Reversible Attachment of Bioactive Molecules to Polymer Surfaces

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Statement of Purpose: Conjugation of biomolecules to polymeric surfaces has been used to control biocompatibility, cell attachment, and proliferation. For example poly(tetrafluoroethylene) (PTFE) scaffolds containing immobilized VEGF on their surfaces have been demonstrated to promote endothelial cell migration (angiogenesis) *in vitro* (1). However, surfaces containing only immobilized VEGF lack the cell adhesion signal that is required at the onset of the healing process (1). Thus, it is desirable to design a scaffold that is capable of expressing various bioactive factors (e.g. RGD, VEGF, etc.) at different times. β-cyclodextrin (CD) has been widely used to form selective and reversible, high affinity inclusion complexes with hydrophobic molecules such as adamantane (Ad) (2). In this study, we examine the potential of using inclusion complexes formed between CD and Ad derivatives to selectively and reversibly bind bioactive molecules to biomaterial surfaces.

Methods: Poly(ethylene-co-vinyl alcohol) (EVOH) was reacted with p-Toluenesulfonyl chloride (Tosyl chloride) in DMF to give EVOH-Tosylate, as confirmed by ^{1}H NMR. The EVOH-Tosylate solution was then added to a tissue culture treated polystyrene dish and a semi-interpenetrating network (SIPN) was formed following solvent evaporation at 80 ºC. The films were then reacted with an aqueous solution containing an excess of 6-Amino β-cyclodextrin giving EVOH-CD films (Fig. 1). Ad-PEG_{3400}-fluorescein and Ad-$mPEG_{2000}$ were synthesized using 1-Adamantanemethylamine and either NHS-PEG_{3400}-fluorescein or $mPEG_{2000}$-NHS, respectively. EVOH-CD films were first reacted with Ad-PEG_{3400}-fluorescein (Fig. 1). The films were subsequently reacted with Ad-$mPEG_{2000}$ in order to determine if previously complexed Ad-PEG_{3400}-fluorescein molecules would be displaced.

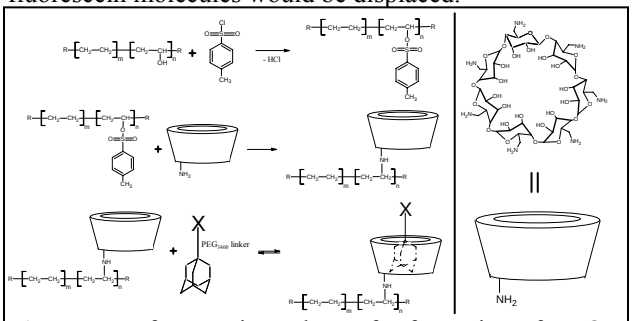

Figure 1. Left: Reaction scheme for formation of EVOH-β-CD films and complexation (X = Biomolecule, e.g. fluorescein). Right: Structure of 6-Amino β-cyclodextrin.

Similarly CD covered surfaces were made by self assembly of CD-thiol on gold substrates. Ad-PEG-RGD and Ad-PEG-RGE derivatives were made as above and associated with CD surfaces. 3T3 fibroblasts were examined for their ability to attach to these RGD and RGE surfaces made by CD-Ad association, as compared to cell attachment on covalent RGD and RGE surfaces.

Results/Discussion: Using the CD inclusion Ad-PEG_{3400}-fluorescein was selectively bound to a circular region of the surface of an EVOH-CD film (Fig. 2).

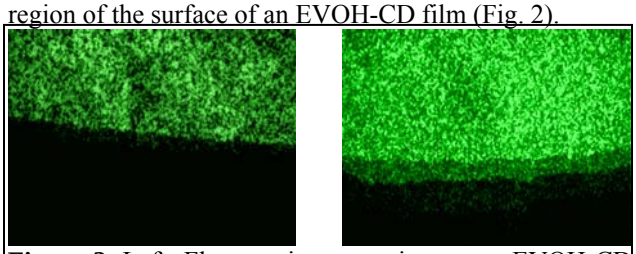

Figure 2. Left: Fluorescein expression on an EVOH-CD film via an Ad-PEG_{3400}-fluorescein inclusion complex formation. Right: Migration of some Ad-PEG_{3400}-fluorescein from initial complexation sites to new CD sites following displacement with Ad-$mPEG_{2000}$.

After incubation with Ad-$mPEG_{2000}$, migration of displaced Ad-PEG_{3400}-fluorescein molecules out of the original circular region was noticeable demonstrating the reversibility of the inclusion complex.

Using the same CD-Ad inclusion complexation bioactive surfaces were made containing either the cell attachment peptide RGD or the non-attaching control peptide RGE, and were characterized by MALDI. GFP labeled 3T3 fibroblasts were observed to attach and proliferate on RGD surfaces, while little cell attachment was observed on RGE or surfaces with only Ad-$mPEG_{2000}$ (Fig. 3).

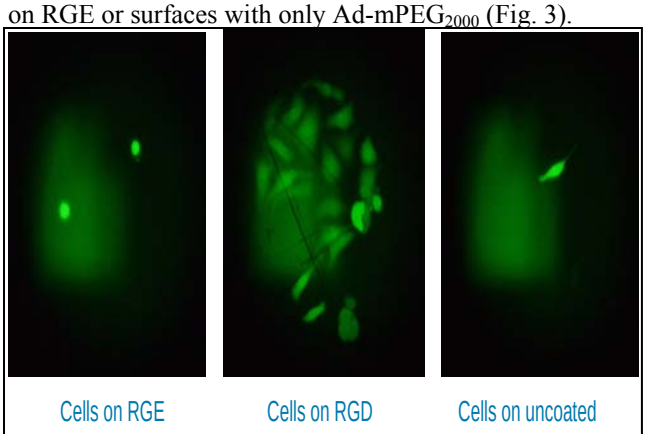

Figure 3. GFP labeled 3T3 cells are grown on surfaces modified with RGE peptide, RGD peptide or no peptide.

Conclusions: We have demonstrated a new method for reversibly attaching molecules to biomaterial surfaces using a CD-Ad inclusion complex. Initial studies demonstrated reversible attachment of fluorescent molecules. Cell culture studies demonstrated that this observation could be translated to incorporation of biomolecules influencing cell attachment. Future research will examine using more complex bioactive molecules (e.g. EGF, bFGF, VEGF, etc.), with which we can examine how spatial and temporal arrangements influence cell proliferation, migration and differentiation.

References:

1. Crombez, M. Biomaterials. 2005; 26: 7402-7409.
2. Park, IK. Langmuir. 2006; 22: 8478-8484.