Published in final edited form as:

ASAIO J. 2015 ; 61(1): 61–70. doi:10.1097/MAT.0000000000000158.

Engineering 3D Bio-artificial Heart Muscle: The Acellular Ventricular Extracellular Matrix Model

Nikita M. Patel, Ze-Wei Tao, PhD, Mohamed A. Mohamed, Matt K. Hogan, Laura Gutierrez, PhD, and Ravi K Birla, PhD

Department of Biomedical Engineering, Cullen College of Engineering, University of Houston, Houston, TX, 77204

Abstract

Current therapies in left ventricular systolic dysfunction and end stage heart failure include mechanical assist devices and/or transplant.^[1] The development of a tissue-engineered integrative platform would present a therapeutic option which overcomes the limitations associated with current treatment modalities. This study provides a foundation for the fabrication and preliminary viability of the Acellular Ventricular Extracellular Matrix (AVEM) model. AVEM were fabricated by culturing 4 million rat neonatal cardiac cells around an excised acellular ventricular segment. AVEM generated a maximum spontaneous contractile force of 388.3 μ N and demonstrated a Frank-Starling relationship at varying pretensions. Histological assessment displayed cell cohesion and adhesion within the AVEM as a result of passive cell seeding.

Keywords

Acellular Ventricular Extracellular Matrix; Cardiac Tissue Engineering; Twitch Force; 3D Bio-artificial Heart Muscle

1. Introduction

Left ventricular systolic dysfunction (LVSD) is a primary component of end-stage heart failure (ESHF), which most commonly occurs in conjugation with ischemic heart disease and myocardial infarction. Resultant physiological outcomes include heart wall thinning, ventricular dilation and cardiac remodelling. These outcomes are irreversible due to the nominal reparative capacity within myocardial tissues.^[2]

Current drug therapies aim to maintain cardiac output by reducing the decline in myocardial function. They are often unable to sustain declining ESHF heart function, and are, therefore, coupled or replaced with cardiac assist devices.^[1] Mechanical left ventricular assist devices (LVADs) pump blood from the left ventricle to the aorta.^[1] Biocompatibility presents the predominant limitation in mechanical device therapy applications. Additional limitations include lack of functional interaction and coupling with host tissue, thrombogenicity, risk of

Corresponding Author: Ravi K. Birla, PhD, Associate Professor, Department of Biomedical Engineering, Cullen College of Engineering, University of Houston, 3605 Cullen Blvd, Rm. 2005, Houston, TX, 77204, Phone: 713-743-8861, Fax: 713-743-0226, rkbirla@uh.edu.

infection, device control complexity and long-term durability.^[3] Transplant remains the precedent treatment option for ESHF, however, a shortage of donor hearts and the accompanying need for long term immunosuppression, presents a major limitation.

Cell-based cardiac tissue engineering strategies may provide regenerative therapeutic options of equivalent function to mechanical devices. If these strategies utilized autologous cells and extracellular matrix (ECM), surmounting biocompatibility and biomimetic considerations would be more feasible. This natural alternative encourages functional coupling and interaction with native tissue.

Previous research has focussed on characterizing acellular platform feasibility to bioengineer functional cardiac constructs. Ott et al^[4] utilized native rat acellular cardiac tissue matrices, onto which cardiac cells may be loaded, to promote functional differentiation. Eitan et al^[5] characterized acellular porcine ventricle ECM mechanical properties, and its propensity to functionally couple cardiac cells as measured by protein expression. Potpova et al^[6] and Godier-Furnemont et al^[7] characterized acellular urinary bladder matrices as potential cardiac cell delivery platforms. These cited studies provide platforms to develop cardiac-tissue specific engineered models in the treatment of ESHF.

Hydrogels ^{[8], [9], [10]}, alginates ^{[11], [12]} and collagen gels ^{[13],[14],[15]} have also been reported to be appropriate self-organizing scaffolds with ECM properties. Constraints associated with synthetic ECM platforms emanate around biocompatibility and biomimetic considerations. Use of acellular ECM as a cell delivery platform alleviates these considerations.

We propose the use of a cardiac patch as a treatment method to either replace damaged native tissue or facilitate regeneration in LVSDs and ESHF, as a viable alternative to LVADs and transplant. This study focuses on re-cellularization of Acellular Ventricular Extracellular Matrix (AVEM) constructs to provide a foundational assessment of applicability. The rationale for the use of ventricular ECM as the biomaterial platform is derived from the whole heart study carried out by Ott et al^[4]. Ott et al^[4] demonstrated the retention of collagen structure in the acellularized whole heart, with the preservation of ECM fibre orientation and composition. Ventricular ECM was chosen as the decellularized scaffold material for the AVEM model, to utilize the complex cardiac ECM architecture. The inherent bioactivity and mechanical integrity of the ECM may better promote the viability of the transplanted cells. The suitability of the optimal loading density was determined to be the cell loading quantity at which macroscopic contractions were observable. The rationale for this study develops the whole heart study carried out by Ott et al^[4]. The aim of this study is to provide a model to determine the potential of cell loading acellular ventricular tissue to yield function and cell-scaffold integration.

Rat hearts were detergent decellularized to produce an acellular total organ. Excised ventricles were repopulated with neonatal cardiac cells. Construct functionality and cell distribution of the AVEM was characterized by twitch force measurements and histology. The particulars of the presented concept are novel, with the potential to have a significant

impact in both the field of tissue engineering and to be developed into a therapeutic option in the treatment of LVSDs and ESHF.

2. Materials and Methods

All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication 86-23, 1986). All materials were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

2.1 Decellularization of Rat Hearts

The decellularization protocol for rat hearts has been adapted from previously described methods.^[16] Rat hearts were obtained from 3-6 month old Sprague-Dawley rats. The hearts were washed with phosphate buffer solution (PBS) to remove extraneous debris and blood. The whole hearts were then detergent decellularized as described in Table 1, over a 14 day period.

2.2 Ventricular Scaffold Excision and Preparation

Acellular ventricular tissue segments were excised (Figure 1). To circumferentially isolate the left, right and septal myocardium, an incision was made along the longitudinal direction of the decellularized heart and continued along the pulmonary axis, symmetrically about the apex.

AVEM constructs were passive-seeded in conical tubes to increase the surface area in contact with the cell solution. Natural cell migration populates the interior of the AVEM constructs. A 50ml conical was trimmed to a volume of 5ml, coated with 2ml of SYLGARD (PDMS, type 184 silicone elastomer) (Dow Chemical Corporation, Midland, MI) and air dried for 2 weeks. The conical was sterilized with 80% ethanol, exposed to UV light for 20 minutes and air dried in a laminar flow hood prior to scaffold transference. Scaffolds were pinned, using four minuten pins of 0.1mm diameter (Fine Science Tools, Foster City, CA), sterilized in 80% ethanol and air dried, as shown in Figure 1B and 2B. Pinned scaffolds were washed three times in sterile PBS to remove residual decellularization detergents and preservatives. Scaffolds were then submerged in 3ml of culture medium, composed of M199 (Life Technologies, Grand Island, NY), with 20% F12k (Life Technologies, Grand Island, NY), 10% foetal bovine serum, 5% horse serum, 1% antibiotic-antimycotic, 40 ng/ml hydrocortisone and 100 ng/ml insulin. Culture medium was renewed at 24hour intervals over a 48hour period, in preparation for cell loading. Throughout the preparatory scaffold media wash step, pinned constructs were incubated at 37°C supplied with 5% CO₂.

2.3 Isolation of Primary Cardiac Myocytes

Cardiac cells were isolated from the hearts of 2-3 day old neonatal Sprague-Dawley rats using an established method.^[17]

2.4 Determining optimal cell load for the acellular ventricular extracellular matrix (AVEM)

Three different concentrations of rat neonatal cardiac cells were passive-seeded onto each scaffold; 2×10^6 , 4×10^6 and 6×10^6 cells. Cells were isolated on two separate occasions within a 24hour period and loaded onto the superior and anterior surface of the acellular scaffolds; half the total population on each surface. The superior surface was cell loaded first and incubated for 24hours. The minuten pins were removed, the scaffold was rotated 180° to expose the anterior surface for secondary cell loading and re-pinned (Figure 2Ai and Bi).

The cellularized constructs were cultured as shown in Figure 2Aii and Bii. A 35mm tissue culture plate was coated with 2ml of SYLGARD. The plate was air dried for 2 weeks and sterilized, with 80% ethanol and exposure to UV light for 20 minutes before use. The cellularized tissue construct was transferred from the conical tube to the tissue plate and pinned at 4 corners using minuten pins. A 3ml volume of culture medium was added and the construct was manually elevated to be suspended on the pins (Figure 2Aii and Bii), to increase the surface area during cell culture. Tissue cultures were maintained in an incubator at 37°C supplied with 5% CO_2 , with culture medium changes every 48hrs.

Constructs were assessed to derive the optimal cell loading quantity of the final AVEM construct. Macroscopic contractile measurements were obtained for 15second intervals and extrapolated to estimate the number of contractions per minute for each cell loaded quantity. To determine porosity and differentiate cell deposition between cell loading quantities, after 4days of culture, constructs were place in peel-a-way disposable embedding molds (VWR International), immersed in Tissue-Tek OCT compound (VWR International) and frozen at -80°C for 24hours. Planar sections of $10\mu\text{m}$ and $20\mu\text{m}$ thickness were cut using a Cryotome (ThermoScientific) and placed onto VWR Microslides for staining with Masson's Trichrome reagents, according to the manufacturer's protocol, to determine pore size and cell deposition, respectively. Masson's Trichrome images were obtained using a light microscope (Olympus, Center Valley, PA). ImageJ was used to analyse pore size.

2.5 Contractile Force Measurement

From days 2-3, at macroscopic contraction observation, twitch force of the constructs was measured using a high sensitivity isometric force transducer (MLT0202, ADInstruments), connected to a quad bridge amplifier (FE224, ADInstruments). Data acquisition was through a 16 channel PowerLab system (PL3516/P, ADInstruments). The force transducer arm was attached to one free corner of the AVEM, while the other three ends were held fixed by minuten pins; spontaneous measurements were recorded for 20-60 seconds. The contractility of AVEM patches was collated over the 14 days culture period, at 4 to 5 day intervals, to map the contractile frequency behaviour of the construct. In order to obtain the Frank-Starling relationship, pretension was adjusted using a micro-manipulator (Radnoti LLC, Monrovia, CA) and measurements of spontaneous contraction were recorded. LabChart was used for data analysis with the peak analysis module, to calculate maximum twitch force and baseline force (pretension).

2.6 Structural, Contractile and ECM Histological Analysis

Fourteen days after plating, AVE M tissues were prepared as per the method outlined in Section 2.4, to produce planar tissue sections of 20µm thickness. Immunohistochemistry was used to compare native rat ventricular tissue, decellularized scaffolds and AVE M, with regards to desmin, troponin I, α-actinin and collagen type I.

Non-specific epitope antigens were blocked with 10% goat serum at room temperature for 1 hour. To show desmin, sections were incubated with mouse anti-desmin, 1:100 (Cardiomyocyte Characterization Kit, EMD Millipore, MA). To show contractile composition, sections were incubated with mouse anti-troponin I, 1:100 (Cardiomyocyte Characterization Kit, EMD Millipore, MA). To show collagen-structure contrast, sections were incubated with mouse anti-α-actinin (Sigma, Catalog No A7811) 1:200 and rabbit anti-collagen type I (Abcam, ab34710) 1:100. All sections were incubated with antibodies, for 1 hour at room temperature. All sections were counterstained for nuclei with 4,6-diamidino-2-phenylindole (DAPI) (2.5 µg/ml) for 5 minutes at room temperature. Fluorescent images were obtained with a Nikon C2+ confocal laser scanning microscope (Nikon Instruments Inc., Melville, NY). ImageJ was used to analyse cell count.

3. Results

3.1 Proof of Decellularization And Determining Optimal AVE M Cell Load

Figure 1B shows the decellularized heart; the tissue is translucent and structural features, such as the coronary vessel, remain visible. Detergent decellularization resulted in a comprehensively acellular cardiac matrix (Figure 3); no nuclei (blue) are evident in the decellularized tissue. Figure 3 shows the retention of tissue structural integrity (yellow collagen Type I) and ECM porosity post-decellularization. Using Masson's Trichrome stained tissue sections, the average pore size of native and decellularized ventricular tissues (n=4 for both), for a 0.37mm² tissue section, were calculated to be 0.0019mm² ± 0.0018mm² (mean ± standard deviation) and 0.1355mm² ± 0.078mm², respectively. The diameter of cardiac cells ranges from 10-35µm^[4] (~80-960µm² area). The calculated ECM pore size indicates that the scaffold would be amenable to cardiac cell migration.

Constructs loaded with different cardiac cell quantities, were assessed for macroscopic contraction and cell count, to derive the optimal cell load for AVE M. Macroscopic contractions were observed across all cell loading ranges (n=2 for each), following a 4 day culture period (Figure 4A; video data not provided). The native rat heart has a contractile rate of 330-480beats per minute (bpm)^[4]. Scaffolds loaded with 2×10⁶ and 4×10⁶ cells, exhibited the highest average contractile rate of 36bpm (culture day 3 of 4). The cell loading density of 4×10⁶ macroscopically demonstrated more synchronous contraction throughout the entire construct and the stronger observable contraction, comparatively. ImageJ was used to calculate cell content per 1.3mm² of tissue, to determine which cell density was appropriate for the final AVE M (n=4 for each) (Figure 4B). The acellular average cell count was 0, to prove the efficacy of the decellularization process. Constructs loaded with 4×10⁶ had the highest average cell count per area, of 66cells ± 23cells. Compared to the native tissue, cell content was significantly lower in the recellularized constructs.

The AVEM is defined as an acellular ventricle scaffold loaded with 4×10^6 cardiac cells, based on the results presented above. It is this defined AVEM which is referred to throughout the remainder of the study.

3.2 Fabrication of acellular ventricular extracellular matrix (AVEM)

Observable contractions occurred within 48-72 hours of cell loading, primarily at the construct perimeter. Subsequently, contractions dissipated throughout the construct during the remaining culture period. Contraction began as localized arrhythmic events, developing to become more pervasive, synchronous and rhythmic.

3.3 Contractile Force Measurement

In total, 23 AVEM constructs were analyzed in this study; 10 (43%) demonstrated measurable contractions. Figure 5 shows representative spontaneous twitch force results for the acellular ventricular ECM (negative control) and for recellularized AVEM, measured 72 hours after cell loading at pretensions ranges of $\sim 800 \mu\text{N}$ to $\sim 2900 \mu\text{N}$, as 5 second excerpts to demonstrate relative base-to-peak amplitude and frequency. Previous studies^[18] have extensively characterized fresh rat ventricular twitch force (42 mN). Figure 6 represents the Frank-Starling relationship, as pre-tension versus twitch force. The force generated by AVEMs ($n=10$) is sub-divided into pretension ranges (Table 2).

3.4 Characterization of AVEM Regenerated Myocardial Tissues

Figure 7 shows histological characterization of AVEM constructs, in comparison to healthy native heart and acellular ventricle scaffold tissues, for a range of cardiac specific factors.

The native ventricle demonstrates the presence of desmin, showing defined sarcomeric striations. Comparatively, the acellular ventricle lacks clear sarcomeric organization shown by desmin. The AVEM shows desmin presence around nuclei with a small degree of sarcomeric organization.

The native ventricle demonstrated high levels of troponin I whereas the acellular ventricle showed a complete absence. The AVEM demonstrated a greater degree of troponin I peripherally, where most contractile activity was observed. Comparatively, the central part of the AVEM was more sparsely cellularized, observed to be less contractile and therefore showed little presence of troponin I.

α -actinin was abundant in the native tissue alongside underlying collagen. Only collagen remained in the acellular tissue. The AVEM images highlight the presence of α -actinin, both centrally and peripherally, in conjugation with cell clusters; coupled with desmin observations, the presence of sarcomeres, and therefore cardiomyocytes is reinforced.

Cells predominantly cluster at the AVEM periphery to form a mostly cohesive layer, with retention of the underlying ECM-Collagen structure. The presence of α -actinin and desmin indicate the preservation of myocyte expression to promote sarcomeric organization. Contractile macroscopic observations are validated by the presence and absence of troponin I at AVEM periphery and centre, respectively.

4. Discussion

The foundational stages of AVEM development have been described within this study. AVEM present a potentially biocompatible and biomimetic LVSD and ESHF treatment method to either replace damaged native tissue or facilitate regeneration. The AVEM presents a dual purpose; as a cell platform for cardiac myocyte delivery and as a 3D bio-artificial support structure for host integration.

Present research exploits a niche in using acellular ventricle ECM. Natural acellularized matrices have been characterized as cell delivery platforms for various organs and species. Ott et al^[4] utilized whole rat acellular heart, loaded with cardiac cells, to promote functional differentiation, as a comparison of native and acellular ECM. Eitan et al^[5] characterized acellular porcine ventricle ECM mechanical properties and its propensity to functionally couple cardiac cells, measured by protein expression. Figure 3 demonstrates the validity of acellular ECM viability as scaffolds, as porosity is retained following decellularization. The average pore size of tissue sections, native (0.0019mm^2) and acellular (0.1355mm^2), compared to the size of the cardiac myocyte ($80\text{-}960\mu\text{m}^2$ area) validates the suitability of acellular ventricular tissue to encourage myocyte infiltration. The differences in pore size between native and acellular tissue are explained by the relative density of cellular material and sarcomeric activity observable in each; native heart includes more structural components such as desmin and α -actinin to augment the underlying collagen structure. Viability of primary cardiac myocytes, and other cardiac cells, within the decellularized ventricle validates its retention of biocompatibility.

The AVEM was defined to be the acellular scaffold loaded with a total of 4×10^6 cardiac cells. This cell quantity was chosen since it demonstrated the highest macroscopic contractile frequency ($36\text{bpm} \pm 2\text{bpm}$, $n=2$, culture day 3) during the optimal cell load studies (Figure 4A) and showed a greater qualitative force and coherence of contraction, comparatively. Additionally, this cell load had the highest cell count per area scaffold ($66\text{cells} \pm 23\text{cells}$ per 1.33mm^2 , $n=4$) compared to the other cell loads. The lower cell count in tissues loaded with 6×10^6 cells may be explained by the short 4 day period allotted to passive-seeding, and the technique of passive-seeding inefficiently encouraging more cell infiltration into the scaffold, despite the use of a higher cell load. However, the cell content remained significantly lower than that of the native tissue (native vs cell load, t-test, $p < 0.05$ for all) (Figure 4B).

Patch contraction was observed 48-72hours after cell loading. The twitch force and Frank-Starling relationship, shown in Figures 5 and 6, are experimentally validated by the acellular ventricle tissue negative control data (Figure 5A). Values for native tissue have been described extensively in previous studies (42nM)^[18,19]. The maximum spontaneous twitch force observed within this study was $388.3\mu\text{N}$. The twitch force of fresh rat ventricular tissue is 42mN ^[18]. The twitch force of mammalian tissue ranges over 25mN to 44mN , and is dependent on contractile frequency^[19]. Contractile frequency plateaued between the pre-tension ranges of ~ 1200 - $\sim 2400\mu\text{N}$. The magnitude of contraction, displayed as the Frank-Starling relationship in Figure 6, was initially exponential in response to an increased pre-tensile force, with a plateau at $\sim 2400\mu\text{N}$. AVEM tissues responded to pre-tensions as they

do in native tissues. In order to simulate these biomechanical outputs, the AVEM would need to be optimised for cell loading and pre-tension.

Figure 7 characterizes the AVEM. Cell permeation and clustering were observed both at the AVEM periphery and centre. Presence of interior cell clusters and the measured porosity of the AVEM validate its conduciveness for cell migration and infiltration. Structurally, desmin and α -actinin staining, shows some observable myocyte sarcomeric organization in the AVEM both centrally and peripherally, clustered around nuclei. Contractions were predominantly observed at the scaffold periphery. This is explained by the presence of troponin I, in greater amounts at the scaffold periphery, in congruence with nuclei clusters. Although cells permeated further towards the centre of the AVEM, no troponin I was seen. Collagen staining shows retention of the underlying acellular scaffold architecture and the relative arrangement of nuclei.

This study provides a foundation to validate the applicability of AVEM, however numerous optimizations are required. Cell retention, cell-matrix infiltration and construct functionality represent the primary optimization variables. For any natural tissue engineered concept, biocompatibility and immune response remain significant variables. Autologous cardiac myocytes or stem cells must be made available to address these considerations. ECM sourcing must also consider these variables despite the acellular nature of the structure. AVEM may overcome the limitations brought about by the complexities of storage and transport facing organ transplantation.

AVEM cell loading was a passive process; a cell solution was loaded onto both the superior and anterior surface of the scaffold, respectively. The cell loading process may be described as passive migration-seeding; diffusion represents the active process of cell migration with a 3D matrix, without bioreactor mediated control. This diffusion process relies on static culture conditions and matrix characteristics. Passive-seeding limits the degree to which myocytes can permeate the scaffolds, thus limiting AVEM twitch force and contractile frequency, due to the diminished number of functional interactions occurring towards the centre of the construct. A more effective cell seeding and dynamic culture protocol must be developed, to ensure cell-matrix infiltration. Cell loading techniques of direct scaffold injection coupled with diffusion loading or fibrin gel cell entrapment may be employed.

AVEM must be characterized to define mechanical and electrical properties, to ensure comprehensive function as regenerative scaffolds for cardiac myocyte delivery. Chronological studies to define time to peak AVEM function in culture and time at which AVEM cells interact with the native heart. This information would serve to define variables to condition the tissue in future bioreactor studies. Following AVEM optimization, clinical implantation presents two options for application. The first is determination of *in vivo* model integration and vascularization capabilities; patch overlay on existing tissue to provide a regenerative cell delivery mechanism. Another application would be as 3D bioengineered heart muscle for complete replacement of non-functional tissue.

This foundation AVEM model has shown feasibility as a unique platform concept in bioengineering 3D artificial heart muscle. There are multiple validation considerations to

further optimize the AVEM model. Primarily, optimization is required on a cellular level, through seeding methodology, with further mechanical and electrical validation by chronological and bioreactor development studies. AVEM has the potential to be a tissue-engineered cell delivery platform for the treatment in LVSD and ESHF.

References

1. Packer M. The impossible task of developing a new treatment for heart failure. *J Card Fail.* 2002; 8:193–196. [PubMed: 12397564]
2. Baig MK, McKenna WJ, Caforio ALP, Bonow RO, F GS. The pathophysiology of advanced heart failure. *Am Heart J.* 1998; 135:S216–S230. [PubMed: 9630087]
3. Jacobsen, GM.; Easter, D, MD, FACS. Allograft vs. Xenograft: Practical Considerations for Biologic Scaffolds. Musculoskeletal Transplant Foundation; 2008.
4. Ott HC, Mattiesen TS, Goh SK, et al. Perfusion-Decellularized Matrix: using nature's platform to engineer a bioartificial heart. *Nature Medicine.* 2008; 14:213–221.
5. Eitan YP, Sarig Uri PhD, Dahan Nitsan PhD, Macluf Marcelle PhD. Acellular Cardiac Extracellular Matrix as a Scaffold for Tissue Engineering: In Vitro Cell Support, Remodeling and Biocompatibility. *Tissue Engineering: Part C.* 2010; 16:671–683.
6. Potpova I, Doronin SV, Kelly DJ, Rosen AB, Schuldt AJT, Lu Z, Kochapura PV, Robinson RB, Rosen MR, Brink PR, Gaudette GR, Cohen IS. Enhanced Recovery of mechanical function in the canine heart by seeding an extracellular matrix patch with mesenchymal stem cells committed to a cardiac lineage. *Am J Physiol Heart Circ Physiol.* 2008; 295:H2257–H2263. [PubMed: 18835924]
7. Godier-Furnemont A, Martens TP, Koeckert MS, Wan L, Parks J, Arai K, Zhang G, Hudson B, Homma S, Vunjak-Novakovic G. Composite scaffold provides a cell delivery platform for cardiovascular repair. *PNAS.* 2011; 108:7974–7979. [PubMed: 21508321]
8. Malchesky PS. Artificial Organs 2005: A Year in Review. *Artif Organs.* 2006; 30:192–207.
9. Vara DS, S H, Kannan RY, Bordenave L, Hamilton G, Seifalian AM. Cardiovascular tissue engineering: state of the art. *Pathol Biol (Paris).* 2005; 53:599–612. [PubMed: 16364812]
10. Soonpaa GK, Klug MG, Field LJ. Formation of nascent intercalated disks between grafted fetal cardiomyocytes and host myocardium. *Science.* 1994; 264:98–101. [PubMed: 8140423]
11. Jen WM, Mikos AG. Review: Hydrogels for cell immobilization. *Biotechnol Bioeng.* 1996; 50:357–64. [PubMed: 18626984]
12. Shapiro L. Novel alginatesponges for cell culture and transplantation. *Biomaterials.* 1997; 18:583–590. [PubMed: 9134157]
13. Eschenhagen T, Remmers U, Scholz H, et al. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system. *FASEB J.* 1997; 11:683–694. [PubMed: 9240969]
14. Zimmermann WH, Schubert P, Didie M, et al. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res.* 2002; 90:223–230. [PubMed: 11834716]
15. Zimmermann WH, Wasmeier G, Didie M, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med.* 2006; 12:452–458. [PubMed: 16582915]
16. Gilbert T, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials.* 2006; 29:3675–83. [PubMed: 16519932]
17. Huang YC, Khait L, B RK. Contractile three-dimensional bioengineered heart muscle for myocardial regeneration. *J Biomed Mater Res A.* 2007; 80:719–31. [PubMed: 17154158]
18. Kiriazis H, G C. Effects of aging on the work output and efficiency of rat papillary muscle. *Cardiovasc Res.* 2000; 48:111–119. [PubMed: 11033113]
19. Mulieri LA, H G, Leavitt B, Allen PD, Alpert NR. Altered myocardial force-frequency relation in human heart failure. *Circulation.* 1992; 85:1743–1750. [PubMed: 1572031]

Fig. 1.

A, Schematic of ventricular scaffold excision from acellular heart construct; **B**, Ventricular scaffold excision from acellular heart construct differentiated by relative physiological position

Fig. 2.

A, i) Surface and anterior cell loading of ventricular scaffold; **ii)** Cell-culture construct with elevated suspended cellularized ventricular construct; **B, i)** Primary and secondary anterior surface cell loading of ventricular scaffold in conical; **ii)** Cell-culture construct process to produce elevated suspended cellularized ventricular construct.

Fig. 3. Representative tri-stained native and acellular ventricle tissues to define Collagen Type I (Yellow), α -actinin sarcomeres (Green) and nuclei (Blue) components at 40 $\times$ objective.

Fig. 4.

Determining optimal cell load of AVEM; **A**, Macroscopic contractile frequency at differential cell loading densities in beats per minute (bpm). Native contractile frequency rate of 330-480bpm^[4]; **B**, Average cell count per 1.3mm² tissue section. Acellular cell count = 0±0 (mean±standard deviation). P-values represented as native vs. cell load; p<0.05 for all.

Fig. 5.

AVEM representative twitch force over 5 second intervals at differential pre-tensions; **A**, Twitch force for negative control acellular ventricular ECM of pre-tension ~1000μN, ~2000μN and ~3000μN; **B**, Twitch force at pre-tension baselines ~800μN, and ~1120μN; **C**, Twitch force at pre-tension baselines ~1300μN, ~1700μN and ~2000μN; **D**, Twitch force at pre-tension baselines ~2400μN and ~2800μN.

Fig. 6. Defined AVEM representative Frank-Starling Length Tension relationship of pre-tension versus twitch force.

Fig. 7.

Representative immunofluorescent AVEM staining at 40× objective for native heart, acellular and AVEM peripheral and central tissue sections; **Column Stains, A**, desmin (green) and DAPI (blue); **B**, troponin I (red) and DAPI (blue); **C**, α-actinin (green), collagen type I (yellow) and DAPI (blue).

Table 1
Decellularization Protocol

Solution/Properties	Components	Schedule
Solution 1: Fixing agent/Detergent	80% Glycerol (by volume) 0.9% NaCl 0.05% NaN ₃ 25 mM EDTA	Day 0-2
Solution 2: Detergent	4.2% sodium deoxycholate 0.05% NaN ₃	Day 2-4
Solution 3: Detergent	1% SDS 0.05% NaN ₃	Day 4-6 Day 8-10
Solution 4: Detergent	0.05% NaN ₃	Day 12-14
Solution 5: Detergent	3% Triton X-100 (by volume) 0.05% NaN ₃	Day 6-8 Day 10-12

Components of Decellularization Key: Glycerol: Solvent; NaCl: Isotonic component; NaN₃: Bacteriostatic; EDTA: Ionic surfactant for celly lysis and membrane/cellular component solubilisation; SDS: Denaturing agent for non-covalent protein bonds to make tissue quasi-transparent ; Triton-X 100: non-ionic surfactant for membrane solubilisation and cell lysis

Table 2
Force generated by AVEM at pre-tensile ranges (total n=10)

n	Pre-tension (μN)	Twitch Force (Mean ± Standard Deviation) (μN)
3	<2500	320.851 ± 108.201
6	2500-5000	431.599 ± 225.756
4	5000-7500	224.892 ± 76.536
4	7500-10,000	92.728 ± 44.562
2	10,000-12,500	152.800 ± 12.869
4	12,5000-15,0000	79.583 ± 5.713
3	>15,000	77.683 ± 10.776