Controlled Delivery of the Mitochondrial Targeted SPN-15 Peptide for OA Prevention

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Introduction: Mitochondrial respiratory dysfunction is a hallmark of several chronic diseases[1]. A critical feature of this dysfunction is the depolarization of the inner mitochondrial membrane (IMM), which arises from the loss of cardiolipin, a phospholipid exclusive to the IMM. Cardiolipin depletion destabilizes the IMM, leading to reduced energy production, elevated reactive oxygen species generation, and activation of apoptotic pathways that worsen with disease progression[2]. While several FDA-approved mitochondrial therapies exist, few can enter the IMM to repair or protect cardiolipin[1,3]. The Szeto-Schiller (SS-31) peptides are a unique class of positively-charged molecules that localize to the IMM and stabilize cardiolipin[1,4]. In an *in vitro* model of post-traumatic osteoarthritis (OA), super-physiological loading caused mitochondrial depolarization and tissue death within cartilage tissue. Tissue pre-treatment with SS-31 preserved IMM polarization after loading, suggesting that SS-31 is a promising therapy to prevent tissue death leading to OA. However, clinical translation remains challenging because maintaining therapeutic concentrations within the joint would require repeated and frequent intra-articular injections, which are invasive and impractical for long-term OA treatment. Therefore, a delivery platform capable of sustained SS-31 release is needed to improve clinical feasibility. SPN-15, a novel SS-31 derivative containing a biotin functional group, has been shown to preserve mitochondria health in preclinical OA models with daily dosage[6], and additionally offers a method for controlled release through protein-ligand interactions[5]. Streptavidin, a protein with high affinity biotin-binding domains, can reversibly bind biotin to achieve long-term controlled release[7]. Alginate hydrogels are promising polymers to serve as SPN delivery vehicles, as they contain tunable physical and chemical properties that have been used in tissue engineering applications to control drug release[8]. We hypothesize that increasing the binding abilities of the alginate vehicle will slow the release of SPN peptides. To test our hypothesis, we set out to establish two versions of alginate vehicles: (1) physically modified alginate designed to electrostatically modulate SPN release at early timescales (days) and (2) streptavidin-modified alginate that is capable of long-term (months) of delivery.

Methods: Chemically-Modified Alginate Preparation: Maleimide functional groups were conjugated to alginate using previously established methods[9], and thiol-streptavidin was conjugated to alginate using maleimide-thiol click chemistry. The substitution for the final Streptavidin-Alginate was 10% of available maleimide groups. Alginate Bead Preparation: Alginate beads containing equal amounts of SPN peptide were crosslinked in calcium chloride at various mass ratios for 30 minutes before being placed in tubes filled with Hank's Balanced Salt Solution at 37°C. For physically modified alginate, alginate densities varied between 2.5-4% w/v (weight/volume). For chemically modified alginate, alginate density was 2% w/v, and the SPN peptide was loaded at 1:1 with available binding sites. Release Measurements: Release samples were taken from all tubes at various time points and SPN release was measured using a fluorescent fluoroaldehyde assay. Release data were normalized to initial peptide and fit with custom Matlab script using bi-exponential release curves to capture fast and binding mediated SPN release [Release = A_1*exp(-t/τ_1) + A_2*exp(-t/τ_2)]. A_1 and τ_1 describe the faster release weighting and time constants, while A_2 and τ_2 describe the slower release weighting and time constants, respectively.

Results: At all concentrations, unmodified alginate exhibited a fast release profile at early time points (~0.3 hour), and a slower-phase release at time points greater than 2 hours (**Figure 1a**). Bi-exponential curve-fit analysis on all plots described the data well ($R^2 > 0.97$) and indicated that increasing alginate density did not modify the fast release kinetics (**Table 1, A1**). This indicated that the fast release was diffusion-mediated and was largely unaffected by alginate properties. Interestingly, increasing the alginate density both increased the time constant of the slow release from 51 to 370 hours and increased the relative proportion of SPN peptides that follow the slow release profile, which indicated that alginate electrostatically controls SPN release on the order of hours (**Table 1**). In a separate experiment (**Figure 1b**), inclusion of streptavidin shifted the proportion from a release mediated entirely through diffusion and electrostatic interaction (A_1 = 1, **Table 2**) to a release that was heavily influenced by SPN release from streptavidin (A_1 = 0.11, A_2 = 0.73, **Table 2**). Additionally, SPN release was further extended from a release of 3.6 days to 133 days (**Table 2**).

Discussion: These release data indicates that streptavidin functionalization extends SPN delivery from alginate on timescales of hours to weeks through reversible binding interactions. In early OA, extracellular matrix degradation precedes irreversible chondrocyte damage, leaving viable cells vulnerable to subsequent mechanical stress[1]. As such, the ability to tune the release of SPN peptides at early and long timescales has promising applications for OA therapy. Here, we demonstrated tuning binding abilities of an alginate hydrogel to control the release of SPN peptides at different time scales. By increasing the alginate density, we slowed the release of SPN peptides over several hours. By including streptavidin, we shifted the SPN release that was mediated mainly through diffusion and electrostatic interaction to a release that is largely controlled by reversible binding over several weeks, with curve-fitting trends indicating potential SPN release over months. Our findings agree with previous research that has shown that streptavidin-functionalized polymers slow the release of other biotin payloads over months[10]. Future work will evaluate the customizability of tuning the ratio between fast and slow release of SPN peptides with streptavidin-alginate as well as evaluate their therapeutic efficacy at long timepoints *in vitro*.

Significance: We developed a customizable alginate vehicle to control the release of a mitochondrially active peptide over the course of hours to months, which has the potential to enhance efficacy of OA therapies.

References: [1]Bartell+ *Orthop Res* (2020), [2]Paradies+ *Cells* (2019), [3]Zong+ *Signal Transduct. Target. Ther.* (2024), [4]Szeto+ *Pharm Res*. (2011), [5]Sanchez+ *ORS* (2025), [6]Szeto+ (2022), [7]Hirsch+ *Anal. Biochem* (2002), [8]Aguilar+ *Acta Biomater*. (2017), [9]Madl+ *Biomacromole.* (2014), [10]Sharp+ *ORS* (2025)

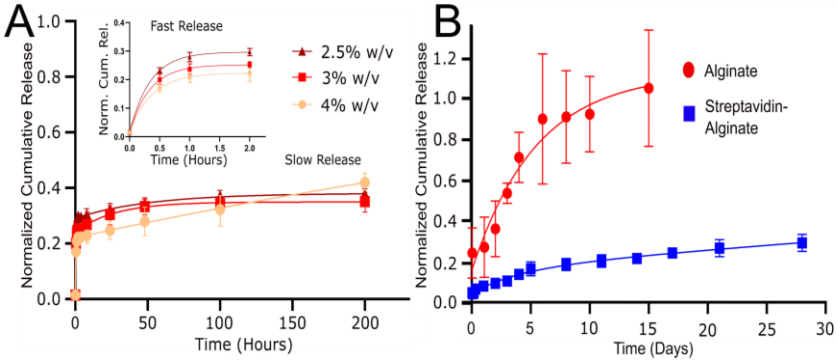

Figure 1: **(A)** Normalized cumulative release measurements of SPN peptides from beads with different alginate densities (2.5-4% w/v) for 200 hours. The inset depicts the fast peptide release peptide release up to 2 hours, while the full graph depicts the slow release kinetics. **(B)** Normalized cumulative release measurements of SPN peptides from alginate and streptavidin-conjugated alginate (blue) beads for up to 28 days. All trendlines are the mathematical fit detailing the bi-exponential release curve for the respective groups. Data are listed as mean ± st. dev. ≥ 10 beads/group, 3 groups.

Table 1: SPN Release Fits From Various Alginate Densities

Group (w/v)	A_1	τ_1 (Hours)	A_2	τ_2 (Hours)
2.5%	0.28	0.35	0.10	51
3%	0.24	0.34	0.11	23
4%	0.22	0.36	0.49	370

Table 2: SPN Release Fits From Strep-Alginate

Group	A_1	τ_1 (Days)	A_2	τ_2 (Days)
Alginate	1	3.6	0	0
Strep-Alginate	0.11	4.0	0.73	133

Table 1: Release fit of SPN peptides from alginate at various densities. **Table 2**: Release fit of SPN peptides from alginate vs streptavidin(strep)-alginate. τ_1 and τ_2 represent time constants for the fast and slow release patterns, and A_1 and A_2 represent weightings for fast and slow release, respectively.