AROMATIC PEPTIDES AS COMPONENTS OF POTENTIAL SCAFFOLDS FOR REGENERATIVE MEDICINE

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Introduction

Over the past few years, applications of the peptides were continued and extended in regenerative medicine. For instance, Thomas P. J. Knowles and co-workers have reported that in the naturally occurred β-amyloid deposits complex proteins, have been identified common motif composed of phenylalanine residues (FF) [1]. Additionally, Ehud Gazit proved that its structural feature upholding β-sheet structures is the relatively weak interactions between aromatic rings [2]. On the basis of the studies, a hypothesis was put forward that short-aromatic (hydrophobic) peptides could be used in medicine [3]. The aim of this study was to characterize biological effect of nonamer and hexamer peptides in cell culture and check whether the peptides could be used as components of scaffolds for regenerative medicine.

Materials and Methods

Materials

Six types of peptides with different composition (TABLE 1) were designed and synthesized in Institute of Organic Chemistry, Lodz University of Technology.

TABLE 1. Composition of peptides used in research.

3-letters structure representation	Symbol
H-PhePhePhePhePhePhe-OH	$(FFF)_2$
H-TrpTrpTrpTrpTrpTrp-OH	$(WWW)_2$
H-TrpTrpCysTrpTrpCys-OH	$(WWC)_2$
H-TrpTrpCysTrpTrpCysTrpTrpCys-OH	$(WWC)_3$
H-TryTyrCysTryTyrCys-OH	$(YYC)_2$
H-TryTyrCysTryTyrCysTryTyrCys-OH	$(YYC)_3$

Preparation of peptide layers

In the first step, 100 mg of peptides were dissolved in 100 ml of 70% ethanol. This Stock solution (0.1%) was diluted into two other concentrations (which were respectively 0.05% and 0.025%). In the second step, 300 µl of each solution was added into independent wells of the 96-well culture-plates. After that, the solvent was evaporated, resulting in the formation of peptide layers. All this procedure was conducted under sterile conditions.

Cell study

L929 mice fibroblasts (ATTC, USA) were cultured in Dulbecco's modified Eagle's medium DMEM (ATTC, USA) supplemented with 10% fetal bovine serum (ATTC, USA). The cells were cultured in optimal conditions at 37°C, 5% CO_2, and 95% humidity. After passage 3rd, cells were seeded on layers of peptides at a density of 5 x 10^3 cells per well (200 µl/well) and kept under culture conditions. The biocompatibility of the peptides was analyzed after 3 and 7 days of the culture with the use of PrestoBlue™ assay (Invitrogen, USA). The test was used to determine the amount of intracellular redox reaction of resazurin into fluorescent resorufin which corresponded with cells viability. In compliance with the manufacturer's protocols, 20 µl of the PrestoBlue reagent was added per well and plates were returned to an incubator for 1 h. The fluorescence was read at an excitation/emission wavelength of 560/590 nm on the microplate reader POLARstar Omega (BMG Labtech, Germany). Cells morphology was controlled using an optical microscope. All results were obtained by performing three independent repetitions of each measurement. All data were given as mean ± standard error of mean (SEM).

Results and Discussion

In this work, viability test was chosen to determine the biocompatibility of studied materials. The PrestoBlue assay confirmed the positive influence of studied peptides on cells viability. Obtained data are given in FIG. 1. In FIG. 2, the morphology of cells cultured in contact with the most effective peptides is shown.

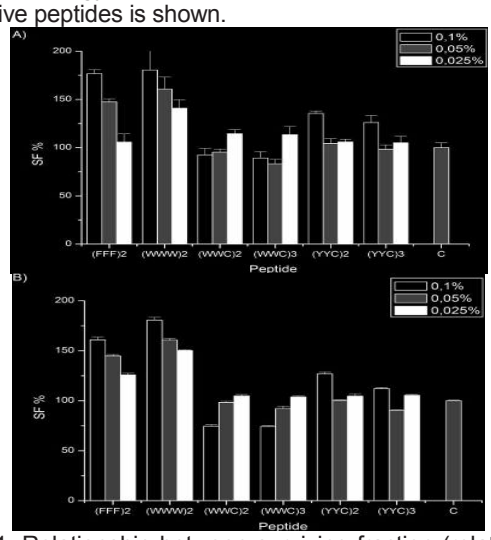

FIG. 1. Relationship between surviving fraction (relative to control) and the concentration of each peptide contacted with L929 fibroblasts for a) 3 days, and b) 7 days.

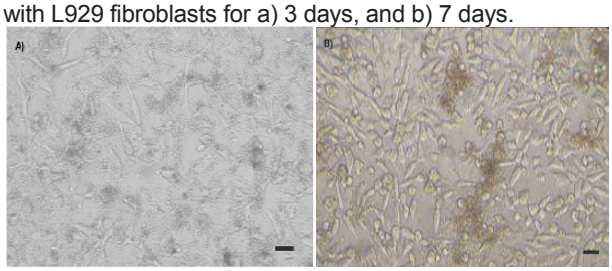

FIG. 2. Morphology of L929 cells cultured 3 days with a) $(FFF)_2$ and b) $(WWW)_2$. Scale bar 100 µm.

Conclusions

Based on the data gathered from viability test and obtained cells' morphology pictures, it is proven that the synthesized materials are not cytotoxic and do not negatively influence the growth of cells. Moreover, homohexamer peptides ($(FFF)_2$, $(WWW)_2$) are very effective in accelerating the proliferation and stimulating the activity of L929 fibroblasts.

Acknowledgments

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References

[1] T. P. J. Knowles, M. J. Buehler, Nat. Nanotechnol. 6 (2011) 469-479.
[2] E. Gazit, Nanobiotechnol. 1(3) (2005) 286-288.
[3] S. Marchesan, A. V. Vargiu, K. E. Styan, Molecules 20(11) (2015) 19775-19788.