Modified collagen with DOPA as a tissue repair matrix

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Objectives: The repair of musculoskeletal tissue defects including cartilage, bone, tendon, muscle, nerve, skin is still a challenging clinical problem. Collagen, either alone or in combination with other materials, is an important natural biomaterial used in tissue engineering applications. The disadvantages of using collagen as a biomaterial for tissue repair are its low biomechanical stiffness and rapid biodegradation. This can be achieved by chemical cross-linking methods. Mussel adhesive proteins are remarkable materials that display an extraordinary capability to adhere substrates under water. 3, 4-dihydroxyphenylalanine (DOPA) is found in most known adhesive marine mussel proteins with about 11% high levels [1]. In the present study, we modified collagen with DOPA to develop a biodegradable tissue repair matrix in which curing would occur through free radical polymerization of the DOPA end groups initiated by the Ag^{+}/peroxdiphosphate redox pair. The toxic of the degradation products was also investigated.

Methods: DOPA modified collagen matrix was prepared by three-step reactions [2, 3]. First, amine group of DOPA was protected by trifluoroacetate, Second, N-trifluoroacetyl-DOPA was activated by N-hydroxysuccinimide. Finally, activated DOPA was conjugated to collagen to produce a DOPA-rich collagen matrix. The structure of activated DOPA was confirmed by mass spectrum. The concentration of DOPA in collagen matrix was determined by HPLC, and collagen was detected by electrophoresis and Sirius red stain. The toxic of the DOPA-modified collagen matrix on the cell proliferation was studied using primary human skin fibroblasts. The cells were cultured on the collagen gels with or without modified DOPA for 21 days. The growth of the cells was checked using a light microscope every 12 hours and the viability of living cells were measured using the MTT assay.

Results / Discussion: Tissue repair matrix was prepared by activated DOPA and collagen using trifluoroacetate and N-hydroxysuccinimide. The structure of activated DOPA was confirmed by electrospray mass spectrometry. Tandem mass spectrometric analysis of m/z peak 392 gave daughter ion peaks consistent with the expected fragmentation pattern of this compound. Amino acid analysis indicated that 76 nmol DOPA has combined with 5 mg of collagen. Electrophoresis showed the collagen sample modified with DOPA had larger molecular weight bands of proteins than that of the collagen without DOPA treatment. Collagen was stained by Sirius red in the DOPA-collagen gel and DOPA-based polymer collagen gel. As expected, no any red color was stained in the gel made from DOPA-based polymer only. Degradation testing indicated that DOPA-based polymer-collagen gel was degradable and the collagen fiber was found in the DOPA-collagen polymer gel during the gel degradation. The gel made by DOPA-based polymer-collagen degraded slowly than the gel made by collagen only. Growth of fibroblast on the DOPA-containing polymer gel showed similar behavior to that on a tissue culture plate. Furthermore, human skin fibroblasts grown on the collagen-containing gel showed an enhancement on cell proliferation than that grown in the tissue culture plate only. The similar result was found in the DOPA-based polymer-collagen gel. MTT result indicated that the proliferation rate of human skin fibroblasts grown on the gels made by collagen only or collagen-containing polymers was significantly increased compared to the tissue culture plate or DOPA-base polymer alone ($p<0.02$).

Conclusions: Our experiments suggested that an activated collagen has been modified with DOPA. This DOPA-modified collagen could react with DOPA-containing polymer to give a polymer gel initiated by silver and peroxydiphosphate. This collagen-containing polymer gel was more flexible and compatible than the polymer gel without collagen. It was biodegradable and nontoxic to the cells. It should be a good matrix for tissue repair.

References:

Waite JH. Evidence for a repeating 3,4-dihydroxyphenylalanine- and hydroxyproline-containing decapepride in the adhesive protein of the mussel, mytilus edulis L. J Biol Chem 258, 2911-2915, 1983.

Hu BH, Messersmith PB. Protection of 3,4-dihydroxyphenylalanine (DOPA) for Fmoc solid-phase peptide synthesis. Tetrahedron Letters 41, 5795-5798, 2000.

Curphey TJ. Trifluoroacetylation of amino acids and peptides by ethyl trifluoroacetate. J Org Chem 44, 2805-2807, 1979.

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