An in vitro examination on the effectiveness of commercial toothpastes in the prevention of tooth decay, using eggshell as a substitute for human tooth material.

SADJ August 2018, Vol 73 no 7 p446 - p451
SC Onwubu¹, PS Mduli², S Singh³, V Bharuth⁴

ABSTRACT
Background: Despite improvements in oral health status, dental caries remains a public concern. This study examines the effectiveness of commercial toothpastes in the prevention of tooth decay, using eggshell powder as a substitute for human tooth material.

Methods: Colgate, Aquafresh, Colgate Sensitive, Sensodyne, and Oralwise were tested. An enamel model was simulated by adding eggshell powder to beakers containing dissolved toothpastes. The contents were agitated for eight hours at 800rpm, filtered and oven dried. Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDX) were used to characterise the surface morphology and the calcium and phosphate composition of the toothpastes pre- and post- agitation with eggshell powder. Gas-displacement and pressure sensor tests evaluated the rate of reaction between the substitute “tooth enamel” and acids.

Results: EDX analysis confirmed the presence of calcium phosphate ions, while SEM revealed the formation of an enamel-like layer after agitation. Pressure sensor tests confirmed Colgate Sensitive as most effective in protection against acid attacks, with Oralwise least effective.

Conclusions: Eggshell can be used as a substitute for the human tooth in an in vitro experiment. All the tested brands of toothpaste effectively reduced the acid reaction, which would contribute to the prevention of tooth decay.

INTRODUCTION
Dental caries, also known as tooth decay, is a common bacterial disease characterised by demineralisation and destruction of hard tissues, usually by the production of acid by bacterial fermentation of undisturbed food debris which has accumulated on the tooth surface.1-3 Despite the global improvement in oral health status, Inetianbor et al.4 point out that dental caries remains one of the most chronic and prevalent infectious diseases in the world.1,5,6 Although dental caries has multifactorial causes,1 the dietary lifestyles of the individual2 as well as sub-optimal oral hygiene habits7 help facilitate the onset of this disease. For instance, it has been reported that the consumption of sweets with high concentrations of glucose, saccharine, or fructose, especially if taken in processed juices, and over a prolonged period play an important role in caries development in children.8 Dental caries occurs as a result of the metabolism of bacteria lodging in plaque attached to the tooth, and hence toothpaste and tooth brushing should help reduce the adherence of these microorganisms within the plaque biofilm in the mouth. This is supported by some clinical studies9,10 which have shown that regular tooth brushing with well-formulated fluoride toothpaste can reduce the incidence of dental caries. Significantly, the dental health organisation in Nigeria has advocated preventive and prophylactic measures in the management of dental caries through regular hygiene and dietary modifications.4 The increased availability and consumption of soft drinks, fruit juices, and sports drinks,11 however, make it difficult for individuals to alter their dietary habits. As such, promoting good oral habits through regular brushing with the use of toothpaste may become the most viable option for the oral

ACRONYMS
SEM: Scanning Electron Microscope
EDX: Energy Dispersive Spectroscopy

Keywords: eggshell powder, toothpaste, dental caries, tooth enamel

1. Stanley Chibuzor Onwubu: MHSc. Dental Sciences, Durban University of Technology (DUT), Durban, South Africa.
2. Phumlane Selby Mduli: PhD. Chemistry, Durban University of Technology (DUT), Durban, South Africa.
3. Shenuka Singh: PhD. Discipline of Dentistry, University of KwaZulu-Natal (UKZN), Durban, South Africa.
4. Vishal Bharuth: MSc. Microscopy and Microanalysis Unit, University of KwaZulu-Natal (UKZN), Durban, South Africa.

Corresponding author
Stanley Chibuzor Onwubu:
Dental Sciences, Durban University of Technology (DUT), Durban, South Africa. Cell: +27 71 793 0652. E-mail: 214455999@durutlife.ac.za.

http://dx.doi.org/10.17159/2519-0105/2018/v73n07a3
health care provider in the management of dental caries. Brushing of teeth using toothpaste is recognised as the most commonly practiced form of oral hygiene in most countries. Toothpaste serves as an abrasive which helps in removing dental plaque and food particles from the teeth, as well as assisting in suppressing halitosis and releasing active ingredients, mainly fluoride. 

Goldman, Yee (15) Strong arguments have been presented that toothpaste is the only realistic fluoride strategy in many low-income countries where lack of infrastructure renders fluoridation of water or salt not feasible. In recent years, the oral health care market has witnessed a boom of different brands of toothpaste, with some claiming to be more effective in protection against acid attack and the prevention of dental caries. But just how effective they actually are remains a question since the continued prevalence of dental caries is still a public health concern. Thus, it is desirable to evaluate the claims that these commercial toothpastes completely prevent the acid attacks that result in caries.

Previous studies suggested that bovine and enamel models be used when examining the effectiveness of commercial toothpaste against acidic attacks. Also considered as the sole test model was the dissolution of hydroxyapatite (HA). It is generally well known that tooth enamel consists mostly of calcium hydroxyapatite with a molecular formula of Ca$_{10}$(PO$_4$)$_6$(OH)$_2$. Although hydroxyapatite is a hard and resistant compound, acid (H+) that is produced, especially after a high-sugar meal, attacks the apatite causing the enamel to dissolve. In this in vitro study, eggshells were used as a substitute for human teeth in the examination of factors associated with tooth decay. Eggshells are natural structures composed of both inorganic and organic components such as ~97% of calcium carbonates in the form of calcite; 1% magnesium carbonate; 1% apatite [Ca$_3$(PO$_4$)$_2$]; and ~5% of organic matter. Owing to this unique chemical composition, eggshells have been used either directly or indirectly as a bone substitute in maxillo-facial surgery and as source of hydroxyapatite in bone regeneration.

To our knowledge, there is limited research on the use of eggshells in examining the effectiveness of commercial toothpaste in the prevention of dental caries. This in vitro study was therefore designed to test that premise. It also aimed to evaluate whether commercial toothpaste can slow down the rate of reaction between dental enamel and acids.

### MATERIALS AND METHODS

Five different brands of toothpaste were bought from a popular shopping mall located in Durban (South Africa). These toothpastes included: Sensodyne, Colgate Sensitive toothpaste, Aquafresh, Oral-wise and Colgate. The composition, active ingredients and manufacturers of the toothpaste are shown in Table 1. The listed brands of toothpaste were selected on the criteria that they: (1) contained no whitening or herbal formulation that may react with acids and: (2) did contain fluoride in their formulations.

<table>
<thead>
<tr>
<th>Table 1: Brand, composition, and manufacturer of toothpastes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Products</strong></td>
</tr>
<tr>
<td>Colgate</td>
</tr>
<tr>
<td>Oralwise</td>
</tr>
<tr>
<td>Colgate Sensitive</td>
</tr>
<tr>
<td>Sensodyne</td>
</tr>
<tr>
<td>Aquafresh</td>
</tr>
</tbody>
</table>
of the samples and to examine the surface morphology. Prior to SEM observation, the surface was coated with a thin, electric conductive gold film to prevent a build-up of electrostatic charge. As a proxy measure, samples of pre and post agitation of eggshell powder with toothpaste were characterised to establish the formation of a calcium hydroxyapatite layer.

**Methods of evaluating the effectiveness of the toothpaste in prevention of tooth decay**

The effectiveness of the commercial toothpaste against acid attack was evaluated using 2 M hydrochloric acid. This was prepared by diluting 9.6 mL of acid with deionised water in a 500 mL volumetric flask.

**Gas displacement test**

A gas-displacement test was used to quantify and measure the rate of reaction by observing the displacement of gas formed during the experiment. This entailed using a cylindrical tube of two cm inner diameter and of 60 cm in height as the gas displacement test set-up. As illustrated in Figure 1, a 250 mL Schott bottle, sealed using an airtight stopper, was used for the experiment. A tube was firmly attached through the stopper and connected to a cylindrical tube placed in a reservoir of deionised water. With the aid of a vacuum pump, water from the reservoir was drawn into the cylindrical tube. Prior to the gas displacement test, the initial height of the water in the cylindrical tube was marked using a permanent marker. 0.5g from each samples were placed in the Schott bottle, while 50mL of the prepared 2 M HCl was used as the acid reactant. After 20 minutes, the volume of gas displaced was measured again as the final height of displacement. The amount of gas formed was calculated using equation 1 below.

\[ V = \pi r^2 h \]  

Where “V” is the volume of the cylinder, “\( \pi \)” is a constant, approximated as 3.142, “r” the radius of the cylinder and “h” is the displaced height of the cylinder.

**Pressure sensor test**

A gas pressure sensor was used to monitor pressure changes (kPa) against time (s) during the reaction of the toothpastes samples with 2 M HCl acid. As shown in Figure 2, an Erlenmeyer flask (250 mL) served as the reaction container during the pressure test, with 0.5g of each samples being placed sequentially in the flask, while 25mL of the prepared 2 M HCl was used as the acid reactant. A stopper fitted with plastic tubing was inserted into the flask to provide an airtight container. The tubing lead to a gas pressure sensor (Order Code GPS-BTA). With the aid of an interface system (Vernier LabPro) attached to a computer, the pressure readings were collected and analysed using LoggerPro 3 software.

**RESULTS**

**Characterisation of eggshell powder tooth substitute model**

Table 2 illustrates the elemental composition of the various toothpastes brands pre and post agitation with eggshell powder. Significant differences in the amounts of calcium and phosphorus content in the brands of toothpaste were observed between pre and post agitation. In particular, excluding Colgate Sensitive brand of toothpastes, there was a noticeable increase in phosphorus content in the toothpaste samples after agitation with eggshell powder. All the brands of toothpaste containing hydrated silica (Table 1) recorded the presence of calcium after agitation.

The SEM micrograph shown in Figure 4-7 revealed differences in the surface morphology pre and post agitation with eggshell powder. For the Colgate brand of toothpaste shown in Figure 3, for example, it can be observed that the surface morphology of toothpaste
became more compact and dense post agitation with eggshell powder (Figure 3B), compared with the brand pre agitation (Figure 3A). Similar patterns and compactness in the toothpaste after agitation were observed for the other brands of toothpaste (Figures 4 to 7).

Figure 4: SEM micrographs of Colgate Sensitive toothpaste (A) pre agitation; (B) post agitation with eggshell powder.

Figure 5: SEM micrographs of Sensodyne toothpaste (A) pre agitation; (B) post agitation with eggshell powder.

Figure 6: SEM micrographs of Oralwise toothpaste (A) pre agitation; (B) post agitation with eggshell powder.

Figure 7: SEM micrographs of Aquafresh toothpaste (A) pre agitation; (B) post agitation with eggshell powder.

Acid resistance properties of commercial toothpastes
Figure 8 illustrates the results of the gas-displacement test from the various brands of toothpastes after reaction with 2 M HCl acids. In contrast to the control sample (eggshell powder dissolved in water), the various brands of toothpastes generated a lesser volume of gas. The amount of gas produced is in accord with the average mean pressure of gas measured in the gas pressure tests (Figure 9). Overall, it can be gathered that the Aquafresh brand of toothpastes generated the least amount of gas in reaction to the acid when compared with the rest of the brands.

The amount of pressure (kPa) generated during the gas test was plotted against time (seconds) (Figure 10). It may be observed that reaction to acid was low in the various brands of toothpaste after 100 seconds. Thereafter,
increased acid reaction could be observed. After 500 seconds, all the test samples recorded near plateau-like graph lines suggesting no significant changes were occurring in the quanta of gas pressure generated. The Oralwise sample produced the lowest curve (d), while the control had the widest curve (a). Notwithstanding this, Colgate Sensitive brand of toothpaste had the lowest initial rate of acid reaction, while the eggshell powder (control) had the highest initial rate (Figure 11).

**DISCUSSION**

Despite general improvements in oral health status, dental caries still remains a significant public concern posing a grave challenge for the oral health care provider in mitigation and prevention. Published literature suggests that brushing regularly with well formulated fluoride toothpastes can prevent the onset of this disease.\(^3\)\(^4\)\(^5\)\(^6\)\(^7\) In the present *in vitro* study, eggshell powder was used as a substitute for human tooth material to verify the effectiveness of five commercially available toothpastes in the prevention of dental caries. SEM and EDX were used to study the surface morphology and the calcium and phosphate composition of the brands of toothpastes before and after agitation with eggshell powder.

Calcium phosphate is regarded as being fundamental for the formation of teeth.\(^2\)\(^5\) Based on the EDX elemental analysis (Table 2), calcium phosphate ions were evidently present post agitation with eggshell powder. Notably, the SEM micrographs of the tested brands of toothpastes (Figure 4-7) were able to show net differences pre and post agitation with eggshell powder, which suggests the formation of enamel like hydroxyapatite layer in all brands of toothpastes. In light of the differences in the surface morphology as well as the elemental composition of the tested brands of toothpastes, it can be inferred that eggshell will be suitable as a human tooth substitute for in vitro assessment of erosive attack. This is in agreement with Haghgoo, Mehran (29) that eggshell powder could be used as an alternative for hydroxyapatite since it contains calcium, phosphorus and other mineral elements.

Furthermore, effectiveness the toothpaste against HCl acid attacks was evaluated using both gas-displacement and pressure sensor tests. The data generated from the gas tests suggest that Aquafresh brand of toothpaste is more likely to produce the less volume of gas when reacted with acids (Figure 8 and 9). However, the volume of gas generated from the test samples is not indicative of the effectiveness of the brands of toothpaste in the prevention of acid attacks. As reported in Figure 11, Colgate Sensitive had the lowest initial rate of acid attack, followed by Sensodyne, hence their ability to prevent acid reaction with the tooth that causes dental caries. Overall, all the tested brands of toothpaste tend to be effective in the prevention of dental caries by reducing the reaction of the tooth enamel to HCl acid exposure.

Importantly, and in respect to time of protection, clear differences in the prevention of acid attacks were observed between the tested brands of tooth pastes and control (eggshell powder dissolved in water). From the graphical results shown in Figure 10, as expected, the various brands of toothpastes resulted in less acid reaction than the control. For instance, the tested toothpastes provided greatest protection to the tooth surface in less than 2 minutes (100 seconds) after acid attacks. In contrast, more acidic reaction could be observed for the control sample even after 8-10 minutes (above 500 seconds) exposure to the acids. This suggests that human tooth can react with acid more easily without any protection offered by toothpastes. Hence, it can be gathered that the composition of the tested toothpastes, particularly the fluoride content is responsible for the protection of the tooth against acid attacks. Kallahalli, Sanjay (30) reported that fluorides are abundantly used in many oral health products including toothpastes and mouth rinses as they help in caries prevention by reducing dental caries between 30 and 70% compared with no fluoride therapy. The findings from this study therefore confirmed the effectiveness of fluoride-based toothpastes in the prevention of dental caries.

The protective effect against erosion observed for Colgate Sensitive and Sensodyne, for example, is in line with previous studies. Lombardini, Ceci\(^2\)\(^1\) reported that Colgate Sensitive Pro Relief was more effective than Sensodyne in the protection of enamel against acid attacks. Kato, Lancia,\(^2\)\(^6\) who used bovine enamel, observed that Colgate Sensitive and Sensodyne Original had the best protective effect against erosive acids.

Drawing from the above discussion, it is sufficient to say that the eggshells model was successfully used to evaluate the protective effect of commercial toothpaste against acid attacks. This may be important, particularly in the context of oral health care, as eggshells present a suitable, cheaper, and readily available alternative source to the use of bovine and human enamel models in examining the protective effect of commercial toothpaste. The study suggests that gas displacement and the use of a pressure sensor present a new method of evaluating the rate of reaction between enamel and acids.

**LIMITATIONS**

The gas displacement and pressure tests cannot measure mineral dissolution and surface changes in the eggshell model. Future research could be directed at an examination of the surface of the eggshell model post acid exposure. This would help explain the mechanisms associated with the effective protection of the tested toothpastes by evaluating the mineral dissolution in the eggshell model.

**CONCLUSION**

Eggshell powder can be used as a substitute for human tooth in an *in vitro* experiment. Notably, this study conclusively showed that of the test products, Colgate Sensitive is the most effective brand of toothpaste against acid attacks, while Oralwise is the least effective. However, all brands of the tested toothpastes did lower the rate of reaction between HCl acid and eggshell tooth powder substitute, suggesting their ability to protect the tooth from acids that cause dental caries.

**Acknowledgment**

The authors acknowledge the financial support from the National Research Foundation of South Africa (No. 104824).

**References**

2. Maharani DA, Rahardjo A. Mothers’ dental Health Behaviors...


