MOBILITY OF TYPE II COLLAGEN RECEPTOR ON MSC CELLS STIMULATED OR NOT WITH TGF-β BY FLUORESCENCE CORRELATION SPECTROSCOPY

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Fluorescence Correlation Spectroscopy (FCS) is mainly used for biological applications as a sensitive technique for measuring dynamic processes (number density, interaction fractions and molecular dynamics) in a fluorescent signal on the nanosecond to second time scales. Several works have recently shown that Mesenchymal Stem Cells (MSC) collected from bone marrow can differentiate in vitro into cartilage cells under the effects of transforming growth factor-β (TGF-β), others growth factors or critical transcription factors. The effect of TGF-β, a multifunctional cytokine, has been described as a potent stimulant of the synthesis of the matrix molecules. In this work, the mobility of type II TGF-β receptor in the MSC membranes has been compared for MSC TGF-β stimulated or unstimulated.

The autocorrelation functions G(t) from the fluorescence intensity fluctuations of diluted suspensions of Fab2 fragments conjugated with Alexa Fluor 488) were determined to give the average diffusion time taken by the molecule to cross the observation volume, along with the average number of molecules present. For calibration on TCS SP2 AOBS- FCS2 (Leica Microsystems, 63x/NA water immersion), a diffusion coefficient for Fab2 fragments of \( D = (164 \pm 6) \mu m^2/s \) were obtained with an effective volume of 0.85 ± 0.03 fl (\( w_r = 0.48 \mu m; \ w_z = 0.5 \mu m \)), in agreement with others data obtained with the traditional methods of calibration. There is an increase in the decay time of the FCS curves from before stimulation indicating that receptor diffuse more quickly through the focal volume and are thus shown to have higher mobility after stimulation (Figure 1).

Figure 1. Fluorescence autocorrelation curves on a single Mesenchymal Cells before and after stimulation with TGF-β1 (10 ng/ml TGF-β for 12h) and indirect immuno-labelling of RII collagen receptor (A11017 Invitrogen, Alexa488™). Each curve is the average of five measurements on a single focal spot (target laser) at 488 nm.