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Electrophoresis of Myocardial Cells

Ying Zhou

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1. Introduction

Mammals are composed of a large number of surface charged cells. The cell structure and functions in different tissues and organs are different. Through the method of cell electrophoresis the information of the cell surface structure can be obtained and is valuable for the function study of cells, tissues and organs. Many studies have been reported on the fluidic electric phenomena of cells (Ertan & Rampling, 2003; Aki et al., 2010; Brown et al., 1985; Pimenta & de Souza, 1982), but quite limited on the separation of myocardial cell electric phenomena possibly due to the short of myocardial cell electrophoresis technology to conduct the experiments. The methods only observing through separation and investigation of myocardial cell surface complex sugars and plasma membrane phospholipid composition on the cell contraction within the ion flow (inward ionic current) may not actually take the cell membrane and membrane structure as a whole but insularly highlight the single component in achieving cardiac function. Integration is not equal to the simple sum of the single components. The heart is the vital organs of humans and animals, its interfacial electric phenomena (such as electrocardiogram) and its response to the pacemaker have revealed the hints on the close relationship between the myocardial cell membrane structure and function of the heart (Podrid et al., 1995). For this reason, electric phenomenon of the myocardial cells was systematically studied. Although electrophoresis does not directly measure the cell surface charge density and zeta potential, it can help to find a trace to elucidate the mentioned relationship by exploring the classic knowledge of colloidal particles and to set up a electric double layer model for insight into the structural characteristics of the myocardial cell surface and their variations. It opens a way to realize the mechanism in respect of the cardiac function. We found that the myocardial cell is rich and complex internal and external membrane structure, and the distribution and variation of the adsorbed ions on cell surfaces are completely different from the colloidal particles, these should be the basis for completion of the cardiac function.

2. Experimental

2.1. Cell preparation

Ventricular myocardial cells were isolated from adult Sprague-Dawley rats (2–3 months old, weight 225–300 g) using standardized enzymatic techniques (Zhou et al., 2000). Freshly isolated single cells were stored in Tyrode's solution containing (in mM) 137 NaCl, 5.4 KCl, 1.2 MgCl₂, 1 NaH2PO₄, 1 CaCl₂, 20 glucose, and 20 HEPES (pH 7.4). (Fig. 1)
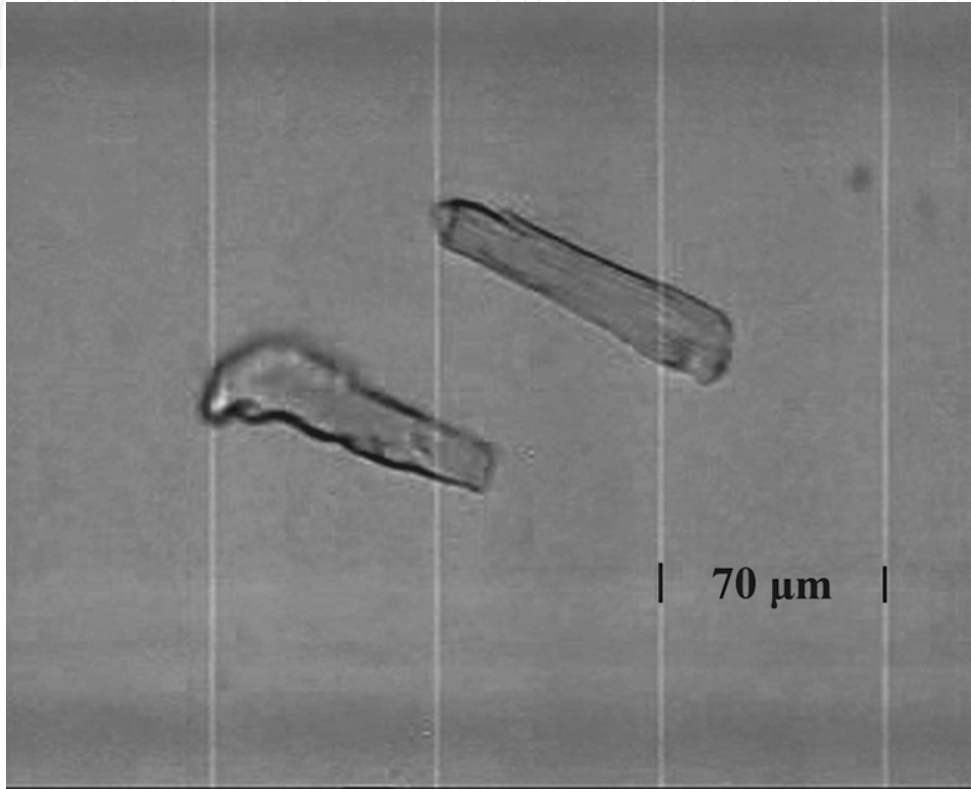

Figure 1. Photo of isolated myocardial cells under low power lens.

2.2. Preparation of cell suspensions

Cell suspensions were prepared in isotonic solutions composed of an aqueous glucose solution and 60%, 30%, 15%, 10%, 7.5%, 5% and 2.5% (v/v) of a stock solution of 145 NaCl and 2.97 CaCl₂ (referred to in Table 1). To adjust the transmembrane potential, which is determined primarily by the K⁺ transmembrane equilibrium potential (Fozzard et al., 1991), K⁺ was added at a required concentration depending on the potential needed which can be calculated using the Nernst equation: $E_K = -\dfrac{RT}{F}\ln\left(\dfrac{c_{Kin}}{c_{Kout}}\right)$, where E_K is the K⁺ transmembrane equilibrium potential, R is the universal gas constant, T the absolute temperature, F the Faraday, and c_{Kin} and c_{Kout} are the concentration of K⁺ inside and outside the cell, respectively (Liu, 2005). Aqueous Dextran 40 (free of ion, transmembrane potential not known) was from Shijiazhuang No.4 Pharmaceutical (China). A proper amount of

hydroxypropyl methyl cellulose (HPMC) was added into all suspensions to adjust the viscosity of the final suspensions at 3.8 mPa·s (24°C, Table.1).

2.3. Removal of sialic acid from myocardial cells

Myocardial cells were suspended, at 0.5%(v/v), in the Tyrode's solution containing 0.25U/mL neuraminidase (Sigma Chemical, St. Louis, Mo) at pH 6.0, continually shaken at 37°C for 90 minutes, and then washed with the Tyrode's solution (Post, 1992) .

2.4. Procedure of electrophoresis

The prepared myocardial cells were washed three times with the suspending medium and then suspended in the same medium at 0.1% (v/v) measured at 24°C. The used media were collected in Table 1 where the No. 10-14 solutions were used for the suspension of enzyme-treated myocardial cells while the No. 1-9 for non-treated cells. The suspended cells were loaded on a cell electrophoresis system (Beijing Warder Biomedicine Instrument Company, China) and electrophoresed, within 2-3 minutes, at 2-7V/cm depending on the ionic strength of the suspension. Only the perfect cells were recorded. Each sample solution was determined in parallel for 12 times. All the measurements were finished within eight hours after the myocardial cells were isolated.

№	Ionic strength mM	Glucose mM	Dextran mM	E_K [d] mV	HPMC mM	Viscosities mPa.s	HEPES mM	pH
1	154~163 [a]	5		-96.8~-61.9	0.57	3.8	20	2.5~7.35
2	93.6~102.6 [a]	108.1		-96.8~-61.9	0.54	3.8	20	2.5~7.35
3	48.3~57.3 [a]	180.1		-96.8~-61.9	0.52	3.8	20	2.5~7.35
4	25.7~34.7 [a]	214.5		-96.8~-61.9	0.5	3.8	20	5.0~7.35
5	18.1~27.1 [a]	227.1		-96.8~-61.9	0.5	3.8	20	5.0~7.35
6	14.3~23.3 [a]	233.4		-96.8~-61.9	0.5	3.8	20	5.0~7.35
7	10.6~19.6 [a]	239.7		-96.8~-61.9	0.5	3.8	20	5.0~7.35
8	6.8~15.8 [a]	246		-96.8~-61.9	0.5	3.8	20	5.0~7.35
9	151 [b]	8		0	0.57	3.8	20	5.0~7.35
10		252	1.5		0.2	3.8	20	5.0
11	12.6 [c]	239.7		-83.9	0.5	3.8	20	5.0
12	20.1 [c]	227.1		-83.9	0.5	3.8	20	5.0
13	156 [c]	5		-83.9	0.57	3.8	20	5.0
14	151 [b]	8		0	0.57	3.8	20	5.0

a) Total-3~12mM KC=96%NaCl+4%CaCl$_2$.

b) 140mM KCl+5mM NaCl+3mM CaCl$_2$.

c) Total-5mM KCl=96%NaCl+4%CaCl$_2$.

d) Theoretical value.

Table 1. The suspensions used in this study.

2.5. Note

2.5.1. Cell separation should be to reduce the loss of the charged matter on cell surface

a. Single collagenase (collagenase II, 1mg/ml) can be used as the first choice for myocardial cell separation enzyme; b. enzymatic digestion time of myocardial tissue control in about 15 minutes (not more than 20 minutes), c. the same experimental animals age and enzyme digestion time should be consistent.

2.5.2.

Select the electrophoresis tank stationary layer as the level of observation and measurement of the electrophoretic velocity, to ensure the accuracy of the measured values.

2.5.3.

When the high ionic strength suspensions were studied, the electrophoresis voltage is better selected an proper high value in compromising with the electrophoresis time (making the cells migrate at <10μm • s-1).

2.5.4.

The experiment should be completed within 6-8 hours after the cell separation, to ensure the normal activity of the cells.

3. Data processing and interpretation

Myocardial cells showed different features under an electric field depending on the nature of the suspensions used. Although the cells may contract at high ionic strength or may not at low ionic strength, the impact of the contraction on the electrophoretic speed did not affect the analysis and judgment of experimental results measured from different conditions. Commonly, myocardial cells show negative charges in the electric field. But their electrophoretic speed may have obvious difference even in same a suspension, suggesting that the composition of the charged layer on myocardial cell membrane is variable. In fact, cells were possibly affected by enzyme action (time-dependent), mechanical damage and so on in the preparation. However, this also did not impact on the judgment of variation tendency of the cell mobility.

In the case that the type and proportion of adions on cell surface were maintained, the cell electrophoretic mobility (EPM) showed a zigzag increase (Fig. 2A). At an ionic concentration from 9.7 to 33.2mM, no electromigration of some cells were observed at some conditions, mostly at pH 5.0 and 6.0, rare at pH 7.35 (Table 2). However, reversed electromigration has not yet been observed. The variation of the mobility was largely dependent of the suspension pH. The mobility was found to be in common greater at low pH (2.5-4.5) than at high pH (4.5-7.35) with the minimum at pH 5.0 (Fig. 2C). The mobility commonly decreased as pH increased but changed to fast increase when pH value was above 7.0. The curves of

the EPM against pH values under the conditions of the same transmembrane potential and different ionic strength was basically the same as (Fig. 3).
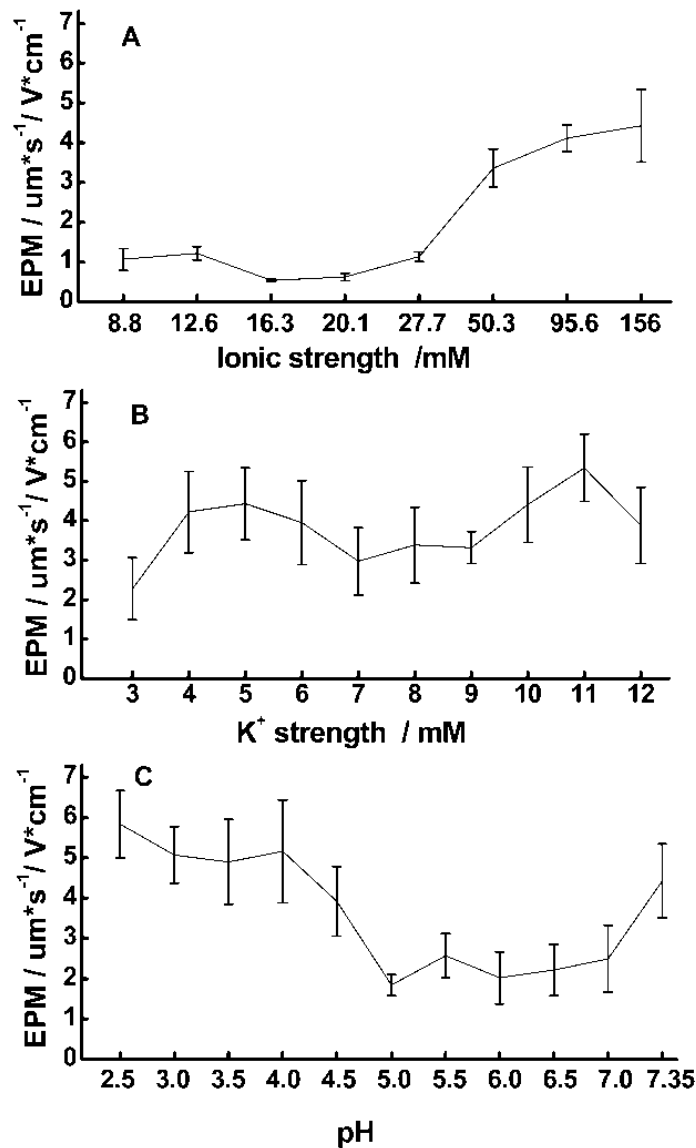

Figure 2. A: Zigzag increase of EPM measured in the suspensions of 5 mM K+, pH 7.35 and ionic strength 8.8-156mM (P<0.01). B: an obvious zigzag increase of EPM in the suspension of 5mM K+ at 156mM ionic strength and pH 2.5-7.35 (P<0.01). C: increasing tendency of EPM in the suspensions of ionic strength 151-160mM, pH 7.35 and K+ strength 3-12mM (P<0.01).

The zigzag increase of cell mobility was also measured when the transmembrane potential or K+ increased (from 3 to 12mM K+, Fig. 2B). This suggests that the electromigration of myocardial cells may be imposed largely by the transmembrane potential. Such a zigzag variation of mobility was found in different conditions with somewhat similar features except for some point as shown in Figure 4. Myocardial cells tended to die at the ionic strength < 6.7mM and pH < 5.0 or at the ionic strength <151mM and pH < 2.5. The death could largely be avoided when the cells were suspended in Dextran solution at pH≥5.0.

K+ strength	Ionic strength	pH		
mM	mM	5.0	6.0	7.35
3	10.4	4	0	5
	25.2	0	6	0
4	11.4	0	6	0
5	12.4	0	4	0
	16.1	0	5	0
6	9.7	5	0	0
8	11.7	4	0	0
	15.4	7	0	0
9	16.4	3	0	0
	20.1	0	4	0
	23.8	0	6	0
10	13.7	6	0	0
	21.1	0	4	0
	24.8	4	0	0
11	25.8	0	4	0
	33.2	0	7	0
12	19.4	6	0	0

Table 2. Cells with zero mobility found in various experimental groups
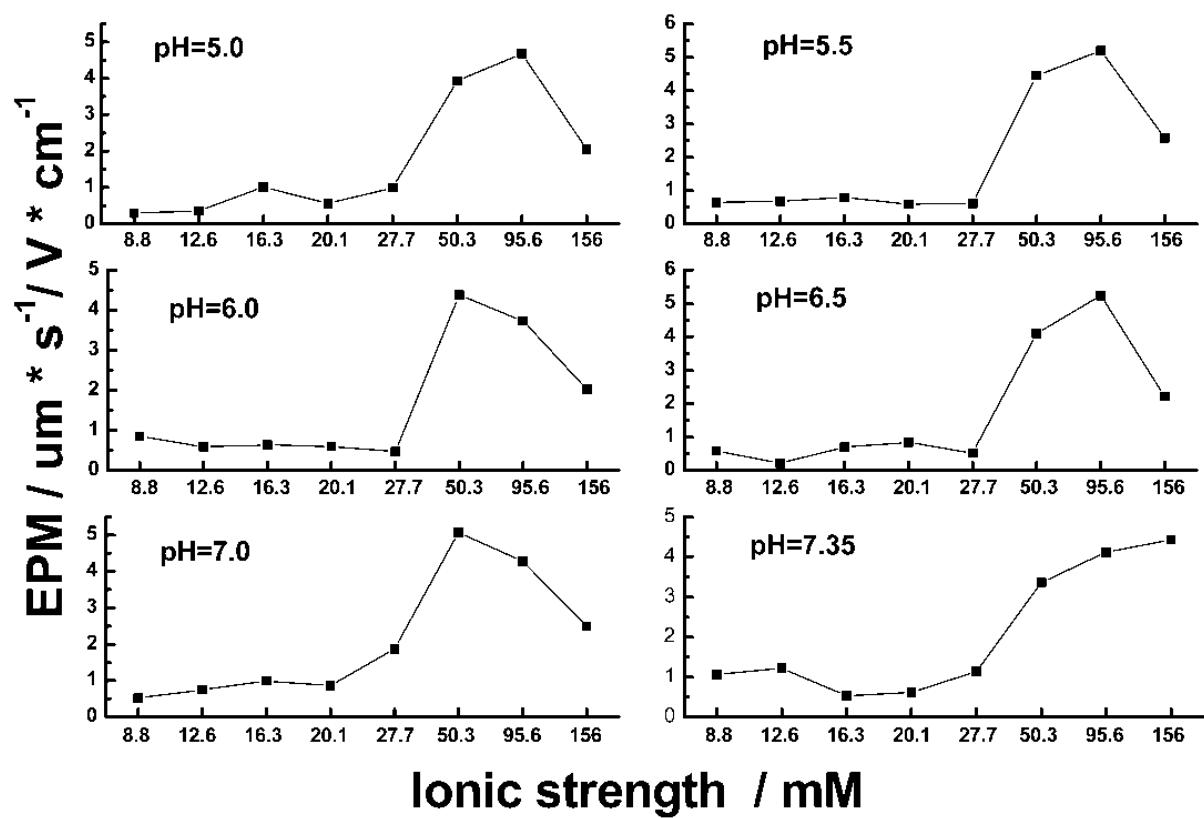

Figure 3. Plot of EPM against ionic strength measured in the suspensions of 5 mM K+ and 8.8-156mM ionic strength at pH values as shown in the figure.
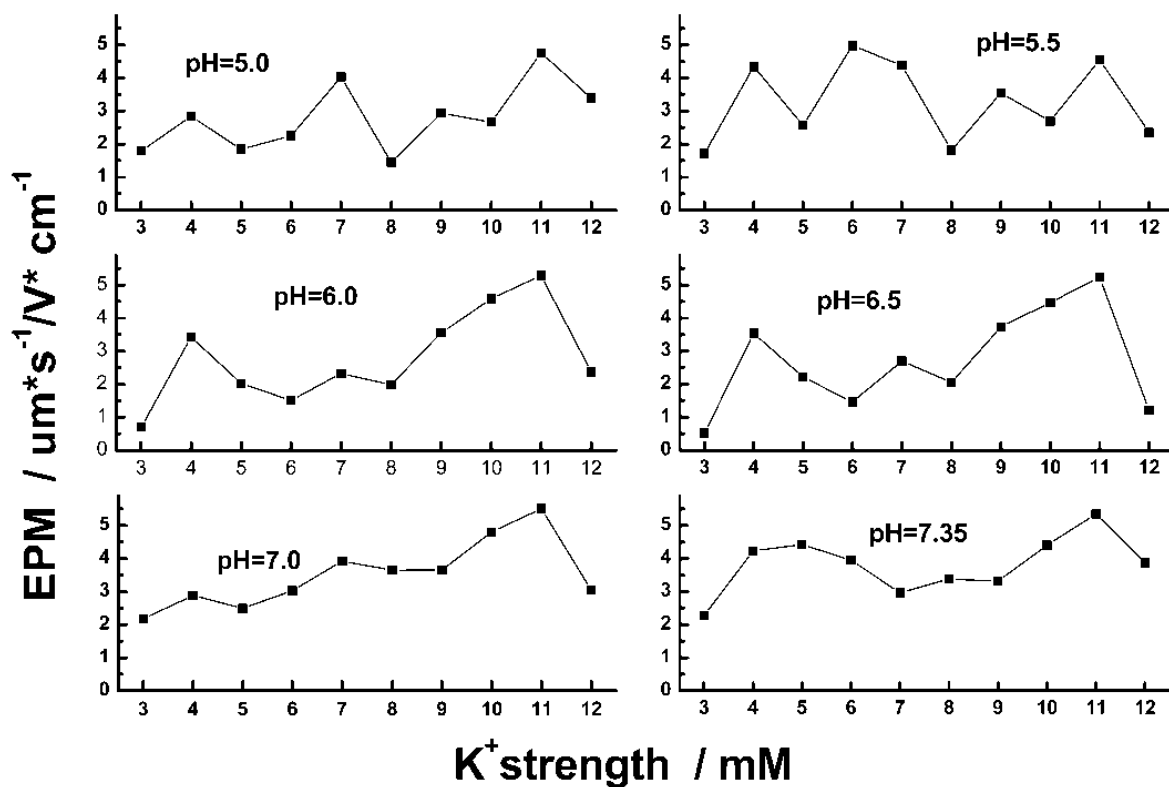
Figure 4. Plot of EPM against the concentration of K+ from suspensions with ionic strength of 151-160mM at pH values as shown in the figure.

At zero transmembrane potential (140 mM K+), the variation tendency of cell mobility along with the pH change was still the same but the range changed to 3.09 - 6.00, the highest and the lowest value appeared at the pH value of 6.5 and 6.0. At a transmembrane potential of approximately - 83.9mV (5 mM K+) and in 153mM ionic strength suspension, the cell mobility generally increased with an exception at pH7.35 (Fig. 5).

After the surface sialic acid was cut off, the mobility of myocardial cells decreased at pH 5.0 as expected (Fig. 6, the first three bars). The decreasing extent was found to depend on the ionic strength. Unexpectedly, the mobility increased when they were suspended in dextran medium (Fig. 6, the 5th bars) or at transmembrane potential of 0 (Fig. 6, the 4th bars).

In conclusion, the zigzag mobility variation (over a range from 0 to 8.67) of myocardial cells was observed for the first time, and the variation was found to depend not only on the suspension's pH and ionic strength but also on K+ or transmembrane potential.

4. Electric double layer on myocardial cell surface

4.1. Characteristics of myocardial cell mobility

This aberrant change could have four characteristics: First, the cell mobility changes in parallel to the ionic strength and becomes large as the charged layer shrinks. Second, the undulant mobility repeats in a certain range as the surface negative electric field is enhanced

or weakened. Third, the cell mobility rises abnormally as a component of the comprehensive surface negative electric field decreases to its minimum or is equal zero (at pH 2.5 or zero volt of transmembrane potential), Fourth, zero mobility appeared several times, especially under the conditions of relatively low ionic strength where the mobility should originally be slow (diffusion layer shrinks).
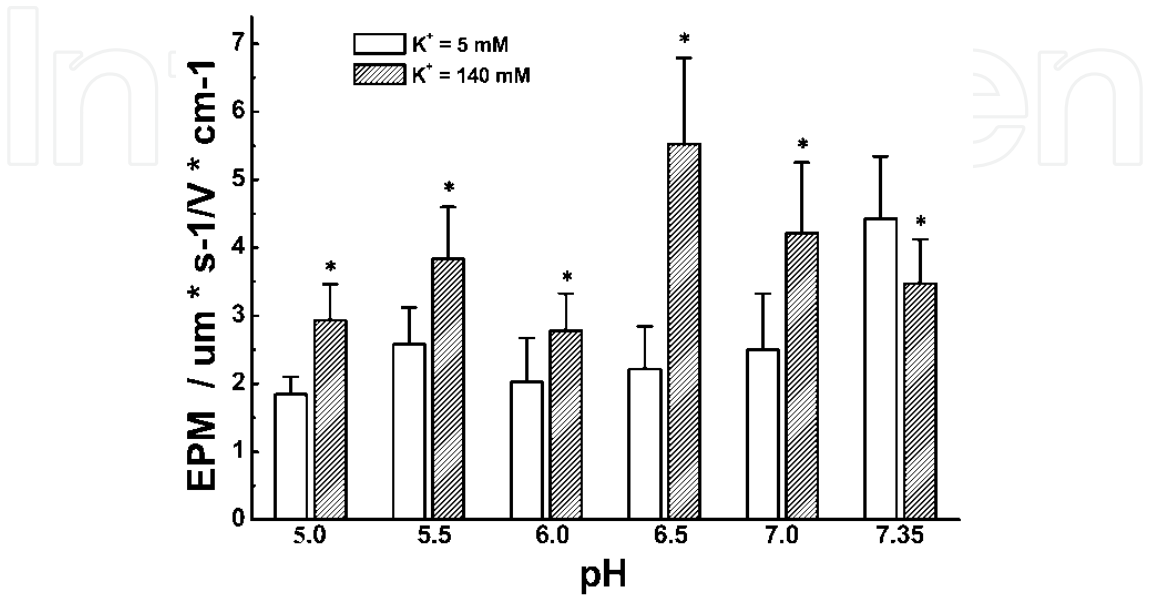

Figure 5. Plot of EMP against pH from the suspension of 140 mM K+ (transmembrane potential was zero). EMP at pH 7.35 decreased compared with that at 5mM K+ (transmembrane potential was -83.9mV) and 153mM ionic strength, and the others were increased (*: $P<0.01$).
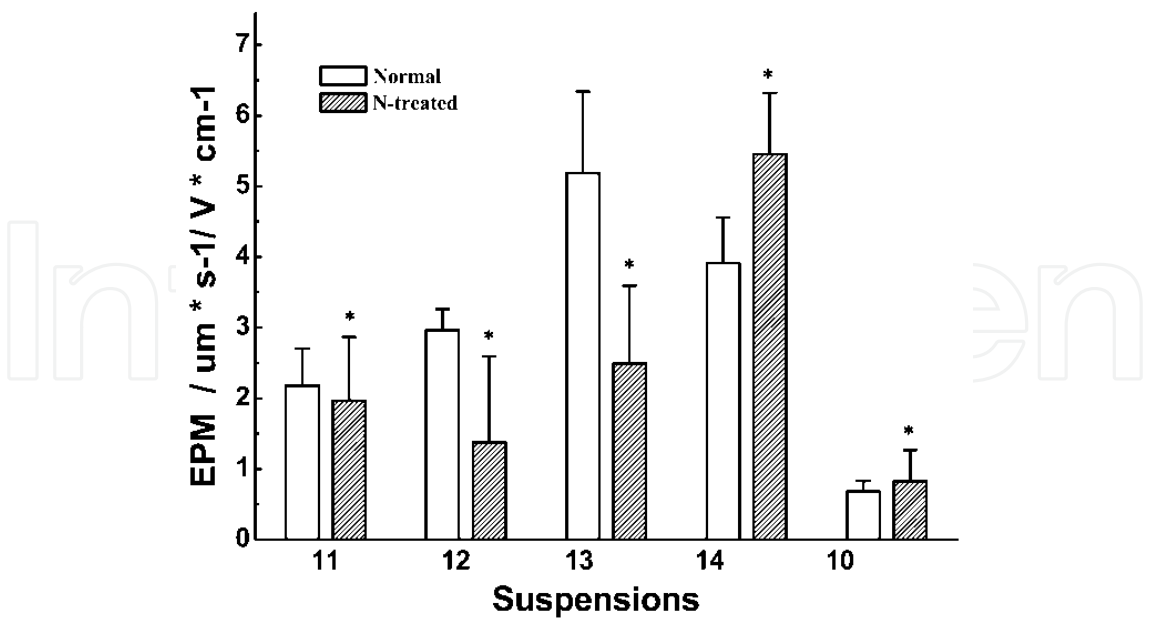

Figure 6. Effects of neuraminidase treatment on EMP of myocardial cells. EMP were measured in suspensions at pH 5.0 and ionic strengths of 12.4mM (Table 1, № 11), 19.8mM (Table 1, № 12) and 153mM(Table 1, № 13), 140 mM K+ (Table 1, № 14) and Dextran 40 (Table 1, № 10), respectively (*: $P<0.01$).

4.2. Composition of the cell surface electric field

The fact that K^+ strength influenced on the cell EPM demonstrated that the transmembrane potential participation should be considered to impose on the surface negative electric field of myocardial cells, this conforms to the theoretic expectation. However, when the transmembrane potential changes to zero, the characteristics of the EPM of myocardial cells are still different from the common particles. After removing of the sialic acid on the surface of cells, and at pH 5.0, which is lower than the isoelectric point of phospholipids (pI=6.5±) (Yamada et al., 1981) and changed the isoelectric point of membrane protein (Braun et al., 2007; Popot & Engelman, 2000; Cho & Stahelin, 2005), or when the transmembrane potential is zero (at 148mM ionic strengths), the EPM showed a strange rise, they may have very complex contribution to the surface charges.

First, the cell mobility changes in parallel to the ionic strength and becomes large as the charged layer shrinks. The role of transmembrane potential is complex, there should be a complex negative electric field from intracell which concerns with the charged layer but has an effect different from the charged layer. It enhances the half layer of the adsorbed ions and reduces the diffusion layer at the same time or causes redistribution of the absorbed surface ions, making the cell mobility changed zigzag. Second, the undulant mobility repeats in a certain range as the surface negative electric field is enhanced or weakened. This is just an effect of the complex negative electric field inside the cells: the adsorption layer of cell surface varies as the charged layer does, causing part of the ions entering from the diffusion layer. Third, the cell mobility rises abnormally as a component of the comprehensive surface negative electric field decreases to its minimum or is equal zero (at pH 2.5 or zero volt of transmembrane potential), implying that the effect of the complex intracellular negative electric field is reduced as the charged layer shrinks significantly. Fourth, zero mobility appeared several times, especially under the conditions of relatively low ionic strength where the mobility should originally be slow (diffusion layer shrinks). The intracellular complex negative electric field act thus in a limited space within the diffusion layer. The zero mobility concerns only with a zero diffusion layer, not necessarily a zero value for cell surface negative electric field and the adsorption layer. Therefore, the negative electric field of myocardial cell surface is composed by the surface sialic acid, plasmalemma of phospholipids, proteins and transmembrane potential (complex intracellular negative electric).

Each component of negative electric field on the myocardial cell surface has different influence on adions because its amount, isoelectric point and affect characteristics are not the same. Sialic acid on the extracellular surface is about 50 nm away the plasma membrane proteins, phospholipids and transmembrane potential, and in between the negative charges sparsely distributes, including some penetrated adsorbed ions (Langer, 1978). More specifically, in myocardial cells there are many of the same or similar membrane structures besides the negative electric field (transmembrane potential). The surface area of these structures is about 1/3 or more of the cell surface area. The special structure and composition make the electric double layer of the myocardial cell surface different from the general

particles: There exist the intersection of the surfaces and inside/outside infiltration of matter, and unique or even strange changes of the distribution characteristics of the cell surface adsorbed ions and mobility. The so-called electric double layer structure is thus not sterling, may be better called an aberrant electric double layer.

4.3. Analysis of mobility change

The one way declining of the cellular mobility after removal of only the extracellular surface sialic acid, which is different from the zigzag mobility vibration, suggests the removal of sialic acid has almost no impact on the complex intracellular negative electric field. At zero transmembrane potential of zero, the mobility was not found to have an obvious relationship with the phospholipid isoelectric point as the pH varied, implying that the pH value may influence on all the membrane composition of sialic acid, protein, phospholipids, and the intracellular complex negative electric field. The mobility is a result of multiple integral ation. Thus the plasma membrane phospholipid may have only weak contribution to the distribution change of adsorbed ions on a myocardial cell surface. After complete removal of the effect of the transmembrane potential, the mobility almost allways increased except for at pH7.35. This indicates the significant effect of the transmembrane potential changes on the intracellular complex negative electric field: The more is the transmembrane potential reduced, the weaker becomes effect of intracellular complex negative electric field and the less are the ions entering from the diffusion layer into the adsorption layer. (In case of pH7.35 there may be other charged components enhancing the effect). The fact that removal of sialic acid and plasma membrane phospholipids and suppression of the transmembrane potential could cause high mobility at high ionic strength, which should also be an integral result, reveals that these factors have some effect on the intracellular complex negative electric field.

By these imitated in vivo, broadly varied suspension conditions, the myocardial cells were shown to have a complex aberrant electric double layer structure on the membrane surfaces. The related surface electric field is a cellular character integrated from the complex combinations of membrane sugars, phospholipids, proteins and transmembrane potential (intracellular complex negative electric field). The ion layer is composed of a large adsorption layer and a large cyclicly varied diffusion layer. This is a special structure, making mobility and its related features completely different the general colloidal particles. Interestingly, the contraction and Ca^{2+} influx (slow inward calcium current) of a living myocardial cell surface, which is chared only negatively, is not significantly affected after removal of the negative sialic acid composition (Yee et al., 1991; Langer & Nudd, 1983). This is due to the contribution of other powerful components in addition to the sialic acid. The aberrant electric double layer structure, which is also distinguished from non-excitable cells, is dependent on the variation of each complex membrane composition such as sugars, membrane proteins, phospholipids and transmembrane potential (intracellular complex negative electric field). The change may be the normal physiological performance but can also be a result of pathology. The charged layer in combination with the intracellular complex negative electric field connects the intra and extra cell environments as a whole

body. This point is very important for not only realizing the myocardial cell function (contraction) but also for studying the cardiovascular disease mechanisms.

5. Challenges and prospects of myocardial cells electrophoresis

Animal and human cells are a complex system. According to the basic essence of systems biology research--structure determines function we believe that the study of cell electric phenomena is a key point to crack the hard shells of some diseases and related mechanism. This may unroll all the true information on finding the formation and changing mechanism and inside/outside cellular connecting routes of the cell surface electric double layer, which should be different from the ordinary colloidal particles and on the integration of the double layer's characters into the overall cellular structure and natural biological behaviors. In fact, the occurrence of some diseases is not only caused by one abnormal gene or protein by the unusual results of the electric double layer structure of the cell membrane surface. Therefore, the electric double layer structure of cell membranes or surfaces should be a coherent part in biological researches, and the related methods and materials selected should be able to imitate body conditions as much as possible during performing the investigation of cell electric phenomena. It is also critical to establish cell model capable of re-diplaying the cell functions.

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