

# Characterizing Cell-Material Interactions When Chemical Cues are Presented Locally to Human Mesenchymal Stem Cells

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## Introduction

- Cell-laden hydrogels are designed to enhance cell delivery to and create structure for damaged tissues in wound healing applications.
- Human mesenchymal stem cells (hMSCs) are chosen for cell delivery due to their importance to tissue regeneration in signaling to other cells during wound healing
- Cytokines, which are present in the native wound environment, are tethered to the hydrogel network to determine their effect on hMSC remodeling

## Cytokines and Wound Healing

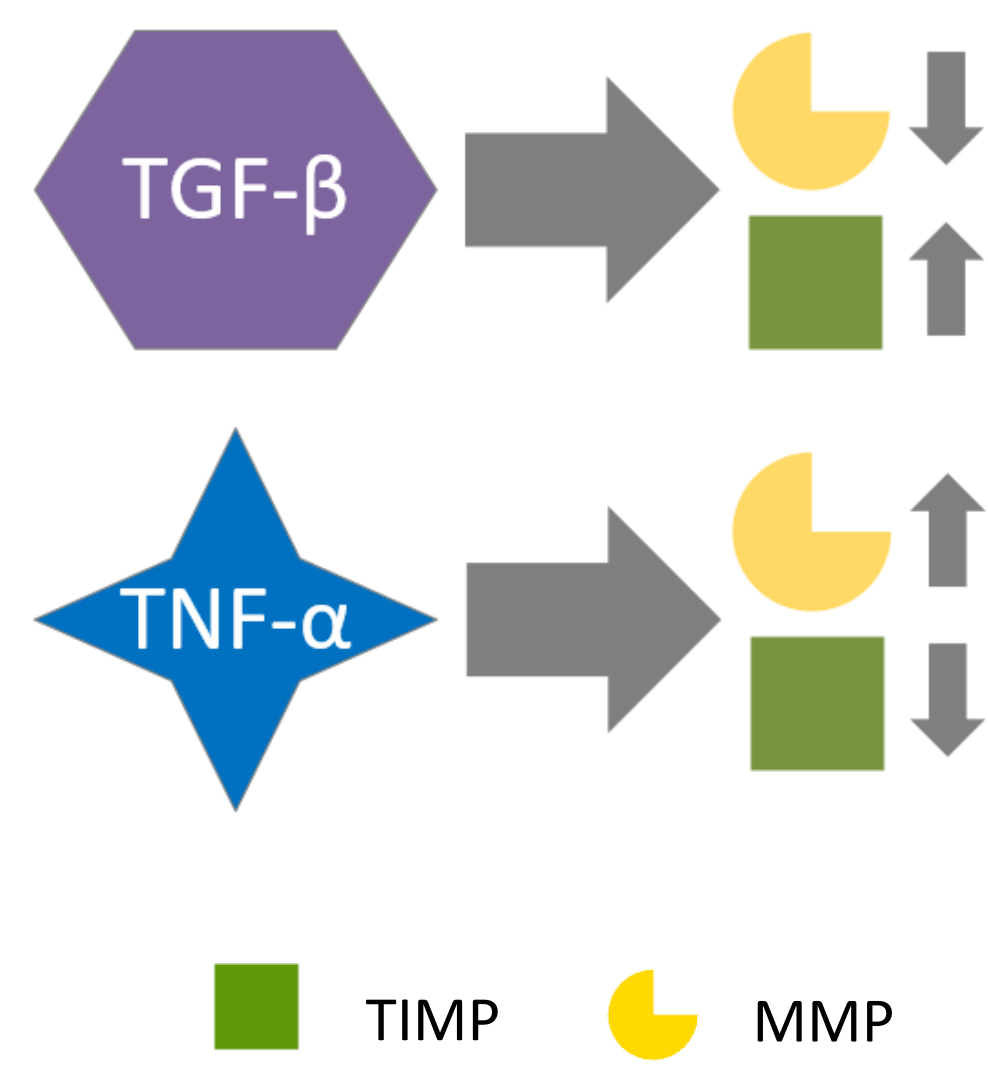
TNF- $\alpha$  and TGF- $\beta$  are cytokines which signal to hMSCs during wound healing

### TGF- $\beta$

- Present from inflammation to remodeling
- Promotes ECM structure by increasing TIMP secretions which inhibit MMP activity

### TNF- $\alpha$

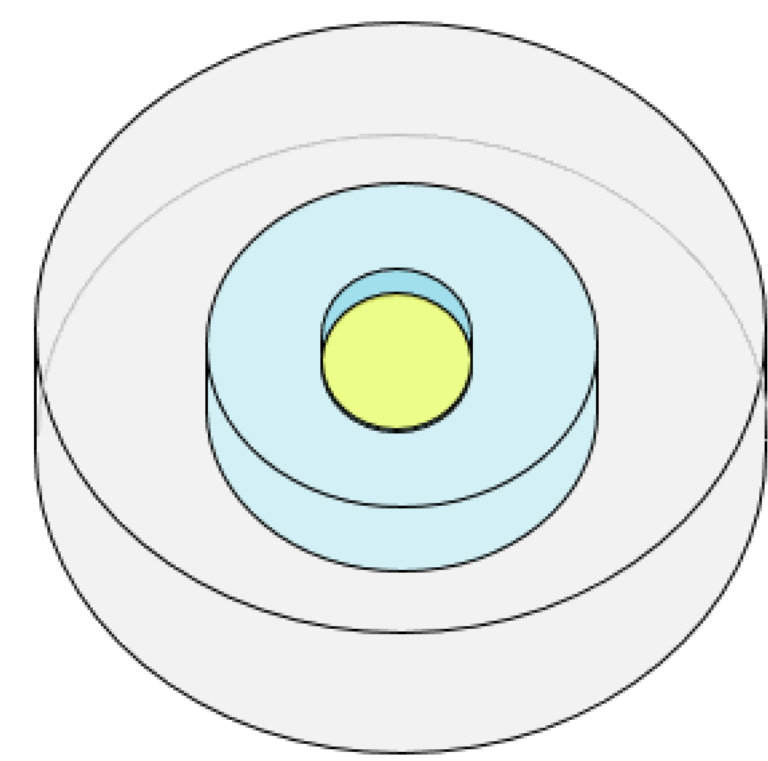
- Present primarily in the inflammatory stage of wound healing
- Increases network degradation by increasing hMSC secretion of MMPs and inhibiting production of TIMPs



## Poly(ethylene-glycol) Hydrogels

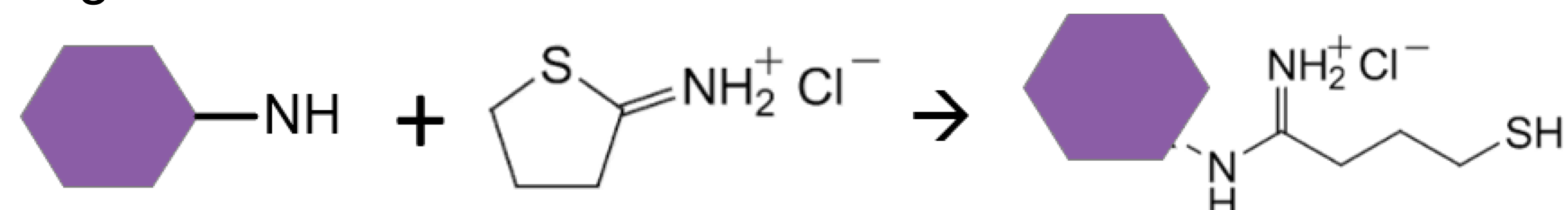
hMSCs are encapsulated into a poly(ethylene-glycol)-norbornene (PEG-N) hydrogel.

- Gels formed via a step-growth photopolymerization
- Gels are kept at 37 °C and 5% CO<sub>2</sub>
- Hydrogels have a MMP degradable crosslinker



## Cytokine Thiolation

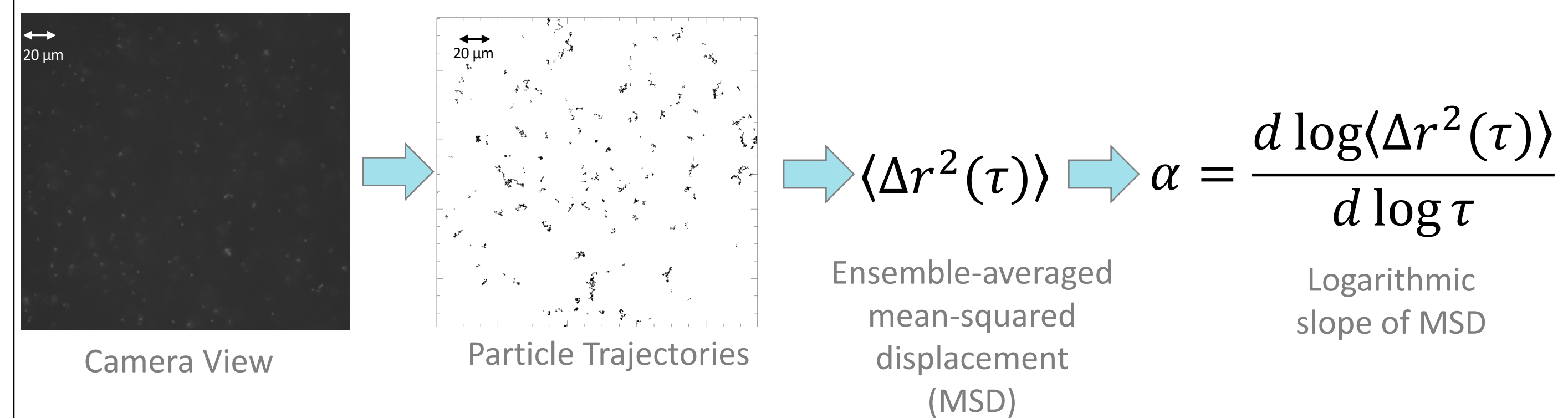
Cytokines (symbolized with hexagon) were thiolated using Traut's reagent.



The presence of cytokines in the hydrogel is confirmed using an enzyme-linked immunosorbent substrate assay (ELISA). The blue color in the gel (left) indicates that the cytokines are successfully tethered into the network.

## Multiple Particle Tracking Microrheology (MPT)

MPT is a passive microrheological technique that measures the Brownian motion of fluorescent probes embedded in the hydrogel network to obtain bulk rheological properties.



$\alpha$  is the logarithmic slope of the MSD and is used to quantify the state of the material

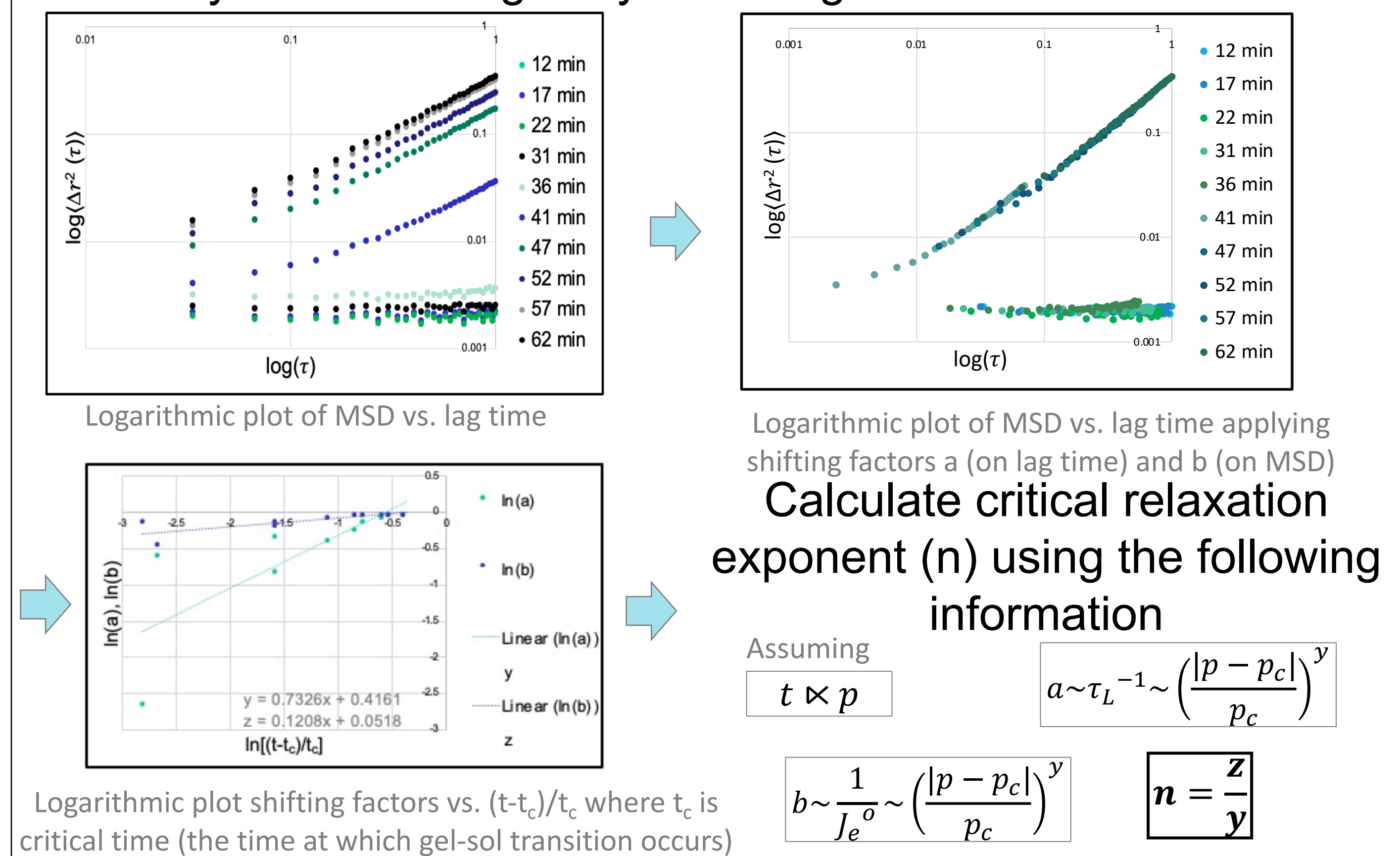
$\alpha \rightarrow 0$ : gel  
 $0 < \alpha < 1$ : viscoelastic gel or liquid  
 $\alpha = 1$ : liquid

Transition from gel to sol is determined by comparing  $\alpha$  to the critical relaxation exponent,  $n$

$\alpha > n$ : sol  
 $\alpha < n$ : gel  
 $n = 0.25 \pm 0.05$  for our hydrogels

## Enzymatic Degradation in the Absence of Cells

Time cure superposition (TCS) was used to determine the critical relaxation exponent of gels with tethered TGF- $\beta$ , tethered TNF- $\alpha$ , and no cytokines during enzymatic degradation.

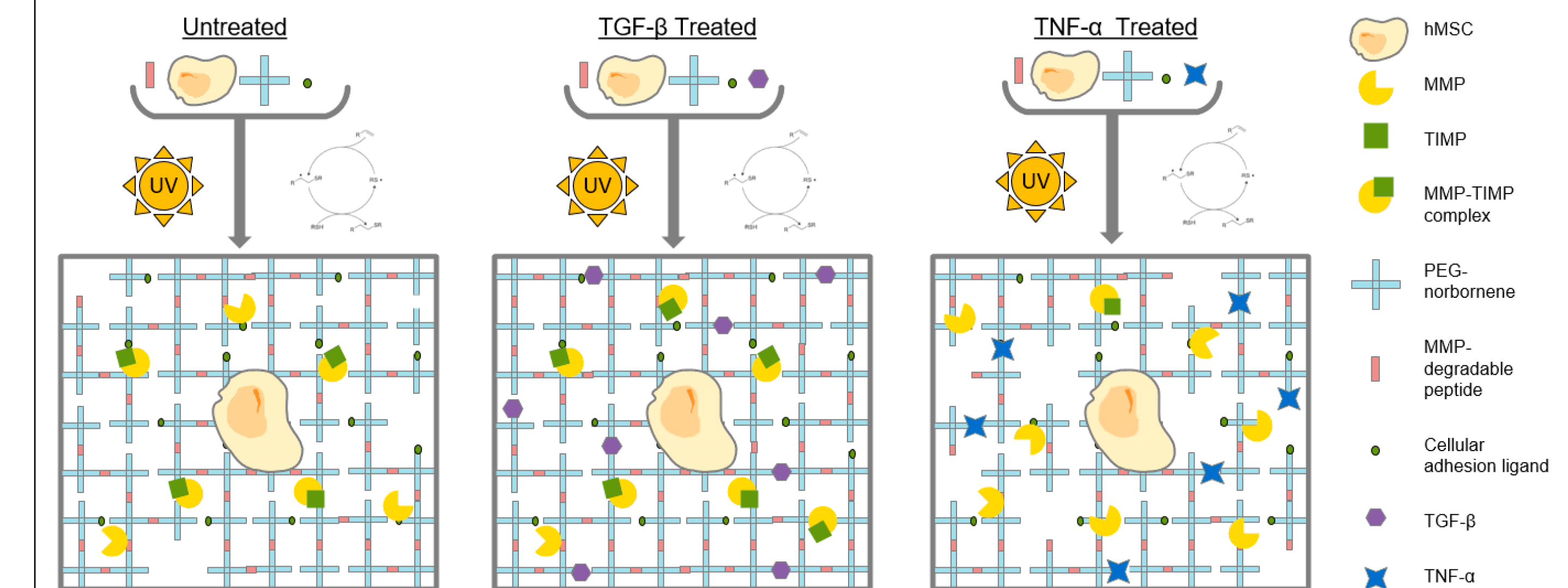


Using TCS, the following values were determined for  $n$  for gels with tethered TNF- $\alpha$ , tethered TGF- $\beta$ , and untreated PEG-N hydrogels. A t-test determined the difference in  $n$  between the 3 treatment groups is not statistically significant. This means that tethering cytokines to the network does not affect the material structure during enzymatic degradation.

Treatment	$n$
Untreated	0.212 $\pm$ 0.11
TGF- $\beta$	0.236 $\pm$ 0.18
TNF- $\alpha$	0.233 $\pm$ 0.12

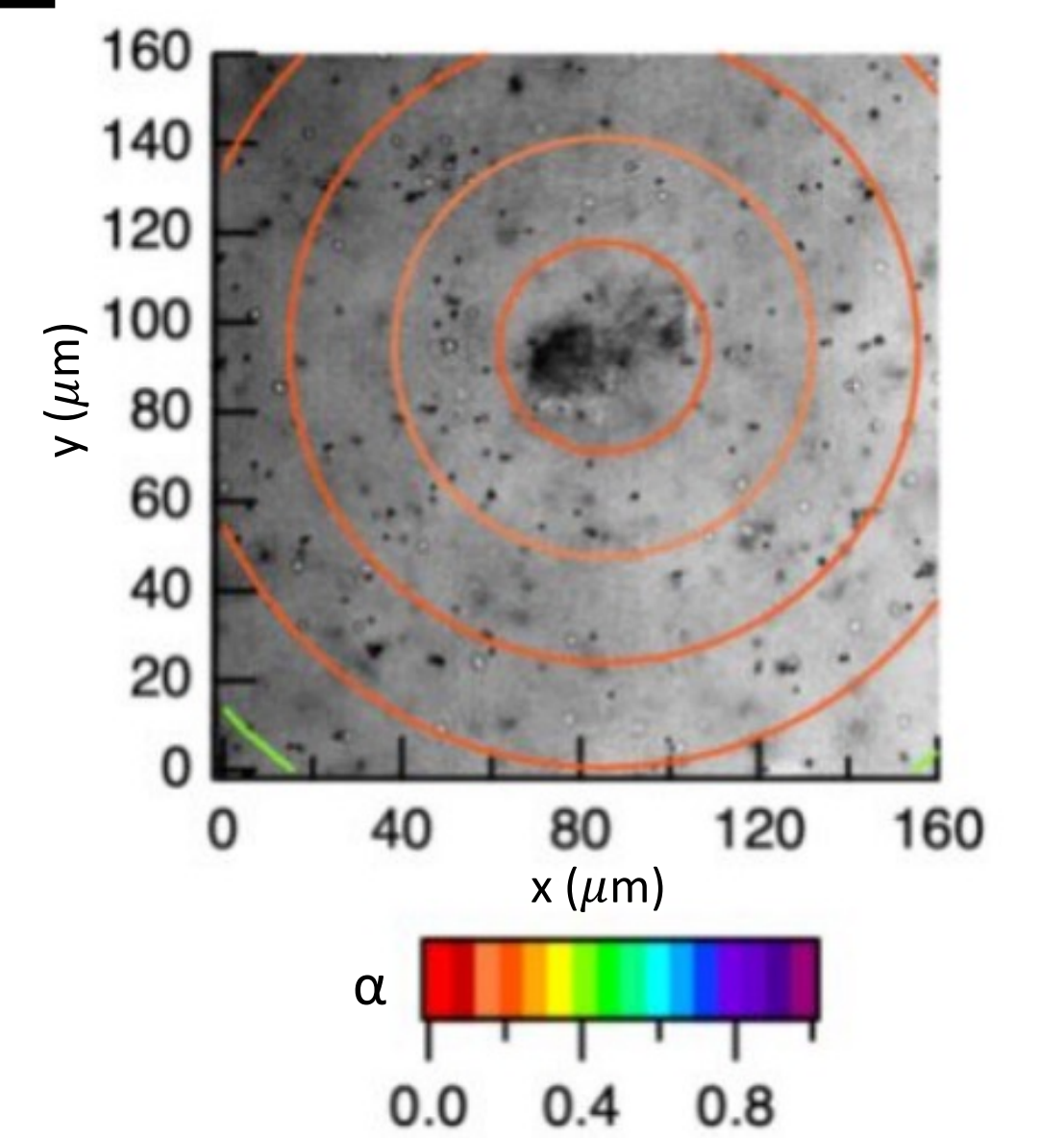
## Cell-Mediated Degradation

TNF- $\alpha$  and TGF- $\beta$  alter cell-mediated degradation of the hydrogel network



### Untreated Group Preliminary Results:

Day after hMSC Encapsulation	Average Field of View $\alpha \pm$ Standard Deviation
2	0.02151 $\pm$ 0.02455
3	0.02856 $\pm$ 0.02697
4	0.05753 $\pm$ 0.06933



Because the most activity was seen in day 4 after encapsulation, a plot is shown with radially averaged  $\alpha$  values for a sample cell from day 4 in control treatment group (left). The circles are colored according to their respective averaged  $\alpha$  values (see key below figure). These measurements show little remodeling directly around cells and increasing degradation as the distance from the cell increases.

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