## BOSTON UNIVERSITY

# Patient-specific Internalization Rates Mediate TGF-β Delivery in Cartilage Tissue Engineering



Sedat Dogru <sup>1</sup>, Kirk Pierce<sup>1</sup>, Tianbai. Wang<sup>2</sup>, Michael. B. Albro <sup>1,2</sup>
<sup>1</sup>Boston University, Department of Mechanical Engineering
<sup>2</sup>Division of Material Science & Engineering

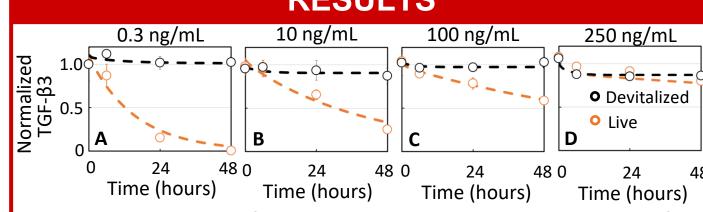
## INTRODUCTION

- Autologous cell implantation (ACI) is a promising cartilage tissue engineering (TE) strategy to treat osteoarthritis (OA). Primary chondrocytes from patients are seeded in scaffolds and used to generate neocartilage in OA defect sites [1].
- Transforming growth factor-β (TGF-β) is a prominent mediator of TE cartilage growth due to its efficacy in accelerating extracellular matrix (ECM) synthesis [2].
- For TE protocols where neocartilage is initially cultivated *in vitro*, TGF-β is supplemented in the culture medium. For protocols where neocartilage is directly implanted in the *in vivo* defect site, TGF-β can be directly loaded into and controlled-released scaffolds.
- Recently, we have demonstrated that a uniquely high rate of cell-receptor mediated internalization kinetics of TGF-β can dramatically influence its delivery to TE cartilage from immature bovine chondrocytes, yielding severe concentration gradients when delivered in media or short residence times when delivered via scaffold loading.
- However, TGF-β internalization rate measurements and assessments of its effect on delivery to cartilage has not been performed for clinical human cell populations.
- In the current investigation, we perform novel characterizations of:
  1) the dose dependent internalization rate of TGF-β in TE constructs generated from human cell populations, 2) the influence of internalization rates on TGF-β uptake into constructs during in vitro media-supplementation, and 3) the influence of internalization rates on TGF-β residence time after loading in affinity scaffold-based constructs in vivo.

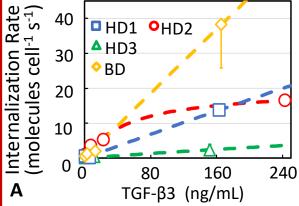
#### **METHODS**

- Human chondrocytes were isolated from the femoral condyles of three autopsy human donors (HD; age/sex; HD1: 36/ , HS2: 60/ , HS3: 60/ ; NDRI procured) or immature bovine donor (BD), passaged [5], and cast in agarose scaffolds (Ø2x1mm) at 30 M cells/mL.
- Internalization rate: TE Constructs from each of the BD and HDs (live and devitalized) were exposed to a TGF- $\beta$ 3 bath dose,  $C_F$ , of 0.3, 3, 10, or 150 ng/mL in chondrogenic medium. For each dose, the transient decrease in  $C_F$  for live constructs (from cell internalization) was monitored via ELISA (TGF- $\beta$ 3 Duoset, R&D Systems) and subsequently curve-fit with a FE model (FEBio solver) to extract the TGF- $\beta$ 3 internalization rate constant,  $R_{TGF}$  [6]. TGF- $\beta$ 6 internalization rates were computed as the product of  $R_{TGF}$ 1 x  $C_F$ 1 and curve-fit for using the Michaelis-Menten relation:  $R_{TGF}$ 1 x  $C_F$ 1 =  $V_{max}$ 1 /( $V_{M}$ 1 +  $V_{F}$ 1).
- In vitro TGF-β uptake model: For each donor, predictions of the uptake of media supplemented TGF-β (10ng/mL) into constructs (Ø5x2mm) were generated using our recently validated FE model [5,6], while incorporating the acquired donor-specific Michaelis-Menten TGF-β internalization constants and previously characterized agarose scaffold binding properties [3].
- In vivo TGF-β retention model: For each donor, predictions of the retention of scaffold-loaded TGF-β (100ng/mL) in heparin-affinity scaffold-based constructs (Ø5x2mm) were generated using the donor-specific Michaelis-Menten TGF-β internalization constants and heparin scaffold-TGF-β binding properties (K<sub>D</sub>=2.3µg/mL, Nt=250µg/mL [7]).

#### RESULTS

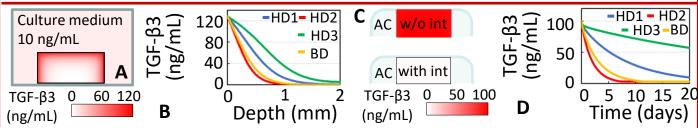


**Fig 1:** For all donors, TGF- $\beta$  bath levels,  $C_F$ , decreased in the presence of live constructs due to cell internalization. As  $C_F$  increases, transient decreases become less pronounced. Dashed curves represent curve-fits for internalization rate constant extraction.



	В	Km (ng/mL)	Vmax (molecules cell <sup>-1</sup> s <sup>-1</sup> )
	BD	1.0E+3	2.4E+3
	HD1	5.3E+3	4.6E+2
	HD2	5.4E+1	2.0E+1
)	HD3	1.4E+3	2.4E+1

**Fig 2**: (A) Donors exhibit varied dose-dependent TGF-beta internalization rates (B) and Michaelis-Menten parameters. Dashed curves represent curve-fit of Michaelis-Menten relation.



**Fig 3**: (**A-B**) Donors exhibit varied steady-state uptake distributions of media supplemented TGF- $\beta$  (rached at ~48 h) (**C-D**) Donors exhibit varied retention of loaded TGF- $\beta$  in affinity heparin scaffold. In the absence of internalization kinetics, loaded TGF- $\beta$  exhibits only minor loss over 20 days.

#### This study demonstrates two important new findings:

- Human chondrocytes exhibit high rates of TGF-β internalization that substantially impact the uptake and retention of TGF-β in constructs, and
- Internalization rates vary considerably with human donor cell populations, giving rise to disparate TGF-β delivery outcomes.

The influence of TGF- $\beta$  internalization rates on the delivery of TGF- $\beta$  to constructs has important clinical implications for ACI platforms.

For in vitro delivery, the generation of steady-state gradients during TGF-β uptake can give rise to constructs with undesirable heterogeneities, increasing the likelihood of failure after implantation [6].

For in vivo delivery, the diminished residence time of TGF-β in scaffold-loaded constructs may give rise to insufficient biosynthetic enhancements. As such, novel TGF-β delivery platforms may be needed to overcome these challenges.

An important implication of this variability is that ACI outcomes may vary considerably between patients due to variability in TGF-β internalization rates and ensuing limitations in TGF-β delivery

### **CONCLUSIONS**

The work suggests that novel platforms may be needed to overcome cell-internalization-induced TGF- $\beta$  delivery limitations to patient TE constructs and that these platforms may need to be patient-specific to account to TGF- $\beta$  internalization variability.

### **REFERENCES**

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### **ACKNOWLEDGEMENTS**

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