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

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ABSTRACT

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.

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 (α1–α5), 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,

Abbreviations: ECM, Extracellular matrix; BME, Basement membrane extract; EHS, Engelbreth-Holm-Swarm; Fmoc, 9-Fluorenylmethoxycarbonyl; MBS, *N*-(*m*-maleimidobenzoyloxy) succinimide; DMF, *N,N'*-dimethylformamide; FBS, Fetal bovine serum; BSA, Bovine serum albumin; HDF, Human dermal fibroblast; NGF, Nerve growth factor; DAPI, 4,6-Diamidino-2-phenylindole; FAK, Focal adhesion kinase; ESC, Embryonic stem cell.

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and contain multiple and diverse receptor binding sites within the laminin chains. The regulation of these laminin–receptor interactions is important for many biological processes, including cell adhesion, migration, angiogenesis, tumor progression, and neurite outgrowth [10,12]. Laminin-111 is the major component of Matrigel/BME and contains laminin α1, β1, and γ1 chains. We previously have identified about sixty cell adhesive sequences from laminin-111 using 673 synthetic peptides covering the entire sequence [13–17]. These active peptides showed various biological activities, including promotion of cell spreading, cell differentiation, neurite outgrowth, angiogenesis, and wound healing [18–22].

Recently, we developed easy-handling peptide-chitosan matrices and examined potential biomaterial applications using in vitro and in vivo assays [23–26]. Chitosan, a deacetylated polysaccharide chitin, is biodegradable and improves wound healing. Chitosan matrices have been used for medical applications, such as suture thread and artificial skin. However, chitosan matrix alone adheres to tissues but dose not show cell attachment [27]. To add the biological function of cell adhesive activity, we conjugated laminin-derived cell adhesive peptides to the chitosan matrix as a scaffold. For example, we conjugated laminin α1 chain active peptides, AG73 (RKRLQVQLSIRT, mouse laminin α1 chain 2719–2730) and EF1zz (ATLQLQEGRLHFXFDLGKGR, X: Nle, mouse laminin α1 chain 2749–2767) on a chitosan matrix. AG73 binds to syndecan, a transmembrane proteoglycan, and EF1zz binds to integrin α2β1. AG73-chitosan matrix promotes cell adhesion with membrane ruffling and neurite outgrowth, and EF1zz-chitosan matrix promotes cell spreading with well-organized actin stress fibers [23,25]. We also demonstrated that AG73-chitosan matrix could deliver cells, such as keratinocytes to the wound bed [24,26], and that the AG73-chitosan matrix sustains its angiogenic activity as well as AG73 solution [28]. These results suggest that the peptide-chitosan scaffold is a powerful tool for cell and tissue engineering, including cell transplantation. Further, when AG73 and EF1zz were conjugated on a chitosan membrane with 1:9 M ratio, the mixed peptide-chitosan membrane promoted strong cell attachment and neurite outgrowth similar to that on a recombinant laminin protein contained the AG73 and EF1 sites [29]. The mixed peptide-chitosan approach has potential as a multifunctional biomaterial for mimicking the multifunctional ECM molecules. Molecular and biological database of peptide-chitosan matrices have not been established yet. In this paper, we screened the biological activity of sixty biologically active laminin-111 peptides conjugated to a chitosan matrix to construct the database of laminin-111 derived peptide-chitosan matrices. Further, we conjugated multiple active peptides onto chitosan matrix to enhance the biological activities of peptide-chitosan matrices.

2. Materials and methods

2.1. Synthetic peptides

All peptides were manually synthesized using the 9-fluorenylmethoxycarbonyl (Fmoc) strategy and prepared in the C-terminal amide form as previously described [17]. For conjugation to a chitosan matrix, a cysteine residue was added at the N-terminus and two glycine residues were used as a spacer between the cysteine and the active peptide sequence. Purity and identity of the peptides were confirmed by an analytical HPLC and an electrospray ionization mass spectrometer at the Central Analysis Center, Tokyo University of Pharmacy and Life Sciences.

2.2. Cells

Human dermal fibroblasts (HDFs; Cell Applications Inc., San Diego, CA) were maintained in DMEM containing 10% fatal bovine serum (FBS), 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA). Rat pheochromocytoma PC12 cells [30] were cultured in DMEM containing 7.5% horse serum (HS; Invitrogen), 7.5% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Table 1
60 biologically active peptides derived from laminin-111.

Peptide	Sequence	HT-1080 cell attachment activity	
		Sepharose beads	Plate coat
A3	LWVTVRSQQRGLF	+	++
A10	GTNNWWQSPSIQN	++	++
A12	WVTVTLDLRQVFQ	+	++
A13	RQVFQVAYIIIKA	+++	+++
A18	LTRYKITPRRGPPT	++	−
A24	LLEFTSARYIRL	−	+++
A25	YIRLRLQRIRTL	++	+
A51	SINNTAVMQRLT	+++	+
A54	LGNKLTAFGGFL	+	+
A55	GGFLKYTVSYDI	+	++
A64	RDQLMTVLANVT	++	++
A65	ANVTHLLIRANY	−	++
A99[a]	AGTFALRGDNPQG	+++	−
A99a[a]	ALRGDN	n.t.	n.t.
A112	VLIKGGRARKHV	+++	−
A119	LSNIDYILIKAS	−	++
A121	LQQSRIANISME	−	++
A167	NLLLLVKLNLK	++	++
A174	HRDELLLWARKI	+	++
A177	KRRARDLVHRAE	++	−
A194	PGGMREKGRKAR	++	−
A206	LSEIKLLISRAR	−	++
A208	AASIKVAVSADR	−	+++
AG6	LAVEMRRGKVAF	−	++
AG10	NRWHSIYITRFG	++	+
AG22	SSFHFDGSGYAM	++	−
AG28	LSIELVRGRVKV	−	++
AG31	TDRRYNNGTWYK	++	−
AG32	TWYKIAFQRNRK	++	+
AG39	SKAVRKGVSSRS	+++	−
AG47	FATKNSSGILLV	++	++
AG53	GTSLRKALLHAP	++	−
AG56	SLVRNRRVITIQ	+	+
AG63	IKNVVLDAQLLD	−	+++
AG73	RKRLQVQLSIRT	+++	+++
AG75	GLIYYVAHQNQM	+++	−
AG101	YQPRAARAL	+++	−
AG103	AHKSKHRIVLTV	++	++
AG111	AHKSKHRIVLTV	+++	−
EF1[b]	DYATLQLQEGRLHFMFDLG	+	n.t.
EF1zz[b]	ATLQLQEGRLHFXFDLGKGR	n.t.	n.t.
B7	AFGVLALWGTRV	+++	+++
B20	HLIMTFKTFRPA	+	++
B23	KTWGVYRYFAYD	+	++
B30	RIQNLLKITNLR	++	+
B31	TNLRIKFVKLHT	+	++
B34	REKYYYAVYDMV	+	+
B43	HFDMAVFIATG	−	++
B54	KRLVTGQR	+++	−
B62	GPGVVVVERQYI	+	++
B123	AAEPLKNIGILF	+	+
B133	DSITKYFQMSLE	−	+++
B160	VILQQSAADIAR	−	+++
C3	LWPLLAVLAAVA	++	+
C16	KAFDITYVRLKF	+++	+++
C18	RPESFAIYKRTR	+	+
C28	TDIRVTLNRLNTF	−	++
C35	LPFFNDRPWRRAT	++	+
C57	APVKFLGNQVLSY	++	+
C59	SFSFRVDRRDTR	+	+
C64	SETTVKYIFRLHE	−	+++
C68	TSIKIRGTYSER	−	++

60 peptides derived from laminin-111 promote HT-1080 cell attachment activity on plate and/or beads assay [28–34,41,43]. When setting "+" as 1 point, more than 2 points of peptides were chosen.
n.t.: not tested.
[a] A99a sequence arranged A99 sequence short. A99a-chitosan matrix promotes HDF cell attachment activity. A99a was used for this experiment.
[b] EF1 is different from other peptides in evaluation method. EF1zz-chitosan matrix promotes HDF cell attachment activity. EF1zz was used for this experiment.