High tetragonula sp honey addition reduce cell proliferation on fibroblast preputium culture

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Abstract. Induced pluripotent stem cells can be engineered by protein transduction from fibroblast cell, that has been obtained from skin tissue. To increase the protein transduction, needs optimization culture medium. Fetal Bovine Serum was replaced by honey from Tetragonula sp. to reduce the protease and increased the protein transduction efficiency. The purpose of this preliminary research we re to determine the comparison between DMEM media-free serum addition of Tetragonula sp honey on fibroblast preputium cell proliferation. The design of this study used true experimental method. The sample of preputium skin was taken from a healthy child <13 years old. The fibroblast cells isolated from tissue explants, were cultured by variations (0,1%, 1%, 5%) honey concentration, then measured using MTT assay. Fibroblast cell culturing in medium supplemented with 5% Tetragonula sp honey showed significant difference of less proliferation than standard medium with fetal bovine serum (p=0.000). Medium supplement with 0,1% Tetragonula sp honey was significant difference of higher proliferation compare to 1% (p=1.000) and 5% (p= 0.000) treatment medium, but still cannot overlap standard medium with fetal bovine serum. Abundant sugar (dominant in honey) in the culture medium can inhibit fibroblast cells growth. The search for a safe and effective Fetal Bovine Serum (FBS) substitution in fibroblast preputium cell cultures must continue to be developed.

1. Introduction

Somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs) by the introduction of pluripotency related transcriptional factors OCT4, KLF4, SOX2, and cMYC.1 A large number of these iPSC lines were generated by retroviral transduction. However, in this process, small sequences of the retroviral vector are integrated in the host genome along with the genes coding for reprogramming factors. As a result, these permanent insertions potentially increase the risk of tumor formation.2

Proteins can be delivered into cells both in vivo and in vitro if they are fused in frame to cell-penetrating peptides (CPP) or protein transduction domains.3 The advantage of reprogramming by protein transduction is that no genetic integration occurs; thus, allowing the generation of exogene-free iPSC lines, which is an important safety advantage for human therapy.

Stem cells are defined as cells that have not differentiated so that they have the potential to multiply and grow into certain cells.4 According to the origin, stem cells are divided into 3 major parts, namely embryonic stem cells (ESC) obtained from blastocysts, extraembryonic stem cells obtained from the umbilical cord, placenta and amniotic fluid and adult stem cells (adult stem cells, ASC) obtained from adult tissue according to the type of tissue where cells are obtained, for example bone marrow, blood, fat and skin.5

Potential stem cells that can proliferate and differentiate into any cell that forms the body in large numbers causes stem cells to be considered more valuable for use in cell transplants. Topical and
Systemic cell transplantation can improve wound healing on skin in experimental animals. The dermis cells are very accessible and there is increasing evidence that the dermis contains ASC. Stem cells obtained from the dermis of the skin have been shown to have the capacity to produce nerve and mesodermal derivatives.

One part of the skin that can be used as a source of stem cells is the preputium skin. Indonesia as a country with a majority of its population embraces Islam is routinely doing circumcision to boys. This circumcision process contains medical waste, which is quite a lot of preputium, because this preputium can be used as a source of fibroblast cells.

Cell isolation from routine preputium is done to obtain primary fibroblasts and keratinocytes for research purposes. Fibroblasts are the most common cells found in connective tissue and these cells synthesize several extracellular matrix components such as collagen, reticular and elastin. In addition, it also synthesizes anionic macromolecules namely glycosaminoglycans and proteoglycans as well as multiadesif glycoproteins laminin and fibronectin which can encourage cell adhesion on the substrate.

In vitro cultures of fibroblast cells were reported to secrete about 175 types of protein, including cytokines and several growth factors such as basic fibroblast growth factors that can stimulate cell proliferation and inhibit cell differentiation.

Culture systems can use a variety of media. At present, Dulbecco's modified Eagle medium (DMEM) plus Fetal Bovine Serum (FBS) are usually used for human fibroblast cell culture. A study showed that continuous human ESC culture in animal serum and high ascorbate levels produced ectopic expression from CD30. Inappropriate miHA peptides can be presented directly to self-MHC class I to CD8 + T cells which destroy graft therapy or through APCs that process and present miHA peptides to T cells, resulting in an alloresponse. Therefore, research is needed which aims to avoid immune rejection, serum-free cell culture medium is prepared to replace FBS.

Sell et al (2012) reported that Manuka honey accelerated wound closure produced in human monolayer fibroblasts. Honey from Stingless bee has also been used in traditional medicine in Central and South America, and Africa, showing that Stingless bee honey may have therapeutic properties similar to drug honey currently used such as manuka honey from New Zealand. But there is still little research using Indonesian Stingless bee honey which is Tetragonula sp as one of the serum substitutes for FBS for skin fibroblast cell cultures. Therefore, the authors are interested in conducting a comparative study of the effectiveness of serum-free DMEM media, adding Tetragonula honey to the isolation of preputium skin fibroblasts.

2. Literature review

The skin consists of two main layers, namely the epidermis and dermis. The subcutaneous layer which is the layer below the dermis is not considered part of the skin. The outermost layer, the epidermis, consists of a collection of specific cells known as keratinocytes, which are responsible for the synthesis of keratin. Keratin is a long protein that has a protective role. The second layer is called the dermis. The dermis layer is composed of fibroblasts, which produce collagen, elastin and proteoglycans. The dermis is located above the subcutaneous tissue or referred to as the hypodermic layer (panniculus) which contains a small lobe of fat cells known as lipocytes.

In the reprogramming process, the cells most commonly used come mainly from connective tissue (fibroblasts). Fibroblast cells are obtained from the dermis of the skin, capsules and stroma of various mucous or serous organs and membranes.

Honey is a sweet and flavorful natural product, which is consumed for its high nutritional value and for effects on human health, with antioxidant, bacteriostatic, anti-inflammatory and antimicrobial properties, as well as wound healing effects. Regarding its nutritional composition, honey is a natural source of macro and micro nutrients, which consist of saturated sugar solutions, where fructose and glucose are the main contributors, but also of various minor constituents, especially phenolic compounds.

3. Research Methods

3.1. Research design

This study uses a true experimental study design with the research design used is post test only control group design. The results of the study were seen from a comparison of the proliferation of fibroblast cells from the preputium skin that grew on the media.
3.2. Population and Sample
The population in this study was preputium skin from healthy pediatric patients aged <13 years. The sample of this study was preputium skin taken from circumcision waste that met the inclusion criteria. The criteria in this study were preputium skin taken from the circumcision of healthy children aged <13 years and preputium skin in good condition, there were no diseases or abnormalities. The exclusion criteria in this study were preputium skin taken by laser method.

3.3. Sampling
In this study sampling was conducted using non-probability sampling method. The non-probability sampling design used is consecutive sampling, i.e., all subjects who meet the selection criteria are included in the research until the required number of subjects is met.

3.4. Data collection
The data used in this study is primary data taken from the circumcision clinic in the form of circumcision waste, namely preputium skin.

3.5. Research procedure
The study was conducted using isolated cells in an incubator (37 °C, 5% CO2) for at least 1 week using DMEM medium (Gibco) with 10% FBS supplementation (Gibco) and 1% antimicrobial antibiotic solution in T-flask 25 (TPP). Treatment of fibroblast cell cultures was carried out with 5 different treatments. Treatment a) cells were cultured in DMEM with Tetragonula honey concentrated at 0%, b) cells were cultured in DMEM with Tetragonula honey concentrated 0.1%, c) cells were cultured in DMEM with Tetragonula honey concentrated 1%, d) cells cultured in DMEM with concentration of 5% Tetragonula honey, and e) cells were cultured in DMEM with 10% Fetal Bovine Serum (FBS) then determined fibroblast cell proliferation using MTT Assay methods on days.

4. Result and Discussion
Results of the fibroblast cell proliferation studies are shown in Figure 1. Fibroblast cell proliferation was observed over 3 days in response to test media containing varying concentrations of Tetragonula honey, and cell numbers ranged from near 0 for the higher Tetragonula honey concentrations to greater than 70 for the 0.1% Tetragonula honey on day 3. Additionally, the presence of FBS alone had a greater impact on cell proliferation than Tetragonula honey alone, as 10% FBS had higher cell numbers than the 0.1 or 1% Tetragonula honey. This can be caused because abundant sugar in the medium can also inhibit the growth of fibroblast cells. Addition of substances other than sugar is also needed to balance fibroblast cell growth, rather than just sugar addition alone.

![Cells Proliferation](image)

Figure 1. Fibroblast percentage after treatment with or without addition honey in the medium
Anova test results have a significance value of 0.000. Because of the significance value <0.05, from the results of this test it can be concluded that there are significant differences in the results between the concentration groups tested and the fibroblast cell proliferation. The data analysis was followed by a Bonferroni post-hoc test to identify significant differences on different treatment groups. Bonferroni test statistics showed that each group of Tetragonula honey concentration had a significant difference. It can be seen from the significance value less than 0.05 (Table 1.)

### Table 1. Bonferroni Post-hoc Test

| Treatment Group | 0%   | 0,1%  | 1%   | 5%   | 0%   | 0,1%  | 1%   | 5%   | 0,1% | 1%   | 5%   | 0,1% | 1%   | 5%   |
|-----------------|------|-------|------|------|------|-------|------|------|------|------|------|------|------|------|
| Positive Control| 1.000| 0.002 | 0.001| 0.000| 0.003| 0.001 | 0.000| 0.000| 1.000| 1.000| 0.000| 1.000| 1.000| 1.000|

**Figure 2.** (a) Samples with 10% FBS concentration where the cells looks more dense and longer. (b) Samples with 0% Tetragonula honey concentration. (c) Samples with 0.1% Tetragonula honey concentration. (d) Samples with 1% Tetragonula honey concentration where the cells appear less frequently. (e) Samples with 5% Tetragonula honey concentration where almost all cells are dead.

### 5. Conclusion

Tetragonula honey in 0,1%, 1%, and 5% shows little to no effect in the proliferation of fibroblast cell even though Tetragonula honey contains some necessary sugars and antibacterial properties. The search for a safe and effective Fetal Bovine Serum (FBS) substitution in skin fibroblast cell cultures must continue to be developed.
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