In vivo monitoring of degradation of ICG-labeled polymer implants using optical clearing

Apukhtina U.A. [1], Astemir R. Likhov [1], Victoria V. Zherdeva [1]*

[1] Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation; vjerdeva@inbi.ras.ru; Correspondence: vjerdeva@inbi.ras.ru (V.Z.)

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Introduction

The problem of low signal level and poor contrast while obtaining epifluorescence images of subcutaneous fluorescent objects in animals (tumors, composite materials) arises often. The use of optical clearing agents (OCA) helps to improve both the signal output and equalize the refractive indices, thereby increasing the fluorescence intensity (FI) output and the contrast of objects.

The goal of our work was to select conditions for OC during imaging of ICG-labeled polyester copolymer materials implanted in Balb/c mice.

Materials and Methods

Polyester copolymer disks of 5 mm size were implanted subcutaneously subdorsally in mice. Measurements were performed for 50 days after implantation. A 70% aqueous solution of glycerol with 5% DMSO was used as OCA, application time 10 min. Imaging was performed before OCA application, immediately after removal of the application, at 15 min and at 30 min after removal of the application.

The images obtained were analyzed using ImageJ software (Fiji). ROIs were selected and fluorescence profiles were analyzed before and after using different exposure times (30-120s). To take into account the background signal nearby skin areas. FI profiles were built to analyze different exposure times of the brightening agent and the time elapsed from the day of implantation.

Results

We took the data from 12 mice and averaged them to get one 'average' mouse. All data points that matched in date, exposure time, and clearing agent duration were combined and averaged.

It was shown that in general starting form the day of surgery, the signal dropped, sometimes giving an increase on day 10-14. In most cases, OC demonstrated the maximum at time 0 minutes and 15 minutes after removal of the applanation.
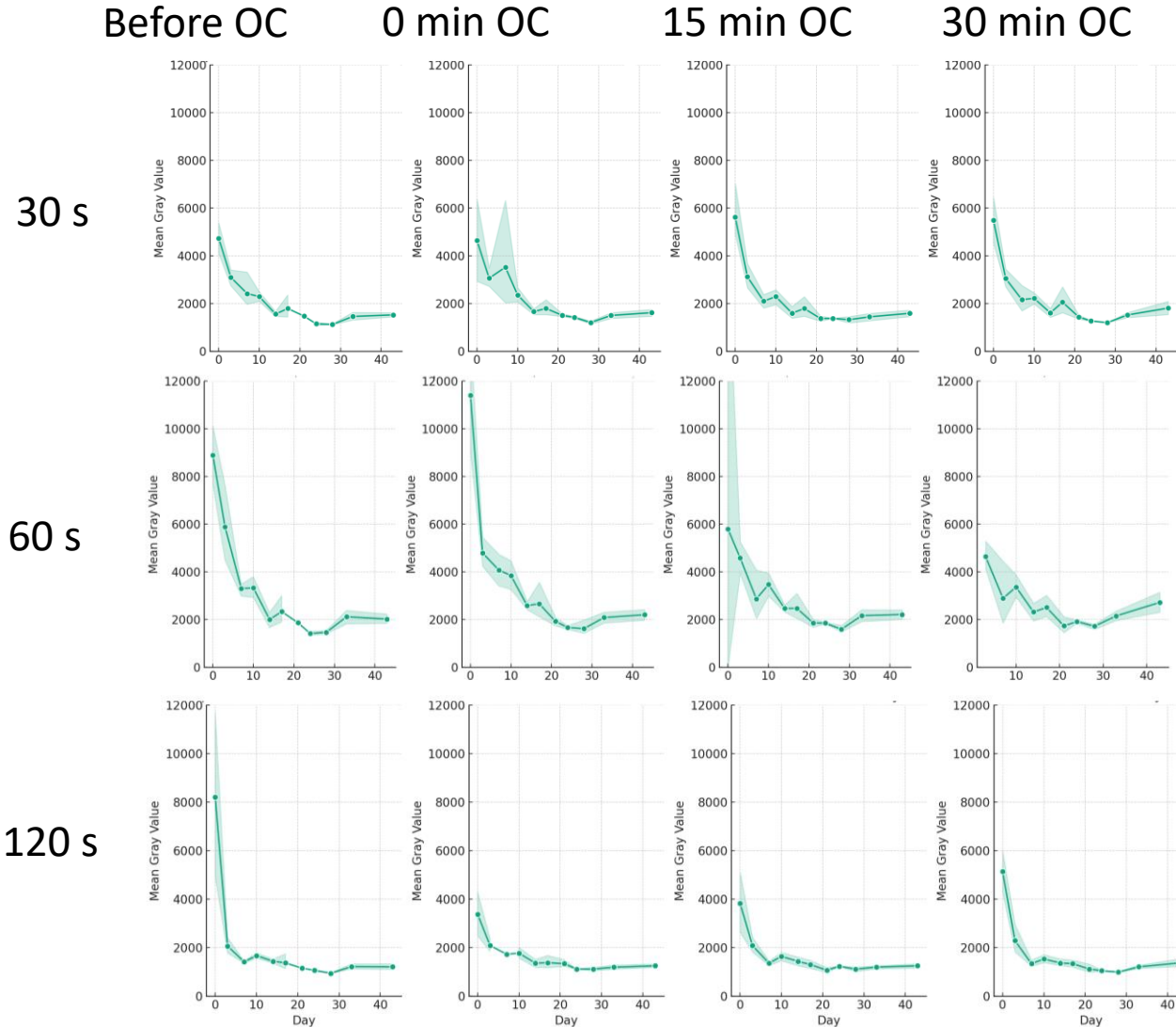
The highest OC level was achieved with an initially low fluorescence signal (about 2000a.u. gray value) and the OC reached 110-115% based on the maximum signal value. In the case of higher levels of FI values, the changes were not pronounced.
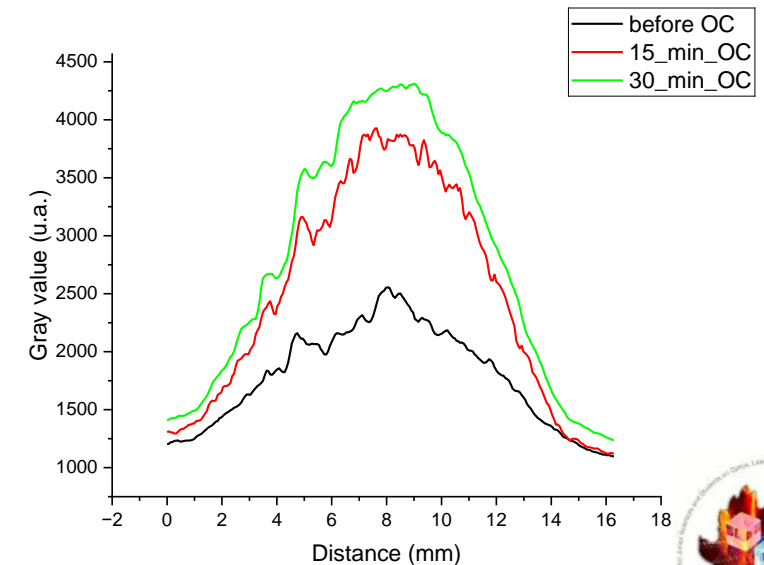
Conclusions

Thus, the glycerol-based OC for ICG fluorescence demonstrated the same time intervals of optical clearing as in the fluorescence analysis of weakly fluorescent tumors obtained earlier, which indicated the redistribution of interstitial fluid in the skin layers under the influence of high-osmolar glycerol solutions.