Development of an Innovative Urease-Aided Self-Healing Dental Composite

Mostafa Seifan, Zahra Sarabadani and Aydin Berenjian *

School of Engineering, Faculty of Science and Engineering, The University of Waikato, Hamilton 3216, New Zealand; mostafa.seifan@waikato.ac.nz (M.S.); salvlin@gmail.com (Z.S.)

* Correspondence: aydin.berenjian@waikato.ac.nz

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Abstract: Dental restorative materials suffer from major drawbacks, namely fracture and shrinkage, which result in failure and require restoration and replacement. There are different methods to address these issues, such as increasing the filler load or changing the resin matrix of the composite. In the present work, we introduce a new viable process to heal the generated cracks with the aid of urease enzyme. In this system, urease breaks down the salivary urea which later binds with calcium to form calcium carbonate ($CaCO_3$). The formation of insoluble $CaCO_3$ fills any resultant fracture or shrinkage from the dental composure hardening step. The healing process and the formation of $CaCO_3$ within dental composites were successfully confirmed by optical microscope, scanning electron microscopy (SEM), and energy-dispersive X-ray (EDS) methods. This research demonstrates a new protocol to increase the service life of dental restoration composites in the near future.

Keywords: self-healing; dental composite; crack; urease; calcium carbonate; dental caries; restorative composite

1. Introduction

Dental restorative composites are one of the most commonly used materials in modern dentistry. These materials play a key role in restoring the teeth due to exceptional aesthetics, direct filling capabilities and a continuous improvement in mechanical and physical properties, which resulted in an increasing of dental composites over the past 30 years [1]. However, the current restorative materials suffer from shrinkage and cracking, which in turn results in secondary caries formation, crack ad fracture and, in some cases, failure of the restoration. These cracks are extremely hard to detect and impossible to be repaired, and consequently lead to decreasing the service life of composite and finally treated teeth. The results from clinical studies show that restoration failures in the first 5 years are mainly due to restoration fracture and secondary caries, while from 6 to 17 years, secondary caries is the primary reason for replacement of fillings [2–10]. Therefore, the durability and performance of composite restorations need to be improved. The most available solution is to enhance the properties of dental composites by improving the chemistry of the resin and fillers to produce high-performance materials. However, this approach cannot inhibit the likelihood of shrinkage, microcrack formation and crack propagation, and subsequently secondary caries formation.

The development of restorative composites with a self-healing capability is another viable alternative approach to substantially enhance the functionality of dental composites. Recently, the development of self-healing mechanisms has emerged as a promising approach to overcome the issues associated with composite materials in terms of recovering the load-bearing capabilities after cracking. To date, progresses have been made by the use of monomer-catalyst self-healing models to address the cracking issues of the restorative materials. A common technique to produce a self-healing material is encapsulating liquid healing materials into polymeric shells and then incorporating it into a

dental composite. Once cracking occurs, its propagation ruptures the shells and finally the healing materials flow as a result of capillary action to seal the crack. In this context, Wu et al. [11] employed urea-formaldehyde to encapsulate *N,N*-Dihydroxyethyl-p-toluidine (DHEPT) and triethylene glycol dimethacrylate (TEGDMA) for developing a self-healing dental composite. It was found that the incorporation of 7.5% healing microcapsules results in only 64%–70% recovery. In another investigation, Wertzberger et al. [12] evaluated the effectiveness of self-healing dental composites by encapsulating TEGMA, urethane dimethacrylate (UDMA), bisphenol-A-glycidyl dimethacrylate (BisGMA), and Grubbs' catalyst. The incorporation of a self-healing microcapsule resulted in a 57% average recovery rate of the original fracture toughness. Although the microencapsulation self-healing method can partially recover caries, this technique is not a repetitive and permanent self-healing mechanism. Additionally, the remained microcavities after releasing healing materials increase the stress concentration and initiate secondary cracks and eventually leads to failure. More importantly, these approaches raised concerns about their potential toxicity and negative impacts on human health, as well as their cost.

As the literature clearly demonstrates, the efficacy of self-healing in dental composites needs to be substantially improved. Calcium carbonate ($CaCO_3$) is one of the novel inorganic materials with a range of biomedical applications due to its biocompatibility properties. The precipitation of $CaCO_3$ to develop self-healing materials has been previously explored in different fields including construction [13–16]. For example, microbially induced $CaCO_3$ precipitation (MICP) via hydrolysis of urea as a consequence of the urease positive microorganism's metabolism has been examined for designing self-healing cementitious composites [17,18], improvement of soil properties [19], and remediation of environmental contaminants [20]. Urease is a high molecular weight enzyme, and the salient feature of it is the active site that contains a bi-nickel center [21]. Therefore, urease exerts a catalytic function which can hydrolyze urea according to Figure 1. As a result of these series of reactions, carbonate is formed, and the available calcium ions can attach to carbonate to produce $CaCO_3$ (Equation (1)).
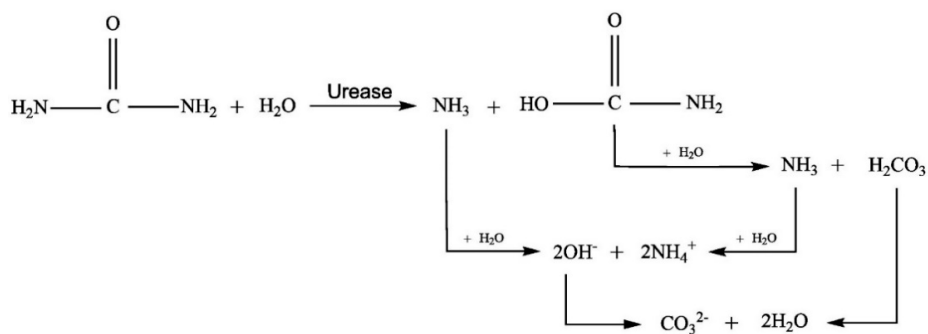
$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\ (s) \tag{1}$$

Figure 1. Schematic reactions representing the hydrolysis of urea by the urease enzyme [22].

Therefore, the main aim of the present investigation was to develop a method to address the current issues associated with dental composites by evaluating the possibility of $CaCO_3$ biomineralization to heal the gaps and cracks in dental restorative materials.

2. Results

2.1. Viability of Producing CaCO3 by Enzyme in Liquid Media

The possibility of inducing $CaCO_3$ crystals via the decomposition of urea by urease in an aqueous solution of calcium chloride was tested. For this purpose, an optimum liquid medium for the biological precipitation of $CaCO_3$ was prepared. Two hours after the addition of urease to the potent medium under a gentle agitation at 37 °C, white insoluble crystals gradually precipitated and collected/attached to the bottom/side of the shake flasks. No additional precipitates were visually formed after 24 h,

indicating all urea was decomposed to form carbonate. To visualize the produced crystals, they were collected using a 0.45 μm filter paper under vacuum pressure (Figure 2a). To determine the morphology of the precipitates, they were washed three times with distilled water to remove the residual media and then oven-dried for SEM analysis. As shown in Figure 2b the precipitates were rhombohedral, demonstrating the successful formation of calcite particles.
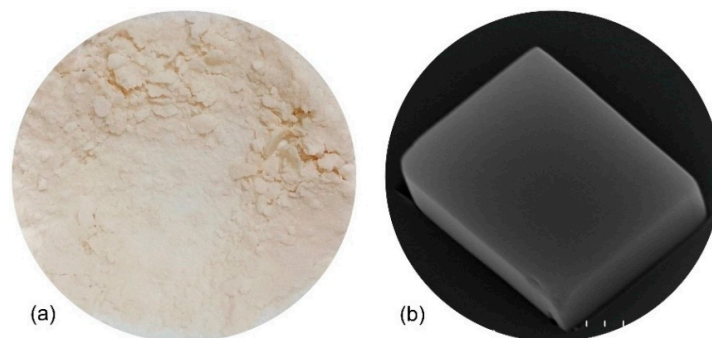

(a)　　　　　　　　　　(b)

Figure 2. (**a**) Collected precipitated crystals from shale flask and (**b**) SEM micrograph of the collected precipitates from shake flask.

2.2. Possibility of Producing CaCO₃ in Dental Composite Matrix

After successful precipitation of $CaCO_3$ via urease in liquid media, the possibility of mineral formation in the dental composite matrix was investigated. In this context, the urease was introduced into the composite, while the other two healing materials (calcium chloride and urea) were supplemented in the surrounding environment by dissolving into the distilled water. As shown in Figure 3, the white precipitates were formed on the cured composites while no precipitation was observed for the control samples (without healing materials). The observation confirmed that the light-curing does not affect the properties of the designed healing materials. This shows the functionality of the designed healing materials in solid matrices.
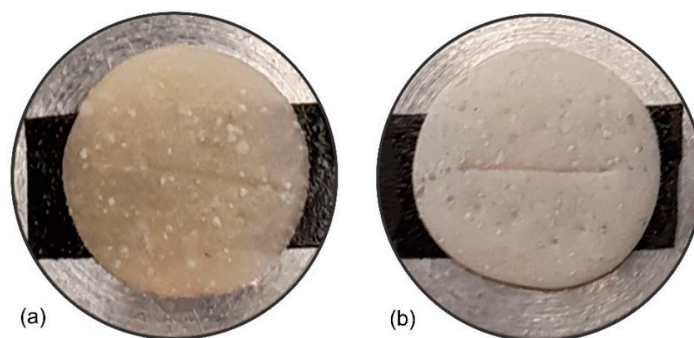

(a)　　　　　　　　　　(b)

Figure 3. Optical microscopic images: (**a**) control composite (without healing materials) and (**b**) self-healing composite.

To gain an insight into the role of the healing compound on filling cavities and cracks, the samples were visualized using an optical microscope. Figure 4a presents the artificial crack with a width of 0.7–1 mm in control and self-healing composite. The images clearly show that the presence of healing compound resulted in the formation of insoluble precipitates. Figure 4b shows the view of a cavity (Φ = 1 mm) in the control and the self-healing composite. The result shows that the fabricated self-healing composite could successfully fill the holes to prevent deterioration of composite upon the formation of cavities.

To obtain an insight into the microstructure of the induced crystals and composites, the samples were examined using SEM. Figure 5a,b shows the micrographs of the induced $CaCO_3$ crystals. It was observed that the precipitation $CaCO_3$ by the urease enzyme had a mostly rhombohedral shape

indicating the mineralization of stable calcite. On the other hand, no such crystal was observed on the control samples (without urease) showing the successful functionality of the urease to induce mineralization in a solid matrix (Figure 5c,d). To determine the elemental composition of the precipitated crystal, EDS analysis was performed and the spectra for the self-healing and control sample are illustrated in Figure 6a,b, respectively. The EDS analysis confirmed that calcium, carbon and oxygen atoms are the main compositions of the precipitated crystals in the self-healing composite and the spectra were similar to the pure $CaCO_3$, previously reported in the literature studies [23–25]. Moreover, a trace of platinum was also detected because of coating to minimize charging and thermal damage prior to SEM-EDS analysis. However, silicon, aluminum, carbon and oxygen were found as the main elements in the control samples. Similar to the self-healing specimen, because of the coating of a conductive layer of metal on the sample, a platinum peak was also detected. The results confirm that the addition of healing materials could successfully coat the composite matrix and compensate the resultant shrinkage during the hardening stage.
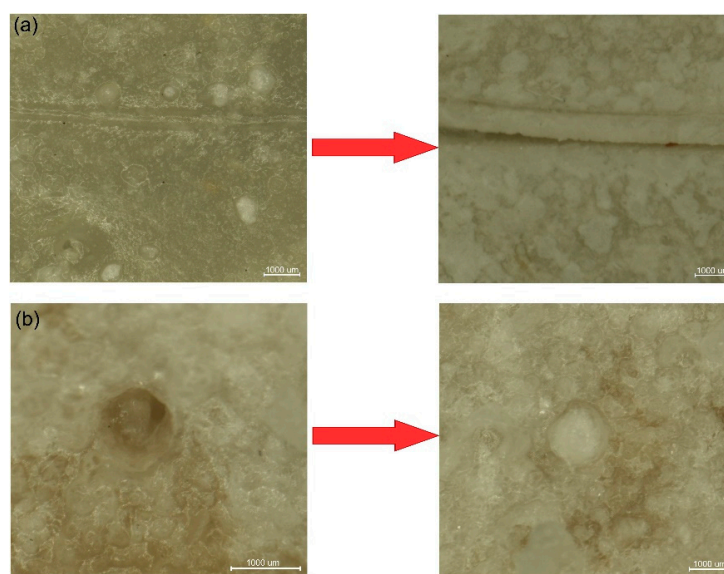

Figure 4. Optical microscopic images before and after healing: (**a**) crack (width 0.7–1 mm) and (**b**) cavity (Φ = 1 mm).
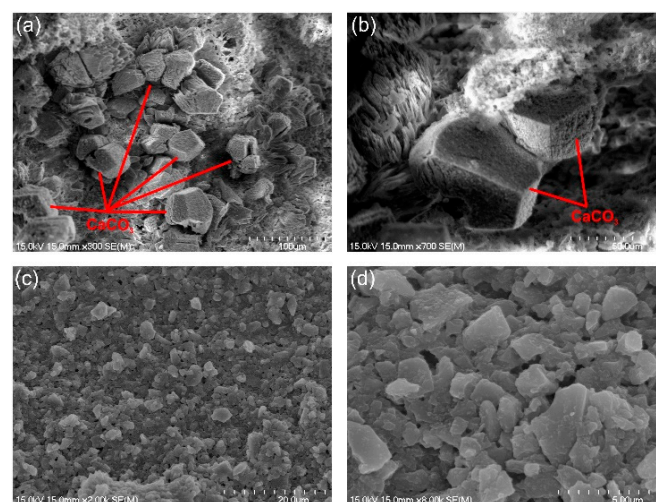

Figure 5. Scanning electron micrographs of dental composite: (**a**,**b**) supplemented with urease enzyme (self-healing composite) and (**c**,**d**) without urease enzyme (control).
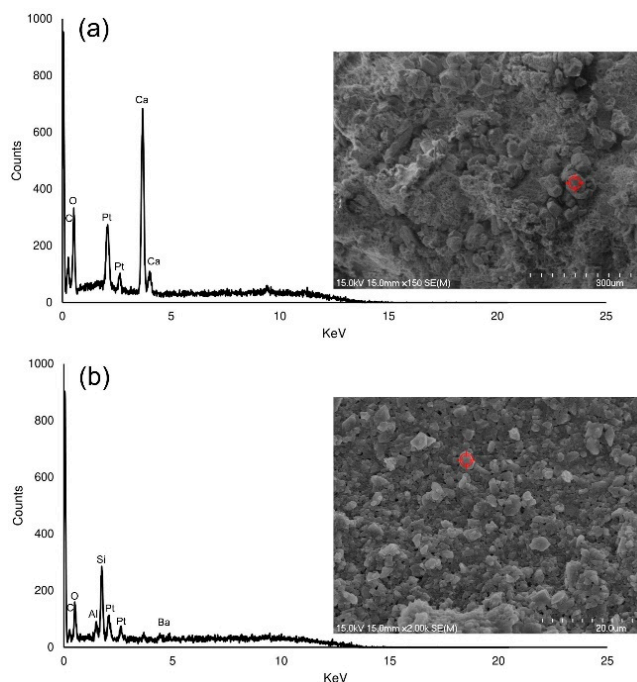
Figure 6. Energy-dispersive X-ray (EDS) spectra of the dental composite: (**a**) supplemented with urease enzyme (self-healing composite) and (**b**) without urease enzyme (control).

2.3. Behaviour of Self-Healing Dental Composite in Saliva

Once the capability of inducing $CaCO_3$ precipitation in a solid composite has been demonstrated, in the next step the effects of enzyme concentrations along with two levels of healing media were investigated. For this purpose, three different concentrations of urease (enzyme to composite ratio: 0.1, 0.3 and 0.5% wt/wt) were mixed with the composite. The composites were incubated into a standard level of other healing materials (40 g/L calcium chloride and 65 g/L urea). In another case, the prepared composites were submerged in a low concentration of healing materials (90 mg/L calcium chloride, 200 mg/L urea) to simulate the salivary compositions. The optical observation indicated that all composites submerged in a standard level of calcium chloride and urea formed insoluble $CaCO_3$. There was no significant difference in the amount of precipitates produced with the increase in the concentration of urease. In contrast, no visual precipitation was detected on the samples that submerged into a low concentration of calcium chloride and urea. However, a small amount of precipitate was observed on the surface of the composite with the highest level of urease cured in a low concentration of calcium chloride and urea. After drying, the microstructures of the samples were studied using SEM and the results are shown in Figure 7. The precipitation of abundant $CaCO_3$ was noticeable in all samples submerged in a standard solution of calcium chloride and urea. Although a trace of $CaCO_3$ was found in the composite mixed with the highest urease and submerged in a low level of calcium chloride and urea, the morphology of the crystals was different from the same composite incubated in a standard concentration of calcium chloride and urea. Despite the similarity in the morphology of $CaCO_3$ produced in a standard solution of calcium chloride and urea, the textures of crystal surface were rough for the composites mixed with 0.1% wt/wt and 0.3% wt/wt urease and immersed in a low concentration of calcium chloride and urea.
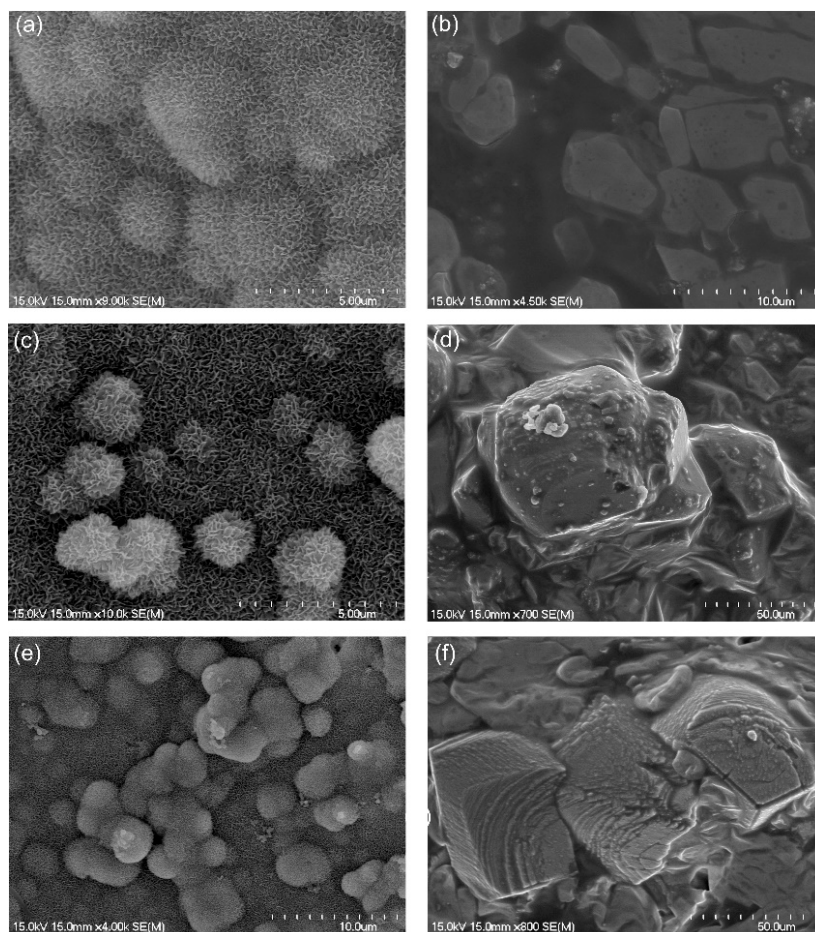
Figure 7. Scanning electron micrograph: composite with 0.1% wt/wt urease submerged in (**a**) low concentration of calcium chloride and urea and (**b**) standard concentration of calcium chloride and urea; composite with 0.3% wt/wt urease submerged in (**c**) low concentration of calcium chloride and urea and (**d**) standard concentration of calcium chloride and urea; composite with 0.5% wt/wt urease submerged in (**e**) low concentration of calcium chloride and urea and (**f**) standard concentration of calcium chloride and urea.

3. Discussion

Dental composites or resin restorative materials are an essential part of restorative dentistry nowadays. Similar to other materials, composites have a limited lifetime and degrade due to different reasons such as physical, chemical and/or biological stimuli [26]. These can contribute to exerting internal stress (thermal), external stress (creep, bending and fatigue), corrosion, erosion, dissolution and biodegradation which gradually lead to failure of the composites. This becomes more important when the composites are embedded in humans and the consequences directly impact the health of the individual. Despite a decreasing trend in replacing restorative composites, the literature shows that approximately 50% of restoration replacements in adults are still due to failed restorations [27–29]. Therefore, there is an urgent need to develop an efficient and easy to use self-healing dental composite.

Currently, there are different methods to address these issues such as increasing the filler load or changing the resin matrix of the composite from methacrylate to a non-methacrylate one. However, their potential toxicity as well as their cost has raised concerns. Inspired by natural biological systems, continuous efforts have been made to incorporate self-healing capabilities into polymeric matrices to significantly improve the service life and functionality of composite materials. The synthesis of inorganic materials by natural organisms is one of the most promising approaches and can be used as a self-healing mechanism in different fields. Among the synthesized materials, $CaCO_3$ has shown an advantage

as it is a stable chemical and is compatible with most matrices. In this context, the precipitation of $CaCO_3$ via microorganisms which is known as MICP has drawn researchers' attention due to its efficiency in converting soluble form of calcium to insoluble minerals. The concept of self-healing using microorganisms has been successfully tested for different applications. In this method of inducing $CaCO_3$, bioprecipitates are formed through two primary routes including sulfur and nitrogen cycle. Although the productivity in the nitrogen cycle varies from pathway to pathway, the efficiency of biosynthesis in the nitrogen cycle is mostly higher than the sulfur cycle. The hydrolysis of the urea or ureolysis pathway is the most employed pathway to address engineering and environmental issues. In this pathway, urease-positive bacteria facilitate the hydrolysis of urea, and consequently carbonate is formed. Depending on the genre of microorganisms, types of calcium ions and environmental conditions, different polymorphs of $CaCO_3$ can be formed. However, to implement the concept of self-healing through $CaCO_3$ precipitation for health applications such as dental composites, microorganisms must be eliminated from the process. Since the role of bacteria in the ureaolysis pathway is to promote the enzyme to break down urea, the replacement of bacteria with urease can resolve the issue. Urease-catalyzed $CaCO_3$ precipitation is known for its efficiency as well as its capability of inducing a high quantity of stable crystals in a short period of time. This feature has enabled a urease-aided self-healing system to quickly fill the resultant cracks/defects and therefore minimize the chance of further damage and failure. An investigation performed by Antipov et al. [30] confirms that urease-aided $CaCO_3$ crystals start growing inside polyelectrolyte capsule shells after 4 min. As compared with MICP approaches, the formation of $CaCO_3$ crystals in the urease-aided technique is less dependent on the pH of the environment. This is mainly due to absence of microorganisms which require a favorable pH and temperature for growth and metabolism. Unlike other proposed self-healing approaches, the developed urease-aided self-healing composite is capable of healing itself repeatedly without compromising the healing efficacy and functionality of composite. Price-wise, eliminating the encapsulation process and the utilization of a single chemical to produce self-healing dental composite has made the urease-aided technique an economically viable approach. Moreover, it has been reported that bacterial- and plant-derived ureases have high sequence similarity [31]. Both of them have similar 3D structures and conserved catalytic mechanism [32,33]. This similarity offers a cheaper source of urease for the development of the self-healing dental composite. Another feature of the urease-aided self-healing composite is the ease of preparation and application. These unique features for urease-aided self-healing help to improve the service life, durability and performance of dental composites by minimizing the crack propagation and the likelihood of secondary caries.

4. Materials and Methods

4.1. Materials

Calcium chloride anhydrous, urea and urease enzyme were purchased from (Sigma-Aldrich Co., St. Louis, MO, USA). The chemicals used in this research were all analytical grade. The dental composite was purchased from SDI Co, Victoria, Australia.

4.2. Self-Healing Composite Preparation

Initially, the potential application of urease for the production of $CaCO_3$ in a liquid medium was tested. In this step, the optimum biological liquid $CaCO_3$-inducing medium (65 g/L urea and 40 g/L calcium chloride) was chosen according to our previous studies [34,35]. A total amount of 5 mg/mL of urease was added to the autoclaved medium and the shake flasks were kept in an incubator at 37 °C with an agitation speed of 50 rpm. In the next step, the effectiveness and performance of healing materials in dental composite were evaluated. For the solid matrix, the healing material was composed of 40 mg/g calcium chloride, 65 mg/g urea and 5 mg/g urease enzyme. To investigate the effect of healing materials on the performance of composites, only urease was introduced to the matrix, while the other healing materials (urea and calcium chloride) were supplemented into the distilled water where the

cured composites were incubated to trigger the healing process. The healing materials were ground separately using a pestle and mortar and then autoclaved separately to ensure the aseptic conditions were maintained. After autoclaving, urease was mixed with composite, formed into a coin shape and then a few holes and artificial cracks were made on one side of the samples. Once the samples were light-cured under laminar flow, they were immersed into an autoclaved solution of urea and calcium chloride in 20 mL beakers to activate the healing agent. All beakers were covered using cotton wool to avoid contamination and placed in an incubator at 37 °C to simulate body temperate under a static condition.

4.3. Microscopic Observation Using Optical and Scanning Electron Microscopy

The formation of minerals on the composites was observed using a motorized stereomicroscope (Nikon, SMZ25, Tokyo, Japan) and the images were analyzed using NIS-Element software. In this sense, the treated composites were oven-dried at 50 °C for 24 h and then placed onto microscope slide. Further characterization was performed using scanning electron microscopy (SEM, Hitachi S-4700, Tokyo, Japan) to evaluate the possibility of $CaCO_3$ precipitation and to visualize the morphology of the precipitated crystals in composites. Energy-dispersive X-ray (EDS) analysis was also carried out to identify the elemental composition of the precipitated crystals in composites. To perform SEM-EDS analysis, the composites were dried in an oven overnight at 50 °C. Thereafter, they were attached onto sticky carbon tape connected to an aluminum stub. After coating the samples with a thin layer of platinum using a sputter coater machine (Hitachi, E1030, Tokyo, Japan), they were introduced into the high vacuum chamber for analysis. Both SEM imaging and EDS spectrum were collected at an acceleration voltage of 15 KV.

5. Conclusions

In this investigation, a new type of viable self-healing dental composite was developed and successfully tested by the incorporation of urease. Both optical and SEM analysis confirmed the precipitation of insoluble crystals. To identify the composition of precipitated crystals, the samples were analyzed using EDS and the resulting spectra matched that of pure $CaCO_3$. This shows the successful catalytic decomposition of urea by urease enzyme in a solid matrix of dental composite. The developed technology can significantly increase the service life of restorative composite by filling the defects (cracks and cavities) when they form as a result of thermal or mechanical stresses. It is expected that the utilization of this technology would provide an enhanced functionality and performance of dental composites as it acts in a short period of time and is available in the entire part of the composite.

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