Supplementary Information

Production and optimization of multifunctional living microfibres for tissue engineering applications by microfluidic chip technology

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Table S1 ANOVA Production of alginate based microfibres by microfluidic chip technology (chip #1): ANOVA analysis of variance of the model for DoE approach.

	N	**R^2**	**R^2Adj.**	**Q^2**	**Model validity**	**Repr.**
Mean diameter	17	0.97	0.93	0.81	0.62	0.99
SD	17	0.76	0.41	0.61	0.81	0.72

N is the number of experiments; R^2 is the percent of the variation of the response explained by the model; R^2 Adj. is the fraction of the variation of the response explained by the model adjusted for the degree of freedom; Q^2 is the percent of the variation of the response predicted by the model; Repr. is the model reproducibility.

All the data obtained fitted well with the model, suggesting a good reproducibility of the studied model. We got a large regression coefficient R^2 that resulted in a necessary condition for a validity model with a significant power of prediction Q^2

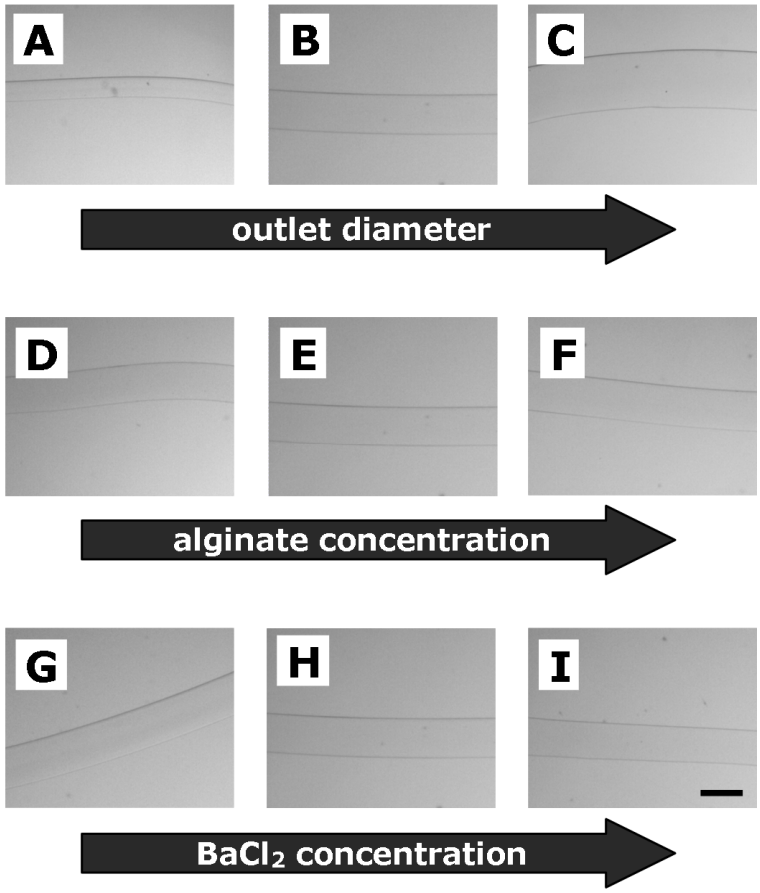

Fig. S1 Optical stereo photomicrographs of alginate microfibres, showing the effect of the experimental setup (following a COST approach) on microfibre dimension and morphology. Microfibres were produced by the single inlet linear microchip (#1). The investigated factors were: the diameter of the outlet tube (A-C), the polymer concentration (D-E) and the concentration of gelling bath, expressed as percentage (w/v) of $BaCl_2$ (G-I). The complete experimental conditions are reported in Table 2. Bar corresponds to 400 μm.

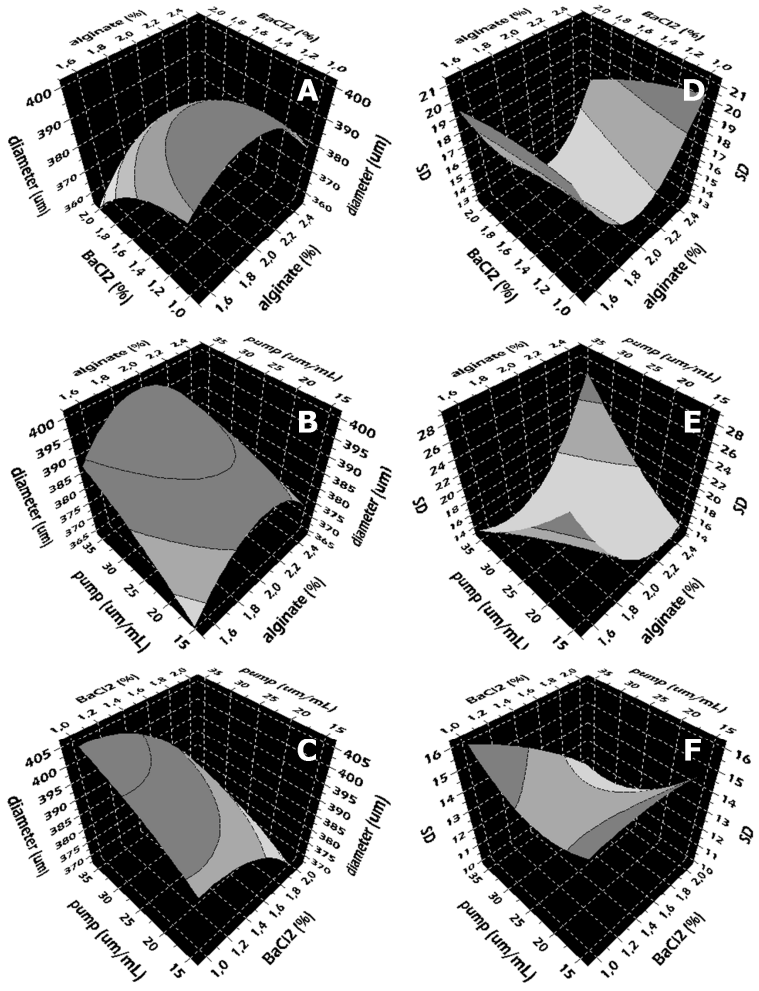

Fig. S2 DoE analysis of the alginate microfibre production. Surface plots of the responses "diameter (A-C)" and "SD" (D-F) for the interactions between "alg" and "$BaCl_2$" (A, D); "pump" and "alg" (B, E); "pump" and "$BaCl_2$" (C, F). Factors, levels and responses employed for the DoE analysis are reported in Table 3.

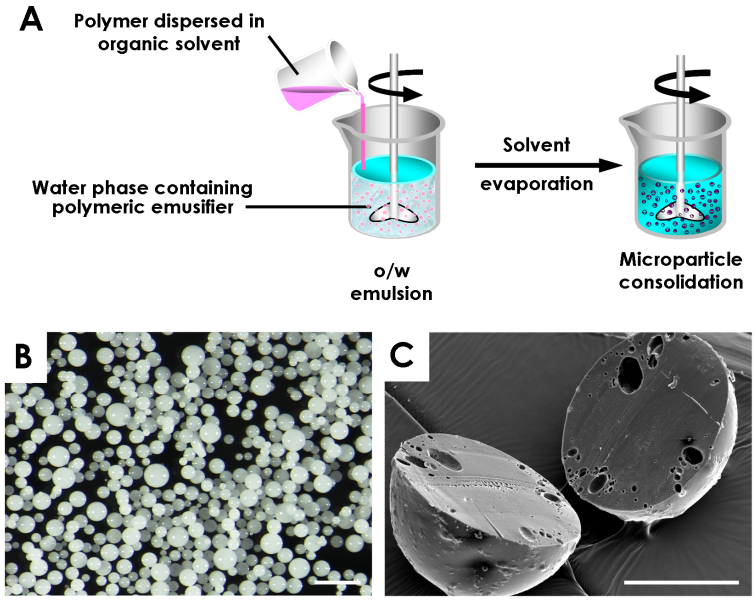

Fig. S3 Schematic representation of the solvent evaporation technique used for cellulose acetate microspheres (CAM) production. Optical stereo photomicrograph (B) and SEM image (C) of the produced CAM. Scale bars correspond to 100 and 40 µm, in panel B and C, respectively. Typically, 1 to 2 g of polymer (cellulose acetate (CA) CA-398/10-NF from EASTMAN, Tennessee USA) were dissolved in 5 mL of CH_2Cl_2. The organic solution was emulsified with an aqueous phase containing hydrolysed poly(vinyl alcohol) (PVA) (Airvol 205, Air Products Corp., PA, USA) as stabilizer. The obtained emulsion was maintained under continuous stirring with a four-blade turbine impeller, typically at 350 rpm. At different time intervals, samples were observed microscopically throughout complete evaporation of CH_2Cl_2, usually occurring in 3–5 h. Once the consolidation of CAM was complete, microspheres were isolated by filtration and extensively washed.

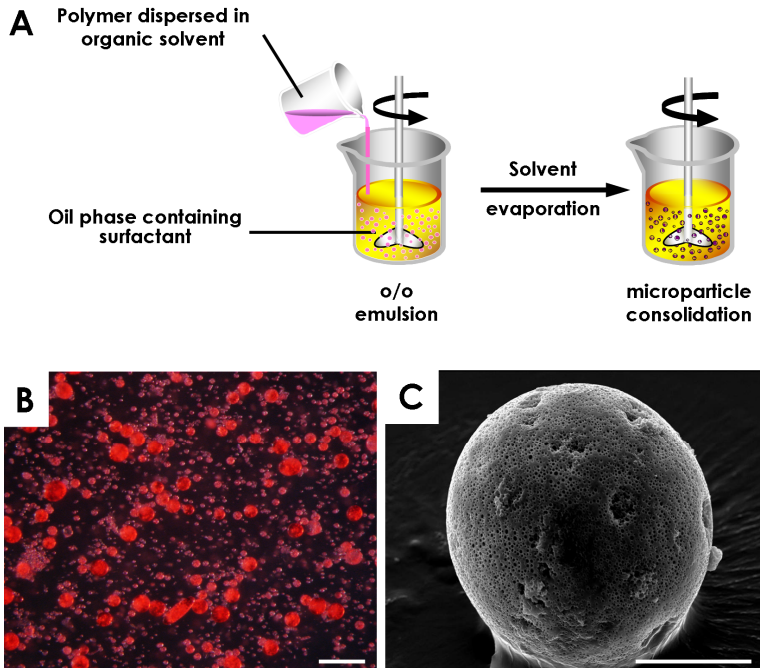

Fig. S4 Schematic representation of the "in-liquid drying process from an oil-in-oil system" technique used for Eudragit RS microspheres (ERSM) production. Optical stereo photomicrograph (B) and SEM image (C) of the produced ERSM. Scale bars correspond to 150 and 40 μm, in panel B and C, respectively. Typically, 500 mg of polymer (acrylic resin Eudragit® RS 100) were dissolved in 15 ml of methylene chloride. The solution was then poured into 100 ml of light mineral oil: d=0.84 g/ml (Sigma Chemical Co., St. Louis, Missouri, USA) containing 0.5% (w/v) soybean lecithin as emulsifier. The mixture was stirred at 350 r.p.m. by a four blade turbine impeller. The system was then heated at 35°C, in order to evaporate methylene chloride. After 5 hours, the microspheres were isolated by filtration, and extensively washed with n-hexane in order to remove residues of mineral oil.

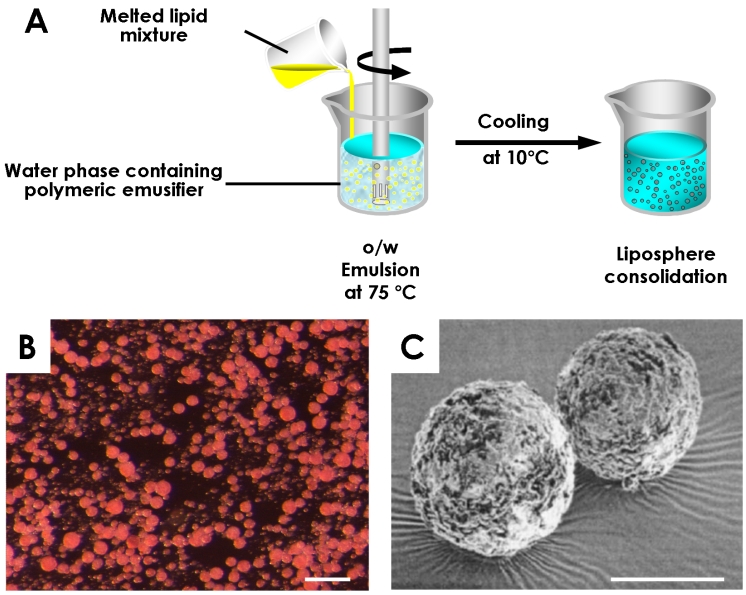

Fig. S5 Schematic representation of the melt dispersion techniques used for liposphere (LS) production. Optical stereo photomicrograph (B) and SEM image (C) of the produced LS. Scale bars correspond to 100 and 20 μm, in panel B and C, respectively. A lipid mixture constituted of glyceryl tristearate/glyceryl monostearate) from Fluka (Buchs, Switzerland) was melted at 70–75 ◦C and then emulsified into 15 mL of an external aqueous phase containing 2% (w/v) of PVA as the dispersing agent. The emulsion was stirred at 8000 rpm using an IKA T25 Ultra-turrax (IKA Labortechnik, Germany) for 2 min. Once the agitation was stopped, the milky emulsion was rapidly cooled to about 10°C by immersing in a cool water bath. The obtained LS were isolated by centrifugation (10 min at 5200×g) and the pellet was washed three times with water and finally dried under vacuum. For the preparation of coloured LS, the dye oil red O from Aldrich (Germany), was used.

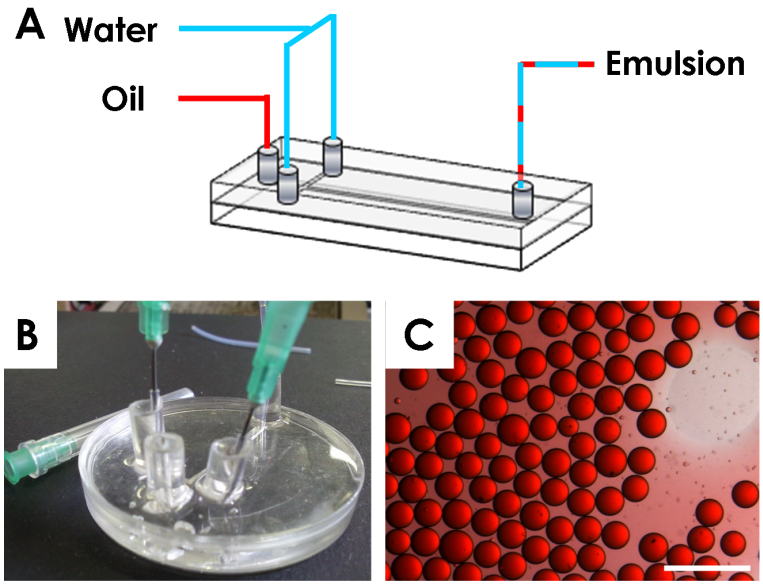

Fig. S6 Schematic representation of the microfluidic flow focusing technique used for the production of corn oil based-emulsion (COBE). Photographs of microfluidic chip (B) and of produced COBE (C). Scale bar corresponds to 250 µm. The used microfluidic chip was constituted of polydimethylsiloxane (PDMS) and contains channels with a square section of 200 × 200 µm. Both dispersed and continuous phases were injected into the microfluidic chip by a syringe pump (KDS Model 100 Series, Kd Scientific). The microchip was connected to syringes through fluorinated ethylene propylene FEP tubes. Corn oil (Gattefossé, SaintPriest Cedex, France) was used as oil internal phase and slowly injected into the central inlet of the flow focusing geometry. The second immiscible liquid (water) was injected into the two lateral inlets as continuous phase. Corn oil was forced into the water phase at the junction of the focusing channel, forming a multiphasic flow (droplets) represented by a o/w emulsion. For the preparation of coloured COBE, the dye oil red O from Aldrich (Germany), was used.

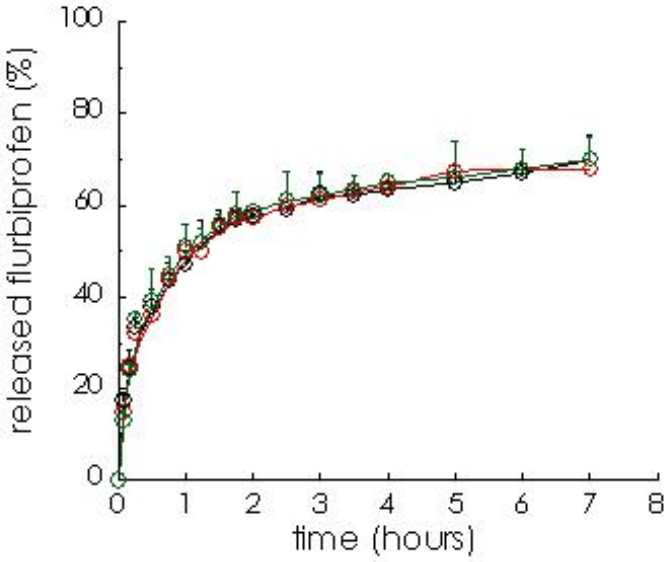

Fig. S7 Flurbiprofen from COBE embedded into alginate microfibres with a mean diameter of 180 (green symbols), 398 (black symbols) and 510 μm (red symbols). Data represent the average of four independent experiments ± SD.

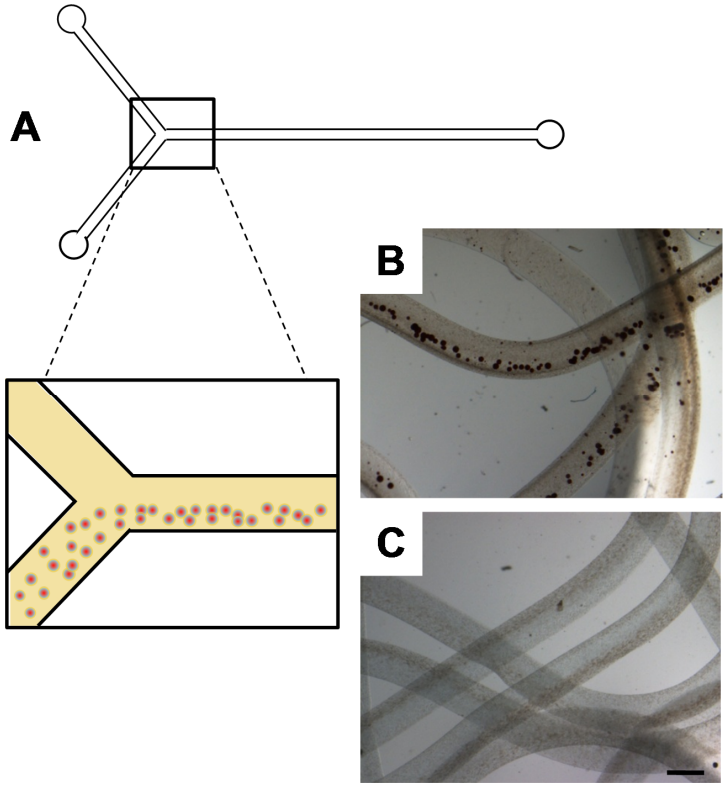

Fig. S8 Schematic representation of the microfluidic device with two inlets and a single straight channel used for the production of multifunctional alginate microfibres containing DDS or cells. The inset shows the mechanism of segregation of the particulate matter within the chip channel. Optical stereo photomicrographs showing alginate microfibres containing CAM (B) or K562 cells (C). Microfibres were produced by two independent syringe pumps, one pumping alginate solution (Pump #1), the other pumping alginate solution containing microparticles (80 mg/mL) or cells (18 x 10^6 mg/mL) (Pump #2). Microfibres were prepared with a Pump #1/Pump #2 rate ratio of 50/200 µl/min. Bar corresponds to 400 µm.

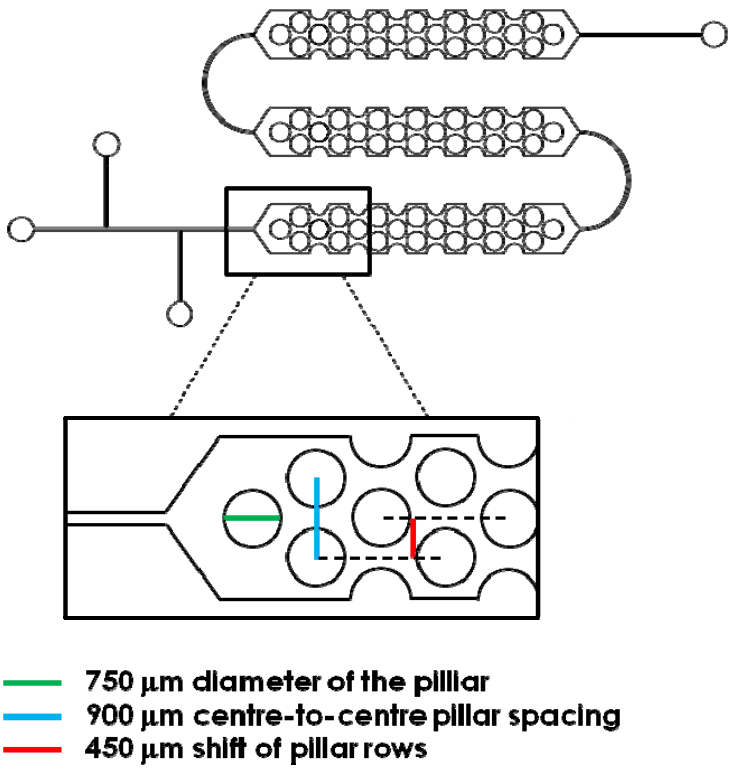

Fig. S9 Schematic representation of the microfluidic Chip #2. Inset reports the characteristic dimensions of the particulate dispersing chamber with pillar obstructions.

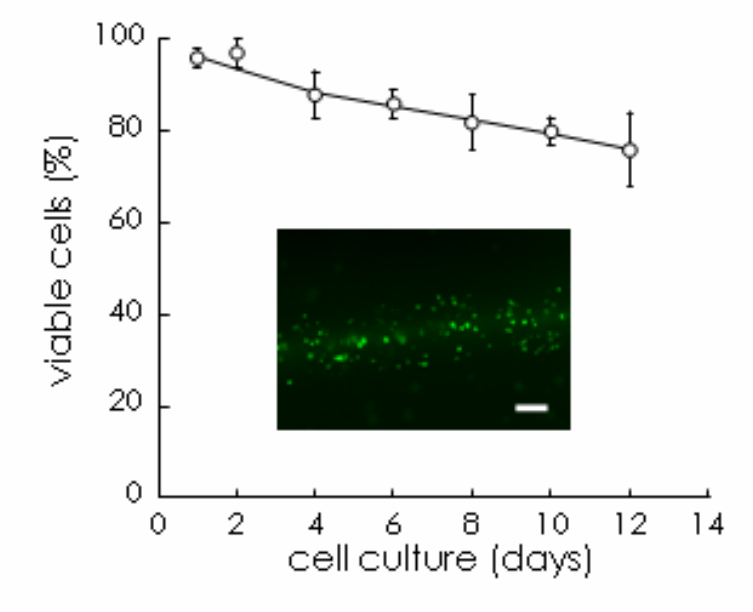

Fig. S10 Graph reporting the percentage of viable K562 cells determined by double staining, after different length of time of *in vitro* cell culture. Values are expressed as percentages of viable cells respect to total entrapped cells and represent the mean of three independent samples analyzed in quadruplicate ± SD. In the inset is reported a fluorescence microphotograph of multifunctional alginate microfibres containing K562 cells. Microfibres were produced by Chip #2. Fluorescence photomicrograph was taken after double staining with Calcein-AM and propidium iodide, after 4 days of cell culture. Bar corresponds to 200 μm.