CHAPTER I

INTRODUCTION

Background and Rationale

Peritoneal dialysis (PD) is one of the effective renal replacement therapies (RRT). It has become a successful and widely-used treatment for end-stage renal disease patients for over 30 years worldwide [1-6]. It was first introduced to a patient with uremia in 1923 by Granter. The flexibility of a plastic bag was applied in 1977; afterwards, there was introduced that reduced the incidence of peritonitis [3, 7].

Later, continuous cyclic PD (CCPD) and other forms of automated PD were also used. These methods provide benefits to lifestyle and facilitate the prescription management of more individualized dialysis regimens [8]. With an excellent survival rate at lower cost, the quality of life of PD patients has improved compared to hemodialysis (HD)[9-12].

Recent evidence has shown a favorable outcome with PD, especially during the first two years. Hence, it is an alternative to hemodialysis (HD) [2]. Utilization of PD as renal replacement therapy (RRT) has inherent advantages and does not require highly trained staff or complex technology compared to HD. It is inexpensive and easy to use. PD can be performed intermittently or continuously, and either manually or via an automated device, it has been recently accepted as a long-term renal failure therapeutic option [13-15].

Glucose is used as an osmotic agent in peritoneal dialysis for more three decades [16, 17]. However, one major disadvantage of this solution is bio-incompatibility and one of the most important current issues in PD therapy is how to minimize the use of glucose as an osmotic agent because of its several disadvantages.

1) Changes to the peritoneal membrane and function during PD: the glucose concentration in PD solutions exceeds by 15–40 times the physiologic concentration, until intra-peritoneal equilibration, when the glucose concentration remains at 6–16 times that of physiologic concentration [18-21]. Factors driving these changes are thought to

involve hypertonic glucose exposure. The mechanism of glucose toxicity is presented in Fig. 1.1 and the changes can lead eventually to treatment failure in peritoneal dialysis.

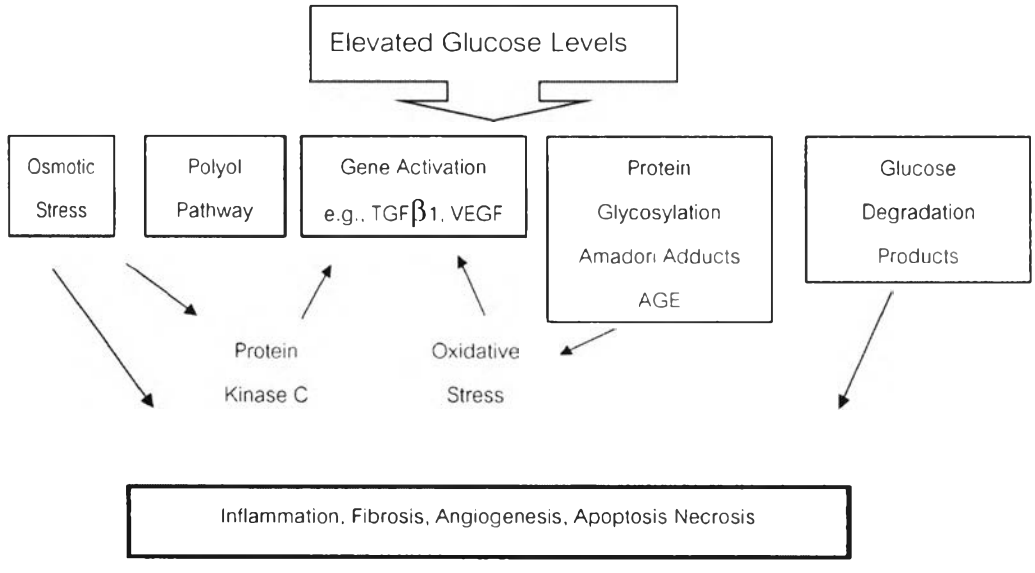


Figure 1.1: Mechanism of peritoneal gluco-toxicity [22].

Moreover, heating glucose leads to generation of glucose degradation products (GDPs) in the heat sterilization process, and GDPs will be promoted with the formation of advanced glycation end-products (AGEs) by cross-linking to structural protein. The AGEs can induce compromise cellular function, in both resident and infiltrating cells, and peritoneal membrane integrity (Table 1.1). These harmful agents can influence cell viability, interrupt leukocyte cell functions and initiate an inflammatory cytokine cascade. A low pH, hyperosmolality can damage the peritoneum and viability of peritoneal mesothelium lining cells, with deposition of extracellular matrix and AGEs within the media of arterioles and arterial wall. There are many concerns about peritoneal dialysis treatment failure [23-27].

2). A high glucose exposure on the peritoneum: excessive glucose load produces systemic adverse effects including hyperinsulinemia, hyperglycemia, which can induce hyperglycemic stress episodes four times per day, obesity and satiety. A

quick glycemic increase can alter the physiologic homeostasis of various organs and systems, probably by production of free radicals [28].

3) The limited ultra-filtration from rapid dissipation over a long period. Patients have the risk of developing permanent ultrafiltration (UF) loss: 3%, 10%, and 31% after 1, 3, and 6 years respectively [14, 29-31]. With loss of UF capacity because of its rapid absorption across the peritoneal membrane and loss of osmotic gradient of glucose over longer periods, the fluid will be reabsorbed, with fluid removal (negative ultrafiltration) particularly in patients with high peritoneal transport characteristics. Ultrafiltration failure (UFF) continues to be a major complication of PD, particularly long-term PD (23-25).

Table 1.1: Effect of GDPs and AGEs [32-35]

Agent	Effect on peritoneal resident or infiltrating cells	Effect on peritoneal membrane
GDPs	Mesothelial cells: reduced cell viability, decreased synthesis of matric proteins and cellular attachment and expression of pro-inflammatory cytokines Fibroblasts: inhibition of cell growth Leukocyte: increase number of rolling leukocytes	Mesothelial cells: Mesothelial denudation, impair cellular function, inflammation Fibroblasts: impaired wound healing Leukocyte: inflammation
AGEs	Mesothelial cells: Increased VEGF and TGF-beta synthesis, increased secretion of pro-inflammatory cytokines and accumulation in cells	Mesothelial cells: peritoneal fibrosis, inflammation, fibrosis

VEGF= vascular endothelial growth factor, TGF-beta= transforming growth factor-beta

Continuous exposure to bio-incompatible PD solutions causes inflammation of the peritoneal membrane as diagramed in Fig. 2; this progressively undergoes fibrosis and angiogenesis and leads to ultrafiltration failure [36, 37] as illustrated in Fig. 1.2.

Perturbations in normal cellular physiology can be detected in chronic exposures to high glucose solution. Membrane structure and function changes result to the declining peritoneal transport and ultrafiltration, and worsening clinical outcomes.

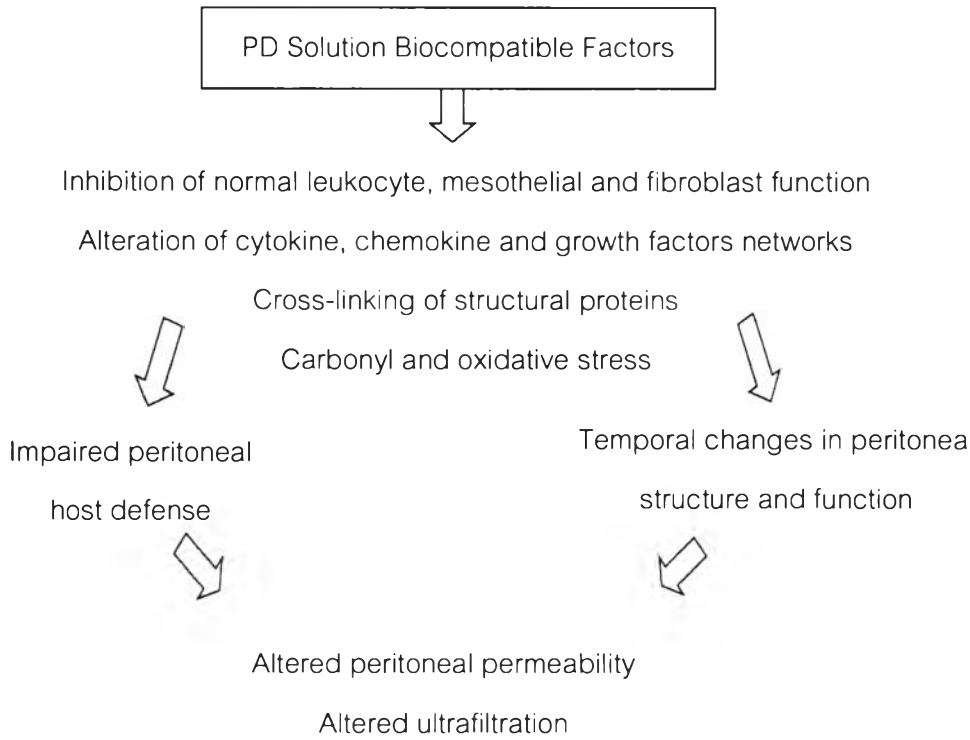


Figure 1.2: Potential role of biocompatibility in clinical outcomes.

The unphysiologic concentrations of glucose and the GDPs in conventional peritoneal dialysis fluids are major contributors towards induction of structural and functional abnormalities in the peritoneum during PD. Therefore a more physiological fluid, such as corn-derived peritoneal dialysis fluid (CPDF), has been introduced to improve bio-compatibility and to overcome the negative ultrafiltration effects in repetitive long-term users.

Characteristic of a glucose polymer CPDF is the molecular weight structure, a heterogeneous complex mixture of corn-derived water soluble glucose polymer, with

difference chain length distribution. This polymer is obtaining from partial hydrolysis of corn (maize) starch with the chemical named "dextrin." The molecular weight characteristic of the polymer is a mixture of various chain lengths (heterogeneous fractions) with molecular weight by weight (Mw) ranging from 12,000-20,000 Daltons and the molecular weight by number (Mn) ranging from 5000-6500 Dalton [38, 39].

Glucose-based peritoneal dialysis fluid (GPDF) contains varieties of 1.5, 2.5, 4.25% dextrose, which is equivalent to 1.36, 2.27, and 3.86% of anhydrous glucose respectively. The GPDF composition containing the electrolytes compared to polygluocse corn-based peritoneal dialysis fluid (CPDF) is presented in Table 1.2. The advantages and disadvantages of glucose-based PDF and glucose polymer corn derivative-based PDF are compared in Table 1.3.

Table 1.2: Composition of glucose and glucose polymer based commercially PDFs [40]

Components	Commercial available PDF	
	Glucose based (GPDF)	Corn derivative based (CPDF)
Glucose (g/dL)	1.36, 3.86	-
Corn-derivative (g/dL)	-	7.5
Sodium (mmol/L)	132	132
Calcium (mmol/L)	3.5	3.5
Magnesium (mmol/L)	0.5	0.5
Chloride (mmol/L)	96	96
Lactate (mmol/L0	35-40	40
pH	5.5	5.2-5.6
Osmolality (mOsm/Kg)	358, 511	282

Table 1.3: Comparison of effects of GPDF and CPDF [41-51]

Function	Glucose based PDF	Corn-derived PDF
Cost	inexpensive	expensive
Mesothelial cell	Abnormal morphology	Normal morphology
% Atypical cells after *in vitro* mesothelial culture	60%	20%
Cell proliferation	inhibition	improved
polymorphic blood mononuclear cells (PBMC), macrophage	Cytotoxicity	no significant cytotoxicity
Cell viability	decreased	improved
TGF beta1/ Mitochondrial damage	Increased	improved
Mesothelium	denudation	preserved
AGE deposition	increased	moderately decreased
Peritoneum exposure to glucose/ GDPs/ AGEs	High	Low
Ultrafiltration	decreased	improved

CPDF has been an important achievement in PD therapy, and has been successfully used for decades [52-56]. The chains of this corn based-derivative polymer with an average molecular weight of 17000 Da can be absorbed through the peritoneal membrane over an up-to 12 hour period with greater fluid removal than with GPDF. The MW distribution of non-used corn-derived PDF is illustrated below in black, compared to the first long period in white bars.

There was an increase in the medium MWW fraction 9.89-21.4 kDa as shown in Fig. 1.3. HMW profiles of CPDF fractions over a 4-hour period in human and non-uremic rats are presented in Fig. 1.4

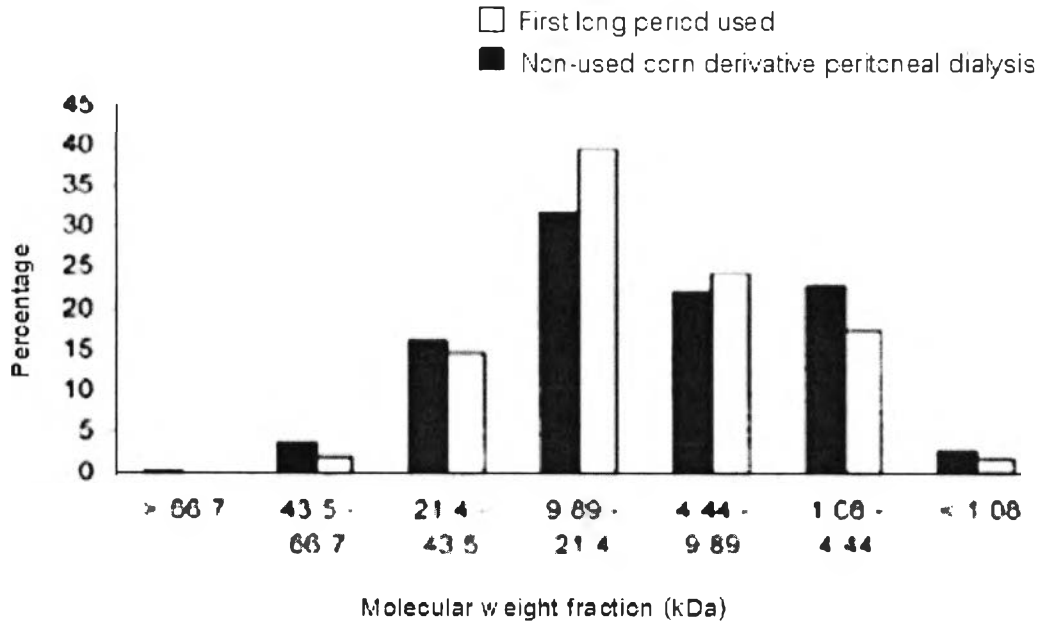


Figure 1.3: The MW distribution of corn-derived PDF [49]

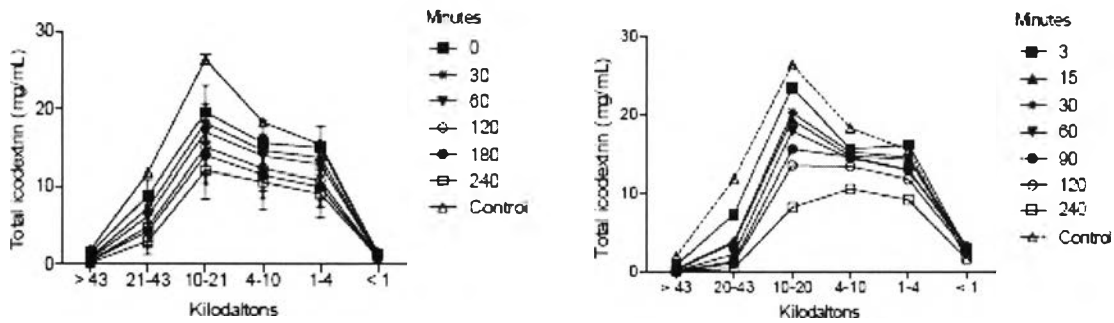


Figure 1.4: The molecular weight distribution. Pattern of corn-derivative fractions after 4 hours peritoneal dialysis dwelled from patients (left) and non-uremic rats (right) [49, 57]

It contains relatively low levels of GDPs and it is iso-osmolar to plasma but sustains longer ultrafiltration. Many advantages of CPDF have been described, such as healing of the peritoneal mesothelial cell lining, and reduction of intra-peritoneal

inflammation, as well as the maintenance of macrophage functions (Table 3). Its iso-osmolar properties reduce peritoneum damage [58] and decrease glucose load, and its ability to sustain a long period of exchange in patients, both CAPD and APD offers patients a better survival benefit, especially for diabetic CAPD patients, allowing better glycemic condition control. In addition, it enhances salt and water management by removal of free water, control of extracellular water and total body fluid, and better control of fluid balance. It is well-established for patients with poor UF or high transporters [59, 60]. It is described as having the undisputed advantage of volume control by exerting a colloid osmotic pressure gradient [61] .

However, some disadvantages, such as skin rash reaction and vasculitic papule, have been reported after using CPDF[62] and some clinical data has implicated systemic activation and peritoneal inflammation in patients using CPDF[18, 63].

Statement of the Problem

In Thailand, there are approximately 100,000 Thai ESRD patients who need therapeutic care. This is a relatively expensive lifesaving cost in the South East Asia region [64, 65]. However, PD has long been established as a major option for renal replacement therapy in patients with ESRD. It is a substantial burden on health care expenditure, but the Thai government has launched a nationwide health policy to reimburse totally the expense of PD therapy. This support will abruptly increase the number of PD patients under treatment, with a concomitant need for a larger total public health budget in coming years.

To avoid the use of bio-incompatible glucose based peritoneal dialysis fluid, to prevent a negative balance of Thai import and export in consumption of the PD products, an alternative new glucose polymer must be generated. Tapioca is one of the well-known major products of Thai agricultural and economic industries. It may well be possible to produce a modified tapioca derivertive in Thailand.

Hypotheses Mechanism

Glucose based peritoneal dialysis fluid proven the cost effective, however, undesired side effects related to systemic glucose absorption and the long-term peritoneal side effects and rapid absorption problem have become sources of serious concern. Corn derivative based peritoneal dialysis shows more advantage for limiting glucose exposure and improve ultrafiltration. However, the cost remains high, which limits utilization because of Thailand's need to import from a monopoly manufacturing company, while this increases the numbers of PD-needing patients. The ideal osmotic agents from this study would be physiological substances, easily available and cheap, easily metabolized, high dialysis efficiency, and no toxicity. The use of an alternative new glucose polymer-based peritoneal dialysis fluid, whether, tapioca derivative peritoneal dialysis will be associated with a reduction in glucose and GDP exposure and improves biocompatibility and effectiveness in volume status control.

Research Methodology

To prove hypotheses whether tapioca derivative can be applied as alternative as glucose polymer based peritoneal dialysis, a new glucose polymer tapioca derivative based peritoneal dialysis was developed. The chemical and physical with molecular characteristics have been intensively reviewed. The efficiency in term of safety and effectiveness of this new product are evaluated. *In vitro* peritoneal cytotoxicity and *in vivo* animal toxicity testing have been performed. The cytotoxicity in human mesothelial peritoneal cells, fibroblast cell line and peripheral mononuclear cells has been investigated. The toxicity effects in small animals' mice and rats are also examined. This study is also to prove the effectiveness of this osmotic agent, whether can this new fluid able to induce water transport and what mechanism of how molecular weight characteristics effect on water transportation have been demonstrated through in vitro water osmosis and computer simulation studies.

Research Questions

Objectives

1. To establish proper preparation of a new non-glucose polymer tapioca derivertive-based PDF (TPDF).
2. To investigate the cytoxicity effects of prepared TPDF in cell culture models using mesothelial cells, fibroblast and immune cells.
3. To test whether TPDF is safe in animal models (Toxicity Testing).
4. To examine the effectiveness of TPDF in water transportation.
5. To investigate the mechanism of TPDF in ultrafiltration.

Hypothesis

1. Tapioca-derivative peritoneal dialysis fluid has better biocompatibility than glucose-based PDF and similar effects as corn-based PDF.
2. Tapioca-derivative peritoneal dialysis fluid is an effective osmotic agent than glucose-based PDF and has similar capability to induce water transportation when compared to glucose polymer corn-based PDF.

Key Words

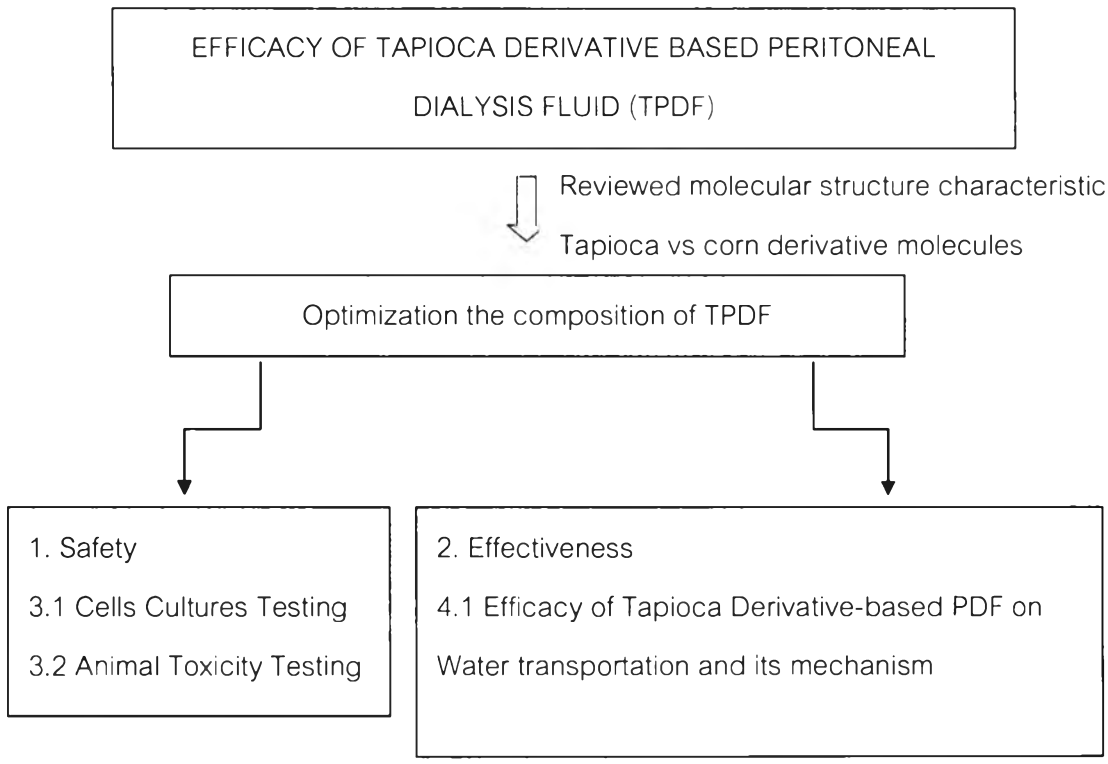
Corn-derivative, tapioca-derivative, glucose, peritoneal dialysis, and efficacy

Expected Benefits

The result of this study will generate a basic knowledge of this new alternative glucose polymer-based PDF product, and whether TPDF is bio-compatible compared to CPDF and GPDF. As well, it will help an understanding of the mechanism of ultrafiltration induced by glucose polymer-based PDFs, TPDF and CPDF. Furthermore, the results of this study will offer very important evidence towards extension of the possibility of applying this Thai aquiculture product in the area of health care research. Importantly, this research study is interdisciplinary, applying not only to health science areas, but creating multi-lateral research cooperation between pure science and biotechnology, including working with the private sector in order to strengthen research capability and

develop sustainable socio-economic development. Lastly, if this product is shown to be safe, with sufficient efficacy, it will benefit not only Thai people, but also the world chronic renal failure population.

Conceptual Frameworks



To investigate the efficacy of tapioca derivative-based glucose polymer, derived from hydrolysis of tapioca starch, with a similar structure or characteristics to a corn-base derivative; this modified derivative is a novel alternative to corn derivative-based glucose polymer PD solutions.

To assess whether this solution is safe and effective compared to glucose-based and corn-based solutions.

The present study aims to investigate the major efficacies in terms of safety and effectiveness.

The studies scope was presented in conceptual framework as follow:

(i) Optimization of the TPDF composition (ii) safety - cell culture models and animal testing; (iii) effectiveness: efficacy of tapioca-derivative PDF induces water transportation and its mechanism

Review of molecular structure characteristics of tapioca-derivative molecules

The molecular characteristics of tapioca-derivative molecules were analyzed at Cassava and Starch Technology Research Unit/ National Center for Genetic Engineering and Biotech, Kasetsart University, and the results reviewed and compared to corn-based derivative molecules.

1. TPDF composition testing

Tapioca-derivatives powder and varied concentration to 5%, 7.5%, 10%, 20%

↓ +Electrolytes & measure pH, osmolality

Autoclave, Filter

↓ Measure pH, osmolality

Selected the optical tapioca derivative-based PD (TPDF)

↓

Storage at 4°C, 25°C, and 37°C

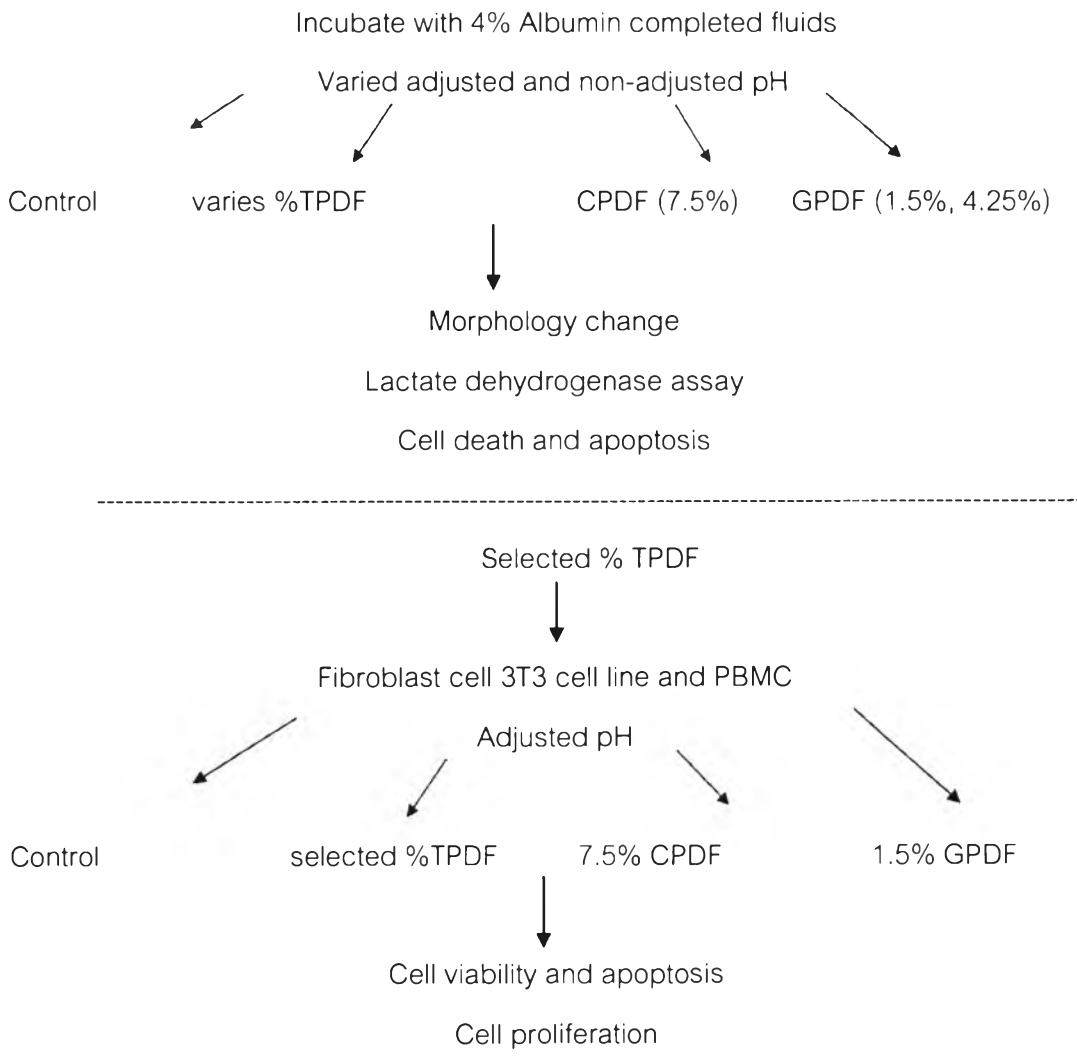
1. Examination of GDPs by HPLC at Cassava and Starch Technology Research Unit/ National Center for Genetic Engineering and Biotech, Kasetsart University
2. Reviewed the changes of GDPs levels
3. Compared to glucose-based PDF
4. Compared to glucose polymer corn-based PDF

2. Bio-compatibility profiles of TPDF compared to CPDF and GPDF

The biocompatibility profile in terms of its potential to avoid glucose and hyperosmolarity-mediated cytotoxicity, GDPs mediated cellular alteration. Most cell culture models involve a study of mesothelial cells, fibroblasts, or leukocytes in vitro to assess the effects on cell function [42, 43, 66, 67]. Choice of incubation periods and study time-points vary according to the parameter of interest and the clinical context [68, 69]. In this respect, short exposure times are commonly used for analysis of rapid responses such as those to pH and buffer components. Assessing the effect of osmolality often takes 1–4 hours.

3.1 Cytotoxicity was tested in human mesothelium cells

Use passage 2-3 of phenotype confirmed-primary c/s mesothelial cells



3.2 Toxicity testing in mice was carried out.

The study protocol was approved by the Faculty of Medicine Committee and the NIH guide on Humane Care and Use of Laboratory Animals was followed.

3.2.1 Acute toxicity in mice

11 Bal/C 4-6 wk olds with body weight 20-40 gm

0.9% Normal saline solution (NSS) 15% TPDF

Intravenous (twice daily for 14 days)

Reevaluation clinical aspect

Gross morphology

Microscopic pathology examination

3.2.2 Repeat safety test in mice

20 Bal/C 4-6 wk olds with body weight 20-40 gm

0.9% NSS 20% TPDF

Intraperitoneal (twice daily for 14 days)

Reevaluation clinical aspect

Gross morphology

Microscopic pathology examination

3. Effectiveness of this TPDF-induced water transportation

To test the hypothesis that TPDF can induce water transportation, the *In vitro* osmosis of water was performed, and computer modeling was simulated. In addition, the effect of membrane pore sizes and molecular weight (MW) distribution on water transportation was investigated.