INJECTABLE THERMORESPONSIVE HYDROGELS FOR CARDIAC REGENERATION

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BACKGROUND & AIM

Following myocardial infarction, because of the limited regenerative ability of the heart tissue, damaged tissue is replaced by a fibrotic scar, which is a non-conductive tissue that limits the passage of heart synchronized electrical signals, thus resulting in the loss of heart muscle function [1]. Scaffolds loaded with conductive materials, such as graphene, have emerged, as they mimic the physiological role of electrical signalling in the native myocardium [2]. Given these premises, the aim of this research project was the design and development of thermosensitive hydrogels based on poloxamer (PL) and pullulan (PULL) or hyaluronic acid (HA) and loaded with graphene (Gr) for pericardial cavity injection, intended as a bridge to connect the functional myocardial areas, bypassing the fibrotic area.

METHODS

HYDROGEL PRODUCTION

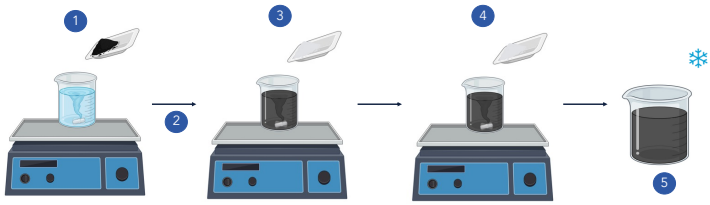


Figure 1. Schematic representation of hydrogel preparation: (1) addition of Gr powder at different concentrations to MilliQ water; (2) sonication for 2 hours; (3) addition of PULL or HA to the blend; (4) addition of PL to the blend; (5) overnight dissolution at 4°C

Table 1. Quali-quantitative composition of Gr-doped hydrogels

Blend	PL (%w/w)	PULL (%w/w)	HA (%w/w)	Gr (%w/w)
PL20	20	-	-	-
PL-PULL				-
PL-PULL-Gr0.05				0.05
PL-PULL-Gr0.1	20	0.4	-	0.1
PL-PULL-Gr0.5				0.5
PL-HA				-
PL-HA-Gr0.05				0.05
PL-HA-Gr0.1	20	-	0.4	0.1
PL-HA-Gr0.5				0.5

RESULTS

VISCOSITY

An increase in viscosity was registered at 37°C for all formulations in comparison to 4 and 20°C, indicating a transition to a gel-like state at higher temperature due to the presence of PL.

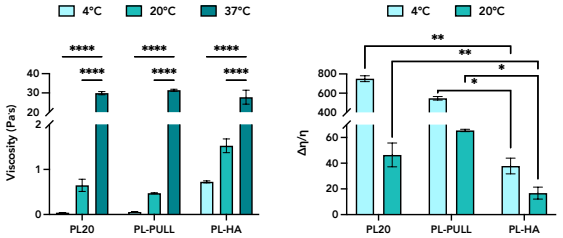


Figure 2. Viscosity at 4°C, 25 °C and 37 °C in a range of strain between 0 and 100 1/s (mean values ± SD; n = 3). ANOVA one-way; Scheffé test (*p ≤ 0.05, **p ≤ 0.01, ****p ≤ 0.0001).

GELATION TEMPERATURE

All formulations demonstrated a gelation temperature below 37°C, which is ideal for easy injection or administration into the target site, as the material would transition into a gel-like state at body temperature, ensuring optimal mechanical support and stability upon injection.

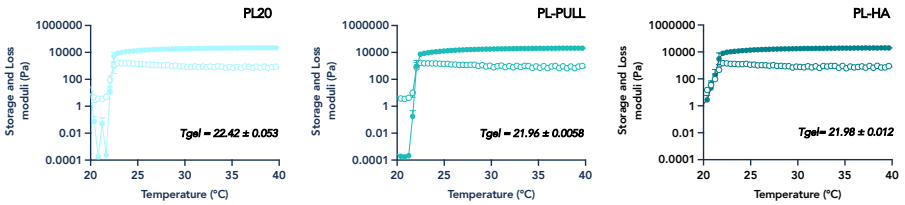


Figure 3. Temperature sweep and variation in G' and G'' moduli of PL20, PL-PULL and PL-HA, measured at 1 Hz and 25 Pa in a temperature range of 20–40 °C; in the inserts, the temperature of gelation of PL20, PL-PULL and PL-HA corresponding to the crossover point where G' equals G'' (mean values ± SD; n = 3)

PENETROMETRY

The systems were characterized by very low consistency at 20°C, while storage at 37 °C caused an increase in system consistency as evidenced by an increase in the work of penetration, demonstrating that gelation was not impaired by the addition of Gr.

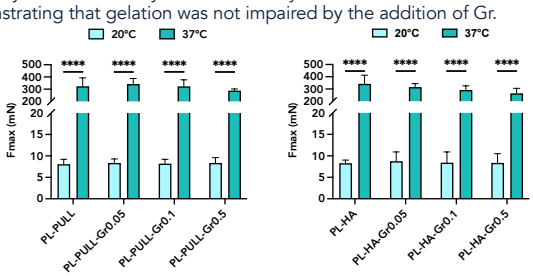


Figure 4. Maximum penetration force profile (Fmax) of PL-PULL and PL-HA and Gr-doped PL-PULL and PL-HA hydrogels, reflecting the stiffness and internal structure of the gel (mean values ± SD; n = 6)

ELECTRICAL CONDUCTIVITY

All the hydrogels exhibited conductivity within the range of native myocardium (0.1 mS/cm to 10,000 mS/cm). Specifically, Gr-doped PLHA hydrogels exhibited an enhanced conductivity, suggesting their potential for restoring electrical communication in damaged cardiac tissue.

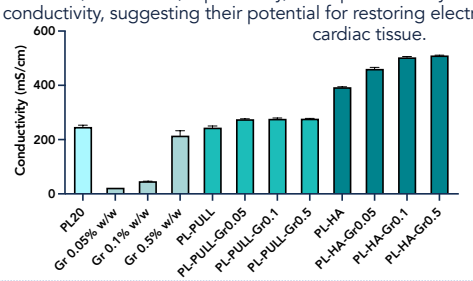


Figure 5. Electrical conductivity (mS/cm) of undoped PL20, PL-PULL and PL-HA and Gr-doped PL-PULL and PL-HA hydrogels (mean values ± sd; n = 3)

SYRYNGEABILITY

As the values of extrusion force measured for all systems is significantly lower (between 1500 and 23000 mN) than the average force of extrusion which is normally applied by clinicians during injections, they can be considered to have excellent injectability.

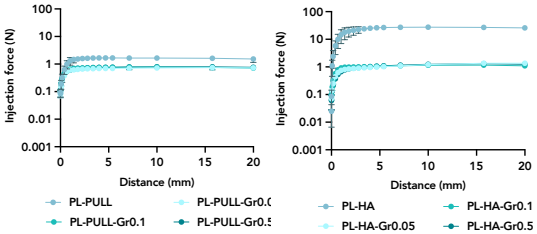


Figure 6. Injection force profile obtained from the extrusion of PL-PULL and PL-HA and Gr-doped PL-PULL and PL-HA hydrogels (mean ± s.d.; n = 6).

BIOADHESION

The hydrogels doped enriched with HA and PULL exhibited strong adhesive properties, as demonstrated by their higher force of detachment in comparison to the blank and PL20, ensuring stable integration with the damaged cardiac tissue and preventing displacement.

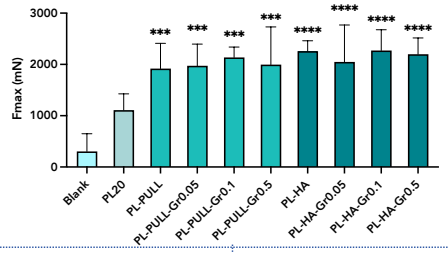
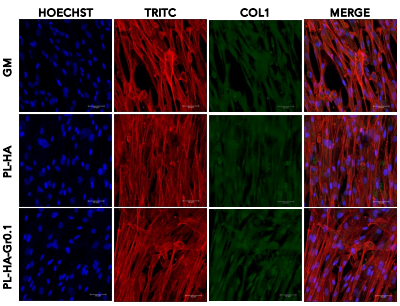


Figure 7. Fmax values in presence and in absence (blank) of an in-vitro biological substrate of undoped PL20, PL-PULL and PL-HA and Gr-doped PL-PULL and PL-HA hydrogels measured at 37 °C after 180 s of contact (mean value ± s.e.; n = 6)

IN-VITRO ASSAYS

Cell proliferation was comparable to GM up to 10 days, without fibroblast overactivation. Cells showed proper cytoskeletal organization and physiological Col 1 deposition, with no signs of overproduction.



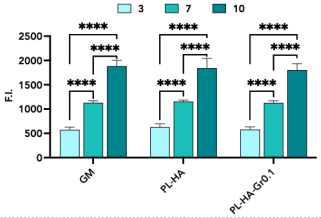


Figure 8. AlamarBlue® assay at 3, 7 and 10 days of HCFs (mean values ± sd; n = 4). ANOVA one-way; Scheffé test; CLSM images of HCFs after 10 days (in blue nucleiin red cytoskeletons in green collagen I matrix) (scale bar: 50 µm).

CONCLUSION

Thermoresponsive hydrogels, loaded with Gr, were developed as a promising treatment for cardiac regeneration, exhibiting excellent injectability and strong bioadhesion. HCFs showed preserved viability, proliferation, and morphology, without signs of excessive remodelling *In-vivo* safety was confirmed on a murine model.

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FORCE REPAIR

REFERENCES

[1] Talman V. et al., 2016, 10.1007/s00441-016-2431-9; [2] Saghebasl S. et al., 2022, 10.1002/pat.5669

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