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UNIVERSITY OF HACETTEPE
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WET WEIGHT TOTAL VIABLE BACTERIAL COUNTS LACTOBACILLI STREPTOCOCCUS MUTANS AND pH OF PLAQUE ACCUMULATED ON THREE DIFFERENT TYPES OF POLISHED AND UNPOLISHED COMPOSITE RESINS

A THESIS SUBMITTED FOR THE DEGREE OF
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IN
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I N T R O D U C T I O N

Dental caries in the present day is the most prevalent chronic disease of the human race. It results in destruction of tooth tissue, changes in the physiology and psychology of the sufferer, causes pain and discomfort and is a cause of wastage of much time both for skilled man-power and the patient. At the same time, it is expensive in terms of the money spent on the individual as well as on national levels.

From very ancient times, people have been suffering from this disease. Different scholars have tried to investigate its cause and to treat it. However it was not until the time after Von Leewonhoek, Lister and Magitot, that the nature of the disease was understood. Miller was the first to study the disease comprehensively and G.V. Black was the first to give a detailed treatment plan.

As the lost part of teeth cannot be replaced by natural healing processes as happens in other tissues, it becomes necessary to restore the morphology by artificial means to eradicate disease and to re-establish function and aesthetics.

Several, quite different materials have been and are being used for the purpose. A material being used to replace a vital tissue, in an area of vital importance such as the oral cavity, must have a set of certain properties. For example, its physical and mechanical properties such as hardness, resilience, thermal conductivity, co-efficient of thermal expansion, and contraction, resistance to stress and strain, the colour

and translucency etc. must be comparable to that of tooth tissue. Chemically it must not be irritant to pulp or oral tissues, it must not be soluble in the oral environment and it must not corrode or tarnish. There must be a chemical bond between the tooth structures and the filling materials. And last but not least, it must not encourage plaque accumulation which is the cause of occurrence and recurrence of caries and periodontal diseases.

No available material fulfils all these requirements. But those in much use are, gold and its alloys, amalgam, unfilled resins and different cements including the silicate cements. During the last two decades. Composite resins are being used and are increasing in popularity day by day.

Plaque accumulates on teeth as well as on all types of filling materials[28]. Some studies[52,67] show that chemical composition, surface free energies and zeta potentials of a surface have some role in bacterial accumulation. Even minor differences of constitutents in amalgam cause a decrease in plaque accumulation[11]. It has been observed in a study[30] that the plaque from approximal areas of sound teeth and those having class II composite fillings differed with respect to the nature of bacteria. It has also been observed that more plaque accumulates on rough surfaces of composite fillings[18,44,71]. The type of matrix material of the composites affect the amount of plaque on their surfaces[18,52]. It is now well established that macrofilled resins give a rougher surface as compared to microfilled resins[43,68,73] while it has been shown that smooth surfaces of restorations attract less plaque[18,44,45,71].

Today's literature is full of studies about composite materials. Their physical and mechanical properties[4,32], composition[29], methods of placement[21], adaptability to teeth and microleakage[6,50,56], color stability[22], shrinkage[13],

water absorption and polishability etc.[8,37,48,68,73] have been the main topics of investigations. But the quantity and quality of plaque on composite materials (having different types of fillers, matrix materials and curing mechanisms) have not yet been given sufficient attention.

In addition, for the composite resins, there is a controversy among different researchers, whether the fillings be polished or left as such when cured under a matrix.

The present study was planned to evaluate the amount (wet weight) and the nature of bacteria (by cultivating the CFU, lactobacilli, the Streptococcus mutans and pH measurement) of plaque accumulated on three different types of polished and unpolished (cured under a transparent matrix band) samples of composite resins, having the same size and under similar conditions.

R E V I E W

DENTAL PLAQUE

The dental plaque may be defined as a soft concentrated mass of micro-organisms, tenaciously adherent to exposed surfaces of teeth and gingival margins. The micro-organisms are embedded into the inter-cellular matrix which is mostly of microbial origin. The cross-section of plaque, when examined by electron-microscope can be divided into two distinct layers, a granular, acellular inner layer - the pellicle, and an outer layer consisting of densely packed microbial cells - the plaque proper[62].

Pellicle means a thin skin or film and, in dentistry, it is generally described as the acellular, structureless, bacteria-free thin layer which covers all of the exposed surfaces of teeth. It is usually 0.1-1.0 μm in thickness but may become 10 μm thick in certain cases.[62]

After thorough prophylaxis certain salivary proteins get adsorbed onto the dental surfaces with in a few seconds. There seems to be a definite mechanism for selection of proteins which may depend upon the surface structure of the enamel. Pellicle formed on enamel in vivo and in vitro shows similarities in composition, while the quantity and structure of pellicle formed on other materials differ markedly[58,63].

These differences are important in the formation of bacterial plaque[24] on those surfaces. Altohugh saliva is supposed to provide most of the organic constituents of pellicle, some bacterial products, gingival crevicular fluid and haemorrhage from gingivae may contribute to its formation[16,36,62]. The main body of pellicle consists of acidic amino acids - namely aspartic and

glutamic acids while basic amino acids like lysine, histidine and arginine are also present. In addition, the presence of glucose, galactose, mannose, glucosamine, galactosamine and rhamnose have also been confirmed[36,62]. Pellicle may serve as a diffusion barrier for ions and may give resistance to acid demineralization and also may help remineralization[62] by storing ions such as Ca, PO_4 and F^- etc. However at the same time it provides a medium for the attachment of bacteria which eventually give rise to dental plaque.

The oral cavity is never free from micro-organisms, the first invaders being those from maternal vagina, others gaining entery via foods and still others from the persons in close contact.

Few of them get a chance to establish in the oral cavity due to the presence of typical habitat which changes with age, at different times of the day, affected by flow of saliva, eating and drinking and the oral hygiene measures[35].

The oral microbiota is composed of a number of distinct ecosystems due to the presence of quite different types of habitats namely the teeth, mucous membranes, saliva, tongue and gingival crevices. The largest biomass of bacteria is present on teeth and tongue. Coronal plaque consists of 90 % bacteria and the number of bacteria range between 10^{11}-10^{12} organisms per gram wet weight. Each ecosystem differs in composition due to differences of habitat.

Dental plaque is a community of organisms living together and existing with their abiotic constituents on various surface areas of tooth which differ in certain aspects from one area to another. Plaque from coronal

areas differ from the plaque of subgingival areas or from pits and tissues. Again it varies with age of the mass and certain host factors such as the type and frequency of foods, salts present in drinking water and the life style of the subject[34].

Formation of the typical plaque may be divided into four stages[36,38].

1- Formation of acquired pellicle which starts within seconds after prophylaxis of teeth and is due to adsorption of certain salivary proteins caused by surface charges of teeth.

2- Initiation of pioneer community : Soon after pellicle formation, certain bacteria, usually the streptococci, start invading and forming local colonies on teeth. After eight hours, a rapid multiplication can be seen and microbes make a single layer on all the surfaces. The most commonly found bacteria at this stage are S. sanguis and A. viscosus. The bacteria continue multiplying and forming multilayers. Some filamentous organisms may also be present at this stage.

3- Formation of an intermediate community : This stage is characterized by invasion of secondary invaders which is caused by changes in local environment e.g. lack of oxygen in deeper layers. A. naeslundii, veillonella and peptostreptococci become more prominent. Filamentous forms appear to invade the underlying bacterial mass-gradually replacing the coccal forms. Secondary invaders adhere to the organisms already present or to the matrix material of the plaque.

4- Establisment of a climax community : After about ten days, a stage is reached where a few new organisms are introduced. At this stage, the inner layer consists of densely packed anaerobic/microaerobic cocci and bacilli and other rather loosely packed filamentous bacteria oriented perpendicular to the surface.

Certain factors may encourage the bacterial growth which include carbohydrates, amino-acids, peptides and proteins from saliva and gingival crevicular fulids etc. While exogenous carbohydrates, proteins, fats and interbacterial nutritional relationships also help the establishment of plaque whereas lysozymes, salivary immunoglobulins, glycoproteins and lactoferrin as well as oral hygiene measures inhibit the growth[34].

Biochemical Activities of Plaque[36] : Certain bacteria of plaque-like S. mutans give rise to extra-cellular dextrans from sucrose which is the main binding matrix material and is insoluble in the oral environments.

Fermentation of carbohydrates "glycolysis" is caused by the anaerobes of the plaque giving rise to certain organic acids. Lactic acid is the most important while acetic, propionic and formic acids may also be formed.

A drop in pH of plaque is seen if any person with established plaque rinses with glucose solution and this drop is proportional to the concentration of the solution and will take 20-30 minutes to return to normal limits. pH below 5.2 is said to be critical as demineralization starts below this limit. The opposite happens if the oral cavity is rinsed with urea. An elevation in plaque pH is seen due to the production of ammonia.

It can be concluded that the plaque and changes in plaque are the net result of different salivary factors, foods habits and oral hygiene measures etc.

COMPOSITE RESINS

A composite can be defined as "a three dimensional combination of at least two chemically different materials with a distinct interface". The resultant product exhibits properties which can never be achieved by the individual constituents.

The dental composite resin filling materials consist of three phases [29,31] - organic phase, dispersed phase and the coupling phase.

ORGANIC PHASE : This is also known as the matrix-phase. It consists of an oligomer, usually the Bis-GMA which is a combination of bisphenol-A and glycedyl methacrylate. Bis-GMA is a viscous material so some diluents are added to obtain the desirable working properties. Some modified forms of Bis-GMA, urethane-diacrylate, TEG-DMA etc. are also being used as matrix material of the composite resins.

DISPERSED PHASE : This consists of different fillers which may be different glasses, quartz or colloidal silica. The type and ratio of the filler dictates the properties of a particular product.

COUPLING AGENTS : The fillers are immissible to the organic matrix so a coupling agent is layered upon them. These agents - usually "silanes", have double binding sites, so they bind the two of the other phases together.

CLASSIFICATION : The composite resins can be classified depending upon 1) the type of fillers, 2) the curing mechanism.

Depending upon the fillers, composites may be classified into three groups : 1) Macrofilled, 2) Microfilled and 3) Hybrid.

MACROFILLED COMPOSITE RESINS : The filler content of this type of materials consists of particles of quartz, glass, borosilicates and ceramics etc. which range from 0.1-100 μm in size. But now-a-days there is a trend to use smaller particles of softer materials. These materials contain fillers upto 80 % by weight. These types of materials have certain advantages which are the high compressive and tensile strength, low co-efficient of thermal expansion, low water absorption, low polymerization shrinkage and high modulus of elasticity. The disadvantages include unpolishability (which cause more plaque accumulation and early staining of filling) irritation of gingiva abrasion of adjacent teeth and fillings etc.

MICROFILLED COMPOSITE RESINS : To accomplish better aesthetics, very small-sized filler particles, namely the colloidal or pyrogenic silicas, were added to the organic matrix. The size of this filler particle is 0.04 μm which is smaller than the wave length of visible light, so they can be polished to a very good finish. But the physical and mechanical properties are not comparable to those of the conventional resins because the filler, being very small in size, cannot be added to a sufficient quantity to the matrix without compromising the handling properties.

To increase the ratio of filler, some modifications have been made. The modified forms are called the "Hetrogenous Microfilled Composite Resins (H.M.C.R.)" and they may be divided into three groups.

A- Splinter shaped H.M.C.R. : A large amount of filler is added to an organic matrix which is polymerized into large bulks which are then ground to splinter shaped particles and are thus used as fillers.

B- Spherical Shaped H.M.C.R. : Partially polymerized spherical organic

(matrix) particles are added with high concentrations of microfillers and are used for loading organic matrix after completion of polymerization.

C- Agglomerated H.M.C.R. : Pure inorganic fillers are agglomerated by heat treatment and ground to small particles which are then used as fillers.

The microfillers impart some favourable properties to the composite resins. The most important is the high polishability with permanence of aesthetics. Due to relatively smaller percentage of filler, their physical and mechanical properties are poorer as compared to macrofilled composite resins.

HYBRID COMPOSITE RESINS : To obtain good aesthetics with better physical and mechanical properties, still another modification was made. Each of the two types of fillers (i.e. macro- and micro-) were added to the same organic matrix. These types of composites exhibit a combination of properties of the other two types of materials. Their properties are improving due to the availability of smaller and softer types of macrofillers.

The second types of classification depends upon the mode of polymerization :

A- CHEMICALLY CURED RESINS : They are available in the form of two pastes, one is a base while other contains the activator, they react upon mixing and give rise to a hard mass within a certain time period specific to that particular product.

Advantages : The main advantage is the simplicity of use i.e. just mixing of the two pasters is required to start the polymerization. And also polymerization is uniform throughout the bulk of the set mass.

Disadvantages : Air is trapped during mixing which causes porosity, irregularities on the surface, loss of strength and increase in microleakage. Marginal adaptation is poor. Polymerization is never complete. And they have a very limited working time.

B- ULTRAVIOLET LIGHT CURED RESINS : They consisted of one paste and were polymerized upon exposure to ultra-violet rays. This system has been abandond due to health hazards and low efficacy of the curing system as compared to visible light curing system.

C- VISIBLE LIGHT CURED RESINS : These types of composite resins are polymerized when exposed to visible light of certain specific wavelength. They are available in one paste system.

The advantages of this system include the long working time, smooth and homogenous surfaces, less porosity and improved marginal adaptation. The main disadvantage is that the deep layers of the materials may not be cured as the light can reach only to a limited depth. Also a special instrument is needed for the operation.

All these different types of composite resins, with their advantages and disadvantages have some particular place of use. Some are preferred for the filling of anterior teeth while others have been suggested for filling of posterior teeth.

The surface of a filling must be as smooth as possible. Since it has been shown experimentally that smooth fillings accumulate less plaque[18,44, 55,71] and cause less gingival inflammation[44].

To achieve surface smoothness of conventional composite resin fillings,

a glaze was coated on their surfaces and it was found, in an in vivo study[72] that the coated surfaces were smoother than un-coated surfaces even after 23 months.

It has been seen that the composite resins cured under a matrix give the smoothest surface[8,33,48,71]. However in clinical situations, it is usually necessary to reshape the filling and thus polishing is needed to achieve proper smoothness.

To evaluate the efficacy of different polishing procedures McLundi and Murrey[33] studied the surface smoothness after use of Tungston carbide burs, Diamond burs, Garnet disks, sand paper disks, Al_2O_3 strips, Zirconium silicate strips green and white stones, steel finishing burs and some combinations of these instruments. They used "Adaptic" (a macrofilled resin) for their study. They concluded that Tungston Carbide bur was good for gross reduction whereas the disks or strips may be used for final finishing and polishing. They concluded that the maximum smoothness could only be achieved when composites were cured under polyester strips.

Polishability of four microfilled composite resins was studied by Dennison et al.[8]. They concluded that the white stones and rubber polishers gave the worst results and 'Soflex' disks the best, whereas the smoothest surface was obtained when resin was cured under a mylar strip.

Reinhardt et al.[51] used six different systems to polish two microfilled and two hybrid resins. They evaluated their results by SEM, surface profilometry and refractive brightness readings. They recommended that medium Al_2O_3 disks may be used for gross reduction while the vivadent polishing system or Moore's paste may be used for final finishing and polishing.

Another study was conducted by Noort and Davis[37]. They used four macrofilled, two hybrid and one microfilled resin. They used "Soflex" disks or "Alumina" paste (of three different sized particles in sequence) with the help of a rubber cup. They observed that the Soflex disk was suitable for macrofilled resins, and Alumina paste was good for hybrid resins while both systems were equally effective for microfilled resins.

Zaimoğlu and Ulusoy[73] used the vivadent polishing system to evaluate the smoothness of three visible light cured composites (Heliomolar, Heliosit and Durafil) and two chemically cured composites (Adaptic and Miradapt). They observed by SEM that after the same procedures of polishing, microfilled resins gave the smoothest surfaces and macrofilled gave the roughest. In another report by the same authors[68], profilometric readings confirmed the SEM results.

Another study was performed by Önen[43] who compared Adaptic (chemically cured macrofilled resin), Isopast (chemically cured microfilled resin) and Heliosit (visible light cured microfilled resin). Using two polishing systems - silicone rubbers in sequence and Flexi (Al_2O_3) disks in sequence, Önen concluded by SEM examination that Flexi disks were the better of the two, and also that microfilled resins gave the smoothest surfaces after use of any of the two systems.

An SEM study in addition to profilometry was carried out by Pratten and Johnson[48] to evaluate smoothness of one posterior and one anterior composite resin. They compared the effect of mylar strip, carbide and diamond burs, Soflex and Shofu disks, white stone, Quasite rubber point and Kerr luster polishing paste. They found no statistically significant

difference in smoothness of the two composites. But Soflex and Shofu disks and Kerr luster paste gave the best results. The smoothest surfaces were obtained when resins were cured under a mylar strip.

It has been suggested by Weitman and Eamas[71] that, for polishing a composite resin, the polishing agent must be harder than the surface being polished and the size of the polishing particles must be small. Use of Al_2O_3 has been proposed as this has got Mohs hardness number 9, as compared to 7. of quartz (a filler material) or to 7.5 that of Zirconium silicate (a polishing agent). The Mohs hardness of fluor of pumice is only 5-6 and it must never be used as a polishing agent for composite resin Al_2O_3 in the form of disks or slurries may be used to achieve the smoothest finishes.

MATERIALS AND METHODS

This study was performed using intra-oral appliances worn by ten (5 male and 5 female) healthy volunteers who were all students of the Faculty of Dentistry, University of Hacettepe, Ankara. Their ages ranged from 18-31 years the average being 21.11 years. An oral examination of the volunteers was performed and all the active carious lesions were treated (if present). Periodontal treatment (scaling and polishing) was also provided for all of them. The DMF-T ranged from 2-13, average being 5.67.

The appliances were made in the orthodontics department of the same faculty. The samples of composite resins were made and fixed to the appliances in the operative dentistry department. The wet weight measurement was performed in the Quality Control Section of the Faculty of Pharmacy, while microbiological procedures and pH measurement were performed in the Microbiology Department of the Faculty of Medicine. The data obtained was evaluated in the Biostatistics Department of the Faculty of Medicine, University of Hacettepe.

PREPARATION OF APPLIANCES : Impressions of upper and lower jaws of the volunteers were taken in alginate and hard plaster models were made. Appliances (as used by Özgünaltay[45]) were constructed in cold cured acrylic resin around $\overline{765|}$ teeth on the models (Fig. 1). The appliances had two windows (each 4x5x2 mm in size) in lingual flanges.

The Study consisted of three experimental periods. Each period lasted 7 days. One type of material was tested in each period and total of three

different composite resins (Table I) were tested individually. Twenty samples from each materials were prepared one day before their use.

TABLE I. Types of composites resins used in the study.

	NAME OF MATERIAL	TYPE	MECHANISM OF CURING	TYPE OF MATRIX	MANUFACTURER
1.	EXPRESS	MACRO-FILLED	CHEMICALLY CURED	Bis-GMA	ALCOS, USA
2.	BRILLIENT	HYBRID	VISIBLE LIGHT CURED	Bis-GMA	COLTENE, SWITZERLANDS
3.	HELIOSIT	MICRO-FILLED	VISIBLE LIGHT CURED	URETHANE	VIVADENT, LIECHTENSTEIN

Sample Preparation : For the first experimental period samples were made from EXPRESS. The material was mixed on paper pads according to the manufacturer's instructions and packed into properly sized moulds. A transparent matrix was adapted on the surface of the resin and then the moulds were held between two glass slabs for five minutes. Prepared samples were stored in distilled water for 24 hours at room temperature. Half of them were then polished using Flexi† polishing disks in sequence at low speed under constant water spray. The samples were then fixed into the appliance with the help of sticky wax. The polished samples were always fixed in the mesial window and the unpolished in the distal. This procedure was performed carefully so that samples neither intruded nor extruded from the windows.

† Quality Dental Products manufacturing Co. USA

For the second experimental period, samples were made from BRILLIENT. The material was extruded directly into the moulds from the tube, matrix was adapted on the surface and every sample was exposed to curing light (HELIOMAT)† for 40 seconds. All the samples were stored in distilled water and half of them were polished after 24 hours. These samples replaced the Express samples (polished sample in mesial and unpolished in distal window) and the appliances were used in the second period.

In the third period, samples were prepared from 'HELIOSIT' in the way described for 'Brillent' and they replaced the old samples of the appliance for use in next test period.

Test Procedure : Every set of samples was worn by the valunteers for seven days continuously during which they continued their normal food habits. The appliance was removed only once a day to facilitate the brushing of natural teeth. Volunteers used a fluoride-free tooth-paste during the study period.

Collection of Plaque Samples : On completion of the 7 days' periods, the appliances were taken out of the oral cavities (two hours after breakfast during which no foods were consumed), washed under tap water for five seconds and gently dried with a chip-blower. Plaque was scraped with a sterilized GRACEY currette from each sample and was put on a pre-weighed sterilized galss-sheet. The weight measurement was made on a digital - balance‡ (Fig. 2). The weighed samples were put into small, sterilized. capped vials containing 2 ml of normal saline (of 6.85 pH).

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† Vivadent, Liechtenstein

‡ Libror : Shimadzo, JAPAN

Microbiology : To homogenize the plaque 0.1 ml of sterilized glass beads were added to each vial and was shaken on a vortex vibrator† at speed number 9, for 2 minutes. Serial ten fold dilutions were made from the original to 10^{-2} dilution. Three types of culture media were inoculated with a platinum wire loop of 0.01 ml capacity. The first was Blood-Agar (BA) to evaluate the total colony-forming units (CFU). This medium was inoculated from 10^{-2} dilution in duplicate. The second medium used was the Rogosa SL Agar to evaluate the Lactobacilli. The third medium used was the Mitis - Salivarius - Bacitracin (MSB) to cultivate the Streptococcus mutans. The later two media were inoculated from the original solution in duplicate. All the media were incubated at $37^{o}C$ in a candle-jar (Fig. 3) for 48 hours. The colonies were counted and calculations were made for number of bacteria per milligram of plaque.

pH Measurement was performed after completion of culturing procedures. A Beckman digital pH meter model 3500 with an ingold semimicro combined pH electrode (ingold no: 2) was used for the purpose. The pH meter was checked and standardized by a solution of known pH-7 before and during the operation. There was a time lapse of approximately 1 1/2 hours between sample collection and pH measurement.

Once during the period of study, a three-days old plaque was collected from the lingual surface of first molar around which the appliance was worn for comparison purposes. This plaque was sampled and processed in the same way as described before (for plaque from composite samples).

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†Vortex Genie Mixer. Scientific Product : American Hospital Supply Co. Evanston III, USA.

The data was analysed by "Analysis of variance with repeated measurements" (RMAOU). And the differences among the groups were analysed by 'Tukey Test'.

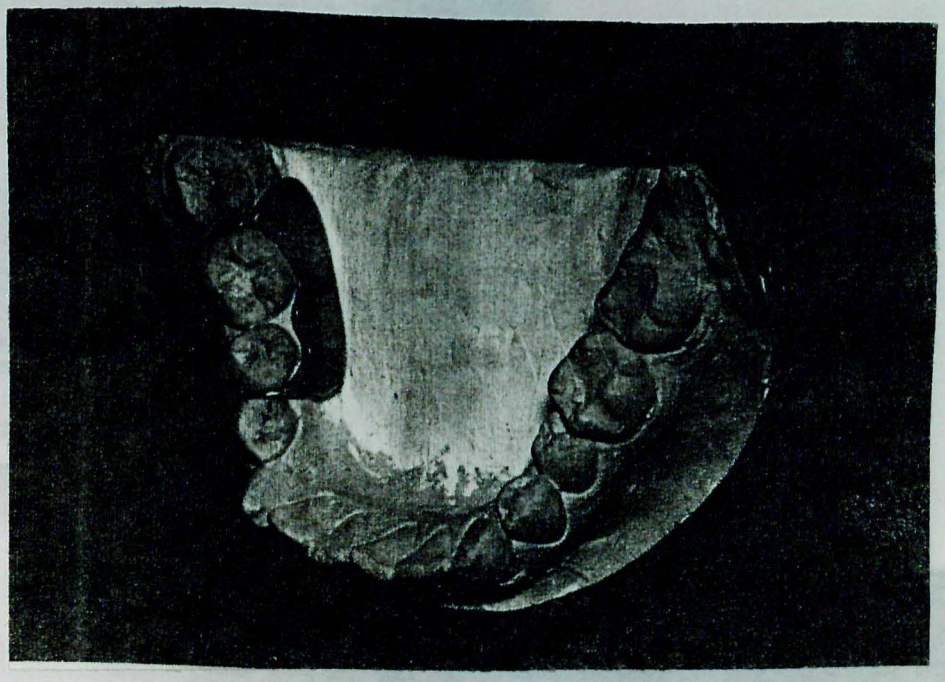

Fig. 1 : The appliance used in the study on plaster model.

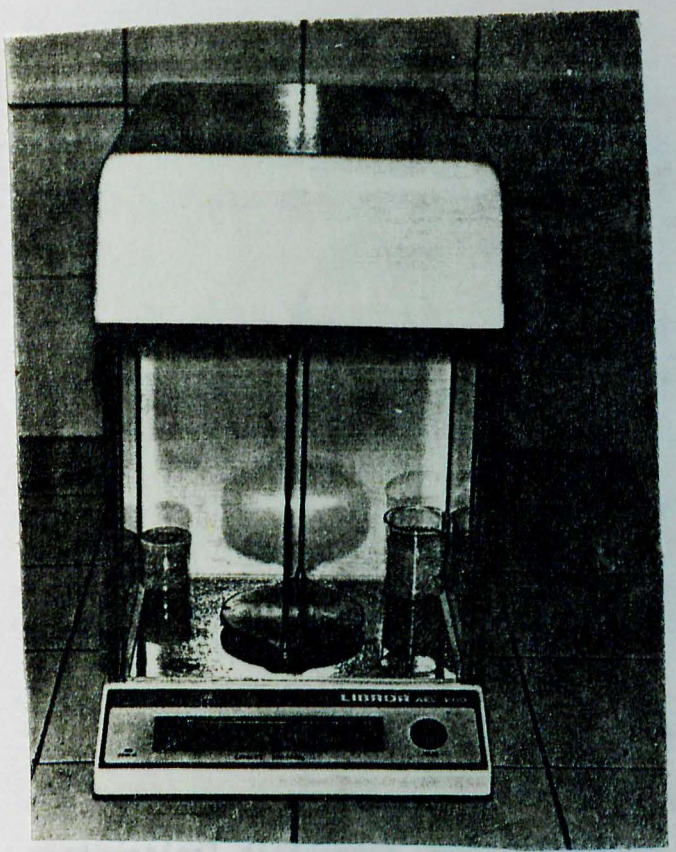

Fig. 2 : Digital balance (Shimadzo-Librar) having measuring capacity upto 0.1 mg.

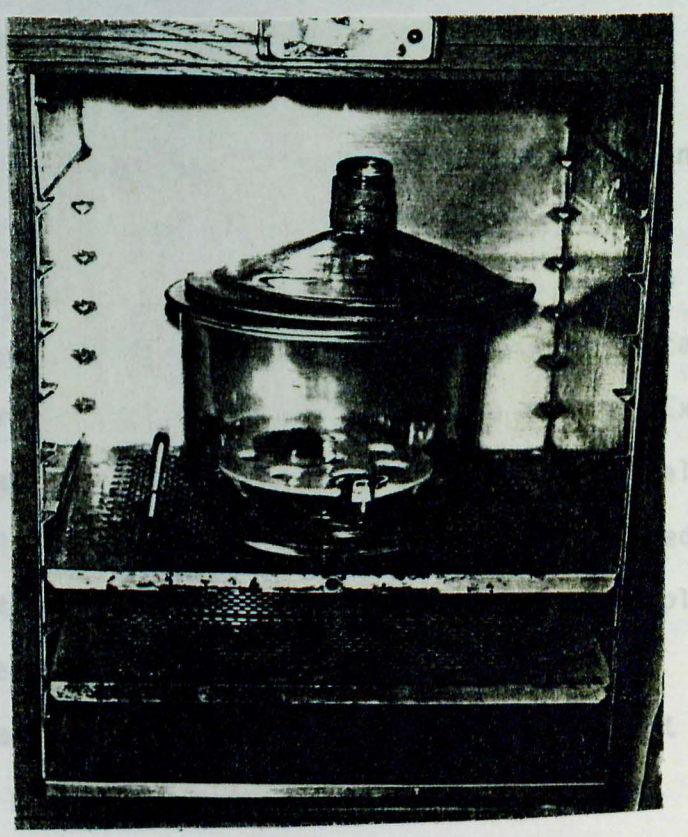

Fig. 3 : The Candle-jar (in an incubator).

F I N D I N G S

The study was carried out with ten appliances worn by ten volunteers. The study was divided into three experimental periods of seven days each. One material was tested in each period. One of the volunteers could not complete all of three experimental periods, so she was dropped from the study. The results comprising average of the nine appliances are given in tables II-VII.

The first objective of the study was to measure the amount of plaque collected by different materials Table II shows that there is a marked arithmetical difference among the wet-weight of plaque from different types of materials, and also the polished and unpolished samples of the same material collected different amount of plaque. Maximum amount of plaque was collected by Express. Polished samples collected more plaque than unpolished samples. Polished Brillient and polished Heliosit accumulated almost same amount of plaque. Again the unpolished samples of these two accumulated approximately equal amount of plaque. There was a statistically significant difference between the wet-weight of plaque from polished Express and that collected by polished Brillient and also that from polished Heliosit. The plaque from Express is also statistically greater in amount compared to the plaque collected from either unpolished Brillient and Heliosit samples. Other differences are not statistically significant. It may be worth mentioning that three polished and four unpolished samples of Brillient were free of any collectable plaque.

The second criterion was the estimation of total viable colony forming unit (CFU) of plaque collected from different types of composite resins and that from natural teeth. Table III shows the total CFU and Table IV gives the CFU per mg of the plaque wet weight. A comparison can be made among CFU of plaque samples from different types from natural teeth. As can be seen from the Table III, there are differences among different samples of plaque, but the differences between the total CFU of plaque from un-polished Express and unpolished Brillient and that from un-polished Heliosit reached a level of statistical significance (Heliosit containing the maximum number of bacteria). Other differences are not statistically significant.

The CFU per mg of plaque wet weight also showed variances among different materials and the polished an un-polished samples of the same materials. Statistically significant differences were found between the CFU per mg of plaque from unpolished Express and unpolished Brillient and that from unpolished Heliosit. The differences between the CFU per mg of plaque from polished Express and polished Brillient and that of unpolished Heliosit were also statistically significant. The CFU per mg of Plaque from unpolished Heliosit showed a great difference from its polished counterpart and also that of natural teeth. Only the difference between unpolished Heliosit and that from natural teeth was statistically significant. Difference of CFU per mg between the polished Heliosit and unpolished Heliosit was not statistically significant. Also the differences of CFU per mg between the polished and unpolished samples of Express and Brillient and that of the natural teeth are not statistically significant.

The third part of the study was the evaluation of Lactobacilli (Table V). Plaque from three polished and three unpolished samples of

Express, five polished and two unpolished Brillient, and four polished and five unpolished samples of Heliosit gave rise to growth of lactobacilli on Rogosa SL Agar. Plaque from natural teeth also gave inconsistent growth. The unpolished Brillient and Heliosit appeared to attract maximum number of Lactobacilli per mg of plaque wet weight, but due to scarcity of data, statistically significant differences could not be calculated.

The evaluation of Streptococcus mutans in different plaque samples was the fourth objective of our study (Table VI). Only one plaque sample from polished and one from unpolished Express contained S. mutans. Five plaque samples collected from polished and four from unpolished Brillient showed the presence of S. mutans. Heliosit was the best attractor of S. mutans among the composite resins. Eight plaque samples collected from polished Heliosit and all those collected from unpolished Heliosit gave rise to growth of S. mutans. All plaque samples collected from natural teeth showed the presence of S. mutans. The number of S. mutans from plaque samples of polished and unpolished, Express and Brillient showed no statistically significant differences among them and when compared with those from the plaque of natural teeth. The number of S. mutans from plaques of Heliosit samples was greater than any other plaque sample including that from natural teeth. Statistically significant differences were found between samples of unpolished Express and unpolished Heliosit and also between unpolished Express and polished Heliosit. All other differences were statistically insignificant.

The last aim of the study was the evaluation of pH changes caused by bacterial plaque, in the transport medium(Table VII). Plaque collected from samples of polished and unpolished samples of Express and Brillient and also

from the natural teeth, caused an elevation of pH. The elevation was different for every type but was not statistically significant. The plaque from Heliosit (polished and unpolished) caused a reduction in pH of the transport medium. Although the reduction was not much, when compared to those of the other samples, there was a statistically significant difference from all other samples. There was no statistically significant difference between the pH of plaque from polished and that form un-polished Heliosit.

Tables II-VII give the results of the study :

Abbreviations used are :

n	:	number of samples
$\bar{x}$	:	average
SD	:	standard deviation
$S\bar{x}$	:	standard error
G.S.S.D.	:	pairs of groups having statistically significant differences (pairs not given in the list did not show any statistically significant differences).

TABLE II : Wet weight (in milligrams).

GROUP NUMBER	NAME OF SAMPLE	n	$\bar{x}$	SD	$S\bar{x}$	$\bar{x} \pm S\bar{x}$
1	POLISHED EXPRESS	9	1.3222	0.646	0.22	1.3222 ± 0.22
2	UN-POLISHED EXPRESS	9	0.7666	0.951	0.32	0.7666 ± 0.32
3	POLISHED BRILLIENT	9	0.5333	0.646	0.22	0.5333 ± 0.22
4	UN-POLISHED BRILLIENT	9	0.2333	0.250	0.08	0.2333 ± 0.08
5	POLISHED HELIOSIT	9	0.5111	0.344	0.11	0.5111 ± 0.11
6	UN-POLISHED HELIOSIT	9	0.2000	0.150	0.05	0.2000 ± 0.05
7	PLAQUE FROM NATURAL TEETH	9	1.0000	0.000	0.00	1.0000 ± 0.00

$F = 8.03044$, $p < 0.05$ G.S.S.D.: 1-3, 1-4, 1-5, 1-6

TABLE III : CFU Total count from the samples ($x10^6$).

GROUP NUMBER	NAME OF SAMPLE	n	$\bar{x}$	SD	$S\bar{x}$	$\bar{x} \pm S\bar{x}$
1	POLISHED EXPRESS	9	1.2944	0.802	0.27	1.2944 ± 0.27
2	UN-POLISHED EXPRESS	9	0.7811	0.880	0.29	0.7111 ± 0.29
3	POLISHED BRILLIENT	9	1.3170	1.581	0.53	1.3170 ± 0.53
4	UN-POLISHED BRILLIENT	9	0.4417	0.790	0.26	0.4417 ± 0.26
5	POLISHED HELIOSIT	9	1.9366	0.859	0.29	1.9366 ± 0.29
6	UN-POLISHED HELIOSIT	9	2.4788	2.477	0.83	2.4788 ± 0.83
7	PLAQUE FROM NATURAL TEETH	9	2.0866	0.656	0.22	2.0866 ± 0.22

$F = 3.62419$, $p < 0.05$ G.S.S.D. : 2-6, 4-6

TABLE IV : CFU per milligram wet weight ($x10^6$).

GROUP NUMBER	NAME OF SAMPLE	n	$\bar{x}$	SD	$S\bar{x}$	$\bar{x} \pm S\bar{x}$
1	POLISHED EXPRESS	9	1.0676	0.515	0.17	1.0676 ± 0.17
2	UN-POLISHED EXPRESS	9	1.4132	1.700	0.57	1.4132 ± 0.57
3	POLISHED BRILLIENT	9	1.1022	1.314	0.44	1.1022 ± 0.44
4	UN-POLISHED BRILLIENT	9	0.8213	1.286	0.43	0.8213 ± 0.43
5	POLISHED HELIOSIT	9	6.4944	6.567	2.19	6.4944 ± 2.19
6	UN-POLISHED HELIOSIT	9	12.8411	9.145	3.05	12.8411 ± 3.05
7	PLAQUE FROM NATURAL TEETH	9	2.0866	0.656	0.22	2.0866 ± 0.22

$F = 9.0505$, $p < 0.05$ G.S.S.D.: 1-6, 2-6, 3-6, 4-6, 6-7

TABLE V : Lactobacilli per mg wet weight ($x10^2$).

GROUP NUMBER	NAME OF SAMPLE	n	$\bar{x}$	SD	$S\bar{x}$	$\bar{x} \pm S\bar{x}$
1	POLISHED EXPRESS	9	1.5677	3.974	1.32	1.5677 ± 1.32
2	UN-POLISHED EXPRESS	9	4.4577	11.510	3.84	4.4577 ± 3.84
3	POLISHED BRILLIENT	9	12.6289	24.351	8.12	12.6289 ± 8.12
4	UN-POLISHED BRILLIENT	9	80.0778	226.796	75.60	80.0778 ± 75.60
5	POLISHED HELIOSIT	9	0.6188	1.191	0.40	0.6188 ± 0.40
6	UN-POLISHED HELIOSIT	9	32.4444	89.184	29.73	32.4444 ± 29.73
7	PLAQUE FROM NATURAL TEETH	9	1.1111	1.833	0.61	1.1111 ± 0.61

$F = 0.930832$, $p > 0.05$ No pair of groups have statistically significant differences

TABLE VI : S. mutans per mg wet weight ($x10^3$).

GROUP NUMBER	NAME OF SAMPLE	n	$\bar{x}$	SD	$S\bar{x}$	$\bar{x} \pm S\bar{x}$
1	POLISHED EXPRESS	9	0.8511	2.553	0.85	0.8511 ± 0.85
2	UN-POLISHED EXPRESS	9	0.1477	0.443	0.15	0.1477 ± 0.15
3	POLISHED BRILLIENT	9	2.0555	4.035	1.34	2.0555 ± 1.34
4	UN-POLISHED BRILLIENT	9	1.6866	2.393	0.80	1.6866 ± 0.80
5	POLISHED HELIOSIT	9	7.3288	9.235	3.08	7.3288 ± 3.08
6	UN-POLISHED HELIOSIT	9	6.9555	5.052	1.68	6.9555 ± 1.68
7	PLAQUE FROM NATURAL TEETH	9	2.1111	1.075	0.36	2.1111 ± 0.36

$F = 3.66031$, $p < 0.05$ G.S.S.D. : 2-5, 2-6

TABLO VII : pH measurements.

GROUP NUMBER	NAME OF SAMPLE	n	$\bar{x}$	SD	$S\bar{x}$	$\bar{x} \pm S\bar{x}$
1	POLISHED EXPRESS	9	7.5077	0.448	0.15	7.5077 ± 0.15
2	UN-POLISHED EXPRESS	9	7.5933	0.414	0.14	7.5933 ± 0.14
3	POLISHED BRILLIENT	9	7.2622	0.381	0.13	7.2622 ± 0.13
4	UN-POLISHED BRILLIENT	9	7.3811	0.407	0.14	7.3811 ± 0.14
5	POLISHED HELIOSIT	9	6.5377	0.141	0.05	6.5377 ± 0.05
6	UN-POLISHED HELIOSIT	9	6.7777	0.117	0.04	6.7777 ± 0.04
7	PLAQUE FROM NATURAL TEETH	9	7.2533	0.405	0.14	7.2533 ± 0.14

$F = 13.4978$, $p < 0.05$ G.S.S.D.: 1-5, 1-6, 2-5, 2-6, 3-5, 3-6, 4-5, 4-6, 5-7, 6-7

D I S C U S S I O N

This study was designed to evaluate the quantity (wet weight) and quality (total viable CFU, Lactobacilli, S. mutans and pH) of plaque accumulated an three different types of polished and un-polished composite resins (having different types of matrix materials, different fillers and different types of curing mechanisms) in oral conditions. An intraoral appliance was used for this study and ten volunteers were selected for the use of appliances constructed individually for each of them. Ten volunteers were used because Weitman and Eames[71] quoted from Kaslick[25] that at least ten subjects were necessary for the validity of the results.

All the volunteers were students of the Dental Faculty. This selection was intentionally made because they would be more co-operative and considerate about the ethics of the experiment. They would also always be available for every type of check-up, control, discussion and/or collection of samples at the proper time.

The volunteers underwent an oral examination. Some of them had active carious lesions and some had mild tartar/stain deposits with gingivitis. Carious lesions were treated where necessary while scaling and polishing was performed for every volunteer. Although it has been suggested that past or present caries experience of the volunteers has no effect on the amount and type of bacteria in the ICT (intra-oral cariogenicity test) models[22], we provided this prophylaxis to minimize any chance of error due to this variable.

G.V. Black was perhaps the first to use an intra-oral appliance. Recently it has been used by many[9,22,26,27] for ICT. The one used in this study was a modification of the modified form used by Özgünaltay[45]. The modification was made due to the observation that maximum amount of plaque accumulates on the lingual side of lower molars[3].

Our pilot study also gave similar results. Therefore the composite samples were put into the windows in lingual flange of the appliance.

There are certain advantages in using an intra-oral appliance method instead of in vivo or an in vitro experiment. Intra-oral appliance takes the test materials into the oral cavity (the in-vivo conditions) and the test is performed without harm to any of the natural tissues while it has the advantage of being more standardized. For example, if the material of our study were to be tested by a classical in vivo method, it may had been impossible to find such a large number of patients requiring fillings in the same area and of same size. With the help of the appliance it is possible to make samples of a material standard in size under specific conditions which is impossible in the oral cavity. The collection of plaque from the sample is easy and more reliable. As has been indicated by Creanor et al.[5] plaque was more consistent in nature from ICT appliances than that from natural teeth. The in-vitro method has the biggest disadvantage since natural (oral) conditions can never be reproduced outside the oral cavity. Saliva, the micro-organisms, the foods and drinks taken by a subjects, teeth, tongue, gingivae, mucous membranes, different hormonal and emotional factors etc. are very much unique to the human oral cavity and cannot be duplicated outside in a laboratory even by use of experimental animals. Also the bacteria change their adherence properties when cultivated outside the oral cavity[41].

The disadvantage of the appliance (used for an examination like ours) may be more friction from tongue to the samples on the appliance (as compared to teeth) due to larger contour of the appliance.

There were three types of materials tested in our study. First "EXPRESS" a chemically cured composite resin, second (BRILLIENT - a visible light (VL) cured hybrid and third was the "HELIOSIT" which is a VL cured microfilled resin. All of these three differ in one way or another. The first two have Bis-GMA as their main matrix material, but Express is macrofilled and chemically cured while Brillient is a VL cured hybrid composite resin. The third resembles the second in that it is also light cured but differs in the matrix material which is a urethane dimethacrylate and is filled with agglomerated microfine particles.

The three of the above represent one from each of the three main types (according to classification depending upon the filler size) of the composite resins. In our study, samples of these materials were prepared by curing the material in properly sized moulds under transparent matrix bands. The surface of the set material thus obtained is said to be the smoothest possible surface of that particular product[8,33,48,71]. At the same time however microfilled resins give smoother surfaces as compared to macrofilled resins[8,43,66,68,73]. None of the available polishing procedures produce and surface as smooth as one cured under a strip, but still when different systems of polishing are compared, Aluminium oxide disks (Shoufu, Soflex, Flexi etc.) give the best results[8,33,37,43,48]. In this study half the samples were polished by Flexi disks. A set of samples (one polished

and one unpolished specimen from the same material) was fixed in one appliance, which was used in one experimental period. Before polishing all the samples were stored in distilled water for 24 hours at room temperature. This was done to decrease the chances of crazing the surface during polishing[22].

Placement of samples was done carefully so that no sample or a part of it was intruded or extruded from the window of the flange. Protruded sample may had suffered more friction from the tongue, resulting in less plaque, while the intruded samples would had caused more retentive sites specially at the borders of the windows. Either of the situations would had affected the results. The unpolished sample was placed in the distal window of the appliance to further decrease the friction of the tongue on it.

Before the start of the actual experiment, appliances were worn by the volunteers for three days to become familiar with the "forign-body" inside the oral cavity (the idea was taken from our pilot study). After the start of the study, with composite samples in situ, volunteers wore the appliances continuously for 7 days except while brushing the teeth. Volunteers were advised to use a fluoride-free tooth-paste to eliminate the supposed antibacterial effect of fluorides. All the volunteers continued their own food habits throughout the study.

A seven days period has been used by many authors to evaluate the plaque in ICT[5,39,42,47]. Although it has been suggested that the types of bacteria do not differ much from 3-days to 3 weak period[61], we used the standard regime of seven days.

The appliances were taken out of the oral-cavity at the end of each

experimental period, washed in tap water for about five seconds to remove debris (in the way other authors did[42,61]) and was then dried with a gentle stream of air from a chip blower (as described in the literature[23]). Plaque was scraped from the composite samples with a Gracey curette applying moderate pressure. The sample was put on a sterilized pre-weighed glass sheet and weighed again immediately. The whole of the procedure was done quickly (within two minutes) to avoid loss of viability due to drying of plaque or due to long contact with air. All the samples were then put into vials containing 2 ml of Normal Saline.

In our experiment, normal saline was used as transport medium. The most widely used media are PBS[23,42], RTF[19,39] etc. RTF has been claimed to be the best (for human dental plaque samples) by Syed and Loeshe[64] but they did not include N. saline in their comparison and their main aim was to find a medium for storage of samples. Normal saline has been used by Schaeken et al.[53] and we preferred it because we had to measure the changes in pH of the solution after addition of the plaque. Our pilot study proved saline to be suitable for pH measurement, and also that the viability does reduce even in RTF (which is claimed to be the best by some[64]) in as short a period as one hour. A study carried out by Greger and Eisenberg[17] has shown a reduction of 12.5 % in one hour and upto 40 % in three hours as compared to viability if the cultures were made within fifteen minutes.

For microbiology, the plaque was homogenized by shaking the vial on a Vortex vibrator after addition of 0.1 ml of glass beads. Although ultrasonication is said to give better dispersion and has been used by many[5,10,26,53], it has been pointed out that sonication may cause rupture of bacteria[42] and thus reduce the viable counts.

We wanted to evaluate the total CFU (colony forming units), the lactobacilli and the S. mutans because the lactobacilli and specially the S. mutans have been related with initiation and propagation of dental caries[1,10,23,69]. We used Blood Agar (BA) for CFU counts because it is widely used for the purpose[5,17,30,39,65]. Rogosa SL Agar was used to cultivate Lactobacilli and this medium has been used by many[5,10,42] for the purpose. For evaluation of S. mutans, MSB (mitis-salivarius-Bactracin with 20 % sucrose) was used. This medium has been developed and recommended by Gold et al.[15] and has been used by many researchers[1,5,10,19,30,41]. Shoeken et al.[54] observed that MSB gives less viable counts as compared to (their own developed) TSY 20B medium. They claimed that this reduction may be as much as one log. Serotype "d and g" was said to be very depressed while serotype "a" may also be affected. Another author proved that recovery on MSB was reliable[10].

The culture plates of all three media (in duplicate for every specimen) were incubated in a candle jar at 37^{o} for 48 hours. Many authors[10,17,26,39,60] have used a mixture of different gases without oxygen while traces of oxygen are left in candle-jar. The bacteria we were interested in, are micro-aerophilic, so the recoveries were supposed to be reliable and the results of our pilot study confirmed our supposition.

The first aim of our experiment was to study the amount of plaque (measured in mgs.) accumulated on a unit area of a particular composite surface. Results (Table II) show that polished and unpolished samples of composites accumulated different amount of plaque and also there was a difference in accumulation of plaque on different types of materials. Maximum amount was collected from Express while HELIOSIT collected the least amount

and BRILLIENT collected a little more than Heliosit.

The differences may be explained in many ways. It has been demonstrated that rough surfaces attract more plaque[55]. Express, being a macrofilled resin, has an inherent rough surface as compared to hybrids or microfilled composite resins[8,18,43,46,66,68]. Again Express traps air when mixed (due to it's being a chemically cured type, it is available in two paste system). The trapped air remains in the set material as small bubbles which may cause an increase in surface area of the filling after polishing[59,71].

Kjeld et al.[59] studied 15 different composite resins for porosities and estimated 1.2-28.4 % porosities by volume but they could not correlate it with surface roughness on profilometry, further-they found in an in-vitro study, using S. sanguis that bacteria adhered to all the surfaces in a similar way. In our study there was a marked difference between the plaque accumulated on different types of composite resins.

Weitman and Eames[71] polished Adaptic by four different polishing procedures. They found that more plaque accumulated on rougher surfaces (although not statistically significant). They used tracings from coloured photographs for evaluation of results. Our study gave similar results.

Önen and Nohutçu[44] studied plaque accumulation on smooth and rough surfaces of composite fillings. Plaque indices were used for evaluation. They concluded that there was more plaque on rough fillings (differences were statistically significant). Bacterial attachment on different types of composite resins was studied in vitro by Gürgan and Alaçam[18]. Using S. mutans for study, they found that larger number of bacteria adhered to rough surfaces.

BRILLIENT and HELIOSIT are at an advantage in that they are light cured so air entrapment is least suspected. Both of them have smaller filler particles as compared to Express. Heliosit is a microfilled resin, so it gives the smoothest surface[8,43,66,68,73].

Tullberg[67], in a report, has stated that plaques accumulate less on polished surfaces although he observed that PM acrylates and gold attract more bacteria than amalgam.

Another aspect may be the antibacterial action of the composite resins [40]. This does not seem to be very important because the materials are antibacterial only when they are fresh, whereas we used the samples after storage of 24 hours.

Other factors which may affect the plaque formation are the surface free energies[14,24,49], hydrophic interactions, zeta potentials[52] and chemical composition of the surfaces[63]. All these factors change the adherence properties of a surface. And the same factors change the nature of pellicle on different substances when put in saliva[16]. So the composition of pellicle differs from one substance to another[58] which may be one of the causes of the change in amount and nature of plaque on that particular surfaces.

Pratt et al.[49] have investigated the effects of different surfaces (of different sustances) on the nature of the pellicle. They found that the substrata influence the bacterial adherence. The number of adherent bacteria decreases after pellicle formation but still the surfaces exert their specific influence. Sönju and Glanz[63] in a study have demonstrated that the chemical composition of the surface of a material affects the type of proteins (pellicle) they absorb, which may change the nature of bacteria which adhere later.

Malcome et al.[24] have shown that the adherence properties of different materials become similar after placement in oral cavity but still the underlying material imparts certain features to the surface of pellicle which influence the type of bacteria which are later attracted to it.

Processing processes of chemically similar substances may change the surface chemistry[63] which may then change the nature of pellicle and plaque.

Tullberg[67] observed in his study that unpolished amalgam attracted less plaque as compared to polished amalgam inspite of the fact that the polished amalgam has a smoother surface. This can be explained on the basis of surface changes due to accumulation of mercury in the upper layers of the fillings during polishing which may then change the surface charges etc. causing a changed accumulations of bacterial plaque.

Express and Brillient, used in our study are based on the same matrix material in Bis-GMA but fillers and other ingridients e.g. diluents etc. are different which may change the surface characteristics. Heliosit has quite different type of matrix and filler, which may explain the differences in type and amount of plaque on its surface.

The recovery of CFU, lactobacilli and S. mutans showed a great variation when the different types of materials and polished and unpolished samples of the same material were compared. Recovery of CFU ranged from (0.44-2.4) $x10^6$ while the number of bacteria per mg ranged from (1.7 to 12.84)$x10^6$. Again if the number of bacteria per mg were compared to the wet weights, there was an inverse relationship i.e. the samples having maximum wet weight of plaque harboured less number of bacteria per mg while those having less wet weight contained maximum number of bacteria on per mg basis (unpolished Brillient was the only exception).

Statistical calculations did not find significant differences in the number of bacteria per mg when polished and unpolished samples of Express and Brillient were compared. On the other hand there was a statistically significant difference when the above mentioned samples were compared to those of Heliosit. The number of bacteria per mg on unpolished Heliosit was the highest while the amount of plaque on it was the minimum of all the tested samples. The polished Heliosit was next to its unpolished counterpart in this respect.

The differences in number of bacteria per mg can be explained on the basis of the types of bacteria in plaque. Different types of bacteria differ in their ability to produce extracellular material. The S. sangius and the A. viscosus make up the major part of the early plaque and they seem to produce maximum amount of Extracellular Matrix Material (EMM)[61]. Our study did not evaluate the quantity of bacteria other than S. mutans and the lactobacilli, and our results showed that S. mutans and lactobacilli were rarely found on Express and not much on Brillient while the amount of these bacteria was maximum on Heliosit (17 out of 18 samples of Heliosit contained S. mutans). The differences in number of S. mutans on Express and Brillient from that on Heliosit were statistically significant. It can be expected that the plaque on Heliosit did not contain much of the EMM producing bacteria.

Distler et al.[9] found that the number of bacteria did not correlate to the wet weight of plaque and our study confirmed their results. They have suggested that the wet weight depends upon the water content carbohydrates and the minerals present in the plaque, and it can be seen from earlier studies[49,58,63] that the surface properties of the materials influence the type of accumulates on that particular material.

The diet available to the EMM producing bacteria affect the amount of EMM and thus the wet weight of plaque. In an in vivo - in vitro study Van Houte et al.[70] found that the amount of sucrose present in the culture medium of S. mutans changed the amount of EMM. But this dietary factor does not seem to be an influencing factor in our case because all the volunteers continued their own food habits in all the three experimental periods.

It has been suggested that plaque differs form person to person - as a result of a study conducted by Ashley et al.[2]. The factors most important in causing such differences have been investigated by Simonsson et al.[57]. They found that saliva induced aggregation of of S. sanguis, content of glutamic acid in acquired pellicle and the retention depth of dentogingival angle were the most important (Three of our volunteers were constantly heavy plaque formers). This cannot however explain the differences of wet weight of plaque on the different type of composite in our experiment.

Oral hygiene measures improve gingival health, decrease the amount of plaque but increase the number of S. mutans[2,27]. Again this was not a factor in our study.

Plaque accumulated on the Heliosit contained the maximum number of S. mutans and it caused a decrease in the pH of the transport fluid while the plaque samples from Express and Brillient raised the pH of the transport fluid and gave rise to a very few numbers of S. mutans.

S. mutans on a sucrose rich medium produced more EMM and caused a more rapid and prolonged decrease in pH[70]. The results of our study show that S. mutans when present in sufficient numbers in any type of plaque cause a decrease in pH, while plaque containing EMM without S. mutans does not cause a decrease in pH.

Satou et al.[52] investigated the hydrophobic interactions, zeta potentials and electrostatic interactions of certain bacteria and filling materials. They concluded that different types of interactions play a part in adhesion of different bacteria to different materials. They concluded that Bis-GMA based resins attracted the least amount of bacteria as compared to gold, amalgams and other composites. S. mutans and S. sanguis depended upon different types of mechanisms for attraction. This study may explain why less and different type of bacteria were found on Bis GMA based Express and Brillient as compared to Heliosit which is a microfilled composite and a urethane type of material makes its' matrix. In another study conducted by Kjeld et al.[60] plaque accumulation on a Bis-GMA based composite "Adaptic", amalgam and gold alloys was investigated. In a 2 1/2 hours study they recovered a maximum number of CFU from Adaptic and also maximum number of S. mutans was present on it when compared to other materials. But we could not find many S. mutans from the Bis-GMA based composites in our study.

Our experiment attempted to evaluate the difference in number of S. mutans on the three different composites (Express, Brillient and Heliosit) used in our study. The differences in the numbers of bacteria after polishing were also noted. Special consideration to S. mutans was given because it is the only bacteria closely related with dental caries[1,10,23,69] (while the lactobacilli were not closely related to the carious lesions in the above quoted studies).

Orstavik et al.[39] found that the filling materials when placed in contact with inflammed mucosa attracted less plaque but the number of S. mutans was more. It may be due to certain changes caused by exudation eg. antibacterial factors and the pH changes etc. An other study conducted by

Lundin and Emilson[30] showed that approximal surfaces having class II composite fillings contained more S. mutans when compared to normal approximal surfaces or to those having an amalgam filling. They did not investigate the type of composite material. The increase in S. mutans may had been due to the changes in exudation in that area due to faulty margins or due to the nature of the composite.

Schaeken et al.[53] found a positive correlation between the number of bacteria to the Löe plaque index. They found that as the plaque matures, there is a rapid increase in the number of S. sanguis and A. viscosus whereas S. mutans did not increase much. This type of change has also been reported by Socransky et al.[61]

In their study Gürgan and Alaçam[18] compared the attachment of S. mutans to Heliosit, Brillient and Adaptic. The results of this in-vitro study have shown that maximum numbers of bacteria were attached to Adaptic while least to Heliosit, and the rough surfaces attracted more bacteria. The results of their study are quite opposite to that of ours. In our study, although the wet weight of plaque was greater on rough surfaces, the number of bacteria per mg was less whereas the smooth surfaces collected less wet weights of plaque but the number of bacteria per mg was more. Again, Heliosit attracted the maximum number of S. mutans in our study. This may be due to change of environmental conditions i.e. our study was an intra-oral experiment, and the adhesion properties of S. mutans have been shown to change when they are cultivated outside the oral cavity[41].

The lactobacilli showed an inconsistent recovery from the plaque samples in our study. Maximum adherence was seen on unpolished Brillient, next to which was the unpolished Heliosit. Their presence could not be

related to pH changes in the transport fluid and no statistical significance could be calculated.

Recovery of Lactobacilli was also inconsistent with the plaque samples of natural teeth. The best Habitat of Lactobacilli are the naturally protected areas of teeth. They are recovered mostly from plaque samples of pits and fissures, from deep carious lesions and not from the plaque of smooth surfaces[1,10,23,26,34,36]. They are acidogenic, aciduric and rely on the constant supply from extracellular source e.g. the carbohydrates retained in pit and fissures. So their inconsistent recovery was very much expected.

Comparisons were also carried out on CFU, Lactobacilli, S. mutans and pH of plaque accumulated on natural teeth to those from the composite samples. A three days old plaque was collected from the lingual surfaces of the lower first molar. Although the plaque from composite samples was 7 days old it was not wise to prevent the volunteers from continuing oral hygiene for 7 days as it has been reported by Socronsky et al.[61] that total viable counts remain constant after 2 days of refraining from oral hygiene measures. By culturing the plaque in aerobic, microaerobic and anaerobic conditions, they found the recovery in the range of 10^7-$5x10^8$. They reported that after 8-12 hours of starting plaque formation, there is a rapid increase in the number of S. sanguis. Some anaerobes and spirocheates also are added which multiply, increasing the total number of bacteria. So the percentage of Streptococci may decrease but the actual number remains the same or may even increase.

Another study performed by Schakens et al.[53] has revealed that the number of bacteria increase as the plaque matures but the increase in number of S. mutans is not very pronounced. Keeping in view our preferences we used a three days plaque for comparasion purposes.

pH changes of the plaque were evaluated by measuring the pH of homogenized samples of plaque in (transport fluid i.e.) the normal saline. It was seen that plaque samples removed from Heliosit caused a decrease in pH of solution whereas plaque recovered from Adaptic and Brillient samples or from the natural teeth caused an elevation in pH of the solution. The differences in pH of plaque from Heliosit to pH of plaque of all other origins was statistically significant. S.mutans was present in plaque removed from Express and Brillient also (but v. small in number). The plaque from natural teeth contained S. mutans comparable to those from Heliosit but the decrease in pH was caused only by Heliosit plaque.

These differences may be explained by the presence of different serotypes of S. mutans[7,20]. It has been reported that serotypes "c and e" are most acidogenic while alkalies may be produced by serotype "b". Veillonella which is a strict anaerobe and is present in deep layers of plaque (where the structure of plaque produces absolute oxygen depletion). EMM of plaque on Adaptic and that on natural teeth may had caused such conditions. Veillinolela[12,34] utilizes acids produced by other bacteria and this may explain why the pH of plaque samples other than those from Heliosit did not cause a decrease in pH of the solution inspite of the presence of S. mutans and Lactobacilli in some of them.

C O N C L U S I O N S

The results of our study indicate that the polished samples of the composites collected more plaque as compared to the unpolished samples of the same material. The macrofilled resins collected more plaque than hybrid or the microfilled resins. On the other hand there was an inverse relationship between the number of bacteria to the wet-weight i.e. the samples which collected the maximum amount of plaque wet weight contained the minimum number of bacteria per mg. Lactobacilli gave inconsistent recovery while S. mutans were cultivated mostly from the microfilled resin samples, although some of the samples of other two types also gave growth of S. mutans.

The polished samples of the resins gave more S. mutans number than unpolished samples. Accordingly a similar type of relationship was found in pH changes. The plaque samples from unpolished resins were more alkaline than those collected from polished samples of the same materials.

Keeping in view the amount, number and type of bacteria, the pH changes of plaque collected from the composite resins, and comparing the results to those of the plaque collected from natural teeth and also the aesthetic requirements, we may say that hybrid (BRILLIENT) resins may be used as a material of first choice. The macrofilled (EXPRESS) resins may be used in cases, where they can be cured under a matrix and left as such or be polished to a high quality finish. The microfilled (HELIOSIT) resins may be used as the last choice.

It must not be forgotton that we tested just one material from each

type of composite resins, whereas different products from different manufacturers may differ markedly with respect to type of matrix, diluents or fillers which may change the surface properties of that particular product. Also, our samples were placed in the form of a smooth surface filling-away from gingiva and were subjected to more friction from the tongue. The situation in routine clinical practice is usually different. The fillings are near or under the gingiva where they can neither be cured under a matrix nor be polished properly. More protected and retentive areas may exist in the approximal fillings where natural and even routine prophylaxis measures cannot remove plaque. There may be other factors eg. the strength of a material or more often the aesthetics which play a bigger role in daily decisions in clinical practice.

S U M M A R Y

This study was designed to evaluate the wet weight, total CFU, Lactobacilli, S. mutans and pH of plaque accumulated on three different types of polished and unpolished composite resins. Express (macrofilled - chemically cured), Brillient (hybrid-VL cured) and Heliosit (microfilled - VL cured) were chosen for the test. The test was performed by fixing two 4x5x2 mm sized (one polished and other unpolished) samples of the one material into lingual flanges of intra-oral appliances which were worn by ten volunteers. After a 7-days period (for plaque formation) in mouth, plaque was scraped off the samples. Wet weight was measured on a digital balance. After homogenizing the plaque in normal saline, cultures were made on Blood Agar for total CFU, Rogosa SL Agar for Lactobacilli and Mitis - Salivarius-Bacitracin for S. mutans. pH of the solution was also measured by a Beckman pH meter. The culture media, after incubation of 48 hours at $37^{o}C$ in a candle-jar, were counted for colonies. The same procedures were repeated for the other two types of composites.

Results of the study showed that maximum plaque accumulated on macrofilled resins while hybrid and microfilled resins accumulated approximately the same amount. Plaque on the polished samples was more as compared to their un-polished counterparts. Number of bacteria (CFU) was maximum on microfilled resins. CFU per mg was less on samples which accumulated more wet weights. The recovery of Lactobacilli was inconsistent. S. mutans were found on all types of materials but maximum number was cultured from Heliosit samples. The number of S. mutans was less on unpolished samples

as compared to polished samples of all the materials and also the plaque from unpolished samples was more alkaline. Plaque from Heliosit was the only one to cause a decrease in pH of the normal saline solution. The CFU, Lactobacilli, S. mutans and pH changes of plaque from polished and unpolished samples of macrofilled and hybrid types composite resins are comparable to those of plaque from natural teeth.

Considering all the above-cited facts, it may be said that hybrid resins may be used as the first choice, the macrofilled the second while microfilled the third. Whenever possible, the resins may be allowed to be cured under a matrix band and left undisturbed. And where necessary, after recontouring, the best possible polishing may be performed.

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C O R R E C T I O N S

Page	Line	Written	Corrected
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10	24	pasters	pastes
14	4	Eamas	Eames
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29	2	(ref.) 9,22,26,27	5,39,42,47
29	22	subjects	subject
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34	9	Kjeld	Skjørland
42	4	Adaptic	Express
42	16	Adaptic	Express
49	Ref.No: 24	First author is MALCOME, R.	
53	Ref.No: 60	First author is KJELD, Kr.	