Original Article

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Physical Properties of A New Hydroxyapatite-Based Endodontic Sealer Containing Silicon and Strontium Ions

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ABSTRACT

BACKGROUND AND OBJECTIVE: Producing a new sealer using local technology helps to produce dental materials with ideal properties in the country and at a lower cost. The present study was performed to investigate the physical properties of a calcium silicate- and hydroxyapatite-based endodontic sealer in three different mass compositions.

METHODS: In this laboratory study, a new sealer was made in Mashhad Dentistry School. Three different weight ratios in the experimental groups S_1= Cs Si Sr, S_2= Cs Si_2 Sr and S_3= Cs Si Sr_2 were evaluated in terms of setting time, working time, film thickness, solubility, and flow. Experiments were performed according to ISO/FDIS6876:2012 standard. To determine the crystal structure of the sealer in three different formulas, the samples were examined by X-ray and Energy-dispersive X-ray Spectroscopy.

FINDINGS: The highest amount of flow was related to S_1 (17.08 mm) and the lowest amount was related to S_2 (16.2 mm). The lowest solubility was related to the formula S_1 (1.83%) and the highest was related to S_2 (3.6%). Experiments also showed that the amount of film thickness in the formula S_2 and S_1 have the highest and lowest levels, respectively. Setting time in the samples was 330 minutes in S_1, 428.33 minutes in S3 and 1030 minutes in S_2. S_1 had the longest working time, followed by S_3 and S_2, respectively.

CONCLUSION: Based on the results of this study, sealers S_1 and S_3 showed good results in accordance with the standards in setting time, working time, film thickness, solubility, and flow tests, but sealer S_2 showed results close to the standard.

KEY WORDS: *Sealer, Hydroxyapatite, Root Canal Treatment.*

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Introduction

So far, many sealers have been produced and considerable efforts have been made to investigate their biological and physical properties. Considering that endodontic treatments are part of routine treatments Iran and in these treatments, sealers play an essential role in sealing the root canal and preventing the penetration of microorganisms, the use of more ideal sealers allows us to have better quality treatments and more success (1-3). The use of local technology, in addition to the possibility of making dental materials with ideal properties, leads to better supply of these materials in the country and at a lower cost. The new generation of sealers are based on calcium silicate, which have very good mechanical and chemical properties, including good sealing (4), antibacterial activity (5), tissue compatibility (6) dentin bonding (7), differentiation of dental pulp stem cells and biomineralization (8).

Hydroxyapatite is the main building block of teeth and bones. This compound induces the formation of hard tissue in contact with the bone and periapex. One of its disadvantages is its fragile structure. To eliminate this defect, hydroxyapatite is combined with various elements such as silicon and strontium, which improves its physical properties (9). Considering the properties of bioceramic sealers as well as the properties observed in strontium and silicon in previous studies, a hydroxyapatite-based sealer was produced in Mashhad University of Medical Sciences. After producing any type of dental material, several in vivo and in vitro studies should be performed. Evaluation of mechanical properties is an essential part of testing. These studies pave the way for clinical trials.

In this study, the purpose of laboratory experiments was to investigate the setting time, working time, film thickness, solubility and flow of a new sealer produced in Iran, which is based on hydroxyapatite materials containing silicon and strontium ions with three different weight ratios and the results were compared with ISO/FDIS6876:2012 standard. Based on the results of previous studies on the effects of strontium and silicon in medicine, as well as similar studies in dentistry, we decided to produce a new hydroxyapatite-based sealer that contains strontium and silicon ions, and tried to adopt a new design and formulation to eliminate the defects of other existing sealers while introducing a chemical composition different from the sealers available in the market. The aim of the three formulas adopted in this study was to achieve the best composition and the most ideal properties. For this reason, the materials were combined in different ratios to create the three main formulas for this study.

Methods

This laboratory study was approved by the ethics committee of Mashhad University of Medical Sciences with the ethics code IR.MUMS.DENTISTRY.REC.1397.104 and aimed to investigate the physical properties of a new domestically produced ceramic sealer. Tests related to working time, setting time, film thickness, solubility, and flow were performed based on ISO/FDIS6876:2012 standard tests.

Sealer production procedure:

Production of silicon-hydroxyapatite: Synthesis of silicon-hydroxyapatite was performed by sol-gel process in aqueous/alcoholic medium, assuming the substitution of silicate ions with phosphate. For this purpose, 0.02 mol of tetraethyl orthosilicate in 100 cc of aqueous/ethanol solution was placed on a magnetic stirrer until hydrolysis process is done completely. Then 0.28 mol of sodium dihydrogen phosphate was dissolved in 100 cc of deionized distilled water and added to a container containing tetraethyl orthosilicate. The pH of the solution was adjusted to 10 using normal sodium hydroxide. As a source of calcium ions, 0.5 mol of calcium chloride in 200 cc of water was used. Finally, the following molar ratio was established:

$$[Ca^{+2}]/ [P+Si]= 1.67$$

Calcium chloride solution was gradually added to the solution containing phosphate and silica. The resulting material was placed in a stirrer at 80 °C for 12 hours. After 12 hours, the liquid phase was separated and the resulting solid was dried at ambient temperature. In order to remove residual nitrate and complete the crystallization reaction, the resulting powder was exposed to 800 °C for 2 hours. After heat treatment, the resulting ceramic mass was ground by a mortar. After drying, the resulting powder was sieved with a sieve with 37-micron pores.

Preparation of strontium-hydroxyapatite: Synthesis of strontium-hydroxyapatite was also performed by sol-gel process in aqueous medium, assuming the substitution of strontium ions with calcium. For this purpose, 0.05 mol of strontium chloride and 0.45 mol of calcium chloride were first dissolved in 200 cc of deionized distilled water. Then, 0.3 mol of sodium dihydrogen phosphate was dissolved in 200 cc of deionized distilled water. The pH of the solution was

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adjusted to 10 using 1 N sodium hydroxide. Finally, the following molar ratio is established:

$$[Ca^{+2}+Sr^{+2}]/[Pi]= 1.67$$

Then all the mentioned steps for making silicon-hydroxyapatite were repeated.

Production of tricalcium silicate: Synthesis of tricalcium silicate was performed by sol-gel process in aqueous/alcoholic medium. First, 0.5 mol TEOS was mixed in 200 cc of water and nitric acid (as a catalyst) to complete the hydrolysis process. Then 1.5 mol of water-soluble calcium nitrate was added to it and stirred at 80 °C to become a gel. The resulting gel was dried in an oven at 120 °C and the resulting white powder was placed at 1200 °C for 10 hours. Then sieving the ceramic powder was done as mentioned above. All three powders were examined by X-ray and Energy-dispersive X-ray Spectroscopy to evaluate the crystal structure.

Composition of sealers: After successful production of C_3S, Si-HA and Sr-HA, it was necessary to achieve the desired percentage of these materials for sealer composition. By testing the different mass compositions of these materials and using different liquid compositions, it was found that the three mass compositions S_1= Cs Si Sr, S_2= Cs Si_2 Sr and S_3= Cs Si Sr_2 in combination with the solution of monosodium phosphate as sealer fluid, offers acceptable properties.

Flow calculation: 0.05±0.005 ml sealer with a graduated syringe was poured in the center of one of the glass plates and 180±5 seconds after the start of mixing, the second glass plate was placed centrally above the sealer. An additional weight was placed on top of that glass plate to reach a total mass of 120±2 grams. Ten minutes after mixing, the weight was removed and the maximum and minimum diameters of the compressed circular sealer were measured. Three measurements were performed and its mean was recorded as flow.

Calculation of working time: To determine the working time of seals, the flow test process was used, except that the sealer was evaluated in a longer time; in fact, until the sample diameter was 10% less than the sample diameter in 180 seconds, this evaluation continued. Three time points were determined and its average was calculated and recorded as working time.

Calculation of setting time: Every 5 minutes, the setting of the sealer was checked by placing the tip of a syringe needle on its surface. When it was time of setting, the Gilmore index was carefully lowered vertically on the horizontal surface of the sealer. If serration or jaggedness was observed, the needle was raised and the head was cleaned and placed in a new position on the sealer surface. This action was repeated so that the serration or jaggedness were no longer visible. The time of completion of mixing was recorded. Three time points were determined and its mean was recorded as setting time.

Calculation of film thickness: A part of the sealer was placed in the center of one of the glass plates and the other glass plate was placed centrally on the sealer. After 180±10 seconds from the start of mixing, a force of 150 N was applied vertically to the upper glass. After 10 minutes from the start of mixing, the thickness of two glass plates and a thin layer of sealer between them were measured by micrometer. The thickness of the sealer thin layer was calculated based on the difference between the thickness of the two glass plates with and without sealer and was recorded as film thickness.

Solubility calculation: The sample was placed in a shallow container A. 50±1 ml of water was added and placed in the chamber for 24 hours. An oil filter was placed in the funnel and the funnel was placed in container B and water along with the samples was poured into the oil filter. Container B was placed with the collected water in an oven at 110±2 °C and its water was evaporated to obtain a constant mass. The difference between the main mass of the shallow container and its final mass was recorded with an error of 0.001 g as the amount of sealer removed from the samples. Mean size was recorded as solubility. According to ISO/FDIS6876:2012, all experiments were repeated three times and the results were expressed using the mean and standard deviation.

Results

Findings of this study include 6 parts in the sections of XRD analysis and EDS analysis to determine and compare film thickness, solubility, setting time, working time and flow.

Result of X-ray diffraction (XRD): XRD pattern analysis confirmed the formation of apatite structure in the presence of silicon and strontium ions and the substitution of strontium and silicon ions in the hydroxyapatite structure. The XRD pattern for silicate composition also showed that the reaction product was a mixture of dicalcium silicate and tricalcium silicate (Figure 1).

EDS Analysis: Figure 2 shows the SEM image of the surface of calcium silicate particles. As can be seen, the size of calcium silicate crystals is a maximum of 1

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micrometer and has a structure close to hexagonal. EDS analysis indicates the presence of calcium, silicon and oxygen. Figure 3 shows the SEM image of Si-HA ceramics, showing hexagonal crystals similar to apatite crystals. EDS analysis shows the presence of silicon ions in the synthesized ceramic structure. Figure 4 shows the SEM image of Sr-HA ceramic, which shows hexagonal crystals similar to apatite crystals. EDS analysis shows that Sr ions have replaced calcium ions. The highest amount of flow is related to the formula S_1 with a value of 17.08 mm and the lowest amount is related to S_2 with a value of 16.2 mm. The lowest solubility is related to formula S_1 with a value of 1.83% and the highest amount is related to S_2 with a value of 3.6%. Experiments showed that the amount of film thickness in the formula S_2 and S_1 with the values of 67.33 µm and 34.66 µm, respectively, have their highest and lowest levels. Regarding the setting time, the test results showed that the setting time in the samples is 330 minutes in S_1, 428.33 minutes in S_3 and 1030 minutes in S_2. The results showed that S_1 gives us the maximum working time, which is equal to 33.33, followed by S_3 and S_2 with the working times of 27.33 and 17.33, respectively (Table 1).

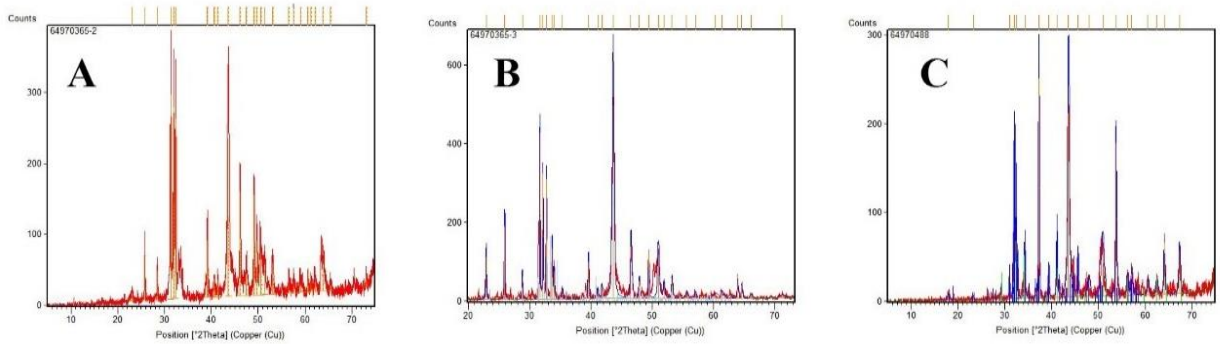

Figure 1. XRD pattern of silicon hydroxyapatite (A), strontium hydroxyapatite (B) and tricalcium silicate (C)

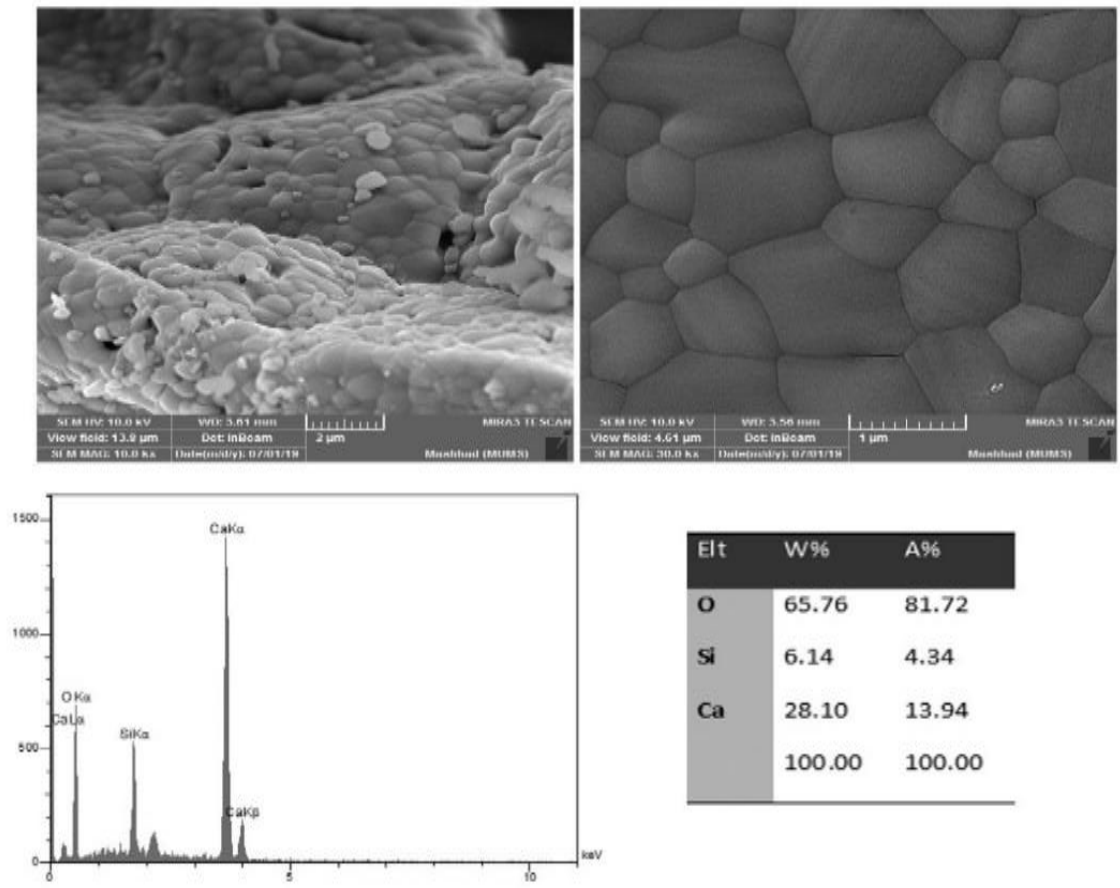

Figure 2. SEM image and EDS analysis of tricalcium silicate/dicalcium silicate particles

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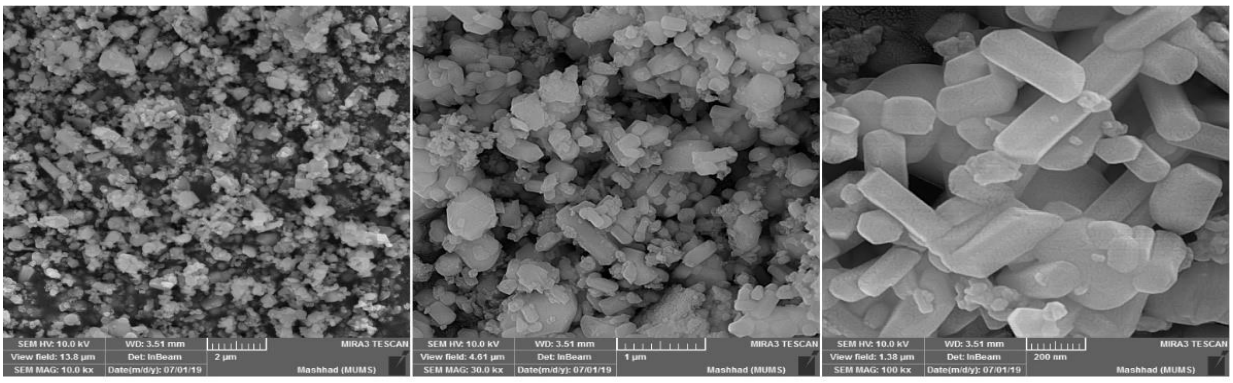
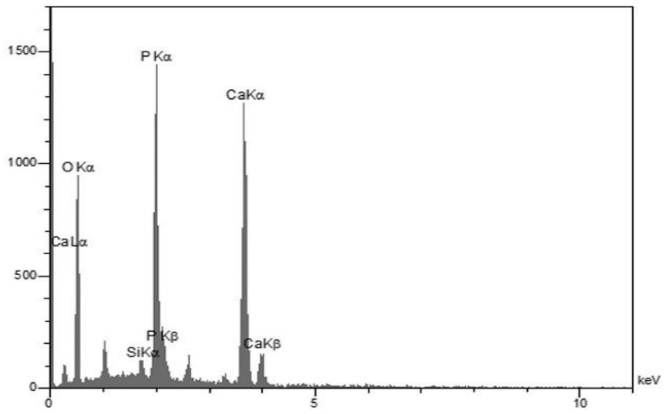
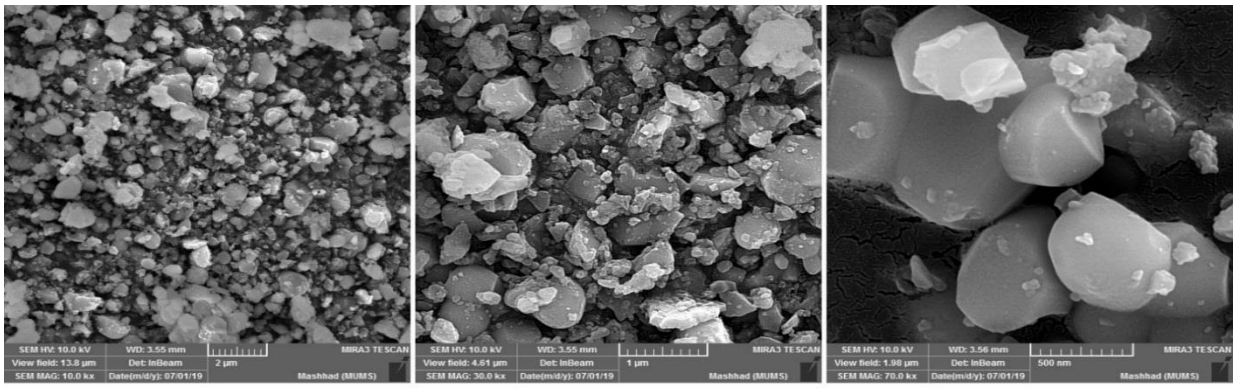
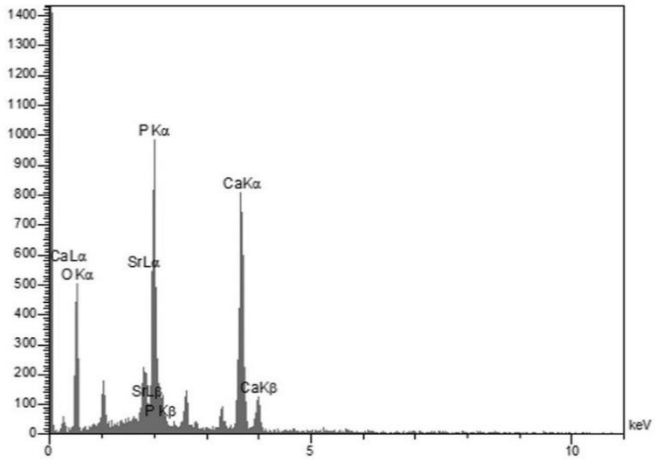




Elt	W%	A%
O	64.53	80.14
Si	1.04	0.73
P	14.10	9.05
Ca	20.33	10.08
	100.00	100.00

Figure 3. SEM image and EDS analysis of silicon hydroxyapatite particles





Elt	W%	A%
O	58.54	77.37
P	15.57	10.63
Ca	20.13	10.62
Sr	5.76	1.39
	100.00	100.00

Figure 4. SEM image and EDS analysis of strontium hydroxyapatite particles

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Table 1. Values of mean and standard deviation of flow, solubility, film thickness, setting time and working time for 3 hydroxyapatite sealer formulas

Sealer	Flow Mean±SD	Solubility Mean±SD	Film Thickness Mean±SD	Setting Time Mean±SD	Working Time Mean±SD
S_1	17.080±0.227	1.883±0.152	34.666±0.577	330.00±2.645	33.333±1.154
S_2	16.203±0.058	3.666±0.115	67.333±0.577	1030.00±10.000	17.333±0.577
S_3	17.033±0.072	2.933±0.152	46.333±0.577	428.333±57.951	27.333±0.577

Discussion

The results showed that sealer S1 with equal ratios of Si-HA and Sr-HA with the formula C_3S (50%)/ Sr-HA (25%)/ Si-HA (25%) and with equal ratios of Sr and Si showed better results compared to other formulas. These results also indicate that this sealer has results in accordance with ISO/FDIS6876:2012. In the study by Xing et al., which examined the effects of Sr and Si on the proliferation of human bone marrow stem cells (hBMSCs), the results showed that the effects of Sr and Si not only cause osteogenic differentiation in cells but also lead to angiogenesis (10).

Furthermore, the study of Mao et al. on the effects of Sr and Si in bioceramics on bone regeneration showed that these ions affect osteogenesis, osteoclastogenesis and angiogenesis as well as osteoporotic bone regeneration (11). The results of the study by Gao et al. showed the effect of these substances on the growth and adhesion of osteoblasts (12). The study by Rodríguez-Valencia et al. showed similar results regarding the function of Si and Sr ions in stimulating osteoblasts (13). These studies all emphasize the effect of Si and Sr on the stimulation and increase of osteoblast activity.

If Sr is adjacent to the hard tooth tissue, it replaces the structures of hydroxyapatite and forms strontium apatite, which forms structures with high acid resistance (14, 15). To prevent the cytotoxic effects of older sealers, silicon-based sealers have been introduced to the market with better sealing and adaptability (16). In addition, studies have shown that silicon reduces the number of osteoclast cells and increases vascularization. Studies by Han et al. showed that using sufficient amounts of silicon with PBS (Phosphate buffered saline) in the vicinity of the tooth leads to the formation of silicon-rich apatite crystals inside the tooth (17, 18).

One of the first steps in the application of new materials in dentistry is to study the physical and chemical properties of these materials. The experiments performed in this study are in accordance with the ISO/FDIS6876:2012 standard, which allows the obtained results to be compared with the results obtained from other sealers in similar studies. According to these results, the highest amount of flow is related to the formula S1 with a value of 17.08 mm and the lowest amount is related to S2 with a value of 16.2 mm, which is an acceptable value when compared with the ISO/FDIS6876:2012 standard which states that the diameter of each disc after the flow test should be 17 mm or more. Regarding the solubility test, the lowest value is related to formula S1 with a value of 1.83% and the highest value is related to S2 with a value of 3.6%. In the values reported in ISO/FDIS6876:2012, the solubility standard is less than the 3% of stated mass, and when compared with this, the results obtained from S1 are considered excellent. The standard values of ISO/FDIS6876:2012 state that the film thickness of sealers must be 50 µm or less. According to the results of the present study, the amount of film thickness in formula S_1 and S_2 was 34.66 µm and 67.33 µm, respectively. Compared to the standard values, S1 has shown considerable results.

The results of the setting time in the present study showed the values of 330 minutes in S_1, 428.33 minutes in S_3 and 1030 minutes in S_2. In ISO/FDIS6876:2012 standard values, the setting time must be exactly in the range of 30 minutes and less than 72 hours. As it turns out, the values obtained are at this time range. The results showed that S_1 has the highest working time, which is equal to 33.33 minutes, followed by S_3 and S_2 with times of 27.33 and 17.33 minutes, respectively, which are in the standard values specified in ISO/FDIS6876:2012.

Lee et al. investigated the physical properties of flow, final setting time, radiopacity, and dimensional stability in 3 bioceramic sealers (EndoSequence BC, EndoSeal MTA and MTA Fillapex) and 3 epoxy resin-based sealers (AHplus, AD Seal and Radic-Sealer). The results showed that the values obtained from the flow test regarding the formula S_1 with a value of 17.08 mm and S_3 with a value of 17.033 mm in the present study are considered acceptable values in comparison with the ISO/FDIS6876:2012 standard, but compared to the values obtained in the study by Lee et al. on conventional sealers, they have lower flow (24).

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Moreover, in the present study, the results showed that the obtained setting time falls within the range of conventional sealers (24).

Reasonable manufacturing cost, possibility of manufacturing the material inside the country, formulation and chemical composition different from other common endodontic materials are the positive points of the new cement. Among the advantages of this study is the evaluation of 5 series of physical properties of the new sealer together. In addition, all tests in this study were performed in accordance with ISO/FDIS6876:2012 guidelines, which makes all results comparable to international standards. It is recommended that additional studies be performed to evaluate cytotoxicity and genotoxicity, biocompatibility and other physical and chemical tests including push out test and test of dimensional changes, and if approved, clinical studies must be performed in this area. It seems that the new sealer based on hydroxyapatite with equal ratios of Si-HA and Sr-HA and formula S_1= C_3S (50%)/ Sr-HA (25%)/ Si-HA (25%) is in accordance with ISO/FDIS6876:2012 standards.

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