Articular cartilage protection via IGF-I targeted fluorescent silica nanoparticles

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INTRODUCTION: Disruption of chondrocyte homeostasis by interleukin-1β (IL-1β) and other pro-inflammatory cytokines in osteoarthritis (OA) leads to degradation of glycosaminoglycans (GAGs) and collagen in the extracellular matrix (ECM)[1]. Insulin-like growth factor-I (IGF-I) is a potent anabolic protein synthesized endogenously that protects cartilage integrity and counteracts cytokine-induced catabolic responses[2,3]. Despite great therapeutic promise, the translation of IGF-I for OA has been constrained by its short intra-articular half-life (on the order of hours), necessitating frequent dosing[4,5]. Nanoparticle-based delivery systems have emerged as promising platforms to improve intra-articular and intra-tissue IGF-I retention[4,5,6] however, most existing carriers covalently attach IGF-I which may limit its bioavailability and efficacy. Ultrasmall (d_h ~ 6 nm) poly(ethylene glycol)-coated fluorescent silica nanoparticles, known as Cornell Prime Dots (C' Dots), have demonstrated exceptional biocompatibility and clinical translatability in oncology applications[7,8,9,10]. The surface of C' Dots has been decorated with small molecules, peptides, proteins, and antibodies using facile click chemistry[8,11]. In a rat model for OA, C' Dots were investigated as a cartilage-penetrating drug delivery vehicle and demonstrated long-term joint retention (> 3 months) after a single injection[12]. Recently, an IGF-I-binding peptide was derived from the insulin-like growth factor-binding protein-5 (IGFBP-5) binding pocket. Polymers modified with this peptide exhibited high affinity for free IGF-I (K_D ~ 50 nM) and may enable noncovalent IGF-I delivery by mimicking the native IGFBPs[13]. In this study, we engineered IGF-I-binding peptide-functionalized C' Dots (IGF-I targeted C' Dots). We hypothesized that modifying C' Dots with an IGF-I-binding peptide would extend the efficacy of IGF-I for cartilage protection. To test this hypothesis, we developed an IL-1β-based inflammatory model to mimic moderate stage of OA using whole cartilage explants and simultaneously treated them with a single dose of untargeted (plain) C' Dots or IGF-I targeted C' Dots with or without exogenous IGF-I.

METHODS: *C' Dot Synthesis:* Cyanine 5 fluorophore-encapsulating C' Dots were synthesized following previously established methods[7]. Post-PEGylation, heterobifunctional PEGs were employed to introduce IGF-I-targeted functionalization via efficient, biorthogonal click chemistry, with resulting experimental groups containing 20 IGF-I binding peptides per particle[8]. *Therapeutic Studies:* Cylindrical articular cartilage explants (d = 4 mm, h = 1 mm; n = 12-18 per group; 5 experiments) were harvested from the patellofemoral groove of neonatal bovine stifles (n = 11 joints) and randomly assigned to each group. Explants were equilibrated overnight in high-glucose DMEM with 1% serum concentration. Following equilibration, IL-1β (100 ng/mL) was added with each media change (every 2-3 days) to model a moderate stage of OA. Simultaneously, explants were treated with a single (S) dose of either untargeted or IGF-I-targeted C′ Dots (132 nM) with or without a single (S) dose of free IGF-I (100 ng/mL). Parallel controls received a single (S) or continuous (C) free IGF-I in the media (100 ng/mL). Explants were treated for 7 days, after which tissues were collected. *Biochemical Assays:* Sulfated glycosaminoglycan (GAG) content was quantified using a standard DMMB assay. GAG content was first normalized to the wet weight of each explant and further normalized to the mean of untreated controls for each experiment. *Histological Analyses:* Cartilage explants were fixed in 10% formalin for 48 hours, sectioned, and stained with Safranin-O/Fast Green to evaluate GAG distribution and matrix integrity. *Statistical Analysis:* GAG content was compared in each group using a custom nested-ANOVA and Tukey's post-hoc pairwise comparisons (α = 0.05).

RESULTS: IL-1β treatment reduced GAG content by ~50% in cartilage explants compared to untreated controls ($p < 0.001$). A single dose of free IGF-I did not protect GAG content ($p = 0.61$), while continuous free IGF-I treatment reduced GAG loss and maintained GAG content at ~75% of untreated controls ($p = 0.04$). Untargeted C' Dots alone did not maintain GAG content ($p = 1.0$) and IGF-I supplementation was needed to have some protection ($p = 0.003$). Explants treated with single dose of IGF-I targeted C′ Dots alone demonstrated prevention of GAG loss ($p = 0.014$), with IGF-I addition enhancing this effect further up to ~83% of untreated controls ($p < 0.001$) (**Fig. 1** and **Table 1**). Safranin-O/Fast Green staining corroborated these biochemical results, showing pronounced proteoglycan depletion in IL-1β-treated tissues and near-complete protection and uniform staining through the full cartilage depth in both the IGF-I targeted C′ dot groups (**Fig. 2**). Notably, explants treated with IGF-I targeted C' Dots (without additional IGF-I) demonstrated uniform GAG distribution.

DISCUSSION: A single dose of IGF-I delivered with IGF-I targeted C' Dots prevented substantial GAG loss from cartilage after exposure to IL-1β. This treatment was nominally even more effective than a continuous dose treatment of free IGF-I. The beneficial effects of IGF-I targeted C' Dots alone which protected cartilage despite the lack of exogenous IGF-I was noteworthy. These results are consistent with our idea that the covalent attachment of IGF-I-binding peptide to the C' Dot surface likely increases the affinity of C' Dots towards exogenous and endogenous IGF-I enabling them to function as a local IGF-I depot. We believe that IGF-I targeted C' Dots passively diffused into the ECM and mimicked native IGFBPs allowing for localized docking-undocking of IGF-I for sustained effects. Our previous results that demonstrate extended presence of C' Dots in cellular and extracellular space[12] combined with our current approach enables extended bioavailability of IGF-I to chondrocytes. The significant efficacy of single dose IGF-I targeted C' Dots ± IGF-I highlights the potential of this delivery system to reduce dosing frequency and improve translational feasibility for OA and cartilage repair applications.

SIGNIFICANCE: A single dose of IGF-I-targeted C' Dots enable sustained efficacy of IGF-I within cartilage, effectively preserving matrix integrity under inflammatory conditions.

REFERENCES: [1]Martel-Pelletier+ *Nat Rev Dis Primers* 2016; [2]Fortier+ *Clin Orthop Relat Res* 2011; [3]Rosenfeld+ *Pediatrics* 1999; [4]Miller+ *Arthritis Rheum* 2010; [5]Geiger+ *Sci Transl Med* 2018; [6]Vedadghavami+ *Arthritis Res Ther* 2022; [7]Ma+ *Chem Mater* 2015; [8]Ma+ *Chem Mater* 2017; [9]Phillips+ *Sci Transl Med* 2014; [10]Zanoni *JAMA* 2021; [11]Wu+ *Chem Mater 2022;* [12]Fortin+ *Acta Biomater* 2025; [13]Aguilar+ *Acta Biomater* 2017

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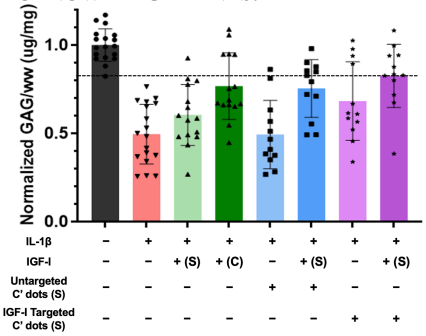

Figure 1. Evaluation of GAG content in IL-1β treated bovine explants following respective therapeutic treatments showed significant protection with IGF-I targeted C' Dots alone and enhanced protection with IGF-I addition.

Treatment comparisons for GAG content in explants		p-value
IL-1β treatment		< 0.001
Effect of IGF-I addition	Single dose	0.613
	Continuous dose	0.004
	Untargeted C' Dots	0.003
	IGF-I Targeted C' Dots	< 0.001
Effect of C' Dot addition	Untargeted	1.000
	IGF-I Targeted	0.014
IGF-I targeted C' Dots compared to untargeted C' Dots		0.050

Table 1. Statistical comparisons for GAG content across treatment groups, indicating significant protection with IGF-I targeted C' Dots ($p = 0.014$) and enhanced protection with the addition of IGF-I ($p < 0.001$).

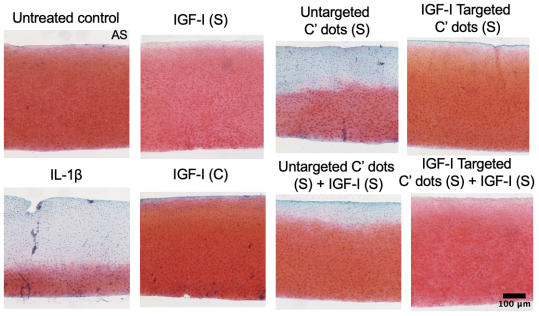

Figure 2. Safranin-O stained histological sections demonstrated preservation of GAG distribution in explants treated with IGF-I targeted C' dots ± IGF-I compared to IL-1β treated controls (AS = articular surface, Scale bar = 100 µm).