ESTABLISHMENT OF ENCAPSULATED HUMAN DENTAL PULP STEM CELL-DERIVED INSULIN PRODUCING CELLS (hDPSC-IPCs) FOR TRANSLATIONAL STEM CELL BASED DIABETES THERAPY

Mr. Suryo Kuncorojakti

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การพัฒนาวิธีการห่อหุ้มเซลล์สังเคราะห์อินซูลินที่ผลิตจากเซลล์ต้นกำเนิดจากเนื้อเยื่อโพรงฟันของมนุษย์เพื่อการรักษาโรคเบาหวาน

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Thesis Title	ESTABLISHMENT OF ENCAPSULATED HUMAN DENTAL PULP STEM CELL-DERIVED INSULIN PRODUCING CELLS (hDPSC-IPCs) FOR TRANSLATIONAL STEM CELL BASED DIABETES THERAPY
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เซอโย คุนโคโรจัคติ : การพัฒนาวิธีการห่อหุ้มเซลล์สังเคราะห์อินซูลินที่ผลิตจากเซลล์ต้นกำเนิดจากเนื้อเยื่อโพรงฟันของมนุษย์เพื่อการรักษาโรคเบาหวาน. (ESTABLISHMENT OF ENCAPSULATED HUMAN DENTAL PULP STEM CELL-DERIVED INSULIN PRODUCING CELLS (hDPSC-IPCs) FOR TRANSLATIONAL STEM CELL BASED DIABETES THERAPY) อ.ที่ปรึกษาหลัก : รศ. ดร. ศยามณ ศรีสุวัฒนาสกุล, อ.ที่ปรึกษาร่วม : ผศ. ดร.เจนภพ สว่างเมฆ

การรักษาโรคเบาหวานในปัจจุบันนั้นยังมีความเสี่ยงต่อภาวะแทรกซ้อนในหลาย ๆ รูปแบบตั้งแต่อาการในระบบทางเดินอาหารไปจนถึงอาการที่รุนแรงอื่น ๆ จนกระทั่งสามารถทำให้เสียชีวิตได้ การรักษาแบบฟื้นฟูโดยการใช้หลักการEdmontonแม้ว่าจะจะมีประสิทธิภาพและมีความปลอดภัยแต่ยังมีข้อจำกัดอื่น ๆ ที่ทำให้การใช้หลักการนี้ยังคงไม่แพร่หลาย ดังนั้นการศึกษานี้จึงมีจุดประสงค์เพื่อที่จะหาเซลล์ทางเลือกอื่น ๆ ในการผลิตอินซูลิน รวมทั้งแบบแผนในการขนส่งเซลล์เหล่านั้น โดยการใช้เซลล์ต้นกำเนิดจากโพรงรากฟันมนุษย์ที่มีการห่อหุ้มและเหนี่ยวนำให้เป็นเซลล์ที่ผลิตอินซูลิน(hDPSC-IPCs) โดยใช้สารอัลจิเนท รวมทั้งสารอัลจิเนทร่วมกับพลูโรนิคเอฟ127 เป็นตัวห่อหุ้มเซลล์ต้นกำเนิด ผลการศึกษาพบกว่าทั้งสารอัลจิเนทและอัลจิเนทร่วมกับพลูโรนิคเอฟ127สามารถรักษาสภาพของเซลล์ต้นกำเนิดจากโพรงรากฟันได้ และสามารถเกิดการแพร่ของกลูโคสและอินซูลินเข้าและออกได้ โดยที่การใช้ตัวห่อหุ้มอัลจิเนทและอัลจิเนทร่วมกับพลูโรนิคเอฟ 127 สามารถคงสภาพเซลล์ไว้ได้อย่างต่ำ336ชั่วโมง รวมทั้งสามารถคงสภาพการแสดงออกของตัวกำหนดเซลล์ชนิดเอนโดเดิร์มของตับอ่อน หรือ NGN3, ตัวกำหนดเซลล์ตับอ่อนซึ่งได้แก่*NKX6.1, MAF-A, ISL-1, GLUT-2*และ*INSULIN*เป็นเวลาอย่างน้อย14วัน ในกรณีของการวิเคราะห์การทำงานของเซลล์พบว่าเซลล์ต้นกำเนิดสามารถหลั่ง glucose-responsive C-peptideผ่านการห่อหุ้มด้วยอัลจิเนทและอัลจิเนทร่วมกับพลูโรนิคได้เป็นระยะเวลา14วัน โดยสรุปพบว่าการใช้สารอัลจิเนทและอัลจิเนทร่วมกับพลูโรนิคสามารถคงสภาพเซลล์และหน้าที่ของเซลล์ต้นกำเนิดจากโพรงรากฟันมนุษย์เพื่อเหนี่ยวนำให้เป็นเซลล์ผลิตอินซูลินได้ในระดับห้องทดลอง และการศึกษาในครั้งนี้สามารถต่อยอดไปเป็นแบบแผนในการขนส่งเซลล์ต้นกำเนิดเพื่อการรักษาฟื้นฟูโรคเบาหวานต่อไปได้ในอนาคต

สาขาวิชา	วิทยาศาสตร์ทางการสัตวแพทย์และเทคโนโลยี	ลายมือชื่อนิสิต .....
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Suryo Kuncorojakti : ESTABLISHMENT OF ENCAPSULATED HUMAN DENTAL PULP STEM CELL-DERIVED INSULIN PRODUCING CELLS (hDPSC-IPCs) FOR TRANSLATIONAL STEM CELL BASED DIABETES THERAPY. Advisor: Assoc. Prof. Dr. SAYAMON SRISUWATANASAGUL, DVM., MSc., PhD. Co-advisor: Asst. Prof. Dr. Chenphop Sawangmake, DVM., MSc., PhD.

Current approach for diabetes treatment remained several adverse events varied from gastrointestinal to life-threatening symptoms. Regenerative therapy regarding Edmonton protocol has been facing serious limitations involving protocol efficiency and safety. This led to the study for alternative insulin-producing cell (IPC) resource and transplantation platform. In this study, evaluation of encapsulated human dental pulp stem cell (hDPSC)-derived IPCs (hDPSC-IPCs) by alginate (ALG) and pluronic F127-coated alginate (ALGPA) was performed. The results showed that ALG and ALGPA preserved hDPSC viability and allowed glucose and insulin diffusion in and out. ALG- and ALGPA-encapsulated hDPSC-IPCs maintained viability for at least 336 hours and sustained pancreatic endoderm marker *(NGN3*), pancreatic islet markers (*NKX6.1, MAF-A, ISL-1, GLUT-2* and *INSULIN*), and intracellular PRO-INSULIN and INSULIN expressions for at least 14 days. Functional analysis revealed a glucose-responsive C-peptide secretion of ALG- and ALGPA-encapsulated hDPSC-IPCs at 14 days post-encapsulation. In conclusion, ALG and ALGPA encapsulations efficiently preserved the viability and functionality of hDPSC-IPCs *in vitro* and could be the potential transplantation platform for further clinical application.

Field of Study:	Veterinary Science and technology	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

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CHAPTER I

INTRODUCTION

Importance and rationale

Diabetes mellitus (DM) is classified as a group of chronic metabolic diseases caused by incapability of pancreas to produce/secrete insulin or inability of the body to use insulin effectively. Hyperglycemia is the common clinical sign in diabetic patient and leads to diabetes-related complications. There are two main types of DM. Type I DM, formerly classified as insulin dependent diabetes in veterinary field, is associated with immunological disorder. The defect of pancreatic islet cells is mediated by autoimmune disorders resulting in the absence of insulin. Type II DM, formerly classified as non-insulin dependent diabetes in veterinary field, is caused by the ineffectiveness of pancreatic insulin secretion and/or insulin usage by the body. Type I DM requires prolong and consistent exogenous insulin administration to address hyperglycemia that can increase the risk of diabetes complications, such as

retinopathy, nephropathy, neuropathy, and cardiac disorder (Iqbal et al., 2018; Rattananinsruang et al., 2018). The development of potential therapy for DM during the past decade has mainly focused on the generation of effective insulin-producing cells from various sources of stem cells that can be transplanted into the body (Korsgren, 2017). The success of pancreatic islet transplantation using Edmonton protocol was firstly introduced in 2000 (Shapiro et al., 2006). It can maintain production of endogenous insulin and stabilize blood glucose of patient with type I DM (Shapiro et al., 2006). The long term evaluation of this protocol showed that the patients with type I DM in United States who did the allograft islet transplantation for more than ten years ago had no experience with severe hypoglycemia, opportunistic infection, or lymphoma, showing the safety aspect of long-term application of the Edmonton protocol (Brennan et al., 2016). However, the obstacle for application of the Edmonton protocol also was the limited number of cadaveric pancreas donors, since the patient with type I DM required at least 2-4 islet donors that caused the islet pancreatic transplantation impractical (HealthQualityOntario, 2003). The long-term use of immunosuppressive agent also becomes another negative issue. Side

effects due to the long term use of steroid or immunosuppressants raised a big concern of many investigators (HealthQualityOntario, 2003; Brennan et al., 2016) Therefore, the necessity of an effective protocol to overcome the limitations of Edmonton protocol in aspect of inadequate pancreas islet donor and the long term use of steroid or immunosuppressants should be done. The study should be focused on the exploring the alternative sources of beta cells by generating insulin-producing cells (IPCs) from stem cells and the delivery device that can protect the generated IPCs from immune attack by encapsulation.

Investigation in the field of regenerative medicine over the past few years is mainly focused on adult and extra-embryonic stem cells. Unlike pluripotent embryonic stem cells, adult stem cells have some advantages such as they have no ability to generate tumors (Gauthaman et al., 2012), the ability to modulate immune system that make a good potency for allotransplantation, easy to manipulate and they have no ethical concern (Kalaszczynska and Ferdyn, 2015). Mesenchymal stem cells are characterized as non-hematopoietic, multipotent which have the self-

renewal and multilineage differentiation capability (Al Madhoun et al., 2018). Human dental pulp stem cells (hDPSCs) are dental tissue-derived mesenchymal stem cells that already reported as an alternative source of stem cells (Egusa et al., 2012). The multipotency capability of hDPSCs has beneficial aspect in regenerative medicine. The study showed that hDPSCs have a good potency to differentiate toward insulin-producing cells (IPCs) through chemical induction protocol (Sawangmake et al., 2014).

Another approach for successful stem cell application in type I DM therapy, a huge effort was aimed toward the effective method to transplant generated IPCs. The host immune system may recognize transplanted IPCs through direct or indirect antigen presentation. Encapsulation can be offered as an alternative strategy to protect the IPCs from the body rejection by blockage the interaction between recipient T-cell receptor and transplanted major histocompatibility complex (MHC) of IPCs (Korsgren, 2017). The encapsulation systems use the constituents that contain permeable characteristic, allowing the diffusion of glucose and other nutrients to come inside the encapsulation and also the diffusion of insulin and IPCs' by-product

to come out from the encapsulation. (Richardson et al., 2014). The encapsulation materials that have widely been used in biomedical field, drug delivery, and tissue engineering is alginate (Wang et al., 2009). Alginate encapsulation system has many advantages as it is easy to be applied and contains immuno-isolation property (Paredes Juárez et al., 2014). The material also supports the cells viability (Wang et al., 2009). Previous study had been conducted to evaluate alginate encapsulation enhancing embryonic stem cells differentiation toward IPCs (Wang et al., 2009; Richardson et al., 2014). Another encapsulation material that have been successfully used is pluronic F127. This synthetic hydrogel has various beneficial properties such as non-toxic, biocompatible, and biodegradable (Tam et al., 2011b; Goh et al., 2012; Gasperini et al., 2014). Other advantages of this material are the capability to enhance cell attachment, induce the formation of collagen, and increase the angiogenesis level (Löhr et al., 2003; de Vos et al., 2014). The usage of pluronic F127 as the encapsulation had been reported before, this type of hydrogel can maintain DPSCs viability and also adipogenic and osteogenic differentiation (Diniz et al., 2015).

Therefore, it was shown that pluronic acid has a good potency for stem cells encapsulation system (Diniz et al., 2015)

Until present, the encapsulation technique using combination of alginate and pluronic acid for transplantation platform of stem cell-based diabetes therapy has not been established. Therefore, the present study aimed to establish and validate the encapsulation platform by aliginate/pluronic F127 combination using hDPSC-IPCs model. The obtained know-how information may fulfill the supporting platform for translational stem cell-based diabetes therapy.

Objective of the study

1. To establish the alginate/pluronic F127-based encapsulation technique for serving transplantation platform of translational stem cell-based diabetes therapy.

2. To prove the feasibility of the improvement of cell delivery system production using human dental pulp stem cell-derived insulin-producing cells (hDPSC-IPCs) model.

Keywords (Thai)

เบาหวาน การห่อหุ้ม เซลล์ต้นกำเนิดจากเนื้อเยื่อโพรงฟันมนุษย์ เซลล์สังเคราะห์อินซูลิน การรักษาด้วยเซลล์ต้นกำเนิด

Keywords (English)

diabetes, encapsulation, human dental pulp stem cell, insulin-producing cell, stem cell therapy

Hypothesis

The improvement of cell delivery system using alginate/pluronic F127-based encapsulation technique supports transplantation platform for translational stem cell-based diabetes therapy in human dental pulp stem cell-derived insulin-producing cells (hDPSC-IPCs) model.

CHAPTER II

LITERATURE REVIEW

Pathophysiology of diabetes mellitus

Diabetes mellitus is a complicated metabolic disorder that caused not only by single factor, the most common clinical sign of this disease is associated with hyperglycemia as a consequence of the defect of beta-cells pancreas caused impaired insulin production and/or the action of insulin. The developing number of serious life-threatening health problems will increase in patients with diabetes. Moreover, long-term hyperglycemia can cause various complications in heart, eyes, kidney, nerves, etc. due to generalized vascular damage (Siddiqui et al., 2013). Until present, this disease still become a global concern, International Diabetes Federation (IDF) reported that estimated global prevalence of DM in human medicine is dramatically increase, from 151 million in 2000, to 415 million in 2015. The newest study regarding the projection of estimation worldwide prevalence of diabetes from

2017 until 2045 based on epidemiological data, showed that people with diabetes will be projected increase from 451 million in 2017 to 693 million in 2045 (Cho et al., 2018). Regarding veterinary field, the prevalence of diabetes mellitus in dog in United Kingdom (UK) from August 2009 – June 2012 was 0.34%. This data obtained from 128,210 dogs that were attending in veterinary clinic in UK. The higher risk of DM was reported on neutered male dogs (Mattin et al., 2014). In cat, the data recorded from 193,435 cats in UK (2009-2014) showed that the prevalence of DM was 0.58%, the risk factors of this disease are obesity (above 4 kg of body weight) and age (above 6 years old) (O'Neill et al., 2016).

There are two main types of diabetes mellitus in human medicine, type I diabetes mellitus (type I DM) and type II diabetes mellitus (type II DM), this classification also was used in veterinary field (Gilor et al., 2016). Type 1 DM, formerly known as insulin dependent diabetes mellites which associated with autoimmune disease that cause the inability of beta cell pancreas to produce insufficient insulin. Genetic and environment factors might play the important role in this type of

disease. Investigators proposed that combination of environment factors, such as enteroviruses, diet or toxin can trigger the development of T-cell dependent autoimmunity in sensitive individual with genetic predisposition. Insulin autoantibody (IAA), ICA512/1A-2 and glutamic acid decarboxylase (GAD) antibody are commonly detected in autoimmunity. At the first stage, insulitis with progressive beta cell destruction might be occurred as a pre-diabetes manifestation and changed to overt diabetes. The developing of type I DM is associated with a number of genetic loci in the major histocompatibility (MHC) in human also known as human leukocyte antigen (HLA) region, including the alleles DR3/4, DQ0201/0302, DR 4/4 and DR 0300/0302 (Siddiqui et al., 2013). Type 2 DM, previously known as non-insulin dependent, is complicated metabolic and endocrine disorder. Progressive disorder with variable degree of insulin resistance and pancreatic beta cell dysfunction may cause by interaction between several genetic and environmental factors. Major factor for developing of type 2 DM is overweight and obesity, the insulin resistance will be occurred through several pathways, such as hormonal imbalance (e.g, increasing of leptin and glucagon, reducing of adiponectin), increasing or reducing both pro or anti-

inflammation cytokine (Siddiqui et al., 2013). The physiological state between pro and anti-inflammation cytokine should be maintained to avoid the destruction of beta cell pancreas. The increase of pro-inflammatory cytokine such as IL-11β, TNF-α and IFN-γ will lead the elevation of MnSOD and Caspase-3 expression level that will cause pancreatic beta cells destruction, whereas that pathway can be inhibited by elevation of anti-inflammatory cytokines such as IL-4, IL-13 and IL-10 (Souza et al., 2008; Barlow et al., 2018).

Dental tissue-derived mesenchymal stem cells

Mesenchymal stem cells (MSCs) are characterized as undifferentiated, heterogeneous, non-hematopoietic and have immunosuppressive capacities in both innate and adaptive immunity. Recently, many studies are aimed to find the strategy to reconstruct of damaged tissue and restoring organ malfunction. Until now, many investigators have been successfully isolated and characterized eight types of dental tissue-derived MSCs, leading by the isolation of dental pulp stem cells (DPSCs) from

pulp tissue in 2002 by Gronthos, followed by stem cell from human exfoliated deciduous teeth (SHEED), periodontal ligament stem cell (PDLSCs), dental follicle progenitor cells (DFPCs), alveolar bone-derived MSCs (ABMSCs), stem cells from apical papilla (SCAP), tooth germ progenitor cells (TGPCs), and gingival MSCs (GMSCs) (Liu et al., 2015; Stanko et al., 2018). DPSCs is one of the MSCs population isolated from dental-derived tissue that have unique characteristic, they have high proliferative potential and can differentiate toward osteoblasts, adipocytes, and chondrocytes (Stanko et al., 2018). The MSCs surface marker also expressed in DPSCs such as CD29, CD90, CD105, CD73, and CD44 but, the did not express hematopoietic lineage markers such as CD14, CD34, CD45, CD11b, CD79α, CD19, and HLA-DR (Li et al., 2014; Liu et al., 2015; Stanko et al., 2018). Another study also reported the immunomodulatory activity of DPSCs. This study showed that the ability of DPSCs inhibit the proliferation of stimulation T-cells was higher than BM-MSCs. This action is happened due to the finding that the DPSCs can suppress the proliferation of PBMCs through the TGF-β secretion. In addition, Toll-like receptors (TLRs) also have an

important role to trigger the expression of TGF-β and IL-6. This finding promised that DPSCs are suitable to set the allogenic transplantation by suppressing T-cell-mediated reaction (Li et al., 2014). *In vivo* study, it was showed that both of DPSCs and PDLSCs can maintain their MSC characteristics such as colony-forming ability, cell surface antigen, and multi-differentiation potential. The stability of DPSCs under *in vivo* condition is better when compared with the PDLSCs (Lei et al., 2014). Long term post transplantation of DPSCs has already been done in diabetic animal model (diabetic polyneuropathy), and the results showed that DPSCs may contribute the neuropathological recovery in that animal model (Omi et al., 2017).

New endocrine islet cells production strategy

Nowadays, islet transplantation, as a promising cell-based therapy in DM, becomes a concern of many investigators. The success of Edmonton protocol application for islet transplantation in 2000's raised the opportunities for diabetes mellitus treatment. However, this promising strategy still faced up many burdens, the

lack of cadaveric pancreatic islet donors and the use of long term immunosuppressive agent remained a big problem (Shapiro et al., 2006). To overcome these obstacles, finding alternative source of pancreatic islets is very necessary. Endogenous reprogramming of non-beta-cells toward beta-cells is one of the strategy that can be achieved for this purpose (Zhou and Melton, 2018). The converting of pancreatic acinar cells toward beta-cells involved the combination of three developmental regulators of beta-cells such as, NGN3, PDX1 and MAFA, (Zhou et al., 2008). Another study also showed the success of endogenous reprogramming of alpha-cells toward beta-cells involving adeno-associated virus carrying *PDX1* and *MAFA* (Xiao et al., 2018).

After human embryonic stem cells (hES cells) were successfully cultured in 1998, it leads various study how to convert hES cells toward pancreatic islets. In 2006, new concept was established regarding the induction of somatic stem cells toward induced pluripotent stem cells (iPS cells), triggering the development of adult stem cells investigation (Zhou and Melton, 2018). Basic concept of genetic evidence

of pancreatic endocrine lineage development showed that many transcription factors were involved for islet differentiation. *PDX1* and *Ptf1a* are the representative of the multipotent progenitor markers which will gradually restricted with the expression of *Neurog3 (NGN3)* (all cell type endocrine progenitor). Finally, several transcription factors that involved in beta-cell differentiation are *Nkx2.2, Nkx6.1, Rfx6, Glis3, Insm1,* and *Neurod1*. From this various beta-cell specification transcription factors, *Nkx2.2* may play as a critical regulator. *Nkx2.2* not only involved in the first step of pancreatic progenitors' phase, but also has additional function during endocrine progenitor phase. The absence of *Nkx2.2* in both pancreatic progenitor and endocrine progenitor will cause the absence of beta-cell differentiation (Churchill et al., 2017).

During the last decade, several studies *in vitro* differentiation of MSCs have been conducted by many investigators. Comparative study of chemical induction between BM-MSCs and AT-MSCs differentiation toward IPCs showed that there was no difference in the terms of gene expression level, c-peptide and insulin productions (Gabr et al., 2017). Another study involved combination of differentiation culture medium and adenovirus-mediated expression pancreatic endocrine transcription

factors (*PDX1, MAFA, NGN3* and *PAX1*) could induce gallbladder and cystic duct primary cells (GBCs) toward pancreatic beta-cell-like (Galivo et al., 2017). The Differentiation study of IPCs from DPSC that have been conducted by Sawangmake et al. (2014), showed that hDPSCs had a better differentiation ability compared to hPDLSCs. Similar study on human natal dental pulp stem cells (hNDPSCs) also showed the differentiation ability toward IPCs (Suchánek et al., 2017).

Encapsulation in regenerative medicine

In this past decade, massive study on stem cell-derived insulin producing cells as an alternative strategy to substitute the use of cadaveric pancreatic islet transplantation. Nowadays many obstacles for translational research into clinical application still remain difficult. The establishment of suitable delivery device platform for IPCs is one of the issues that might be a concern of many investigators. Encapsulation as a promising strategy in cell-based therapy offers many advantages and can be applied in various chronic diseases. It aimed to immobilize the cells and

provide immunoisolation ability. Immunoisolation can be achieved by preventing cell-to-cell contact in order to avoid the activation of cytotoxic $CD8^+$ cells, thus transplant rejection can be avoided (Hwang et al., 2016; Saenz Del Burgo et al., 2018) and the negative effect of prolong administration of immunosuppressing agent can be eliminated (de Vos et al., 2006). For the success of encapsulation, the hydrogel polymer should be biocompatible and permeable, allowing oxygen and nutrients to diffuse in and metabolic wastes to diffuse out across the membrane. The earlier study showed that in 0.5 -1% alginate provided the pore diameter range from 7.2 – 8.0 nm, therefore, it will restrict of 21 – 25 kDa for dextran and 78 – 103 kDa of protein, based on this data, it will sufficient to protect the capsule from immunoglobulin G protein (Dembczynski and Jankowski, 2001). Moreover, the protective ability can be increase by increasing the concentration of alginate (Wang et al., 2009).

Currently, two major types of encapsulations are available, macroencapsulation and hydrogel microencapsulation. Macroencapsulation has

limitation with low mass transfer capacity due to the small surface area, while the microencapsulation have larger surface area, thus have better ability of mass transfer capacity (An et al., 2018). Alginate is a material that have been widely used in biomedical science as well as in regenerative medicine. Initial study of alginate microencapsulation was done in 1980, but the result were not satisfactory due to poor biocompatibility (Lim and Sun, 1980). In 1992, another investigator reported that the use of purified alginate can improve the biocompatibility of the materials (Soon-Shiong et al., 1992). Using dog as an animal model for transplantation with pancreatic islet, the result of this study showed that the alginate encapsulation of pancreatic islet could maintain its function for a long term (174 days) without immunosuppression administration (Hwang et al., 2016). The similar result was also performed by applied alginate-encapsulated IPCs in mice (Vegas et al., 2016). *In vitro* study of alginate encapsulation in term of immunological reaction, when the alginate-encapsulated rat MSCs was co-cultured with lymphocytes, the less secretion of IL-2 was reported (Ramezanzadeh Andevari et al., 2018). Several studies related MSCs encapsulation in alginate hydrogel reported that, this platform could maintain

the cell viability in hASCs (Swioklo et al., 2017), enhance osteogenic differentiation (Chan et al., 2013), maintain viability and function of BM-MSC derived IPCs (Sabek et al., 2016).

Another aspect for cell encapsulation is avoiding protrusion of cells from capsules that can lead rejection and fibrotic responses followed by necrosis of encapsulated cells. Multilayer immunoisolation encapsulation of alginate and poly-L-lysine (PLL) could prevent cell protrusion and improve surface of capsules (Bhujbal et al., 2014). Double layer alginate encapsulation using PLL, chitosan or PEG also has another benefit, they provide second barrier against host immune system by decreasing the permeability of larger molecules (Tam et al., 2011a; Ramezanzadeh Andevari et al., 2018). Double encapsulation study using alginate and pluronic acid in hMSCs reported that its platform could maintain cell viability and provide protection from 70 kDa molecules invasion to come inside to the capsule (Chan et al., 2013). Pluronic acid is a synthetic polymer which contain polyethylene oxide (PEO) and polypropylene oxide (PPO) that characterized as a thermosensitive polymer, less-

toxic, biocompatible and biodegradable. Pluronic F127 can also enhance cell adhesion, formation of collagen and have the ability to initiate angiogenesis (Diniz et al., 2015).

Conceptual framework

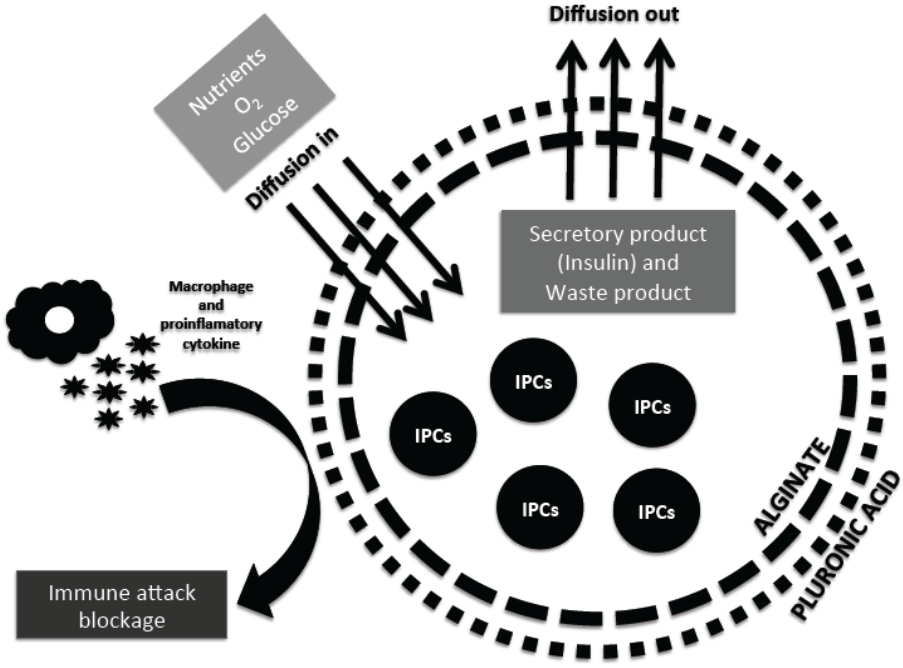


Figure 1 Conceptual framework of insulin-producing cells encapsulation

CHAPTER III

METHODOLOGY

Materials and Methods

According to the proposed objectives, the methodology of the study will be focused as described below.

Specific aim 1: To establish the alginate/pluronic acid-based encapsulation technique for serving transplantation platform of translational stem cell-based diabetes therapy.

Strategy: 1a) Establishment of the alginate/pluronic F127-based encapsulation technique for serving transplantation platform of translational stem cell-based diabetes therapy.

The alginate polymerization was occurred when the alginate solution extruded into $CaCl_2$ solution. Gelling formation was achieved by using Ca^{2+} (divalent cations) as a cross linker agent. Whereas pluronic F127 is thermosensitive hydrogels,

the gelling formation was achieved based on temperature, it will be on liquid phase at 4°C and semi-solid phase at 37°C. In this part of this study, the generation of alginate/pluronic F127-based encapsulation technique was performed.

Firstly, for alginate hydrogels polymerization, the devices that was used for the beads production will be prepared. Sterilized polystyrene syringe and 22G, 24G and 26G needle on the top were used as a bead-generator. Sterile alginate solutions with concentration of 2.0% (w/v) were driven away trough the polystyrene tube, the drops of beads were collected in the autoclaved beaker glass containing sterilized 100 mM $CaCl_2$ under stirring condition for completing the polymerization and avoiding coalescence of beads.

Secondly, to perform double encapsulation of alginate beads with pluronic F127, cell strainer was used to put the alginate beads, then 30% (w/v) of cold (4°C) pluronic acid were immersed into the alginate beads in the cell culture plate in room temperature until polymerization was occurred. This processes were repeated

for several times. Each sample in each process was collected and analyzed under microscope to determine the diameter of the beads.

Strategy: 1b) Efficiency validation of the alginate/pluronic F127-based encapsulation technique for serving transplantation platform of translational stem cell-based diabetes therapy.

For successful encapsulation, the diffusion ability of capsules should be achieved. The encapsulation platform should allow the diffusion in of nutrients (e.g. glucose) and diffusion out of secretory product (e.g. insulin). In the other hand, the encapsulation platform should have ability to maintain cell viability. To prove this concept, diffusion study was performed by using trans well diffusion assay. The layer in the trans well insert barrier will be modified by placing polymerization of alginate/pluronic F127, the thickness of each polymer layer was based on data from the previous strategy. Enzyme-linked immunosorbent assay (ELISA) method was used

to determine the insulin level, while glucose oxidase assay was used to determine the glucose level.

To evaluate the encapsulated cell viability, encapsulated hDPSCs were stained with live/dead staining kit containing blue fluorescent cell permeable-nucleic acid dye for all cells and red fluorescent cell impermeable-nucleic acid dye for dead cells, the results were analyzed under florescent microscope. AlamarBlue™ assay was also performed as a confirmation assay.

Specific aim 2: To prove the feasibility of the improvement of cell delivery system production using human dental pulp stem cell-derived insulin-producing cells (hDPSC-IPCs) model.

Strategy: 2a) Production and encapsulation of human dental pulp stem cell-derived insulin-producing cells (hDPSC-IPCs).

For the IPCs production, hDPSCs were induced for IPCs differentiation by three-step differentiation protocol as performed by Sawangmake et al. (2014).

hDPSCs were induced toward definitive endoderm, pancreatic endoderm, and pancreatic endocrine lineage, respectively. Gene expressions referring pancreatic endoderm (*PDX1* and *NGN3*), pancreatic islets (*NKX6.1, MAF-A, ISL-1, GLUT-2 and INSULIN*) and pancreatic related genes (*GLP-1R and GLUCAGON*) were measured by RT-qPCR. Insulin and C-peptide protein expression was analyzed by immunofluorescent staining.

Obtained IPCs were encapsulated with alginate/pluronic acid-based encapsulation with the adjusted protocols in the previous part of this study.

Strategy 2b) Efficiency validation of the encapsulated hDPSC-IPCs.

To validate the efficiency of encapsulation platform for hDPSC-IPCs, functional and viability assay were performed in this study. Glucose stimulated C-peptide secretion assay was done to prove this concept, C-peptide production from encapsulated hDPSC-IPCs were measured by ELISA. Whereas to evaluate cell viability,

live/dead staining was performed, and the results were analyzed under fluorescent microscope.

Summary of experiments

The summary of experiments in this study is presented in the following flowchart.

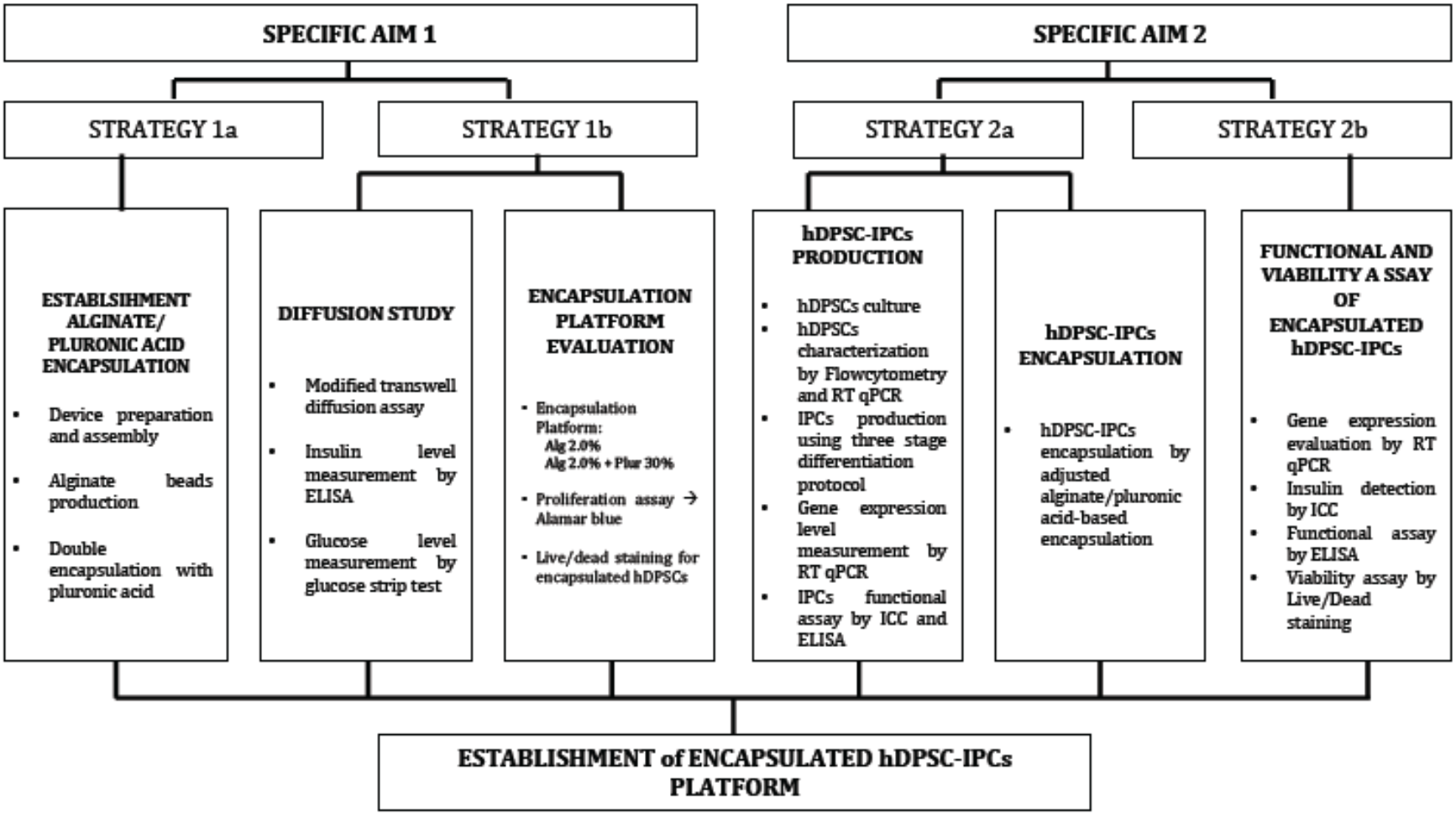


Figure 2 Experimental flowchart of the study

Detailed materials and methods

Establishment the alginate beads production protocol

The alginate beads production used manual extruding method. Sterile polystyrene syringe and 22G, 24G and 26G needle on the top was used as a bead-generator. Sterile alginate solutions with concentration of 2.0% (w/v) were driven away through the polystyrene syringe, the drop of beads were collected in the autoclaved beaker glass containing sterilized 100 mM $CaCl_2$ under stirring condition for completing the polymerization and avoiding coalescence of beads. The number of generated beads per-minute were recorded as well as the speed of peristaltic pump and stirrer. The distance of the tip of needle into surface area of $CaCl_2$ solutions and the diameter of the beads also were recorded.

To perform double encapsulation of alginate beads with pluronic F127, cell strainer was used to put the alginate beads, then 30% (w/v) of cold (4°C) pluronic F127 were immersed into the alginate beads in the cell culture plate in room temperature until polymerization occurred (4 minutes). Each sample in each process

was collected and analyzed under microscope to determine the diameter of the beads.

Trans well diffusion assay

Costar brand (Ref: 3422; Corning, New York, USA) 24-well 8 μm-pore size trans wells were used in this study. Trans well insert barriers were modified by pouring alginate and/or pluronic F127 and leaving it to allow the polymerization. The experimental design of this study was categorized into two types of platform. The first platform used alginate coating modified trans well and the second platform used alginate in combination with pluronic coating modified trans well.

For diffusion studies, trans well insert barrier was assembled into respective well, then 100 μL of 22 mM glucose solution and insulin standard solution 100 μU/mL were placed in the upper side of compartment, in the lower compartment 600 μL PBS were filled in. The trans well culture plates were maintained in 37 ^{o}C incubator for transport study. After 5, 15, 30, and 60 minute-incubation, the solution

in the lower compartments were collected and quantified using glucose liquocolor GOD-PAP method (Human, Wiesbaden, Germany) to evaluate glucose level and to measure insulin level, ELISA kit (Cat. #EZHI-14K; EMD Millipore, Burlington, Massachusetts, USA) was used to quantify after 30 and 60 minute-incubation.

hDSPCs culture

hDPSCs were isolated from human dental pulp tissues of extracted premolars and molars according to wisdom teeth issues under patients' informed consents and ethical approval from the Human Research Ethic Committee, Faculty of Dentistry, Chulalongkorn University (HRE-DCU 2018-054). Tissue explant technique was used, based on previous protocol (Sawangmake et al., 2014). Cells were seeded and maintained in high glucose Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific Corporation, USA) with supplementation of 1% of Antibiotics-Antimycotic (Thermo Fisher Scientific Corporation, USA), 1% GlutaMAX^{TM} (Thermo Fisher Scientific Corporation, USA), and 10% fetal bovine serum (FBS) (Thermo Fisher

Scientific Corporation, USA) under 37 ^{o}C in humidified environment with 5% CO_2 condition. Culture medium was changed every 48 hours. Cells were sub cultured when 80% confluence reached. Four different cell lines in passage 2-5 were used in the experiments.

hDPSCs encapsulation

For the encapsulation platform evaluation, hDPSCs were used in this study. Each type of cells was encapsulated by single encapsulation (alginate – ALG) and double encapsulation (alginate and pluronic F127 - ALGPA). The concentrations of alginate used in this study was 2.0% (w/v) while 30% (w/v) for pluronic. In briefly, 5 x 10^6 cells/mL density was resuspended with 2.0% (w/v) alginate solution. To make alginate beads, the cell suspensions were driven away through polystyrene syringe and 22G needle in the top as a bead's generator. The result of alginate beads were maintained in a stirring of 100 mM $CaCl_2$ solution until the polymerization being completed, the encapsulated cells were washed using Krebs-Ringer Hepes (KRH)

buffer (pH 7.22) containing 2.5 mM $CaCL_2$ (132 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2.6H_2O$, 25 mM HEPES and 2.52 $CaCl_2.2H_2O$). Some alginate encapsulated cells were treated as double encapsulation using 30% (w/v) pluronic F127. Alginate encapsulated cells were placed in cell culture plate, then cold pluronic F127 solution were immersed until polymerization occurs. The polymerization of pluronic F127 solution occurred if the solution temperature increases until the room temperature. All of the encapsulation procedures were performed under aseptic condition. All of encapsulated cells were maintained in culture medium under 37 ^{o}C and 5% CO_2 condition.

Live/Dead staining

Encapsulated hDPSCs were evaluated for their viability using qualitative live/dead fluorescent staining kit, The NUCLEAR-ID® Blue/Red cell viability reagent (GFP-CERTIFIED®) (Enzo Life Science, Farmingdale, New York, USA), according to the

manufacture protocol. The result were interpreted using fluorescent microscope equipped with Carl Zeiss™ Apoptome.2 apparatus (Carl Zeiss, California, USA).

alamarBlue™ assay

The alamarBlue™ (Invitrogen, California, USA) were used to evaluate the encapsulated hDPSCs. Briefly, encapsulated hDPSCs were maintained in normal medium containing 10% (v/v) alamarBlue™ for 20 hours. To determine the percent reduction of alamarBlue™, the solution was measured using spectrophotometer at 570 and 600 nm wavelength.

hDPSCs characterization

hDPSCs were characterized according cell morphology, stemness and proliferative mRNA marker expression, and surface marker analysis. Cell morphology was captured by phase-contrast microscope. RT-qPCR was used to analyzed mRNA

marker expression regarding stemness property (*REX1, NANOG*, and *OCT4*) and proliferative marker (*Ki67*). Cells were then characterized by flow cytometry against MSC surface markers. In brief, the cells were stained with FITC-conjugated anti-human $CD105^+$ antibody (Bio Legend, California, USA), FITC-conjugated anti-human $CD73^+$ (Ecto-5'-nucleotidase) antibody (Bio Legend, California, USA), FITC-conjugated anti-human $CD90^+$ (Thy1) antibody (Bio Legend, California, USA), FITC-conjugated anti-human $CD44^+$ antibody (Bio Legend, California, USA), and FITC-conjugated anti-human $CD45^-$ antibody (Bio Legend, California, USA). FITC-conjugated Mouse IgG1**K** iso-type Ctrl (FC) antibody (Bio Legend, California, USA) was used as an iso-type control for this assay.

hDPSC-IPCs production

IPCs were obtained from hDPSCs that were induced using 3-stage differentiation protocol as performed by Sawangmake et al. (2014). Briefly, 10^6 of hDPSCs were seeded in 60 mm non-treated culture dish (Eppendorf, Hamburg,

Germany) as a single cell suspension. First, the cells were maintained in serum-free medium (SFM)-A for 3 days, after 48 hours media should be changed with the fresh SFM-A for another one day. In the next step, cell was maintained in SFM-B for 48 hours (2 days). The final step of this protocols was the cell were maintained in SFM-C for 5 days and the media should be changed every 48 hours. The main composition of media was SFM-DMEM (Thermo Fisher Scientific Corporation, USA) with different supplementation was added in each media. The supplementation of each media were respectively as follows; SFM-A: 1% BSA (Cohn fraction V, fatty acid free; Sigma, Missouri, USA), 1X insulin-transferrin-selenium (ITS) (Invitrogen), 4 nM activin A (Sigma, Sigma, Missouri, USA), 1 nM sodium butyrate (Sigma, Missouri, USA), and 50 μM beta-mercaptoethanol (Sigma, Missouri, USA); SFM-B: 1% BSA, 1X ITS, and 0.3 mM taurine (Sigma, Missouri, USA); and SFM C: 1.5% BSA, 1X ITS, 3 mM taurine, 100 nM glucagon-like peptide (GLP)-1 (Sigma, Missouri, USA), 1 mM nicotinamide (Sigma, Missouri, USA), and 1x non-essential amino acids (NEAAs) (Sigma, Missouri, USA).

Reverse transcription-quantitative real time PCR

Cellular RNA was extracted using Trizol reagent (Invitrogen), then 1 μg RNA was converted into cDNA using reverse transcriptase enzyme kit (Promega, Wisconsin, USA). Gene expression level was detected by reverse transcription-quantitative real time PCR (RT-qPCR) by FastStart® Essential DNA Green Master® (Roche Diagnostic, Risch-Rotkreuz, Switzerland) Using CFX96™ real time PCR detection system (Bio-Rad, California, USA). The mRNA value was presented as relative mRNA expression by normalized to 18S ribosomal RNA and the control. The formula $2^{-\Delta\Delta Ct}$ was used to calculate normalization and fold change. The primer sequences that were used in this study are based on the previous study (Sawangmake et al., 2014) (presented in Table 1).

Table 1 Primer sequences

Genes	Accession number	Forward Primer Reverse Primer	Length (bp)	Tm (°C)
NANOG	NM_024865.4	5' – ATGCCTCACACGGAGACTGT – 3' 5' – AAGTGGGTTGTTTGCCTTTG – 3'	103	61.19 57.31
OCT-4	NM_002701.6	5' – TCGAGAACCGAGTGAGAGG – 3' 5' – GAACCACACTCGGACCACA – 3'	125	58.14 59.56
REX-1	NM_174900.5	5' – TGGGAAAGCGTTCGTTGAGA -3' 5' – CACCCTTCAAAAGTGCACCG – 3'	90	59.89 59.97
Ki67	NM_001145966.1	5' – TCAGAATGGAAGGAAGTCAACTG – 3' 5' – TCACTCTCATCAGGGTCAGAAG – 3'	105	58.35 58.90
PDX-1	NM_000209.4	5' – AAGCTCACGCGTGGAAAGG – 3' 5' – GGCCGTGAGATGTACTTGTTG – 3'	145	57.89 52.38
NGN-3	NM_020999.3	5' – CGGTAGAAAGGATGACGCCT – 3' 5' – GGTCACTTCGTCTTCCGAGG – 3'	138	59.54 60.11
NKX-6.1	NM_006168.2	5' – TTGGCCTATTCGTTGGGGAT – 3' 5' – GTCTCCGAGTCCTGCTTCTTC – 3'	125	59.08 60.14
MAFA	NM_201589.3	5' – GCACATTCTGGAGAGCGAGA – 3' 5' – TTCTCCTTGTACAGGTCCCG – 3'	102	59.83 58.74
ISL-1	NM_002202.2	5' – TCCCTATGTGTTGGTTGCGG - 3' 5' – TTGGCGCATTTGATCCCGTA – 3'	200	60.32 60.39

GLUT-2	NM_000340.1	5' – GGTTTGTAACTTATGCCTAAG – 3' 5' – GCCTAGTTATGCATTGCAG – 3'	211	52.25 54.24
INSULIN	NM_000207.2	5' – CCGCAGCCTTTGTGAACCAACA – 3' 5' – TTCCACAATGCCACGCTTCTGC – 3'	215	64.34 64.45
GLP-1R	NM_002062.4	5' – TCGCTGTGAAAATGAGGAGGA – 3' 5' – TCACTCCCGCTCTGTGTTTG – 3'	189	59.38 60.25
GLUCAGON	NM_002054.4	5' – TTATTTGGAAGGCCAAGCTGC – 3' 5' – GTCTGCGGCCAAGTTCTTCA – 3'	110	59.45 60.88
18S	NR_003286.2	5' – GTGATGCCCTTAGATGTCC – 3' 5' – CCATCCAATCGGTAGTAGC – 3'	233	55.04 54.86

Immunocytochemistry study

For immunocytochemistry study, cell colony (hDPSC-IPCs) were fixed in cold methanol, then permeabilized with 0.1% Triton®-X100 (Sigma, Missouri, USA) in PBS and incubated with 10% donkey serum in PBS for 1 hour. Mouse anti-human pro-insulin (EMD Millipore, Burlington, Massachusetts, USA) and mouse anti-human insulin (EMD Millipore, Burlington, Massachusetts, USA) at the dilution 1:100 were used as primary antibody, and Alexa Fluor Plus 488-conjugated Goat Anti-Mouse IgG (Termo

Fisher Scientific, Waltham, Massachusetts, USA) at dilution 1:1000 was used as secondary antibody. For nucleus staining, DAPI (0.1 μg/mL) was used. Interpretation of this assay was performed by analyzing under fluorescent microscope equipped with Carl Zeiss™ Apoptome.2 apparatus (Carl Zeiss, California, USA). Microscopic evaluation were performed by detection the localized antigen in the cell colony.

Functional test for hDPSCs-IPCs

For the functional test of generated IPCs, glucose-stimulated C-peptide secretion assay was performed. The IPCs were incubated in normal KRH $NaHCO_3$ (KRBH) pH 7.22 (120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2.2H_2O$, 1.1 mM $MgCl_2.6H_2O$ and 25 mM $NaHCO_3$) 5.55 mM of glucose anhydrous (Sigma, Missouri, USA) in KRBH and 22 mM glucose anhydrous (Sigma, Missouri, USA) in KRBH. Enzyme-linked immunosorbent assay (ELISA) was used to detect the amount of C-peptide level based on manufacturing protocol. In briefly, this assay was based on the binding of human C-peptide in the sample by coated monoclonal antibody in the 96 well-

plate. Biotinylated anti-human C-peptide monoclonal antibody will bind into captured human C-peptide. This detection system of this assay will use poly-HRP-labeled streptavidin enzyme and TMB substrate. Finally, the enzyme activity was measured by ELISA reader with the absorbency 450 nm. Reference standard curve (r^2 = 0.9918) was generated by using standard solution provided by manufacture.

hDPSC-IPCs encapsulation

For IPCs encapsulation, generated IPCs from 10^6 hDPSCs were collected and resuspended with 2.0% (w/v) alginate solution. Alginate encapsulation procedure was the same protocol as described in the previous step. Some of encapsulated hDPSC-IPCs was used for double encapsulation with 30% (w/v) of pluronic F127 as mentioned in previous step. All of the encapsulation protocols were performed under aseptic condition, then encapsulated cells were maintained in SFM-C under 37 ^{o}C and 5% CO_2 condition for further evaluations.

The experimental design of this study was described as follow: 1) non-encapsulated hDPSC-IPCs; 2) encapsulated hDPSC-IPC using 20% alginate (ALG) and 3) encapsulated hDPSC-IPC using 20% alginate and 30% pluronic (ALGPA).

Capsule dissolution

Prior to post encapsulation evaluation, encapsulated hDPSC-IPCs were dissolved by incubate in dissolving buffer (0.1 M EDTA and 0.2 M $C_6H_5Na_2O_7.2H_2O$, pH 7.4) for 5 minutes in 37°C. hDPSC-IPCs from degraded alginate beads were washed in PBS three times 3 minutes each and processed for further analysis (RT-qPCR and Immunocytochemistry staining).

Glucose-stimulating C-peptide secretion assay

For the functional test of encapsulated hDPSC-IPCs, glucose-stimulated C-peptide secretion assay was performed in this study. The encapsulated hDPSC-IPCs

were maintained in normal KRBH, 5.5 mM and 22 mM of glucose anhydrous (Sigma, Missouri, USA) in KRBH. Enzyme-linked immunosorbent assay (ELISA) was used to detect the amount of C-peptide level based on manufacturing protocol. In brief, this assay is based on the binding of human C-peptide in the sample by coated monoclonal antibody in the 96 well-plate. Biotinylated anti-human C-peptide monoclonal antibody will bind into captured human C-peptide. This detection system of this assay used poly-HRP-labeled streptavidin enzyme and TMB substrate. Finally, the enzyme activity was measured by ELISA reader with the absorbency 450 nm. Reference standard curve (r^2 = 0.9918) was generated by using standard solution provided manufacture.

hDPSC-IPCs viability assay

Encapsulated hDPSC-IPCs were evaluated for their viability using qualitative live/dead fluorescent staining kit, The NUCLEAR-ID® Blue/Red cell viability reagent (GFP-CERTIFIED®) (Enzo Life Science, Farmingdale, New York, USA), according to the

manufacture protocol. The results were interpreted using fluorescent microscope equipped with Carl Zeiss^{TM} Apoptome.2 apparatus (Carl Zeiss, California, USA).

Statistical analyses

The results of this study were presented as box plot with whisker bar. Non-parametric statistical analysis was used. Four replicates (n=4) were used in the study. The statistical analysis was performed by using SPSS Statistics. To compare two independent groups, the Mann Whitney U test was employed. Kruskal Wallis test and pairwise comparison was used for three or more group comparison. Statistically significant difference was recognized when *p*-value < 0.05.

CHAPTER IV

RESULTS AND DISCUSSION

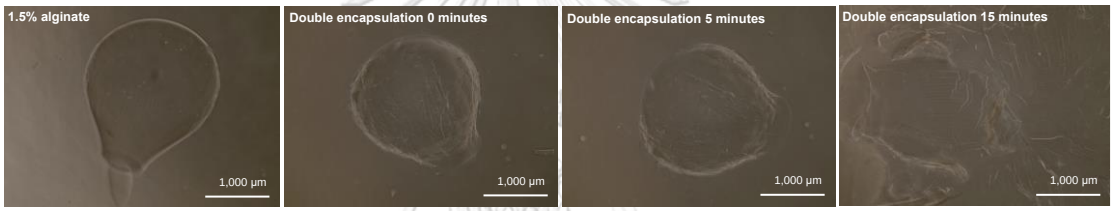
Results

Alginate and Pluronic F127 Concentration Determination

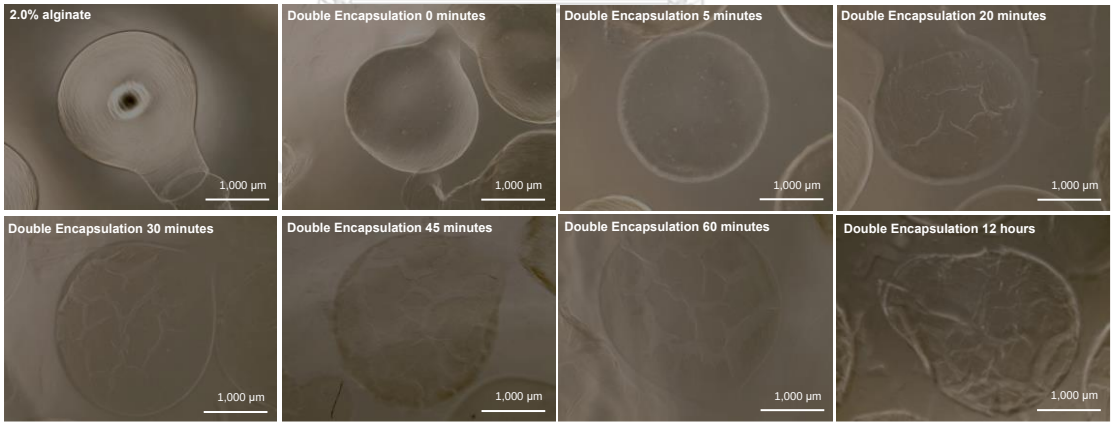
The initial step of this study was to determine the appropriate protocol for cell encapsulation. In this step, four platforms of encapsulation were tried to obtain the best results. These four platforms were 1) 1.5% alginate bead with 20% pluronic F127 as a coating; 2) 2.0% alginate bead with 20% pluronic F127; 3) 1.5% alginate bead with 30% pluronic F127 and 4) 2.0% alginate bead with 30% pluronic F127. The result of this study is described in **Figure 3**. Based on this study, the best encapsulation platform was evaluated based on the production of beads after the application of pluronic F127 as coating material. Stable beads were evaluated time by time until 72 hours postproduction. The beads were unstable when 1.5% alginate and 20% pluronic F127 was used, the beads destroyed 15 minutes post coating.

Similar results were also shown by using same concentration of alginate and 30% pluronic F127. The best platform was achieved by using 2.0% alginate for beads production and 30% pluronic F127 as coating material for double encapsulation, this platform was used for further step of this study.

1.5% Alginate and 20% Pluronic F127



2.0% Alginate and 20% Pluronic F127



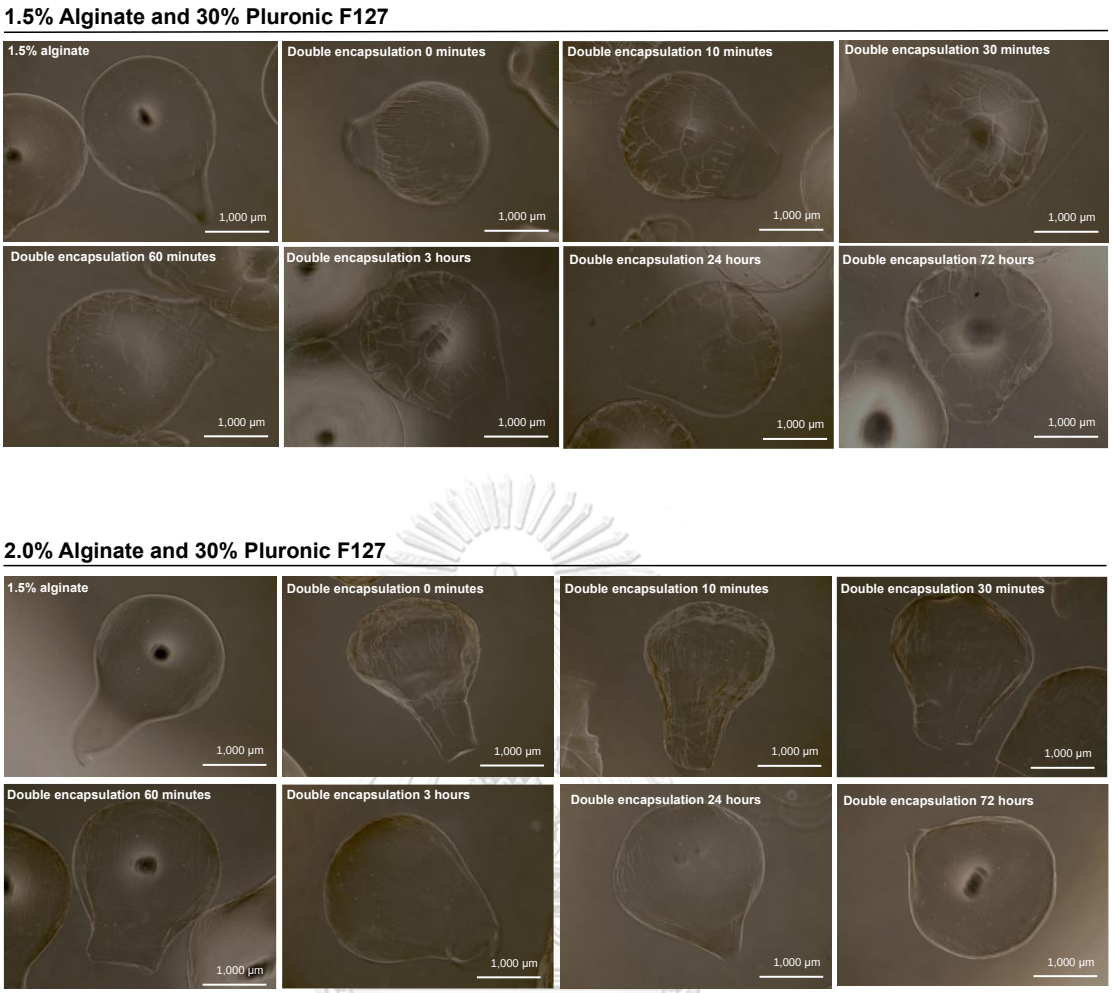


Figure 3 Aginate/pluronic F127-based encapsulation protocol trial.

Morphological appearances of ALG and ALGA beads using different concentration of alginate (1.5 and 2.0%) and pluronic F127 (20 and 30%) was evaluated by light microscope.

Effect of Alginate/Pluronic F127-based encapsulation toward viability of hDPSCs

For serving the hDPSC-IPC encapsulation, the diameter of needle plays an important role. **Figure 4** visualized the effect of different size of needle (22G, 24G and 26G) on the diameter of the beads. The measurement of diameter was assessed at day 0, 7, 14 and 28. The results showed that manual extruding protocol using different needle size resulted in different beads diameter (**Figure 4**). At day 0 there is no significant difference on bead diameter produced from all different size of needle between alginate (ALG) and pluronic F127 coated alginate (ALGPA) encapsulation. The 22G needle resulted in the highest diameter in both encapsulation platform, respectively 2,732.27 ± 44.62 mm and 2,770.16 ± 34.08 mm for ALG and ALGPA, while 26G resulted the lowest diameter size, 2,355.22 ± 32.41 mm and 2,379.70 ± 70.44 mm for ALG and ALGPA. Similar trend was also shown in day 7, 14 and 28, but with significant differences of diameter was noted between ALG and ALGPA encapsulation. The shrinkage was not observed during this experiment (from all needle size), however in the end of experiment the percentage swelling of both encapsulations were 2.61 – 4.50 % and 7.64 – 10.91% for ALG and ALGPA

respectively. The swelling percentage was assessed by calculating the percentage of final diameter at day 28 minus initial diameter at day 0, then compared to the baseline (diameter at day 0).

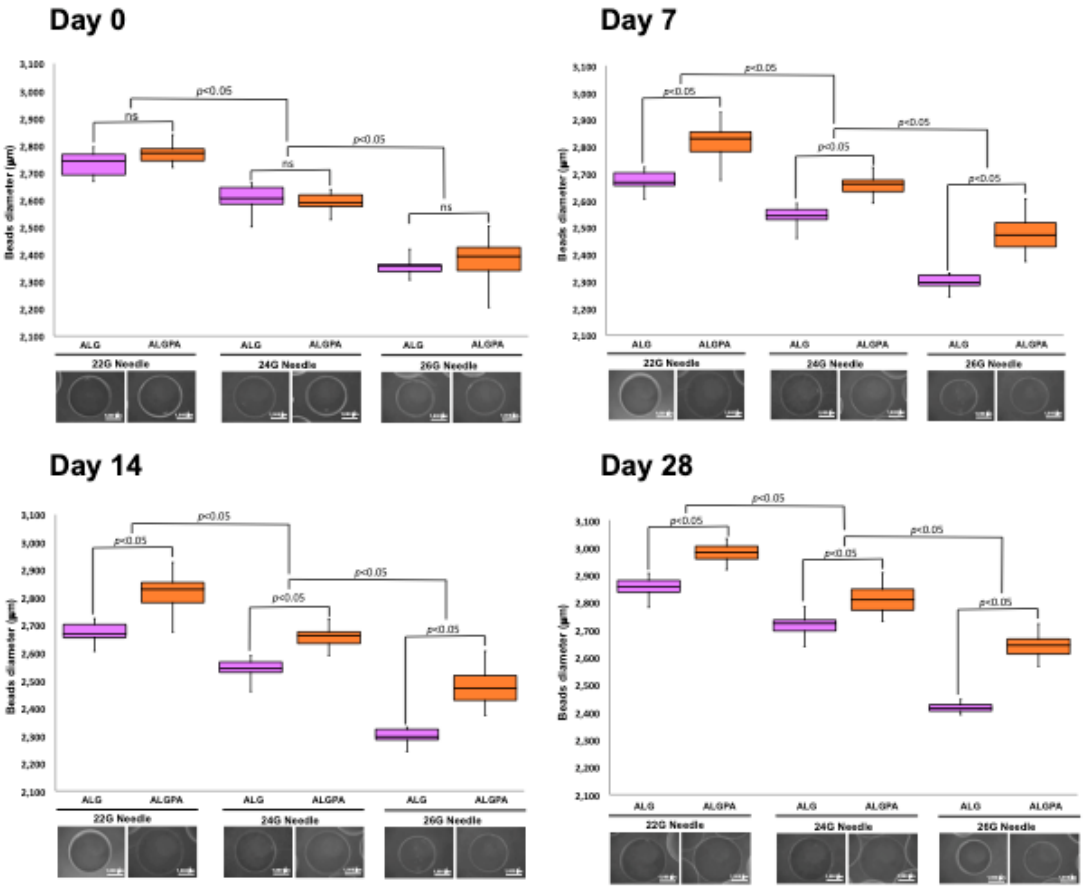


Figure 4 Bead diameter morphology evaluation of ALG and ALGPA.

Bead diameters and morphological appearances of ALG and ALGPA generated by manual extrusion through different sizes of needle (22G, 24G and 26G) at day 0, 7, 14 and 28 were illustrated. Bars indicated statistical relationship, $p<0.05$ or not significant (ns).

To evaluate the diffusion ability of ALG and ALGPA, modified trans well diffusion assay was performed. **Figure 5a** showed the glucose diffusion in both encapsulation platforms, after 60 minutes incubation. The percentage of glucose diffusion were 62.90 – 76.10% and 64.50 – 78.40% for ALG and ALGPA respectively, these results were significantly different compare to the baseline (at 15 minutes). Significant difference was noted on insulin diffusion study. As shown in **Figure 5b**, the percentage of insulin diffusion in ALG and ALGPA encapsulation were 44.00 – 54.40% and 48.00 – 61.90%.

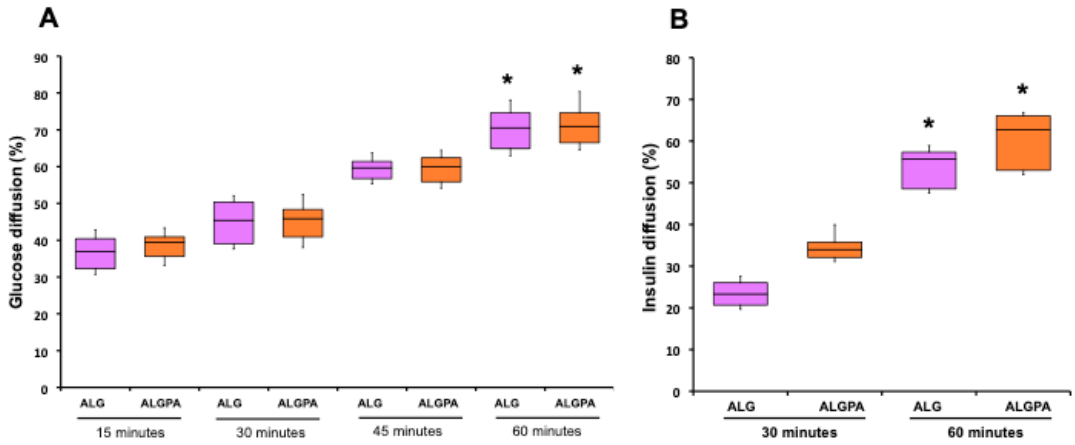
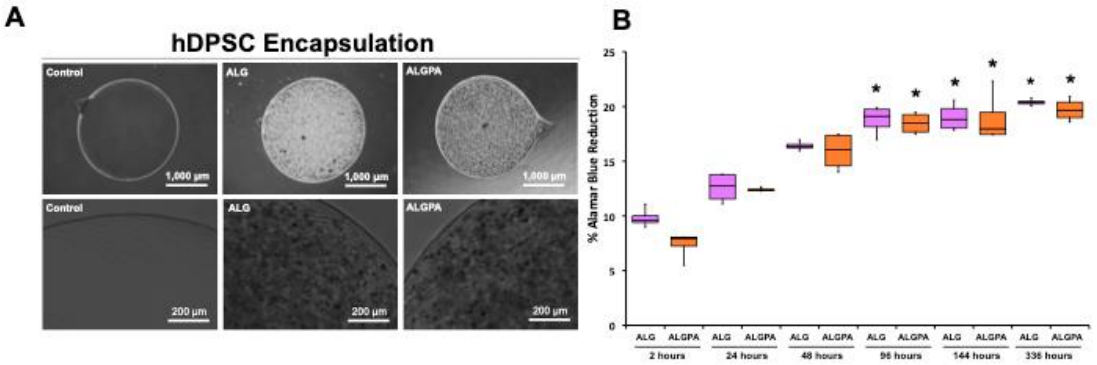


Figure 5 Glucose and insulin diffusion efficiency of ALG and ALGPA.

The percentages of glucose diffusion (A) and insulin diffusion (B) across ALG and ALGPA as determined by modified trans-well diffusion assay were illustrated. The asterisks indicated significant difference, comparing with the initial condition at 15 minutes (glucose diffusion) and 30 minutes (insulin diffusion) ($p<0.05$).

Visualization of encapsulated hDPSC morphology under inverted microscope showed that the hDPSCs were dispersed into single cells inside the alginate capsule (**Figure 6a)**. Viability assessment of post encapsulation of hDPSCs was performed by quantitative method of alamarBlue™ assay and live/dead staining. Metabolic activity

of hDPSCs was assessed by alamarBlue™ assay. **Figure 6b** showed that the metabolic activity of hDPSCs in both encapsulations were slightly increase at 24 - 96 hours post encapsulation and tend to be stable until 336 hours. Consistent result was visualized in **Figure 6c** when encapsulated hDPSCs were stained by using live/dead staining kit.



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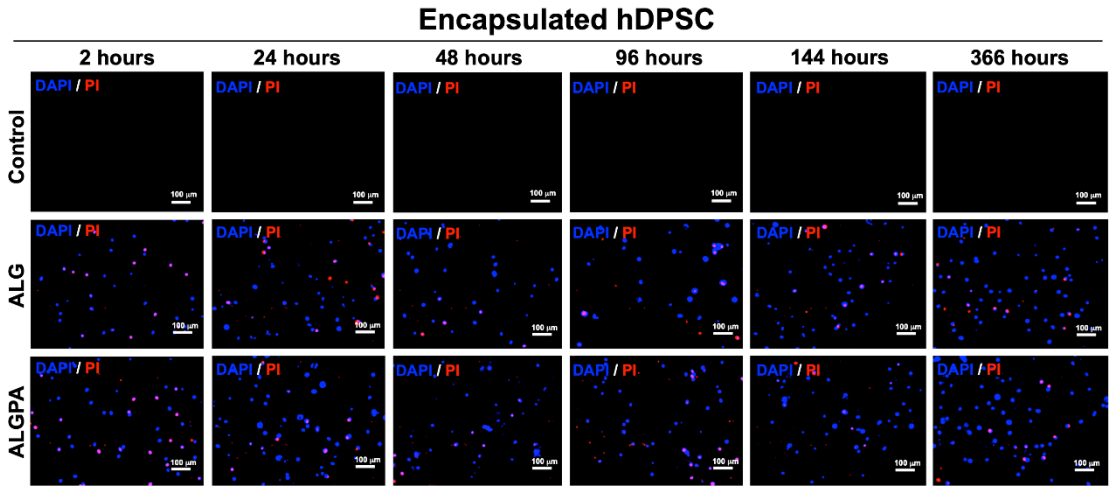


Figure 6 Morphology and viability evaluation of encapsulated hDPSCs using ALG and ALGPA.

ALG- and ALGPA-encapsulated hDPSC morphologies were evaluated under light microscope (A). The viability of encapsulated hDPSCs was also determined by alamarBlue™ assay (B) and live/dead staining (C). The asterisks indicated significant difference, comparing with initial condition at 2 hours ($p<0.05$).

hDPSC Characterization

Morphological assessment of hDPSCs under normal culture medium were performed. Plastic adherent and fibroblastic cells were observed from hDPSCs used in this experiment (**Figure 7a**). In addition, RT-qPCR was employed to analyse the expression of stemness and proliferative genes. The results showed that the stemness genes which were *REX1, NANOG* and *OCT4* and proliferative gene, *Ki67* were expressed by hDPSCs (**Figure 7b**). The surface markers of hDPSCs were determined by using flow cytometry. Several mesenchymal stem cell surface

markers CD90 (99.97% ± 0.03), CD73 (99.87% ± 0.12) and CD44 (99.93% ± 0.08) was strongly expressed. However, CD105 (20.80% ± 11.39) expression was relatively lower in hDPSCs. Furthermore, CD45 surface marker expression in hDPSCs were extremely low (**Figure 7c**).

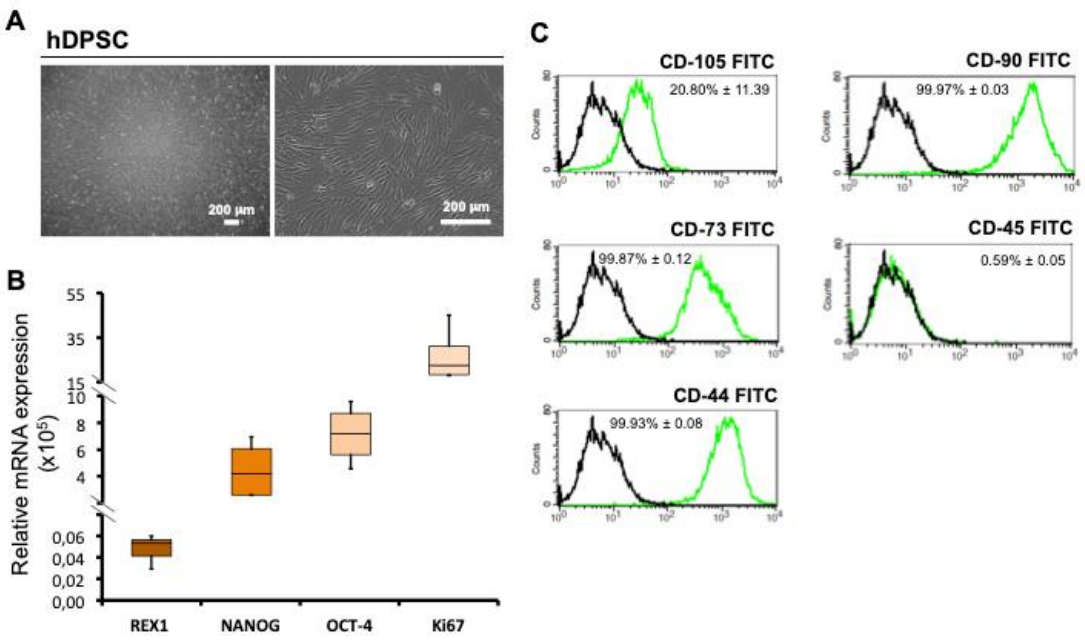


Figure 7 Morphology and characterization of hDPSCs.

Morphological features of hDPSCs with low and high magnification were evaluated under light microscope (A). The mRNA expression of stemness property genes (*REX1, NANOG* and *OCT4*) and proliferation gene (*Ki67*) were determined by RT-qPCR (B).

Expression of surface marker reflecting MSC property was also determined using flow cytometry (C).

In vitro differentiation hDPSCs toward IPCs

Ten-day induction protocol was employed in this experiment. Initial differentiation was observed at day 3, where the single cell suspension hDPSCs at day 0 were changed into cell aggregates. Further, the development of cell aggregates was noted at day 5, day 7 and day 10. In the end of induction protocol, big and dense cell aggregates were observed (**Figure 8a**). The total colony count of cell aggregates obtained from this experiment were 424 – 581 colonies in which 60.68 – 74.70% that had diameter more than 100 μm (100. 81 – 303. 43 μm), while small colonies (diameter less than 50 μm) were only 1.55 – 12.10% (**Figure 8b and 8c**). The results were further analyzed by immunocytochemistry staining (**Figure 8d**). Cell aggregates derived from hDPSCs expressed both intracellular pro-insulin and insulin.

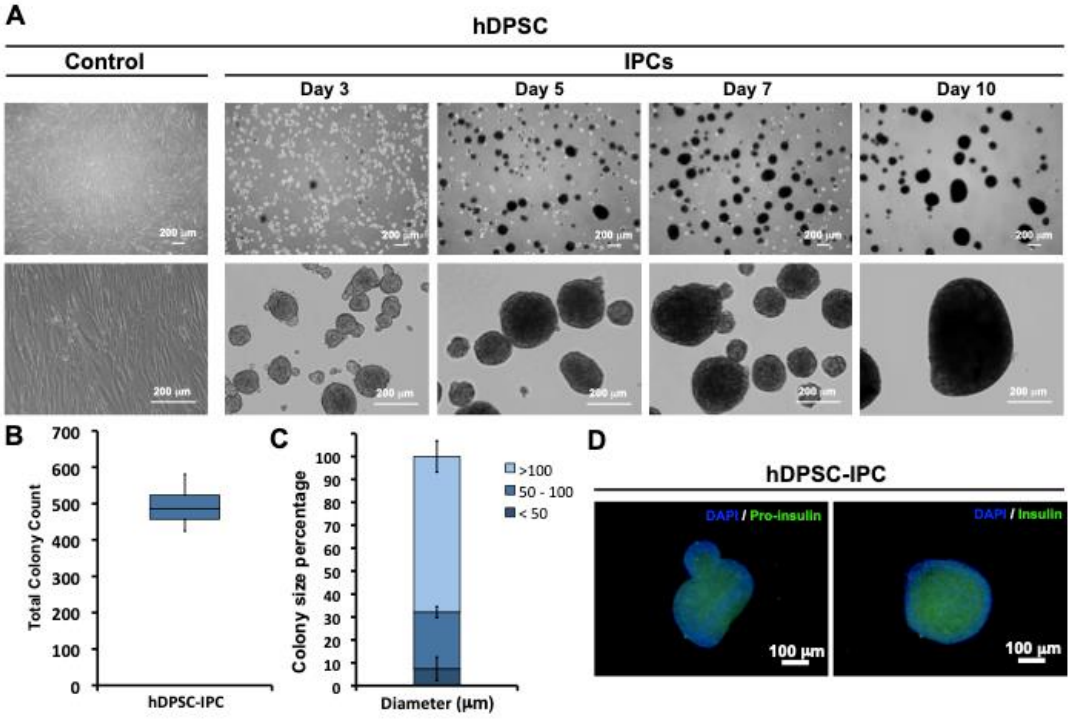


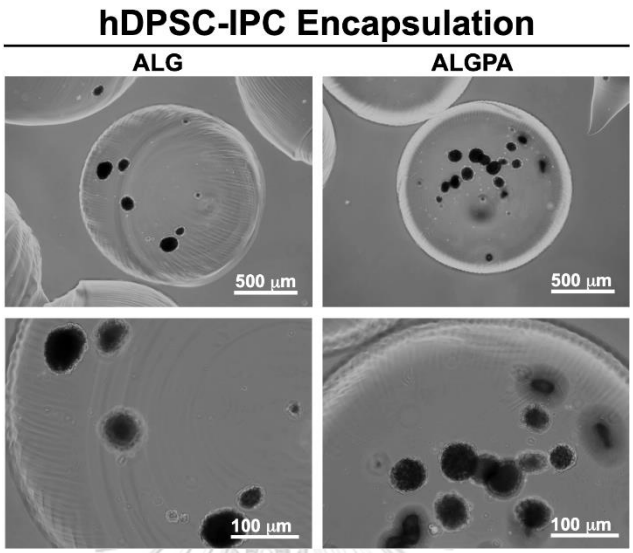
Figure 8 *In vitro* differentiation of hDPSCs toward IPCs.

Different morphological appearances of hDPSCs after induction using three-stage differentiation protocol were illustrated at day 3, 5, 7 and 10 (A). The total colony number (B) and the colony size distribution (C) were also investigated. The expression of PRO-INSULIN and INSULIN were evaluated by immunocytochemistry staining (D).

In Vitro evaluation Alginate/pluronic F127-based encapsulation on viability of hDPSC-IPCs

Microscopic observation of encapsulated hDPSC-IPCs in both encapsulation platform was performed (**Figure 9a**). The findings showed that cell aggregates derived from hDPSCs were unequally dispersed into the alginate beads, cell aggregates damage was not observed. Further analysis to evaluate the viability of encapsulated hDPSC-IPCs was done by using live/dead staining (**Figure 9b**). Post encapsulation staining showed that hDPSC-IPCs were capable to survive at least 336 hours. However, small mass of non-viable cells was observed in the core of encapsulated cell aggregates.

A


hDPSC-IPC Encapsulation
ALG
ALGPA
500 µm
500 µm
100 µm
100 µm

B

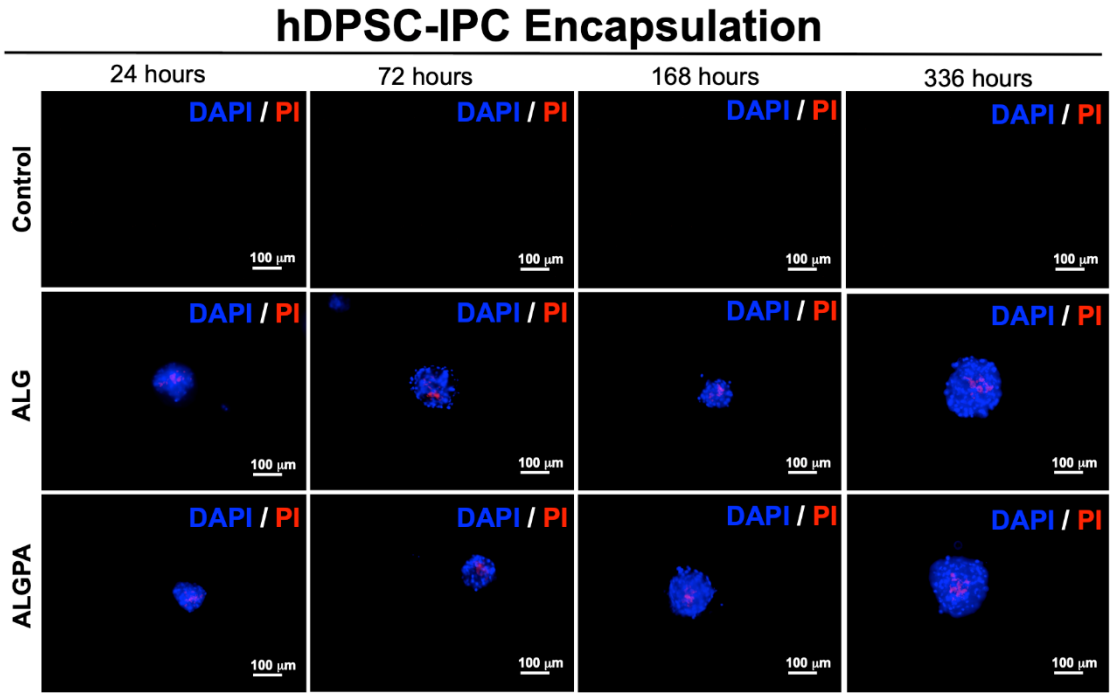

hDPSC-IPC Encapsulation
24 hours
72 hours
168 hours
336 hours
Control
ALG
ALGPA
DAPI / PI
100 µm

Figure 9 Morphology and viability evaluation of encapsulated hDPSC-derived IPCs using ALG and ALGPA.

Encapsulated hDPSC-derived IPCs morphologies in both ALG and ALGPA were illustrated (A). The viability evaluation of encapsulated hDPSC-derived IPCs was also determined by live/dead staining (B).

Alginate/pluronic F127-based encapsulation mainained functionality of hDPSC-IPCs

Post encapsulation evaluation of hDPSC-IPCs was performed by RT-qPCR to analyze the expression of pancreatic endoderm, pancreatic islet and pancreatic related genes. Encapsulated hDPSC-IPCs in ALG and ALGPA expressed pancreatic endoderm gene marker (*NGN3*) at day 7 and day 14 post encapsulation. Unfortunately, *PDX1* gene was not observed in this study (**Figure 10a**). The expression of pancreatic islet genes was noted at day 7 and 14. *NKX6.1, MAF-A, ISL-1, GLUT-2* and *INSULIN* genes were expressed in both encapsulated hDPSC-IPCs with

ALG and ALGPA (**Figure 10b**). Further, at day 7 and 14 in both encapsulation platform, the pancreatic-related gene, *GLP-1R* was detected, while the expression *GLUCAGON* gene was not significantly different compare to undifferentiated cells (**Figure 10c**). All gene expression patterns regarding pancreatic endoderm, pancreatic islet and pancreatic-related genes of encapsulated hDPSC-IPCs were not significantly different compared to initial condition before encapsulation (non-encapsulation).

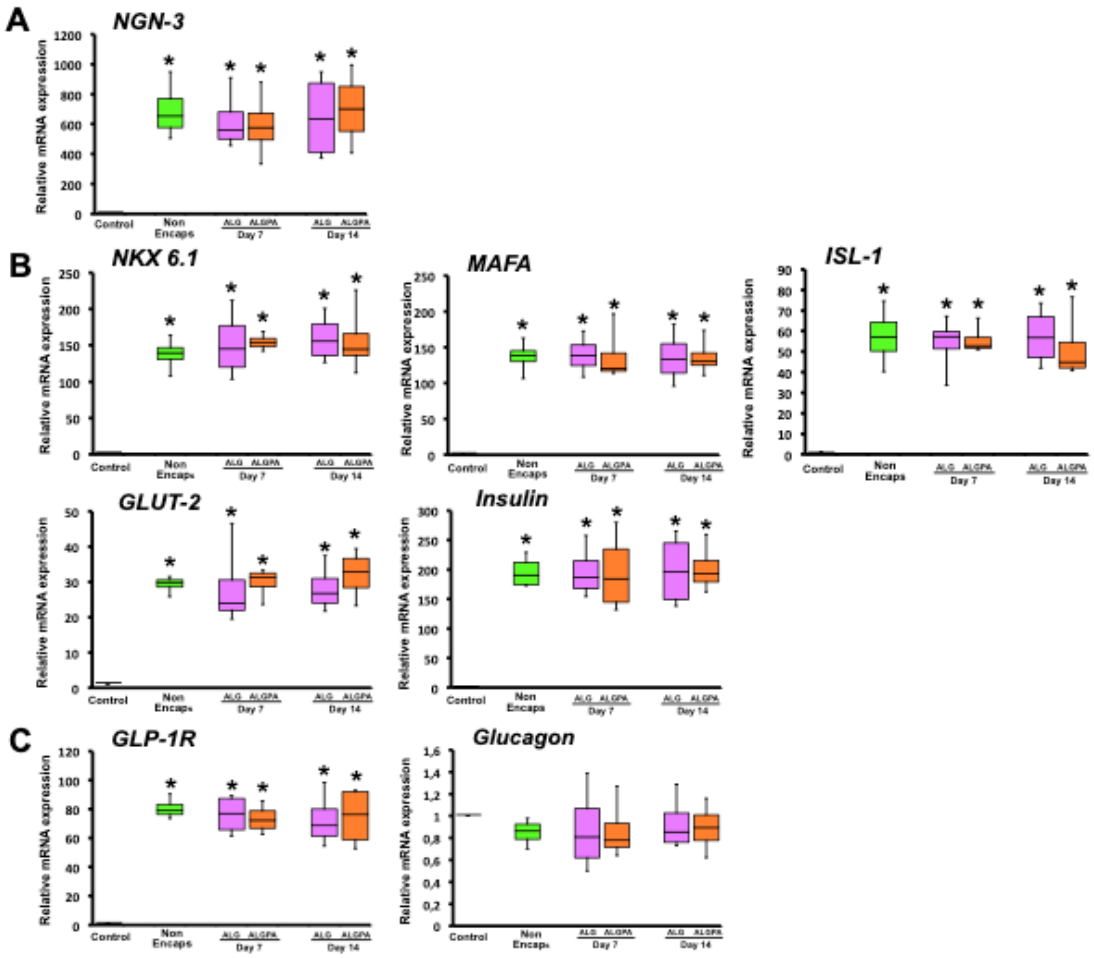


Figure 10 Pancreatic gene expression analysis of encapsulated hDPSC-derived IPCs using ALG and ALGPA.

The mRNA expression of pancreatic endoderm marker (*NGN3*) (A), pancreatic islet markers (*NKX 6.1, MAFA, ISL-1, GLUT-2* and *INSULIN*) (B) and pancreatic related markers (*GLP-1R* and *GLUCAGON*) (C) by ALG- and ALGPA-encapsulated hDPSC-derived IPCs were determined by RT-qPCR at day 7 and 14 post encapsulation. The

asterisks indicated significant difference, comparing with undifferentiated cell ($p<0.05$).

Immunocytochemistry staining was employed to confirm the results. hDPSC-IPCs were stained before encapsulation and post encapsulation (7 and 14 days). **Figure 11** visualized that in both encapsulation ALG and ALGPA, intracellular pro-insulin and insulin were expressed at day 7 and 14 post encapsulation. The similar expression of intracellular pro-insulin and insulin also was observed in hDPSC-IPCs at the initial condition (before encapsulation).

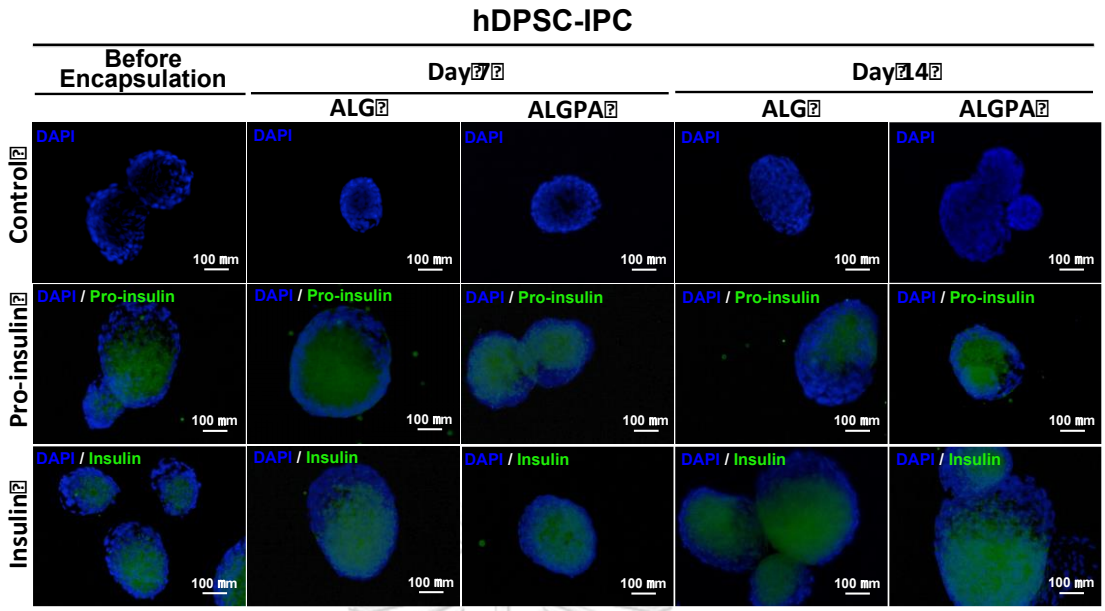


Figure 11 Post encapsulation evaluation by using Immunocytochemistry staining.

The expression of PRO-INSULIN and INSULIN by ALG- and ALGPA-encapsulated hDPSC-IPCs were evaluated by immunocytochemistry staining at day 7 and 14 post encapsulation.

In vitro evaluation regarding the function of encapsulated hDPSC-IPCs was assessed by using glucose-stimulated C-peptide secretion (GSCS) assay. The results of this experiments were compared with the initial condition of hDPSC-IPCs (before encapsulation). At day 14 post encapsulation, hDPSC-IPCs in both ALG and ALGPA

were challenged with three different condition, normal KRBH, 5.5 mM and 22 mM glucose in KRBH. The result showed that C-peptide was secreted by hDPSC-IPCs in both ALG and ALGPA encapsulation conditions when incubated with 22 mM glucose in KRBH. This significant difference was observed when compared with the C-peptide secretion in normal KRBH and 5.5 mM glucose in KRBH conditions, suggesting trend of glucose-responsive function. In this study, no significant difference was observed regarding to the *in vitro* functional evaluation of hDPSC-IPCs before and after encapsulation (**Figure 12**).

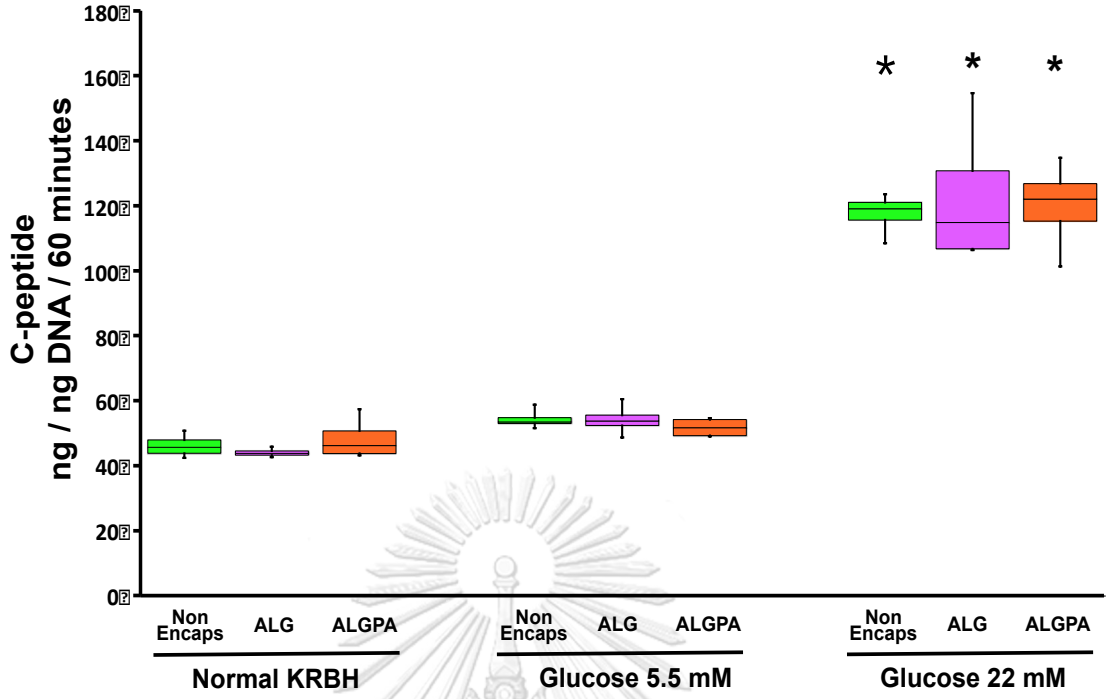


Figure 12 Glucose stimulated C-peptide secretion assay.

C-peptide secretion was also determined by glucose-stimulated C-peptide secretion (GSCS) assay. The asterisks indicated significant difference, comparing with normal and glucose 5.5 mM in KRBH ($p<0.05$).

Discussion

The concept of cell encapsulation was first introduced almost nine decades ago. Thus, some clinical trials regarding the application of cell encapsulation have not led any approval for clinical application (Ashimova et al., 2019). Type I diabetes mellitus (T1DM) is an intractable disease caused by the defect of pancreatic β-cells. Currently, stem cell-based diabetes therapy offers promising strategy for T1DM and eliminate the obstacles of Edmonton protocols (Dimitrioglou et al., 2019). Encapsulation platform can serve to provide viable allogeneic or xenogeneic cells for its purpose (Ashimova et al., 2019).

The first part of this study, the main objective is focused on the establishment and validation of encapsulation platform. Four different protocols were evaluated to obtain the best platform to serve IPCs encapsulation. When 1.5% alginate incorporated as a bead production followed by pluronic F127 as a coating material, this platform produced unstable beads. The produced beads cracked and dissolved within 15 minutes after the application of 20% pluronic F127 and the

similar result was obtained after the application of 30% pluronic F127. Another encapsulation platform with 2.0% alginate beads that could produce more stable beads, but the result was not satisfactory when 20% pluronic F127 was applied. When the 30% pluronic F127 was applied, the stable coated beads could be produced, and this platform showed stable beads until 72 hours under phosphate buffer solution in 37°C and 5% CO_2 condition. Based on the results of this study the best platform for encapsulation was obtained by using 2.0% alginate as a bead production and 30% pluronic F127 as coating material. Therefore, this selected platform will be used for the further study. Based on previous studies regarding the biocompatibility of alginate, concentration ranging from 0.5 – 10% are the most preferable in scafold production (Hoesli et al., 2017; Zareifard et al., 2018; Li et al., 2019; Chawla et al., 2020) and that, 2.0% alginate could maintain the best cell viability rate (Bijan Nejad et al., 2017; Mitry et al., 2017; Safley et al., 2018; Zareifard et al., 2018; Xiang et al., 2019). The concentration of 30% pluronic F127 was chosen in this study because it has shorter gelling time compare to 20% pluronic F127. This finding was in aggrement with previous study that the gelling time can be reduced by

increasing the concentration of pluronic F127 (Gioffredi et al., 2016). Furthermore the acceptable concentration of pluronic F127 for tissue engineering construction varied between 20 – 40% (Khattak et al., 2005; Diniz et al., 2015; Gioffredi et al., 2016; Kaisang et al., 2017). To ensure the reproducibility of selected protocol, the reproducibility test was performed in this study. The beads production with selected protocol was repeated for five times and kept in the PBS under 37°C and 5% CO_2 condition. The production of alginate beads showed similar and consistent result in each batch and the stable beads still could be observed until 49 days after production. This result showed the reproducibility aspect of selected protocol.

In this study, both ALG and ALGPA encapsulations were validated. The encapsulation method in this study is based on the manual nozzle extrusion, hDPSC-IPCs will be extruded through needle as a nozzle tip. The size of needle plays an important role since the suitable needle size should be fulfilled to avoid the cell aggregate damage (Hoesli et al., 2017). In present study, needle size 22G with the inner diameter 0.413 mm was chosen because it provided suitable inner diameter for

generated hDPSC-derived IPC size. Permeability is one of important factors for cell encapsulation application (Farina et al., 2018). The ALG and ALGPA permeability against glucose and insulin was reported in this study. Similar studies demonstrated the permeability of alginate using mouse insulinoma and glucose-responsive rat cell line, the results of these studies reported that encapsulation of both cell lines could keep the ability of insulin secretion in response to extra-capsular glucose stimulation (Bertolotti et al., 2009; Duruksu et al., 2018). Gautier et al. (2011) reported that glucose, ammonia, vitamin B12 and another low to middle molecular weight substance can easily diffuse across the alginate (Gautier et al., 2011). The results regarding bead swelling assay from the present study showed that, both encapsulation by ALG and ALGPA were relatively stable. Assessment of bead swelling was aimed to determine the stability of the beads. In some conditions, alginate beads can swell resulted the increasing of porosity and bead damage (Montanucci et al., 2015). During this experiment, ALG and ALGPA beads were incubated in PBS at 37^{o}C for 28 days. The swelling behavior can occur mainly due to the osmotic factors. In PBS which have high concentration of Na^{+} can cause bead swelling, while, in some

studies, alginate beads showed the elasticity ability without showing membrane breakage (Montanucci et al., 2015). In this experiment high purity of alginate powder was employed to avoid over-swelling that might cause bead defect. Substances i.e. proteins or endotoxins were not detected in high purity of alginate, these substances could increase the chemical potential of the solvent (negative charge) inside the capsule. Capsule deformation can be caused by the rapid change of the environment, in consequence the adaptation to a new environment will be occurred to achieved the equilibrium of chemical potential of the solvent inside and outside the capsule (Hong et al., 2009). Though, pore morphology was not reported in this study, similar study with the same alginate type and ionic crosslink solution showed that concentration of 0.5 – 1% alginate solution could produce a bead with pore diameter 7.2 - 8.0 nm, so it inhibited the permeability of 21 – 25 kDa dextran and 78 – 103 kDa protein including immunoglobulin G (Dembczynski and Jankowski, 2001). In addition the pore size of the beads can be reduced by increasing the alginate concentration (Wang et al., 2009).

In the present study, biocompatibility of both encapsulation ALG and ALGPA was assessed. AlamarBlue™ assay, a redox indicator was employed in this study. In three-dimensional (3D) culture system using alginate matrix, choosing the most reliable and precise assay to assess the viability plays an important role. Tetrazolium salt-based assay is widely used to evaluate the cell viability in two-dimensional (2D) culture system, however the highly toxic of DMSO or HCl/isopropanol used in the assay led an obstacle (Bonnier et al., 2015). Moreover, multiple metabolic reactions in both cytoplasm and mitochondria can be assessed by alamarBlue™ assay. This assay is based on the oxidation reduction caused by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), flavin adenine dinucleotide hydrogen ($FADH_2$), flavin adenine mononucleotide hydrogen ($FMNH_2$), nicotinamide adenine dinucleotide hydrogen (NADH), cytochromes and all cellular respiration metabolic reaction (Ahmed et al., 1994). During the first 48 hours post encapsulation, the metabolic activity of hDPSCs was slightly increase, it might cause by the absorption, ion and protein exchange was slower due to the encapsulation compare with 2D culture system. Cell to cell communication was also limited by this platform,

consequently the cells need several time for acclimatization (Sarker et al., 2015). Further, after 96 hours post encapsulation the metabolic activity of hDPSCs was relatively stable, evidence from another study showed that alginate encapsulation limited the cell proliferation and remains the cell into G0 stage (Razavi et al., 2015). Moreover, qualitative observation incorporates live/dead staining visualized similar condition. Numerous viable cells were more visible compared with non-viable cells after 96 hours post encapsulation.

Based on The International Society for Cellular Therapy, the minimum criteria of MSCs were established in 2005. The criteria that should be fulfilled for MSCs included fibroblastoid in morphology, adhere in culture plate/flasks under normal culture condition and exhibit CD73, CD90 and CD105 surface markers whereas the MSCs should lack the express of CD45 marker (Horwitz et al., 2005). These minimum criteria were fulfilled by the hDPSCs used in this study. The results of present study is consistent as previous study reported by Karamzadeh et al. (2012) that showed the similar expression of MSCs surface markers (Karamzadeh et al., 2012). CD73 or 5'-

ecto-nunucleotidase was known as one of major classical MSC surface marker that have involve in osteogenic and chondrogenic differentiation. The expression of this marker is regulated by Wnt-β catenin signaling (Ode et al., 2013). Another study conducted by Tan et al. (2019) reported that the expression of CD73 in MSCs is related with the function of modulation of immune response (Suto et al., 2017; Tan et al., 2019). CD44 or homing cell adhesion molecule (HCAM) is also widely found in may type of MSCs. It's function related with cell adhesion, migration, homing, proliferation and stemness maintenance (Maleki et al., 2014). Another MSC marker, CD90 that also known as Thy-1 played an important role in cell-cell or cell-matrix interaction as well as in cell migration (Maleki et al., 2014). The endoglin or CD105 is involved in cell proliferation, migration and differentiation (Maleki et al., 2014). A study conducted by Roura et al. (2006) showed that the CD105 expression in MSCs involved in adipogenic, osteogenic and chondrogenic differentiation (Roura et al., 2006). However different result was showed by another study (Cleary et al., 2016) that the expression of CD105 did not related with chondrogenic differentiation. In

addition to cell surface markers, pluripotency transcription factors *REX1, NANOG* and *OCT4* was expressed in hDPSCs from the present study which is in agreement with the previous study conducted by Shivakumar et al. (2019) (Shivakumar et al., 2019).

Currently, various trans differentiation protocols of IPCs were reported. Non integrative methods are widely used in MSC-based differentiation protocol, while integrative methods, which foreign sequences were transduced into host genome are mainly used in iPSC (Nakanishi and Otsu, 2012). Further, the risk of tumor formation and gene alteration still become the concern regarding safety aspect in clinical application (Schlaeger et al., 2015). Regarding this, several studies reported the safety aspect of adenoviral and Sendai viral integrative methods (Zhou and Zeng, 2013). In the present study, non-integrative method using small molecules and peptides was employed for IPC induction. In MSC-based protocols, two main steps of IPC differentiation were involved. The initial stage, MSCs were induced into pancreatic progenitor followed by β-cell maturation (Pavathuparambil Abdul Manaph et al., 2019). In addition, three-stage differentiation protocol was involved in this study. The

initial stage of IPC differentiation was the induction of hDPSCs into definitive endoderm. The combination between activin A and sodium butyrate was used in this study. A pioneer study reported the successful of the adipose derived stem cells (ASCs) induction into definitive endoderm by using these combinations of small molecules. The results was confirmed by the expression of definitive endoderm markers i.e. *SOX17, Foxa2, HNF-1β, CK-19* and *GATA-4* (Chandra et al., 2009). Definitive endoderm differentiation also was achieved in hESCs after activin A treatment (Massumi et al., 2016). In present study, L-taurine was employed in the pancreatic endoderm differentiation stage. Similar substance was used for hASC differentiation toward pancreatic endoderm (Chandra et al., 2009) and human placenta derived mesenchymal stem cells (hPDMSCs) (Kadam et al., 2010). In hASC study incorporated physiological dose of L-Taurine, the expression of *PDX-1, NGN3, NeuroD, Pax4* and *NKX2.2* was achieved in day 5 post induction (Chandra et al., 2009). However, *PDX1* was not expressed in this study, instead *NGN3* expression was noted at day 10. Final step of β-cell maturation was achieved by adding

combination of small molecules and peptides i.e. high dose of L-taurine, nicotinamide and glucagon-like peptide (GLP)-1. The β-cell-related markers, *NKX6.1, MAFA, ISL-1, GLUT-2* and *INSULIN* were expressed in hDPSC-IPCs. PDX1 and NKX6.1 are the important transcription factors for β-cell maturation. (Abdelalim and Emara, 2015). In this study, the matured and functional β-cell still could be achieved in the absence of *PDX1* expression, it might cause by the expression of *NKX6.1* that could maintain *MAFA* as a transcription factor for insulin gene expression (Pavathuparambil Abdul Manaph et al., 2019). Additionally, GLP-1 was reported to increase and stabilize the expression of *INSULIN* mRNA, subsequently the secretion and stimulation of insulin was enhanced (Chandra et al., 2009; Pavathuparambil Abdul Manaph et al., 2019).

In the present study, qualitative *in vitro* viability assessment of encapsulated hDPSC-IPCs in ALG and ALGPA showed that both encapsulation platforms can provide suitable environment for hDPSC-IPCs, which were consistent with previous studies by using pancreatic islet, ESC and iPSC-derived IPC (Richardson et al., 2014;

Montanari et al., 2017; Westenfelder et al., 2017; Alagpulinsa et al., 2019; Liu et al., 2019; Vethe et al., 2019). The essential requirements for cell culture i.e. porosity, stability and permeability were fulfilled by alginate (Barati et al., 2019). Another study on pancreatic islet cryopreservation incorporated alginate encapsulation reported that alginate encapsulation could maintain both viability and functionality of pancreatic islets (Kojayan et al., 2019). According to the functionality evaluation of hDPSC-IPC encapsulation in ALG and ALGPA, the results of this study showed that this encapsulation platform could maintain the expression of pancreatic endoderm and pancreatic β-cell gene markers at least for 14 days. In undifferentiated ESC and iPSC encapsulation study, post encapsulation induction in alginate could enhance both gene and protein expression of mature β-cell markers (*PDX1, MAFA* and *INSULIN*) compared to induction in tissue culture plastic (2D system) (Richardson et al., 2014; Vethe et al., 2019). However, in MSC-derived IPC study using trabecular meshwork MSCs (TM-MSCs), the mature and functional IPCs in tissue culture plates and alginate microfiber induction did not show significant difference (Barati et al.,

2019). In addition, encapsulation of differentiated cells (mature β-cell) was remained difficulties especially in how to maintain the viability of differentiated cells. Study in hESC-derived IPCs, showed that mature β-cell encapsulation viability still could be maintained, but some mature phenotype was slightly decreased. Post-encapsulation induction in undifferentiated hESCs resulted adequate mature β-cell. However, *in vitro* differentiation of post-encapsulation hESCs seemed impractical. For clinical application, to achievement of adequate mature β-cell was difficult in static culture system (Vethe et al., 2019). Moreover, the contact of undifferentiated cells under induction media should be minimized to avoid the antigen contamination from dead cells (Richardson et al., 2014). This present study demonstrated for the first time regarding the mature MSC-derived IPCs encapsulation. In both encapsulation ALG and ALGPA, intracellular pro-insulin and insulin expression were detected in day 7 and 14 post encapsulation which were consistent with the pancreatic endoderm and pancreatic islet marker gene expression maintained by ALG and ALGPA

encapsulation. Finally, the functional encapsulation hDPSC-IPCs in both ALG and ALGPA was confirmed by the ability of C-peptide secretion.

CHAPTER V

CONCLUSION

In conclusion, this an *in vitro* study proofed that the alginate/pluronic F127-based encapsulation technique can support the viability of hDPSCs, thus it can serve as transplantation platform of translational stem cell – based therapy. Furthermore, this encapsulation platform can maintain the viability and functionality of hDPSC-IPCs. This study provided information regarding encapsulation platform as an alternative strategy for hDPSC-IPC delivery systems, including diffusion evaluation of alginate and pluronic F127, the evaluation of encapsulation system for cell viability, generation of *in vitro* hDPSC-IPCs using small-molecule induction protocol, and functional assay of encapsulated hDPSC-IPCs. The findings of this study can be used as a scale-up prototype for translational stem cell-based diabetes therapy for human and veterinary medicine.

REFERENCES

Abdelalim EM and Emara MM 2015. Advances and challenges in the differentiation of pluripotent stem cells into pancreatic beta cells. World J Stem Cells. 7(1): 174-181.

Ahmed SA, Gogal RM, Jr. and Walsh JE 1994. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H]thymidine incorporation assay. J Immunol Methods. 170(2): 211-224.

Al Madhoun A, Alkandari S, Ali H, Carrio N, Atari M, Bitar MS and Al-Mulla F 2018. Chemically Defined Conditions Mediate an Efficient Induction of Mesodermal Lineage from Human Umbilical Cord- and Bone Marrow- Mesenchymal Stem Cells and Dental Pulp Pluripotent-Like Stem Cells. Cell Reprogram. 20(1): 9-16.

Alagpulinsa DA, Cao JJL, Driscoll RK, Sirbulescu RF, Penson MFE, Sremac M, Engquist EN, Brauns TA, Markmann JF, Melton DA and Poznansky MC 2019. Alginate-microencapsulation of human stem cell-derived beta cells with CXCL12

prolongs their survival and function in immunocompetent mice without systemic immunosuppression. Am J Transplant. 19(7): 1930-1940.

An D, Chiu A, Flanders JA, Song W, Shou D, Lu YC, Grunnet LG, Winkel L, Ingvorsen C, Christophersen NS, Fels JJ, Sand FW, Ji Y, Qi L, Pardo Y, Luo D, Silberstein M, Fan J and Ma M 2018. Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes. Proc Natl Acad Sci U S A. 115(2): E263-e272.

Ashimova A, Yegorov S, Negmetzhanov B and Hortelano G 2019. Cell Encapsulation Within Alginate Microcapsules: Immunological Challenges and Outlook. Front Bioeng Biotechnol. 7: 380.

Barati G, Nadri S, Hajian R, Rahmani A, Mostafavi H, Mortazavi Y and Taromchi AH 2019. Differentiation of microfluidic-encapsulated trabecular meshwork mesenchymal stem cells into insulin producing cells and their impact on diabetic rats. J Cell Physiol. 234(5): 6801-6809.

Barlow J, Solomon TPJ and Affourtit C 2018. Pro-inflammatory cytokines attenuate glucose-stimulated insulin secretion from INS-1E insulinoma cells by restricting

mitochondrial pyruvate oxidation capacity - Novel mechanistic insight from real-time analysis of oxidative phosphorylation. PLoS One. 13(6): e0199505.

Bertolotti A, Borgogna M, Facoetti A, Marsich E and Nano R 2009. The effects of alginate encapsulation on NIT-1 insulinoma cells: viability, growth and insulin secretion. In Vivo. 23(6): 929-935.

Bhujbal SV, de Haan B, Niclou SP and de Vos P 2014. A novel multilayer immunoisolating encapsulation system overcoming protrusion of cells. Sci Rep. 4: 6856.

Bijan Nejad D, Azandeh S, Habibi R, Mansouri E, Bayati V and Ahmadi Angali K 2017. Investigation of the role of alginate containing high guluronic acid on osteogenic differentiation capacity of human umbilical cord Wharton's jelly mesenchymal stem cells. J Microencapsul. 34(8): 732-743.

Bonnier F, Keating ME, Wrobel TP, Majzner K, Baranska M, Garcia-Munoz A, Blanco A and Byrne HJ 2015. Cell viability assessment using the Alamar blue assay: a comparison of 2D and 3D cell culture models. Toxicol In Vitro. 29(1): 124-131.

Brennan DC, Kopetskie HA, Sayre PH, Alejandro R, Cagliero E, Shapiro AM, Goldstein JS, DesMarais MR, Booher S and Bianchine PJ 2016. Long-Term Follow-Up of the Edmonton Protocol of Islet Transplantation in the United States. Am J Transplant. 16(2): 509-517.

Chan HF, Zhang Y, Ho YP, Chiu YL, Jung Y and Leong KW 2013. Rapid formation of multicellular spheroids in double-emulsion droplets with controllable microenvironment. Sci Rep. 3: 3462.

Chandra V, G S, Phadnis S, Nair PD and Bhonde RR 2009. Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. Stem Cells. 27(8): 1941-1953.

Chawla D, Kaur T, Joshi A and Singh N 2020. 3D bioprinted alginate-gelatin based scaffolds for soft tissue engineering. Int J Biol Macromol. 144: 560-567.

Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW and Malanda B 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 138: 271-281.

Churchill AJ, Gutierrez GD, Singer RA, Lorberbaum DS, Fischer KA and Sussel L 2017. Genetic evidence that Nkx2.2 acts primarily downstream of Neurog3 in pancreatic endocrine lineage development. Elife. 6.

Cleary MA, Narcisi R, Focke K, van der Linden R, Brama PA and van Osch GJ 2016. Expression of CD105 on expanded mesenchymal stem cells does not predict their chondrogenic potential. Osteoarthritis Cartilage. 24(5): 868-872.

de Vos P, Faas MM, Strand B and Calafiore R 2006. Alginate-based microcapsules for immunoisolation of pancreatic islets. Biomaterials. 27(32): 5603-5617.

de Vos P, Lazarjani HA, Poncelet D and Faas MM 2014. Polymers in cell encapsulation from an enveloped cell perspective. Adv Drug Del Rev. 67-68: 15-34.

Dembczynski R and Jankowski T 2001. Determination of pore diameter and molecular weight cut-off of hydrogel-membrane liquid-core capsules for immunoisolation. J Biomater Sci Polym Ed. 12(9): 1051-1058.

Dimitrioglou N, Kanelli M, Papageorgiou E, Karatzas T and Hatziavramidis D 2019. Paving the way for successful islet encapsulation. Drug Discovery Today. 24(3): 737-748.

Diniz IM, Chen C, Xu X, Ansari S, Zadeh HH, Marques MM, Shi S and Moshaverinia A 2015. Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. J Mater Sci Mater Med. 26(3): 153.

Duruksu G, Polat S, Kayis L, Ekimci Gurcan N, Gacar G and Yazir Y 2018. Improvement of the insulin secretion from beta cells encapsulated in alginate/poly-L-histidine/alginate microbeads by platelet-rich plasma. Turk J Biol. 42(4): 297-306.

Egusa H, Sonoyama W, Nishimura M, Atsuta I and Akiyama K 2012. Stem cells in dentistry--part I: stem cell sources. J Prosthodont Res. 56(3): 151-165.

Farina M, Alexander JF, Thekkedath U, Ferrari M and Grattoni A 2018. Cell encapsulation: Overcoming barriers in cell transplantation in diabetes and beyond. Adv Drug Deliv Rev.

Gabr MM, Zakaria MM, Refaie AF, Abdel-Rahman EA, Reda AM, Ali SS, Khater SM, Ashamallah SA, Ismail AM, Ismail HE-DA, El-Badri N and Ghoneim MA 2017. From Human Mesenchymal Stem Cells to Insulin-Producing Cells: Comparison

between Bone Marrow- and Adipose Tissue-Derived Cells. BioMed Res Int. 2017: 3854232.

Galivo F, Benedetti E, Wang Y, Pelz C, Schug J, Kaestner KH and Grompe M 2017. Reprogramming human gallbladder cells into insulin-producing β-like cells. PLoS One. 12(8): e0181812.

Gasperini L, Mano JF and Reis RL 2014. Natural polymers for the microencapsulation of cells. J R Soc Interface. 11(100).

Gauthaman K, Fong C-Y, Suganya C-A, Subramanian A, Biswas A, Choolani M and Bongso A 2012. Extra-embryonic human Wharton's jelly stem cells do not induce tumorigenesis, unlike human embryonic stem cells. Reprod Biomed Online. 24(2): 235-246.

Gautier A, Carpentier B, Dufresne M, Vu Dinh Q, Paullier P and Legallais C 2011. Impact of alginate type and bead diameter on mass transfers and the metabolic activities of encapsulated C3A cells in bioartificial liver applications. Eur Cell Mater. 21: 94-106.

Gilor C, Niessen SJM, Furrow E and DiBartola SP 2016. What's in a Name? Classification of Diabetes Mellitus in Veterinary Medicine and Why It Matters. J Vet Intern Med. 30(4): 927-940.

Gioffredi E, Boffito M, Calzone S, Giannitelli SM, Rainer A, Trombetta M, Mozetic P and Chiono V 2016. Pluronic F127 Hydrogel Characterization and Biofabrication in Cellularized Constructs for Tissue Engineering Applications. Procedia CIRP. 49: 125-132.

Goh CH, Heng PWS and Chan LW 2012. Alginates as a useful natural polymer for microencapsulation and therapeutic applications. Carbohydr Polym. 88(1): 1-12.

HealthQualityOntario 2003. Islet transplantation: an evidence-based analysis. Ont Health Technol Assess Ser. 3(4): 1-45.

Hoesli CA, Kiang RLJ, Raghuram K, Pedroza RG, Markwick KE, Colantuoni AMR and Piret JM 2017. Mammalian Cell Encapsulation in Alginate Beads Using a Simple Stirred Vessel. J Vis Exp. (124).

Hong W, Liu Z and Suo Z 2009. Inhomogeneous swelling of a gel in equilibrium with a solvent and mechanical load. International Journal of Solids and Structures. 46(17): 3282-3289.

Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS and Keating A 2005. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy. 7(5): 393-395.

Hwang PT, Shah DK, Garcia JA, Bae CY, Lim DJ, Huiszoon RC, Alexander GC and Jun HW 2016. Progress and challenges of the bioartificial pancreas. Nano Converg. 3(1): 28.

Iqbal A, Novodvorsky P and Heller SR 2018. Recent Updates on Type 1 Diabetes Mellitus Management for Clinicians. Diabetes Metab J. 42(1): 3-18.

Kadam S, Muthyala S, Nair P and Bhonde R 2010. Human placenta-derived mesenchymal stem cells and islet-like cell clusters generated from these cells as a novel source for stem cell therapy in diabetes. Rev Diabet Stud. 7(2): 168-182.

Kaisang L, Siyu W, Lijun F, Daoyan P, Xian CJ and Jie S 2017. Adipose-derived stem cells seeded in Pluronic F-127 hydrogel promotes diabetic wound healing. J Surg Res. 217: 63-74.

Kalaszczynska I and Ferdyn K 2015. Wharton's jelly derived mesenchymal stem cells: future of regenerative medicine? Recent findings and clinical significance. BioMed Res Int. 2015: 430847.

Karamzadeh R, Eslaminejad MB and Aflatoonian R 2012. Isolation, characterization and comparative differentiation of human dental pulp stem cells derived from permanent teeth by using two different methods. J Vis Exp. (69).

Khattak SF, Bhatia SR and Roberts SC 2005. Pluronic F127 as a cell encapsulation material: utilization of membrane-stabilizing agents. Tissue Eng. 11(5-6): 974-983.

Kojayan GG, Flores A, Li S, Alexander M and Lakey JRT 2019. Cryopreserved Alginate-Encapsulated Islets Can Restore Euglycemia in a Diabetic Animal Model Better than Cryopreserved Non-encapsulated Islets. Cell Med. 11: 2155179019876641.

Korsgren O 2017. Islet Encapsulation: Physiological Possibilities and Limitations. Diabetes. 66(7): 1748-1754.

Lei M, Li K, Li B, Gao L-N, Chen F-M and Jin Y 2014. Mesenchymal stem cell characteristics of dental pulp and periodontal ligament stem cells after in vivo transplantation. Biomaterials. 35(24): 6332-6343.

Li L, Chen Y, Wang Y, Shi F, Nie Y, Liu T and Song K 2019. Effects of concentration variation on the physical properties of alginate-based substrates and cell behavior in culture. Int J Biol Macromol. 128: 184-195.

Li Z, Jiang CM, An S, Cheng Q, Huang YF, Wang YT, Gou YC, Xiao L, Yu WJ and Wang J 2014. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells. Oral Dis. 20(1): 25-34.

Lim F and Sun AM 1980. Microencapsulated islets as bioartificial endocrine pancreas. Science. 210(4472): 908-910.

Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A and Liu S 2015. Concise reviews: Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells. 33(3): 627-638.

Liu Q, Chiu A, Wang LH, An D, Zhong M, Smink AM, de Haan BJ, de Vos P, Keane K, Vegge A, Chen EY, Song W, Liu WF, Flanders J, Rescan C, Grunnet LG, Wang X and Ma M 2019. Zwitterionically modified alginates mitigate cellular overgrowth for cell encapsulation. Nat Commun. 10(1): 5262.

Löhr M, Hoffmeyer A and Kröger JC 2003. Safety, feasibility and clinical benefit of localized chemotherapy using microencapsulated cells for inoperable pancreatic carcinoma in a phase I/II trial. Cancer Ther. 1: 121-131.

Maleki M, Ghanbarvand F, Reza Behvarz M, Ejtemaei M and Ghadirkhomi E 2014. Comparison of mesenchymal stem cell markers in multiple human adult stem cells. Int J Stem Cells. 7(2): 118-126.

Massumi M, Pourasgari F, Nalla A, Batchuluun B, Nagy K, Neely E, Gull R, Nagy A and Wheeler MB 2016. An Abbreviated Protocol for In Vitro Generation of Functional Human Embryonic Stem Cell-Derived Beta-Like Cells. PLoS One. 11(10): e0164457.

Mattin M, O'Neill D, Church D, McGreevy PD, Thomson PC and Brodbelt D 2014. An epidemiological study of diabetes mellitus in dogs attending first opinion practice in the UK. Vet Rec. 174(14): 349-349.

Mitry RR, Jitraruch S, Iansante V and Dhawan A 2017. Alginate Encapsulation of Human Hepatocytes and Assessment of Microbeads. Methods Mol Biol. 1506: 273-281.

Montanari E, Meier RPH, Mahou R, Seebach JD, Wandrey C, Gerber-Lemaire S, Buhler LH and Gonelle-Gispert C 2017. Multipotent mesenchymal stromal cells enhance insulin secretion from human islets via N-cadherin interaction and prolong function of transplanted encapsulated islets in mice. Stem Cell Res Ther. 8(1): 199.

Montanucci P, Terenzi S, Santi C, Pennoni I, Bini V, Pescara T, Basta G and Calafiore R 2015. Insights in Behavior of Variably Formulated Alginate-Based Microcapsules for Cell Transplantation. BioMed Res Int. 2015: 965804.

Nakanishi M and Otsu M 2012. Development of Sendai virus vectors and their potential applications in gene therapy and regenerative medicine. Curr Gene Ther. 12(5): 410-416.

O'Neill DG, Gostelow R, Orme C, Church DB, Niessen SJM, Verheyen K and Brodbelt DC 2016. Epidemiology of Diabetes Mellitus among 193,435 Cats Attending Primary - Care Veterinary Practices in England. J Vet Intern Med. 30(4): 964-972.

Ode A, Schoon J, Kurtz A, Gaetjen M, Ode JE, Geissler S and Duda GN 2013. CD73/5'-ecto-nucleotidase acts as a regulatory factor in osteo-/chondrogenic differentiation of mechanically stimulated mesenchymal stromal cells. Eur Cell Mater. 25: 37-47.

Omi M, Hata M, Nakamura N, Miyabe M, Ozawa S, Nukada H, Tsukamoto M, Sango K, Himeno T, Kamiya H, Nakamura J, Takebe J, Matsubara T and Naruse K 2017. Transplantation of dental pulp stem cells improves long-term diabetic

polyneuropathy together with improvement of nerve morphometrical evaluation. Stem Cell Res Ther. 8(1): 279.

Paredes Juárez GA, Spasojevic M, Faas MM and de Vos P 2014. Immunological and Technical Considerations in Application of Alginate-Based Microencapsulation Systems. Front Bioeng Biotechnol. 2(26).

Pavathuparambil Abdul Manaph N, Sivanathan KN, Nitschke J, Zhou XF, Coates PT and Drogemuller CJ 2019. An overview on small molecule-induced differentiation of mesenchymal stem cells into beta cells for diabetic therapy. Stem Cell Res Ther. 10(1): 293.

Ramezanzadeh Andevari R, Hashemi-Najafabadi S and Bagheri F 2018. Immunoisolation of stem cells by simultaneous encapsulation and PEGylation. Prog Biomater. 7(1): 55-60.

Rattananinsruang P, Dechsukhum C and Leeanansaksiri W 2018. Establishment of Insulin-Producing Cells From Human Embryonic Stem Cells Underhypoxic Condition for Cell Based Therapy. Front Cell Dev Biol. 6: 49.

Razavi S, Khosravizadeh Z, Bahramian H and Kazemi M 2015. Time-Dependent Effect of Encapsulating Alginate Hydrogel on Neurogenic Potential. Cell J. 17(2): 304-311.

Richardson T, Kumta PN and Banerjee I 2014. Alginate encapsulation of human embryonic stem cells to enhance directed differentiation to pancreatic islet-like cells. Tissue Eng Part A. 20(23-24): 3198-3211.

Roura S, Farre J, Soler-Botija C, Llach A, Hove-Madsen L, Cairo JJ, Godia F, Cinca J and Bayes-Genis A 2006. Effect of aging on the pluripotential capacity of human CD105+ mesenchymal stem cells. Eur J Heart Fail. 8(6): 555-563.

Sabek OM, Farina M, Fraga DW, Afshar S, Ballerini A, Filgueira CS, Thekkedath UR, Grattoni A and Gaber AO 2016. Three-dimensional printed polymeric system to encapsulate human mesenchymal stem cells differentiated into islet-like insulin-producing aggregates for diabetes treatment. J Tissue Eng. 7: 2041731416638198.

Saenz Del Burgo L, Ciriza J, Espona-Noguera A, Illa X, Cabruja E, Orive G, Hernández RM, Villa R, Pedraz JL and Alvarez M 2018. 3D Printed porous polyamide

macrocapsule combined with alginate microcapsules for safer cell-based therapies. Sci Rep. 8(1).

Safley SA, Kenyon NS, Berman DM, Barber GF, Willman M, Duncanson S, Iwakoshi N, Holdcraft R, Gazda L, Thompson P, Badell IR, Sambanis A, Ricordi C and Weber CJ 2018. Microencapsulated adult porcine islets transplanted intraperitoneally in streptozotocin-diabetic non-human primates. Xenotransplantation. 25(6): e12450.

Sarker B, Rompf J, Silva R, Lang N, Detsch R, Kaschta J, Fabry B and Boccaccini AR 2015. Alginate-based hydrogels with improved adhesive properties for cell encapsulation. Int J Biol Macromol. 78: 72-78.

Sawangmake C, Nowwarote N, Pavasant P, Chansiripornchai P and Osathanon T 2014. A feasibility study of an in vitro differentiation potential toward insulin-producing cells by dental tissue-derived mesenchymal stem cells. Biochem Biophys Res Commun. 452(3): 581-587.

Schlaeger TM, Daheron L, Brickler TR, Entwisle S, Chan K, Cianci A, DeVine A, Ettenger A, Fitzgerald K, Godfrey M, Gupta D, McPherson J, Malwadkar P, Gupta M, Bell

B, Doi A, Jung N, Li X, Lynes MS, Brookes E, Cherry AB, Demirbas D, Tsankov AM, Zon LI, Rubin LL, Feinberg AP, Meissner A, Cowan CA and Daley GQ 2015. A comparison of non-integrating reprogramming methods. Nat Biotechnol. 33(1): 58-63.

Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J and Lakey JR 2006. International trial of the Edmonton protocol for islet transplantation. N Engl J Med. 355(13): 1318-1330.

Shivakumar SB, Lee HJ, Son YB, Bharti D, Ock SA, Lee SL, Kang YH, Park BW and Rho GJ 2019. In vitro differentiation of single donor derived human dental mesenchymal stem cells into pancreatic beta cell-like cells. Biosci Rep. 39(5).

Siddiqui AA, Siddiqui SA, Ahmad S, Siddiqui S, Ahsan I and Sahu K 2013. Diabetes: Mechanism, pathophysiology and management-A review. Int J Drug Dev & Res. 5(2): 1-23.

Soon-Shiong P, Feldman E, Nelson R, Komtebedde J, Smidsrod O, Skjak-Braek G, Espevik T, Heintz R and Lee M 1992. Successful reversal of spontaneous diabetes in dogs by intraperitoneal microencapsulated islets. Transplantation. 54(5): 769-774.

Souza KL, Gurgul-Convey E, Elsner M and Lenzen S 2008. Interaction between pro-inflammatory and anti-inflammatory cytokines in insulin-producing cells. J Endocrinol. 197(1): 139-150.

Stanko P, Altanerova U, Jakubechova J, Repiska V and Altaner C 2018. Dental Mesenchymal Stem/Stromal Cells and Their Exosomes. Stem Cells Int. 2018: 8973613.

Suchánek J, Nasry SA and Soukup T 2017. The differentiation potential of human natal dental pulp stem cells into insulin-producing cells. Folia Biologica (Czech Republic). 63(4): 132-138.

Suto EG, Mabuchi Y, Suzuki N, Suzuki K, Ogata Y, Taguchi M, Muneta T, Sekiya I and Akazawa C 2017. Prospectively isolated mesenchymal stem/stromal cells are enriched in the CD73(+) population and exhibit efficacy after transplantation. Sci Rep. 7(1): 4838.

Swioklo S, Ding P, Pacek AW and Connon CJ 2017. Process parameters for the high-scale production of alginate-encapsulated stem cells for storage and distribution throughout the cell therapy supply chain. Process Biochem. 59: 289-296.

Tam SK, Bilodeau S, Dusseault J, Langlois G, Hallé JP and Yahia LH 2011a. Biocompatibility and physicochemical characteristics of alginate–polycation microcapsules. Acta Biomater. 7(4): 1683-1692.

Tam SK, Dusseault J, Bilodeau S, Langlois G, Halle JP and Yahia L 2011b. Factors influencing alginate gel biocompatibility. J Biomed Mater Res A. 98(1): 40-52.

Tan K, Zhu H, Zhang J, Ouyang W, Tang J, Zhang Y, Qiu L, Liu X, Ding Z and Deng X 2019. CD73 Expression on Mesenchymal Stem Cells Dictates the Reparative Properties via Its Anti-Inflammatory Activity. Stem Cells Int. 2019: 8717694.

Vegas AJ, Veiseh O, Gurtler M, Millman JR, Pagliuca FW, Bader AR, Doloff JC, Li J, Chen M, Olejnik K, Tam HH, Jhunjhunwala S, Langan E, Aresta-Dasilva S, Gandham S, McGarrigle JJ, Bochenek MA, Hollister-Lock J, Oberholzer J, Greiner DL, Weir GC, Melton DA, Langer R and Anderson DG 2016. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. Nat Med. 22(3): 306-311.

Vethe H, Legøy TA, Abadpour S, Strand BL, Scholz H, Paulo JA, Ræder H, Ghila L and Chera S 2019. Encapsulation boosts islet-cell signature in differentiating human induced pluripotent stem cells via integrin signalling. bioRxiv. 791442.

Wang N, Adams G, Buttery L, Falcone FH and Stolnik S 2009. Alginate encapsulation technology supports embryonic stem cells differentiation into insulin-producing cells. J Biotechnol. 144(4): 304-312.

Westenfelder C, Gooch A, Hu Z, Ahlstrom J and Zhang P 2017. Durable Control of Autoimmune Diabetes in Mice Achieved by Intraperitoneal Transplantation of "Neo-Islets," Three-Dimensional Aggregates of Allogeneic Islet and "Mesenchymal Stem Cells". Stem Cells Transl Med. 6(7): 1631-1643.

Xiang G, Lippens E, Hafeez S, Duda GN, Geissler S and Qazi TH 2019. Oxidized alginate beads for tunable release of osteogenically potent mesenchymal stromal cells. Mater Sci Eng C Mater Biol Appl. 104: 109911.

Xiao X, Guo P, Shiota C, Zhang T, Coudriet GM, Fischbach S, Prasadan K, Fusco J, Ramachandran S, Witkowski P, Piganelli JD and Gittes GK 2018. Endogenous Reprogramming of Alpha Cells into Beta Cells, Induced by Viral Gene Therapy, Reverses Autoimmune Diabetes. Cell Stem Cell. 22(1): 78-90.e74.

Zareifard N, Soleimani A, Talaei-Khozani T and Bahmanpour S 2018. Improved BALB/c mice granulosa cell functions using purified alginate scaffold. Iran J Vet Res. 19(3): 182-188.

Zhou Q, Brown J, Kanarek A, Rajagopal J and Melton DA 2008. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature. 455(7213): 627-632.

Zhou Q and Melton DA 2018. Pancreas regeneration. Nature. 557(7705): 351-358.

Zhou YY and Zeng F 2013. Integration-free methods for generating induced pluripotent stem cells. Genomics Proteomics Bioinformatics. 11(5): 284-287.

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