Guidance of postinfarct myocardial remodeling processes by an epicardial patch system may alleviate the consequences of ischemic heart disease. As macrophages are highly relevant in balancing immune response and regenerative processes their suitable instruction would ensure therapeutic success. A polymeric mesh capable of attracting and instructing monocytes by purely physical cues and accelerating implant degradation at the cell/implant interface is designed. In a murine model for myocardial infarction the meshes are compared to those either coated with extracellular matrix or loaded with induced cardiomyocyte progenitor cells. All implants promote macrophage infiltration and polarization in the epicardium, which is verified by in vitro experiments. 6 weeks post-MI, especially the implantation of the mesh attenuates left ventricular adverse remodeling processes as shown by reduced infarct size (14.7% vs 28–32%) and increased wall thickness (854 µm vs 400–600 µm), enhanced angiogenesis/arteriogenesis (more than 50% increase compared to controls and other groups), and improved heart function (ejection fraction = 36.8% compared to 12.7–31.3%). Upscaling as well as process controls is comprehensively considered in the presented mesh fabrication scheme to warrant further progression from bench to bedside.

1. Introduction

Following myocardial infarction (MI), the mammalian heart has a very limited—if any—ability to recover and/or regenerate. In order to limit the consequences of MI, cells, bioactive compounds, implants, or combinations of these have been extensively studied,[1] but most concepts failed in clinical applications.[2] This is also true when employing bioactive compounds such as insulin-like growth factor,[3] vascular endothelial growth factor,[4] episomes, and noncoding RNAs. More recently, combinations of “biologics” with biomaterials have been investigated for cardiac applications,[5] but there is no consensus about the most promising strategy.

From a regulatory perspective, medical devices have the clear advantage of an efficient clinical translation process when compared to cell therapeutics or pharmaceuticals. The challenge in the design of implants promoting tissue regeneration is the implementation of the required biofunctionality, as it must rely predominantly on physical cues. The classical approach for conceptualization of surgical implants is primarily paying attention to their macroscopic structural functions, e.g., to close wounds by contracting wound limbs or to keep a blood vessel open by a skeleton structure. This strategy was also applied in cardiac patch systems consisting of elastic polymer films, which were bridging the healthy cardiac tissue over the infarcted area, acting as restrain to reduce or prevent the undesired left ventricular remodeling or dilation observed after myocardial infarction. Although some positive effects on the tissue remodeling could be found, the biological effect had its limitations.[6]
Meanwhile fundamental research on cell-material interaction has advanced especially taking into view the 3D microscopic structure surrounding cells,[5] the nano/micromechanics[6] of the cell-biomaterials interaction as well as the spatial distribution of anchor points for cell receptors[7] at the biomaterial surface. Furthermore, the dynamics and the changes in material properties/geometries[8] during implant degradation have been understood as an opportunity to control cellular behavior.[9]

In the heart, degradable polymers,[10] derivatives of the extracellular matrix (ECM)[11] and injectable hydrogels[12] have been used to support cardiac function and regeneration, and we found that a cell-free epicardial patch consisting of decellularized amniotic membrane helps preserve heart function in a mouse model.[13] Supporting myocardial regeneration processes via the epicardial surface may provide physical and biochemical cues to activate epicardial tissue by mobilizing endogenous macrophages and progenitor cells, which subsequently might lead to cardiac regeneration. Interestingly, tissue regeneration does not necessarily depend primarily on cells that form the damaged tissue, but rather on the immune response, and specifically macrophages, that especially upon polarization seem to orchestrate regeneration, e.g., by the release of hydrolytic enzymes and paracrine signaling molecules.[14] Macrophage polarization associated with regeneration typically involves mainly the M1 phenotype in the initial state and a subsequent shift to the M2 phenotype. It has been postulated that materials can support such transient polarizations[15] with fitting elasticity, pore size,[16] and surface properties, though the exact values are still in discussion.[17] Our approach to gain the desired bioinstructivity of polymer mesh is based on the interactivity between monocytes and the designed microenvironment provided by the mesh (Figure 1a). Its processability to nonwovens with different fiber sizes and organization[18] as well as its promising effect on endothelial cell tube formation supporting neovascularization of pericardium (PEEU) as a promising multifunctional biomaterial forming the basis of our mesh implant. The specific, elastic and degradable PEEU employed are multiblock copolymers consisting of poly(ε-dioxanone) (PPDO) and poly(ε-caprolactone) (PCL) segments, which are interlinked by diurethane junction units.[20] Their mechanical properties and degradation behavior can be tailored by adjusting segment lengths, the weight ratio of the two segments, the type of junction unit, overall molar mass as well as the shape of the formed material. PEEU, which comprises a PPDO:PCL weight ratio of 70:30 and an L-lysine based junction unit, is not cytotoxic and supports the growth of various cell types.[21] Our decision to focus on a synthetic medical grade polymer material for epicardial application was also supported by the given translational limitations when working with a material.

In order to realize the PEEU mesh design and to advance this system toward translation, i) synthesis and processing have to be upscaled and adjusted to the requirements of medical grade polymers (fabrication scheme); ii) a relevant benefit in vivo has to be demonstrated; iii) components that are required for performance need to be identified; and iv) the mechanism underlying the in vivo effects should at least be partially understood. The bioinstructivity of the system acts through physical principles and in this way qualifies as a medical device. Consequently, the physicochemical and morphological characterization of a PEEU mesh was performed in detail. The interactions of the material with relevant cardiac cell populations and inflammatory cells are shown (Figure 1b). Finally, the outcome of the in vivo evaluation in a mouse model of MI is reported, studying cardiac performance (Echo), infarct size, heart wall thickness, angiogenesis, arteriogenesis, and cell types in the infarcted area over time. Our approach with the pure PEEU mesh will be compared to a PEEU mesh coated with ECM or loaded with induced cardiomyocyte progenitor cells (iCMs).

2. Results

2.1. Fabrication and Characterization of the PEEU Mesh

PEEU synthesis was based on coupling the two macrodiol precursors with lysine diisocyanate ethyl ester (LDI) giving multiblock copolymers with a 70:30 w/w composition regarding the PPDO and PCL components determined by $^1$H NMR and an $M_n$(GPC) = 271 kg mol$^{-1}$ with a polydispersity index (PDI) = 3.2 determined by gel permeation chromatography (GPC) with universal calibration. Fourier transform infrared (FT-IR) spectroscopy (Figure 2g) did not show a band at ≈2245 cm$^{-1}$, at which isocyanate groups show absorption, so that it can be stated that no residual isocyanate was present in the final polymer. The electrospinning process gave the desired meshes with random fiber orientation (Figure 2a–e, Table 1, and Table S1, Supporting Information) for full characterization) and pore sizes of 2.51 ± 1.27 µm. All data reported in the study were determined on the ethylene oxide sterilized samples. The sterilization process did not change the fiber structure, and did not have a strong impact on the mechanical properties, with only a minor reduction of the Young’s modulus (Figure 2f and Figure S1 and Table S2, Supporting Information).

To test the effect of providing additional, tissue-specific cues to the PEEU mesh, the material was coated with human myocardial ECM in separate groups (PEEU + ECM). ECM hydrogel was derived from surgical samples of myocardium, which were processed as previously described.[22] The ECM completely covered the fiber mesh as shown in Figure 2b, resulting in a surface with hidden fiber morphology. A tilted cross-section of the ECM coated mesh shows the ECM remained as a coating layer on top of the mesh, without much penetration into the mesh (Figure 2c–e).

The mechanical properties of both the PEEU and PEEU + ECM meshes were characterized in tensile tests in a hydrated state at 37 °C. Coating of the PEEU mesh with ECM changed the surface morphology drastically, whereas the macroscale mechanical properties were only slightly affected. Both samples showed a similar profile in the stress–strain curves (Figure 2f), however, the noncoated PEEU fiber meshes had lower tensile strength and longer elongation at break than the PEEU + ECM mesh. The elastic modulus of PEEU and PEEU + ECM meshes were 4.69 ± 0.16 and 5.64 ± 0.52 MPa, respectively. As the coating led to a continuous hydrogel surface, the local elasticity was determined by atomic force microscopy (AFM) indentation and gave a Young’s modulus $E_{(AFM)} = 264 ± 91$ kPa. Single fiber mechanics was previously studied by AFM as reported in ref. [23].
Figure 1. a) Proposed mechanism and working hypothesis. Rather than mechanically stabilizing the heart wall with the idea to reduce dilation and offer a scaffold for tissue formation, the PEEU mesh is implanted to guide macrophage behavior. In the first step, invading macrophages Mφ are guided by material elasticity on the micro/nanoscale, and the geometry of the 3D microenvironment to differentiate into the M1 phenotype. This leads to the secretion of cytokines and enzymes and M1 macrophages are active in efferocytosis and material degradation. The paracrine signaling and/or changed material properties support polarization of the local group of macrophages to the M2 phenotype that supports regeneration. b) The experimental work in the in vivo evaluation consists of infarct induction, material epicardial one-point attachment on the left ventricle, further fixation by mounting pericardial adipose tissue and the initially opened pericardial sac, through evaluation of cardiac function by echos (2, 4, and 6 weeks) and histological evaluation of cardiac regeneration (2 and 6 weeks). The in vitro evaluation consists of the compatibility with relevant cardiac cells (cardiac fibroblasts, epicardium-derived cells, and induced cardiomyocyte progenitor cells) as well as the interaction of the material with macrophages, studying the cellular phenotype, secretome, and material degradation/infiltration.
2.2. Compatibility of PEEU Mesh with Cardiac Cells

Compatibility with regeneration/repair-relevant cells present in the myocardium is important for the in vivo applicability of a PEEU mesh. Therefore, adhesion, cell proliferation, and cell death of human cardiac fibroblasts (hCF), epicardial derived cells (EPDC) and iCMP on both coated and uncoated meshes were studied. At the early state (2 h), PEEU+ECM facilitated attachment of iCMP, but not of hCF or EPDC (Figure 3a). hCF, EPDC, and iCMP proliferated on both PEEU and PEEU+ECM under normoxia and ischemia conditions (Figure 3b). A low level of cell death was retained on PEEU and PEEU+ECM for 7 d, as illustrated by lactate dehydrogenase (LDH) release (Figure 3c). Under ischemia, ECM coating reduced iCMP death, but did not significantly affect the death of hCF and EPDC (Figure 3c). Altogether, these results demonstrate high cell compatibility of PEEU mesh for cardiac cells as well as the functionality of ECM for initial cell adhesion and iCMP survival.

2.3. Interaction between Macrophages and PEEU Mesh

Macrophage-material interactions were evaluated in vitro by culturing macrophages directly on PEEU meshes and ECM coated meshes. Macrophages remained in spherical shape without flattening at 12 h after seeding (Figure 4a,e). It was shown that during three weeks of hydrolytic degradation in medium, both PEEU and PEEU+ECM have similar degradation behavior in terms of change of mechanical properties and morphology (Figures S2 and S3a, Supporting Table 1). Mechanical properties and morphology of PEEU and PEEU+ECM meshes. Mechanical properties were determined by tensile tests of the swollen material at 37 °C.

| Mesh type       | $E$ [MPa] $b)$ | $\sigma_{\text{max}}$ [MPa] $b)$ | $\varepsilon_{\text{b)}$ [%] | $\varnothing$ [µm] $c)$ | Porosity [%] | Thickness [µm] |
|-----------------|----------------|-------------------------------|-----------------------------|-------------------------|--------------|---------------|
| PEEU            | 4.7 ± 0.2      | 4.4 ± 0.5                     | 475 ± 50                    | 1.7 ± 0.4               | 67 ± 5       | 49.2 ± 2.4    |
| PEEU + ECM      | 5.6 ± 0.5      | 4.6 ± 0.6                     | 425 ± 40                    | n.a.                    | n.a.         | 51.3 ± 5.3    |

$E =$ Young's modulus; $^a$ local Young's modulus determined by AFM indentation experiment in PBS at 37 °C; $^b$ Tensile strength; $^c$ Elongation at break; $^d$ Fiber diameter. n.a.: not applicable.

Figure 2. Material characterization. a–d) Scanning electron microscopy (SEM) images of electrospun meshes. a) PEEU and b–d) PEEU + ECM, different points of view. b) Top view, c,d) cross-section, d) dashed area indicates the dehydrated ECM layer, around 3 µm. Scale bar: a,b,d) 10 µm; c) 50 µm. e) Illustration of the SEM view points on PEEU + ECM samples in relation to (b)–(d). Gray area: cross-section from cutting, yellow area: ECM coating layer. f) Tensile tests performed at 37 °C in a water tank of the PEEU mesh (black), PEEU + ECM (red), and a replicate of the PEEU mesh before (blue) and after (green) ethylene oxide sterilization. g) FT-IR spectra of LDI (black) and the PEEU mesh (red).
Figure 3. Compatibility of PEEU mesh and ECM coated mesh with various cardiac cells under normoxia and ischemia conditions. a) Quantitative analysis of initial cardiac cell adhesion for 2 h after cell seeding. b) Proliferation of hCF, EPDC, and iCMP cells on the PEEU and PEEU + ECM meshes over 7 d. c) Cell death of cardiac cells through analysis of LDH release during 7 d of culture on PEEU and PEEU + ECM meshes. n ≥ 3. All data are mean ± SD.

*P < 0.05 **P < 0.01 *** P < 0.001 ****P < 0.0001.
Figure 4. Penetration and polarization of macrophages on PEEU and PEEU + ECM meshes. Immunostaining of CD68 (red) and nucleus (blue) on macrophages on PEEU mesh a) at 12 h and b) at 48 h, and d) staining of CD86 (red) and CD206 (green) on day 7. b,d) Top view showing the cluster of cells on a PEEU mesh and the side view showing the depth of the cavity formed by macrophages. White dash line indicating the top level with observable fiber structure, the yellow dash line indicating the cross section of the side view on the right. c) Macrophages on an PEEU + ECM mesh at 48 h. SEM images of macrophages on a PEEU mesh e) at 12 h, f-i) at 48 h, and j) on day 7. g,h) Zoom in of boxes in (f) to show the (g) macrophage wrapping around the degraded fiber structure and the (h) degradation of PEEU fibers by macrophages. k) The representative SEM image shows a cavity with diameter of approx. 50 μm left by macrophages. l) Macrophages on PEEU + ECM mesh on day 7. m) Heatmap of cytokine levels from macrophages on normal culture plate (TCP) versus PEEU and PEEU + ECM meshes with normalization to the actual cell number by the amount of DNA. n) Quantification of pixel density (shown in arbitrary units) of the levels of cytokines in fold change compared to normal culture on well plate is also shown. The array assay contains duplicates.
results indicated the regulatory effect of mesh composition level (Figure S6 and Table S3, Supporting Information). These dramatically increased F-actin expression but not the peroxide with different fiber diameters and pore sizes. ECM coating the F-actin level and peroxide secretion. High expression enhanced M2 polarization on PEEU meshes, we examined whereas degradation of PEEU + ECM was characterized by a relatively homogeneous thinning of the ECM layer (Figure S3b lower panel, Supporting Information). In this way, macrophages penetrated deeply into the material within 48 h (Figure 4b,f) forming colonies (Figure 4i). The generated cavity in the PEEU mesh could reach a depth of 20 μm (Figure 4b,d). The erosive pit became deeper, larger and softer with extended degradation time (Figure 4d,j,k, Figure S3b upper panel and Figure S4, Supporting Information). During degradation, macrophages wrapped around the fibers (Figure 4g) and the observed presence of macropores was always colocalized by (Figure 4f,g) or neighbored structural defects (Figure 4h). Given the high production of reactive oxygen species from macrophages, we further studied the oxidative degradation of PEEU and its PPDO composition. The erosion and cleavage of both PEEU and PPDO fiber meshes was observed within 24 h, whereas the ECM on PEEU showed no observable changes (Figure S5, Supporting Information). Therefore, our results demonstrate different degradation behaviors of ECM and PEEU in response to the surrounding microenvironments.

Macrophage polarization was assessed by examining M1 and M2 marker expression. Without cytokine treatment, the macrophages on PEEU mesh expressed M2 markers (CD206, anti-inflammatory surface marker, green) much stronger than M1 markers (CD86, proinflammatory surface marker, red) (Figure 4d), indicating the successful transition of M1 macrophages into an M2 phenotype on the PEEU mesh. In contrast, macrophages remained in cubical shape on the ECM and PEEU mesh. Seeding of iCMP onto PEEU in response to the surrounding microenvironments.

Cardiac function was assessed at 2-week intervals by echocardiography up to six weeks after implantation of the mesh. The left ventricular area of PEEU mesh treated hearts was much smaller than that of PBS control hearts (27% larger than PEEU hearts), or PEEU + ECM and PEEU + ECM + iCMP hearts (10% and 5% larger than PEEU mesh treated hearts, respectively), which were significantly dilated, compromising cardiac pump function (Figure 6a). Similarly, the ejection fraction of PEEU mesh implanted mice (wk2: 38.3%; wk4: 34.0%; wk6: 36.8%) was significantly increased compared to PBS control mice (wk2: 16.2%; wk4: 16.9%; wk6: 19.9%) throughout the follow-up period (Figure 6b). Interestingly, the ejection fraction of the PEEU mesh group is also larger than the PEEU + ECM group at week 2 and week 6 (wk2: 21.6%; wk4: 20.6%; wk6: 20.9%), indicating that ECM coating compromised the positive effect of the PEEU mesh. Seeding of iCMP onto PEEU + ECM mesh resulted in an increase in ejection fraction in week 2 and week 4 (wk2: 33.5%; wk4: 33.9%; wk6: 31.3%); however, this increase was still lower than in the PEEU mesh group. There was no synergistic effect detected between the iCMP and the PEEU + ECM mesh. Less pronounced effects were seen for the cardiac output, where PEEU mesh treated mice had significant higher values than PEEU + ECM mesh treated mice at week 6 (16.6 and 9.6 mL min⁻¹, respectively), reaching similar levels as normal mice (Figure 6c). Furthermore, left ventricular end diastolic (LVEDV) and -end systolic volumes (LVESV) showed significant improvements upon treatment with the PEEU mesh. LVEDV of PEEU mice was decreased compared to PBS mice at week 2 (73.3 μL vs 127.8 μL) and week 4 (81.6 μL vs 141.1 μL), while LVESV was decreased up to week 6 (PEEU vs PBS: wk2 48.0 μL vs 109.1 μL; wk4 26.7 μL vs 120.1 μL; wk6 65.5 μL vs...
2.6. PEEU Mesh Promotes Angiogenesis and Arteriogenesis in the Border Zone

The capillary and arteriolar densities were measured after 6 weeks in the border zone of the infarction for all groups. A significant increased capillary density was observed in PEEU mesh treated hearts compared to hearts of other groups (Figure 7a). This observation was corroborated by the quantitative assessment, which showed a 50% increase in comparison to PBS, while this increase was not seen with the other groups (Figure 7b). PEEU mesh implantation also resulted in a significantly increased number of arterioles in the peri-infarction area compared to PBS (3.4 fold increase) (Figure 7c). Therefore, epicardial application of the PEEU mesh alone is sufficient to induce angiogenesis and subsequently increase arteriolar vessel density.

2.7. Macrophage Infiltration in the Infarct and Border Zone is Increased with PEEU Mesh

Given the in vitro upregulation of macrophage chemoattracants on PEEU meshes, we next examined cardiac macrophages at the site of infarction 14 d post-MI (Figure 8a). There was a noticeable increase in macrophages in both the infarct zone and the border zone in mesh treated animals (Figure 8a). Indeed, quantitative analysis showed a significant increase of macrophages in the border zone of the mesh treated animals compared to the other groups (macrophages per field: PEEU mesh = 18, PBS = 6, PEEU + ECM mesh = 5, 120.7 µL) (Figure 6d,e). Taken together, these results show that the PEEU mesh promotes left ventricular function and attenuates undesired cardiac remodeling.
PEEU + ECM + iCMP = 6) (Figure 8b). A similar effect was seen in the infarct zone, where macrophage number in the mesh group was significantly higher than in the PBS group (macrophages per field: PEEU mesh = 41, PBS = 16, PEEU + ECM mesh = 32, PEEU + ECM + iCMP = 26) (Figure 8c). Further, there was a strong enrichment of T_{reg} (Figure S7a, Supporting Information) and eosinophil cells (Figure S7b, Supporting Information), particularly in the infarct zone, the region where M2 macrophages (CD206^+ Ly6c-low) were recruited to (Figure S7c, Supporting Information). Altogether, myocardium regeneration and promoted left ventricular function induced by PEEU meshes could have arisen from the recruitment of anti-inflammatory and proreparative T_{reg} cells, eosinophils and M2 macrophages.

3. Discussion
We designed, fabricated, and characterized an electrospun PEEU mesh and demonstrated that its application to the epicardial surface of the infarcted mouse heart preserves left ventricular function and limits pathologic remodeling processes. In clinical translation, simplicity is a virtue, and thus our strategy...
has several advantages over the injection of cells or bioactive compounds into the heart: the mesh modulates postinfarct inflammation processes, provides mechanical reinforcement to the left ventricular wall, and can be produced on industry scale for immediate off-the-shelf availability. By designing a system whose bioinstructivity is based on physical principles such as elasticity on the nano/microscale and 3D microenvironment (here characterized by pore sizes), a medical device approach is followed, which simplifies clinical translation as compared to ATMPs of pharmaceutical approaches. An electrospun mesh was chosen because its fibers are similar to the dimensions of cells and their 3D arrangement offers cavities (pores), which allow cells to migrate into the mesh, but also adhere to more than one fiber and in this way recognize the spatial 3D arrangement. The selection of the material system was based on earlier studies, in which various PEEU meshes based on PCL and PPDO segments showed excellent compatibility with several cell types. A PEEU with 70:30 PPDO:PCL ratio and an L-lysine diisocyanate ethyl ester derived diurethane junction was chosen because it promotes tube formation of endothelial cells and has suitable mechanical properties and degradation rates.

In the first step, the polymer synthesis procedure was improved compared to previous protocols, including repeated process controls by GPC and FT-IR spectroscopy in order to ensure a high molar mass. Also, 1,8-octandiol was added in the final reaction step to convert residual isocyanate groups that may otherwise impair histocompatibility. In this way, a medical-grade polymer was generated that is free from potentially toxic compounds and also did not show any cytotoxicity in corresponding assays. Polymer synthesis could be performed on a 100 g scale, sufficient for producing more than 750 meshes of clinically applicable size (~5 × 5 cm²). The electrospinning process for the material has already been established and the meshes can be stored for more than one year at −20 °C without

![Figure 7](image-url)

**Figure 7.** PEEU mesh stimulates angiogenesis and arteriogenesis in the border zone of the infarction after 6 weeks. a) Representative fluorescence images of capillaries stained for CD31 (green) and arterioles stained for αSMA (red) in the infarct border zone. Nuclei are stained with DAPI (Blue). Scale bar = 50 μm. Quantification of b) capillary density and c) arteriolar density in the border zone of the infarction. Density of both capillaries and arterioles was determined in five random fields in the border zone per mouse. PBS n = 7, PEEU mesh n = 11, PEEU + ECM mesh n = 14, PEEU + ECM + iCMP n = 8. All data are mean ± standard error of mean. ** P < 0.01 **** P < 0.0001.
changes to mesh structure or polymer properties. Ethylene oxide sterilization did not considerably change fiber diameter, pore size, or mechanical properties of the mesh, which is particularly relevant for an easily hydrolysable polymer such as PEEU.

The pore size of polymer systems has a strong influence on cellular processes\cite{29} including macrophage polarization.\cite{30} Although the optimal pore size for a specific application is still unknown, smaller pore sizes (<50 µm) are believed to support tissue repair processes\cite{7a,31} by favoring M2 over M1 polarization of macrophages. The pore size of our PEEU mesh is within this range (≈3 µm). While the size of macrophages is species- and organ-dependent,\cite{32} with human macrophages having a diameter of ≈21 µm, and rodent macrophages being smaller (≈13 µm),\cite{33} macrophages can traverse openings as small as 1 µm.\cite{34} Macrophage-mediated material degradation increases in situ pore size, so that M2 polarization is facilitated as soon as the initial inflammatory response has been abated.

The mechanical properties of the mesh are relevant for surgical handling, for maintaining material integrity over time in a moving environment, and for guiding cell function.\cite{8} The macroscale properties of the PEEU and PEEU + ECM meshes are similar, and with a Young's modulus \( E \) in the low MPa region and an elongation at break >400% suitable for application on the heart. The higher \( E \) of the PEEU + ECM samples measured in tensile tests can be explained by the crosslinking hydrogel layer in the pores of the mesh, providing additional elastic resistance. Establishing defined material property-cellular function relationships is difficult, and comparison with previously published data is limited by the differences in experimental setups. The local elasticity of the PEEU + ECM mesh, however, is within a range that should not induce proinflammatory cytokine expression/secretion\cite{35} which would be expected on substrates with higher Young's moduli. Individual cells on the PEEU mesh are likely to probe the properties of individual fibers that have much higher moduli than the hydrogel.

The adhesion of different types of cardiac cells on PEEU meshes was evaluated to address two questions: i) Can PEEU meshes support early attachment of cardiac-derived cells and ii) is the cell attachment cell type-specific? (Figure 3). Synthetic...
polymers generally do not offer cell adhesion sites. Hence, surface functionalization of polymers with ECM or ECM components has been shown to support cellular function,\cite{39} and cellular behavior is guided by tissue-specific ECM components.\cite{40,41} Therefore, we hypothesized that coating a PEEU mesh with processed myocardial ECM provides additional cues that support postinfarct reverse remodeling or “regeneration” processes, though such interaction may also be mediated via proteins adsorbed from cell culture medium or blood. The coating of the mesh was achieved by adding a solution of the myocardial ECM to the mesh surface followed by nonhumid incubation to achieve a thin layer of ECM. An alternative design to incorporate specific adhesion sites in electrospun polymeric patches would be incorporating adhesion epitopes (i.e., arginyl glycy1 aspartic acid) in individual fibers.\cite{42} The behavior of cardiac fibroblasts (CF) and epicardium-derived (progenitor) cells (EPDC), key components of the epicardium that come in direct contact with the mesh, were studied. In addition, induced cardiomyocyte progenitor cells (iCMP, generated by transcription factor-guided direct reprogramming of cardiac fibroblasts followed by mRNA-expression-dependent enrichment using synthetic hairpin DNA oligonucleotide molecular beacons) were investigated with the idea to enhance the local pool of cells that contribute to the postinfarct reconstitution of contractile tissue (Figure 1b).

We found that the PEEU mesh especially supports the proliferation of EPDC (Figure 3), and these results were confirmed by in vivo experiments. It may be assumed that the mesh activates the epicardium locally, recruiting more macrophages by stimulating paracrine effects (Figure 5). The epicardium is crucial for heart development and possibly repair. During early embryo development, macrophages are dependent on the epicardium for their recruitment to the fetal heart as residential cardiac macrophages.\cite{43} Genetic ablation of epicardial cells results in diminished myocardial macrophages and impaired recruitment of sub-epicardial macrophages.\cite{44} Reactivation of the epicardium after the injury is critical for macrophage infiltration, modulation of the inflammatory response, and recruitment of cardiac progenitor cells. In acute myocardial infarction alone, the mammalian epicardium is only activated to a limited extent, which is not sufficient to support myocardial repair.\cite{45}

In contrast, the PEEU mesh provided a suitable microenvironment for macrophages. Macrophages adhered, spread, and were capable of penetrating the mesh and initiate macrophage-driven material degradation. Macrophages were observed predominantly on junction zones of several fibers and/or attached to several fibers, so that they were within a 3D environment also in cell culture. After 1 week, macrophages were predominantly found close to the PEEU degradation sites, where fibers were broken down, and the newly formed cavities (~50 μm) correspond to a pore size associated with polarization to the M2 rather than the M1 phenotype.\cite{46,47} The defect in morphology differed from that seen in hydrolytic degradation,\cite{48} with larger irregular erosive pits (Figure 4i) instead of the cracks, suggesting the occurrence of oxidative degradation, whereas lamellae or clear cuts are typical for hydrolytic degradation.\cite{49}

In PEEU + ECM meshes, however, macrophages remained on the hydrogel surface and did not penetrate into the mesh (Figure 4). Even though the ECM coating may promote cell attachment, cellular activity obviously did not result in detectable matrix degradation during the same fiber exposure time so that cell penetration did not occur. We conclude that the pure, uncoated PEEU mesh effectively activates the epicardium, recruits macrophages, and stimulates macrophage cytokine secretion. The PEEU mesh was functionally integrated into the host left ventricular free wall 6 weeks after implantation (Figure 5) and markedly improved neangiogenesis and arteriogenesis in the infarct border zone (Figure 7). In vivo, macrophages were mainly detected in the peri-implantation area of the PEEU mesh but not directly in the mesh. By contrast, in vitro cell culture tests, macrophages were found within the mesh (Figure 4). This difference in macrophage localization may stem from the different points in time: 2 weeks in vivo versus 48 h in vitro. We recognized that PEEU also triggered the engagement of other immune cells (Figure S7, Supporting Information), which was in line with previously published literature. It was reported that PEEU also triggered the engagement of other immune cells (Figure S7, Supporting Information), which was in line with previously published literature. It was reported that PEEU also triggered the engagement of other immune cells (Figure S7, Supporting Information), which was in line with previously published literature. It was reported that PEEU also triggered the engagement of other immune cells (Figure S7, Supporting Information), which was in line with previously published literature.
Our cardiac patch without biological active substance may provide a straightforward and off-the-shelf solution to satisfy the regulatory requirement of medical device for clinical translation, with the ease in upscaling, processing, sterilization and quality control.

4. Conclusion

We designed a multifunctional PEEU fiber mesh for "cardiac regeneration" and established a fabrication scheme paying attention to medical grade production quality as well as to sufficient capacity by upscaling. This bioinstructive mesh system, with tailored micromechanical properties and fiber arrangement, facilitates epicardial-derived cell adhesion and macrophage infiltration as well as cardiac tissue engraftment. In addition, it supports the M1/M2 transition of endogenous macrophages, and the release of proangiogenic factors, improved neovascularization, and contractile function. Neither the addition of cardiac extracellular matrix nor of induced cardiomyocyte progenitor cells further enhances the cardioprotective effect of the mesh. This cell- and factor-free system for treatment of postischemic myocardial dysfunction greatly simplifies manufacturing as well as regulatory processes and warrants further translational advancement toward a first-in-human clinical study.

5. Experimental Section

Materials: Poly(ε-caprolactone) (PCL, M_0 = 3.6 kg mol⁻¹) was purchased from Solvay (Hannover, Germany), hexafluorisopropionol (HFIP) from Abcr GmbH (Karlsruhe, Germany), and porcine pepsin, glutaraldehyde, dibutyltin dilaurate (DBSnDL), 1,8-octanediol (≥ 98 Vol%), poly(paraoxanone) (PPDO, M, = 60–100 kg mol⁻¹), ethanol, hexamethyl disilazane (HMDS), Dulbecco’s modified Eagle’s medium (DMEM)-high glucose, 10 wt% v/v fetal bovine serum, and mounting medium were added. The reaction was ended after 5 d when a number average molar mass of 50 kg mol⁻¹ (standard calibration) was reached by adding further 150 mL DMF and 0.3 g 1,8-octanediol under stirring at 250 rpm for one hour. The polymer was precipitated by pouring the solution into liquid nitrogen and drying the polymer till constant weight at room temperature and about 100 mbar. The PEEU had a PPD0 to PCL weight ratio of 70:30, and an Mₑ(pPDC parm(T)=11) = 27.1 kg mol⁻¹ with a PDI = 3.2.

The electrosprinning setup (Linari Engineering, Pisa, Italy) included a voltage supply, a moving rotatory drum collector, and a syringe pump. A customized transparent plastic chamber with air source connected via tubing for humidity control was used for the electrosprinning process. 11 wt% PEEU was dissolved in HFIP and filtered with 1 μm pore size glass fiber filter into a syringe. The syringe was connected to a 19-gauge blunt tip needle. During the electrosprinning, the PEEU solution was supplied with a flow rate of 2.121 mL h⁻¹ at a distance of 25 cm from the rotary drum collector under a relative humidity of 20% at room temperature at a voltage of 10–15 kV. The drum collector with a polypropylene wrap was rotating at a speed of 5 rpm for a duration of 4 h electrosprinning. For PEEU meshes with fiber diameters 1.2 and 2.0 μm, the flow rates were set to 1.535 and 2.687 mL h⁻¹, respectively. For the PCL mesh, 15 wt% PCL was dissolved in chloroform/ethanol (7:3 by volume) and filtered with 1 μm pore size glass fiber filter into a syringe. The PCL solution was supplied with a flow rate of 1.06 mL h⁻¹ at a distance of 30 cm from the rotary drum collector at a voltage of 20 kV. The drum collector with a polypropylene wrap was rotating at a speed of 5 rpm for 3 h. PEEU films were prepared by compression molding. 0.6 g of PEEU was placed in between two metal plates with polypropylene films. The plates were compressed at 5 bar and heated up to 100 °C for 15 min. Then pressure was applied to 200 bar at 100 °C for 15 min followed by cooling to 25 °C at 200 bar. The PEEU and PCL fiber meshes and PEEU films were sterilized with ethylene oxide at 45 °C for 3 h at 1.7 bar before ECM coating and/or use for cell culture.

Coating of PEEU Mesh with Human Cardiac ECM: Human cardiac ECM was obtained as previously described.[48] Human tissue collection was approved by the Institutional Review Board and ethics committee of Charité-Universitätsmedizin Berlin (EA4/028/12). Briefly, the lyophilized human cardiac ECM particles were dissolved in the porcine peptic solution (1 mg mL⁻¹ in 0.01 M HCl, pH 2.0) to a concentration of 10 mg mL⁻¹, followed by homogenization for 48 h at 27 °C at 1200 rpm in a shaker. The mixture was brought to ice. 0.1 m precooled NaOH was then added to the ECM solution in a volume ratio of 1:10. The ECM solution was further diluted in PBS to a final concentration of 8 mg mL⁻¹. The ECM solution was stored at 4 °C and used for coating within 24 h. The mesh was first cut to fit the size of the culture dish. An autoclavable silicone ring was used to fix the mesh onto the dish bottom. ECM solution was added to cover the mesh with 150 µL. The mesh was then transferred to the sample holder and placed into the SEM. The fiber mesh porosity was calculated by the scaffold dimension from measurement and the actual volume from measured sample mass and the material density, which is 1.044 g cm⁻³. SEM of samples from cell culture were taken from PEEU mesh and mesh with ECM coating after fixation with 4 wt% PFA for 10 min at room temperature. The samples were further fixed with 2.5 wt% glutaraldehyde for 30 min at room temperature and washed three times in PBS for 5 min. The samples were subsequently dried by incubation in ethanol with increasing
concentration, starting from 30 Vol%, then 50, 70, and 90 Vol%, each for 5 min, by up to 100 Vol%, two times for 5 min each, followed by incubation in HMDS, two times for 10 min each, and finally air-drying, overnight in a chemical fume hood. The dried samples were placed on the sample stub with conducting tape and incubated in vacuum overnight followed with 5 nm gold coating in a sputter coater.

**Tensile Test:** A tensile test machine with water tank (Zwick GmbH, Ulm, Germany) was used to determine the mechanical properties of PEEU mesh and mesh with ECM coating at 37 °C in the hydrated state. Four randomly selected samples per group were measured. The samples were cut into 20 × 20 × 1 mm³ stripes, measured for thickness with a thickness gauge, clamped on the grip, and hydrated in water at 37 °C for 10 min before the measurement. The measurements were performed at a constant speed of 5 mm min⁻¹ until the sample underwent elongation of break. The elastic modulus was calculated from the slope at linear elastic deformation by Hooke’s Law.

**AFM:** The surface elastic modulus of different meshes were measured on an MFP-3D (Asylum Research, Santa Barbara, CA, USA). A silicon cantilever (SD-Sphere-FM-M, from NANOSENSORS, Switzerland), having a driving frequency of around 75 kHz and a spring constant of 4.1 N m⁻¹ was utilized for force mapping. The force set point was set to 100 nN and randomly measured five to ten positions with 10 × 10 or 20 × 20 points at each position. The reduced elastic modulus was calculated from the force–distance curves according to the Johnson-Kendall-Roberts model using the data processing software Igor Pro 6.38 (WaveMetrics, Inc., Portland, OR, USA).

**GPC:** Weight average molecular weights of PEEU raw material and mesh were determined on a multidetector GPC Security 2 1260 Infinity system (Polymer Standards Service GmbH, Mainz, Germany). The concentration of PEEU in chloroform was 0.2 wt%. GPC measurements were performed at a solvent flow rate of 1 mL min⁻¹ at 35 °C using chloroform as eluent and 0.2 wt% toluene as internal standard. The system was equipped with three SDV gel columns (50 mm × 8 mm, two 300 mm × 8 mm), an isocratic pump, an automatic injector, an refractive index detector, a UV detector, and a viscosity detector. The size exclusion chromatography software WINGPC UniChrom V8.33 Build 9050 (Polymer Standards Service GmbH, Mainz, Germany) was used to determine the molecular weight distributions by universal calibration with polystyrene standards from 1.82 to 975 kDa (Polymer Standards Service GmbH, Mainz, Germany). The error of the measurement method was estimated as 10% based on measurements of polystyrene standards.

**Contact Angle Measurements:** Dynamic contact angle (DCA) measurements were conducted in ultrapure deionized water provided by an Ultra Clear UV clean water system (EC Water purification, Regenerierung) with a conductivity of 0.055 µS cm⁻¹ at ambient temperature on a drop shape analyzer (DSA 100, Krüss GmbH, Hamburg, Germany) using captive bubble method. The contact area between the surface and the bubble was increased with each measurement cycle from 2 to 5 mm in diameter. Advancing and receding contact angle measurements were performed by stepwise withdrawing/adding of air from/to the captured bubble. Prior to the DCA measurement, all samples were preconditioned for 24 h in ultrapure deionized water at ambient temperature for equilibration. At least five measurements for advancing and receding angle on three different locations were performed for each sample. The median ± standard deviation (SD) is stated.

**Oxidative Degradation of PDDO, PEEU and PEEU + ECM Meshe:-** PEEU and PEEU + ECM meshes were prepared as above. The PDDO mesh was prepared with the same electrosprinning setup. PDDO (11 wt%) in HFIP was electrosprun at a flow rate of 1.909 mL h⁻¹ at a distance of 25 cm from the rotatory drum collector under a relative humidity of 20% at room temperature at a voltage of 5–11 kV. The drum collector was rotating at a speed of 5 rpm for a duration of 4 h of electrosprinning. Fentons reagent (5 wt% H₂O₂/FeSO₄ 147 µM, pH 5) was used. The fiber samples (1 cm × 4 cm) were placed in polypolyylene Falcon tubes with screw cap followed with the addition of 5 mL Fentons reagent. The tubes containing the samples and the Fentons reagents were placed in a shaker (150 rpm) at 37 °C for 24 and 48 h. The samples were collected and followed with a washing procedure of potassium iodide (1 × 10⁻³ M) solution washing (5 mL for 5 min) and water washing (5 mL for 5 min, two times). The washing procedure underwent two times and the samples were dried on PP film at room temperature overnight before further characterizations.

**Culture of Macrophages on PEEU Mesh:** The macrophage J774A.1 cell lines (purchased from MERCK KGaA, Germany, Cat. No. 91051511-TLV) was cultured according to the recommended protocols in T75 flask with DMEM-high glucose with 10 Vol% fetal bovine serum. The PEEU mesh was cut into circular shape with a diameter of 10 mm using a biopsy punch. PEEU meshes were transferred into a 48 well plate and held at bottom by inserting an autoclaved silicon ring cut from a silicon tubing forming a well similar size to a 96 well plate. Macrophage cells were then seeded at 2 × 10⁴ cm⁻² in 200 µL of medium.

**Degradation of PEEU and PEEU + ECM Meshe-:** PEEU and PEEU + ECM meshes were cut into 1 cm × 4 cm specimens and underwent ethylene oxide sterilization before the degradation experiment. Specimens were then transferred from the sterilization bag to petri dishes (60 mm in diameter, 21.5 cm²; MERCK KGaA, Germany). 5 mL culture medium with and without J774A.1 macrophela Bartha (USA cm⁻²) was added to each dish. After 16 h culture in the incubator at 37 °C with 5 Vol% CO₂, specimens with and without cells were transferred into new petri dishes and cultured for 3 weeks. The medium was changed every other day. At designated points in time (week 0, 1, 2, and 3), all specimens with and without cells were treated with nonenzymatic macrophage detachment solution (PromoCell GmbH, Germany) and then washed with deionized water three times. Cells were dissociated and underwent osmotic lysis during this process. Specimens were air-dried for tensile test and SEM analysis.

**Peroxide Production from Macrophages:** Conditioned medium from J774A.1 macrophages growing on different materials was collected in 15 mL conical tubes and then centrifugated at 1000 × g for 10 min at 4 °C to remove the cell pellets. Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Thermo Fisher Scientific, Germany) was used to determine the hydrogen peroxide level. In brief, 50 µL conditioned medium from each sample was loaded into the 96-well flat bottom black microplate (MERCK KGaA, Germany). The Amplex Red working solution was freshly prepared by mixing 50 µL of 10 × 10⁻³ M Amplex Red stock solution, 100 µL of 10 U mL⁻¹ horseradish peroxidase stock solution and 4.85 µL reaction buffer. 50 µL Amplex Red working solution was immediately added to each well of microplate loaded with the sample and incubated at room temperature for 15 min. The fluorescent intensity of samples was measured (excitation: 530 nm, emission: 590 nm) using a microplate reader (Infinite 200 Pro, Tecan Group Ltd.). To normalize the peroxide level, macrophage nuclei were stained with NucBlue Live ReadyProbes (Thermo Fisher Scientific, Germany) and the fluorescence images were taken using laser scanning confocal microscopy (LSM 780, Carl Zeiss, Germany). Cell nuclei were automatically recognized and the cell number was counted by customized OpenCV computer vision software.

**Cytokine Secretion Profile of Macrophages:** Macrophages on PEEU patches were fixed with 4 wt% PFA for 10 min and washed three times with PBS. The samples were incubated in 1 wt% bovine serum albumin (BSA) in PBS for 30 min and then incubated with AlexaFluor 488 conjugated CD206 or primary antibodies CD68 and CD86 (Table S4, Supporting Information) overnight at 4 °C. The samples were rinsed 3 × 5 min in PBS and incubated with secondary antibodies for 1 h followed by a PBS wash. F-actin and cell nuclei were counter-stained with ActinRed 555 and NucBlue Live ReadyProbes, respectively (all from Thermo Fisher Scientific, Germany). The samples were transferred onto a glass slide and covered with mounting medium and a glass coverslip. The fluorescence images were captured using LSM 780 (Carl Zeiss, Germany).
analyzed by using the ImageJ plug-in Protein Array Analyzer (C. Carpentin, 2010). The remained cells on mesh or culture plate underwent freeze-thaw lysis for extraction of DNA with the PureLink Genomic DNA kits. The amount of purified DNA was measured using the NanoQuant Plate together with Infinite M200 PRO (TECAN) at 260 nm using average blanking. The DNA amount was used for normalizing the cytokine secretion.

**Culture of Cardiac Derived Cells:** HCF were isolated and characterized by S. van Linthout, Berlin-Brandenburg Center for Regenerative Therapies, Berlin, following the established protocol.[9] HCF were then cultured in DMEM containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Human EPDC were isolated and cultured as described previously.[10] Briefly, cardiac tissue was isolated by separating the epicardium from the underlying myocardium. The tissue was further cut into small pieces and digested with Trypsin 0.25 wt%/ethylene-diaminetetraacetic acid (EDTA) for a total of 30 min at 37°C. The cell suspension was passed through a 100 µm cell mesh filter (Sigma-Aldrich), collected and plated on 0.1 wt% gelatin-coated dishes (Corning, BioCoat). The established EPDC were cultured in a mixture of 1:1 low glucose DMEM and Medium 199 (M199; Invitrogen) supplemented with 10% fetal bovine serum (Gibco), and 100 U/mL penicillin/streptomycin (Gibco). In order to maintain EPDC in an epithelial state, the transforming growth factor (TGF), TGF-beta receptor inhibitor SB431542 (5–10 µM; Sigma-Aldrich) was added. Induced cardiomyocyte progenitor cells (iCMP) were generated via direct reprogramming of primary neonatal C57BL/6J mouse cardiac fibroblasts (Pelobiotech, cat. no. PB-C57-6049) by transduction with lentiviruses encoding the cardiac transcription factors Gata4, Mef2c, Tbx5, and Myocd. Two weeks after induction, the proliferated cells were sorted for mRNA expression of cardiomyocyte-specific Myh6/7 using molecular beacon technology. All cells were maintained at 37°C and 5% Vol% CO2. Hypoxia treatment was conducted in modular incubator chamber (1 Vol% O2/5 Vol% CO2, Binder CB150 incubator (CB150, Binder, Tuttingen, Germany). Cells were cultured on PEEU or TCP (CELLSTAR) for 24 h before the start of glucose/serum deprivation for 5 h (glucose- and FBS-free DMEM).

**Cardiac Cell Adhesion, Proliferation, and Cytotoxicity:** Cells were labeled with 5 × 10^10 M Calcein following the manufacturer’s instructions and 2 × 10^6 cells/cm² were used. Cells were seeded onto the TCP and PEEU meshes with and without ECM coating and incubated at 37°C with 5% Vol% CO2 for 120 min. Supernatants containing unbound cells were collected. Both unbound cells and attached cells were lysed with 1 wt% Triton-X100 for 15 min. Fluorescence intensity was analyzed using the microplate reader (MithrasLB940, Berthold Technologies). The number of attached cells was calculated using the formula: Attached cells = 100 × (Total number of attached cells) / (Total number of cells seeded number). For cell proliferation analysis, all types of cells growing under various culture conditions were collected at the desired points in time (day 1, 3, and 7) using 0.25 wt% Trypsin/EDTA (5 min at 37°C). Living cell number was then determined by Countess Automated Cell Counter (Thermo Fisher Scientific). Cell death on the materials was determined using the plate reader (MithrasLB940, Berthold Technologies). Supernatants from various culture conditions were collected for LDH measurements according to manufacturer’s instructions. The total cellular LDH for each cell type and different culture conditions was extracted by adding 10 Vol% cell lysis solution for 30 min at desired points in time. The absorbance at 450 nm (reference wavelength: 650 nm) of the samples was measured using a microplate reader (Infinite 200 Pro, Tecxan Ltd.). The LDH level was calculated by the following formula: LDH release (% of total) = (Test sample LDH – medium control) / (total cellular LDH – medium control) × 100.

**Induction of Acute Myocardial Infarction and Patch Implantation:** All animal experiments were approved by the LaGeSo (G028/16) and carried out in accordance with the Guide for the Care and Use of Laboratory Animals. Male C57BL/6J mice (Jackson), 10–12 weeks old, were anesthetized by intraperitoneal injection (i.p.) of 150 µL anesthesia mixture (fentanyl 0.08 mg kg⁻¹, midazolam 8 mg kg⁻¹, medomidin 0.4 mg kg⁻¹) and subsequently intubated. The left anterior descending artery (LAD) was permanently ligated as described previously.[5] In sham-treated animals, all the surgical steps were conducted with the exception of the tightening of the ligature around the LAD. In the control groups, PBS solution (2 injections of 5 µL each) were injected into the border zone of the infarction. For all patch implanted groups, the patch was attached to the knot of the ligation and placed over the infarcted area. The patch was retained in place by the surrounding pericardial sac.[10] Treatments were randomly assigned. The mice were awakened by i.p. injection of an antagonist mixture (100 µL; flumazenil 0.3 mg kg⁻¹, atipamezol 1.7 mg kg⁻¹, buprenorphine 0.1 mg kg⁻¹). Additionally, pain medication (carprofen 3 mg kg⁻¹; 100 µL and 5 wt% glucose (100 µL) for rehydration were injected i.p.

**Echocardiography:** Cardiac function was assessed at week 2, 4, and 6 with the Vevo 2100 system (Fujifilm Visualsonics, Toronto, Canada). Images were analyzed with 4 Vol% isoflurane and positioned onto the echo table. Anesthesia was maintained at 1.5–2 Vol% isoflurane. Body temperature and heart rate were monitored during cardiac function measurements. Long axis and short axis data were collected—B-Mode, M-Mode, and electrocardiogram-gated kilohertz visualization. Analyses were performed with the Vevo LAB software.

**Immunostaining and Histological Analyses:** The hearts were explanted and incubated overnight in 15 wt% sucrose before embedding in OCT (TissueTec). Transversal sections of mouse hearts were prepared from apex until ligation site with 10 µm sections. H&E and iridium staining were performed for infarct size and left ventricular wall thickness.[22] Infarct size was determined as the total red area in the left ventricle per total area in the left ventricle with ImageJ software (v. 1.53e). Wall thickness was determined as the average thickness of five measured points in the left ventricle, in all sections. Furthermore, the hearts were stained for immunofluorescence. Sections were fixed with 4 wt% PFA, washed with PBS and blocked 1 wt% BSA in PBS with 0.3 wt% Triton X-100. Primary antibody was added (Table S3, Supporting Information) in blocking solution (1 wt% BSA in PBS) and incubated overnight. The slides were then washed and incubated with secondary antibody (1:500) in blocking solution for 1–2 h at room temperature. Subsequently, the slides were washed with PBS and mounted with mounting medium with 4’,6-diamidino-2-phenylindole (DAPI). Capillary and arteriolar densities were determined in sections stained for smooth muscle actin (αSMA) and CD31. Images were taken randomly in the infarct border zone (5 per section, at 400×). Macrophages, M2 macrophages, T[H]T[1], B and eosinophil cells were stained with antibodies against F4/80, Ly6C/C2D266, CD4/CD25, and Siglec F (see Table S4, Supporting Information for detailed information).

**Statistical Analysis:** All values are presented as mean ± SD or mean ± standard error of mean. Differences between more than two groups were tested by one-way analysis of variance (ANOVA) with Bonferroni post hoc test for multiple comparisons. For two-group comparisons, a two-tailed Student’s t-test was performed if the normality test was passed. The Mann–Whitney test was performed if non-normality was detected. Changes over time were tested by two-way ANOVA with Bonferroni’s correction. GraphPad Prism v. 9.0.0 was used for data analysis and plotting. Sample size (n) and P-value are specified in the text of the paper or in figure legends.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

bioinstructive materials, cardiac regeneration, function by structure, modulation of in vivo regeneration, multifunctional biomaterials

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