Characterization of Cells Migration through Cardiac Tissue using Advanced Microscopy Techniques and Matlab Simulation

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Abstract

Mesenchymal stromal cells (MSC) and neutrophils (NP) migration are important factors of the postinfarcted hearts remodeling. These both types of the cells can migrate through cardiac extracellular matrix to the central ischemic region. The quantitative description of MSC and NP migration through collagen matrix is important aim of modern bio-medicine.

NP and MSC migration through peri-infarct zone was simulated in a custom-made microphantom: two chambers (bottom 10×20 mm) connected by a collagen tunnel. Bottom of the system was constructed from glass plate, compatible with confocal microscopy. System was heated at 37° C in 5-21 % O_2 environment. The first chamber was starting point of migrating MSC and NP. The second chamber included living or apoptotic myocytes (model of central infarcted). Monitoring of migrating cells was performed on the confocal laser scanning microscope Leica. Chemotaxis movement of MSC through collagen tunnel between two chambers was approved. Speed was significantly modulated by collagen fiber orientation and hypoxic condition. The speed constants of cell motility were quantified by originally-made microphantomMatlab utility and basic equations for cell motility was proposed, usable for future creating of in-silico simulator of real cell invasivity in patients.

1. Introduction

MSC and NP migration are important factors of the postinfarcted heart remodeling. MSC and also NP are cells which are normally circulating in blood flow. When infarcted heart produce chemoattractants, MSC and NP are naturally landed to internal surface of microvessels and after that they are migrating via gradient of chemoattractants into the tissue. This phenomenon of nature migration is known more than 30 years, many histological analysis have shown neutrophils infiltration of border zones of ischemic myocardium and another histological analysis also proved migration of MSC towards the center infarcted tissue [1]. The impact of this massive neutrophil infiltration is mainly in production of proteolytic enzymes, on the other hand the MSC have main impact in producing and delivery of prosurviving and antiapoptotic factors [2], direct contact and supporting of surviving myocytes [3] and anti-inflamatory and mechanical stabilization of myocardium [4]. However until today a little is known about interplay of biophysical factors and quantitative rules of these MSC and NP migration kinetic. In-vitro model in literature are focusing mainly to migration of cell on 2D plane dish and this result are far from real in-vivo 3D structure in infarcted heart. Developing of original 3D phantom for cell migration, and its combination with precise microscopic monitoring, was basic aim of our work. Second aim was basic quantification of speed constant of MSC and NP motility and proposing of basic equation for creating of in-silico simulator in future.

2. Materials and methods

2.1. Preparation of cells

Cardiomyocytes were obtained from young adult Wistar rats (250 ± 50 g). Rats were anesthetized. After the loss of reflex, the sternum was quickly cut, the ribcage opened, and the beating heart cut from circulation were extracted. We proceeded cardiomyocyte isolation using the method of enzymatic dissociation [5]. The supernatant of enzymatic digestion (more than 50 % rod-shaped cells) was collected, filtered, and exposed to 0.9 mmol/l $CaCl_2$

solution. Morphology and viability of the cells was recorded (see Figure 1).

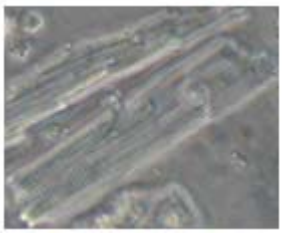


Figure 1. Freshly isolated myocyte from rat heart (brightfield image).

Rat mesenchymal stromal cells (MSCs) were harvested and cultured, as previously described [4]. Briefly, cells were isolated from the bone marrow of femurs by flushing medium into the bone shaft (method of anesthesia of the rat based on ketamine and xylazine was the same as for the heart isolation protocol described above). Cell suspension was filtered through a 40 μm nylon cell strainer (BD Falcon, USA) and layered on Histopaque at 500 g for 30 min, mononuclear cell layer was plated in chambers of 24-well plates. Cells were grown in Iscove's Modified Dulbecco's Medium (IMDM) with 10 % fetal bovine serum (FBS) at 37° C and 5 % CO_2. Rat neutrophils were collected via standard protocol [6].

2.2. Preparation of collagen matrix sample and confocal slides

3D collagen matrix with isotropic fibers was prepared by methods adopted from [7]. The steps are illustrated on Figure 2. Briefly, the sterile silicon tube (internal diameter 1 mm) was fixed to microscopical glass (200×200 mm; 0.2 mm) by silicon glue. The collagen (C8897, Sigma-Aldrich, solution 1.5 mg/ml) was injected through silicon tube, the laminar flow induce the isotropic arrangement of collagen fibril. The collagen „block“ was cut by sterile scalpel blade. We have prepared two variants of collagen blocks: 1. with parallel fibers and 2. with orthogonal fibers (Figure 2 E-F) to axis of silicon tube. We arranged „starting chamber“ and „chemoattractive chamber“ near the collagen box (see Figure 3).

The collagen block (horizontal platform 1 mm x 5 mm) was fixed by additive silicon drop (Figure 2) and 1 cm microcopies wall with central permeable collagen region was created. On the first side of the wall the cells were arranged and keep to adhere in plastic ring (pentagram in Figure 3), 2 hours later the source of chemoattractant was arranged (M-point in Figure 3). All elements on the microscope glass was enclosed by plastic square and both chamber (on both sides of collagen-silicon block) was covered by 1 mm layer of culture medium (DMEM with 1 % of antibiotic).

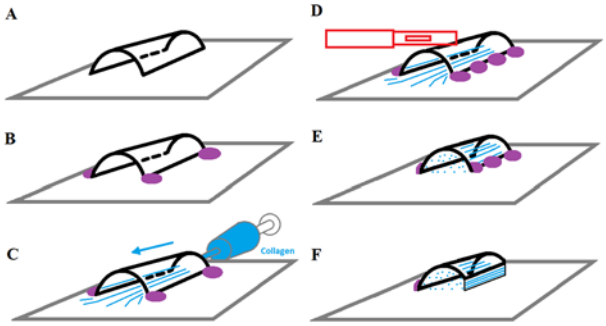


Figure 2. Preparation of collagen blocks.

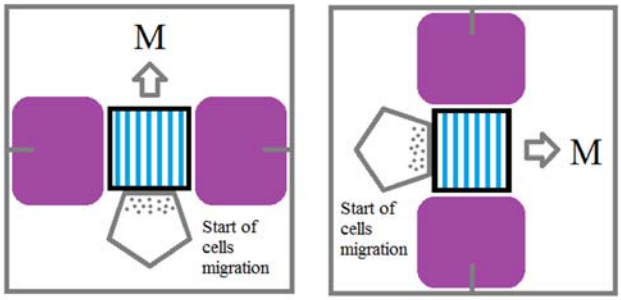


Figure 3. Two variants of arrangement of cell sample and collagen block on confocal slides. Pentagram = starting point of cells with migratory potential, blue fibers = orientation of fibers in collagen block, violet block = isolation created by silicon drop; M = myocyte or source of chemoattractants in chemoatractive chamber, arrow = direction of gradient of chemoattractants and supposed direction of cell migration.

The glass with block and chamber were inserted into the plastic holder (compatible with confocal microscope Leica TCS SP8 X), MSC was stained by biocompatible nanoparticles [8], which were upgraded by biocompatible fluorescence probes (probe with maximum of excitation/emission = 530/575 nm), cells were injected to the starting chamber (variants with 500 or 1000 cells). The “chemoattractive chamber” was filled by live or apoptotic cardiomyocyte (heat shock preparation) with drop of PDGF-BB (final difference between “starting chamber” and „chemoattractive chamber“ was 100 pg/ml). The set was incubated in 37° C and 5 % CO_2 for 6 hours and the initial scan of MSC localization (red fluorescence positive dots) was obtained by confocal microscopy. The setting of Leica TCS SP8 X confocal microscopy including HCPL APO CS2 objective with 10x magnification, and White Light Laser (WLL), which was used for the excitation by 530 nm. The tunable spectral filter was opened in emission range of 550-600 nm (Figure 4).

The original analytical utility created in Matlab was presented in [9], based on the 3D reconstruction of morphologically complex plant cell culture systems and

this analytical utility has been used also in this work. It was tested for our data sets (scan of collagen tunnel in actual experiments, scanning space maximal area 5012×6591 μm with spatial resolution of 512×512 pixels, thickness of sample 90 μm implicates the necessity of multiple-plane scanning, optimal setting was 21 horizontal plane with distance about 4.5 μm).

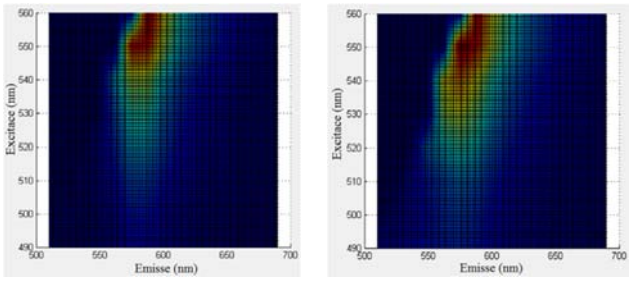
Figure 4. Setting of confocal microscope.

Then, the acquired confocal microscopy image data were exported in lossless TIFF image format for further processing. For the detecting migration cells the using version software was improved. Halving the data sets of chamber with cells has been created. It was necessary for searching the position of center of mass of aggregate consisted of all cells bodies in these sections, at start and after migration. It made possibility to calculate the distance between the centers of mass and the speed of cell migration.

Initial scan was followed by 3-days long incubation of setup with migrating cells in CO_2 incubator. At day 3 and 6 the next scans were obtained.

3. Results

First experimental prerequisite, which had to be tested before scanning of the cell migration, was stability of excitation/emission spectra of labeled MSC cells and NP (Figure 5).



A B

Figure 5. Lamda scans measured by Leica TCS SP8 X.

The detection of migrating cells can not be done without approving of the spectral stability and evaluation of adequate setting of confocal microscope detectors.

The result of the evaluation was promising. The shift of the spectrum after 6 days was minimal. Thus, the setting (530 nm / 550-600 nm) can be applied in longtime scanning experiments. Comparison of two spectral lambda scans is on Figure 5. The results of migration assay, can be divided in 3 main blocks: experiments with MSC and live myocyte (myocyte in chemoattractive chamber), experiments with MSC and apoptotic myocyte, experiments with NP and apopatoic myocytes.

The first block displays zero chemoattractive activity and locomotion to "chemoattractive chamber". The motility of the MSC was only diffusive (inside and very near the area of starting chamber), without main direction.

The second block displays significant migration in one direction (toward chemoattractive chamber). Typical scan of cell distribution in collagen block are on Figure 6 and Figure 7.

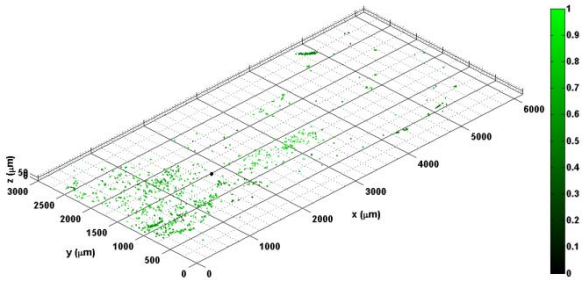


Figure 6. 3D visualization of cell migration in collagen block (generated by Matlab utility). Green dots represent position of cell bodies and blue one represents the center of mass of these cells aggregate.

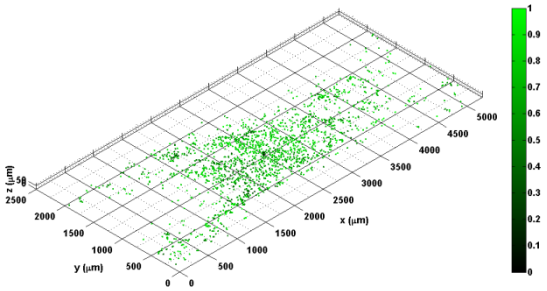


Figure 7. Same visualization for cells migration experiment in experiment hypoxic condition, The higher migration distance and migration speed is evident.

The migration distance in parallel-oriented fibers structure reached several millimeters in 3 days, and there is visible higher speed of migration after application of hypoxic atmosphere. The collagen tissue with orthogonal-oriented fibers structure display several time lower speed of migration. The statistical quantification of pathways is in Table 1.

The average speed of cells was quantified as: $V = \Delta S / \Delta t$, where ΔS is distance between center of mass of the cell cluster in starting and final position.

Table 1. Average pathway and average speed of MSC cells in 6 day migration assay.

Parallel fibers to gradient of chemoattractant. Normoxic variant	Parallel fibers to gradient of chemoattractant. Hypoxic variant	Orthogonal fibers to gradient of chemoattractant. Normoxic variant
1380 ± 938 μm	2300 ± 692 μm	700 ± 260 μm
16.5 ± 9.8 μm/h	22.5 ± 6.8 μm/h	4.2 ± 1.8 μm/h

The third block of experiments was focused to NP migration. These migrations were measured only under normoxic condition and in tissue with parallel-oriented collagen fiber to chemoattractive gradient. The disadvantage of this measurement is time-limited vitality of NP in-vitro. The scan had to be done in 24 hours after start of migration. Result is 80.0 ± 25.5 μm/h.

4. Discussion

Understanding of cell migration is critical to the mechanistic description of physiology and pathology, and also to the design of modern curative methods. In this work we have developed hardware and software for tracking of cells migration (MSC and NP) in collagen phantom tissue. The results show that orientation of collagen fibril has the main impact to cell speed, these corresponds with experimental and in-silico analysis of another cell types in several recent publications [10]. Quantification of speed constant for different physiological condition will be continued. Our future aim is integration of basic equation of single cell´s migration distance $dS = v\,(coll, stim, int\text{-}en)\cdot dt$ to the software simulator of massive cell infiltration to the infarcted heart (where coll = local parameters of collagen concentration and angle of collagen fiber, stim = gradient of chemoatractants and inhibitors of migration, int-en = parameters of internal energy deposit in cell and cell metabolical activity).

Acknowledgment

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