Sustained Release of Bioactive Compounds from Polymer Microcapsules for Smart Dental Composites

M. YOURDKHANI\(^1\), N. SOTTOS\(^1,2\) and S. WHITE\(^1,3\)

ABSTRACT

Natural plant extracts have been shown as effective and attractive dental biomaterials with promising properties for restorative/reparative procedures. These bioactive extracts, however, may lose their stability when exposed to air, moisture, or light, if not well-protected. Here, we present a novel technique for sequestering bioactive extracts within polymer microcapsules for long-term stability and controlled release of active compounds as needed. We use a combination of water-oil-water (W/O/W) double emulsion and solvent evaporation techniques to encapsulate water-soluble bioactives within polymer microcapsules. Polylactide acid was used as the encapsulating polymer to provide sustained release of bioactive compounds from microcapsules. Manufactured microcapsules possess a polynuclear inner structure as a result of two-step double emulsion technique. We demonstrate the ability to control the size and core loading of microcapsules with varying the key microencapsulation parameters.

INTRODUCTION

Dental composite resins are commonly used as restorative material or adhesive to replace carious or missing dental tissue (i.e. dentin and enamel). These resins exhibit superior mechanical properties, ease of handling, and oral stability; however, their service life still remains limited to only a few years (e.g. 5-7 years in posterior teeth) [1]. Furthermore, it has been reported that around 50% of all restorations carried out by dentists are to replace existing restorations [1]. Although several reasons are known to promote the failure of restoration materials, the primary reason identified is the failure of dentin-resin interface caused by dentin demineralization over time [1, 2].

Natural extracts such as cinnamon bark, pine bark, and grape seed extract have been shown to significantly enhance the strength and stability of dentin organic matrix

\(^1\) Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
\(^2\) Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
\(^3\) Department of Aerospace Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
Despite these promising results, direct integration of natural extracts at the dentin-resin interface results in gradual loss of their chemical stability and biocompatibility in the moist dental environment. In this work, we present a novel approach to sequester and stabilize natural extracts within polymer microcapsules, and release them on demand when required for optimal efficacy. Microencapsulation is a common technique used for isolation, protection, and controlled delivery of active materials across a number of industries from food to pharmaceutics. Encapsulated compounds can be released from microcapsules through a variety of external stimuli, including pH, temperature, mechanical force, and time. Here, we manufacture microcapsules that exhibit time (sustained) release for controlled delivery of natural extracts during the service of restorative composite materials. These microcapsules will be incorporated into dental composites or adhesives to deliver bioactive compounds to the dentin-resin interface, aiming at enhancing the dentin matrix strength, stability, and ultimately, the longevity of dental restorations.

Time release is generally achieved by encapsulating or embedding active payloads in a hydrophobic matrix. We use polylactide (PLA), a biodegradable and biocompatible polymer, to encapsulate bioactive compounds. Gradual degradation of shell wall polymer ensures sustained release of encapsulated materials in the exposed dental regions. The release rate of the capsules can be tailored by controlling various parameters, including partition coefficient of payloads, pore size, degradation rate of the shell, chemical nature of shell wall, and environmental conditions.

Majority of bioactive compounds with proven bioactivity are water-soluble. Extensive research has reported on encapsulation of hydrophobic core materials due to relatively simple encapsulation process and availability of various compatible polymer systems; however, encapsulation of water-soluble materials has been found to be more limited and challenging, especially in development of nano- and micron-sized capsules. Herein, we manufacture microcapsules using a novel water-oil-water (W/O/W) double emulsion technique developed in our lab to sequester selective water-soluble compounds. W/O/W double emulsions are produced first to encapsulate the active compounds, and then, the middle layer of the double emulsions is consolidated to form solid polymer shell using a solvent evaporation method. In this microencapsulation technique, the water-soluble compound of interest is first dissolved inside water to produce the inner water solution (Wi). This solution is then emulsified into an “oil” solution (O), which consists of the encapsulating polymer material (i.e. PLA) in a volatile, water-immiscible organic solvent such as dichloromethane (DCM). The prepared emulsion is then emulsified into outer water solution (Wo), which contains surfactants to stabilize the emulsified droplets. After forming the double emulsion, the organic solvent is evaporated. As a result, the solid polymer phase-separates and encapsulates the inner water droplets. An overall schematic of W/O/W double emulsion technique is shown in Figure 1.

In this study, we used tannic acid as the control water-soluble compound to develop and optimize the microencapsulation technique. We studied the influence of surfactant type on microcapsules morphology, and explored the role of key encapsulation parameters on microcapsule size as well as core loading. Same technique was applied to encapsulate grape seed extract (GSE), another natural extract with proven bioactivity, within PLA microcapsules and to verify the ability to encapsulate various water-soluble compounds.
METHODS

Manufacturing of PLA Microcapsules by Mechanical Agitation

In a typical experiment, 300 mg of tannic acid was dissolved in 3 mL of distilled water and mixed well for 3-4 min to prepare \( W_1 \) solution. Unless otherwise specified, \( W_o \) solution consisted of 300 mL of 2.5 wt.% polyethylene glycol (PEG) and 1 wt.% sodium dodecyl sulfate (SDS) in DI water. The middle oil solution was prepared by mixing 0.3 mL of Span 85 oil-soluble surfactant in 20 g of 7.5 wt.% PLA in dichloromethane (DCM) solution. The first emulsion (i.e. \( W_1/O \)) was formed by mixing the tannic acid solution into the oil phase while stirring at 1200 rpm using mechanical agitation. This stirring rate was fixed for all trails. The mixing continued for 10 min. For second emulsion, the \( W_1/O \) emulsion prepared in the previous step was transferred to the outer aqueous surfactant solution, and stirred at 800 rpm to form the double emulsion. The 800 rpm agitation rate was chosen as the baseline, and was varied to investigate the size dependency of microcapsules on agitation rate. The end product was collected after five hours of mixing to ensure the evaporation and removal of DCM from microcapsules. Microcapsules were then collected using vacuum filtration and washed at least five times with distilled water to remove the remaining surfactants. Manufactured microcapsules were air-dried for at least 24 hours to remove any encapsulated water.

The above microencapsulation procedure was repeated by changing the water-soluble surfactant to select for a surfactant that yields microcapsules with acceptable surface morphology and core loading. Following surfactant solutions were examined: (i) 2.5 wt.% polyvinyl alcohol (PVA) in DI water, (ii) 2.5 wt.% PVA + 1 wt.% SDS in DI water, (iii) 2.5 wt.% SDS in DI water, (iv) 2.5wt.% PEG + 1 wt.% SDS in DI water.

Manufacturing of PLA Microcapsules by Homogenization

Preparation of microcapsule-based self-healing dental resins necessitates manufacturing of microcapsules that are smaller than \( \sim 10-15 \mu m \) in diameter. It is not quite feasible to reach this size limit via mechanical agitation; instead, we used a homogenization technique to significantly reduce the size scale and range of microcapsules. Similar encapsulation procedure as the previous part is followed here with few adjustments in emulsification steps while using the same concentration and quantity of solutions and chemicals. The first emulsion (i.e \( W_1/O \)) was prepared by adding \( W_1 \) solution dropwise to the oil phase while stirring at 1500 rpm and simultaneously

Figure 1: Schematic illustration of water-oil-water (W/O/W) double emulsion technique. \( W_1 \) is an aqueous solution containing bioactive compound, O is the middle oil phase (a solution of polymer in an immiscible volatile organic solvent), and \( W_o \) is the outer aqueous solution.
sonicating the emulsion via an ultrasonic horn (20% power, 0.2 ON and 0.2 OFF). The first emulsion was then added to the W₀ solution and the mixture was emulsified using a homogenizer for three minutes. The formed double emulsion was then stirred at 800 rpm for five hours to remove DCM. Microcapsules were collected and washed four times with distilled water by centrifugation at 4000 rpm for 10 min. Collected microcapsules were freeze-dried for 24 hours to remove any residual water.

**Characterization of Microcapsules**

Microcapsules morphology was studied using scanning electron microscopy (SEM). The inner structure of microcapsules was determined by preparing cross-sections of representative microcapsules after embedding them in an epoxy resin and slicing the cured resin via a diamond knife using an ultramicrotome instrument. The prepared cross-sections were then viewed under SEM.

Core loading of microcapsules was evaluated using UV-Vis spectroscopy technique. First, weighted amount of microcapsules was completely dissolved in 10 mL of tetrahydrofuran (THF), which is a good solvent for both PLA and tannic acid. Then, the peak absorption intensity of the prepared solution at 280 nanometer wavelength (specific absorption wavelength of tannic acid) was measured and compared to a calibration curve to determine the tannic acid content. By knowing the concentration of tannic acid in initial Wᵢ solution, we could measure the quantity of water entrapped within microcapsules, and compare it to the quantity of microcapsules used to estimate the encapsulated water volume fraction.

**RESULTS AND DISCUSSIONS**

SEM micrographs of microcapsules prepared by different surfactants in the W₀ solution are presented in Figure 2. Various morphologies and surface properties were observed for the tested aqueous surfactant solutions. Use of PVA alone (Figure 2a) as the surfactant resulted into microcapsules with a porous surface, whereas fused capsules or irregular morphologies were observed for SDS or combination of SDS and PVA as the surfactants (Figure 2b-c). Combination of PEG and SDS resulted into microcapsules with spherical morphology and acceptable surface properties, indicating more efficient microcapsule formation compared to other tested surfactant options (Figure 2d). In addition, it was not possible to make microcapsules at various size ranges using other surfactants. Therefore, combination of PEG and SDS was selected as the suitable aqueous surfactant choice for the rest of this study.

Manufactured microcapsules exhibit a polydisperse distribution with diameters ranging from few hundred nanometers to few hundred microns (Figure 2d). It is not feasible to make monodisperse microcapsules using mechanical agitation, but the size range can be controlled by varying the agitation rate of the second emulsion. In Figure 3, variation in the size distribution of microcapsules with respect to the applied agitations rate is shown. For each trial, the maximum, minimum, and weighted average diameter of produced microcapsules are presented. Increasing the agitation rate results into higher shear forces being applied on the emulsified droplets, breaking them into smaller droplets, and therefore, producing smaller microcapsules.
SEM micrographs from the cross-section of a representative microcapsule manufactured at 800 rpm is shown in Figure 4a. A polynuclear structure is observed where numerous water-based pores are randomly distributed within the polymer shell. Water pores were generated during the first emulsion, in which inner water solution was emulsified within the oil phase. Because of the relatively high viscosity of the oil phase and presence of hydrophobic Span 85 surfactant, the emulsified water droplets could not move and merge to form a core-shell structure during the microencapsulation process. The volume fraction of water pores present in microcapsules was estimated based on the tannic acid loading determined by UV-Vis spectroscopy measurements. In general, pore volume fraction of around 35-50% was calculated for microcapsules manufactured at various agitation rates (i.e. 250-1200 rpm).

Figure 3: Plot of maximum, minimum, and weighted average diameter for microcapsules prepared at various agitation rates.
Figure 4: (a) SEM micrograph from the cross-section of a representative microcapsule indicating the polynuclear structure of microcapsules; (b) dependency of pore volume fraction of microcapsules on the volume of inner water solution. SEM micrographs show cross-sections of representative microcapsules from each formulations.

The pore volume fraction is directly related to the volume of inner water solution (Wi) used during the emulsification process. Typically, we used 3 mL of Wi solution to manufacture our microcapsules, however, we also examined other volumes of Wi solution (i.e. 1 mL, 2 mL, and 4 mL) to investigate the variation in the volume fraction of water pores inside microcapsules. Microcapsules were manufactured at 800 rpm and all other microencapsulation parameters were maintained unchanged. The relationship between pore volume fraction and volume of Wi solution is plotted in Figure 4b. As expected, pore volume fraction decreases proportionally as the volume of inner water solution is reduced.

Further reduction in microcapsules size was achieved via homogenization technique and adjusting the microencapsulation procedure. SEM micrographs of manufactured microcapsules and cross-sectional view of a representative capsule are shown in Figure 5. Obtained microcapsules had an average diameter of around 4 μm with minimum and maximum diameter of ca. 900 nm and 26 μm, respectively. Tannic acid content and pore volume fraction of microcapsules were estimated to be around 3.75 wt.% and 31%, respectively. We used the same encapsulation procedure to sequester GSE as an alternative bioactive compound within PLA microcapsules. Microcapsules contained around 4.4 wt.% GSE as the core material with estimated pore volume fraction of around 36%.

Figure 5: SEM micrographs of microcapsules prepared by homogenization: (a) size distribution of microcapsules; (b) cross-sectional view of an individual capsule indicating the polynuclear structure.
CONCLUSIONS

A combination of W/O/W double emulsion and solvent evaporation technique was used to encapsulate water-soluble natural extracts within PLA microcapsules. PLA was selected as the shell material to provide sustained release of the core compound due to its biodegradable characteristic in various environmental conditions. Manufactured microcapsules were polydisperse in size and possessed polynuclear structure.

We examined the effect of various aqueous surfactants on the morphology and surface porosity of microcapsules. A combination of PEG and SDS was found to yield microcapsules with tunable size, spherical morphology, and non-porous surface property. The size range of microcapsules was shown to be directly related to the agitation rate of second emulsion/solvent evaporation step. Besides, the pore volume fraction of microcapsules was varied by using different volume of inner water solution.

In this work, we showed the ability to encapsulate tannic acid and GSE within PLA microcapsules. This technique can be applied for encapsulation of any water-soluble compounds within polymer microcapsules for controlled delivery of active materials. Sustained release of microcapsules can easily be tuned by utilizing a suitable grade of PLA based on the molecular weight and degree of isomerism. Other triggering methods can be obtained by using a different type of polymer. For instance, pH-responsive polymers can be used as the shell material to manufacture aqueous core pH-responsive microcapsules using the developed technique.