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Articular cartilage- and synoviocyte-binding poly(ethylene glycol) nano-composite microgels as intra-articular drug delivery vehicles for the treatment of osteoarthritis

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Supplementary Information:

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1. Supplementary materials and methods

1.1. Materials

Poly(lactic-co-glycolic) acid (50:50, MW: 24,000 - 38,000 Da), poly-vinyl-alcohol (PVA, MW: 31,000 - 50,000 Da, 87-89% hydrolyzed), dichloromethane (DCM, anhydrous, ≥99.8%), mineral oil, SPAN80, bovine serum albumin (BSA, ≥98%), rhodamine B (≥95%), Optiprep™ density gradient medium and methanol (anhydrous, 99.8%) were purchased from Sigma-Aldrich. Four-arm poly(ethylene-glycol)-maleimide (PEG-4MAL (20 kDa) was acquired from Laysan Bio, phosphate buffered saline with Ca++ and Mg++ ($PBS^{+/+}$) from Corning, HEPES buffer (1M) from Life Technologies. Ultra-pure dithiothreitol (DTT), GibcoTM Ham's F12 nutrient mix, CellTracker Green™ and Alexa Fluor™ 488-NHS ester were purchased from Thermo Fisher Scientific. Articular cartilage-, synoviocyte- or integrin-binding peptides (WYR, HAP-1 and RGD) and their scrambled sequences (WYRsc, HAP-1sc and RDG) were custom-synthesized by GenScript. Near-infrared (NIR) dyes cyanine 7 (Cy7), sulfo-cyanine 7 and cyanine 7-NHS ester were purchased from Lumiprobe Life Science Solutions, and Conray contrast agent (60%) was obtained from Henry Schein™. HIG-82, C2C12 and SW982 cell lines were purchased from ATCC and MC3T3-E1 from RIKEN. Male Lewis rats (250-300g) were acquired from Charles River Laboratories.

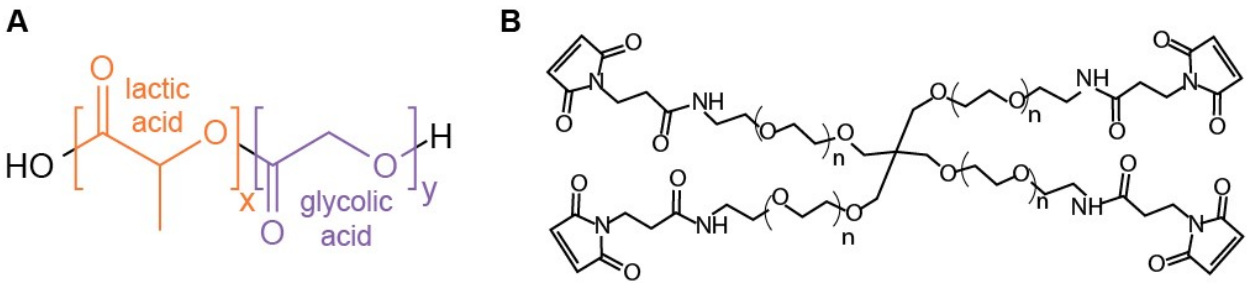

Figure S1. Chemical structure of A) poly(lactic-co-glycolic) acid and B) 4-arm poly(ethylene glycol) maleimide.

1.2. PLGA NP distribution within PEG-4MAL microgels

Confocal microgel images containing either 0.25, 0.50 or 1.00% w/v PLGA NPs were used to determine their distribution within PEG-4MAL microgels using a customized MATLAB script. Rhodamine B-loaded PLGA NPs content inside individual microgels was measured as the microgel's cross-sectional area fraction corresponding to fluorescent pixels. For each formulation, a distribution of the PLGA NPs content per microgel was generated using at least 200 microgels per group.

1.3. WYR-functionalized PEG-4MAL/PLGA NPs microgel in vivo stability

To determine the ability of PEG-4MAL/PLGA NPs microgels to withstand the IA mechanical loads, fluorescently labeled microgels were synthesized by conjugating WYR peptide to AlexaFluor 488-NHS ester, prior to microgels formation according to manufacturer's instructions. Then, microgels containing 0.5% w/v PLGA NPs were injected bilaterally in healthy knee joints of male Lewis rats (50µL/injection, microgels suspended in sterile saline). A knee lavage using 100 µL of sterile saline was performed on days 4, 8, 14 and 21 (2 knees per time point). Then, 100 µL of the recovered lavage were incubated with 50 µL of lysis buffer for 2 min to remove any cells and imaged using confocal microscopy. The size of at least 80 microgels per time point was calculated using a customized MATLAB script (MATLAB, v 9.7 (R2019b), Natick, Massachusetts: The MathWorks Inc.).

2. Supplementary data

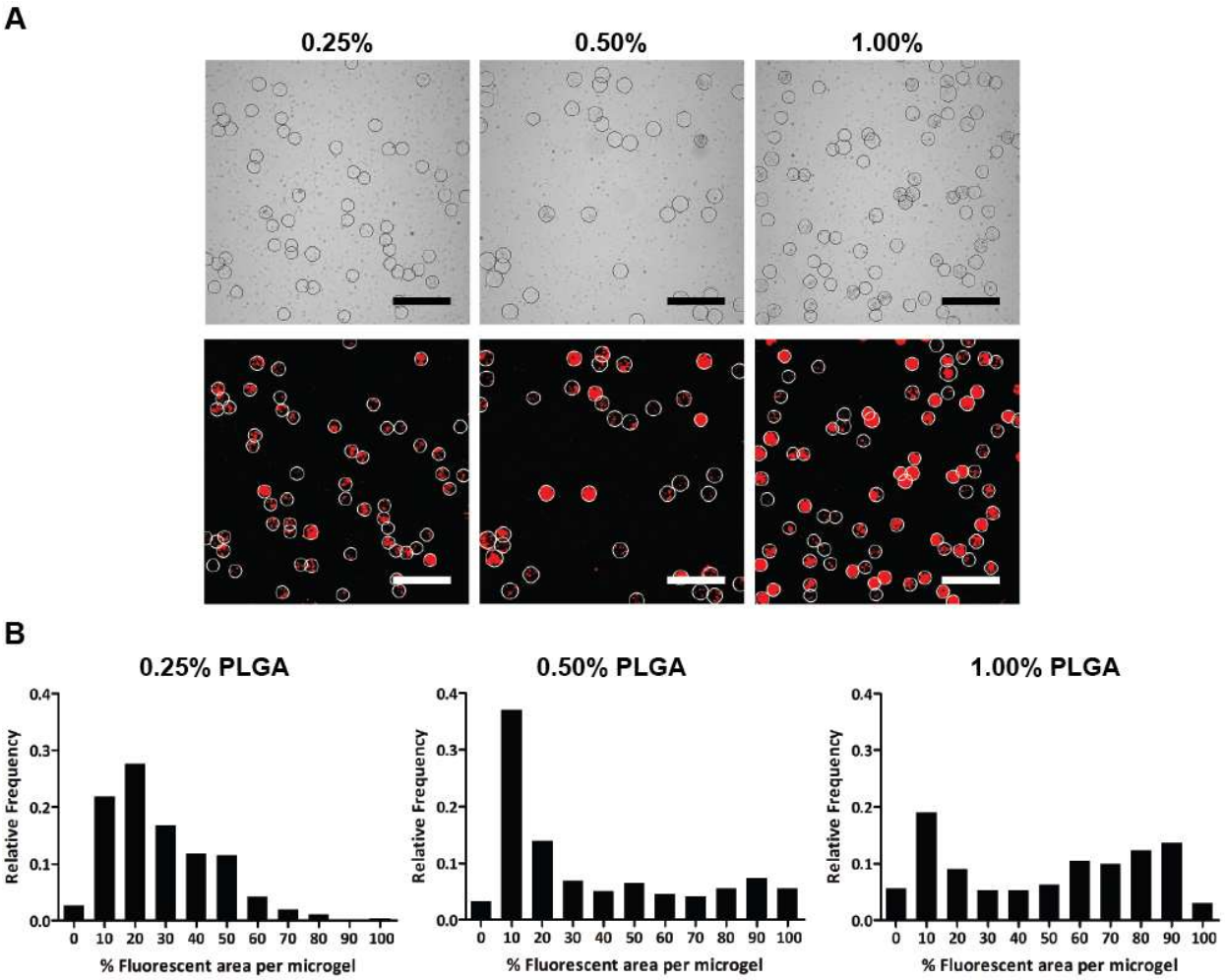

Figure S2. PLGA NP encapsulation into PEG-4MAL microgels. A) Representative confocal images of microgels containing 0.25, 0.50 or 1.00% w/v PLGA NPs. Bright field (top) and red fluorescence (below) channels, with microgels outlined with white circles. Scale bar: 200 µm. B) Distribution of PLGA NPs inside PEG-4MAL microgels measured as the microgels area fraction corresponding to fluorescent pixels. Results demonstrate that PLGA NPs distribution into PEG-4MAL microgels shifts from a unimodal to a bimodal distribution as the PLGA NP concentration increases.

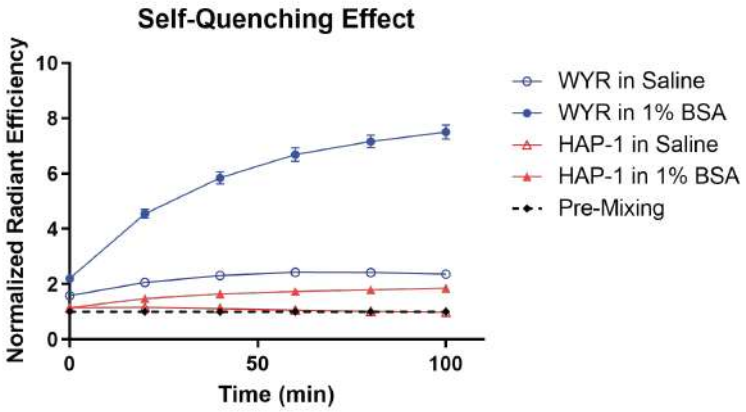

Figure S3. Self-quenching effect of peptide-functionalized PEG-4MAL microgels was evaluated *in vitro*. WYR and HAP-1 peptide were conjugated with Cy7-NHS ester dye prior to microgels synthesis. Microgels (50 µL) were placed in a 96-well plate and their radiant efficiency was measured (n=8). 50 µL of saline (control) or a solution of 1% BSA was added to the microgels formulations (n=4). This was done to simulate the *in vivo* IA injection scenario where microgels suspended in saline mix with synovial fluid, represented here by the 1% BSA solution. Microgels radiant efficiency was measured via IVIS immediately after mixing and every 20 min up to a total of 100 min. Radiant efficiency at each time point was normalized to the pre-mixing fluorescence. Results demonstrate that an apparent increase in fluorescence occurs after diluting both microgels formulations with either saline or 1% BSA. Interestingly, this effect is more pronounced when diluting the microgels in 1% BSA. This occurs because the solution of 1% BSA is a better solvent than saline only and helps solubilize Cy7. Also, WYR-functionalized PEG-4MAL microgels presented a considerably higher degree of self-quenching compared to HAP-1 microgels as measured by a difference in the plateau values obtained after a one-phase association curve fit (Welch's ANOVA, $p<0.0001$).

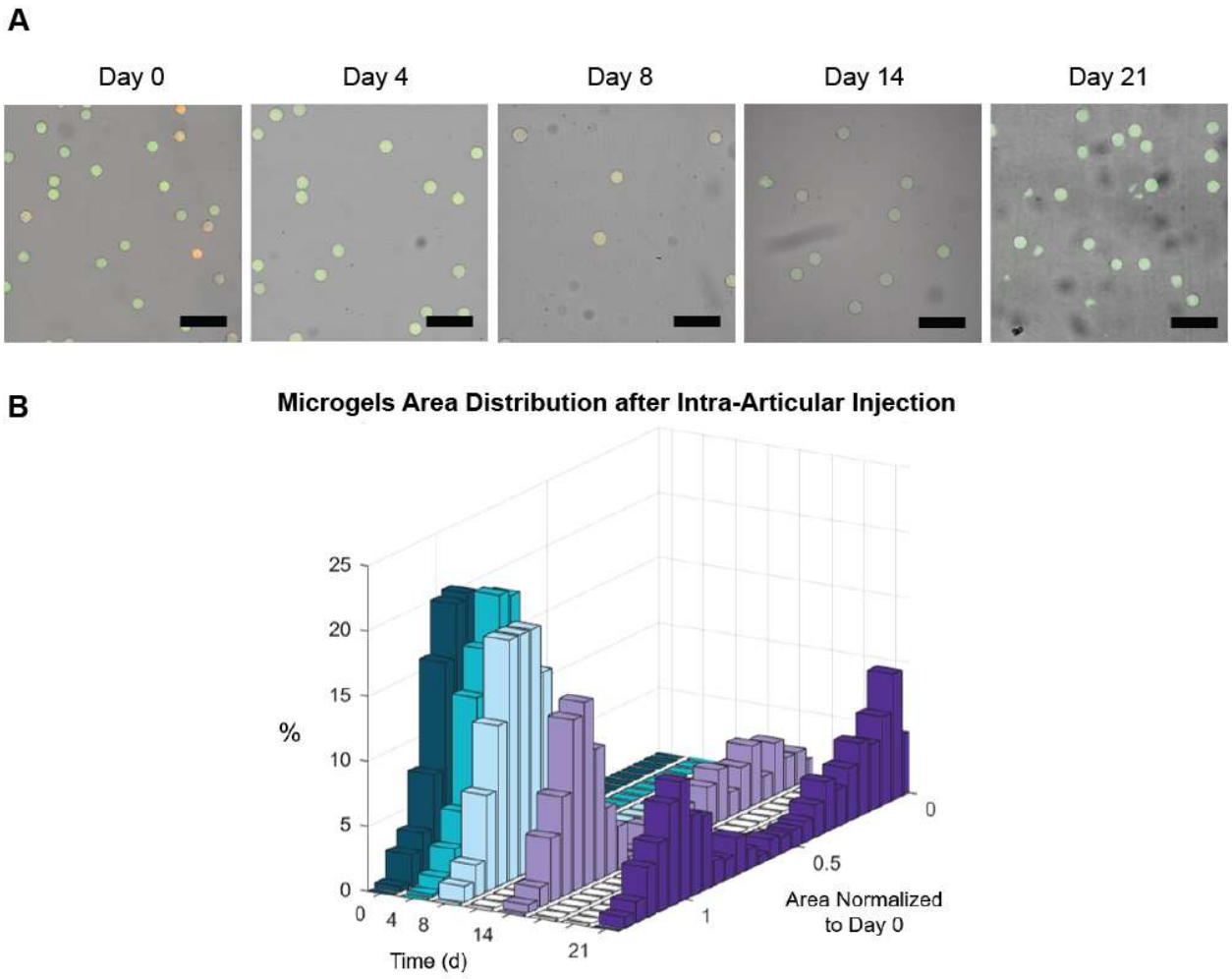

Figure S4. Stability of WYR peptide-functionalized PEG-4MAL nano-composite microgels *in vivo*. A) Representative confocal images of PEG-4MAL microgels (green) recovered via intra-articular lavage. Microgel size is reduced by day 14 leading to presence of smaller, irregular hydrogel fragments. Scale bar: 200 µm. B) Microgel area histogram at different time points after intra-articular injection demonstrate that at days 14 and 21 smaller microgels are observed (Distributions obtained from the lavage of 2 knees at every time point).