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Adherence of Salivary Proteins to Various Orthodontic Brackets

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The principal aims of this study were to identify the composition of salivary pellicles formed on various orthodontic brackets and to obtain a detailed information about the protein adsorption profiles from whole whole saliva and two major glandular salivas. Four different types of orthodontic brackets were used. All were upper bicuspid brackets with a 022 × 028 slot Roth prescription; stainless steel metal, monocrystalline sapphire, polycrystalline alumina, and plastic brackets. Bracket pellicles were formed by the incubation of orthodontic brackets with whole saliva, submandibular-sublingual saliva, and parotid saliva for 2 hours. The bracket pellicles were extracted and confirmed by employing sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western transfer methods, and immunodetection. The results showed that low-molecular weight salivary mucin, α-amylase, secretory IgA (sIgA), acidic proline-rich proteins, and cystatins were attached to all of these brackets regardless of the bracket types. High-molecular weight mucin, which promotes the adhesion of *Streptococcus mutans*, did not adhere to any orthodontic brackets. Though the same components were detected in all bracket pellicles, however, the gel profiles showed qualitatively and quantitatively different pellicles, according to the origins of saliva and the bracket types. In particular, the binding of sIgA was more prominent in the pellicles from parotid saliva and the binding of cystatins was prominent in the pellicles from the form plastic brackets. This study indicates that numerous salivary proteins adhere to the orthodontic brackets and these salivary proteins adhere selectively according to bracket types and the types of the saliva.

Key words : Saliva, Pellicle, Orthodontic bracket

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In the patients who wear fixed orthodontic appliances, teeth and orthodontic appliances are covered by a thin film that is called by salivary pellicle.[1] The pellicle is generally 1 to 2 μm thick and formed mainly by the selective adsorption of salivary components.[1] Since oral bacteria interact with the salivary components of pellicles in order to adhere to a surface, saliva plays an important role in the adherent process. Considerable evidences support that the initial

colonization on the tooth involves the binding of bacteria to salivary components within the acquired pellicle and saliva modulates bacterial adhesion to a variety of surfaces. It has been also known that the bacterial ability to adhere to saliva-coated surfaces is a prerequisite for plaque formation.[1,5-8]

Among many orthodontic appliances, orthodontic brackets may play a role in the enamel demineralization, because they attach continuously to dentition during almost all periods of orthodontic treatment and their complex design provides a unique environment that impedes proper access to the tooth surfaces for cleaning. Metallic orthodontic brackets have been found to induce specific changes in the oral environment, such as decreased pH, increased plaque accumulation, and elevated *Streptococcus mutans* colonization.[9,10] Recently, a new generation of ceramic and plastic brackets became available due to patients' demands for an improved esthetic appearances. Their mechanical properties including frictional resistance and bond strength, as well as their morphologic nature and structure, have been studied extensively.[11-17] But, fewer studies have investigated the biological aspects and biocompatibility of these brackets.

This study was undertaken to identify the composition of salivary pellicles formed on the surfaces of various kinds of the orthodontic brackets and to obtain a information about the protein adsorption profiles from the whole saliva and two major glandular salivas.

MATERIALS AND METHODS

1. Saliva collection

Saliva collection was performed from 9 : 00 A.M. to 11 : 00 A.M. to minimize the effects of diurnal variability in the salivary composition. Saliva was collected from a 31-year-old man of good oral health to avoid donor-specific variation in the salivary samples. This volunteer was refrained from eating, drinking, and brushing for at least 2 hours before saliva collection.

Unstimulated whole saliva (UWS) was collected by the spitting method. Parotid saliva (HPS) was collected via a modified Lashley cup placed directly over the Stensen's duct orifice. Submandibular-sublingual saliva (HSMSL) was collected by using a custom-made mouthpiece of rubber base impression material, as described by Block and Brottman.[1] Each saliva sample was collected into a chilled sterile tube and centrifuged at 3500g for 10 minutes to remove cellular debris. The resulting supernatants were used immediately for pellicle and adhesion experiments.

2. Preparation of orthodontic brackets

Four different kinds of orthodontic brackets were used; (1) Stainless-steel metal (Korean smart, Dae-Seung, Seoul, Korea), (2) Monocrystalline sapphire (Inspire, Ormco/A Company, Glendora, CA, USA), (3) Polycrystalline alumina (Transcend 6000 series, 3M/Unitek, Monrovia, CA, USA), and (4) Plastic (Silkon m, American orthodontics, Sheboygan, WI, USA). All were upper bicuspid brackets of Roth prescription with a 022×028 slot. Fifty of each type of bracket were washed 3 times in Tris-buffered saline (TBS) (10 mmol/L of Tris-HCl, 154 mmol/L of NaCl ; pH 7.5), then incubated in 2 mL of each saliva with agitation for 2 hours at room temperature. The specimens were washed 3 times by addition of TBS to remove unbound salivary components from the bracket pellicle. The bracket pellicles were removed by using 0.5% sodium dodecyl sulfate (SDS) and then incubated at 100°C for 5 minutes.

3. Electrophoresis and Western Transfer (Immunoblotting)

Fresh saliva samples and the bracket pellicles were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli[19] with the use of an electrophoresis unit (Hoefer SE 280 Tall Mighty Small ; Hoefer Scientific Instruments, San Francisco, CA, USA). For the detection of cystatins, 15% SDS-PAGE

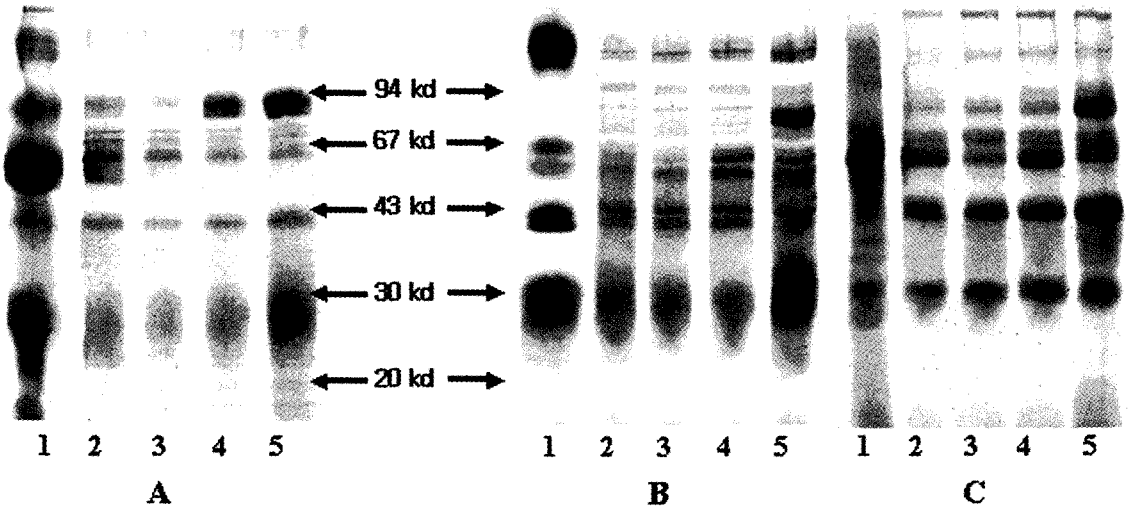

Fig. 1. SDS-PAGE (10%) of pellicles formed on the orthodontic brackets from UWS(panel A), HSMSL (panel B), and HPS (panel C). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets. The gel was stained with 0.25% Coomassie brilliant blue.

was used. Following electrophoresis, gels were stained for 2 hours with 0.25% Coomassie brilliant blue (Sigma chemical, St. Louis, MO, USA). Molecular weights were determined by the comparison of mobilities of unknown components with standard low-molecular weight markers (Amersham Pharmacia Biotek, Piscataway, NJ, USA).

Western blotting was performed through the transfer of pellicle proteins to polyvinyledene difluoride membranes (Millipore, Bedford, MA, USA) using a Semi-Dry Blotting Unit 2020 (Fisher Scientific, Pittsburgh, PA, USA) at 250 mA for 45 to 55 minutes according to the methods of Burnette[20] and Towbin et al.[21]

Unbound sites on the membrane were blocked in TBS containing 2% bovine serum albumin (BSA) for 3 hours and then incubated with rabbit antisera to a panel of salivary proteins (high-molecular weight mucin [MG1], low-molecular weight mucin [MG2], α-amylase, heavy chain of secretory immunoglobulin A [sIgA], acidic proline-rich proteins [PRPs], and cystatins) for more than 5 hours at room temperature.

After being washed 3 times with TBS containing 0.01% BSA, the blots were incubated with a 1:2500 dilution of alkaline phosphatase conjugated affinity purified goat anti-rabbit IgG (Bio-Rad Laboratories, Hercules, CA, USA) for 3 hours, washed 3 times with TBS containing 0.01% BSA, and visualized with nitroblue tetrazolium/5-bromo- 4-chloro- 3-indolyl-phosphate alkaline phosphatase color developing reagents (Bio-Rad Laboratories). For negative control tests, the same experiments were performed with rabbit preimmune sera as a primary antibody. All test samples were assayed at least 3 times.

RESULTS

1. Results of the electrophoresis

The results of the electrophoresis showed that the protein adsorption profiles of the bracket pellicles were similar to those of their original saliva (Fig. 1). The bracket pellicles from UWS and HSMSL appeared to be mainly composed of 4 salivary components about 30

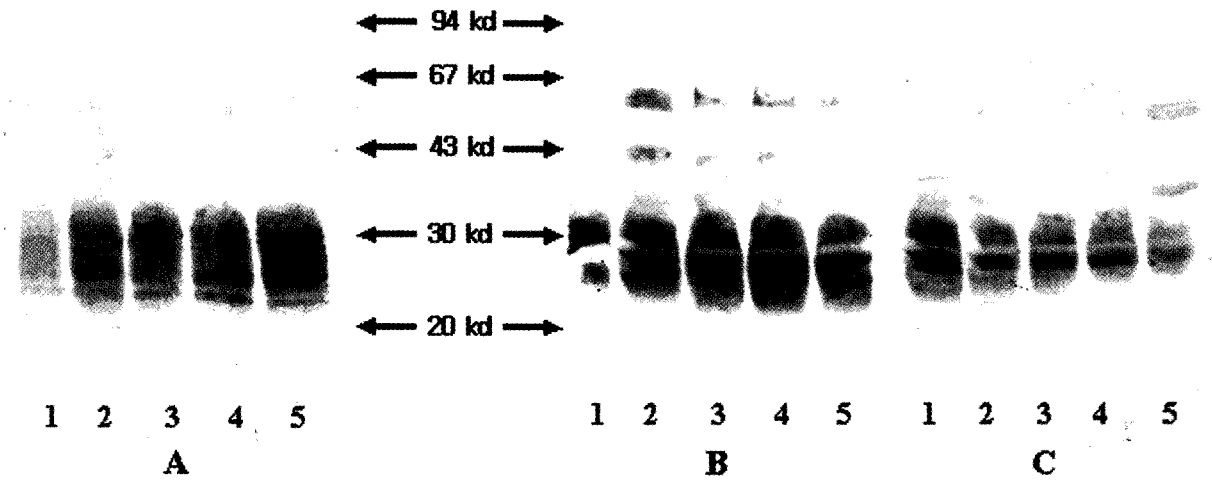

Fig. 2. SDS-PAGE (10%) and immunoblotting of experimental pellicles formed from UWS (panel A), HSMSL (panel B), and HPS (panel C). The membrane was probed with rabbit antisera to human salivary acidic PRPs (1:1000 dilution). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets.

kilodalton (kd), 43 kd, 60 kd, and a high-molecular weight larger than 94 kd in size. The bracket pellicles from HPS appeared to be the same as those from UWS and HSMSL except the high-molecular weight component larger than 94 kd.

There are quantitative differences in the patterns of protein adsorption according to the kinds of orthodontic brackets, though their adsorption profiles are qualitatively similar. Generally, some of the salivary components attached more to the surfaces of plastic brackets than those of other brackets. 30 kd- and smaller than 94 kd- molecules were more evident in the experimental pellicles from plastic brackets (Fig. 1(A, B, and C) : lane 5).

2. Identification of salivary components in the pellicles from UWS

Immunodetection of the bracket pellicles from UWS with monospecific antisera identified the 30-kd molecule as acidic PRPs (Fig. 2(A)), the 55-kd molecule as secretory IgA (Fig. 3(A)), the band about 60-kd molecule as α-amylase (Fig. 4(A)), and the high-molecular weight molecule larger than 94 kd as low-molecular weight mucin (Fig. 5(A)). High-molecular weight mucin was detected only in the unstimulated whole saliva (Fig. 6(A)). Cystatins were not detected clearly in the 10% SDS-PAGE due to their light molecular weight. The 13- to 20-kd low-molecular weight molecule was identified as cystatins in the 15% gel (Fig. 7(A)). Compared with other bracket pellicles, cystatins were detected very strongly in the plastic brackets (Fig. 7 (A) : lane 5).

3. Identification of salivary components in the pellicles from HSMSL

There was no marked difference in protein adsorption profiles between the bracket pellicles from fresh ductal HSMSL and those from UWS. The bracket pellicles were composed of acidic PRPs (Fig. 2(B)), sIgA (Fig. 3(B)), α-amylase (Fig. 4(B)), MG2 (Fig. 5(B)), and cystatins (Fig. 7(B)). Cystatins showed a more evident adsorption

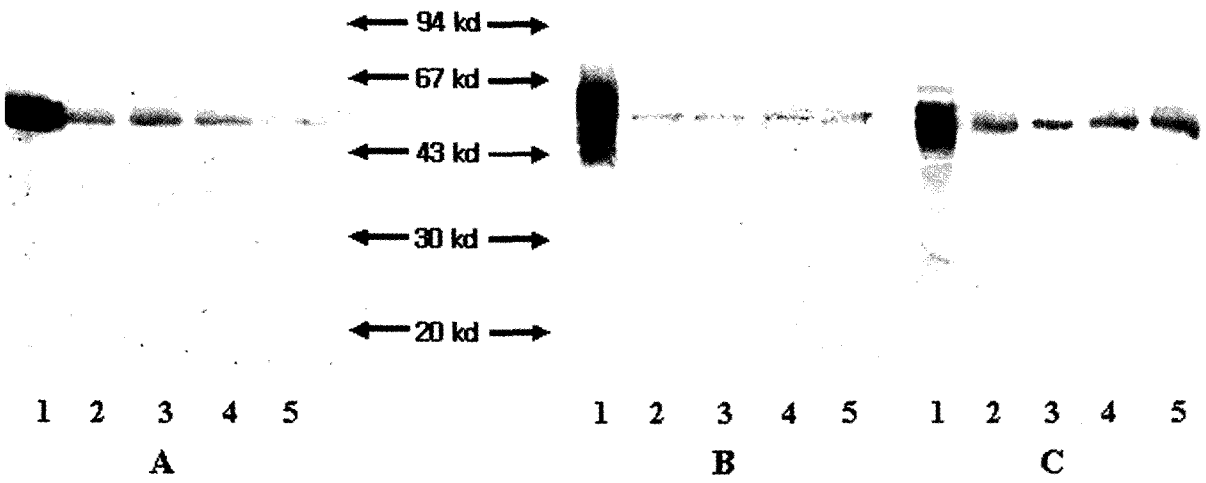

Fig. 3. SDS-PAGE (10%) and immunoblotting of experimental pellicles formed from UWS (panel A), HSMSL (panel B), and HPS (panel C). The membrane was probed with rabbit antisera to human salivary low-molecular weight mucin (MG2, 1:1000 dilution). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets.

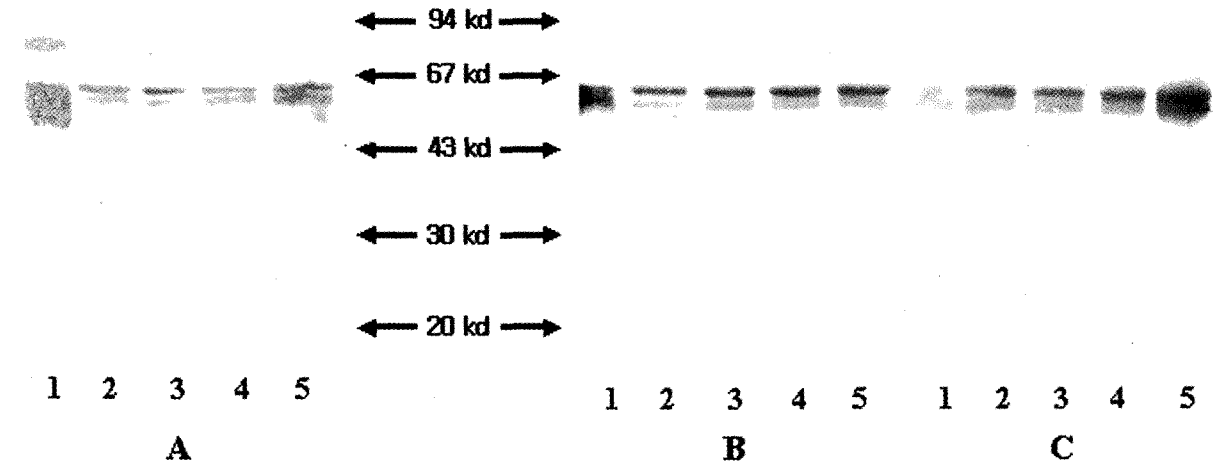

Fig. 4. SDS-PAGE (10%) and immunoblotting of experimental pellicles formed from, UWS (panel A), HSMSL (panel B), and HPS (panel C). The membrane was probed with rabbit antisera to human salivary α-amylase (1:1000 dilution). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets.

onto the plastic brackets (Fig. 7(B) : lane 5). MG1 was not detected in the bracket pellicles from HSMSL (Fig. 6(B)).

4. Identification of salivary components in the pellicles from HPS

In the case of HPS, protein adsorption profiles were

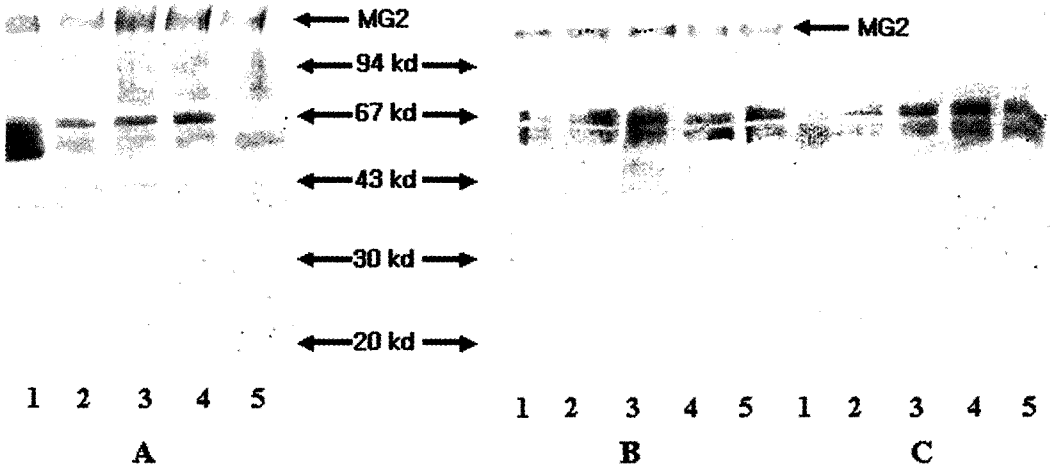

Fig. 5. SDS-PAGE (10%) and immunoblotting of experimental pellicles formed from UWS (panel A), HSMSL (panel B), and HPS (panel C). The membrane was probed with rabbit antisera to heavy chain of human IgA (1:1000 dilution). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets.

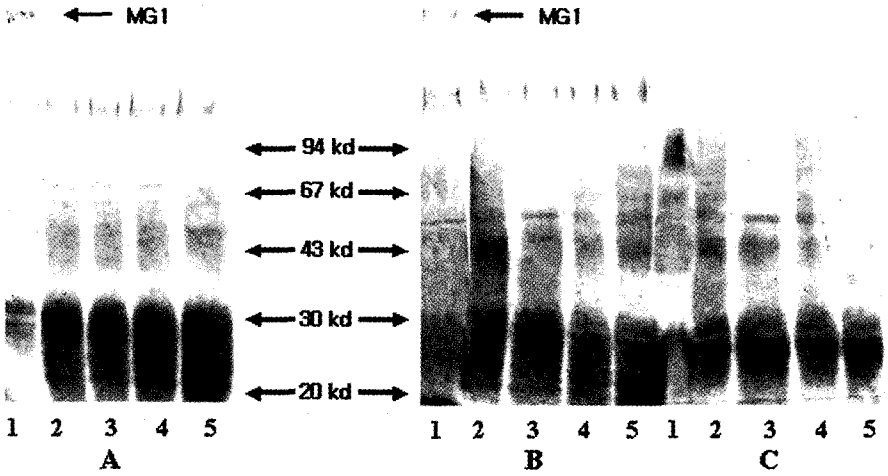

Fig. 6. SDS-PAGE (10%) and immunoblotting of experimental pellicles formed from UWS (panel A), HSMSL (panel B), and HPS (panel C). The membrane was probed with rabbit antisera to human salivary high-molecular weight mucin (MG1, 1:1000 dilution). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets.

different from those of UWS and HSMSL. The bracket pellicles from fresh ductal HPS were mainly composed of acidic PRPs (Fig. 2(C)), sIgA (Fig. 3(C)), and α-amylase (Fig. 4(C)). Mucins and cystatins were not detected in either fresh HPS or its bracket pellicles (Figs. 5, 6, and 7). Compared with the bracket pellicles from HSMSL and UWS, the affinity of sIgA showed a more evident adsorption on the bracket pellicles from HPS (Fig. 3(C)).

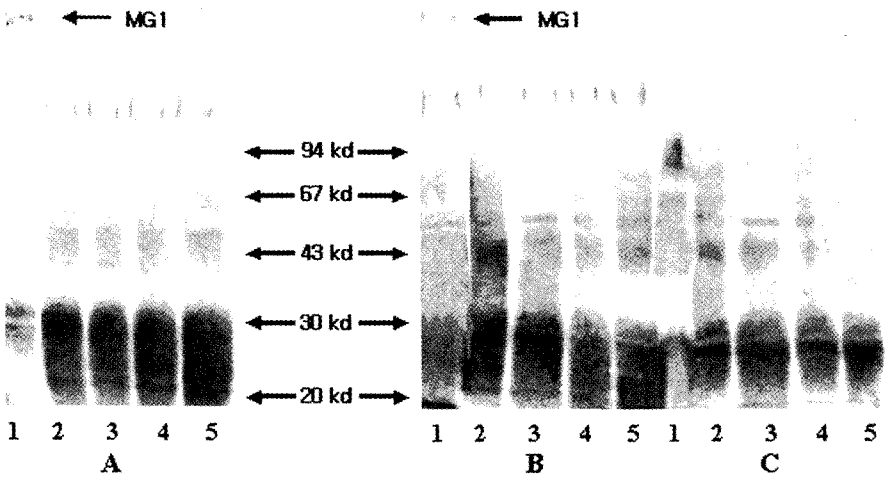

Fig. 6. SDS-PAGE (15%) and immunoblotting of experimental pellicles formed from UWS (panel A), HSMSL (panel B), and HPS (panel C). The membrane was probed with rabbit antisera to human salivary cystatins (1:1000 dilution). Lane 1, fresh saliva (UWS or HSMSL or HPS); lane 2, experimental pellicle formed on metal brackets; lane 3, experimental pellicle formed on PCA brackets; lane 4, experimental pellicle formed on MCS brackets; lane 5, experimental pellicle formed on plastic brackets.

DISCUSSION

Many clinical reports have shown that patients who receive orthodontic treatment are more susceptible to enamel white spot formation and demineralization.[7,22-24] In particular, enamel white spot formation adjacent to orthodontic brackets has been of great concern to many orthodontists. Orthodontic brackets play a major role in enamel demineralization because their complex design increases the retention of food particles and dental plaque by impeding proper access to the tooth surfaces for cleaning. Nevertheless, few studies have investigated the biological aspects of the orthodontic brackets associated with dental plaque and dental caries formations until recent days.

Since the edgewise mechanism was originally presented by Dr. Angle, various orthodontic brackets have been applied across the orthodontic fields. Over the years, many changes and modifications have been made in their basic design and composition. Currently, a new generation of ceramic and plastic brackets have become available due to patients' demands for improved esthetics. To examine the biocompatibilities of these various orthodontic brackets including the role of saliva-derived pellicle, we have investigated specific salivary proteins present in the bracket pellicles.

In vivo pellicle is formed by the adsorption of degraded proteins as well as intact proteins from glandular secretions, because many of the proteins in the whole saliva are thought to be degraded forms of secreted salivary proteins.[25] Thus, the division of whole saliva into original glandular secretions permits the determination of the relative affinities of intact proteins for the orthodontic brackets and a more detail comparison of the protein adsorption. In addition, saliva from the major salivary glands dominates different areas of the mouth. HPS and HSMSL differ in their protein composition and different areas of the mouth are characterized by different types of glandular saliva. Local variation in the pellicle composition by these reasons was reported before.[26] Thus, it may be meaningful to identify specific experimental pellicles formed by different types of glandular saliva as well as whole saliva at the same time.

Common proteins found in all bracket pellicles were acidic PRPs, α-amylase, and sIgA. Mucins (MG1 and MG2) and cystatins were not found in the bracket

Table 1. Comparison of salivary components found in bracket pellicles and other materials

	Previous studies							Present study		
	Enamel (89)[39]	Denture (92)[2]	Titanium (96)[1]	Elastic ring (2001)[4]	Bonding resin (2001)[4]	Bracket metal	Metal bracket (2001)[4]	MCS bracket	PCA bracket	Plastic bracket
MGI	+	+	+	−	−	+	−	−	−	−
MG2	+	+	+	−	−	+	−	−	−	−
-Amylase	+	+	+	−	−	+	−	−	−	−
sIgA	+	+	+	−	−	+	−	−	−	−
Acidic PRPs	+	+	+	−	−	+	−	−	−	−
Cystatins	+	+	+	−	−	+	−	−	−	−

− ; absent, + ; present

MG1 : high-molecular weight salivary mucin

MG2 : low-molecular weight salivary mucin

MCS (Mono-Crystalline Sapphire Brackets) : Inspire brackets

PCA (Poly-Crystalline Alumina Brackets) : Transcend 6000 series brackets

Plastic : Silcon m brackets

Metal : Korean Smart brackets

pellicles from HPS. This is due to the facts that mucins and cystatins are only present within a little in the parotid saliva, and are present plentifully in the submandibular-sublingual saliva. Whole saliva exists on the mixed state from submandibular-sublingual saliva and parotid saliva. Therefore, mucins and cystatins were also found in the bracket pellicles from UWS (Figs. 5, 6, and 7).

Salivary proteins like α-amylase, MG2, and acidic PRPs were known to act as specific receptors for bacterial adhesion.[27-32] Therefore, the presence of these proteins in the bracket pellicles may offer various binding sites and further colonization opportunities for oral bacteria. This means that orthodontic brackets may provide a potential risk for dental plaque formation during orthodontic treatment.

Though qualitatively the same components may be present from antibody detection, there are some differences quantitatively in the gel profiles in Fig. 1. This was particularly evident in the experimental pellicles from plastic brackets. Generally, some of the salivary components adhered more to the plastic brackets (Fig. 1(A, B, anc C) : lane 5). This is also supported by their immunoblotting procedure that showed an increase in cystatins in these pellicles (Fig. 7(A and B) : lane 5). Additionally, bracket pellicles were different from pellicles formed on enamel, denture, and titanium previously reported by others (Table I). For example, MG1 that has been detected in the enamel, denture, and titanium, was not detected in the bracket pellicles. This means that orthodontic brackets have unique surface properties and structures compared with enamel and other dental materials. This distinct composition of the bracket pellicles may promote the development of a different plaque by selecting for bacteria with different adhesive properties.

The bindings of sIgA and acidic PRPs showed considerable differences between the bracket pellicles from HSMSL and HPS (Figs. 2 and 3). The binding of sIgA in bracket pellicles from HSMSL was weaker than that from HPS, while the binding of acidic PRPs in bracket pellicles from HSMSL was stronger than that from HPS. This can be explained by the unique property of mucins. Mucins were known to form heterotypic complexes, interacting with sIgA, lysozyme, and

cystatins.[3] The low affinity of sIgA in HSMSL samples may be due to the formation of the mucin-sIgA heterotypic complexes. sIgA binding sites may be substituted by acidic PRPs and MG2, which presented a stronger binding pattern in HSMSL samples (Fig. 2). In HPS, which has no micin, the binding affinity of IgA was increased, while that of acidic PRPs was decreased.

An interesting finding is the different adsorption profile between metal bracket and bracket metal (Table I). MG1 was not detected in the experimental pellicles formed on the metal bracket, but detected in the experimental pellicles formed on the bracket metal (the same raw material). This may result from changes of the surface free energy during bracket fabrication procedures, such as milling and surface polishing. This is confirmed by the previous finding that the physical and chemical nature of materials significantly affected the relevant physico-chemical surface properties, the composition, packing, density, and the configuration of the pellicle coating. It is so desirable that MG1 did not adhere to the orthodontic brackets, because MG1 has been known to promote the initial adhesion of *Streptococcus mutans*,[3] which is one of the major cariogenic bacteria.

When orthodontic brackets are placed in the oral cavity, a layer of saliva is rapidly adsorbed to the surface. Characterization of the acquired bracket pellicle could provide a basis for understanding its function either by the prevention or promotion of the adhesion of pathogenic microorganisms, because it's possible to decrease binding of pathogenic bacteria by means of surface modification and change of chemical composition. In this situation, this study will provide a primary step toward finding means to interfere with the process of adherence of pathogenic bacteria to the pellicle on the orthodontic appliances.

CONCLUSIONS

In the present study, experimental pellicles that formed on the 4 different orthodontic brackets were analyzed by SDS-PAGE, Western transfer methods, and immunodetection. From these assays, the following results were obtained.

1. MG2, α-amylase, sIgA, acidic PRPs, and cystatins adhered to all kinds of orthodontic brackets. MG1 which promotes the adhesion of S. *mutans*, did not adhere to any orthodontic brackets.
2. Protein adsorption profiles of the bracket pellicles were different quantitatively and quantitatively according to the origins of saliva. In particular, the binding of sIgA was more prominent in the bracket pellicles from parotid saliva.
3. Gel profiles also showed the selective adsorption of salivary proteins according to bracket types. In particular, cystatins were more prominent in the pellicles form plastic brackets.
4. The adsorption profiles of major proteins on the orthodontic brackets were different from those on teeth and other dental biomaterials. This result means the selective adsorption of salivary proteins to the specific surfaces.

Collectively, this study indicates that numerous salivary proteins adhered to the orthodontic brackets and that these salivary proteins adhered selectively according to the origin of saliva and the types of brackets.

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국문초록

다양한 교정용 브라켓 표면에 부착하는 타액단백질에 관한 연구

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본 연구의 목적은 다양한 교정용 브라켓의 표면에 형성되는 타액성 피막의 조성을 확인하고, 전타액, 악하선타액 및 이하선타액에서 유래하는 타액성 피막의 성분을 비교하는 것이다. 네가지 서로 다른 종류의 교정용 브라켓을 본 연구에 사용하였다. 이들은 022×028 Roth prescription의 상악 소구치 브라켓으로 조성은 다음과 같다; 스테인레스 스틸, 단결정 사파이어, 다결정 알루미나 및 플라스틱 브라켓. 교정용 브라켓을 각각 전타액, 이하선타액 및 악하선타액에 2시간 배양하여 타액성 피막을 형성시켰다. 브라켓 피막의 타액성분은 sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Western transfer method 및 면역검출법을 통해 확인하였다. 이 결과 low-molecular weight salivary mucin, α-amylase, secretory IgA (sIgA), acidic proline-rich proteins, cystatins 등이 모든 브라켓의 타액성 피막에 존재하였으며, 치아우식증의 원인균인 *Streptococcus mutans*의 부착을 촉진시키는 타액단백질인 high-molecular weight mucin은 어떤 브라켓에도 부착하지 않았다. 그러나, 비록 동일한 타액단백질이 모든 브라켓에서 발견되었지만, 타액단백질 부착 양상은 타액의 종류 및 브라켓의 종류에 따라 양적 및 질적으로 다르게 나타났다. 특히 sIgA는 이하선타액에서 유래한 브라켓 피막에 더 많이 부착하였고, cystatins의 경우는 플라스틱 브라켓에서 유래한 브라켓 피막에 더 많이 존재하였다. 본 연구는 다양한 타액단백질이 교정용 브라켓에 부착하며, 타액단백질이 타액의 출처 및 브라켓의 종류에 따라 교정용 브라켓의 표면에 선택적으로 부착함을 나타내었다.

주요 단어 : 타액, 타액성 피막, 교정용 브라켓