WBC2020 - Late Breaking

Biomaterials for tissue engineering applications

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Glycerylphytate-crosslinked chitosan lactate microgels as MSC-delivery platform improve cell survival and upregulate secretory profile

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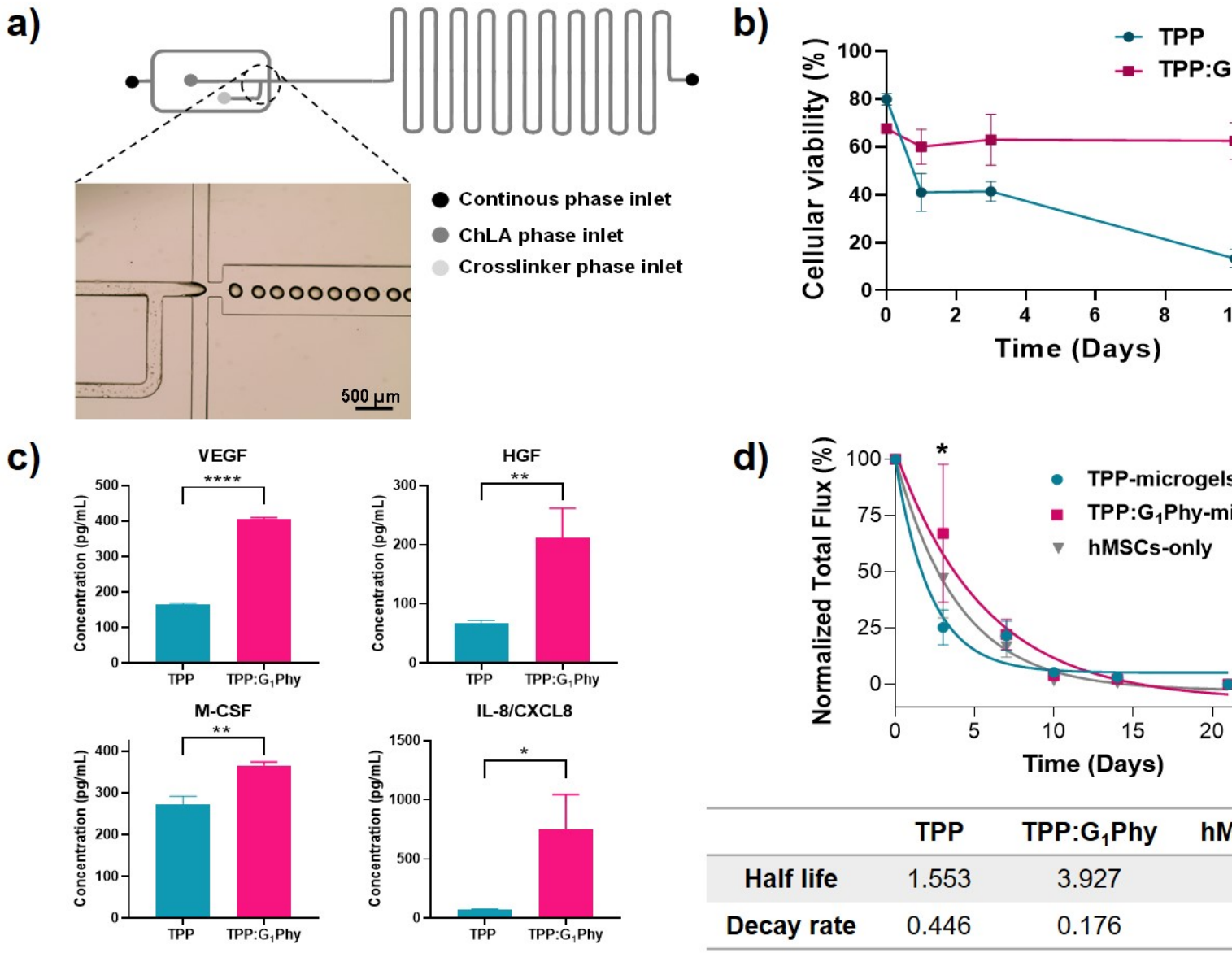
Introduction: Mesenchymal stem cells (MSCs) exhibit multiple beneficial properties, most notably potent immunomodulation via their secretory factors that render them as an attractive cell source for cell therapy. However, their clinical application remains limited due to their low survival and persistence *in vivo*, highlighting the need for new cell-carriers[1]. Chitosan is a widely applied biomaterial, but its poor solubility has constrained its use as a cell carrier[2]. We examined the fabrication of chitosan-based MSCs-microcarriers using a water-soluble chitosan lactate (ChLA) derivative and *in situ* crosslinking in a microfluidic device using tripolyphosphate (TPP) and glycerylphytate (G_1Phy) as ionic crosslinkers. G_1Phy is a powerful antioxidant,[3] whose crosslinking ability has been evinced[4]. Our microgel synthesis allows for cell encapsulation, and G_1Phy provided beneficial features to microgels in terms of MSCs survival, persistence and secretome modulation.

Experimental methods: ChLA microgels were synthesized by *in situ* crosslinking reaction in a microfluidic device (Fig. 1a). Two crosslinker compositions were analyzed: TPP:G_1Phy-microgels, and TPP-microgels, which did not contain G_1 Phy. Cellular viability was assessed by live/dead assay. To evaluate the antioxidant effect of G_1Phy on MSCs, cells were cultured under oxidative stress and their secretome was analysed using a multiplex LUMINEX® assay. *In vivo* cell persistence and viability were studied by tracking microgels containing luciferase-transfected MSCs, subcutaneously injected in immunocompromised mice using an IVIS Spectrum CT.

Image:



	TPP	TPP:G_1Phy	hM
Half life	1.553	3.927	
Decay rate	0.446	0.176	

Fig. 1: a) Microfluidic device and microgel synthesis approach; b) Cellular viab
over time; c) Cytokine secretion of encapsulated MSCs under oxidative str
Normalized bioluminescence flux (%) over time.

Table:

ChLA microgels were successfully synthetized through *in situ* crosslinking reaction in microfluidic devices. For both microgel compositions, our fabrication method allowed efficient cell encapsulation with over 70% cellular viability due to the use of: (i) water-soluble chitosan derivative; and (ii) biocompatible ionic crosslinkers with fast crosslinking kinetics. MSCs viability in TPP-microgels decreased up to 13±4% after 10 days of culture, while it remained stable (62±8%) for TPP:G_1Phy-microgels (Fig. 1b). This result indicates that G_1Phy maintained cell survival over time.

We observed an upregulated secretion of pro-survival and pro-angiogenic cytokines (Fig. 1c) for TPP:G_1Phy- in comparison to TPP-microgels under oxidative stress. Thus, the immunoregulatory effect of G_1Phy was demonstrated on injured-like tissue environment, which are characterized by a high concentration of oxidative species.
Benefits of G_1Phy were also demonstrated *in vivo*. Bioluminescence tracking showed higher half-life values for TPP:G_1 Phy- than for TPP-microgels, indicating an enhanced cell persistence and survival (Figure 1d).
Results and discussions: ChLA microgels were successfully synthetized through *in situ* crosslinking reaction in microfluidic devices. For both microgel compositions, our fabrication method allowed efficient cell encapsulation with over 70% cellular viability due to the use of: (i) water-soluble chitosan derivative; and (ii) biocompatible ionic crosslinkers with fast crosslinking kinetics. MSCs viability in TPP-microgels decreased up to 13±4% after 10 days of culture, while it remained stable (62±8%) for TPP:G_1Phy-microgels (Fig. 1b). This result indicates that G_1Phy maintained cell survival over time.
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Conclusions: Given all beneficial properties provided by G_1Phy, we envision that our G_1Phy-crosslinked ChLA microgels will have promising applications as MSCs-delivery platforms.
References/Acknolwedgements: 1. G. Choe et al. Polymers 10(9), 2018
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Disclosure of Interest: None Declared

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