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厦门大学

硕 士 学 位 论 文

核酸的电、磁纯化新方法及其在微流控芯片中的应用研究

Study on Electric and Magnetic Methods of Nucleic Acid Purification and Application in Microfluidic Chip

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摘　要

微全分析系统(μ-TAS)具备在芯片实验室上实现分析过程集成化、自动化和缩微化的特点，能够极大地减少试剂的消耗量、缩短分析时间、提高分析检测效率。因此，在疾病诊断、生化分析、临床检测等领域获得了普遍关注。通过不同功能芯片的使用，达到分析全过程多种功能的集成，是实现微全分析系统集成化的有效途径之一。针对复杂生化样品体系，样品预处理是实现整个μ-TAS 分析的前提，而目前样品预处理技术正成为μ-TAS 向集成化、高效率、连续化发展必须突破的瓶颈之一。

在基因分析和疾病检测中,常常需要进行 DNA 提取、DNA 扩增和 DNA 分离检测等步骤，其中 DNA 的提取是决定基因分析成败与质量的关键步骤。传统的核酸提取方法具有操作繁琐，难以自动化等缺点。固液双相分离富集是一种既可实现待测物的浓缩富集又能够去除杂质组分干扰的样品预处理技术，而且它比较容易与微流控芯片系统结合实现在线预处理。

本论文发展了两种可用于捕获浓缩生物样品中 DNA 的固液双相分离富集方法——电渗析分离富集与磁珠法固相萃取，并把两种方法集成至微流控芯片中，实现生物样品的在线预处理，芯片易与其他芯片联用。论文共分为四章。

第一章为绪论，首先总结了 DNA 提取的一般过程与几种传统的 DNA 提取方法；然后介绍了膜分离技术尤其是电渗析技术及其在生物分离领域的应用；接着概述了磁性复合纳米颗粒的制备方法及其在 DNA 萃取中的应用；最后，论述膜分离技术与固相萃取技术在样品预处理微流控芯片中的集成和应用。

第二章构建一个可用于浓缩生物大分子(DNA)的电渗析装置。基于电渗析技术，利用阳离子交换膜在电场中对负电性物质的截留特性，在流路死体积中捕获并浓缩负电性物质。以阴性染料达旦黄作为探讨富集机理的模型化合物，研究了负电性物质在装置中的运动特点，并初步优化实验条件。生物大分子样品的实验结果表明：本装置在低电压条件下成功地实现无损伤捕获浓缩 DNA 分子，单体系的 λ-DNA 实验回收率约 47%，浓缩液可直接用于 PCR 实验；装置具备较好的分离效能：从 DNA-血红蛋白的混合体系中选择性地捕获浓缩 DNA，去除绝大部分的蛋白质，浓缩液可直接用于 PCR 实验；从全血破碎液中成功地

萃取出基因组 DNA，二次浓缩液可用于 PCR 实验。本电渗析装置适用于分离具备不同电性的生物大分子，如等电点不同的蛋白质或核酸，实验操作自动简便，使用简单的有机试剂；也可用于一般的无机离子和生物小分子的纯化与预富集以降低对仪器的检测限要求；微型化可与微流控芯片联用或集成于芯片上，在生物样品的捕获、脱盐和分离等预处理过程中有很大的应用空间。

第三章发展了可控表面电性的　超顺磁 氨基化 $SiO_2@Fe_3O_4$ 复合 纳米颗粒(Amino-Si-MNPs)用于核酸的分离纯化 。采用水热法制备超顺磁的 Fe_3O_4 纳米粒子，通过SEM、TEM、XRD和MPMS超导量子干涉磁强计对其进行表征；运用Stöber法对 Fe_3O_4 纳米粒子进行包覆得到粒径约300nm，具有核壳结构的 $SiO_2@Fe_3O_4$ 复合纳米颗粒；基于硅烷试剂的水解，利用3-氨丙基三乙氧基硅烷 (APTES)对 $SiO_2@Fe_3O_4$ 复合纳米颗粒进行氨基化表面改性，得到Amino-Si-MNPs。借助ZETA电位研究磁珠的表面电性，发现氨基化磁珠的表面电性对溶液的pH敏感，通过研究磁珠在萃取DNA体系中的电性，提出磁珠与DNA分子结合的机理。利用该磁珠成功地从全血中萃取得到基因组DNA，实验仅使用简单的、不对PCR产生干扰和抑制的试剂，萃取效率约70%，洗提液可直接用于PCR试验。本磁珠除了具有超顺磁性、分散性好等优点之外，其表面电性可通过调节溶液的pH得到控制、磁珠与DNA分子作用时，吸附和脱附速率快。这些优势使其成为一种良好的固定相，在DNA萃取等生物分离过程、在样品预处理芯片中具有广阔的应用前景。

第四章制作了两个基于固液双相分离富集技术的样品预处理芯片。其一把第二章基于电渗析技术的装置微型化并集成至微流控芯片中。以 PMMA 为材料，用激光刻蚀沟道，制作一个“三明治”式的分离浓缩芯片，阳离子膜依靠机械力固定于两层芯片间 。在线观测达旦黄在芯片上的富集特性 ；以花菁类染料(Gene-finder)为荧光指示剂，在线监测 DNA 在该芯片中的富集特性。利用单体系 DNA 与 DNA-血红蛋白混合体系研究芯片的浓缩和分离效能，结果表明：该芯片能够无损伤地捕获浓缩单体系与混合物体系中的 DNA，浓缩液可直接供给 PCR 试验；在全血中基因组的提取实验中，该芯片能够捕获浓缩裂解液中的基因组 DNA，但是纯度较低。芯片自动化程度高，试剂用量少等特点使具有与其他芯片联用的潜力，如与 PCR 芯片联用，用于分离 PCR 产物中的蛋白质；或与其他萃取芯片联用用于提取液的脱盐，浓缩等处理。

另一芯片基于第三章表面电性可控的氨基化磁珠技术，磁珠作为固定相填充于 DNA 萃取芯片中，借助外磁场固定于微通道中。芯片成功地从全血裂解液中萃取得到基因组 DNA,洗提液可直接用于 PCR 实验。与传统的固相萃取芯片相比，磁珠芯片制作工艺要求低，磁珠填充与去除方便，试剂用量少、洗提液可直接用于进一步的生物分析等优势使该芯片具有与细胞破碎芯片、PCR 芯片等联用的发展前景。

本论文发展的两种固液双相分离富集方法：把电渗析技术用于生物大分子的分离浓缩，并集成至微流控芯片，有效地实现在线富集与分离；制备表面电性可控的 MNPs 作为固定相用于核酸萃取，在芯片上实现 DNA 在线纯化与浓缩。在样品的预处理领域具有较大的应用空间。

关键词： DNA 萃取，电渗析，氨基化磁珠，固相萃取，微流控芯片

Abstract

Miniaturized total analytical system(μ-TAS) has the great potential to realize analytic process integration, automatization and minimization on chip, it can greatly reduce reagent volume, shorten analysis time and improve testing efficiency, which earns its popularity in the fields of disease diagnoses, biochemical analysis,clinic test and so on. One of effective approaches to realize integration is using chips with different function to constitute the whole analysis process. For μ-TAS analysis, sample pretreatment is one of the most important step and actually has become a bottleneck that must be addressed for μ-TAS.

A typical gene analysis and desease detection usually involves DNA-extraction, DNA-amplification and DNA-separation. Extracting DNA from sample is the first and most important step for gene analysis. Traditional approach of DNA-extraction suffers disavantages such as imcompactible to PCR, tedious and labours. Solid-liquid phase separation and enrichment is a sample pretreation technology which can not only remove impurity but also concentrate target substance. More importantly, it can be easyly used in microfluidic chip to acturize on line pretreation.

This thesis developed two kinds of solid-liquid phase seperation and enrichrment which can be use to capture and concentrate DNA. One was based on electrodialysis and the other based on solid-phase extraction with magnetic nanoparticles (MNPs). Both mothods were demonstrate its usefulnees on microchip for onchip DNA extraction. The thesis consists of four chapters.

In chapter one, firstly, several ways and the general process of DNA extraction were reviewed. Then, membrane separation technique especially the electrodialysis and its application in biochemcal separation field was introduced. Nextly, the preparation of nanopaticles and magnetic nanopaticles were discussed, SPE technique especially that based on MNPs, and its application in DNA extraction was summerized. And finally, a variety of DNA extraction techniques including SPE,

membrane separation for microfluidic applications were compared.

In the second chapter, a device based on electrodialysis for separating and enriching DNA was developed. It utilized comprehensively cation-exchange membrane's property of trapping electronegative substance in constant-current electric field and dead volume in flow path. As a model compound, Titan Yellow was used to study the enrichment mechanism, movement feature of the electronegative and optimize experimental conditions. The experimental result of capturing and enriching biomacromolecule indicated that the device could be used to capture and enrich DNA non-destructively. The recovery of λ-DNA in single system was about 47%, and extraction product can be used directly to polymer chain reaction(PCR) without any inhibition effection. The device can also capture λ-DNA selectively for PCR ampliction, from mixture of λ-DNA and hemoglobin which were of different electric property. On the basis of above work, we further used the device to extract genenomic DNA from whole blood and direct PCR experiment. The device was able to separate biomacromolecule of different electric property,such as proteins with different PKa and nucleic acid, it can also be used for puring and enriching amino acid and other micomolecules.The advantages of simplicity, automation, low consumption of reagent made it win large space of application in the fields of extraction,desalting and purification and other pretreation process. It had potential to be miniaturized and integrated on microchip.

The third chapter of the thesis developed a charge switching DNA technology using superparamagnetic Amino-SiO_2@Fe_3O_4 complex nanopaticles (Amino-Si-MNPs). Superparamagnetic Fe_3O_4 nanopaticles was prepared by hydro-thermal method and characterized by SEM,TEM,XRD and MPMS. Stöber method was adopted to coat Fe_3O_4 nanopaticles and SiO_2@Fe_3O_4 complex nanopaticles with core-shell stucture and size of about 300nm was acquired. APTES was used to modify the surface of SiO_2@Fe_3O_4 complex nanopaticle with amino groups. ZETA potential measurement was performed to study the surface electric property of amino-MNPs. The Zeta potential results showed that the surface electric property of amino-MNPs was senstive to pH of the system and the mechenism of amino-MNPs

binding DNA was investigated. Genenomic DNA was extracted from whole blood, using little reagent, the extraction rate was about 70%, and the elution could be used for PCR directly. Except of being superparamagetic and water-dispersible, beads owned its property of electric property could be controled by changing pH of solution, short time of binding and desorption. These made it be a good solid phase for SPE in DNA extraction and other bioseparation, also in chips for pretreatment.

In the forth chapter, two microfluidic chips for DNA sample pretreatment based on the technique of solid-liquid phase separation and enrichment were fabricated. We miniaturized and integrated the device based on electrodialysis in chapter2 to microchip. A chip with sandwich structure for separation was fabricated whose microchannal was etched on PMMA by laser ablation, the cation-exchange membrane was sandwitched by screws.Titan yellow was used to observe its enrichment property on line. Combining with genefinder(a gene dye), DNA was observed on line by fluorescent microscope. λ-DNA and mixture of λ-DNA and hemoglobin were both applied to study the effect of enrichment and separation, the result showed that the chip could not only capture and enrich DNA in pure buffer but also separate DNA from mixture, both concentration can be directly used for PCR. Though with low purity, the chip had extracted genomic DNA from whole blood successfully. Automation, little comsumption of reagent earned it convenience to coupled with other chips such as PCR chip or other chips for extraction.

Based on charge swithing amino-MNPs technique, Amino-MNPs was filled into the microchannel etched by laser in PMMA and fixed by magnet. Genomic DNA was extracted from whole blood with the elution could be used for PCR directly. With the simple manufacture process, convenience to fill and remove, little comsumption of reagent and the direct using for PCR elution, the chip was easy to couple with other chips such as those for cell discruption or PCR.

The two methods of serapation and enrichment in this thesis had an extensive space of application in pretreatment. Electrodialysis was applied to separate and concentrate biomacromolecule and integrated into microfluidic chip to realize

enrichment and separation on-line. Charge switching MNPs was prepared to extract DNA and used in microfluidic chip to actualize purification and enrichment on line.

Key words: DNA extraction, electrodialysis, amino-MNPs, SPE, microfluidic chip

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