METHOD FOR DETECTING CALCIUM PHOSPHATE MINERALIZATION ON DENTAL COMPOSITES

ADM Conference

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Abstract

Purpose: To develop a procedure for determining the in-vitro calcium phosphate mineralization potential of dental composites.
Methods: This study used Activa Presto, a dental composite containing 3wt.% MCP (3MCP), and a control with the same chemical formulation as Presto, but without MCP (0MCP).
Six cylindrical samples (three of 3MCP and three of 0MCP) were created with an approximate diameter of 9.5mm and height of 4mm. Each sample was fabricated with an embedded nylon thread, which was used to suspend each sample in 25mL of phosphate buffered saline (PBS) in individual plastic bottles. The samples were stored in a 37°C incubator for time periods of one, two and four weeks, and the PBS solution was replaced twice weekly. Once the designated time period elapsed, the samples were removed from the solution, placed back in the 37°C incubator and desiccated for scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) evaluation.
Results: The SEM of the 0MCP sample was uniform throughout. At 3000x magnification the surface was granular and showed no sign of crystal formation. All of the SEMs of the 3MCP samples had areas of crystal formation and areas where crystals had not yet formed (nucleation areas). The nucleation areas on the 3MCP sample looked granular, similar to the control, but EDS revealed a different surface composition.
Conclusion: The SEM and EDS proved to be valuable tools in evaluating the mineralization of the samples. The calcium and phosphorus content increased from the 0MCP sample, to the 3MCP nucleation area, and increased further in the 3MCP crystal area. This increase, along with the SEM images confirmed that the surfaces had been mineralized.
The results of this study confirm that this procedure is suitable to determine the in-vitro mineralization potential of dental composites.

Purpose/Aim

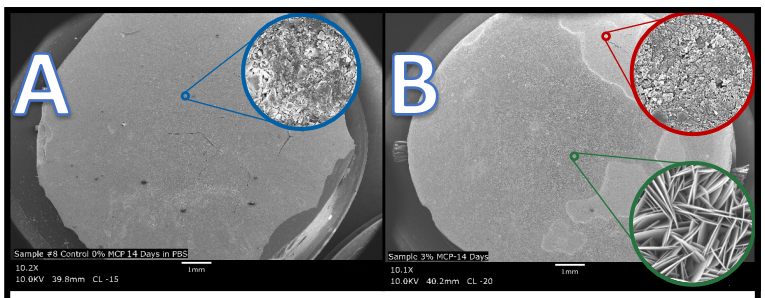
To develop a procedure for determining the in-vitro calcium phosphate mineralization potential of dental composites, and to evaluate the methods for characterizing the mineralization.

Methods and Materials

Composite disc preparation: A dental material containing Methacrylate-Functionalized Calcium Phosphate (MCP), was studied to determine a procedure to identify and quantify the material's capacity to create calcium phosphate mineralization. This study used Activa Presto (3MCP), and a control with the same chemical formulation as Presto, but without MCP (0MCP).
Using ISO 23317:2007 as a guideline, six cylindrical samples were created with an approximate diameter of 9.5mm and height of 4mm. A silicon mold with a diameter of 9.5mm and height of 2mm was placed on a Mylar sheet on a flat glass plate, the uncured composite was extruded into the mold with slight excess and a second sheet of Mylar film followed by a second glass plate was placed on top and pressure was applied to displace any excess material. The glass plate was removed and the sample was cured for 20 seconds on each side. A nylon thread, approximately 8mm long, was placed on the cured sample and a second silicon mold with the same dimensions was placed on top. Additional material was extruded into the second mold, and the sample was cured using the same steps as above, effectively embedding the nylon thread between the two samples. The sample was removed from the molds and polished with 600-grit SiC paper, rinsed with distilled water and dried. This procedure was repeated for each of the six samples, three were made using the 3MCP composite, while three were made using the 0MCP composite.
Sample Storage: Each sample was suspended in 25mL of PBS (Dulbecco's Phosphate Buffered Saline, Sigma-Aldrich) by securing the nylon thread to the lid of a 60mL plastic bottle. The bottles were stored in a 37°C incubator (Boekel Industries, Feasterville, PA, USA) for time periods of one, two and four weeks. Twice a week the PBS in each bottle was replaced with a fresh solution. Once the designated time period had elapsed, the samples were removed from the solution, placed back in the 37°C incubator and desiccated for scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) evaluation.
Evaluation: The samples were analyzed using scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) to examine the changes in surface appearance and to determine the composition of the surface layer. The samples were mounted on aluminum SEM stubs using double sided carbon adhesive tape. To mitigate charging in non-conductive samples, the SEM stubs were sputter-coated with Au-Pd for 90-95 seconds in a Denton Vacuum Desk II (Moorestown, NJ, USA). The samples were analyzed using a RaySpec light element detector (High Wycombe, UK) for 120 – 130 seconds to determine the elemental composition of the material. SEM images were captured at 10x, 400x, 3,000x and 6,000x.

Results

Surface Composition Comparison
The SEM images of all of the 0MCP samples were uniform throughout. At 3000x magnification the surface was granular and showed no sign of crystal formation. All of the SEM images of the 3MCP samples had areas of crystal formation and areas where crystals had not yet formed (nucleation areas). The nucleation areas on the 3MCP sample looked granular, similar to the control, but EDS revealed a different surface composition.
Figure 1, Section A shows the SEM of the 0MCP after 14 days in PBS. The surface of the sample looks generally uniform, and the insert at 3,000x shows that the surface is granular with no crystal development.
Section B shows the SEM of the 3MCP sample after 14 days in PBS. There are two distinct areas on the sample, the lighter grey areas are granular, which is shown by the upper 3,000x insert, while the darker areas have crystal formation which is shown in the lower 3,000x insert.
Section C shows the percentage of elements detected by EDS of the three 3,000X inserts. Although the 0MCP (Section A) sample and the 3MCP nucleation area (Section B, upper insert) are both granular surfaces, the EDS reveals that the calcium and phosphorus values are much higher in the 3MCP nucleation area. The crystal area (Section B, lower insert) of the 3MCP sample has even higher percentages of calcium and phosphorus than the nucleation area.
The silicon values are the highest in the 0MCP sample, slightly lower in the 3MCP nucleation area, and not detectable in the 3MCP crystal area. Silicon values do not contribute to mineralization, but may give insight into to the density in the crystals, as silicon is inherent in the underlying composite material.



Element	0% MCP Control	3% MCP Nucleation Area	3% MCP Crystal Area
Calcium	0.6%	6.7%	24.6%
Phosphorus	1.4%	7.0%	20.9%
Silicon	15.4%	13.0%	0.0%

*Figure 1: **A:** SEM of the 0MCP sample at 14 days at 10x, with insert at 3,000x magnification. **B:** SEM of the 3 MCP sample at 14 days at 10x with inserts at 3,000x magnification of the nucleation area (top) and crystal apatite crystal area (bottom). **C:** Element composition of each of the inserts described above*

Crystal Surface Area Coverage
The SEM images of the 3MCP samples show a clear increase in crystal surface area coverage over time. The surface area covered by crystals was estimated to be 33% after 7 days in PBS, 87% after 14 days and 95% after 28 days. Figure 3 shows the SEM images of the 3MCP samples over time. A program (SketchAndCalc, 2021, Palm Coast, FL.) was used to estimate the surface area of the crystalized areas. These images are at low magnification to show the entire sample surface, although the crystals may not be obvious in these images, the existence of crystals was confirmed with higher magnification images.

7 DAYS	14 DAYS	28 DAYS
Crystal Surface Area = ~33%	Crystal Surface Area = ~87%	Crystal Surface Area = ~95%

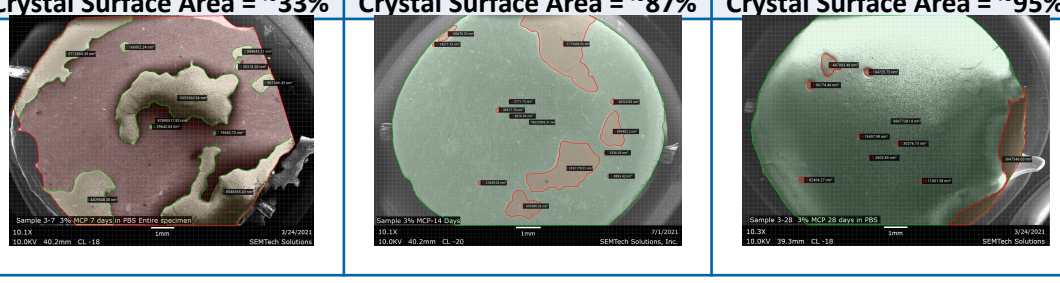


Figure 2: The areas highlighted in green indicate the areas with fully formed crystals, while the areas in red indicate nucleation areas, where there is increased calcium and phosphate content, but crystals are not yet visible.

Conclusions

The EDS shows that the 0MCP sample had minimal calcium and phosphorus, elements essential for mineralization. The nucleation area of the 3MCP sample had over five times the amount of calcium and phosphorus compared to the control, which indicates that there is an increase in potential for mineralization.
The crystal area of the 3MCP sample shows about three times the amount of calcium and phosphorus when compared to the nucleation area in the same sample, these values, along with SEM images confirm the surface has been mineralized. Additionally, silicon was not detectable in this area, which suggests that the crystals are dense, and completely cover the underlying silica.
The increase in crystal surface area coverage over time demonstrates that the crystal growth is an ongoing process, and the samples need to be monitored over time to get a true picture of what is happening to the surface. It shows that the increase in calcium and phosphorus in the granular areas of the 3MCP samples is a true nucleation area, and over time the entire sample will eventually be covered with crystals.
The results of this study confirm that this procedure is suitable to determine the in-vitro mineralization potential of dental composites.