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Supplementary information

Metabolic labeling of secreted matrix to investigate cell–material interactions in tissue engineering and mechanobiology

In the format provided by the authors and unedited

Supplementary Method

LC-MS/MS analysis

Samples were analyzed using a Thermo Scientific Dionex UltiMate 3000 RSLCnano System coupled to a Thermo Scientific Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap Mass Spectrometer. After normalization, 5 mL of sample was loaded onto a Waters 75mm i.d. ´ 250mm rpC18 nanoEase column and washed for 16 minutes with 2% acetonitrile, 0.1% formic acid in LC/MS grade water at a flow rate of 0.4 mL/minute. Peptides were separated using a 145 minute gradient elution method at a flow rate of 0.3 mL/minute. Peptides were gradient eluted with 0.1% formic acid from 2% to 20% (v/v) acetonitrile over 105 minutes then 20% to 32% (v/v) acetonitrile over 20 minutes. The column was washed with 95% (v/v) acetonitrile for 4 minutes and re-equilibrated at 2% (v/v) acetonitrile for 15 minutes at 0.4 mL/minute. Peptides were ionized and introduced to the HF-X using a Thermo Scientific Nanospray Flex™ Ion Source fitted with a Fossil Ion Technologies nanoESI emitter. Data acquisition was performed by selecting the top 12 precursors ions from 380 to 1580 m/z at 120,000 resolution with a AGC target of 3E6 and a maximum fill time of 45 ms. MS2 fragment ion spectra were collected with a 1.4 m/z isolation window and a normalized collision energy of 27 at 7,500 resolution with an AGC target of 1E5 and a maximum fill time of 100 ms. Only charge states of 2 to 6 were considered. Dynamic exclusion was set for 25 seconds.