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Research article

Spatial arrangement of the heart structure: Application of second-order stereology in diabetic rats

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ABSTRACT

The present study explored three-dimensional spatial arrangements of the cardiomyocytes and microvessels within the heart of rats and evaluated the arrangement for differences after diabetes using second-order stereology. Isector method was applied to obtain isotropic uniform random sections of the heart. The pair correlation $g(r)$ and cross-correlation functions were estimated by counting dipole probes (with length of 3.57 μm) superimposed on histological sections of the heart. The co-variograms indicated that the curve of $g(r)$ for the cardiomyocyte showed a gap between cardiomyocytes at r = 21–25 μm in the control rats and a wider gap at r = 18–50 μm in diabetic hearts. Estimates of $g(r)$ for the vessels also showed a wider gap (at r = 25–39 μm) in diabetic hearts compared to the control rats (r = 25–32 μm). These indicate a negative correlation (repulsion) between the cardiomyocytes and microvessels in the diabetic hearts. Evaluation of the cross-correlation function of the cardiomyocytes and microvessels showed that at 32–36 μm, both structures had a negative correlation in the control group, but not in the diabetic rats.

Conclusion: Dissociation of the cardiomyocytes at some places can be seen in diabetic heart. This can be seen also in microvessels. Neither cardiomyocytes nor microvessels are arranged normally after diabetes.

1. Introduction

In general, cardiovascular disease is more prevalent among the patients with diabetes in comparison to the non-diabetic population. Pathological, physiological, and molecular bases of this condition have received great attention (Mandavia et al., 2012). Hitherto, to the best of our knowledge, there has been no quantitative information concerning the three-dimensional arrangement of cardiomyocytes, microvessels, and connective tissue. First-order stereological methods include estimation of the numbers, volume, surface, and length of the histological parameters. However, with the development of modern quantitative methods, such as second-order stereology, it is possible to evaluate various descriptors of spatial arrangement, including covariance, $C(r)$, and pair correlation function, $g(r)$ (Reed et al., 2010; Reed and Howard, 1999; Krasnoperov and Stoyan, 2006, 2004; Mattfeldt et al., 2006).

Second-order stereology has been used in evaluation of some tissues after different normal or pathologic conditions. Spatial arrangement of the of the human mammary gland in benign and malignant conditions (Mattfeldt et al., 1993), organelles in the salivary gland acinar cell (Mayhew, 1999a), microglia in the mouse hippocampus (Jinno et al., 2007), oligodendrocytes in the cingulum bundle and superior frontal gyrus in schizophrenia (Segal et al., 2009; Hof et al., 2003), the villi of the placenta (Mayhew, 2006) and dorsal root ganglion cells after sciatic nerve crush (Noorafshan and Omidi, 2011) are the examples. There are limited studies on diabetic tissues including the spatial arrangements of tissue compartments within glomeruli of normal and diabetic kidneys (Mayhew, 1999b), but, to our knowledge the spatial arrangement of the heart structure in diabetic models has not been evaluated.

Covariance is a measure of how much two variables change together. Pair correlation function is related to the probability of exploring the center of a particle at a given distance from the center of another particle. For short distances, the parameter is related to how the particles are packed or clustered together (Fig. 1) (Crocker and Weeks). For example, if some hard spheres, like marbles, are considered, the spheres cannot overlap; therefore, the closest distance the two centers can be is equal to the diameter of the spheres.

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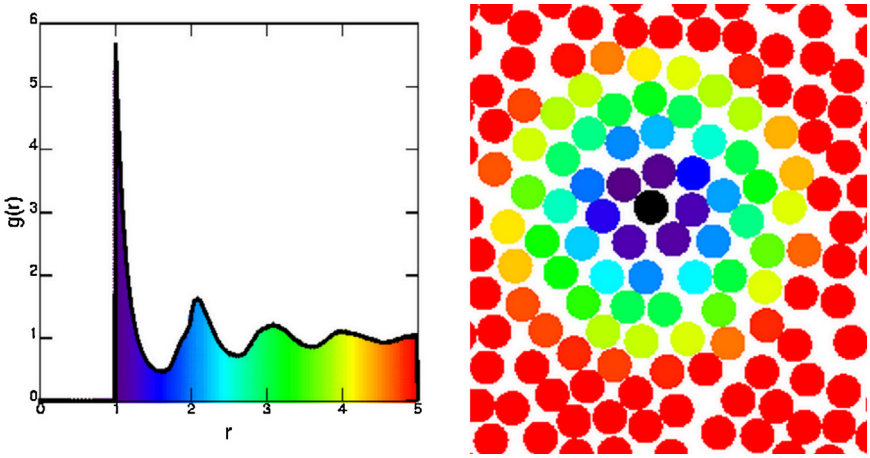

Fig. 1. Concept of pair correlation function. Left. It shows $g(r)$ calculated for a simple simulation of two-dimensional disks. The function is calculated based on all pairs of the particles, but to make it clear, one reference black particle in the right picture has been highlighted. Right. The surrounding particles are colored based on their distance from the black particle. Five particles are colored purple and correspond to the prominent peak at a separation of 1 diameter. A couple of particles are dark blue corresponding to the less-likely position about 1.5 diameters away. Several more particles are light blue corresponding to the 2nd nearest neighbor peak at about 2 diameters. Further away, the green particles form the third nearest neighbors, while the yellow particles form the fourth nearest neighbors. All of the particles farther away are colored red and $g(r)$ tends toward a uniform value of 1 for great values of r. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
Source: Reproduced from http://www.physics.emory.edu/~weeks/idl/gofr.html.

When the layers of the particles show a more diffuse form, the probability of finding two particles with a given separation is essentially constant. In this case, it is related to the density (Crocker and Weeks). A more dense system has more particles and, consequently, it is more likely to find two of them with a given distance. Therefore, the pair correlation function, $g(r)$, accounts for these factors by normalizing by the density; thus, at large values of r, it goes to 1; i.e., uniform probability (Reed et al., 2010; Reed and Howard, 1999; Krasnoperov and Stoyan, 2006, 2004; Mattfeldt et al., 2006, 1993; Mayhew, 1999a,b).

In the case of heart structure, clustering of the cardiomyocytes, microvessels, and connective tissue could be evaluated by computing the pair correlation function. Heart is mainly a muscular structure and it needs microvessels for its blood supply. It is of great importance to explain the normal spatial arrangement of the above-mentioned structures of the heart and also any possible changes after diabetes. Using this approach, we can explore the covariance of a volumetric particle at a distance of "r" units into the reference space. The pair correlation function $g(r)$ explains the arrangement by normalizing by the density. Therefore, at great values of r, it moves toward 1; i.e., a uniform probability. The term cross-correlation function is often used to show how different particles are clustered or dispersed together (Reed et al., 2010; Reed and Howard, 1999; Krasnoperov and Stoyan, 2006, 2004; Mattfeldt et al., 2006, 1993; Mayhew, 1999a,b).

The present investigation extends the earlier works by comparing the arrangement of the cardiomyocytes, microvessels, and connective tissue within the diabetic heart of the rats. It is known that diabetes affects certain aspects of heart morphology, including cell number, volume, and surface (Noorafshan et al., 2013). Therefore, the present research aims to evaluate the effects of experimental type I diabetes on the spatial arrangement of cardiomyocytes, microvessels, and connective tissue in the animal model of streptozotocin injected rats.

2. Materials and methods

2.1. Experimental animals and diabetes induction

Ten adult male Sprague-Dawley rats weighing 200–250 g were divided into two groups of five rats. The control and experimental animals received only vehicle (citrate buffer) and streptozotocin (60 mg/kg) dissolved in citrate buffer, respectively (Kim, 1994). All the rats were housed under standard conditions at constant temperature and a 12-h light/dark cycle with free access to food and water. All the procedures were approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran.

2.2. Sectioning of the heart

After 2 months, the animals were sacrificed and their hearts removed and immersed in neutral buffered formaldehyde. The rats with blood glucose greater than 300 mg/dl were considered diabetic (Kim, 1994). The heart was embedded in a sphere and randomly sectioned into the slabs according to the isector method (Nyengaard and Gundersen, 1992). Isector was used to have isotropic uniform random sections which are necessary for estimating the pair correlation and cross correlation function (Fig. 2). Overall, 8–12 slabs were obtained and embedded in a paraffin block.

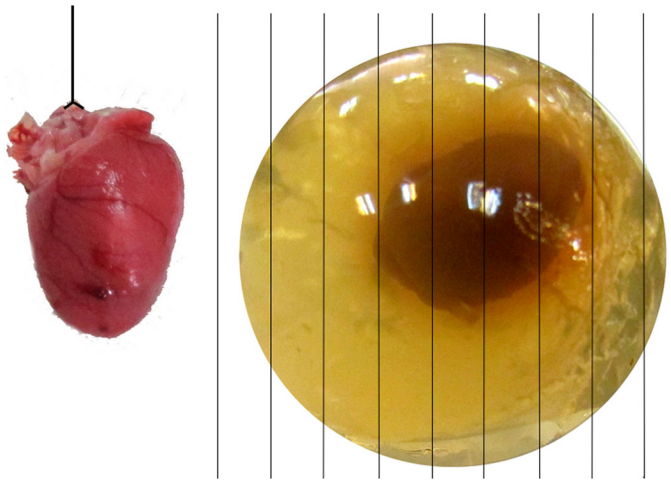

Fig. 2. Isector method for generating the isotropic uniform random sections. The heart was suspended using a thread (left) and embedded in a spherical block and rotated randomly on the laboratory bench. Then, it was cut done in the random orientation of the sphere.

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