Reconstitution of laminin-111 biological activity using multiple peptide coupled to chitosan scaffolds

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A B S T R A C T

Laminin-111, a multifunctional matrix protein, has diverse biological functions. Previously, we have identified various biologically active sequences in laminin-111 by a systematic peptide screening. We also demonstrated that peptide-conjugated chitosan matrices enhance the biological functions of the active sequences and are useful as a scaffold. Here, we conjugated sixty biologically active laminin-111 peptides onto chitosan matrices. The twenty-nine peptide-chitosan matrices promoted various biological activities, including cell attachment, spreading, and neurite outgrowth. The biological activities of peptide-chitosan matrices depend on the peptide. These peptide-chitosan matrices are categorized into six groups depending on their biological activities. Next, we conjugated five active peptides, which showed strong cell attachment activity in the each group, onto a single chitosan matrix to mimic the multiple activities of laminin-111. The mixed peptides-chitosan matrix significantly promoted cell attachment and cell spreading over that observed with the individual peptides. We also demonstrated that a mixed peptides-chitosan matrix, using four neurite outgrowth-promoting peptides each from a different group, enhanced the activity. These data suggest that the mixed peptides synergistically induce laminin-like biological activities on a chitosan matrix. The active peptides-chitosan matrices described here have potential for use as biomaterial for tissue engineering and regeneration.

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1. Introduction

Potential therapeutic applications for regenerative medicine include cell-based tissue engineering. There are a number of biomaterials that are suitable for cell culture in vitro and for transplanting the cells in vivo [1]. These biomaterials required two major functions to promote tissue regeneration and repair: physically support as a scaffold and biological activity for cell binding [2,3]. In native tissues, endothelial and epithelial cells are separated form underlying stroma by a basement membrane matrix, which has critical roles in maintaining tissues, guiding development, regeneration, and homeostasis. Basement membrane, a thin extracellular matrix (ECM), consists of type IV collagen, laminin, nidogen, and perlecan [4]. These molecules bind each other by protein–protein and protein–polysaccharide interactions to form a mesh-like structure and physically support for the cells. The basement membrane components bind to cells via multiple and specific cell surface receptors which maintain and promote many cell functions [4,5]. Matrigel/BME (basement membrane extract), a functional soluble ECM complex derived from the mouse Engelbreth-Holm-Swarm (EHS) tumor, is an ideal cell culture substrate for both 2D and 3D culture [6]. However, it is difficult to use this matrix for tissue engineering, since Matrigel/BME is derived from the mouse tumor and cannot be used in humans as a biomaterial. Mimicking the functions of basement membrane is a valid approach in biomaterial studies for tissue engineering [1,2,7].

Laminins, a major component of basement membrane, contain α, β, and γ chains [8–10]. Five α chains (ζ1–ζ5), three β chains (β1–β3), and three γ chains (γ1–γ3) have so far been identified and they comprise at least 15 different laminin isoforms (laminin-111 to laminin-523) [11]. Each laminin isoform is expressed tissue- and/or developmental stage-specifically and promotes laminin isoform-specific functions. Laminins bind various kinds of cell surface receptors, such as integrin, syndecan, sulfatide, and dystroglycan,