Supplementary materilas

Chitosan cryogels cross-linked with 1,1,3-triglycidyloxypropane: mechanical properties and cytotoxicity for cancer cell 3D cultures

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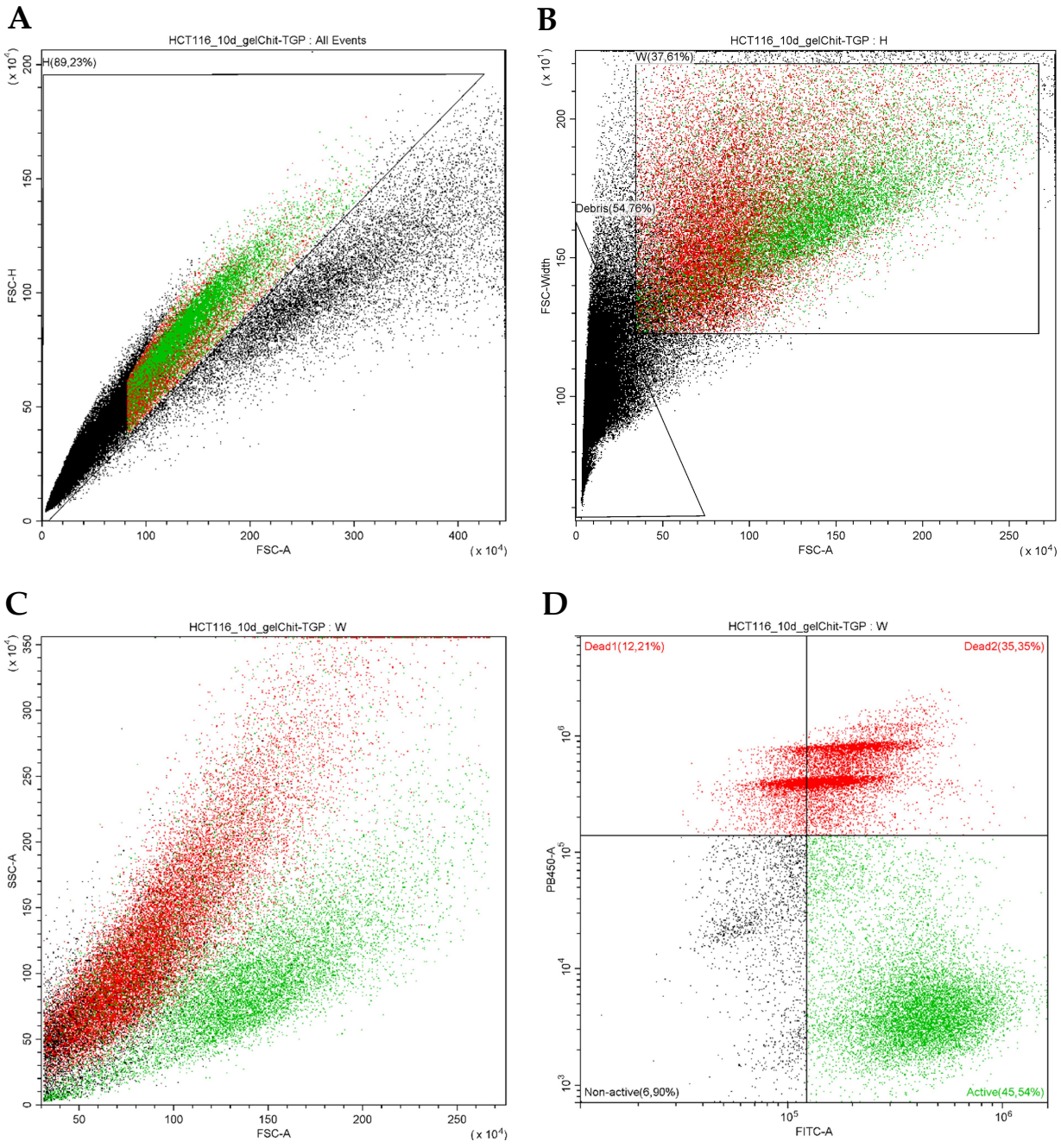

Figure S1. The algorithm of analysis of flow cytometrical data of stained cells. The presented example are cells from spheroids cultivated in cryogel disk of chitosan-TGP (1:4) for 10 days, disaggregated and detached from the disk, and stained for 10 min at room temperature in the dark with 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (excitation in FITC fluorescent channel) to assess the mitochondrial activity, and 1 μg/mL 4′,6′-diamidino-2-phenylindole (PB450 fluorescent channel) to stain dead cells.

Single events (about 90% of total events in each sample) were determined by triangle gating on FSC-A against FSC-H plot in order to exclude cell aggregates from the following analysis (A). The gate of analyzed cells ("W") was separated from debris by gating on FSC-A against FSC-W (B). FSC-A against SSC-A plot represents the relative size and granularity of events, respectively (C). The proportion of dead cells (4′,6′-diamidino-2-phenylindole-positive events) is determined as a sum of "Dead1" and "Dead2" gates from the chosen at the previous step gate "W" (D). The proportion of active cells includes all 2′,7′-dichlorodihydrofluorescein diacetate-positive events from the chosen at the previous step gate "W" (D).