Optimizing the Bioactive Modification of Alginate Films for MSC Proliferation

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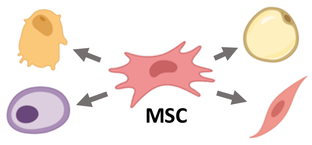
MRSEC materials research science + engineering center

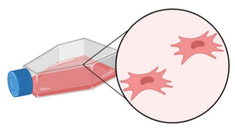
Background and Motivation

Mesenchymal Stem Cell (MSC) Treatments

- Used for cell therapies and tissue regeneration
- Treatments require billions of MSCs with multiple doses



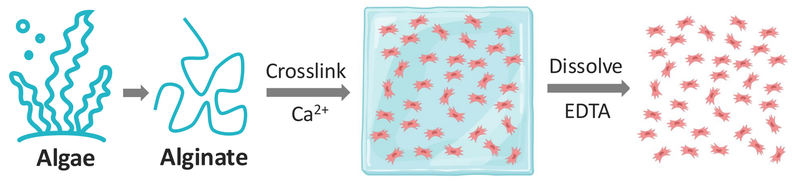
Issues with Current Production: Proteolytic Enzyme Detachment



Issue 1: Quantity
Only ~50% of cells detached

Issue 2: Quality
Cell stress, premature differentiation

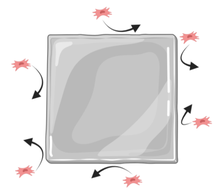
Introduction: Alginate Hydrogel Films



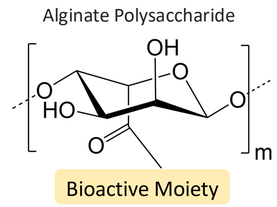
Biodegradable and Non-toxic

Bioactive Alginate Film

Efficiently harvested MSCs



Problem: Alginate is naturally bioinert

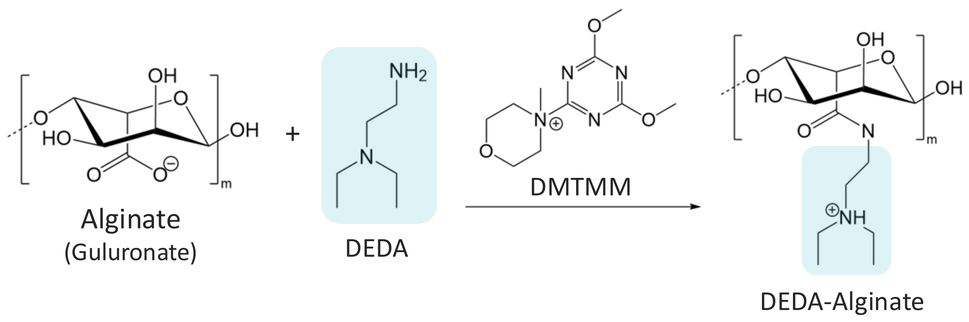


Solution: Introduce bioactivity to alginate

Objective: Chemically modify alginate for biofunctionalization to improve MSC attachment and production
Target Degree of Substitution (DS): 0.3-10%

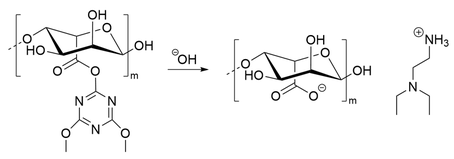
I. DMTMM Coupling Chemistry Optimization

Goal: Optimize to 0.3-10% DS with model amine for biofunctionalization
Varied Conditions: pH, Buffer presence, Salt presence, Limiting reagent



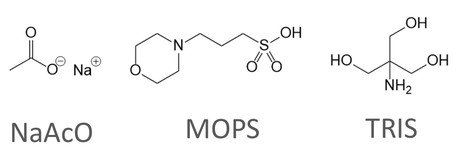
pH Study

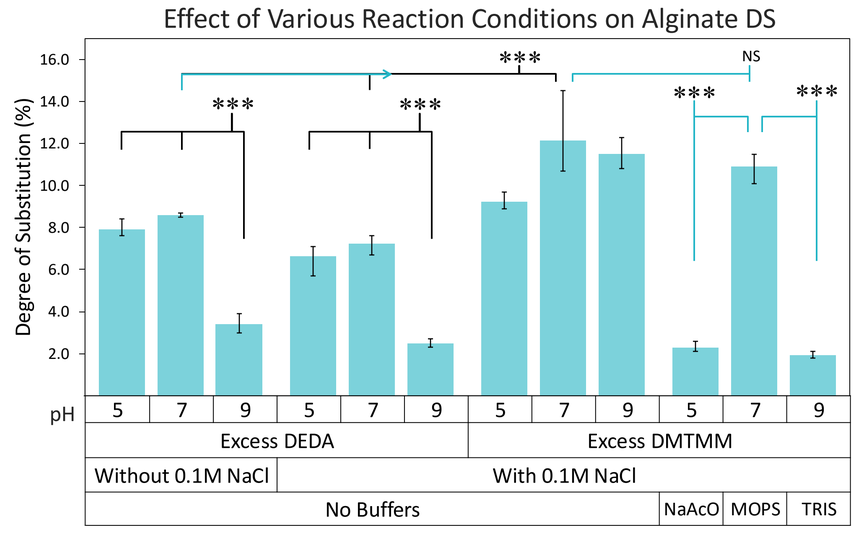
pH-dependent side reaction:
Premature hydrolysis of intermediate



Buffer Study

pH stabilization to prevent alginate degradation; potential side reactions





DEDA-Alginate DMTMM coupling at varied conditions. Using 0.3 eq DEDA, 0.1 eq DMTMM (excess DEDA) or 0.1 eq DEDA, 0.2 eq DMTMM (excess DMTMM). Statistics with One-way ANOVA Bonferroni multiple comparisons (black), Two-way ANOVA Turkey (blue).

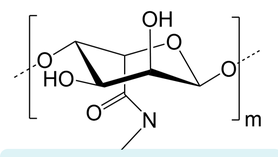
Results

- Limiting reagent is DEDA
- pH significant with excess DEDA
- NaAcO & TRIS cause side reactions

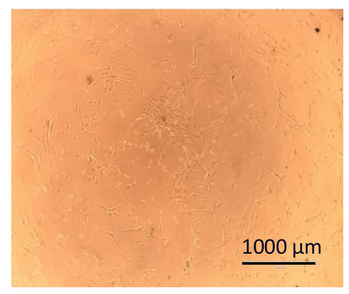
Optimized Conditions

- Excess DMTMM
- No Buffer

MSC Attachment to DMTMM coupled Alginate-Peptide Film



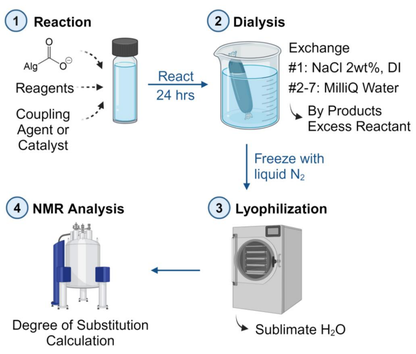
DS 0.4%



MSCs remain attached to ECM-mimicking peptide coupled alginate after 8 days.
Study and image courtesy of Pranati Mondkar.

Methods

Process: Elucidating DS



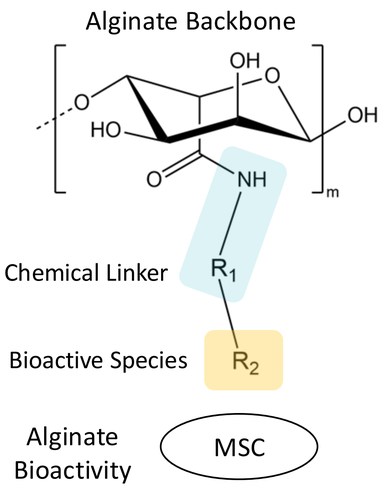
NMR: Degree of Substitution

$$\frac{\frac{I_S}{N_H}}{(G{:}M+1)\times I_G} = DS$$

I_S = Intensity of moiety signal
N_H = #protons represented by IS
G:M = alginate G, M blocks ratio
I_G = G anomeric peak intensity

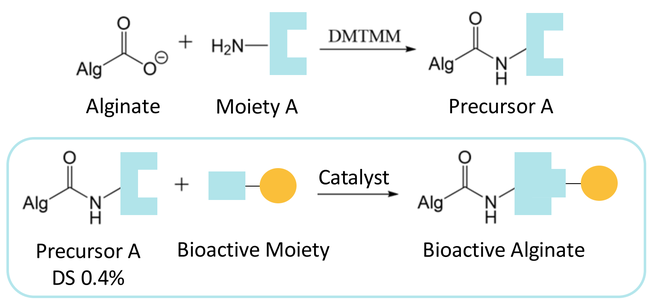
II. Multi-Step Addition Chemistry*

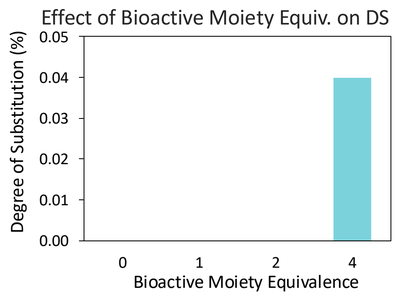
Study how the chemical linkage between alginate and bioactive moiety affects cellular behavior



Addition Chemistry A

Goal: Optimize bioactive moiety addition equivalence





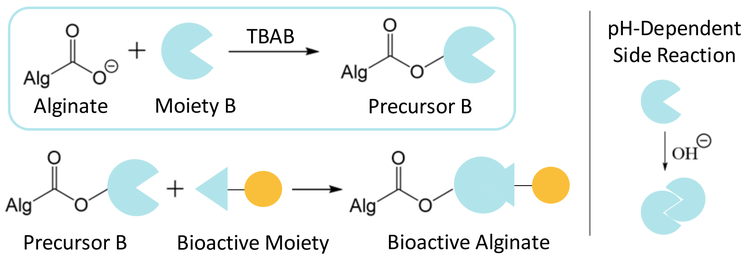
Addition of bioactive moiety, equivalence respective to 1 eq precursor A (2% alginate solution with 0.4% DS of Moiety A). Using 1 eq catalyst

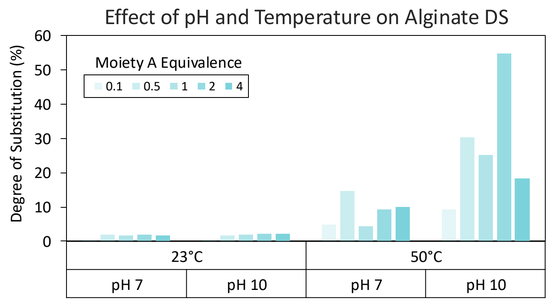
Results

- Negligible DS at lower bioactive moiety eq.
- Higher equivalences needed to reach target DS

Addition Chemistry B

Goal: Screen pH and temperature to mitigate side reactions





Addition of moiety B, equivalence respective to 1 eq alginate. Using 0.06 eq TBAB. Bad NMR baseline for 50°C and pH 7 study, underestimates DS.

Results

- Highest efficiency at higher temp.
- Minimal pH effect on DS

Conclusion and Future Work

I. DMTMM Coupling Chemistry

- Opt. conditions: excess DMTMM, no buffer
- Peptide-Alginate hydrogel showed MSC attachment
- Future Work: Use optimized conditions to study various bioactive moieties

II. Multi-Step Addition Chemistry A

- Low bioactive moiety equivalence had negligible DS
- Future Work: Study the reaction using a higher bioactive moiety equivalence, and vary catalyst equivalence

II. Multi-Step Addition Chemistry B

- High temp had higher substitution
- pH had less effect on DS than temp
- Future Work: Use smaller equivalences at pH7, 50°C to achieve materials with target DS

The tuned chemical pathways from this work can be widely applied to various moieties, foundational for future biomodification studies.

References and Acknowledgements

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This work was supported partially by the Research Experiences for Undergraduates (REU) Program of the National Science Foundation under Award Number **DMR-2011401** and **DMR-2244111**

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