WBC 2020 virtual
11TH WORLD BIOMATERIALS CONGRESS
11 - 15 December 2020

GLYCERYLPHYTATE-CROSSLINKED CHITOSAN LACTATE MICROGELS AS MSC-DELIVERY PLATFORM IMPROVE CELL SURVIVAL AND UPREGULATE SECRETORY PROFILE

A. MORA-BOZA [1], L. M. MANCIPE CASTRO [2, 3], R. S. SCHNEIDER [3, 4], W. M. HAN [2, 3], J. SAN ROMÁN [1], B. VÁZQUEZ-LASA [1], A. J. GARCÍA [2, 3]

[1] Institute of Polymer Science and Technology, and CIBER-BBN, Madrid, Spain
[2] Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA
[3] Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA
[4] School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA

INTRODUCTION

Therapies based on human mesenchymal stem cells (hMSCs) offer promising approaches for the treatment of diverse degenerative and inflammatory diseases. [1] Therapeutic interest in hMSC originally focused on their self-renewal capacities and ability to differentiate into different cell lineages. [2] Recently, paracrine signalling has been recognized to play an essential role in hMSCs therapeutic efficacy via secretion of bioactive factors. [3-5] Nevertheless, the effective clinical application of hMSCs-based therapies remains limited due to low cell survival and persistence *in vivo*, **highlighting the need for developing new cell-carriers.**

Herein, **chitosan lactate (ChLA) microgels are fabricated in a flow-focusing microfluidic device via *in situ* gelation using the biologically active compound glycerylphytate (G_1Phy)** combined with tripolyphosphate (TPP) as ionic crosslinkers. G_1Phy is a natural derivative compound with reduced cytotoxicity and powerful antioxidant activity [6] that could provide biological benefits to the microgels. Its crosslinking capacity has been applied in 3D printing technology, but it has never been applied before in hMSCs encapsulation microfluidic processes. [7] The proposed microgel formulation and fabrication approach provide novelty at two different levels: **G_1Phy will act not only as a cytocompatible and natural-occurring crosslinker with powerful gelation properties, but also as a biologically active component** of the developed microcarriers in comparison to other traditionally applied crosslinking agents that lack bioactivity.

Specifically, **hMSCs encapsulation is realized via the use of: (i) ChLA, a water-soluble chitosan derivative synthetized in our laboratory; and (ii) a reactive mixture of G_1Phy combined with TPP as crosslinkers providing bioactivity and optimum gelation kinetics**. The novel microgel composition (i.e. G_1Phy:TPP-microgels) is studied and compared with the microgels formed with only TPP (i.e. TPP-microgels) as control to evaluate the effect exerted by G_1Phy regarding hMSCs viability, paracrine factor secretion, and *in vivo* persistence.

AIM

Microfluidics generation of bioactive ChLA microgels containing G_1Phy crosslinker for *in situ* hMSCs encapsulation that can be directly administered by minimally invasive injection for cell therapy applications.

METHOD

1. **ChLA derivative synthesis:** ChLA was synthesized through a condensation reaction with lactic acid as shown in Figure 1a. Lactic conjugation was confirmed by ^{1}H-Nuclear Magnetic Resonance (NMR) (Figure 1b).

2. **ChLA microgels microfluidics generation:** ChLA microgels were obtained by ***in situ* ionotropic gelation in a flow-focusing microfluidic device** (Figure 2a). ChLA phase consisted of ChLA solution (1.0 wt-%) in PBS, pH 7.4, and crosslinker phase contained a G_1Phy:TPP mixture or TPP as control (Table 1). For encapsulated-hMSCs microgels, cells were resuspended in ChLA solution at a final density of $2x10^6$ cells mL^{-1}. Flow rates were adjusted to 1.5 µL min^{-1} for both polymer and crosslinker phases, and 20 µL min^{-1} for continuous phase (mineral oil containing 3% (v/v) SPAN80). Microgel morphology was evaluated by **optical microscopy.**

Table 1: Sample nomenclature as function of crosslinker phase composition.

Sample	TPP (wt-%)	G_1Phy (wt-%)
TPP-microgel	0.50	0
G_1Phy:TPP-microgel	0.40	0.10

3. ***In vitro* encapsulated hMSCs characterization:** *In vitro* cell viability was evaluated by **Live/Dead assay** and confocal imaging. **Paracrine factor secretion** of encapsulated hMSCs in microgels was evaluated under two conditions: oxidative stress and interferon-γ (IFN-γ) activation. Samples were analysed using a custom Luminex® Assay (R&D Systems).

4. **Pilot *in vivo* study:** Luciferase-expressing hMSCs (hMSCs^{Luc}) were generated by transducing hMSCs with lentivirus encoding for firefly luciferase and used for pilot *in vivo* study. **Cell persistence and survival** were evaluated *in vivo* by **tracking the bioluminescence** of encapsulated hMSCs^{Luc} that were injected into dorsal subcutaneous spaces of immunocompromised mice. Bioluminescence signal was measured using an IVIS Spectrum CT System.

RESULTS

1. ChLA derivative synthesis: The characteristic poor solubility of chitosan at physiological pH limits its use for *in situ* cell encapsulation processes. [8] Thus, we synthetized ChLA derivative (Figure 1a), which showed improved solubility at pH 7.4. Lactate conjugation was confirmed by ^{1}H-NMR (Figure 1b) due to lactate groups appearance.

ChLA showed ***in situ* gelation ability against ionic crosslinking agents containing phosphate groups at physiological conditions**, based on electrostatic interactions between phosphate groups present in the crosslinkers (G_1Phy and TPP) and protonated amino groups of chitosan (Figure 1c).

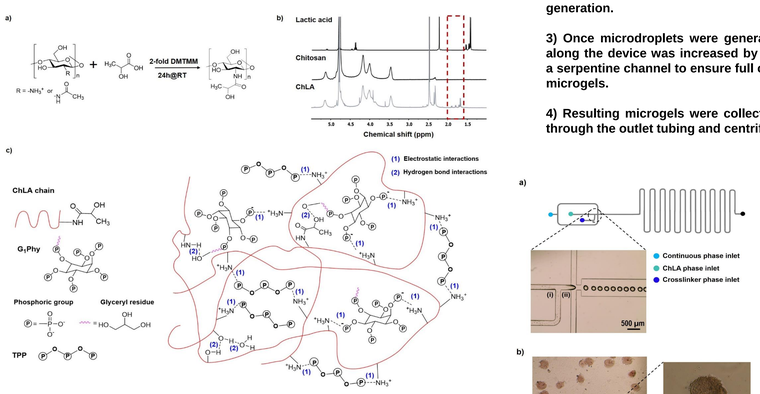


Figure 1: (a) Scheme of condensation reaction between chitosan and lactic acid; (b) ^{1}H-NMR spectra of lactic acid, chitosan, and ChLA recorded in D_2O; in ChLA spectrum red box denotes the chitosan conjugated lactate groups; (c) Molecular structure of G_1Phy and TPP, and schematic illustration of the network showing interactions that take place during ChLA gelation with crosslinkers.

ChLA provided an adequate environment for cell encapsulation and the suitable amount of ionizable groups that can be successfully crosslinked at physiological conditions using phosphate-based agents.

2. Generation of ChLA microgels using microfluidics: Flow-focusing devices with 3 independent flow inlets (ChLA, crosslinker, and continuous phases) were used (Figure 2) to produce ChLA microgels with and without encapsulated hMSCs as follows:

1) ChLA and crosslinker phase (Table 1) were merged at a T-junction to enable polymer-crosslinker interaction.

2) The reactive mixture was focused to the continuous phase to allow water/oil emulsion and droplet generation.

3) Once microdroplets were generated, residence time along the device was increased by the incorporation of a serpentine channel to ensure full crosslinking of ChLA microgels.

4) Resulting microgels were collected in culture media through the outlet tubing and centrifuged.

Figure 2: (a) Microfluidic device design that allowed: (i) the mixture of ChLA and crosslinker phases; and (ii) the subsequent water/oil emulsion with continuous phase to generate microdroplets; (b) Light microscopy images of G1Phy:TPP-microgels at different magnifications after purification;

The blend G_1Phy and TPP represents a new strategy to obtain stable microgels containing G_1Phy that provided the microgel formulation with bioactive properties

3. *In vitro* viability of encapsulated hMSCs: High cell viabilities (79±2% and 67±2%, for TPP- and G_1Phy:TPP-microgels, respectively) were observed after synthesis, demonstrating the **suitability of encapsulation and fabrication methods regarding cell survival (Figure 3a).**

For prolonged culture periods, **cell viability of encapsulated hMSCs in G_1Phy:TPP-microgels remained constant over time** in comparison to encapsulated cells in TPP-microgels (Figure 3b)..

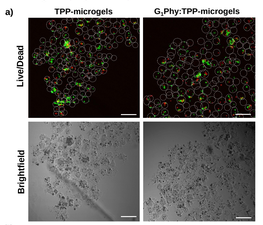


Figure 3: (a) Live/Dead staining and brightfield confocal images of G_1Phy:TTP- and TPP-microgels after hMSCs microencapsulation. Scale bars correspond to 500 µm; (b) Cell viability percentages (mean ± sd) over time for G_1Phy:TPP- and TPP-microgels. Two-way ANOVA analysis was performed at significant levels of **p<0.001 and ****p<0.0001.

The presence of G_1Phy exerts a positive effect on the survival and maintenance of encapsulated hMSCs in ChLA microgels over time.

4. *In vitro* paracrine secretory profile of encapsulated hMSCs: G_1Phy incorporation into microgel composition had a modulatory effect in the secretome of encapsulated hMSCs by enhancing the secretion of different pro-survival and pro-angiogenic factors (e.g. VEGF, HGF, FBF basic, among others) and immunoregulatory factors (e.g. CXCL10, IL-8, MCP-1) when exposed to oxidative and inflammatory environments.

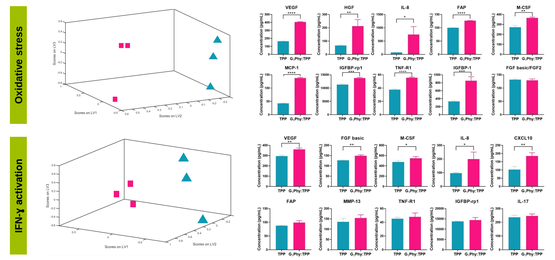
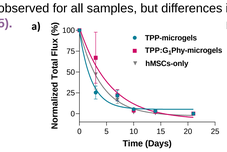


Figure 4: PLS-DA of the total set of analyzed paracrine factors. Blue triangles and pink squares correspond to TPP-microgels and G1Phy:TPP-microgels groups, respectively. Individual analysis of secreted analytes from encapsulated hMSCs in TPP-and TPP:G_1Phy-microgels studied using Luminex® assay. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

G_1Phy could be considered as a promising compound for enhancing paracrine signalling related to tissue repair capacities of encapsulated hMSCs in oxidative/inflammatory environments.

5. Pilot *in vivo* study: Microgel-encapsulated cells delivery process was as simple as the injection of unencapsulated hMSCs (hMSC-only). Equivalent signal intensities were observed between microgel-encapsulated cells and unencapsulated cells in saline, **demonstrating no adverse effects in initial cell survival.** Similar bioluminescence profiles over time were observed for all samples, but differences in the decay rates were observed among groups (Figure 5).



b)

Sample	Half-life (days)
G_1Phy:TPP-microgel	3.7±1.9
TPP-microgel	2.2±1.5
Unencapsulated cells	2.8±1.3

Figure 5: *In vivo* bioluminescence signal represented as normalized total flux (%) over time n = 7 per group; mean ± sd (a); Calculated half-life values for each sample from single decay fit of the normalized total flux. mean ± sd (b).

These results suggest an improved persistence tendency since the found half-life values for G_1Phy:TPP-microgels were relatively higher than those obtained for TPP-microgels and unencapsulated cells.

CONCLUSIONS

→ **A microfluidics approach for *in situ* hMSC encapsulation in bioactive chitosan microgels has been developed.**

→ **ChLA microgels incorporating the bioactive G_1Phy combined with TPP offer significant advantages as a hMSC delivery platform:**

- → Minimally invasive delivery by injection.
- → Cell viability maintenance over time.
- → Upregulation of paracrine signalling at adverse conditions (e.g. oxidative stress and inflammation).

The as-obtained G_1Phy-crosslinked microgels emerge as a suitable and novel cell delivery platform since its therapeutic effect is not only due to support of encapsulated hMSC viability but also modulation of hMSCs secretome.

REFERENCES

1. **Facklam AL**, Volpatti LR, Anderson DG. Biomaterials for Personalized Cell Therapy. 2020;32(13):1902005.
2. **Choe G,** Park J, Park H, Lee JY. Hydrogel Biomaterials for Stem Cell Microencapsulation. Polymers (Basel). 2018;10(9).
3. **Ascencio González D**, Hernández Pando R, Ángel Gómez Lim M, Ayala Fraustro S, Torres Garcia A. Therapeutic Strategies of Secretome of Mesenchymal Stem Cell. Stromal Cells - Structure, Function, and Therapeutic Implications2019.
4. **Gnecchi M**, Ciuffreda MC, Mura M. Mesenchymal Stromal Cell Secretome for Tissue Repair. Cell Engineering and Regeneration2019. p. 1-26.
5. **Harrell CR**, Fellabaum C, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Molecular Mechanisms Responsible for Therapeutic Potential of Mesenchymal Stem Cell-Derived Secretome. Cells. 2019;8(5).
6. **Mora-Boza A,** López-Donaire ML, Saldaña L, Vilaboa N, Vázquez-Lasa B, San Román J. Glycerylphytate compounds with tunable ion affinity and osteogenic properties. Scientific Reports. 2019;9(1):11491.
7. **Mora-Boza A**, Włodarczyk-Biegun MK, del Campo A, Vázquez-Lasa B, Román JS. Glycerylphytate as an ionic crosslinker for 3D printing of multi-layered scaffolds with improved shape fidelity and biological features. Biomaterials Science. 2020;8(1):506-16.
8. **Chen X-G**, Liu C-S, Liu C-G, Meng X-H, Lee CM, Park H-J. Preparation and biocompatibility of chitosan microcarriers as biomaterial. Biochemical Engineering Journal. 2006;27(3):269-74.

ACKNOWLEDGEMENTS

The authors thank the **Spanish Ministry of Economy, Industry and Competitiveness** (project, MAT2017-84277-R) and **U.S. National Institutes of Health** (R01 AR062368) for financial support. B. Vázquez-Lasa and J. San Román are members of the SusPlast platform (Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy) from the **Spanish National Research Council** (CSIC). Ana Mora-Boza was supported by "**La Caixa" Foundation** (ID 100010434, scholarship code LCF/BQ/ES16/11570018) and **CIBER-BBN (Health Institute Carlos III)** Travel grant.

CONTACT INFORMATION

Ana Mora-Boza, PhD
Researcher at Biomaterials group (ICTP-CSIC)
ana.mora@ictp.csic.es
@Ana_mboz