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Zeta-Titration of SiO₂-Nanoparticles Amino-80 functionalized 60 Calcined 40 Zetapotential 20 0 -20 -40 -60 -80 рΗ 9 4 5 7 8 3 6 10



С



(A) The mesopore size and the specific surface areas of the MSNs were determined by nitrogen sorption at 77 K. (Qadrasorb, Micromeritics) and the isotherm is shown below. The BET surface

area was 1121 m²/g and the BJH desorption pore diameter distribution peaked at 3,2 nm. (B) Only one reflection with a maximum intensity corresponding to a d-spacing of 3.6 nm was observed in SAXRD (Bruker Nanostar equipped with a Cu source) indicative of a disordered pore structure. (C) The iso-electric point (IEP) was determined by titration in pure water using HCl and NaOH for pH adjustment (Malvern Zetasizer). The IEP of the calcined particles was about 4, while the amino-functionalised MSNs had an IEP of 7.8. (D) The hydrodynamic particle diameter was measured by dynamic light scattering (DLS)





Supplementary Information 2. Biocompatibility of C2C12 myoblasts on MSN films. (A) Light microscopy of C2C12 myoblasts on MSN films at 3, 6, 24 and 72 hrs. (B) Fluorescent microscopy images of Phalloidin labeled C2C12 cells cultured on MSN films for at 3, 6, 24 and 72 hrs. (C) SEM images of C2C12 cells cultured on MSN films for at 24, 48, 72 and 96 hrs.









Supplementary information 3. Quantification of MHC, nestin and Notch protein levels on western blots of cells cultured on cover slips and MSNs based films.



time/hrs

В



С



Supplementary information 4. Prolonged incubation of MSNs-based films in biological medium does not coincide with toxicity. (A) ICP analyses of the SiO₂ content in medium collected at 24, 48 and 72 hrs from the following samples: coverslips, coverslips with cells, films, films with cells and only medium. (B) SEM images of films incubated in serum containing-cell medium at 37°C for 1,2,3, and 4 days. (C) C2C12 cells were incubated with conditioned or control, normal medium for 48 hrs and viewed by light microscopy. No evidence of necrotic or apopotic cell death could be observed and the cells reached the same confluency over a period of 48 hrs. The time point in the images denote the time the films been incubated in the conditioned medium.

S5



Supplementary information 5. Cell internalization and intracellular release of a model drug. C2C12 cells were cultured on films consisting of TRITC (red) labeled MSNs loaded with the model drug DiO (green). Cell internalization and drug release was analyzed by fluorescence and confocal microscopy. DiO drug release was evident by the green labeling of cells in line with

efficient DiO-release and incorporation of DiO into cellular membranes. Particles in red was observed compartmentalized in intracellular vesicles



Supplementary information 6. (A) A black and white confocal images C2C12 cells grown on polymer scaffolds impregnated with DiI loaded particles. Cell labeled with DiI are observed on the fibers (B) Confocal image of part of a cell cultured on the particle impregnated scaffold. Asterix denote intracellular particles (DiI-red loaded and FITC-green labeled). As the particles accumulate in endosomal vesicles the intracellular dots are larger than the individual particles covering the scaffolds (small clearly aligned green dots). Note the reflective image of the fibers in red. FITC is a pH sensitive dye that is quenched in the lower pH of late endosomes-lysosomes

where the drug is released from the particles and hence the green signal weakens at places

whereas the red DiI retains its intensity^{1,2,3}

¹Rosenholm JM, Peuhu E, Eriksson JE, Sahlgren C*, Linden M* (2009) Targeted intracellular delivery of hydrophobic agents using mesoporous hybrid silica nanoparticles as carrier systems. *Nano letters* **9**: 3308-3311

²Rosenholm JM, Peuhu E, Bate-Eya LT, Eriksson JE, Sahlgren C*, Linden M* (2010) Cancer-cellspecific induction of apoptosis using mesoporous silica nanoparticles as drug-delivery vectors. *Small* **6**: 1234-1241

³Mamaeva V, Rosenholm JM, Bate-Eya LT, Bergman L, Peuhu E, Duchanoy A, Fortelius LE, Landor S, Toivola DM, Linden M, Sahlgren C (2011) Mesoporous Silica Nanoparticles as Drug Delivery Systems for Targeted Inhibition of Notch Signaling in Cancer. *Molecular Therapy* **19**: 1538-1546