578c Pegylated Bacteriophage for Identification of Tissue Homing Peptides

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Combinatorial chemistry approaches for identifying cell and tissue-specific peptides have wide applicability in numerous contexts, ranging from fundamental to clinical applications. Recent in vivo uses of phage-displayed peptide libraries have shown promising results (in both mouse and human models) to identify peptide epitopes capable of homing to specific understood, but has nevertheless allowed remarkably selective targeting of therapeutic agents to tissues, and in several cases, tumors.

On bacteriophage, displayed peptides and even proteins are presented in a manner very similar to their native state. In addition, bacteriophage are easily amplified in vitro by infection of E. coli. All of these strengths, however, have been considerably limited by the rapid clearance of phage particles by the reticuloendothelial system (RES). Extensive studies have now shown that poly(ethylene glycol) (PEG) attachment to surfaces and drug delivery vehicles can reduce protein deposition in vivo, and thereby help avoid the RES – prolonging circulation times. By combining the ability of PEG to extend in vivo lifetimes with the ability of peptide-on-phage libraries to identify novel targeting epitopes, we intend to open new possibilities in targeting and therapy. Two main strategies have been employed: (i) non-specific attachment to primary amines using PEG vinyl sulfone and (ii) specific modification to cysteines using PEG acrylates.

In vitro tests quantify any adverse effects of PEG attachment on bacteriophage infectivity and therefore establish a common basis for establishing the biodistribution. Preliminary results show a modest decrease in phage infectivity upon random PEGylation, suggestive of modification of the minor coat protein necessary for infection. Using a chromophoric and/or fluorescamine assay, one can estimate the degree of PEGylation of the bacteriophage to reach values approaching the Flory regime for ideal polymers, providing further evidence of a “Stealth” character. To decouple PEG attachment from adverse effects on infectivity, solvent-accessible single cysteines are introduced via site-directed mutagenesis of the phage major coat protein. Use of PEG-acrylate will then allow for selective attachment to the bacteriophage major coat proteins while preserving the infectivity of the minor coat proteins. This modular approach sets the stage for in vivo studies of peptide-on-phage libraries to identify novel peptide homing epitopes.

Initial in vivo studies verify the ability of PEG to dramatically prolong bacteriophage circulation time in mice compared to wild-type phage. Further work aims to establish the optimal PEG surface coverage and chain lengths. Circulation time and biodistribution are determined via phage amplification of the tissue homogenate in E. coli, thus eliminating the need for any fluorescent or radio-labeling. Independently, real-time PCR quantifies the amount of viral DNA recovered, enabling a distinction between surface-associated and cell-uptaken or otherwise inactivated phage.