Controlled Immobilization of Bioactive Peptides via the N-Terminus

J.L. Sharon and D.A. Puleo
Center for Biomedical Engineering, University of Kentucky, Lexington, KY 40506

INTRODUCTION

A variety of approaches are being investigated for controlling tissue-biomaterial interactions. For example, proteins and bioactive peptides can be attached to biomaterial surfaces to affect adhesion and/or subsequent responses of cells. Adsorption is the simplest way to deposit biomolecules, but it offers little control over their amount and orientation. Methods for covalent immobilization generally involve formation of bonds between surface-exposed functional groups, such as amino, carboxyl, and thiol groups, of amino acids with suitable substrates. These reactions typically lead to multiple bonds between the surface and protein molecule, resulting in a heterogeneous population of bound biomolecules. Residues involved in attachment may also be in/near regions needed for bioactivity of the molecule. Improper spacing of the immobilized biomolecule or insufficient distance from the surface can lead to steric problems and further reductions in bioactivity.

The objective of this study was to develop a method for controlling the orientation of immobilized biomolecules via the N-terminus.

MATERIALS AND METHODS

Glass coverslips were coated with acid-encapped 50:50 PLGA. Samples were then derivatized with dihydrazides of increasing length using single-step carbodiimide chemistry. Between the two hydrazide end groups, the spacer arms contained 2 (C2), 4 (C4), 6 (C6), and 10 (C10) carbon atoms. Based on previous studies, respective dihydrazide concentrations of 0.018, 0.057, 0.018, and 0.011 mM were used to give a similar surface density of hydrazide groups for each treatment. The N-terminal serine of Ser-Gly-Arg-Gly-Asp-Ser (Peptides International) was oxidized with periodate to create an aldehyde moiety, which was then allowed to react with the hydrazide-derivatized surfaces. For comparison, peptide was directly immobilized without an intervening spacer. To assess availability of the peptide for interaction with cells, adhesion of MC3T3-E1 osteoblastic cells (ATCC CRL-2593) was determined by measuring DNA content between two and six hours of incubation.

RESULTS AND DISCUSSION

The immobilization scheme is shown in Figure 1. After attachment of the spacer molecule to carboxyl groups on PLGA, one of the hydrazide groups was available for binding to peptide. Under neutral conditions, oxidation of peptides is specific for N-terminal serine groups. The resulting aldehyde then reacts with hydrazide groups at neutral pH to form stable hydrazone bonds. For the controls, the peptide was bound via the only amino group, which was also at the N-terminus, but a spacer was not present.

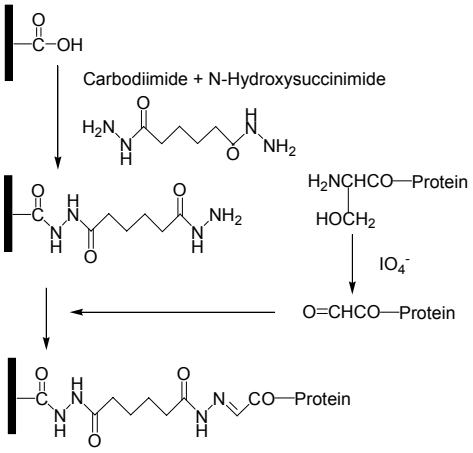

Figure 1. Immobilization scheme.

An RGD-containing peptide was used as a model biomolecule for demonstrating the immobilization scheme. At two hours, all substrates had similar numbers of cells attached (Figure 2). After six hours of incubation, some differences became apparent. The C4, C10, and direct surfaces showed slight decreases in cell attachment, but peptide bound to the shortest (C2) and intermediate (C6) length spacers had 70-120% more cells. Flexibility of the spacer may have played a role in making the peptide more available for interaction with the cells. This interesting finding is being investigated further.

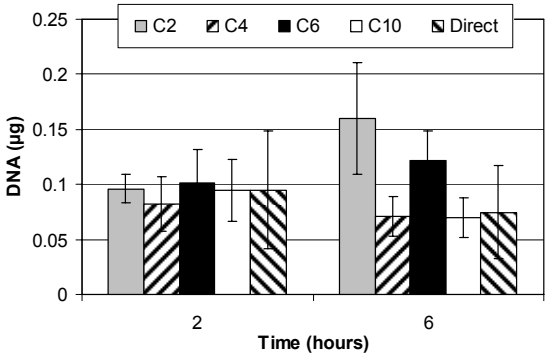

Figure 2. Cell adhesion to RGD peptide immobilized by different spacers.

CONCLUSIONS

This simple, controllable two-step method for attaching bioactive peptides is useful for modifying biomaterial surfaces. The procedure is applicable to any substrate with carboxyl groups and any biomolecule with an N-terminal serine, which can be native or introduced during expression or synthesis.

ACKNOWLEDGEMENT

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