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Electronic Supplementary Information

3D Structured Capillary Cell Suspensions Aided by Aqueous Two-Phase Systems

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Experimental

Materials

Saccharomyces cerevisiae cells, Tryptone, D-glucose, yeast extract and polyethylene glycol PEG (*M*w 200 kDa), Tetramethyl rhodamine isotiocyanate-dextran (DEX-TRITC) and phosphate-buffered saline (PBS), dimethylsulfoxide (DMSO), poly(allylamine hydrochloride) (PAH) and Acridine Orange base (AO) with λ_{max} = 488 nm were purchased from Sigma-Aldrich; dextran DEX (*M*w 40 kDa) was obtained from Fisher Scientific, UK; 5(6)-Carboxyfluorescein diacetate succinimidyl ester (CFDA-SE), generational stain with λ_{max} = 491 nm, was purchased from Cayman Chemical Company; Deionized water was used through the study.

Methods

Preparation of PEG and DEX aqueous solutions

10 wt.% aqueous solution of PEG were prepared and equilibrated for 24h for full hydration. Then PEG solution was centrifuged at 8000 rpm for 1 h to remove the residual silica from manufacturer and filter sterilized with 0.45 μm filter. 10 wt.% of DEX was separated easily in water and filter sterilized with 0.45 μm filter.

Culturing and staining of yeast cells

The yeast cells were initially hydrated with 5 mL of water and then grown in (Tryptone, D-glucose, yeast extract) sterilized medium for 24 h at 37 °C. Mean diameter of the cells was evaluated using a particle analyzer (Anton Paar Litesizer 500, Germany) as shown in Figure S1. Cells were then washed three times with water and centrifuged at 1800 rpm for 5 minutes. For florescence microscopy, yeast cells were stained with Acridine Orange base at (1mg/mL in water) by adding 100 μL AO to the 10 wt.% cells in water then cells centrifuged at 1800 rpm for 5 minutes. For CLSM, cells were stained with CFDA-SE. First a 10 mM of the CFDA-SE stock solution was prepared in DMSO, then 5 μL from the stock was diluted up to 5 mL PBS to obtain 10 μM working solution. For staining at 5 μM, 1:1 (v/v) of working solution and cell suspension in PBS were mixed and incubated for 10-15 minutes at 37 °C. The stained cells were then centrifuged, washed with water and PBS to be ready for dispersion in PEG.

Preparation of yeast cells capillary suspension

DEX was stained with DEX-TRITC (1 mg/ml in water) by mixing using 40 μL of the stain with 1 mL of pure 10 wt.% freshly prepared DEX solution. Next, 10 wt. of the stained yeast cells were dispersed in 1 mL of 10 wt.% fresh PEG solution using vortex for 2 minutes. Then a desired amount of the secondary phase DEX was added to the cell suspension and mixing by a mini vortex mixer (Fisher scientific, UK) for 1 minute at 1800 rpm. The formed suspensions were examined as required and then kept for 12h at 25 °C for further examinations.

Rheological (oscillation mode) measurements

An Anton Paar MCR 72 rheometer (Germany) was used with 25 mm parallel spindle (PP25) at 25 °C. The "zero gap" was set to 1 mm. Oscillatory amplitude sweep was done first using stress mode at fixed frequency of 1 Hz to identify the limit of the linear viscoelastic (LVE) range to avoid irreversible structure changes when carrying out further measurements. A series of capillary suspensions with different vol% DEX were tested with optimum parameters based on the initial assessment to correlate the rheological parameters with the capillary structure. A shear stress amplitude sweep test was carried out using oscillatory mode with oscillating stress ranging from 0.01 to 10000 Pa in ramp logarithmic profile at constant frequency of 1 Hz and at 25 °C. The yield stress is defined as the stress at which the mixed system starts to flow and deform plastically and it was measures as the point at crossover between storage and loss moduli. It was also confirmed by the drastic decrease of the suspension viscosity.[1] All samples were allowed to stabilize for 5 minutes after equilibrating the temperature and before the test. No pre-shear setting was used. All data were collected and processed using (RheoCompass® software).

Optical, fluorescence and Confocal laser scanning microscopy (CLSM) and Scanning electron microscopy (SEM)

An Olympus fluorescence microscope CKX53SF (Germany) fitted with an FITC filter set was used for optical and fluorescence images. Images or videos were captured and processed using Olympus cellSens software.

CLSM was carried out using (Carl Zeiss LSM780, Germany) fitted with different laser lines (405/458/488/514/561/633 nm). Samples were examined in 8 well plate- 0.27 mm glass bottomed. Fluorescence was collected at laser excitation lines at 488 nm (2%) and 561 nm (2%) with DIC in Plan-

Apochromat 20x/0.8 M27 objective lens. Images in XY and Z-stack mode were recorded and processed using ZEISS ZEN 3.9 software (ZEISS, Germany). For better visualization of the formed capillary bridges connecting the cells and because of the complex structure of the cell suspensions, channels were split and recorded using the software. For SEM analysis, a ZEISS Crossbeam 540 Scanning electron microscopy (Germany) was used. Prior examination, the cells were fixed as described elsewhere.[2]

Contact angle, surface and interfacial tensions measurements

A Drop Shape Analyzer, DSA25S, (Krüss, Germany) was used for contact angle, surface and interfacial tensions measurements at 25 °C. For contact angle measurement a sessile drop (with tangent fitting method) was used, and the contact angle was measured after 10 minutes equilibrium using as an average of the left and right values of 15 measurements. For preparation of yeast cells layer, a 10 wt.% yeast cells in water were deposited on square cut clean glass slides coated with poly(allylamine hydrochloride) (PAH) to help adhering the cells to the glass and allowed to dry at room temperature. For three phase contact angle measurements, a square glass slide deposited with yeast layer was inserted into a quartz cuvette and the PEG solution was added then a 5 μL DEX drop was deposited on the yeast layer using a glass syringe with 0.5 mm needle. Surface and interfacial tensions were measured using a pendant drop profile (with Young-Laplace fitting method) of 15 μL fresh drop of the desired liquid. Data were collected and processed with (Krüss ADVANCE software).

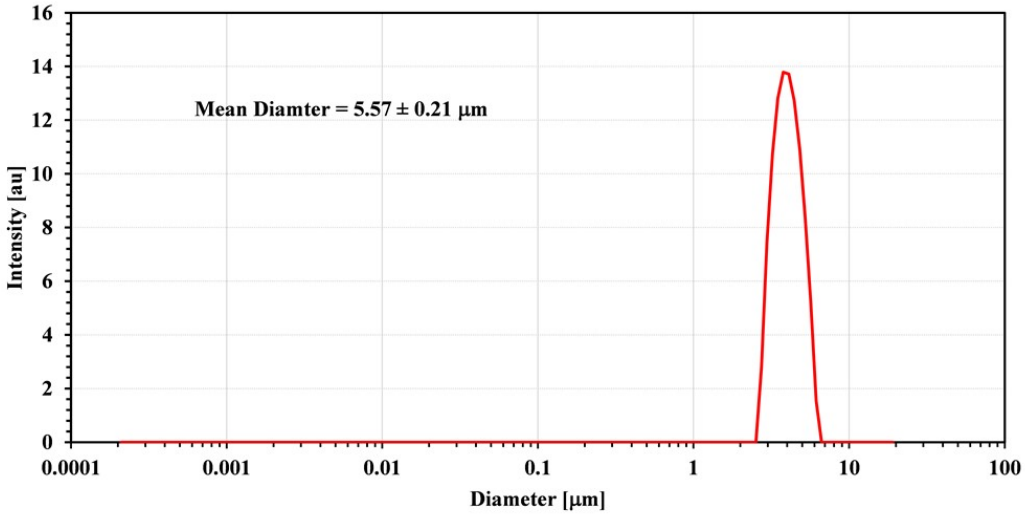

Figure S1. Size distribution of 10 wt.% yeast cells in 10 wt.% PEG aqueous solution.

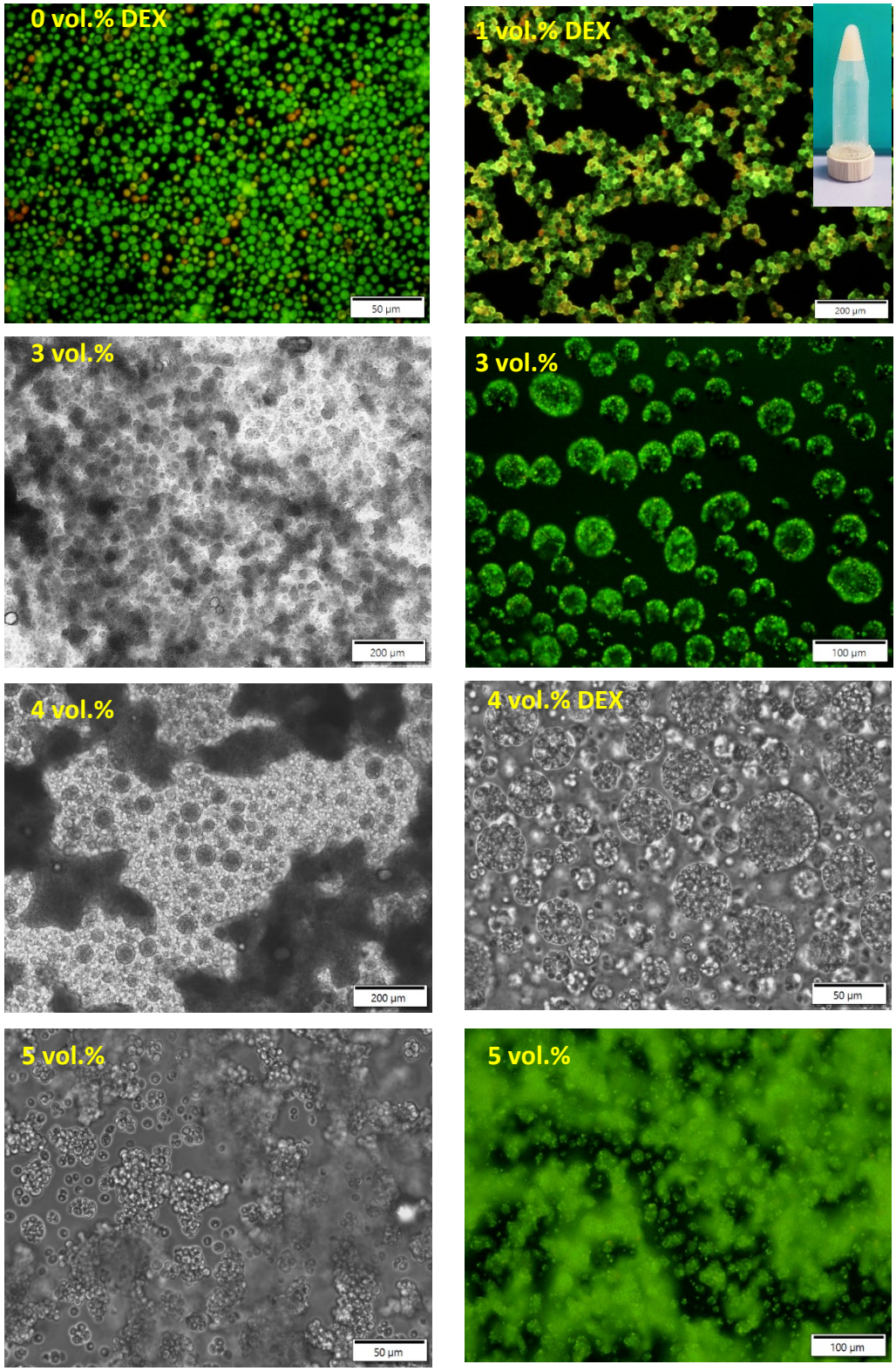

Figure S2. Optical and fluorescence microscopy images of cell microstructures formed by the addition of the secondary liquid, DEX (in vol. % shown), to suspensions of 10 wt.% yeast cells in the bulk phase, PEG, after 24 h at room temperature. Cells were stained with Acridine Orange. The inset of the first raw is a digital image of the formed capillary suspension with 1 vol% DEX after 24h showing the consistency of the formed structure.

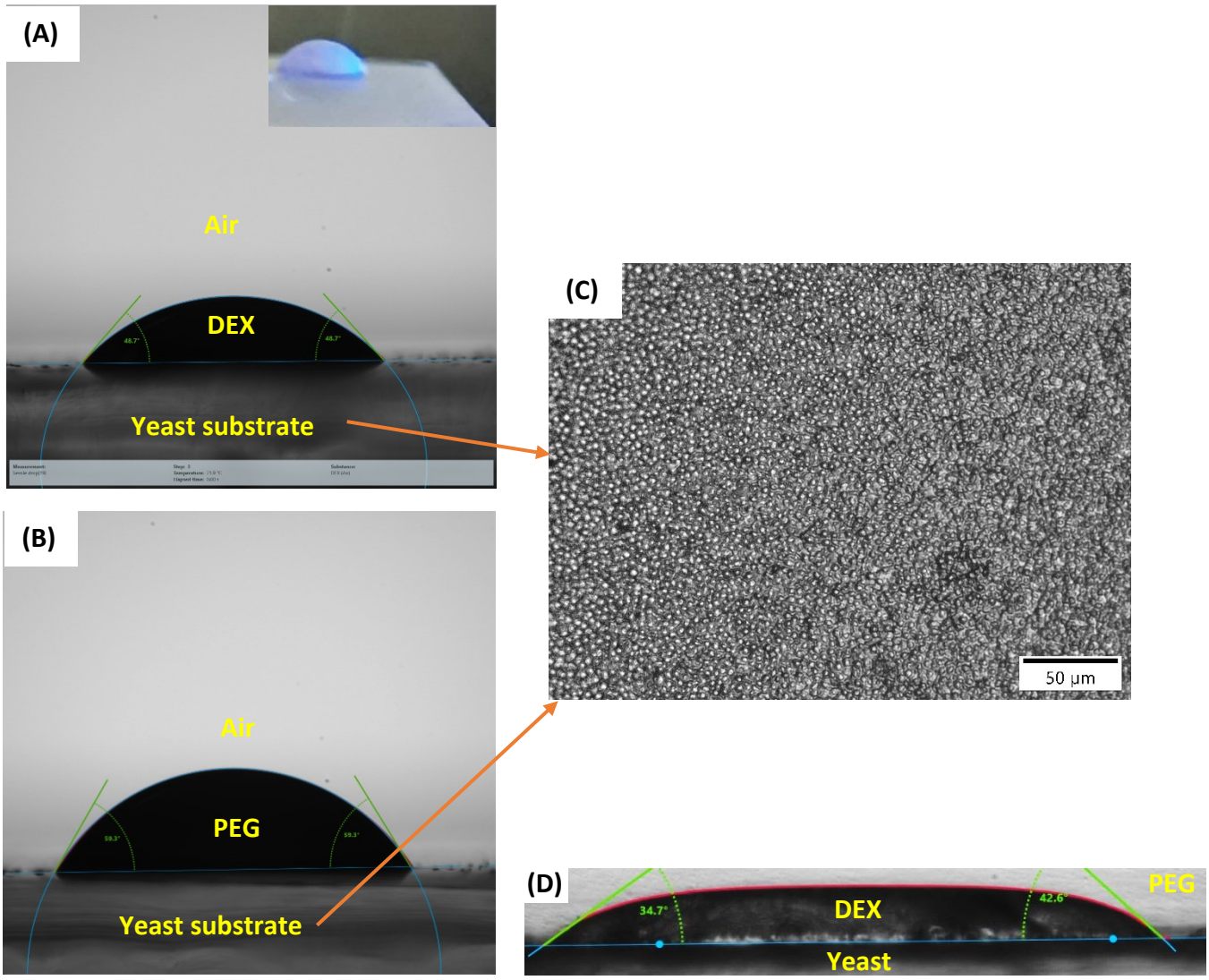

Figure S3. Contact angles of (A) DEX and (B) PEG on a deposited dry layer of 10 wt. % yeast cell shown in (C) against air using sessile drop method (inset in A is a digital zoomed image of the DEX drop. (D) The three phase contact angle of PEG-DEX-Yeast layer measured through the secondary DEX liquid phase at room temperature.

Table S1. Surface/interfacial tensions and contact angles of the phases used.

Surface/interface	Surface/interfacial tension mN/m	Contact angel/ θ
DEX-PEG	0.41 ± 0.12	----------
PEG-air	53.76 ± 0.07	----------
DEX-air	46.80 ± 0.17	----------
DEX-PEG-Yeast	----------	38.65 ± 0.16
PEG-yeast-air	----------	59.78 ± 0.26
DEX-yeast-air	----------	48.40 ± 0.08

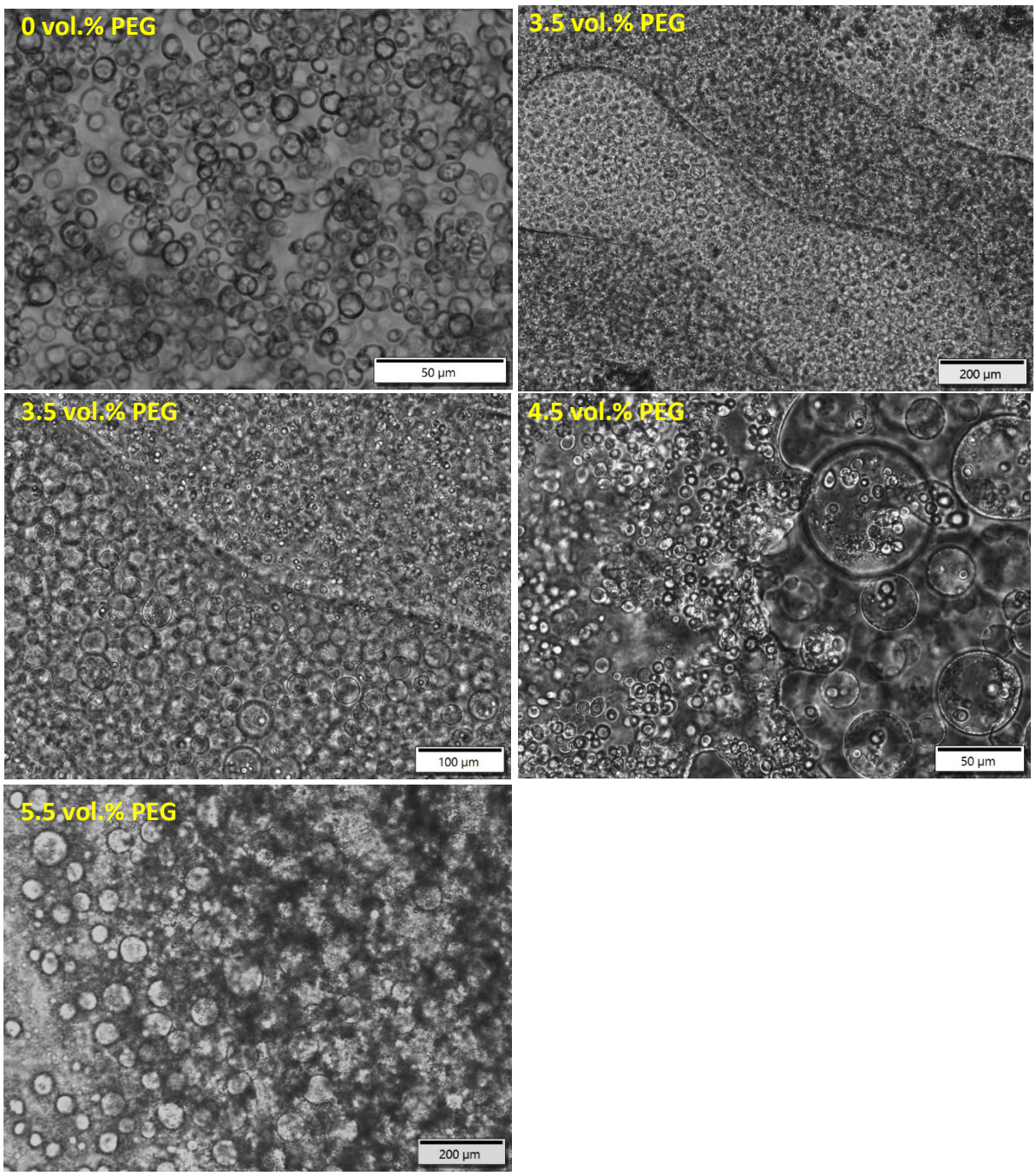

Figure S4. Optical images of microstructures formed by adding different volume % of PEG as a secondary liquid to a 10 wt.% yeast cells suspension in DEX. Bi-phases and large DEX emulsion drops can be seen with no spanning structure observed.

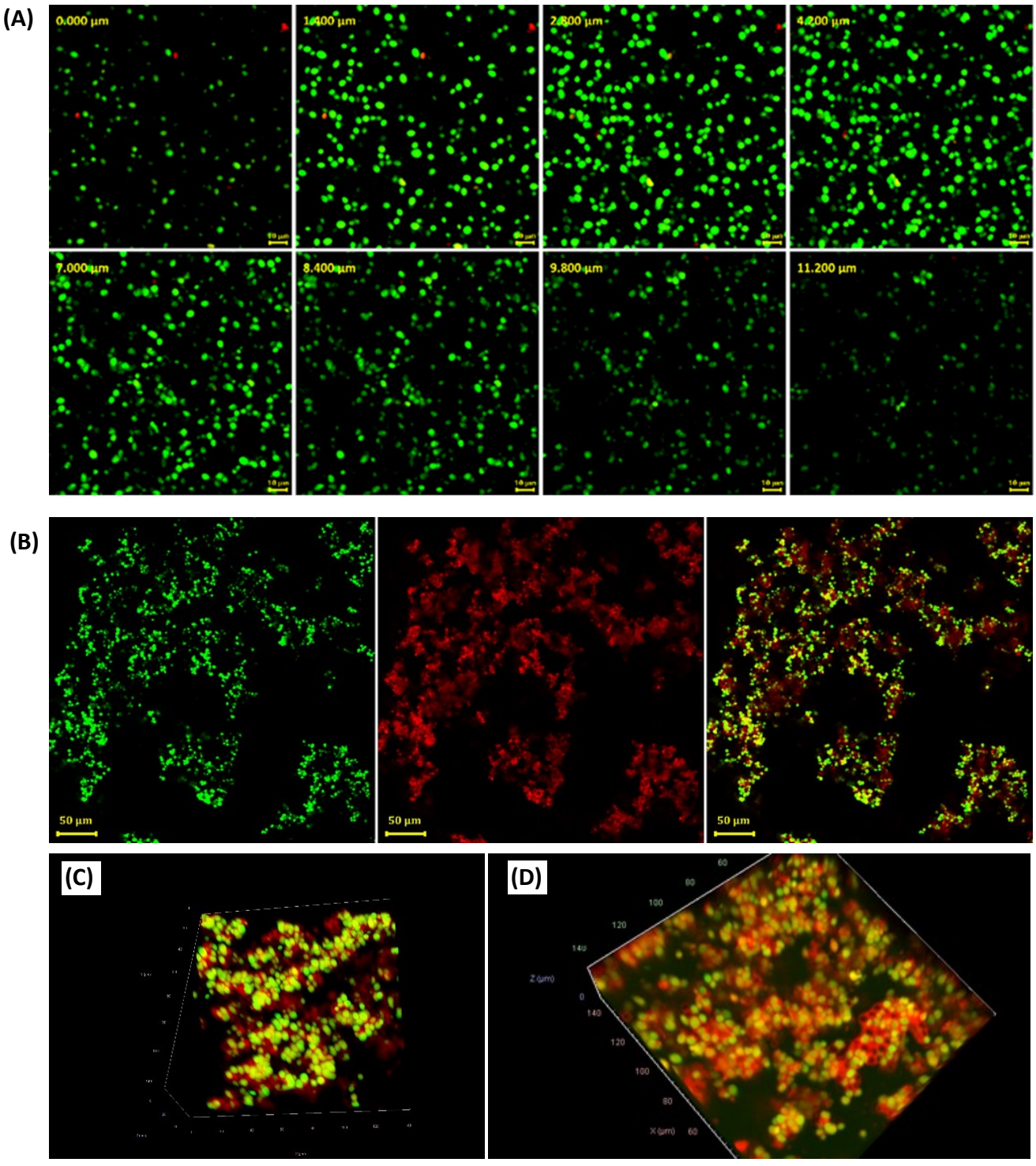

Figure S5. CLSM images of capillary suspensions of yeast cells. (A) Overlay images sequence of different slices of 10 wt.% yeast suspension in PEG without added DEX phase. (B) Three channel images for general structuring morphology of cells by capillary force by adding 1 % (v/v) of the secondary phase (DEX) to the cell suspension in PEG. (C,D) reconstructed 3D images for connecting clusters formed as in sample shown in (B) depicting the complex structuring of the cells via the capillary bridges.

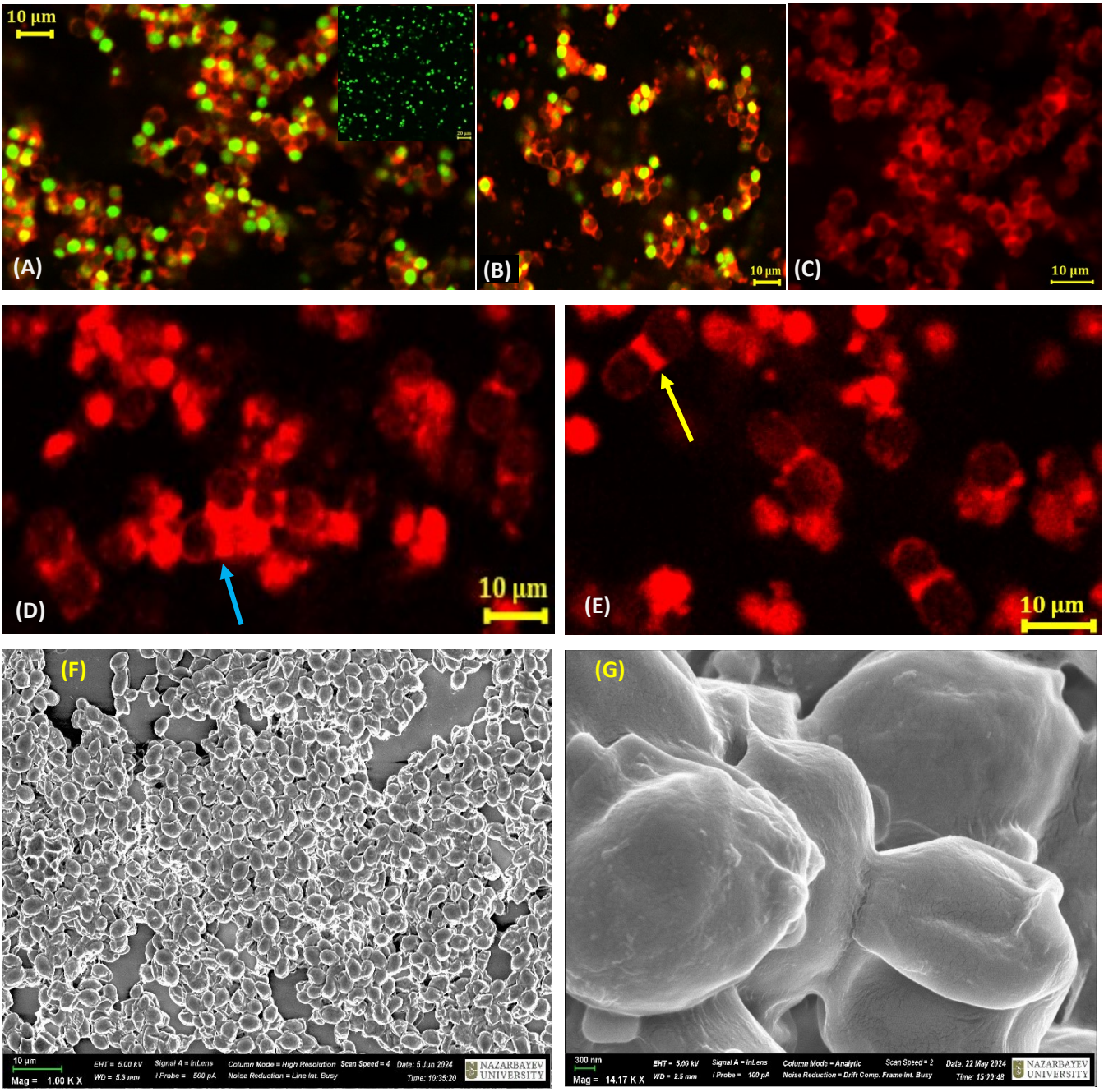

Figure S6. CLSM images of capillary suspensions of yeast cells. (A,B) show XY slices of green/red channels overlay of 10 wt.% yeast suspension in PEG with 1 % (v/v) of the secondary phase (DEX). The inset in (A) represents yeast suspension without DEX. The cells are shown in green color, DEX is shown in red color and the continuous phase (PEG) is black regions. (C) Section of image (A) with a single red channel showing the capillary DEX bridges. (D,E) Close images of same sample as in (A) showing the formation of capillary aggregates (blue arrow) and pendular state (yellow arrow) structures with pairwise bridging. (F,G) SEM images of morphology of a cluster of fixed yeast cells as in (A), showing the adhesion of cells.

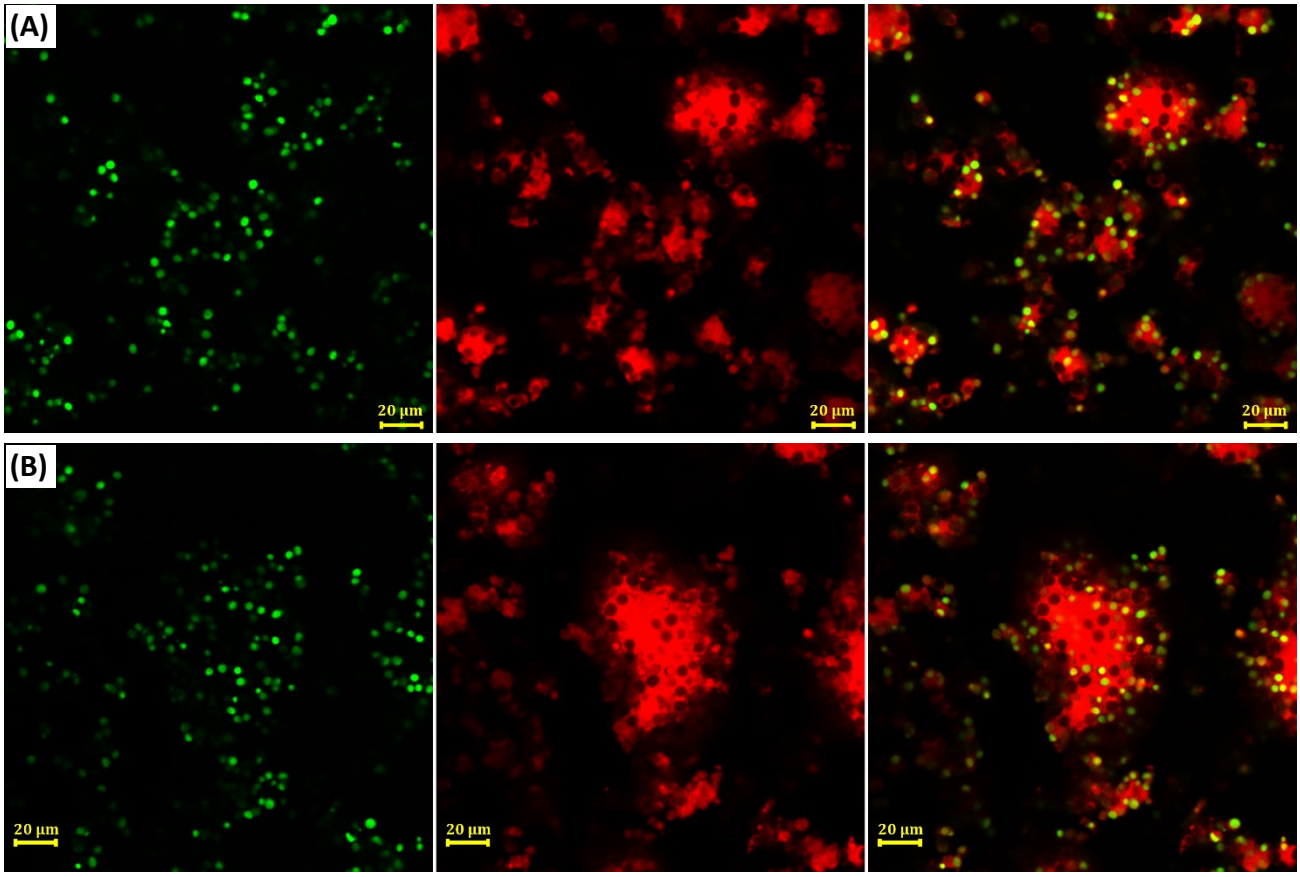

Figure S7. CLSM images of microstructures of capillary aggregates in suspensions of yeast cells formed by the addition of: (A) 3 vol% (v/v) of the secondary phase (DEX) to the cell suspension in PEG phase and (B) with 4 vol% DEX phase. The aggregates appeared separate, and no spanning structuring was observed.

Videos descriptions

Video V1: CLSM scan of cells aggregates in DEX-n-PEG emulsion drops at 3-5 vol% of DEX phase.

Video V2: CSLM scan of cells suspension in PEG without DEX as a secondary phase**.**

Video V3: CLSM scan of a capillary cells suspension in a PEG phase at 1 vol% of DEX phase.

Video V4: CLSM scan of a capillary cells suspension in PEG phase at 1 vol% of DEX phase (Red channnel for DEX bridging phase.

Video V5: CLSM scan of a capillary cells suspension in PEG phase at 2 vol% of DEX phase.

References

1 E. Koos, W. Kannowade and N. Willenbacher, *Rheol. Acta*, 2014, **53**, 947–957.

2 A. A. K. Das, B. W. Filby, D. A. Geddes, D. Legrande and V. N. Paunov, *Mater. Horizons*, 2017, **4**, 1196–1200.