SUPPLEMENTARY INFORMATION

Dual-functional injectable hydrogel as antimicrobial and angiogenic therapeutics for dental pulp regeneration

Nhu-Quynh Thi Nguyen [a,1], Cuong Hung Luu [b,c,1], Ngoc-Dan Ho Nguyen [d], Duy Quoc Vo [a], Ngoc Hong T. Luu [e], Nhu-Y Ngoc Ha [a], Thanh-Truc Ngoc Vo [d], Que-Phuong Le Huynh [a], Thavasyappan Thambi [f], Chau T.T. Ngo [d,*], Giang V.H. Phan [a,*]

[a] *Biomaterials and Nanotechnology Research Group, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam*

[b] *School of Environment and Science, Griffith University, Nathan, QLD 4111, Australia*

[c] *Queensland Micro- and Nanotechnology Centre, Griffith University, Nathan, QLD 4111, Australia*

[d] *Group of Applied Research in Advanced Materials for Sustainable Development, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam*

[e] *School of Biomedical Engineering, International University, Ho Chi Minh City, Vietnam*

[f] *Graduate School of Biotechnology, College of Life Sciences, Kyung Hee University, Yongin si, Gyeonggi do 17104, Republic of Korea*

[1] These authors contributed equally.

[*] Corresponding authors:

Chau T.T. Ngo, Ph.D., email: ngothituongchau@tdtu.edu.vn

V.H. Giang Phan, Ph.D., email: phanvuhoanggiang@tdtu.edu.vn

Crosslinking density calculation

The Flory-Rehner equation was employed to calculate the crosslinking density, following the approach described by Leach et al., to determine the average molecular weight between crosslinks and the mesh size [1]. The Flory-Rehner equation is presented below.

$$Crosslinking\ density\ (mol \cdot mL^{-1}) = -\frac{\ln(1-\varphi_p) + \varphi_p + \chi\varphi_p^2}{2\varphi_s\left(\varphi_p^{1/3} - \frac{\varphi_p}{2}\right)} \tag{S1}$$

Here, φ_p represents the polymer volume fraction in the swollen state, while φ_s denotes the molar volume of the solvent ($mL \cdot mol^{-1}$). For PBS, this value was approximated to that of water, which is 18 $mL \cdot mol^{-1}$. The polymer-solvent interaction parameter (χ) was estimated to be approximately 0.473, as described in [1]

To determine φ_p, the swelling ratio was utilised in calculations based on the equation presented below, which was adapted from the study by Richbourg et al. [2].

$$\varphi_p = \frac{1}{1 + \frac{\rho_p}{\rho_s}Q} \tag{S2}$$

In this equation, ρ_p denotes the density of the nonswollen polymer network, which was estimated to be approximately 1.7 $g \cdot mL^{-1}$, while the polymer-solvent interaction parameter (ρ_s) corresponded to the density of water (1 $g \cdot mL^{-1}$). The results obtained from these calculations depended upon the volumetric swelling ratio (Q), determined experimentally for each hydrogel sample. All computed values derived from these calculations were subsequently compiled and are summarised in Table S1.

Table S1. Crosslinking density of HACM hydrogels calculated from swelling ratio results

Samples	Crosslinking density ($nmol \cdot mL^{-1}$)
HACM1	23.4 ± 5.9
HACM2	175 ± 14.2
HACM3	94.9 ± 1.9

HACM4	45.9 ± 6.7

Table S2. Drug release kinetic of drug-loaded HACM hydrogels

	HACM/AX		**HACM/EPO**	
	Regression equations	**R^2**	**Regression equations**	**R^2**
Zero-order	$Q = 0.0046\ t - 0.0645$	0.9847	$Q = 0.0046\ t - 0.0618$	0.9778
First-order	$\log\ = 0.0055\ t - 1.0568$	0.9699	$\log Q = 0.0055\ t - 1.0386$	0.9673
Higuchi	$Q = 8.5708\ t^{1/2} - 42.2542$	0.9338	$Q = 8.6170\ t^{1/2} - 42.1000$	0.9244
Hixson-Crowell	$Q^{1/3} = -0.0119\ t + 5.0112$	0.9190	$Q^{1/3} = -0.0124\ t + 5.0406$	0.8870
Korsmeyer-Peppas	$\log Q = 1.0079 \log t - 2.4478$	0.9867	$\log Q = 0.9841 \log t - 2.3951$	0.9946

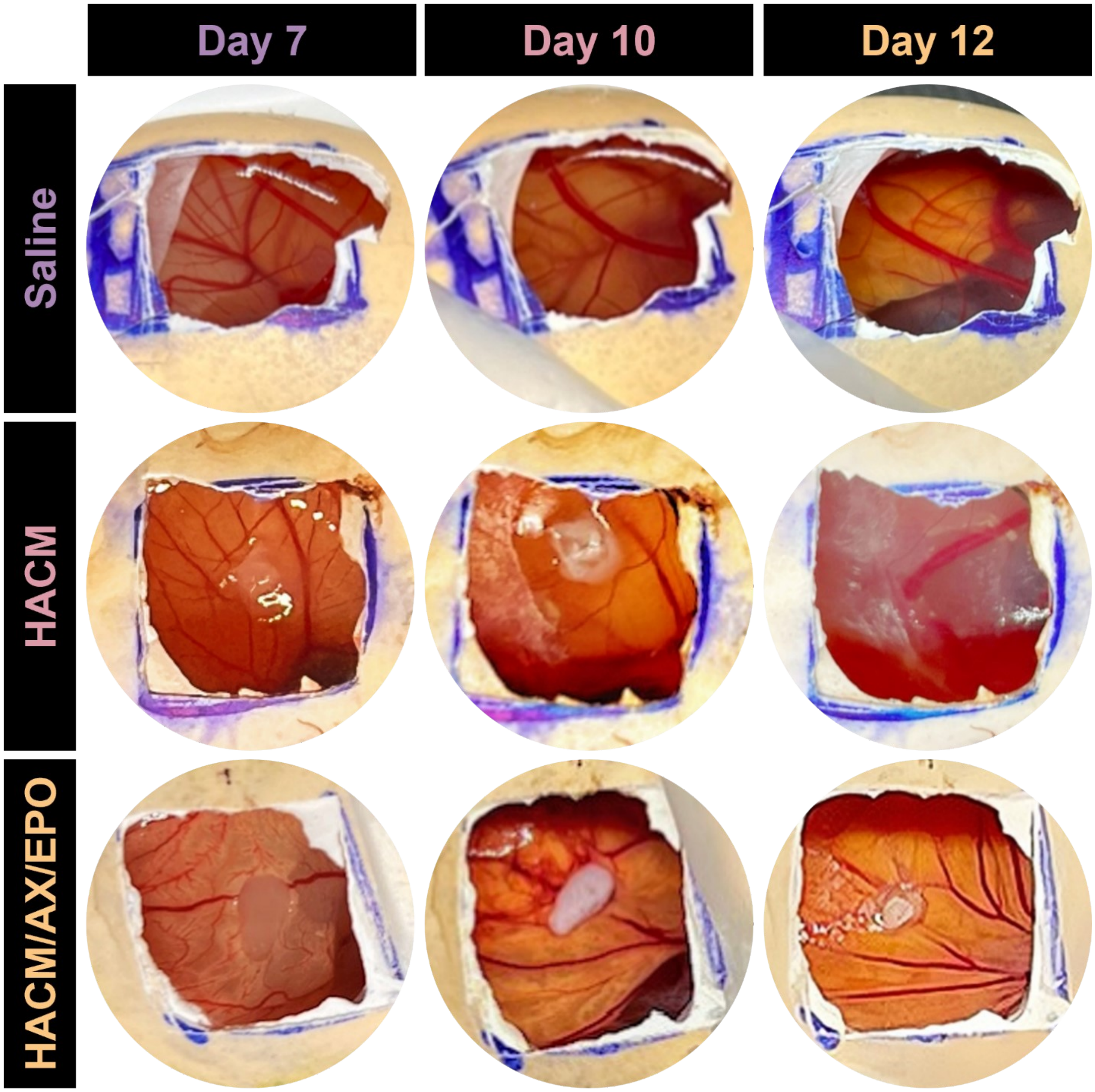

Figure S1. Chick embryo chorioallantoic membrane (CAM) assay performed with HACM and HACM/AX/EPO using saline as control and monitored on days 7, 10, and 12 of embryonic development.

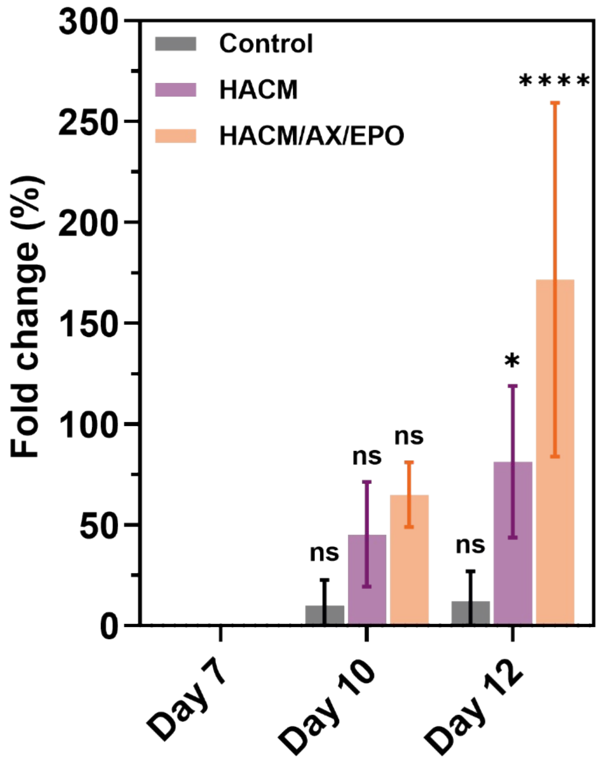

Figure S2. Vascular area expansion quantified via CAM assay results.

References

1. Baier Leach, J., et al., *Photocrosslinked hyaluronic acid hydrogels: Natural, biodegradable tissue engineering scaffolds.* Biotechnology and Bioengineering, 2003. **82**(5): p. 578-589.

2. Richbourg, N.R., et al., Precise control of synthetic hydrogel network structure via linear, independent synthesis-swelling relationships. Science Advances, 2021. **7**(7): p. eabe3245.