Optimization of methacrylated alginate hydrogels synthesis for bone repair and regeneration

Ignacio Reinal[1], Cecilia Traldi[2,3,4], Chiara Tonda-Turo[2,3,4], José A. Gómez-Tejedor[1,5], Gloria Gallego-Ferrer[1,5], Piergiorgio Gentile[1,5]

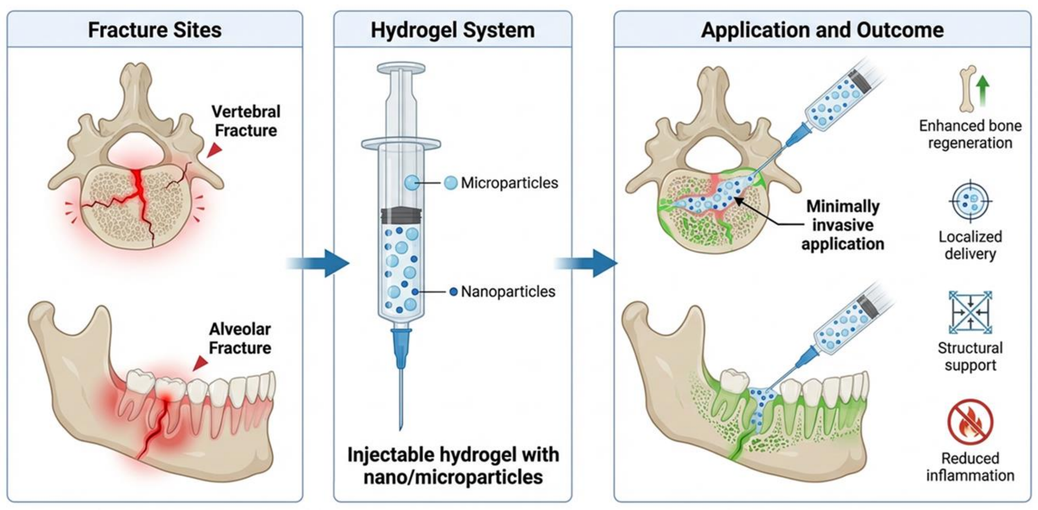
[1]Center for Biomaterials and Tissue Engineering (CBIT), Universitat Politècnica de Valencia, [2]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy, [3]POLITO BIOMedLAB, Politecnico di Torino, Turin, Italy, [4]Interuniversity Center for the Promotion of the 3Rs Principles in Teaching and Research, Italy, [5]CIBER de Bioingeniería, Biomateriales y Nanomedicina, Instituto de Salud Carlos III, Spain

INTRODUCTION

Bone diseases have become increasingly prevalent in recent decades, with fractures caused by osteoporosis, trauma, infections, or cancer significantly impacting patient quality of life and contributing to elevated mortality. In addition, comorbidities can impair the intrinsic regenerative capacity of bone, leading to delayed healing or non-union fractures and imposing a substantial socioeconomic burden[1-2]. Current clinical approaches, including autografts, allografts, and synthetic implants, are limited by donor-site morbidity, restricted availability, immune-related complications, and suboptimal long-term integration[3-4]. In this context, injectable hydrogels have emerged as promising biomaterials due to their minimally invasive administration, adaptability to irregular defect geometries, and ability to support tissue regeneration. In this work, we developed photocurable hydrogels based on methacrylated alginate (AlgMA) with tunable properties and controlled gelation kinetics[5-6]. A Box–Behnken experimental design was employed to optimize the degree of methacrylation, and AlgMA formulations were characterized using FTIR and ^{1}H-NMR to confirm successful functionalization. This optimization and physicochemical characterization represent a key step toward the development of injectable, in situ-forming biomaterials for improved bone fracture repair.

AIM OF THE WORK

The HYDROHEAL Project aims to develop injectable nano/microparticle-loaded hydrogels for bone fracture repair. **This work contributes by developing methacrylated alginate as the base material for these hydrogels.**

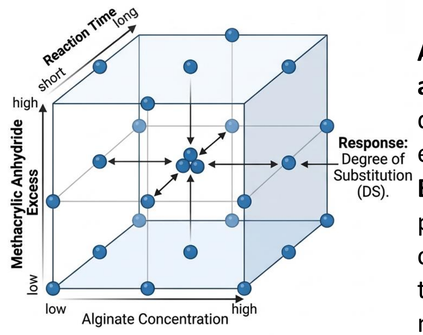


MATERIALS AND METHODS

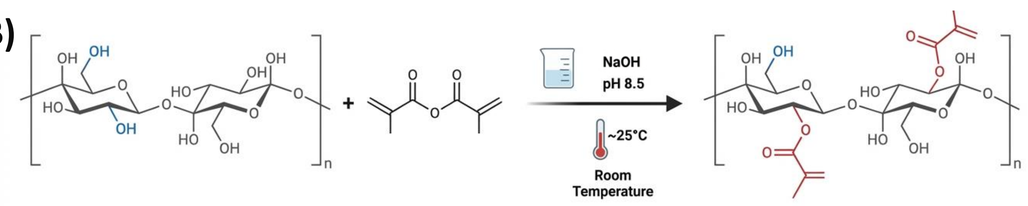
Design of Experiment and AlgMAs

A)

	Minimum	Maximum
Alg conc (%w/v)	0.5	1.5
Meth Anid conc (molar excess)	5	15
Reaction time (hours)	1	8



B)



Sodium Alginate — Methacrylic Anhydride — Methacrylated Alginate (AlgMA)

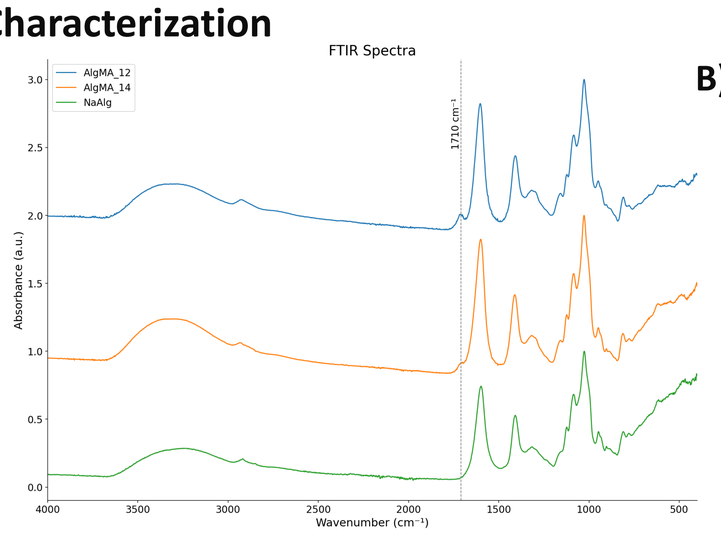
A) A **three-factor, three-level Box–Behnken design** was used to evaluate the effects of **alginate concentration**, **methacrylic anhydride excess**, and **reaction time** on the degree of substitution. The design consisted of 15 experimental runs, including 12 edge points and 3 center point replicates.

B) Reactions were carried out under constant stirring at room temperature, with the pH periodically adjusted to 8.5 using 5 M NaOH. After the reaction, samples were centrifuged to remove unreacted methacrylic anhydride, followed by dialysis for 7 days to eliminate residual reagents. Finally, the purified product was freeze-dried to obtain methacrylated alginate (AlgMA).

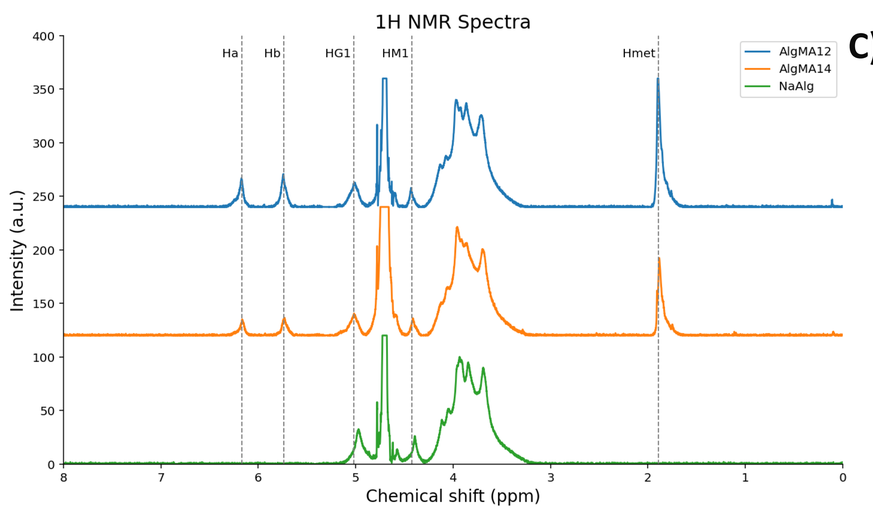
RESULTS

AlgMA Characterization

A)



B)



C)

Sample	Alg conc (%w/v)	Meth Anid conc (molar excess)	Reaction time (hours)	DS(%)
AlgMA_1	0.5	10	1	9.5
AlgMA_2	0.5	10	8	12.2
AlgMA_3	1.5	10	1	6
AlgMA_4	1.5	10	8	34.5
AlgMA_5	1	5	1	13.6
AlgMA_6	1	5	8	1.1
AlgMA_7	1	15	1	19.1
AlgMA_8	1	15	8	38
AlgMA_9	0.5	5	4.5	6.8
AlgMA_10	1.5	5	4.5	33
AlgMA_11	0.5	15	4.5	17.9
AlgMA_12	1.5	15	4.5	50.3
AlgMA_13	1	10	4.5	25.9
AlgMA_14	1	10	4.5	34.6
AlgMA_15	1	10	4.5	30.3

$$DS\% = \frac{\frac{Ha + Hb}{2}}{HG1 + HM1} * 100 \quad \text{(Eq.1)}$$

Figure A shows the **FTIR spectra**, where the **characteristic peak of alginate** appears at **~1710 cm^{-1}**. Figure B presents the **^{1}H-NMR spectra**, in which the **vinyl protons of the methacrylate group** are identified as **Ha** and **Hb** at **6.17** and **5.74 ppm**, respectively. The signal at **5.02 ppm (HG1)** corresponds to the **anomeric proton of guluronate units**, while the peak at **4.43 ppm (HM1)** corresponds to the **anomeric proton of mannuronate units**. Additionally, a signal at **1.89 ppm** is attributed to the **three protons of the methacrylate methyl group**. Figure C summarizes the **experimental conditions** and the **degree of substitution (DS)**, calculated using Eq. 1.

Box-Behnken model

A)

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
8.8875	72.25%	61.15%	30.27%

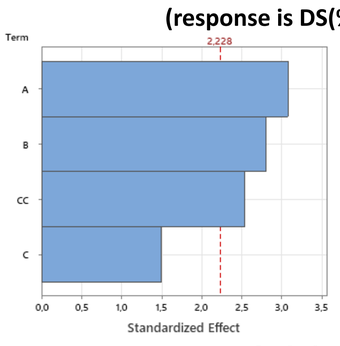
Analysis of Variance

		F-Value	P-Value
Model		6.51	**0.008**
Linear		6.54	0.01
	Alg conc(%w/v)	9.47	**0.012**
	Meth Anid conc (molar excess)	7.88	**0.019**
	Reaction time (hours)	2.25	0.164
Square		6.43	0.03
	Reaction time (h)*Reaction time (h)	6.43	**0.03**

Regression Equation in Uncoded Units

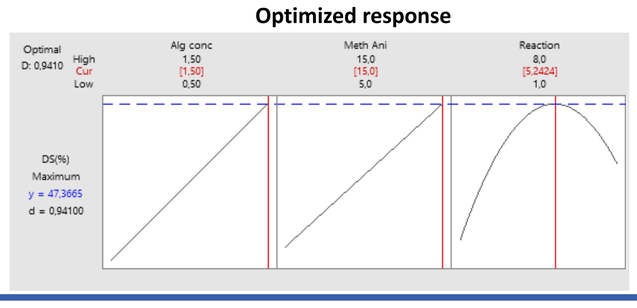
DS(%) = -33.9 + 19.34 Alg conc (%w/v) + 1.764 Meth Anid conc (molar excess) + 9.91 Reaction time (hours) - 0.952 Reaction time(h)*Reaction time(h)

B)

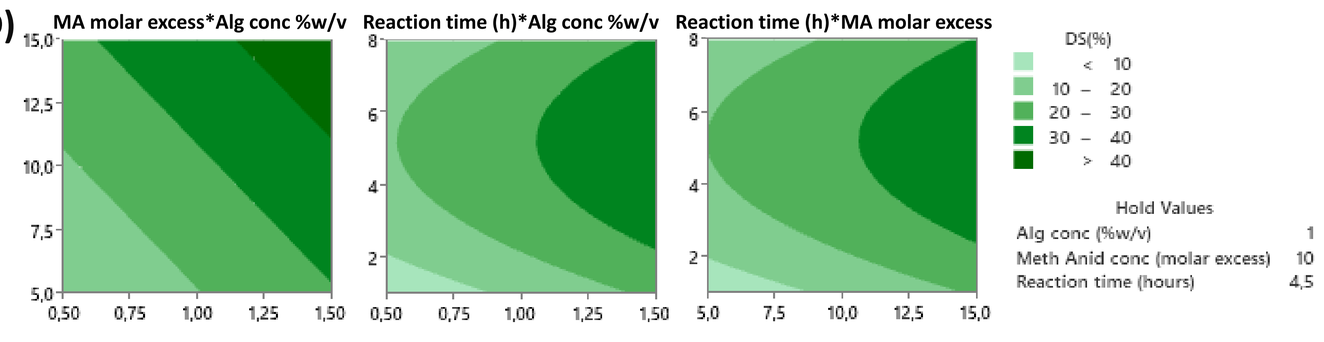


Factor	Name
A	Alg conc (%w/v)
B	MA conc (molar)
C	Reaction time (h)

C)



D)



The Box–Behnken model results are summarized in Figure A, including the **model summary**, the **analysis of variance (ANOVA)**, and the **regression equation**. ANOVA indicated that **alginate concentration (A)**, **methacrylic anhydride concentration (B)**, and the **quadratic term of reaction time (C²)** were **statistically significant**, supporting a **reduced model** including linear terms (A, B, C) and the quadratic term (C²). Figure B shows the Pareto chart, confirming the **significance of A, B, and C²**. In Figure C, the response optimizer illustrates the effect of each factor, highlighting the **non-linear behavior of reaction time**. Finally, Figure D presents contour plots for each pair of variables while keeping the third factor constant, providing a **visual representation of the response surface and factor interactions**.

CONCLUSIONS

This study demonstrates that methacrylated alginate can be successfully synthesized and its degree of substitution effectively modeled using a Box–Behnken design. Alginate and methacrylic anhydride concentrations were identified as the main factors influencing the reaction, while reaction time exhibited a non-linear effect, indicating the presence of an optimal intermediate value. Although the model showed good statistical significance and no lack of fit, its predictive capability remains moderate, suggesting that further refinement is possible. Future work will focus on incorporating additional experimental points, particularly around the predicted optimum, to improve model accuracy and robustness. Once the model is optimized, subsequent studies will evaluate gelation behavior and rheological properties to identify formulations with suitable injectability and mechanical properties for bone tissue repair applications within the HYDROHEAL project.

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