Production and Surface Modification of Protein Microspheres

Gregory S. Kufner Final Seminar July 19, 2000

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 Rosenblatt 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.

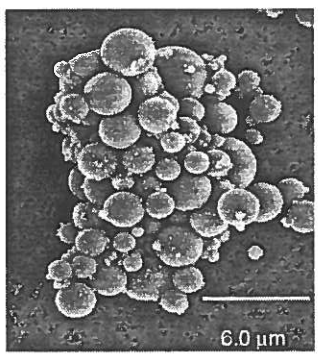

Figure 1

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 nasopharangeal 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)[10,11], 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 of the microspheres to the T cells *in vitro*. 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 vitro* (measured by the up-regulation of CD69 very 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 ($K_d \sim 10^{-9}$). 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[12]. 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 subunits 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).

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