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Microdialysis Sampling of Macro Molecules

Fluid Characteristics, Extraction Efficiency and Enhanced Performance

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Abstract

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In this thesis, fluid characteristics and sampling efficiency of high molecular weight cut-off microdialysis are presented, with the aim of improving the understanding of microdialysis sampling mechanisms and its performance regarding extraction efficiency of biological fluid and biomarkers.

Microdialysis is a well-established clinical sampling tool for monitoring small biomarkers such as lactate and glucose. In recent years, interest has raised in using high molecular weight cut-off microdialysis to sample macro molecules such as neuropeptides, cytokines and proteins. However, with the increase of the membrane pore size, high molecular weight cut-off microdialysis exhibits drawbacks such like unstable catheter performance, imbalanced fluid recovery, low and unstable molecule extraction efficiency, etc. But still, the fluid characteristics of high molecular weight cut-off microdialysis is rarely studied, and the clinical or in vitro molecule sampling efficiency from recent studies vary from each other and are difficult to compare.

Therefore, in this thesis three aspects of high molecular weight cut-off microdialysis have been explored. The first, the fluid characteristics of large pore microdialysis has been investigated, theoretically and experimentally. The results suggest that the experimental fluid recovery is in consistency with its theoretical formula. The second, the macromolecule transport behaviour has been visualized and semi-quantified, using an in vitro test system and fluorescence imaging. The third, two in vitro tests have been done to mimic in vivo cerebrospinal fluid sampling under pressurization, using native and differently surface modified catheters. As results, individual protein/peptide extraction efficiencies were achieved, using targeted mass spectrometry analysis.

In summary, a theory system of the fluid characteristics of high molecular weight cut-off microdialysis has been built and testified; Macromolecular transport of microdialysis catheter has been visualized; In vivo biomolecules sampling has been simulated by well-defined in vitro studies; Individual biomolecular extraction efficiency has been shown; Different surface modifications of microdialysis catheter have been investigated. It was found that, improved sampling performance can be achieved, in terms of balanced fluid recovery and controlled protein extraction efficiency.

Keywords: microdialysis, high molecular weight cut-off, fluid characteristics, fluid recovery, extraction efficiency, biomarker, microporous membrane, macromolecule transport, transmembrane, large pore, surface modification, pluronic, dextran, in vitro, microdialysis catheter

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To My Family

献给我亲爱的家人

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Chu, J.**, Hjort, K., Larsson, A., Dahlin A.P. (2014) Impact of static pressure on transmembrane fluid exchange in high molecular weight cut off microdialysis. *Biomedical Microdevices*, 16: 301-310

II **Chu, J.**, Koudriavtsev, V., Hjort, K., Dahlin, A.P. (2014) Fluorescence imaging of macromolecule transport in high molecular weight cut-off microdialysis. *Analytical Bioanaytical Chemistry*, 406 (29): 7601-7609

III **Chu, J.**, Undin, T., Lind, S.B., Hjort, K., Dahlin, A.P. (2015) Influence of surface modification and static pressure on microdialysis protein extraction efficiency. *Biomedical Microdevices*. In press.

IV **Chu, J.**, Undin, T., Lind, S.B., Dahlin, A.P., Hjort, K. (2015) Influence of different pluronic surface modifications and pressure on microdialysis protein extraction efficiency. Submitted to *Biomedical Microdevices*.

V **Chu, J.**, Undin, T., Dahlin, A.P., Wang, C., Park, J., Hjort, K. (2015) Protein Desalination Chip for Mass Spectrometry Sample Preparation. *Proceedings of μTAS 2015*. In press.

VI Dahlin, A.P., Purins, K., Clausen, F., **Chu, J.**, Sedigh, A., Lorant, T., Enblad, P., Lewen, A., Hillered, L. (2014) Refined Microdialysis Method for Protein Biomarker Sampling in Acute Brain Injury in the Neurointensive Care Setting. *Analytical Chemistry*, 86(17): 8671-8679

In addition, the following paper is included to give an overview of new aspects in cerebral microdialysis biomarker sampling:

A. Hillered, L., Dahlin, A.P., Clausen, F., **Chu, J.**, Bergqvist, J., Hjort, K., Enblad, P., Lewen, A. (2014) Cerebral microdialysis for protein biomarker monitoring in the neurointensive care setting – a technical approach. *Frontiers in Neurology*, 5: 245

Author's contribution

Paper I Major part of planning, all experiments, most of analysis and writing.
Paper II Part of experiments, major part of analysis and writing.
Paper III Most of planning, experiments, analysis and writing.
Paper IV Most of planning, experiments, analysis and writing.
Paper V Part of planning, experiments, analysis and writing.
Paper VI Performed the PCA analysis.

Contents

Abbreviations

CSF	Cerebrospinal fluid
Da	Dalton, molecular mass unit
EE	Extraction Efficiency
FITC	Fluorescein isothiocyanate
FR	Fluid Recovery
ICP	Intracranial pressure
kDa	kilo Dalton , 1000 Da
LC	Liquid chromatography
MD	Microdialysis
MS	Mass spectrometry
MW	Molecular weight
MWCO	Molecular weight cut-off
PCA	Principal Component analysis
PEO	Polyethylene oxide
PPO	Polypropylene oxide
RR	Relative Recovery
TBI	Traumatic Brain Injury
TM	Trans-membrane
μ	micro, 1x 10^{-6}

1. Introduction

授人以鱼，不如授人以渔。
Give a man a fish and you feed him for a day. Teach him how to fish and you feed him for a lifetime.

-老子　Lao Tzu

Microdialysis (MD) is a fairly novel sampling technique, introduced by Swedish researchers in 1974. It has experienced continuous development over these years. Its various applications have been developed and its commercial market has expanded.

MD is a brilliant technique that functions on the basis of a simple principle: the target species in the extra cellular space of living organisms can be sampled by a tiny needle like catheter, which mimics the capillary function in living tissue. The key of the sampling is the MD membrane, which is usually a micro- or nano-porous membrane. It can be defined in its pore size, or molecular weight cut-off (MWCO), as to MD sampling parameter. The MWCO of the membrane determines the MW range of the molecules: which can pass through the membrane, and which are excluded by the membrane. Often the perfusate passing inside the MD catheter is a physiological fluid that has the same properties regarding ion strength, salt composition and pH as the body fluid, to help sampling target molecules without osmotic pressure difference influence or introducing possible contamination from/to the sampling environment.

The advantages of MD over other similar techniques lay in six aspects: First, by mimicking a capillary in the tissue, the dialysate provide a direct representation of the extracellular compartments of living organisms. Second, if the dialysate is connected directly after collection to an on-line analysis instrument, real-time monitoring of the target molecules can be achieved. Third, MD functions as sampling and sample preparation methods in the same time. Fourth, MD operation and sampling procedures are relatively non-intrusive compared to similar techniques. Fifth, MD uses a simple principle that employs fluidics with passive driving force, which does not require outside power input of any kind, except pumping of the physiological perfusion fluid. The simplicity of the non-complex instrumentation and operating procedure makes its popularity over other similar methods. Sixth, MD can

work on a small targeted area, with targeted molecule species, samples small amount of analytes, and is able to do fast detection and monitoring if coupled to capable analysis methods.

In past years, MD with membrane MWCO below 20 kDA has been well investigated in various aspects and routinely used in clinical setting as the monitoring tool for small biomarkers such like neurotransmitters and metabolites. Efforts have also been put on to link the analysis of the collected analytes in MD dialysates to their physiological significance. The MD sampling method contributed to the research mostly in the biochemistry, neurology and brain functions areas. The examples include blood-brain transport mechanism, Parkinsonism, Alzheimer disease, stroke, depression, drug abuse, etc.

The performance of the MD sampling is often characterized by fluid recovery (FR) and extraction efficiency (EE). The standard for a good performance is marked by the balanced FR and the high EE of the target analytes, preferably as close as to represent their real concentrations in extracellular spaces. The studies of the past years suggest that the low MWCO MD provides stable performance and acceptable EE yield. However, along with the development of the MD membranes, targeted molecules range, dialysate analysis methods and MD sampling operations parameters (like push-pull pumping and adding osmotic agents into the perfusate, etc.), the traditional MD are with various drawbacks and facing several challenges, which are unfortunately rarely studied or provided with alternatives/solutions. As examples, five aspects have been investigated in this thesis:

1. The fluidic mechanisms of MD are not thoroughly studied. Especially when the flow regime enters the ultrafiltration range for the high MWCO MD, previous diffusion driven dominant theory cannot explain the fluid characteristics of the high MWCO MD, which could be ultrafiltration, nanofiltration or mixture of diffusion and filtration, or even microfiltration. Without clear understanding of fluid characteristics, control over FR to achieve a balanced sampling fluidics, cannot be realized. **Paper I** corresponds to this aspect. It builds a theory system for high MWCO MD and presents a formula which includes most of the FR influencing factors. Moreover, the experimental FR results are consistent with the theory and the formula.

2. The MWCO of MD is a loosely defined term by statistics; the sampling performance is hard to predicate and to be comparable. A study on macromolecule transport over a high MWCO MD membrane has been done in **Paper II**, by direct visualization of fluorescence tagged macromolecules and semi-quantitation of their concentration.

3. The EE of individual proteins/peptides has been investigated by a pressurized, in vitro test which mimics in vivo conditions, in **PAPER III and IV**. The results are able to quantify the concentration for each individual analyte in the dialysate, along with varying test conditions.

4. Various surface modifications have been applied to MD membrane and their effects on FR and individual EE of protein/peptides have been in-

vestigated, in **PAPER III, IV and VI**. The results suggest that different pluronics as surface modification alternatives have good potential to improve FR and EE of high MWCO MD, either in vitro (**PAPER III, IV**) or in vivo (**PAPER VI**).

5. A microchip device which is able to separate salts from the protein sample has been presented and tested in **PAPER V** on MD sample. Its performance suggests that it has good potential to be used on desalting the MD dialysate prior to analysis process, or on other desalination applications.

2. Microdialysis

2.1 Principle

In MD sampling, a tubular shaped, semipermeable hollow fiber membrane is placed in the sampling area of interest. The perfusate which flows continuously in constant rate through the inside of the tubular membrane, carries away the collected analytes for further analysis [1, 2, 3], as shown in Figure 2.1. In principle, the concentration gradient that occurs between the perfusate and the sampling environment drives analytes, smaller than the membrane molecular weight cut-off (MWCO), to diffuse across the membrane. This basic principle of MD is governed by Fick's first law of passive diffusion. The driving force for the analytes to penetrate the membrane is the concentration gradient.

The basic structure of the MD membrane is an anisotropic structure which has a well-defined porous surface layer attached to a much more open 'network' of microporous substrate. The well-defined membrane porous surface layer performs the initial rejection and separation of differently sized molecules, the microporous substrate functions as mechanical strength support and secondary screening of the analytes passing through. The structure and MWCO of the MD membranes are explained in details in section 4.1.

In general practice, the MD probe is implanted into the region of interest (either a tissue or a medium). The perfusate enters through the inner tube and perfuse at a constant flow rate and then enters the space between the innertube and the MD membrane [1, 2, 3]. The molecular exchange occurs around the membrane, which is located at the tip of the MD catheter. The perfusate is usually a physiological fluid which mimics the human body fluid, for instance Ringer solution.

The concept is that ideally the implanted MD catheter is to mimic a normal tissue capillary, which can provide time resolved snapshots of the place where the membrane is placed with minimal effect on the microenvironment. If the collected dialysate is connected to an on-line analysis instrument, a real-time monitoring of the sampling site can be achieved.

There are two most crucial characterizing parameters of MD sampling performance: fluid recovery (FR) and relative recovery (RR). FR is defined as the ratio of the volume of the dialysate that is collected divided by the

volume of the perfusate that is delivered into the MD catheter. Its formula is expressed as:

$$\boldsymbol{FR = Q_{out} / Q_{in}} \qquad (2.1)$$

Q_{out} is the volume of the dialysate and Q_{in} of the perfusate. FR is often expressed in percentage (%).

RR is defined as the ratio of a target analyte's concentration in the dialysate C_{out}, divided by its concentration in bulk external medium C_{ext}, providing that its concentration in perfusate C_{in} is zero. Its formula is expressed below:

$$\boldsymbol{RR = C_{out} / C_{ext}} \qquad (2.2)$$

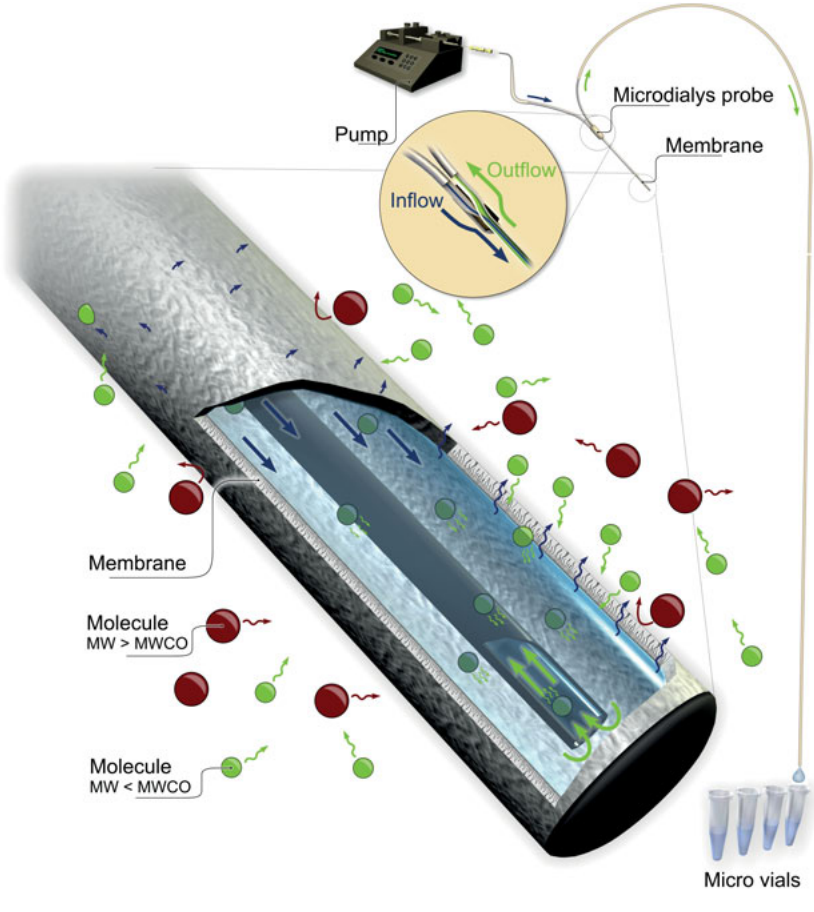

Figure 2.1 Principle of microdialysis sampling. Courtesy Andreas Dahlin.

Another commonly used factor characterizing MD performance is extraction efficiency (EE). From the MD theory [4], the slope of the concentration difference curve is termed the 'in vivo extraction efficiency' of the MD probe. In another word, the rate at which an analyte is exchanged across the semipermeable membrane is the analyte's extraction efficiency [5].

The EE is defined as the ratio between the loss/gain of analyte during its passage through the probe (C_{in}/C_{out}) and the difference in concentration between perfusate and distant sampling site (C_{in}/C_{ext}). The various formulation of EE is not going to be presented here. What worth mention is that RR is commonly viewed to be the same factor as EE. This equality was demonstrated in the works of Bungay et al. [4]. RR and EE as two terms are commonly accepted as a same factor which characterizes the MD sampling efficiency for a certain target analyte.

2.2 Trend

Previously, MD is designed for sampling small and hydrophilic molecules [1, 6]. In recent years, MD is well-established as a clinical sampling tool for monitoring small hydrophilic molecules such as lactate, puruvate and glucose in neurointensive care settings [6, 7]. Recently, interest has been raised in sampling molecules with larger masses [8, 9, 15], such as neuropeptides [10, 11, 12, Paper III and IV], cytokines [8, 13], and proteins [9, 14-18, Paper III and IV]. With the increased popularity in using high MWCO (over 100 kDa) membranes for MD sampling, high MWCO MD is routinely used as a clinical sampling tool for neurochemical brain monitoring of neuro intensive care patients [3, 8, 9, 19, 20]. In such cases, the in vivo intra cranial pressures could differ between 2-7 mmHg (torr) [21] in healthy humans to more than 50 mmHg in treated patients.

High MWCO MD membranes with such large pore sizes are designed to transmit fluid rather than diffusion-dominant traditional low MWCO MD. The fluid characteristics of the high MWCO MD membranes enter ultrafiltration or even microfiltration regime, where convective flux dominates the molecular transport mechanism. The detailed MD fluid characteristics are presented later in section 3.2 and 3.3.

As a consequence of ultrafiltration and convective flow, the sample volume collected is different from the sample volume pumped into the MD system. In this case, the FR is not 100%. A change of FR will alter the concentration of the analytes [22]. Also, a FR that differs from 100% is unfavorable since the idea of MD is to provide time resolved snapshots of the place where the membrane is placed with minimal effect on the microenvironment. For example, if there is a perfusion fluid leakage from the membrane, there will be a dilution effect in the microenvironment and the collected dialysate resulting in a MD sample that is a less representative image of the dynamic

processes that are happening in vivo. Hence, there are good reasons to gain understanding on the high MWCO MD fluid characteristics and improve its sampling performance by keeping a balanced FR and controlled EE/RR.

2.3 Challenges

The large pore size of the high MWCO MD affects the hydrodynamic equilibrium in the catheter by making it more sensitive to pressure changes exerted by static, osmotic and dynamic pressures [23, Paper I]. This sensitivity to changes in TM pressure leads to higher uncertainties in dialysate volumes and analyte concentrations. It is therefore important to tune in the MD system in order to obtain near 100% FR and thereby avoiding leakage, or drainage of, the surrounding sampling environment [24, Paper II].

Typically, MD TM pressure is adjusted by changing the passive pressure components- Perfusion pressure and osmotic pressure. Perfusion pressure could easily be varied by changing perfusion flow rates or by adjusting the altitude in where the dialysate is collected in respect to the membrane position [25]. The most common way of adjusting osmotic pressure is by adding osmotic agent [26] such like albumin [27] or large dextran molecules (60-500 kDa) [18, 23, 28] to the perfusion fluid. However, albumin rules out consecutive proteomic applications using liquid chromatography (LC) in combination with mass spectrometry (MS) based detection since the added albumin would completely dominate the sample loading. Albumin will also diffuse through a 100 kDa membrane, mix into the tissue and contaminate the sample environment. Compared to albumin, dextrans of higher MWs will not diffuse through a 100 kDa membrane and are compatible with LC/MS [29].

There are very few studies on how the TM pressure and FR are affected by changed outside static pressures. Siaghy et al. [30] presented an in vitro MD system where the outside static pressure was varied by increasing or decreasing the sampling volume where the MD membrane was placed. However, in his study, a simple definition of two pressure components during the MD process was considered: external and internal pressure. It is not clear what the origin of these two pressures is; and there is no identification of any pressure component with a specific type or nature, which should be responsible for the TM pressure.

In pre-clinical and in vitro studies, one way to control the TM flow balance is by using different perfusion pumping methods such as push, pull or push-pull [25, 31] and receive FR of 100%. However, until now such pumping systems have been considered not suitable for clinical use.

Another challenge to consider when sampling proteins with MD is its intrusive interaction with the organism. The MD catheter will cause irritation and often an inflammatory response, which will change the biological sam-

pling environment. The reason for this reaction is the well-known protein adsorption process to foreign material (or called foreign body reaction) that eventually leads to bio-fouling and encapsulation [32]. This is a severe problem in MD since it alters the pore size of the membrane and also initiates response actions that make result interpretation more complicated [33].

Recently our group presented an approach to decrease the protein adsorption onto the MD catheter [18, paper III, paper IV]. The catheters were surface modified with the triblock co-polymer, Poloxamer 407 (a.k.a. Pluronic F-127) prior to MD-sampling. The Poloxamer 407 coated MD-membranes adsorbed 33% less proteins than membranes without coating [34]. The coating has shown to increase the FR precision both in vitro [18, paper III, paper IV] and in vivo [28]. Combined use of 500 kDa dextran in the perfusate and Poloxamer 407 surface modified MD catheters has indicated more consistent and responsive EE in vitro [paper III, paper IV] and in vivo, when tested in porcine brain injury model [28]. But issues remain with fouling and low EE when using 100 kDa membranes.

3. Microdialysis fluid characteristics

3.1 Molecule transport models

The well-established MD nowadays is mostly with a MWCO of 20 kDa or below (low MWCO), it functions on sampling small molecules. As described in previous sections, during dialysis the concentration gradient across the membrane causes a flow of solute and solvent from one side of the membrane to the other side, which can be described as diffusion. As the result of diffusion, a net transport of solute will happen, from the high concentration to the low concentration region.

The law of diffusion was established by Fick in 1855 [35]. It is called Fick's law of diffusion, and can be formulated as the below formula:

$$J_i = -D_i \frac{dc_i}{dx} \tag{3.1}$$

Where J_i is the rate of transfer of component i or flux *(*g/cm^2 · s*)* and dc_i/dx is the concentration gradient of component i. The term D_i is called the diffusion coefficient (cm^2/s) and is a measure of the mobility of the individual molecules. The minus sign shows that the direction of diffusion is down the concentration gradient.

Low MWCO dialysis is a perfect example of the solution-diffusion model of membrane transport mechanism. In ideal MD sampling situation, there will be no solvent passing through the membrane, only concentration gradients are concerned.

The other membrane transport model is the pore-flow model. As the name of the model suggests, the permeants are transported by the convective flow through the pores in the membrane. During the transportation, some permeants were filtered by some of the pores, while other permeants pass through the pores and move to the other side of the membrane. The two membrane transport models are illustrated in Figure 3.1.

The pore-flow model is often used to represent a capillary or porous medium, for which the pressure driven fluid flow dominates the fluid characteristic. Darcy's law is the basic governing rule of this transport model. The formula of Darcy's law is:

$$J_i = K' c_i \frac{dp}{dx} \qquad (3.2)$$

Where dp/dx is the pressure gradient existing in the porous medium, c_i is the concentration of component i in the medium and K' is a coefficient reflecting the nature of the medium, sometimes expressed as membrane resistance R_m.

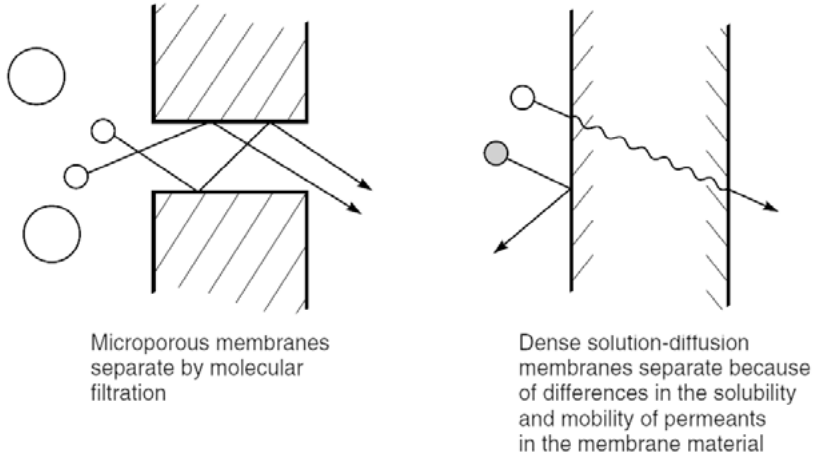

Figure 3.1 Membrane molecule transport models. Left: flow-pore model. Right: solution-diffusion model. Reprinted with permission from John Wiley and Sons.

3.2 Membrane fluid characteristics

The transport mechanism of the membrane is mainly determined by the relative size of the pores, also by other factors such like permanence of the pores. Membranes in the solution-diffusion model are with tiny pores (from 3 to 5 Å in diameter, within the range of thermal motion of the polymer-chains that form the membrane). Membranes in the pore-flow model are with relatively large pores. Larger the pores, the more likely they are to produce pore-flow characteristics in the membrane [36].

'The average pore diameter in a membrane is difficult to measure directly, and must often be inferred from the size of the molecules that permeate the membrane or by some other indirect technique.' [36], with this in mind, membranes can be divided into four general groups, as shown in Figure 3.2.

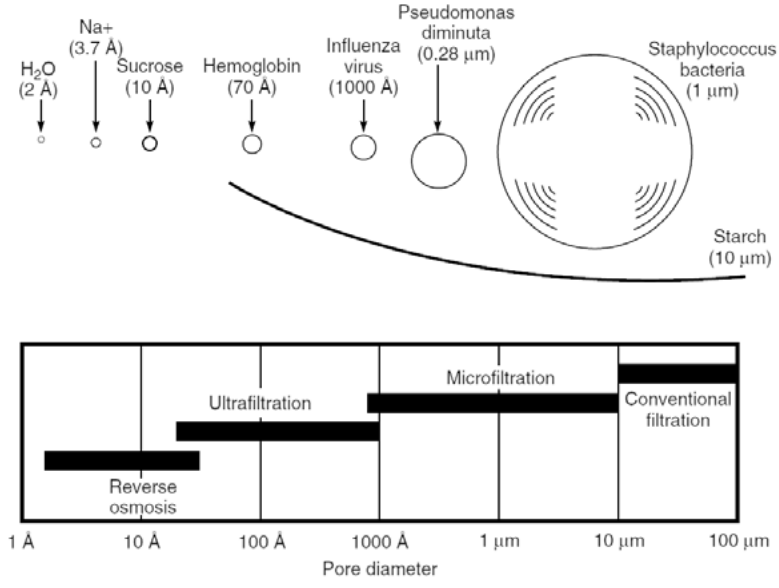

Figure 3.2 Schematic of four groups of membrane differing in fluid characteristics. Below scale shows their average pore diameter range. Upper examples are different solutes which can be removed by corresponding class of membranes, with the solutes' sizes presented. Reprinted with permission from John Wiley and Sons.

Four classes of membrane are grouped: reverse osmosis, ultrafiltration, microfiltration, and conventional filtration. Ultrafiltration, microfiltration membranes are microporous, molecular transport follows pore-flow model. There will be convective fluxes of permeants. For reverse osmosis, transport follows the solution-diffusion model, solute diffusion process dominates, and the fluxes through the membranes are much lower than through the microporous membranes.

Although reverse osmosis, ultrafiltration and microfiltration are conceptually similar processes, due to that basically their mode of separation is very similar-'molecular sieving through increasingly fine pores.' [37]. But their difference in pore diameter makes their distinctive range of applications. As can be seen from Figure 3.2, a typical pore diameter of the microfiltration membrane is 10,000 Å, which are 100 fold of the average ultrafiltration pore size and 1,000 fold of the pore diameter of osmosis membranes [38].

The primary use of reverse osmosis membrane is desalination of groundwater or seawater. The main application of ultrafiltration membranes is to filter dissolved macromolecules, such as proteins, from solutions. Microfiltration membranes can be used to filter colloidal particles or bacteria (from 0.1 to 10 μm in diameter).

3.3 Microdialysis fluid characteristics

MD catheter utilizes a hollow fiber semi-permeable membrane, with different MWCOs. MWCO is a statistical measurement of the MD sampling efficiency to certain size ranged molecules, and does not present the actual membrane average pore size. For instance, 20 kDa MWCO MD membranes have a statistical efficiency that is 80-90% of the molecules with a MW of 20 kDa will be rejected by the MD membrane [39]. See detailed definition of MD MWCO in section 4.1. MD catheter used in the studies of this thesis uses a cylindrical membrane design, to have similarity as a capillary.

As far as fluid characteristics are concerned for MD, membranes with low MWCO (20kDa or below) mainly follows the solution-diffusion model, the permeants diffuse through the membrane with densely-packed small pores, due to concentration gradient. The solvent fluxes through the membrane are mainly due to osmotic pressure-driven osmosis.

On the other hand, high MWCO (100 kDa or above) MD membranes follow the pore-flow model. Their fluid characteristics enter ultrafiltration regime or even to microfiltration. In ultrafiltration sampling, permeants are mainly carried through the membrane by convective fluxes, which are mostly pressure-driven flow.

Due to the fact that the pores in the membrane are increasingly finer from outside to the inside of the catheter (most design follows finer pore from outside to inside of the membrane, but in some cases the catheter uses the opposite design), during the transportation, some permeants are filtered by some of the pores, while other permeants pass through the pores and move to the other side of the membrane, carried by the perfusate inside the catheter and eventually collected in the dialysate.

The well-established low MWCO MD membranes are designed to have a low ultrafiltration coefficient, which leads to minimal transport of the solvent (fluid) across the membrane. The reason lays in that, ‘In the ideal case, the MD sampling is meant to be based solely on the diffusion of molecules driven by the concentration gradient, with no fluid being taken from, or delivered into, the MD sampling area.’ [40], which unfortunately is not the case for high MWCO MD anymore, since it enters ultrafiltration regime and has high ultrafiltration coefficient.

To compare high MWCO MD in ultrafiltration/microfiltration regime to low MWCO MD in osmosis diffusion driven regime, high MWCO MD has larger pores in the membrane, much more fluxes through the membrane due to convective flow. Due to the fact that most convective flow is pressure-driven, high MWCO MD is more sensitive to pressure influence than low MWCO MD. The sampling principle of high MWCO MD is based on filtering than diffusion for low MWCO MD. Due to that the basis of filtering is more statistical than diffusion, thus the sampling efficiency of high MWCO MD is more statistically measured and described.

As a summary, the difference of sampling targets, sampling principle and efficiency between high and low MWCO MD, lies in their distinctively different fluid characteristics, which is due to primarily their big difference in pore sizes, and also have to do with the structure of the membrane, and the design of the specific MD catheters. To have in mind that, the MD sampling experimental settings, e.g. perfusion rate, solvent or perfusate osmosity, fluid temperature , sampling environment pressure etc., each factor that can alter the fluid characteristics of the MD catheter, will influence the sampling performance of the MD catheter.

What about the MD membrane with intermediate MWCO (20-100 kDa), what are their fluid characteristics? There is so far no definite conclusion about their membrane fluidic mechanism. A reasonable statement would be that their fluid characteristics should be an intermediate between solution-diffusion and pore-flow, more like a combination of both mechanisms. In fact, 20 kDa for low MWCO and 100 kDa for high MWCO, are not definite classification point for solution-diffusion and pore-flow model.

For low MWCO MD, there should be convective fluxes exist as well alongside the diffusion driven flux. It's just that in such model diffusion driven flux dominates.

The same for high MWCO, convective driven and diffusion driven permeants co-exist.

For MD membrane with an intermediate MWCO, both membrane fluid mechanisms should contribute to the molecular transport, it is just a matter of degree.

The diffusion of large molecules through the MD membrane becomes progressively reduced as MW increases, for example, the effective MWCO of a nominal 30 kDa MD membrane is found to be typically below 10 kDa in vivo [41].

In fact, there is a group of membranes have pores with diameter between 5-10 Å. They are called nanofiltration membranes. Their fluid mechanism is intermediate between solution-diffusion and microporous. The study results of Cadotte et al. [42] suggests that this kind of membranes can filter some molecules with diameter of 10–13 Å, but allow some molecules with diameter of 5-6 Å to pass with ease. This is interesting since this kind of nanofiltration mechanism shows useful selectivity (filter out/pass) over molecules with very close molecular sizes, which could have potential for interesting usage on MD sampling.

3.4 Theory: high MWCO microdialysis fluidics

As discussed above, high MWCO MD enters the ultrafiltration fluidic regime. The large pore size of high MWCO MD allows the convective fluxes of fluid across the membrane, which could lead to a net gain or loss of perfusate in the MD catheter. For example, if a high MWCO MD probe is running normal procedure but without let the membrane part in the actual sample instead let it in the air, after a while, the membrane part will 'sweat' with a few tiny fluid drops presented on the outside of the membrane. This would be the net loss of the perfusate, meaning a FR less than 100%. On the opposite case, under some MD experimental setting, under static pressure of the MD sample for instance, the collected dialysate volume will be large than the volume of the pumped in perfusate, which is a net gain of fluid from the MD sample, meaning a FR over 100%.

In fact, in **Paper I, III and IV**, all FR vs. Pressure results suggest that the FR of high MWCO MD can be control via shifting the MD sample pressure and other means. As result, a FR ranging from 70-150% could be achieved with shifting pressure in the range of 0-25 torr.

For MD sampling, the shift in FR is unfavorable. A FR around 100% is preferred, to maintain fluid balance around MD membrane. A change of FR will alter the concentration of the analytes [22]. Also, a FR that differs from 100% is unfavorable since the idea of MD is to provide time resolved snapshots of the place where the membrane is placed with minimal effect on the microenvironment. For example, if there is a perfusion fluid leakage from the membrane, there will be a dilution effect in the microenvironment and the collected dialysate resulting in a MD sample that is a less representative image of the dynamic processes that are happening in vivo. Hence, it is necessary to investigate the factors which influence the fluid balance of MD sampling, and find ways to achieve a FR around 100%.

TM pressure represents the pressure across the MD membrane and is dependent on both the inside and outside pressure components. The three main components of the TM pressure are: inside perfusion pressure of the perfusate, TM osmotic pressure difference, and the outside static pressure originating from the MD sampling environment.

Typically, MD TM fluid balance is adjusted by changing various pressure components. Perfusion pressure could be varied by changing perfusion flow rates or by adjusting the altitude in where the dialysate is collected in respect to the membrane position [25]. TM osmotic pressure could be adjusted by adding colloids agent [26], for instance dextran, in different concentrations in the perfusion fluid, to affect the MD FR [18]. In pre-clinical and in vitro studies, one way to control the TM fluid balance is by using different perfusion pumping methods such as push, pull or push-pull [25, 31] and receives FR of 100%. However, until now such pumping systems have been consid-

ered not suitable for clinical use, due to its complex setting. The use of osmotic agent to achieve fluid balance is widely investigated, but far from effective, since the choice of the agents is undetermined and the leakage of the agents would contaminate the MD sampling.

After MD enters ultrafiltration regime with high MWCO, the membrane is more sensitive to pressure-driven convective fluxes. This makes the external static pressure of the MD sample an important influencing factor of fluid balance. During in-vivo sampling, many pressurized sampling conditions could occur, for example intracranial pressure and external pressure in other body parts like the in vivo experimental setting of **Paper VI.** As its results suggest, pressure did clearly affect the fluid balance and the performance of the high MWCO MD.

The efforts on achieve MD fluid balance are all related to pressure factors: perfusion pressure, osmotic pressure and sample static pressure. Previous studies [12, 22, 25, 26] show that the TM pressure is the key influencing parameter on FR.

Therefore, pressure is the most important factors on MD fluidics. It is important to understand the influence of different pressure components on MD fluid balance, i.e. FR. In another word, how pressure affects FR.

A novel MD in vitro test system has been developed and evaluated, **Paper I**. As illustrated in Figure 4.2, it holds four MD probes under static pressure set by a precisely controlled sample injection rate into a chamber. Thus the FR of the in vitro MD system can be accurately studied as a function of external chamber pressure. This well-controlled in vitro experiment has the advantage of screening out unnecessary factors but focusing only on the factors to be studied.

Figure 3.3 gives a schematic presentation of the pressure system in the chamber. All pressure components which affect the fluid exchange across the membrane directly or indirectly are considered. They can be divided into external pressure, P_{ext}, outside the membrane and towards its inside; and internal pressure, P_{in}, pressure inside the probe and towards the outside of the membrane. The TM pressure ΔP is given by $\Delta P = P_{ext} - P_{in}$. Hence, with a positive ΔP there will be a TM net flow, Q_r, into the MD catheter.

If we keep the relative positions constant in the system and assume the atmospheric pressure constant, we can simplify the model and get the dependence of ΔP on the different system parameters as:

$$\Delta P = P_{chamber} - P_{outlet} + \Delta P_{osmotic} \tag{3.3}$$

where $P_{chamber}$ is the static pressure in the chamber monitored by a pressure sensor, P_{outlet} is the pressure drop of the dialysate along the outlet tubing and $\Delta P_{osmotic} = P_{osmotic\text{-}in} - P_{osmotic}$.

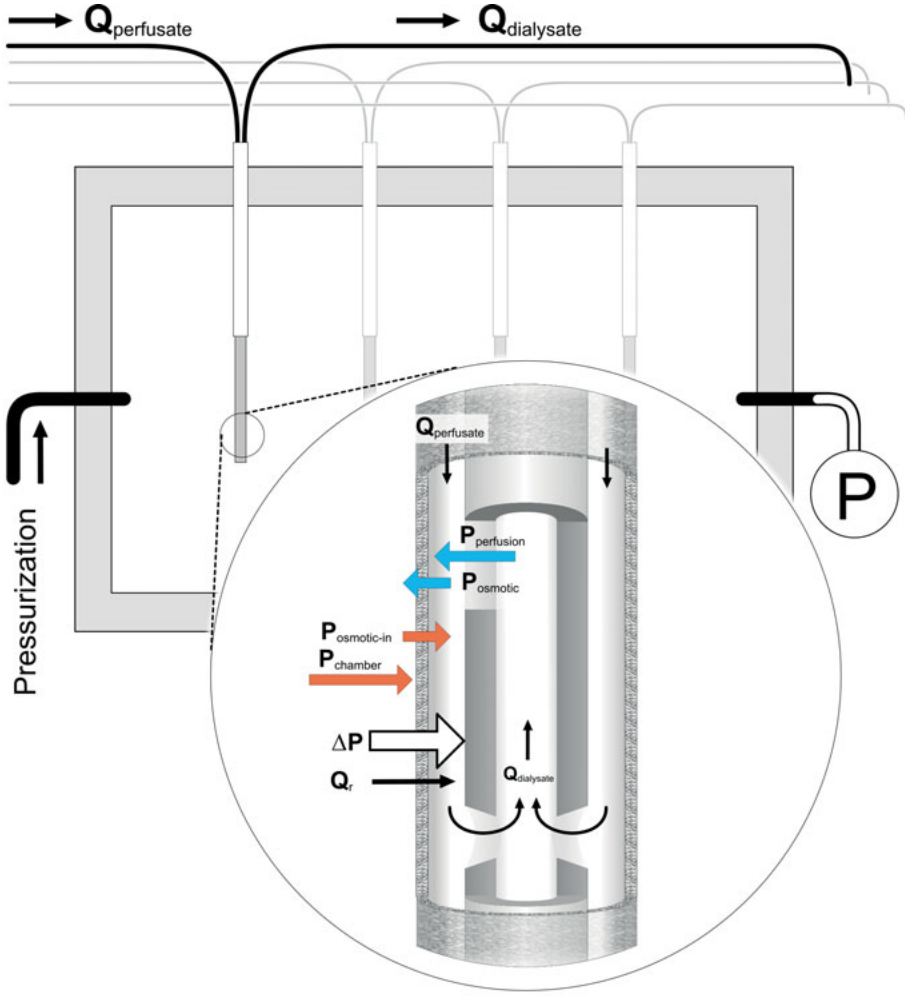

Figure 3.3 The experimental set-up of the pressurized in vitro MD sampling in **Paper I**. Four MD probes perform sampling in the pressurized chamber, driven by four syringes on a syringe pump; MD sample is filled in the chamber and can be injected through a port into the chamber at controlled rate, also driven by a syringe pump; The port on the right side is connected to a pressure sensor, where the chamber pressure is monitored. The enlarged part of the catheter tip shows a sketch of the pressure system around the membrane, when the MD catheter is running the in vitro test with pressurization condition. The details of the pressure components and description on the TM pressure system can be found in **Paper I** and its supplementary materials. They are related to Equation 3.3. Courtesy Andreas Dahlin.

We can measure the FR, in this case denoted as $FR = Q_{dialysate} / Q_{perfusate}$, which has a direct coupling to the TM net flow since $Q_r = Q_{dialysate} - Q_{perfusate}$. By using Poiseuille's law (equation 3.6) to model the P_{outlet} and Darcy's law (equation 3.2) to model ΔP, we can express the FR as Equation 3.4 below. For detailed evolution from the MD TM pressure system to Equation 3.4, refer to the theory section in the supplementary materials of **Paper I**.

$$FR = a + \frac{b}{Q_{perfusate}} \Delta P_{osmotic} + \frac{b}{Q_{perfusate}} P_{chamber} \tag{3.4}$$

where the constants are given by:

$$a = \frac{1}{1 + \dfrac{256lL}{R_m d^4 \ln \dfrac{r_o}{r_i}}} \quad \text{and} \quad b = \frac{1}{\dfrac{\mu R_m}{2\pi L} \ln \dfrac{r_o}{r_i} + \dfrac{128\mu l}{\pi d^4}} \tag{3.5}$$

with l being the length of the outlet tubing, L the length of the membrane, R_m the MD membrane resistance to the TM permeate, d the inner diameter of the outlet tubing of MD probe, r_0 the external radius of the cylindrical membrane, r_i the inner radius of the cylindrical membrane, and μ the dynamic viscosity of the TM permeate. For detailed definition and unit information of TM pressure system components and experimental parameters, see **Paper I** appendix.

From Equation 3.4, it is clear that by setting the osmotic condition, and hence $\Delta P_{osmotic}$, and the MD perfusion flow rate, $Q_{perfusate}$, the fluid recovery, FR, can be studied as a function of the chamber pressure, $P_{chamber}$. This is the basis for the FR- static pressure studies of **Paper I, III and IV**.

3.5 Influence of static pressure

The experimental results from **Paper I** are consistent with the theoretic model of the TM fluid exchange, formulated as Equation 3.4. Figure 3.4 presents in vitro MD test FR plotted with chamber static pressure.

The experimental results in Figure 3.4 shows that the FR is linearly proportional to the applied static sample pressure, while other influencing factors in Equation 3.4 are set to be constant.

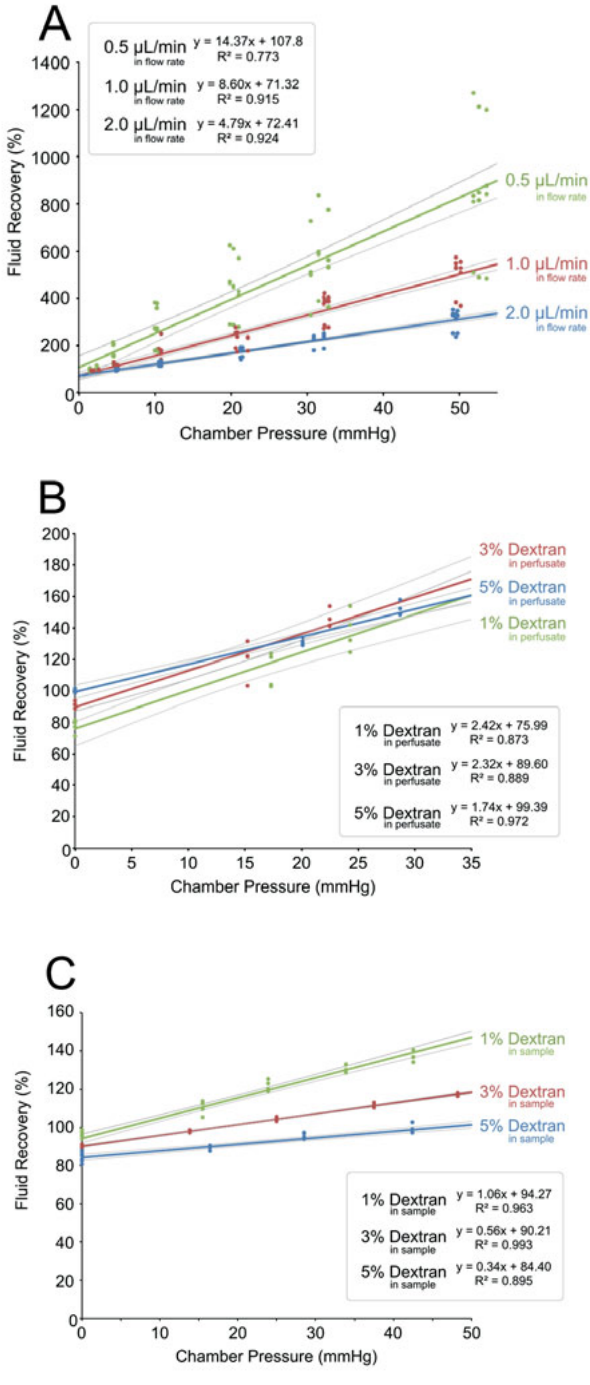

Figure 3.4 In vitro MD pressurization test results: FR vs. Chamber pressure. The series in the same figure varies in **(a)** MD perfusion flow rate. **(b)** MD perfusion fluid compositions. **(c)** MD test sample compositions. For details refer to **Paper I**.

As all three graphs in Figure 3.4 suggest, FR has a linear increase with chamber pressure, just as the theoretic model predicted, no matter the varying factors are MD perfusion rate (a), MD perfusate osmotic agent concentration (b), or MD sample osmotic agent concentration (c).

Following the linear model of Equation 3.4, the FR experimental data of each test set was linearly fitted with the chamber pressure, presenting the corresponding linear regression. The two curves besides each linear fit line marked out a 95% confidence zone for the experimental data points.

Figure 3.4 (a) suggests that FR also increased faster with lower MD perfusion flow rate which is in agreement with Equation 3.4 where the linear fit slope is a representation of the value $\boldsymbol{b}/Q_{perfusate}$. The ratio of the linear fit slope values 14.4: 8.6: 4.8 is a reasonably good representation of 2(1/0.5): 1: 0.5(1/2). According to Equation 3.5, the linear fit intercept $\boldsymbol{a}$, is unaffected by $Q_{perfusate}$, which is the case in Figure 3.4 (a).

In order to find out if the linear fit is the most suitable fit to the experimental data, higher order regressions (quadratic, cubic) were performed. The R^2_{adj} value in the regression model is often used to determine which order of regression is the best fit. The criterion is that the order of the best regression model is determined when the R^2_{adj} value stops increasing. By comparison of the R^2_{adj} values among the linear, quadratic, cubic regression models of each test set, it was found that: for perfusion rate as 1 μL/min, the R^2_{adj} value achieves highest value 0.994 at linear model; for perfusion rate as 2 μL/min, the R^2_{adj} value achieves highest value 0.999 at linear model; for perfusion rate as 0.5 μL/min, the R^2_{adj} value achieves highest value 0.991 at linear model. These suggest that for all three test sets, the linear fit is the best regression model.

3.6 Influence of osmotic pressure

In Figure 3.4 (b) the effect of colloid osmotic pressure inside membrane $P_{osmotic\text{-}in}$ was analyzed by varying the amount of dextran with MW 500 kDa (1, 3 and 5% conc. w/v.) in the Ringer's perfusate and keeping the perfusion fluid flow rate (1 μL/min) and sample (Ringer's solution) unchanged.

As Equation 3.4 suggests, the term $\boldsymbol{a} + (\boldsymbol{b}/Q_{perfusate})\,\Delta P_{osmotic}$ represents the intercept value, while $\boldsymbol{b}/Q_{perfusate}$ represents the slope value in the linear fit equation. According to this analysis, the contribution of $\Delta P_{osmotic}$ to FR is on the intercept value and the contribution should be additive which was the case. The FR at no pressurization injection increased from 76% when 1% dextran was added compared to 90% when 3% dextran was added and finally reaching 99% when 5% dextran was added. These results are in agreement with the findings of previous studies [12, 26]. The slopes decreased slightly when more dextran was added to the perfusion fluid which could be ex-

plained by increased membrane resistance R_m since all the other parameters of the membrane and outlet tubing (l, L, d, r_o and r_i) stay unchanged.

In Figure 3.4 (c) The effect of colloid osmotic pressure outside membrane was evaluated by adding dextran (1, 3 or 5%) to the Ringer's sample in the chamber. Also, dextran in a concentration of 3% was added to the Ringer's perfusate that was kept at 1 µL/min flow rate, for all three tests.

The intercept is clearly decreased with increased amount of dextran in the sample since the increased osmotic pressure outside the membrane caused the decrease on $\Delta P_{osmotic.}$(since the positive direction of the TM osmotic pressure is defined as towards the inside of membrane) and according to Equation 3.5, the decrease of $\Delta P_{osmotic}$ leads to the decrease on the intercept value.

Stepwise changes have been seen in the slope value where increased colloid osmotic pressure of the sample decreases the slope value. The reason for this is that first, the viscosity of the dextran solution increases and hence $\boldsymbol{b}$ in Equation 3.5, decreases. Secondly, and more prominently, the overall membrane resistance R_m increases due the concentration polarization of the colloidal dextran molecules. Solutes and colloids (dextran) are transported towards the surface of the membrane from the sample. The solutes pass through the membrane while the large dextran colloids accumulate at the surface. The concentration of the colloids at the surface have been reported to be 20-50 times higher than in the sample solution and therefore form a gel layer that becomes denser with increased chamber pressure and the concentration of colloids [43]. This gel layer eventually leads to membrane fouling.

Furthermore, in **Paper I**, the investigation of gel layer formation using human plasma sample is presented. The result Fig.5 in **Paper I** shows that, the FR behavior of complex biological sample along with changed pressure, are similar to in-house made sample with added osmotic agent such like Dextran T500 3% w/v.

When comparing plasma to 3% dextran T500, they are similar in viscosity and particle movement. This result clearly shows two things:

1- The in vitro MD systems with a pressurized biological sample could be simulated using this chamber. Complex biological events such as different traumatic brain injuries (TBI), where the intracranial pressure (ICP) could raise and fall dramatically, could be mimicked and evaluated by such in-vitro test system. This becomes the basis for the study of **Paper III and IV**.

2- Dextran as a macro molecule can be used to mimic the behavior and fluidics of macro molecules which are targets of high MWCO MD. This has been successfully implemented in the study of **Paper II**.

3.7 Other influence factors

Other factors which could influence the FR of high MWCO MD includes:

R_m - the MD membrane resistance to the transmembrane permeate, μ -the dynamic viscosity of the transmembrane permeate, and the catheter design parameters (all dimension parameters in Equation 3.5).

When discussing the pressure influence on FR, variables ***a*** and ***b*** in Equation 3.4 are set constant, to allow only perfusion rate, static pressure and osmotic pressure as variants. In fact, the experiment setting of **Paper I** are intentionally set to make all variants in variables ***a*** and ***b*** stay constant. However, as can be seen from the expression of Equation 3.5, ***a*** and ***b*** can vary in a large extent if any of the included variants varies. For example, the membrane resistance of a 20 kDa MWCO membrane could be 10,000 fold of the membrane resistance of a 100 kDa MWCO membrane. This would vary variable ***b*** greatly. The dynamic viscosity of the TM permeate could easily vary in 1,000 fold range among different permeate as well, which would change variable ***b*** a lot.

At the last, the catheter design parameters, as the listed dimension parameters in Equation 3.5, seems to be not so crucial since there are so many different MD catheter designs. In fact, they are crucial. First of all, for Equation 3.5, every one of the dimension parameters will influence both ***a*** and ***b***. From the length of the outlet tubing and inlet tubing, to the length of the membrane, the inner diameter of the outlet tubing, then to the inner and external radius of the cylindrical membrane, the combination of their numerical values in a whole, decides and influences the variables ***a*** and ***b***, then in turn decides the *FR* value.

This has been verified by the author by experiments whose results are not shown in this thesis. For instance, one experiment has been done to shorten the catheter outlet tubing into several lengths, and compare their FR response.

The result shows that the FR increased with shorter outlet tubing length. This is consistent with Equation 3.5 which suggests the drop in l will give bigger ***a*** and ***b***. Another way to explain this is by the Poiseuille's law:

$$P_{outlet} = \frac{128\mu l}{\pi d^4} Q_{dialysate} \tag{3.6}$$

with l being the length of the outlet tubing, μ the viscosity of the dialysate, and $Q_{dialysate}$ the flow rate of the dialysate. With the decrease of l, P_{out} decreases in linear fashion. According to Equation 3.3, decrease in P_{out} will lead to increase of overall TM pressure ΔP, which of course , will contributes to the increase of the inflow into the MD catheter, and subsequently , the *FR*.

In a word, the catheter design will definitely influence the FR of the MD catheter. To extend further, even the material choice or fabrication method should be considerate.

For example, the choice of the material for the tubing should follow the rule that its inner diameter should not be varying too much. And the cylindrical membrane should be fabricated in the way that its inner and external radius will not be easily altered by pressure, etc. These design details could hopefully contribute to the stability of the MD sampling performance.

4. Microdialysis extraction efficiency

4.1 Molecular weight cut-off

MD catheters vary in catheter design and membrane geometry. The membranes can differ in material, surface property, pore structure and pore size. For MD membranes, their pore size is defined as the MWCO.

The MWCO is a loosely defined term which is generally taken as the MW of the molecule that is 80-90% rejected by the membrane. Here in the definition, the molecules are assumed to be globular, as an approximate model to their real 'shape'. To clarify the definition further, the nominal MW of MWCO is an average point where 80-90% of molecules in such size will be rejected or retained by the membrane. This loosely defined statistic nature of the MWCO, become one of the reasons for the study of **Paper II**.

The MWCO is often expressed in unit as Dalton (Da), and kilo-Dalton (kDa). Dalton is unit of mass, also called 'the unified atomic mass unit'. It is the standard unit that is used for indicating mass on an atomic or molecular scale (atomic mass) [44].

One dalton is approximately the mass of one nucleon (either a single proton or neutron) and is equivalent to 1 g/mol [45]. It is defined as one twelfth of the mass of an unbound neutral atom of carbon-12 in its nuclear and electronic ground state [46]. For examples, a hydrogen atom has a mass of 1.0078250 Da; a carbon-12 atom has a mass of 12 Da. One molecule of Aspirin has a mass of 180.16 Da. One molecule of glucose weighs 180 Da.

Masses of proteins are often expressed in daltons. For example, a protein with a MW of 64,000 $g \cdot mol^{-1}$ has a mass of 64 kDa [45]. Neurotensin has a MW of 1,672 Da [47]. Serum albumin has a MW of 69.7 kDa. Titin, the largest known protein, has an atomic mass of 3-3.7 megadaltons (3,000 kDa) [48].

As mentioned previously, high MWCO MD utilizes ultrafiltration membranes. The exact fabrication method and process of the MD membranes used in the studies of this thesis are unknown, due to limited information. But their basic structure is known, that is they have an anisotropic structure which has a well-defined porous surface layer attached to a much more open 'network' of microporous substrate.

The well-defined membrane porous surface layer performs the initial rejection and separation of differently sized molecules, the microporous sub-

strate functions as mechanical strength support on one hand, and on the other hand, the complex porous 'network' in the substrate retains some molecules and become another stage of separation. The molecules which pass through the microporous 'network' can finally be collected into the MD dialysate.

Ultrafiltration membranes are generally considered to have pore diameters from 10 to 1,000 Å [49]. Figure 4.1 shows an example of the membrane surface porous structure of 100 kDa MD membrane, with SEM images. As can been seen from the most magnified Figure 4.1 last image, the majority of pores are with diameter 1-1.6 μm, the largest pore can reach to 4 μm. Approximate average pore diameter estimated from the image to be about 2.3 μm, which is 2,300 Å.

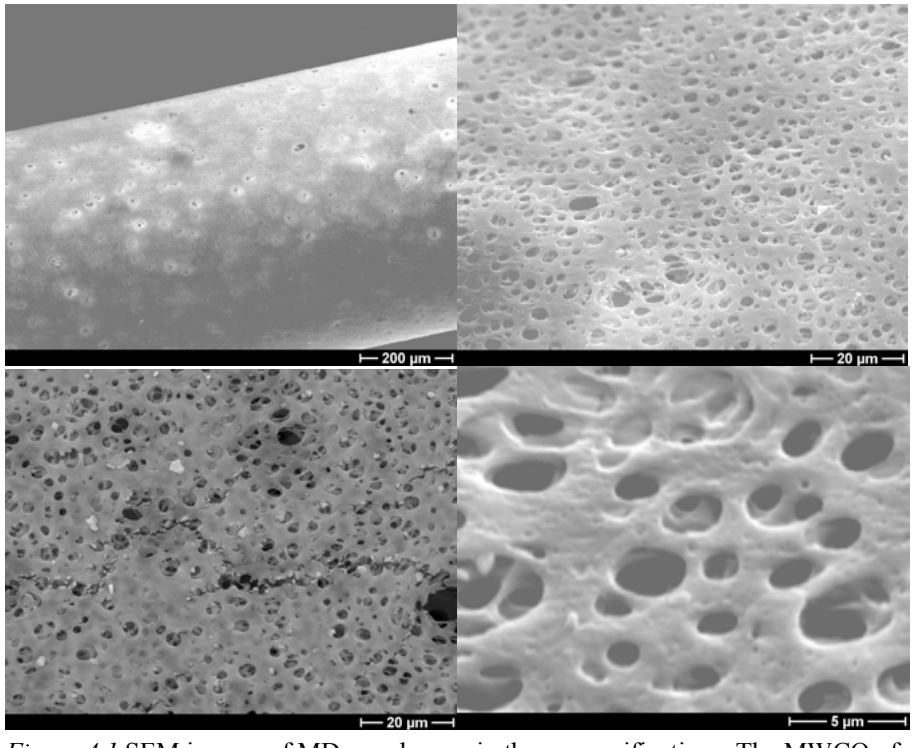

Figure 4.1 SEM images of MD membranes in three magnifications. The MWCO of the membrane is 100 kDa. After usage on the in vitro test, the used membranes are dried and then sputter coated with Pt/Au alloy to make its surface conductive, to be suitable for SEM analysis.

4.2 Imaging of macromolecule transport

As described in previous section 3.6, dextran as a macromolecule has the possibility to mimic the transport behavior of complex biological samples such like blood plasma. And Fluorescein isothiocyanate (FITC) tagged dextran is suitable for fluorescence imaging. Also dextran is widely-used in MD sampling practice. These facts make dextran an ideal macromolecule model to study the macromolecule transport in High MWCO MD.

In **Paper II**, the transport of FITC dextrans with different MW during High MWCO (100 kDa) MD sampling is studied with fluorescence microscopy and Matlab image processing, to analyze both qualitatively and quantitatively the dextran molecular transport in the MD catheter and their leakage over the membrane. In addition, an in vitro MD test chamber specially designed for fluorescent microscope imaging was fabricated and validated, shown in Figure 4.4.

The results of **Paper II** suggests that, fluorescence imaging provides direct revelation of molecular transport properties of individual MD catheters and increases the understanding of the variations in MD sampling when high MWCO membranes are used.

The developed in vitro chamber and imaging method suggests that fluorescence imaging could be used as a method for qualitative (shown as Figure 4.2) and semi-quantitative (shown as Figure 4.3) molecular transport and fluid dynamics studies for MD membranes and other hollow fiber catheter membranes.

Figure 4.2 shows that the imaging method could cover most details of the qualitative molecular transport over entire MD membrane. Figure 4.3 shows the semi-quantitative images suggesting that dextran 40 and 250 kDa leak through a 100 kDa MWCO membrane but dextran 500 kDa do not leak.

4.3 In vitro test systems

For the studies in this thesis, three in vitro test systems were designed and validated, Figure 4.4 presents them. Temperature control and biological samples were used in the in vitro tests, all three systems exhibited potential to mimic in vivo MD test.

Similarly, Stenken et al. [50] developed an in vitro MD hydrodynamic system, in which the flow of both the perfusate and the sample through a cross-flow MD probe can be well characterized. Their study results showed that the in vitro calibration of MD probe is an appropriate method for use in hydrodynamic in vivo environments.

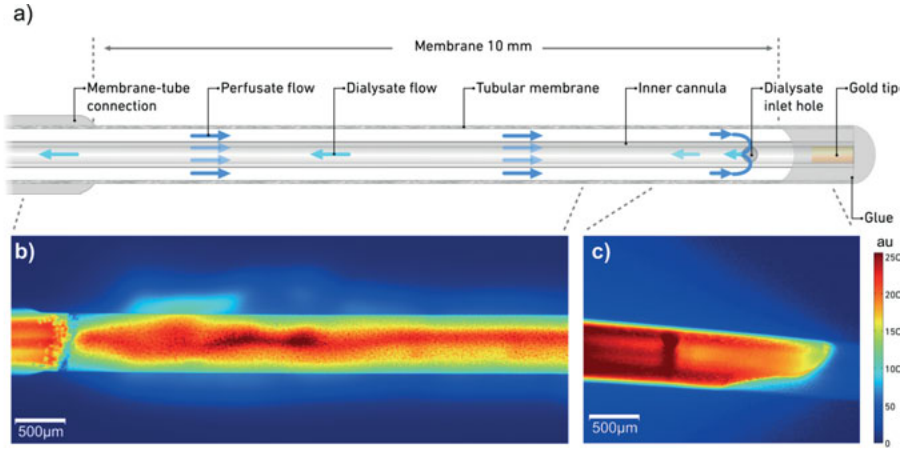

Figure 4.2 Schematic illustration of **(a)** the detailed structure of the MD capillary and its fluidic macromolecule transport. **(b)** Fluorescence image of a 100 kDa MD membrane in the beginning of perfusion. **(c)** Fluorescence image of the perfusate inlet opening at the end of the concentric 20 kDa MWCO capillary. [**Paper II**]

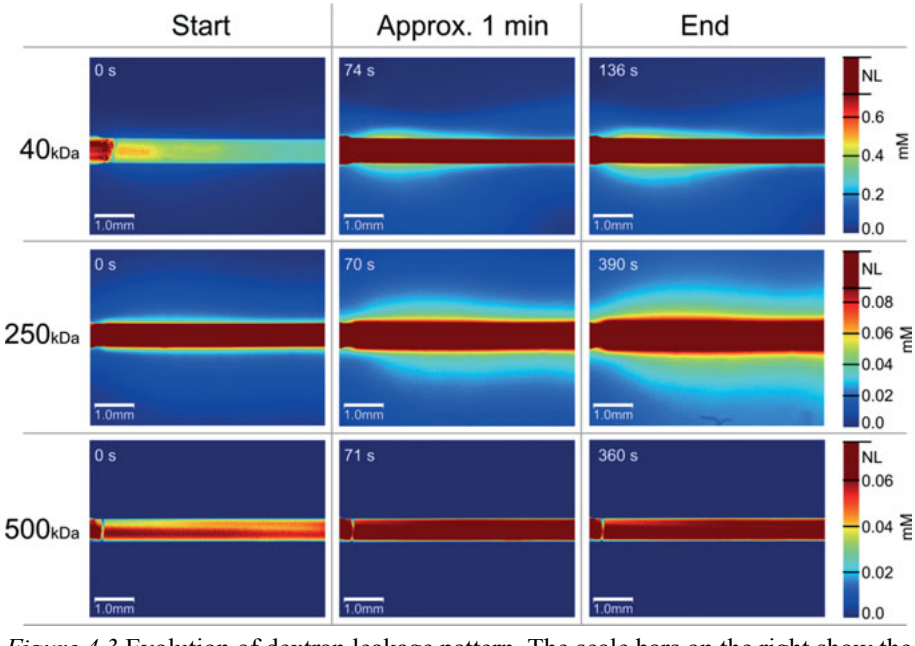

Figure 4.3 Evolution of dextran leakage pattern. The scale bars on the right show the dextran molar concentration (mM) depending on the pixel color. The pixels which have color over the linear zone are all marked as non-linear ('NL'), which is displayed in dark red. 100 kDa MWCO membranes are used. [**Paper II**]

Three chambers are designed to fit the aim of its study, and are validated in the experiment with all other experimental settings. Their respective advantages are:

System no.1: able to facilitate four probes in a chamber; small size of the chamber needs small volume of test sample; nearly identical test conditions for four probes; direct coupling between sample injection-chamber pressure validated.

System no.2: thinnest among the three (also the least chamber volume among the three); microscopic grade glasses cover; fully compatible with a fluorescence microscope; easy sample filling and removal. Effective removal of the trapped air bubbles.

System no.3: improved version of System no.1; similar function but more suitable for biological test samples; still with more advantages: smaller chamber volume than no.1; ensures identical test condition among four individual test catheters; effective chamber filling and sample removal; effective removal of the chamber air dead volume; the chamber is easy to fabricate and clean/reuse; the cap can fit to all bottles with standard dimensions.

4.4 Protein extraction efficiency and membrane surface modification

In **Paper III and IV**, a protein standard with similar concentrations as in human cerebral spinal fluid (CSF) was used as sampling matrix. The individual protein or peptide EE was analyzed using targeted MS and Dot-it spot-it protein assay. The influence of external sample pressure and surface modification of MD catheter are examined as well.

The catheters were surface modified with the triblock co-polymer, Poloxamer (a.k.a. Pluronic) prior to MD-sampling. The polymer consists of one hydrophobic polypropylene oxide (PPO) group flanked by two hydrophilic polyethylene oxide (PEO) strands. The triblock co-polymer forms a cilia-like brush layer of PEO strands on the surface of the hydrophobic membrane and tubing of the MD catheter [51], which reduce protein absorption and membrane fouling.

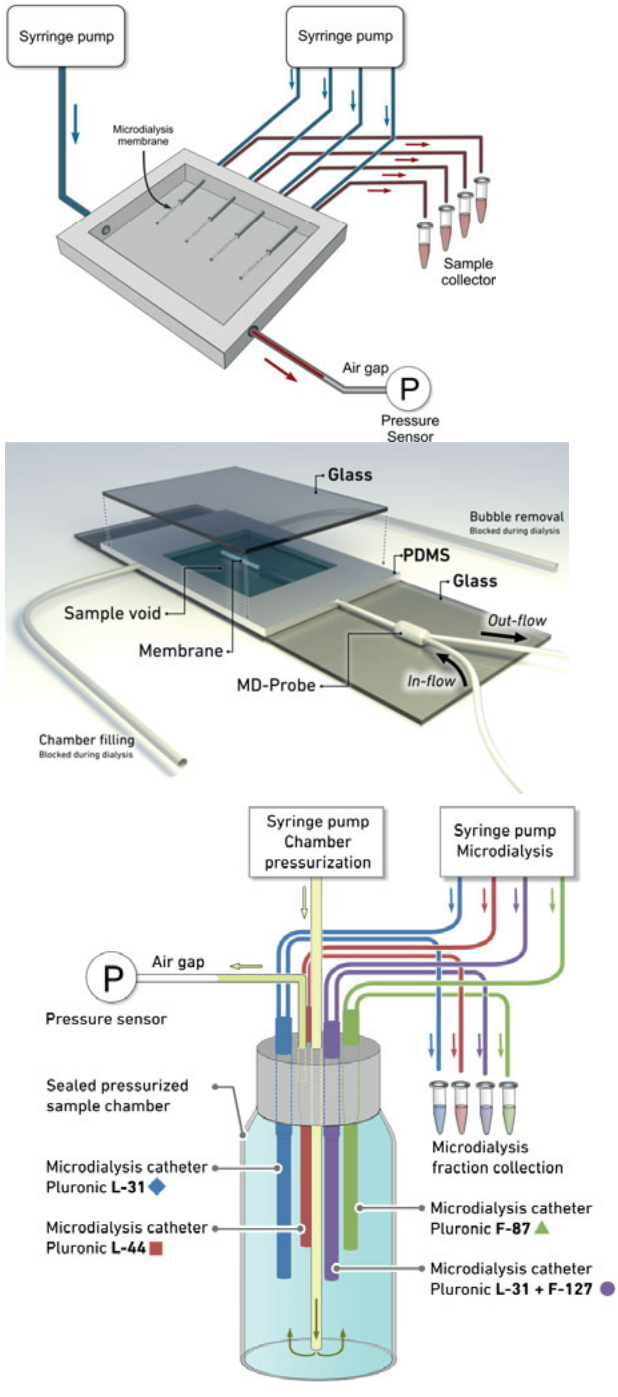

Figure 4.4 In vitro systems used in the studies of this thesis. **Top**: Pressurization system in **Paper I**. **Middle**: Fluorescence imaging system in **Paper II**. **Bottom**: Pressurization system with protein sample matrix and four differently surface modified MD catheters in **Paper III and IV**. Courtesy Andreas Dahlin.

The EE behavior of individual proteins was compared between native and pluronic 127 coated catheters in **Paper III**, the corresponding results are shown as Figure 4.5. In **Paper IV,** four different pluronic surface modifications were evaluated and their performance was assessed, as shown in Figure 4.6.

The study results of **Paper III** suggests that the FR of surface modified catheters were less sensitive to the pressure and provide higher precision, and provided a FR closer to 100%. The surface modification did not show a significant effect on the protein EE. The average total protein EE of surface modified catheters was slightly higher than that of the native ones. The MS EE data of individual proteins showed a clear trend of complex response in EE with pressure.

The study results of **Paper IV** suggests that the trend of FR among the four pluronic modifications follows the theory that the thicker the pluronic adsorption layer, the less the hydrodynamic diameter of the membrane pores, leading to lower and more stable FR. The EE behavior of the four pluronic modifications can be classified as three groups: Pluronic F127 + L31 showed similar behavior to the Pluronic F127 and the native original membrane; Pluronic F87 showed a continuous EE increase with pressure; Pluronic L31 and L44 showed similar EE values, which were stable with pressure. Different surface modifications are clearly selective to different proteins and peptides.

This provides a potential of choosing a specific pluronic surface modification depending on the objective of the sampling, *e.g.*, reaching high EE at high FR, stable EE over different pressures, or specificity between certain proteins. It's also possible that the pluronic surface modification could provide MD sampling with either stable or improved sampling performance regarding both EE and FR. More pluronics and combinations of them as MD surface modification are worth being investigated.

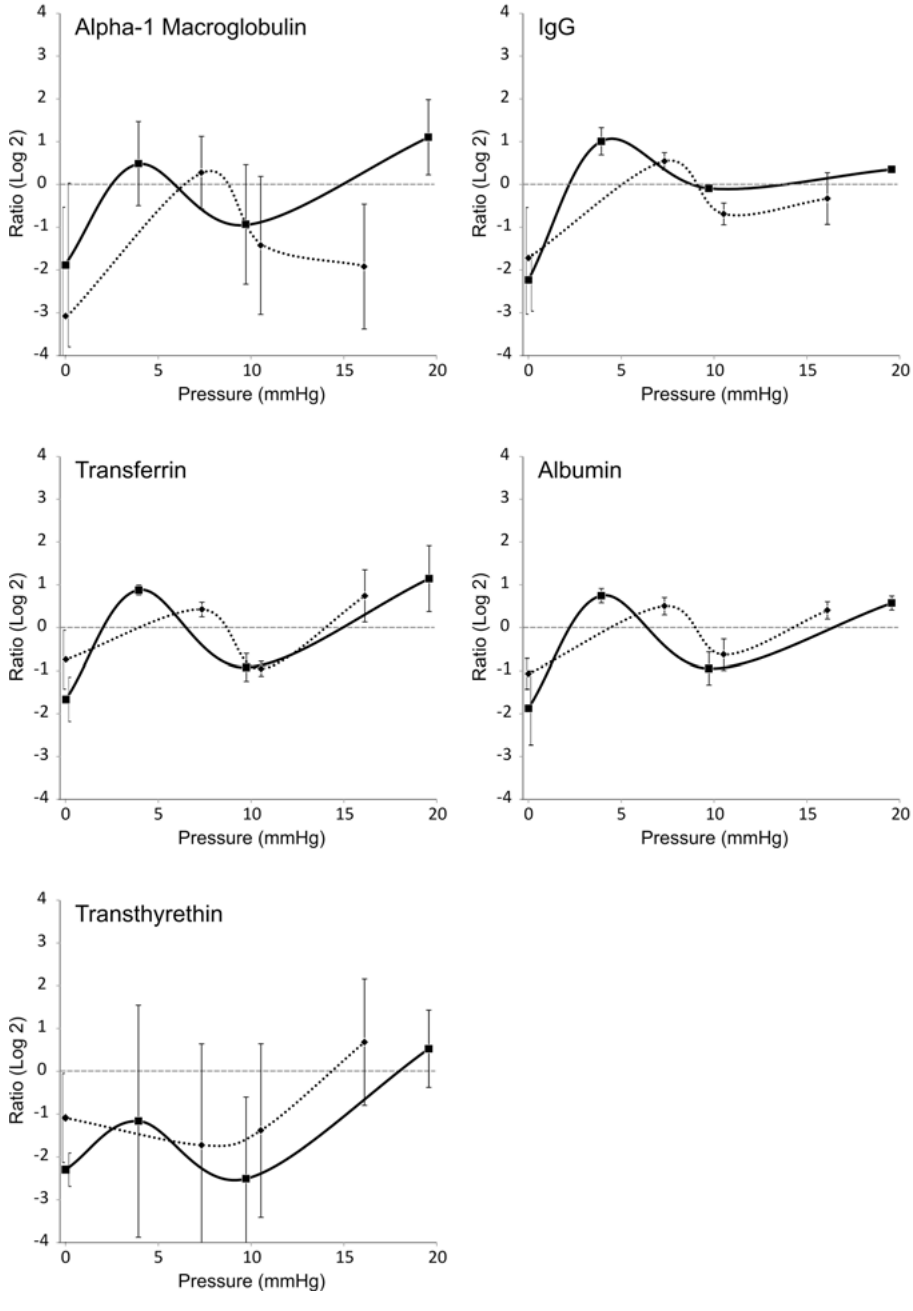

Figure 4.5 EE of proteins from the MD dialysates in response to the chamber pressure, for native (dashed line) and surface modified (solid line) catheters. The EE value is the Log 2 ratio of the concentration data from the MS measurement (after normalization). Each data point is the average of three MS runs on the same MS sample (each represents certain pressure). The four data points are fitted to a line to show any trend. [**Paper III**]

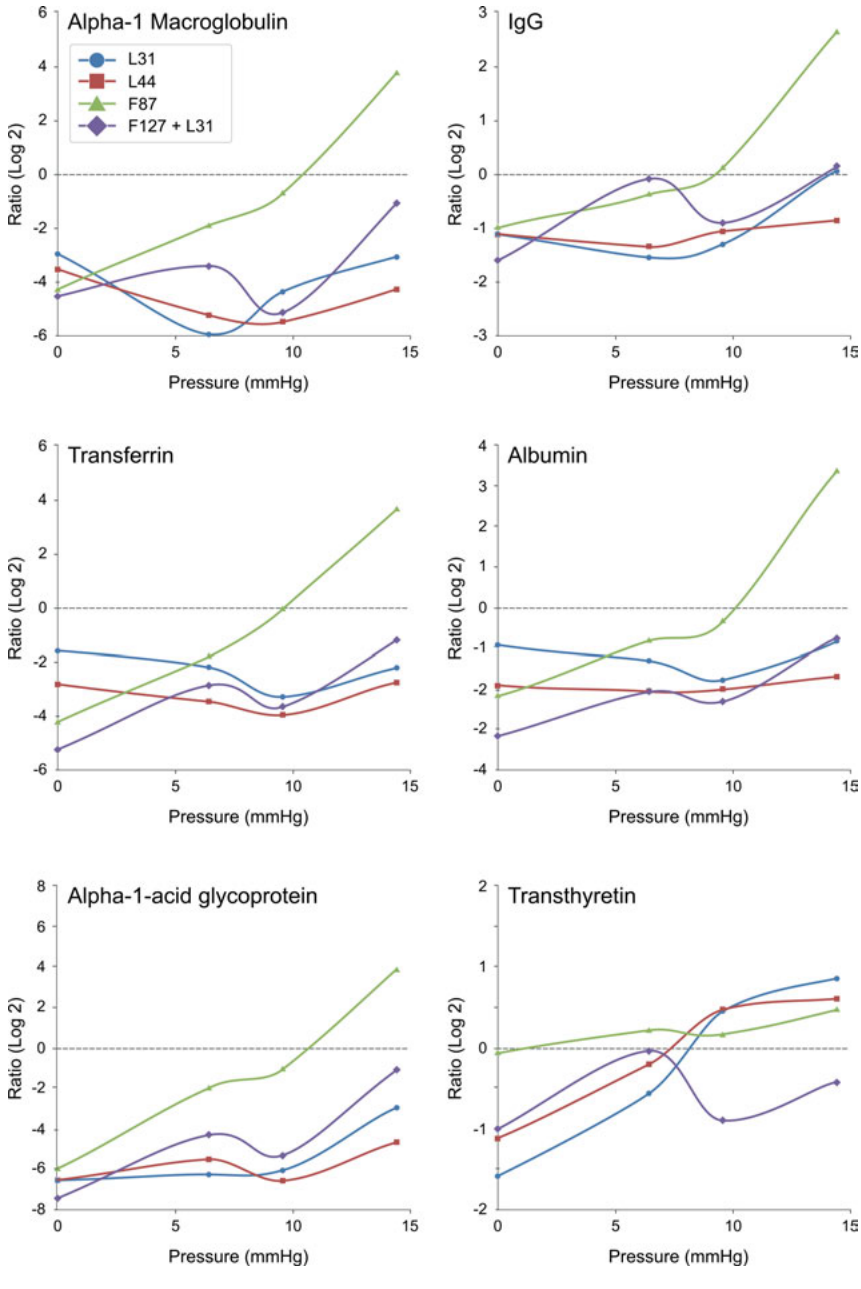

Figure 4.6 EE of proteins from the MD dialysates in response to the chamber pressure, for the four different pluronics modified MD catheters (blue circle-L31, red square-L44, green triangle- F87, purple diamond- F127+L31). The EE value is the Log 2 ratio of the concentration data from the MS measurement (after normalization). Each data point is the average of three MS runs on the same MS sample (each represents a dialysate fraction at a certain pressure range). For each series, the four data points are fitted with a line to show any trend. [**Paper IV**]

5. Conclusion and outlook

This thesis studied High MWCO MD sampling on following aspects: MD fluid characteristics theory; MD FR under pressurization; Fluorescence imaging of MD membrane macromolecule transport; in vitro MD test systems; In vitro MD protein/peptide sampling FR and EE; Pluronic surface modification of MD catheters.

A summary of the main studies and their results in the form of images is presented as Figure 5.1. Table 5.1 describes the topics that each paper is focusing on, and their corresponding results/contributions to the field of high MWCO MD sampling.

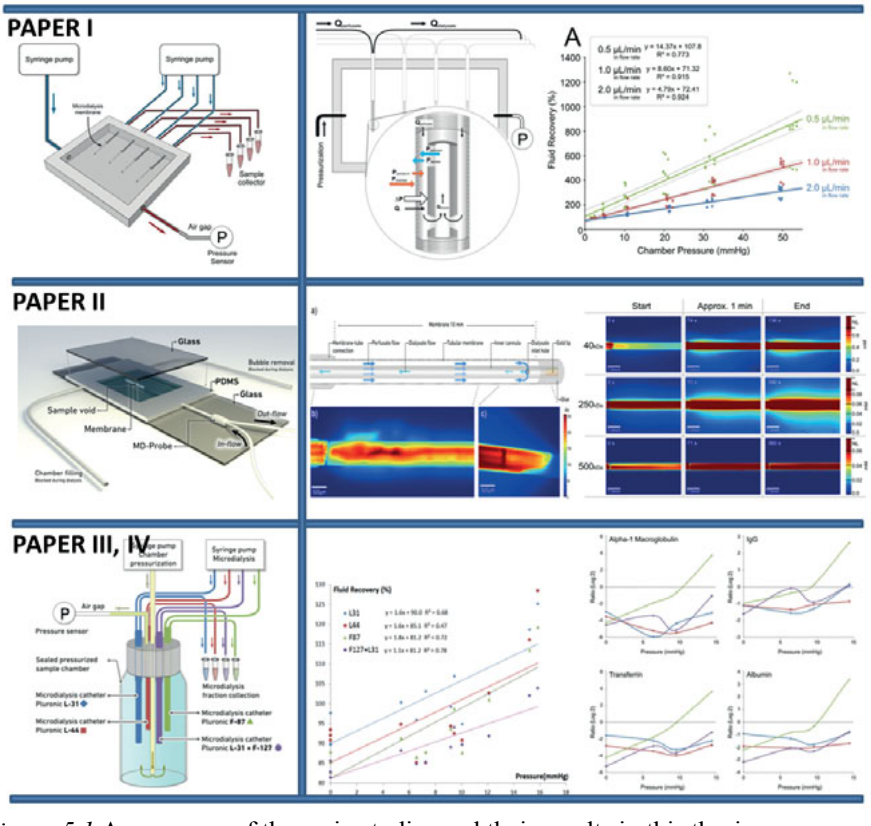

Figure 5.1 A summary of the main studies and their results in this thesis.

Table 5.1 A summary of the investigated topics and their results in this thesis.

Paper	Topics	Results
I: High MWCO MD FR Under static pressure	-MD fluid characteristics -MD pressure system -FR vs. static chamber pressure	Experimental consistent with theory and formula: FR in linear relation with pressure
II: Macromolecule transport in high MWCO MD	-FITC dextran as macro biomolecule -Visualization of molecular transport -Quantification of molecular concentrtion	-Macromolecule transport through MD membrane visualized -Semi-quantification of the molecular concentration -Fluorescence imaging as a tool for MD sampling study
III , IV: In vitro protein sampling efficiency of surface modified MD catheters	-In vitro protein matrix sampling to mimic in vivo CSF sampling -Individual protein/peptide extraction efficiency -Dialysate analysis by targeted Mass Spectrometry method -Influence of surface modification on sampling efficiency	- Individual protein/peptide extraction efficiency quantified by targeted Mass Spectrometry -More stable FR for surface modified catheter than native catheter -Complex behaviors of EE in response to pressure depending on pluronic coating types -Due to selectivity of EE behaviors, surface coating has potential to be customized to achieve EE control.
V: Microchip for MD protein sample desalination	-Microchip desalination device based on ion concentration polarization -desalination efficiency on MD protein matrix mimicking CSF	- The protein sample was desalinated and the protein concentration ratio between the desalinated and the salted fractions could be adjusted by applying different voltages - The concentration of a protein in the desalinated fraction could be three times higher than that of the original sample

Through the studies in this thesis, it is clear that many aspects of high MWCO MD sampling can be improved to enhance MD sampling of macromolecules. Here, five are listed:

1. MD pumping control. First of all, push-pull has a potential to deliver precisely controlled FR. In future, a miniaturized automatically adjusted push-pull system which can be carried conveniently and operated with ease should be realized. Second, the MD sampling pressure should be monitored and corresponding feedback control mechanism could be considered.

2. Osmotic agent to be added into the MD perfusate. By the studies in this thesis, it was demonstrated that large size osmotic agent such like dextran T500 (500 kDa) has functions like balancing FR and no leakage across the membrane. Its usage should be promoted.

3. Micro device for MD dialysate analysis and MD sample preparation. The studies in this thesis used targeted MS to analyze MD dialysate with

very low amount of analytes. **Paper V** presents a micro device which could perform desalination of MD protein sample. Micro devices like microfluidic chip device or MEMS have great potential to be integrated into the MD sampling system.

4. MD membrane improvement. It can be improved in three aspects: its intrinsic porous structure, pore design; its material; its surface modification by e.g. pluronics. The aims will be to reduce membrane bio-fouling and make its MWCO statistically stable and trustworthy.

5. MD catheter design. As discussed in section 3.7, MD catheter design details matter for the MD sampling performance. For instances, the length and inner diameter of the outlet tubing; the inner and outer diameter of the cylindrical membrane; the membrane resistance; even to the material choice of the outlet tubing, etc. MD catheter has to be carefully designed to meet the desired performance.

At last, may all the study efforts open-up a bright future for the microdialysis sampling technique.

6. Svensk sammanfattning

Mikrodialys är en ganska ny provtagningsteknik som infördes av svenska forskare 1974. Den har haft en kontinuerlig utveckling sedan dess. Dess olika tillämpningar har utvecklats och dess kommersiella marknad har expanderat.

MD är en lysande teknik på grundval av en enkel princip: molekyler utanför celler i levande organismers vävnad kan samlas in av en liten nålliknande kateter som härmar en kapillärs funktion i kroppens vävnad. Nyckeln till provtagningen är dialysmembranet, som vanligtvis är mikro- eller nanoporöst strukturerad. Membranet definieras ofta av dess porstorleken, d.v.s. efter vilken molekylvikt som inte kan passera igenom membranet. En vätska som har samma fysiologiska egenskaper som kroppsvätska pumpas kontinuerligt igenom katetern innanför membranet för att tillåta provtagning av målmolekyler utan att det blir en tryckskillnad på grund av olika saltmängd.

Fördelarna med mikrodialys gentemot andra liknande tekniker ligger i sex aspekter: För det första, genom att härma en kapillär i vävnaden kommer provet att ge en direkt representation av vad som finns i vävnaden. För det andra, om utloppsslangen till katetern är ansluten direkt till ett analysinstrument så kan realtidsövervakning uppnås av målmolekylerna. För det tredje, mikrodialys fungerar både som provtagning och provberedningsmetoder simultant. För det fjärde, mikrodialysens är i relation till andra liknande tekniker mindre störande för vävnad. För det femte så använder mikrodialys en enkel princip som utnyttjar vätskors flödesmekanismer med passiv drivkraft, vilket medför att yttre styrning förutom den nödvändiga pumpningen av fysiologisk vätska, krävs. Enkelheten i driftförfarandet och den icke-komplexa instrumenteringen har stor del i dess popularitet jämfört med andra liknande metoder. För det sjätte kan mikrodialys användas på ett litet fokuserat område med valda molekyler och små provvolymer, som ger snabb respons vid klinisk övervakning.

Under de senaste åren, har mikrodialys med membran med små porer undersökts väl och dessa katetrar används numera rutinmässigt i klinisk miljö som övervakningsinstrument för små biomarkörer som t.ex. signalmolekyler och energimolekyler. Mikrodialys har bland annat bidragit till utökad förståelse inom områden sombiokemi, neurologi och hjärnforskning. Exemplen inkluderar bättre förståelse för transportmekanismer mellan blod och hjärna, Parkinson, Alzheimers sjukdom, stroke, depression, drogmissbruk, för att nämna några.

Provtagning med mikrodialys beskrivs ofta av vätskebalansen och extraktionseffektiviteten. Ett gott resultat präglas av en stabil vätskebalans och hög extraktionseffektivitet, företrädesvis så att man kommer nära koncentrationen i vävnaden. Mikrodialys av små molekyler med membran som har små porer ger stabil prestanda och acceptabel effektivitet. Men under de senaste åren har det blivit klart att det är mycket svårt att nå samma kvaltet i provtagningen av större molekyler som proteiner med hjälp av membran med stora porer. Mikrodialys av proteiner har flera utmaningar, som tyvärr sällan har studerats. Som exempel har fem aspekter undersökts i studierna till denna avhandling:

1. Vätskors rörelse vid mikrodialysprovtagning har inte studerats ingående. Speciellt när flödesregimen når ultrafiltration-intervallet för vid större porer i membranet, kan den tidigare teorin där diffusion dominerar inte förklara proteinernas transport. Utan en tydlig förståelse av vätskors rörelse, kan man inte kontrollera vätskebalansen. I **Papper I** bygger vi en teori för membran med stora porer och presenterar en ekvation som innehåller de flesta av de faktorer som påverkar vätskebalansen. De experimentella resultaten överensstämmer med teorin.

2. Definitionen av membranets porstorlek bygger på en löst definierad statistik vilket gör att provtagningens prestanda är svår att förutsäga och jämföra mellan olika membran. En studie av stora molekylers transport över ett membran med stora porer gjordes i **Papper II**, genom direkt visualisering av fluorescensinmärkta molekyler och relativ bestämning av deras koncentration.

3. **Papper III** och **IV** studerar vi extraktionseffektiviteten av proteiner och peptider i ett trycksatt test som efterliknar förhållandet i människokroppen. Vi var i stånd att kvantifiera koncentrationen för varje enskild protein i dialysatet och studerade hur dessa varierar med provtagningsförhållanden.

4. Olika ytmodifieringar har använts på mikrodialyskatetern och deras effekter på vätskebalansen och extraktionseffektiviteten för enskilda proteiner/peptider har undersökts i **Papper III, IV** och **VI**. Resultaten tyder på att olika Pluronics ger olika ytskydd som i sin tur ger möjligheter att förbättra både vätskebalansen och effektiviteten både laborativt (**Papper III, IV**) och i levande vävnad (**Papper VI**).

5. Ett mikrochip som är i stånd att skilja salter från proteiner i provet har tagits fram och testats i **Papper V**. Dess prestanda tyder på att det har god potential att användas för avsaltning av provet från mikrodialysen innan den ska analyseras, men även att användas för annan provpreparering av proteiner där avsaltning behövs.

Det finns många möjligheter att öka kvalitén hos proteinprover vid mikrodialys. Här listar jag fem:

1. För att få kontroll på vätskebalansen finns push-pull pumpar (då en pump trycker in vätska och en annan suger upp den) för experiment men inte till kliniken. I framtiden bör ett miniatyriserat och automatiserat push-pull-system som är enkelt att använda tas fram. Annars bör alltid provtagningstrycket kontrolleras och noteras tillsammans med värdet för vätskebalansen.
2. Om dextran tillsätts i perfusatvätskan som pumpas i katetern bör man använda molekyler som är mycket större än membranets porer.
3. Papper V presenterar ett mikrofluidalt chip som kan utföra avsaltning av proteinprov från mikrodialys. Sådana mikroflödessystem har stor potential att integreras i provtagningssystemet.
4. Mikrodialysmembranet kan förbättras på tre viktiga punkter: dess porösa struktur; dess material; dess ytmodifiering. Målet är att minska möjligheten för proteiner att fastna på membranet och därmed göra provtagningen av proteiner mer stabil och pålitlig.
5. Mikrodialyskateterns design och material spelar stor roll för provtagningsprestandan. Som exempel är det viktigt att skydda utloppsröret så att proteiner fastnar för lätt på ytan.

Till slut vill jag önska att studierna i denna avhandling kan hjälpa till att skapa en ljus framtid för mikrodialys som provtagningsteknik.

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