The pro-angiogenic factor CCN1 enhances the re-endothelialization of biological vascularized matrices in vitro

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Aims
A problem in generating artificial tissues is supplying nutrients to cells within 3D constructs. The use of a decellularized biological vascularized matrix with preserved pedicles (BioVaM), as a scaffold, appears to aid the generation of perfusable tissue constructs in vitro. To prevent vessel occlusion upon implantation, a functional endothelium must line the graft vessel bed. Here we tested whether the pro-angiogenic factor CCN1 could improve the re-endothelialization of BioVaM in vitro.

Methods and results
BioVaM vessel beds were incubated with 100 ng/mL recombinant human CCN1. Human cord blood endothelial cells (hCBEC) were analysed with respect to adhesion behaviour upon CCN1 exposure and seeded onto vessel structures of CCN1 exposed BioVaM (cBioVaM). BioVaMs were fixed in a bioreactor and perfusion cultured for 4 and 14 days (d). BioVaM without CCN1 treatment served as controls. Initial seeding success and endothelialization progression were monitored by fluorescence-labelled hCBEC. During construct cultivation, pH and lactate production were measured. Degree of endothelialization and characterization of seeded cells, with respect to endothelial markers, were investigated histologically. BioVaM vessel structures showed a 78% increase of attached cells when pre-treated with CCN1. Evaluation of re-endothelialization (arbitrary units) was 4.0 and 0.8 after 4 d, and 5.0 and 0.5 after 14 d in cBioVaM vs. BioVaM, respectively. On day 14, lactate concentration, an indicator of metabolic activity, was increased 12-fold in cBioVaM relative to BioVaM. A preserved endothelial phenotype of seeded cells was verified in all cultures by acetylated low density lipoprotein uptake and positive immunohistochemistry against von Willebrand factor, endothelial nitric oxide synthase, and CD31.

Conclusion
Coating of decellularized vessel structures with CCN1 supports adhesion of hCBEC and enhances re-endothelialization of BioVaM. Perfusable, endothelialized constructs may aid in solving the problem of nourishing cells inside 3D tissue-engineered constructs.

Keywords
Endothelium • Tissue engineering • Extracellular matrix • CCN1

1. Introduction
Tissue engineering (TE) is a promising technique for future therapeutic approaches especially for cardiac reconstruction, as myocardial infarct and co-morbidities remain one of the leading causes of death in modern society.

A persistent obstacle to the generation of 3D tissues for therapeutic application is the efficient substrate supply and subsequent
removal of culture waste products of cells seeded inside complex cell matrix constructs. Depending on the architecture and stiffness of the reverse engineered tissue, a more or less intense supply of substrates and oxygen must be provided to the constructs, as inherent diffusion processes are limiting with increases in tissue thickness. Indeed, this is a substantial problem in cardiac TE, due in part to the fact that cardiac muscle is dense and has the highest oxygen consumption of all organs, but has little extracellular matrix content. Thus, in vivo, each individual cardiomyocyte is connected to at least one capillary. As a result, only cardiac tissue thicknesses ranging from $40\mu m$ to $100\mu m$ can be perfused using available TE methods. Several efforts have been made to solve this problem: (i) porous scaffolds or channelled matrices to promote diffusion processes inside tissues after seeding cells, (ii) employment of devices, for example, rotating wall vessels or stirrers to enhance convection in the tissue surrounded by media; and (iii) the use of oxygen carrying molecules or moieties to enhance oxygen concentration within polymeric scaffolds. Despite improvement, these approaches have not solved the problem of constricted diffusion inside the artificial cardiac tissue.

Since therapeutically relevant ischaemic situations in the heart usually occur in the left ventricle, an applicable TE myocardial patch would need to have a thickness of millimeters. However, to date, no complex cardiac tissue has been created with a thickness greater than $100\mu m$. Overcoming this obstacle would be best accomplished by supplying nutrients via in vitro and/or in vivo vascularization, a system already established in nature.

To this point, we have employed biological vascularized matrix (BioVaM), a highly perfusable matrix generated from decellularized porcine small intestine segments with preserved pedicles and vessel network, as an in vitro vascularization system. In 2005, first steps towards the re-seeding of BioVaM vessel structures were conducted at our institution. Despite promising results, it was evident that the seeding process required improvements, as an incomplete endothelial lining of the vessel bed of the BioVaM would be highly thrombogenic when connected to the blood stream in vivo. To enhance the re-seeding process, we generated a new perfusion bioreactor system that uses BioVaM as a patch. Furthermore, we investigated whether the coating of the BioVaM vessel structures with the secreted matrix associated protein, CCN1, would additionally support and promote adhesion, migration, proliferation, and survival of endothelial cells as previously reported (reviewed by Brickett et al. and Chen et al.). Herein, we report an improvement in the repopulation properties by coating BioVaM vessel structures with CCN1.

2. Methods

All reagents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (Munich, Germany). All experiments were conducted at room temperature unless otherwise indicated.

2.1 Cell culture: isolation, differentiation, and culture of human cord blood-derived endothelial cells

Mononuclear cells were obtained from heparinized human umbilical cord blood (obtained from healthy newborn donors after informed parental consent) by density gradient centrifugation on Ficoll-Hypaque (Seromed). CD34$^+$ progenitor cells were isolated by immunomagnetic separation using anti-CD34 antibody-linked immunomagnetic microbeads and double separation on a magnetic column (MACS system, Miltenyi Biotec) according to a protocol described previously by Muromara et al. Isolated CD34$^+$ endothelial progenitor cells were cultured using a protocol described by Gehling et al. with slight modifications. In detail, cord blood-derived CD34$^+$ cells of 2–3 donors were pooled, seeded on fibronectin-coated plastic plates, and cultured in IMDM with both 10% horse serum and foetal calf serum supplemented with 50 ng/mL recombinant human stem cell factor (R&D), 50 ng/mL vascular endothelial growth factor (R&D), 20 ng/mL fibroblast growth factor-2 (R&D), and 100 ng/mL stem cell growth factor (SCGF-β, PeproTech). Adherent endothelial cell clusters were observed at week 1–2 of culture. Cells were adapted to endothelial cell basal medium (EBM-2) with 2% FCS and growth factor supplement (EGM-2, Lonza). For all experiments, human cord blood-derived endothelial cells (hCBEC) were cultured in gelatin-coated culture flasks in EGM-2/10% FCS/1% antibiotics. Medium was exchanged every 3 days.

2.2 CCN1 influence on hCBEC attachment

As hCBEC have been extensively characterized previously, we sought to determine the effect of CCN1 on hCBEC attachment in culture plates, prior to initiating BioVaM experiments. In brief, six-well plates were pre-coated with 1% gelatin alone or with 1% gelatin enriched with either 10 or 100 ng/mL CCN1. After overnight incubation, wells were rinsed with phosphate buffered saline (PBS). hCBEC were then seeded onto wells with an average density of $3.5 \times 10^5$ cells/cm$^2$. Cells were incubated for 10, 20, 30, or 75 min, trypsinized, and counted using CASY rexcell (Innovates AG, Reutlingen, Germany). To confirm that CCN1 binds via integrins, cells were pre-treated for 30 min at 37°C with 1 mM RGD peptide (Arg-Gly-Asp, Bachem), a non-specific integrin inhibitor, prior to seeding on above described six-well plates. Data are presented as the % of total cells initially seeded into the wells.

2.3 BioVaM preparation

Segments of small intestine with preserved pedicles (BioVaM) were isolated from German landrace pigs (18–25 kg) as previously described. All animals used in this study received humane care in compliance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). The study was approved by the local Animal Care and Research Advisory Committee and was conducted according to local government policy (#05/937). In brief, a median laparotomy on anaesthetized animals (propofol, 10 mg/kg i.v.; thiopental sodium, 5 mg/kg i.v.; fentanyl citrate, 0.1 mg/kg i.v.) was used to isolate a 10–15 cm long segment of jejunum, including the artery and vein pedicle. Anticoagulation was achieved by systemic application of heparin (150 I.E/kg). Following insertion of a 6 F catheter into the artery and an 8 F catheter into the vein, the vascular system was perfused with saline (0.9% NaCl supplemented with vancomycin (1 g/L; ratio-pharm). After excision of the segment, the explants were stored in undiluted Braunol (B. Braun) at 4°C for 60 min until further processing (described below). The animal was then euthanized with sodium-pentobarbital (90 mg/kg, i.v.).

The mucosa of the BioVaM grafts was removed mechanically by scraping the inner lumen of the intestine. This was followed by the detergent decellularization of the BioVaM (2 h: 0.5% sodium deoxycholate and 0.5% sodium dodecylsulfate), seven wash cycles at 4°C...
(24 h each) with PBS supplemented with Nebactin (100 μg/mL, Biochrom) for removal of remaining detergents and cell debris were performed. Decellularized matrices were stored in PBS/Nebactin at 4°C.

For seeding, the BioVaM was cut open along the longitudinal axis opposite to the hanging mesentery, after the serosa was removed mechanically. The resulting patch was sutured (Mersilene 5/0; Ethicon) to a silicon rubber cushion made from a two-component silicon mixture (RTV 71556A and B, Rhodia Silicones, France) cast with the dimensions of 5 x 3 cm. The orientation was such that the serosa side with mesentery was turned up towards the environment, whereas the submucosa side was turned down towards the cushion. The constructs were then sterilized by γ-irradiation (150 Gy) and stored in PBS/Nebactin at 4°C.

Constructs treated with CCN1 (cBioVaM; n = 7) were compared with constructs without CCN1 (BioVaM; n = 8). For CCN1 exposure, the vessel system of the BioVaM was infused through the arterial cannuula with 100 ng/mL recombinant human CCN1 (Abnova) in culture medium (EBM-2, Lonza) and incubated overnight (12 h; 37°C). Unbound CCN1 was removed 12 h after incubation by rinsing the vessel system with warm DMEM containing 1% penicillin–streptomycin (37°C). After rinsing, the seeding procedure was initiated.

2.4 Seeding and culture of hCBEC in BioVaM

Constructs were seeded by the injection of 0.8 × 10⁷ hCBEC in 10 mL EBM-2 through the arterial cannuula, twice in a 4 h interval (0.5 mL of in-flow and 0.5 mL of off-flow was used to determine the seeding efficiency, see below). In some cases (BioVaM; n = 4 and cBioVaM; n = 4), cells were fluorescence labelled prior to seeding, by incubation with 25 μM S(6)-TAMRA, SE (Molecular Probes) in PBS for 20 min at 37°C, 5% CO₂ and 100% relative humidity. Perfusion cultivation in a bioreactor device was initiated 12 h after seeding (medium: EGM-2/10% FCS/1% PS; medium volume: 200 mL; flow: 2 mL/min). As the mucosa was removed, submucosal vessels (arterioles and capillaries) were leaky and thus, the outflow was re-circulated through a pump (Ismatec). BioVaM and cBioVaM constructs were cultivated for 4 d (n = 4, each) and 14 d (n = 3, each).

2.5 Seeding efficiency

The ratio of the number of cells cultivated from 0.5 mL in-flow and 0.5 mL out-flow at each seeding, cultivated for 12 h in a 25 T culture flask was used to determine the efficiency of living cells retained in both the BioVaM (n = 3) and cBioVaM (n = 3).

2.6 Dil-Ac-LDL assay

At the end of cultivation (4 and 14 d), a Dil-Ac-LDL uptake test was performed.²⁹ Constructs (n = 1 from each condition) were perfused with 10 μg/mL Dil-Ac-LDL in EGM-2 and cultivated statically for 4 h at 37°C, washed with PBS, and fixed with 4% paraformaldehyde (PFA) for 20 min in the dark. Results were evaluated microscopically (fluorescence microscope Observer A1, Zeiss).

2.7 DNA content

DNA was isolated with the DNA Blood and Tissue Easy Kit (Qiagen) from homogenized constructs (BioVaM: n = 3 and cBioVaM: n = 3) (FastPrep²⁴ System, MP Biomedicals) according to the manufacturer’s protocol. DNA content was measured photometrically.

2.8 Measurement of cultivation parameters

Oxygen saturation and pH were measured using a CDI 500 blood gas analyser (Terumo) according to the manufacturer’s protocol. Lactic acid measurement (n = 3 BioVaMs/group) was assessed with a YSI 2300 STAT Glucose and Lactate Analyzer.

2.9 Histology and immunohistochemistry

Cryosections (6 μm) of constructs were stained with either haematoxylin–eosin (H&E) stain or pentachrome stain and visualized in a bright field (OlympusBX41 microscope). Cryosections were also assessed immunohistochemically with the following primary antibodies: Rabbit anti-laminin, mouse anti-collagen IV, mouse anti-von Willebrand Factor (vWF), mouse anti-CD31 (all DAKO), mouse anti-endothelial nitric oxide synthase (eNOS) (Beckton Dickinson and Company), goat anti-CCN1 (Santa Cruz Biotechnologies). Cyanine-conjugated donkey anti-mouse, anti-goat, and anti-rabbit antibodies (Jackson Research) served as secondary antibodies. Negative controls with the omission of the primary antibodies were used. Nuclear staining was performed with 4′,6-diamidino-2-phenylindole, dichloride (DAPI). Sections were analysed and documented with fluorescence microscopy (Observer A1, Zeiss).

2.10 Statistics

Data are reported as mean ± standard deviation. The t-test analyses were performed in Excel (Microsoft Office) and two-way ANOVA was performed using GraphPad Prism. A P-value of less than 0.05 was considered significant.

3. Results

3.1 CCN1 increases hCBEC adhesion

The positive effect of CCN1 on hCBEC adherence with respect to concentration (CCN1) is given in Figure 1. Gelatin coating enriched...
with 100 ng/mL CCN1 increased the amount of hCBEC adhering to the wells. Specifically, a significant increase in adhered cells was observed after 30 and 75 min relative to gelatin coating only. As expected, RGD inhibited the adhesion effect of CCN1 via inhibiting the interactions with integrins (Figure 1).

3.2 Characterization of decellularized BioVaM

Detergent-treated BioVaM was free of nuclei as demonstrated by H&E stains (Figure 2A) and showed a well-preserved ECM as determined by pentachrome staining (data not shown). These data correlate with positive immunohistochemical stains for the basal lamina proteins: collagen IV (Figure 2B) and laminin (Figure 2C), further indicative of structure preservation. Adherence to the vessel bed of CCN1 by overnight incubation was shown by immunohistochemistry against CCN1 in cBioVaM. Positive fluorescence signals were detectable on the luminal side of the vessel bed, but not inside the matrix structure (Figure 2D and E).

3.3 CCN1 lining of the vessel bed in decellularized BioVaM improves repopulation dynamics of hCBEC

Upon seeding hCBEC into the vessel structures, significantly more cells were retained in cBioVaM than in BioVaM (84 ± 9% vs. 47 ± 4%, n = 3, P < 0.05) (Figure 3A). Consistently, significantly more

![Figure 2](https://academic.oup.com/cardiovascres/article-abstract/85/4/806/297428/854808272428/download)

**Figure 2** Histological appearance of decellularized BioVaM. H&E staining of decellularized BioVaM shows no remaining nuclei or cell residues inside the matrix (A). Immunohistochemistry against collagen IV (B) and laminin (C) revealed the preservation of both basal lamina proteins on the luminal side of the vessel structures after decellularization. Successful coating of the vessel walls with CCN1 was demonstrated by immunohistochemistry against CCN1 in cBioVaM (D), whereas uncoated BioVaM showed no immunoreactive CCN1 protein (E).

![Figure 3](https://academic.oup.com/cardiovascres/article-abstract/85/4/806/297428/854808272428/download)

**Figure 3** Re-seeding efficiency of BioVaM is increased by the CCN1 lining of vessel structures. A higher percentage of hCBEC is retained in BioVaM structures coated with CCN1, **P < 0.01** cBioVaM (n = 3) vs. BioVaM (n = 3) (A). Similarly, 12 h after re-seeding, cBioVaM (n = 3) had a higher DNA content when compared with BioVaM (n = 3), **P < 0.01** (B).
DNA was isolated 12 h after seeding from cBioVaM compared with BioVaM (37 ± 2 μg/g vs. 11 ± 3 μg/g, n = 3, P < 0.001) (Figure 3B). There was no DNA detected from the decellularized BioVaM.

The effect of CCN1 and cultivation duration (4 vs. 14 d) on vessel endothelialization was evaluated. On the basis of an arbitrary quantification system, the microscopic appearance of the repopulation success was rated (Table 1). As a result, both pre-treatment with CCN1 and perfusion duration had a significant effect on vessel status (two-way ANOVA: *P_{CCN1} = 0.028, †P_{time} = 0.002; n = 3 for all groups).

After 4 d of perfusion cultivation in a bioreactor, fluorescent microscopy revealed linear, re-populated vessel structures with a maximum length of 1000 μm within BioVaM (Figure 4A), whereas in cBioVaM vessel structures with a maximum length of 2500 μm were observed (Figure 4B). The observational assessment of re-endothelialization after 4 d in BioVaM resulted in a mean value of 2.6 ± 0.8 AU (n = 3) and in cBioVaM 4.0 ± 0.8 AU (n = 3) (Table 1). Lactate production, an indicator of cell metabolism, on day 4 was 1.1 ± 0.1 and 5.4 ± 1.2 mmol/L in BioVaM

| Table 1 Analysis of the re-endothelialization of BioVaM and cBioVaM |
|-----------------|----------------|----------------|
| Time of cultivation (d) | BioVaM | cBioVaM |
| 4                | 2 ± 0.5 | 4.0 ± 0.8* |
| Mean ± SD        | 3.3 ± 0.5† | 5.0 ± 0.0† |
| 14               | 4      | 5      |
| 3 ± 0.5          | 5      | 5      |
| Mean ± SD        | 5.0 ± 0.0† | 5.0 ± 0.0† |

Degree of BioVaM and cBioVaM endothelialization characterized by arbitrary units defined as follows: 0, no visible cells; 1, single cells; 2, small cell clusters; 3, short, re-populated segments up to 500 μm; 4, long, re-populated segments over 500 μm; 5, broad, cross-linked vessel sections (mm); 6, broad, cross-linked vessel sections (cm); 7, complete re-endothelialization. n = 3 for all groups.

*Two-way ANOVA: P_{CCN1} = 0.028.
†Two-way ANOVA: P_{time} = 0.002.

Figure 4 Re-endothelialization of vessel beds in BioVaM and cBioVaM by TAMRA pre-stained hCBEC after cultivation in a perfusion bioreactor for 4 and 14 d, respectively. A representative BioVaM from each group (n = 3/group) is shown. (A) BioVaM and (B) cBioVaM after 4 d. (C) BioVaM and (D) cBioVaM after 14 d.
and cBioVaM, respectively (Figure 5A). Despite the increase in lactate in cBioVaM, no differences in oxygen saturation, which was 99% throughout the experiment, nor pH values, decreasing from $7.4 \pm 0.2$ to $7.2 \pm 0.3$, were observed between either BioVaM or cBioVaM cultivation systems.

The endothelial cell type character of seeded hCBEC, tested by a Dil-Ac-LDL uptake assay, revealed a vesicular staining pattern inside all adherent cells in both BioVaM and cBioVaM (Figure 6A).

After 14 d of perfusion cultivation, extensive repopulated vessel structures were found. In BioVaM, mainly linear structures with a maximum length of 6 mm were present ($n = 3$) (Figure 4C), whereas in cBioVaM ($n = 3$) the vessel structures were widely interconnected covering an area up to 56 mm$^2$ (Figure 4D). Comparison of the repopulation process in coated and uncoated patches after 14 d of cultivation revealed an arbitrary value of $3.3 \pm 0.5$ AU for BioVaM and $5.0 \pm 0.0$ AU for cBioVaM (Table 1). In parallel to an increase in re-endothelialization, lactate concentrations increased as well (Figure 5B). After 14 d in cBioVaM systems, the lactate concentration was seven times higher than in BioVaM and reached $16.5 \pm 12.3$ and $1.6 \pm 0.6$ mmol/L, respectively.

Similar to data collected after 4 d, the Dil-Ac-LDL assays after 14 d, resulted in positive stains for all constructs, e.g. a representative vesicular pattern inside all adherent cells (Figure 6A), indicative of the maintenance of an endothelial cell character 14 d post-seeding of hCBEC. Supporting evidence for this observation was determined using immunohistochemical analysis against eNOS, CD31, and vWF (Figure 6B–G).

4. Discussion

Herein, we present the successful repopulation of a decellularized vascular bed with endothelial cells differentiated from hCBEC. This construct, i.e. a carrier matrix with a perfusable endothelial cell lined vessel system, may serve as a basis for many approaches to generate artificial tissue by means of TE.

Despite several efforts, a satisfactory solution for the nourishment of 3D tissue has not yet been elucidated. In particular and with respect to the reconstruction of highly nourished native tissues with high oxygen and substrate demand like the myocardium, an efficient supply is indispensable. In the case of reconstructing cardiac muscle, only an efficient, hollow fibre-based perfusion system can provide the required demands. Such a system would minimize the distance between medium and cells within the 3D construct, thereby enhancing the exchange of nutrients, oxygen, and metabolites between the cells and medium.

The solution to the nourishment of generated artificial tissue for in vivo TE remains equivocal. Morrill et al. reported the in vivo vascularization of cardiac tissue by ectopic transplantation to the groin of rats. By this method, it was possible to increase the thickness of the construct up to 2 mm. Although such an approach is promising, the required thickness for constructs to be ultimately used in the future therapeutic application in humans has not yet been achieved.

Beyond the demand for highly vascularized 3D engineered tissue, defined tubes for anastomosis must be provided for in situ application in therapeutic approaches. Such anatomical connections are necessary to assure rapid and immediate nourishment of the artificial tissue to prevent necrosis after transplantation. BioVaM matrices, our approach to solving these problems, are promising to assure both the creation of an in vitro vascularized vessel bed, in addition to providing defined vessels for anastomosis in situ.

Already in 2005, the feasibility of repopulating vessel bed structures of decellularized BioVaM was demonstrated; however, an incomplete lining of the vessels was observed. Targeting a revascularized BioVaM with non-thrombogenic properties for in vivo applications, we pursued an alternative strategy using re-endothelialized vessel structures. First, we conducted the re-seeding process on a longitudinally opened BioVaM in an advanced bioreactor system, and second, we exposed the lumen of the vessel bed to CCN1, which we believed would additionally support and promote the repopulation process of the vessel bed.

CCN1 is a 42 kDa, secreted cystein-rich, heparin binding, extracellular matrix, and cell surface-associated protein. It has been shown that CCN1 mediates adhesion, migration, proliferation of endothelial cells, and in vivo, the angiogenic effect of CCN1 has been demonstrated in a rat corneal micropocket angiogenesis model and the rabbit ischaemic hindlimb model. Furthermore, high CCN1 expression is found in atherosclerotic plaques and post-myocardial infarction and may induce neovascularization of hypoxic areas. Here we demonstrated that recombinant CCN1 enhanced adhesion of hCBEC to matrix components, i.e. collagen IV and laminin present in decellularized vessel walls of BioVaM in cell culture dishes in vitro. In addition, we show that CCN1 in the
absence of these matrix components is not able to induce hCBEC adherence to cell culture dishes, suggesting that CCN1 only improves endothelial cell adherence to vessel walls with intact matrix components. It has been demonstrated previously for various cell types that CCN1 improves cell angio- and vasculogenic properties via integrins.\(^{21,37,38}\) In the present study, we confirmed that enhanced adhesion of hCBEC by CCN1 was mediated by integrins since this effect was abolished by the unspecific integrin inhibitor RGD. The specific integrin types involved in hCBEC adhesion will be explored in future studies.

In TE, a cell free matrix with well-preserved ECM, specifically an intact basal membrane, might be a prerequisite for successful repopulation with cells, in order to yield the best results. Therefore, we analysed the decellularized matrix, as well as the integrity of the basal membranes of the BioVaM prior to in vitro seeding with endothelial cells. H&E stains indicated the absence of cells and debris emphasizing an effective mechanical and detergent decellularization protocol. Furthermore, both basal lamina proteins, laminin and collagen IV, were found in the matrix on the luminal side of the vessel bed as indicated by immunohistochemical stains. Thus, seeded hCBEC are presented with a well-preserved basal membrane representing an appropriate surface for endothelial cell adhesion.

Immunofluorescence staining provided evidence of the successful coating of the BioVaM vessel structures by CCN1. The increased number of attached cells on the CCN1-enriched collagen, relative to the population of adhered cells on the collagen coating alone, illustrated the impact of CCN1 on the adhesion behaviour of hCBEC. Furthermore, the increased seeding efficiency and increased DNA content of the cBioVaM vessel structures relative to the BioVaM also indicate improved hCBEC adhesion.

Employing an arbitrary quantification system, results clearly show that the coverage of the vessel structures exposed to CCN1 with endothelial cells is superior to those without CCN1 exposure both at 4 and 14 d. This comparison of cBioVaM vs. the untreated BioVaM suggests at least, a more rapid process of repopulation. Since the ‘time’ factor is an essential issue in TE, the use of CCN1 represents a very important improvement towards the in vivo application of such constructs. However, even with CCN1 coating, a complete re-endothelialization was not achieved in our experimental settings by 14 d of culture. Here, the additional time for creating an implantable, functional tissue, i.e. contractile heart tissue by adding cardiomyocytes in vitro, has to be accompanied by an additional (neo)-vascularization process.

Characterization of the seeded BioVaMs suggested that the cells lining the vessel structures were of an endothelial nature, as the uptake of Dil-Ac-LDL resulted in a typical dotted, vesicular pattern. This suggests that the cultivation system used in our study perpetuates the differentiation of hCBEC. Furthermore, eNOS, CD31, and vWF, typical endothelial cell-specific markers, could be detected in vessel structures of cultivated BioVaM further supporting a maintained endothelial differentiation as well. An approximate seven-fold increase in lactate concentration in cultures obtained from cBioVaM patches suggests a higher metabolic activity relative to those in uncoated patches. One can speculate that in addition to a reduced time for re-endothelialization, CCN1 may also positively affect viability of cells seeded onto
Constructs for therapeutic applications. The system will significantly improve the success of engineered tissue. A perfusable, endothelialized matrix for the TE of artificial tissues. These data suggest an enhanced quantity of cells within the vessel. An overall endothelialization for successful implantation without reaching a steady state level. Targeting an improvement of these genic factor CCN1 promotes adhesion and migration of circulating CD34+ progenitor cells. Potential role in angiogenesis and endothelial regeneration. Blood 2007;110:877–885.

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