Abstract

Design and synthesis of peptide scaffolds with potential pro-regenerative properties

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The skin is an organ which serves protective functions and separates the internal organs from the external, environmental factors. It is formed by three main layers: epidermis, dermis and subcutaneous tissue. The skin injuries are hazardous and may lead to severe consequences for the whole organism. They involve mechanical injuries such as wounds and grazes. The risk resulting from such injuries are caused by the occurrence of an open wound oozing blood and body fluids. The cases of open wounds are frequently the cause of bacterial infections and nerve, tissue and bone infractions. Such trauma is often very painful and its treatment relays mainly on cleansing, disinfection and securing the wound. Therefore, securing the skin continuity is crucial for health. Reconstruction of the lost tissue structures and re-establishing their function and architecture is the key factor in restoring the skin continuity.

Regenerative medicine and tissue engineering contribute to the medical sector specializing in the search for methods of mending and/or replacement of the damaged tissue. When combined, the three most important elements of these studies form the tissue engineering triad. It consists of cells (e.g. stem cells), scaffolds and signaling factors. The simplified idea of combining the three elements is expressed by a scaffold, which links the architecture of extracellular matrix, active substances functioning as signaling factors and cells suspended in them. The tissue engineering steel exhibits high demand on such materials (scaffolds). Therefore, in my thesis I decided to design peptide scaffolds, which would fulfill the above mentioned requirements.

For this purpose, I designed and studied two types of scaffolds which were hybrids of peptides containing self-organizing, enzymatic and biologically active sequences. As a self-organizing peptides, I choose fibrylogenic (QAGIVV) and gel forming (RADA) peptides. Peptides exhibiting pro-regenerative potential included: GHK, KGHK and RDKVYR. Self-organizing peptides were separated from biologically active peptides by the insertion of elastase specific sequence. This procedure was used to obtain constructs, which would release pro-regenerative peptide fragments from the scaffold, when exposed to the enzyme occurring in the wound. Biologically active peptides GHK and KGHK were recognized as molecules

with pro-regenerative properties before. The third molecule – trade name Imunofan (BIONOX), on the other hand, is a drug available in Russia and Ukraine. It is known to possess anti-inflammatory properties and assist the immune system. In my thesis I confirmed its pro-regenerative properties. Another peptides exhibiting pro-regenerative properties, which I studied as part of my thesis, included peptides with multiplicated active sequence (GHK, KGHK or RDKVYR). The sequences were separated from one another with the elastase specific sequence.

The research presented in this dissertation are interdisciplinary and involve chemical synthesis and physicochemical and biological studies. For the purpose of structural studies, I used circular dichroism technique and confirmed that the scaffolds form a β-sheet structures. I also used liquid chromatography and mass spectrometry to evaluate the stability of the peptides in water and blood serum, to determine the affinity of the peptides to albumin and to verify the location of the enzymatic cleavage of the peptides. To describe the morphology of the formed peptide fibers I used microscopy techniques such as AFM, TEM and Cryo-SEM. The biological studies i.e. cytotoxicity and proliferation of the dermal cells and the immunogenicity studies were performed as a cooperation with the Medical University of Gdańsk. The molecules exhibiting the highest pro-regenerative properties were selected for the *in vivo* studies on mice with two different models of skin damage. The studies were performed as a cooperation with the Technical University of Gdańsk and Tri-City Academic Laboratory Animal Center,

The obtained results will broaden the World knowledge in the field of peptide scaffolds used in tissue engineering. They may also be used in treatment of difficult to heal skin wounds.