THE EFFECT OF THIN FILMS MADE OF CHITOSAN/COLLAGEN, POTASSIUM SILICATE AND TANNIC ACID ON VIABILITY OF CANCER AND HEALTHY CELLS

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Introduction

Composites consisting of a matrix and structural component are a promising and quickly-expanding branch of materials and tissue engineering. Current ecological issues call out for changes toward more natural lifestyle and this trend is also observed within the field of biocomposites – composite materials in which at least one component comes from the natural origin [1]. There is also substantial need of the medical market for innovative and natural biocomposites serving as drug and cell delivery vehicles and/or medical implants. This study was carried out with the composites prepared with the chitosan and collagen matrix supplemented with potassium silicate as an inorganic component. The latter was added to improve material properties. In addition, a small amount of tannic acid was added to stabilize polymers. The obtained composite materials were tested in human cancer cell lines: MNT-1 (highly pigmented melanoma), SK-MEL-28 (malignant melanoma) and Saos-2 (osteosarcoma) as well as in healthy cells: HaCaT (immortalized keratinocytes) and human bone marrow stromal cells (BMSC).

Materials and Methods

Collagen (COLL), chitosan (CTS) and tannic acid (TA) were dissolved separately in 0.1M acetic acid, first two at 1% concentration and the last one at 2%. COLL and CTS were mixed at 50/50 wt/wt% ratio. Then, 5 and 20 wt% of 2% TA was added followed by supplementation with 5 and 10 wt% of potassium silicate (PS). Polymer solution with inorganic additive was stirred to obtain a homogeneous mixture. Then it was placed in 24-well cell culture plates for 48h to form films after solvent evaporation. The films were sterilized for 10 minutes in 70% ethanol (water solution) and then rinsed with PBS. All types of cells used in this study were seeded directly on the material's surface in serum-containing media at a density of $1x10^4/cm^2$. Culture media were exchanged on day 2 culture. On day 6 culture, CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) was performed to assess cells viability, accordingly to the manufacturer's protocol. Values of MTS absorbance (λ = 492 nm) were averaged and recalculated to a percent change in the metabolic activity of cells on the surfaces consisting of chitosan, collagen and either 5% or 10% potassium silicate and tannic acid vs. cell viability on the materials without tannic acid (assumed as 100%). Results were statistically analyzed with one-way ANOVA and post-hoc Tukey; $p<0.05$ was considered significant.

Results and Discussion

TABLE 1. Cell metabolic activities (viabilities) are presented for MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. Asterisk (*) stands for statistically significant results vs. material composed of CTS/COLL 50/50 wt/wt% ratio with either 5 or 10% PS without TA (assumed as 100% viability).

	5% PS 5% TA	5% PS 20% TA	10% PS 5% TA	10% PS 20% TA
MNT-1	* 51% ± 5%	* 128% ± 17%	* 51% ± 7%	* 64% ± 4%
SK-MEL-28	105% ± 5%	* 157% ± 13%	107% ± 3%	* 145% ± 5%
Saos-2	104% ± 1%	* 231% ± 13%	86% ± 4%	* 190% ± 13%
HaCaT	82% ± 6%	137% ± 22%	81% ± 5%	135% ± 19%
BMSC	133% ± 18%	* 294% ± 18%	102% ± 16%	* 219% ± 24%

Significant decrease in viability (up to 50%), was observed for MNT-1 cells on materials containing 5 or 10% PS and 5% TA. The addition of 20% TA to 5% PS materials increased the viability of all cell types except HaCaT, in which no difference was observed. BMSC cells showed the highest, three-fold increase in the viability on materials containing 5% PS and 20% TA. 20% TA added to 10% PS materials caused an increase in the viability of all cell types except MNT-1, where this addition caused a decrease in viability.

Conclusions

The addition of inorganic component – potassium silicate (PS) and tannic acid (TA) to thin films composed of chitosan and collagen affects cell viability of MNT-1, SK-MEL-28, Saos-2, HaCaT and BMSC. The most significant changes in cell viability were observed for cells cultured on composites prepared with either 5 or 10% PS and 20% TA. Considering the cancer field, it is of interest that the materials tested in this study showed a significant decrease of MNT-1 (melanoma) viability. The apoptotic effect of tannic acid (TA) on estrogen receptor-positive cancer cells has been previously shown [3] and melanoma cells have been shown to express estrogen receptors [4]. This preliminary assessment of PS and TA supplemented chitosan/collagen-based materials suggests they may display anti-cancer properties but further research is required to verify this thesis.

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