유의하며 pocket내로 삽입하였으며 이때 16개의 관을 대조군으로 사용하기 위해 봉함제를 넣지 않은 상태로 삽입하였다. 이 후 절개 부위를 surgical gut suture로 봉합하였으며 1주일 후에 발사하였다.

Rat을 1, 2, 4, 12주 후에 각 군 당 세 마리 씩 에테르 흡입을 통해 희생하였으며 이 때 한 마리씩의 대조군도 포함시켰다. 이 후 매식된 관을 주위 조직과 함께 제거하고 포르말린에서 48시간 고정시킨 후 파라핀에 포매한 다음에 microtome을 사용하여 6㎛로 serial section을 시행하였다. 정중선 부위의 시편에 Hematoxylin-Eosin staining을 시행한 후 Olsson, Orstavik 그리고 Mjor 등의 방법에 따라 조직학적 변화를 관찰한 후 slight(1), moderate(2), severe inflammation(3)의 단계로 분류하였다. 얻어진 결과를 통계처리 프로그램인 Jandel사의 Sigmastat을 이용하여 Kruskal Wallis Test로 통계처리를 하였다.

결 과

The Relationship between ideal access placement and incisal wear (n=200)

봉 함 제	평균 ± 표준편차				생체친화성
	1 주	2 주	4 주	12 주	
Control	1.50±0.50	1.00±0.00	1.00±0.00	1.00±0.00	양 호
AH 26	2.67±0.65	2.33±0.77	1.92±0.79	1.66±0.65	양 호
AH Plus	2.42±0.66	2.25±0.62	1.58±0.67	1.50±0.67	양 호
Pulp Canal Sealer	2.58±0.51	2.33±0.49	1.75±0.62	2.08±0.67	불 량
Adseal-1	2.17±0.58	1.75±0.75	1.58±0.51	1.50±0.67	양 호
Adseal-2	2.08±0.67	2.00±0.73	1.58±0.67	1.41±0.66	양 호

*시편수 : 실험군당 12개, 대조군당 4개

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결 론

1) Pulp Canal Sealer를 제외한 모든 군에서 시간이 지남에 따라 유의성 있게 염증이 감소되는 양상을 보였다 ($p < 0.05$).
2) Pulp Canal Sealer는 1주, 2주, 12주에서 강한 염증반응을 보였다.
3) AH 26과 AH Plus에서는 1주, 2주에서 강한 염증반응을 보였으나 12주에서는 염증이 감소하였다.
4) 새로 개발된 봉함제 Adseal-1,2는 1주, 2주에서는 가장 약한 염증반응을 보이나 4주, 12주 후에는 AH Plus와 비슷한 수준의 염증 반응을 보였다.
5) Pulp Canal Sealer를 제외한 모든 군에서 인정할 만한 생체친화성을 보였다.
6) Adseal-2가 Adseal-1에 비하여 전반적으로 낮은 염증반응을 보였다.
7) 각 군간 결과의 차이에 통계적 유의성은 없었다 ($p > 0.05$).

주요어 : 근관봉함제, 생체친화성, 피하조직, 매식

Ⅰ. Introduction

Endodontic materials are used for the permanent obturation of root canals and consist of gutta percha core and sealer combinations. Grossman has adressed the requirements for the ideal root canal system filling materials[1]. They include ease of introduction, adequate sealing to canal wall, impermeability to moisture, lack of shrinkage, radiopacity and bacteriostasis. Materials must be nonirritating to apical tissue, not stain the teeth, and be easily sterilized and removed from the root canal system.

Endodontic filling materials are peculiar in that they are placed directly onto vital tissue. Therefore, the tissue response to these materials becomes of importance and may influence the outcome of the endodontic treatment[2]. In clinical practice, it is the sealer cement that comes into contact with the periapical tissue for a long time. So, one of the requirements of an ideal root canal sealer is that it should be nonirritating to the periapical tissues and should be compatible with living connective tissue[3].

The biological properties of the sealers are therefore considered to be especially important, and several in vitro and in vivo tests have been applied to assess these properties.

Several methods have been used to evaluate tissue responses to endodontic materials. One of the most practical and widely used methods is the implantation of the material into the subcutaneous connective tissue of rodents[4-6]. The irritative effect of endodontic materials is evaluated by the histopathological examination of the tissue response around the implants[7-10].

The subcutaneous implantation test possesses several of the features of a secondary test for the biological evaluation of endodontic materials[11]. In this study we used Teflon tubes because of their inert nature and suitability for bringing a test material in contact with living tissue in a controlled and effective manner[12]. Torneck firstly used polyethylene tube. The thin, cellular response alongside the tube serves as a negative control[13].

The purpose of the present, in vivo, research was to study the biocompatibility of five root canal sealers. Two of them (AH 26, AH Plus) contain in their formula epoxy resin. A third, Pulp Canal Sealer is a typical zinc oxide-eugenol sealer. The forth and fifth materials are newly developed resin-based root canal sealers.

Ⅱ. Materials and Methods

1. Materials

Sixty-four white male Sprague-Dawley rats were used in this study. They were 3 to 4 months old and 200 to 250 g. There were five groups of three animals each for every experimental period. The used sealers, composition and manufacture were Table 1. For every period there was also a control group.

Table 1. Composition of materials

Name/Manufacturer		Compositions
Adseal-1 Meta dental, Seoul, Korea	Paste A	Epoxy resin Ethylene glycol monosalicylate Calcium phosphate Zirconium oxide Bismuth subcarbonate
	Paste B	4-aminobenzonate Calcium phosphate Zirconium oxide Bismuth subcarbonate
Adseal-2 Meta dental, Seoul, Korea	Paste A	Oligomer Ethylene glycol monosalicylate Calcium phosphate Zirconium oxide Bismuth subcarbonate
	Paste B	4-aminobenzonate Calcium phosphate Zirconium oxide Bismuth subcarbonate Calcium oxide
AH 26 silver-free DeTrey Dentsply, Zurich, Switzerland	Powder	Bismuth oxide Methenamine
	Liquid	Epoxy resin
AH Plus DeTrey Dentsply, Zurich, Switzerland	Paste A	Epoxy resin Calcium tungstate Zirconium oxide Silica Iron oxide pigments
	Paste B	Adamantane amine Diamine calcium tungstate Zirconium oxide Silica Silicone oil
Pulp Canal Sealer Kerr, Detroit MI, USA	Powder	Zinc oxide Precipitated molecular silver Oleo resins Thymoliodide
	Liquid	Oil of cloves, Canada Balsam

2. Preparation of Specimens

The polyethylene teflon tubes, 5mm in length with an inner diameter of 1.5mm, were washed in ethanol and distilled water and autoclaved before being filled with the sealers[14].

Table 2. Criteria for scoring of inflammatory tissue response

Scoring Category Ordinal Scale	Extent	Verbal Descriptions
1	No/slight inflammation	Thickness of reaction zone similar or only slightly wider than along side tube; none or few inflammatory cells.
2	Moderate inflammation	Increased reaction zone; presence of macrophages and/ or plasma cells.
3	Severe inflammation	Increased reaction zone; presence of macrophages and plasma cells; occasional foci of neutrophil granulocytes and/or lymphocytes

3. Implantation Procedure

After ether inhalation, the animals were anesthetized by intraperitoneal administration of ketamine (0.001g/kg body wt). After shaving the back of animal and disinfecting it with 5% iodine in ethanol, incisions were made in the dorsum by blade and four subcutaneous pockets were carefully prepared by blunt dissection to a depth of l0mm above.

The tubes containing freshly mixed sealers were then placed into the pockets prepared in each rat. It is important to prevent spilling of the materials into the tissue. Empty teflon tubes were used as controls. The incisions were closed with surgical gut sutures.

4. Implants and Tissue Removal

The observation periods lasted 1, 2, 4 and 12 weeks. By the end of each period the animals were killed by ether inhalation. After the skin overlying the implants was shaved, the tubes were removed with the surrounding tissue and immersed in 10% buffered formalin. After fixing the tissue for 48 h. it was processed for paraffin embedding. Paraffin blocks were cut in serial sections with the microtome set at 6μm. The sections were mounted on glass slides and then stained with hematoxylin and eosin.

During experiment bacterial infection was observed in two animal. Five animals died after anesthesia. These animals were excluded and replaced by others so that the total number of healthy experimental animals remained constant.

5. Evaluation of Histological Tissue Response

For state of the surrounding tissue, the below biological parameter were examined[14].

① Extent of fibrosis/fibrous capsule and inflammation
② Degeneration as determined by changes in tissue morphology
③ Number and distribution as function of distance from the material/tissue interface of the inflammation cell type, namely polymorphonuclear luecocytes, lymphocyte, plasma cell, eosinophils, macrophages and multinucleated cells
④ Presence of necrosis as determined by nuclear debris and/or capillary wall breakdown
⑤ Other parameter such as material debris, fatty infiltration, granuloma

Tissue reactions were graded as mild, moderate, and severe, according to the criteria suggested by Olsson et al.[15] and Orstavik and Mjor[16]. This criteria is represented in Table 2.

6. Evaluation of Biocompatibility

Interpretations of the results shall be based on appropriate statistical analysis of the data to demonstrate acceptance or rejection of the material[11]. These mehtods were to know the biocompatibility of the materials.

① No to slight reaction at both 2 and 12 weeks is acceptable. No to slight reaction at 2 weeks which increases to moderate or severe reaction at 12 weeks is not acceptable.

② Moderate reaction at 2 and 12 weeks is not acceptable. Moderate reaction at 2 weeks which diminishes at 12 weeks is acceptable.
③ Severe reaction at any period is unacceptable.

Ⅲ. Results

All specimens were undergone blinded examination by the single examiner who did not know which sealer or which period was being examined. Histopathological results are summarized in Table 3. Data were statistically analyzed with the Kruskall-Wallis test. There was no statistically significant difference among the test materials ($p > 0.05$).

To know the tendency with experimental period within group, data were statistically analyzed with Kruskall-Wallis test. There was statistically significant difference in all sealer groups ($p < 0.05$). These results are summarized in Table 4 and Figure 1.

1. Control

Slight reactions generally were observed in the control group (Fig 2). These reactions were characterized by the presence of macrophages and of few plasma cells next to fibrous connective tissue, especially at 1 week specimens. The inflammation was reduced further with time (2, 4 and 12 weeks). But only two specimens at 1 week showed moderate inflammation.

2. AH 26

Severe inflammation was observed in the AH 26 specimens at 1 week (Fig. 3). The intensity of the reaction decreased little at 2 weeks. The tissue was infiltrated with many histiocytes and lymphocytes. And there are foreign body reactions around the spilled material (Fig. 4). At 4 weeks, the inflammatory reaction had diminished, compared with the 1 and 2 weeks. There were very few inflammatory cells (Fig. 5). Fibroblasts and collagen fibers with very few inflammatory cells were observed (Fig. 6).

3. AH Plus

Moderate to severe inflammatory reaction was observed with AH Plus at 1 week. Inflammatory cell infiltration was around spilled sealer (Fig. 7). The inflammatory response was similar at 2 week. It was characterized by the presence of macrophages, plasma cell, lymphocyte and fibroblasts (Fig. 8). Reduction of inflammation continued progressively to 4 and 12 weeks. The tissue was almost mild to moderate. The connective tissue became almost normal architectures (Fig. 9). Very thick fibrous capsule was observed (Fig. 10).

4. Pulp Canal Sealer

Severe inflammatory reaction was observed at 1 week after the implantation of sealer. Accumulation of macrophages, lymphocytes, plasma cells, and foreign body giant cells were seen in the specimens (Fig. 11). The inflammatory response was similar at 2 weeks (Fig. 12), whereas at 4 weeks it was graded as mild to moderate. It was characterized by the

Table 3. Histological findings

Sealer	AH 26 (n=12/wk)			AH Plus (n=12/wk)			Pulp Canal Sealer n=12/wk)			Adseal-1 (n=12/wk)			Adseal-2 (n=12/wk)			Control (n=4/wk)		
*Grade	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1 wk	1	2	9	1	5	6	0	5	7	1	8	3	2	7	3	2	2	0
2 wks	2	5	5	1	7	4	0	8	4	5	5	2	3	6	3	4	0	0
4 wks	4	5	3	6	5	1	4	6	1	5	7	0	6	5	1	4	0	0
12 wks	5	6	1	7	4	1	2	7	3	7	4	1	8	3	1	4	0	0

* Grade 1 : No/slight inflammation, 2 : moderate inflammation, 3 : severe inflammation

Table 4. The mean and interpretation

Sealers	Mean ± S.D.				Interpretation
	1 wk	2 wks	4 wks	12 wks	
Control	1.50±0.50	1.00±0.00	1.00±0.00	1.00±0.00	
AH 26	2.67±0.65	2.33±0.77	1.92±0.79	1.66±0.65	acceptable
AH Plus	2.42±0.66	2.25±0.62	1.58±0.67	1.50±0.67	acceptable
Pulp Canal Sealer	2.58±0.51	2.33±0.49	1.75±0.62	2.08±0.67	unacceptable
Adseal-1	2.17±0.58	1.75±0.75	1.58±0.51	1.50±0.67	acceptable
Adseal-2	2.08±0.67	2.00±0.73	1.58±0.67	1.41±0.66	acceptable

*Number of specimens: 4 for control group, 12 for experimental group

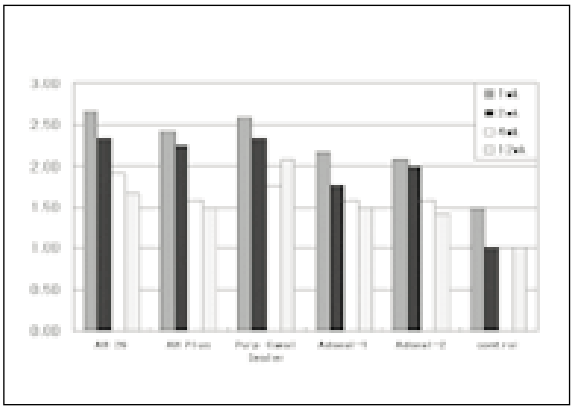

Fig. 1. Means of the each period

presence of a few lymphocytes, fibroblasts and fibrous capsule (Fig. 13). But at 12 weeks the severity of inflammation increased to moderate. There were plasma cells and macrophages as much as at 2 weeks (Fig. 14).

5. Adseal-1

Moderate to severe inflammation with concentration of macrophages and lymphocytes was observed at 1 week after implantation (Fig. 15). Some foreign body giant cells with engulfed particles of the material were seen in the areas surrounding the ends of the tubes. The intensity of the reaction was diminished at 2 weeks (Fig. 16). Inflammation consisted of lymphocytes. Fibrous capsule and normal architecture were also observed at 4 weeks (Fig. 17). In the tissue adjacent to the material, fibroblast and collagen fiber were detected. Inflammation almost subsided and thin fibrous capsule was observed (Fig. 18).

6. Adseal-2

Moderate to severe inflammation was observed in the implant area at 1 week. The connective tissue was infiltrated by lymphocytes, plasma cells and macrophages (Fig. 19). The intensity of inflammatory reaction at 2 week was similar with 1 week. Macrophages, plasma cells, and lymphocytes were also present (Fig. 20). In the specimens of 4 weeks, the intensity of the inflammtion was diminished very much (Fig. 21). At 12 weeks fibrous connective tissue infiltrated with few plasma cells and lymphocytes was observed (Fig. 22).

Ⅳ. Discussion

Many methods have been used to evaluate the biocompatibility of endodontic sealers. But, in vivo methods correlate better than the in vitro tests[17]. Because the direct contact between root canal sealer and periapical tissue is important, the subcutaneous implantation was used in this study.

And this study used an ordinal scoring system for tissue response evaluation[15,16]. The results of the ordinal scale and mean of them provided numerical illustration of the impressions and histopathological findings.

A common feature of the tissue response to implantation was the development of a well-defined reaction zone. Tissue response to the materials showed the increase in the width of the reaction zone and local-

ized infiltration by inflammatory cells. In addition, inflammatory responses may occur around spilled material fragments. The filling tubes with freshly-mixed sealers happened spillage of them. It is could be prevented if the tubes were implanted after the sealers had set, but high toxicity that most endodontic material have observed before setting [15].

AH 26 seems to be the most irritative material at 1 and 2 weeks. But the intensity of the inflammation was decreased by 4 and 12 weeks. These results are agreement with those of other researches showed high initial and dramatically declining irritation surrounding AH 26[17,18].

These toxic effects of AH 26 could be caused mainly by formaldehyde, which is released primarily during the initial setting reaction[19]. The powder, however, contains hexamethylenetetramine which could be decomposed in acid environment, yielding ammonia and formaldehyde. The concentration of formaldehyde has increased two-fold 12 hours after mixing and increased until 2 days of setting. The concentration of formaldehyde has increased to nearly 200 times over the concentration of freshly mixed AH 26. The approximate setting time for AH 26 is 2 days. The cytotoxicity due to formaldehyde is supported by other researches [20,21]

Additionally, mutagenic substances, epoxy derivatives of bisphenol-A-diglicidylether which may be also cytotoxic, have been found in the set material [20,22]. It is known that epoxy-bis-phenol resin (liquid component of AH [26] relate to its severe cytotoxicity [23]. So other researcher pointed to the possibility that mixed silver-free AH 26 might contain small amounts of two mutagenic substances : bisphenol A diglycidyl eter and formaldehyde[24].

AH Plus represented the moderate to severe inflammation at 1 and 2 weeks and the intensity of the inflammation was decreased by 4 and 12 weeks. But it looked like milder inflammation than AH 26 overall periods. In another research, AH 26 and AH plus exhibited severe reactivity by agar diffusion test [25]. The amount of formaldehyde released by AH 26 and AH plus has been measured and found to be 1347 ppm and 3.9 ppm, respectively. The fact that epoxy-bis-phenol resin was related to cytotoxicity could explain why AH Plus, which release minimal formaldehyde, is also as cytotoxic as AH 26.

On the other hand, other study indicated that AH Plus indeed exhibited a lower cytotoxic potential compared to AH 26[26]. The new formulation, AH Plus, uses new types of amines and a setting reaction based on thermal epoxide-amine addition reaction. It was said that the new sealers may represent progress in the development of more biocompatible endodontic materials.

Pulp Canal Sealer is a zinc oxide-eugenol sealer. The toxic effects of zinc oxide-eugenol sealers have been studied extensively. The cause of cytotoxicity may be free eugenol remaining in the zinc oxide and eugenol mixture. The free eugenol can also be released from mixture subject to hydrolysis. The rate of releasing eugenol is decreased very slowly. So small amounts of eugenol continue to be released from mixture for at least 1 year[27]. Eugenol may have a beneficial effects at concentration ranging from 10^{-8}M to 10^{-5}M, but may be cytotoxic above 10^{-3}M concentration by cell respiratory inhibition[28].

In the present study, severe inflamation was observed at 1 and 2 weeks and the severity was decreased to moderate at 4 weeks. But at 12 weeks increased inflammation was detected. It should be noted that free-eugenol is still present even after the sealer has been set and released over an extended period. But no positive relationship between the release of eugenol and cytotoxicity has been proven. This fact is supported by other study showed that the pattern of eugenol release did not concide with the cytotoxicity expressed by the test solution[29]. So, other investigator suggested that the cytotoxicity of zinc oxide eugenol sealer may be based on the possible toxic effect of zinc ions[30]. Adseal-1 and adseal-2 had the least inflammatory reaction at 1 and 2 weeks. But the severity became same with AH Plus at 4 and 12 weeks. Adseal-2 had lower inflammation than Adseal-1 at 1 and 12 weeks. Adseal-1 and Adseal-2 are resin based sealer. Calcium phosphate is added to Adseal-1 to improve the biocompatibility of resin-based root canal seaelr. And 4-aminobenzonate is used for amine as curing agent. This is a kind of polymer which is less irritative than monomer. Ethylglycol monosalicylate used for good flowability remains as liquid which appear to be

cytotoxic in mixtures. To remove Ethylglycol monosalicylate, Adseal-2 contains calcium oxide as chelating agent.

Considering the variations between the in vitro and in vivo studies, it is difficult to compare our results with others. But, It is necessary to investigate more extensively.

V. Conclusions

1. The inflammation of all sealer groups except Pulp Canal Sealer decreased when time elapsed with significant difference ($p < 0.05$).
2. Pulp Canal Sealer was strongly inflammatory at 1, 2 and 12 weeks.
3. AH 26 and AH Plus were strongly inflammatory at 1 and 2 weeks. But it had the decreased inflammatory reaction after 12 weeks.
4. Adseal-1 and Adseal-2 had the least inflammatory reaction at 1 and 2 weeks. But the severity became same with AH Plus at 4 and 12 week.
5. Adseal-2 had lower inflammation than Adseal-1 at 1 and 12 weeks.
6. All materials except Pulp Canal Sealer showed the acceptable biocompatibility.
7. There was no statistically significant difference among the test materials ($p > 0.05$).

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Explanation of Figures

Fig 2. Control 2 weeks (×40)
Fig 3. AH 26 1 week (×40)
Fig 4. AH 26 2 weeks (×400)
Fig 5. AH 26 4 weeks (×400)
Fig 6. AH 26 12 weeks (×400)
Fig 7. AH Plus 1 week (×100)
Fig 8. AH Plus 2 weeks (×400)
Fig 9. AH Plus 4 weeks (×400)
Fig 10. AH Plus 12 weeks (×400)
Fig 11. Plup Canal Sealer 1 week (×400)
Fig 12. Plup Canal Sealer 2 weeks (×400)
Fig 13. Plup Canal Sealer 4 weeks (×400)
Fig 14. Plup Canal Sealer 12 weeks (×400)
Fig 15. Adseal-1 1 week (×400)
Fig 16. Adseal-1 2 weeks (×400)
Fig 17. Adseal-1 4 weeks (×400)
Fig 18. Adseal-1 12 weeks (×400)
Fig 19. Adseal-2 1 week (×400)
Fig 20. Adseal-2 2 weeks (×400)
Fig 21. Adseal-2 4 weeks (×400)
Fig 22. Adseal-2 12 weeks (×400)

사진부도 ①

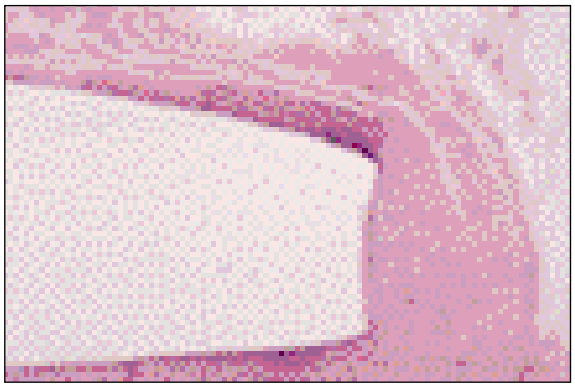
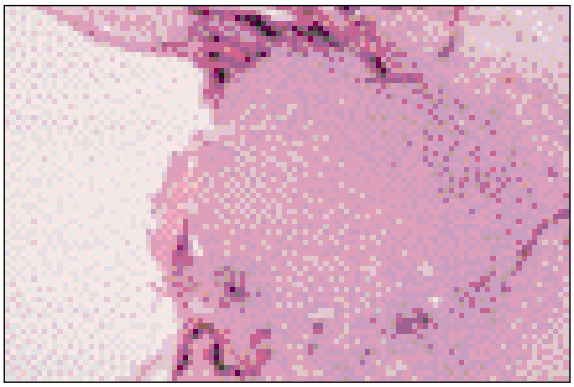


Fig. 2



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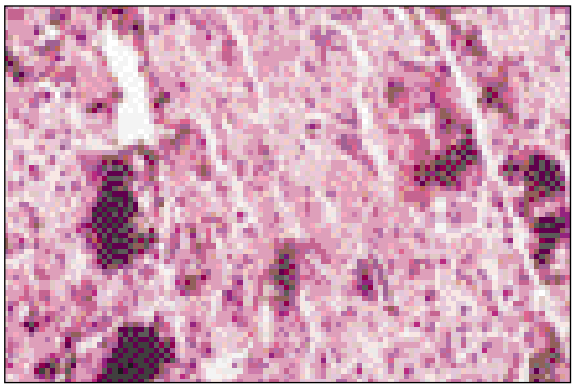

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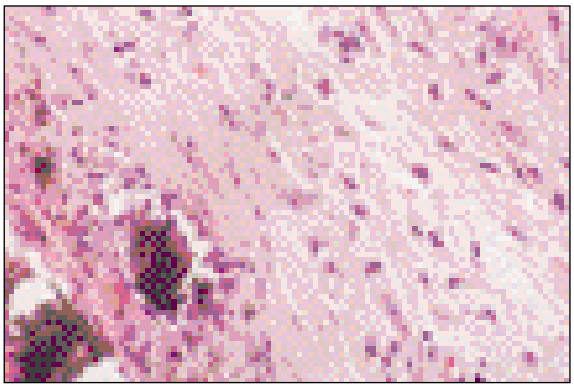

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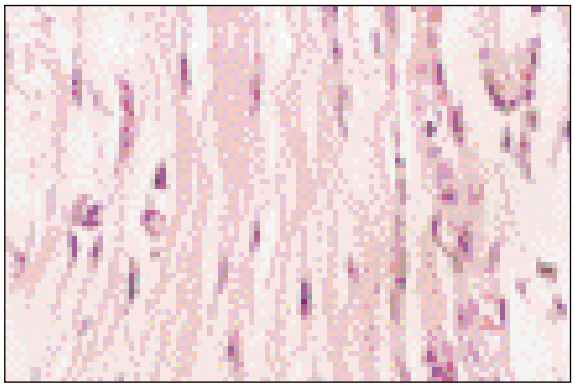

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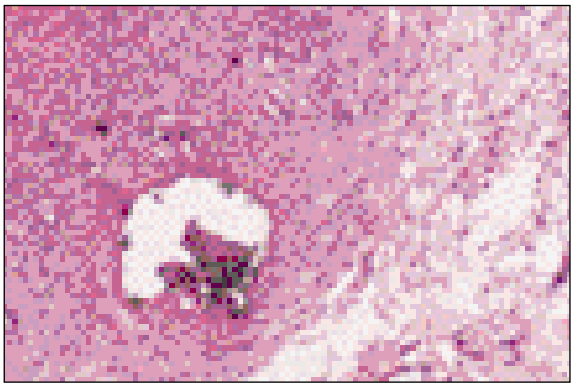

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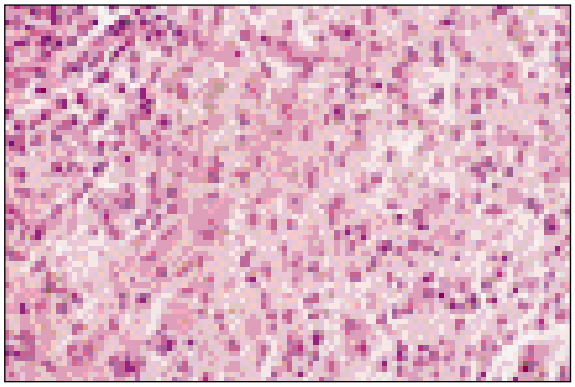

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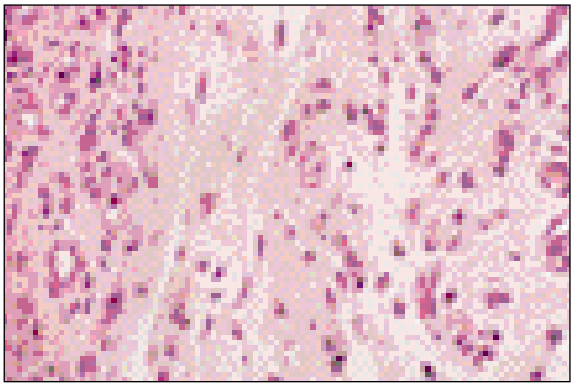


Fig. 9

사진부도 ②

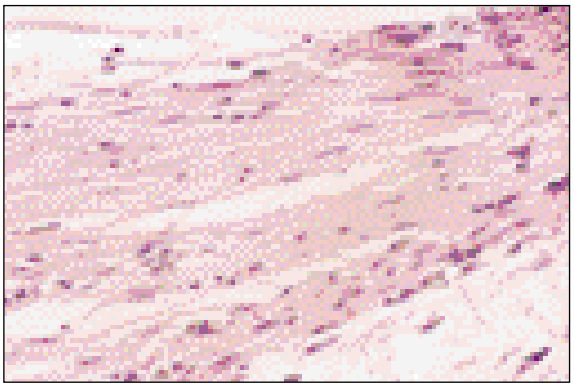
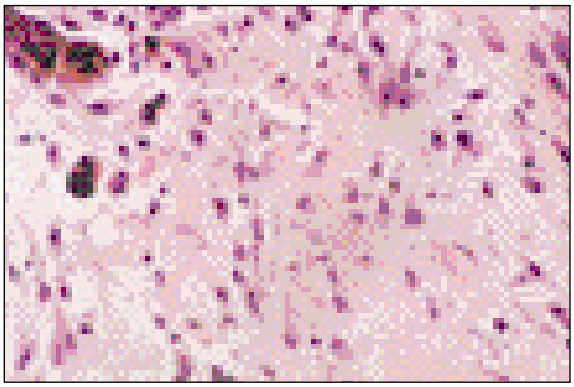


Fig. 10



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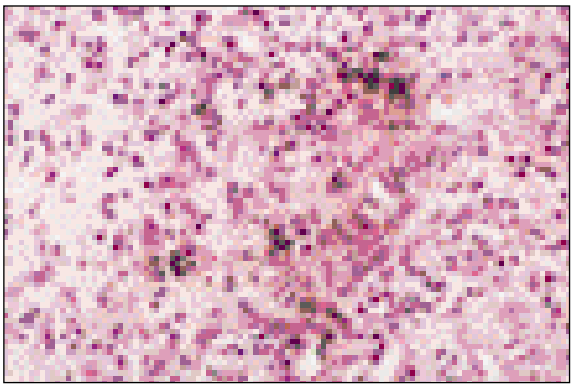
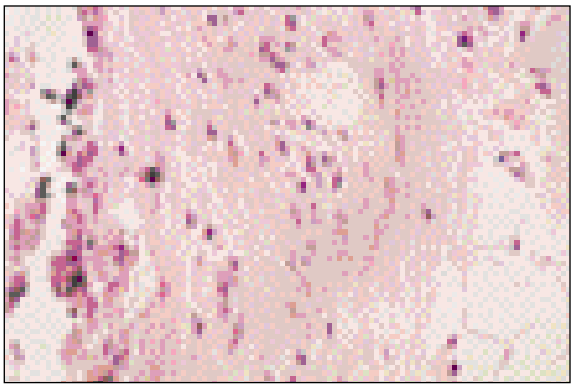


Fig. 12



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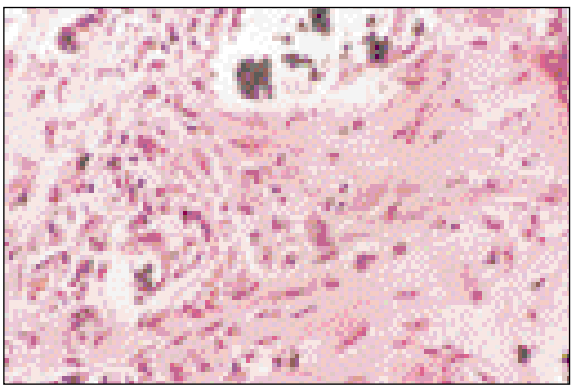

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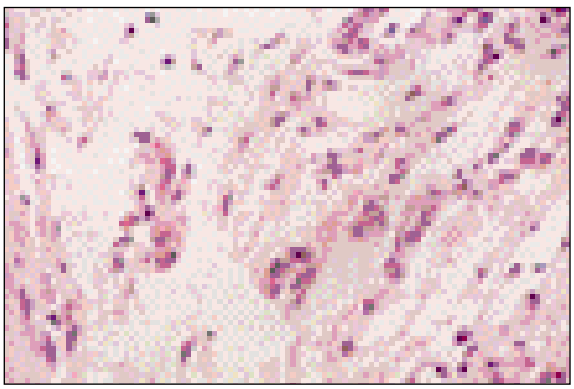

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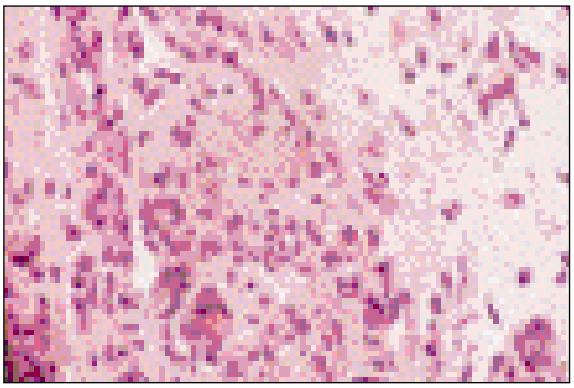

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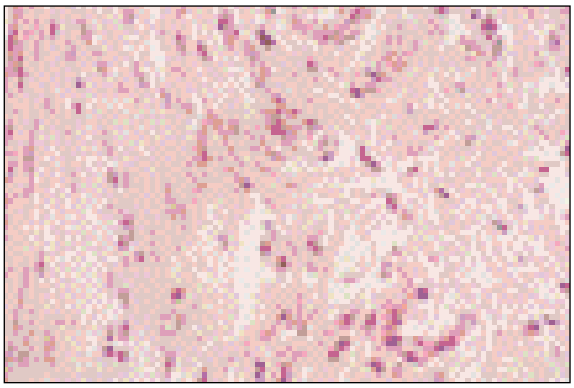
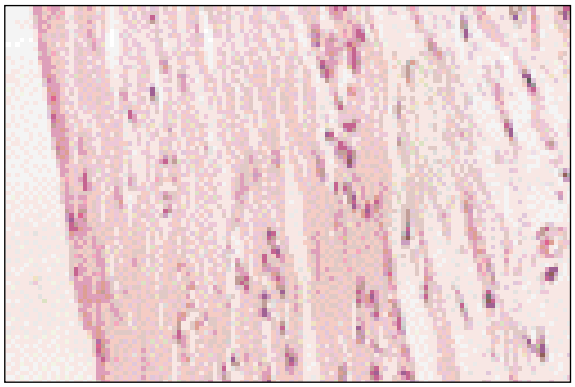


Fig. 17

사진부도 ③



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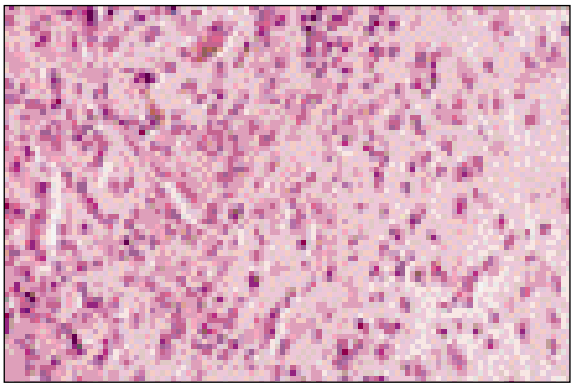

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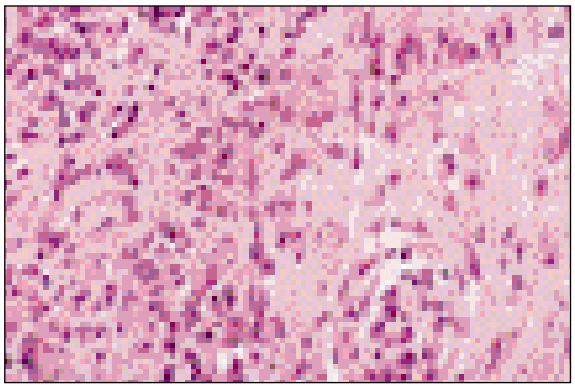

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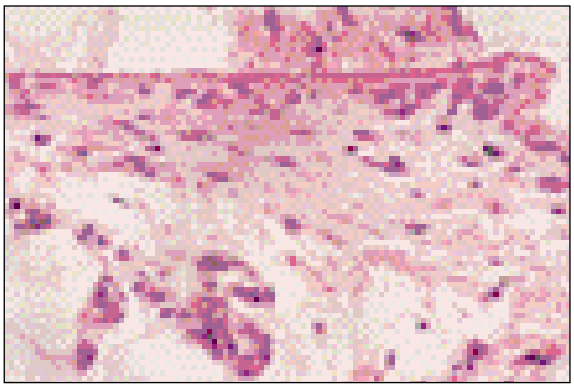

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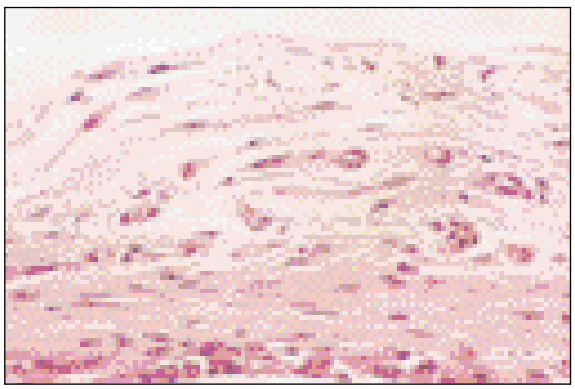

Fig. 22