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Original Article

Surface Degradation of Resin-based Materials by Pediatric Syrup Containing Amoxicillin under Erosive Challenge

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Abstract

Objective: To investigate the effect of a pediatric syrup containing Amoxicillin on resin-based on the surfaces of resin-based materials under pH cycling. **Material and Methods:** Cylindrical samples (n=60) of a compomer (Freedom) and a microhybrid composite (TPH Spectrum) were prepared following the manufacturer's instructions. The specimens were divided into two groups and treated for 30min, twice a day, for 14 days with a pediatric syrup containing Amoxicillin, AmoxilTM 500mg/5ml (experimental group) or Distilled water (control group). During the 14 days, the samples were submitted to pH cycling (3h in demineralizing, 20h in remineralizing saliva, and 1h for treatment). The surface roughness was evaluated at baseline, on the 7th and 14th days of erosive challenge using a profilometer and illustrated by SEM. The data were statistically analyzed by one-way ANOVA, Tukey HSD and paired T- tests ($p < 0.05$). At baseline, the mean $Ra_{TPH} = \text{mean } Ra_F$ ($p > 0.05$). **Results:** After 7 days, it was observed no erosion ($p = 0.674$) for THP Spectrum (0.19 Ra) and an increasing of Ra ($p = 0.02$) for Freedom (19.95 Ra). After 14 days, it was observed an increasing of Ra for both THP Spectrum (0.56 Ra) and for Freedom (3.44 Ra), demonstrating that the degradation was treatment and time-dependent ($p < 0.001$). The pediatric syrup containing Amoxicillin increased the surface roughness of the TPH Spectrum surface one and a half times ($p = 0.003$) and the surface roughness of Freedom was increased by more than two times ($p < 0.001$). SEM images showed different aspects of the surfaces of the two materials with a loss of integrity to both. **Conclusion:** The pediatric syrup containing Amoxicillin under pH cycling increased the roughness of the both tested resin-based restorative materials.

Keywords: Tooth Erosion; Dental Materials; In Vitro Techniques; Medicine.

Introduction

Resin-based materials have been widely used for restoring primary and permanent teeth [1] following the concept of minimal intervention in dentistry with conservative cavity preparations. The compomer has intermediate characteristics between the microhybrid composite resin and the glass ionomer cement [2]. It is available in a single paste, encapsulated, pre-dosed and are photopolymerizable [3]. The composition is similar to composite resin with the addition of a molecule with carboxylic acid groups. This condition allows the occurrence of acid-base reaction, but this material does not contain water, so this reaction will only occur after contact of the material with saliva [4]. Its ease of use justifies the interest of the investigation.

The degradation of resin-based materials can occur by many factors, including liquid oral medicines. The longevity of these restorations depends on their abilities to withstand the harsh conditions of the oral environment such as biodegradation [5]. Gopferich et al. [6], described the degradation as a “chain scission process during which polymer chains are cleaved to form first oligomers and then monomers”. Monomers have different functional groups compared to polymers. Among the mechanisms of polymer degradation are hydrolytic, hydrothermal, chemical and chemo-mechanical [7]. These mechanisms may result in a reduction of the physical-mechanical properties of restorative materials [8-10].

Liquid oral medicines form a significant proportion of the medicines used by the population as a whole, either prescribed or over the counter [11]. The palatability of these medicines is increased with the addition of sugars such as sucrose, glucose or fructose to foster patients' acceptability and thus compliance [12,13]. Furthermore, the pH is formulated to optimize their efficacy, and, usually acidic preparations are often necessary for drug dispersion [5,14,15]. Thus, liquid oral medicines may act as extrinsic agents causing biodegradation of resin-based materials, especially if consumed frequently [5].

Among acids medicines, anti-histaminic liquid oral medicines have been reported to promote surface degradation of composite resins in an *in vitro* protocol [5]. Other liquid medicine widely used in infancy is syrups containing amoxicillin, in the majority of cases, the chosen drug for antibacterial therapeutic purposes [16]. Especially in children that presents chronic diseases that need to use this medicine for a long term, it is important to evaluate the effects of pediatric syrups containing amoxicillin on the integrity of the composite resin restorations. The effects of pediatric syrups containing amoxicillin on the surface of resin-based materials are still unknown. It is important to investigate the behavior of restorative materials in the erosive challenge since it presents differences regarding the properties and volumetric fractions of the composition, inorganic phase distribution and dispersion, size, porosity and interaction between phases.

Therefore, the aim of this study was to evaluate the surface changes of two resin-based restorative materials caused by a pediatric syrup containing amoxicillin under pH cycling. The null hypothesis tested was that erosive challenge thought the exposure to pediatric syrup containing amoxicillin would not influence the degradation and surface erosion of the two resin-based materials.

Material and Methods

Sample Preparation

Sixteen disk-shaped specimens (7mm diameter x 2 mm high) of each resin-based restorative material, being Freedom (n= 12; SDI, Bayswater, Australia) and TPH Spectrum (n = 12; Dentsply, USA) (Table 1) were prepared in metal molds. All materials were used according to the instructions as outlined by the manufacturers. The materials were injected into the molds with a Centrix syringe (Have Neos, USA) and transparent cellulose strips with glass slabs were placed on the top and bottom of the molds. Pressure was applied (500g) for 20 seconds to extrude excess material. All samples were light-cured on both sides (top and bottom) for 40 seconds through the glass slab with a halogen lamp/ light-curing unit (Optilux Demetron, Demetron Research Corporation, USA) emitting 550mW/cm² power density measured with a curing radiometer (Demetron radiometer, Model 100 P/N – 10503/Demetron Research Corporation, USA). After that, the surfaces were immediately examined under a metallographic microscope (100 x) to assure the absence of any defects, such as ditches and bubbles. Specimens were removed from molds and maintained in a humid media at 37° C for one week in order to allowing post-irradiation hardening of the resin-based materials being tested.

Table 1. Resin based materials evaluated in the study.

Material	Brand Name (Sample size)	Manufacturer	Batch #	Basic Composition			
				Resin matrix	Filler	Mean particle size (µm)	% Vol Weight
Polyacid modified composite resin TM	Freedom (n=16)	SDI ¹	045658	urethane dimethacrylate resin, photoinitiator, acrylic monomer	strontium fluorosilicate glass filler sodium fluoride	1.0	77%
Microhybrid composite resin TM	TPH Spectrum (n=16)	DENTSPLY ²	305449	Bis-EMA, Bis-GMA-adduct, TEDMA, UDMA	Barium-aluminium-borosilicate Highly dispersed silicon dioxide,	mean particle size < 1.5 µm 0.04 µm)	77%

¹Bayswater, Victoria 3153, AUSTRALIA; ²Dentsply, Petropolis, Rio de Janeiro, BRAZIL; TEGDMA (3,6-Dioxaoctamethylene-dimethacrylate) Bis-EMA (2,2-Bis[4-(2-methacryloyloxyethoxy)-phenyl]propane) Bis-GMA-adduct (adduct of 2,2- Bis).

Baseline Roughness Evaluation

After one week, the baseline surface roughness (Ra) was measured for each sample using a profilometer (Dektak IIA profilometer). A square with 2 diagonal lines of 3mm was marked centrally on the surface of the specimens so that the surface roughness could be reevaluated in the same area. Two measurements were made of 0.2 mm on each line with the profilometer to determine the value of surface roughness. The mean value of the four measurements was recorded as the surface roughness for each sample. Roughness (average Ra of the 16 samples) was obtained for each restorative material.

pH Cycling and Erosive Challenge Protocol

The samples of groups (Freedom control, Freedom experimental, TPH spectrum control, and TPH spectrum group) were submitted to a modified pH cycling [18]. For the pH cycling, each group was placed alternately in 5 mL of artificial remineralizing saliva [20] (1.54 mmol/L calcium, 1.54 mmol/L phosphate, 20 mmol/L acetic acid and 0.308 g ammonium acetate, adjusted to pH 6.8 with KCl; VETEC, Rio de Janeiro, RJ, Brazil) and 5 mL of demineralizing saliva [20] (3 mmol/L calcium, 3 mmol/L phosphate, 50 mmol/L acetic acid and 0.308 g ammonium acetate; VETEC, Rio de Janeiro, RJ, Brazil) with pH adjusted to 4.5 with NaOH. The solutions of pH cycling were changed daily.

The samples, both control and experimental group, remained in the remineralizing saliva daily for 20 hours consecutively, and after rinsing with deionized water, they were kept in contact with demineralizing saliva for 3 hours, completing a cycle of 23 hours. The 24-hour experiment was completed with the 1 hour of treatment time.

After 23 hours of pH cycling, the samples were removed from the pH cycling solutions, rinsed with 10 ml of deionized water for 1 minute and dried and were immersed in treatment solutions. The treatment was divided into two protocols, as follows:

- Experimental group – Use of 5 mL of pediatric syrup containing Amoxicillin - 500mg/5ml (Table 2) for 30 minutes, twice a day, for 14 days;
- Control group – Use of 5 mL of distilled water, for 30 minutes, twice a day, for 14 days.

After 30 minutes, the samples were rinsed with 10 ml of deionized water for 1 minute. The treatment solutions (pediatric syrup containing Amoxicillin and deionized water) were replaced for every immersion.

Table 2. Pediatric syrup used in the present study.

Medicine Brand Name	Batch Number	Composition	Titrateable Acidity V NaoH (mL)	pH (cP)	Viscosity
Amoxil pediatric suspension	134374	Amoxicillin tri-hydrated 500mg Silicon dioxide, sodium benzoate, dyes, sodium citrate, di-hydrated, gum, flavoring and sucrose. 5ml	2.53 ±0.11	5.74 ±0.01	90

GlaxoSmithKline from Mexico - Importada by GlaxoSmithKline Brazil Ltda. MS:1.0107.0003.

Scanning Electronic Microscopy (SEM) Analysis

The surface aspects of the resin-based materials before and after erosive challenges and treatment protocol were analyzed by SEM. Initially, two disc-shaped specimens of each material were randomly selected and set aside before erosive challenge and treatment protocol, for qualitatively examination. After 14 days, one pair of each group was also randomly selected to evaluate their surfaces. For SEM analysis, the specimens were rinsed with deionized water to remove and debris and mounted on aluminum stubs, sputter-coated with gold, and examined at 4,000X magnification using a scanning electronic microscope (Jeol/EO JSM- 6460, version 1.1, 20 -

acceleration voltage - signal SEI, 5 μ m, Tokyo, Japan) operating at 20kV and the images were registered.

Statistical Analysis

Data analysis was carried out using the SPSS 16.0 (SPSS Inc, Chicago, IL, USA) statistical package. The assumption of equality of variance and normal distribution of errors were checked through Shapiro-wilk test. Based on homogeneous distribution, baseline surface roughness values were statistically analyzed by one way ANOVA with of the homogeneity of variances test and Tukey HSD *post hoc* test. Paired T- test was applied to evaluate each material in comparison to treatment protocol on the 7th and 14th day of erosive challenge. All analyses were performed at 95% confidence level.

Results

Surface Roughness

The surface degradation analyzed by erosion could be detected by a profilometer. Table 3 shows the analyses of Freedom and TPH Spectrum with regard to surface roughness taking into account the period of time and treatment protocol under an erosive challenge.

Table 3. Surface roughness of resin based materials in the experiment.

Restorative Material	Surface Roughness (Ra)				
	Erosive Challenge				
	Baseline	07 days		14 days	
		Treatment (G I)	No treatment (G II)	Treatment (G I)	No treatment (G II)
TPH Spectrum™	0.28 (0.07) ^A	0.47 (0.18) ^A	0.42 (0.11) ^A	0.84 (0.29) ^E	0.52 (0.14) ^G
Freedom™	0.46 (0.15) ^A	2.41 (0.65) ^B	1.95 (0.50) ^C	3.90 (1.26) ^F	1.88 (0.29) ^H

Statistical analysis between restorative materials: ANOVA and Tukey test; Statistical analysis between days in the same restorative materials: paired t test; Same letters indicate no statistically significant difference ($p > 0.05$); Different letters indicate statistically significant difference ($p < 0.05$).

At the baseline, surface roughness was not statistically different between Freedom and TPH Spectrum ($p = 0.937$). After 7 days of treatment and/or pH cycling, a significantly increase in the surface roughness values of Freedom ($p = 0.002$) were noted, especially when the pediatric syrup containing amoxicillin was applied to it. The surface roughness of Freedom was doubled ($p < 0.001$) in comparison to the non-treated group. On the other hand, for the TPH Spectrum group, this short 7-day period under erosive challenge was unable to provoke erosion, neither by pH cycling alone nor by the pediatric syrup containing Amoxicillin. ($p = 0.522$; $p = 0.674$, respectively). After 14 days, both materials Freedom and TPH Spectrum became rougher ($p < 0.001$). Changes on their surface roughness were found to be material and treatment (media) dependent. The pediatric syrup containing Amoxicillin potentiated the degradation of the TPH Spectrum surface ($p = 0.003$). However, Freedom was even more susceptible to the pediatric syrup containing Amoxicillin, which increased its surface roughness by more than two times ($p < 0.001$).

Surface Integrity

Considering the initial images, changes to the surface texture of Freedom and TPH Spectrum were seen (Figure 1) after erosive challenge. SEM images showed a rough surface with pits, voids, depression areas and individual glass particles protruding from the resin matrix. Freedom exhibited great changes to the surface with particle losses. The changes of the surface texture of the TPH Spectrum material were lower. There was slight degradation after 7 days of pH cycling and more evident after 14 days of pH cycling, suggesting a cumulative degradation effect.

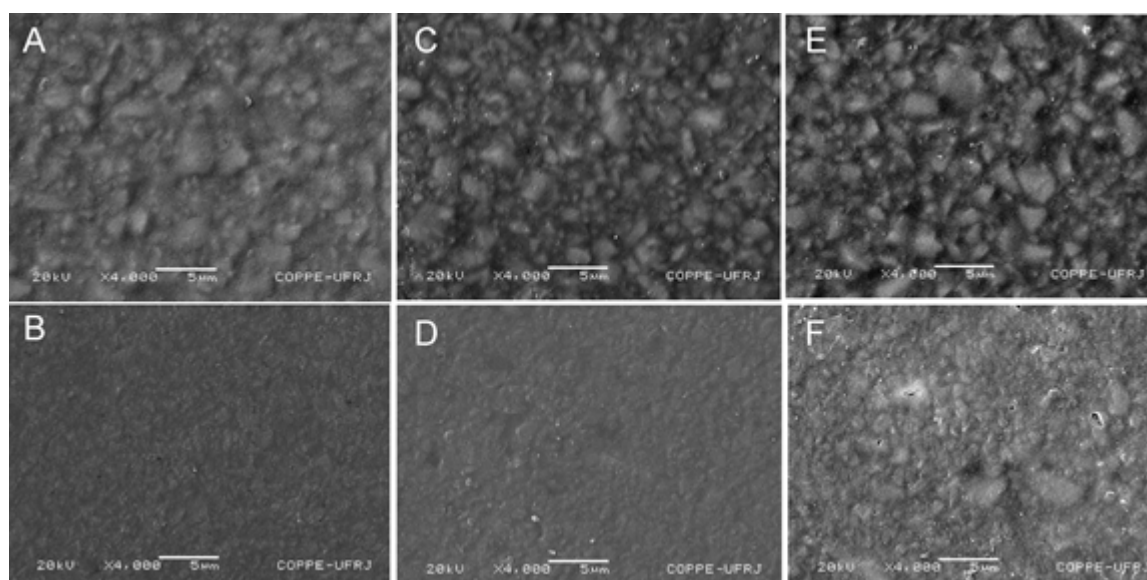


Figure 1. (A) Surface characteristics of Freedom and (B) TPH Spectrum before erosive challenge; and (C) Freedom and (D) TPH Spectrum after erosive challenge when treated with distilled water; or (E) Freedom and (F) TPH Spectrum after erosive challenge and treated with Pediatric syrup containing Amoxicillin during the 14 days of the experiment.

Discussion

In an attempt to simulate oral conditions as closely as possible, this experiment was performed under pH cycling where the samples remained for longer periods in an acidic media as suggested previously [18] and other published studies [21,22]. Acidic conditions are mediums of choice to mimic some *in vivo* conditions. Moreover, the samples were treated with pediatric syrup containing Amoxicillin twice a day as prescribe for acute respiratory infections in children [11] and finally, the period of contact (30 minutes) between restorative materials and medicine was chosen based on Stephan's curve in which saliva takes 30 minutes to return to a for resting neutral pH level.

The results of the present study denied the null hypothesis since the pediatric syrup containing Amoxicillin under erosive challenge was able to increase the surface roughness of both resin-based restorative materials causing polymer degradation and surface erosion after two weeks. Moreover, the results point out that the polymer degradation was material, media storage and time dependent because the velocity of the polymer degradation was influenced by pH or storage media,

by type of chemical bond within the polymer backbone, copolymer composition, water up take and by storage time as suggested by some author [9,10].

It is clear that both the erosive challenge as well the pediatric syrup containing Amoxicillin under erosive challenge were able to promote remarkable changes in surface texture of both materials tested. The authors of the present study suggested the pediatric syrup containing Amoxicillin acted as reservoir for H^+ ions, since this medicine has carboxylic acid trihydrate radicals, among other chemical components which not only decrease the pH but also increase the titratable acidity of the media [17] despite having hydroxide magnesium in its composition that decreases degradation rates [6].

In parallel, this can explain why Freedom demonstrated a significantly higher rate of degradation and erosion than TPH Spectrum. The latter restorative material appeared to be chemically more stable [23]. In Freedom composite resin, the interface between filler particle and resin matrix was the route for water penetration as a consequence of incomplete filler particle silanization. The long period (20 hours) in demineralizing artificial saliva (low pH media) seemed to provoke chemical erosion by etching the surface resin matrix. As a result, individual particles were dissociated from each other. Moreover, there was a softening of the resin matrix, which became rougher. The erosive challenge seemed to provoke a “denudation” on the TPH surface, removing part of the resin matrix and filler particles from the material surface, which left particles protruding from the resin matrix. The degradation and erosion of the materials seemed to be related to their hydrophilic properties and chemical composition of the resin matrix and the particle filler. According to the manufacturer Freedom has similar volume fraction (77%w) filler as TPH Spectrum but the resin matrix, filler type/size and silane treatment are different. Furthermore, the comparatively low surface roughness for TPH Spectrum was probably a result of a more hydrophobic matrix due to the resin matrix lacking hydrogen bonding groups, which leads to lower sorption and consequently, less degradation [24]. Freedom which took up large quantities of water since it has hydrophilic polymers was more affected by the erosive challenge. Therefore, it was cleaved into carboxylic groups and, the degradation rates increased [6]. It is also important to point out that compomer has an incomplete filler silanization [25] which in an erosive media allows ion diffusion (Na^+ , Si^{++} , P , F^- , Ca^{++} , Al^{+++} , OH^-) from the resin matrix [26]. This process caused pores and other defects on the surface of the restorative material, increasing the surface roughness.

The results should not underestimate the influence of dental pellicle in potentially inhibiting or reducing the occurrence of erosion on the material surfaces. According to some authors [27], there is, *in situ*, a two-hour protective effect of the dental pellicle against enamel erosion by an acid beverage. In oral conditions, differently from an *in vitro* environment, acids are neutralized by saliva flow and salivary components. Saliva has a buffering, diluting, and remineralizing capacity and also allows for the acquisition of a salivary pellicle on tooth and restoration surfaces. This protein-based pellicle may behave as a diffusion barrier or a perm-selective membrane, preventing direct contact between acids and the tooth and restoration surface, and thus inhibiting its demineralization [27].

On the other hand, it should be noted that salivary enzymes also promote biodegradation [28]. Furthermore, some chronic diseases and liquid medicines can reduce the salivary flow rate affecting its protective potential [29-31].

It is well known that rougher materials can increase staining susceptibility and dental biofilm accumulation contributing to secondary caries [33,34]. This could happen especially at a lower pH value of pH 5, as occurs during the ingestion of sweeter liquid oral medicines [35], when there is a higher adhesion force between the dental biofilm and resin-based materials. According to Bollen et al. [32], a threshold surface roughness for bacterial retention is $R_a = 0.2$ micron below which no further reduction in bacterial accumulation can be expected. As seen in this study, the surface roughness values of both materials were higher after treatment with pediatric syrup containing Amoxicillin under erosive challenge. The surface roughness value after erosive cycling was below the surface roughness roughness caused by caries in an *in vivo* study [32]. This suggests that in addition to structural loss caused by erosion, surface changes in restorative materials may be comparatively worse than those caused by caries, and therefore subject to microbial colonization. Furthermore, in oral cavities, resin-based restorations are permanently affected by other agents such as topical fluoride treatments, alcohol solutions, dietary habits, thermal and mechanical stress that contribute to the degradation of resin-based restorations. Some researchers [7], conducted an *in situ* study and suggested that toothbrushing can increase the effects of an erosive challenge. A limitation of the present study was the absence of frictional force. Further studies should be conducted to evaluate the surface changes caused by a pediatric syrup containing amoxicillin under erosive challenge concomitant to the use of toothbrushing.

Despite of allows the researcher to control the involved variables in this process resulting in low variability as well as the sample size required is reduced, *in vitro* erosive cycle models presents a limitation in reproduce the clinical conditions. The pH is the most important parameter for determine the erosive rate [36]. However, the authors highlight that under clinical conditions, there is no clear critical pH for erosion. In clinical conditions, possibly other factors present protective hole against erosion, such as the presence of acquired pellicle [37]. In the present study, with the limitations of an *in vitro* study, it was tried to simulates some clinical parameters, for example, the time of contact with amoxicillin was similar to the real condition in order to simulate the time of clearance of this antibiotic in the mouth. Even thought, *in vitro* models as present limitations, such as the impossibility to completely simulate the complex intraoral conditions; they cannot simulate solid surface area/solution ratios because the different oral surfaces are bathed in different volumes and source combinations of saliva; they cannot mimic topical use and clearance of substances from the oral cavity and, in many times, cannot simulate the tooth brushing as it happens in clinical environment [15]. This mentioned limitations must be highlighted when *in vitro* studies are performed, in these cases, the conduction of *in situ* studies are suggested to minimize the limitations of *in vitro* protocols.

Amoxicillin has been recognized as a priority essential medicine and it has been recommended by WHO as first choice for infections as well as many systemical diseases. For example, for the treatment of Pneumonia. Unfortunately, Pneumonia still remains the biggest killer worldwide of children under five years of age. Another systemic disease that can affect children's health, is Sick Cell Disease (SCD) which is the most prevalent hereditary hemoglobinopathies not only in Brazil, but also, around the world. In both systemic disorders, oral Amoxicillin is the drug for treatment [38]. In SCD, Amoxicillin is used as a prophylactic strategy to reduce by up to 80% of cases of infant mortality at earlier ages. When liquid formulations are adopted, children with SCD ought to make use of Amoxicillin suspension from birth to five or seven years old, according to the severity of the disease, every day for two or three times / day, depending on the concentration of the drug [13]. Based on these, the authors of the present study decided to use an extremely long intervals time of immersion in the solutions. Thus, the samples were immersed in Amoxicillin syrup for 30 minutes, spite of the literature show that erosive challenges should be performed at exposures in reduced period of time [37,39]. Although it is worthwhile mention that this particular medium, such as Amoxicillin syrup, for eroding these restorative materials used in the study, have not been tested yet. The *in vitro* erosive potential of Amoxicillin suspension on deciduous teeth has already been demonstrated [40], but its effect on restorative material it was underexplored. Therefore, the present study adds knowledge once show that the Amoxicillin syrup was also able of provoking degradation in restorative materials used in the experiment. To take into account the dynamic factors present in the oral cavity, the findings of this study point to the need further studies combining qualitative and quantitative evaluations to indicate more precisely the effects of Amoxicillin syrup, chronically use, on the clinical integrity of the aesthetic restorative materials.

The findings of the present study, within its limitations, is relevant in relation to the oral health of children, especially those chronically ill, who take more liquid medications than healthy children do and, consequently, have high probability to damage their restorations as suggested by the results of this study. It is important to emphasize that a single acidic attack is of minor importance; however if repeated, the ability of the substrate to deal with the acid becomes less and less [41]. Within the limitations of these preliminary *in vitro* results, we suggest that the health care professional who is involved with prescribing or recommending pediatric syrups containing Amoxicillin must be fully aware of the erosive disorders that may arise such as the side-effects on the resin-based restorative materials. In addition, in order to lessen these side-effects adequate mouth rinsing with water should be performed after taking such medication. However, more clinical studies are necessary in order to confirm the present findings.

Conclusion

With the limitations of an *in vitro* study, it can be concluded that the degradation and erosion of resin-based materials was time and material dependent. The pediatric syrup containing

Amoxicillin had a significant influence on the erosion of resin-based materials after 14 days by increasing their surface roughness.

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