Propolis Microgel as Protective Agent for Odontoblast Cell in External Bleaching

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Abstract

To evaluate the effects of 10% propolis microgel (PM) on the MDPC-23 odontoblast cell line viability in external bleaching.

The cells were seeded in well plate with Dulbecco's Modified Eagle medium (DMEM) and incubated for 48 hours. Enamel dentin disc is placed in acrylic transwell above the MDPC-23 odontoblasts cell line and slightly contacted with the cells in well plate. 40% Hydrogen peroxide (H_2O_2) and 10% propolis microgel were applied over enamel-dentin disc during treatment. Six groups of cells (n=10) were treated as follows: P0: no treatment (control); P1: H_2O_2 /20 min; P2: H_2O_2 /20 min, flushed, incubated/30 min; P3: H_2O_2 /20 min, flushed, PM/30 min; P4: PM/30 min, flushed, H_2O_2 /20 min; and P5: PM/30 min. The cell viability was evaluated by MTT assay. The data obtained were analyzed by ANOVA test (α=0.05).

The percentages of cell viability were as follows: P0 (control)= 69.063%; P1=23.844%, P2=31.579 %, P3=50.964 %, P4=53.349 %, and P5=57.628 %. Group P3 and P4 presented a statistically higher cell viability than did group P1 and P2. PM decreased cell death percentage caused by H_2O_2, demonstrating its protective effect against the toxic components of this bleaching agent.

It was concluded that 10% propolis microgel could maintain the viability MDPC-23 odontoblastic cells, so it can be used to protect these cells against the cytotoxic effects of H_2O_2.

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Introduction

Bleaching is a dental procedure to brighten or remove stains on tooth surface chemically by using a strong peroxide solution. Bleaching is divided into internal bleaching which performed on nonvital teeth and external bleaching which performed on vital teeth.[1,2] One of the most widely used materials for external bleaching is hydrogen peroxide. Hydrogen peroxide (H_2O_2) is an unstable chemical with strong oxidative properties, which can be split into free radicals and other reactive oxygen species (ROS). This material has the ability to degrade organic molecules that play a role in tooth discoloration, and eliminate pigments that cause tooth discoloration.[3,4,5]

During the external bleaching procedure, a direct contact between hydrogen peroxide and teeth for a few minutes will release H_2O_2 and some reactive oxygen species (ROS) that can diffuse through enamel and dentin structures until they reached the pulp tissue. The contact between pulp cells and ROS causes oxidative stress that can damage cell membranes and leads to decreased cell viability, extracellular matrix degradation, cellular necrosis, and even pulp tissue necrosis.[5,6,7]

In order to protect the pulp cells from damage, studies on the effects of antioxidant protection on odontoblast cells have been conducted. Some previous studies suggested that the used of antioxidants before bleaching procedures, effective to protect the pulp cells from cell necrosis caused by free radical

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bleaching materials.[8,9,10] In addition, some other studies also report that the antioxidants activity plays a role in cell proliferation process to achieve homeostatic conditions.[10,11] One of the natural substance with antioxidant content which is currently used in dentistry is propolis. Propolis or bee's glue is a natural resin material produced by honey bees from various plant species.[12,13,14]

Propolis has been known to contain phenolic substances such as phenolic amino acids and flavonoid which act as antioxidants that are capable of against free radicals. Several studies indicated that propolis possessed biological and pharmacological activity such as antibacterial, anti-inflammatory and antioxidant.[12,13,14] Nevertheless, until now no study has been conducted on the antioxidant effect of propolis on odontoblast cell line. Based on this matter, this study aims to know the effect of application of 10% propolis microgel to viability of odontoblast cell line on external bleaching in vitro.

Material and Methods

Cell Culture. The cells used in this study were odontoblast-like cells MDPC-23. The cells were cultured and subcultured into acrilyc transwell with DMEM medium (Dulbecco's Modified Eagle medium) containing 10% foetal bovine serum (FBS; Gibco) at 37 °C and 5% CO_2 to obtain sufficient quantities of cells to be put into the medium.

Test Medium. The test medium were made using well-plate containing DMEM and cultured cell, Enamel-dentin disc with 3.5 mm thickness and 5.6 mm diameter, acrylic transwell with 8 mm pore size.

A total of 20 enamel-dentin discs from Premolar tooth were prepared with carborundum disc until the thickness of the enamel-dentin disc was 3.5 mm and the diameter of 5.6 mm measured by using a caliper. Then the enamel-dentin disc and trans-well acrylic were placed above the odontoblast cell line which has been embedded on 300 IL DMEM cultured medium. The enamel-dentin disc was placed so that enamel can accept application of 10% propolis microgel and 40% H2O2 gel while dentin can be in contact with odontoblast cell line.

Treatment. The sample was divided into 6 treatment groups.

1. Group P0. Well-plate containing MDPC-23 and enamel-dentin discs as controls
2. Group P1. Well-plate containing MDPC-23 and enamel-dentin disc then 40% H_2O_2 was applied above the enamel-dentin disc and incubated for 20 minutes, then the enamel-dentin disc was lifted and an MTT assay examination was performed .
3. Group P2. Well-plate containing MDPC-23 and enamel-dentin disc then 40% H_2O_2 was applied above the enamel-dentin disc and incubated for 20 minutes, then the enamel-dentin disc was lifted and cleaned. The cells were incubated again for 30 minutes in a CO_2 incubator at 37 °C, after which an MTT assay was performed.
4. Group P3. 40% H_2O_2 application above enamel-dentin disc and incubation for 20 minutes, then the enamel-dentin disc was lifted and cleaned. Further application of 10% Propolis Microgel and incubation for 30 minutes, then enamel-dentin disc was lifted and cleaned, and then the cell viability was examined with MTT assay.
5. Group P4. 10% Propolis Microgel application above enamel-dentin disc and incubation for 30 minutes, then cleaned, followed by 40% H_2O_2 application and incubation for 20 minutes. Next we checked the cell viability with MTT assay.
6. Group P5. 10% Propolis Microgel application above enamel-dentin disc and incubation for 30 minutes, then enamel-dentin disc was removed and the cell viability was checked with MTT assay.

Cells viability examination (MTT Assay)

Methyl Tertratholium (MTT) reagents of 100 µL for the treatment of 1 24 well-plate, by taking 1 ml of MTT diluted with 10 ml cultured medium. Then remove the PBS washer media, add 100 ml MTT reagent to each well including the media controls. Incubate the cells during treatment time in CO_2 incubator. After that, examine cell condition with a microscope. If formazan has been clearly formed, as a stooper add 100 ml SDS 10% in 0.1 N HCl. Then wrap the plate with paper or aluminum foil and incubate at room temperature

overnight. After that open the wrap and insert the plate into the ELISA reader. Furtherly, formazan absorbance will be read spectrophotometricly with ELISA reader. The more concentrated the color, the higher the absorbance value and the greater the number of cells. The living cells were calculated using the following formula:

$$\% \text{ living cell} = \frac{\text{Treatment Absorbance - media control Absorbance x 100\%}}{\text{Negative control absorbance - media control Absorbance}}$$

Results

From cells viability examination from each control and treatment group, the obtained results are as follows:

Table 1 shows the mean viability of cells in each treatment group. From the cell viability test, P0 group showed the highest mean value of 69.063%, P1 group had the lowest mean value of 23.844%, then P2 group with the mean value of 31,579%, P3 group with the mean value of 50.964%, P4 group with the mean value 53,349 %, and group P5 with mean value 57.628%.

Treatment Groups	n	Mean ± SD	Control (P0) (n=4)	*P Value*
P1	4	23.844 ± 1.466	69.063 ± 3.226	0.000
P2	4	31.579 ± 1.108	69.063 ± 3.226	0.000
P3	4	50.964 ± 2.768	69.063 ± 3.226	0.304
P4	4	53.349 ± 6.127	69.063 ± 3.226	0.505
P5	4	57.628 ± 9.262	69.063 ± 3.226	0.507

Table 1. Comparison of Cells Viability between in treatment groups and control groups.

Treatment Groups	n	Mean ± SD	*p value*
P1	4	23.844 ± 1.466	0.505
P2	4	31.579 ± 1.108	
P1	4	23.844 ± 1.466	0.000
P4	4	53.349 ± 6.127	
P2	4	31.579 ± 1.108	0.000
P3	4	50.964 ± 2.768	
P3	4	50.964 ± 2.768	0.505
P4	4	53.349 ± 6.127	

Table 2. Comparison of Cells Viability in P1 dan P2, P1 dan P4, P2 dan P3, P3 dan P4 group. Uji one way anova ($p<0.05$); Uji independent sample t-test ($p<0.05$)

The comparison between group to which H2O2 were only given for 20 minutes (P1) and group to which propolis microgel were applied before H_2O_2 (P4) applications can be seen in Table 2. The P4 group had a significantly higher percentage of cell viability compared to P1 (53,349) with p = 0.00 ($p <0.05$).

From the results of this study, there was a significant difference between treatment groups incubated for 30 min after H_2O_2 (P2) application with treatment group receiving Propolis Microgel application after H_2O_2 (P3) application as shown in table 2. P3 group was statistically higher of 50.964% compared with the P2 group of 31.579% with a p = 0.00.

Table 2 also shows the comparison between treatment group receiving Propolis microgel application prior to the H_2O_2 (P3) application and treatment group receiving the Propolis microgel application after H_2O_2 (P4) application. There were no significant differences found between the 2 groups. Where the average percentage of cell viability in the P4 group was 53.349 while in the P5 was 50.964 with p = 0.505. (Figure 1)

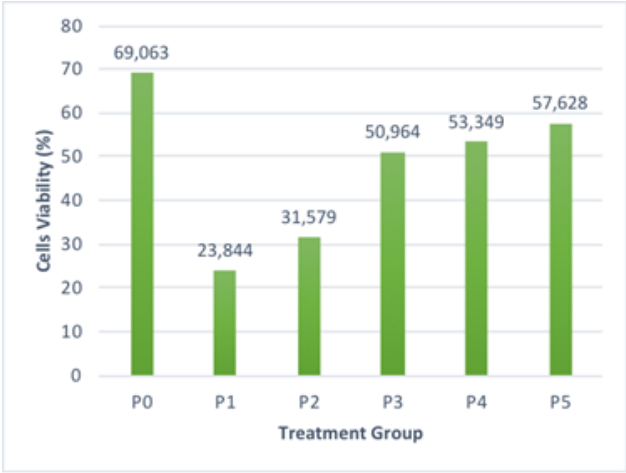

Figure 1. The Average Percentage of Cells Viability in each group.

Discussion

The odontoblast cells are part of dentin and pulp because cell body lies inside the pulp chamber and cytoplasmic processus cells extend into the dentinal tubules in mineralized dentin, thus these both tissues are included as living tissues which are capable of reacting to stimuli and pathological conditions.[15,16]

The odontoblast cell is the first cell to come into contact with a toxic component that can diffuse through dentin, this cell initiates an

inflammatory reaction in pulp tissue to protect the underlying pulp cells from further damage.15,16 One of dental material which has a toxic effect is hydrogen peroxide.[5,6,7]

Hydrogen peroxide is one type of ROS with highly reactive molecules and can cause defect to some cell components, such as plasma membranes, cell organelles, and cell DNA. An imbalance between endogenous cell antibodies and ROS can trigger oxydative stress that causes cell defect from reversible lesions to cell death. This reaction is triggered by an oxidation reaction, which lead to the excessive production and release of ROS.[5,6,7,17] Local and systemic oxidative changes can trigger inflammation and result the antioxidant system to be totally destroyed, and unable to address the ROS at the final stages of injury. The increased oxidants and decreased non-enzymatic antioxidant activity will cause lipid peroxidation in cell membranes and decreased cell energy. Cell membrane lipid peroxidation may cause changes in the liquidity and permeability of cell membranes, and increase the rate of proteins and nucleic acids degradation, leading eventually to lysis cells.[18,19] The 40% H_2O_2 toxic effect on odontoblast cell line is shown in this study. The P1 group had the lowest mean value compared with all groups with 23.844% of living cells (p = 0.000). These result is consistent with the study conducted by Lima et al (2011) who found the viability of odontoblast cell by 20.56% after 38% H2O2 gel application in vitro.[10] The toxic effect observed when 40% H2O2 was applied to cells (P1) could be attributed by the high activity of dissolved ROS in DMEM media along with odontoblast cell line.

Given the fact that the high toxicity of 40% H_2O_2 to odontoblast cell line, several studies on the antioxidant protective effect have been performed. The protective effect of microgel propolis can be seen in table 2, which shows that the percentage of living cells in group P4 is significantly higher than P1 which is 53.349% with p value = 0.00 (p <0.05). This is associated with the antioxidant properties of propolis microgel which are capable of against free radicals and protecting odontoblasts cells. Cell protection during bleaching is associated with ROS inactivation by antioxidant compounds contained in propolis microgel, converting highly reactive radicals into stable molecules.[9,10] Propolis contains various antioxidants such as flavonoids and phenolic acids that have many positive electrons. Propolis stops free radical activity by donating one electron to unpaired electrons in free radical group so that oxidation activity becomes more stable.[20,21] Some studies regarding on antioxidants as external pretreatment bleaching are in accordance with this study found that administration of antioxidant agents effectively protects odontoblasts cells from the toxic effects of bleaching materials.[5,6,7]

The results also showed comparison between treatment group that received propolis microgel application before H_2O_2 application (P4) and treatment group that received Propolis microgel application after 40% H_2O_2 (P3) application, as seen in table 2. There was no significant difference between the 2 groups . Where the mean percentage of cell viability in group P4 was 53.349 and P3 was 50.964 with p value = 0.505. However the P4 group had higher value. This indicates that administration of 10% propolis microgel either before or after application of 40% H_2O_2 has an important role to the viability of odontoblast cell line. Chemically, propolis structure is very complex and contains a variety of potent substances. Propolis is also rich in flavonoids, phenolic acids and caffeic acid phenyl esters (CAPE) that have strong antioxidant properties. The highly active substance in propolis is CAPE which has antioxidant and antiinflammatory activity. CAPE also contains as much as 50% of the total compounds contained in propolis. Nevertheless, some studies believed that there is no single dominant active substance in propolis, all substances work sinergically as a holistic product.[20,21,22,23]

The results showed that P3 group was significantly higher by 50.964% compared with P2 group by 31.579% (p = 0.000). This means that microgel propolis can stimulate the proliferation of odontoblast cell lines, in which the cell proliferation aims to achieve normal conditions. Under normal circumstances, cell proliferation leads to an increase cells number. Where cell numbers not only depend on cell proliferation but also on cell death.[24,25] The

application of aquades propolis extract can kill cancer cells and stimulate the proliferation of normal cells. In addition, the author reported that propolis contained substances that work in synergically and selectively.[26,27,28] Therefore, it can be concluded that 10% of propolis microgel has a good effect in achieving cellular homeostasis condition.

Conclusions

Based on the results, it can be concluded that aplication of 10% of Propolis Microgel could maintain viability of MDPC-23 odontoblast cells, so it can be used to protect these cell againts the cytotoxic effect of H_2O_2 in external bleaching

Conflict of Interest

The authors state that there were no conflicts of interest related to this study.

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