

Supporting Information

Early Chondrogenic Differentiation of Spheroids for Cartilage Regeneration: Investigation of the Structural and Biological Role of a Lactose-Modified Chitosan

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S2. Materials and methods

S2.1 Release studies of CTL

CTL-FITC solution (2% w/v in PBS; 0.4 mL) was used to coat the well of cell culture plate (24-well), following by air-drying. PBS (1.5 mL) was added on the air-dried CTL-FITC coating. The supernatant was collected at different time points (1, 3, 5 and 24 hours) and the fluorescence was read (excitation: 485nm; emission: 520nm) by a fluorimeter (FLUOStar Omega-BMG Labtech spectrophotometer). To calculate the concentration of fluorescent CTL released in the samples, a standard curve prepared with a CTL-FITC solution in PBS was used.

S2.2 Dynamic light scattering (DLS)

Dynamic light scattering (DLS) measurements were performed using a Zetasizer Nano ZS system (Malvern Instruments, UK). A CTL solution was employed as a well-coating layer as reported in section 2.2. PBS was added on the CTL-coated wells and the samples were collected from the wells after 24 and 72 hours. As reference, PBS and a CTL solution (0,53%) were used. DLS measurements were performed at 25 °C and each sample was analyzed in triplicate.

S3. Results

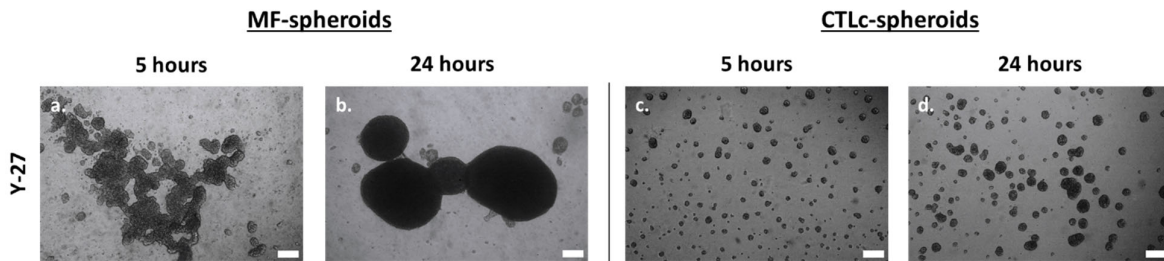


Figure S1. MF-spheroids (a-b) and CTLc-spheroids (c-d) treated with Y-27 for 5 and 24 hours.

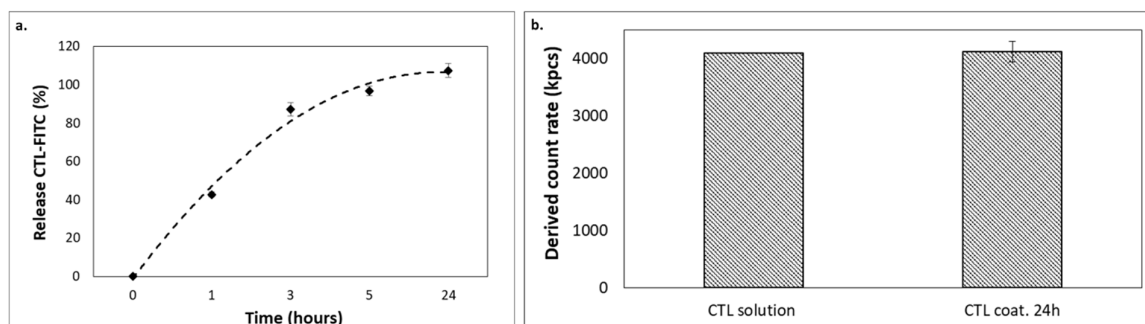


Figure S2. Release of CTL-FITC after rehydration of CTL-coating (a); dynamic light scattering (DLS) analysis of CTL solution and rehydrated CTL-coating (b).

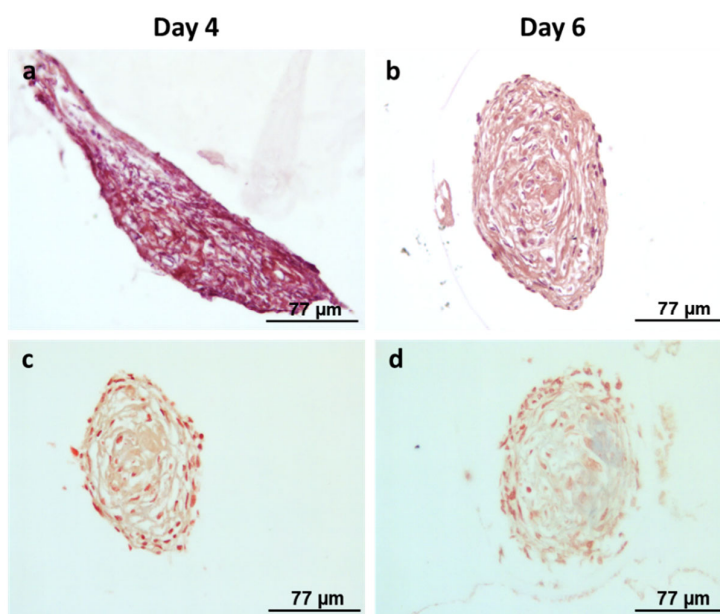


Figure S3. Haematoxylin-eosin staining (a-b) and Masson's Trichrome staining (c-d) of MF-spheroids at days 4 and 6. Scale bar bar 77 μm; magnification: 40x.

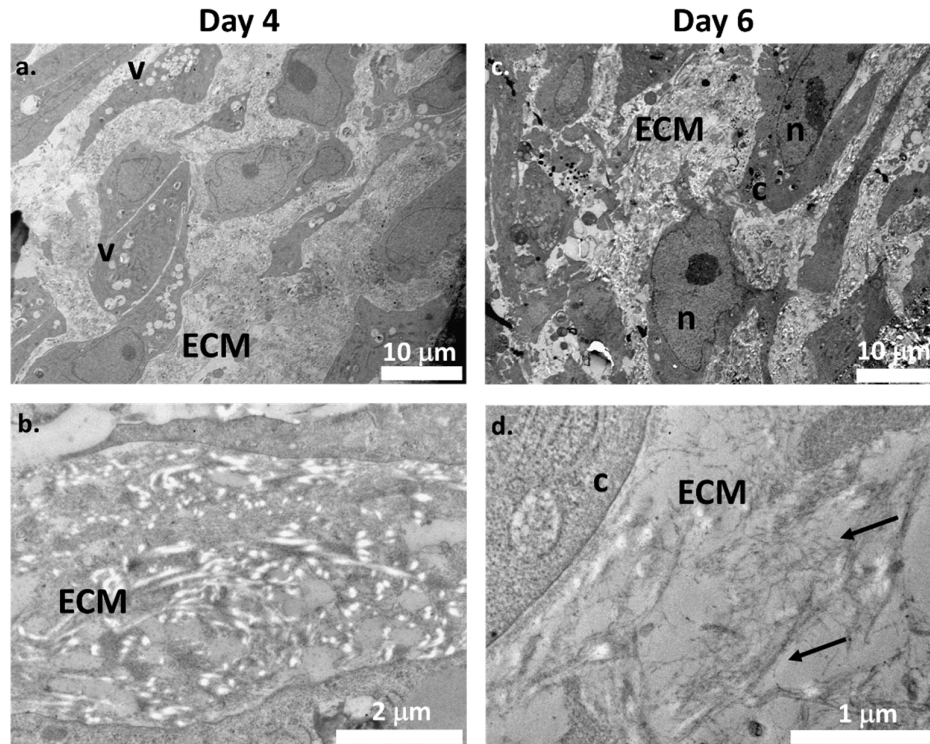


Figure S4. TEM images of MF-spheroids at day 4 (a-b) and day 6 (c-d). At day 4, MSCs in MF-spheroids show a polygonal shape surrounded by extracellular matrix (ECM). Several vesicles (v) are detected in the cytoplasm (a.). No mature fibrillary components can be detected in the ECM (b.). At day 6, MSCs maintain their polygonal shape surrounded by extracellular matrix (ECM). Nucleus (n); Cytoplasm (c) (c.). Thin filaments (arrows) corresponding to precursor components of the extracellular matrix are detected between cells (d.). Scale bar: a: 10 μm; b: 2 μm; c: 10 μm; d: 1 μm.