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硕士学位论文

液相芯片中羧基功能性聚苯乙烯微球的制备、表征及其在生物检测中的应用

Preparation, Characterization of Functionalized Polystyrene Microsphere and its Application in Biological Detection

许艳军

指导教师姓名：颜晓梅 教授

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摘要

流式微珠分析技术，又称液相芯片技术或悬浮芯片技术，是将溶液中的可溶性待测物质通过生物分子之间的特异性亲和反应结合在类似于细胞大小的经光学编码的微球体上，利用流式细胞仪对同一个微量样本中的多元待测组分同时进行快速定性、定量分析的新一代分子诊断技术平台。核酸杂交，免疫反应，酶学反应等都可以在同一个平台上实现。检测时，富集有待测物质的微球体逐一、顺次、高速地通过流式细胞仪的激光探测区，微球体本身带有的光学编码可用来区分不同的特异性结合，对待测物进行定性分析；微球体上报告分子所携带的荧光可对待测物进行定量分析。

羧基功能性聚苯乙烯微球是流式微珠技术的重要载体，微球表面的羧基通过耦联剂活化之后可与蛋白、核酸等探针分子结合。结合有探针分子的聚苯乙烯微球经亲和反应与待测物质结合，进而与标记有荧光物质报告分子结合，通过测定微球表面荧光强度来检测生物样本中待测物的含量。

本论文的主要工作是单分散、粒径可控且表面羧基密度较高的聚苯乙烯功能性微球的合成及应用，以及包被有荧光染料的交联聚苯乙烯微球的初步制备。论文共分五章，第一章为绪论，第二章为粒径可控的聚苯乙烯微球的合成，第三章为羧基功能性聚苯乙烯微球的合成及其在甲胎蛋白检测中的应用，第四章为交联型聚苯乙烯微球的合成及荧光染料的包被，第五章为工作总结与展望。

论文的第一章首先介绍了生物芯片的历史、发展及优势，重点介绍生物芯片中的一种——液相芯片的原理及应用。随后探讨了液相芯片载体聚苯乙烯微球合成的反应机理，并列举了不同的聚合方法及各方法的优势与局限性。最后讨论了分散聚合法合成聚苯乙烯微球中影响微球的粒径及单分散性的主要因素。绪论部分同时讨论了对微球进行表面羧基功能性修饰的不同方法，并介绍了羧基功能性微球在生物样本检测中的应用。

第二章为粒径可控的聚苯乙烯微球的合成。由于不同粒径的微球具有不同的应用范围，在液相芯片中所应用的微球粒径为微米级，因此我们合成的目标微球的粒径为微米级的粒径可控的微球。本章实验通过调整单体质量分数、甲

基丙烯酸的质量分数（与苯乙烯相比）、稳定剂的浓度等实验条件合成了粒径在2.0 μm~7.0 μm 范围内且粒径可控的微球。

论文第三章是羧基功能性微球的制备。实验尝试了制备表面带有羧基的聚苯乙烯微球的各种方法，包括分散共聚合法、分步加料法、种子聚合法、二步聚合法和后修饰法。实验证明分散共聚合法、分步加料分散聚合法、种子聚合法、二步聚合法合成表面羧基密度较高且粒径均一的微球均具有局限性，而采用后修饰法对分散聚合合成的微球进行羧基功能性修饰不会影响微球的粒径及单分散性，且大大提高了微球的表面羧基密度，因此该方法是制备羧基功能性聚苯乙烯微球的较好途径。

论文的第四章尝试了交联型聚苯乙烯微球的初步合成，并成功的将罗丹明荧光染料包被在微球内，不足之处是微球的单分散性不是很好，有待改进。

本论文的主要成果是合成了粒径均一可控的微米级聚苯乙烯微球；对聚苯乙烯微球进行表面羧基功能性修饰，其表面耦联探针的能力达到国际同类商品微球的水准。将合成的羧基化聚苯乙烯微球应用于生物样品的检测，得到较低的检测限，具有较高的实用价值。

关键词：液相芯片；功能性微球；生物检测

Abstract

Microsphere-based array, also named as liquid chip or suspension array, is a powerful platform for multiplexed analysis of complex samples. Nucleic acid hybridization, immuno reaction, enzyme reaction can all be carried out on this platform. The suspension array is created by immobilizing capture reagents on the surfaces of encoded microspheres in conjunction with flow cytometric analysis. The array elements, the microspheres, are classified by their distinct optical properties, such as light scatter or fluorescence from an internal dye. When analyzed by the flow cytometer, the microspheres are passed through the laser detection volume one by one. The fluorescence produced from the internal dye is used to decode the microspheres for quanlitative analysis, meanwhile the fluorescence signal from the analyte is used for quantitative analysis.

Carboxyl functionalized polystyrene particle is an important carrier in the Microsphere-based array. The external carboxyl group can be activated to couple with probes such as proteins or nucleic acids. The microspheres coupled with probe molecules capture the analytes which then bind with fluorescently labelled reported molecules. Through the detection of the microsphere fluorescence intensity, the analyte concentration can be determined.

The main work in this thesis is the synthesis of mono-dispersed and size-controllable polystyrene particles rich in surface carboxyl groups. We also explored the synthesis of cross-linked polystyrene microspheres embeding fluorescent dye. This thesis is composed of four chapters. Chapter one is the overview. Chapter two decribes the synthesis of size-controllable polystyrene microspheres. Chapter three discusses the preparation of carboxyl functionalized polystyrene particles and their application in the detection of alpha-foetal protein (AFP). Chapter four decribes the synthesis of cross-linked polystyrene microspheres and their fluorescent dyeing.

In Chapter one, the history, development and advantages of biochip are

introduced with emphasis on the principle and applications of liquid chip. There are many methods to synthesize carboxyl functioanlzied polystyrene microspheres, the main carrier of liquid chip. The polymerization mechanism, advantages and limitations of several important synthesis methods were reviewed. For the disperse polymerization approach, factors affecting microsphere diameter and monodispersity were discussed. In the end of the introduction section, different approaches for surface carboxyl functionalization of polystyrene particles were discussed, along with the their applications in biological detection

Chapter two describes the synthesis of size-controllable polystyrene particles. Polystyrene microspheres with different sizes are used in different fields. The microspheres employed in the liquid chip are in the micron size. So, we aimed to synthesize micro-sized polystyrene particles. In this part of work, through adjusting the wt% of the monomer, the wt% of the MAA (to St), and the concentration of the stabilizer PVP, we were able to successfully synthesize polystyrene microspheres with controllable size in the range of 2.0 μm to 7.0 μm with good monodispersity.

In chapter three, the carboxyl functionalized polystyrene microspheres are prepared. Disperse polymerization, stepwise disperse polymerization, seeded polymerization, two-step polymerization and post-modification were all explored to synthesize the carboxyl functionalized polystyrene microspheres. The first four methods were all limited in synthesizing mono-dispersed polystyrene microspheres with high surface carboxyl density. It was demonstrated that during the post-modification process, the uniformity or diameter of the microsphere was not affected while enhancing the carboxyl groups density on the surface. Therefore, post-modification was recommended as the best approach for obtaining carboxyl functionalized microsphreres.

In Chapter four, exploration of preparing cross-linked polystyrene microspheres was carried out. Rhodamine B was embedded in the cross-linked microspheres successfully.

The main contribution of this thesis is the synthesis of the size controllable, monodispersed polystyrene microspheres. After post-modification for surface