Research article

# *In silico* mechanics and TGF-ß expression of stem cells intramyocardially transplanted with a biomaterial injectate for treatment of myocardial infarction

YD Motchon[1]*, KL Sack[1,2], MS Sirry[1,3], NJ Nchejane[1], T Abdalrahman[1], J Nagawa[1], M Kruger[4], E Pauwels[5], D Van Loo[5,6], A De Muynck[5], L Van Hoorebeke[5], NH Davies[4], T Franz[1,7]*

[1]Biomedical Engineering Research Centre, Division of Biomedical Engineering, Department of Human Biology, University of Cape Town, Observatory, South Africa
[2]Cardiac Rhythm Management, Medtronic Inc, Minnesota, USA
[3]Department of Biomedical Engineering, School of Engineering and Computing, American International University, Al Jahra, Kuwait
[4]Cardiovascular Research Unit, University of Cape Town, Observatory, South Africa
[5]Centre for X-ray Tomography, Department of Physics and Astronomy, Ghent University, Ghent, Belgium
[6]Current affiliation: XRE nv, Bollebergen 2B box 1, 9052 Ghent, Belgium
[7]Bioengineering Science Research Group, Department of Mechanical Engineering, Faculty of Engineering and Physical Sciences, University of Southampton, Southampton, United Kingdom

*Corresponding authors: Dodzi.Motchon@uct.ac.za, Thomas.Franz@uct.ac.za

ORCIDs:

| | |
|---|---|
| YD Motchon: 0000-0002-6222-3483 | E Pauwels: 0000-0002-3296-8011 |
| KL Sack: 0000-0002-8253-1504 | D Van Loo: 0000-0001-6127-1996 |
| MS Sirry: 0000-0003-3735-0963 | A De Muynck: - |
| NJ Nchejane: 0000-0003-3983-7476 | L Van Hoorebeke: 0000-0002-1943-1359 |
| T Abdalrahman: 0000-0002-0320-811X | NH Davies: 0000-0003-0432-4515 |
| J Nagawa: 0000-0002-3712-9647 | T Franz: 0000-0002-1504-3842 |
| M Kruger: - | |

## Abstract

Purpose: Biomaterial and stem cell delivery are promising approaches to treating myocardial infarction. However, the mechanical and biochemical mechanisms underlying the therapeutic benefits require further clarification. This study aimed to assess the deformation and resulting transforming growth factor β (TGF-β) expression of stem cells injected with the biomaterial into the infarcted heart.

Methods: A microstructural finite element model of a mid-wall infarcted myocardial region was developed from *ex vivo* microcomputed tomography data of a rat heart with left ventricular infarct and intramyocardial biomaterial injectate. Nine cells were numerically seeded in the injectate of the microstructural model. The microstructural and a previously developed biventricular finite element model of the same rat heart were used to quantify the deformation of the cells during a cardiac cycle for a biomaterial elastic modulus ($E_{inj}$) ranging between 4.1 and 405,900 kPa. The cellular TGF-β expression was determined with a mathematical relationship of deformation and TGF-β expression developed from existing experimental data and single-cell finite element analysis.

Results: The transplanted cells' deformation and TGF-β expression were largest for $E_{inj}$ = 7.4 kPa, matching that of the cells, and decreased for an increase and decrease in $E_{inj}$. Cell deformation and TGF-β expression were more sensitive to $E_{inj}$ changes for softer ($E_{inj} \leq 738$ kPa) than stiffer biomaterials.

Conclusions: Combining the microstructural and biventricular finite element models enables quantifying micromechanics and signalling of transplanted cells in the heart. The approach offers a broader scope for *in silico* investigations of biomaterial and cell therapies for myocardial infarction and other cardiac pathologies.

**Keywords**: Biomaterial injection therapy; Cell mechanics; Cellular signalling; Cell therapy; Finite element method; Mechanotransduction

## Symbols

| Symbol | Description |
|---|---|
| $\bar{a}$, b | Material parameter for the isotropic response in the myocardium |
| $\bar{a}_{fs}$, $b_{fs}$ | Material parameter for the coupling stiffness in the fibre and sheet directions |
| $\bar{a}_I$, $b_i$ | Material parameter for additional stiffness in the fibre and sheet directions for i = f, s |
| B | Governs the shape of peak isometric tension-sarcomere length relation |
| $C_{10}$ | Material parameter for the isotropic response in the Neo-Hookean model |
| $Ca_0$ | Peak intracellular calcium concentration |
| D | Myocardium material parameter for incompressibility properties |
| $D_1$ | Material parameter for incompressibility properties in the Neo-Hookean models |
| E | Elastic modulus for the isotropic responses in the Neo-Hookean model |
| $ECa_{50}$ | Length-dependent calcium sensitivity |
| **F** | Deformation gradient tensor |
| h | Parameter defining the pathological state of the tissue (healthy or infarcted) |
| $I_{4f}$ | Transversely isotropic invariant in the fibre direction |
| $I_{4s}$ | Transversely isotropic invariant in the sheet direction |
| $I_{8fs}$ | Orthotropic invariant from coupling in fibre and sheet direction |
| $I_i$ | Isotropic invariants in principal directions |

| | |
|---|---|
| $J$ | Third deformation gradient invariant describing the volume change of a compressible material |
| $l$ | Sarcomere length (time-varying model in active contraction) |
| $l_r$ | Initial sarcomere length (time-varying model in active contraction) |
| $p$ | Parameter scaling the isotropic response of the infarcted tissue |
| $\mathbf{T}$ | Stress tensor defining the mechanics of the myocardium |
| $\mathbf{T_a}$ | Active stress tensor describing the myocardium responses during active contraction |
| $T_{max}$ | Constitutive law scaling factor (time-varying model in active contraction) |
| $\mathbf{T_p}$ | Passive stress tensor defining the additional stress from the passive response during the active contraction |
| $t$ | Time |
| $t_r$ | Linear function of sarcomere length |
| $t_0$ | Time to reach peak tension (time-varying model in active contraction) |
| $W$ | Strain energy density function |
| $\varepsilon_{Cell}$ | Cell strain |
| $\varepsilon_{Sub}$ | Substrate strain |
| $\kappa$ | Bulk modulus |
| $\lambda$ | Stretch |
| $\nu$ | Poisson's ratio |
| $\omega$ | Variable in the internal variable function depending on time and sarcomere length |
| $\sigma$ | Stress |

## 1. Introduction

Cardiovascular disease-related deaths are predicted to rise from 17.5 million (i.e. 31% of global deaths) in 2012 to 22.2 million by 2030 [1, 2]. In low- and middle-income countries, such as South Africa, the number of affected people in the working-age population is increasing [3, 4]. Myocardial infarction (MI) causes cardiac cell death in the affected myocardial region, leading to scar formation, ventricular remodelling, and heart failure in the long term. Transplanting stem cells into the myocardium with injectable biomaterials has gained substantial interest in MI therapy research. The delivered stem cells are expected to promote regenerative reactions in the impaired tissue [5] and can differentiate into cardiac-like cells and endothelial cells (EC) necessary for revascularisation and functional recovery of the heart [6, 7].

Pre-clinical studies have demonstrated the beneficial effects of adult stem cell transplantation on MI. For example, Tomita et al. [8] demonstrated that intramyocardial transplantation of bone marrow-derived stem cells in the rat model improved angiogenesis and cardiac function and reported differentiation into cardiac-like cells (*in vitro* and *in vivo*) in the infarcted myocardium. Wang et al. [9] reported an increase in the expression of endothelial-related proteins, improved ejection fraction, reduced infarct size and a high vessel density by using a biochemically and genetically activated hydrogel loaded with adipose-derived stem cells in rats.

Clinical studies also reported the benefits of cell transplantation in MI. These benefits include the differentiation into cardiac-like cells [10-12] and enhanced neovascularisation and angiogenesis with reduced cardiomyocyte death through paracrine or autocrine signalling [13, 14]. Strauer and Steinhoff [15] observed improved cardiac function in patients with MI six months after human mesenchymal stem cell transplantation.

Pre-clinical and clinical studies support the hypothesis that paracrine signalling drives the beneficial effects of cell transplants in MI [8, 9, 13-19]. During cellular communication in cell-based therapies, proteins such as growth factors are released by a cell and attached to membrane receptors of the same or another cell through autocrine and paracrine effects. These processes are essential in the mechanotransduction of the injected cells in the dynamic mechanical environment of the heart.

Cyclic strain has been reported to play an essential role in the cellular production of growth factors and proteins that promote angiogenic phenotypes in bone-marrow-derived mesenchymal stem cells [20, 21]. Zheng et al. [22] found an increase in vascular endothelial growth factor (VEGF) mRNA and its associated protein concentration in cardiac cells exposed to cyclic stretching. They also showed a 2.5-fold increase in transforming growth factor-beta (TGF-β) one hour post-stretching. More importantly, they observed that neutralising TGF-β inhibited the stretch-induced production of VEGF, confirming the mediatory role of TGF-β in VEGF production. The beneficial effects of mechanically stimulated growth factor secretion in the infarcted heart were also demonstrated *in vivo* by several pre-clinical studies [23-25].

The physical environment of cells transplanted in the infarcted heart is essential in cell therapies. A biomaterial as a carrier and scaffold for the transplanted cells constitutes the immediate microenvironment that transmits mechanical stimuli from the myocardium to the cells. The biomaterial delivered into the infarct contributes to the mechanics of the surrounding myocardium and the embedded cells. Hence, the biomaterial offers a vehicle for improving wall mechanics and ventricular function and enhancing paracrine cardioprotective signalling through cellular mechanotransduction.

Several computational studies have investigated biomaterial delivery in the infarcted heart [26-31]. These studies focussed on determining the mechanical effects of the injected biomaterials on myocardial and ventricular mechanics that contribute to inhibiting post-infarction adverse remodelling. Wall et al. [29] first reported a reduction in the wall stress and changes in ventricular function in the presence of an injected biomaterial that depended on the volume and stiffness of the delivered material and the delivery location in a finite element model of an ovine left ventricle. Other studies expanded on the investigation of treatment parameters to guide the optimisation of the injectate therapy using computational models of different species, including ovine [26, 30], canine [28], porcine [32, 33], human [34], and rat [31, 35, 36]. Wenk et al. [27] proposed an automated computational method to optimise injectate patterns for biomaterial delivery to treat heart failure, and Sirry et al. [35] was the first study presenting a microstructurally detailed *in situ* geometry of a hydrogel injectate in an infarcted rat heart. These studies exclusively focused on acellular biomaterial injectates, and there are no computational studies on the mechanobiology of the cells transplanted with the biomaterial into the infarcted heart.

Therefore, this computational study aimed to determine the mechanics and associated signalling of cells transplanted in a carrier biomaterial intramyocardially in a rat heart with a left ventricular infarct and quantify the relationship between the stiffness of the carrier biomaterial and the TGF-β production of the embedded stem cells.

## 2. Materials and methods

The deformation and associated biochemical signalling of intramyocardially transplanted stem cells were investigated using a multi-scale approach linking the ventricular and wall mechanics

to the cellular deformation and mechanically induced growth factor release (Figure 1). First, a biventricular finite element model of a rat heart with an infarct and intramyocardial biomaterial injectate in the left ventricle (LV) was developed and used to determine the ventricular and myocardial mechanics during a cardiac cycle (organ and tissue length scale). Second, a microstructurally detailed finite element model of a mid-wall region in the LV infarct with biomaterial injectate, referred to as the microstructural model, was developed (cellular length scale).

Nodal displacements of the LV mid-wall region of the microstructural geometry were recorded from the biventricular model during a cardiac cycle. The recorded displacements were applied as mechanical load boundary conditions to the outer surface of the microstructural model containing the transplanted therapeutic cells.

The deformations of the transplanted cells predicted with the microstructural model were used to quantify cellular signalling in the infarcted heart. An experiment of stretch-induced cellular TGF-β expression [37] was simulated using a finite element model to determine the relationship between cell deformation and TGF-β expression (Figure 2). This relationship was used to estimate the TGF-β production associated with the predicted deformation of the transplanted cells in the biomaterial injectate.

## 2.1 Geometrical modelling

### 2.1.1 Biventricular geometry of an infarcted rat heart with biomaterial injectate

The current study utilised a three-dimensional biventricular geometry of a rat heart with infarct and polymeric intramyocardial injectate in the left ventricle (LV) developed in a previous study [36]. In brief, myocardial infarction was induced in a male Wistar rat by permanent ligation of the left anterior descending coronary artery and 100 μl of radiopaque silicone rubber containing

lead chromate (Microfil® MV-120) was injected into the infarct region seven days later. The heart was explanted and underwent *ex vivo* microcomputed tomography (μCT) (custom scanner with Feinfocus X-ray tube and Varian 2520V Paxscan a-Si flat panel detector, Centre for X-ray Tomography, Ghent University, Belgium [38]). The biventricular geometry was reconstructed from the μCT image stack using semi-automated segmentation tools, including region-growing, level-set thresholding, and manual actions (Simpleware, Synopsys). The resulting geometry captured the essential morphology of the left and right ventricles and microstructural details of the dispersed injectate in the LV free wall (Figure 1 a). The meshed geometry comprised 147,240 (mesh density 302.8 $mm^3$) and 58,902 (3,852.3 $mm^3$) quadratic tetrahedral elements in the myocardial and injectate region, respectively (Figure 1 b). The meshed geometry was imported in Abaqus 6.14-3 CAE (Dassault Systèmes, Providence, RI, USA) and the infarct region was approximated by identifying the nodes surrounding the biomaterial injectate (Figure 1 c). The myofibre orientation varying from -50° at the epicardium to 80° at the endocardium [36] was implemented with a rule-based approach.

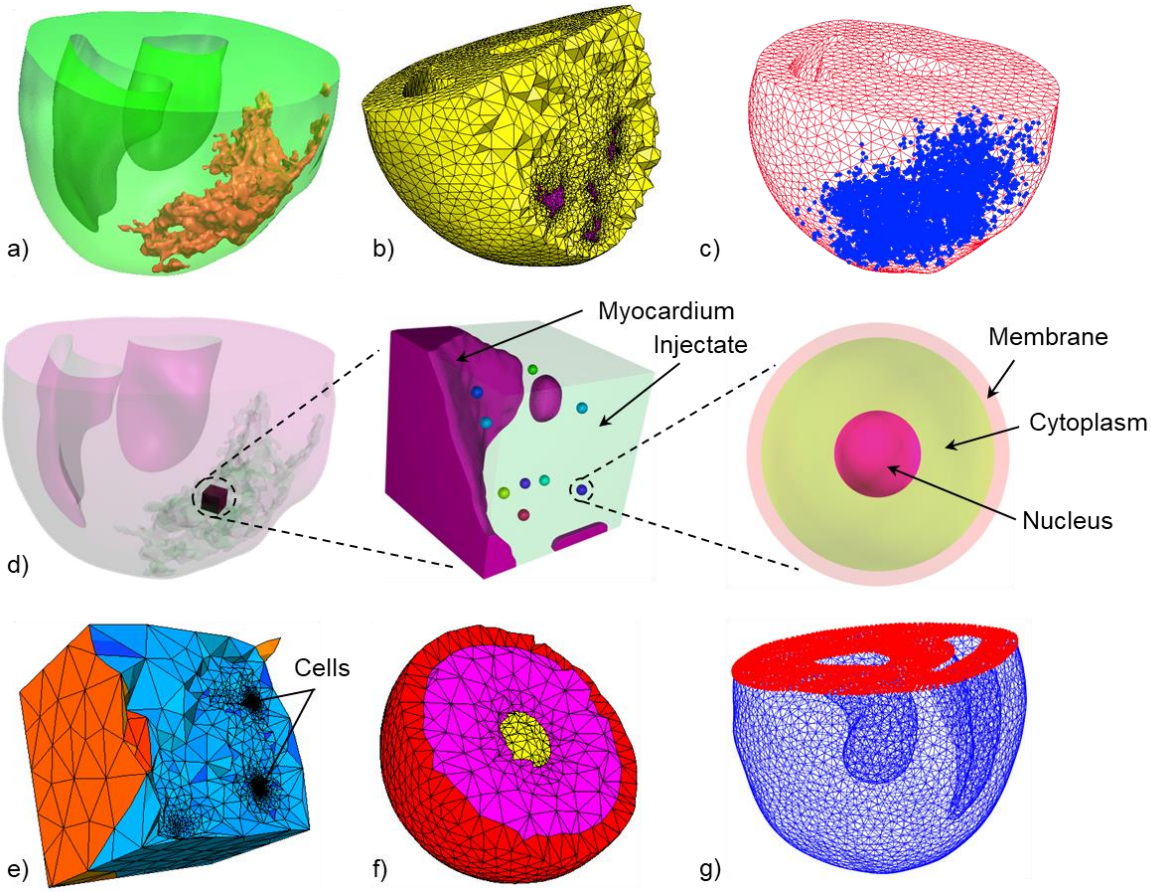


Figure 1. Geometries and models developed from the biventricular rat geometry to the microstructure extracted from the left ventricle mid-wall with transplanted cells in the injectate region. a) Biventricular geometry showing myocardium (green translucent) and injectate (beige). b) Meshed biventricular geometry with a cross-section illustrating the difference in mesh density between myocardium (green) and injectate (red). c) Meshed biventricular geometry illustrating the infarct region (red). d) Development of a microstructural model of a left ventricle mid-wall region. Left: Biventricular geometry showing the size and location of the microstructural region. Middle: Microstructural geometry comprising myocardium, injectate and nine transplanted cells. Right: Cellular geometry with membrane, cytoplasm, and nucleus. e) Meshed microstructural geometry with coarse mesh for the myocardium and injectate and fine mesh for the cells. f) Cross-sectional view of meshed geometry of single cell showing membrane (red), cytoplasm (pink) and nucleus (yellow). g) The biventricular FE mesh's basal nodes (red) were fixed as the boundary condition for simulations.

### 2.1.2 Microstructural LV mid-wall geometry and transplanted cells

A microstructural geometry of mid-wall volume of 748 µm x 748 µm x 722 µm in the LV infarct (Figure 1 d) with higher spatial resolution was reconstructed by resampling of the cardiac µCT image data with reduced spacing (7.8 µm compared to 30 µm in x, y, and z direction for the biventricular geometry) (Simpleware).

Fifteen cells comprising membrane, cytoplasm and nucleus were numerically seeded at random locations in the injectate region of the microstructural geometry with a custom Python script in Simpleware ScanIP. For each cell, three concentric spherical surfaces with diameters of 60, 55, and 20 µm were created for the membrane, cytoplasm, and nucleus (Figure 1 d right). A Boolean subtraction defined a thickness of 5 µm and 35 µm for the membrane and the cytoplasm, respectively. The tree-component assembly was placed in the injectate. Of these 15 cells, six cells were located in the myocardium or near model boundaries and interfaces and were not considered for the analysis.

The resulting micro-structural geometry containing myocardium, injectate, and nine cells with membrane, cytoplasm and nucleus was meshed and imported into Abaqus. The mesh comprised 320,653 10-node tetrahedral elements (C3D10M), i.e. 2,552, 168,746, and 149,355 elements for the myocardium, injectate and the nine cells, respectively. The mesh size varied among the nine cells based on the automated meshing process (Simpleware), and element numbers were 6,101 ± 42 for the membrane, 8,710 ± 299 for the cytoplasm, and 1,784 ± 82 for the nucleus.

## 2.2 Constitutive laws

### 2.2.1 Healthy and infarcted myocardium

The passive mechanical behaviour of the myocardium was represented by a hyperelastic anisotropic law using a modified strain energy density function from Holzapfel and Ogden [39], Eqn. (1). Changes were introduced to describe the pathological stage of the myocardium [40]. The passive mechanical response of infarcted myocardium depends on the stage of infarct healing [41-44]. A one-week infarct was modelled, corresponding to the delayed biomaterial delivery into the infarct. The associated increased stiffness in the fibre, circumferential, and longitudinal directions [45] was implemented using parameters h and p in Eqn. (2). The tissue health parameter $0 \leq h \leq 1$ corresponds to infarcted ($h = 0$) and healthy ($h = 1$) myocardium [40]. The pathological scaling parameter $p = 4.56$ [40, 46] adjusts the passive material response of the myocardium according to pathology.

$$W = \frac{\bar{a}}{2b} e^{b(I_1-3)} + \sum_{i=f,s} \frac{\bar{a}_i}{2b_i}\left\{e^{b_i(I_{4i}-1)^2} - 1\right\} + \frac{\bar{a}_{fs}}{2b_{fs}}\left\{e^{b_{fs}(I_{8fs})^2} - 1\right\} + \frac{1}{D}\left(\frac{J^2-1}{2} - \ln(J)\right) \qquad (1)$$

with

$$\begin{aligned} \bar{a} &= a(h + (1-h)p), \\ \bar{a}_i &= a_i(h + (1-h)p) \text{ and} \\ \bar{a}_{fs} &= a_{fs}(h + (1-h)p). \end{aligned} \qquad (2)$$

The active contraction of the myocardium was represented with a time-varying elastance approach with an additional term to consider the passive response during the contraction [47-49] and the tissue health parameter h described above [40]:

$$T_a(t, E_{ff}) = \frac{T_{max}}{2} \frac{Ca_0^2}{Ca_0^2 + EC_{a_{50}}^2(E_{ff})} \left(1 - \cos\left(\omega(t, E_{ff})\right)\right) h \quad (3)$$

The additive approach was used to compute the total tension in the myocardium where the time-varying active tension $\mathbf{T_a}$ (Eqn. (3)) was combined with the tension derived from the passive response $\mathbf{T_p}$:

$$\mathbf{T} = \mathbf{T_a} + \mathbf{T_p} \quad (4)$$

The active and passive constitutive parameter values were adopted from [40] and are provided in Supplemental Tables S1 and S2.

**2.2.2 Injectable biomaterial**

The injectable biomaterial, e.g. a polyethylene glycol (PEG) hydrogel, was represented as a hyperelastic isotropic incompressible Neo-Hookean material:

$$W = C_{10}(I_1 - 3) \quad (5)$$

where $I_1$ is the first deviatoric strain invariant, and $C_{10}$ characterises the material stiffness obtained from the elastic modulus $E_{inj}$ and the Poisson's ratio $\nu_{inj}$:

$$C_{10} = \frac{E_{inj}}{4(1 + \nu_{inj})} \quad (6)$$

with $\nu_{inj}$ = 0.5 defining incompressibility and $E_{inj}$ varied between 4.1 and 405,900 kPa (see section 2.5 FE simulations and data analysis for more details).

**2.2.3 Cells**

Cell membrane, cytoplasm and nucleus were treated as hyperelastic isotropic compressible material using a Neo-Hookean strain energy density function [50, 51]:

$$W = C_{10}(I_1 - 3) + \frac{1}{D_1}\left(J^{el} - 1\right)^2, \quad (7)$$

where $J^{el}$ is a measure of volume change of the compressible material, and $C_{10}$ and $D_1$ depend on the elastic modulus and the material compressibility. The material parameter values for the cellular components are given in Supplemental Table S3.

## 2.3 Boundary conditions and loading

The boundary and loading conditions for the biventricular model were described in detail previously [36]. In brief, zero displacement in the longitudinal direction was applied to the nodes at the basal surface to prevent the rigid body motion of the biventricular geometry (Figure 1 g). Additionally, a basal node in the LV ring was fixed in the circumferential and radial directions to prevent lateral motion and rotation. Passive filing was implemented with a linearly increasing pressure load on ventricular cavity surfaces from 0 to 3.0 mmHg for the LV and 0 to 0.75 mmHg for the RV.

Displacement boundary conditions for the microstructural model were obtained from the biventricular model employing the node-based sub-modelling technique in Abaqus. The displacements of sets of nodes near the surfaces of the microstructural region were recorded in the biventricular geometry during the simulation of one cardiac cycle. These recorded displacements were applied as boundary conditions to surface nodes of the microstructural FE mesh and recapitulated the surface displacements and deformations during a cardiac cycle.

## 2.4 Cell deformation and TGF-β production

The mechanical cues for the transplanted cells were assumed to originate from their environment, i.e. the biomaterial injectate and its deformation during a cardiac cycle. A mathematical model was developed to predict the TGF-β expression in the transplanted cells due to the deformations of the surrounding biomaterial injectate. The model is based on

experimentally measured TGF-β concentration in mesangial cells exposed to substrate stretch [37] and the corresponding cellular strain predicted with a single-cell finite element model.

**2.4.1 Mechanics of a single cell on an elastic substrate subjected to stretch**

A finite element model of a single cell attached to an elastic substrate was developed to simulate biaxial substrate stretch experiments with mesangial cells [37]. The cell was represented by a spherical cap with a diameter of 20 µm and a central thickness of 2 µm. The spherical cap was attached to the centre of a planar substrate (100 µm x 100 µm, thickness 2 µm) using 37 disks representing focal adhesions (FA) (diameter 2 µm, thickness 0.5 µm). The FA disks were equally distributed over the basal cell surface with a distance of 2.8 µm to the nearest adjacent disk (Figure 2). The cell-FA and FA-substrate contacts were modelled using tie constraints to ensure permanent adhesion and prevent sliding.

The geometries were meshed (Abaqus 6.14-3, Dassault Systèmes, Providence, RI, USA) with 7,697 ten-node tetrahedral elements (C3D10) for the cell, 1,186 six-node linear triangular elements (C3D6) for each FA disk, and 122,694 eight-node hexahedral elements (C3D8H) for the substrate, following a mesh sensitivity study (Supplemental Figure S1). The cell and the FA disks were represented as hyperelastic material with a Neo-Hookean strain energy density function, Eqn. (7). The substrate was described using a linear-elastic model. The material parameter values are provided in Supplemental Table S4.

Two substrate edges were fixed, and displacements were applied to the other two edges to generate an equibiaxial strain of up to 20% (Figure 2 d). The average strain in the cell and the focal adhesions were recorded and related to the substrate strain, see Eqn. (9).

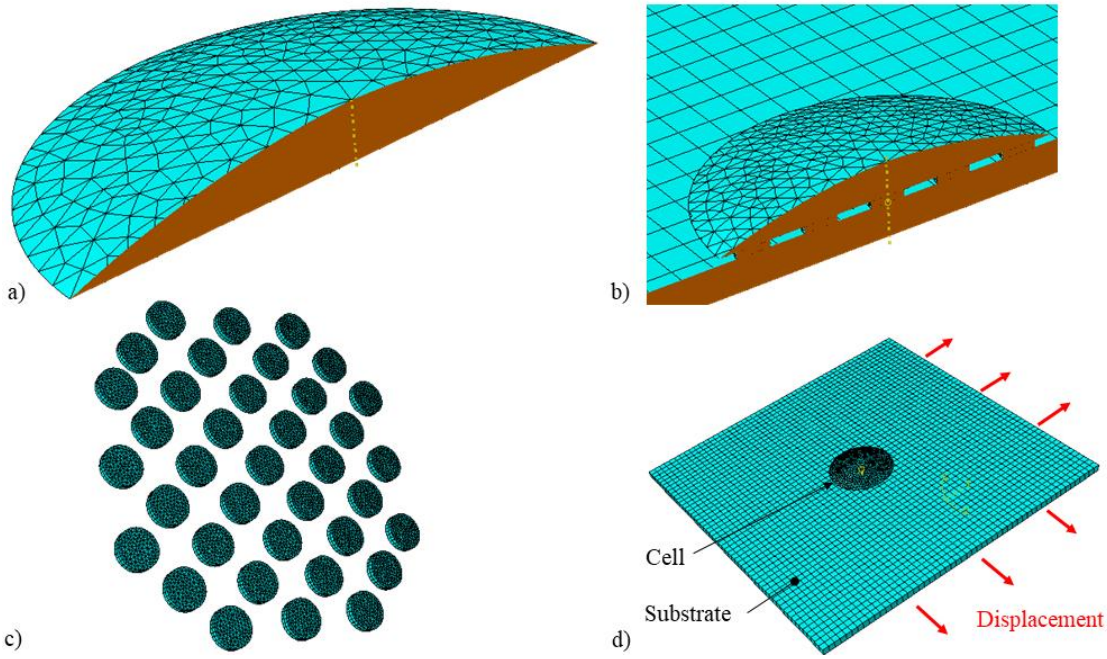


Figure 2. Single-cell stretching finite element model. a) Spherical cap representing the cell. b) Cross-sectional view of the cell attached to the substrate with focal adhesion disks. c) Arrangement of focal adhesion disks. d) Cell and substrate assembly with a schematic illustration of displacement of two substrate edges to generate biaxial strain.

### 2.4.2 TGF-β production in cells exposed to substrate stretch

Several experimental studies have quantified the mechanically induced growth factor release in cells [23, 37, 52]. Hirakata et al. [37] measured mRNA expression for TGF-β in rat mesangial cells cultured on an elastic substrate and subjected to cyclic biaxial substrate strain of different amplitudes (5%, 10%, 15% and 20%). The concentration of TGF-β mRNA expression as a function of the substrate strain amplitude [37, Fig. 3] was utilised in the current study to express TGF-β expression as a function of substrate strain $\varepsilon_{Sub}$:

$$\text{TGF-}\beta = a_0\, \varepsilon_{Sub} + b_0, \tag{8}$$

with $a_0 = 0.06$ and $b_0 = 0.08$.

A relationship between the average strain in the cell $\varepsilon_{Cell}$ and the substrate strain $\varepsilon_{Sub}$ can be derived from predictions of the FE model described in the previous section:

$$\varepsilon_{Cell} = a_1 \varepsilon_{Sub} + b_1, \tag{9}$$

with $a_1$ and $b_1$ to be obtained from a linear fit.

Combining Eqns. (8) and (9), the expression of TGF-β mRNA as a function of cellular strain is:

$$\text{TGF-}\beta = a_2 \varepsilon_{cell} + b_2 , \tag{10}$$

where $a_2$ and $b_2$ are determined by eliminating $\varepsilon_{Sub}$ in Eqns. (8) and (9).

## 2.5 FE simulations and data analysis

A parametric study with the variation of the elastic modulus of the biomaterial injectate in the range of $E_{inj}$ = 4.1 to 405,900 kPa (i.e. 4.1, 7.4, 40.6, 73.8, 405.9, 738, 4,059, 7,380, 40,590, 73,800 and 405,900 kPa) was conducted to determine the impact of biomaterial stiffness on the deformation of the transplanted cells in the biomaterial injectate in the infarct.

The analysis of the deformation of the transplanted cells comprised the following strain measures.

i) The element strain as mean strain at the integration points of an element:

$$\varepsilon_{El,j} = \frac{1}{P} \sum_{i=1}^{P} \varepsilon_{IP,i} , \tag{11}$$

where $\varepsilon_{IP,i}$ is the strain at integration point *i*, and P is the total number of integration points of element *j* in the cell mesh.

ii) The volume-averaged strain in a cell component (i.e. membrane, cytoplasm or nucleus) of cell *k*:

$$\varepsilon_{CC,k} = \frac{1}{V}\sum_{j=1}^{N} v_j\, \varepsilon_{El,j} \tag{12}$$

where V is the total volume of the cell component considered, N is the total number of elements in the cell component, $v_j$ is the volume of element $j$, and $\varepsilon_{El,j}$ is the element strain of element $j$ calculated with Eqn. (11).

iii) The mean strain in a cell component for all cells in the model:

$$\varepsilon_{CC} = \frac{1}{n}\sum_{k=1}^{n} \varepsilon_{CC,k}\,, \tag{13}$$

where $\varepsilon_{CC,k}$ is the volume-averaged strain in a component of cell $k$ according to Eqn. (12), and n is the total number of cells in the model (n = 9 in the current study).

iv) The mean strain in the entire cell for all n cells in the model, also referred to as cell strain:

$$\varepsilon_{Cell} = \frac{1}{3}\sum_{l=1}^{3} \varepsilon_{CC} \tag{14}$$

for the three cell components, i.e. membrane, cytoplasm, and nucleus, where $\varepsilon_{CC}$ is the mean strain in a cell component for all cells in the model according to Eqn. (13).

Descriptive statistical analysis was performed on the strain data in the different model components to determine the normality (Shapiro-Wilk normality test) and variability (SciPy, https://scipy.org/ and NumPy, https://numpy.org/). Data are presented as mean and standard deviation if normally distributed; otherwise, they are presented as median and interquartile range.

## 3. Results

The end-systolic deformation and maximum principal strain of a cross-section of the microstructural model and the transplanted cells are illustrated in Figure 3 for an injectate elastic modulus of $E_{inj}$ = 7.4 kPa. The highest maximum principal strain was observed in the cellular membrane with the lowest elastic modulus (E = 1.7 kPa), whereas the strain in the cytoplasm (E = 8.0 kPa) and nucleus (E = 5.0 kPa) was similar to that in the biomaterial surrounding the cells.

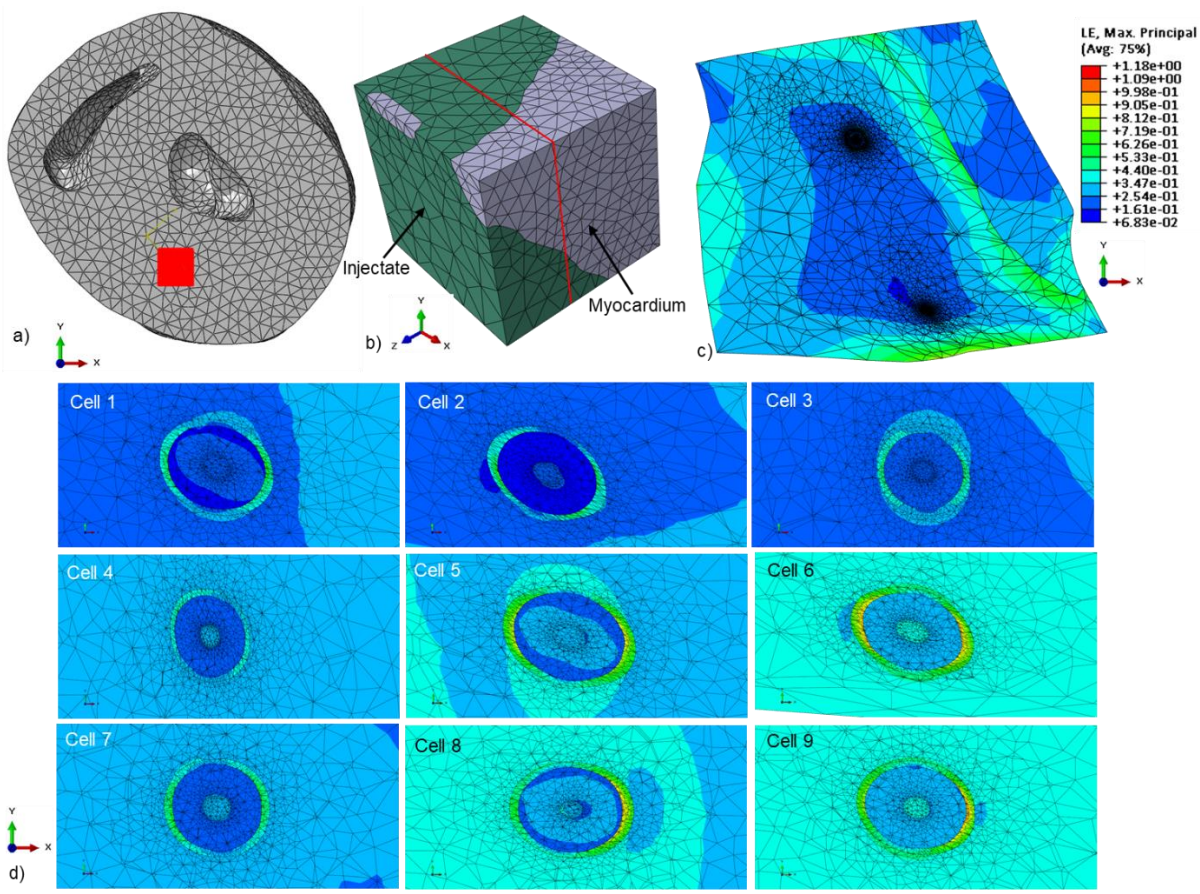


Figure 3. Illustration of deformation in infarcted myocardium, biomaterial injectate and transplanted cells at end-systole predicted with the microstructural FE model for $E_{inj}$ = 7.4 kPa. a) Short-axis view of the undeformed biventricular geometry demonstrating position and orientation of the hexahedral region of the microstructural geometry in the LV free wall. b) Perspective view of the undeformed microstructural geometry containing infarcted

myocardium and biomaterial injectate. c) Contour plot of maximum principal stress in a cross-section of the deformed end-systolic configuration of the microstructural model with colour legend of strain. The two regions with high mesh density are the locations of transplanted cells. (The location of the cross-section is indicated by red lines in b). d) Contour plots of maximum principal stress in a cross-section of the nine transplanted cells at different locations in the biomaterial injectate region of the microstructural model. The magnitude of the cellular deformation varied amongst the different cell locations. Generally, higher strain levels were predicted in the cellular membrane than in the cytoplasm and nucleus. The colour legend from figure c applies to these contour plots.

### 3.1 Deformation of injectate in the microstructural mid-wall model

At end-diastole, the median maximum principal strain in the injectate decreased from 6.5% to 0.9%, and the median minimum principal strain decreased in magnitude from -7.3% to -0.9% with increasing injectate elastic modulus from $E_{inj}$ = 4.1 to 405,900 kPa (Figure 4 a and b). At end-systole, the median maximum principal strain decreased from 43.8% to 1.4%, and the median minimum principal strain decreased in magnitude from -38.0% to -1.5% with increasing injectate elastic modulus (Figure 4 c and d). The ratio of end-systolic to end-diastolic maximal principal strain decreased from 6.7 (for the softest injectate with $E_{inj}$ = 4.1 kPa) to 1.6 (for the stiffest injectate with $E_{inj}$ = 405,900 kPa). A similar decrease from 5.2 for the softest injectate to 1.6 for the stiffest injectate was observed for the ratio of end-systolic to end-diastolic minimum principal strain.

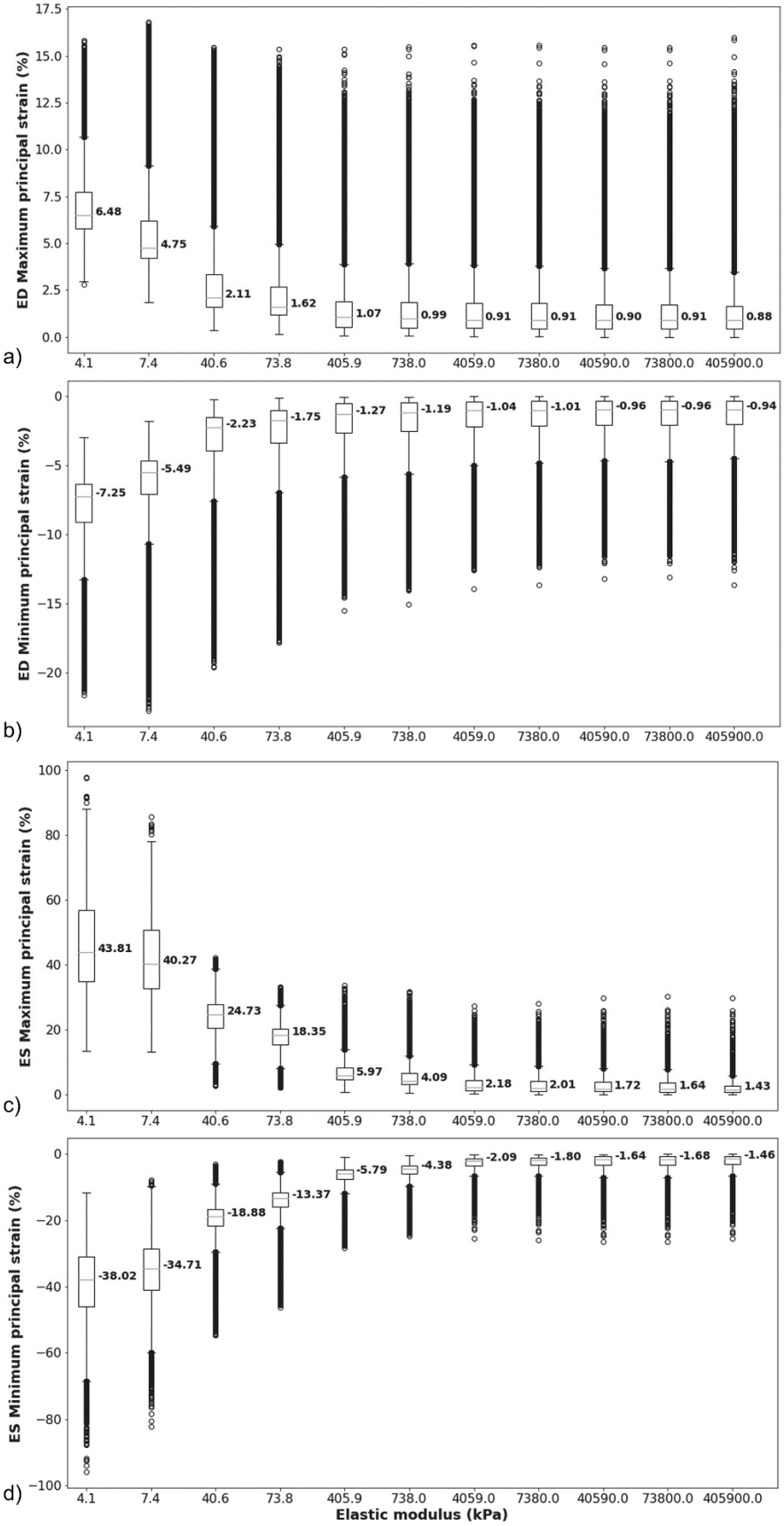
a)
ED Maximum principal strain (%)
6.48
4.75
2.11
1.62
1.07
0.99
0.91
0.91
0.90
0.91
0.88
b)
ED Minimum principal strain (%)
-7.25
-5.49
-2.23
-1.75
-1.27
-1.19
-1.04
-1.01
-0.96
-0.96
-0.94
c)
ES Maximum principal strain (%)
43.81
40.27
24.73
18.35
5.97
4.09
2.18
2.01
1.72
1.64
1.43
d)
ES Minimum principal strain (%)
-38.02
-34.71
-18.88
-13.37
-5.79
-4.38
-2.09
-1.80
-1.64
-1.68
-1.46
4.1
7.4
40.6
73.8
405.9
738.0
4059.0
7380.0
40590.0
73800.0
405900.0
Elastic modulus (kPa)

Figure 4. End-diastolic and end-systolic principal strains in the injectate of the microstructural model for different values of the injectate elastic modulus. Maximum (a) and minimum principal strain (b) at end-diastole, and maximum (c) and minimum principal strain (d) at end-systole. (Box and whiskers indicate the median (red line in box), interquartile range (IQR) between first and third quartile (lower and upper box bound), 1.5x IQR (lower and upper whisker), and data points smaller or larger than 1.5x IQR (open circles). Each data point represents the strain value $\varepsilon_{El}$ in an element of the finite element mesh. Data larger or smaller than 1.5x IQR are considered actual data and not outliers.)

### 3.2 Deformation of transplanted cells in the injectate

The volume-averaged maximum principal strain in the cell components decreased for increasing injectate elastic modulus at end-systole and end-diastole (Figure 5 a-c). The strain decrease was larger in the lower range of the injectate elastic modulus from $E_{inj}$ = 7.4 kPa to 738 kPa than for higher values of the injectate elastic modulus $E_{inj}$ > 738 kPa. The membrane displayed higher strain than the cytoplasm and nucleus, which exhibited similar strains. Similar results were observed for the minimum principal strain, exhibiting negative values indicative of compression (Figure 5 d-f). The strain magnitude decreased for $E_{inj}$ = 7.4 kPa to 738 kPa and levelled off for $E_{inj}$ > 738 kPa.

Increases in maximum and minimum principal strains at end-systole were observed for an increase of the injectate elastic modulus from $E_{inj}$ = 4.1 kPa to 7.4 kPa.

The ES-ED range of the median volume-averaged maximum and minimum principal strains in the cell components decreased in magnitude for increasing injectate elastic modulus $E_{inj}$ = 7.4 kPa to 405,900 kPa (Figure 5 d). The ES-ED range of the principal strain reflects the deformation range to which the transplanted cells are exposed during a cardiac cycle.

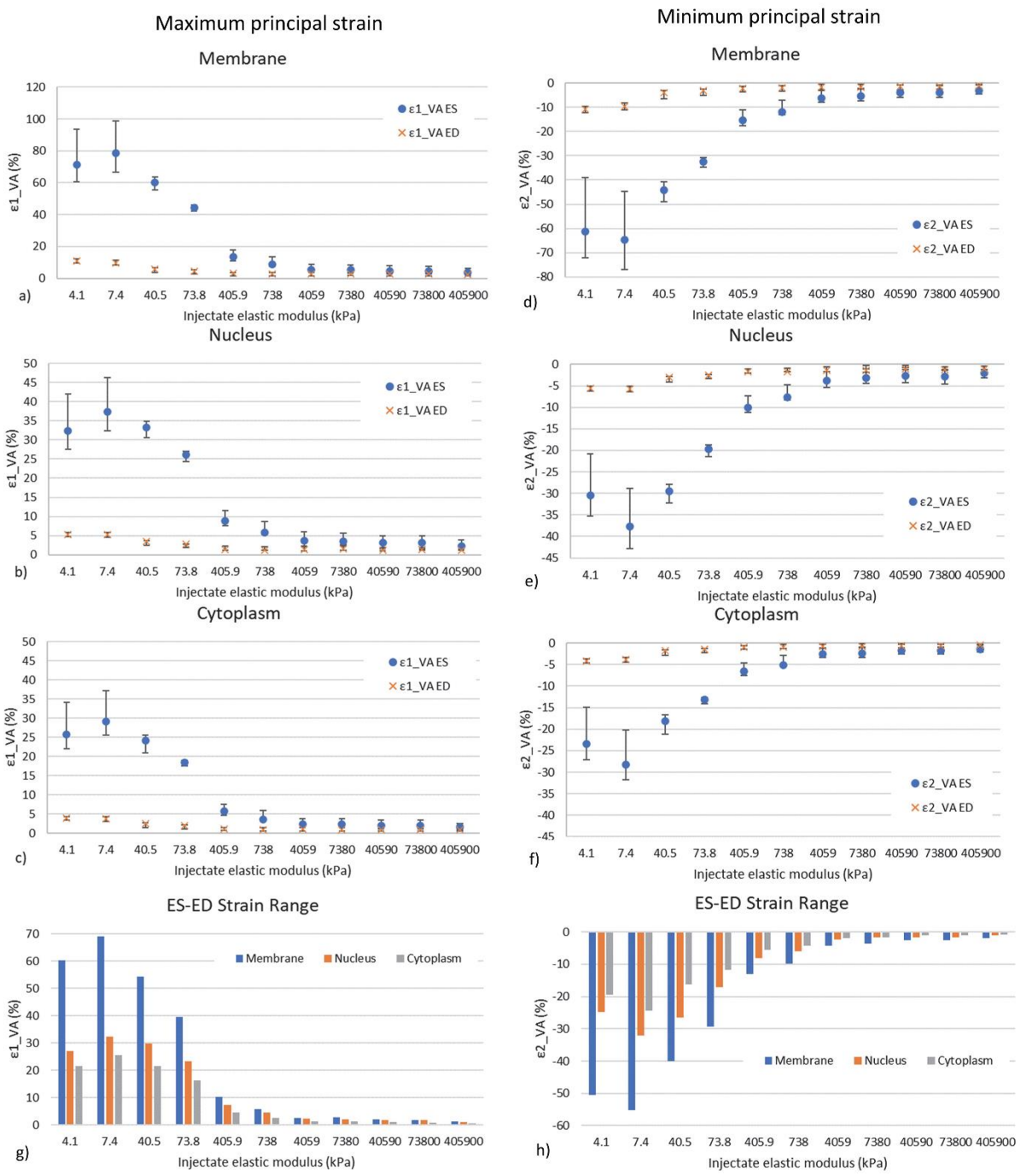


Figure 5. Maximum (a-c) and minimum (d-f) principal strains in the membrane, nucleus, and cytoplasm of transplanted cells at end-systole (ES) and end-diastole (ED) versus injectate elastic modulus. ES-ED range of maximum (g) and minimum (h) principal strain versus injectate elastic modulus for the three cell components. (Data in a to f are median; error bars represent interquartile range).

### 3.3 Mechanically induced cellular TGF-β expression

The distribution of the maximum principal strain in the cell at a biaxial substrate strain of 15% predicted with the FE model is illustrated in Figure 6.

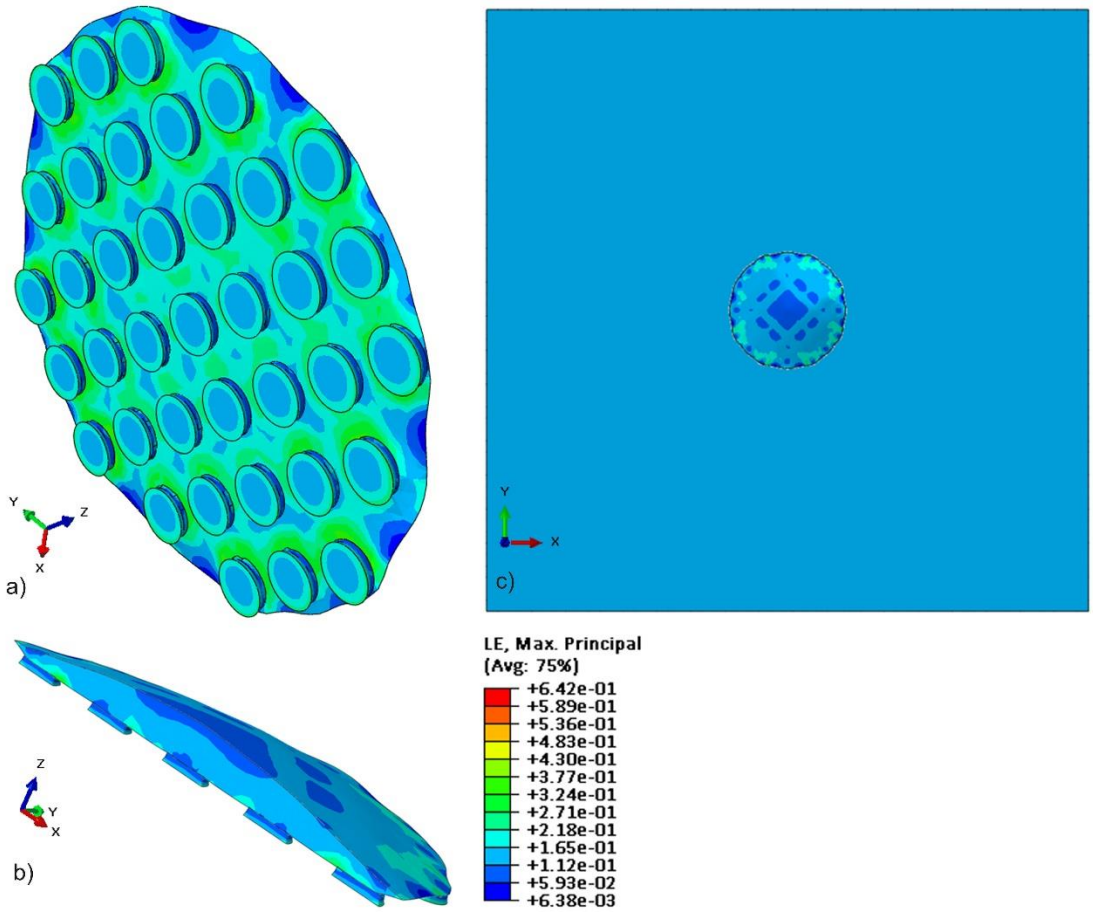


Figure 6. Contour plots of maximum principal strain in (a) the focal adhesions, (b) cell body, and (c) substrate-cell assembly at equibiaxial strain of 15% in the substrate.

The volume-averaged maximum principal strain in the cell body and focal adhesions, $\varepsilon_{Cell}$, increased linearly with increasing substrate strain $\varepsilon_{Sub}$ = 5% to 20% (Figure 7 a) and resulted in the relationship:

$$\varepsilon_{\mathrm{Cell}} = 0.84\, \varepsilon_{\mathrm{Sub}} + 0.37\,. \tag{15}$$

Quantitative data of TGF-β expression as a function of substrate strain extracted from Hirakata et al. [37] (Figure 7 b) was expressed as:

$$\text{TGF-}\beta = 0.06\,\varepsilon_{\text{Sub}} + 0.08\,. \tag{16}$$

Employing Eqn. (10), the TGF-β mRNA expression could be expressed as a function of cell strain (Figure 7 c):

$$\text{TGF-}\beta = 0.07\,\varepsilon_{\text{Cell}} + 0.11\,. \tag{17}$$

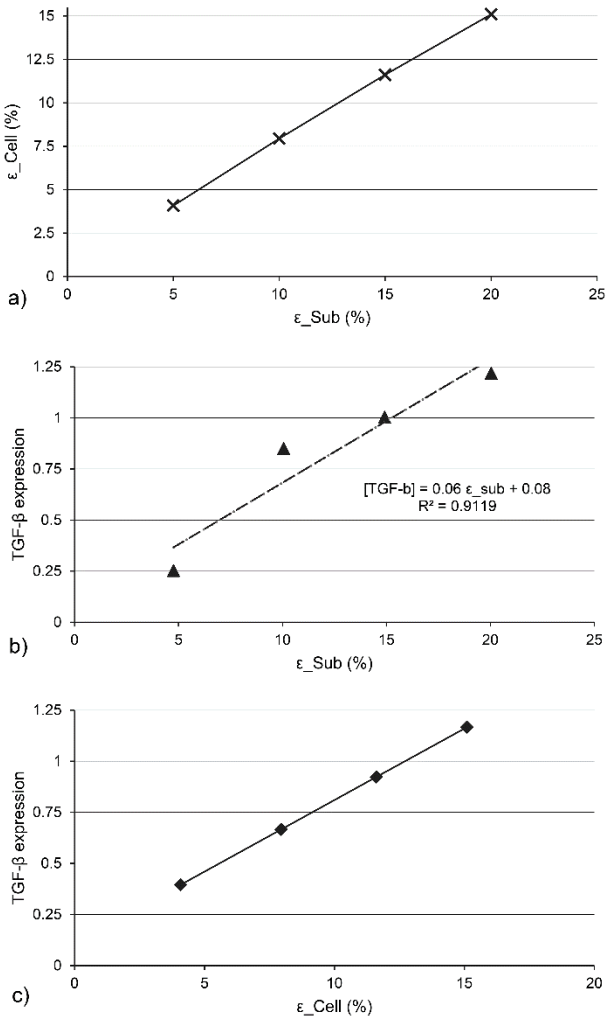


Figure 7. a) Volume-averaged maximum principal strain in a single cell stretched on an elastic substrate versus substrate strain. The finite element model computed volume-averaged strain (crosses) is shown with the strain predicted in the cell for increasing substrate strain with Eqn. (15) (solid line). b) TGF-β mRNA expression in a stretched cell on an elastic substrate versus substrate strain showing experimental data of TGF-β mRNA expression from Hirakata et al. [37] (solid triangles) and a linear fit (dashed line). c) Mechanically induced TGF-β mRNA expression versus volume-averaged maximum principal strain in the cell.

The TGF-β mRNA expression, determined with Eqn. (17) using the ES-ED range of maximum principal strain in the transplanted cells (Figure 5 g), was predicted to increase with an increase of the substrate elastic modulus from 4.1 kPa to 7.4 kPa and to decrease with further increasing

injectate modulus (Figure 8). However, the extrapolation beyond the strain range considered by Hirakata et al. [37] was required, and it was assumed that the linear relationship is valid in the ES-ED strain range domain.

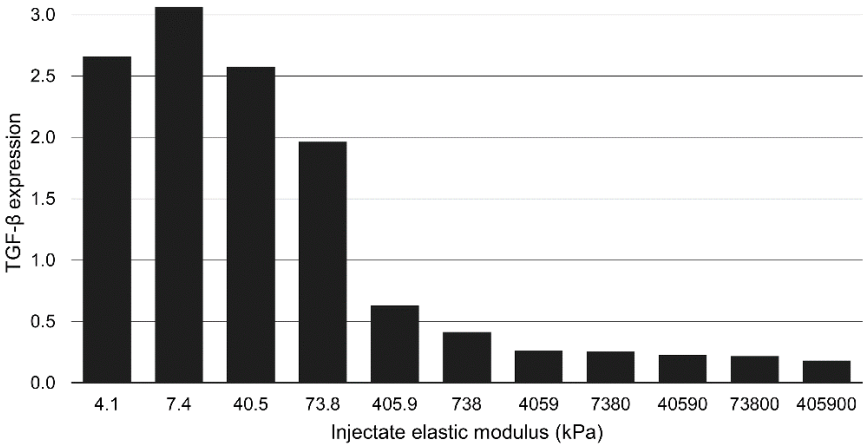


Figure 8. Predicted mechanically induced TGF-β mRNA expression in transplanted cells in the injectate versus injectate elastic modulus.

## 4. Discussion

Understanding mechanotransduction involved in cardioprotective paracrine signalling of cell therapies for MI is critical for these therapies. The present computational study demonstrated the effect of the stiffness of the biomaterial injectate on the mechanics and TGF-β expression of cells intramyocardially transplanted with the biomaterial. The variation of the injectate stiffness can be used to guide deformation and associated biochemical signalling of the transplanted cells in the injectate. Soft injectates promise the most beneficial effects as the cell deformation is more sensitive to a change in injectate stiffness similar to the transplanted cells' stiffness than higher injectate stiffnesses.

A decrease in strain in the injectate was predicted for an increasing injectate elastic modulus. Similar observations were reported by Sirry et al. [35], where stiffer injectate resulted in less

deformation within an acellular injectate region of the microstructural model. The current study investigated a wide range of injectate stiffness, with the upper range of the elastic modulus of $E_{inj}$ > 738 kPa being unrealistic for PEG hydrogels and other injectable biomaterials [53, 54]. It was shown that the deformations in the injectate are negligible from a particular threshold of the injectate elastic modulus.

A decrease in ES and ED strains and the associated ES-ED strain range in the transplanted cells were predicted for increasing injectate stiffness. Additionally, the cell mechanics were considerably more sensitive to environmental stiffness changes for soft ($E_{inj} \leq 738$ kPa) than stiff injectates ($E_{inj}$ > 738 kPa).

Interestingly, an increase in cell strain was observed for an increase in injectate stiffness from $E_{inj}$ = 4.1 kPa to 7.4 kPa. This contrasting cell behaviour may reveal a threshold of the injectate stiffness at which the mechanical response of the cells changes. This change may be explained when considering the elastic modulus of the cellular components, namely E = 1.7 kPa for the membrane, 5 kPa for the nucleus and 8 kPa for the cytoplasm. The injectate with $E_{inj}$ = 4.1 kPa had a lower elastic modulus than the bulk of the cellular structure (i.e. cytoplasm and nucleus) and transferred less deformation to the cells than the injectate with $E_{inj}$ = 7.4 kPa that more closely matches the elastic modulus of cytoplasm and nucleus.

It is well established *in vivo* and *in vitro* that cyclic mechanical loads induce biochemical responses in cells [21, 22, 55, 56]. Bhang et al. [20] showed that cyclic strain promotes the expression of cardiomyogenic markers in bone marrow-derived mesenchymal stem cells and, combined with TGF-β, leads to enhanced expression of cardiac-specific genes.

The single-cell stretching finite element model, replicating experiments from Hirakata et al. [37], predicted a linear relationship between substrate and cell deformation, similar to the

results of Abdalrahman et al. [57]. Data from Hirakata et al. [37] demonstrated a linear relationship between biaxial substrate strain (between 5% and 20%) and cellular TGF-β expression. Combining these two relationships predicted a linear increase in TGF-β expression with increased cell strain. Based on the effect of injectate stiffness on cellular deformation, a higher sensitivity of TGF-β expression to a change in the environmental stiffness was predicted for a low injectate stiffness ($E_{inj} \leq 738$ kPa) than a high injectate stiffness ($E_{inj} > 738$ kPa). The increase in TGF-β combined with mechanical deformation of the transplanted cells can enhance the expression of cardiac-specific genes [20, 58].

The numerical cell seeding algorithm randomly placed cells in the microstructural model's entire volume. Six of the 15 seeded cells were not used for the mechanical analysis due to their location in the tissue domain and near model boundaries and interfaces. Expanding the seeding algorithm to monitor the location of the cells will provide more control over the seeding process and the number of cells available for analysis. An increase in the number of seeded cells will allow a more detailed investigation of the impact of cell location and density on their mechanics and therapeutic signalling. Such a comprehensive study can help design cell patterns within the injectate at the culture stage and when targeted spatial delivery into the heart becomes available.

The microstructural model used a single mesh configuration as the mesh variation was challenging due to the small thickness of the cellular membrane. Future studies may benefit from different meshing strategies or mesh sensitivity investigations. Using shell elements instead of three-dimensional elements for the cell membrane can be considered.

The cellular components were treated as isotropic and compressible materials with Neo-Hookean strain energy density functions. The constitutive equations did not consider the active

processes in the cell (e.g. actin polymerisation and depolymerisation). Including these dynamic cytoskeletal processes will allow for assessing the medium- and long-term structural responses of the transplanted cells.

The data on mechanically-induced growth factor production available in the literature are commonly from experiments performed over several hours [22]. The kinematics of TGF-β expression were not considered in the current model because the time scale (microseconds) was beyond that of the biochemical reactions involved in signalling pathways. A quantitatively accurate model will require information on the entire biochemical pathway of TGF-β in mesenchymal stem cells.

Additional growth factors and other biochemical factors can be included to extend the application of the current work, such as VEGF, known for its role in the neovascularisation of the damaged tissue and is responsible for neovascularisation in the infarcted tissue. The mathematical model for TGF-β production was based on experiments with mesangial cells. The lack of replication of the current mechanotransduction results in mesenchymal stem cells is a limitation of the current study. The predictions will benefit from the adaption of the model to cells that are more relevant to the cardiac application.

The microstructural model predicted deformations of therapeutic cells embedded in the injectate beyond the strain range obtained from the experiment by Hirakata et al. [37]. Employing the developed relationship of the deformation-induced cellular TGF-β expression assumed that the relationship is valid beyond the experimental strain range (20%). Further investigations or experiments are needed to confirm the validity of the linear increase in TGF-β expression in the ES-ED strain range covered in the cell deformation at the cellular scale. In

addition, considering the TGF-β expression of cells in a three-dimensional environment will more closely mimic the application in the heart.

The biventricular FE model utilised structural boundary conditions at the truncated base demonstrated in previous studies [32, 33, 36, 40]. Future studies can extend the biventricular model by including the atria and the pericardium in the geometry and representing the tissue environment of the heart in the chest in the boundary conditions.

The effect of the biomaterial injectate on the ventricular function of the infarcted heart has not been considered in the current study. However, functional improvement is essential for optimising biomaterial and stem cell-based therapy for MI and will be included in future work.

## 5. Conclusions

The current study is the first to quantify the mechanotransduction of therapeutic cells intramyocardially transplanted into an infarcted rat heart using a biomaterial injectate. The developed microstructural finite element model of the myocardium and biomaterial injectate at cellular length scale enables quantifying micromechanics and signalling of transplanted cells during a cardiac cycle. The coupled microstructural and biventricular cardiac finite element models can provide a point of departure for an *in silico* method with a broader scope of advancing therapeutic biomaterial and cell injections for MI, e.g. timing of the injection after infarction and cellular expression of other biochemical factors and for other cardiac conditions such as heart failure.

## Data availability

Computational models, custom code, and data supporting this study are available on the University of Cape Town's institutional data repository (ZivaHub) at

http://doi.org/10.25375/uct.22673884 as Motchon YD, Sack KL, Sirry MS, Nchejane NJ, Abdalrahman T, Nagawa J, Kruger M, Pauwels E, Van Loo D, De Muynck A, Van Hoorebeke L, Davies NH, Franz T. Data for "In silico mechanics and TGF-ß expression of stem cells intramyocardially transplanted with a biomaterial injectate for treatment of myocardial infarction". ZivaHub, 2023, DOI: 10.25375/uct.22673884.

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## Competing Interests

The authors declare that they have no competing interests.

## CRediT Author Contributions

YDM: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

KLS: Methodology, Software, Supervision, Writing – Review & Editing

MSS: Methodology, Software, Supervision, Writing – Review & Editing

NJN: Methodology, Writing – Review & Editing

TA: Methodology, Supervision, Writing – Review & Editing

JN: Methodology, Writing – Review & Editing

MK: Investigation, Writing – Review & Editing

EP: Investigation, Methodology, Writing – Review & Editing

DVL: Investigation, Methodology, Writing – Review & Editing

ADM: Investigation, Writing – Review & Editing

LVH: Resources, Methodology, Supervision, Writing – Review & Editing

NHD: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – Review & Editing

TF: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing

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