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Original Article

MICROENCAPSULATION OF INDONESIAN POLYMER BIODIVERSITY IN WARTHON'S JELLY MESENCHYMAL STEM CELL (WJMSC)

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ABSTRACT

Objective: WJMSC is a stem cell-derived from Wharton's Jelly the umbilical cord of pregnant women which has the ability to differentiate into other cells that are osetogenic, myogenic, neurogenic, and hematopoietic. Stem cell microencapsulation is a cell coating technique that is expected to act as a delivery vehicle. This study aims to make stem cell microencapsulation using various types of natural polymers.

Methods: WJMSC (Wharton's Jelly Mesenchymal Stem Cell) was cultured with supplemented Modified Eagle Medium (MEM) Alpha in incubator with 37 °C and 5% of CO_2. In this study, we investigated various of natural polymers (chitosan, glucomannan, inulin, fucoidan and amylopectin) in WJMSC microencapsulation. Viability cell of WJMSC microencapsulate was measured using MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay, and the condition medium (CM) was determined the measure EGF, IL-6, VEGF with ELISA.

Results: WJMSC microencapsulation using chitosan, oxidized glucomannan, inulin, fucoidan and oxidized amylopectin showed viability cell up to 100%. The EGF, IL-6, VEGF levels were increased in all tested polymers compared to negative control.

Conclusion: Tested polymers (chitosan, oxidized glucomannan, inulin, fucoidan, oxidized amylopectin) were not toxic to WJMSC and cell microencapsulation was successfully carried out.

Keywords: WJMSC, Microencapsulation, Polymers, Stem cells, Drug delivery

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INTRODUCTION

Currently, especially in the pharmaceutical field, stem cells are developed into various medicinal preparations. Due to the diverse potential of stem cells and their medical benefits, stem cells provide hope for the treatment of diseases in the future. One of the stem cells that can be developed is mesenchymal stem cells (MSC). MSC can be obtained from Wharton's jelly contained in the umbilical cord and blood in the placenta after the baby is born [1-3].

The engineering of the delivery system of MSCs is important in clinical applications to prevent unwanted cell migration. In addition, delivery system engineering also ensures that MSCs enter the site of inflammation. Encapsulation is one way that can be used to immobilize cells as a delivery system and can control therapeutic materials from cells [4]. MSCs encapsulation can improve macrophage activation *in vitro* and stimulate tissue regeneration *in vivo*. Simultaneously, the cell immobilization system can develop administering MSCs and has protective properties against tissues [5]. Several factors secreted by MSCs act as immunomodulators and provide a protective effect on tissues [6]. Microencapsulation of MSCs is known to maintain the secretory function of MSCs, including secretion that plays a role in growth. Encapsulation can also protect stem cells from friction forces, microenvironment, and immune response [7].

Alginate is an anionic polymer compound obtained from brown algae, Pseudomonas, and Azotobacter. Alginates include a group of non-branched and non-recurrent exopolysaccharides consisting of two monomers, namely β-D-mannuronic acid and α-L-guluronic acid [8]. Several studies have microencapsulated MSCs using alginate. For example, microencapsulation using 1.7% and 2.2% alginate can improve the secretion of cultured MSCs activated using the inflammatory factors TNF-α and IFN-γ [5]. Encapsulation of embryonic stem cells with alginate has been reported to maintain cell viability for 110 d without differentiation and without requiring subculture maintenance [9]. In addition, research conducted by [10] stated that encapsulation of MSCs with alginate-$CaCl_2$ can maintain viability under hypothermic conditions. Furthermore, this research has aim to formulate the microencapsulated stem cells using various types of natural polymers which are part of Indonesia's biodiversity, including alginate, chitosan, glucomannan, inulin, fucoidan, and amylopectin.

MATERIALS AND METHODS

WJMSC cell culture

WJMSC (Wharton's jelly mesenchymal stem cell) was obtained from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, West Java, Indonesia. The WJMSC cell was cultured by medium with composition: Modified Eagle Medium (MEM) Alpha (Biowest, L0475-500), fetal bovine serum (FBS) (Biowest, S1810) as much as 10% (v/v), antibiotic-antimycotic (Gibco, 15240062) as much as 1% (v/v), also nanomycopulitine (Biowest, LX16) as much as 1% of (v/v). When the cells were confluent, they were rinsed using PBS and separated using trypsin-EDTA (Gibco, 25200072). The cells were then used for microencapsulation [11-13].

WJMSC microencapsulation

Microencapsulation was prepared according to the method used by Zhang et al., (2007) with slight modifications [14]. 4 ml of 1.75% alginate and 1 ml of WJMSC cell suspension were added to 1.2 CaCl2 solution. The mixture was stirred using a magnetic stirrer at 500-700 rpm. The beads formed were washed using PBS. The periodate oxidation was applied on glucomannan and amylopectin. 0.2%

chitosan/oxidized glucomannan/inulin/oxidized amylopectin/ fucoidan solution was dripped onto the resulting beads and incubated for 5-10 min. Afterwards, they were washed again using PBS. In addition, the beads with inulin/oxidized amylopectin/fucoidan were added with 0.15% inulin/oxidized glucomannan/chitosan, respectively and incubated for 3 min. The microencapsulated cells were put into six well-plates with the growth medium. The generated microencapsules were observed under a light microscope. The negative control used was the microencapsulation without the cells inside. The positif control used was the microencapsulation without the additional polymers after the beads were formed.

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Cell viability assay

A cell viability test was carried out to see the percentage of live cells that were microencapsulated. This viability test was carried out on microencapsulation with different cell numbers, namely empty beads (negative control), alginate beads (positive control), chitosan beads, glucomannan beads, inulin beads, fucoidan beads, and amylopectin beads. Observations were made on the first day after microencapsulation. Each test was repeated three times. After treatment, 20 µl of MTS was added to each well and incubated for 3 h at 37 °C with 5% CO_2. The absorbance was obtained using a UV-Vis spectrophotometer at a wavelength of 490 nm [15].

Measurement of EGF, IL-6, VEGF and IGF-1 levels

The microencapsulation condition medium was used to measure EGF, IL-6, VEGF, and IGF-1. The EGF (Elabscience, E-EL-H0059), IL-6 (Elabscience, E-EL-R0015), and VEGF (Elabscience, E-EL-R0578) assays were performed according to the manual kit with minor modifications. Standard solution, blank, and sample of 100 µl each were put into the well. In the blank well, the reference standard and sample diluent was added. The well plate was closed using a sealer and incubated for 90 min at 37 °C. After incubation, the solution in the well was removed. Immediately, the Biotinylated Detection Ab working solution was added 100 µl to each well. The plate was again sealed and incubated for 1 h at 37 °C. After that, each well was washed three times using Wash Buffer (350 µl). Then, 100 µ of HRP Conjugate working solution was added to each well. The plate was again sealed and incubated for 1 h at 37 °C. The wells were washed five times after incubation. Afterwards, 90 µl of the substrate was added to each well. The well plate was closed with a new seal and incubated for 15 min at 37 °C. Lastly, the Stop Solution was added 50 µl to each well. The absorbance was measured using a microplate-reader at a wavelength of 450 nm [16].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) software version 10 was used to analyze the data. One-way analysis of variance (ANOVA) was used to verify the results of different treatments followed by Tukey's HSD Post Hoc Test.

RESULTS

Microencapsulation morphology

The microencapsulation was prepared according to the previously described method with several kinds of polymers. Fig. 1 shows the morphology of the microencapsulation viewed under a light microscope. The results showed that WJMSC microencapsulation using several kinds of polymers were successfully carried out, and WJMSC cells were successfully encapsulated in them.

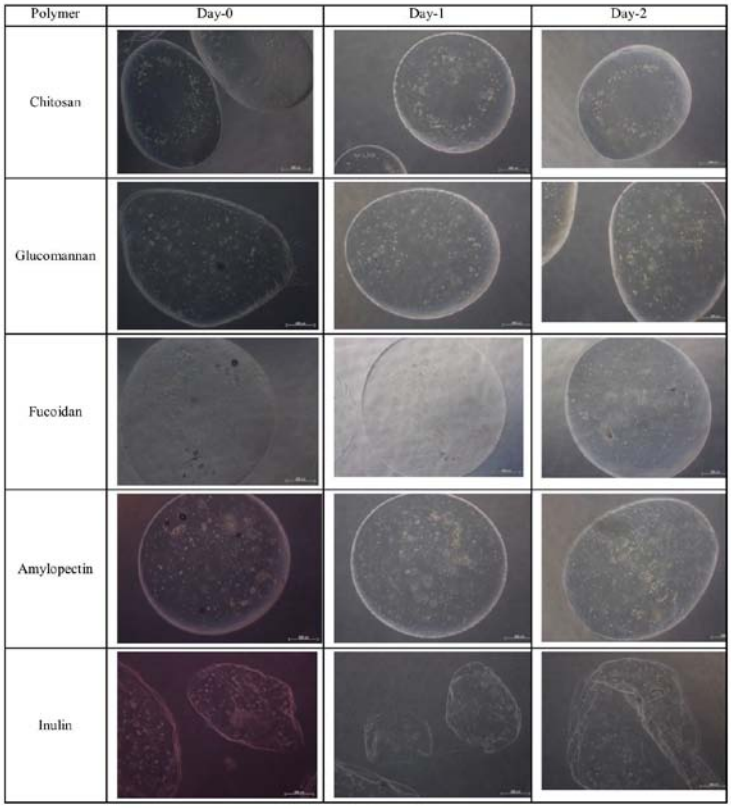

Fig. 1: WJMSC microencapsules morphology using various polymers: chitosan, glucomannan, fucoidan, amylopectin, and inulin at 4× Magnification

Microencapsulation cell viability

The results of the viability test can be seen in fig. 2. Fig. 2 shows the percentage of cell viability in several different natural polymers, namely chitosan, glucomannan, inulin, fucoidan, and amylopectin. On the second day after microencapsulation, the cell viability was almost 100%, so it could be said that all the polymers used were safe for cells. Fig. 3 shows that the inhibition result was less than 30%. These results indicated that chitosan, glucomannan, inulin, fucoidan, and amylopectin polymers were not toxic to WJMSC cells.

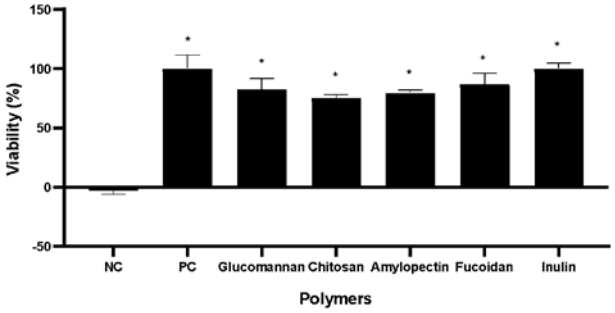

Fig. 2: Viability assay of microencapsulation using natural polymers, *The data was presented as mean±SD, n=3. The experiment had three replications. The asterisk symbol (*) shows significant differences between treatments based on Tukey HSD post hoc test (p<0.05)

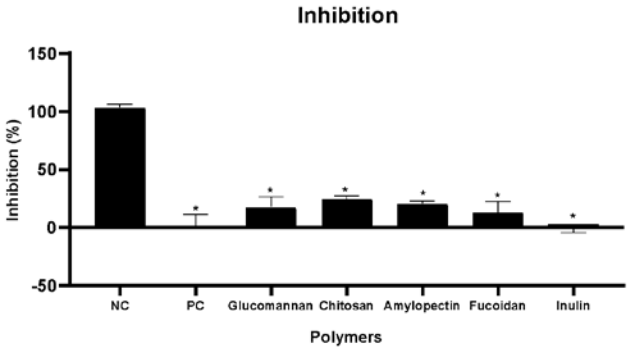

Fig. 3: Inhibition assay of microencapsulation using natural polymers, *The data was presented as mean±SD, n=3. The experiment had three replications. The asterisk symbol (*) shows significant differences between treatment based on Tukey HSD post hoct test (p<0.05)

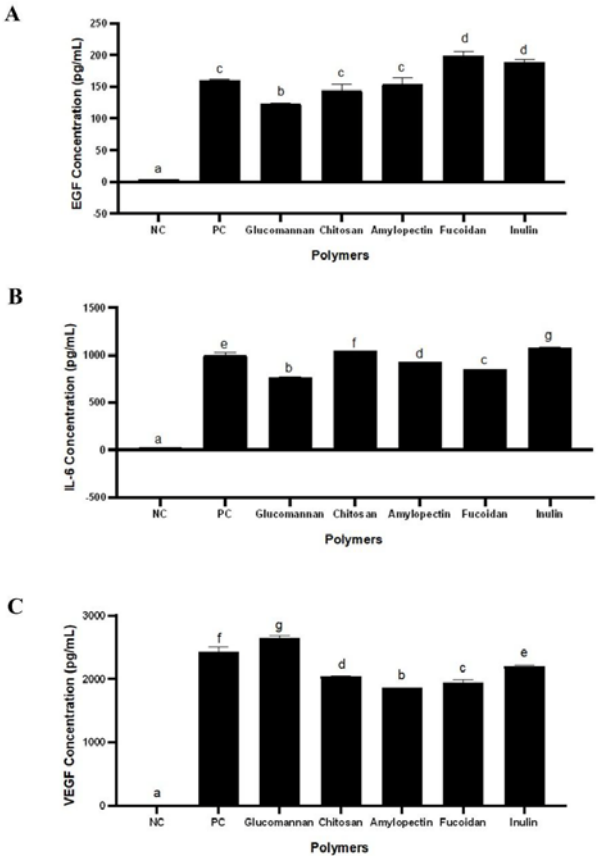

Fig. 4: The effect of microencapsulation using natural polymers on the levels of EGF, IL-6, and VEGF, *The data was presented as mean±SD, n=3. The experiment had three replications. The superscript letter (a-g) shows significant differences between treatment based on Tukey HSD post hoct test (p<0.05)

The effect WJMSC microencapsulation toward the levels of EGF, IL-6, and VEGF

Fig. 4 shows the levels of VEGF, IL-6, and EGF. Based on the results, WJMSC microencapsulated with chitosan, glucomannan, inulin, fucoidan, and amylopectin polymers still secreted the growth factors of VEGF, IL-6, and EGF. In the negative control where no WJMSC cells were in the capsule, there's was no growth factor secretion.

DISCUSSION

Cell microencapsulation is a cell coating technique that is expected to act as a delivery vehicle. WJMSC is known to have some potential for disease treatment. This study aimed to find other natural polymers that can support alginates. The polymers were chitosan, glucomannan, inulin, fucoidan, and amylopectin. Hence, alginate was used as a positive control in this study. As shown in fig. 2 and fig. 3, positive control has 100% of viability and 0% of inhibition. In the previous research has been reported that encapsulation of stem cells with alginate could maintain cell viability [9]. Chitosan, glucomannan, inulin, fucoidan, and amylopectin are polymers that are not toxic to cells. In fig. 2 and fig. 3 it can be seen that the viability of these polymers is more than 75% and the inhibition is less than 25%. Based on previous research, several natural polymers such as chitosan are known to be biocompatible, biodegradable, non-toxic, and have excellent film-forming abilities [17].

MSCs secrete a variety of growth factors and cytokines that contribute to wound healing and tissue regeneration. A hypoxic condition can be created through stem cell microencapsulation. The hypoxic condition promotes MSC production of growth factors and cytokines. These released components then induce fibroblasts in wound sites to produce collagen and migrate, increasing the wound healing process even further [18]. Based on fig. 4, MSC microencapsulation can secret some growth factors and cytokines including EGF, IL-6, and VEGF. According to prior study, hypoxia increases the synthesis of VEGF and bFGF by MSCs in an HIF-dependent way. By stimulating epithelial cell migration and angiogenesis, IL-6 also plays an important role in MSC-mediated enhanced wound healing. MSCs release IL-6 through the p38MAPK pathway. Although IL-6 is one of the primary mediators promoting wound healing, excessive IL-6 production by MSCs may result in keloid development [18]. Based on the results of the ELISA assay, it is useful for further research, where MSC microencapsulation will be used as wound healing therapy.

CONCLUSSION

Based on the results, microencapsulation of WJMSC cells using various types of natural polymers was successfully carried out. The results of the cell viability test showed that the polymer used in this study was not toxic to WJMSC cells. The WJMSC microencapsulated also secreted growth factors such as VEGF, IL-6, and EGF.

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AUTHORS CONTRIBUTIONS

Study concept and design: DR, AF, WW, IMN, DP; Acquisition of data: WW, DR, AF, and DP; Analysis and interpretation of data: IMN, DP, and HSWK; Drafting of the manuscript: DR, EA, AB, WW; Critical revision of the manuscript for important intellectual content: TMZ and WW; Statistical analysis: AB, EA.

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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