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"Strategic Achievement of Oral Sciences and Promotion of Quality of Life and Professional Education for Oral Hygienists by Using Information and Communication Technology"

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Iwan Dewanto
Department of Preventive and Community Dentistry, School of Dentistry, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia

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Mayty Rahma Fitria and Sartika Puspita
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Pipiet Okti Kusumaristi, Haryo Mustiko Dipoyono, M.Esti Tjahjanti
1School of Dentistry, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia
2Faculty of Dentistry Universitas Gadjah Mada Yogyakarta, Indonesia
to have a role in the marketing process of Asri Medical Center (AMC) and Rumah Sakit Gigi Mulut Pendidikan-RSGMP UMY (Educational Dental Hospital of Universitas Muhammadiyah Yogyakarta) because it has advantages in information dissemination. **Objective:** This study aims to determine the effectiveness of DSM as a means of marketing strategy of AMC in Yogyakarta. **Material and Method:** A descriptive study with case study design was carried out on samples of the member of DSM that was treated in AMC during the period of November 2009 to April 2010, aged 18 years and over. In assessing the effectiveness of DSM as a means of marketing strategy, a questionnaire that was based on expectation-reality of Parasuraman's Servequality was administered to the samples. Data was analyzed by comparing the results of the SWOT analysis (TOWS analysis) and Cartesian expectation-reality diagram. **Results:** TOWS analysis shows that threats and opportunities (external factor) are the complaints of patients that might result in contract termination and patient treatment delayed. The main strength of AMC is the strategic place and satisfactory facilities. The weakness is the tiered referral system. Results from Cartesian diagram shows that the role of physicians in dealing with the patient's recovery, a friendly attitude (assurance), service facilities (tangible), attention to the needs of the patient (empathy), the fast and accurate service and the skills of health personnel (reliability) are in conformity between expectations and reality. While the expectation to get immediate specialist care and the ability of doctors in delivering care which is not optimum are factors that not show conformity between expectation and reality. **Conclusion:** Dana Sehat Muhammadiyah (DSM) can be used as part of AMC's and RSGMP UMY's marketing strategy with some improvement of services as expected by the participants. As part of the Muhammadiyah Community, the participants of DSM is able to be the social connect by using the word of mouth as a marketing strategy, and this strategy can be chosen as a low-budget high impact model which has a mass impact in building brand awareness of AMC. **Keywords:** Marketing Strategy, Dana Sehat Muhammadiyah (Muhammadiyah Health Fund) Asri Medical center.

P28

Cytotoxicity of Sea Cucumber Extract to Human Gingival Fibroblast Stem Cell

Kristanti Parisihni, Syamsulina Revianti,

Department of Oral Biology, Faculty of Dentistry Hang Tuah University, Surabaya, Indonesia

**Objective:** Holothuria atra and Holothuria scabra are species of sea cucumber which has been known have antibacterial and antifungal properties thus potentially explored as therapeutic agent in oral infection. The aim of this study is to evaluate the cytotoxicity of two sea cucumber extract Holothuria atra and Holothuria scabra to human gingival fibroblast stem cell. **Material and Method:** Methanolic extract of Holothuria atra and Holothuria scabra in concentration of 1%, 0.5%, 0.25%, 0.125%, 0.006%, 0.003%, 0.0015%, 0.0007%; and 0.0003% were tested its cytotoxicity on human gingival fibroblast stem cell. Cell viability was measured by MTT assay. Data were analyzed by ANOVA and LSD. **Result:** Result showed significant toxicity of Holothuria atra Holothuria scabra only on the concentration of 1% (p<0.05) while on Holothuria scabra it showed toxicity in the concentration of 1% and 0.5% (p<0.05). **Conclusion:** Holothuria atra extract was not cytotoxic on human gingival fibroblast stem cell in the concentration below 1%, while Holothuria scabra extract was not cytotoxic on human gingival fibroblast stem cell in the
concentration below 0.5%.

Keywords: Holothuria atra, Holothuria scabra, cytotoxicity, fibroblast gingiva stem cell

P29
Antimicroba Effect of Jatropha curcas Latex Etanol Extract toward Streptococcus mutans
Maytv Rahma Fitria1 and Sartika Puspita2,
1Student of School of Dentistry, Faculty of Medicine and Health Sciences Universitas Muhamadiyah Yogyakarta, Indonesia,
2Lecturer, School of Dentistry, Faculty of Medicine and Health Sciences Universitas Muhamadiyah Yogyakarta,

Background: Back to use natural things is the society attention currently or known by back to nature which is believed to have benefit thing. Jatropha curcas latex utilization with active substances composition is able to release toothache, as the mouthwash for bleeding of gum, as hemostatic and treat the lesion. Objective: This research is aimed to know antimicroba effect of Jatropha curcas latex etanol extract toward Streptococcus mutans. Material and Method: Jatropha curcas latex which is extracted by maseration method is divided into four concentrations: 12, 5%, 25%, 50% and 100%. Negative control is aquabest used as comparison. Extract and negative control are dropped in each petri plate which consists of Streptococcus mutans bacteria colony as much as 50 µl. Radical zone measurements after 24 hours using sliding caliper with scale 0, 05 mm. Data analyze uses One-Way Anova and LSD0.05. Results: The result of One-Way Anova test shows that there is a significant effect of Jatropha curcas latex etanol extract toward formation of Streptococcus mutans bacteria radical zone (p<0.005). The result of LSD0.05 test shows that concentration of 100% shows the highest inhabitation towards Streptococcus mutans bacteria growth.

Keyword: Jatropha curcas latex etanol extract, antimicroba effect, Streptococcus mutans

P30
Alginate Impression vs Alginate Impression plus Cassava Starch (Microscopic analysis)
Mimna Febriani,
Dental Material Department Staff, Faculty of Dentistry, Universitas Prof.DR.Moestopo(8), Jakarta, Indonesia

Objectives: Alginate impression material is a material to make impression of teeth and oral cavity. The result will be reproduced by gypsum type III. Alginate impression material can be modified with cassava starch with ratio 1:1. The major composition is algin or alginate acid and cassava starch composition are amyllose 25 % and amylopectine 75 %. The aim of this study was to compare microscopic analysis of alginate impression without cassava starch and alginate impression with cassava starch. Material and Method: Alginate impression type normal setting, cassava starch, aquadestilata, light microscope and polarization microscope. Result: Microscopic structure alginate impression with light microscope showed that birefringence structure less than birefringence structure of alginate impression with cassava starch. When the structure is changed, the crystallity of cassava starch will be changed. Conclusions: The added cassava starch to alginate impression microscopically will not form a new chemical compound, will not change the molecule structure of alginate impression, without any chemical reaction between alginate
CYTOTOXICITY OF SEA CUCUMBER EXTRACT TO HUMAN GINGIVAL FIBROBLAST STEM CELL

Kristanti Parisihni, Syamsulina Revianti

Department of Oral Biology
Faculty of Dentistry Hang Tuah University

Background:
*Holothuria atra* and *Holothuria scabra* are species of sea cucumber which has been known to have antibacterial and antifungal properties thus potentially explored as therapeutic agent in oral infection.

Objective: The aim of this study is to evaluate the cytotoxicity of two sea cucumber extract *Holothuria atra* and *Holothuria scabra* to human gingival fibroblast stem cell.

Method: Methanolic extract of *Holothuria atra* and *Holothuria scabra* in concentration of 1%, 0.5%; 0.25%; 0.125%, 0.006%, 0.003%, 0.0015%, 0.0007%; and 0.0003% were tested its cytotoxicity on human gingival fibroblast stem cell. Cell viability were measured by MTT assay. Data were analyzed by ANOVA.

Result: Result showed significant toxicity of *Holothuria atra Holothuria scabra* only on the concentration of 1% (p<0.05) while on *Holothuria scabra* it showed toxicity in the concentration of 1% and 0.5% (p<0.05).

Conclusion: *Holothuria atra* extract was not cytotoxic on human gingival fibroblast stem cellin the concentration below 1%, while *Holothuria scabra* extract is not cytotoxic on human gingival fibroblast stem cellin the concentration below 5%.

Keyword: *Holothuria atra, Holothuria scabra*, cytotoxicity, fibroblast gingiva stem cell

INTRODUCTION

Indonesian waters in the Indo-Pacific region is a habitat for several types of sea cucumber. There are at least 56 species of sea cucumbers are found in this wiyah. Some of the many commercial species exploited in Indonesia among Stichopus hermanii (Tuwo, 2004; Arlyza, 2009). The potential of marine living resources in Indonesia is in countries such as China and Malaysia have been widely used in some traditional medicines because they contain the active ingredients in the process of wound healing has content (Poh-Sze C, 2004; Arlyza, 2009). Some studies have also suggested that sea urchins have pharmacological effects, such as anti-inflammatory, antibacterial, and serves as an antioxidant. (Zohdi, et al., 2011). Stichopus hermanii has more components glycosaminoglycans, Stichopus hermanii also contains saponins that have fewer than Holothuriae scabra (Rizal, 2012; Sari, 2012). Addition of unsaturated fatty acids such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in Stichopus more hermanii (Sari, 2012). Wounds in the oral cavity is the case, whether it's because of trauma or injury in the form of microorganisms that cause discomfort and even the worst of the decline in appetite. This will affect the nutritional status of a person whose impact on health. The process of tissue remodeling is a complex process and related to one another that aims to reconstruct a network so that
the network as closely as possible to the original (Ibelgaufts, 2002). Where in the process, the network requires extracellular matrix that play a role in making arrangements and create a framework for many of the wound healing process (Fitzgerald and Steinberg, 2009). Glycosaminoglycans components is required in the process of wound healing. This relates because components glycosaminoglycans, such as hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin and heparin sulfate are needed in wound healing (Sari, 2012). Hyaluronic acid is important in the inflammatory process early because it can lead to increased infiltration of inflammatory cells and cytokines proinflami products, as well as important in the control of angiogenesis during tissue repair protects the granulation tissue from damage caused by ROS (Reactive Oxygen Species) (Raoudi, et al., 2007; Sari, 2012).

Chondroitin sulfate plays a role in regulation of granulation tissue formation during wound healing. Chondroitin sulfate has the ability to bind to Fibroblast Growth Factor-2 (FGF-2) that can help FGF-2 in triggering cell proliferation mainly fibroblasts (Zou et al., 2004; Sari, 2012). Heparan sulfate and dermatan sulfate are produced in a state of functionally active. Heparan sulfate and dermatan sulfate is a major kompenen wound fluid and become soluble. Dermatan sulfate soluble has the ability to activate growth factors, such as FGF-2 and FGF-7 and keratinocyte growth factor that triggers cell proliferation. In the event of dermatan sulfate deficiency will result in friability injury (Lee, et al., 2004). The minimum size of dermatan sulfate is needed to trigger the increase FGF-2 is oktasakarida (Taylor and Gallo, 2006; Sari, 2012).

In addition, dermatan sulfate chains may regulate coagulation early in the inflammatory process, because of the bond with HCII dermatan sulfate (heparin cofactor II), a serine protease inhibitor that inhibits the procoagulant effects of thrombin. Regulation can also occur early oagulasi because bond dermatan sulfate with platelet factor-4 (PF-4) (Taylor and Gallo, 2006; Sari, 2012). In response to the body's normal, if there is injury, omega-3 resulting from the release of components of the phospholipid bilayer of the cell membrane, so that the omega-3 that plays a role in controlling the inflammatory process caused the injury (McDaniel, et al., 2008). Role of EPA and DHA common in the inflammatory phase. EPA and DHA is a key mediator in the control of inflammatory processes in the wound healing process. (Collins and Suleweski, 2011). In this research, McDaniel et al (2008) stated that EPA and DHA may lead to an increase in IL-1, in which IL-1 regulates fibroblast proliferation and collagen synthesis that will produce healthy collagen. The advantage gained is to minimize the formation of scar tissue and increase the strength of connective tissue. IL-1 also acts to enhance the growth of keratinocytes and stimulate angiogenesis reepitelisasi.

In addition it contains several minerals contained in the body of Stichopus hermanii role in the wound healing process. Zinc is an essential cofactor in physiological role in normal cell growth and replication and is involved in many different enzyme reactions. Directly zinc plays a role in the process of epithelialization and proliferation of fibroblasts as metal enzymes, such as RNA polymerase, DNA polymerase, and DNA transcriptase (Burns, et al., 2003). Other minerals, such as copper and magnesium trigger VEGF. Copper can accelerate the wound healing process to stimuli angiogenesis. In studies in vitro, copper and zinc stimulates integrin expression on keratinocytes in the basal layer of the unknown is part of the wound healing process (Burns, et al., 2003). Stichopus hermanii including phylum Echinodermata, class Holothuroidea, Stichopodidae family, the genus Stichopus have used about 500 years ago as a
medicine, especially in accelerating the wound healing process (Arlyza, 2009). This is evidenced in an earlier study using mice as experimental animals. Hydrogel made from golden sea cucumber (Stichopus hermanii) may trigger the wound healing process to be faster, because it allegedly could modulate the inflammatory response to injury by reducing proinflammatory cytokines so that the wound healing process is faster (Zohdi, et al., 2011). But for concern that the content of Stichopus hermanii are triterpene glycoside having antiproliferative properties (Yang et al., 2010). Cell culture method is often used for testing the biological effects at the beginning of a material used in dentistry to determine the effects of toxicity. (Yuliati, 2005). It is necessary to test for one of the properties of a material is biocompatible, non-toxic on the network. Gingival fibroblasts (GFs), is the most substantial in the gingival connective tissue plays an important role in wound healing and has a unique tolerance trait in oral mucosa (Egusa et al, 2010; Zhang et al, 2009). The use of stem cells taken on human gingival tissue that is not only easily taken from the oral mucosa, but has more value in the study to see the process of regeneration (Egusa et al, 2010). Based on the above, it is necessary to test the cytotoxicity of whole extract of Stichopus hermanii against stem cell gingival fibroblasts.

**MATERIAL AND METHODS**

**Stemcellcultures**

Stem cell cultures derived from stocks stored in liquid tanks are replaced in a temperature of -196 °C. Culturing stem cells is done in water±37.7°C, then the cells are grown in a few (2-3) small pieces of tissue culture flasks and incubated in an incubator at 37°C with an airflow of 5% CO2 and 95% O2. After 24 hours, the media was replaced and cells were grown until confluent and enough for research. After a sufficient number of cells is confluent (± 70%), the medium was replaced with fresh RPMI 1640 medium as much as 5 mL. Cells are taken equal to 3 x 104 cells/100 mL medium calculated by haemocytometer chamber.

**Cytotoxicity Test Methods MTT (tetrazolium Microculture).**

WiDr cell suspension of 100 mL at a density of 3 x 104 mL cells/100 mL is distributed into wells in a 96-well plate and incubated for 24 hours. After incubation, the wells put into 100 mL test solution at different concentration series starting from concentration of 1% - 0.00390625%. As a control added 100 mL cell culture medium into wells containing 100 mL cell suspension and the control solvent DMSO added 100 mL into wells containing 100 mL cell suspension with delusions according to the concentration of the test solution and then incubated for 24 hours in incubator with 5% CO2 flow and 95% O2. At the end of incubation, the culture medium removed and added to 10 mL solution of MTT (5 mg/mL PBS), and then the cells were incubated for 3-4 hours. MTT reaction was stopped by adding SDS stopper reagent (100 mL). Microplate containing cell suspension diseker ± 5 minutes then wrapped with aluminum foil and incubated for 1 night at room temperature. Living cell reacts with MTT to form purple. The test results are read by ELISA reader at a wavelength of 595 nm. Readings on Elisa Reader accomplished by measuring the absorbance. Large absorbance shows the number of living cells in culture media. (Nursid et al., 2006; Sholikhah, 2010). The results form the absorbance readings are converted into % with a corresponding formula (American Type Culture Collection, 2001; Podolak et al., 2010), ie Cell viability = (ABS MEDIA TREATMENT +) / (Abs Abs + cells control the media control) × 100%.
The data were analyzed by statistical tests in SPSS analytics. If normal distribution and homogeneity, parametric statistical test performed by ANOVA followed by Tukey HSD test. Significance level used was 0.05 (95%).

RESULT
Research data include variable data dependent, then the data is analyzed by descriptive statistics which aim to obtain an overview and summarizing the distribution of data in order to clarify the presentation of results.

Table 1. The results of the mean and standard deviation stem cell viability gingival fibroblasts

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<th>Group</th>
<th>X ± SD</th>
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<tr>
<td>Kontrol Sel</td>
<td>100 ± 0</td>
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<tr>
<td>P1 (1%)</td>
<td>56.07 ± 33.206</td>
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<td>P2 (0.5%)</td>
<td>62.54 ± 24.377</td>
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<td>P3 (0.25%)</td>
<td>78.29 ± 46.223</td>
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<td>P4 (0.125%)</td>
<td>97.84 ± 18.990</td>
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<td>P5 (0.0625%)</td>
<td>81.44 ± 48.632</td>
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<td>P6 (0.03125%)</td>
<td>93.65 ± 43.168</td>
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<td>P7 (0.015625%)</td>
<td>59.21 ± 25.917</td>
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<tr>
<td>P8 (0.0078125%)</td>
<td>113.88 ± 24.300</td>
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<tr>
<td>P9 (0.00390625%)</td>
<td>73.17 ± 24.043</td>
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</tbody>
</table>

Before performing data analysis results with ANOVA, data were tested for normality distribution with Shapiro-Wilk test and Levene's test of homogeneity test. The result showed that the data were normally distributed (p>0.05) and had homogeneous variance between groups (p>0.05). The results of univariate analysis showed that cell viability on the provision of whole cucumbers at various concentrations of gold shows the result p = 0.406, which means that p > 0.05. This indicates that there is no significant difference in the study. After going through the Tukey HSD test (Table 5.5) contained details of the result which states that there is no significant difference between each group. This is indicated by the presence of only one class in a homogenous subset (Table 3).

Tabel 2. Hasil Uji Tukey HSD

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DISCUSSION
The research sample was cultured stem cells derived from human gingival fibroblasts treated with freeze-dried Sea cucumber extract gold from a concentration of 1% - 0.0078125%. In vitro cytotoxic assays are an early stage of the development of new drugs that are more economical than the toxicity tests using animals. In addition, the limitations of animal models related to differences in metabolism correlate with the results in humans and also because of factors moralistic use animals for experiments (Muslim, 2008).

On the results of ANOVA and Tukey HSD test found no significant differences. This suggests that freeze-dried Sea cucumber extract gold safe ingredients. Although some groups found the cell viability of less than 60%. This condition may be caused by freeze-dried results are in the form of particles that are difficult to dissolve in PBS preparations, so that the results of the test solutions not homogeneous, although it was anticipated by using the supernatant of the test solution. In addition, the content of saponin in the extract can be freeze-dried. The decrease in cell viability of fibroblasts allegedly also caused cytotoxicity activity of saponin extract gold freeze-dried Sea cucumber (Susanto et al., 2006). Saponins are natural glycosides that have various pharmacological properties including cytotoxic activity (Podolak et al., 2010). Saponin induces cell apoptosis by affecting mitochondrial membrane potential without going through death receptors pathway found in cell membranes. This situation will induce the release of mitochondrial factors (factors penginduksia apoptosis and cytochrome c), which will activate caspase. Cytochrome c will interact with Apaf-1 in the cytosol. Apaf-1 interaction with adenosine triphosphate (ATP) will activate caspase 9, which then activates caspase 3, which in turn results in cell death (Susanto et al., 2006).

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9. Cong


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Cytotoxicity of Sea Cucumber Extract to Human Gingival Fibroblast Stem Cell

Kristanti Parisihni, Syamsulina Revianti
Faculty of Dentistry, Universitas Hang Tuah

Abstract

Introduction

Extract of Holothuria atra and Holothuria scabra have antibacterial and anti-fungal properties:

Experimental Procedure

Experimental laboratory research with post-test only control group design

Extraction Procedure

Extraction process: sonication with methanolic extract 1:20:1.5, followed by filtration, concentration at 54 °C.

Table 1: Cytotoxicity of Extracted Holothuria atra crude extracts

Graph 1: Cytotoxicity of Extracted Holothuria atra crude extracts

Result and Discussion

Cell culture can be used to screen for toxicity both by:

- Estimation of the basal function of the cell by tests on specialized cell functions
- Identifying sub-cellular structures

Apparatus

- Secondary mediator: xenobiotic metabolism of xenobiotics, evenly distributed in higher plants and marine invertebrates
- Ability to form secondary metabolites. It forms compounds with the environment, which has a beneficial effect on cell proliferation. Alterations in the negatively charged carbohydrates present on the cell surface.

Table 2: Cytotoxicity of Extracted Holothuria atra crude extracts

Graph 2: Cytotoxicity of Extracted Holothuria atra crude extracts

Acknowledgments

This research was supported by a grant from Fundamental Research Program funded by the Ministry of Education and Culture, Indonesia.

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References