Abstract A novel method of protein array immobilization, using micro stamps to pick up proteins from micro wells and deposit them on to a bio-absorption chip, has been developed. This method can potentially transfer several protein spots on to an organized array for applications such as disease diagnosis and drug screening by parallel biological or chemical processes. Fabrication of the micro stamp and the micro well arrays involves thick-photore sist lithography, bulk micromachining, and a molding process, whereas fabrication of the bio-absorption chip involves amino-modification by use of APTS (aminopropytrimethoxysilane) and surface activation by use of BS³ (bis-sulfo succinimidyl suberate). Successful transfer of protein on to the bio-absorption surface using the micro stamp-well array has been demonstrated. The size variation between different stamping spots has been shown to be less than 10%, and the APTS–BS³ surface has also been proved to bind the protein efficiently. Appreciable protein retention was achieved during 6-h washing, which shows the binding strength of the bio-absorption surface is sufficient for protein processing.

Introduction

The ability to prepare and test numerous biological samples or chemical compounds simultaneously and rapidly is crucial to modern biomedical and analytical chemical applications, such as disease diagnosis, DNA sequencing, and drug screening and discovery. Because bio-reagents and some other chemical compounds are expensive, difficult to obtain, and time consuming to process, assay technology characterized by batch processing and trace sample usage has become increasingly important. Among the various technologies developed for the aforementioned needs, micro-array technology is one of the most promising, because of its capacity to immobilize and process huge numbers of reagents in a short time. There are two major aspects of array-based technology – transfer of large numbers of biological samples or reagents to substrates in an organized format, and immobilization of the biological samples or reagents on the substrates for further processing.

A complete array-based protein-diagnosis process used in this study is illustrated in Fig. 1. In Fig. 1a, protein arrays can be deposited by a variety of micro deposition methods on to a bioassay chip. The bound protein arrays then go through a bio-process including bio-blocking for reducing background noise and two-step antibody binding for fluorescent expression, as shown in Fig. 1b. The image of the expressed protein spots is then taken by the microscope system for image processing – to identify the quantity expression for diagnosis. In this diagnosis system, bio-processing is the most time-consuming procedure – it usually takes hours for the whole task. As a result, a tremendous amount of time can be saved by processing many samples in arrays. There are, however, not many means suitable for the preparation of protein arrays. This paper introduces a novel approach – use of a micromachined stamp and well system for protein immobilization.

Micro array methods

Many arraying methods have been proposed [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. The conventional micro-array technique uses a computer-controlled robot to manipulate several needles for selecting and spotting biological samples in an array format [1, 2]. This arraying technique, however, has limitations such as high-cost positioning equipment and large variation of spot size, and is time consuming, which might increase the danger of the protein drying out during the stamping process. The need to wash the needle after each sampling is, in addition, not only time-consuming, but also poses cross-contamination problem.
The advanced techniques currently available for preparing micro arrays employ a variety of micro-system technologies, including photolithography [3, 4, 5, 6], inkjet printing [7], spray printing [8], contact printing [9, 10, 11], and protein dispensing on to a gel matrix [12, 13], etc. Photolithography [3, 4, 5, 6] uses photoresist techniques to define the locations of attachment of biological samples, and this might involve chemicals detrimental to the samples. In addition, only a couple of protein samples can be immobilized at one time, which is not practical for the preparation of thousands of proteins. Inkjet printing [7] uses inkjet heads, similar to those employed in inkjet printers, to eject micro droplets of biological samples onto a surface, thereby forming arrays. Spray printing [8] uses nozzles to eject and deposit sample droplets on selected regions of a masked substrate. These two methods have the common shortcomings of irregular spot shape, and they limit the number of samples which can be prepared. Protein dispensing on to a gel matrix [12, 13] deposits a limited number of samples at one time, again a time-consuming process for the preparation of large number of proteins. In a word, most of the aforementioned techniques are not suitable for high-throughput and parallel bio-sample transfer and immobilization in large arrays. The purpose of this study was to develop a novel stamping method by using micro-stamp array for parallel spotting and immobilization of proteins on to a bio-absorption surface. This stamping method, characterized by simple mechanical contact, can simultaneously and rapidly spot a variety of proteins on to the bio-absorption surface in seconds, therefore avoiding dry-out and denaturation of the proteins during the deposition process. The micro stamp and well system is designed to be disposable and low cost,