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Original Article

CHARACTERIZATION OF NANOPARTICLES CONDITIONED MEDIUM ADIPOSE TISSUE MESENCHYMAL STEM CELL (CM-ATMSC)

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ABSTRACT

Objective: The aims of this study were to formulate and characterize the nanoparticles containing conditioned medium adipose tissue mesenchymal stem cell (CM-ATMSC).
Methods: Adipose Tissue Mesenchymal Stem Cell (AT-MSC) was cultured with supplemented Modified Eagle Medium (MEM) Alpha in an incubator with 37 °C and 5% CO2. The conditioned medium was collected when the cells were confluent. Nanoparticles were characterized in the term of morphology using transmission electron microscopy (TEM), particle size, zeta potential, loading capacity, entrapment efficiency, and drug release.
Results: CM-ATMSC nanoparticles had the smallest particle size, namely chitosan-inulin nanoparticles with particle size of 128 nm, and the largest particle was modified chitosan (thiomer)-fucoidan nanoparticles with particle size of 254.3 nm. Based on zeta potential results, it is known that the resulting nanoparticle suspension was stable.
Conclusion: The resulting nanoparticle suspension was stable and the smallest CM-ATMSC nanoparticle demonstrated the particle size of 128 nm. The results showed that the nanoparticles of CM-ATMSC have been successfully prepared.
Keywords: Nanoparticles, Drug delivery, Conditioned medium, Stem cells

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INTRODUCTION

Stem cells can be derived from embryonic and extraembryonic tissues and adult organs and are being used in clinical applications. Stem cells can self-renew indefinitely and develop into adult cells of many lineages, making them valuable cell sources for tissue engineering. Many strategies are explored to improve the quality and quantity of stem cells since clinical therapies require a high number of cells. In various disorders, mesenchymal stem cells (MSCs) are used as cellular carriers for the targeted delivery and local production of biologic medicines [1].

However, stem cell therapy has limitations. Therefore, a conditioned stem cell (CM) medium was employed to overcome this issue. MSCs-CM (mesenchymal stem cells conditioned media) is a CM that allows undifferentiated mesenchymal stem cells to develop. According to some evidence, stem cell paracrine signals are involved in the secretion of several growth factors involved in angiogenesis. Therefore, CM treatment can be an alternative therapy [2].

Apart from the ability of MSC as a drug delivery system (DDS), MSC has several limitations. For example, MSC has poor drug loading capacity, limited homing efficiency, and potential living cell risk. These issues restricted their practical applications for disease treatment. Insufficient drug loading is currently a major obstacle to the practical application of MSCs-DDS. As a result, plenty of efforts has been made to increase the drug loading capacity of MSCs carriers [3].

So far, many technologies have been applied to address the MSCs-DDS challenges mentioned above. One of the technologies applied in nanotechnology. Research conducted by [4] showed that spheroid nanoparticles internalized by MSC could increase loading capacity compared to MSC alone. These nanoparticles also increase the efficiency of drug targeting for gliomas.

In addition to increasing the drug loading capacity of MSCs, the nanoparticles also increase the targeting efficiency of MSCs-DDS. For example, in a rabbit model, superparamagnetic iron oxide NPs were employed to drive AT-MSCs through an external magnetic field, increasing AT-MSC accumulation at a meniscal defect site [5]. Furthermore, the use of iron oxide NPs allows MSCs to express high levels of therapeutic genes and improves their homing potential by up-regulating CXCR4 [6]. Therefore, based on the studies that have been carried out, this nanoparticle technology can improve the performance of MSC-based carriers for targeted drug delivery [3].

One of the drug carrier matrix materials used in nanoparticle technology is chitosan. It aims to increase penetration power, prolong contact time to increase effectiveness. The use of chitosan in the form of nanoparticles was chosen because it has biocompatible, biodegradable, low toxicity, and has mucoadhesive properties [7].

Based on the explanation above, this study aimed to prepare CM-ATMSC nanoparticles constructed from several polymers, including chitosan, glucomannan, inulin, and fucoidan, which were then be characterized for morphology, particle size, zeta potential, loading capacity, entrapment efficiency, and drug release.

MATERIALS AND METHODS

Cell culture

ATMSC (Human Adipose tissue mesenchymal stem cell) was obtained from Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung, West Java, Indonesia. The cells were cultured by medium with composition: Modified Eagle Medium (MEM) Alpha (Biowest, L0475-500, Nuaille France), fetal bovine serum (FBS) (Biowest, S1810, Nuaille France) as much as 10% (v/v), antibiotic-antimycotic (Gibco, 15240062, Thermo Fischer Scientific, Massachusetts, USA) as much as 1% (v/v), also nanomycopulitine (Biowest, LX16, Nuaille France) as much as 1% of (v/v) [8-10]. When the cells were confluent, the conditioned medium was collected.

Nanoparticles preparation

Nanoparticles were prepared based on the method used by [11] with slight modifications. Briefly, 0.4 gram of chitosan was dissolved

in 100 ml of 1% glacial acetic acid using a magnetic stirrer to obtain a concentration of 0.4% chitosan. Afterwards, 15 ml of conditioned medium of ATMSC were mixed with 40 ml of 0.4% chitosan solution. The mixture was stirred using a magnetic stirrer for 10 min and 40 ml of 0.1% aqueous solution of glucomannan/fucoidan/inulin was added. 0.1% Na-TPP was dripped into the mixture at rate of 1 drop/3 seconds until the nanoparticles were formed and characterized for homogeneous turbidity. The mixture was stirred for another 15 min to obtain a stable suspension of conditioned medium nanoparticles.

Nanoparticles evaluation

Particle and zeta sizers were used to determine the size and zeta potential value of the produced nanoparticles, while transmission electron microscopy (TEM) was used to investigate the morphologies. Before all of the tests, 1 ml of nanoparticles were diluted in 99 ml pure water. Within 5 d, the stability of the nanoparticle suspension was tested, including color, turbidity, and sedimentation. All the nanoparticles were determined loading capacity and entrapment efficiency by centrifugation of the nanoparticles and the measurement of CM phenol red absorbance using a UV-Vis spectrophotometer [11].

Drug release assay

The nanoparticles (75 ml) were poured into a dialysis cassette which was then put in 500 ml 0.1 M phosphate buffer as medium release in beaker glass. 5 ml of the medium was withdrawn every 30 min within 180 min to measure the absorbance of CM phenol red using a UV-Vis spectrophotometer. 5 ml of fresh medium were then added. Afterwards, the release (%) was determined quantitatively and recorded.

RESULTS AND DISCUSSION

Nanoparticles preparation

The use of cell therapies such as MSC-sourced secretomes for regenerative efforts will provide better benefits than the use of stem cells: (a) the use of secretomes provides safety considerations associated with transplantation of live and proliferative cell populations including immune response, tumor, embolism, and transmission. Infection; (b) the secretions of which MSCs can be regulated for safety, dosage, and potency as drugs; (c) storage of secretions without the need for toxic cryopreservatives (d) secretomes from MSCs, such as conditioned media (CM), are more economical and more clinically practical because they avoid the use of invasive cells; (e) time and cost for cultured stem cells can be greatly reduced [6].

The ionic gelation method involves a cross-linking process between polyelectrolytes in the presence of multivalent ion pairs. Ionic gelation is often followed by complexation of the polyelectrolyte with the opposing polyelectrolyte. The formation of these crosslinks will strengthen the mechanical strength of the particles formed.

Nanoparticles evaluation

The results for particle size, zeta potential, loading capacity, and entrapment capacity were shown in table 1. The resulting nanoparticles have sizes in the range of 128-254 nm and are included in the nano size. . These indicated that the CM was successfully entrapped within the nanoparticles. The result for zeta potential showed that the nanoparticles of chitosan-glucomannan, chitosan-inulin, and modified chitosan (thiomer)-fucoidan exhibited zeta potential of 28.1 mV; 24.1 mV; 26.8 mV, respectively.

The results of CM-ATMSC nanoparticles morphology shown by TEM can be seen in fig. 1. CM-ATMSC nanoparticles and had the smallest particle size with chitosan-inulin, and the largest nanoparticles, modified chitosan (thiomer)-fucoidan, demonstrated 254.3 nm in the particles size. Moreover, the nanoparticles of CM-ATMSC met the requirements as the particles in the range of 1-1000 nm in the diameter.

Drug release assay

Table 2 shows the drug release from the prepared nanoparticles. The results showed that drug release increased with increasing time, up to 180 min. The best nanoparticles to release drugs are chitosan-glucomannan nanoparticles.

CM can provide a complete regenerative environment of the secretome and cell-derived vesicular components. The soluble components of the secretome can be separated from the microvesicle fraction by centrifugation, polymer deposition, filtration, ion-exchange chromatography. These two components can independently provide regeneration and repair tissue engineering organs. CM-ATMSC nanoparticles used several polymers, including chitosan, glucomannan, inulin, and fucoidan with ionic gelation method. The resulting nanoparticles were characterized for particle size, zeta potential, loading capacity, entrapment efficiency, morphology, and drug release.

Table 1: Evaluation of nanoparticles

Parameters	Chitosan-glucomannan	Chitosan-inulin	Modified chitosan-fucoidan
Particle size (nm)	212±22	128±42	254.3±54
Zeta potential (mV)	28.1±5	24.1±4	26.8±4
Loading capacity (%)	1.3±0.2	1.8±0.2	1.7±0.2
Entrapment efficiency (%)	12.6±2.08	11.33±1.53	19.67±2.53

The data was shown in mean+SD, n = 3

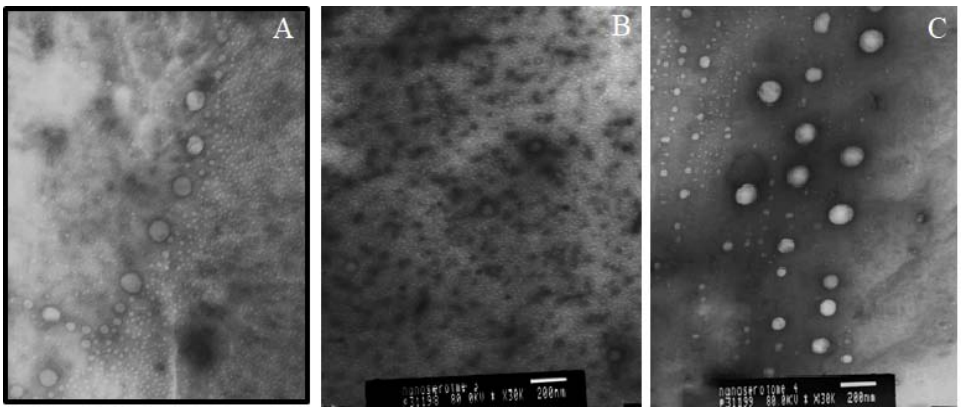

Fig. 1: Morphology of the nanoparticles A) Chitosan-glucomannan; B) Chitosan-Inulin; C) Modified chitosan-fucoidan

Table 2: Drug release of nanoparticles

Time (min)	Drug release (%)		
	Chitosan-glucomannan	Chitosan-inulin	Modified chitosan-fucoidan
0	0±0	0±0	0±0
30	15±3	4±1	10±2
60	25.33±3.05	14.33±3.21	19.67±3.06
90	32.67±3.05	20.67±2.51	27±2.64
120	40±2	32.3±2.51	36±4
150	49.3±6.11	41.33±1.52	49.67±1.53
180	61.3±3.05	50.33±1.52	54.67±2.52

The data was shown in mean+SD, n = 3

Chitosan and fucoidan will crosslink with the contribution of tripolyphosphate because the positive charge of chitosan will interact with the negative charge of the tripolyphosphate and fucoidan. Meanwhile, the active components will be entrapped in a cross-linked chitosan matrix and glucomannan. By adjusting the stirring speed and adding the tripolyphosphate solution, nanoparticles will be formed [12].

Based on the results from morphology (TEM) and in table 1, the nanoparticles from CM-ATMSC generated have met the requirements,

where a particle is recognized as a nanoparticle if the size is in the range of 1-1000 nm. There is a similarity of each positively (+) charged particle from the CM-ATMSC nanoparticles, the greater of repulsion between the particles so that the possibility of the formation of aggregates of nanoparticles dispersed in the suspension system is reduced. This effect is related to the binding of anionic groups by amine groups of chitosan resulting in high electrical values to prevent aggregation.

CONCLUSION

The resulting nanoparticle suspensions were stable and displayed various particle sizes. The characterization results showed that the nanoparticles of CM-ATMSC were successfully prepared and render a promising strategy to deliver secretome.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

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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