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Carbon Based Solid-State Calcium Ion-Selective Microelectrode and Scanning Electrochemical Microscopy: A Quantitative Study of pH-Dependent Release of Calcium Ions from Bioactive Glass

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Abstract

Solid-state ion-selective electrodes are used as scanning electrochemical microscope (SECM) probes because of their inherent fast response time and ease of miniaturization. In this study, we report the development of a solid-state, low-poly(vinyl chloride), carbon-based calcium ion-selective microelectrode (Ca²⁺-ISME), 25 μ m in diameter, capable of performing an amperometric approach curve and serving as a potentiometric sensor. The Ca²⁺-ISME has a broad linear response range of 5 μ M to 200 mM with a near Nernstian slope of 28 mV/log [a_{Ca²⁺}]. The calculated detection limit for Ca²⁺-ISME is 1 μ M. The selectivity coefficients of this Ca²⁺-ISME are log K_{Ca²⁺,A} = -5.88, -5.54, and -6.31 for Mg²⁺, Na⁺, and K⁺, respectively. We used this new type of Ca²⁺-ISME as an SECM probe to quantitatively map the chemical microenvironment produced by a model substrate, bioactive glass (BAG). In acidic conditions (pH 4.5), BAG was found to increase the calcium ion concentration from 0.7 mM ([Ca²⁺] in artificial saliva) to 1.4 mM at 20 μ m above the surface. In addition, a solid-state dual SECM pH probe was used to correlate the release of calcium ions with the change in local pH. Three-dimensional pH and calcium ion distribution mapping were also obtained by using these solid-state probes. The quantitative mapping of pH and Ca²⁺ above the BAG elucidates the effectiveness of BAG in neutralizing and releasing calcium ions in acidic conditions.

Graphical Abstract

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SUPPORTING INFORMATION

Cyclic voltammograms of dual SECM probe and calibration curves of pH and Ca²⁺ sensor probes.

INTRODUCTION

A variety of materials, such as amalgam, resin, and polymer composites, are widely used as dental filling materials. Recently, bioactive glass (BAG), which has historically been used in bone repair and tissue engineering, has been introduced as a next-generation “smart” dental filling material.^{1,2,3} A BAG-resin composite is capable of responding to its immediate surroundings by neutralizing the local pH drop and releasing calcium ions (Figure 1).^{4,5} Later, the calcium ions (in the presence of phosphate ions) precipitate as calcium phosphate, depending on the solubility at a given pH. Thus, there is a need to develop new analytical methods to characterize the local chemical environment produced by BAG materials, especially to quantify the calcium ion release pattern at different local pH values.

Scanning electrochemical microscopy (SECM) has been widely used to map the local chemical environment by determining Na^+ , K^+ , Ag^+ , and other relevant ion concentrations on a wide variety of biological and material samples.^{6,7,8,9} The most common strategy for potentiometric detection of ions of interest is the use of a liquid/liquid interface (ITIES) SECM probe or an ionophore-based nonaqueous commercial cocktail as an SECM probe.^{10,11,12,13} However, the major obstacle in such a strategy is positioning the SECM probe at a known distance with the aid of an approach curve. To overcome such challenges, investigators have used a dual-electrode SECM probe comprising a potentiometric liquid membraned-based K^+ ion sensor and an amperometric gallium electrode for positioning and mapping ion release from a 0.1 M KCl-filled glass capillary.¹⁴ In several studies,^{15,16} shear force has also been used to position the potentiometric SECM tip. A high-resolution pH profile was measured with a 500 nm diameter SECM tip composed of a commercially available proton ionophore II liquid membrane cocktail. A solid-state fast-response pH-sensing SECM probe composed of an iridium oxide layer was reported in which the probe was positioned with an optical microscope because of its inability to perform an amperometric approach curve.¹⁷ Alternative strategies, such as polyaniline-coated carbon fiber electrodes, have also been demonstrated to act as pH sensors with a large working range from pH 2 to 12.5.¹⁸

Similar solid-state calcium ion-selective electrodes have been reported^{19,20,21,22,23,24} that used conducting polymer as a solid contact between electrode materials and the ion-sensing membrane. For example, Michalska et al.¹⁹ reported an improvement in the detection limit of close to 10^{-5} M by using polyethylene dioxy thiophene as a solid contact between glassy carbon and a poly(vinyl chloride) (PVC)-based calcium ion-selective membrane containing ETH 1001 ionophore. However, similar challenges remain regarding the positioning of the potentiometric Ca^{2+} -sensing probe for SECM applications. Etienne et al.²⁵ reported the use of a shear-force-based technique to position the SECM probe at a known distance from the substrate, as their Ca^{2+} probe was purely a PVC-based commercially available cocktail from Sigma-Aldrich. However, no quantitative mapping of $[\text{Ca}^{2+}]$ distribution from the dissolution of calcite crystals was reported. An alternative solution to the SECM probe positioning effort is to use a dual-function SECM probe, *i.e.* the same probe capable of functioning as an amperometric (to perform an approach curve) and as a potentiometric sensor. Horrocks et al.²⁶ reported one such dual-functioning (amperometry and potentiometry) SECM probe made of antimony to map the pH variation due to water reduction above a platinum ultramicroelectrode (Pt-UME), as well as the pH gradient produced by the metabolism of the immobilized yeast cells.²⁶ Similarly, carbon paste could act as a dual-function SECM probe, as demonstrated by the use of a 25 μm diameter Cu^{2+} -selective electrode composed of thiuram-containing carbon paste reported in one study.²⁷ The probe was used to map Cu^{2+} dissolution from a copper substrate. However, to our knowledge, no SECM study using a dual-function (*i.e.* potentiometric measurements besides performing amperometric approach curve), solid-state calcium ion-selective microelectrode (Ca^{2+} -ISME) has been reported.

In the present study, we used a solid-state pH and Ca^{2+} -ISME SECM probe to quantitatively map the local pH gradient and the corresponding calcium ion release from BAG dental composites. A dual electrode, consisting of two 25 μm diameter Pt electrodes, served as an SECM pH probe. One of the two Pt electrodes of this probe was modified with polyaniline and used as a pH sensor, and the other bare Pt electrode was used as a redox sensor. In addition, a new carbon paste containing ETH 129 (Ca^{2+} ionophore) was used as a Ca^{2+} sensor to detect calcium ions and to perform an amperometric probe approach curve to determine the tip-substrate (BAG) distance.

MATERIALS AND METHODS

Chemicals

ETH 129 (Ca^{2+} ionophore) and high molecular weight Poly (vinyl chloride) (PVC) were purchased from Sigma Aldrich. 1-Nitro-2-(n-octyloxy)benzene (NPOE) and aniline ($\text{C}_6\text{H}_5\text{NH}_2$) were purchased from Alfa Aesar and Macron, respectively. Bis(2-ethylhexyl)sebacate (DOS) and potassium tetrakis(4-chlorophenyl)borate (KTPIB) were purchased from TCI America. Tetrahydrofuran (THF) was purchased from EMD. HEPES, free acid, was purchased from OmniPur. Vulcan carbon was a kind gift from the Cabot Corporation. Deionized water (18 $\text{M}\Omega$) was used to prepare all solutions.

Artificial saliva solution (0.70 mM CaCl_2 , 0.427 mM MgCl_2 , 4 mM NaH_2PO_4 , 20 mM HEPES, 30 mM KCl) was freshly prepared and stored at room temperature.²⁸ The pH was adjusted to 4.5, 6, and 7.2 by additions of NaOH or HCl.

Instrumentation

Electrochemical measurements were performed with a CHI model 920D bipotentiostat and an SECM instrument (CH Instruments, Austin, TX, USA). An Ag/AgCl reference electrode and a 0.5 mm Pt counter electrode were used in all electrochemical experiments. All potentiometric measurements were performed with a high impedance potentiometer (Lawson Labs, Malvern, PA, USA and EA Instruments, Middlesex, UK).

Fabrication of pH-sensing dual SECM tip

A theta pipette was used to fabricate the dual-tip UME. The pipette was pulled with a pipette puller (Sutter Instruments, Novato, California, USA), and 1.5 cm long, 25 μm diameter platinum wires were inserted into each cavity of the pipette before sealing. Platinum wires were connected to copper wires by conductive silver epoxy. The electrode surface was then polished on a polishing pad via the successive use of 1.0, 0.3, and 0.05 μm alumina powder. The electrode was tested by cyclic voltammetry in a 1 mM ferrocene methanol and 0.1 M KCl solution. The electrode was further characterized by running probe approach curves (with each electrode, independently) above an insulating substrate in the presence of ferrocene methanol solution. The pH sensor was fabricated by electrochemically depositing polyaniline onto one of the two Pt surfaces by cycling the potential from -0.2 to 1.0 V (vs. Ag/AgCl reference) in 0.1 M aniline and 1 M HCl at 100 mV/s for 50 cycles. A schematic of the dual-tip pH sensor is shown in Figure 2A (*left*). pH calibration was performed with a high impedance potentiometer at room temperature (23°C) by addition of 1 M NaOH to artificial saliva to bring the pH from 4.5 to 8.

Fabrication of calcium-sensing SECM tip

A cocktail of the following composition was prepared in THF (w/w): ETH 129 (5%), KTIPIB (2%), NPOE (30 %), PVC (3%), and Vulcan carbon powder (60%). The composition was mixed thoroughly with a glass rod in a watch glass and an additional 40% of NPOE was added to obtain a pasty consistency. The resulting paste was then packed into the back of a pulled borosilicate capillary tube (o.d. 1.5 mm; i.d. 0.86 mm). The pulled capillary tubes were polished to obtain an internal tip diameter of 25 μm and an RG (ratio of glass insulation diameter to electrode diameter) < 4 . The cocktail was pushed with a copper wire with pressure until the cocktail squeezed out of the capillary tip. To make the backside contact, the back end of capillary was filled with a 2.6 (w/w %) of Vulcan carbon in DOS. A copper wire was subsequently inserted from the back and glued with epoxy. The electrode was wiped with lens paper to obtain a clean and smooth surface and later cured/soaked in artificial saliva for at least 12 h before use. A schematic of the Ca^{2+} -ISME is shown in Figure 2A (*right*).

Testing the potentiometric and amperometric function of the Ca^{2+} -ISME

The sensors were calibrated by using the standard addition method in artificial saliva and in 0.1 M KCl solution respectively. The pH-dependent potentiometric response of the sensors was also tested in HEPES buffer and artificial saliva solution. Later, the sensors were calibrated in potentiometric mode separately in the presence of 1 mM of RuHex, ferrocene methanol, and ferrocyanide, as well as in varying concentrations of RuHex solution, to determine the effect of redox active molecules on potentiometric Ca^{2+} sensing.

The amperometric response was characterized by calibrating the sensors with artificial saliva solution that contained hexaammineruthenium (RuHex) by square wave voltammetry and the constant potential method. The effect of the amperometric method, i.e. applying potential to the Ca^{2+} -ISME, on the potentiometric capability of the sensor was measured in the presence of both calcium ions and redox molecules. A potential was applied to the sensor for 1 h and precalibration and postcalibration curves were obtained to test the effect of passing current on the potentiometric response of Ca^{2+} -ISME. In addition, the sensors were calibrated by the addition of Ca^{2+} standards in artificial saliva solution before each SECM experiment. We used the calibrated sensors with $\text{RG} < 4$ to perform amperometric approach curves on the surface of the exposed BAG, as well as on Kapton tape (Figure 2B). The experimental approach curves were later fit with the theoretical approach curves to determine the tip-substrate distance.⁶

Preparation of dental composite samples

BAG powder was synthesized by using the sol-gel method as previously described.^{29,30} The BAG disks were prepared by pressing the BAG powder into steel molds in a universal testing machine. The typical resin composite formulation was as follows (mol %): 65% Si, 31% Ca, and 4% P in the form of their elemental oxides. These composites were pressed at 6,000 PSI into 12.5 mm discs and cured within plastic molds by using an LED dental curing unit with 20 mJ/cm^2 total energy to ensure adequate cure. BAG disk composites were polished with rough sandpaper (120 grit) prior to each use. Kapton tape was used to cover the composite surface, leaving an exposed surface of 1.6 mm diameter when necessary (Figure 2B).

SECM experiments with dental composites

The BAG disk was secured inside a 30 mm petri dish and attached to the SECM stage (Figure 2B). The BAG or the SECM substrate tilt over the distance of 5,000 μm was fixed by using a 25 μm diameter Pt-UME before all pH and Ca^{2+} mapping. The Pt-UME was then replaced by a Ca^{2+} -ISME and the probe approach curves were performed in the presence of 1 mM potassium ferrocyanide solution to determine the probe-substrate distance. The redox-containing artificial saliva solution was later replaced with 15 mL artificial saliva solution at the designated pH before potentiometric measurements were recorded. Probe scans in the z-direction began at 2,020 μm above the BAG surface and approached at a speed of 3 $\mu\text{m}/\text{s}$. X-direction scans of 3,000–5,000 μm were performed at a constant distance of 150–820 μm above the substrate. SECM images were taken at 50 $\mu\text{m}/\text{s}$ at heights of 100, 200, and 1,000 μm above the BAG surface. A pH sensor and a Ca^{2+} -ISME were used as an SECM probe to map pH and Ca^{2+} ions, respectively, in separate experiments.

Bulk pH and calcium ion measurements

Composites were submerged in 15 mL of artificial saliva solution at a designated pH and left undisturbed at room temperature. The supernatant was removed, mixed thoroughly, and tested at various times by using a glass pH probe. Similarly, the bulk concentrations of free calcium ions were measured by the Ca^{2+} -ISME. BAG substrate was submerged for 4 h in artificial saliva solution of different pH values between 4.5 and 7.2 and then supernatants were collected to determine the Ca^{2+} present in the solution.

RESULTS AND DISCUSSION

Electrochemical characterization of dual pH-sensing electrode

The dual electrode comprised two 25 μm Pt electrodes: one for amperometry (bare Pt electrode) and the other for potentiometry (pH sensor). Cyclic voltammetry and probe approach curve experiments using bare Pt electrodes showed no appreciable difference at the electrode surfaces (Figure S1A). The approach curve was later fit with the theoretical curve,⁶ as shown in Figure S1B. The potentiometric pH-sensing electrode (25 μm polyaniline modified Pt electrode) was calibrated by the standard addition method, as shown in Figure S2. Only sensors with a slope of -59 ± 2 mV/pH were used for further SECM studies. The bare 25 μm Pt electrode was used for performing approach curves to fix the tip-substrate distance.

Electrochemical characterization of Ca^{2+} -ISME

The potentiometric response of the Ca^{2+} -ISME and the corresponding calibration curve are shown in Figures S3A and 3A. The sensor shows a broad dynamic range response over the concentration range of 5 μM to 200 mM with a slope of 28 ± 2 mV/log $[\text{a}_{\text{Ca}^{2+}}]$. The limit of detection was calculated to be 1 μM according to the IUPAC definition.³¹ The Ca^{2+} -ISME also showed a very fast response time of ~ 0.2 s (Figure 3B) in the presence of 500 μM of calcium ions and thus can be used as a scanning probe for SECM experiments.

The pH interference test was conducted both in 0.1 M KCl and in artificial saliva solution with a constant concentration of 10 μM Ca^{2+} (Figure 3C). The pH was varied from 4.5 to 7.2 and the potential response of the Ca^{2+} -ISME was recorded. The Ca^{2+} -mediated potentiometric signal was constant from pH 4.5 to 6.8, followed by a small 2 mV change in the pH range of 6.8 to 7.2 for a 0.1 M KCl and 10 mM HEPES solution. A similar constant potential response was observed for artificial saliva solution (pH 4.5 to 6.2), except a potential variation of 4 mV was recorded from pH 6.2 to 7.2. This small change in potential beyond a near neutral pH value is due to the change in solubility of the 10 μM free Ca^{2+} in the presence of artificial saliva solution containing phosphate ions. Further tests were done to record the response of Ca^{2+} -ISME in increasing initial concentrations of Ca^{2+} (Figure 3D). These tests confirm the differential solubility of Ca^{2+} with changing pH and show that the Ca^{2+} -ISME was unresponsive to a change in pH. This observation is significant, as we could use this sensor for SECM experiments to map the $[\text{Ca}^{2+}]$ above the BAG surface, where the change in calcium ion concentration is accompanied by a change in pH.

Figure 4A shows the calibration curve of the calcium ions in the presence of 1 mM ferrocene methanol, RuHex and ferrocyanide in artificial saliva solution. The Ca^{2+} -ISME shows Nernstian response in the presence of all of the above-mentioned redox species, as well as before and after applying the potential to the electrode (Figure S3B). However, the presence of ferricyanide redox molecules affects the response of Ca^{2+} -ISME in the low Ca^{2+} concentration range (< 0.01 mM) but still shows the Nernstian slope in the higher Ca^{2+} concentration range (0.1 to 1 mM). The non-specific adsorption of ferricyanide on the sensor surface might contribute to such behavior. The absolute potential shift for various calibration curves shown in Figures 4A is because of the use of different electrodes. In addition, the sensor showed no change in potential in presence of increasing amount of RuHex confirming its insensitivity towards the redox molecules. (Figure 4B).

The selectivity of the Ca^{2+} -ISME was tested by the matched potential method. A solution of 0.1 M KCl at pH 4.5 with a concentration of $5\ \mu\text{M}$ Ca^{2+} ions was chosen as the starting point. The response of the Ca^{2+} -ISME to a small increment from $5\ \mu\text{M}$ Ca^{2+} to $6\ \mu\text{M}$ Ca^{2+} was measured as 3 mV. Later, the concentration of the interfering ion was increased on top of the $5\ \mu\text{M}$ Ca^{2+} and the response of the Ca^{2+} -ISME was recorded. The selectivity coefficient was determined by $K_{pot} = (a_{\text{Ca}^{2+}} - a'_{\text{Ca}^{2+}})/a_A$, where $a_{\text{Ca}^{2+}}$, $a'_{\text{Ca}^{2+}}$ are the activities of $5\ \mu\text{M}$ Ca^{2+} to $6\ \mu\text{M}$ Ca^{2+} , respectively, and a_A is the activity of the interfering ion, which showed an equivalent response of 3 mV. Table 1 shows the selectivity against common interferents in artificial saliva. The selectivity coefficients of this Ca^{2+} -ISME are reported as $\log K_{\text{Ca}^{2+},A} = -5.88, -5.54, \text{ and } -6.31$ for Mg^{2+} , Na^+ , and K^+ , respectively, in 0.1 M KCl at pH 4.5 (Table 1). The selectivity coefficients were also measured by using the fixed interference method. A fixed concentration of the interfering ion and 10 mM HEPES was chosen as the background solution and calcium ions were added to increase the concentration from 1 nM to 100 mM. The calculated selectivity coefficients for Ca^{2+} -ISME is comparable to earlier reports ($-\log K_{\text{Ca}^{2+},A} = -4.5$ to -6.0).^{32,33,34} The limit of detection

of the calcium ions was used to calculate the selectivity as $K_{\text{Ca}^{2+},\text{int}}^{\text{pot}} = a_{\text{Ca}^{2+}}/a_{\text{int}}^{\left(\frac{z_{\text{Ca}^{2+}}}{z_{\text{int}}}\right)}$, where z represents charge of the corresponding species.

After potentiometric characterization, we tested whether the Ca^{2+} -ISME could also be used as an SECM tip by obtaining a negative feedback approach curve on an insulator. Hence, the calibration curve using square wave voltammetry and the constant potential method were first performed by using RuHex as a redox mediator to obtain a linear relationship (Figure 5A and 5B) between the redox couple concentration and the tip current. Subsequently, a cyclic voltammetry was obtained (Figure 5C) and a negative feedback approach curve was recorded by using RuHex as a redox mediator and was fit with the theoretical approach curve (Figure 5D). The good fit of the experimental approach curve indicates that this Ca^{2+} -ISME could be used in future SECM experiments.

pH profile above BAG using SECM

Figure 6A shows the schematic representation of the pH profile produced by BAG in an acidic solution (pH 4.5). The local pH remained relatively constant when more basic (pH 6.0 and 7.2) artificial saliva solution was added to the BAG sample dish. The z-direction scan

(Figure 6B) shows the neutralization zone extending 600 μm above the surface for the acidic solution (pH 4.5) and the bulk solution remaining constant at pH 4.5. A similar neutralization zone was observed during x-scans over the BAG surface at various heights (Figure 6C) in the same acidic solution (pH 4.5). Figure 6D shows the x-scans over the BAG surface at same height ($z=220\text{ }\mu\text{m}$) for pH 4.5, 6.0, and 7.2 solutions. Figure 6C also confirms that the BAG substrate was able to neutralize the local pH. However, little to no neutralization zone was observed in the presence of solution at pH 6.0 and 7.2, as shown in Figures 6B and 6D. To estimate the extension of this neutralization zone in the x-y plane, we obtained constant height pH mapping (Figure 7) at a distance of 100 and 1,000 μm above the BAG surface.

Calcium ion profile above the BAG using SECM

Ca^{2+} -ISME was used to map the pH-dependent calcium ion release from BAG, as shown in Figure 8A, B, and C. The z-direction calcium ion release profiles in the presence of various pH solutions (pH 4.5, 6.0, 7.2) are shown in Figure 8A. The z-direction scans show that the calcium ion concentrations remained constant in bulk solution, 1,000 μm from the surface of the BAG. The Ca^{2+} concentrations in artificial saliva solution increased by 0.7 mM in pH 4.5, by 0.3 mM in pH 6.0, and by 0.02 mM in pH 7.2, indicating that the calcium ions released from the BAG are pH dependent. Figure 8B shows x-scans at different heights above the BAG in the presence of an artificial saliva solution at pH 4.5. The x-scans show that the concentration of the Ca^{2+} ions increased over the exposed BAG and there was a more pronounced effect in the x-scans taken closer to the surface. A Ca^{2+} profile image was obtained with artificial saliva at pH 4.5 at a fixed height of 200 μm above the BAG, as shown in Figure 8C. The 3-D image shows that the calcium ion concentration increased to 1.2 mM at 200 μm above the BAG. The calibrations obtained before and after applying the potential to the electrode are reported in Figure S3B. The data from Figure S3B shows that the change in slope is less than 1 mV/log [Ca^{2+}] before and after applying potential to the Ca^{2+} -ISME. Since the data over the BAG were collected after positioning the tip in amperometric mode, the calcium ion concentrations during the SECM experiments were calculated from a post-SECM experiment calibration curve for the appropriate pH solution.

Correlation of pH and calcium ion profile above the BAG surface

The main function of the BAG material is to neutralize the local environment when it is exposed to a low pH solution ($\text{pH} < 5.5$). The calcium oxide present inside the BAG reacts with the acidic solution, neutralizing the local pH and subsequently releasing Ca^{2+} . Our results (Figures 6 and 7 and Table 2) show that the BAG is able to neutralize the local pH to 6.0 ± 0.3 even though the bulk solution remains at pH 4.5. This leads to the release of calcium ions, which can then react with the phosphates that are present in the buffer and precipitate out of the solution. However, precipitation of calcium phosphate highly depends on the pH of the solution. Hence, when we map the calcium ions in solutions of pH 4.5, we are essentially mapping the change in the solubility of calcium phosphate, along with the change in the pH profile (i.e. pH 6.0 to 4.5, or the amount of free calcium ions in equilibrium with calcium phosphate at a particular pH). It is for this reason that we observed the decrease in calcium ions in close proximity (less than 50 μm distance) of the BAG, where the local pH was observed to be 6.0. As the pH decreased with the increasing distance

from the BAG surface (Figure 6B), the amount of free calcium ions changed from 1.4 mM (20 μ m above BAG) to 0.7 mM bulk ($\sim$ 1,000 μ m above BAG) (see Figure 8A). As reported in Table 2, when acidic artificial saliva of pH 4.5 was added to BAG surface, the local pH changed from 4.5 to 6.0 whereas the local $[\text{Ca}^{2+}]$ changed from 0.7 to 1.4 mM. i.e. a change in $[\text{H}^+]$ of 31 μ M is accompanied by change in $[\text{Ca}^{2+}]$ of 700 μ M. According to the reaction mechanism (considering same volume), $[\text{H}^+]: [\text{Ca}^{2+}]$ should be in 1:0.5 ratio in contrast to our observed value of 1:23, which indicates a significantly higher concentration of free Ca^{2+} ions. This is admissible if we consider the initial contact of acidic solution of pH 4.5 and the subsequent burst of release of Ca^{2+} from BAG surface. In addition, the solubility of Ca^{2+} increases as the pH becomes more acidic from the surface to bulk above BAG until the system came to a dynamic equilibrium. Also, the buffering capacity of the artificial saliva solution becomes more dominant as the pH of the solution becomes more basic (pH 6.2) close to the BAG surface. This phenomenon was confirmed by the bulk free calcium ions experiment done in artificial saliva solution (see figure 3D). As expected, we observed a lower calcium ion concentration profile of 0.9 mM to 0.6 mM (20 μ m to 1,000 μ m) in the presence of a solution at pH 6.0. In Figures 6B and 8A, as expected, no such neutralization phenomenon occurred and the free calcium ion profile was not observed when a neutral solution of pH 7.2 was added to the BAG. The bulk (500 μ m above the BAG surface) concentrations of the calcium and proton ions did not change after 4 h of exposure of the artificial saliva at pH 4.5, 6, and 7.2, as shown in Table 2. This quantitative mapping of pH and calcium ions will aid us in predicting the local chemical environment above BAG and thus will help us to optimize the composition of dental materials.

CONCLUSIONS

The newly developed fast response carbon based Ca^{2+} -ISME and dual electrode pH sensors allowed us to quantitatively map the neutralizing capacity of BAG while estimating the amount of calcium ions that it releases. Testing revealed that exposure of BAG to acidic conditions (pH 4.5) results in a change of local pH and $[\text{Ca}^{2+}]$ (6.0 and 1.5 mM, respectively) extending 500 μ m above the BAG surface. The release and reprecipitation of the Ca^{2+} ions from the smart dental composite provides a means to neutralize acidic pH while reducing the erosion of material, thereby enhancing the overall longevity of the filling. The ability to measure this local pH and calcium ion concentration will open opportunities to characterize the ion-releasing capacity of new dental materials and their effects on the surrounding in vivo oral environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic diagram of pH-dependent calcium ion release from bioactive glass (BAG) when exposed to acidic conditions (pH 6.0 or lower).

Figure 2.

(A) Schematic representation of the (*left*) dual-electrode pH sensor (25 μm diameter Pt wire for each tip) and (*right*) 25–30 μm diameter carbon based solid-state calcium ion-selective microelectrode. (B) SECM-BAG experimental setup. A 0.5 mm Pt wire and an Ag/AgCl were used as counter and reference electrodes, respectively (not shown).

Figure 3.

Potentiometric characterization of the Ca^{2+} -ISME. (A) Calibration curves of all solid-state Ca^{2+} sensors in artificial saliva (slope = 28 mV/ $\log a_{\text{Ca}^{2+}}/\text{M}$) and 0.1 M KCl (slope = 27 mV/ $\log a_{\text{Ca}^{2+}}/\text{M}$) solutions at pH 4.5 with no initial Ca^{2+} . (B) Potentiometric response time of the sensor in the presence of $500 \mu\text{M}$ Ca^{2+} . The response time was 0.17 seconds. (C) Potential response of the sensor with a change in pH from 4.5 to 7.2 in artificial saliva (red) and 0.1 M KCl and 10 mM HEPES (blue) with a constant Ca^{2+} concentration of $10 \mu\text{M}$. (D) Ca^{2+} solubility change with change in pH in artificial saliva with different initial Ca^{2+} concentrations.

Figure 4.

(A) Potentiometric calibration curves of Ca^{2+} -ISME in the presence of 1 mM each of RuHex, ferrocene methanol, ferricyanide and ferrocyanide. (B) Potentiometric response of Ca^{2+} -ISME, in the presence of 1 mM calcium ions, to increments in concentrations of redox molecules.

Figure 5. Amperometric characterization of Ca^{2+} -ISME in artificial saliva solution (pH 4.5). (A) Square wave voltammetry response of Ca^{2+} -ISME with varying concentrations of RuHex. (B) Calibration of Ca^{2+} -ISME in RuHex with amperometric i - t curve (blue dots) and square wave voltammetry (red dots). (C) Cyclic voltammetry of Ca^{2+} -ISME in 1 mM RuHex (vs. Ag/AgCl). (D) Fitting of the negative feedback approach curve of the dual-function Ca^{2+} sensor (RG = 1.5) with the theoretical curve.

Figure 6.

(A) Schematic diagram of the pH profile above the exposed bioactive glass (1.5 mm diameter) in acidic conditions (pH 4.5). (B) Z-direction pH profile above the BAG surface after exposure to artificial saliva solution at various pH values. X-direction pH profile (C) at various heights above the BAG surface in the presence of artificial saliva solution at pH 4.5 and (D) at 220 μm above the BAG in artificial saliva at various pH values. Vertical dotted lines represent the exposed BAG surface.

Figure 7.

Three-dimensional SECM image of pH distribution above the BAG surface. pH imaging was performed over 1.6 mm of the exposed BAG surface (100 and 1,000 μm above) in artificial saliva at pH 4.5. The transparent purple sphere represents the neutralized zone produced by the BAG.

Figure 8.

(A) Z-direction Ca^{2+} profile above the BAG substrate in the presence of artificial saliva of pH 4.5, 6.0, and 7.2. (B) X-direction Ca^{2+} profile at different heights above the exposed BAG in the presence of artificial saliva at pH 4.5. (C) Three-dimensional SECM image of calcium ion release from the BAG surface when exposed to artificial saliva (pH 4.5). The image was taken at a constant height of 200 μm from the BAG surface.

Table 1Interference of Ca^{2+} -ISME with Mg^{2+} , Na^+ , and K^+

Interfering ion, A	$\log K_{\text{Ca}^{2+},\text{A}}$	$\log K_{\text{Ca}^{2+},\text{A}}$
	Matched potential method	Fixed interference method
Mg^{2+}	-5.88	-5.5
Na^+	-5.54	-6.7
K^+	-6.31	-6.1

Table 2

Comparison of local and bulk pH and calcium ion concentrations.

Saliva solution	pH measurements ^a		Ca ²⁺ concentration (mM) ^a	
	Bulk	Local (z=20 μ m)	Bulk	Local (z=20 μ m)
pH 4.5	4.58 $\pm$ 0.05	6.04 $\pm$ 0.3	0.70 $\pm$ 0.05	1.40 $\pm$ 0.10
pH 4.5	6.05 $\pm$ 0.01	6.37 $\pm$ 0.1	0.51 $\pm$ 0.05	0.85 $\pm$ 0.05
pH 4.5	7.15 $\pm$ 0.01	7.30 $\pm$ 0.1	0.35 $\pm$ 0.05	0.35 $\pm$ 0.10

^a All concentrations are reported after 4 h of exposure.