

Enzyme-activated degradation of microparticles with enzyme-activated degradation to enhance cell proliferation for 3D-bioprinting

Charlène Dupré^{1*}, Alexandre Sperh², Guéric Bolze², Quentin Muller³, Eric Mathieu¹, Bruno Vincent⁴, Michiya Matsusaki⁵, Youri Arntz¹, Dominique Vautier¹.

¹Inserm, UMR_S 1122, CNRS EMR 7003, Biomaterials & Bioengineering, Strasbourg University, France.

²CNRS UMR 7199 CBST, Strasbourg University, France.

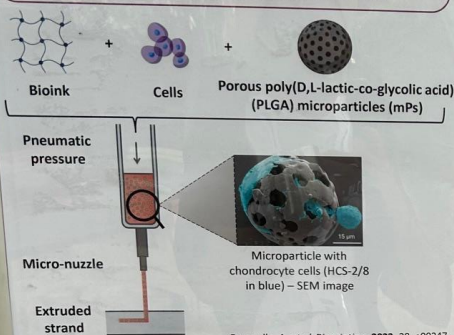
³Consortium for Future Innovation by Cultured Meat, Osaka University, Osaka, Japan.

⁴CNRS UAR 2042, Fédération de Chimie LeBel, Strasbourg University, France.

⁵Joint Research Laboratory for Advanced Cell Regulatory Chemistry, Graduate School of Engineering, Osaka University, Osaka, Japan.

3D Bioprinting method

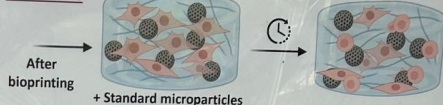
- Limitations** → Non-self-supporting soft hydrogel
→ Shear stress can damage cell membrane
- Solution** → Bioink charged with PLGA microparticles
▪ Increase cell adhesion
▪ Cell protection against mechanical stress



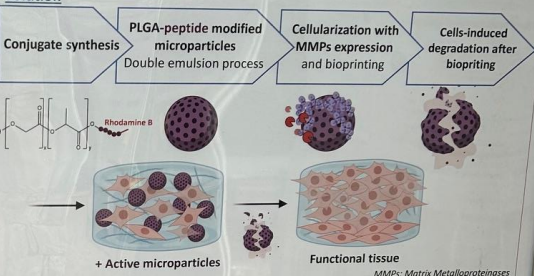
Rousselle, A. et al. Bioprinting, 2022, 28, e00247.

Active degradation of microparticles strategy

Limitations

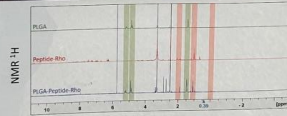
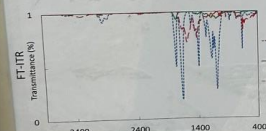


Solution



PLGA-peptide conjugate synthesis

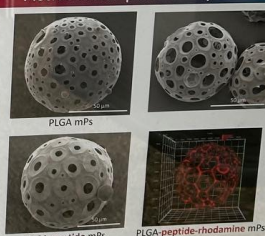
DOSY NMR	Diffusion coefficient (m ² .s ⁻¹)		
	PLGA	2.40x10 ⁻¹¹	
	Peptide	1.27x10 ⁻¹⁰	
		Peptide	PLGA
	Conjugate	3.90x10 ⁻¹¹	2.20x10 ⁻¹¹
	Chain end	3.00x10 ⁻¹¹	2.10x10 ⁻¹¹



	Peptide-rhodamine	Sample
	Peptide	Conjugate
Diffusion coefficient (m ² .s ⁻¹)	2.02x10 ⁻¹⁰	2.03x10 ⁻¹⁰
Fraction (%)	100	0
	100	100

MaxEnt distribution

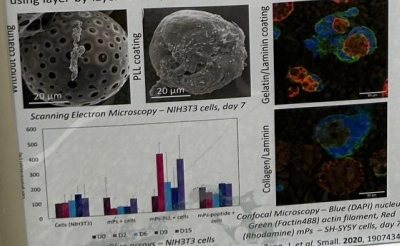
Active microparticles synthesis



Scanning Electron Microscopy & Confocal Microscopy

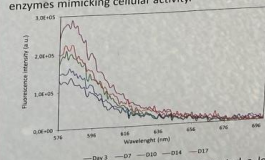
Cell adhesion improvement

→ To improve cell adhesion, different coating strategies are being tested: poly-L-lysine (PLL), peptide, collagen/laminin or gelatin/laminin using layer-by-layer technique.



Kinetic monitoring of microparticles degradation

→ Microparticles were incubated in a dialysis system with enzymes mimicking cellular activity.



→ After 30 days, SEM analysis demonstrated a loss of sphericity and an increase of surface porosity, reflecting structural degradation.



Conclusion & outlook

PLGA-peptide coupling and microparticles synthesis have been validated. Their degradation is being tested with synthetic enzymes, and further cellularization optimizations are expected to promote enzyme-activated degradation, enabling the formation of functional bioactivated tissues.