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APPLICATION OF GLASS IONOMER CEMENT (GIC) FOR REPAIRING DENTAL PULP BY MEASURING EXPRESSION OF DENTIN MATRIX PROTEIN-1

(APLIKASI SEMEN IONOMER KACA UNTUK PERBAIKAN PULPA GIGI DENGAN MENGUKUR EKSPRESI DENTIN MATRIKS PROTEIN-1)

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Abstract

Glass ionomer cement (GIC) has a potential to improve the pulp by inducing Dentin Matrix Protein-1 (DMP-1) release that can mineralize dentin. This study used three types of glass ionomer cements; Conventional GIC i.e. GC Fuji IX; resin-modified glass ionomer cement (RMGIC) i.e. GC Fuji II LC and Nano particle of RMGIC i.e. Ketac™ N100 3MEspe. The three materials were applied to the tooth pulp of Macaca nemestrina. Expression of DMP-1 extract-dental pulp of the subjects was investigated by using ELISA. This study was statistically analyzed by using Mann-Whitney test. The result showed that GICs has a potential to induce the expression of DMP-1 and there was no significant differences among the three groups of GIC to induce DMP-1($p \geq 0,05$). In conclucion, all tested materials have a potential in dental pulp repair by expressing DMP-1.

Key words: glass ionomer cement, dental pulp, dentin matrix protein-1

Abstrak

Semen ionomer kaca (SIK) mempunyai potensi untuk memperbaiki pulpa dengan menginduksi pelepasan Dentin Matrix Protein-1 (DMP-!) untuk mineralisasi dentin. Tujuan penelitian ini adalah untuk menguji tiga jenis SIK yaitu SIK konvensional (Fuji IX- GC), SIK modifikasi resin (SIKMR- Fuji II LC-GC) dan SIKMR nano (SIKMRn-Ketac™ N100-3M Espe). Ketiga material tersebut diaplikasikan pada pulpa gigi hewan coba (Macaca). Ekspresi DMP-1 ekstrak pulpa gigi Macaca dianalisis dengan metode ELISA. Uji statistik menggunakan Mann-Whitney. Hasil penelitian ketiga jenis SIK mempunyai potensi menginduksi ekspresi DMP-1 dan tidak ada perbedaan yang bermakna di antara ketiga kelompok ($p \geq 0,05$). Sebagai kesimpulan, semua bahan percobaan memiliki kemampuan dalam merangsang perbaikan pulpa dengan penanda DMP-1.

Kata kunci: semen ionomer kaca, pulpa gigi, dentin matrix protein-1

INTRODUCTION

Dentin mineralization requires a transcription mechanism that will be used for the development of odontoblast phenotype. Some protein had been known to be involved in dentin mineralization, and one of them is dentin matrix protein-1 (DMP-1). Dentin matrix protein-1 is produced by odontoblast which is undergoing its last phase of differentiation, and its functions as modulator in dentin mineralization. It is a noncollagenous protein and specific gene derived from extracellular matrix of bones and dentin. Dentin matrix protein-1 is used to take part in regulating the bio-mineralization, and also in the initiation of hydroxyl apatite nucleation. The primary location of DMP-1 is in the nucleus, and Ca^{2+} ion is responsible for the process of releasing DMP-1 into the extracellular matrix during the odontoblast differentiation. It has a high level of acidity, therefore it may bind easily to calcium and initiate the nucleation process, which will be followed by the development of hydroxyl apatite crystals.[1,2]

Material that is commonly used in dentistry and now being developed is glass ionomer cement

(GIC), and their development is mainly affected by the unique properties such as the ability to bind chemically to the tooth structure, fast setting, no shrinkage and temperature rising, fluoride release, flexural strength equal to dentin, bacteriostatic, and biocompatibility. In addition to the aforementioned advantages of GIC, it also has some disadvantages, easily wears-off, low compressive strength, and long setting time. In order to overcome its clinical disadvantages, a new formulation of GIC is being developed gradually, aiming to improve the physical properties of GIC. Resin composite modified glass ionomer cement (RMGIC) is developed form of the conventional GIC, the difference lies on the setting process which involves a combination of chemical and acid-base reactions. In the mean time, in order to improve the esthetic factor and its physicomechanical properties, a new RMGIC is developed involving nano-sized particles, known as resin composite modified glass ionomer cement with nano particles (nRMGIC).[3,4]

These superior properties have made GIC to be used as pulp capping material, perforation coverage, and retrograde apex closure. This development was based on research conducted by Tarim et al. A research conducted in human tooth and Mausavinasab et al.[5] conducted the same type of research to monkeys and resulted in a conclusion that GIC could induce the formation of reparative dentin. Meanwhile Nourmohammadiet et al.[6] and PutriAT,[7] who soaked GIC in the simulated body fluid for 4 weeks, and it was concluded that GIC has the ability to induce the hard tissue formation.

The presence of Si-OH,-COOH and SiO_2 molecules in GIC which is less accounted for less than 60% of its content, acting in the formation of heterogenous apatite nucleation.[6-9] Meanwhile, according to Kamikatahara et al.[17] GIC does not induce the formation of hard tissue due to the presence of polyacrylic acid in its content. The aim of study was to test the ability of GIC, RMGIC and nRMGIC in inducing the expression of DMP-1which served as a marker of the differentiation of odontoblast-like cells.

MATERIALS AND METHODS

A total of 27 carious-free teeth of 4 Macacas, aged 5-8 years old, weight 10-12 Kg with fully erupted dentition were used in this study. On each of the buccal surface of 24 teeth was made a 3 mm cavity, with depth reaching the pulp, using round diamond bur # 013. A high-speed handpiece and water coolant, as well as suction were used during the entire experiment. The diamond bur was replaced after being used for 4 cavities preparation, and all cavities were then washed with saline solution and chlorhexidine; to attempt of controlling the bleeding was continued with blotting the tooth with sterile cotton. The 24 teeth were then divided into 4 groups (containing 6 teeth each); the GIC group (Fuji IX from GC Corp), the RMGICgroup (Fuji II LC from GC Corp), the nRMGIC group (Ketac™ N100 from 3 MESPE) and the $Ca(OH)_2$ group (Dycal from Dentsply). Every treatment group was divided further into two subgroups (containing 3 teeth each), which represented the time variable, namely,GIC-1week, GIC-2week, RMGIC-1week, RMGIC-2 weeks, nRMGIC-1week, nRMGIC-2weeks, Ca $(OH)_2$-1 week, $Ca(OH)_2$-2weeks; and the remaining 3 teeth were used as control group. In accordance with the variable of tooth extraction time, pulp of each tooth was removed and immersed in 0,5 ml phosphate-buffer saline (PBS) solution and freezed in -80^0C. Each freezed pulp was thawed for making an extract and pulp powder in the tube was added with 0,5 mL PBS and was ready to be analyzed.

The analysis of DMP-1 was completed using the ELISA method and then determine each Optical density (OD) well using the microplate readerat 450 nm.

RESULTS

Determination of DMP-1 concentration was based on the value of Optical Density (OD) using the standard regression equation: OD DMP-1= 0,086 + 0,020 the concentration of DMP-1 with R2 was 98,3%

Table 1. Distribution DMP-1 concentrations between GIC, RMGIC, nRMGIC and control group

Treatment	n	Minimum	Maximum	Mean	SD
GIC-1W	3	22,059	53,030	33,748	16,824
GIC-2W	3	5,970	41,379	26,721	18,474
RMGIC-1W	3	12,952	20,408	17,438	3,953
RMGIC-2W	3	6,332	200,000	88,916	99,930
nRMGIC-1W	3	5,442	25,833	12,737	11,366
nRMGIC-2W	3	4,265	76,923	30,794	40,099
$Ca(OH)_2$-1W	3	26,230	135,714	79,815	54,779
$Ca(OH)_2$-2W	3	127,273	304,545	216,488	88,642
Control	3	14,731	37,121	22,592	12,597
Total	27	4,265	304,545	58,805	75,487

1W= one week, 2W= two week

Table 1 showed that in the GIC, RMGIC & nRMGIC groups, the highest mean value was observed in RMGIC-2W, mean while the mean value between the first and the second week of all samples in the RMGIC group showed a marked increase (5x), whereas in nRMGIC groups the increase did

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not exceed twice of its baseline, while the GIC groups showed a decrease. In comparison with Ca $(OH)_2$-2 was the standard material, the three GICs groups showed much lower DMP-1 concentration. However, compared with the control group, the concentration of DMP-1 was higher except for the RMGIC-1W and nRMGIC-1W groups.

Table 2 showed the highest mean differences between Ca(OH)2-2W and nRMGIC-1W, while lower mean differences between GIC-1W and nRMGIC-2W.

Table 2. Mean differences, standard error, p-value of DMP-1 concentration between GIC, RMGIC, nRMGIC and control group

Treatment	mean Δ	SE	p
GIC-1W			
GIC-2W	7,027	41,615	1,00
RMGIC-1W	16,310	41,615	0,10
RMGIC-2W	-55,169	41,615	0,70
nRMGIC-1W	21,010	41,615	0,20
nRMGIC-2W	2,954	41,615	0,70
$Ca(OH)_2$-1W	-46,067	41,615	0,20
$Ca(OH)_2$-2W	-182,741	41,615	0,10
Control	11,156	41,615	0,40
GIC-2W			
RMGIC-1W	9,283	41,615	0,70
RMGIC-2W	-62,196	41,615	0,40
nRMGIC-1W	13,983	41,615	0,40
nRMGIC-2W	-4,073	41,615	1,00
$Ca(OH)_2$-1W	-53,094	41,615	0,40
$Ca(OH)_2$-2W	-189,768	41,615	0,10
Control	4,129	41,615	1,00
RMGIC-1W			
RMGIC-2W	-71,478	41,615	0,70
nRMGIC-1W	4,701	41,615	0,70
nRMGIC-2W	-13,356	41,615	0,70
$Ca(OH)_2$-1W	-62,377	41,615	0,10
$Ca(OH)_2$-2W	-199,050	41,615	0,10
Control	-5,154	41,615	1,00
RMGIC-2W			
nRMGIC-1W	76,179	41,615	0,40
nRMGIC-2W	58,122	41,615	0,70
$Ca(OH)_2$-1W	9,102	41,615	1,00
$Ca(OH)_2$-2W	-127,572	41,615	0,20
Control	66,324	41,615	0,70
nRMGIC-1W			
nRMGIC-2W	-18,057	41,615	1,00
$Ca(OH)_2$-1W	-67,077	41,615	0,10
$Ca(OH)_2$-2W	-203,751	41,615	0,10
Control	-9,855	41,615	0,40
nRMGIC-2W			
$Ca(OH)_2$-1W	-49,021	41,615	0,20
$Ca(OH)_2$-2W	-185,694	41,615	0,10
Control	8,202	41,615	0,70
$Ca(OH)_2$-1W			
$Ca(OH)_2$-2W	-136,674	41,615	0,20
Control	57,223	41,615	0,20
$Ca(OH)_2$-2W			
Control	193,897	41,615	0,10

DISCUSSION

To analyze the odontoblast differentiation which is a part of the process of dentin matrix protein formation in the cell cycle, DMP-1 was chosen in this study. Dentin matrix protein-1is a macro molecule of extracellular matrix found in mineralized tissue,that undergoes specific cell differentiation into odontogenic strand.The component of extracellular matrix may increase the attachment and differentiation of various type cells, and it also has the ability to change the morphology of cell.Supposedly, dentin sialophosphoprotein (DSPP) is the single odontoblast specificgene that should be chosen for this study, however, due to the very low amount of its expression in odontoblast, it would be hard to detect the presence of this gene. DMP-1,which is located in the nucleus, may bind specifically to dentin sialophosphoprotein (DSPP) during the early differentiation of odontoblast. The interaction between the DMP-1 and DSPP will provide basic overview of how DMP1regulates the expression of DSPP. DSPP transcription is regulated by several positive and negative regulators of gene transcription with various signaling path. One of several negative factors that might regulate the activity of DSPP is transforming growth factor β1 (TGFβ1). Over expression of TGf β1 will decrease the expression of DSPP, it is found that the expression of DSPP is regulated by TGf β1. Whereas DMP-1 is acid protein, secreted by and related to extracellular matrix, will regulate DSPP positively.Several studies had shown that DMP-1 is located in the nucleus, during the early phase of odontoblast cytodifferentiation when is the form of gene that does not undergo the phosphorylation, it serves as the element that increases the transcription of tooth-specific DSPP gene. During the biomineralization, DMP-1 underwent phosphorylation and was transfered to the extracellular matrix, it showed that DMP-1 played a role in the nucleation process of hydroxyapatite crystal in the arrangement of dentin matrix.[1-2]

The selection of GIC as tested materials in this study was due to its biocompatibility, making it frequently used as capping material and covered a perforation. Therefore an analysis of its performance in regenerating hard tissue should be done. Due to the development of GICs in their compositions as well as their clinical applications, therefore in order to represent each of its types, Fuji IX GIC, Fuji II L Cand Ketac™ N100 were selected to represent the conventional, RMGIC and nRMGIC, respectively. Meanwhile Dycal was chosen to represent $Ca(OH)_2$ for comparison purposes. The selection of those commercially available GIC was due to their po-

pularity in dentistry and their frequent use in many clinical situations by dentists.The selection of 1 week and 2 week time variables in this study was in accordance with the statement of Mauth et al.[9] who mentioned that the molecular marker of pulp cells' differentiation process into odontoblastic strands might be able to be detected starting from day 4-14 of the experiment.

The selection of $Ca(OH)_2$ as the material used for comparison purposes was because $Ca(OH)_2$ is the standard material that has been used to induce the hard tissue formation in tooth, although the exact mechanism has not been fully understood. The disadvantage of $Ca(OH)_2$as hard tissue-inducing material lies in its instability that limits the length of time of this material to be placed safely in dental tissue.[10-11]

Initially, statistical analysis was completed by using the ANOVA, however after data was analyzed, normal data distribution was observed but it is lack of homogenity, therefore non-parametric statistical analysis was applied and the Mann-Whitney test was chosen .

In Table 1, the standard error of DMP-1 concentration between groups showed a great value, this might be due to the very small sample size in this study (n= 3), caused the variation of DMP-1 concentration to show much less difference between groups. Meanwhile the concentration of DMP-1 in $Ca(OH)_2$ -2W group showed the highest mean value among all other groups. This demonstrated that the ability of $Ca(OH)_2$in inducing the differentiation of pulp cells into odontoblastic strands was in its best on the second week.

Among the tested material groups, the highest mean value was observed in RMGIC-2W group; the mean value between the first and the second week of the RMGIC group demonstrated a marked elevation five times (5x); the elevation of the nRMGIC group was only two times bigger of its baseline, whereas in GIC group, a decrease would be observed. Of the three treatment groups, the greatest ability was possessed by RMGIC-2Wgroup, followed by nRMGIC and GIC group. When compared to Ca $(OH)_2$ -2W group as the standard material, the three GIC groups showed a much lower DMP-1 concentration, but when compared to control group, the value of DMP-1 concentrations was the highest in all tested groups, except for RMGIC-1W and nRMGIC-1W group. This study demonstrated that the release of ions in RMGIC and nRMGIC groups was considered low during the first week, and showed a slight increase in the following week. The highest mean value of DMP-1 concentration in GIC group on RMGIC-2W showed much lower than $Ca(OH)_2$-2W and the lower value on RMGIC-1W. The variation of concentration value was in accordance with statistical analysis demonstrating that there was no difference between GIC groups. The highest mean differences between on $Ca(OH)_2$ -2W and nRMGIC-1W, while lower mean differences between GIC-1W and nRMGIC-2W. This condition was caused by differences seen in the packaging of the three tested materialsthat would allow different amount of ions released by the materials. The expression of DMP-1 occured in the last phase of odontoblast differentiation, which process depended on the early differentiation phase. At the early differentiation process, there was great amount of differentiation inducing ions was released, then the amount of DMP-1 expressed would be greater. This showed that in the second week, RMGIC released the most amount of ions, followed by nRMGIC; while inGICgroup, ion release occured during the first week.

In conclusion, GIC, RMGIC, and nRMGCI were capable of increasing the expression of DMP-1 in Macaca pulp. The ability was not as great as Ca $(OH)_2$ but better than control group. RMGIC and nRMGIC have lower capability among the three tested materials compared to control group during the first week, however in the following week, the value increased greatly.

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