RECOMBINANT ADHESIVE PROTEIN WITH PROSPECTS FOR USE AS STICKY MATRIX IN TISSUE ENGINEERING

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Introduction: Proteins which provide adhesive (sticky) properties and biocompatibility are of high demand for the use as medical glues and as matrixes for the *in vitro* modeling of tissues. Highly impressive adhesive proteins are the "mussel foot proteins" (MFPs) which reside in the mussels' byssus. The MFPs are sticky because of the presence of large mass fraction of an unusual amino acid 3,4-dihydroxyphenylalanine (DOPA). Recombinant mussel adhesive protein (Fp-131) was produced in a system which provides conversion of tyrosine residues to DOPA residues.

Methods: Gene encoding the protein Fp-131 was assembled from synthetic oligonucleotides. Synthetic gene was cloned into the pET28/32 plasmid for bacterial expression and co-expressed with a recombinant tyrosinase. Metal affinity chromatography was used to purify the Fp-131. Presence of the DOPA was revealed in a reaction with the nitroblue tetrazolium (NBT-test). To test the absence of cellular toxicity the Fp-131 was used to cover surfaces which were manufactured to suppress a monolayer adhesion (suspension culture treated Greiner flasks). The Fp-131-treated surfaces were tested to support proliferation of the adhesion-depended cell lines (BHK-21, CHO, HEK293).

Results: The protein Fp-131 has on both ends a repeating 10 amino-acids-long motif from the MFP-1 and the central part of the Fp-131 corresponds to the MFP-3. The expression product also has a 6His-tag. To provide co-translational conversion of the tyrosine residues to DOPA residues we developed a double-transformed expression strain in which the Fp-131 and a recombinant tyrosinase from bacterium *Verrucomicrobium spinosum* are simultaneously produced. The Fp-131 was purified and shown to have DOPA moieties in the NBT-test. Adhesion strength of the Fp-131 was 2.5 MPa which is a significant increase compared to the strengths of the common surgical fibrin blues. The Fp-131 covers surfaces of culture vessels and provide adhesive matrix in which various cell types readily proliferate.

Conclusion: Recombinant adhesive proteins have prospects for use as matrixes for tissue and organ engineering.