Title of dissertation			
Development of microfluidic chip-mass spectrometry system for cell metabolism analysis			
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An analytical system that microfluidic chip and mass spectrometer are hyphenated (Chip-MS) is a versatile tool for online biomolecules detection and cell studies. By combining a microfluidic chip which enables precise regulation of cellular environment and its flexible manipulation, and a mass spectrometer which enables facilitating highly accurate quantitative determination, Chip-MS system has extensive applications in pharmacology, proteomics, metabolomics and single cell analysis. To enhance the sensitivity of detection, a solid phase extraction (SPE) integrated Chip-MS system had been developed for real-time monitoring of cell metabolism. In this thesis, to explore the performance of Chip-MS, a serial of research has been implemented. In consequence, the dissertation consists of five parts.

Chapter 1 This chapter introduced the definition, mechanisms and existing applications of Chip-MS. Traditional methods for Chip-MS were summarized, and the advantages and disadvantages of these methods were discussed. The recent fundamental developments of Chip-MS system for cell analysis were described.

Chapter 2 It is well known that cell can response to various chemical and mechanical stimuli. Therefore, flow pressure variation induced by sample loading and elution should be small enough to ignore the physical impact on cells when we use Chip-SPE-MS system for cells. However, almost existent Chip-SPE-MS systems ignored the pressure alternation because it is extremely difficult to develop the homogeneous flow pressure hyphenated system for application in dynamic monitoring of cell metabolism. In this chapter, the author developed a novel fluidic isolation assisted homogeneous-flow-pressure Chip-SPE-MS system. The homogeneous ambient flow pressure was achieved by fluidic isolation between cell culture channel and SPE column. Furthermore, an automatic sampling probe could accomplish the accurate sample loading and dispensing to achieve online pre-treatment of the sample. In this study, the fluid isolation between the cell culture channel and the SPE chromatographic column could minimize the influence of heterogeneous flow pressure on cell behavior, thereby achieving uniform environmental flow pressure to cells during cell culture and sample loading.

Chapter 3 Long-term therapy with vitamin D_3 is used to reduce the risk of bone diseases associated with vitamin D_3 deficiency. Human cytochrome P450 (CYP) catalyze vitamin D_3 and form corresponding metabolites. 25-hydroxyvitamin D_3 (25(OH)D_3) is typically used as a biomarker for vitamin D_3 metabolic

status. This chapter presented a new method for dynamic monitoring of 25(OH)D_3 changes, which contribute to assess vitamin D_3 supplementation. Thereby, the author could screen out 24,25-dihydroxyvitamin D_3 (24,25(OH)$_2D_3$) as the main metabolic product of 25(OH)D_3, and their biotransformation was evaluated in real-time using the Chip-SPE-MS system. Treatment of human healthy hepatocytes L-02 with 25(OH)D_3 was found to increase significant formation of 24,25(OH)$_2D_3$, but this change was not apparent in hepatoma cells HepG2. Further analysis revealed a downward trend in the ratio of 25(OH)D_3/24,25(OH)$_2D_3$ as 25(OH)D_3 increased, indicating that 24-hydroxylase level varies in response to the change in circulating 25(OH)D_3. Moreover, this metabolic pathway was blocked by the addition of ketoconazole, a selective cytochrome P450 3A4 enzyme (CYP3A4) inhibitor, suggesting that induction of hepatic CYP3A4 was necessary for increasing 25(OH)D_3 metabolism. Thus, this process could normalize elevated vitamin D_3 metabolites in patients who lacked the native pathway for vitamin D_3 oxidation due to loss of function mutations in cytochrome P450 24A1 enzyme (CYP24A1).

Chapter 4 Apoptosis is an event of programmed cell death with different biochemical and genetic pathways that play a critical role in normal tissue development and homeostasis. 1,25-dihydroxyvitamin D_3 (1,25(OH)$_2D_3$), which stimulates calcium entry, transfer and calcium buffering in different cell types, plays a role in the regulation of the apoptosis process that determines the outcome of health and disease in the cell. In this chapter, The Chip-MS system was approved to be a novel approach for dynamic monitoring of extracellular metabolites of cancer cells released by drug stimuli. Since the Chip-MS system can be used in online monitoring of cell metabolism, this system was applied to study the effect and mechanism of 1,25(OH)$_2D_3$ on apoptosis of cancer cell in this chapter. This data together demonstrated that the glucose metabolic activity significantly differed between normal cells and cancerous cells. This result also revealed that 1,25(OH)$_2D_3$ affected the proliferation of colon cancerous cell HCT116 to change the cellular metabolism including decrease of glucose uptake.

Chapter 5 The principal findings and results were briefly summarized, and the future research and applications of Chip-MS are prospected.

The results presented in this thesis have significantly contributed to the advancement in research of proteomics and metabolomics. Considering the excellent and high-quality research presented in this thesis, the candidate deserves the doctoral degree.