Electronic supplementary material

Pushing the envelope in tissue engineering: *ex vivo* production of thick vascularized cardiac ECM constructs

Abbreviated running headline:

'Sarig et al., pushing the envelope in tissue engineering'

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Electronic supplementary material

1. Supplementary methods

1.1. Screening modifiers of matrix cell adhesion foci

Several treatments were assessed for their ability to enhance cell adhesion on the surface of the pcECM, presumably by modifying the cell adhesion foci. These treatments included chemical cross-linking of ECM fibers using an ethyl(dimethylaminopropyl)-carbodiimide:N-Hydroxysuccinimide (EDC:NHS) sequence; collagen binding with a 14-amino-acid-long collagen binding peptide containing the RGD sequence (RGD-CBP peptide sequence: RGD-CBPSCQDSETRTFY, Sigma)[1]; and matrix enrichment with representative sulfated (heparan sulphate, HS) and non-sulfated (hyaluronic acid, HA) glycosaminoglycans (GAGs). In addition, EDC-NHS, known to create amide bonds between adjacent amino acids, was tested either alone or in combination with HA, HS or RGD-CBP. The triple combination of EDC-NHS, RGD-CBP and HS was also tested to assess additive or synergistic effects. Nitrocellulose, previously reported to facilitate cell adhesion to biomaterials via protein absorption,[2] was also evaluated. Non-treated pcECM matrices served as controls. Each group was tested in five biological replicates.

Decellularized pcECM scaffolds were prepared as previously published.[3] For pcECM treatment, each well in a 96-well plate was filled with 200 µl of solution comprising: an **EDC-NHS solution** prepared from 0.5 mg/ml 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (EDC, Sigma) and 0.7 mg/ml N-hydroxysuccinimide (NHS, Sigma) dissolved in phosphate buggered saline (PBS) solution. The pcECM specimens were allowed to react with the EDC/NHS solution for 15 min, followed by several washes with double distilled water (DDW) to remove excess EDC/NHS. An **RGD-CBP solution** was prepared by dissolving either rhodamine labeled (when applicable) RGD-CBPSCQDSETRTFY or a non-labeled peptide equivalent (prior to cell cultivation, Sigma, 1 mg/ml)

in PBS. The specimens were blocked with 5% FBS at room temperature for 30 min followed by soaking in the RGD-CBP solution for 2 hrs and DDW rinses. **HS and HA solutions** (Sigma) were prepared using GAG to matrix ratios of 0.1 mg HS and 1 mg HA per mg dry matrix weight, respectively. Matrices were incubated for 2 hrs and then washed several times with distilled water. Decellularized pcECM scaffolds were also treated with nitrocellulose. PcECM matrices were immersed in a 5 ml volume of 0.1 cm^2/ml of nitrocellulose sheet (Bio-Rad, Hercules, CA) in MeOH for 24 hrs and subsequently washed extensively in sterile PBS containing 2x antibiotic concentration (2% Pen-Strep® and 0.8% Fungizone®) for 1 hr. All specimens were then immersed in complete hMSC culture media for two hours, air dried for 90 min and seeded with $3x10^5$ hMSC/cm^2 in 45 µl of culture media per specimen. Cells were allowed to adhere to the scaffolds for 90 min prior to the addition of culture media to the plates. The viability of the seeded hMSCs cells was evaluated using AlamarBlue™ according to the manufacturer's protocol after 3, 7 and 14 days. The modified samples that revealed the highest viability were further subjected to histological analysis using hematoxylin and eosing stain (H&E).

1.2. Developing a mathematical model

Eq. 1 can be used to describe cell adhesion to the pcECM. [S] denotes the scaffold surface density of unbound (free) cell adhesion foci (CAF) as CAF/cm^2; [C] represents the surface density of unbound cells; and [SC] represents the density of cell-bound CAFs. K_{eq} represents the equilibrium constant of the cell binding to the CAF within the seeding time period permitted for cell attachment.

Eq. 1. $$[S]+[C]\xleftrightarrow{K_{eq}}[SC]$$

Assuming first order kinetics, Eq. 2 can be stipulated to describe the K_{eq} constant:

Electronic supplementary material

Eq. 2. $$K_{eq} = \frac{[SC]}{[S] \bullet [C]}$$

Denoting the density of bound CAFs (SC) as a variable 'x', given known initial seeding cell densities (C_0), and a finite, yet unknown, CAF density (S_0), Eq. 2 could be re-written as follows:

Eq. 3. $$K_{eq} = \frac{[SC]}{[S] \bullet [C]} = \frac{x}{(S_0 - x) \bullet (C_0 - x)}$$

After algebraic rearrangement, a quadratic expression of Eq. 3 can be deduced as follows:

Eq. 4. $$K_{eq}x^2 - x(K_{eq}S_0 + K_{eq}C_0 + 1) + KS_0C_0 = 0$$

The roots of this quadratic equation (Eq. 4) can be found according to Eq. 5 below:

Eq. 5. $$x_{1,2} = \frac{(K_{eq}S_0 + K_{eq}C_0 + 1) \pm \sqrt{(K_{eq}S_0 + K_{eq}C_0 + 1)^2 - 4K_{eq}^2 S_0 C_0}}{2K_{eq}} = [SC]$$

According to Eq. 5, the density of matrix bound cells at steady state [SC=x] can be calculated and expressed as a function of K, S_0 and C_0. With increasing quantities of C_0 towards a saturated SC value (i.e. $C_0 >> S_0 \rightarrow SC_{saturation} \approx S_0$) S_0 could be estimated by x representing the maximal matrix cell-holding capacity per cm^2. Given a known set of C_0 (different seeding densities) and SC values (measured through AlamarBlue™), S_0 can be optimized (Microsoft Excel™ solver) to achieve the best fit (least squares method) of empirical data to model predictions (Eq. 5). Approximate S_0 values were used as boundary conditions (estimated by plotting attached cell density as a function of the seeded cell density). Thus to delineate S_0, hMSCs (Fig. 2) or HUVECs (supplementary Fig. S5) were seeded on the pcECM in different densities ($5x10^4$, $2x10^5$, $4x10^5$ and $1.5x10^7$ cells/cm^2 for hMSC and $5x10^4$, $2x10^5$, $4x10^5$ and $7.5x10^6$ cells/cm^2 for HUVECs) and allowed to adhere for 90 min before 2 ml of

complete culture media were added and incubated for an additional 24 hrs to achieve steady state. To evaluate cell attachment density, the matrices were transferred to fresh 24-well plates containing 2 ml of culture media supplemented with 10% (v/v) AlamrBlue™. Cell quantities were determined against the appropriate calibration curve using five biological replicates.

The measured attachment densities (SC) were plotted as a function of the modeled density (Eq. 5, Fig. 2, supplementary Fig. S5).

1.3. Model verification

Two methodologies were used to evaluate the validity of this model. The first methodology involved the artificial modification of the pcECM adhesion foci quantity, testing model sensitivity to artificially modified S_0 values. In a second methodology, the monitoring of cell proliferation for long-term static cultivation was performed, allowing sufficient time for cell proliferation and reaching the theoretical maximal matrix capacity, proving its long-term cell support ability (>3days).

1.3.1. Testing model sensitivity to artificial adhesion site modification

HA + EDC-NHS treated matrices were re-seeded with the same experimental densities and modeled again according to the steps reported in Section 1.2 above. These matrices served as a distinct empirical set aiming to prove model sensitivity to changes in adhesion foci quantities and suggest applicability of this model for other scaffolding materials as well.

1.3.2. Assessing model prediction for long-term cultures

Acellular pcECM constructs of both HA+EDC-NHS and non-treated matrices seeded with two representative hMSC densities ($5x10^4$ and $1.5x10^7$ cells/cm^2) were monitored for 21 days (n=5 samples per group) with AlamrBlue™ (at t=1,7, 14 and 21 days post seeding).

1.3.3. Evaluating the effect of culture medium volume on the maximal cell quantity

To evaluate the effect of culture medium volume on the proliferation of reseeded cells, pcECM matrices were reseeded with equal densities of $2x10^5$ hMSC/cm^2 (just below the maximal cell capacity predicted by the model for both treated and non-treated matrices) and divided into two culture groups (n=5 samples per group) having different culture medium volumes (2 or 10 ml/well), replaced every other day. Cell density was determined using AlamrBlue™ at t=1, 7, 14 and 21 days post seeding. At t=21 days, the experiment was terminated, matrices were documented and histological assessment was performed using standard H&E (Sigma, USA) staining.

1.4. Assessment of luminal endothelial cell density in the porcine coronary arteries

Freshly isolated porcine hearts (n=3) were harvested from a local abattoir (Soon Hin Food Trading Pre. Ltd., Singapore). They were delivered to the lab on ice and immediately perfused with PFA 4% for 1hr following which hearts were washed with cold PBS. A large slab (similar to the one cut for decellularization) containing the lateral anterior descending coronary artery (LAD) was cut from the heart, perfused and immersed in a DAPI staining solution for 20 min (NucBlue™, Life-Technologies, Singapore). The LAD were then opened from their epicardial side in a longitudinal artery cut. The exposed edges of the LAD were clamped to the sides and the slab was mounted on an inverted confocal microscope (LSM700, Carl Zeiss Germany) equipped with an EC Plan-Neofluar 10x/0.30 M27 air lens. Tile scan was performed for the DAPI signal containing at least a 3x3 fields of view per each artery. From each image, four regions of interest (ROI) were used for image analyses.

Electronic supplementary material

1.5. Proving feasibility for pcECM support of cardiac cells

hESC-CM were expanded and differentiated as previously published using an intermittent rocker system[4]. Following differentiation $1.1x10^7$ hES3-CM cells in RPMI containing 2% Pen-Strep®, 0.8% Fungizone®, B27-insulin (2%, Life Technologies) and the ROCK inhibitor Y27632 (Calbiochem, Merck-Millipore, Singapore) were seeded on each thick pcECM scaffold (n=3, diameter – 1.5cm) for 90 min followed by cultivation in 10ml culture media changed every other day for up to 23 days. Beating was documented using the EVOs phase contrast microscope (Advanced Microscopy Group, Life-Technologies, Carlsbad, CA) equipped with a 4x lens (Olympus) and recorded using the manufacture's provided software (Advanced Microscopy Group). At termination, seeded matrices were fixated in 4% PFA over-night followed by paraffin embedding, sectioning (5µm), histochemical (H&E) and immuohistochemical (IHC) staining (Advanced Molecular Pathology Laboratory, A*Star) for Troponin I. For IHC stains, an antigen retrieval step at pH=9 (40min, 100^{o}C, Bond^{TM} epitope retrieval solution 2, Leica-microsystems, Germany) was performed followed by endogenous peroxidase blocking (45min,3.5% H_2O_2), 10% goat serum blocking for 1hr and primary antibody incubation with a mouse monoclonal anti human Troponin I antibody (ab19165, abcam, 1:200). Anti-mouse poly HRP-IgG in 10% animal serum was then added for 5 min, color was developed with Bond^{TM} Mixed DAB refine solution (Leica-microsystems, auto-stainer reagents, Germany) and counterstained with hematoxylin for 5 min prior to dehydration and mounting in synthetic mounting media.

1.6. ***Bioreactor system installation***

Perfusion chamber materials were chosen to enable maximal biocompatibility. The baths, matrix holders and base plate were made of polyether-etherketone (PEEK), the cover was made of glass and

all connectors were made of food grade stainless steel. Unless otherwise stated, all tubing used was made of medical grade silicone 1.5x3 mm tubing and all tubing connectors were luer connectors (Cole Parmer, Vernon Hills, IL).

Three tube lines were used, one for feeding, a second for drainage and a third for low volume applications (Fig. 1). The tubes entering and exiting the pump head were different than the rest of the tubing as these determined the flow ratio between all the channels, given a particular pump rotating speed. The only non-silicone tubing was that leading from the oxygenator to the perfusion bath (Tygon® R-3603, 0.8x2.4mm) as silicone is gas permeable.

For system installation, a standard CO_2 incubator was located next to a biological safety cabinet (BSC) to enable smooth passaging of the perfusion chamber from the incubator to the hood and back, allowing aseptic handling. Prior to installation the oxygenator, perfusion chamber (with its cover open) and reservoir were autoclaved, sprayed with ethanol 70% and inserted into the biological hood for 15 min. The perfusion baths were connected with ethanol-disinfected 24G catheters to the entry port and the glass cover closed. An air filter was connected to the air entry port from the outside (0.22 µm, Millipore, Billerica, MA) using a standard sterile infusion lengthening tube (50 cm manometer line M/F, Biometrix, Jerusalem, Israel). Tygon® tubing was connected to the oxygenator and the perfusion chamber. The oxygenator was connected to the ethanol-disinfected check valve and to the entry and exit air filters (Millipore, UK). The end connectors of the pre-installed tubing lines were closed at each end with red luer combi M/F stoppers (Biometrix), sprayed with ethanol, inserted into the biological hood, and connected to their relevant matching connectors in the oxygenator and the perfusion chamber. The air tubing was connected to the oxygenator and to a standard “fish tank” air pump actively pushing the incubator air through the oxygenator. Subsequently, the perfusion pump was activated.

For system disinfection, the system was perfused with 500 ml of 70% ethanol for 30 min. The returning line was not allowed to enter into the reservoir; instead, it was directed to a waste container and discarded. This was followed by circulation of an additional 500 ml for 2 hrs, thereafter replaced with fresh 70% ethanol and circulated overnight. The system was then aseptically installed with dynamically prepared pre-cut pcECM matrices, followed by perfusion of the culture media overnight and air drying in the hood for 90 min prior to cell seeding.

1.7. Cell seeding and dynamic cultivation standard operating procedure

For HUVEC-GFP cell seeding, sterile matrices were removed from the perfusion chamber (in the BSC) and transferred into custom-built and ethanol-disinfected (70% ethanol, 30 min) seeding frames (supplementary Fig. S1). For seeding, 1 ml solution of $1x10^7$cells/ml was injected through the entry catheter with a 2 ml syringe and incubated for 60 min in the hood covered by a sterile 20 cm plate cover. During this 60 min incubation, the frames were rotated several times. Seeded matrices were transferred back into the perfusion bath (epicardial side facing upwards), inserted into place and the baths filled with 60 ml of complete HUVEC culture media per bath.

MSC cells were seeded by either injection through the bulk cavities or pipettation on the endocardial surface of dynamically prepared matrices. Injection was used to deliver cells deeper into the matrix for initial assessment experiments or when co-culture experiments were performed with HUVEC-GFPs. Injection was performed throughout the matrix bulk using a 25G syringe in multiple locations until a uniformly inflated matrix appeared ($1x10^6$ cells/ml in 10ml culture media: total $1x10^7$ cells). Pipettation on the endocardial surface (nitrocellulose treated) was used to enable static culture conditions reaching cell density steady state for 30 days. The static cultures were then transferred into and cultivated in the bioreactor system for an additional period of 14 days to evaluate the effect of

dynamic culturing—assessing cell penetration (using histology and specific antibody staining for CD44 (mouse anti human, Cat. No. 555476, BD Biosciences, San Jose, CA), and proliferation (using Alamar Blue™).

Unless otherwise stated, dynamic cultivation was based on the low volume cycle (120 ml per construct). Complete media was replenished every other day. Samples of 100 µl each were taken prior to medium exchange of both old and new culture media to check for contamination. Of these samples, 50 µl were suspended in duplicate in 96-well plates containing 50 µl of liquid highly nutritious general-purpose growth medium for fastidious microorganisms (brain heart infusion broth, BHI, Sigma, USA). Samples were incubated overnight at 37°C under shaking (250 RPM) and were read the next day at 600 nm compared to blank containing sterilized media and BHI mixtures (data not shown).

1.8. Histological assessment and immunofluorescent staining

Histological analyses were performed on cryo sections (10 µm thick, using a CM3050 cryostat, Leica, Wetzlar, Germany) to visualize cell morphology and penetration depth of both static and dynamically cultivated constructs. Unless otherwise stated, representative images are presented out of a total of at least three blocks per construct and are based on several histological cross-sections (n>3) taken from different locations on the OCT blocks. With the exception of HUVEC-GFP seeded matrices (PFA 2% - 5 hrs, PFA 1% – 2hrs and 30% sucrose 48 hrs), samples were not fixated prior to freezing. Cross-sections were methanol (-20°C) fixated to the slides and either H&E stained (Sigma, St. Louis, MO) or mounted with DAPI (Fluoromount G, Southern Biotech, Birmingham, Al) for fluorescent imaging of HUVEC-GFPs (green) and/or MSCs stained with ClaretVue™ far red dye (Sigma, st. Louis, MO, red). For protocols involving static mono-cultures of hMSC, non pre-stained hMSCs were used, which

were counter stained with DAPI following histological sectioning and imaged adjacent to the ECM fibers fluorescent signal (Fig. 3, supplementary Fig. S4).

For specific cell staining, blocking was performed with 5% FBS in PBS for 1 hr at room temperature, followed by incubation in 4^{o}C overnight with mouse anti-human CD44 (555476, BD Biosciences, San Jose, CA) or rabbit anti-rat CD31 (sc-3806, Santa Cruz Biotechnology, Dallas, TX) primary antibodies diluted (CD44: 1:25; CD31: 1:50) in PBS containing 3% FBS overnight. Sections were washed 3x5 min in PBS, followed by incubation with a secondary antibody (goat anti-mouse FITC, 1:300, Sigma F8521, St. Louis, MO or donkey anti-rabbit PE, 1:100, sc-3745, Santa Cruz Biotechnology, Dallas, TX) for 1 hr (room temperature). Slides were then washed 3x5 min with PBS, mounted using DAPI containing fluoromount G (water based) and covered with cover slides that were glued using a transparent nail polish. All fluorescent imaging was performed using an inverted confocal microscope (LSM700, Carl Zeiss Germany) with an EC Plan-Neofluar 10x/0.30 M27 air lens or using a fluorescent inverted microscope (Nikon, Ti-S model, Japan) equipped with a 20x air lens.

2. Supplementary results

Four treatments corresponding to several different factors affecting cell matrix binding were screened for their potential enhancement of the quantity of matrix cell-anchorage sites. The amount of fiber cross-linking is directly related to scaffold compliance and hence to providing physical cues that might be required for cell adhesion and proliferation.[5] The RGD sequence has been widely used in tissue engineering and drug delivery, as it is the recognition site of cell integrin mediated collagen binding. GAGs have been reported to provide an equally important and alternative binding system to integrin mediated RGD binding.[6] Finally, nitrocellulose is frequently used in several commercial applications

and was previously reported to facilitate cell adhesion through protein adsorption.[7] These four treatments were conducted either solely or with various combinations thereof.

Both the nitrocellulose treatment as well as the conjugation of sulfated and non-sulfated GAG demonstrated significant cell support ($p<0.05$, ANOVA, supplementary Fig. S2), suggesting that the major component required for cell adhesion to the acellular thick pcECM matrix is GAG and its respective receptors, and not cross-linking or lack of structural integrity and RGD sequences. Furthermore, collagen binding site integrity was demonstrated by incubating acellular thick pcECM matrices with fluorescently labeled RGD containing collagen-binding protein (supplementary Fig. S3), as compared to a positive control of commercial collagen Type 1 (Sigma, St. Louis, MO). Our results indicated that RGD-CBP binds successfully to both commercial collagen and thick pcECM, even after thorough washes with 5% BSA containing wash buffer (10x5 min each), suggesting specific peptide–ECM interaction and the preservation of the collagenous network structural integrity.

When hMSC seeding was performed at higher than maximal cell holding densities (i.e. $>2.7x10^5$ cells/cm^2) steady-state static culture penetration depth to the pcECM was reached starting from minimum three days post seeding. This penetration depth appeared to be limited to the first 100µm from the surface (supplementary Fig. S4); with similar penetration depth observed also when hESC-CM (supplementary Fig. S6) and human ventricular fibroblasts (data not shown) were cultivated on the pcECM. However, this parameter value was also dependent on the cell type used, as employing the same histological sectioning methodology, revealed that endothelial cells preferred to form a monolayer coating of the surface and did not penetrate into the pcECM any further. Hence when assessed through our developed mathematical modeling ($R^2=0.93$) endothelial cell support-ability of the pcECM was shown at much lower steady state values ($5.4x10^5$ cells/cm^2, supplementary figure S5). This value was surprisingly similar to that measured for endothelial cell density at the luminal

side of the porcine lateral artery descending coronary artery ($5.0\pm0.7x10^5$ cells/cm^2, supplementary Rig. S5) as assessed through image analyses.

Finally, the possibility to promote neo-vascularization *ex vivo* was demonstrated using the dynamic co-cultivation of the hMSC and HUVECs in our novel bioreactor setup. This was evident by bright field images taken from the same location at sequential time points (supplementary Fig. S7). New blood vascular structures appeared to form and connect to previously-established larger conduits within the three weeks of the experiment.

3. Supplementary Fig. legends

Supplementary Fig. S1: Seeding frames setup. Clamped pcECM matrix is shown from its epicardial surface (a) and from the side (b) as used for HUVEC seeding inside the main vasculature. Scale bars: 1 cm.

Supplementary Fig. S2: Screening for optimal artificial modification of pcECM adhesion sites. Cell viability upon treated matrices was measured using the AlamarBlue™ assay at different time points post seeding. Comparison of the least square mean cell density (two-way ANOVA with Tukey's HSD post-hoc correction for interaction and α-level adjustment for multiple comparisons, n=6 per group) was used to test the statistical significance of differences among groups through time (a). Two representative time-curves are shown in the inset in (a), symbols: EDC-NHS combined with hyaluronic acid treatment (EDC-NHS-HA, stars); non-treated control pcECM, triangle; EDC-NHS combined with HA treatment yielded the best result in terms of cell proliferation. Statistical

significance groups at α=0.05 are denoted by capital letters (b). H&E staining 16 days post seeding revealed cells that were aligned along the ECM fibers (c).

Supplementary Fig. S3: Evaluating collagen binding sites and structural integrity via fluorescently labeled collagen binding peptide (CBP). Thick pcECM matrices and commercial collagen were blocked with 5% BSA in PBS and labeled with TAMRA conjugated CBP. Macroscopically, labeled matrices exhibited a color change to pink-red, which was not diluted even after 10 consecutive washes compared to a non-treated control (a). Fluorescent imaging of either the crude labeled pcECM and commercial collagen serving as control (b) or of cross cryo-sections taken out of labeled (c, 14 ms exposure time) and non-labeled (d, 5 sec exposure time) matrices exhibiting a bright signal, suggesting peptide-target specific binding. Scale bars: 100µm.

Supplementary Fig. S4: Steady-state static culture penetration depth of hMSC on pcECM. Histological cross sections were counter stained with DAPI (blue) for hMSC nuclei, ECM fibers appear in red. Representative image is presented our of n=5 biological replicas. Scale bar: 100µm.

Supplementary Fig. S5: Modeling the pcECM support ability for HUVEC cells. Mathematical modeling of empirical data sets (a) and a goodness-of-fit between predicted and measured values (b) for HUVEC seeded on pcECM matrices. The HUVEC loading capacity of the pcECM scaffolds ($5.4x10^4$ cells/cm^2) was five-fold lower than that measured for hMSCs (Fig. 2). H&E staining of representative histological cross-sections (14 days, static culture) revealed that HUVECS form a monolayer coating on the pcECM surface and do not penetrate into it, hence lower cell densities are measured and predicted by the model (c). Confocal image analyses of four regions of interest (ROI) in

at least three representative porcine coronary artery luminal longitudinal tile scans resulted in similar endothelial density values ($5.0\pm0.7x10^4$ cells/cm^2). Scale bars: (c)- 100µm; (d)- 200µm.

Supplementary Fig. S6: Histological stains of beating pcECM repopulated with hESC-CM and statically cultivated for 23 days. Hematoxylin & eosin histological stain (H&E, a); Trooponin I immunohistochemical stains (b, brown) as a marker of the contraction machinery. Nuclei are counterstained with Gill's hematoxyilin (pale blue). Scale bars: 20µm. Yellow arrow points for the span of repopulated pcECM penetration depth (100µm). Additional video showing construct synchronous beating is available as an online supplementary movie M1.

Supplementary Fig. S7: Neo vascularization formed during dynamic cultivation. Bright field images were taken using a stereo-microscope (Olympus, SZX16, Japan). Dashed areas show the same location (i.e., of the same specimen) following 3 (a) and 21 days (b) of dynamic cultivation. Yellow arrows in (b) point to new-vessels which appeared to form and connect to pre-existing vessels. Scale bars: 2 mm.

Supplementary Movie M1: Beating pcECM construct can be achieved by seeding of human embryonic derived cardiomyocytes (hESC-CM) on the pcECM. Beating was observed starting from three days after seeding and lasted for at least 20 more days.

4. Supplementary references:

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Electronic supplementary material

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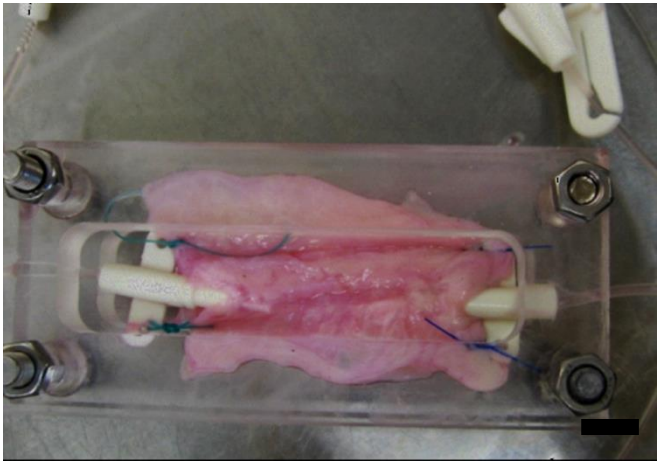
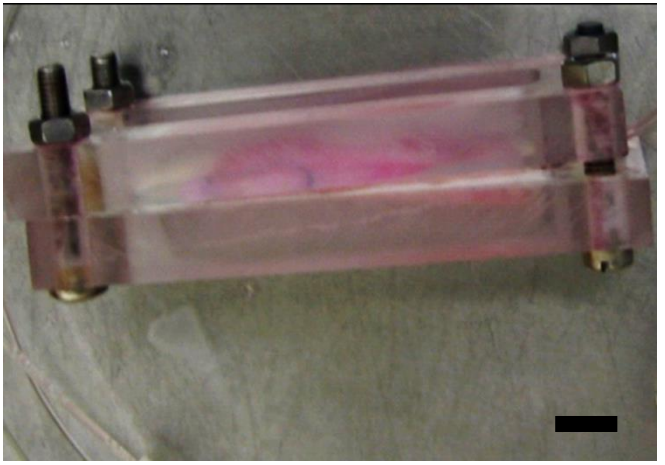
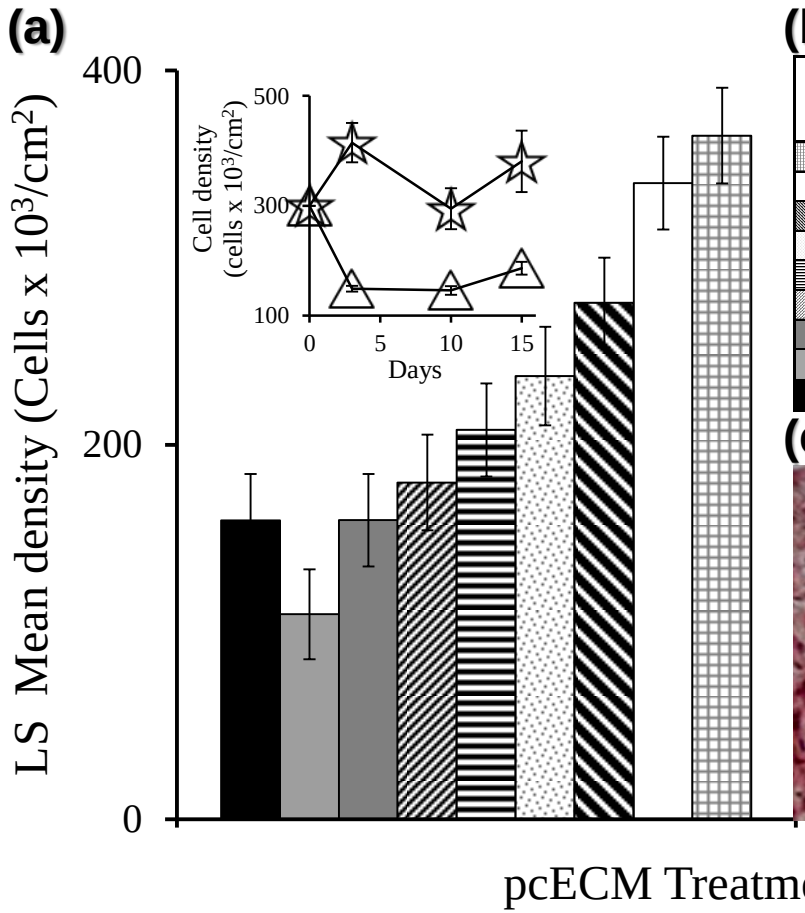
(a)



(b)

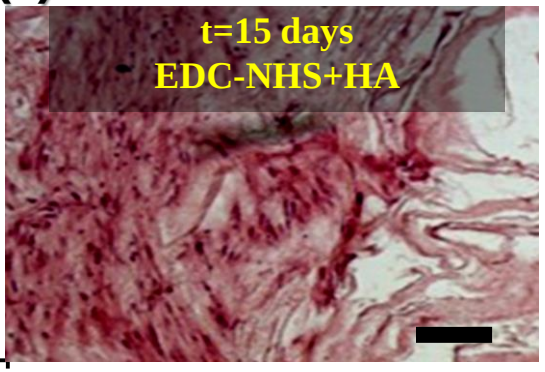


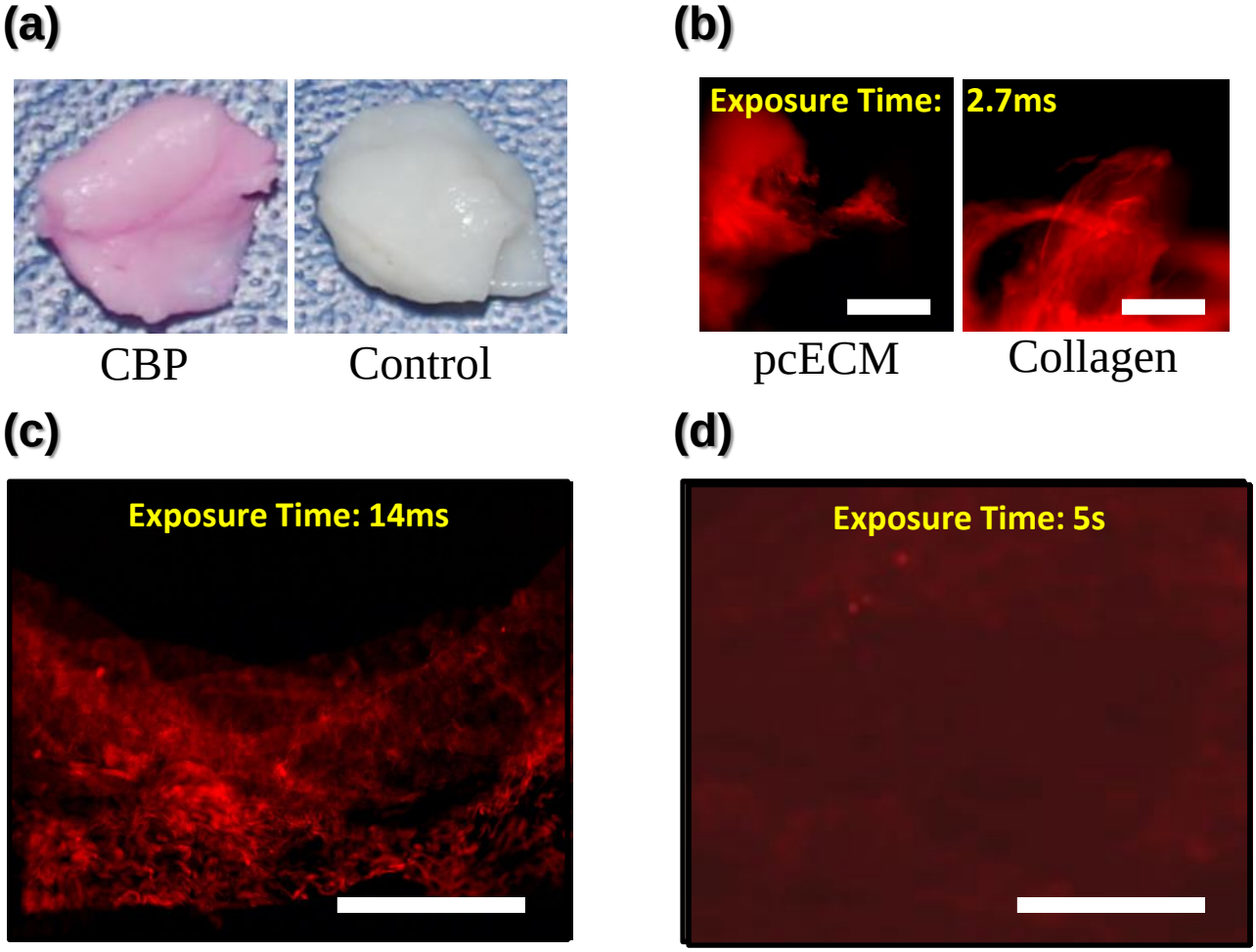


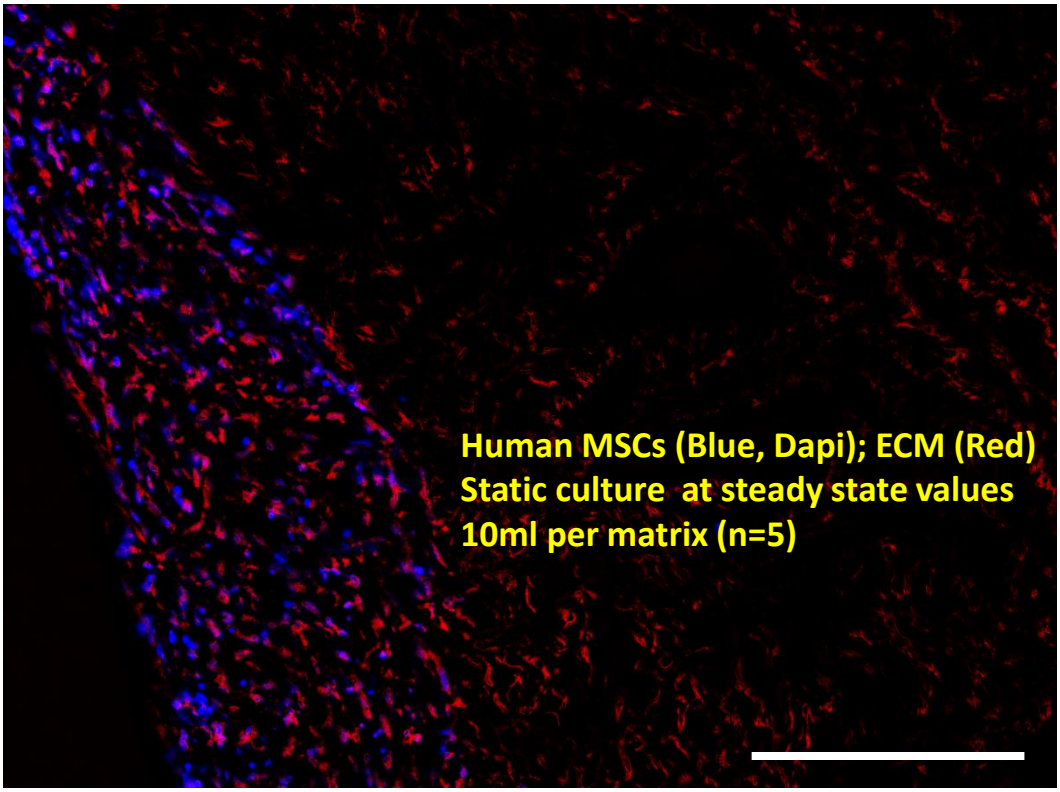
(b)

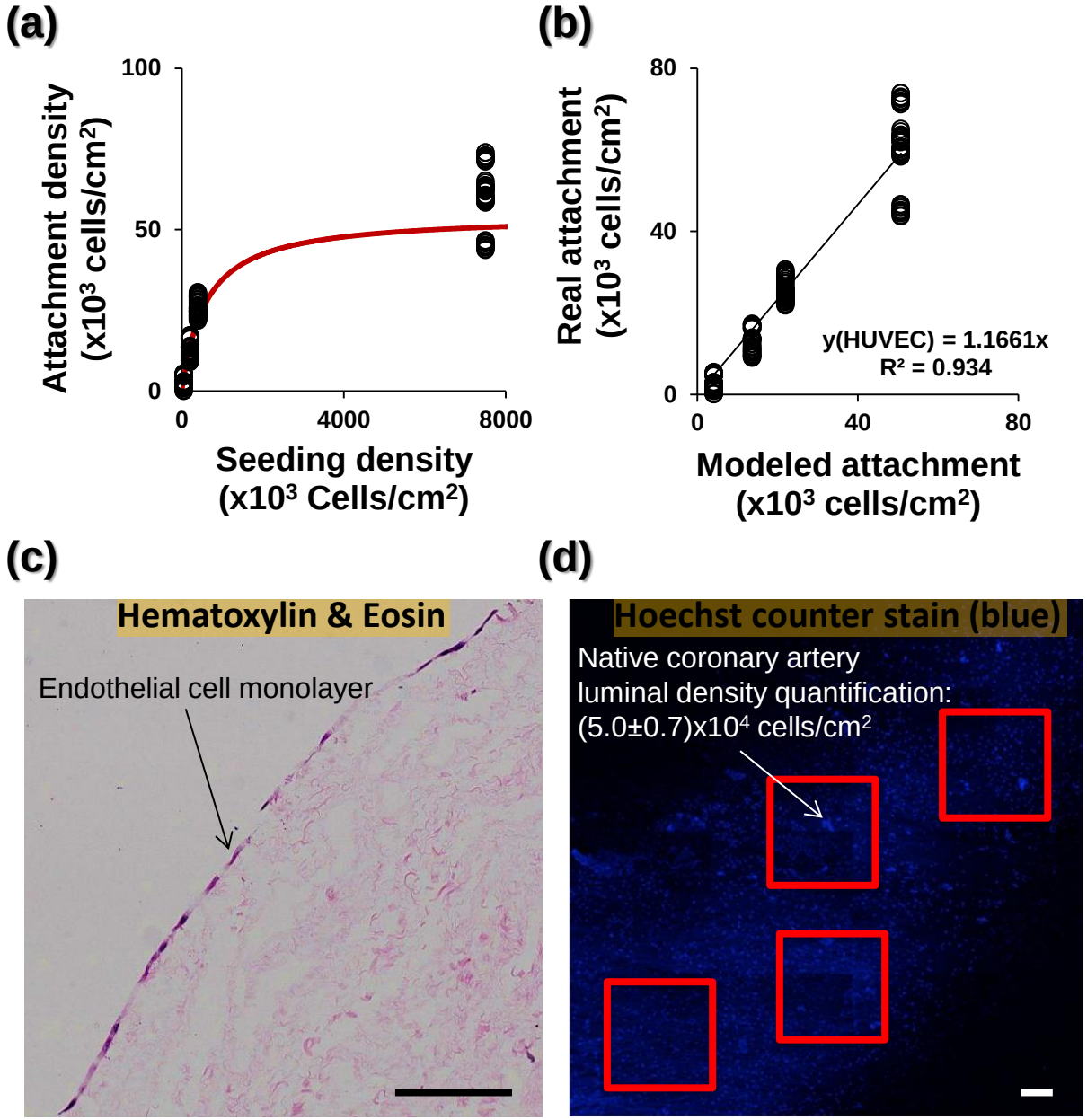
LEVEL	SIGNIFICANCE GROUPS (α=0.05)
EDC-NHS+HA	A
HS	A B
EDC-NHS+CBP-RGD+HS	A B C
NITROCELLULOSE	B C D
EDC-NHS+CBP-RGD	C D E
EDC-NHS+HS	C D E
RGD-CBP	D E
EDC-NHS	E
CONTROL	D E

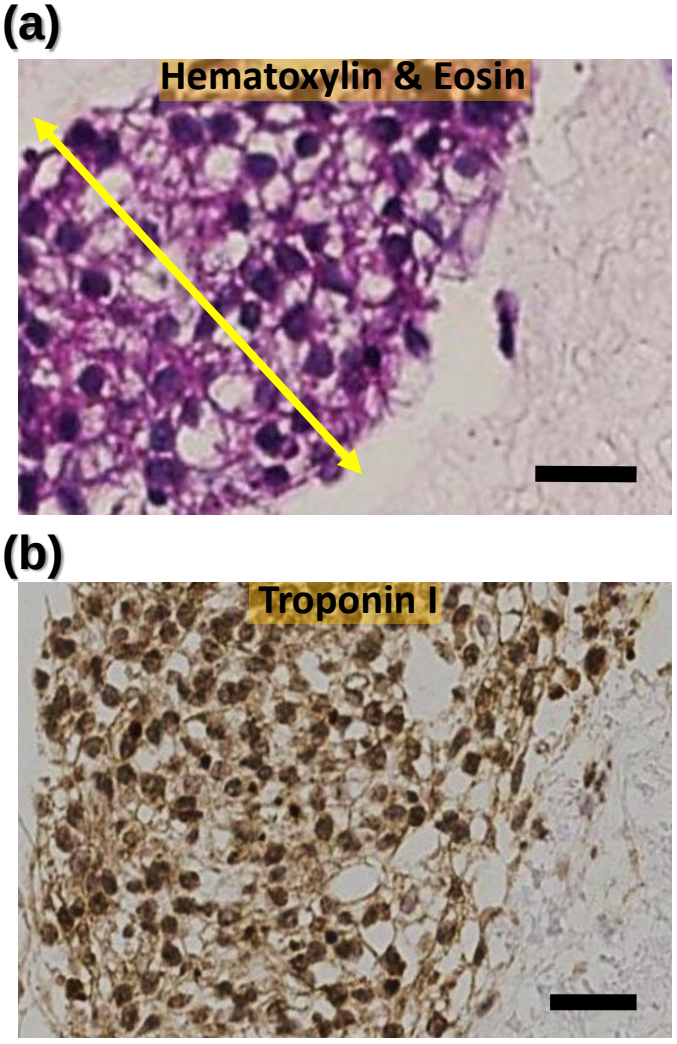
(c)

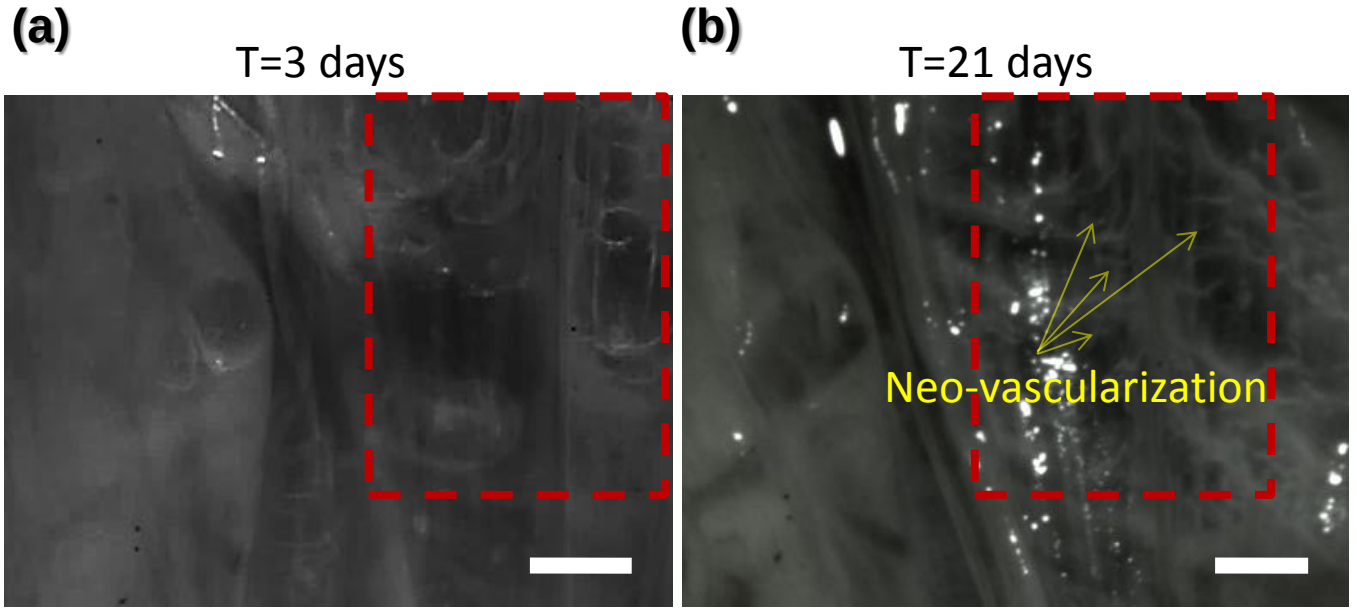



(a)
CBP
Control
(b)
Exposure Time:
2.7ms
pcECM
Collagen
(c)
Exposure Time: 14ms
(d)
Exposure Time: 5s


Human MSCs (Blue, Dapi); ECM (Red)
Static culture at steady state values
10ml per matrix (n=5)


(a)
Attachment density (x10³ cells/cm²)
100
50
0
0
4000
8000
Seeding density (x10³ Cells/cm²)
(b)
Real attachment (x10³ cells/cm²)
80
40
0
0
40
80
y(HUVEC) = 1.1661x
R² = 0.934
Modeled attachment (x10³ cells/cm²)
(c)
Hematoxylin & Eosin
Endothelial cell monolayer
(d)
Hoechst counter stain (blue)
Native coronary artery luminal density quantification:
(5.0±0.7)x10⁴ cells/cm²


(a)
Hematoxylin & Eosin
(b)
Troponin I


(a)
T=3 days
(b)
T=21 days
Neo-vascularization