Towards an Arthritis Flare-Responsive Drug Delivery System

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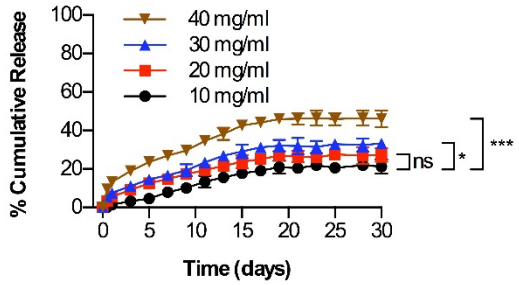
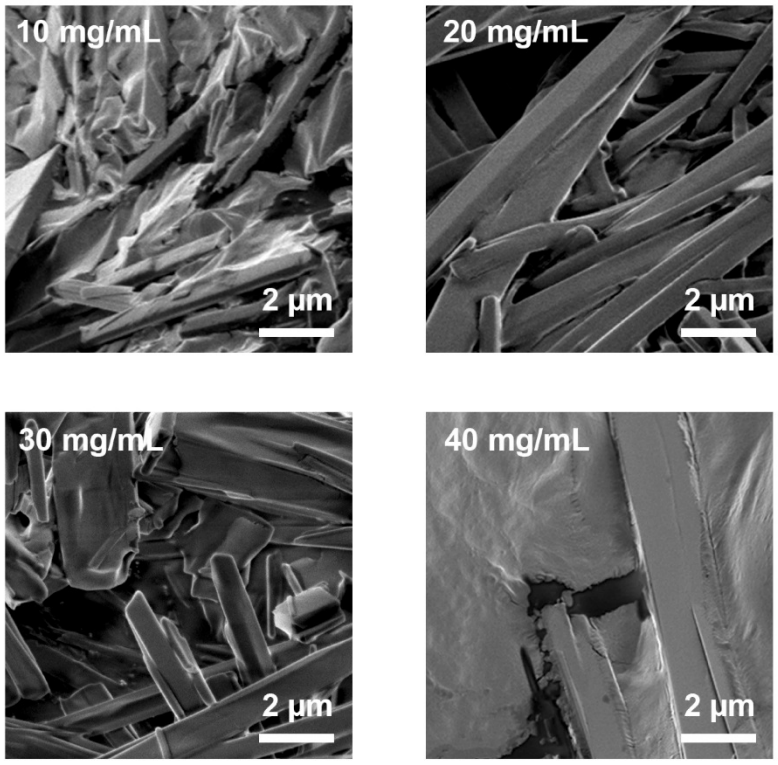
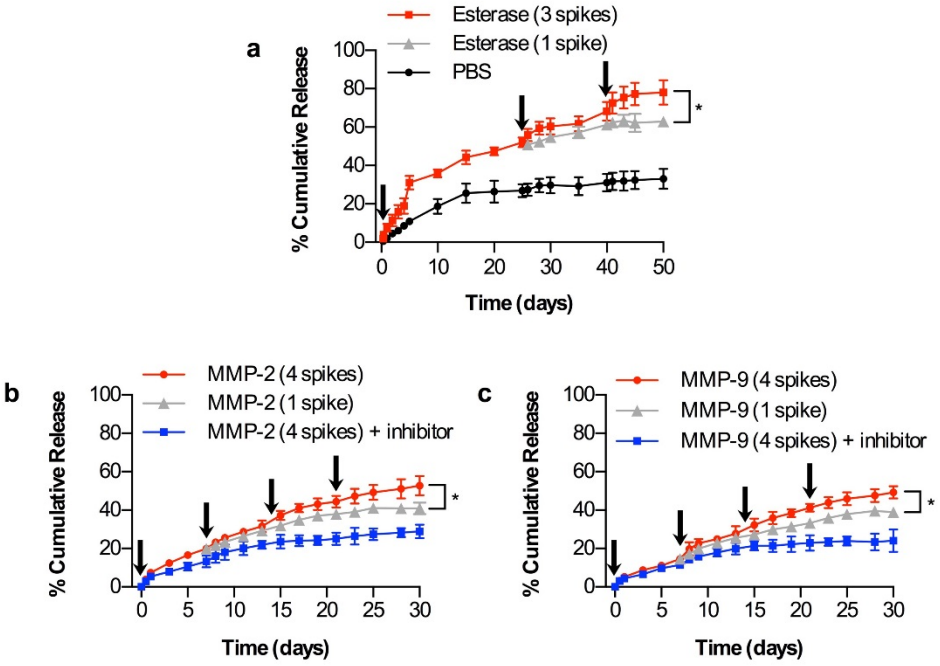
Supplementary Figures



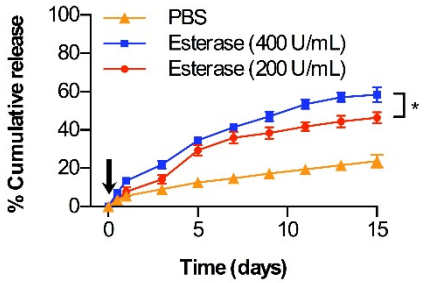
Supplementary Figure 1: TG-18 hydrogel shows dose-response relationship between TA concentration and its encapsulation stability. *In vitro* release kinetics of TA in PBS (pH 7.4) at 37°C comparing hydrogel formulations generated with same amount of TG-18 (10 % w/v), but loaded with different concentrations of TA. *P<0.05 and **P<0.01 on day 30. P values were determined by one-way Anova with Tukey's post hoc analysis. Data are means ± SD for technical repeats (n = 3, experiment performed at least twice).



Supplementary Figure 2: High resolution scanning electron microscopy (HR-SEM) images of TA-loaded TG-18 hydrogels formulated with different TA concentrations. **(a)** 10 mg/ml; **(b)** 20 mg/ml; **(c)** 30 mg/ml and **(d)** 40 mg/ml.

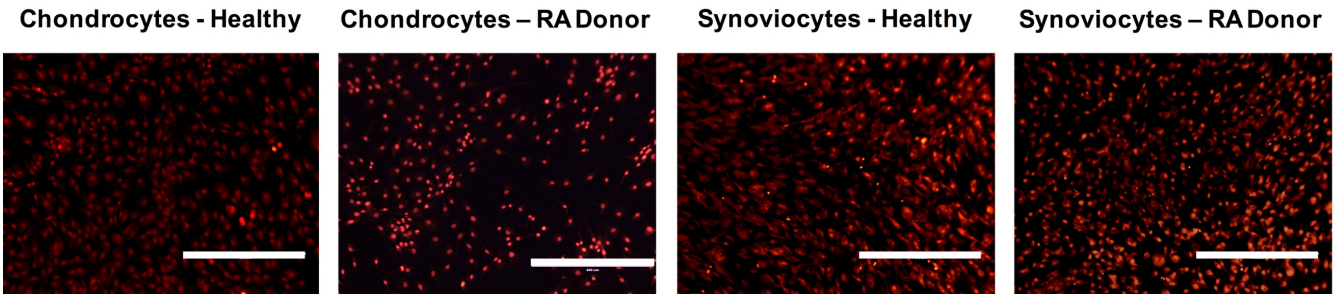


Supplementary Figure 3: A single pulse of enzyme results in less cumulative release of TA compared to multiple pulses. *In vitro* release kinetics of TA from TG-18 hydrogel in PBS without or with a single or multiple pulses of enzymes, including **(a)** esterase (200 U/ml), **(b)** MMP-2 (1.5 µg/ml) and **(c)** MMP-9 (1 µg/ml). Fresh enzyme or enzyme + inhibitor were added at the indicated time points (arrows). For single pulse based releases, enzyme was added only on day 0. For (a), **P*<0.05 on day 50 and for (b) and (c), **P*<0.05 on day 30. *P* values were determined by Students' *t* test. Data are means ± SD for technical repeats (n = 3, experiment performed at least twice).

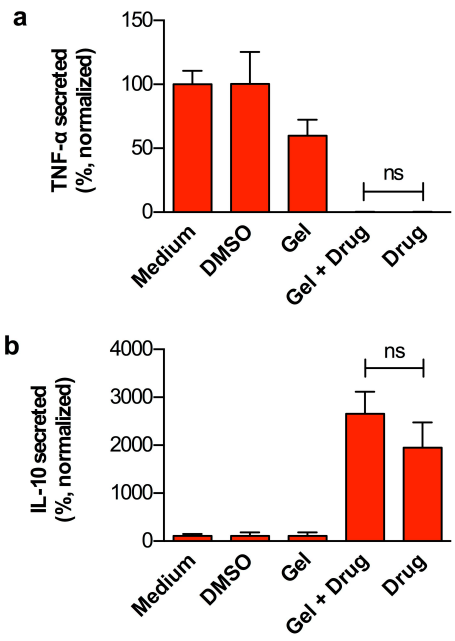


Supplementary Figure 4: TA-loaded TG-18 hydrogel shows dose-response relationship between enzyme concentration and TA release. *In vitro* release kinetics of TA from TG-18 hydrogels in PBS without or with different concentrations of esterase (T. *lanuginosus* lipase) (**P*<0.05). Enzyme was added at the indicated time

point (arrow). *P* values were determined by one-way Anova with Tukey's post hoc analysis. Data are means ± SD for technical repeats (n = 3, experiment performed at least twice).

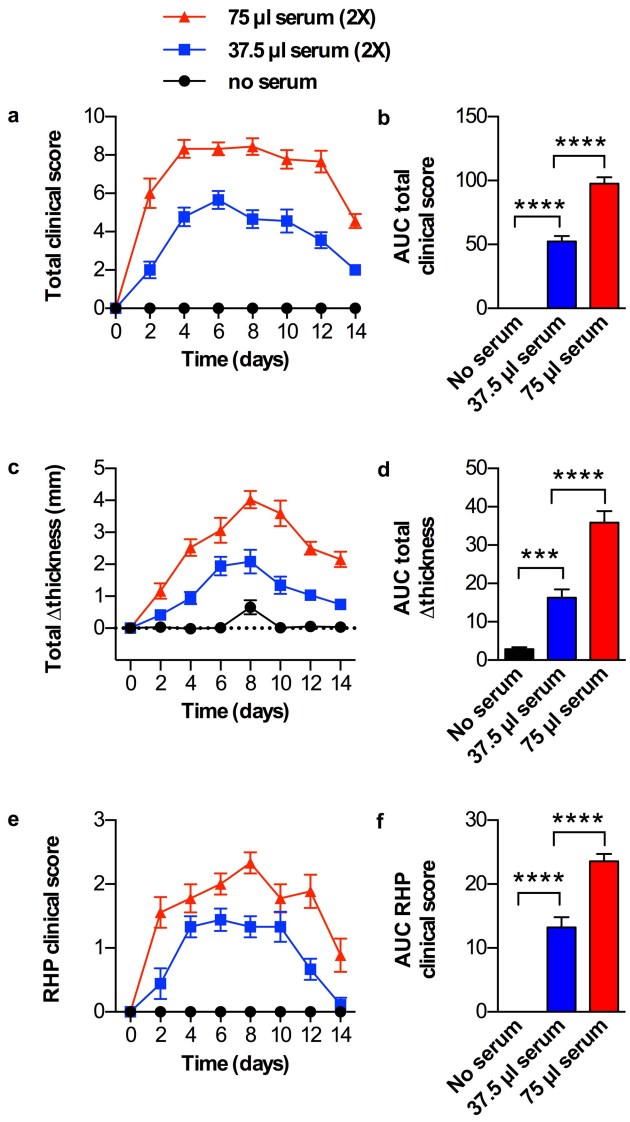


Supplementary Figure 5: Representative fluorescence microscopy images from the live/dead assay of control primary human chondrocytes or synoviocytes treated with 70% methanol solution for 30 minutes. Live/dead assay was performed using calcein-AM/ethidium homodimer-1 dye combination. Live cells stain green with the calcein-AM, whereas dead cells stain red with ethidium homodimer-1 (Scale bar: 400 µm).

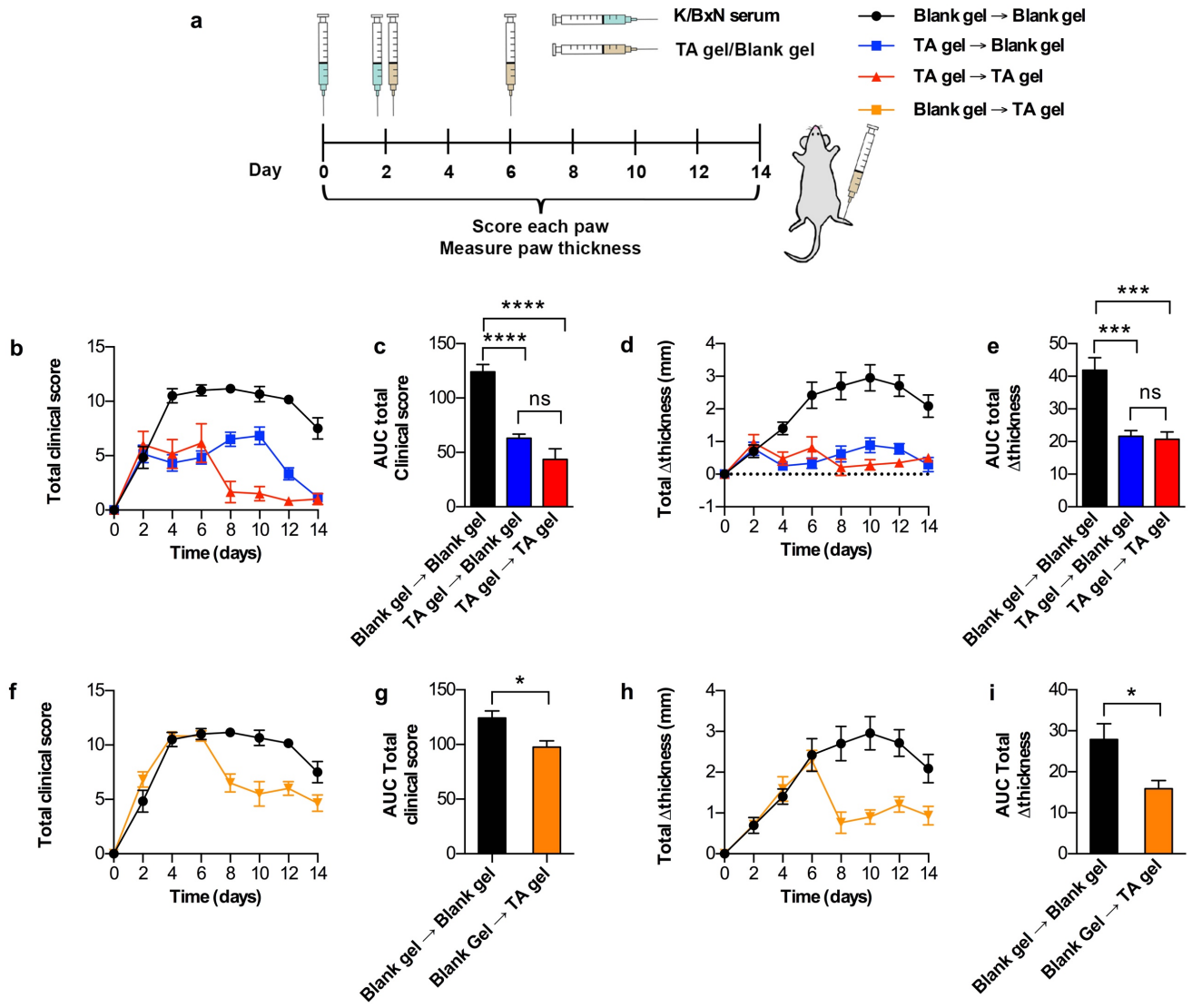


Supplementary Figure 6: TG-18 hydrogel does not compromise the therapeutic efficacy of TA *in vitro*. **(a)** TNF-α secretion and **(b)** IL-10 secretion from LPS stimulated human macrophages incubated in a transwell plate in medium (Medium) or in medium with 100 µl blank hydrogel (Gel), 100 µl TA-loaded hydrogel (Gel + Drug), DMSO (equivalent to 100 µl Gel) or free TA (Drug, equivalent to 100 µl Gel + Drug) added to the upper

chamber of the transwell. Following 24 h incubation, supernatant was assayed for TNF-α and IL-10 levels by ELISA, and normalized to the levels obtained for cells incubated with medium only. *P* values were determined by one-way Anova with Tukey's post hoc analysis. Data are means ± SD for technical repeats (n = 3, performed at least twice).



Supplementary Figure 7: Two doses of 75 µl K/BxN serum resulted in more severe arthritis than two doses of 37.5 µl serum. (a,b) Total clinical score curves and their AUCs (****P<0.0001). **(c,d)** Change in total paw thickness curves and their AUCs (***P<0.001 and ****P<0.0001). **(e,f)** RHP clinical score curves and their AUCs (****P<0.0001). P values were determined by one-way Anova with Tukey's post hoc analysis. Data are means ± SEM (n = 6 mice/group, experiment performed twice).



Supplementary Figure 8: Local delivery of TA loaded TG-18 hydrogel demonstrates therapeutic efficacy in severe arthritis: effect of dosing regimen. (a) Experimental outline: Arthritis was induced by i.p. injections of 75 µl KBx/N serum on day 0 and day 2. Immediately after the second dose of K/BxN serum, animals were randomized into four groups. (Blank gel → Blank gel) group received blank hydrogel (4 µl) on day 2 and 6, (TA gel → Blank gel) group received TA-loaded TG-18 hydrogel (20 mg TA/ml, 4 µl) on day 2 and blank hydrogel on day 6, (TA gel → TA gel) group received two doses of TA-loaded TG-18 hydrogel – one on day 2 and another on day 6 and (Blank gel → TA gel) group received blank hydrogel on day 2 and TA-loaded TG-18 hydrogel on day 6. Treatments were injected into the right hind paw. Every other day, arthritis severity was scored clinically and paw swelling was measured with calipers. **(b,c)** Total clinical score curves and their AUCs for (Blank gel → Blank gel), (TA gel → Blank gel) and (TA gel → TA gel) (****P<0.0001). **(d,e)** Change in

total paw thickness curves and their AUCs for (Blank gel → Blank gel), (TA gel → Blank gel) and (TA gel → TA gel) (***P<0.001). **(f,g)** Total clinical score curves and their AUCs for (Blank gel → Blank gel), (TA gel → Blank gel) and (Blank gel → TA gel) (*P<0.05). **(h,i)** Change in total paw thickness curves and their AUCs for (Blank gel → Blank gel), (TA gel → Blank gel) and (Blank gel → TA gel) (*P<0.05). For (c,e), P values were determined by one-way Anova with Tukey's post hoc analysis and for (g,i), P values were determined by Student's t test. Data are means ± SEM (n = 6 mice per group).