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Normal and cancer cells response on the thin films based on chitosan and tannic acid

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ABSTRACT

A novel aspect of tissue engineering is the material selective influence on the different cell types. The cell viability is a parameter which determine the cell ability to proliferate in the contact with material. In the experimental study the thin films based on chitosan and tannic acid mixture in ratio 80/20, 50/50 and 20/80 were tested. The surface roughness decreases with increasing tannic acid content. The cell culture was established on the proposed films. In vitro tests were carried out for the different cell lines as MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. The result showed the dependence on the material influence on the various cell lines.

1. Introduction

Biopolymers are widely used in biomaterials preparation due to their biocompatibility and non-toxicity. For proper cell response the important factors of obtained materials are the materials surface properties as its roughness. They influence the interactions between the material and host environment composed of body fluids, proteins and various cells (Jaganjac et al., 2012).

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 (Gentleman and Gentleman, 2014). Cell adhesion represents a molecular interplay between cell surface and the extracellular environment (e Silva Filho and Menezes, 2004). 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. Designed materials can be further studied as wound dressings for skin regeneration. The surface roughness was calculated from the atomic force microscopy images. 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).

2. 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).

2.1. Sample preparation

Chitosan and tannic acid were dissolved in 0.1 M 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 obtained films was 0.035 ± 0.003 mm. Results were compared with pure chitosan-based films as control.

2.2. Roughness

Surface images were acquired at fixed resolution (512 × 512 data points) using scan width 1 μm with a scan rate of 1.97 Hz. Silicon tips with spring constant 2–10 N/m were used. Roughness parameter such

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Table 1
Components of SCM used for different cell types.

Cell type	Medium	Serum	Antibiotics	Additions
MNT-1	D-MEM high glucose (Sigma)	20% heat-inactivated Fetal Bovine Serum (Gibco)	1% ZellShield (Minerva Biolabs)	1% L-glutamine (Sigma), 1% HEPES (Sigma), 1% Sodium pyruvate (Sigma), 1% MEM Non-essential Amino Acid (Sigma)
SK-MEL-28	D-MEM high glucose (Sigma)	10% heat-inactivated Fetal Bovine Serum (Gibco)	1% ZellShield (Minerva Biolabs)	1% L-glutamine (Sigma)
Saos-2	Alpha-MEM (Gibco)	10% Fetal Bovine Serum (Gibco)	1% ZellShield (Minerva Biolabs)	–
HaCaT	Alpha-MEM (Gibco)	5% Fetal Bovine Serum (Gibco)	1% ZellShield (Minerva Biolabs)	–
BMSC	Alpha-MEM (Gibco)	10% Fetal Bovine Serum Qualified (Biological Industries)	1% ZellShield (Minerva Biolabs)	–

as the arithmetic average (Ra) and root mean square (Rq) was calculated from 5 µm × 5 µm scanned area using Nanoscope software.

2.3. Materials sterilization for cell culture studies

For cell culture studies the materials were prepared in the form of thin films placed at the bottom of tissue culture plastic (TCP). The films were sterilized with 75% EtOH for 10 min and then rinsed with PBS (BioShop) to get rid of alcohol residues.

2.4. 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 $1 \times 10^4/cm^2$ 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.

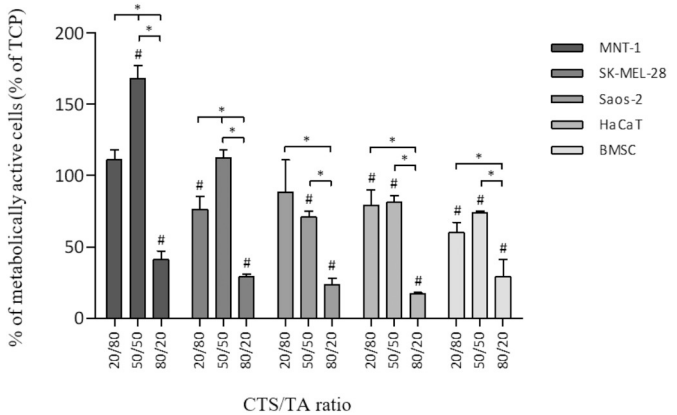

Fig. 1. Metabolic activity of different cell types cultured for 6 days on different material films. Results are displayed as mean +/− STD (i.e. % change of metabolically active cells on the material surface vs. TCP) for cell lines MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. CTS/TA films combined in ratios 20/80, 50/50 and 80/20 were examined. #statistically significant vs TCP, *statistically significant between different material surfaces within particular groups (Table 3).

2.5. Statistical analysis

All MTS tests were performed in triplicates, MTS absorbance values were averaged (mean value) and recalculated to a percentage change in the viability of cells on the given surface vs. cell viability on TCP (assumed as 100%). Results were statistically analyzed with one-way ANOVA and post-hoc Tukey with $p < .05$ considered significant.

3. Results and discussion

3.1. Roughness

The roughness parameter was calculated and showed as arithmetic (Ra) and root mean square (Rq). Ra is determined from deviations about the center line within the evaluation length. Rq is defined as arithmetic mean of the squares of a set of numbers. Both parameters define the surface roughness.

The surface roughness is an important parameter to consider the adhesion possibilities of materials for tissue engineering purposes where the rough surface is a requirement (Richards, 1996). It allows for the cell adhesion to the film surface and inhibits the biofilm formation due to the stiff bacteria cell wall. Films based on pure chitosan had the highest roughness parameters however, they cannot be tested for cell culture because they dissolved in the cell medium. Thereby, it is necessary to detect the chitosan mixtures with other polymers. In can be observed that tannic acid addition to the chitosan decreases the roughness parameters. The use of polymeric mixture to obtain thin films result in the complex formation and changes in the polymeric chains organization (Kaczmarek et al., 2018).

3.2. Metabolic activity of cells cultured on material films

As shown in Fig. 1, the viability of most cell types cultured on material films significantly decreased compared to cell viability on TCP. The highest viability was observed for MNT-1 cells cultured on 20/80 CTS/TA; MNT-1 viability on these films was significantly higher vs. MNT-1 cultured on standard TCP. Nevertheless, 20/80 CTS/TA films decreased the viability of SK-MEL-28, Saos-2, HaCaT and BMSC to the averaged 75% of control TCP (Fig. 1 and Table 2). A similar to MNT-1

Table 2
The arithmetic average (Ra) and root mean square (Rq) parameters.

Specimen	Ra [nm]	Rq [nm]
CTS	7.59	9.50
80/20 CTS/TA	6.55	8.18
50/50 CTS/TA	3.13	3.84
20/80 CTS/TA	1.96	2.57

Table 3
Differences in % change of metabolically active cells on CTS/TA surfaces of 20/80, 50/50 and 80/20 ratios.

	% Change of metabolically active cells vs. TCP	% Change of metabolically active cells vs. 20/80 CTS/TA	% Change of metabolically active cells vs. 50/50 CTS/TA
	20/80	50/50	80/20
MNT-1	111%	↑ 57%	↓ −127%
SK-MEL-28	76%	↑ 36%	↓ −84%
Saos-2	88%	↓ −17%	↓ −48%
HaCaT	79%	↑ 2%	↓ −64%
BMSC	60%	↑ 13%	↓ −44%

Numeric results for 20/80 CTS/TA surfaces show % change of metabolically active cells vs. TCP for different cell types; numeric results for 50/50 CTS/TA show % change of metabolically active cells vs. 20/80 CTS/TA whereas numeric results for 80/20 CTS/TA show % change of metabolically active cells vs. 50/50 CTS/TA. Green upwards arrow shows an increase (red downwards arrow shows a decrease) in % change of metabolically active cells on the selected surface in relation to the compared surface.

trend was observed for SK-MEL-28 viability on different material films (Fig. 1). Compared to respective TCP values, 50/50 CTS/TA films significantly decreased the viability of Saos-2, HaCaT and BMSC but increased viability of MNT-1 and SK-MEL-28 cells by 68% and 12%, respectively (Fig. 1). 80/20 CTS/TA films showed the highest inhibition of cell growth with the averaged 27.6% survival of cancer and healthy cells. Thus, the materials obtained with CTS and TA at 80/20 ratio may prove useful in anti-cancer therapies.

4. 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 be potentially further tested with expanded biological studies for the growth and different cell types, including cancer cells.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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