Supporting Information

Fabrication of hydrogel microspheres via microfluidics using inverse electron demand Diels-Alder click chemistry-based tetrazine-norbornene for drug delivery and cell encapsulation applications

*Rubén Pareja Tello[1], Shiqi Wang[1], Flavia Fontana[1], Alexandra Correia[1] Giuseppina Molinaro[1], Sandra López Cerdà[1], Sami Hietala[2], Jouni Hirvonen[1], Gonçalo Barreto[3,4,5], Hélder A. Santos[1,6,7,]**

[1] Drug Research Program, Division of Pharmaceutical Chemistry and Technology, University of Helsinki, Helsinki FI-00014, Finland

[2] Department of Chemistry, University of Helsinki, Helsinki FI-00014, Finland

[3] Translational Immunology Research Program, Faculty of Medicine, University of Helsinki, PL 4 (Yliopistonkatu 3), 00014, Helsinki, Finland

[4]Orton Orthopedic Hospital, Tenholantie 10, 00280, Helsinki, Finland

[5]Medical Ultrasonics Laboratory (MEDUSA), Department of Neuroscience and Biomedical Engineering, Aalto University, 02150, Espoo, Finland

[6] Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, Ant. Deusinglaan 1, 9713 AV Groningen, The Netherlands

[7] W.J. Kolff Institute for Biomedical Engineering and Materials Science, University Medical Center Groningen, University of Groningen, Ant. Deusinglaan 1, 9713 AV Groningen, The Netherlands

*Corresponding author: h.a.santos@umcg.nl

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Table S1. Calculated parameters obtained from the analysis of the release studies according to Zero-order, First-order, Higuchi and Korsmeyer-Peppas models.

Formulation	Correlation coefficient (r^2)			Korsmeyer-Peppas model	
	Zero-order model	First-order model	Higuchi model	r^2	n
10% PEG-TZ pH 7.5	0.954	0.979	0.988	0.996	0.443
20% PEG-TZ pH 7.5	0.898	0.947	0.985	0.997	0.194
10% PEG-TZ pH 5.5	0.902	0.958	0.980	0.991	0.304
20% PEG-TZ pH 5.5	0.913	0.945	0.832	0.985	0.455

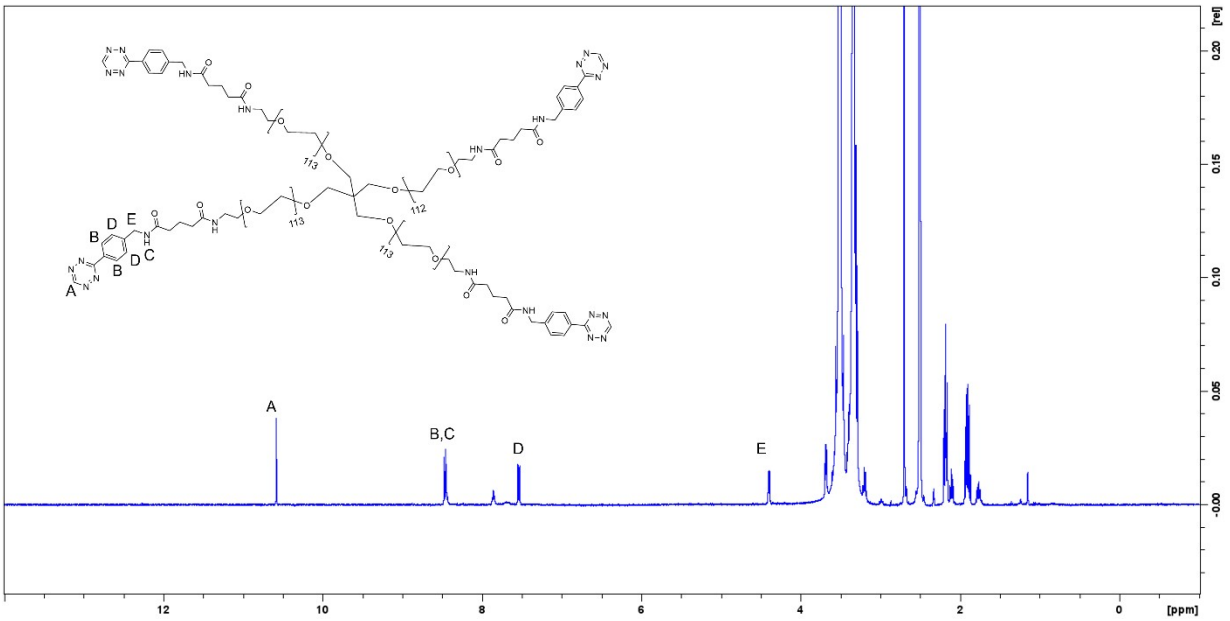

Figure S1. ^{1}H-NMR spectra of 4-arm PEG-TZ

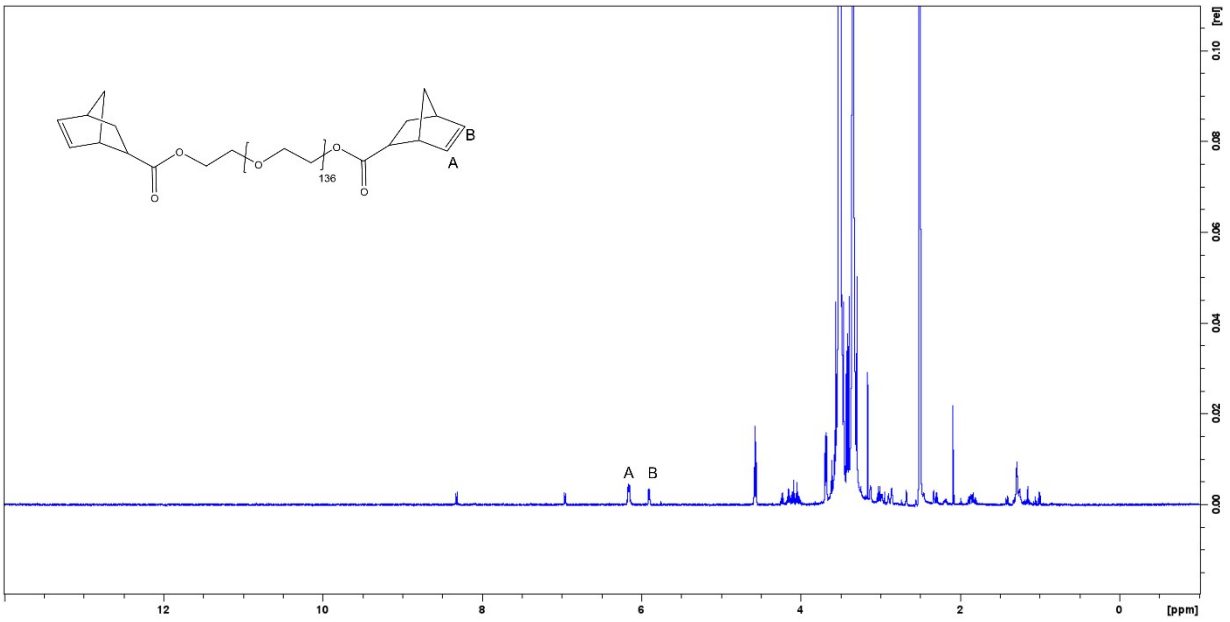

Figure S2. ^{1}H-NMR spectra of 4-arm PEG-NB

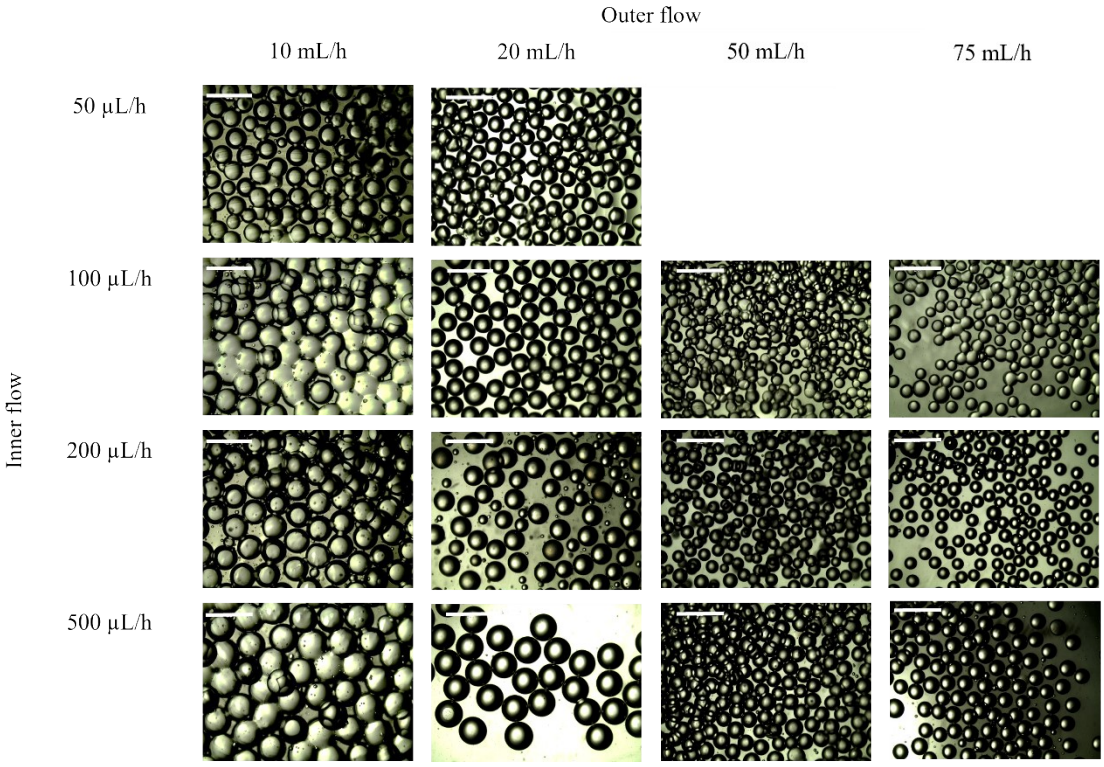

Figure S3. Optimization of MPs production based on inner flow values within 50 and 500 µL/h and outer flow values within 10 and 75 mL/h. Scale bar 1000 µm.

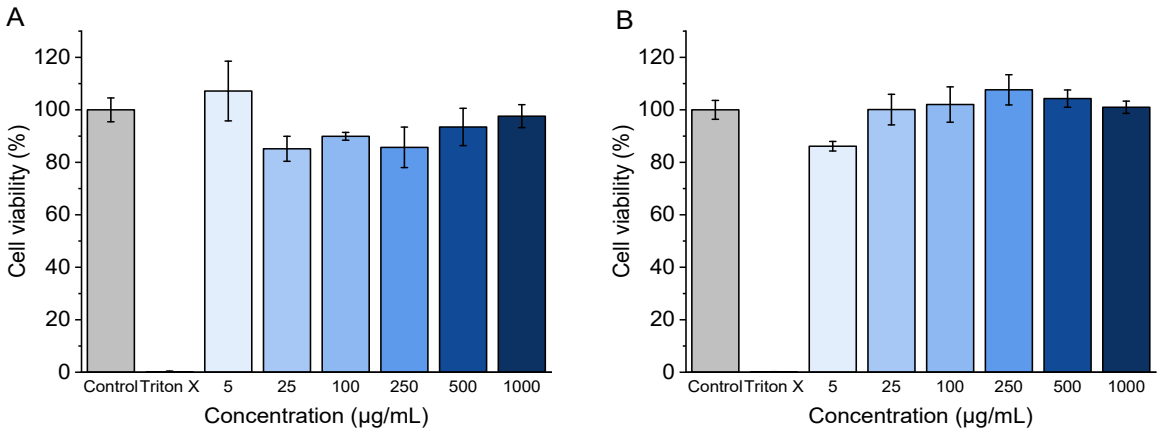

Figure S4. Cell proliferation of primary human tenocytes relative to control at the incubation with MPs' concentrations from 5 µg/mL to 1000 µg/mL at time-points of 24 h **(A)** and 72 h **(B)**. The results were plotted as mean ± standard deviation (n=3 biological replicates in which each time three technical replicates have been used). A one-way ANOVA followed by a Turkey-Kramer post hoc test was used for the statistical analysis for comparison with the medium, which was used as a control in all tests. The significance levels of the differences were set at the probabilities of *$p<0.05$, **$p<0.001$ and ***$p<0.0001$.

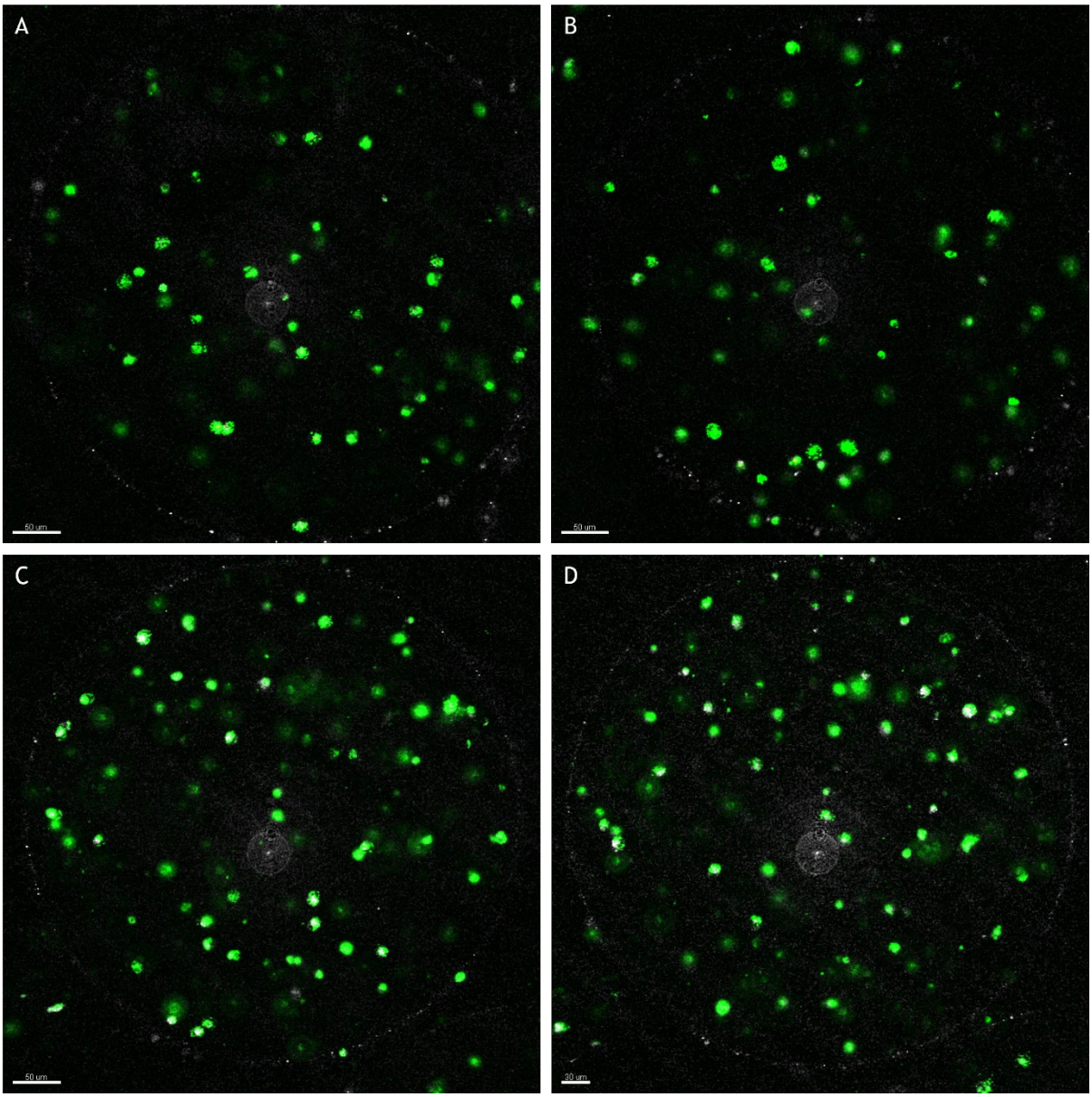

Figure S5. Representative Live/Dead z-stack confocal images of RAW cells encapsulated in 10% of PEG-TZ at 37 °C. Overlay of images of green fluorescence (Calcein AM positive cells), red fluorescence (Ethidium homodimer-1 stained cells) and bright field. **(A-B)** 1 day after encapsulation. Scale bar 50 µm **(C-D)** 7 days after encapsulation. Scale bars 50 and 30 µm.