Studying the Influence of Electroporation on HT29 Cell Line Interaction with Fibronectin and Collagen Protein Micro-Patterned Surface

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Abstract. Micro-contact printing (MCP) is a scheme that allows a substrate or surface to be functionalized freely with extracellular matrix (ECM) protein such as fibronectin and collagen, in a well-defined manner. MCP can be used to regulate cell adhesion geometry on a substrate and in controlling wound healing process by facilitating directed cell migration. In this study, human colon cancer cell line, HT29 were grown on a micro-contact printed pattern of fibronectin and collagen protein with repeat gratings of 25µm, 50µm, and 100µm wide, for 48 hours. The cells alignments to the patterned substrates were then computed, where 0º means 100% alignment to the pattern. This was done with the purpose of finding those pattern that stimulated the best degree of cell alignment. Best alignment and elongation were obtained on 50µm of the two ECM proteins. The quantitative analysis of the results revealed that HT29 cells aligned most readily to the 50µm width pattern with a mean angle of alignment of $5.0^{o} \pm 1.3$ and $16.1^{o} \pm 4.6$, respectively, on fibronectin and collagen pattern surfaces. Contrarily, the cells aligned poorly on the 25µm width pattern of fibronectin, collagen and the control substrates with a mean angle of $33.4^{o} \pm 8.4$, $36.2^{o} \pm 8.9$ and $54.5^{o} \pm 6.0$, respectively. Furthermore, the 50µm stamp pattern was used to investigate the influence of pulse electric field (PEF) on the HT29 alignment to the patterned substrate. The result revealed that there was significant improvement ($P < 0.05$) in the cell alignment between the electrically treated and the untreated cells. The alignment angles of the electrically treated cells were $4.0^{o} \pm 1.2$ and $11.2^{o} \pm 3.5$, respectively, on the 50µm pattern surface of fibronectin and collagen. Therefore, the result of the study revealed that micro-contact printing technique together with pulse electric field could offer a potentially fast method of controlling directed cell migration for wound healing application.

Keywords: Microcontact printing, electroporation, fibronectin, collagen, HT29.

1. Introduction

The Extracellular matrix (ECM) protein is the main regulators of many cellular functions such as cell to cell adhesion, cell to ECM adhesion, cell communication and cell division [1]. Additionally, ECM controls cell migration and cell shape. Understanding the process that controls cell function such as proliferation, adhesion and migration is very significant for wound healing and tissue engineering application and in the development of new tissue in vivo [2, 3]. Fibronectin and collagen are the major components of ECM protein. Each of this protein bind to a specific integrin molecule. The secretion of this protein in the course of development and the level of their expression control many cellular functions and properties [4].

There are different methods used for the deposition of proteins on a surface. This includes plasma-induced micropatterning, soft lithography, ultraviolet radiation micro-patterning and micro-contact printing methods [5]. However, micro-contact printing (MCP) is chosen in this research because it offers a cheap and simple surface patterning technique without denaturing the proteins as in the case of other methods. Additionally, a sub-unit of 0.1µm patterns of protein can be produced with MCP [6]. In this study, MCP was used to produce 25, 50 and 100µm micro-pattern of two different ECM proteins, namely, fibronectin and collagen. The aim of this study is to determine the stamp pattern that will induce the best degree of HT29 cell alignment on different ECM proteins. Subsequently, the best pattern on each protein will be used to examine the influence of PEF on the cell alignment.

Development in multicellular organisms in the course of wound healing process depends on the cell adhesion process which can be influenced by electrostatic charges [7, 8]. Therefore, manipulation of substrate charge, for instance, by patterning the substrate or by application of external electric field to enhance cell adhesion, is a promising scheme that can control cell assembly and migration in wound healing applications [9]. It has been revealed that this surface charge can speed up or slow down cell adhesion and growth on the charged surfaces through repulsion or attraction of positive ions, especially, divalent cations in the cell culture medium [7]. These cations enable interactions between the surface and negatively charged cell membrane and play an important role in creation of focal adhesions [10]. The changes in the substrate charge may modify other properties of the surface, for example, surface chemistry, which in turn can promote cell adhesion process [11]. Thus, in this study, the influence of PEF on the alignment of HT29 cells on guidance cues was investigated which in turn could be used for facilitating cell guidance and directed cell migration in wound healing application [12, 13].

2. Materials and Methods

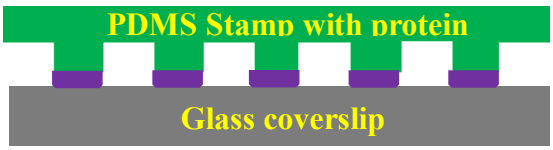
2.1 Cell Culture

The human colon cell line HT29 was used in this study. The HT29 cells were grown in a 25cm^2 culture flask as a monolayer in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (antibiotic). The cells were incubated at a temperature of 37^{o}C in a humidified atmosphere with 5% CO_2 [14], [15], [25]. Cells were harvested and passaged with a tryple express solution (TES) whenever they reached 80-90% confluency. Spent medium was changed every 2 to 3 days [14] if cells were not confluence. For subculture, the old medium was discarded by aspiration. Thereafter, 2ml of phosphate buffer saline (PBS without calcium and magnesium chloride) was added for washing the cells. The added PBS was aspirated and discarded. Next, 2ml of TES was added for cells detachment. The cells plus the TES were incubated for 5-10 minutes at 5% CO_2 at 37^{o}C until all cell become rounded and fully detached from the substrate. When the cells were fully detached, an equal volume of complete growth medium was added to stop the effect of the detachment enzyme used (TES). Next, the cells were re-suspended to form a uniform suspension which is then utilized for experimentation in subsequent sections or seeded in new flasks for further propagation.

2.2 MCP Technique

The PDMS stamps and glass coverslips were washed with 70% ethanol and left to dry totally in a biosafety cabinet before commencing the MCP process. Glass coverslips were then micro-contact printed with two ECM proteins (fibronectin and collagen IV). The dilution used for fibronectin and collagen were 100 and 50µg/ml, respectively [16]. The stamping method used was as follows: a stamp was inked by dipping into different ECM protein solutions for 60 seconds duration [17]. The stamp was then removed from the protein solution and allowed to dry in air for 90 seconds. The dried stamp was then placed in contact with the glass coverslip or substrate and pressed lightly using another glass coverslip for 60 seconds. The process was repeated for each stamp width (25, 50 and 100µm) on different proteins (fibronectin and collagen). Thus, this allowed the glass coverslips to be patterned with 25, 50 and 100µm width of the fibronectin and collagen coated tracks separated by 25, 50 and 100µm width of uncoated tracks, respectively. Table 1 summarizes the micro-contact printing process. A protein free coverslip was used as a control.

Table 1. MCP process on glass coverslip with the ECM protein.

	Sterilized PDMS stamp
	Place the Sterilized PDMS stamp into the protein solution for 60 seconds.
	Remove the PDMS stamp from the protein solution and allow it to air dry for 90 seconds.
	Print the PDMS stamp on a glass cover slip and leave it in contact with the coverslip for 60 seconds.
	Remove the PDMS stamp from the coverslip to obtain protein patterned surface.

2.3 Protocol For Cell Electroporation

In this study, the commercial electroporator ECM830 made by BTX Harvard apparatus was used for electroporating the HT29 cells in suspension before seeding onto the substrates. The low voltage (LV) mode of the BTX ECM 830 electroporator at a voltage of 240V with a 4mm electrode gap was used to achieve a 600V/cm electric field strength which was optimized earlier for HT29 cell line [18]. First of all, cells are detached using the subculture procedures. After neutralizing the effect of the detaching enzyme, 800µl of cells suspension at a concentration of 1×10^5 cells/ml was poured into a 4mm cuvette and then placed in BTX ECM 830 electroporator chamber. Electroporation was executed with an electric field of 600V/cm intensity for 500µs duration. Immediately after electroporation, the cuvette was moved into the biosafety hood.

2.4 Protocol of Plating Cells on Glass Coverslips

The glass coverslip was micro-contact printed with three different grating of the two types of ECM proteins. Next, the glass coverslip was placed, one in each well of a 6-well plate. As control, a protein free glass coverslip was also placed in another well. Next, 2ml of growth media was added to each of the wells. Next, 0.1ml of HT29 cells at concentrations of 100,000 cells/ml was seeded in each well. The six well plate was then incubated at 37^{o}C and 5% CO_2 for 48 hours. After 48 hours, cells were then imaged with standard phase contrast microscope. With the aid of DinoCapture2.0 software, cell alignment to the patterns were computed by measuring the angle between the cell long axis in relation to the stamp pattern, such that an angle of 0º represents 100% alignment to the pattern. Patterns that give the best cell alignment for each protein were also selected and seeded with electroporated HT29 cells of the same concentration as the untreated cells (0.1ml of a 100,000 cells/ml). The cells were also incubated under the same condition.

2.5 Alignment Angle and Cell Length Measurement

The DinoCapture2.0 Software was also used for measuring the alignment angle and cell length. The software is designed by the AnMo Electronic Corporation to work only with their product like the Dino camera. It is a free software program that offers users with quite a number of tools for analysis of both live video images and acquired pictures. It also has numerous features that provide users better skills and experience with a digital microscope.

Line measurement tool: This tool enables the user to measure the distance between two points. In order to measure distance or length with DinoCapture2.0 software, the followings steps were performed:

1. Acquire the images using microscope via Dino camera.
2. Open the image in DinoCapture2.0 software and select the magnification of the objective used in capturing the image.
3. Select the line measurement from the measurement tools as shown below.
4. Select a unit of measurement from software as shown below.
5. Left click on the first point and drag the line measurement tool to the end of the point and left click again to finish the measurement.
6. Repeat step four for all the lengths measurement.
7. Click on MPW icon on the toolbar and export the data to excel by clicking the export to excel icon on the MPW. A sample of a line measurement is shown in Figure 1 with the red line and blue writing.

Figure 1. An example of line measurement with DinoCapture2.0 software.

Point angle measurement tool: This tool enables the user to measure the angle by first starting at the pivot point and then extending out to the desired point. In order to measure the angle with DinoCapture2.0 software, the followings steps were performed:

1. Acquire the images using microscope via Dino camera.
2. Open the image in DinoCapture2.0 software and select the magnification of the objective used in capturing the image.
3. Select the angle measurement from the measurement tools as shown below.
4. Select a unit for measurement in the software.
5. Left click on the first point and drag the angle measurement tool to the end of the point and left click again to finish the measurement.
6. Repeat step four for all the angles measurement.
7. Click on MPW icon on the toolbar and export the data to excel by clicking the export to excel icon on the MPW. A sample of an angle measurement is shown in Figure 2 with the red line and blue writing.

Figure 2. An example of angle measurement with DinoCapture2.0 software.

2.6 Statistical analysis

All experiments in this research were repeated three times and presented as the mean plus or minus the standard deviation (+/-SD). This is because triplicate is the minimum number to have for standard deviation and the number of replicates often depends on other factors such as cost of running the experiment. Data obtained in this research were tested for normality using a Kolmogorov-Smirnov test for normality. Data that showed a normal distribution with P-value greater than 0.05 were analysed through one-way analysis of variance (ANOVA) followed by post Hoc Turkey SHD test using SPSS software. For data that were not normally distributed, the Serial Mann-Whitney test was used. A P-value of less than 0.05 would be taken as data indicating a statistically significant difference.

3. Results and Discussion

3.1 Stamp Construction and Micro-Contact Pattern Printing

Figure 3 depicts the images of the PDMS stamps of different width (25, 50 and 100µm) under microscope. The PDMS stamps were used for the creation of the fibronectin and collagen patterns on the glass coverslips. The various ECM protein pattern was successfully produced as shown in Figure 4.

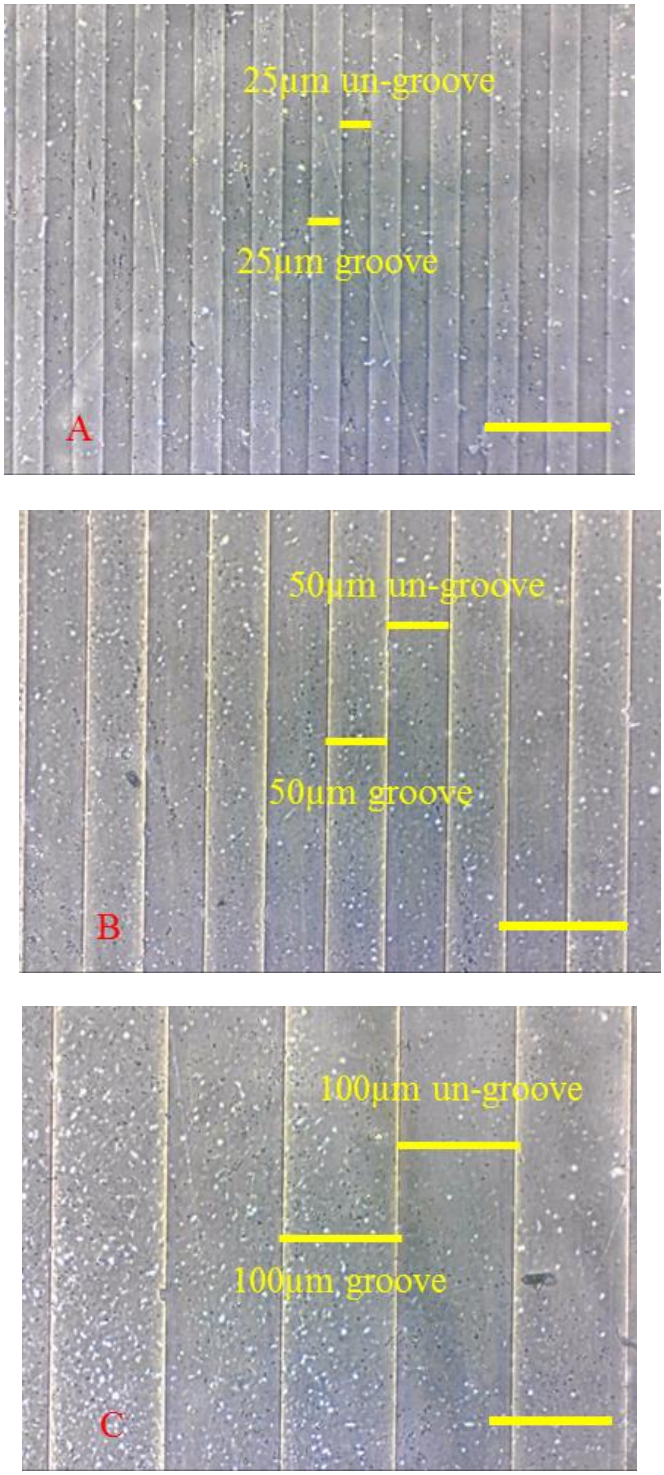


Figure 3. PDMS stamp constructed for the micro-contact printing study: (A) 25μm; (B) 50μm; and (C) 100μm. Scale bar = 100μm

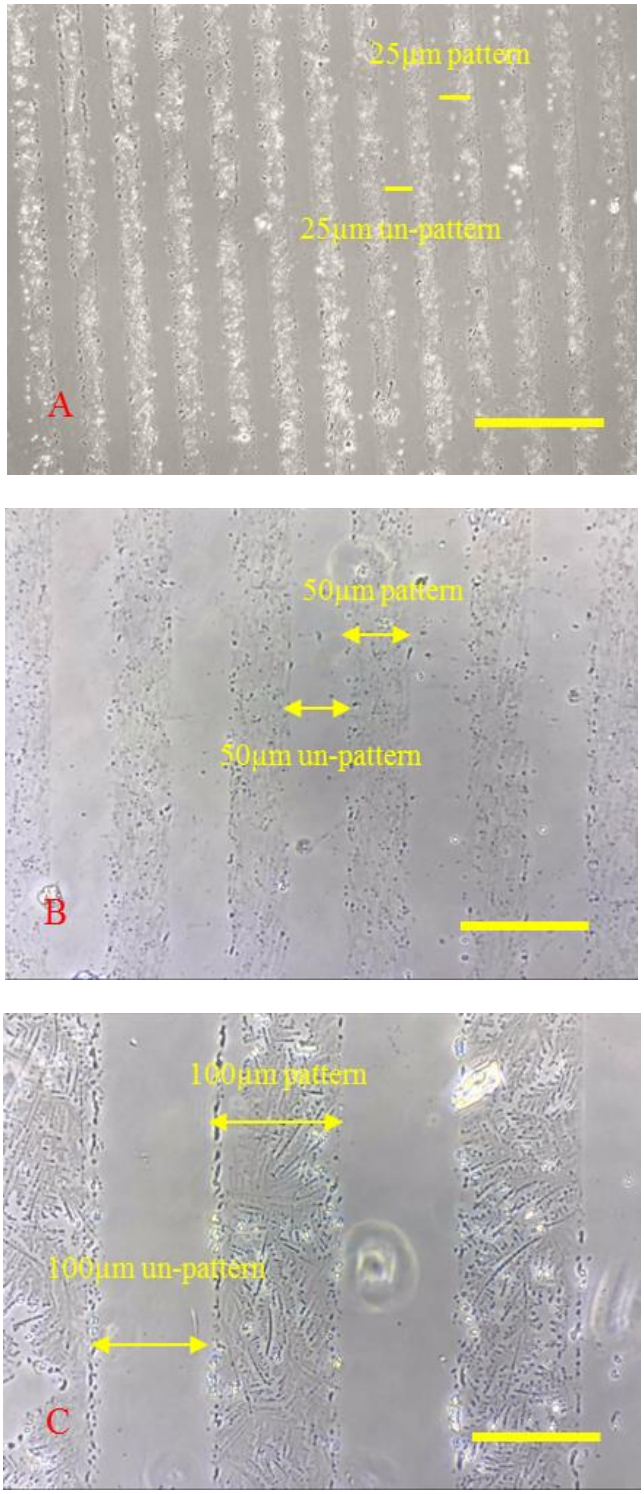


Figure 4. Images of glass coverslips micro-printed with 25, 50 and 100µm width pattern of protein. Scale bar = 100µm

Figure 5 shows the images of HT29 cell line after 48 hours of seeding on the 25, 50 and 100µm width micro-contact printed substrates of fibronectin and collagen and the control group. All substrates were seeded with the same number of cell suspension. The qualitative analysis of the images has revealed that HT29 cell line aligned better on fibronectin pattern than on collagen pattern on all the

stamp sizes used. Similarly, cell elongation reflected the result of cell alignment with fibronectin showing the highest cell elongation followed by collagen.

The result of angular alignment is interpreted as the alignment angle of forty-five degrees (45°) or greater suggesting that orientation of cell to the pattern is random, that is, no alignment [5], [17]. Whereas, an alignment angle of less than 45° implies that cell aligns to the pattern, where zero degrees designate complete alignment (100%) to the pattern [5], [19], [17].

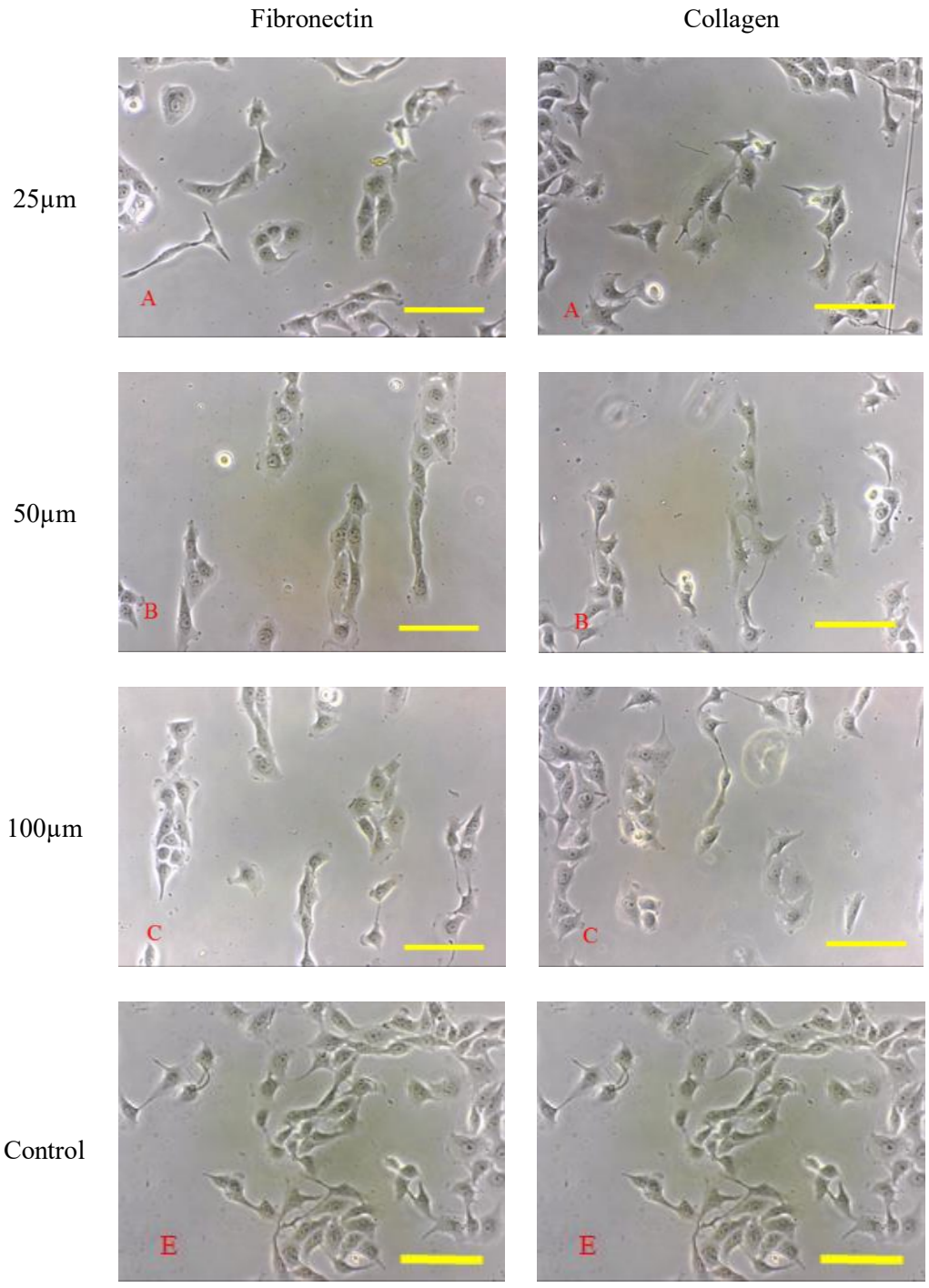


Figure 5. Images of HT29 cell line on glass coverslips micro-contact printed with the ECM proteins and the control after 48 hours. Scale bar =100µm

3.2 Measurement of Cell Alignment

Table 2 contains the data obtained from quantitative analysis of the mean angle of HT29 cell alignment on all the ECM protein used on different patterned substrates (25, 50 and 100µm) and the control substrate. The quantitative analysis of the data obtained revealed that the HT29 cells showed no alignment on control coverslip with mean angle of $54.5^{o} \pm 6.0$. Similarly, the HT29 cells showed good alignment on the 50 and 100µm width of fibronectin patterned surface with alignment angles of $5.0^{o} \pm 1.3$ and $5.8^{o} \pm 1.4$, respectively. The HT29 cells aligned poorly on the 25µm width of fibronectin patterned substrate with an angular alignment of $33.4^{o} \pm 8.4$. Similarly, the cells have shown a relatively good alignment to 50 and 100µm width of collagen patterned substrate with a mean angular alignment of $16.1^{o} \pm 4.6$ and $20.4^{o} \pm 6.3$, respectively. Just as with fibronectin, the cells were poorly aligned on the 25µm width of collagen patterned surface with an angular alignment of $36.2^{o} \pm 8.9$.

Table 2. Angle of alignment for HT29 cell line seeded on coverslip micro-contact printed with ECM protein after 48 hours of seeding.

Protein	Stamp Size	Average Angle of Alignment ± Standard Deviation (SD)	Standard error (SE)
Fibronectin	25µm	$33.4^{o} \pm 8.4$	1.53
	50µm	$5.0^{o} \pm 1.3$	0.23
	100µm	$5.8^{o} \pm 1.4$	0.26
Collagen IV	25µm	$36.2^{o} \pm 8.9$	1.63
	50µm	$16.1^{o} \pm 4.6$	0.84
	100µm	$20.4^{o} \pm 6.3$	1.15
Control		$54.5^{o} \pm 6.0$	1.11

In all experimental data obtained with respect to cells angle of alignment, the data were found to be normally distributed ($P > 0.05$) and therefore one-way analysis of variance (ANOVA) followed by Post Hoc Turkey HSD test was used in analysing the data for statistically significant difference. The results showed that between fibronectin and collagen protein patterns there is a significant difference in angle of alignment on the entire stamp width pattern used ($P < 0.05$). This implies that alignment is better on fibronectin protein irrespective of the stamp size. It could therefore be stated that the HT29 binding to the surface is mostly via fibronectin specific integrin.

The statistical results further revealed that there is no significant difference in alignment angle between 50 and 100µm width pattern of fibronectin protein ($P > 0.05$). Whereas, there was significant difference in alignment angle between 50 and 100µm width pattern of collagen protein ($P < 0.05$). Moreover, there was a significant difference in the angle of alignment between 50µm width pattern and the other pattern width (25µm and control substrates) of the two ECM protein utilised ($P < 0.05$ in all case). Similarly, there is a significant difference in the angle of alignment between 100µm width pattern and the other patterns used (25µm and control substrate) of the two ECM protein used ($P < 0.05$ in all case). Additionally, Figure 6 shows a graphical representation of quantitative data obtained for cell angular alignment. Small angle of alignment was signifying that the cells aligned better on that particular pattern.

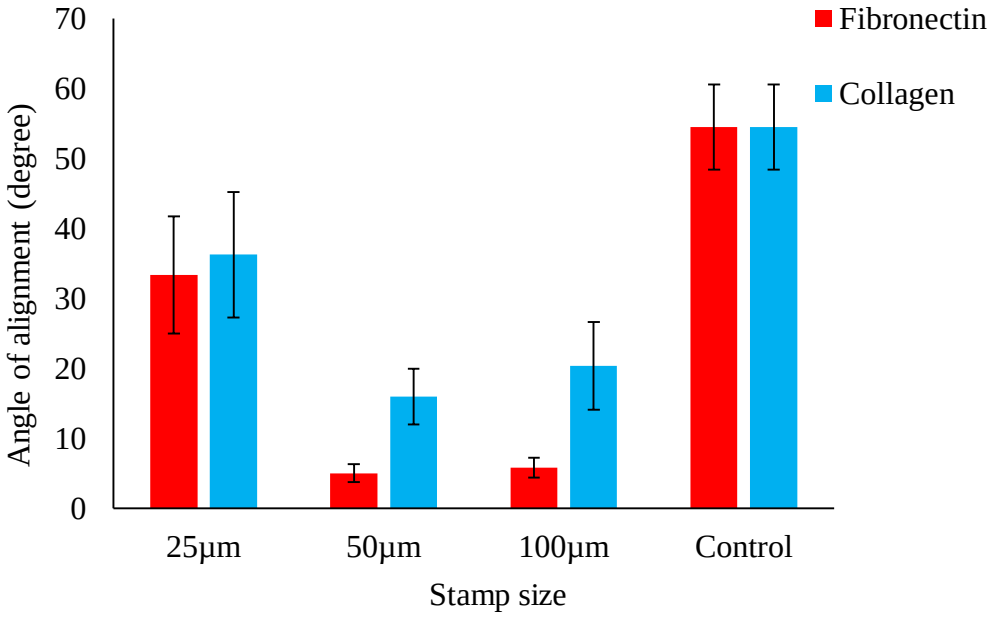


Figure 6. Graph comparing the angle of alignment of HT29 cell line seeded on 25, 50 and 100µm micro-contact printed substrates and control substrate after 48 hours.

3.3 Measurement of Cell Elongation or Cell Length

The result of cell elongation is interpreted as follows: the longer the cell length the better the alignment of the cell to the pattern [5], [17]. The result of cells elongations agrees/reflects well with the result of cell alignment, i.e. cells showing best alignment revealed the highest elongation. Table 3 shows the data obtained from quantitative analysis of the mean cell length of all the ECM protein used on different patterned substrates (25, 50 and 100µm), and control substrate. The mean cell length on the control substrate was 39.6µm ± 5.90. In the case of fibronectin, the mean cell lengths were 53.0µm ± 8.3, 88.8µm ± 13.3 and 71.0µm ± 11.8, respectively, on the 25, 50 and 100µm width patterned substrates. Furthermore, the mean cell lengths on the 25, 50 and 100µm width patterned substrates of collagen were 42.7µm ± 7.8, 58.5µm ± 6.2 and 50.5µm ± 7.7, respectively.

Table 3. Average length of HT29 cell line seeded on 25, 50, 100µm micro-contact printed substrate and the control substrate after 48 hours.

Protein	Stamp Size	Average Cell Length (µm) ± SD	SE
Fibronectin	25µm	53.0 ± 8.3	1.52
	50µm	88.8 ± 13.3	2.43
	100µm	71.0 ± 11.8	2.15
Collagen IV	25µm	42.7 ± 7.8	1.42
	50µm	58.5 ± 6.2	1.13
	100µm	50.5 ± 7.7	1.41
Control		39.6 ± 5.9	1.18

The results showed that between fibronectin and collagen protein patterns, there is statistically significant difference in cell elongation in all the stamp sizes used with $P < 0.05$ in all cases. The

statistical results revealed that there is significant difference in the cell elongation between all other stamp sizes of the two ECM proteins used ($P < 0.05$). Except for the case of 25µm of collagen and control which showed no significant difference. Figure 7 shows the graphical representation of quantitative data obtained for the cell elongation. Longer cell elongation was signifying that the cells aligned better on that particular pattern.

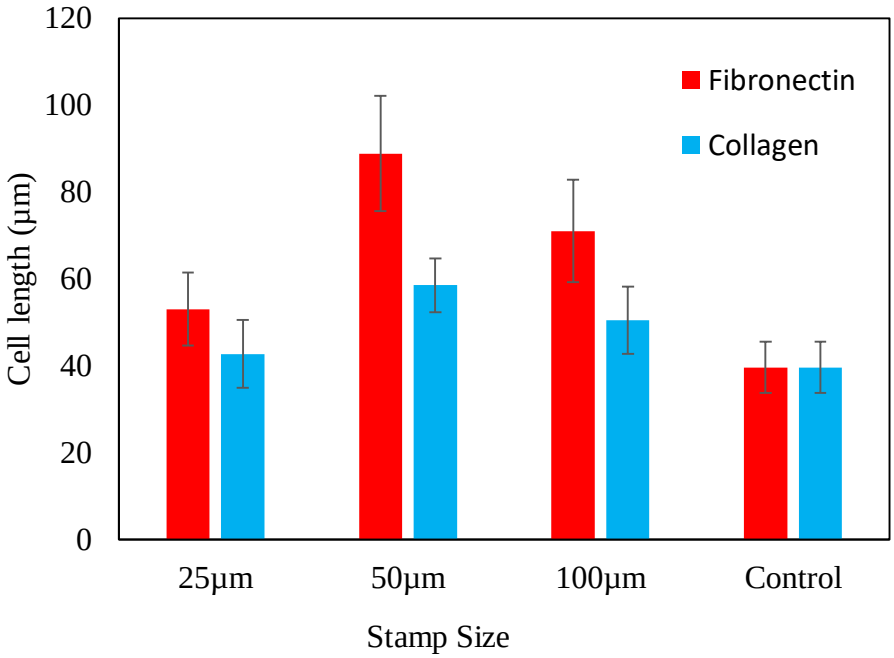


Figure 7. Graph comparing the cell length of HT29 cell line seeded on 25, 50, 100µm micro-contact printed substrates and control substrate after 48 hours.

The result of the study showed that HT29 is most readily aligned to the 50 and 100µm stamp size. This could be connected to the cell size, whereby for smaller stamp size (relative to the average cell length of the HT29 cell line), the cell could not distinguish between the patterns protein lines and un-pattern protein lines, in which case the cell could overlap [16]. Whereas for stamp size greater than the average cell length of the HT29 cell line, as in the case of 50 and 100µm stamp size, the cells could easily distinguish between the protein pattern lines and the protein un-pattern lines. Hence, they could easily follow the guidance cues [17], [19]. The results obtained in this research (results without PEF treatment) are qualitatively in agreement to that of Khaghani et al., (2008), Berend, (2012) and Sefat (2013) that also demonstrated that Chondrocyte, Keratinocyte cells and MG63 osteoblast bone cells, respectively, were most readily aligned to 50 and 100µm [16], [19], [20]. In order to have a better understanding, we further investigated the cell alignment and elongation with application of PEF.

3.4 Influence of PEF on Cell Alignment and Cell Elongation

Cell alignment on 50µm stamp pattern of fibronectin and collagen were the best for the respective protein patterns. Hence, these stamp sizes were used to investigate the influence of PEF on the HT29 cell line alignment on MCP surface. The cells were electrically treated before plating on the patterned substrates of 50µm for fibronectin and collagen. The cells were cultured for 48 hours with the same number of cells as in the case of the untreated cells and under the same physiological environment. Figure 8 shows the images of HT29 cell line after 48 hours of seeding on 50µm stamp pattern of fibronectin and collagen.

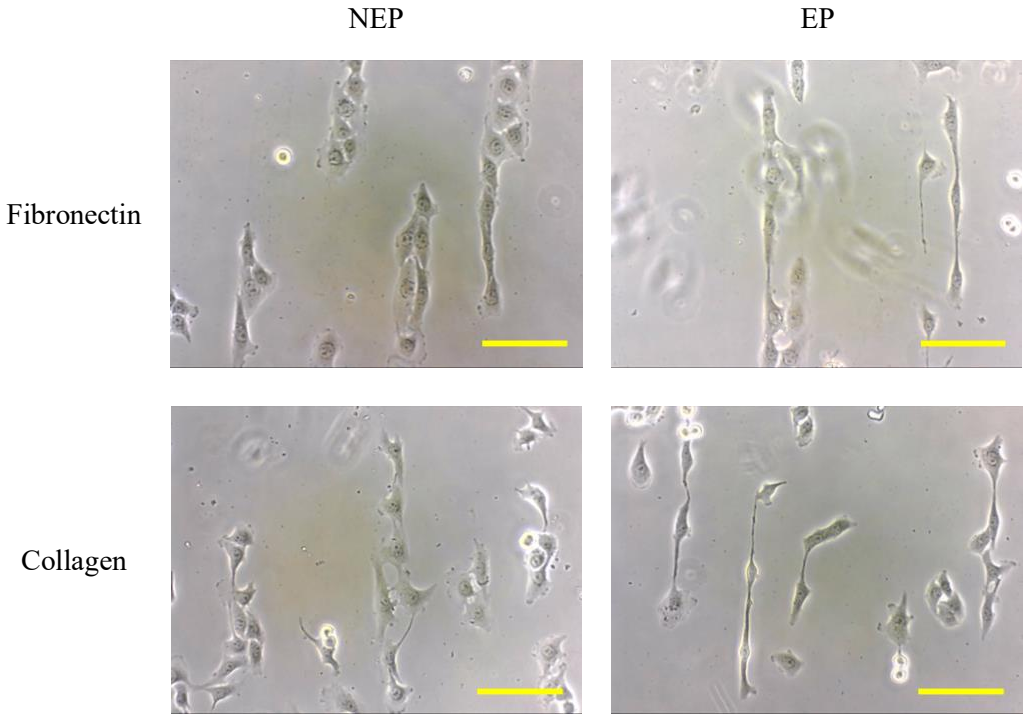


Figure 8. Images of HT29 cell line on 50µm micro-contact printed substrates of fibronectin and collagen respectively. Scale bar = 100µm

The results of the HT29 cell alignment and elongation after exposure to PEF on the 50µm pattern of fibronectin and collagen have shown significant improvement. The angular alignments of HT29 cells were 4.0° ± 1.2 and 11.2° ± 3.5 for the 50µm pattern of fibronectin and collagen, respectively, under PEF treatment as shown in Table 4. On the other hand, the cell lengths of HT29 cells were 101.4µm ± 13.6 and 86.5µm ± 21.2 for the 50µm pattern of fibronectin and collagen, respectively, under PEF treatment as shown in Table 5. Moreover, Figures 9 and 10 show the graphical representation of the cell angular alignment and elongation of the HT29 cell line in the PEF treated and control group.

Table 4. PEF treated HT29 cell line alignment on 50µm micro-contact printed substrates of fibronectin and collagen.

Treatment	EP average angle of alignment (degree) ± SD	SE	NEP average angle of alignment (degree) ± SD	SE
Fibronectin	4.0 ± 1.2	0.22	5.0 ± 1.3	0.23
Collagen	11.2 ± 3.5	0.64	16.0 ± 3.4	0.73

Table 5. Length of PEF treated HT29 cells on 50µm micro-contact printed substrates of fibronectin and collagen.

Treatment	EP Average Cell Length (µm) ± SD	SE	NEP Average Cell Length (µm) ± SD	SE
Fibronectin	101.4 ± 13.6	2.48	88.8 ± 13.3	2.43
Collagen	86.5 ± 21.2	3.87	58.5 ± 6.2	1.13

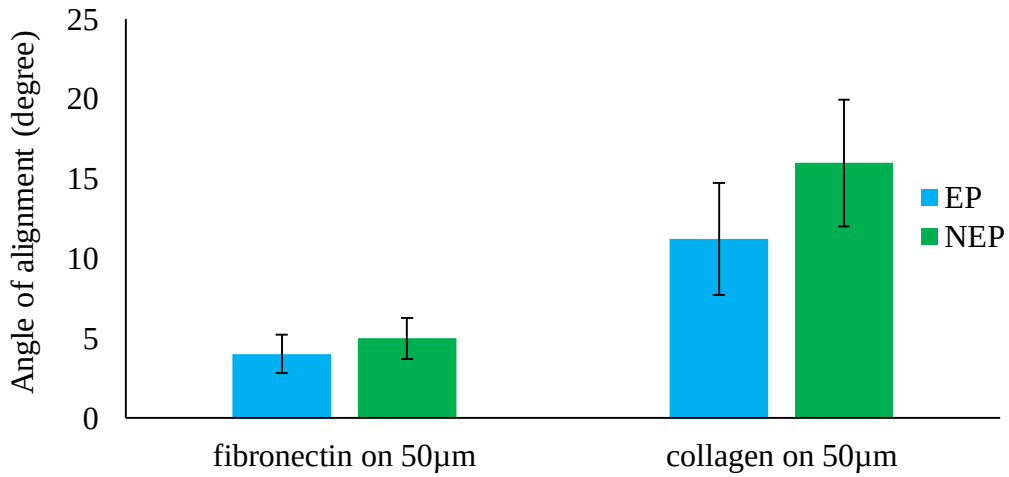


Figure 9. Graph showing PEF treated HT29 cell line alignment on 50µm micro-contact printed substrates of fibronectin and collagen.

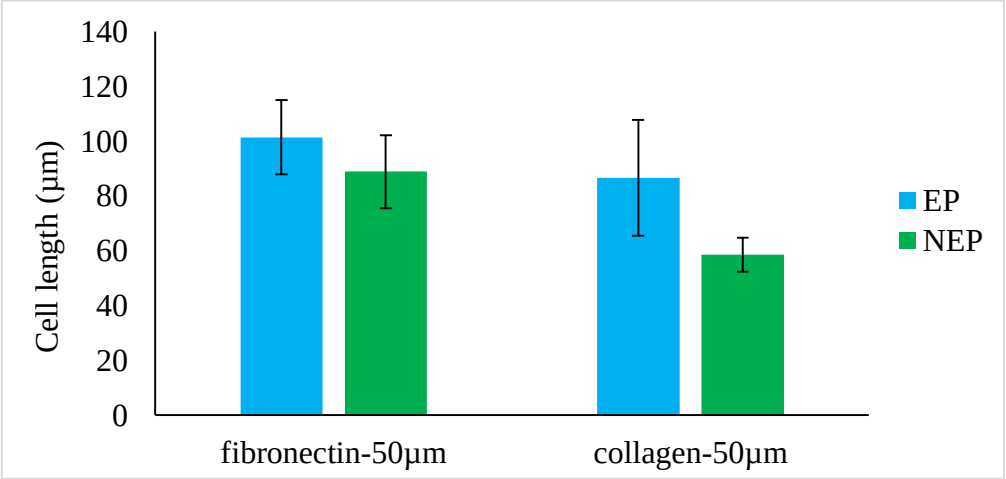


Figure 10. Graph showing length of PEF treated HT29 cells on 50µm micro-contact printed substrates of fibronectin and collagen.

The data obtained from PEF treatment revealed that there was an improvement on the alignment of HT29 cell line on 50µm pattern of fibronectin and collagen. The angles of alignment decreased from $5.0^{o} \pm 1.3$ to $4.0^{o} \pm 1.2$ and from $16.0^{o} \pm 3.4$ to $11.2^{o} \pm 3.5$, respectively, for the fibronectin and collagen under PEF treatment. The results are also statistically significance ($P = 0.003$, i.e. $P < 0.05$) with a mean difference of 1.0^{o} and ($P = 0.0001$, i.e. $P < 0.05$) and mean difference of 4.8^{o} on the 50µm pattern of the fibronectin and collagen, respectively, under PEF exposure. Similarly, the cell elongation under PEF on the 50µm patterned of fibronectin and collagen increased from 88.8µm ± 13.3 to 101.4µm ± 13.6 and from 58.5µm ± 6.2 to 86.5µm ± 21.2, respectively. The results are also statistically significance ($P = 0.001$, i.e. $P< 0.05$) in both cases with a mean difference of 12.6µm and 28.0µm, on the 50µm pattern of fibronectin and collagen, respectively, under PEF exposure.

The result has shown that PEF has great influence on the HT29 cell line alignment and elongation. This could be due to the fact that PEF stimulates the integrin and cadherin which are the molecules responsible for cell-cell and cell-ECM adhesion [21], [22]. In addition, it could be that the PEF

modulated the electrostatic charges on the cell which is also significant and accountable in cell adhesion and alignment [23], [24]. Since the HT29 cell line has shown alignment to all the ECM proteins used in this study, though is better on fibronectin than collagen, we could hypothesis that HT29 attach typically by means of fibronectin-specific integrin signaling pathways [16]. The result of the HT29 cell alignment and elongation via MCP has shown that the cells aligned and elongated most readily on 50µm pattern of fibronectin and collagen.

The HT29 cell have shown strong tendency to direct their movement by contact guidance along the edge of the protein pattern cues. It is possible that the protein patterns provide a suitable surface for the adhesion of the cell lamellipodia and filopodia thereby instigated their projection along the patterns. In addition, the PEF could have further stimulated the filopodia and the cell signaling pathway and facilitated the cell to be aligned better on the pattern cues as compared to the cell that are not exposed to the PEF. Growth and repair in multicellular organism depend solely on cell adhesion which is also affected by electrostatic charges on the cell surroundings [7]. Therefore, controlling the surface charges, for instance by coating the surface with molecules could enhance cell adhesion strength. This could serve as promising technique in manipulating cell migration and assembly in wound healing and tissue engineering application [9].

4. Conclusions

In this work, the influence of PEF on the alignment and elongation of HT29 cell line via micro-contact printing technique has been studied. The HT29 cells were successfully cultured in the presence of various protein patterned surfaces. The cells were found to be aligned and elongated most readily on the 50µm wide stamp pattern of all the ECM proteins used in this work. Best cell alignment and elongation was revealed on the 50µm width pattern of fibronectin protein, followed by the 50µm of collagen protein. The findings suggest that HT29 cells attach mostly via fibronectin specific integrin. Furthermore, when the cells were exposed to PEF before seeding on the pattern surface, the cell alignment and elongation was greatly enhanced. The result in this study, therefore, revealed that PEF can be used to influence the alignment of cells on guidance cues, which in turn could be used for facilitating cell guidance and directed cell migration in wound healing and tissue engineering application. In our next study, the combined influence of transforming growth factor beta-3 (TGF-β3) and PEF on HT29 cell proliferation, spreading and adhesion have been investigated.

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