Supplementary Material

Regeneration of hyaline cartilage promoted by xenogeneic mesenchymal stromal cells embedded within elastin-like recombinamer-based bioactive hydrogels

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SUPPLEMENTARY METHODS

SM-1. hMSC characterization, immunophenotyping and differentiation analyses

The MSC phenotype was confirmed by flow cytometry, and the multi-lineage differentiation potential of the hMSCs used in the study was assessed by inducing adipogenic, osteogenic and chondrogenic differentiation.

SM-2. ELR design

Using recombinant DNA technology as stated in the main text, a previously synthetized block co-recombinamer,[1] was functionalized to include RGD cell-adhesion sequences (Ref. TP20701, TPNBT S.L., Spain). The original block co-polymer was designed to comprise a hydrophobic block (containing isoleucine as the guest residue) with a low Tt and a hydrophilic block (containing glutamic acid with a carboxylic group) with a high Tt.

SM-3. Histological processing

Samples were dehydrated and infiltrated into methyl methacrylate following standardized protocols. The resulting blocks were cut using an automated rotary microtome (Leica Jung Supercut 2065, Leica Biosystems, Germany) into slices with a thickness of 7 µm. Sections were successively stained with Von Kossa and toluidine blue, following the manufacturers' instructions and well-established protocols.

SM-4. Histological image acquisition

Images were taken with a Zeiss Axio Scope A.1 photomicroscope equipped with brightfield and epifluorescence connected to a (Zeiss HRc) digital camera, which, in turn, was coupled to a computer with appropriate software (Zeiss AxioVision 4.8) for the

capture of digital images. Images were processed using Adobe Photoshop CS2 (v. 9.0) in all cases, and only the resolution, brightness and contrast settings were slightly modified to unify the image features.

SUPPLEMENTARY RESULTS

SR-1. hMSC characterization, immunophenotyping and differentiation analyses

SR-1.1. *In vitro* expansion

Cells were isolated from every batch of hMSCs obtained from different donors and expanded appropriately *in vitro*, thus showing the characteristic fusiform morphology and adherence to polystyrene, as observed by phase-contrast microscopy.

SR-1.2. Immunophenotyping

Immunophenotypic analysis of hMSCs collected from iliac crest bone marrow proved that these cells fulfilled the minimal definition criteria for MSCs established by the International Society for Cellular Therapy (ISCT),[2] namely expressing CD90, CD73 and CD105, and being negative for CD45, CD34, CD14, CD19 and HLA-DR.

SR-1.3. Differentiation potential

All samples of human MSCs obtained for this experiment were able to differentiate into osteocytes, adipocytes and chondrocytes, hence fulfilling ISCT requirements (data not shown).

SUPPLEMENTARY FIGURES AND TABLES

Table S-1. Abbreviated amino acid sequence and molecular weight (*Mw*) of the ELR used in this work. Elastin-like blocks are typed in blue, while the 12-mer containing the RGD tripeptide (bold) is represented in red.

	Abbreviated amino acid sequence	***Mw* (Da)**
ELR	MESLLP-{[$(VPGVG)_2$-VPGEG-$(VPGVG)_2$]$_{10}$-$(VGIPG)_{60}$-}$_2$-[$(VPGIG)_{10}$-AVTG**RGD**SPASS-$(VPGIG)_{10}$]$_2$	112270

Table S-2. Modified Wakitani score used for the comparison of the histological outcomes between experimental (ELR + hMSCs) and control (ELR) groups.

Cell morphology	
Hyaline cartilage	4
Mostly hyaline cartilage	3
Mostly fibrocartilage	2
Mostly non-cartilage	1
Non-cartilage only	0
Matrix-staining (metachromasia)	
Normal (compared with host adjacent cartilage)	3
Slightly reduced	2
Markedly reduced	1
No metachromatic stain	0
Surface regularity[a]	
Smooth (>3/4)	3
Moderate (1/2 to 3/4)	2
Irregular (1/4 to 1/2)	1

Severely irregular (<1/4)	0
Thickness of cartilage[b]	
2/3 to 4/3	3
5/3 to 4/3	2
1/3 to 2/3 OR >5/3	1
<1/3	0
Integration of donor with host adjacent cartilage	
Both edges integrated	2
One edge integrated	1
Neither edge integrated	0
TOTAL MAXIMUM SCORE	**15**

[a]Total smooth area of the reparative cartilage compared with the entire area of the cartilage defect. [b]Average thickness of the reparative cartilage compared with that of the surrounding cartilage.

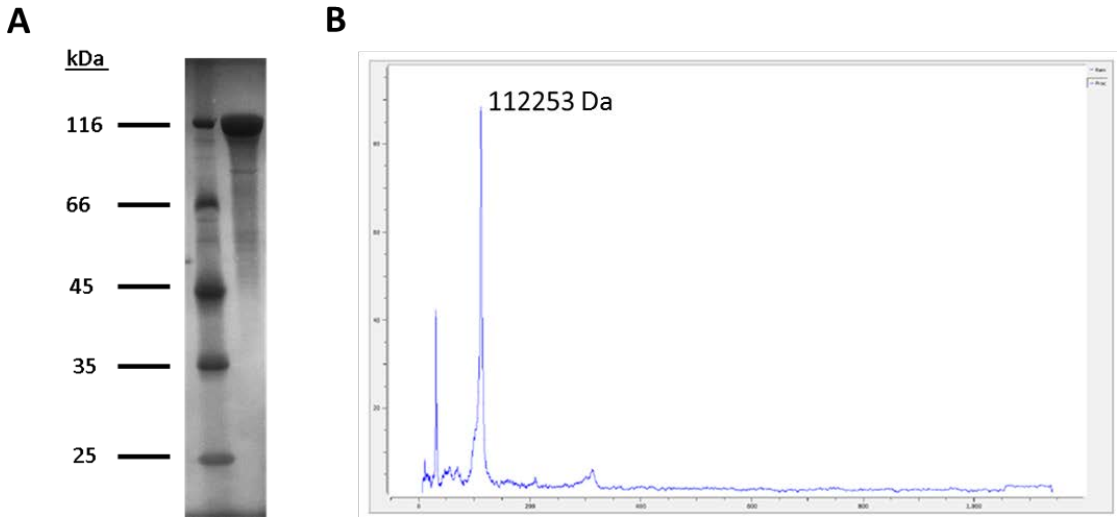

Figure S-1. SDS-PAGE (A) and MALDI-TOF mass spectrometry (B) showing the experimental molecular weight of the ELR used in this work. Both results are in agreement with the expected value, 112270 Da.

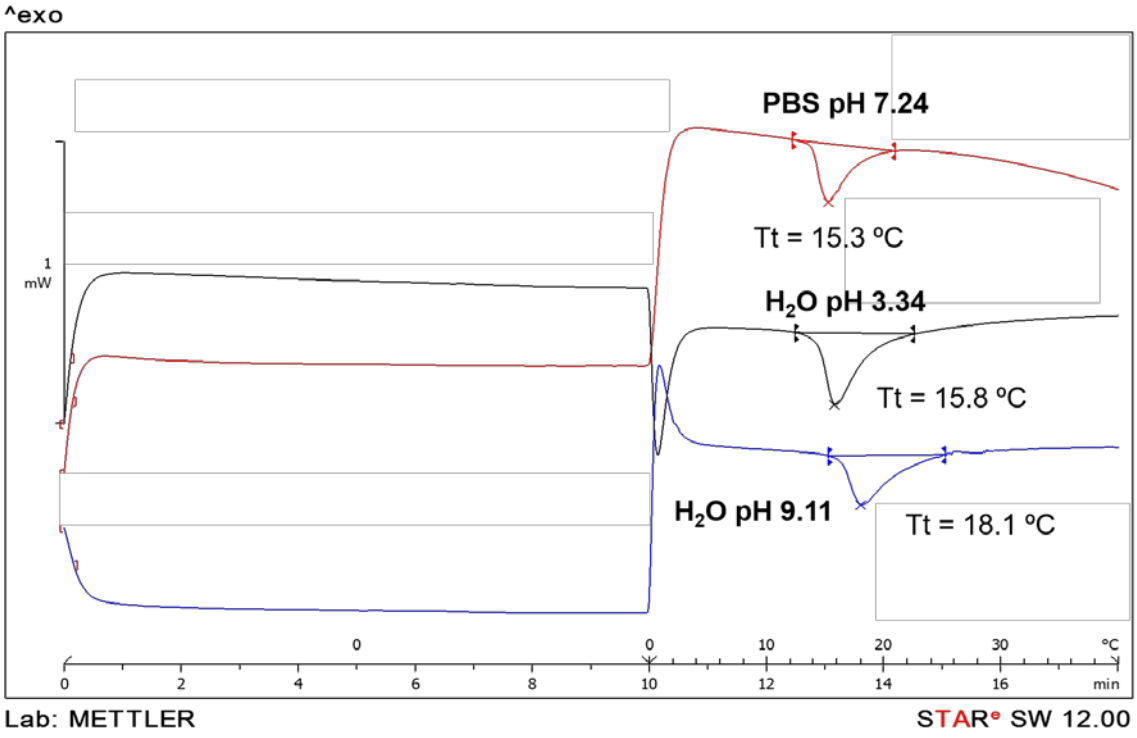

Figure S-2. DSC spectra of the ELR in PBS at pH 7.24 and in ultra-pure water at acid (3.34) and basic (9.11) pH for the evaluation of the thermoresponsive behavior of the ELR.

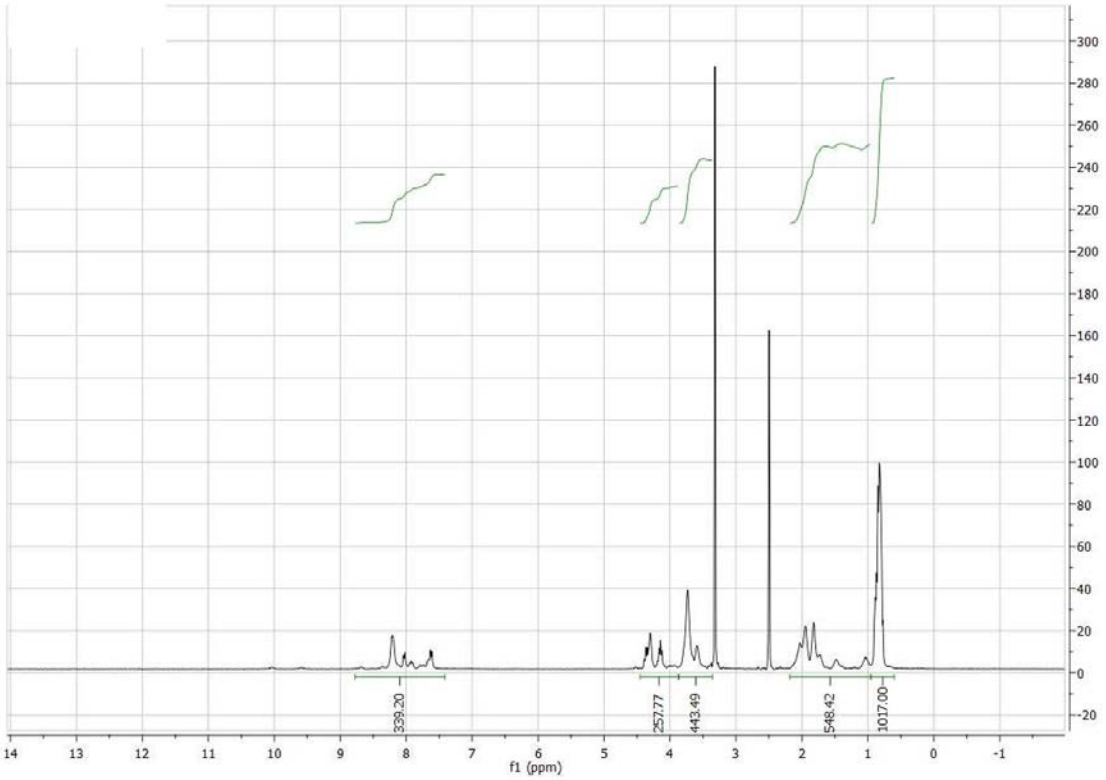

Figure S-3. ^{1}H-NMR spectrum of the ELR used in this study.

Table S-3. Comparison between the predicted value of each type of hydrogen in the ELR used in this study and the experimental values found by integration of each peak in the corresponding ^{1}H-NMR spectrum (see Figure S-3).

Type of hydrogen	Predicted value	Measured value
-CH3	1017	Reference
-CH- and -CH2-	1461	1249.68
-NH2	359	339.20

Table S-4. Predicted and experimental amino acid composition of the ELR used in this work.

Amino acid	Predicted	Experimental

Asp	2	2.6
Ser	7	6.6
Glu	21	20.8
Gly	524	533.1
Thr	2	1.7
Ala	4	5.0
Pro	263	261.3
Arg	2	2.4
Val	343	350.2
Met	1	0.3
Ile	160	145.5
Leu	2	3.8
TOTAL	**1331**	**1333.3**

Table S-5. Scores for specimens from the control (ELR hydrogel) and the experimental group (ELR hydrogel + hMSCs) according to the modified Wakitani grading scale.

	CONTROL GROUP					
Feature	**Rabbit 1**	**Rabbit 2**	**Rabbit 3**	**Rabbit 4**	**Rabbit 5**	**Rabbit 6**
Cell morphology	2	2	1	1	2	2
Matrix-staining (metachromasia)	1	2	1	1	2	2
Surface regularity	2	2	2	1	2	2
Thickness of cartilage	1	1	1	0	1	1
Integration of donor with adjacent host cartilage	2	2	1	0	1	1
TOTAL	**8**	**9**	**6**	**3**	**8**	**8**
AVERAGE	**7**					
	EXPERIMENTAL GROUP					
Feature	**Rabbit 1**	**Rabbit 2**	**Rabbit 3**	**Rabbit 4**	**Rabbit 5**	**Rabbit 6**
Cell morphology	4	4	4	4	4	4

Matrix-staining (metachromasia)	3	3	2	3	3	2
Surface regularity	3	3	2	2	3	2
Thickness of cartilage	3	3	3	3	3	2
Integration of donor with adjacent host cartilage	2	2	1	2	2	1
TOTAL	**15**	**15**	**12**	**14**	**15**	**11**
AVERAGE	**13.7**					

Table S-6. Comparison between our study and other works regarding the regeneration of osteochondral defects after 3 months of treatment, in terms of percentage of regeneration as calculated through the corresponding scoring scales. Relevant features, like injectability and nature of the scaffold (if applicable) are also highlighted.

Animal model	**MSC source**	**% regeneration experimental**	**% regeneration control**	**Scoring scale**	**Hydrogel/ Scaffold**	**Injectable**	**Ref**
Rabbit	Xeno	91	47	Modified Wakitani	ELR-based hydrogel	Yes	This work
Pig	Allo	57	10	Modified Wakitani	-	Yes	34
Rabbit	Allo	89	21	Wakitani	Collagen scaffold	No	43
Rabbit	Allo	66	42	Modified ICRS	Gelatin	Yes (light-mediated cross-linking needed)	44
Rabbit	Allo	50	61	Modified O'Driscoll	Peptide-based hydrogel	Yes	45
Rabbit	-	70	75	O'Driscoll	Hyaluronan-pNIPAAm hydrogel	Yes	46

Rabbit	-	80	44	ICRS	Multi-layered collagen-based scaffolds	No	47

REFERENCES

1. Dominici M, Le Blanc K, Mueller I, et al. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315-317.

2. Martin L, Arias FJ, Alonso M, et al. 2010. Rapid micropatterning by temperature-triggered reversible gelation of a recombinant smart elastin-like tetrablock-copolymer. Soft Matter 6:1121-1124.