SELECTION OF ACTIVE FRAGMENTS OF COLLAGEN

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Introduction

Application of natural building blocks for the preparation of three-dimensional matrix useful for regeneration ensure completely biocompatibility of the temporary ECM equivalent. The advantage of these materials used as a scaffolds after deposition of cells, their adhesion, proliferation and differentiation arise from their steady biodegradation leading solely to natural metabolites. The inherent feature of peptides to form stable, specific spatial structures, cause that they can be evaluated as universal platform for the synthesis of nanomaterials with controllable three dimensional framework [1] bearing the properties stimulating the regeneration process [2]. On the other hand, the presence of polysaccharides assure the formation stable three-dimensional matrix. Considering the properties of both classes of compounds, attempts were made to obtain hybrid materials in which fragments of main ECM proteins were covalently attached to alginate and hyaluronate. The application of collagen derivatives was preferred due to the fact that this protein is the fundamental component of the ECM. The preliminary result of study indicate that synthesized hybrid materials can be useful as a scaffolds for regenerative medicine. On the other hand, it is also possible to use both of these components as scaffolds for use in regenerative medicine. At the Institute of Organic Chemistry of the Lodz University of Technology, research to select biologically active collagen I, II and III fragments able to form a new collagen network derivatives useful as a three-dimensional materials was undertaken. The use of biologically active protein fragments instead of whole proteins eliminates the immunogenic properties of the protein and on the other hand allows to modify their stability.

Materials and Methods

At the stage of selection of biologically active fragments of collagen I, II and III, the SPOT technique of synthesis of protein fragments was used. All tested proteins were divided into decapeptide non-overlapping fragments that were used for synthesis of three libraries of immobilized peptides covering whole collagens I, II and III. Chr-1 Whatman filter paper was used as matrix in the study. Cellulose was modified with 2,4-dichloro-6-methoxy-1,3,5-triazine and and Fmoc-Gly according to standard protocol [3]. Syntheses of immobilized peptides were made by automated SPOT [4] methods using as a coupling reagent DMT/NMM/TosO$^-$ [5]. The synthesized peptide libraries were treated with specific antibodies. A standard Dot-blot procedure was used to visualize the immune complexes. Strong immunological complexes were subjected to epitopic mapping with a shift of the reading frame by five amino acid residues towards the N- and C-terminus.

Results and Discussion

Research on the selection of biologically active collagen I, II and III fragments has been carried out. The process is multi-stage and comprised of synthesis libraries covering the entire proteins in the form of decapeptidic fragments immobilized on cellulose. The synthesis was carried out according SPOT technique by using triazine coupling reagents.

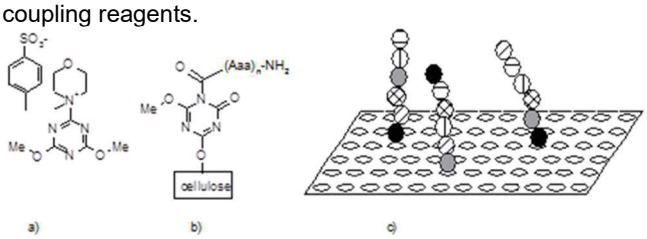

FIG. 1. Coupling reagent a), linker used for anchoring peptides b), and cellulose dot matrix of peptides c) used in the SPOT methodology.

The dot blot assay selected collagen fragments (epitopes) that formed immunological complexes with antibodies. The epitopes feature is the preservation of the proper secondary structure of the native protein.
In the first screening of the 168 element library of collagen I fragments, 33 decapeptides forming strong immunological complexes and 38 fragments with moderate antibody binding capacity were selected (FIG. 2). In the case of collagen II, from the 149 element library of peptide fragments in the dot-blot test, 26 peptides with the ability to interact with antibodies were selected, of which 4 formed strong immunological complexes. From the 147 element library of collagen III fragments, 52 fragments with the ability to interact with antibodies were selected, of which 12 decapeptides formed strong immunological complexes.

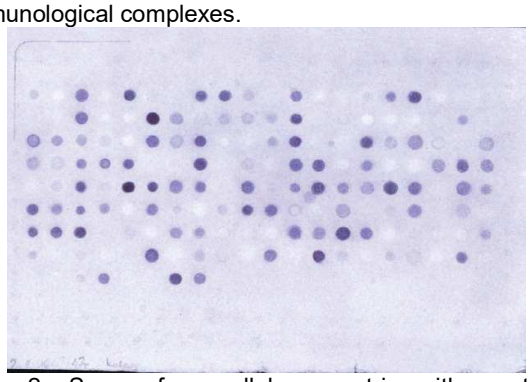

FIG. 2. Scan of a cellulose matrix with peptides embedded on its surface after dot-blot analysis.

After epitope mapping and re-blot testing, a total of 73 fragments derived from collagen I, II, III have been selected, which have the ability to strongly interact with antibodies, and thus these fragments are exposed to the outside of the proteins.

Conclusions

The collagen fragments selected in the screening studies were synthesized on the solid phase and cross-linked to create spatial structures.

Acknowledgments

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