SUPPLEMENTARY MATERIAL

Materials and Methods

Preparation of the poly(ethyl acrylate),PEA

The monomer ethyl acrylate (99%, Sigma Aldrich) was mixed with 1 wt% of benzoin (98%, Scharlau) as photo-initiator and 2 wt% of ethyleneglycol dimethacrylate (98%, Sigma Aldrich) as cross-linker, injected in a porogen template, polymerized for 24h under a UV source, and post-polymerized for 24h more in an oven at 90ºC. The porogenic templates consisted of 8 layers of nylon fabrics with a nominal thread diameter of 150μm and a mesh opening of 300μm (SAATI S.A.). Once polymerized, the nylon templates were removed by dissolution in nitric acid (30%, Sigma Aldrich) [1–3].

Preparation of Poly(caprolactone 2-(methacryloyloxy)ethyl ester), PCLMA

The CLMA monomeric mixture, with the same percentages of photo-initiator and cross-linker employed to prepare the PEA scaffolds, was injected in a porogen template obtained by sintering poly(methyl methacrylate) microspheres of 90 ± 10μm in diameter (PMMA; Colacryl dp 300). After polymerization, the porogen was eliminated by dissolution with acetone (Scharlab) yielding 0.8 mm-thick PEA and PCLMA scaffolds [4].

Adipose tissue biopsy samples processing

Adipose samples obtained from fat pads between skin and sternum were rinsed with PBS (Labclinics) and cut into small pieces, removing visible blood vessels; thereafter, cells were isolated by collagenase II (Gibco) digestion [5,6].

Clonogenic assay

Clonogenic assay was performed as previously described [7]. Briefly, cells were plated at a density of 400 cells/100 cm2 and cloning rings were placed to encircle individual cells. Complete medium (α-MEM + 20% FBS + 1% P/S) was added into the cloning rings and plates

were cultured under standard conditions. Clones were allowed to develop until they reach several mm in diameter.

Immunosupression assay

To analyse the effect of subATDPCs on peripheral blood lymphocyte (PBL) proliferation, $5x10^3$ subATDPCs were plated with $2x10^5$ PBLs and in the presence or absence of the appropriate stimulus (PHA 5 μg/mL). After 4 days of stimulation, BrdU was added to the media for 24h and proliferation was determined by ELISA following the manufacturer's instructions (Cell proliferation ELISA BrdU, Roche). The experiment was conducted in triplicate. Data are shown relative to proliferation of PBLs without progenitor cells.

Immunophenotypical analysis

At passage 2, cells were collected and immunostained with specific mAbs against CD105 (Serotec), CD44, CD166, CD29, CD90, CD106, CD45 and CD14 (BD Pharmingen). Flow cytometry levels of each antigen were defined by the ratio between specific antibody and IgG isotype control (Caltag Laboratories) (1 = no difference). A coulter EPICS XL flow cytometer (Beckman Coulter) was used for all data acquisitions and analyses were carried out using the Expo32 software (Beckman Coulter).

Adipogenic and osteogenic differentiation assays

Expanded primary cell cultures were assessed for adipogenic and osteogenic potentials. Differentiation assays were performed as previously described [8]. Cells were then examined for the presence of lipid vacuoles staining with Oil red O (Sigma) and deposition of calcium matrix was examined by von Kossa staining.

Myocardial infarction model.

Myocardial infarction was created as previously described [9]. Briefly, the animals were intubated and anesthetized with a mixture of O_2/isoflurane and mechanically ventilated. The

heart was exposed and the left anterior descending (LAD) coronary artery was permanently occluded with an intramural stitch (7-0 silk suture). Sham-operated animals were prepared in a similar manner except that the LAD was not occluded. Mice were then randomly distributed into 2 experimental groups: sham and infarcted animals both with bioimplant.

Supplementary References

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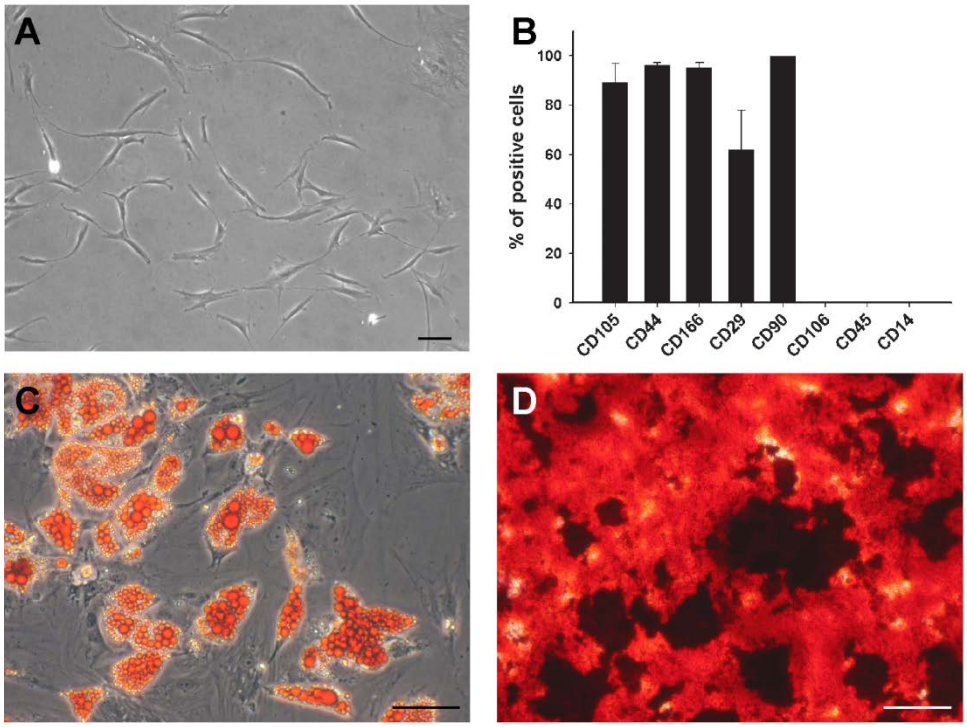
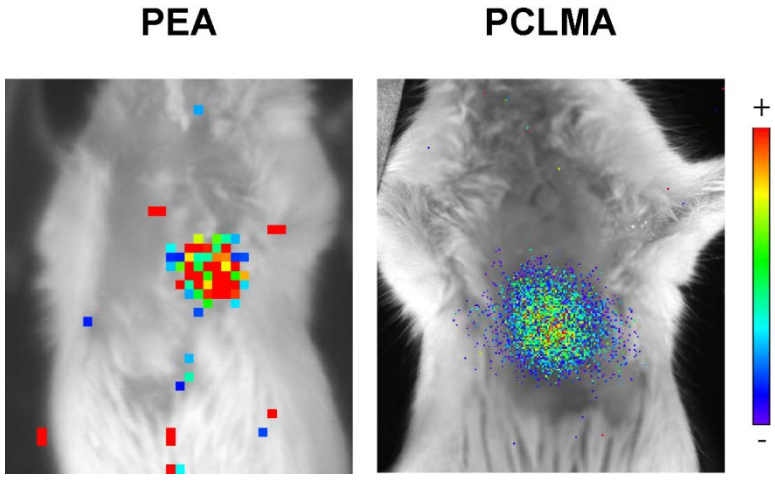


Supplementary Figure 1: *In vitro* characterization of subATDPCs cultured in monolayer. (A) SubATDPCs primary culture at pre-confluence at passage 2. (B) Flow cytometry analysis of subATDPCs immunophenotyping. (C) Oil Red O staining of subATDPCs cultured in adipogenic differentiation medium showing lipid droplets depositions (red). (D) Von Kossa staining of subATDPCs cultured in osteogenic differentiation medium with the presence of calcium depositions (red). Scale bars 100µm.



Supplementary Figure 2: Non-invasive bioluminescence imaging for subATDPCs tracking in living mouse 3 days after implantation. Representative bioluminescent images from constitutively labelled (CMVp-RLuc-mRFP1) subATDPCs within PEA (left) or PCLMA (right) bioimplants. Renilla luciferase images are superimposed on black and white dorsal images of recipient animal. Color bar illustrate the relative light intensities from RLuc: low, blue; high, red.

Supplementary Table1: Primers used to analyse gene expression by RT-PCR.

	NAME		SEQUENCE	bp	Tm (ºC)	Fragment
1	Tbx5	Fw	CATGGAGACATCACCCAGTG	20	60	169bp
		RV	GCAGCTGATGTCCTCTAGGC	20		
2	ACTN1	Fw	GAGAAACTGCTGGAGACCATTGACC	25	63	188bp
		RV	TTGTCGGCATCAGGGAGGGT	20		
3	MEF2C	Fw	CCATTGGACTCACCAGACCT	20	65	139bp
		RV	AGCACACACACACACTGCAA	20		
4	TNNT2	Fw	AGAGGCTGAGACCGAGGAGACCA	23	64	207bp
		RV	TGCAACTCATTCAGGTCCTTCTCCA	25		
5	GJA1	Fw	comercial QT00012684	-	55	92bp
		RV		-		
6	CDH1	Fw	AGCCAAAGACAGAGCGGAAC	20	60	216bp
		RV	AAGCAGGCACTTGGGGATTC	20		
7	SNAI1	Fw	TAGCGAGTGGTTCTTCTGCG	20	60	164bp
		RV	AGGGCTGCTGGAAGGTAAAC	20		