A novel all trans retinoic acid controlled release system for cancer therapy
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Statement of Purpose: Anti-cancer therapeutic drugs like all-trans-retinoic acid (ATRA) for acute promyelocytic leukemia are delivered to their target sites either orally or within liposomal drug delivery systems (1, 2). However, the body already has an endogenous versatile drug carrier in serum albumin that has binding sites for many of the therapeutic drugs in use today(3). Albumin is a 66 kDa water-soluble, monomeric protein and is the most abundant protein in blood plasma (40-50mg/ml). It has three primary domains that are arranged in a heart shape configuration with 17 disulphide bond linkages that stabilize the domains(4). It serves as the primary carrier of various solutes in plasma, including cations, bilirubin, fatty acids, and therapeutic drugs(4). There is extensive literature regarding serum albumin’s affinities to various compounds, denaturation conditions, gelation mechanisms, and current or potential medical uses with many applications investigating covalent linkage of albumin to therapeutic drugs to increase half life(3). In light of albumin’s therapeutic potential, we sought to exploit albumin’s ability to reversibly and drastically change its conformation when exposed to changes in solution pH (5) to form novel hydrogel constructs for ATRA delivery. These constructs could potentially serve as depots for entrapped ATRA, which has a natural binding site to albumin(6).

Methods: Bovine serum albumin (BSA, A6003, Sigma) and human serum albumin (HSA, A3782, Sigma) gel precursor solutions were formed by adding deionized water at 20 wt% concentration (3mM) and stirred until completely dissolved. Precursor solutions were made into solid hydrogels by one of two methods. Electrostatically triggered hydrogels were made by dropwise addition of 2M HCl to pH 3.5 followed by submersion in a water bath at 37°C overnight. Thermally formed hydrogels were made by neutralizing to pH 7.4 by 2M NaOH followed by submersion in a water bath at 80°C. Prior to gelation, all trans retinoic acid (ATRA, R2625, Sigma) pre-dissolved in 1% DMSO was added at a molar concentration of 2:1 ATRA:Albumin. A cylindrical punch was used to make solid ~150mg hydrogel cylinders for 2 week drug release studies in 10mL PBS at 37°C. Released ATRA was quantified by absorbance measurement at 340nm (NanoDrop2000, Thermo Scientific). Auto-dock was used to model ATRA binding to partially denatured albumin structures from previous work(7). Quenching of albumin tryptophan fluorescence signal emitted at 340nm by excitation at 295nm was used to indicate binding of ATRA to albumin. Activity of released ATRA was evaluated by scratch test migration assay on confluent human smooth muscle cells (hSMC). These experiments are designed to demonstrate the potential of using electrostatically triggered partially denatured albumin systems for entrapment of hydrophobic drug molecules.

Results: Serum albumin hydrogels with were successfully formed with incorporated ATRA without gel fracture after handling. Binding of ATRA to albumin was confirmed by measuring tryptophan fluorescence quenching (Fig.1). While not as efficient as native albumin conformations at pH7.4, partially denatured albumin at pH 3.5 retained 70% of the tryptophan binding site affinity to ATRA. The increase in hydrophobic surface area due to partial denaturation results in additional binding site affinities to ATRA over the native albumin structure. ATRA release curves from hydrogels showed that partially denatured albumin was able to increase release 6-fold over thermally denatured gels (Fig.2). Within 1 hour, solution and surface pH of all gels was 7.4, indicating rapid acid leaching. Decreased hSMC migration confirms ATRA activity after release.

Conclusions: Pure partially denatured albumin hydrogels loaded with ATRA have potential for use as a therapeutic drug depot since they retain their binding site for ATRA and display enhanced release profile over thermally denatured albumin hydrogels.

References:
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