Research Article

The Influence of Fucoxanthin Immobilized on Porous Aluminum-Silicon Carrier Surface on the Functional Activities of Immunocytes in Mice

Alexander Lykov[1], Lubov Rachkovskaya[1], Ruslan Gevorgiz[2], Svetlana Zheleznova[2], and Olga Poveshchenko[1]

[1]Research Institute of Clinical and Experimental Lymphology-Branch of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation
[2]A.O. Kovalevsky Institute of Biology of the Southern Seas RAS, Sevastopol, Russian Federation
ORCID
Alexander Lykov; 0000-0003-4897-8676

Abstract. Fucoxanthin is a natural carotenoid obtained from seaweed which exhibits antioxidant properties. This research aimed to assess whether fucoxanthin, immobilized on aluminum-silicon carrier particles, has a toxic effect on immune cells. The viability, proliferation, nitric oxide production and myeloperoxidase activity of thymocytes and splenocytes of mice *in vitro* were studied. It was shown that fucoxanthin, immobilized on aluminum-silicon carrier particles, increased the survival rate and proliferation of mature immunocytes (splenocytes) after 24 hours exposure and increased the survival rate of naïve immunocytes (thymocytes) when exposed for 120 hours. In terms of myeloperoxidase, the activity of the immune cells was not affected by fucoxanthin immobilized on the carrier particles. The obtained results indicated that fucoxanthin, immobilized on particles of an aluminum-silicon carrier, did not have a toxic effect on mouse immunocytes.

Keywords: *Cylindrotheca closterium*, fucoxanthin, γ-aluminum oxide, polydimethylsiloxane, thymocytes, splenocytes, viability, proliferation, nitric oxide, myeloperoxidase activity

Corresponding Author:
Alexander Lykov; email:
aplykov2@mail.ru

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1. Introduction

The search for new therapeutic agents capable of displaying antioxidant properties is increasingly attracting the attention of pharmacologists to the use of plant raw materials, including algae, as an object of research. So, natural carotenoid from brown seaweeds and diatoms fucoxanthin possess antioxidant, anticancer, anti-obesity, and anti-diabetic activities [1, 2]. Fucoxanthin (Fx) administration in animal model of lung cancer decreased tumor growth and increased apoptosis of cancer cells [3]. In dosage 2 micromoleFx have not altered lymphocytes, while in lower dosage possess pro-oxidant properties [4].

The aim of the research was to investigate the influence of the fucoxanthin, immobilized on porous aluminum-silicon carrier surface, on mice immunocytes functional properties.

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2. Materials and Methods

All experiments wereperformed with the approval of the Ethics Research was performed with the prior approval of the Ethics Committee of Research Institute of Clinical and Experimental Lymphology-Branch of the Institute of Cytology and Genetics RAS, and were conducted in accordance with the principles and guidelines of the Declaration of Helsinki.

Male BALB/c mice weighing 20-25 g were obtained from Institute of Cytology and Genetics (Novosibirsk, Russia). The animals were sacrificed by cervical dislocation, thymus and spleen were remote, a single cell suspension was made using homogenization in 5 mL of phosphate-buffer saline.

Fucoxanthin was extracted from *Cylindrotheca closterium* at 30 ^{0}C for 2 hours with ethanol, then concentrated at 25 ^{0}C, purified, and stored at 4 ^{0}C.

To prepare a carrier, porous gamma-aluminum oxide (γ-Al_2O_3) with particle sizes of 0.1 mm (Catalyst, Russia), and predominant pore size of 10-100 nm, and high mechanical strength was used, because losses at attrition made 0.1-0.3%/minute and crushing strength of granules with a large size up to 70 kg. The silica containing polymer polydimethylsiloxane (PDMS; $(CH_3)_3$-Si-O-(Si$(CH_3)_2$-O)$_n$-Si$(CH_3)_2$-O-Si$(CH_3)_3$, Penta, Russia)with a molecular weight of 18000-19000and particle diameter 60 μm was used in the form of an aqueous emulsion (silica content 25-28%). To create a hydrophilic-hydrophobic chemical nature of the carrier surface on alumina, a PDMS emulsion was immobilized at room temperature. The obtained carrier (Al/PDMS) was dried and subjected to short-term heat treatment up to 200 ˚C. Fx was immobilized on γ-Al_2O_3/PDMS in the aqueous phase by physical adsorption, then the obtained matrices was subjected to drying and short-term low-temperature heat treatment up to 120 ^{0}C, the obtained matrices were loose powder materials (Fx@Al/PDMS).

Splenocyte (Sp) and thymocytes (Th) were transplanted in RPMI-1640 (Biolot, Russia) medium with the addition of 80 μg/mL of gentamicin, 2 mmol L-glutamine, 5 mmol HEPES-buffer and 10% FCS at a concentration 10^6 cells/mL. Splenocytes and thymocytes viability and proliferation activity in presence of Fx@Al/PDMS in dosage 0 μg and 0.057 μg was detected by 3-(4,5-dimethylyhiazol-2-yl)-2,5-diphenyltetrazolium bromide

(Merck, USA) assay after 24 h (short-term exposure) and 120 h (long-term exposure) [5]. Nitric oxide (NO) production by splenocytes or thymocytes was measured in supernatants using Griess reagent for nitrite determination (Merck, USA) [5]. Myeloperoxidase activity was detected by mixture of 1 part of 0.6% solution of benzidine (Merck, USA) based on a 96% solution of C_2H_5OH, and 2.5 parts of a mixture of 0.25 ml of 33.4% H_2O_2 (Merck, USA) of 2.25 ml of 70% solution of C_2H_5OH in splenocytes or thymocytes [5].

All measurements were performed in triplicates. Data were analyzed by the Statistica 10.0 for Windows. In this study, the normality of the distribution was determined by the w-Shapiro-Wilkes criterion, in table the obtained data were presented as mean ± standard deviation (SD), the data were analyzed ANOVA with a Bonferroni correction (Bonferroni post hoc test) to analyze differences between groups. If p-values were less than 0.05, it was considered statistically significant.

3. Results

We prepared the composition of fucoxanthin and γ-aluminum oxide with PDMS based on the initial particle sizes of Al/PDMS (0.1 mm), sample were bulk powder materials of yellow color with a uniform distribution of Fx on particles surface (0.057 mg of Fx per 1 g of Al/PDMS).

Unfortunately, only splenocytes viability and proliferative activity was increased by short-term exposure with Fx@Al/PDMS (Figure 1 and Figure 2). While we observed increased survival rate of the thymocytes after long-term exposure with Fx@Al/PDMS and have not any effect on proliferation. Other controls (ethanol, extract of the Fx, and Al/PDMS composition) has a different effect on cell viability and proliferation on 24 hours or 120 hours exposure.

We have estimated significantly changes in NO production by splenocytes on 24 hours exposure with extract of the Fx and Al/PDMS by cells, especially by splenocytes (Figure 3, $p<0.05$). While myeloperoxidase activity of immunocytes was stable to influence of the tested samples (Figure 4).

4. Discussion

PDMS used many fields including medicine, cosmetology [6]. PDMS, in general, inert, non-toxic materials. Fx obtained from *Undaria pinnatifids, Sargassum fulvellum, Hijikia*

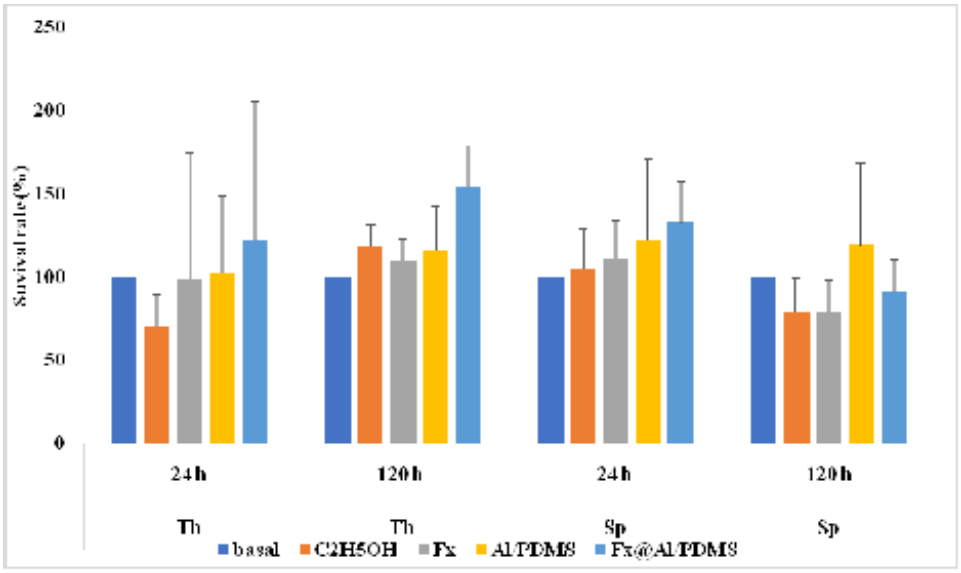

Figure 1: Effect of Fx@Al/PDMS on thymocytes (Th) and splenocytes (Sp) viability.

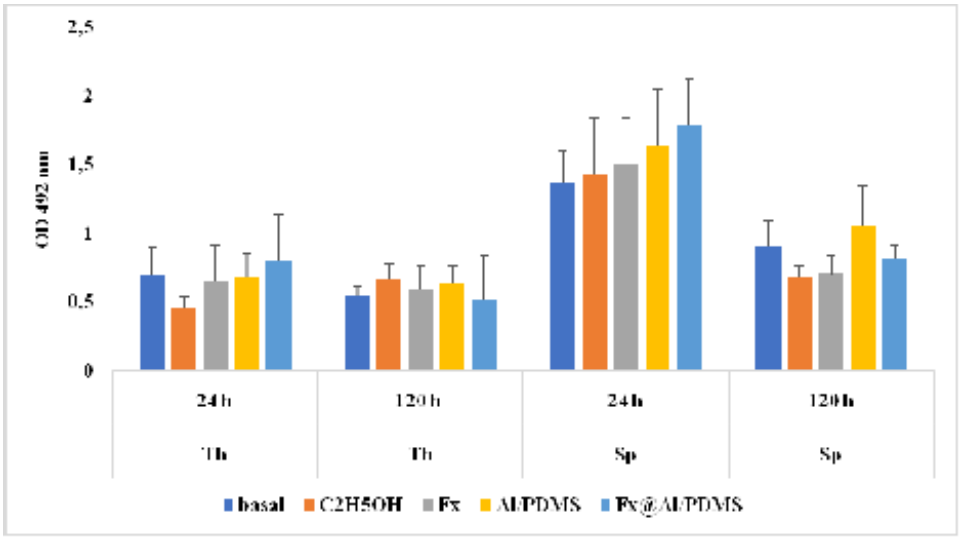

Figure 2: Effect of Fx@Al/PDMS on thymocytes (Th) and splenocytes (Sp) proliferative potential *in vitro*.

fusiformis, etc. [7]. In rat administration of fucoxanthin oil extract from *Chaetoseros sp.* no caused mortality and no has abnormalities [8].

There we present the results of the study of short-term and long-term exposure of immobilized fucoxanthin on porous material based on γ-AL_2O_3 and polydimethylsiloxane particles on mice naïve (thymocytes) and mature (splenocytes) immune cells. We obtained increased survival rate and proliferation by mature immunocytes after a short-term exposure with Fx@Al/PDMS, whereas viability naïve immunocytes increased after long-term exposure with Fx@Al/PDMS.

It is shown that fucoxanthin inhibited T lymphocytes differentiation into Th17 [9], and inhibited NO and reactive oxygen species production by activated macrophages [10].

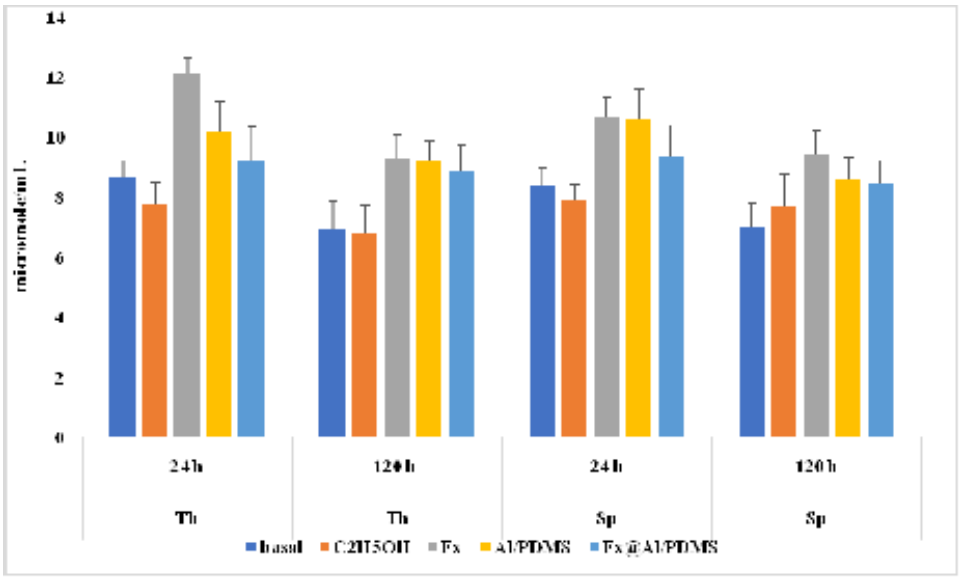

Figure 3: NO production by thymocytes (Th) and splenocytes (Sp) under Fx@Al/PDMS condition.

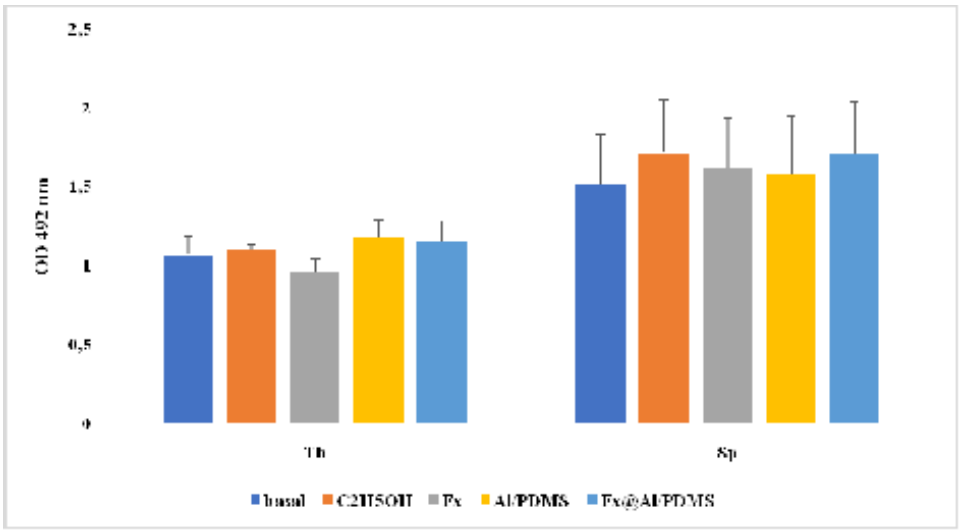

Figure 4: Influence of Fx@Al/PDMS on thymocytes (Th) and splenocytes (Sp) myeloperoxidase activity.

5. Conclusion

This study demonstrated that prepared composition of fucoxanthin immobilization on surface of particle base on gamma-aluminum and polydimethylsiloxane showed not a significantly alteration functional properties of splenocytes and thymocytes of BALB/c female mice.

6. Conflict of Interest

The authors have no conflict of interest to declare.

Abbreviations: Fx-fucoxanthin, Sp-splenocytes, Th-thymocytes, γ-Al_2O_3-gamma- aluminum oxide, PDMS-polydimethylsiloxane, Fx@Al/PDMS-immobilized FX on Al/PDMS surface, NO-nitric oxide

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