Influence of Growth Characteristics of Induced Pluripotent Stem Cells on Their Uptake Efficiency for Layer-by-Layer Microcarriers

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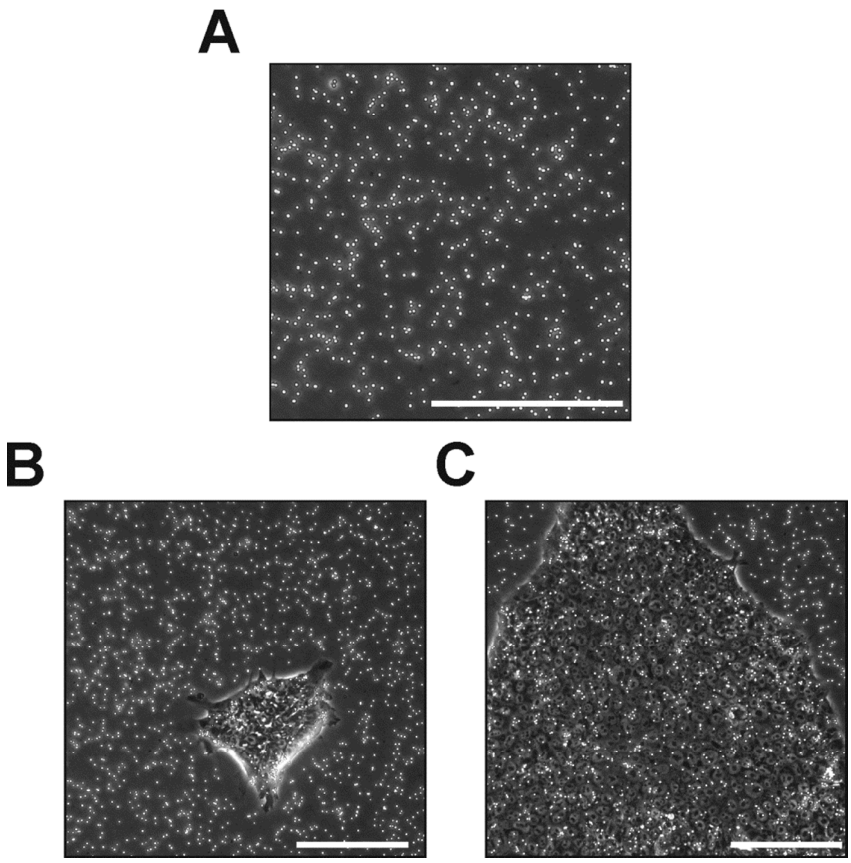
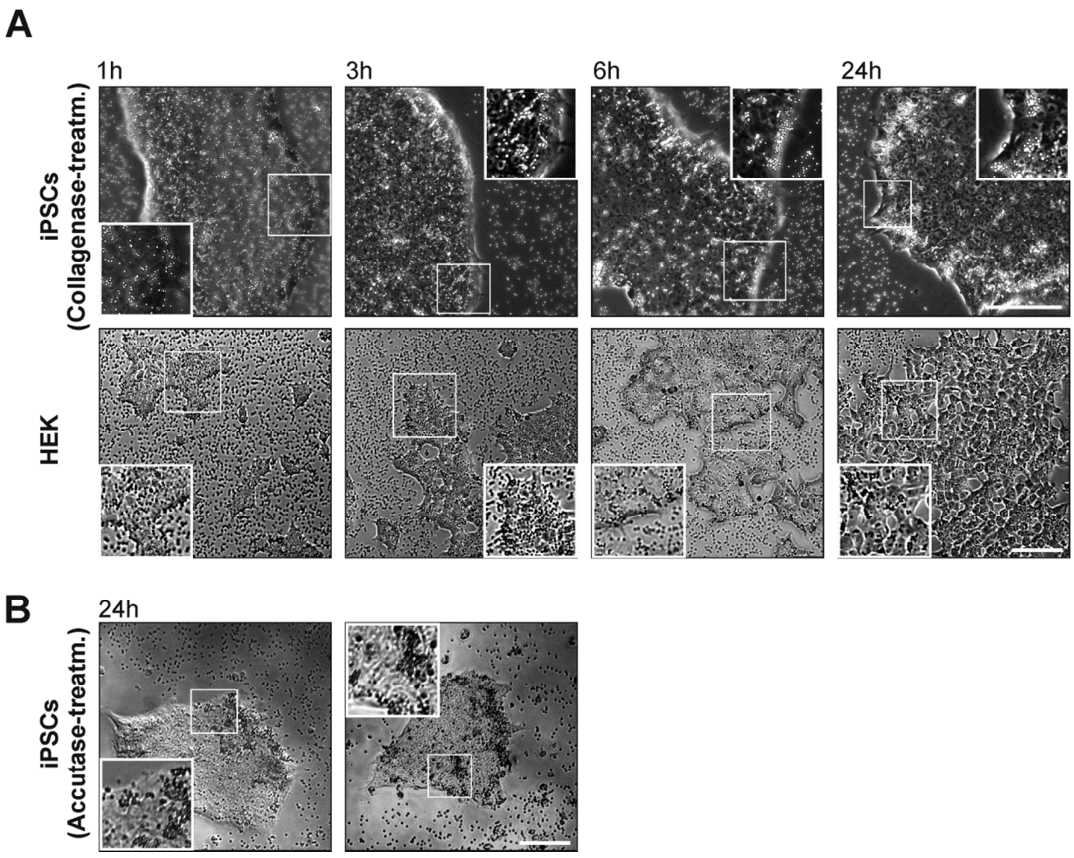
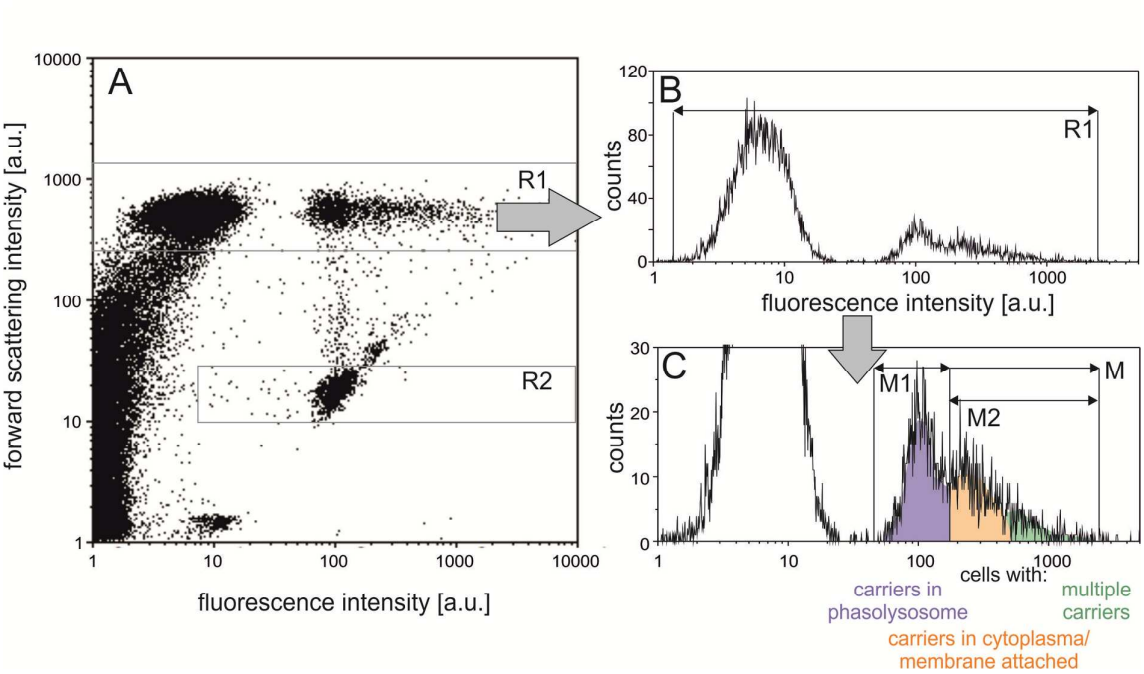


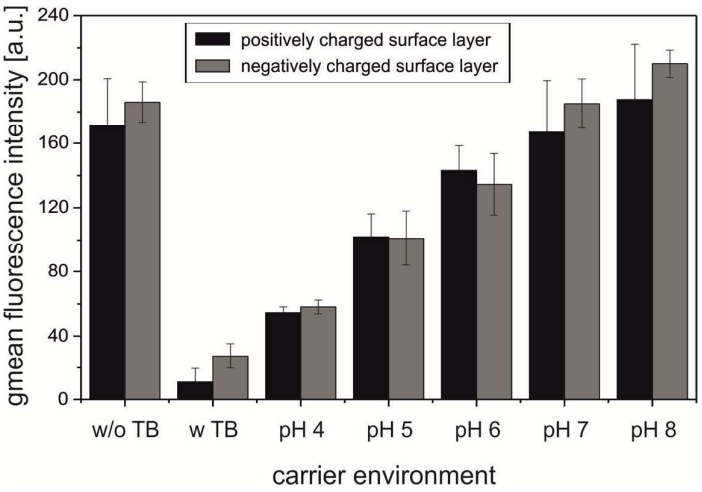
Supplement Figure 1. Figure caption. Initial homogeneous lateral distribution of Lbl carrier on matrigel-coated cell culture vessels, as demonstrated by a very high carrier concentration (2×10^7 negatively charged carriers per well of a six well plate). **(A)** Reverse approach, before cell seeding, (**B**) Direct approach$_{24h}$ after 1 h of co-incubation and **(C)** Direct approach$_{72h}$ after 1 h of co-incubation. Scale bars, 100 µm.



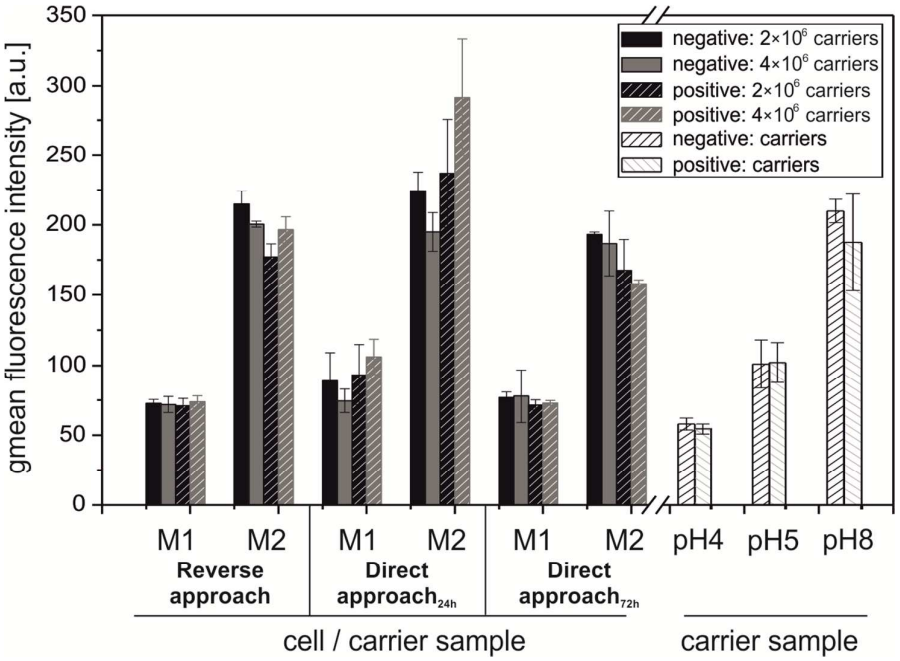
Supplement Figure 2. Figure caption. Microscopic analysis of the distribution of LbL carriers on pluripotent and terminally differentiated cell lines over time of incubation. (A) LbL carriers were applied through the direct approach to collagenase-dissociated iPSCs and HEK293T/17 (human embryonic kidney cell line) as terminally differentiated cell line after being harvested by trypsinization. 24 h after plating 4×10^6 positively charged carriers were applied. Images were taken at the indicated time points. HEK293T/17 were cultivated in Dulbecco´s modified Eagle´s medium (DMEM) with high glucose under supplementation with 10% FCS (fetal calf serum) under standard cell culture conditions. (B) 4×10^6 positively charged carriers were added 24 h after plating of iPSCs. In this case, cells were not collagenase-passaged but separated into single cells through treatment with Accutase (Sigma Aldrich). Images were taken after a further incubation period of 24 h. Insets highlight the marked area for clear visualization of particle distribution. In contrast to collagenase-passaged iPSCs, carriers are more randomly distributed over the colony. Thus, the mode of iPSCs passaging appears to influence their lateral accessibility to LbL carriers. Scale bars, 100 µm



Supplement figure 3. Figure caption. Flow cytometry dot plot of a cell / carrier interaction experiment. In (A), region R1 shows cells either without (low auto-fluorescence intensity, high forward scattering intensity) or with carrier interaction (high fluorescence, high forward scattering intensity). In comparison, carriers in supernatant are labeled as R2 (high fluorescence, low forward scattering intensity). For data interpretation, histogram presentation of R1 was used (B), and sub-region of cell / carrier interaction (M) was further sub-divided into M1 and M2 according to the pH od the cell compartments (C). Compared to carrier investigations of figure S1, M1 represents cells with carriers in phagolysosomes, and M2 contains cells with carriers attached to the outer membrane or located in cytoplasm. Number of cells in M1 and M2 related to the overall cell number in R1 was described as cell / carrier ratio, and geometric mean value (gmean) of M1 and M2 intensity is related to the carrier location within cell.



Supplement figure 4. Figure caption. Flow Cytometry fluorescence intensity investigations of LbL-carriers depending on carrier's surface charge, pH of the surrounding medium (CIP buffer) and trypan blue (TB). Fluorescein (as in FITC labeled PAH) has two properties: (a) Fluorescein intensity is pH dependent and increases with increasing pH value. Thus, carriers in different cellular compartments can be vizualized, e.g. phagolysosomes, pH 4-5, and cytoplasm, pH 7-8. (b) Intensity can be quenched (e.g, by trypan blue (TB)). Thus, internalized carriers by cells can be separated from external, membrane attached carriers, since TB cannot penetrate intact cell membranes. Here, location of carriers at same pH (7-8, carriers attached or in cytoplasm) can be distinguished further. The graph shows the strong influence of both parameters on fluorescence intensity, information was used in cell experiments.



Supplement Figure 5. Figure caption. Geometric mean (gmean) values of carrier / cell interaction regions M1 and M2 after application of positively and negatively charged carriers in different concentrations (filled bars, filled/striped bars). In comparison, carriers exposed to environments with different pH value (CIP buffer) illustrate the pH-dependent fluorescence intensity (empty/striped bars). This allows a classification of different cells compartments containing carriers.