Electronic Supporting Information

Fabrication of 3D Structured Human Cells Networks Using Capillary Cell Suspensions from Aqueous Two-Phase Systems

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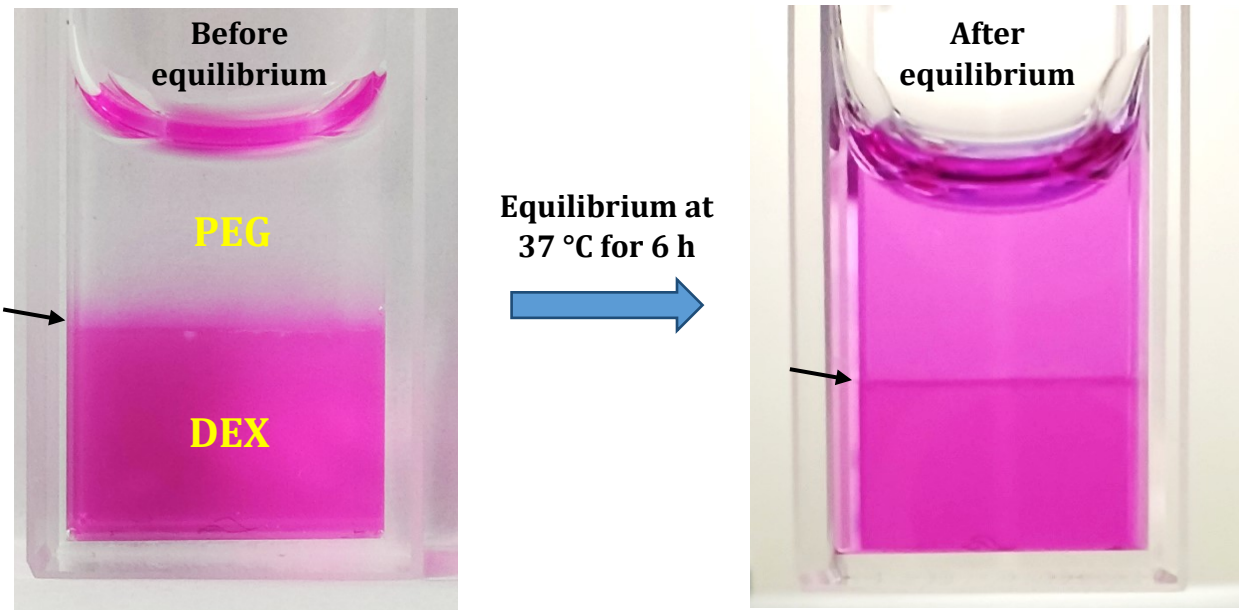


Figure S1. Digital images of equilibrium state between equal volumes of 10 wt.% DEX in DMEM medium and PEG aqueous solution after 6 h. The DEX-PEG interface (black arrows) is clearly seen before and after equilibrium.

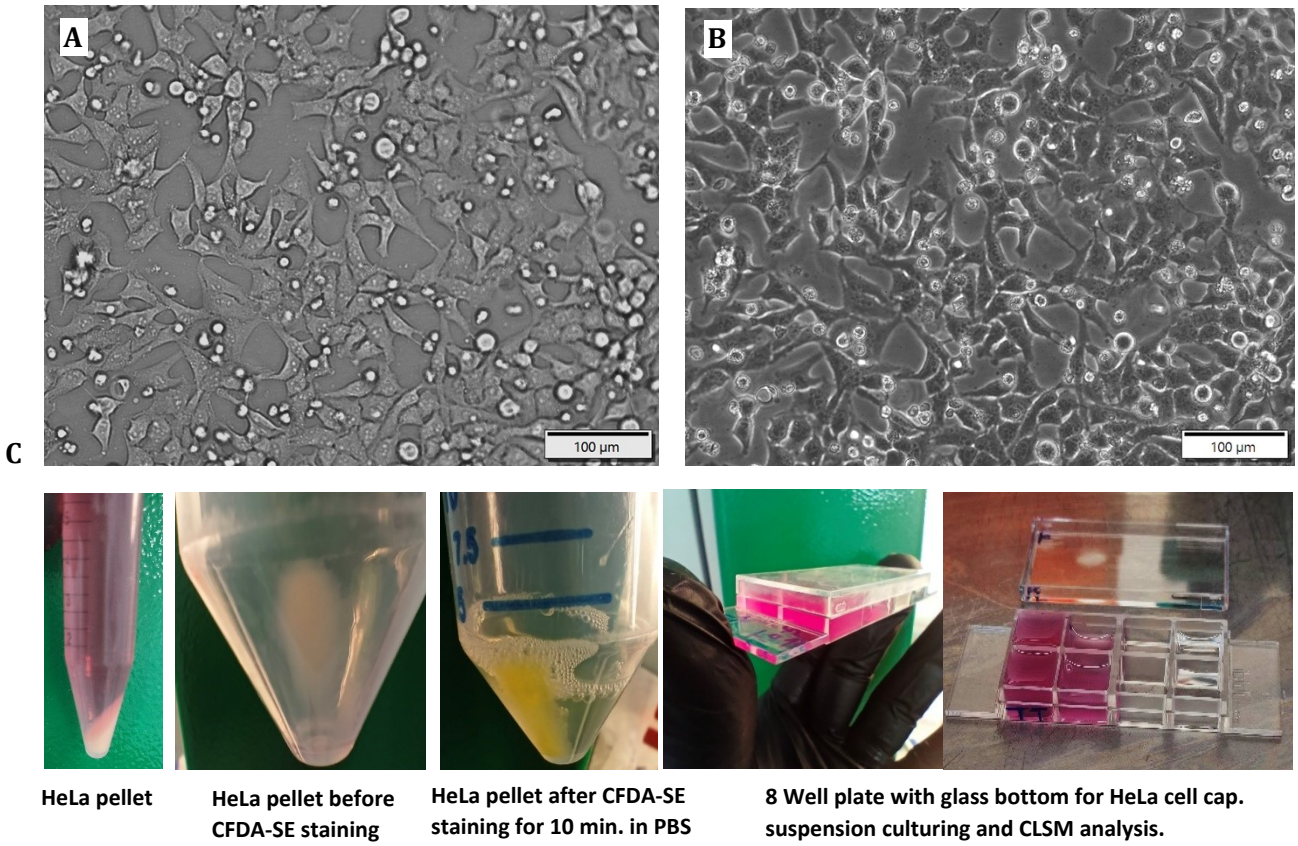


Figure S2. (A) Optical and (B) phase contrast microscopic images of 2D cultures HeLa cells after 80% confluency. (C) Digital images of the process for collecting and preparing HeLa cells suspension and the 8 well plate with glass bottom used for 3D culture of capillary structured cell suspension.

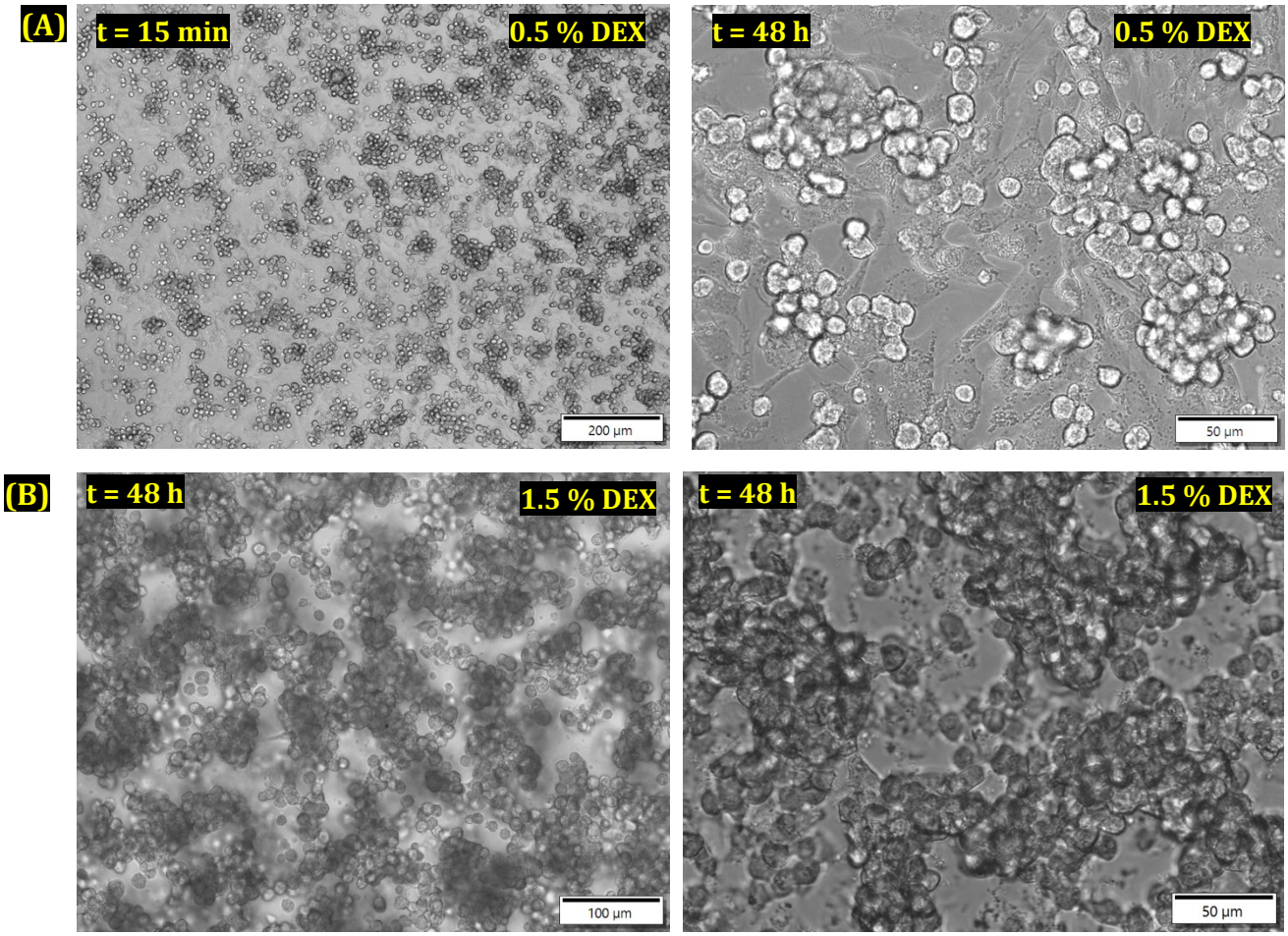


Figure S3. Optical microscopic images of microstructures formed by the addition of (A) 0.5 vol.% DEX to 10 wt.% HeLa cell suspensions in PEG at 15 mins and 48h incubation. (B) Addition of 1.5 vol.% DEX to 10 wt.% HeLa cell suspensions in PEG at 48h using different magnifications.

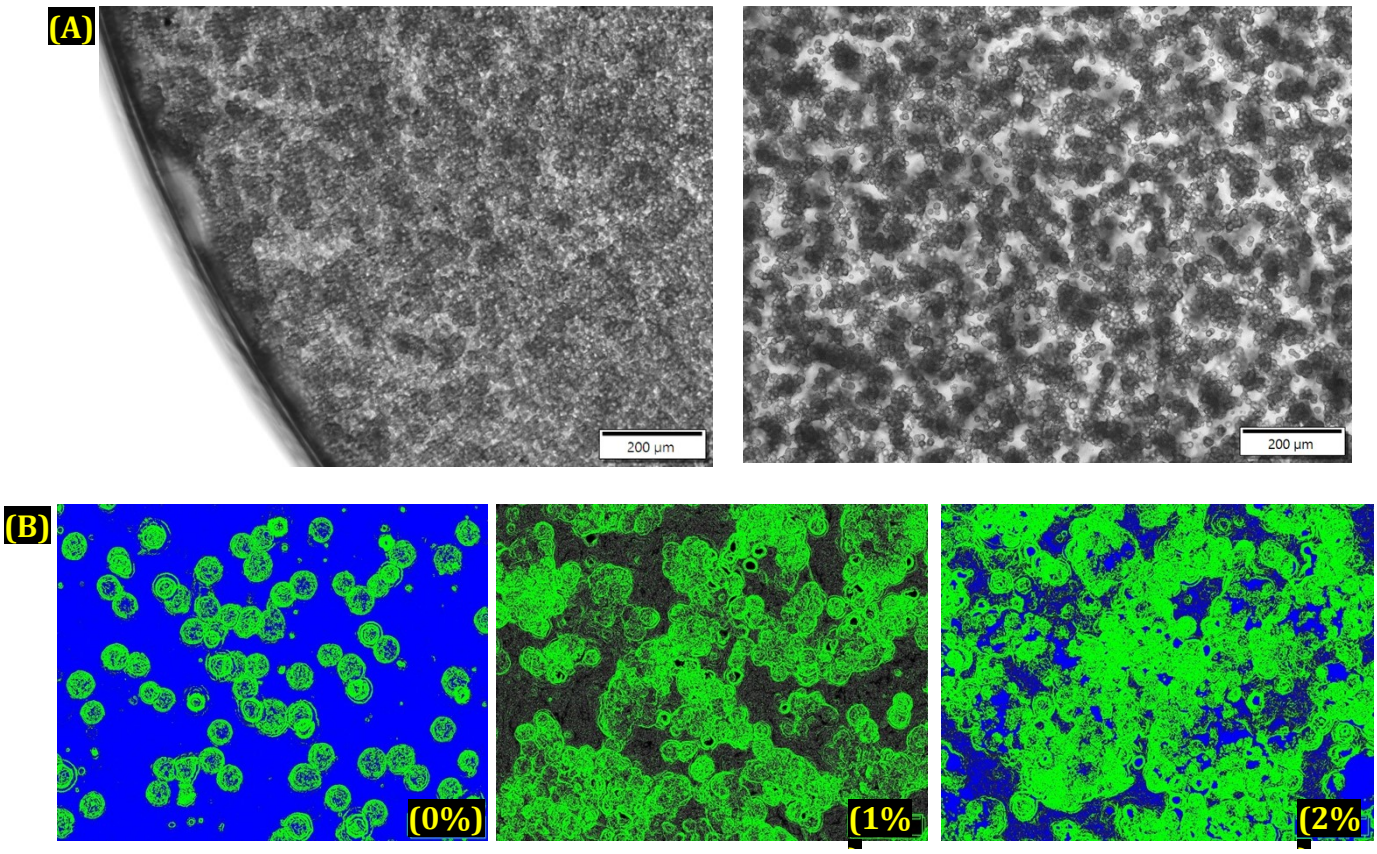


Figure S4. Additional brightfield microscopy images of (A) 2 vol.% DEX after 48h showing the density of the formed 3D clusters of HeLa cells in capillary structured suspension at different magnifications. (B) Processed images using ImageJ tools for analysis of area of clusters formed at different vol.% of DEX second phase, showing the increased density of the clusters at 2 vol.% DEX.

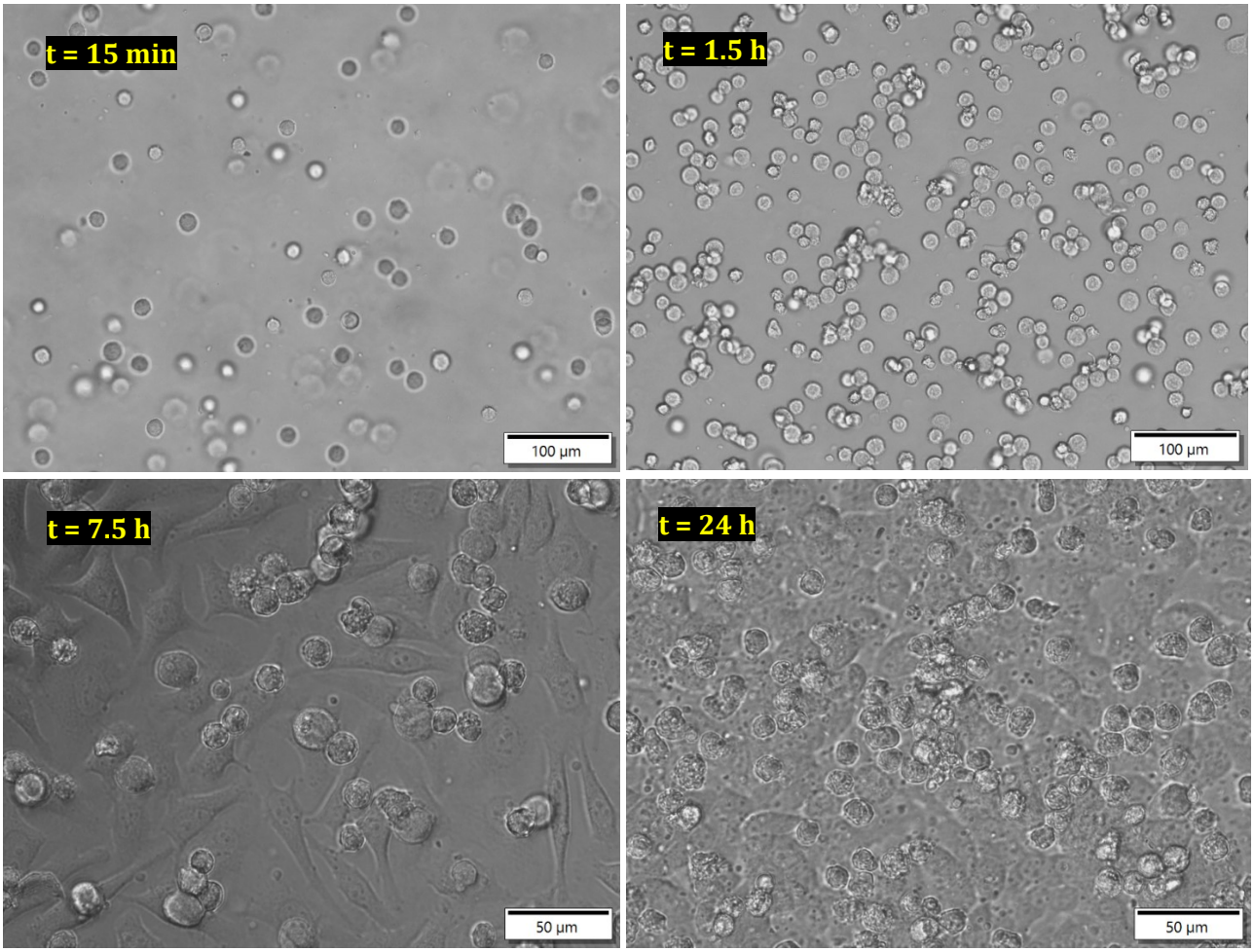


Figure S5. 3D structuring in 4 wt% HeLa suspension in PEG with 2 vol.% DEX after various incubation times (shown).

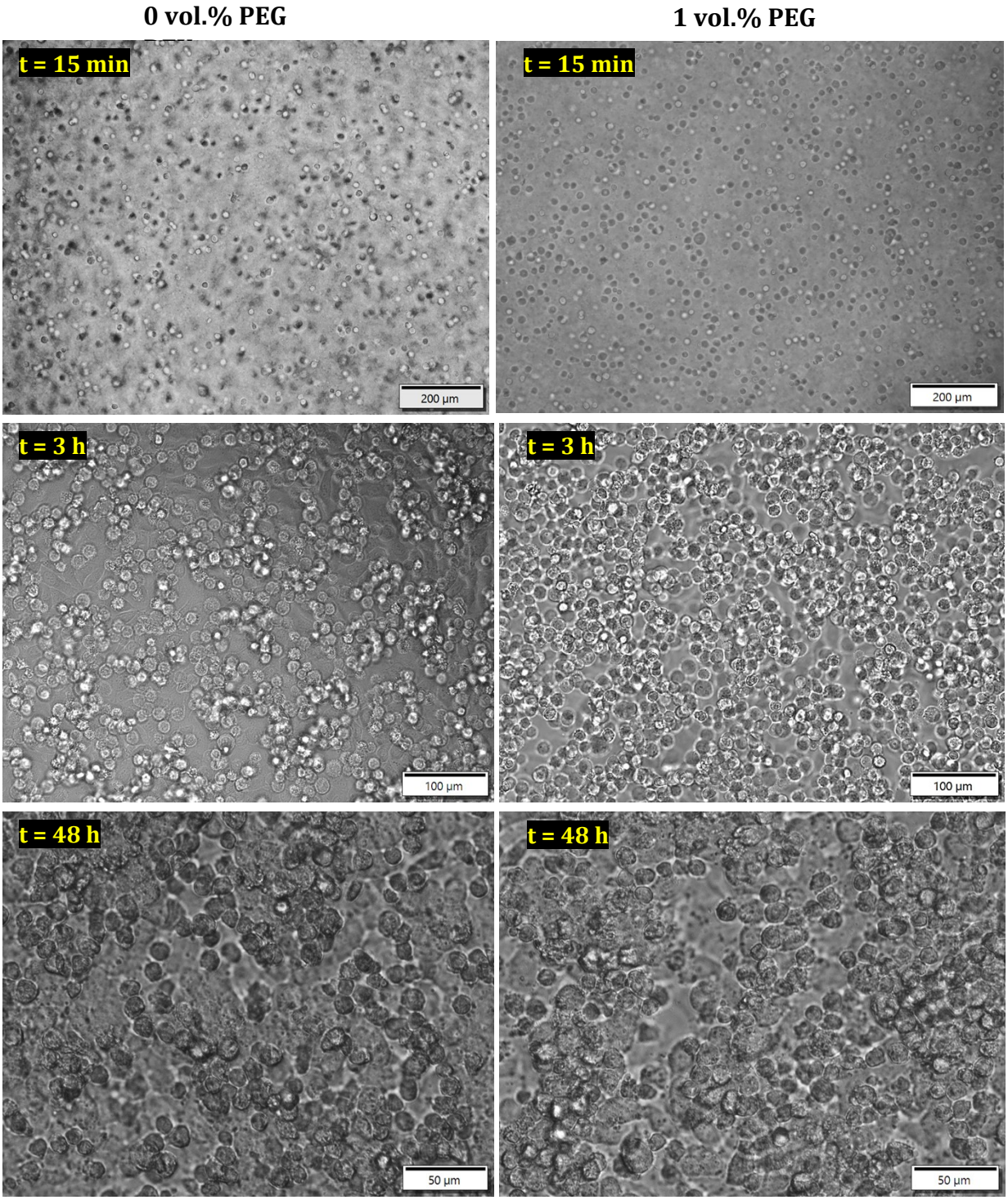
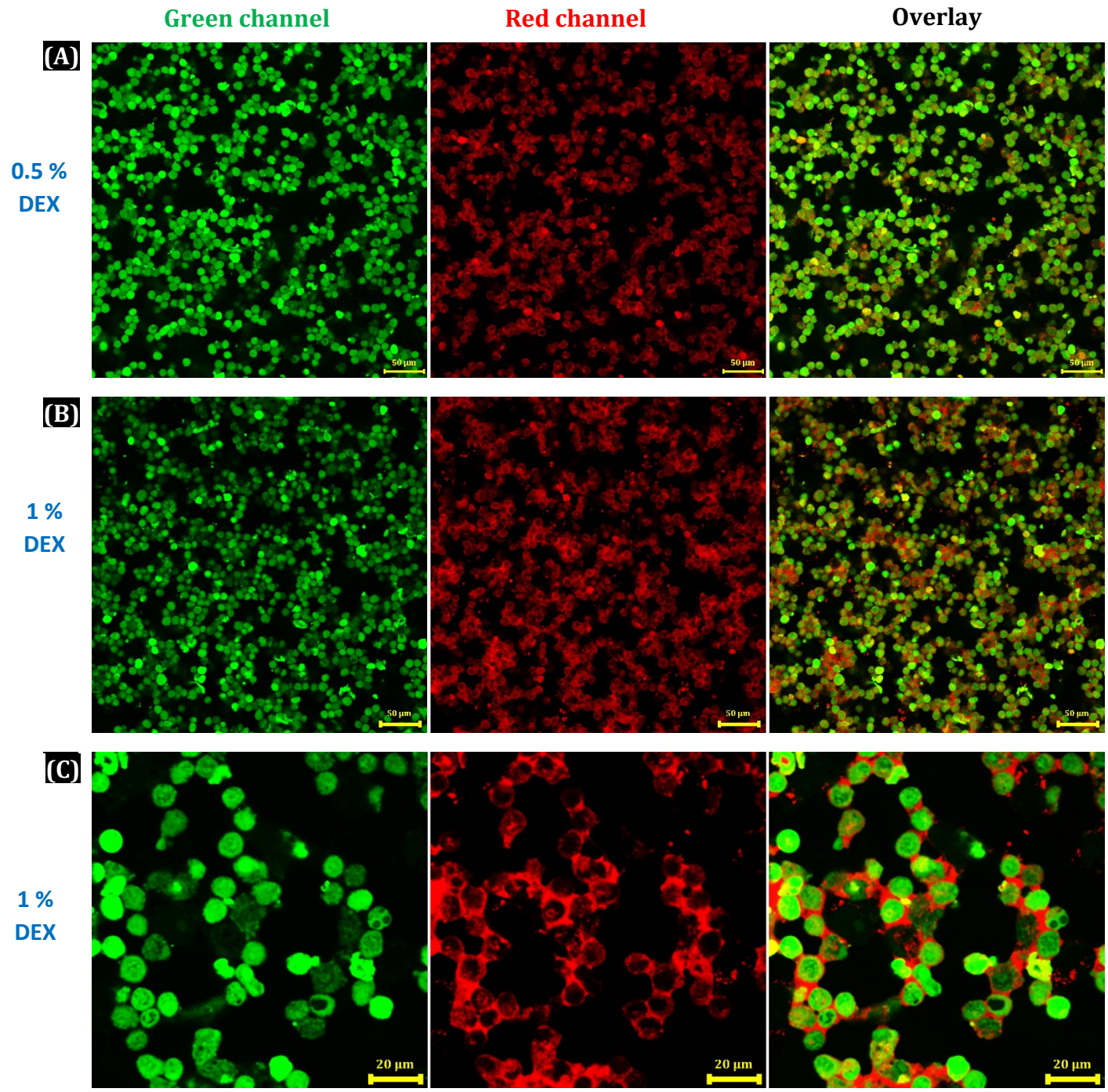


Figure S6. Optical microscopy images of HeLa cells suspensions microstructures obtained by the addition of PEG as a secondary liquid (in vol. % shown) to suspensions of 10 wt.% HeLa cells in DEX as a primary phase after various incubation times (shown).


Green channel
Red channel
Overlay
(A)
0.5 %
DEX
(B)
1 %
DEX
(C)
1 %
DEX
50 µm
20 µm

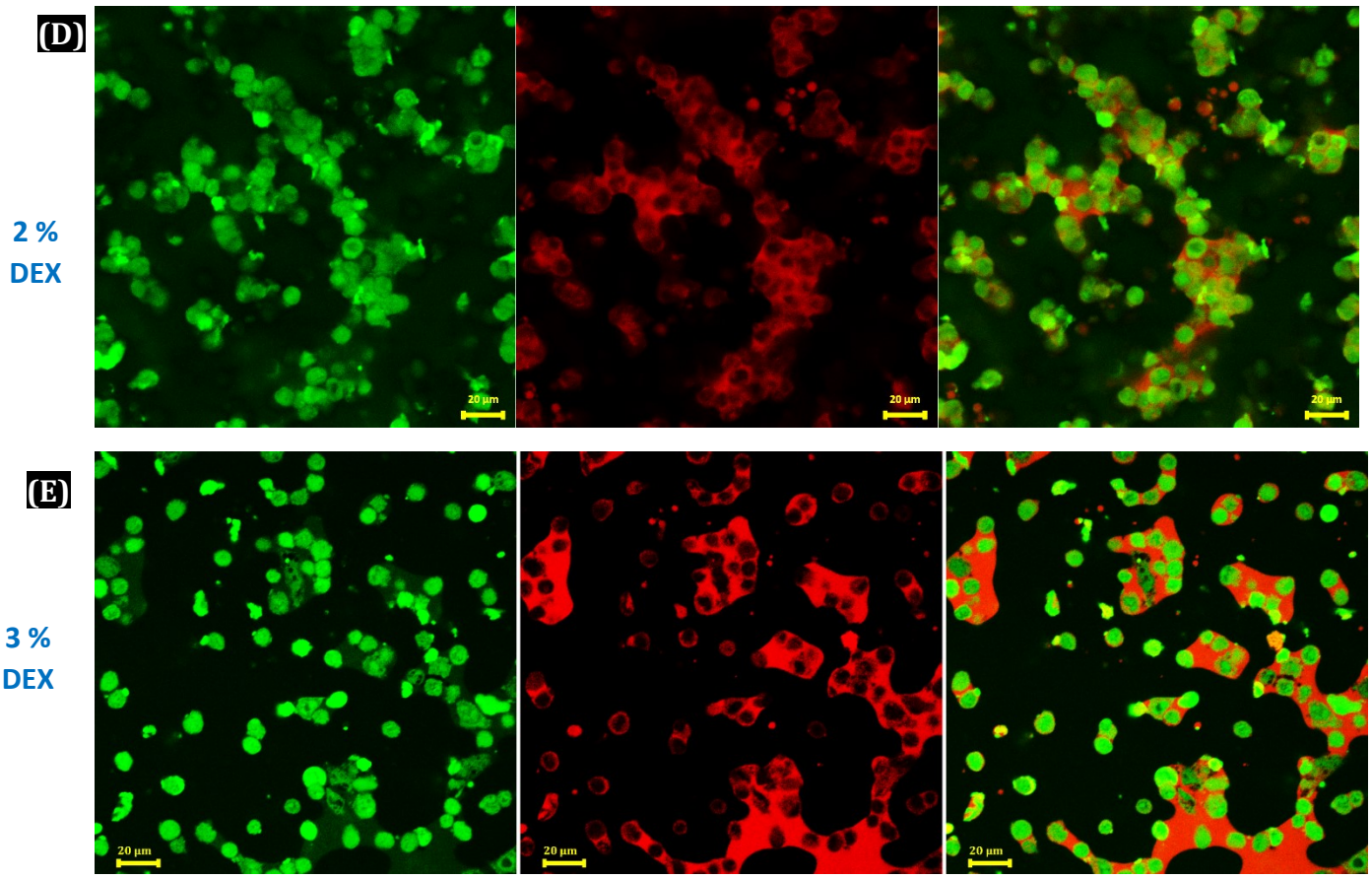


Figure S7. CLSM XY images of morphologies and microstructures of capillary suspensions of HeLa cells in green, red and overlay channels for 10 wt.% HeLa cells suspension the primary phase (PEG in DMEM) treated with different volumes (in vol.% shown) of the secondary phase (DEX in DMEM). Different microstructure can be seen upon increasing the DEX %.vol. HeLa cells were stained with CFDA-SE and appeared in green, DEX was stained with DEX-TRITC and appeared in red while the unstained primary phase (PEG) and shown as black background. All images were recorded after 48h incubation in humidified conditions at 37 °C and 5% CO_2.

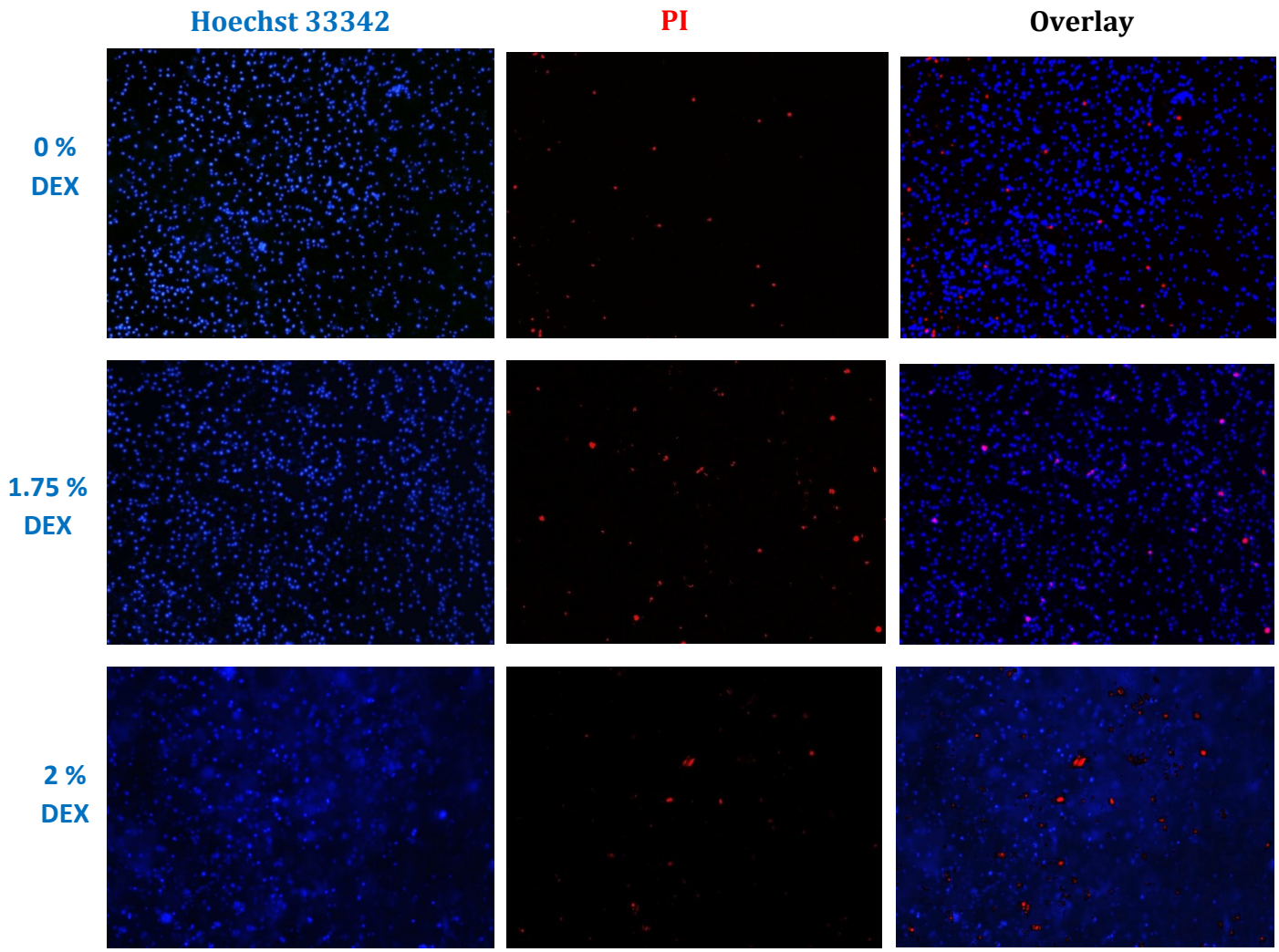


Figure S8. Fluorescence microscopy images for Hoechst 33342/PI dead/live HeLa cell assay. Tests were carried out for HeLa cell capillary suspension obtained by the addition of 0. 1.75, and 2 vol.% DEX to the cell dispersion in PEG after incubation for 48h. Images were captured after re-dispersing the formed clusters using a vortex for 2 min.

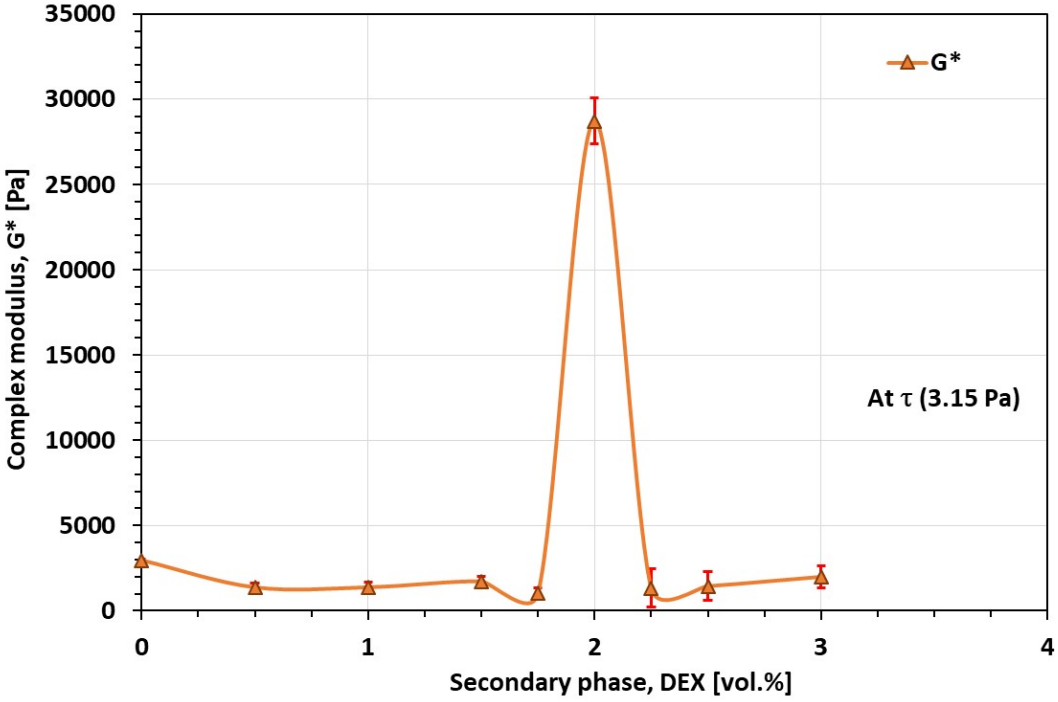


Figure S9. Rheological behavior of capillary structured suspensions of HeLa cells. The complex modulus, *G**, versus the added vol. % of the secondary liquid phase (DEX in DMEM) at an applied stress of (3.15 Pa) for a HeLa cell capillary suspension in (PEG in DMEM) phase at 25 °C and fixed 1 Hz frequency.

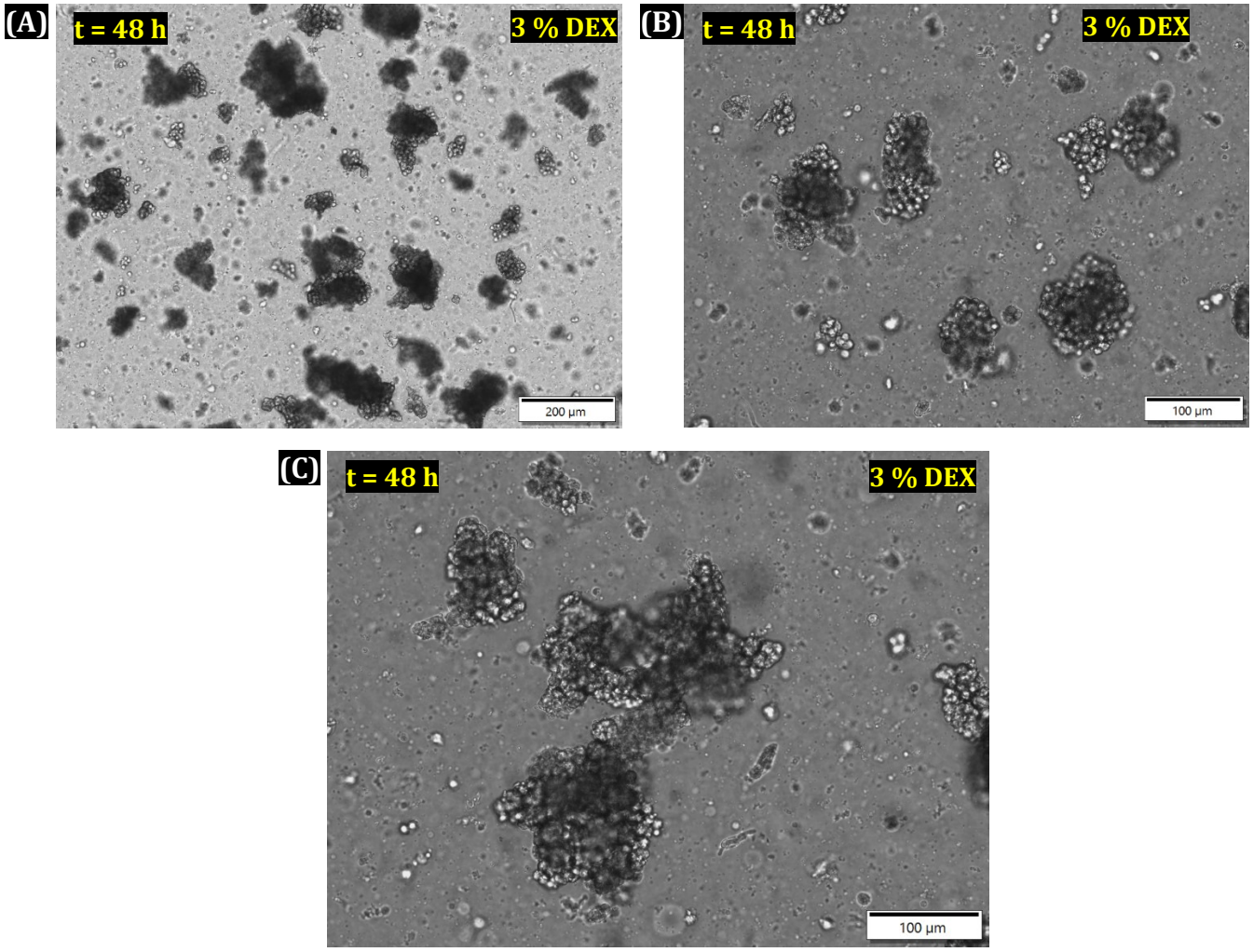


Figure S10. Bright field microscopy images at different magnifications (A,B,C) of capillary structured clusters of HeLa cells observed after performing the oscillatory rheological measurements of 10 wt.% HeLa cells suspensions in PEG treated with 3 vol.% DEX and after 48 h of incubation. Many stable clusters can be seen.