Photopolymerizable Hydrogels made from Poly(ethylene glycol) and Albumin for Drug Delivery: Drug Binding and Release Properties

Liat Oss-Ronen and Dror Seliktar

Faculty of Biomedical Engineering, Technion – Israel Institute of Technology, Haifa 32000, Israel

Introduction: Serum albumin plays a central transport role in blood plasma due to its high capacity for binding endogenous and exogenous compounds, including various drugs molecules. As a drug delivery vehicle, albumin hydrogels combine the high binding capacity of albumin with the structural stability of the hydrogel network to enable controlled release of small drug molecules based on both binding affinity and physical interactions. We developed photopolymerizable hydrogels based on PEGylated albumin for targeted sustained drug release. For this purpose, albumin was covalently modified with functionalized poly(ethylene glycol) (PEG) and formed into a hydrogel network by free radical polymerization. The slow release properties of the hydrogels were designed based on the albumin concentration. Further control in this system is afforded by the molecular chain length of the PEG constituent, which has a substantial influence on the molecular architecture of the resulting hydrogel network. In the current investigation we focus on the drug binding properties of the drug molecule Warfarin to PEGylated albumin hydrogels. The widely used Warfarin drug molecule is a site-specific ligand of albumin that binds with high affinity to a region in the subdomain IIA. Warfarin is also an ideal candidate for drug release studies because of its 10-fold increase in fluorescence emission when associated with its albumin binding site. We now report on the affinity and release characteristics of the Warfarin molecule using PEGylated albumin hydrogels made with different molecular weights of PEG.

Materials and Methods: Bovine serum albumin was PEGylated using a Michael-type addition reaction of free thiols on the denatured albumin molecules and acrylate functional groups on the PEG. PEG diacrylate (PEG-DA) (MW = 10-kDa, 4-kDa, and 1.5-kDA) was synthesized and reacted for 3 hours in high excess with denatured albumin under reducing conditions to form the PEGylated albumin precursors. The precursor solution was purified by acetone precipitation and dialyzed to remove excess unbound PEG-DA. Pure PEGylated albumin was lyophilized and resuspended in PBS at an albumin concentration of 8 mg/ml. Hydrogels were assembled by free radical polymerization of unreacted acrylate groups on the PEG using a photoinitiator and a UV light source (5 mW/cm^2). Warfarin powder was dissolved in DMSO and mixed with precursor solution at a protein to drug molar ratio of 10:1 or 1:10. For binding affinity studies, the mixtures were incubated up to 15 hours, and fluorescence measurements were recorded using a fluorescence plate reader. For release studies, the drug was incubated with precursor overnight before being polymerized into 200 µl hydrogel plugs having an 8-mm diameter and 4-mm thickness. Drug release was measured at 37°C in PBS with 0.1% sodium azide for up to 1 week.

Results: PEGylated albumin was characterized by SDS-PAGE to verify covalent attachment of the PEG to the reduced albumin thiols. Drug binding studies demonstrated a relationship between binding affinity and the PEG molecular weight ($n=7$, $p<0.01$). High MW PEG caused a reduced binding affinity between the Warfarin and the PEGylated albumin, as compared to low MW PEG or unmodified albumin controls (Table 1). Fluorescence measurements also indicated that incubation time did not affect Warfarin binding to albumin or PEGylated albumin. Hydrogels were successfully polymerized using 10-kDa, 4-kDa, and 1.5-kDa PEG-modified albumin with 2.5% additional unreacted PEG-DA. Warfarin release curves show that the drug molecule was released by slow passive diffusion from the PEGylated albumin hydrogels, with a diffusion coefficient of approximately 7.0×10^{-6} cm^2/s.

Table 1: Warfarin binding affinity fluorescence measurements

	PEGylated Albumin			
Time (min)	1.5-kDa	4-kDa	10-kDa	Albumin
10	0.36 ± 0.08	0.26 ± 0.07	0.20 ± 0.07	1.1 ± 0.16
50	0.33 ± 0.08	0.25 ± 0.07	0.19 ± 0.08	1.0 ± 0.08
800	0.37 ± 0.09	0.28 ± 0.1	0.17 ± 0.08	0.99 ± 0.14

Discussion and Conclusions: PEGylated albumin hydrogels are good candidates for local controlled drug delivery, as they combine the drug binding properties of albumin with the ability to control structural properties and release rates by changing the PEG molecular weight and functionality. Although in our studies we found that PEGylation of albumin significantly lowers the binding affinity of Warfarin, we also found that using lower MW PEG helps to retain the drug molecule better than high MW PEG. It is likely that the highly hydrophilic PEG molecules partially block the Warfarin binding site on the albumin and thereby disrupt the association of the hydrophobic drug molecule. Our future work will focus on improving the drug-binding properties of the PEGylated albumin so as to improve the overall drug delivery capabilities of these hydrogel materials.

Reference:

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