SYNTHETIC PEPTIDES FROM LAMININ FOR TISSUE ENGINEERING

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Abstract
We have investigated a potential of biologically active peptides from laminin-l for tissue engineering and therapeutic applications. We utilized a chitosan membrane as a mechanical support for the peptides and tested for biological activity. We covalently conjugated two biologically active peptides from the laminin-1 on chitosan membranes and tested their biological activities using various cells. The peptide-conjugated chitosan membranes promoted cell attachment and spreading in a cell-type specific manner. Morphological differences of fibroblasts were also observed with these peptide-chitosan membranes. Further, the peptide-chitosan membranes promoted neurite outgrowth with PCl2 rat pheochromocytoma cells. Taken together, these suggest that cell adhesive peptides on the membranes are active with cell-type specificity and have a potential ability to serve as bio-adhesives for tissue repair and engineering.

Introduction
Basement membrane matrix plays a critical role in tissue development and repair. Laminin, a major cell adhesive protein of the basement membrane matrix, has multiple biological activities [1]. There are at least fifteen isoforms of laminin, each consisting of three different chains: α, β, and γ [2-5]. The most extensively characterized isoform, laminin-1 (Mr = 900,000) consists of α1, β1, and γ1 chains and promotes cell adhesion, spreading, proliferation, neurite outgrowth, and angiogenesis [1]. Recently, we systematically screened 673 overlapping synthetic peptides for identification of cell binding sites from the laminin-1 molecule [6-9]. Approximately twenty different cell binding sequences with various biological functions were identified. Two peptides (A99: AGTFALRGDNPOG and AG73: RKRLQVQLSIRT) showed strong cell attachment activities, which were comparable to those of the laminin-1 molecule itself. In addition, A99, located on the short arm of the α1 chain and containing the Arg-Gly-Asp (RGD) sequence [10], interacted with...
integrins and promoted cell adhesion and migration\textsuperscript{[11]}. AG73, enhanced cell adhesion, migration, invasion, gelatinase production and neurite outgrowth\textsuperscript{[6,12-15]} and promoted acinar-like development of a human submandibular gland cell line\textsuperscript{[16,17]}. Furthermore, syndecan-1 was identified by peptide affinity column chromatography as a receptor for AG73 on salivary gland cells\textsuperscript{[16,17]}. AG73 also regulated wound healing\textsuperscript{[18]}. These findings suggest that active peptides from laminin have potential therapeutic applications.

Chitin, a mucopolysaccharide composed of N-acetyl-D-glucosamine, is present in the cell wall of fungi and in the outside skeleton of crustaceans and insects. Chitosan, deacetylated chitin, can adhere to proteins, dyes, and cholesterol, due to the presence of a free amino group\textsuperscript{[19]}. Chitosan membranes have been used for medical applications, such as suture thread, artificial skin, and stptic\textsuperscript{[20,21]} Chitosan is a biodegradable natural polymer that has been shown to improve wound healing\textsuperscript{[22]}.

Here, we focus on two laminin-1 derived active peptides for use in tissue engineering and therapeutic applications. Mechanical support of the peptides is required for promotion of cell adhesion and tissue repair. Chitosan membranes are a unique natural biopolymer that can be used as a support for cells. Chitosan membrane alone adheres to tissues but does not show cell attachment. We conjugate two active laminin peptides on chitosan membranes. Using various cells, cell attachment activities of these peptide-conjugated chitosan membranes were examined. We also demonstrate neurite outgrowth on of the peptide-conjugated chitosan membranes.

**Results and Discussion**

Two biologically active peptides (A99, and AG73)\textsuperscript{[11-17]}, were synthesized with Cys-Gly-Gly sequence at the N-terminus (Fig. 1). Chitosan (99% deacetylated) was reacted with N-(maleimidobenzoyloxy)succinimide (MBS) and the obtained MB-chitosan. (A) Photomicrographs of fibroblasts on the peptide-conjugated chitosan membranes. (B) The cells were allowed to attach to the peptide-conjugated chitosan membranes for 2 h and counted.

![Fig. 2. Attachment of fibroblasts to the A99- and AG73-chitosan membranes.](image)

chitosan (1% MB) was coated on tissue culture wells. The peptides were added into the MB-chitosan membrane-coated wells and coupled. Human foreskin fibroblasts were used to evaluate the cell attachment activity of the peptide-conjugated chitosan membranes (Fig. 2). Mercaptoethanol coupled MB-chitosan, which was used as a negative control, did not promote attachment of fibroblasts (Fig. 2). Unmodified chitosan membranes also did not promote cell attachment (data not shown). Fibroblasts attached on the A99- and AG73-chitosan membrane (Fig. 2). Adhesion to the peptide-conjugated chitosan membranes was quantitated (Fig. 2). The number of cells adherent to the peptide-conjugated chitosan membranes depends on the amount of peptide conjugated. When the amount of the conjugated peptides increased, the number of the attached cells increased further. The peptide-conjugated chitosan membranes showed higher cell attachment activity at low doses when compared with attachment to peptide alone coated plates. The A99-, and AG73-, chitosan membranes showed a dose-dependent cell attachment activity.