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DOSE-DEPENDENT CHEMICAL REACTION KINETICS FOR MODELING OF TGF-β DELIVERY IN CARTILAGE TISSUE ENGINEERING

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INTRODUCTION

Cartilage tissue engineering (TE) is a promising osteoarthritis treatment strategy whereby chondrogenic cells are embedded in scaffolds to generate cartilage replacement tissues. TGF-β is prominent cartilage TE growth mediator due to its efficacy in accelerating extracellular matrix (ECM) biosynthesis. An assortment of TGF-β delivery platforms are currently in development, varying by delivery mode (e.g. media supplementation, scaffold loading) and administered dose (0.3-2000ng/mL) [1]. Cells are highly sensitive to TGF-β dose—low doses may be insufficient to induce ECM enhancements and excessive doses can lead to fibrosis [2], hyperplasia [3], or biosynthetic suppression [4] As such, TGF-β delivery optimization may be critical for TE outcomes.

Methodology to optimize TGF-β exposure in delivery systems remain limited. Optimization predominantly consists of empirical measures of TGF-β desorption from acellular scaffolds with the goal of maximizing scaffold retention durations [1]. However, these assessments provide limited predictive capabilities and do not provide quantitative insights into TGF-β activity levels to which cells are exposed. As an alternative approach, reaction-diffusion modeling frameworks that account for chemical reaction kinetics in TE cartilage (e.g. scaffold/ECM binding, cell-mediated internalization, enzymatic degradation) can potentially be used to predict temporo-spatial distribution of different states of TGF-β in TE constructs that are important for clinical outcomes, including: 1) scaffold-bound TGF-β, 2) available free TGF-β, 3) cell-internalized TGF-β, and 4) TGF-β desorbed into synovial joint tissues. As such, modeling can be used to optimize scaffold design parameters to improve TE growth outcomes.

Here, we advance the development of predictive reaction-diffusion modeling frameworks by characterizing chemical reaction constitutive relations that can be influential of TGF-β delivery outcomes, including TGF-β-scaffold/ECM reversible binding and TGF-β cell-mediated internalization reactions. Characterizations are performed over the range of TGF-β doses used in TE applications (0.3-2000 ng/mL) and assessed in the absence or presence of intracellular TGF-β signaling processes. Lastly, finite element (FE) models are implemented to examine the impact of chemical reactions on the spatiotemporal distribution of TGF-β in TE constructs for media supplementation and scaffold loaded delivery platforms.

METHODS

Tissue source: Immature bovine chondrocytes were seeded in 2% agarose (45×10^6 cells/mL) to form ∅2×1mm TE constructs [5]. Freshly-cast constructs were maintained live in chondrogenic media (CM) [5] or subjected to an initial freeze-thaw cycle to induce rapid devitalization for reaction kinetic characterizations.

Binding kinetics: Devitalized constructs were exposed to a range of TGF-β3 doses (0.3-2000ng/mL) for 48h (n=3/dose). Bound TGF-β3 was extracted and measured via ELISA as performed previously [5]. Binding models were curve-fit to experimental data: 1) Langmuir isotherm (Eq.1)—accounting for reversible binding to construct binding sites, and 2) BET isotherm (Eq.2)—accounting for additional aggregation of TGF-β bound to binding sites:

$$C_{B_T} = \frac{C_F N_T}{C_F + K_o} \quad (1) \qquad C_{B_T} = \frac{N_T c x[1-(M+1)x^M + Mx^{M+1}]}{[1-x][1-x+cx-cx^{M+1}]} \quad (2) \qquad c = K_o/K_c \quad x = C_F/K_c$$

where C_{B_T} and C_F: bound and free TGF-β dose, respectively, N_T: surface binding site density, M: maximum number of aggregated TGF-β molecules at a binding site, K_o and K_c: dissociation constant for scaffold-to-TGF-β binding and TGF-β-to-TGF-β binding, respectively. Uptake ratio, R_U, was calculated as the ratio of total TGF-β dose (C_B+C_F) within the construct to free TGF-β dose (C_F).

Internalization rate kinetics: Live constructs were exposed to TGF-β3 in CM (100 μL) at the aforementioned doses. The cell-mediated internalization rate, V_i, of TGF-β3 in constructs was determined by

monitoring the transient TGF-β3 concentration decrease in the bath over 48h and curve-fitting the response with our reaction-diffusion model, which accounts for TGF-β diffusion (Eq.3), scaffold/ECM reversible binding and TGF-β aggregation (Eq.4&5, respectively), and cell-mediated internalization (Eq.6) of TGF-β in constructs:

$$\frac{\partial C_F}{\partial t} = D\nabla^2 C_F \quad (3) \qquad \frac{\partial C_F}{\partial t} = k_r C_B - k_f C_F N_T \quad (4)$$

$$\frac{\partial C_F}{\partial t} = k_{r_1} \sum_{i=1}^{M-1} C_{B_{i+1}} - k_{f_1} C_F \sum_{i=1}^{M-1} C_{B_i} \quad (5) \qquad \frac{\partial C_F}{\partial t} = R_i C_F \quad (6)$$

where D: TGF-β diffusivity, k_r&k_f: reverse and forward binding constant, respectively, R_i: internalization rate constant. The transient TGF-β3 bath decrease was curve fit for R_i using our measured binding parameters as performed previously [5]. The influence of intracellular TGF-β signaling dynamics was further assessed by repeating R_i measurements in the presence of a TGF-β receptor kinase inhibitor (5μg/mL, LY364947). Subsequently, V_i, (internalized TGF-β molecules per cell per second [mol/cell/s]) was calculated as $R_i \times C_F$.

TGF-β transport modeling: Media-supplemented delivery: FE simulations were implemented using measured reaction kinetics parameters to predict the uptake of media-supplemented TGF-β in constructs (∅6×3mm) at a 10 or 800 ng/mL dose ± TGF-β internalization kinetics and ± intracellular TGF-β signaling.

Affinity-scaffold-loaded delivery: FE simulations were implemented to predict the retention of scaffold-loaded TGF-β (800ng/mL) in heparin-affinity scaffold constructs using previously measured heparin -to-TGF-β binding parameters (k_r:$9.2\times10^{-3}s^{-1}$&k_f:$1.0\times10^{5}(M.sec)^{-1}$)[6] and measured internalization rate ± TGF-β internalization kinetics.

RESULTS

Binding kinetics: The TGF-β uptake ratio increased with dose, ranging from R_U=11.4 at 0.9 ng/mL to R_U=32.1 at 1400 ng/mL. While this binding response could not be described by the Langmuir model (R^2<0.01), it could be faithfully described by the BET isotherm model (R^2=0.99, Fig 1A).

Internalization rate kinetics: In the low dose regime (0.3-100ng/mL), V_i exhibited a Michaelis-Menten relationship (R^2=0.96; Fig 1B), saturating at a rate of 2mol/cell/s. In the high dose regime (100-2000ng/mL), V_i increased linearly with dose (R^2=0.96), reaching a rate of 504mol/cell/s at 2000ng/mL. For all doses in the high regime, TGF-β signaling inhibition induced a significant decrease in V_i ($p<0.05$, Fig 1C).

TGF-β transport modeling: Media-supplemented delivery: Media-supplemented 10ng/mL TGF-β exhibited pronounced steady-state gradients in constructs in the presence of cell-mediated internalization whether or not TGF-β signaling dynamics were present (Fig 2). In contrast, 800ng/mL supplemented TGF-β exhibited pronounced steady-state gradients but gradients were mitigated upon TGF-β signaling inhibition (Fig 2). For both doses, gradients were greatly mitigated in the absence of cell-mediated internalization.

Affinity-scaffold-loaded delivery: Cell-mediated internalization led to a rapid loss of loaded TGF-β, exhibited by an 80% loss in the constructs after 2 days. In the absence of internalization, over 40% of TGF-β was retained in the construct after 20 days (Fig 3A&B).

DISCUSSION

This work establishes novel quantitative relations of dose-dependent TGF-β chemical reaction kinetics and provides expanded insights into its biochemical behavior in TE systems. For characterizations of TGF-β binding kinetics, we observe that TGF-β binding to constructs increases with dose. This response contrasts with behavior predicted by Langmuir, in which the uptake ratio is expected to decay to a value near unity as tissue binding sites saturate, thus suggesting the occurrence of TGF-β aggregation events at tissue binding sites. This aggregation phenomenon is consistent with prior examination of TGF-β binding events [7] and can be predicted by a BET isotherm model, that accounts for multiple layers of aggregated TGF-β at each tissue binding site. For characterizations of TGF-β cell-internalization kinetics, we observe an intriguing interplay between TGF-β dose and intracellular TGF-β signaling dynamics. The internalization rate exhibits a unique biphasic response, following Michaelis-Menten kinetics at low doses (<100ng/mL), saturating as a value V_i=2.4 mol/cell/s, but increasing further with a linear relationship at higher doses (>100ng/mL). A striking aspect of this characterization is the remarkably high TGF-β internalization rates measured at high doses—reaching 500 mol/cell/s at 2000 ng/ml—particularly when considering the documented limited number of TGF-β receptors at the cell surface (~10k receptors) [8]. These elevated TGF-β internalization rates can be suppressed at high doses by blocking intracellular TGF-β signaling, thus suggesting a role of TGF-β signaling in upregulating internalization machinery to clear high doses of TGF-β from the extracellular space. Reaction kinetics are predicted to have a significant impact on the spatiotemporal distribution of delivered TGF-β to TE constructs. For conventional 10ng/mL media-supplemented delivery, TGF-β internalization kinetics give rise to pronounced steady state gradients in TE constructs with TGF-β penetrating less than 500μm into the tissue—remarkably, gradients are also present with an 800ng/mL dose due to elevated internalization rates resulting from intracellular TGF-β signaling. For scaffold delivery, while heparin affinity domains can promote long term TGF-β retention, retention is far reduced in the presence of TGF-β internalization kinetics as affinity domains compete for TGF-β with cell internalization machinery. Overall, this work demonstrated the potential significant impact of chemical reactions on the delivery of TGF-β in TE applications and sets the foundation for using reaction-diffusion frameworks to modulate delivery platform parameters to optimize TGF-β delivery to TE constructs.

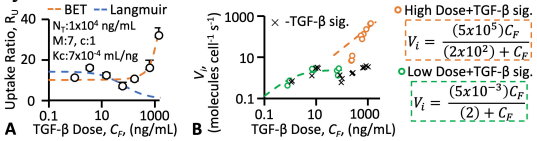

Fig 1: (A)Uptake ratio (R_U) vs free TGF-β (C_F) dose and model fits. (B) Internalization rate for low and high TGF-β dose regime in the absence (+TGF-β sig) or presence (-TGF-β sig) of LY364947.

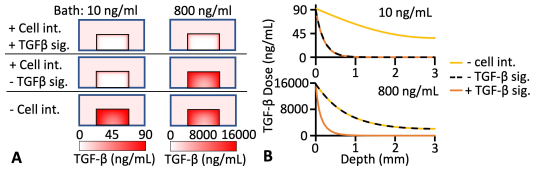

Fig 2: Predicted distribution of media supplemented TGF-β in TE construct after 1-week ±cell internalization ±TGF-β signaling.

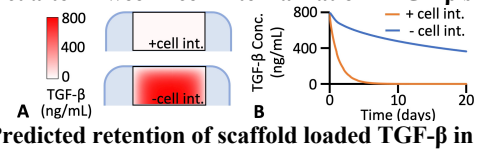

Fig 3: Predicted retention of scaffold loaded TGF-β in heparin-affinity scaffolds after 20 days ±cell internalization

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