Production and Surface Modification of Protein Microspheres

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Microencapsulation of pharmaceutical and imaging agents has many beneficial effects in vivo. Reduction of side effects, increased duration in the bloodstream, and potential drug targeting applications are three such benefits. One particular challenge in the field of microencapsulation has been to develop biodegradable microspheres with surface functionality. Reactive functional groups of the surface of the microspheres allow for the conjugation of many useful molecules, including polymers that extend the lifetime of the microsphere in the circulation and antibodies that can lead to microsphere targeting\(^1\)-\(^2\). Commonly, surface reactive microspheres are made with organic solvents in harsh chemical reactions that can be harmful when applied in vivo without extensive purification.

Previously, Suslick and co-workers have used ultrasound to produce a new type of biodegradable microsphere, protein microspheres\(^3\). Using sonochemistry, a protein solution and a second phase containing the material to be encapsulated are sonicated. Previous studies have shown that chemical cross-linking of the protein subunits through disulfide bonds forms a stable 1-10 micron microsphere with a stable 30 nm thick protein shell as seen in Figure 1. Szewczyk and Rosenblat have demonstrated by circular dichroism and oxygen binding studies that the shell proteins maintain their secondary structure and activity. Since the protein shell retains its functionality, post-synthesis modification of the microspheres is unnecessary.

![Figure 1](image)

Much work has been done to determine the biomedical applications of the protein microspheres\(^4\)-\(^5\), including their use as a potential blood substitute\(^6\), as an X-ray and MRI contrast agent\(^7\), in the in vivo measurement of O\(_2\)\(^8\), and as a temperature probe\(^9\). This work deals with two previously undeveloped aspects of protein microencapsulation, the large-scale production of the protein microspheres and the modification of the surface of the microspheres for other potential biomedical uses.
A novel synthetic procedure for the large-scale production of protein microspheres is described herein. Synthetic procedures were developed to upscale the production process from a batch to a continuous flow process. The resulting protein microspheres were chemically and physically identical to the microspheres produced in the batch process.

Surface modification of the protein microspheres was accomplished by the conjugation of active monoclonal antibodies and a folate-polyethylene glycol to the surface of the microsphere. Potential drug delivery applications were explored with an emphasis on the targeting of T cells and tumor cells (KB, human nasopharyngeal tumor). Surface modified microspheres were analyzed for activity, cell binding, and in the case of T cells, for T cell activation and in vivo biodistribution.

The monoclonal antibody KJ16, which is specific for a T cell receptor (TCR), was successfully coupled to the surface of albumin microspheres. Quantitation of the number of antibodies per microsphere revealed the coupling of 44000 antibodies to the microsphere surface. KJ16 antibodies retained their activity towards water-soluble TCR but showed no specific binding to the microspheres in vivo. Biodistribution data was collected following KJ16 microsphere injection into a mouse. No alteration of the normal biodistribution was observed with the majority of the signal observed in the liver. Despite the lack of T cell binding, the KJ16 microspheres did interact and activate T cells in vivo (measured by the up-regulation of CD69 early activation marker), allowing for their potential use as an immunostimulatory agent.

Certain types of tumors possess a large number of folate binding proteins on their surface. These folate binding proteins have a high affinity for folic acid ($K_a \approx 10^9$). Previous work by Leamon and Lee has shown the successful targeting of folate labeled polyethylene glycol (PEGylated) liposomes to tumors with a large number of folate receptors. PEG provides a longer lifetime in the circulation as well as a spacer arm between the folate and the liposome surface, allowing for better folate to receptor binding.

Adaptation of this technology allowed for the successful conjugation of folate-polyethylene glycol polymers to the surface of the protein microsphere. Approximately 6000 folate units per microsphere are on the surface of the albumin microspheres. In vitro cell binding activity with high folate receptor concentration KB cells indicated no specific targeting of the microspheres under flow cytometry conditions. Currently, examination of potential folate-PEG microsphere binding to KB cells is being performed using light and fluorescent microscopy (much gentler conditions than in flow cytometry).

References


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### References


Soft Lithographic Patterning on Silicon

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Final Defense
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In recent years, chemically modified surfaces have become increasingly important, both for their potential industrial applications, as well as scientific advances. Much of this interest is driven by the need to combine desired bulk properties with tailored surface chemistries. New methods for modifying surfaces allow the exploration of many potential applications, which span a wide variety of research areas. These areas, while ranging widely in their execution and ultimate goal, all bear the common interest of controlling or modifying the chemistry of the desired substrate by engineering surface properties. Areas in which modification of surface properties has proven especially useful include biology/biochemistry and materials science, the latter of which is the focus of this research.

Much work has gone into developing new methods for modifying surface properties through microcontact printing of self-assembled monolayers. Contact printing is a process in which a polymeric stamp, covered with an ‘ink’ appropriate for the given substrate, is brought into contact with the surface. For silicon substrates, alkyltrichlorosilane inks are used, with octadecyltrichlorosilane (OTS) the most commonly studied due to its resist properties. The structural and physical properties of contact printed OTS monolayers were fully characterized using ellipsometry, X-ray photoelectron spectroscopy (XPS), reflection absorption infrared spectroscopy (RAIRS), atomic force microscopy (AFM), and scanning electron microscopy (SEM). These experiments demonstrated that coverages equivalent to a full monolayer were formed after 30 s of stamp contact with the substrate. The structure of the films formed was found to be densely packed, and highly oriented. While OTS was shown to have long range patterning capabilities, migration of OTS islands into underderivatized regions was noted, limiting pattern fidelity and resolution. Two possible mechanisms, lateral diffusion and vapor phase transport, are thought to be responsible for these defects.

In an effort to reduce or eliminate the suspected mechanisms causing island diffusion, a less volatile alkyltrichlorosilane (docosyltrichlorosilane, DTS) was selected for the printing process. Contact printed monolayers of this new ink were fully characterized using ellipsometry, RAIRS, XPS, AFM, and SEM. These experiments found that the monolayer formation was highly influenced by the humidity present in the lab ambient conditions. At low relative humidity, a thickness equivalent to a full monolayer was achieved after 5 minutes of contact time, whereas only 1 min was sufficient for the high relative humidity case. In either case, the films were found to be close packed and highly oriented. SEM and AFM studies of patterned DTS showed little spreading in the non-derivatized regions. The ability of the film to serve as a wet chemical etch resist was also studied, showing marked improvements over OTS films.

The process of contact printing was applied to two novel systems, that of orthogonal self assembly, and mixed monolayer etch resists. Orthogonal self-assembly, a process involving multiple printing steps, which uses the first printing step to serve as a patterned resist to subsequent printings. This methodology is particularly useful when applied to volatile inks that are unable to maintain pattern fidelity using traditional printing methods. In this experiment, two different inks are employed, OTS and octadecyltrichlorosilane (OCT). The structures of these films were analyzed via ellipsometry, XPS, and RAIRS. OTS is shown to be close packed and highly oriented, while the modes in the OCT spectrum were too weak for