

# Extended intra-articular presence of fluorescent C' Dot nanoparticles in naïve and arthritic rat knee joints

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**INTRODUCTION:** Rapid clearance of molecules from synovial fluid on the order of hours is one challenge which prevents injected intra-articular (IA) osteoarthritis (OA) therapeutics from reaching tissue-resident joint cells to halt or reverse progression of the disease<sup>1</sup>. For example, small molecule therapeutics for rheumatoid arthritis, such as anakinra (Kineret) and adalimumab (Humira), are cleared from the joint within 1-3 days and are not effective for OA<sup>1</sup>. Recent studies have extended joint presence by 2-4 weeks using nanomaterials; however, standard clinical recommendations limit the frequency of IA injections to once every 3-6 months<sup>2</sup>. We have shown previously that ultrasmall fluorescent poly(ethylene glycol) coated silica nanoparticles (C' dots) penetrate the articular surface of healthy cartilage within 2 hours to reach tissue-resident chondrocytes *in vitro*<sup>3</sup>. C' dots have been used in multiple investigational new drug (IND) FDA-approved diagnostic and therapeutic human oncology clinical trials showing favorable biodistribution and pharmacokinetics (PK) due to their small size, enabling renal clearance<sup>4</sup>. In this study, we investigate C' dots as a therapeutic delivery platform for OA. We employ a rat surgical model of post-traumatic OA (PTOA) to evaluate joint clearance PK of untargeted C' dots in naïve and arthritic stifles over 8 weeks.

**METHODS:** C' dots covalently encapsulating Cyanine-5 (Cy5) were synthesized following previously established methods<sup>5,6</sup>. Following IACUC approval, adult male Sprague Dawley rats (n=18, 300-325g) were randomly assigned to: unoperated (i.e. naïve) with intra-articular injections of (1) bilateral Cy5 dye (n=2), (2) unilateral C' dot with contralateral saline (n=4), (3) bilateral C' dot (n=4), and (4) animals receiving ipsilateral anterior cruciate ligament transection (ACLT) with contralateral sham surgery and bilateral C' dot injection one-week postoperatively (n=6). Injection volumes of 25 µl of: saline, 10 mM Cy5 dye in saline or 34 µM C' dots in saline were injected into the knee via a parapatellar approach<sup>7</sup>. **Fluorescence imaging:** In vivo total joint fluorescence was captured longitudinally over 8 weeks using the In Vivo Imaging System (IVIS) Kinetic, and images were also obtained post-euthanasia following dissection of the knee joint. Total Radiant Efficiency (TRE) was quantified in region-of-interest boundaries (ROIs) of identical dimensions and TRE of saline-injected knees were subtracted as background. Background-subtracted TRE was normalized in each stifle for clearance analysis. **Analysis:** Models of exponential decay were fit to background-subtracted normalized TRE for each fluorescence-injected stifle. **Statistics:** Fitting parameters were statistically compared using linear mixed-effects model with Kenward-Roger approximation ( $\alpha = 0.05$ ).

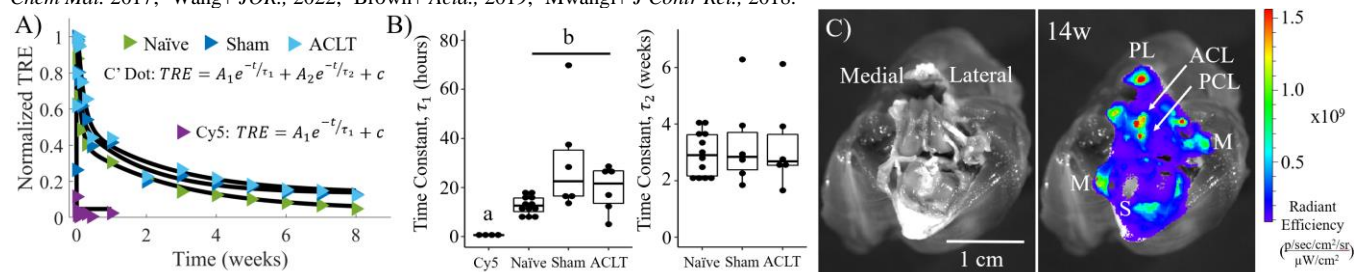
**RESULTS:** In vivo C' dot and Cy5 dye fluorescence were detected in knee joints for more than 8 weeks with signals decreasing over time (**Fig. 1A**). C' dots were detectable following euthanasia and joint dissection at 14 weeks, exhibiting extended presence in the joint (**Fig. 1C**). We found a mono-exponential decay describing Cy5 signal decay ( $\tau = 0.6$  h,  $R^2 > 0.98$ ) and a two-component model of bi-exponential decay describing C' dot signal decay (quick clearance:  $\tau_1 = 18$  h, slow clearance:  $\tau_2 = 22$  d;  $R^2 > 0.95$ ) (**Fig. 1A-B**). Clearance profiles of IA-injected C' dots were not different between naïve and arthritic conditions (Sham and ACLT) ( $p > 0.05$ ), with  $\tau_1$  trending towards longer times in operated stifles compared to naïve stifles (n.s.) (**Fig. 1B**). Fitting parameters  $A_1$ ,  $A_2$ , and  $c$  indicated that 64%/33% of injected C' dots in naïve stifles are described by shorter clearance and longer clearance, respectively, while 3% remains at 8 weeks. Similarly, 56%/37% of C' dots in all operated stifles are cleared in shorter/longer processes, respectively, while 7% remains at 8 weeks. C' dot fluorescence observed in dissected naïve joints 14 weeks post-injection was concentrated in the ACL, PCL, patellar ligament (PL), meniscus (M), and synovium (S). Signal was still present at lower levels in tibial plateau cartilage and condyles (**Fig. 1C**).

**DISCUSSION:** C' dots exhibited slow clearance that was well described with two time constants (bi-exponential decay,  $R^2 > 0.95$ ), indicative of two time-dependent clearance mechanisms. This phenomenon is distinct from the clearance of Cy5 dye, which is modeled by a single time constant. Half-lives of C' dots are more than an order of magnitude longer than Cy5 ( $t_{1/2} = 13$  h and 0.4 h, respectively), with the second half-life of C' dots ( $t_{1/2} = 15$  days) characterizing greatly extended joint presence. Time constants were not different between unilateral and bilateral injection of C' dots in naïve joints ( $p > 0.05$ ) or between unoperated and operated conditions ( $p > 0.05$ ). The shorter time constant describing C' dot clearance is comparable to half-lives of similarly sized solutes<sup>8</sup>. The longer time constant, however, may be describing rate-limited release of C' dots that initially diffused into tissues such as articular cartilage, meniscus, and ligaments, or were internalized by tissue-resident joint cells (**Fig. 1C**). These phenomena may have important beneficial implications for extending total joint presence. In surgically operated stifles, the quicker half-life trended towards longer times compared to naïve stifles, although this effect was not significant. Interestingly, this observation is inconsistent with observations of extended clearance of materials in surgically injured stifles where synovium thickening contributes to longer half-lives of injected dextran<sup>9</sup>. However, the difference in study timescale and evaluation of total joint fluorescence may account for differences in observed results. These observations inform the orthopedic community regarding nanoparticles of this size and design and suggest the potential of even untargeted C' dots for extended delivery of therapeutics in healthy joints and joints with mild arthritis consistent with this surgical model of PTOA<sup>7</sup>.

**SIGNIFICANCE:** Intra-articularly injected C' dot nanoparticles exhibit slow clearance from naïve and arthritic rat stifles that is well described by a bi-exponential model ( $t_{1/2} = 0.5$  days and 15 days) over 8 weeks, indicating two distinct physiologic processes. Untargeted C' dots exhibit extended joint presence at 14 weeks post-injection in ligaments, menisci, and synovium, indicating promise of long-term (> 3 months) delivery of therapeutics using this platform technology.

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**REFERENCES:** <sup>1</sup>Doan+ *Acta.*, 2019; <sup>2</sup>Ayhan+ *World J Orthop.*, 2014; <sup>3</sup>Fortin+ *ORS*, 2023; <sup>4</sup>Phillips+ *Sci Trans Med.*, 2014; <sup>5</sup>Ma+ *Chem. Mat.* 2015; <sup>6</sup>Ma+ *Chem Mat.* 2017; <sup>7</sup>Wang+ *JOR.*, 2022; <sup>8</sup>Brown+ *Acta.*, 2019; <sup>9</sup>Mwangi+ *J Contr Rel.*, 2018.



**Figure 1:** (A) Normalized background subtracted fluorescence quantification from IVIS images of representative naïve rats that received intra-articular injections of C' dots or Cy5 dye, and surgically injured rats (ipsilateral ACL transection, contralateral sham) that received bilateral injection of C' dots one week post-op. One-component (Cy5) and two-component (C' dots) exponential decay model fits are shown in black. (B) Time constants  $\tau_1$  and  $\tau_2$  for model fits shown in (A). Observed  $\tau$  of Cy5 dye was lower than  $\tau_1$  for C' dots ( $p < 0.001$ ) but no differences were observed in C' dot  $\tau_1$  or  $\tau_2$  for unoperated or operated conditions. (C) Representative fluorescence 14 weeks post-injection of C' dots in a dissected naïve stifle. Fluorescence is concentrated in ligaments (PL, ACL, and PCL), synovium (S), and menisci (M) and present at lower levels in the tibial plateau and condylar cartilage.