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Complete List of Authors:	Misra, Devesh; University of Texas at El Paso, Center for Structural and Functional Materials Research and Innovation Kumar, A; University of Texas at El Paso, Biomaterials Nune, VC; University of Texas at El Paso, Biomaterials

Electric Field-Mediated Growth of Osteoblast - The Significant Impact of Dynamic Flow of Medium

A. Kumar, K.C. Nune and R. D. K. Misra*
Biomedical Engineering Department, University of Texas at El Paso, El Paso, TX 79968, USA

Abstract

The endogenous electric field plays an important role in accomplishing various functions including communication with brain and with different parts of the physiological system, wound healing, and cellular functions. Furthermore, the endogenous electric field can be modified using external electric field to induce changes in cell functionality. Given that the cells grow in contact with the dynamic flow of blood and nutrients, the objective of the study is to elucidate the effect of media flow (dynamic condition) on osteoblast functions at pulsed DC (direct current) electric field of strength of 0.5-1 V/cm and compared with the static condition (no flow of media and in the presence of electric field). The electric field provided a guiding cue to cells to move towards the cathode. An interesting aspect of electric field was the migration of cell towards the cathode with axis parallel to the direction of electric field such that the lamellipodia was aligned. Furthermore, there was absence of membrane blebbing or necrosis at cathode and cell growth and expression of proteins (actin and vinculin) was higher than the anode. In contrast, at anode, while the cells were healthy, but cell growth was less such that the expression of vinculin was relatively low together with less densely packed actin stress fibers. It is underscored that the biological functionality is favorably altered in the presence of electrical field in dynamic condition with consequent effect on cell proliferation, growth, and expression level of prominent proteins, actin and vinculin.

Keywords: Biomaterials; Electric field; Dynamic flow; Osteoblast; Cell function

***Corresponding author:** E-mail: dmisra2@utep.edu

1. Introduction

The biological systems such as cells and tissue are responsive to the endogenous cues and external stimuli [1-3]. From this perspective, the external electric field appropriate to the physiological environment, can be used to regulate the cellular and/ or tissue functions [4]. For example, external electric field was observed to be effective in the reorganization of actin filaments [5, 6], cell motility [7], guidance of neural growth cone [8], stem cell differentiation into various lineages [9, 10]. Thus, external stimuli can be effectively used to override the endogenous cues and cure the relevant disease. The electric field was beneficial in tissue and nerve regeneration as well as cancer therapy [11-15]. The effect of electric field on different cells has different effect because of the variation in the physiological characteristics of cells. For example, in differentiated osteoblast, the attachment of plasma membrane with cytoskeleton is stronger than in undifferentiated stem cells. Thus, they respond differently to the external electric field [16]. In one study, Jurkat cells experienced cell membrane damage when short electric field pulse (1-10 sequential pulses) was applied, while human blood polymorphonuclear leucocytes were unaffected under similar conditions [17]. It is important to indicate that the sub-microsecond intense pulsed electric field can lead to permeablization of intracellular membrane without causing electroporation and cells may die through apoptosis [18, 19].

More importantly, short duration pulse elude the probability of overheating of cells as the electric field energy required for the cell lysis is reduced with decrease in pulse duration [20]. An increase in cell proliferation and spreading on biomaterial surface was observed in a narrow window of DC (direct current) pulsed electric field (667 mV/cm and 100 Hz in case of glass) [21]. In the case of hydroxyapatite (HA) and HA-40 wt.%Barium titanate, at 100 Hz frequency, the optimal electric field were 167 and 667 mV/cm [21]. No evidence of heating of medium was noted.

The electric field led to increase in osteoblast proliferation by 72% when applied for 1 h for 5 days [22]. Interestingly, under the effect of electric field, the osteoblast migrated toward the cathode, while osteoclast migrated toward the anode [23].

Given that the cells are dielectric in nature, the frequency and electric field strength influences the strength of electric field experienced by the cells. It is important to mention that the optimal stimulation parameters are a function of biomaterial surface and properties of the culture medium [21, 24]. In regard to the effect of properties of culture medium on the electric field stimulated cell behavior, the electric field led to build-up of electrical charge at the cell membrane in a conducting medium. This induced change in voltage across the plasma membrane and subsequent opening of plasma membrane channels (in the presence of low strength electric field), is referred as voltage gating [20]. This causes cells to be in the state of stress because of change in ion concentration inside the cells induced by flux of ions such as Na^+ and K^+.

Based on the above discussion, it is clear that electric field can be used to modulate cell function. Thus, it is important to understand the cell behavior under the influence of electric field. In physiological environment, flow of blood and nutrients along with endogenous electric field are involved in tissue function. With the above in mind, it is desirable to study the effect of electric field on cell functionality in the dynamic condition, i.e. during the flow of media. The previous studies were focused on cell behavior when the culture medium was static [21, 24, 25]. In the present study, our objective is to study the effect of electric field on osteoblast functions at electric field strength of 0.5 and 1 V/cm under dynamic conditions and compare with the static condition.

2. Materials and Methods

2.1. Starting Material and Electric Field Stimulation

The glass cover slips (Cat no. 63768-01, Gold-Seal, USA) of dimensions (24×40 mm) and ~2 mm thickness were used as a substrate for the cells. Glass cover slips were ultrasonicated in acetone and then in 70% ethanol for 10 min each. The samples were marked for the direction of electric field and flow of media using alcohol proof marker. These samples were autoclaved and kept for further study (Figure 1). The osteoblast (MC3T3-E1, Cat. No. CRL-2593, ATCC, USA) with a cell density of 10,000 cells/well (for sparse cells) as well as 30,000 cells/well (for monolayer) were seeded on the sample surface in a non-treated 8 well plate (Cat. No. 267062, Nunc, USA, well dimension: 3×3.9 mm). After 1 day of incubation, cell seeded on cover slips were stimulated for 10 min by pulsed DC electric field of 0.5 V/cm and 1 V/cm strength (square waveform, 100 Hz frequency and 50% duty cycle). The experimental plan is presented in Table 1. A function generator (Model no. AFG3022C, Tektronix, USA) was used to apply the electric filed. Stainless steel (ASTM 316L) electrodes were used in this study to avoid the degradation of electrode due to corrosion. Electrodes were cleaned in acetone and autoclaved after each experiment. Media (1×PBS) flow (10 ml/min) was maintained during the entire period of electric field application to mimic the dynamic condition of the physiological system. For this, a custom designed bioreactor equipped with a peristaltic pump (Model no. 07522-20, Cole Parmer, USA) was used (Figure 1). For comparison, electric field was also applied on the samples in the static condition, i.e. no flow of medium. The entire process of electric field stimulation in dynamic as well as static condition was repeated every day for total period of 5 days. After 5 days, samples were incubated for 1 day prior to the assessment of cell viability.

2.2. Cell Viability and Cytocompatibility

To study cell viability in the presence of effect of electric field and flow of media, osteoblast were seeded on the glass cover slip for 1 day. 10,000 and 30,000 cells/ml were seeded on individual scaffolds and pre-incubated for 4 h at 37°C in an environment of 5% CO_2 and 95% relative humidity. After the pre-incubation period, complete culture media (89% α MEM, 10% FBS, and 1% antibiotic) was added and samples were incubated for 1 day before the electric field stimulation. Electric field was applied for 10 minutes in both static and dynamic conditions each day and for 5 days. At the end of second day, old media was replaced by fresh media, to avoid cell death because of change in the pH of the media induced by cellular activity. After 5 days of application of electric field, the samples were incubated for another 1 day to normalize the cells. Next, the samples were washed with 1×PBS and fixed with 4% formaldehyde, followed by cell wall permeablization with 0.1% triton×100. To reduce the non-specific binding of dyes, samples were incubated with 5% FBS. After washing with 1×PBS, cells were stained with dyes to see the expression of vinculin (green color) and actin (red color). DAPI was used to stain the nucleus (blue color). After staining, samples were washed with 1×PBS to remove the unreacted dye and stored in 1×PBS at 6 °C for florescence microscopy. Expression of vinculin and actin was used to study the cell adhesion (focal adhesion) and cytoskeleton reorganization, respectively. Cell viability was studied through visualization of nuclei.

3. Results

3.1. Effect of electric field on osteoblast monolayer in static and dynamic conditions

Experiments were carried out in two culture conditions, namely monolayer and sparse. In the case of monolayer, high density of cells (30,000 cells/ml) were seeded, while for sparse

culture, the seeding density was 10,000 cells/ml. In the former case, the high cell density enabled cell-to-cell contact after 1 day of culture. In contrast, in the latter case, because of low cell seeding density, cell-to-cell contact was not observed after first day of incubation. We first summarize the results related to higher seeding density. In this condition, both in static as well as in the dynamic condition, normal cell growth (increase in cell density with time) was observed in the absence of electric field. The cells were healthy even after 5 days of culture. However, after five days of static culture and exposure to 0.5 V/cm electric field for 10 min each day, few unhealthy cells were noted, characterized by fragmented nucleus. Also, low cell number (or nucleus count) (Figure 2a and Table 2) was observed, presumably because of low cell viability. In contrast, no significant damage to cells was induced by 0.5 V/cm electric field in the dynamic condition (Figure 2b and Table 2).

When the electric field was increased to 1 V/cm, a strong detrimental effect of electric field was observed in the static condition. Majority of the cells were dead after the five days of electric field stimulation. This was confirmed by the presence of cell debris and fragmented nuclei (Figure 2c). In contrast, the cells were found healthy after five days of culture and nucleus count was higher in the dynamic condition (Figure 2d).

3.2. Effect of electric field on sparse osteoblast in static and dynamic conditions

As mentioned in the previous section, in the case of osteoblast monolayer, maximum damage was observed at 1V/cm electric field, when static condition was maintained during electric field application. In order to further extend our understanding with respect to cell seeding density and flow of media on electric field-mediated cell growth, we seeded low density of cells to avoid the formation of confluence cell layer on the material surface. Here the absence of electric field and static condition were considered as control. High cell viability was observed

on control samples after 5 days of culture (Figure 3a,b). With extensive reorganization of actin filaments (red color) and higher expression of vinculin (green color) were noted at higher magnification (Figure 3c). Also, no fragmentation of cell nucleus was observed (Figure 3d).

Intriguing observations were made in static and dynamic conditions in the presence of electric field (Figures 4 and 5). In static condition, a low density of cells were observed in the vicinity of anode and cathode (Figure 4). The low density of cells is attributed to cell damage/death induced by the electric field. The damage was more uniform in the proximity of the anode. In contrast, in the vicinity of cathode, a relatively larger number of cells were observed, except in the region close to the electrode (we refer this zone as cell-inhibition zone). In this zone no signature of cells was noted. Interestingly, no cell-inhibition zone was observed in the anode region. Importantly, very distinct cell morphology was found in the three regions of the substrate: (a) vicinity of anode, (b) vicinity of cathode and (c) mid-region of the sample. In the mid-region, cell growth was observed as interpreted from extended lamellipodia. The higher magnification micrographs of regions close to the cathode indicated an alignment of lamellipodia in the direction of electric field, which is a distinct evidence of cell migration. However, no distinct evidence of directional migration of cells was noticed in the mid-region. Also, cell spreading was more in the mid-region as compared to the regions in the vicinity of the electrodes.

In contrast to the static condition, the dynamic condition was observed to be relatively more viable for electric field-mediated cell growth based on the higher density of viable cells in the dynamic condition (Figure 5). However, cell morphology was similar in all the three regions: proximity of anode and cathode, and mid region of the sample. Similar to static condition, lower number of cells were observed in the vicinity of anode. However, in the dynamic condition, cells

were relatively healthier than the static condition. The higher magnification micrographs confirmed organization of actin and noticeable expression of vinculin. In the vicinity of cathode, the cell-inhibition zone was very small and high density of cells were observed near to this zone because of enhanced migration of cells towards the cathode and alignment of cells in the direction of electric field. Low and high magnification micrographs clearly revealed the extension of lamellipodia in the direction of electric field, towards the cathode (Figures 5 and 6). Importantly, in both middle and cathode regions, extensive growth of cells with reorganization of actin and high expression of vinculin was observed. A schematic of the response of cells in the static and dynamic conditions is presented in Figure 7. Figure 8 and 9 summarizes the quantitative data in terms of number of cells and expression level of proteins for static and dynamic culture conditions. Figure 8 shows the number of cells per unit area in static and dynamic conditions. The results were compared with the control sample (in the absence of electric field in static condition). Results revealed reduced proliferation of osteoblast in the presence of electric field strength 1V/cm, irrespective of static or dynamic conditions. However, as compared to static condition, higher number of cells were found in dynamic condition. The quantitative analysis of cumulative expression of vinculin and actin indicated the benefits of dynamic condition over static condition (Figure 9). In general, the higher value of DAPI in dynamic condition confirmed higher cell growth as compared to the static condition. Also, the higher expression level of vinculin and actin confirmed stronger cell adhesion and proliferation of cells in the dynamic condition as discussed in detail in section 4.

4. Discussion

The external electric field has beneficial effects in the context of physiological activity such as enhanced synthesis of proteins. The activity at molecular scale plays an important role in the

cell fate process: cell cycle, proliferation, and apoptosis. The wound healing process can be accelerated by electric field-mediated cell migration towards the wound region. The cell migration is a result of force applied by the electric field on the naturally charged cell surface and the direction of electric field is the direction of force applied on a positive charge. In reality, a live cell is a conducting mass consisting of a conducting cytoplasm (~ 10^{-2} S/cm) and a cell membrane (phospholipid bilayer) of lower conductivity (~10^{-3} S/cm) [26, 27]. Thus, cells get polarized in the presence of electric field and act as a dipole. The application of electric field in orthopedic applications promotes bone regeneration and can be used to treat osteoporosis and fracture of bone [28-30]. In one instance of guinea pig spinal cord, the dorsal column fibres were recovered by electric field (~300-400 mV/mm) [31, 32].

Previous studies on the effect of electric field on cell behavior *in vitro* were performed under static conditions [21, 24, 25]. However, in these experiments, the effect of properties of culture media on cell metabolism was not systematically studied. For instance, during the application of electric field, the variation in pH and denaturing of proteins and the effect of these parameters on the cell behavior were not considered. We have considered these aspects by maintaining the media flow during electric field application, which ensures continuous supply of fresh media to the cells. Moreover, in natural tissue there is continuous flow of blood that ensures homeostatic condition, which was ignored in the previous studies during the application of electric field in the static condition (no flow of media) [21, 24, 25]. Thus, we have maintained a continuous flow of the media to minimize change in pH and denaturing of proteins (Figure 1). In the present study, 1×PBS was used as a medium during electric field application.

In an earlier study, the application of 2 V/cm electric field for 60 min in a serum free buffer saline led to partial disassembly of actin in osteoblast as well as hMSCs [4] and there was two-fold

decrease in cell elasticity. This also led to depletion in intracellular ATP (Adenosine triphosphate). The depletion of ATP resulted in physical decoupling of cell membrane with cytoskeleton. Furthermore, the depletion of ATP led to inhibition of linker proteins that couple the cell membrane with cytoskeleton. Disruption of actin filament is a clear indication of dissociation of cell membrane from the cytoskeleton [33]. The separation of cell membrane from cytoskeleton induced by electric field led to cell membrane blebbing [34]. Similar results were observed in our study when 1 V/cm electric field was applied on sparse cells in the static condition. Interestingly, the effect of membrane blebbing was more prominent near the electrodes (Figure 4). Moreover, a severe damage to the cells was found near anode, which may be considered due to necrosis of cells. In the middle region, although disruption of actin filaments was not noticed, a less densely packed thin bundle of actin fibers were present, which is a clear indication of poor cell adhesion and growth. In contrast, in the dynamic condition, no signature of membrane blebbing or cell necrosis was observed (Figure 5). However, relatively low cell density was observed in the vicinity of the anode. Also, the cells in the vicinity of anode were characterized by healthy nucleus with moderately high expression level of vinculin and less densely packed bundle of acting filaments. In contrast, in the vicinity of cathode and mid-region, the cell growth was higher than anode. Also, the densely packed thick bundle of actin filaments and enhanced expression of vinculin was observed. Importantly, the actin filaments were found arranged in the direction of the electric field in the proximity of the cathode (Figure 6). This confirmed the migration of cells towards the cathode or alignment of cells in the direction of electric field near the cathode region. It is important to mention that electric field affects the direction of cell motility than the speed and provides a guiding cue to cells to move towards the

cathode [7]. As a consequence, the cathode facing the cells move into the wound more rapidly under the effect of electric field, while anode facing cells did not show any movement [7].

Cellular processes such as proliferation depends on the DNA synthesis. The DNA synthesis was found to be accelerated in the presence of electric field [35]. In the context of molecular mechanism involved in cell proliferation, the electric field activates extracellular signaling molecules, which is further transferred to the intracellular signaling proteins via molecular switch [36]. The electric field induced signaling was observed to be effective in the proliferation of fibroblasts on glass, hydroxyapatite, and hydroxyapatite-barium titanate samples in the presence of pulsed DC electric field in the range of 0.3-0.6 V/cm [21]. The number of cells per unit area were higher in dynamic condition (Figure 8). This confirmed higher cell viability and growth in the dynamic condition. The low cell viability and reduced cell growth in the static condition can be related to the variation in pH and is discussed below. The expression level of vinculin (adhesion protein) and actin (skeletal protein) was higher in dynamic condition (Figure 9). Vinculin is involved in the linkage of adhesion receptors with cytoskeleton and is responsible for cell-cell and cell-matrix binding. Also, vinculin contributes to the coordination of cellular signaling along with the transduction of mechanical force to cytoskeleton [37, 38]. Apart from this, actin plays an important role in cellular functions such as cell division, cell signaling, cell motility, cell polarity, and control of cell morphology [39, 40]. Also, the increased turnover of actin filaments increase cell life, while decreased throughput of actin may trigger the cell death through apoptosis [41]. Thus, higher expression of actin and vinculin is an indication of strong cell-cell and cell-matrix binding as well as cell longevity and enhanced cell signaling, required for increased proliferation of osteoblast. Overall, dynamic conditions were favorable for cell proliferation in the presence of electric field.

In static condition, the pH measurement indicated a small decrease in pH (~7) near the anode when the applied electric field was 1 V/cm, which was close to the physiological pH (~7.4). However, in the vicinity of cathode (~8.5) and in the mid-region (~8), a higher pH was measured. Interestingly, as compared to the anode, a higher cell density was found in cathode region with alkaline pH. Moreover, the cell-inhibition zone in the vicinity of the cathode was observed to be associated with H_2 gas evolution. But the evolution of H_2 gas was less in the dynamic condition, which led to smaller cell-inhibition zone. In this case, no effect of pH was considered because the old media was continuously replaced with the new media.

Another aspect of interest is the confluency of cells because confluency of cells affects cell migration under the effect of electric field [42, 43]. The sparse cell motility increases with increase in electric field strength [7]. Furthermore, the reason behind the differential effect of electric field on sparse and monolayer cells viability is that in sparse cells, a smaller voltage drop occurs across individual cells, while in monolayer, voltage drop occurs across the several contacting cells, communicate via gap junction [44, 45]. A higher voltage drop occurs at higher electric field. Under these conditions the membrane permeability reaches to a level that it may require a few hours for the cells to recover, referred as reversible breakdown, while cell death may occur in irreversible breakdown. Thus, in the static condition, a higher damage to the cells in monolayer (confluent) was observed than sparse cells (not confluent).

On the basis of aforementioned discussion, it is obvious that the cellular activity can be tailored using external electric field in the dynamic condition. We believe that in the presence of flow of media and 1 V/cm electric field, the enhanced synthesis of vinculin and actin polymerization followed by extracellular and intracellular signaling is the mechanism behind higher cell proliferation and cell longevity in dynamic condition as compared to the static

condition. Importantly, the electric field stimulation in static condition does not mimic the physiological condition because naturally blood flow maintains the tissue homeostatic, *in vivo*.

To further extend our understanding on the effect of media flow on cell behavior in the presence of electric field and to simulate the condition of blood flow in arteries and capillaries, we are currently studying porous scaffolds. This will help us design suitable porous scaffolds for tissue engineering applications that can be stimulated/activated using external electric field for rapid tissue regeneration.

5. Conclusions

The concept of electric field mediated growth of cells for faster wound healing was studied in static and dynamic conditions. The pulsed DC electric field of 1 V/cm strength had a differential effect on monolayer and sparse osteoblast. In the presence of electric field osteoblast migrated towards the cathode in the direction parallel to the applied electric field such that lamellipodia was aligned. This cell alignment was more prominent in the dynamic condition.

In static condition, 1 V/cm electric field led to strong detrimental effect on sparse osteoblasts, characterized by cell blebbing and disruption of actin filaments. This led to cell apoptosis and decreased proliferation. In contrast, in the dynamic condition, enhanced turnover and polymerization of actin filaments and presence of bundle of stress actin fibers in the vicinity of electrodes and mid-region led to significant proliferation in comparison to the static condition. In comparison to control, higher turnover of actin and thick bundle of actin stress fibers was observed in the dynamic condition in the presence of pulsed electric field of 1 V/cm.

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Table 1. Details of experimental plan and condition used for the electric field stimulation of cells

Culture condition	**Applied electric field (V/cm)**					
	Static			**Dynamic**		
	No Electric Field	Electric Field		No Electric Field	Electric Field	
Monolayer	0	0.5	1	0	0.5	1
Sparse	0		1			1

Table 2. Summary of results of electric field stimulated monolayer of osteoblasts for 5 days

	0 V/cm		**0.5 V/cm**		**1 V/cm**	
	Static	**Dynamic**	**Static**	**Dynamic**	**Static**	**Dynamic**
Day 1	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells
Day 2	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells
Day 3	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells
Day 4	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells	Healthy cells
Day 5	Healthy cells	Healthy cells	**Cells were not healthy**	Healthy cells	**Cells were dying**	Healthy cells

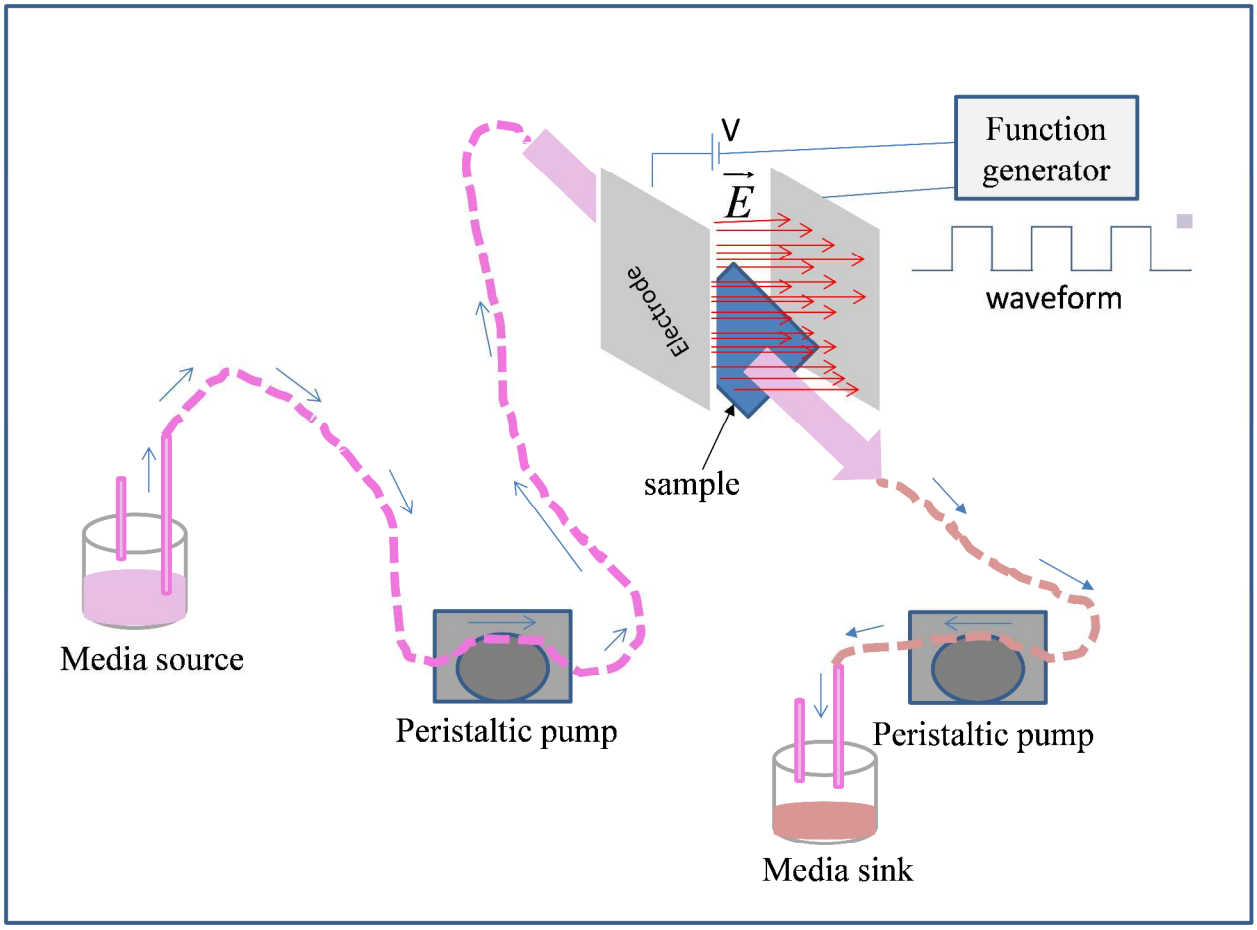

Figure 1. Schematic representation of experimental set-up used for the electric field stimulation of osteoblast.

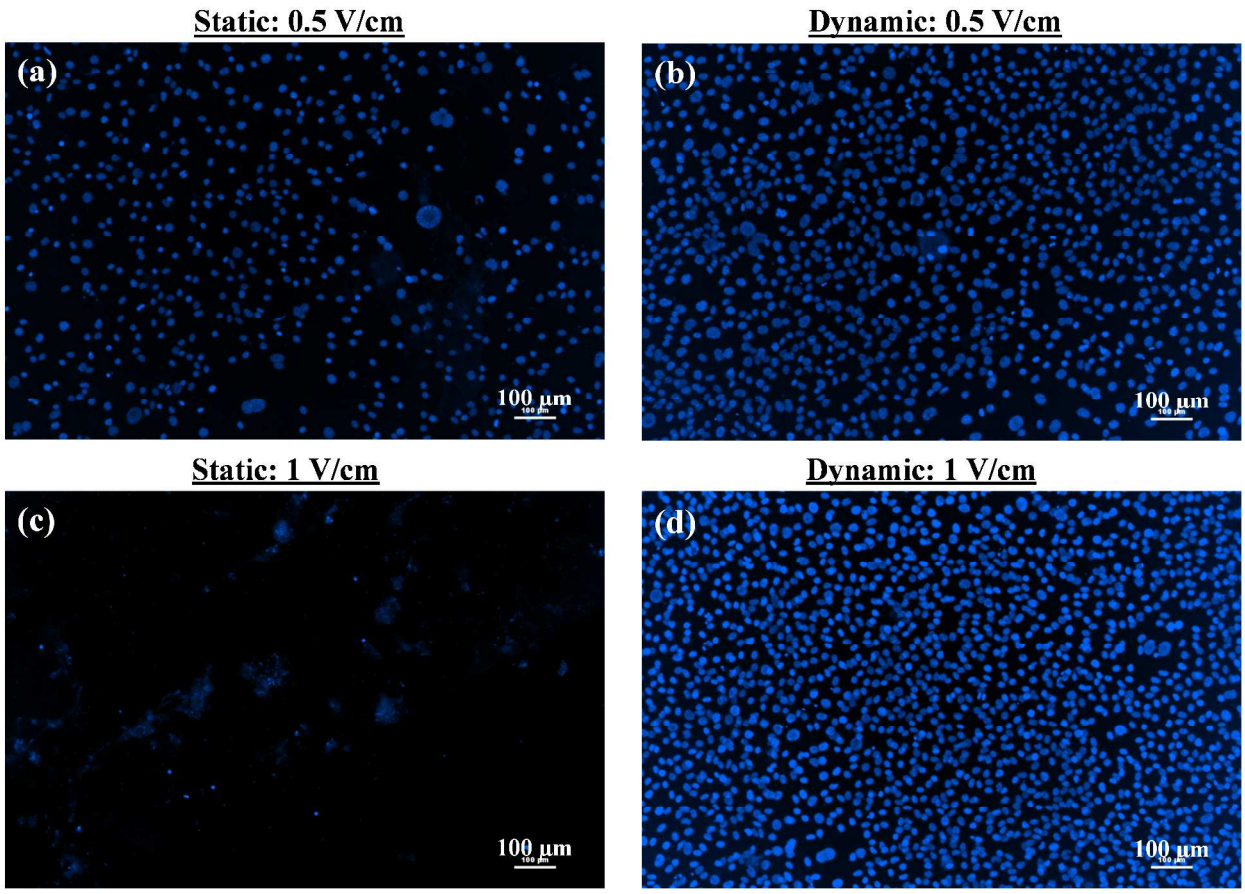

Figure 2. Representative fluoroscence micrographs showing stained nucleus of a monolayer of osteoblast after 5 days of electric field stimulation using a strength of (a) 0.5 V/cm (static), (b) 0.5 V/cm (dynamic), (c) 1 V/cm (static), and (d) 1 V/cm (dynamic). In comparision to static, in the dynamic condition cell viability was higher. The application of 1 V/cm in static conditon was found to be detrimental.

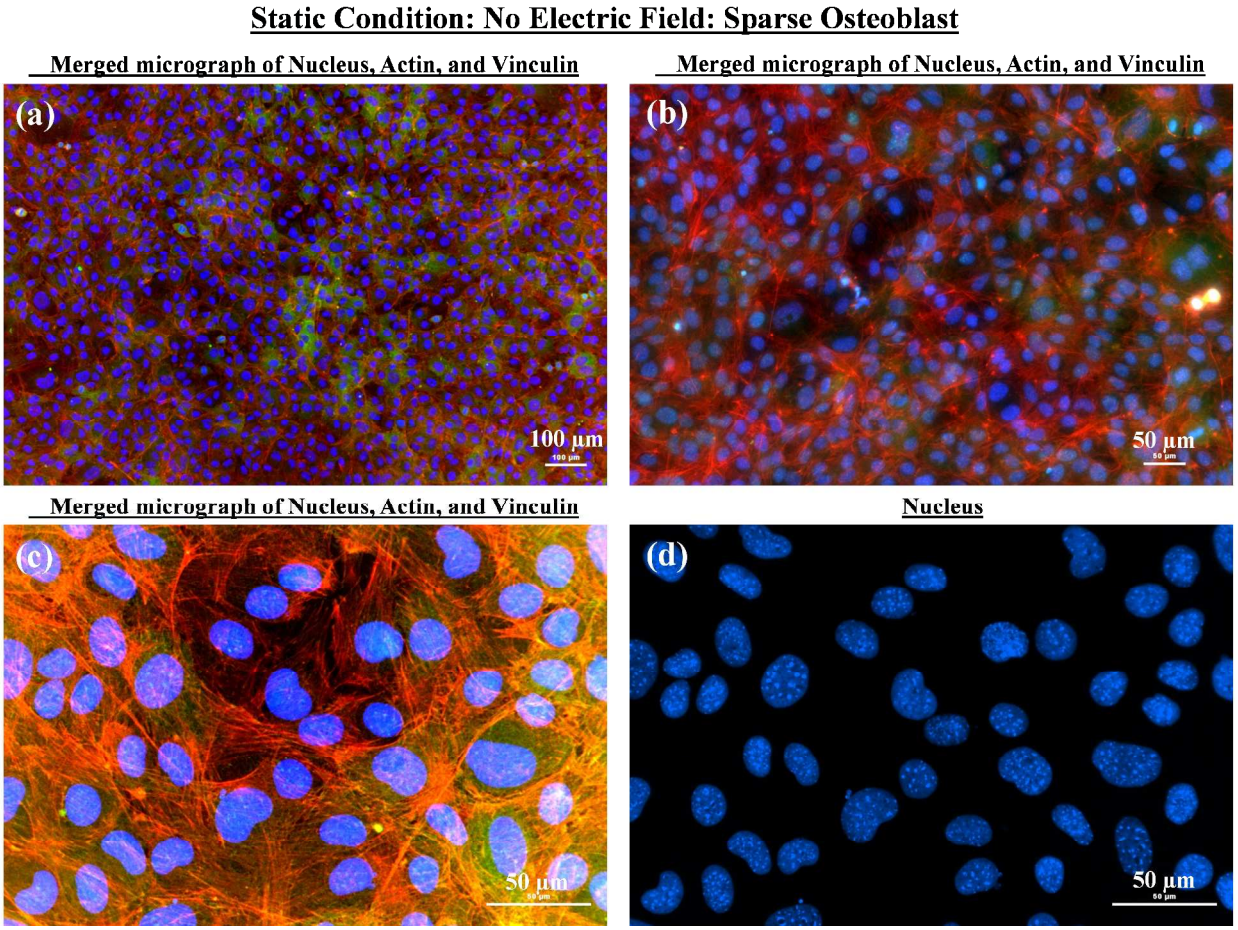

Figure 3. Representative fluoroscence micrographs showing sparse osteoblast after 5 days of culture in the absence of electric field stimulation and in static condition. (a), (b), and (c) are merged images showing nucleus and expression level of actin and vinculin. The extensive reorganization of actin and expresion of vinculin confirmed the cytocomatibility of the glass substrate. (d) The representative high magnification micrograph confirmed healthy nuclues with no visible damage.

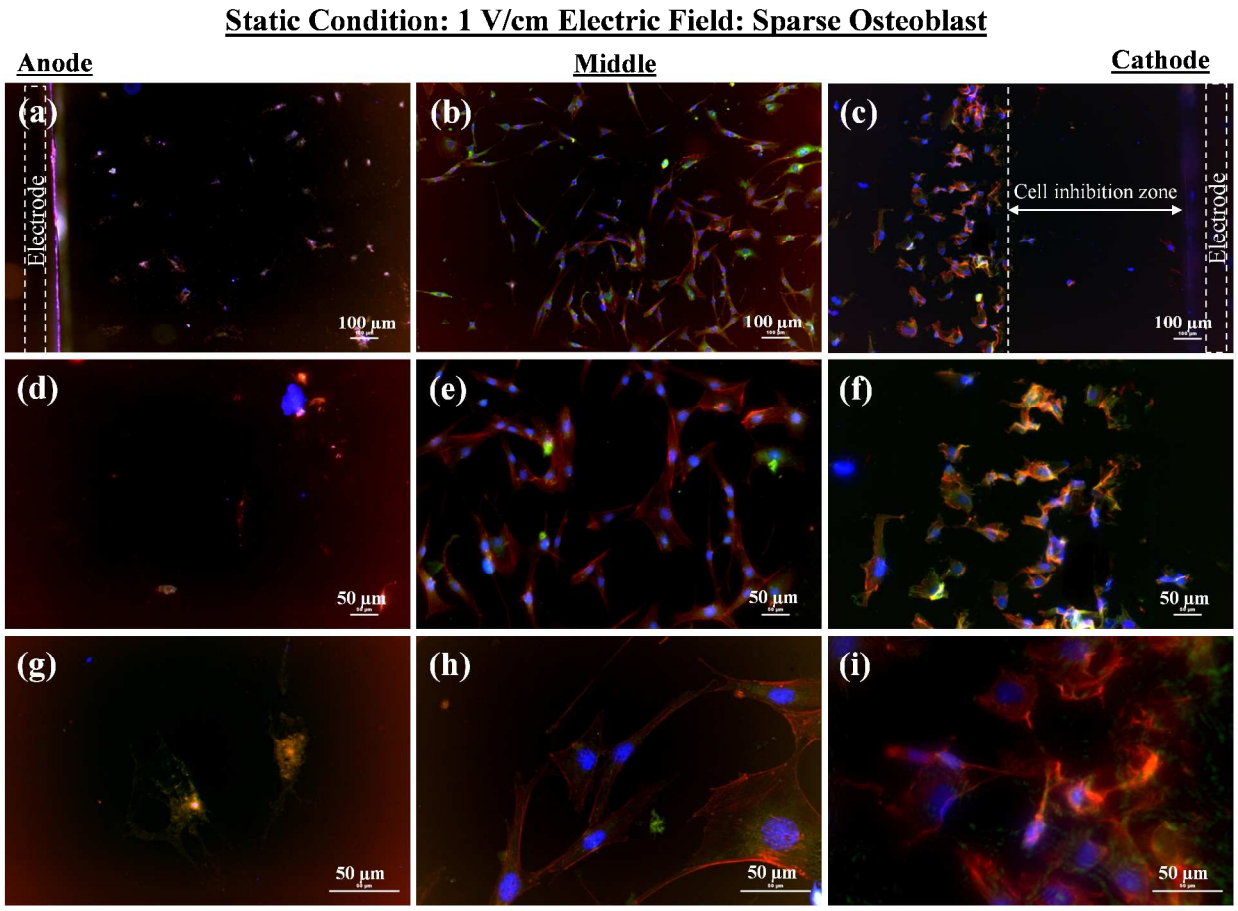

Figure 4. Representative fluoroscence micrographs (merged micrographs of nucleus, actin, and vinculin) showing sparse osteoblasts growing in three different regions of substrate (in the vicinity of anode, mid-section, and vicinity of cathode) after 5 days of electric field stimulation using 1 V/cm in the static conditon. (a), (d), and (g) are merged micrographs showing the nucleus and expression level of actin and vinculin corresponding to cells in the vicinity of anode. (b), (e), and (h) are merged micrographs showing nucleus and expression of actin and vinculin corresponding to cells growing in the mid-region of the glass substrate. (c), (f), and (i) are merged micrographs showing the nucleus and expression level of actin and vinculin, corresponding to cells in the vicinity of cathode. The results show high cell viability in mid-region and in the vicinity of cathode and severe damage to cells in the vicinity of anode.

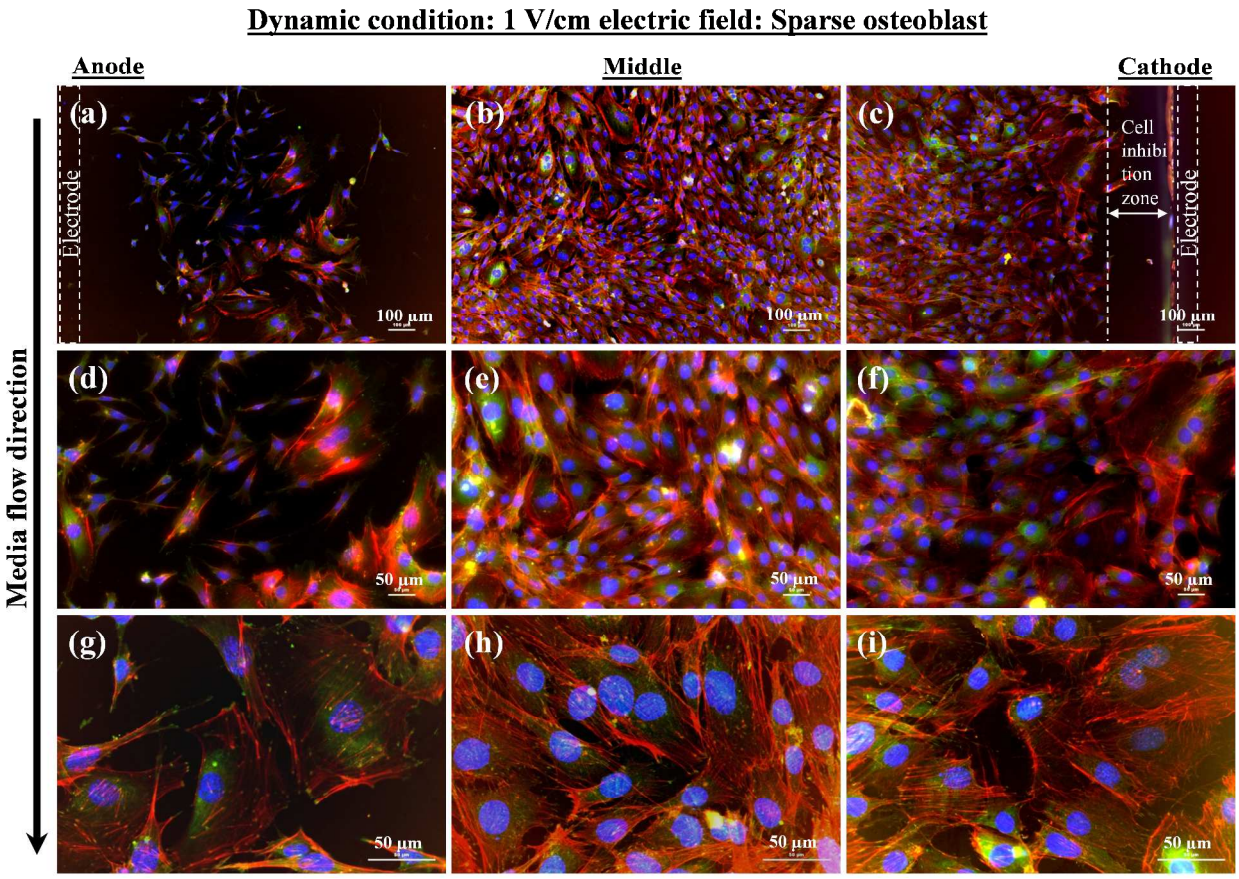

Figure 5. Representative fluoroscence micrographs (merged micrographs of nucleus, actin, and vinculin) showing sparse osteoblasts growing in three different regions of substrate (in the vicinity of anode, mid-section, and in the vicinity of cathode) after 5 days of electric field stimulation using 1 V/cm in dynamic condition. Black arrow shows the direction of medium (1×PBS) flow. (a), (d), and (g) are merged micrographs showing the nucleus and expression level of actin and vinculin corresponding to cells growing in the vicinity of anode. (b), (e), and (h) are merged micrographs showing the nucleus and expression level of actin and vinculin corresponding to cells growing in mid-region of the glass substrate. (c), (f), and (i) are merged micrographs showing the nucleus and expression of actin and vinculin corresponding to cells growing in the vicinity of cathode. The results show the higher cell density in middle and near to cathode as compared to anode. A higher expression of actin (density of actin filaments) was observed in the mid-region and in the vicinity of cathode as compared to anode.

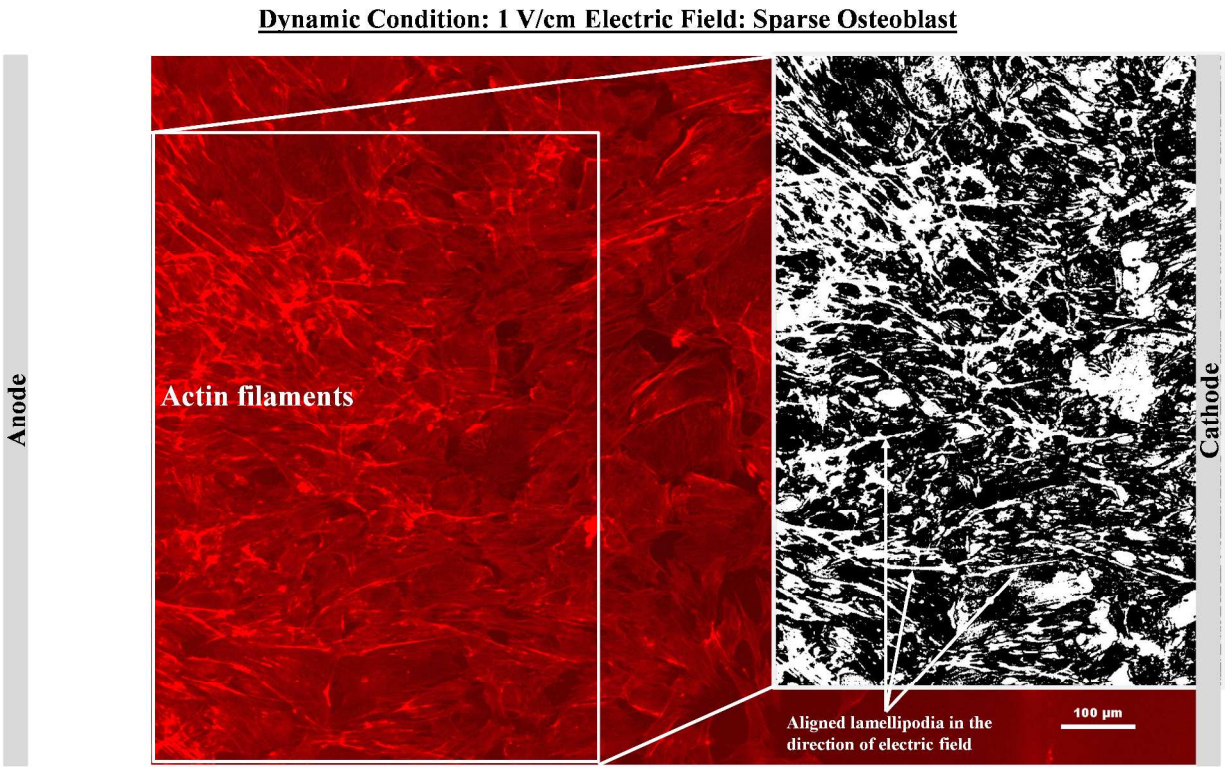

Figure 6. Representative fluoroscence micrographs showing organization of actin filaments of sparse osteoblast in the vicinity of cathode after 5 days of electric field stimulation using 1 V/cm in dynamic condition. The image processing reveals the directional arrangement of the actin (white region in the inset image) in the direction of electric field in the vicinity cathode.

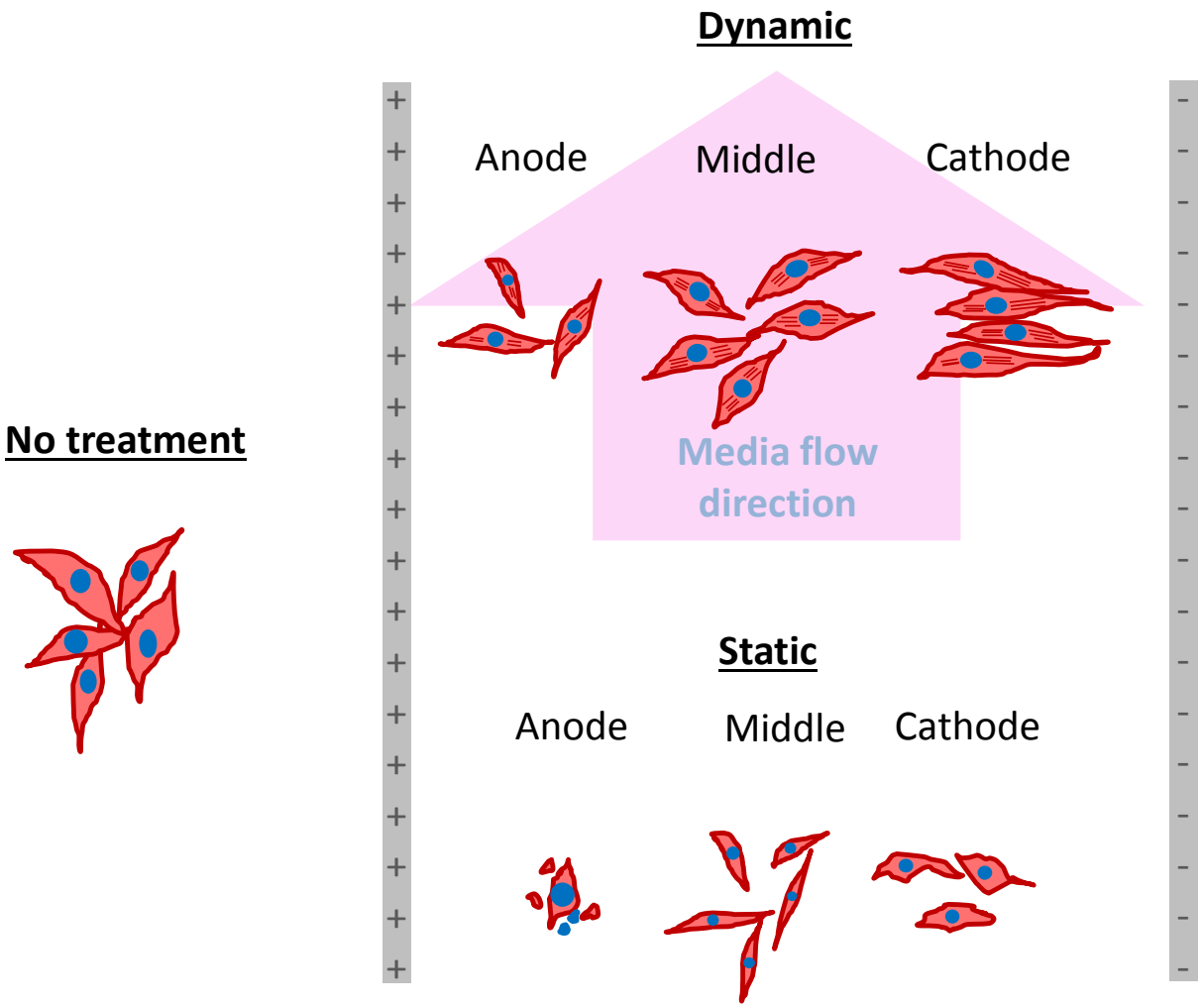

Figure 7. A schematic representation of the cell response in the presene of electric field under static and dynamic conditions. Note the organization of actin filaments and alignment of sparse osteoblast in the vicinity of cathode after 5 days of electric field stimulation using 1 V/cm in dynamic condition.

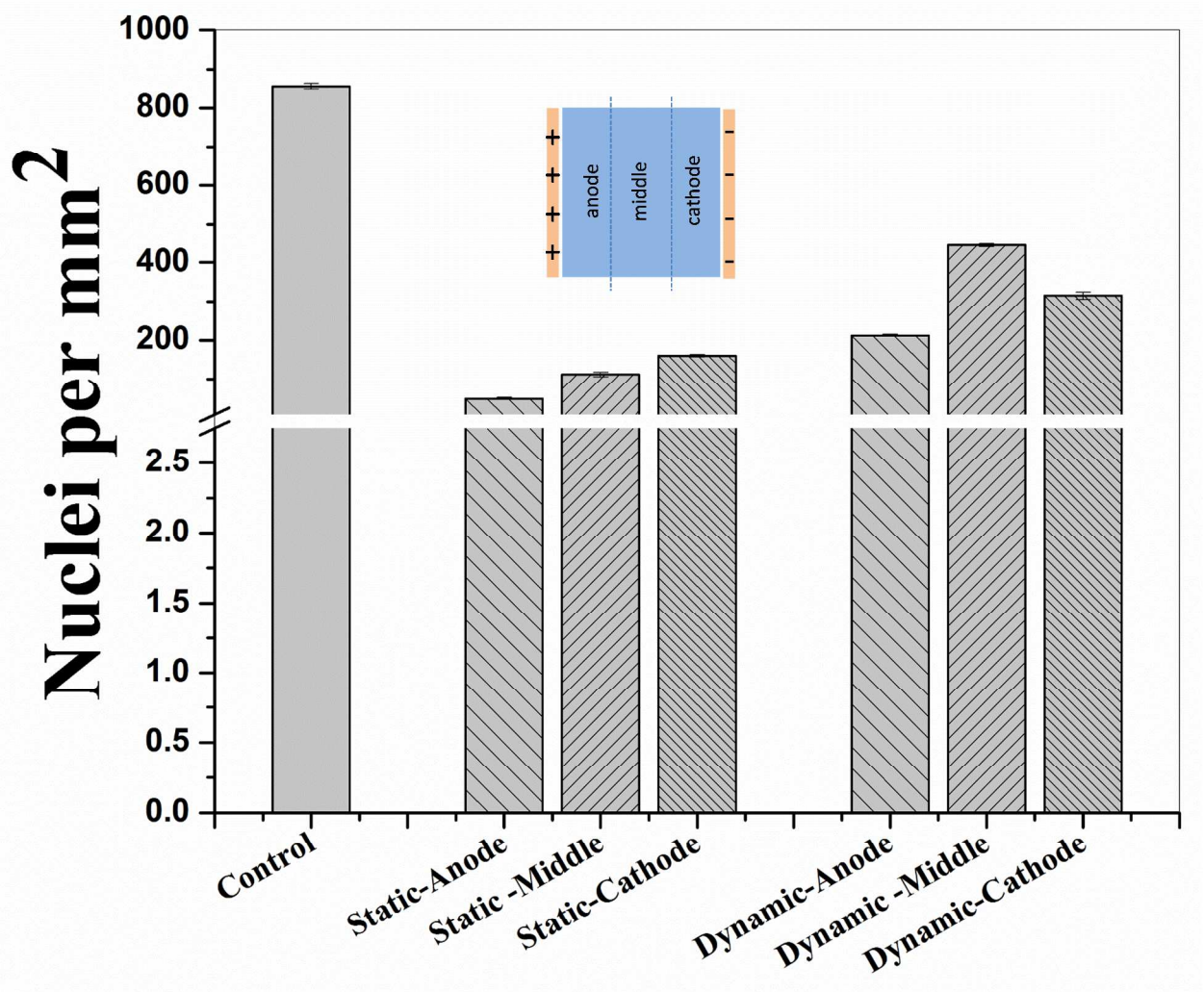

Figure 8. Histogram summarizing the number of cells in the vicinity of anode, mid-region, and vicinity of cathode after 5 days of electric field stimulation of sparse osteoblasts using 1 V/cm in static and dynamic conditions. The data indicates higher cell density in the dynamic condition.

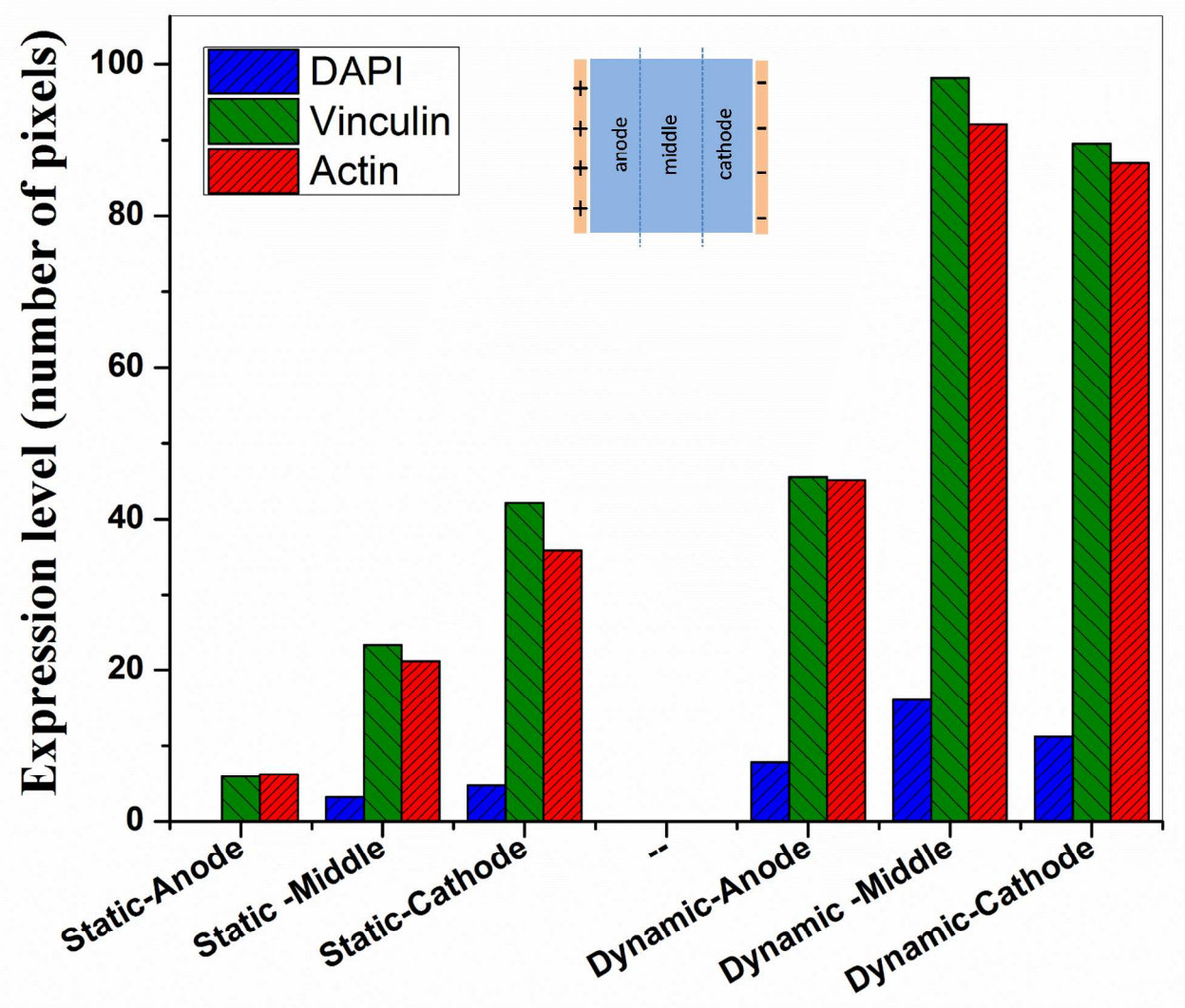

Figure 9. Histogram summarizing the expression level of DAPI, vinculin, and actin in static (1 V/cm electric field) and dynamic (1 V/cm electric field, media flow) conditions in the vicinity of anode, mid-region and vicinity of cathode after 5 days of electric field stimulation of sparse osteoblasts. The data indicates higher expression level of vinculin and actin in electric field stimulated cells and in dynamic condition.