THE IN VITRO ANALYSIS OF THIN FILMS BASED ON CHITOSAN/TANNIC ACID

BEATA KACZMAREK[1]*, OLIWIA MIŁEK[2], KINGA NADOLNA[1], AGATA OWCZAREK[1], KONRAD KLESZCZYŃSKI[3], ANNA M. OSYCZKA[2]

[1] NICOLAUS COPERNICUS UNIVERSITY IN TORUŃ, FACULTY OF CHEMISTRY, DEPARTMENT OF CHEMISTRY OF BIOMATERIALS AND COSMETICS, GAGARIN 7, 87-100 TORUŃ, POLAND
[2] DEPARTMENT OF CELL BIOLOGY AND IMAGING, INSTITUTE OF ZOOLOGY AND BIOMEDICAL RESEARCH, FACULTY OF BIOLOGY, JAGIELLONIAN UNIVERSITY, KRAKÓW, POLAND
[3] DEPARTMENT OF DERMATOLOGY, UNIVERSITY OF MÜNSTER, VON-ESMARCH-STR. 58, 48149 MÜNSTER, GERMANY
*E-MAIL: BEATA.KACZMAREK@UMK.PL

[ENGINEERING OF BIOMATERIALS 153 (2019) 34]

Introduction

The surface roughness affects cells response immediately after the material implantation. Moreover, a rough surface inhibits the biofilm formation, the one of the main problems in implantation surgery [1]. Cell adhesion represents a molecular interplay between cell surface and the extracellular environment [2]. The material properties that affect cell adhesion will also influence cell division and they may either stimulate or inhibit cell growth. Thus, examining cell growth on the material with known surface parameters is the key first step to evaluate clinical potential of experimental biomaterials.
The aim of the study was to examine normal and cancer cells growth on the materials obtained by combining chitosan and tannic acid at 80/20, 50/50 and 20/80 ratios. The cell lines used in this study were the following: MNT-1 (human highly pigmented melanoma), SK-MEL-28 (human malignant amelanotic melanoma), Saos-2 (human osteosarcoma), HaCaT (spontaneously transformed aneuploid immortal keratinocyte cells) and human bone marrow-derived stromal cells (BMSC) obtained from a 56-year-old male patient (Institutional Review Board protocol nr 1072.6120.254.2017).

Materials and Methods

Tannic acid (M=1701.2 g/mol, TA) and chitosan (DD%=78, 1.8 × 10^6, CTS) are commercial compounds purchased from the Sigma-Aldrich Company (St. Louis, MO, USA).

Sample preparation

Chitosan and tannic acid were dissolved in 0.1M acetic acid, separately, at a concentration of 2%. The mixtures of chitosan and tannic acid were prepared in the weight ratios of 80/20, 50/50, 20/80. Mixtures were placed on a plastic holder for solvent evaporation. The thickness of the obtained films was 0.035 ± 0.003 mm. Results were compared with pure chitosan-based films as control.

Establishing cell cultures on the experimental films

All cells used in this study were seeded directly onto material films or tissue culture plastic (control TCP, Nest) at a density of 1x10^4/cm^2 in 1 ml of adequate serum-containing medium (SCM, TABLE 1). SCM was exchanged on day 2. On day 6 MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) was carried out in order to determine the metabolic activity of living cells. Briefly, at the day of MTS assay, cells were rinsed once with PBS (BioShop), supplemented with phenol-free Alpha-MEM (Gibco) containing 10 times diluted MTS reagent in the amount of 200 µl per well, followed by incubation in a CO_2 incubator. The reactions were developed until an apparent color change of the MTS reagent in culture wells vs. MTS reagent in empty (cell-free) well. Afterward, the MTS solutions from culture wells were transferred to individual wells in 96-well plates (Nest) and absorbance was measured at 492 nm using a plate reader (SpectraMax iD3 Molecular Devices). The intensity of the developed color is directly proportional to the amount of metabolically active cells, according to the technical bulletin of CellTiter 96® Aqueous One Solution Cell Proliferation Assay by Promega.

Results and Discussion

Metabolic activity of cells cultured on material films

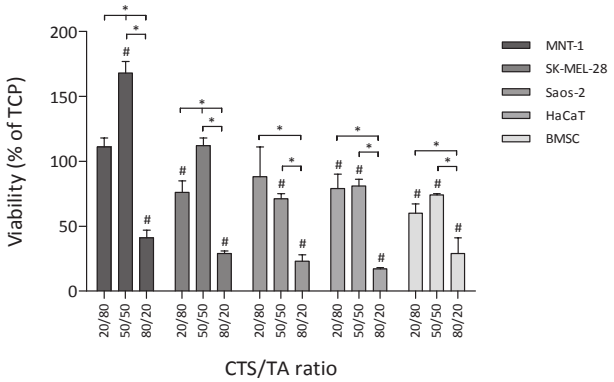

FIG. 1. Metabolic activity of different cell types cultured for 6 days on different material films. Results are displayed as mean ± STD (i.e. % change of cell viability on the material surface vs. cell viability on TCP) for cell lines MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. CTS/TA films combined in ratios 20/80, 50/50 and 80/20 were examined. #statistically significant vs TCP, *statistically significant between different material surfaces within particular groups.

Conclusions

Materials based on chitosan and tannic acid showed different influence on the normal and cancer cells. Films with the lowest tannic acid (CTS/TA 80/20) content inhibit the cell growth. The highest influence was noticed for MNT-1 cells and the lowest for BMSC. It can be observed that those films have higher surface roughness compared to other CTS/TA ratios. Thereby, it can be assumed that materials composed of chitosan and tannic acid may potentially find application in the cancer cells treatment. However, further experiments have to be carried out with expanded biological studies.

References

[1] M.M. Gentleman, E. Gentleman, Int. Mater. Rev. 59 (2014a) 417–429.
[2] F.C. e Silva Filho, G.C. Menezes, Mater. Sci. Eng. C 24 (2004) 637-641.