An Approach for Improvement of Carbon Fiber Technique to Study Cardiac Cell Contractility

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Abstract. The technologies to study cardiac cell mechanics in near-physiological conditions are limited. Carbon fiber (CF) technique is a unique tool to study single cardiomyocyte contractility. However, the CF adhesion to a cell is limited and it is difficult to control CF sliding occurred due to inappropriate adhesion. In this study, we present a CF adhesion quality index – a linear coefficient (slope) derived from "end-diastolic cell length - end-diastolic sarcomere length" relationship. Potential applicability of this index is demonstrated on isolated rat and guinea pig ventricular cardiomyocytes. Further improvement of the approach may help to increase the quality of the experimental data obtained by CF technique.
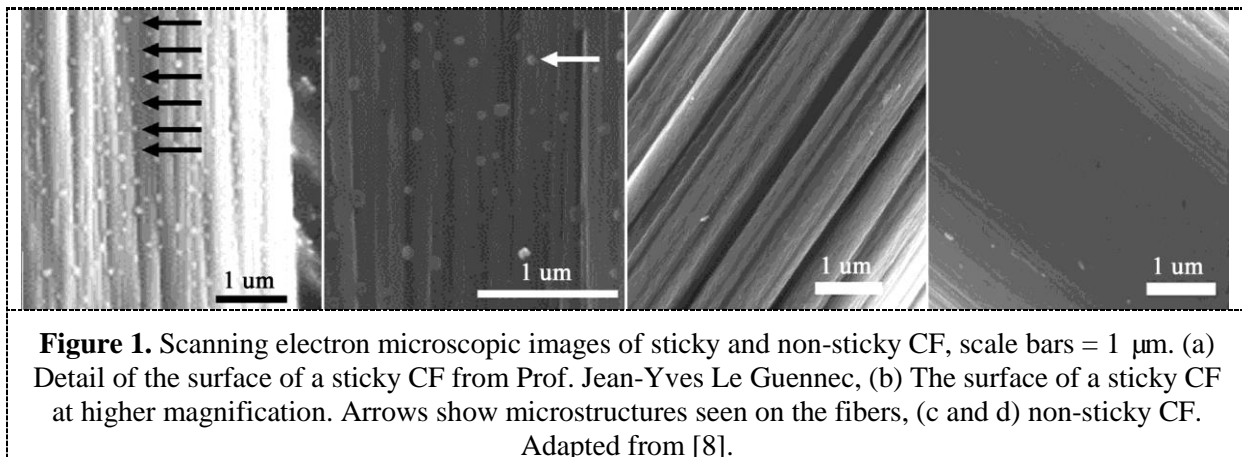
1. Introduction

The heart is a mechanically functioning blood pump and many studies on cardiac mechanics are focused on pressure-volume relation parameters. Increased hemodynamics (volume and load) is associated with increased wall tension, stretching cardiac cells and their sarcomeres. Isolated cardiomyocytes are an important object to study cardiac mechanics in details on cellular and subcellular levels, because they are free from connective tissue, which cause significant modification of tissue contractile properties.

Currently, the tools to study cardiomyocytes contractility in near-physiological conditions are limited, and the majority of research on single intact cardiomyocytes is conducted on mechanically unloaded cells [1]. There are few technologies to apply defined mechanical extensions to cells: hypoosmotic stress approach [2], stress applied by a glass stylus [3], cell-in-a-gel approach [4], and carbon fiber (CF) technique [5-7].

Le Guennec and co-workers were among the first to use thin 12 μm diameter CFs that were produced by high temperature treatment of polyacrilonitryl and oxidized to produce free carboxyl groups. These fibers stick to the cell membrane without the use of glue, probably because of microstructures at the surface of CF (Figure 1a and b) that interact with the amino groups of the membrane surface. Note that smooth fibers without microstructures (Figure 1c and d) do not stick to the cardiomyocytes [8]. This approach allows attachment of carbon fibers of known stiffness to both ends of a cardiomyocyte. CF-based stretching gives rise to a relatively homogeneous increase in sarcomere length (SL) and allows calculation of passive and active forces from optically monitored changes in CF bending [9].

Figure 1. Scanning electron microscopic images of sticky and non-sticky CF, scale bars = 1 μm. (a) Detail of the surface of a sticky CF from Prof. Jean-Yves Le Guennec, (b) The surface of a sticky CF at higher magnification. Arrows show microstructures seen on the fibers, (c and d) non-sticky CF. Adapted from [8].

More recently, to obtain better CF adhesion, graphite-reinforced CFs of higher stiffness were processed by carbonization of rod-shaped graphite granules and resin oligomer mixtures [10]. A higher stiffness allowed recording of higher forces at a small displacements increasing the sensing range.
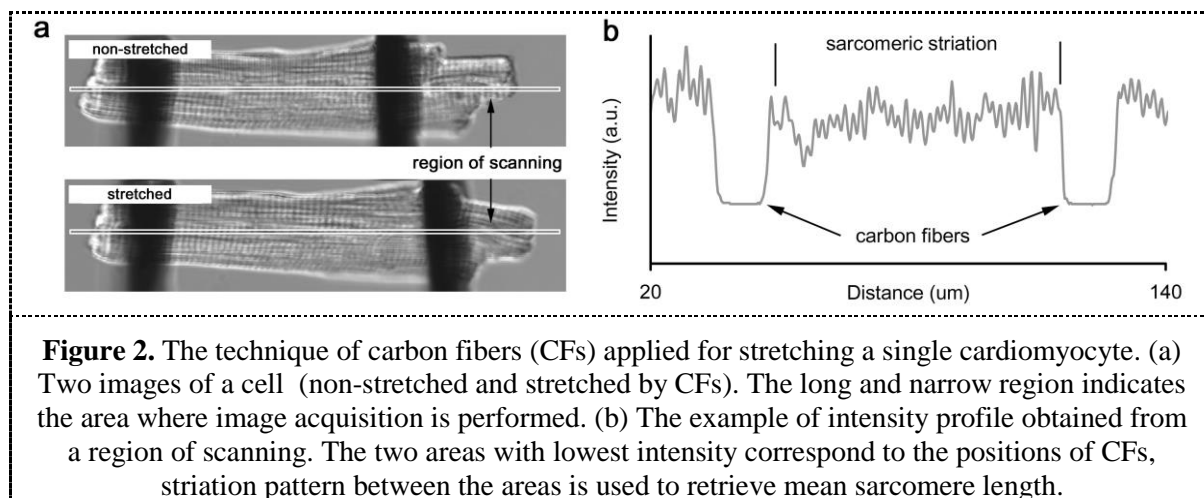
A modification of CF approach – a new CF 'gripping' technique where both ends of a cardiomyocyte are tightly squeezed between a pair of CFs was recently developed by Iribe and co-workers [6]. This improved technique allows one to increase stretch range in isolated cardiomyocytes from ≈ 4% to 16% in SL compared to conventional CF method.

A common limitation of CF technique is the lack of control of CF sliding relative to the cell surface. Since in the CF method the cell length is determined optically as the distance between the fiber edges the sliding causes incorrect recordings of the stretched cell length that does not correspond to changes in SL estimated using Fourier Transform. In this study, we present an approach to assess CF sliding defining a CF adhesion index. We believe that this method and its further improvement can be used to increase the quality of experimental data obtained by CF technique.
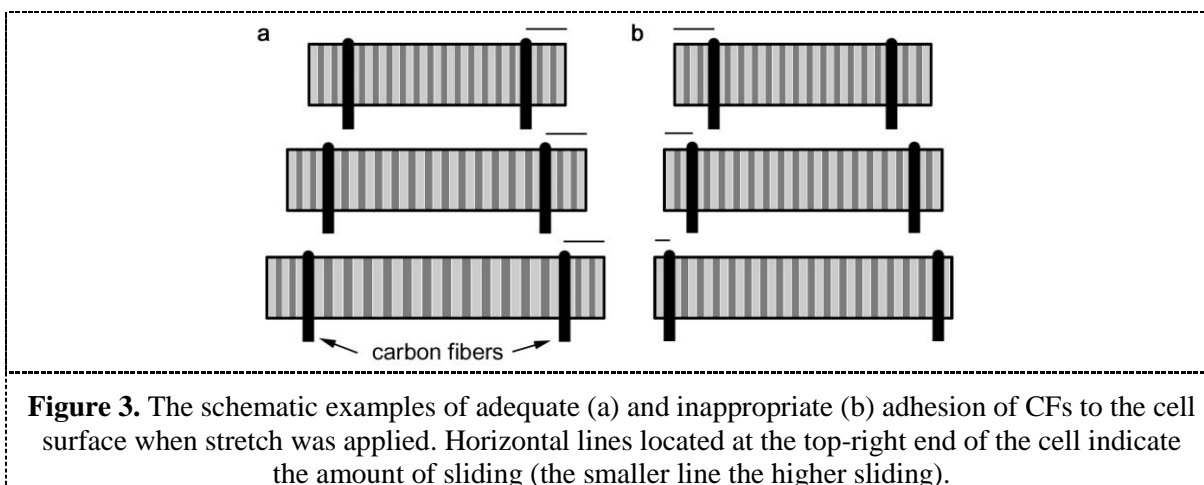
2. Materials and methods

The single myocyte stretch system is described in detail elsewhere [5]. Briefly, thin borosilicate capillaries were pulled from glass tubes and fibers were mounted in the fine tip of the capillaries. The narrow end of the capillary was thermally bent by 45° to allow parallel alignment of fibers with the bottom of the perfusion chamber. The wider end of the glass tube was mounted in the holder of a computer-controlled piezoelectric transducer (PZT) for precise positioning of CF tips. Then two CFs were attached to both cell ends from the top surface of the cell to apply axial stretch to the cell (Figure 1a). Both CFs received the same control command to achieve identical CF position control for stretching the cell end. All experiments were carried out at a stimulation frequency of 1 Hz at room temperature.

The measurement of mechanical activity of isolated cardiomyocyte was performed by the laser confocal scanning microscopy system LSM 710 (Carl Zeiss, Germany) and the software developed for fast acquisition of cell image (ZEN 2010, Calr Zeiss, Germany). Prior to the measurement, a narrow region of scanning was selected on cell image, assuming it contained both CFs (Figure 2a). The intensity profile within the region was then acquired continuously every 1-3 msec. The distance between the CFs was calculated in this profile from the two areas with lowest intensity while striation pattern between the areas was further analyzed to reconstruct mean sarcomere length on the basis of Discrete Fourier Transform (Figure 2b).

Figure 2. The technique of carbon fibers (CFs) applied for stretching a single cardiomyocyte. (a) Two images of a cell (non-stretched and stretched by CFs). The long and narrow region indicates the area where image acquisition is performed. (b) The example of intensity profile obtained from a region of scanning. The two areas with lowest intensity correspond to the positions of CFs, striation pattern between the areas is used to retrieve mean sarcomere length.
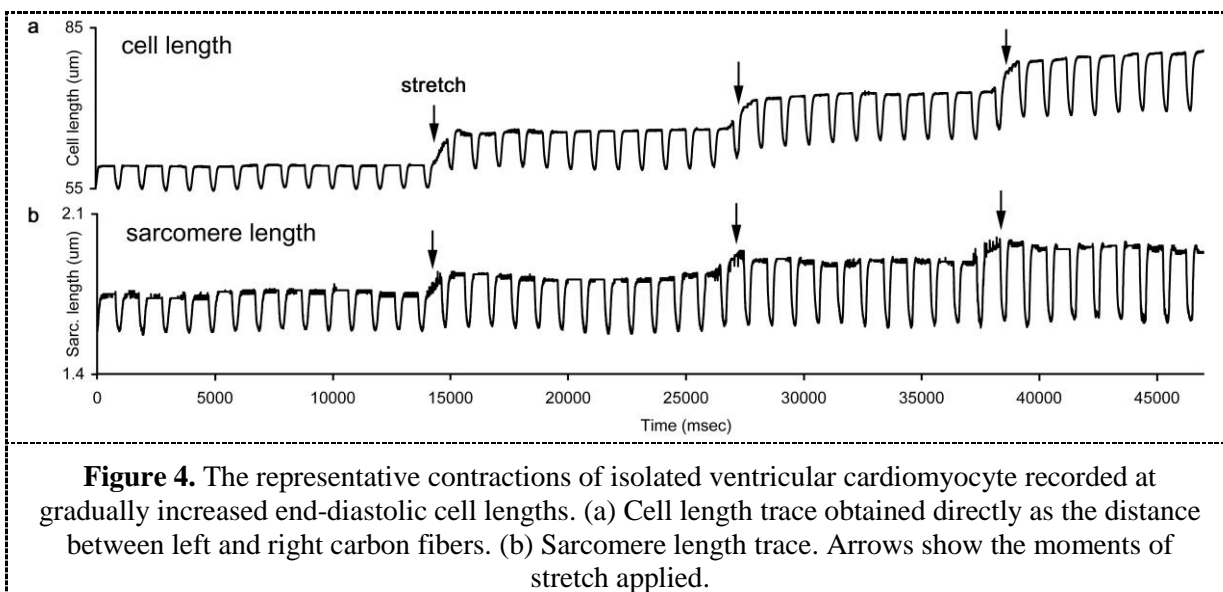
It should be noted that the effectiveness of CF technique strongly depends on the quality of CFs adhesion to cell surface. Figure 3 shows the schematic examples of adequate and inappropriate adhesion of CFs to cell surface. In some cases, adhesion of CFs to the cell surface was sufficiently good to apply adequate stretch to cardiomyocytes and their sarcomeres, i.e. CF sliding to the cell ends is not observed (Figure 3a). In other cases, CF adhesion was not appropriate, so one could see gradual sliding of CFs to the edges of the cell (Figure 3b) when stretch command by PZTs was applied. As provided by the example in Figure 3, the measured cell length, if evaluated as a distance between two CFs, increases with the stretch command in the same extent for both cases. However, in the case of inappropriate CFs adhesion the extent of sarcomere stretch is obviously deficient compared to the measured cell length. In this case, the actual cell length, if obtained directly from the measured length, is overestimated, while actual sarcomere length is much lower than it can be expected from the measured cell length.

Figure 3. The schematic examples of adequate (a) and inappropriate (b) adhesion of CFs to the cell surface when stretch was applied. Horizontal lines located at the top-right end of the cell indicate the amount of sliding (the smaller line the higher sliding).
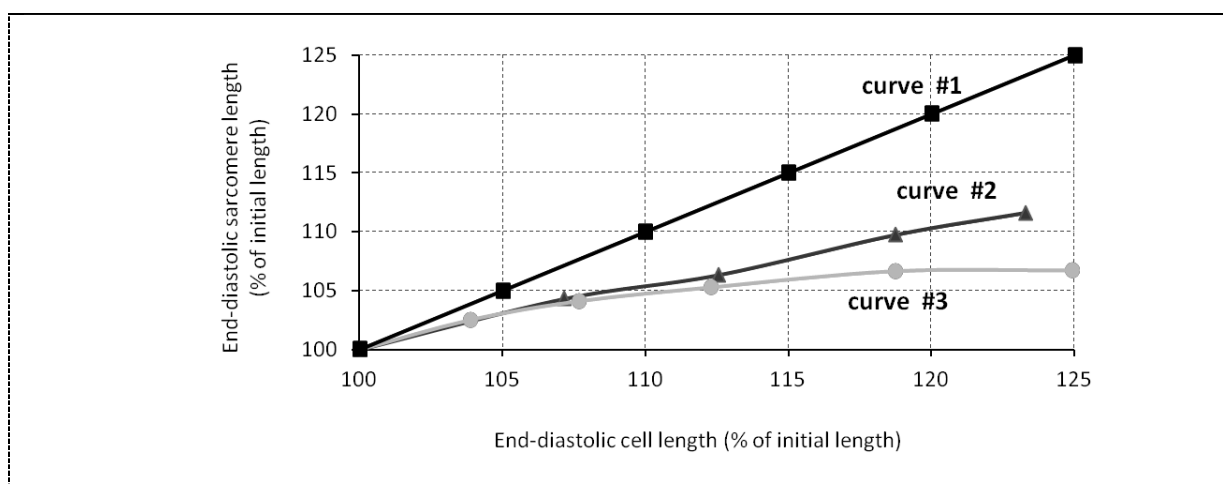
3. Results

Figure 4 shows representative contractions of isolated ventricular cardiomyocyte at gradually increased end-diastolic cell lengths (upon stretch by CFs). The upper trace, i.e. cell length, was obtained directly from the positions of left and right CFs (Figure 4a). The bottom trace, sarcomere length, was retrieved from striation pattern between the two CFs (Figure 4b). As one could see in Figure 4, the extent of cell stretch is somewhat higher than that of sarcomere stretch.

Figure 4. The representative contractions of isolated ventricular cardiomyocyte recorded at gradually increased end-diastolic cell lengths. (a) Cell length trace obtained directly as the distance between left and right carbon fibers. (b) Sarcomere length trace. Arrows show the moments of stretch applied.

In order to estimate the possible discrepancy between the amount of cell stretch and SL stretch, we analyzed the "end-diastolic cell length - end-diastolic SL" relationship in ventricular cardiomyocytes isolated from the rat heart (n = 221) and the guinea pig heart (n = 67). For most cells the dependence had a highly linear shape, so each of them was approximated by a linear function y = kx + b.
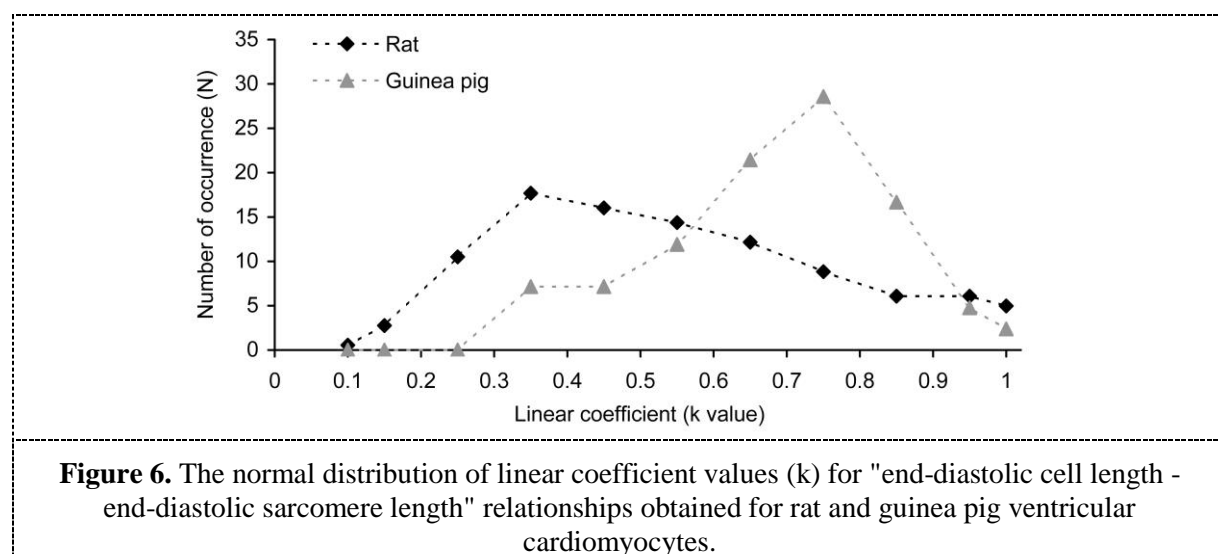
Figure 5 shows representative "end-diastolic cell length - end-diastolic SL" relationships, expressed as a percent of the initial (non-stretched) cell length / initial SL. A theoretical curve has a linear coefficient k = 1, so the stretch amount of the cell strictly corresponds to the stretch amount of its sarcomeres (Figure 5, curve #1). For some cells we observed that an increase in the cell length was always greater than in its sarcomeres but by a constant factor at all displacements (k < 1, Figure 5, curve #2); in this case the curve remains highly linear. For some cells we observed a gradual non-linear deviation from the theoretical curve (k < 1, Figure 5, curve #3); in this case the amount of CFs sliding increased non-proportionally with the extent of stretch command and the curve has highly non-linear behavior.

Figure 5. Representative "end-diastolic cell length - end-diastolic SL" curves. Curve #1 represents a theoretical curve (with a linear coefficient k=1), curve #2 displays a proportional deviation from the theoretical curve and curve #3 displays a gradual non-linear deviation from the theoretical curve; curves #2 and #3 have a linear coefficient k<1.

Prior to the selection of cells for further analysis, the following criteria were applied for the linear regression approach:

- the number of stretch steps for each cell was ≥ 3;
- the maximum percent of cell stretch relative to its initial length was ≥ 10%, so this allowed us to assess the CF adhesion at large displacements;
- the coefficient of determination for linear regression for each cell was ≥ 0.9.

Accordingly to the criteria, further analysis was carried out for n = 181 rat cardiomyocytes and n = 42 guinea pig cardiomyocytes. For each cell, a linear coefficient of "end-diastolic cell length - end-diastolic SL" relationship was calculated and used as a quality index of CF adhesion to the cell. Further, the distributions were checked for normality. We found that mean value of the linear coefficient was ~0.3 for the rat cardiomyocytes and ~0.7 for the guinea pig cells (Figure 6) which means that CFs sliding was observed almost in all experiments.

Figure 6. The normal distribution of linear coefficient values (k) for "end-diastolic cell length - end-diastolic sarcomere length" relationships obtained for rat and guinea pig ventricular cardiomyocytes.

4. Conclusions

CF approach remains a unique and sophisticated technique to study single cardiomyocyte contractility when cells exposed to mechanical load (applied viastretch in our study). A limitation of this technique is the absence of overall control of CF sliding relative to the cell surface, which causes a deviation of the cell length changes against its sarcomeres. In this study, we presented a method to assess CF sliding using a CF adhesion quality index, which can be defined as a linear coefficient from "end-diastolic cell length - end-diastolic SL" relationship. The next step for the development of the method should be an assessment of the allowable range of the index values in order to analyze cell contractility eliminating the cells with huge CF sliding from the data samples but to do not reduce to zero the success rate and to get experimental data using such limited CF technique in itself.

5. Acknowledgements

6. References

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