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Effects of Ca++ and low energy laser on fibroblastic permanent cell lines

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3T3 cells are fibroblastic murine permanent cell lines that grow in a monolayer adherent to the substrate. They constitute a good experimental model for *in vitro* studies, and were used in our work for the study of the effects of chemical compounds and low energy laser upon cellular morphologic characteristics. Low energy laser affects the cicatrization process of connective tissue and is widely used in physiotherapy. The celular mechanisms underlying this activity are however unknown [1,2].

Cell cultures NIH/3T3 (ATCC CRL-161658TM) were used. Semi-confluent cultures were exposed to the concentrations of 3.3 and 40 mM of CaCl2, supplemented with vitamin D at the concentration of 10^{-8}M. The cultures were irradiated with a 5 mW laser operated at 650 nm wavelength for 1 minute at a distance of 100 nm from the liquid surface. Controls were processed without irradiation and/or without supplements. Samples for transmission electron microscopy were collected 8 days after treatment. Samples were fixed in glutaraldehyde 3% in sodium cacodylate buffer 0.1M pH 7.3 and post-fixed in 1% osmium tetroxide in the same buffer and uranyl acetate 0.5% in acetate-acetic acid buffer 0.1M pH 5.0. The dehydration was made in ethanol and followed by passage in propylene oxide. The embedding was made in a mixture of Epon and Araldite polymerized at 70ºC for 48h. Ultrathin sections, made with glass knives and stained with uranyl acetate and lead citrate, were observed and photographed in a JEOL 1200EX electron microscope.

Untreated cells show a round morphology and do not form cellular aggregates. Cells form small aggregates at 3.3 mM and epithelioid cell masses at 40 mM Ca^{++} concentrations (Figs. 1. 1-3). In the epithelioid cell masses the adjacent cell membranes are separated by a regular space (Fig. 1. 3) and focal adhesion points can be seen between more separated cells (Fig. 1. 3 – inset). After irradiation by the laser the epithelioid aggregates dissociate and the cells resume their spherical shape. A fibrillar extracellular matrix is visible between the separated cells (Fig. 1. 1). This matrix disappears between the cells of the epithelioid aggregates but forms again after laser irradiation (Fig. 1. 4). Cellular alterations of mitochondria and Golgi apparatuses were observed together with the modifications of cell adhesion and matrix formation. Our results show that Ca++ concentration modifies cellular adhesion and matrix formation, and is sensitive to laser irradiation, confirming results obtained in other models [3,4,5].

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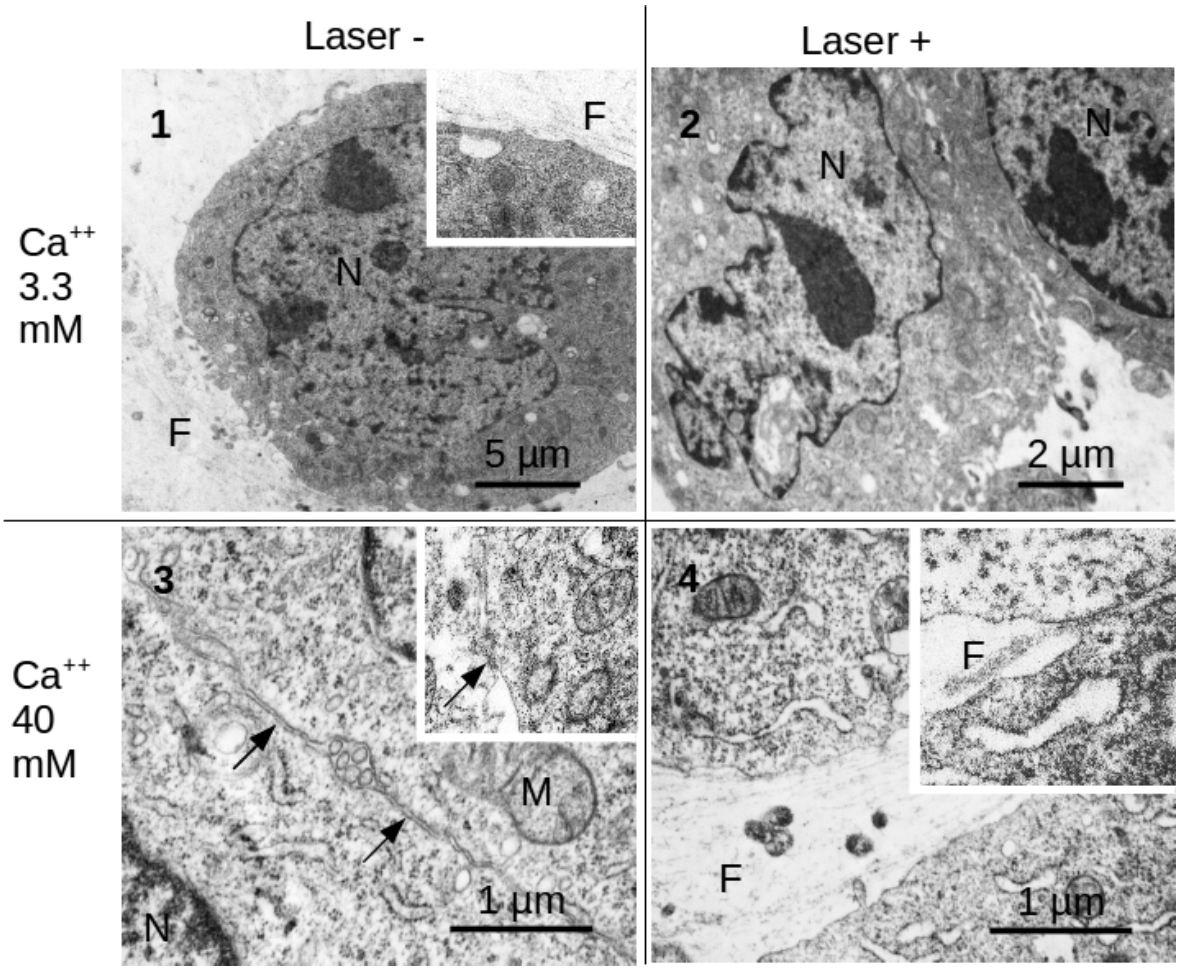

Figure 1. 1-4 3T3 cells treated with Ca^{++} (3.3 and 40 mM) and 5 mW laser operated at 650 nm. F – Fibrillhar extracellular matrix; N – Nucleus; M – Mitochondria; arrows – cell membranes.

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