Evaluating the impact of concentration on anti-fungal property of CEM cement

Evaluación del impacto de la concentración en las propiedades antifúngicas del cemento CEM

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Abstract

Anti-fungal property is regarded as one of the appropriate proprieties of retrograde filing materials. It has been found that the anti-fungal property of MTA is influenced by its concentration. The objective of current research was to evaluate the impact of concentration on anti-fungal property of CEM cement .The anti-fungal properties of CEM cement was evaluated using Tube susceptibility test in the p/l ratios of 1.135, 2. 270, and 3.400 at the time intervals of 0, 1, 24, 48, 72, 168 hours. Data were analyzed using chi-square and fisher exact tests. The growth of Candida albicans was seen in all tubes in all three experimental groups at time intervals of 0 and 1 hour; while the Candida albicans showed no growth in any of the experimental groups at other time intervals. Different p/l ratios in CEM cement are able to prevent the growth of Candida albicans, except in the early hours, and this property is not influenced by p/l ratio.

Keywords: Anti-fungal property, CEM cement, concentration, tube susceptibility test.

Resumen

La propiedad antifúngica se considera una de las propiedades apropiadas de los materiales de presentación retrógrada. Se ha encontrado que la propiedad antifúngica de MTA está influenciada por su concentración. El objetivo de la investigación actual fue evaluar el impacto de la concentración en las propiedades antifúngicas del cemento CEM. Las propiedades antifúngicas del cemento CEM se evaluaron utilizando la prueba de susceptibilidad del tubo en las relaciones p / l de 1.135, 2. 270 y 3.400 en los intervalos de tiempo de 0, 1, 24, 48, 72, 168 horas. Los datos fueron analizados utilizando chi-cuadrado y pruebas exactas de los pescadores. El crecimiento de Candida albicans se observó en todos los tubos en los tres grupos experimentales a intervalos de tiempo de 0 y 1 hora; mientras que Candida albicans no mostró crecimiento en ninguno de los grupos experimentales en otros intervalos de tiempo. Diferentes relaciones p / l en el cemento CEM son capaces de prevenir el crecimiento de Candida albicans, excepto en las primeras horas, y esta propiedad no está influenciada por la proporción p / l.

Palabras clave: Propiedad antifúngica, cemento CEM, concentración, prueba de susceptibilidad del tubo.

Introduction

The microorganisms' role as the main factor in the development of pulpal and periapical diseases and endodontic treatment failure has been proved(1). However, it seems that fungi are not involved in development of early endodontic infections, while their involvement in secondary infections has been well proven(2). Colonization of fungal species in secondary endodontic infections can be due to reaction among the bacteria or reduced certain species of bacteria. These species of fungi may have penetrated into the canal space during or after the endodontic treatment, because of restoration with microleakage(3). Among different fungi species, candida albicans is the most commonly of species found in the endodontic infections(2, 3). In the cases in which endodontic re-treatment fails or re-treatment is not feasible, periapical surgery will be the treatment of choice(4). The appropriate retrograde filing material should have properties such as dimensional stability, resistance against dissolution, and high tissue adaptability(5, 6). In addition, for success of periapical surgery, it is necessary that these materials have high sealing ability and prevent re-contamination of canal and periapicalarea(7, 8). As most of retrograde filing materials do not have hermetic sealing ability, their antibacterial and anti-fungal properties are very important(7, 9, 10). Antibacterial and anti-fungal impact of MTA and CEM cement as two commonly used retrograde filing materials have been proven(11-13, 21). In addition, it has recently been indicated that the anti-fungal property of MTA has direct relationship with its concentration(14). Some research suggests that CEM cement properties are affected by its concentration(15, 20), but as there is little information on the impact of concentration on the anti-fungal properties of CEM cement, the aim of the current research was to evaluate the anti-fungal effect of different concentrations of CEM Cement.

Materials and methods

Fresh colonies of Candida albicans were prepared by culturing Candida albicans (ATCC 10231) on Sabouraud Dextrose Agar plate (Oxoid, UK), incubated at 30 ° C for 2 days. In order to quick access to the fungus, 1×10^6 suspension of cell was prepared and kept at refrigerator by its dissolving in10cc sterile physiologic serum and creating alternate dilutions.

Forty and five sterile test tubes containing 4.5 ml of sterile Sabouraud medium (Oxid, UK) were prepared and divided into three experimental groups (n=10) and three groups (n=5) for positive control, negative control, and Gold standard groups. CEM Cement was prepared with concentrations of 3.40, 1.135, and 2.270 and were added to the test tubes in three experimental groups, respectively. As CEM Cement manufacturer factory has not recommended certain powder to liquid weight ratio(P/L) in instruction of this cement, The conventional consolidation used in the clinic, was selected as normal consolidation in this study. By obtaining the exact weight of the modules, the P/L weight ratio was calculated 2.27 in this consolidation. Two other ratios as ratios related to diluter and thicker consolidation were selected. 1.5 ml of Candida albicans suspension was added into three experimental and gold standard groups. Then, we placed them on a rotary shaker (Labtron-iran) at 100 rpm. The positive control group was without CEM Cement and negative control group was without CEM Cement and Candida albicans. In the gold standard, nystatin was used instead of CEM Cement. In the next step, all groups were incubated for 24 hours at 37 ° C and 100% humidity. blur in tubes was evaluated as a candida growth index at time intervals of 0, 1, 24, 48, 72, 168 hours. Moreover, 0.1 ml of sample of each tube was cultured on Sabouraud dextrose agar plates to confirm the growth of candida albicans. Finally, data were analyzed using Spss 17 software and chi- square and Fisher exact tests.

Results: Negative control group and gold standard group did not show Candida albicans growth at any time, while Candida albicans growth was evident at all times in the positive control group. Findings of examining the experiment groups are shown in Table 1.

Table 1. effect of CEM cement in different concentration on Candida albicans at the different time periods

Concentration time	1.13	2.27	3.40
0	+	+	+
1	+	+	+
24	_	_	_
48	_	_	_
72	_	_	_
168	_	_	_

p-value: 1.000

As seen in table, the candida growth was seen in all tubes in all three experimental groups at time intervals of 0 and 1 hour. However, the growth of candida was not seen in any of tubes in three experimental groups at other time intervals. As no difference was no seen among the experimental groups, conducting the statistical test to compare the data is not necessary.

Discussion

After theEndodontic treatment, change in intracanal oxygen pressure and the interaction among the bacteria can lead to the colonization of species of the intracanal fungus. Fungi are considered as one of the most important factors of failure in endodontic treatment. Low nutritional requirements and the ability to form biofilm are the reasons of fungi survival in the canal(2, 3). retrograde filing materials should have anti-fungal properties(9, 10). Agar diffusion test is the most common method to measure the antifungal properties of retrograde filing materials, but as CEM Cement has a dissolving property, using this method seems not to be reasonable in this research(16-18, 22). As a result, tube susceptibility test allowing the direct contact between Candida albicans and the examined material was used in this study to evaluate the anti-fungal property of CEM Cement.

Findings of our research revealed that CEM Cement in all three P/L ratios at time intervals of 0 and 1 hour is not able to prevent the growth of Candida albicans. However, it prevents the growth of Candida albicans in other time intervals. Kangarlou et al also showed that freshly mixed and completely set CEM cement in two different concentrations at time intervals of 0 and 1 had no antifungal activity, and fungi are lost fully(9).

Appropriate antifungal property of CEM Cement can be attributed to the presence of calcium hydroxide. The calcium hydroxide contained in CEM Cement in contact with the medium increases pH with decomposition to Ca^{++} and OH^{-}. Alkalinity greater than 9 can lead to preventing the cell activity by inhibiting cell membrane enzymes(2, 19). However, Candida albicans are resistant to calcium hydroxide, experiments have shown that calcium hydroxide saturation solutions can inhibit its growth. It has been indicated that candida albicans incubation with calcium hydroxide results in Candida death only after 6 hours(9). This information justifies findings of current research on the growth of Candida albicans in time intervals of 0 and 1 hour. Kangarlou et al showed that the anti-fungal property of CEM Cement is not influenced by the P/L ratio(9), which these findings are in line with findings of current research.

Conclusions

According to findings of this research and given limitations of in vitro studies, different ratios of P/L of CEM Cement have the ability to prevent Candida albicans growth, except in the early days, and this property is not influenced by the P/L ratio. The anti-fungal property of CEM Cement, beside other properties, such as appropriate price, transforms this material to an appropriate option as retrograde filing materials.

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