Supporting Information

Production of a Double Layer Scaffold for the "On Demand" Release of Fibroblast-Like Limbal Stem Cells

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Supplemental methods

Immune- and Limbal phenotyping by cytofluorimetric analysis: the cells were treated with FcR blocking reagent (Miltenyi Biotec) and incubated with primary antibody or appropriate isotype control at 4 °C for 30 minutes in the dark. Cells were then fixed for 15 minutes at 4 °C with 2% paraformaldehyde (PFA) and washed with staining buffer (PBS, calcium and magnesium free, supplemented with 1% BSA (Sigma-Aldrich)).The f-LSC immunophenotype was determined using the following monoclonal antibodies: HLA-DR FITC, CD80 (B7-1) PE, CD86 (B70/B7-2), CD34 FITC, CD45 FITC (BD Biosciences). The f-LSC immunophenotype was determined using human ΔNp63α antibody (unconjugated p63alpha (H-129) and human ABCG2 antibody (CD338-FITC, Miltenyi Biotec GmbH) according to manufacturer instructions. All data were acquired on a FACSCalibur and analyzed using CELLQuest Pro software (BD Pharmingen). Alexa fluor-488 as corresponding secondary antibody (Invitrogen, Thermo Fisher Scientific) was used. Appropriate isotype control and Alexa fluor antibodies were used as negative controls or secondary antibodies, respectively.

Table S1- Primer sequences used in qRT-PCR

GENE	PRIMER SEQUENCE	CODE NUMBER
CD105		QT00033569
NANOG		QT01844808
OCT4		QT00210840
SOX2	F- GGAGACGGAGCTGAAGCCGC R-GACGCGGTCCGGGCTGTTTT	MWG
Thy-1		QT00023569
CD29		QT00068124
ΔNp63α	F:5'-GAGGTTGGGCTGTTCATCAT-3' R:5'-GTGGGAAAGAGATGGTCTGG-3'	Eurofins genomics
SOX17		QT90204099
CK15	F-GGAGGTGGAAGCCGAAGTAT R-GAGAGGAGACCACCATCGCC	
CK12		QT00011949
CK3		QT00050365

Figure S1 - PLLA electrospun scaffold uncovered (on the right) and covered with HA-EDA-C_{18} film (on the left)

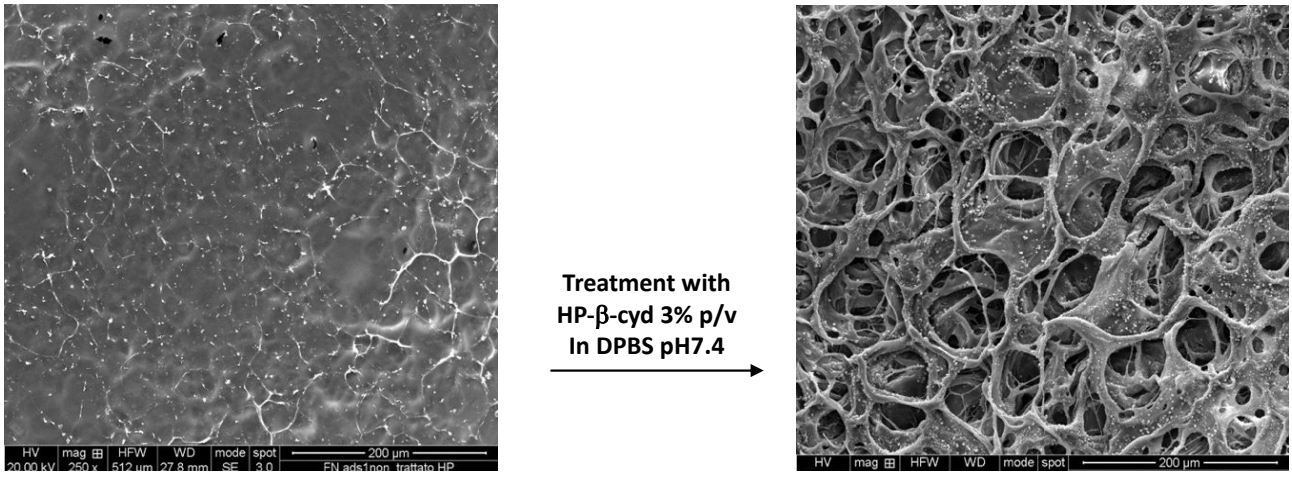

Figure S2 - SEM images of PLLA/HA-EDA-C_{18} scaffold treated with fibronectin before (image on the right) and after (image on the left) 1 hour of incubation with a 3% w/v solution of HP-β-CD in DPBS at pH 7.4

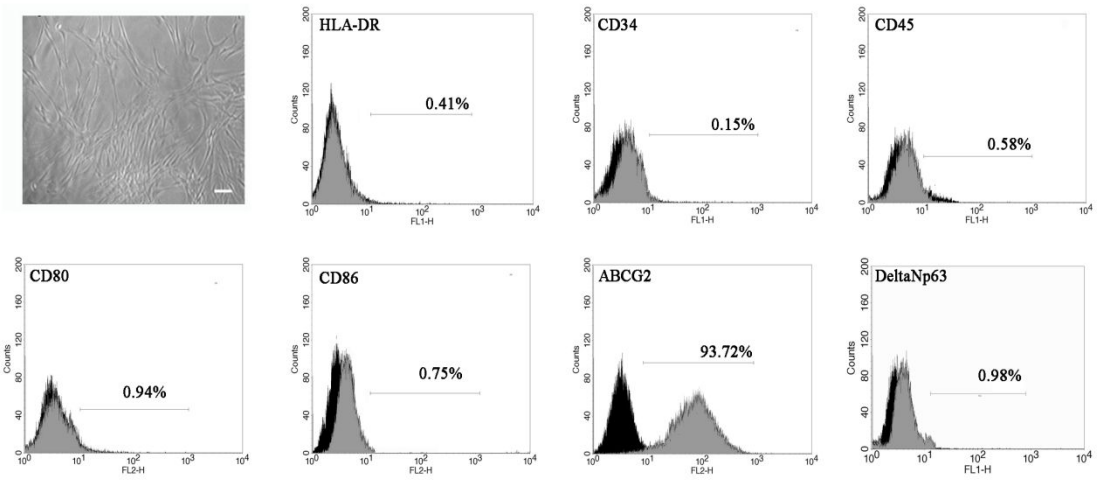

Figure S3 – f-LSC characterization. A representative optical microscope image of typical fibroblast-like morphology of f-LSCs (up left); A representative histogram panel of cytometric analysis for hematopoietic markers (HLA-DR, CD34, CD45), T cell activating molecules (CD80, CD86), limbal specific marker (ABCG2) and LESC marker (ΔNp63α) in f-LSCs. The histogram graphs and quantification analysis were performed by *the CellQuest Pro software.* All pictures are representative of three independent experiments.

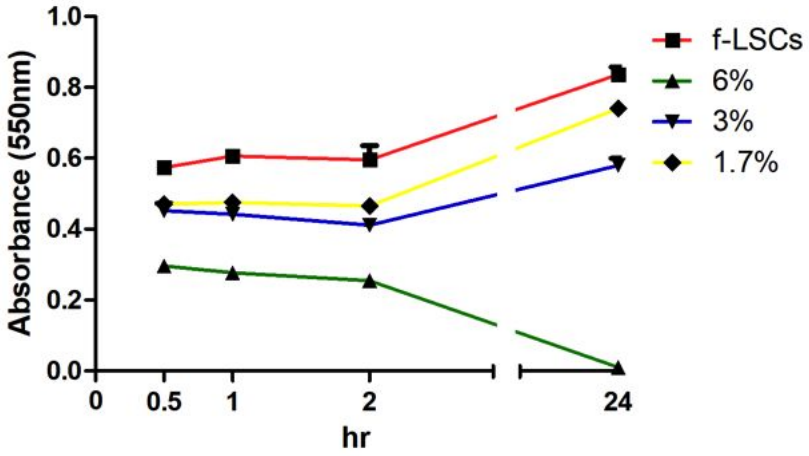

Figure S4 - Cytotoxic effect of (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD). Proliferation assay (MTT) of f-LSCs treated with different HP-β-CD concentration (1.7%, 3% and 6% w/v in culture medium) compared to no-treated f-LSCs. The line graphs (C-D) were performed by GraphPad Software, Inc., California and are represented as ± SD