Selected biodegradable and nonbiodegradable materials – their impact on cells growth and proliferation.

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Abstract: The natural tissue scaffold– Extracellular Matrix (ECM) - is composed of an organic (protein, polysaccharide) and inorganic (i.e. hydroxy-apatite) components that when combined with the cells forms a tissue. A scaffold is an integral part of every tissue that besides providing the environment for cells to grow and exist, it also improves tissue's mechanical properties. It provides elasticity, flexibility and durability for the tissue. Tissue engineering approaches utilize artificial materials (biomaterials) as a substitute of natural ECM. The process of producing tissue scaffolds obtained from biodegradable polymers has become a very intensively researched area for the past several years. Most of the current work focuses on the design and preparation of scaffolds with use of various production technologies and different natural materials like chitosan, collagen, elastine and different synthetic ones, like polymer polycaprolactone (PCL), poly(lactic acid) (PLA), poly(ethylene oxide) (PEO). The objective of this study was to check the impact of the biomaterials on various cell types, and compare their growth pattern. Biodegradable PCL, and five of its hybrids: PCL+SHAP (SHAP, synthetic hydroxyapatite), PCL+NHAP (NHAP, natural hydroxyapatite), PCL+PLGA (PLGA , poly(lactide-co-glycolide), PCL+$CaCO_3$, PCL+SHAP+NAHP+$CaCO_3$ as well as one non degradable biomaterial: polyacrylonitryl (PAN), were tested. For the experiments four different cell types were used: human dermal skin fibroblasts, B16F10 (mouse melanoma cells), HSkMEC (Human Skin Microvascular Endothelial Cells) and HEPC-CB1 (Human Endothelial Progenitor Cells – Cord Blood 1). Impacts of the biomaterials on cells were assessed: 1) by measuring cytotoxic effect of the biomaterials liquid extracts and 2) by direct contact test. The ability of cells to attach to the biomaterials was tested as well as cells' potential to growth and proliferate on the surface of the biomaterials. None of the tested biomaterials was cytotoxic towards the tested cells, making them a potential valuable raw ingredient for 3D scaffold development that would find its applications in tissue engineering. The differences in efficiency of cells attachment and proliferation between tested biomaterials and cells lines were observed. In addition, a stimulating effect of the biomaterials on cells growth was also detected.

INTRODUCTION

The purpose of the study was to evaluate the biocompatibility of the selected biomaterials, as a potential source for artificial tissue scaffolds that may act as an extracellular matrix in regenerative medicine.

Biocampatibilty of the biomaterials was assessed by:
1) liquid extracts test
2) direct contact test
3) cell ability to growth and proliferation on the biomaterials

Evaluated biomaterials:
- PCL (polycaprolactone)
- PCL+SHAP (SHAP, synthetic hydroxyapatite)
- PCL+NHAP (NHAP, natural hydroxyapatite),
- PCL+PLGA (PLGA , poly(lactide-co-glycolide),
- PCL+$CaCO_3$
- PCL+SHAP+NAHP+$CaCO_3$
- PAN (polyacrylonitryl)

PCL is a biodegradable material, while PAN is nondegradable.

Cell lines in the assays:
- human dermal skin fibroblasts,
- B16F10 (mouse melanoma cells),
- HSkMEC (Human Skin Microvascular Endothelial Cells)
- HEPC-CB1 (Human Endothelial Progenitor Cells – Cord Blood

Fibroblasts were used as they are common source for reprogramming and transdifferentiation for regenerative medicne, while HEPC-CB1 represents sesitive cell line.

Production of biomaterials by electrospinning

Electrospinnig is the most common method for the preparation of nanofibers from the solution. The coaxial-electrospinning, a variation of the electrospinning, was employed to produce the biomaterials. Two kinds of solutions were used: core solution and shell solution. After passing the nozzle a double Tylor cone is created. The shape and properties of Taylor cones depend on the characteristic of the respected polymer-forming solution. At the contact-zone both solutions interacts and the nature of this interaction is formative for the coaxial electrospinning process. The shell solution is stretched by electrostatic field within the zone of straight-forward flow. The flow of the core-forming solution is also stretched due to the interactions and friction imposed on it by the coating solution. The shaping (morphology) of nanofibers of the core-shell polymer-type is also strongly influenced by the evaporation rate of solvents used in the process. [1]


Fig. 1A


Fig. 1B

Fig. 1C

Fig.1A: Electrospinning device; Fig.1B Interior of the electrospining device; Fig. 1C Taylor's cone

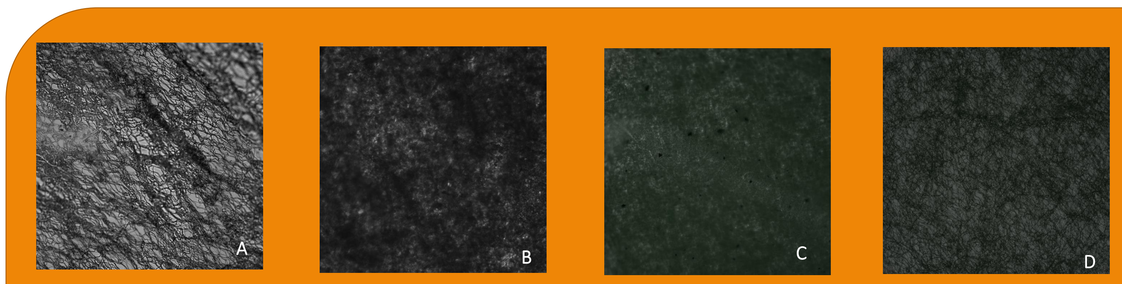


Fig. 2: pictures of the biomaterials structure, *10x magnification*
A. PCL
B. PCL+SHAP
C. PCL+NHAP
D. PCL+PLGA
E. PCL+$CaCO_3$
F. PCL+SHAP+NAHP+$CaCO_3$
G. PAN


Fig. 3A

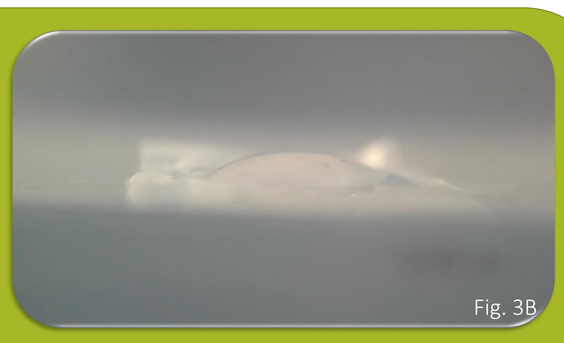


Fig. 3B

Fig. 3A: Drop of water on a surface of a biomaterial. Hydrofobic property of the biomaterials. makes them quite a big challenge in regenerative medicine and has to be overcome.e.g. by cold plasma treatment. [2],

Fig. 3B: Drop of water on a surface of a biomaterial after plasma treatment. The drop changed its shape, flatness is clearly visable.

Cold plasma treatmen performed and pictures taken by dr G. Busco.

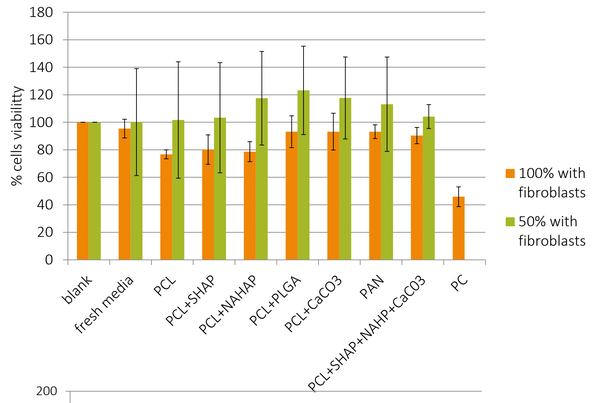
Methods
- 10mm² pieces of each of the biomaterial were prepared. One piece of the biomaterial were placed per well on the plates. Biomaterials were sterilize uder the hood with the 70% alcohol for 1h befor cells seeding or for ~1 min for MTT assay , than dried, washed with PBS and media.
- For MTT assay biomaterials were incubated for 24h with a full grownig media in a standard cell culture conditions,, then 50% and 100% liquid extract was transfered to the plate with cells and incubated for 24h. MTT assay was perfomed as described by Hudecki et al.
- For direct contact test and proliferation 30ul of cells suspension were applied on biomaterials and incubated for 2h in standard cell culture conditions. Then 1,5ml of the proper media was applied and cells were incubated in a standard cell culture conditions. FDA (Sigma), alamarBlue (Invitrogen) and DiD (Invitrogen) staining were performed according to manufacturers instructions.

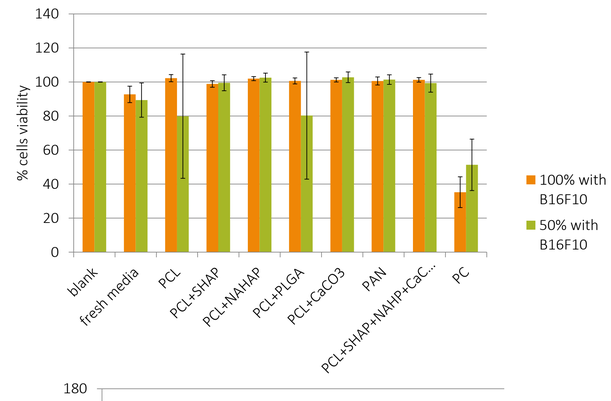
References:
[1] Hudecki A, Gola J, Ghavami S et al.Structure and properties of slow-resorbing nanofibers obtained by (co-axial) electrospinning as tissue scaffolds in regenerative medicine PeerJ.2017 Dec 18;5:e4125
[2] Atyabi SM, Irani S, Sharifi F, et al. Cell attachment and viability study of PCL nano-fiber modified by cold atmospheric plasma. J Cell Biochem Biophys. 2016;74:181–190.

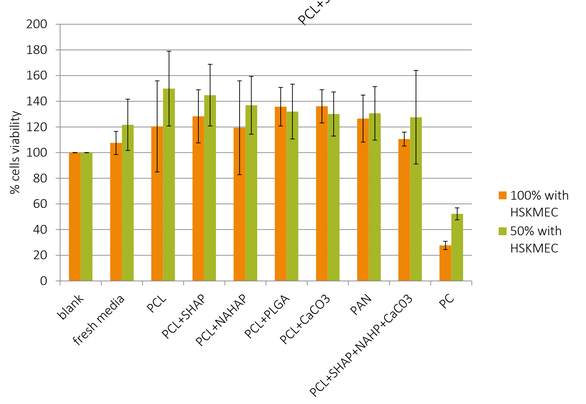
RESULTS OF THE BIOCOMPATIBILITY ASSESSMENT

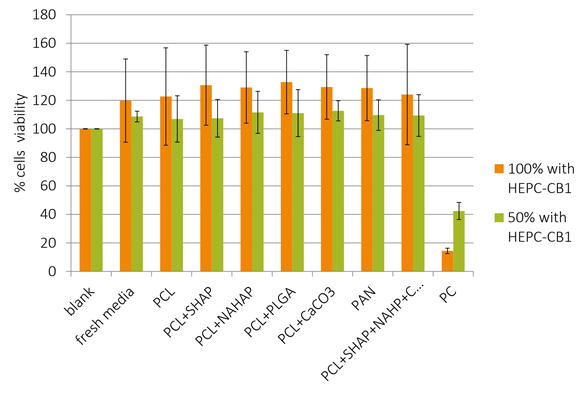
1) Determination of the biomaterials' liquid extract impact on cells viability

Method: MTT assay





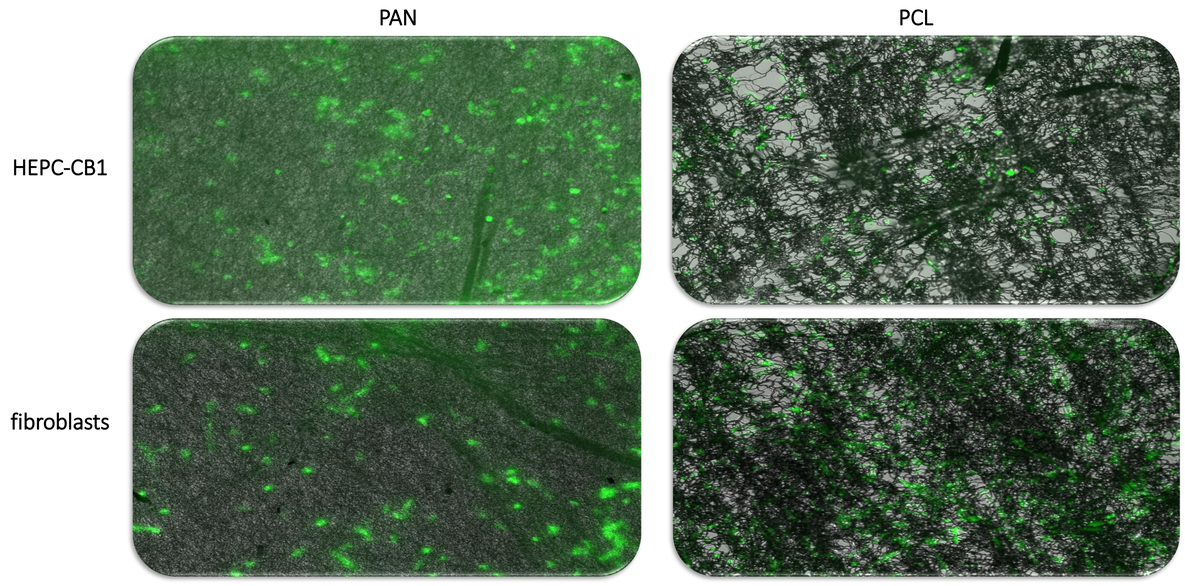




***Conclusions**: The mean viability of the cells didn't decrease below 70%, what means that <u>none of the extracts</u> of the tested biomaterials shown toxic effect to the tested human skin fibroblasts, B16F10, HSK-MEC and HEPC-CB1 cells.*

2) Determination of the biomaterials' direct contact impact on cells viability and attachement

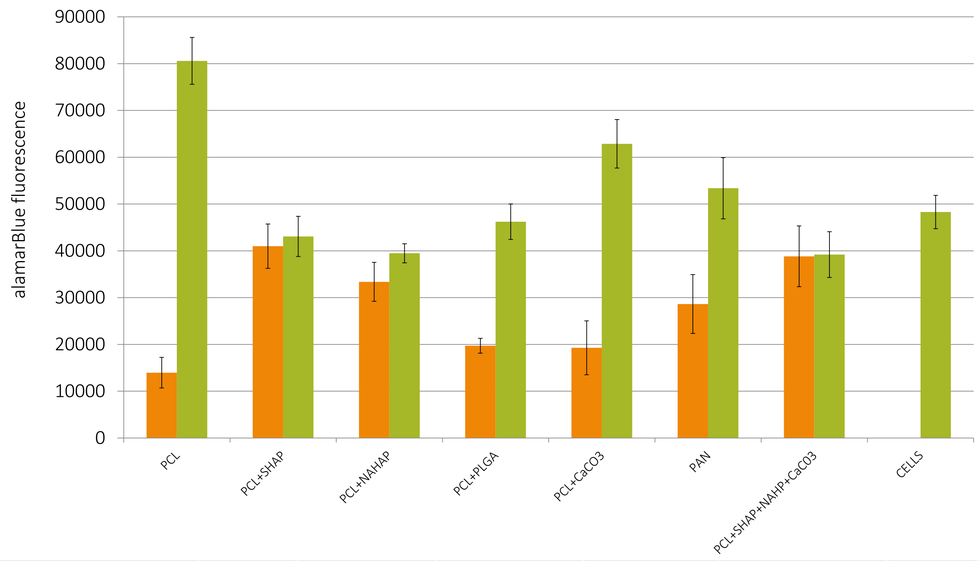
Method: FDA staining



***Conclusions**: Cells attached to all of the biomaterials but with different efficiency. Efficiency of the cells attachment may depend on the kind of the biomaterials and cells type. Representative pictures are shown.*

3) Determination of cells ability to growth and proliferation on the biomaterials

Methods: alamarBlue assay & DiD staining



	PCL	PCL+SHAP	PCL+NAHP	PCL+PLGA	PCL+CaCO3	PAN	PCL+SHAP+ NAHP+ CaCO3	CELLS
TOTAL FLUORESCENCE of alamarBlue	94548	84070	72849	65948	82150	82015	78028	48299
% OF FIBROBLASTS ON BIOMATERIAL	15	49	46	30	23	35	50	N/A
% OF FIBROBLASTS IN WELL	85	51	54	70	77	65	50	100

***Conclusions**: Total fluorescence of the alamaraBlue was higher, even twice, in wells with biomaterials when compared to the well with cells without any biomaterial. That indicated, that the presence of the biomaterial triggered cell proliferation and growth. The same trend was observed for HEPC – CB1 cells (data not shown). Moreover, cells stained with DiD were incubated for 96h and picutres were taken every 4h. Results indicated cells ability to growth and proliferate on the biomaterials with efficiency that vary between cells type and biomaterial type (data not presented).*