Supporting Information

Micropathological chip modelling the neurovascular unit response to inflammatory bone condition

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Figure S1 – 2D tube formation assay and viability assay of endothelial cells on different substrates. Phase contrast images of 2D tube formation assay on Collagen/Fibrin (A), on Fibrin (B) and Matrigel (C). Images of cell viability assay performed with live&dead assay on Collagen/Fibrin (D), on Fibrin (E) and Matrigel (F). Scale bars: 100 µm.
Figure S2 – Osteoclasts culture in microfluidic devices. A – Schematic representation of the closed microfluidic device, with the osteoclasts seeding channel in green. B - Murine osteoclasts seeded within the microfluidic channel, in PDL-coated coverslips; C – Murine osteoclasts seeded within the microfluidic channel, in Collagen I-coated coverslips. Actin stained in green, nuclei in blue. Scale bars: 100 µm.

Figure S3 – Assemble of Vascular and bone units. Optical microscopy images of endothelial cells in the central compartment and osteoclasts in the open microfluidic area. Scale bar 500 µm.
Figure S4 – Control conditions for the characterization of the bone-vascular unit. A – Permeability assay control with bone unit medium; B – Permeability assay control with conditioned media; C – Permeability assay control with empty chambers. Data represented as mean ± SD; * P < 0.05 and **** P < 0.001.

Figure S5 – Axonal growth quantification in microfluidics. Control conditions of DRG sensory neurons exposed to fresh medium without cell contact (VN-medium and VN-medium +IL1b), and conditioned medium from osteoclasts and osteoclasts exposed to IL-1b. A – lambda values; B – A values. Data represented as mean ± SD; * P < 0.05 and ** P < 0.01.
Figure S6 - Ibuprofen-loaded PLGA Nanoparticles treatment. Nanoparticles were not observed within the endothelial cells (A) nor in the axonal projections (B). White arrows heads pointing to FITC PLGA nanoparticles in green. DRG showed a healthy morphology without fragmented neurites under inflammatory IL-1b stimulation (C) and when treated with ibuprofen-loaded nanoparticles (D). Actin stained in red and nuclei in blue, scale bar: 100 µm. (A). NF200 stained in red, nuclei in blue and nanoparticles in green (B, C and D). Scale bars: 100 µm (B), 500 µm (C, D).

Captions Videos:

Video S1: Loading of viscous empty hydrogel (light blue) into the central channel of the microfluidic device. The micropillars allow to constrain the hydrogel in the main channel. At t=80s, aqueous medium (dark blue) was added to the closed bone channel.

Video S2: 3D reconstruction of lumen-like structures of endothelial cells within the microfluidic device at day 1 of culture. Confocal images reconstructed using IMARIS software 3.6.1.

Video S3: 3D reconstruction of lumen-like structures of endothelial cells (green) when cultured with sensory neurons (red) within the microfluidic device at day 6 of culture. Confocal images reconstructed using IMARIS software 3.6.1.