CELL PATTERNING WITH MAGNETIC MANIPULATION

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ABSTRACT

CELL PATTERNING WITH MAGNETIC MANIPULATION

Tissue engineering is a biomedical engineering field that provides solutions to restore, maintain, improve or replace tissues or whole organs. The main goal tissue engineering is to overcome the restrictions of existing treatments that are based on organ transplantation. Cells and biomaterials can be used to form functional tissues and organs. Actually, the goal is to produce structures that resemble and mimic the real tissues. One of the useful mimicking technique is cell patterning. Cell patterning is a technique that provides cell clusters are located at a proper position for function of tissues. Some of the patterning techniques uses cell adhesion ligands, optical tweezers, acoustic tweezers, dielectrophoresis and magnetic force. In addition to the advantages of all these techniques, there are also disadvantages. However, Magnetic force-based cell patterning techniques provide excellent advantages such as low adverse effects to cell. Magnetophoresis is one of the magnetic force-based cell patterning technique that forms cell patterns without labeling cells in a short time using the principle of movement of the cells to lower magnetic field region in a paramagnetic medium. In this study, a cell patterning system was used to form cell patterns via magnetophoresis. Results showed that cell patterns were formed in different shapes in a short time and they maintained integrity even if magnetic force was removed.

ÖZET

MANYETİK MANİPÜLASYON İLE HÜCRE ÖRÜNTÜLEME

Doku mühendisliği dokuları ya da organları onarmak, korumak ya da iyileştirmek için çözümler sunan bir biyomedikal mühendislik alanıdır. Doku mühendisliğinin temel amacı organ nakline dayalı mevcut tedavilerin yetersiz kaldığı durumlarda çözüm sunmaktır. Bu bağlamda hücreler ve biyomalzemeler fonksiyonel doku ve organları oluşturmak için kullanılabilir. Asıl hedef gerçek dokulara benzeyen ve gerçek dokuları fonksiyonel anlamda taklit edebilecek yapılar üretmektir. Gerçek dokuları taklit eden yapılar üretilmesini sağlayan birçok doku mühendisliği tekniği vardır. Bu tekniklerden biri de hücre örüntüleme tekniğidir. Bu teknik, hücre kümelerinin dokuların işlevi için uygun bir konuma yerleştirilmesini sağlayan bir tekniktir. Hücre yapıştırma ligandları, optik cımbızlar, akustik cımbızlar, dielektroforez ve manyetik kuvvet kullanılarak hücre örüntüleme yapılabilir. Tüm bu tekniklerin avantajlarının yanı sıra dezavantajları da vardır. Buna karşın, manyetik kuvvet esaslı hücre modelleme teknikleri hücrelerde diğer tekniklerden düşük yan etkilere sebep olma gibi üstün avantajlar sağlar. Manyetoforez, hücrelerin paramanyetik bir ortamda düşük manyetik alana hareket etme prensibini kullanarak hücreleri etiketlemeden kısa sürede hücre örüntülerini oluşturan manyetik kuvvet esaslı bir modelleme tekniğidir. Bu çalışmada manyetoforez yoluyla hücre örüntüleri oluşturmak için bir hücre örüntüleme sistemi kullanılmıştır. Sonuçlar hücre örüntülerinin kısa sürede farklı şekillerde oluşturulabildiğini ve manyetik kuvvet kaldırılsa bile örüntülerin bütünlüğünü koruduğunu gösterdi.

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CHAPTER 1

INTRODUCTION

1.1. Tissue Engineering

Tissue engineering is a field that applies the principles of life sciences and engineering to the improvement of biological substitutes that restore, maintain or improve tissue function or a whole organ [1], and to the production of realistic tissue constructs for *in vitro* applications. Concerning clinical applications, tissue engineering aims to overcome the restrictions of existing treatments that are based on organ transplantation and biomaterial applications [2].

Tissue engineering is a promising technology for overcoming the organ transplantation crisis that results from donor absence [1]. Forming functional organs by using biomaterials and cells is aimed with tissue engineering (Figure 1). Artificial tissues can be implanted to patients to repair tissue functions that were lost because of heavy injury, disease or a genetic disorder. Tissue equivalents can also be utilized to screen the influences of drugs and toxins and they decrease the use of animals in research [3]. However, it is hard to construct functional organs because tissue-engineered architectures are not completely similar to the *in vivo* organs, in which the cells are allocated exactly and with a high resolution. Therefore, it is required that the produced functional tissue architectures allocate cells as specified above. Tissue engineering deals with the restoration the function of diseased or damaged tissues through the use of cells and biomaterials [3]. Many tissue-engineering approaches include the *in vitro* culture of cells on biomaterial scaffolds to form functional engineered constructs. The working hypothesis is that *in vitro* culture conditions have a significant effect on the structural and mechanical features of engineered tissues, and therefore can be used to manipulate the growth and functionality of engineered tissues. *In vitro* culture conditions will imply to tissue-engineering scaffold systems, signals, growth factors, and mechanical conditioning regimens that mediate cell attitude and functional tissue assembly [4,5]. Mimicking a real tissue is a tissue engineering method and it can be a solution for biomedical problems.

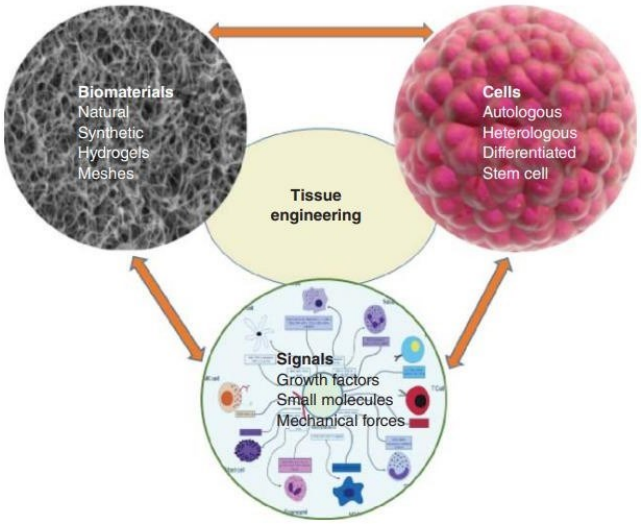


Figure 1.1. The triad of the tissue engineering. The combination of cells, scaffolds and signals is utilized to functionalize tissues (Source: Mhanna and Hasan, 2017) [6]

Specific signals are needed for cells to live and generate their own matrix. Signals are formed by the surrounding cell microenvironment and they contribute to proliferation, apoptosis, migration, differentiation, and matrix synthesis. The most important signals are oxygen levels, mechanical stimulation, growth factors, ECM molecules and other small molecules [6]. Behaviour of cells is also affected from cytokines, growth factors and ECM molecules (Figure 2). For example, "stemness" of stem cells is maintained by fibroblast growth factor (FGF-2) [7], bone formation is provided via bone morphogenic protein (BMP) [8,9], neural differentiation needs nerve growth factor (NGF) [10,11] and chondrogenesis is induced by transforming growth factor beta (TGF-β) [12]. Informations about the role of cellular signals can be used to develop the appropriate treatment method for the identified problem.

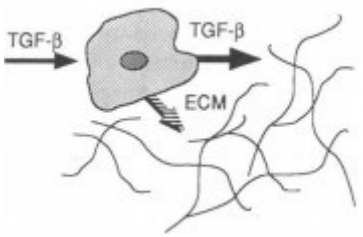


Figure 1.2. Example of the effect of growth factors on cell development: TGF-β induces cell to produce ECM (Source: Border and Ruoslahti, 1992) [13]

Cells are needed to produce engineering tissue resembling the native tissue. The use of primary cells (autologous cells), taken from the patient would be the most successful method but it has some limitations such as invasive nature of cell collection and the potential for cells to be in a diseased state. Therefore, stem cells (Figure 3) such as embryonic stem (ES) cells, bone marrow mesenchymal stem cells (BM-MSCs) and umbilical cord-derived mesenchymal stem cells (UC-MSCs) are used [14]. Embryonic stem (ES) cells could allow the production of type-matched tissues for each patient. ES cells can be maintained for long culture periods, therefore they provide large amounts of cells for tissues that could not be derived directly from a tissue source. ES cells have pluripotent property so this property gives ES cells ability to control differentiation to the desired tissue lineages [15]. Bone marrow-derived mesenchymal stem cells (BM-MSC) are stem cell type that are used to repair bone and cartilage. They are able to differentiate from a generic marrow cell population to an osteogenic lineage [16-20]. These cells can be used to improve materials such as the filler used for stabilizing artificial hip joints or for joining critical sized defects in bone that would not otherwise heal [21]. It was discovered that umbilical cord blood contains MSCs that can differentiate into varying cell types in previous studies. They showed similarities to BM-MSCs with an ability to differentiate into osteoblasts, adipocytes, hepatocytes and neuronal-like cells [22-25].

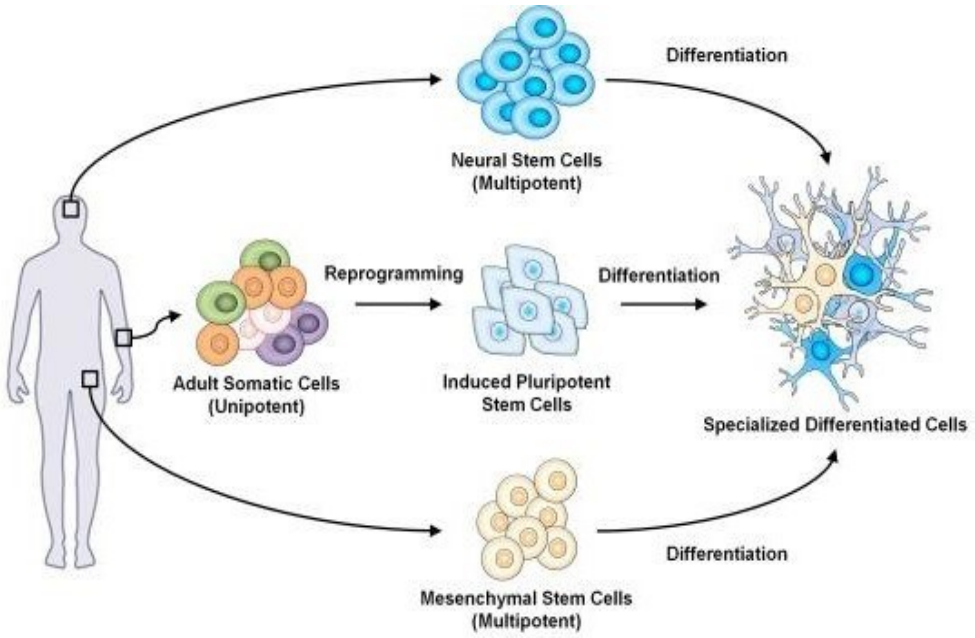


Figure 1.3. Stem cell usage in tissue engineering: Adult stem cells, induced pluripotent cells and mesenchymal stem cells are used in tissue engineering (Source: Lee et al, 2018) [26]

Scaffolds have great role in tissue engineering such as providing structural support and convenient signaling cues for cells so that they can replace the scaffold with their own synthesized matrix (Figure 4). Usually, the aim is to design a scaffold that mimics the composition and structure of the target tissue [6].

Scaffolds can be natural or synthetic. The most known natural scaffolds are collagen, elastin and alginate. Collagens form the basis of ECM of many tissues and give stability and tensile strength to the ECM [27]. Elastins provide improved elastic properties to tissues [28]. Alginate which is not present in the human body, is able to modulate its tensile and viscoelastic properties without resorting to crosslinking methods that can adversely affect cell-binding epitopes [29].

On the other hand, the advantage of synthetic scaffolds in tissue engineering applications is their versatility. Being able to control the ratio of co-polymer structures, the structure of monomeric units, polymer sequence, chain length also enables control of biodegradability and mechanical properties [30,31]. The most known synthetic scaffold materials are PCL (poly-Ɛ-caprolactone) and PLGA (poly(lactic-co-glycolic)acid). PCL is a hydrophobic and semi-crystalline material which has viscoelastic behaviour similar to native tissue [32]. Since it is broken slowly in human body, it provides prolonged scaffold support. Due to this properties, PCL is a good choice for bone TE [33], tracheal TE [34] and intervertebral disc regenaration [35]. Another common synthetic scaffold material is PLGA. PLGA is versatile, biocompatible, aliphatic polymer used for TE applications [36]. PLGA degrades more rapidly unlike PCL.

Synthetic scaffolds provides some advantages such as being customisable and versatile but there are some limitations about them. They lack of the inherent biomimicry that is a feature of natural polymers can limit their application [37]. They have very poor compatibility and their by-products can be toxic when they degrade [38].

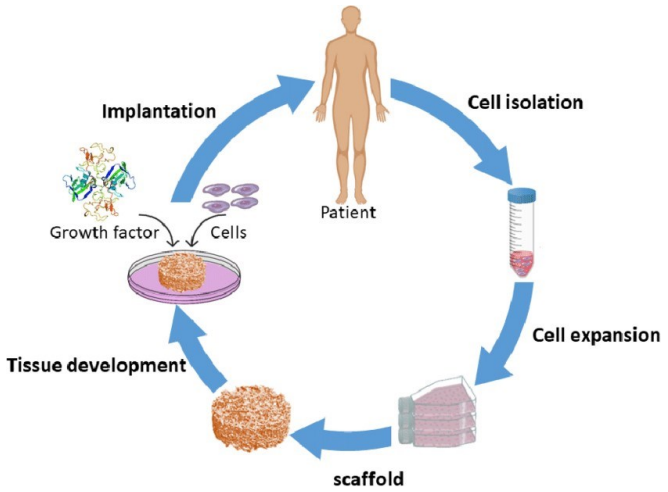


Figure 1.4. Schematic of the scaffold-based tissue engineering approach (Source: Asadian et.al., 2020) [39]

When all tissue engineering applications are evaluated, the common goal of all is mimicking the real tissues. Cell patterning is one of the useful technique of mimicking.

1.2. Cell Patterning

One of the most important tool for mimicking cellular arrangements for tissue engineering applications is cell patterning [40]. In this manner, cell clusters have to be located at proper positions for functions of tissues [3].

Cell patterning first came up with Carter in the 1960s. These early works included metal evaporation and following deposition onto a cell non-adherent surface through a stencil-like mask. The deposited metal formed patterned islands that assisted cell growth. This technique caused a way to form more controlled experiments; the pattern allowed one to track single cells and to monitor their responses for several days *in vitro* over the course of a biological study [41-43]. Then, several cell patterning techniques were developed in time. There are some examples in the literature.

Dielectrophoretic Force

To fabricate micropatterns of living cells at a glass substrate, repulsive dielectrophoretic force can be utilized. An interdigitated microarray (IDA) electrode is placed opposed to the substrate. Application of AC voltages to the IDA electrode forces

the cells to move and rapidly yields a cell pattern which reflects the shape of the electrode array (Figure 5). The IDA electrode can be repeatedly utilized as a template to increase the amount of the cell patterns [44].

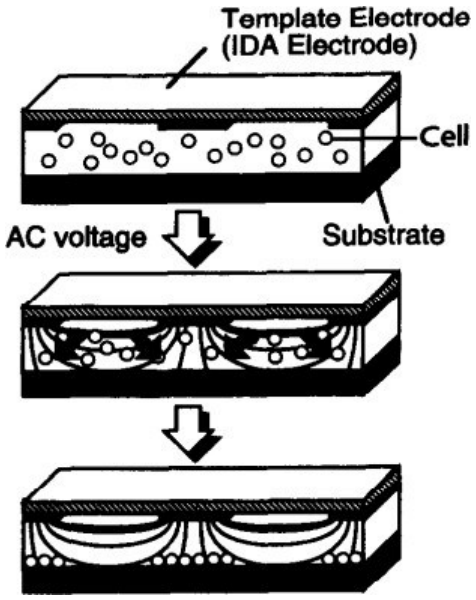


Figure 1.5. Principle of the cell patterning with dielectrophoretic force. When AC voltage is applied to electrode, cells move to glass substrate and patterns occur as a projection shadow of the array pattern of electrode (Source: Matsue et.al., 1997 [44]

A glass substrate is put on the microscope stage, and an IDA electrode is placed above the substrate with the electrode side down. Then, cell suspension is sucked into the space. When an AC voltage is applied to the IDA electrode, the cells suspended in the space are forced to move quickly to the glass substrate and produced lines at the substrate in a few seconds (Figure 6). Since the cell pattern that produced at the substrate is a projection shadow of the array pattern of the IDA electrode, the IDA electrode actes as a template for the cell patterning [44]. The width of cell pattern can be changed by changing the electrode width of the template IDA electrode. Since the movement of cells are administered by the electric field, patterns disappear quickly after removing voltage.

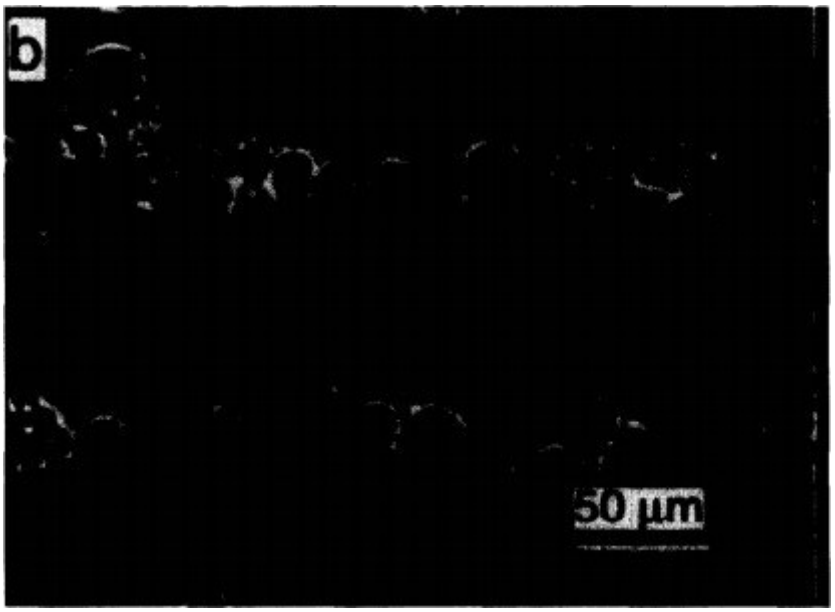


Figure 1.6. Patterned cells after AC voltage is applied to IDA electrode (Source: Guo et. al., 2016) [44]

Surface Acoustic Waves (Acoustic Tweezers)

Using 3D acoustic tweezers is an another patterning method which utilize surface acoustic waves to form 3D trapping nodes for the capture and manipulation of cells. 3D acoustic tweezers can be utilized to pick up, translate, and print single cells and cell assemblies to form 2D and 3D structures in a noninvasive, label-free and contact-free manner (Figure 7). Acoustic vibrations create a pressure gradient in the medium to move suspended cells for cell manipulation. The position of these 3D trapping nodes can be certainly controlled in a 3D environment by varying the input power. Thus, cells are picked and delivered to desired places to form cell patterns [45]. Besides these, acoustic tweezers have minimal impact on cell viability and function.

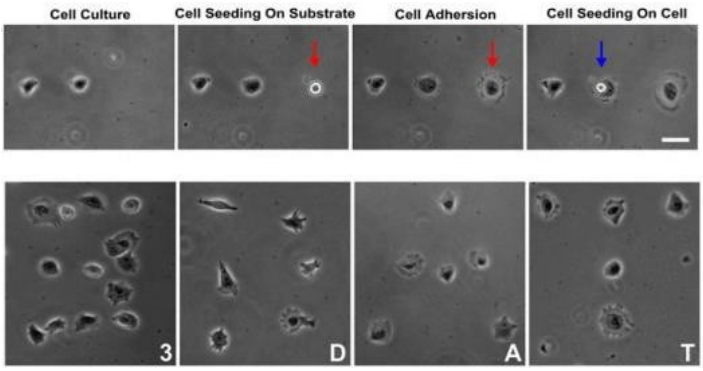


Figure 1.7. Printing living cells with 3D acoustic tweezers. Acoustic vibrations create a pressure gradient to move cells so cells are delivered to certain places to form cell patterns (Source: Guo et. al., 2016) [45]

Adhesive, Soluble and Mechanical Cues

Using adhesive and soluble cues guide patterning locally at a distance. Utilizing this approach, changing the degree of cell adhesion can modulate many cellular processes [46-47]. It is clear that adhesive, soluble, and mechanical cues can arrange cells to direct their functions and thus they could be used to reconstruct functional tissues for regenerative medicine [48]. Since adhesive cues provide cell adhesion to the ECM and neighboring cells, they play a important role in regulating many cellular functions, such as proliferation, differentiation, and migration (Figure 8).

In spite of the importance of adhesive interactions to cell function, it is insufficient defined whether the identity of the matrix protein and respective receptors must be optimized to engage specific cellular attitude. Soluble cues in the form of growth factors and other chemokines regulate numerous cell functions, from proliferation and differentiation to motility and protein synthesis [49].

Recently, studies show that mechanical properties (e.g., rigidity) of the ECM to which a cell adheres can also mediate many aspects of cell function, including proliferation, differentiation, and migration. Increasing ECM rigidity can be correlated with increased proliferation of fibroblasts and vascular smooth muscle cells [50] and greater tumor malignant potential [51]. Changing ECM rigidity also induces MSCs to differentiate into different tissue types corresponding to the tissues' relative mechanical rigidity *in vivo*. Besides all the benefits, while adhesive cues can organize cells on

surfaces within minutes to hours [52], mechanical cues control cell arrangement over days [53].

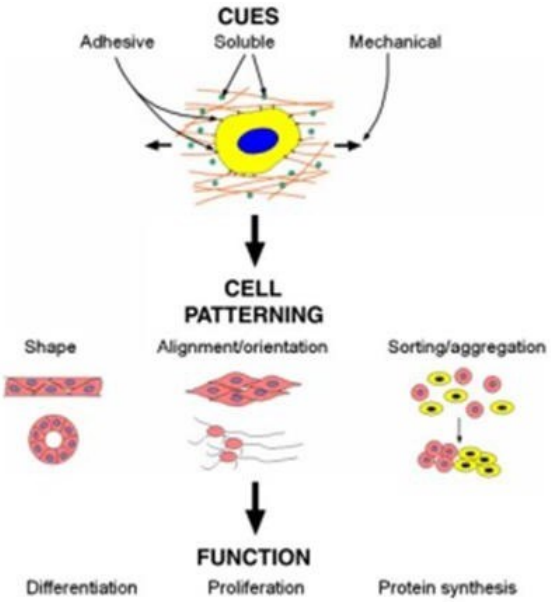


Figure 1.8. Effects of Adhesive, Soluble and Mechanical Cues on Cell Patterning. These factors help to take shape, align and aggregate of cells (Source: Shen et.al., 2008) [48]

Electroactive Mask

This method provides patterning of two cell types by attaching them to a substrate (Figure 9). An electroactive mask directs the attachment of a first cell type, followed by electrochemical modulation of the surface to allow attachment of a second cell type to the previously inert regions. Second, it provides control in defining the features of the substrate [54].

In a study that is used this method, conjugates of cyclopentadiene and the peptide Gly-Arg-Gly-Asp-Ser-NH_2 (RGDCp) were utilized. Since this peptide is a ligand that binds to integrin receptors and mediates cell adhesion [55], the immobilization of this conjugate provides a surface for cells to attach efficiently [56-58]. The glycol groups of the monolayer are crucial to this design because they prohibit the attachment of cells [59]. This gives an electrochemical route to turn on a substrate for the attachment of cells [60].

In summary, this method utilizes an electrical mask [60-62] to control the attachment of a cell population and then allows attachment of a second cell population.

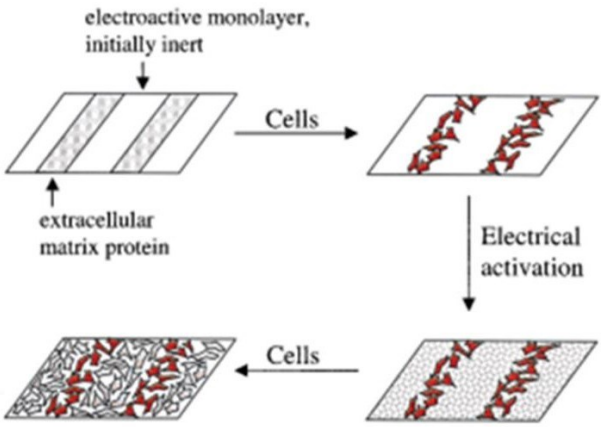


Figure 1.9. Patterning two different cell types with electroactive mask. First type of cells attached to electroactive mask and formed patterns in a place where conjugates of cyclopentadiene and a peptide were already existed. Then, inert regions of mask were electrically activated and cells attached to this regions. Finally, two type of cells were patterned together (Source: Yousaf et.al., 2001) [54]

Microfabrication Technique

While another methods have showed increased viability, function, and tissue stability, the specific mechanisms by which co-cultivation of two cell types modulates tissue function have remained uncertain [63]. Therefore, microfabrication techniques can be used to localize cell populations in patterned configurations on rigid substrates.

Microfabricated co-cultures are formed to control cell-cell interactions by localization of two different cell types on collagen-like patterned substrates (Figure 10). Initially, first cell type is seeded in serum-free media on collagen-like patterned wafers, resulting in a cell pattern due to selective cell adhesion. Addition of second cell type is resulted in formation of micropatterned co-cultures [63].

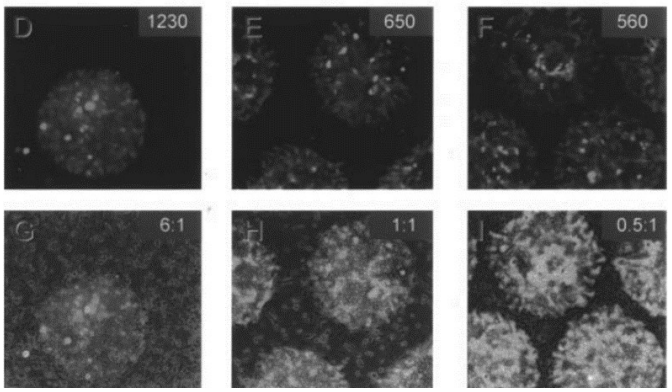


Figure 1.10. Microfabricated Co-cultures. First type of cells were seeded on collagen-like patterned wafers, cell patterns occured due to selective cell adhesion. Microfabricated co-cultures formed with the addition of second type of cells (Source: Bhatia et.al., 1998) [63]

Magneto-Archimedes Effect

Another method is generation of cell aggregates by a label-free three-dimensional '3D' cell assembly method, which is based on the magneto-Archimedes effect. In this method, a paramagnetic salt with high magnetic susceptibility is added to the culturing medium to increase the diamagnetic feature of the cells, by which label-free magnetic manipulation become possible. With this method, cells aggregate into a shape and become a spheroid [64].

In a study, Gadolinium diethylenetriaminepentaacetic acid (GdDTPA) (Sigma-Aldrich) was chosen as a suitable paramagnetic salt with high magnetic susceptibility; its magnetic susceptibility is much higher than the cells and it had been approved for biological use and is already used as the MRI contrast agent Magnevist [65]. Results showed that it is possible to estimate the shape of the cell aggregate and an arbitrarily shaped tissue can be fabricated by designing a convenient magnetic field (Figure 12).

Since this method enables fabrication of a relatively complicated shaped tissue without a scaffold [66,67], it is a promising approach to construct arbitrarily-shaped 3D tissues over the traditional 3D tissue construction methods.

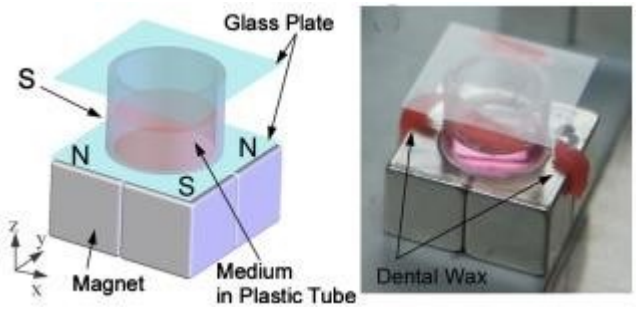


Figure 1.11. The experimental setup to form a cell spheroid that comprise neodymium magnets and a chamber formed by a tube and two plates. Cells were placed in tube and expected to form cell spheroid in the middle of magntes (Source: Akiyama and Morishima, 2011) [64]

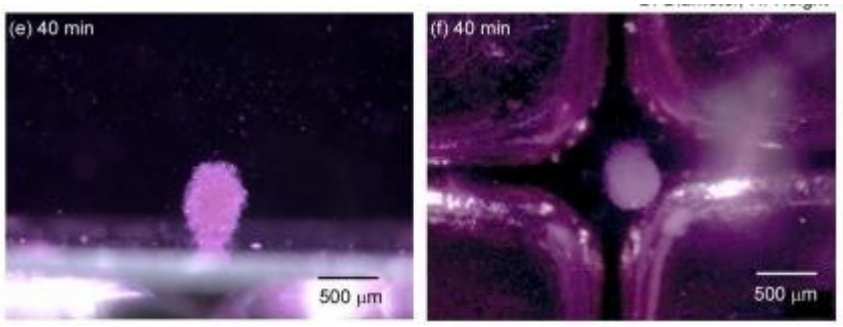


Figure 1.12. Microscope images of patterned cells with Magneto-Archimedes Effect. 40 minutes after cells were placed in tube, cell spheroidc form in the middle of magnets (Source: Akiyama and Morishima, 2011) [64]

Surface Engineering

The challenge for single cell patterning is to produce small islands hosting only single cells and keeping their viability for a long time. For this purpose, surface engineering approach can be utilized. When a covalently bound short peptide is used as a mediator, cells can be patterned better on substrates because covalently bound peptides decrease apoptosis and necrosis of adhered cells [68].

In a study, which this method is used, microarrays of human umbilical cord vein endothelial cells (HUVEC) on gold-patterned silicon oxide substrates were formed utilizing a covalently bound short peptide, LysArg-Glu-Asp-Val-Tyr. Results showed that mediated single cell adhesion and kept cellular viability [68].

1.3. Magnetism of Objects

Magnetism comes up a result of motion of electrically charged particles. Magnetism naturally occurs in all matters because all matters have protons, neutrons and electrons [69-71]. According to reaction of substances to magnetic field, magnetism is divided into five groups: diamagnetism, paramagnetism, ferromagnetism, antiferromagnetism and ferromagnetism [72]. The most known ones are diamagnetism, paramagnetism, ferromagnetism.

Diamagnetism is a type of magnetism and a change in the orbital motion of electrons induces it due to an applied magnetic field. The magnitude of the induced magnetic moment is very small, and its direction is opposite to that of the applied field [73]. Normally, diamagnetic substances includes atoms that net magnetic moments are zero because they do not have unpaired electrons [74,75]. Diamagnetic property is presented in all materials [76]. Diamagnetic materials have negative magnetic susceptibility ($\chi<0$), which is a measure of which matters become magnetized, and it does not change with temperature in diamagnetic materials [75,77].

Besides the diamagnetic substances, paramagnetic ones have unpaired electrons and net magnetic moment. Therefore, they are attracted into the direction of magnetic field. Their magnetic susceptibihty is positive and temperature dependent [78].

Other class is ferromagnetism. The most known property of ferromagnetic substances is being magnetized easily and attracted by magnets. Ferromagnetic substances have higher magnetic suspectibility than paramagnetic and diamagnetic substances. In addition, magnetic suspectibility of ferromagnetic substances changes with applied magnetic field[77]. The most known ferromagnetic substances are iron, nickel, cobalt [79]. When temperature is above their Curie temperature, some ferromagnetic materials lose their ferromagnetic properties and they behave like paramagnetic [77]. For instance, gadolinium is paramagnetic above 20 ˚C although it is a ferromagnetic element [77,80]. Due to its properties, ferromagnetism is used to create permanent magnets [72]. One class of them are Neodymium magnets (known as NdFeB) that made from an alloy of neodymium, iron, and boron. These magnets are the strongest permanent magnets in industry. Neodymium magnets can have several magnetic properties like different magnetic field output and resistance to demagnetization by altering manufacturing

method and their alloy structure [81]. Based on this, there are different available grades of neodymium magnets, ranging from N24 to N52. Among them, N52 grade neodymium has the highest magnetic performance.

1.4. Magnetic Liquids

A magnetic liquid is a fluid that includes small particles of a magnetic material as particular iron oxides in suspension. These magnetic particles are too small and because of that they act like the molecules of the liquid in which they are placed [82]. Ferrofluids and paramagnetic sal solutions are examples to magnetic fluids. Ferrofluids are colloidal suspension of magnetic particles dispersed in a liquid carrier and they have dimensions approximately 10 nm [83-85]. They have relatively high magnetic susceptibility and magnetization and their susceptibility can be controlled by concentration of magnetic particles in the carrier fluid [86].

On the other hand, paramagnetic salts are composed of chelating agents and paramagnetic metals [86]. They have lower magnetic susceptibility than ferrofluids. They can also be manipulated in terms of their susceptibility in the solution by changing their concentration as ferrofluids. There are different types of paramagnetic salt solutions. Gadolinium (III) chloride ($GdCl_3$) is one of the widely used one due to high susceptibility [87]. Living cells might be affected from using of high concentrations of paramagnetic agents might because high concentrations can be toxic. In the previous studies, the range of 0.1-1 M concentration has been used to prevent adverse effects and show biocompatibility [86]. On the other hand, chelated forms of paramagnetic agents can be used to increase biocompatibility**.** Different types of gadolinium-based agents are used [86]88. Gadolinium-based agents are catagorized by their overall charge and ligand framework (ionic, non-ionic, linear and macrocyclic) [89]. The macrocyclic structures are more stable compared to linear forms and it complicates to release free ion in solution [90]. Gadobutrol (GD-BT-DO3A) is macrocyclic and non-ionic structure and it consists of gadolinium (Gd^{3+}) chelated with butrol (Figure 13). In addition it is a paramagnetic medium which is widely used due to be suitable for living cells [91-95].

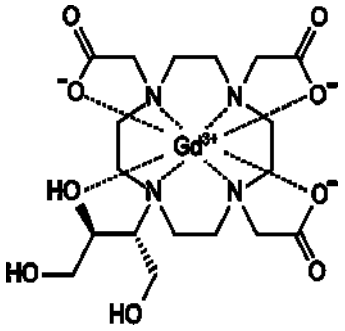
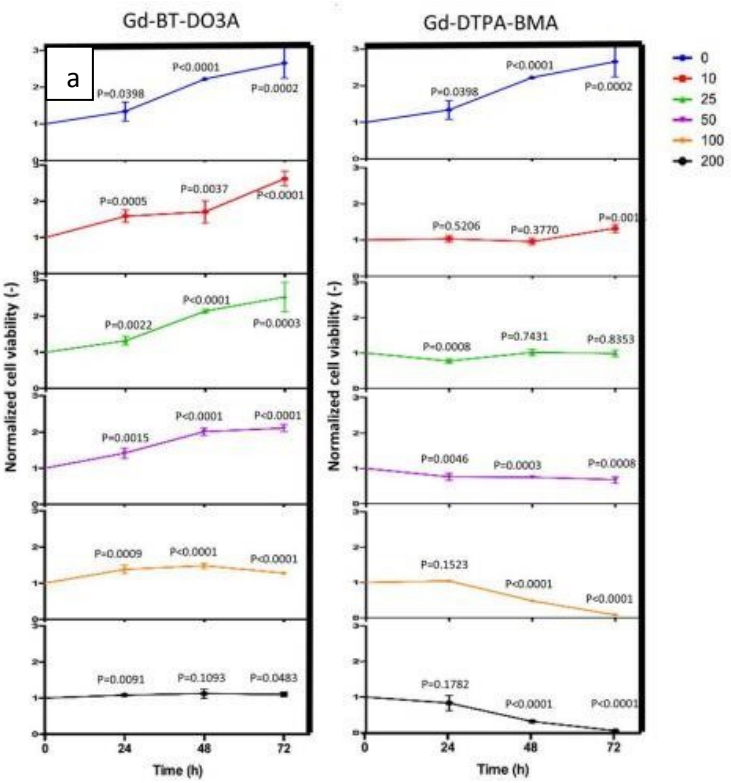


Figure 1.13. Molecular structure of gadolinium contrast agent, Gadobutrol (Gadavist) (Source: Trog et. al.,2019) [93]

In the previous study, it was showed that macrocyclic ligand containing Gd-BT-DO3A provided higher cell viability compared to other gadolinium-based agents [96]. When cell viability (D1 ORL UVA cells) was measured by MTT assay and live/dead assay to compare Gd-BT-DO3A and Gd-DTPA-BMA, Gd-DTPA-BMA inhibited cell growth even at low concentrations (25mM) (Figure 14.a,b). However, cell loss began to form at the concentration of 200mM Gd-BT-DO3A in the culture (Figure 14.b).



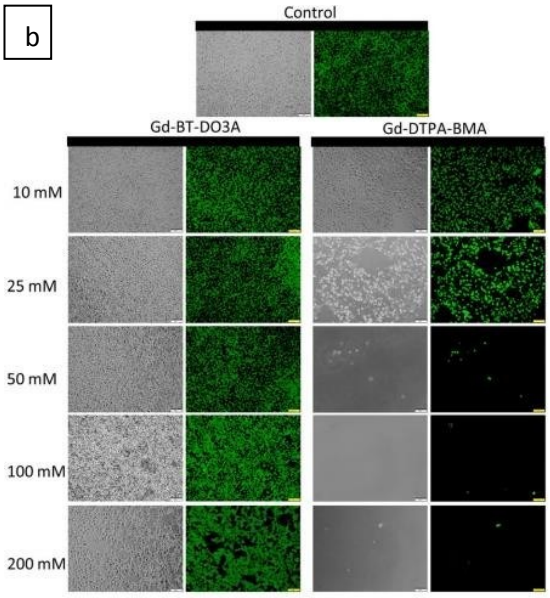


Figure 1.14. Long term cell viability results at different concentrations (10, 25, 50, 100 and 200 mM) of GD-BT-DO3A and GD-DTPA-BMA. a) MTT results. Cells maintained viability longer when Gd-BT-DO3A was used in increasing concentrations. b) Live/dead assay results. Gd-DTPA-BMA showed toxic effect even at low concentrations (Source: Anıl-İlevi et.al., 2018) [96]

In this study, Gd-BT-DO3A was chosen due to providing higher cell viability at all concentrations in long term culturing.

1.5. Magnetophoresis

One of the cell patterning technique is magnethophoresis. Magnethophoresis can be briefly defined as movement of cells under magnetic field. It is divided into two groups as positive and negative magnetophoresis. Cells migrate to the area of high magnetic field in positive magnetophoresis while they escape from high magnetic field in negative magnetophoresis [97]. To separate different cell types, such as blood cells and cancer cells, positive magnetophoresis was utilized. Also, it was demonstrated the technique enables to 3D culture of cells but it needs labeling of cells because most cells are not naturally magnetic. It can require additional experimental steps and it can be toxic to the cells [98]. However, negative magnetophoresis is label free and it reduces adverse effects of labeling [64]. Negative magnetophoresis was also used in the studies which positive magnetophoresis was previously used. It was also used for density measurement, cell

separation, sorting, 3D biofabrication [96,98-106]. For example, negative magnetophoresis was used in magnetic levitation system which simulates weightlessness environment to study the effect of space microgravity on morphology and physiology of the cells [107,108]. Changes in cell growth, cell cycle, and transcriptional profile were determined by using various cell types, such as bacteria and yeast [107-109]. Due to this properties and results, negative magnetophoresis can be more useful for cell patterning.

In this study, it was aimed to pattern cells by manipulating them using negative magnetophoresis. It was supposed that cells will escape from high magnetic field due to higher magnetic suspectibility of paramagnetic liquid than cells and cells settle in a certain region in the patterning material (Figure 15). This method can be further used as a novel bioprinting technique.

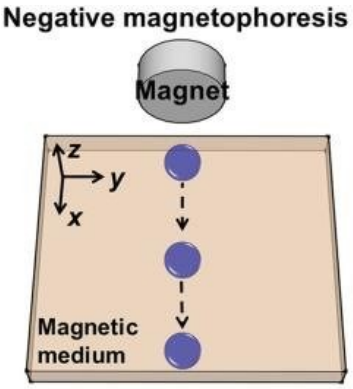


Figure 1.15. Illustration of Negative Magnetophoresis. Cells escape from high magnetic field to low magnetic field due to higher magnetic suspectibility of paramagnetic liquid than diamagnetic cells (Source: Zhao et.al., 2016) [86]

CHAPTER 2

MATERIALS AND METHODS

2.1. Cell patterning System and Experimental Setup

In the paramagnetic medium, cells tend to move towards lower magnetic field region when applying a magnetic field. The dominant force on the system is magnetic force. In the system, the applied magnetic force, where V is cell volume, $\Delta\chi$ is magnetic susceptibility difference between particle/cell and paramagnetic liquid, μ_0 is permeability of free space, B is magnetic induction and ∇ is the del operator (Equation 1).

$$F_{mag} = \frac{V.\Delta\chi}{\mu_0}(B.\nabla)B \quad (1)$$

In the system, magnetic susceptibility plays an active role. By increasing magnetic susceptibility of the paramagnetic liquid, F_{mag} is increased and the cells are positioned at a certain place.

According to above, a patterning material is designed. For this purpose, PDMS was chosen for patterning material. It was constructed as 6 cm length and 2.5 cm width and 3 wells. PDMS (Polydimethylsiloxane) was chosen for patterning material due to its properties such as low cost, being chemically inert, easy handling, biocompatible and non-toxic [110-112].

Preparation of PDMS Patterning Material

1. Firstly, a mold is needed for preparation of PDMS (Polydimethylsiloxane) patterning material. Desired mold was drawn on AutoCAD. Material is designed to have three well for three samples.
2. This drawn mold was printed with 3D printer. Formlabs Clear Resin was used at this 3D printer. This printing process took about four hour.

3. After that, this mold was kept in propanol for one hour to cure. Then, it was allowed to dry.
4. Then, it was kept in detergent diluted with water for better detachment of PDMS.
5. For PDMS, SYLGARD Elastomer kit was used. The elastomer and curing agent were mixed at a 10 parts to 1 part ratio (10:1).
6. The mixture was vacuumed to remove air bubbles.
7. After vacuum, desired cavities of the sample mold was carefully coated with PDMS.
8. PDMS mold was carefully placed into the oven. It was baked for overnight at 60 ^{o}C.
9. Fully cured PDMS sample was removed from the oven. Using a scalpel blade, the PDMS was separated from one side of the mold wall. So, the rest could be pulled gently from the mold.

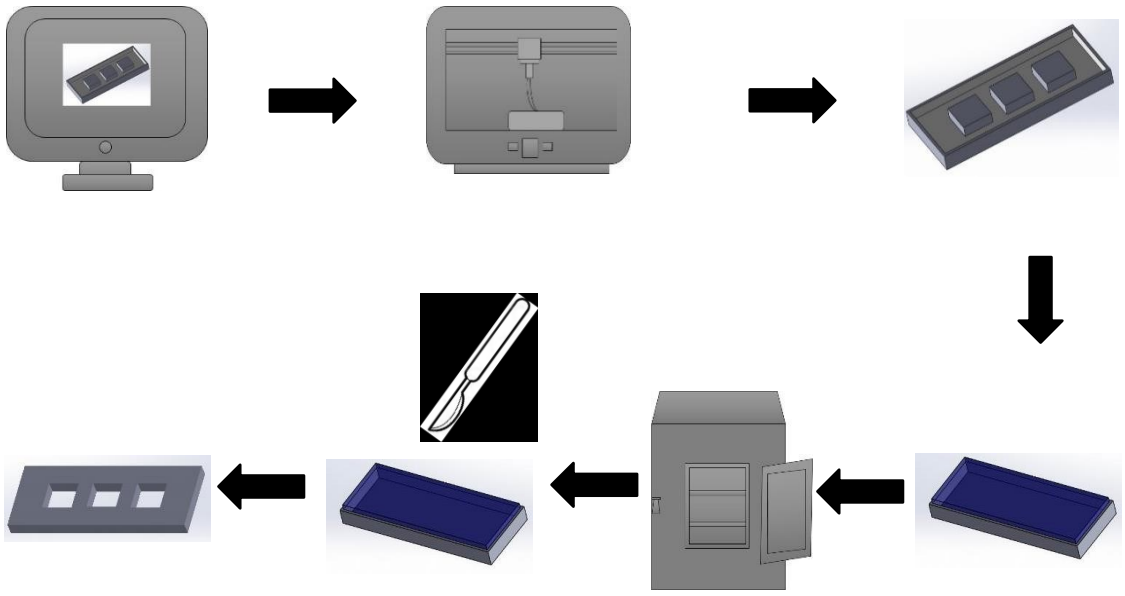


Figure 2.1. Production of Patterning Material. PDMS (Polydimethylsiloxane) was used to product patterning material.

PDMS Bonding with Oxygen Plasma

1. To make patterning material, cover glass (with 0.13-0.17 mm thickness) and PDMS were bonded to each other. For bonding, oxygen plasma was used.
2. Cover glass was cleaned in ultrasonic cleaner with 70% alcohol for 10 minutes.

3. The PDMS surface to be bonded was cleaned.
4. PDMS and cover glass were placed in oxygen plasma generator. Oxygen plasma was applied for 1.5 minutes. Then, they were bonded to each other and fixed by pressing.
5. Finally, newly formed patterning material was kept in oven at 60 ^{o}C for five minutes for better bonding.

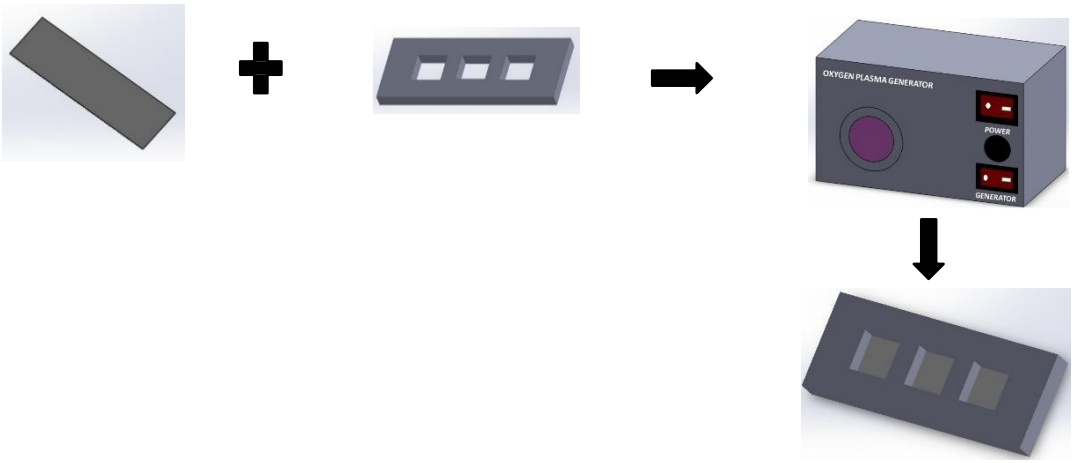


Figure 2.2. Bonding PDMS Patterning Material to Cover Glass. Oxygen plasma was used to bond the PDMS patterning material to cover glass.

2.2. Cell Culture

D1 PMIG (mouse bone marrow stem cells) and 7F2 dsRed (mouse osteoblasts) (ATCC) were cultured in growth medium supplemented with 10% fetal bovine serum (FBS, Biological Industries) and 1% penicillin/streptomycin (Invitrogen) at 37°C in a 5% CO_2 humidified incubator. D1 PMIG was cultured in Dulbecco's Modified Eagle's medium (DMEM high glucose, Gibco) whereas 7F2 cells in alpha minimum essential medium (αMEM, BI) with 2 mM L-glutamine and 1 mM sodium pyruvate. The growth medium was refreshed every 2-3 days and the cells were passaged by using 0.25% trypsin/EDTA solution (Biological Industries) during 5 minutes when they reached approximately 80-90% confluence (every 4-6 days).

2.3. Patterning of Cells

2.3.1. Patterning of 7F2 cells

7F2 cells cultured in growth medium were trypsinized with 0.25% trypsin/EDTA solution, and the cell suspension was centrifuged (Eppendorf) at 125 × g for 5 min. Pellet was resuspended to 1.25×10^5, 2.5×10^5, $5x10^5$cells/mL in the culture medium and each sample with 200 uL/well were loaded into the patterning device after adding Gd^{3+} paramagnetic agent with concentrations of 5, 10, 15, 20, 25 mM, 50 mM and 100 mM. Gd^{3+} is a lanthanide metal and can be cytotoxic due to its similarity to the size of Ca^{2+}, causing competitive inhibition for Ca^{2+} involving biological processes. Cytotoxicity of Gd^{3+} can be suppressed by the utilization of commercially available chelate forms [113,114]. In this study, we used Gadavist with a nontoxic concentration range (≤100 mM) in accordance with previous findings [96].

Patterning material was placed on seven N52 grade neodymium magnets that bound together. Cell suspensions were loaded to wells. Cells examined under microscope after 3 hours, 24 hours and 48 hours. Then, the analysis of patterning images of the cells was done by Image J Fiji software to determine pattern thickness and cell intensity**.** The cells were imaged at 4× under an inverted microscope (Olympus IX-83).

2.3.2. Patterning of 7F2 dsRed and D1 PMIG cells

It was investigated that 7F2 dsRed and D1 PMIG cells formed patterns together. For this purpose, 7F2 dsRed (red labeled with DiI solution) and D1 PMIG (green labeled with DiO solution) were used. Cells were seeded to make patterns in different shapes. 7F2 dsRed cells were seeded into patterning material at first day and D1 PMIG cells were seeded as perpendicular to the other cells at second day. The opposite was also applied. Then, cells were examined under microscope at certain days. Images were captured for analyze by using fluorescent and confocal microscope. In another experiment, 7F2 cells were seeded into patterning material at first day and D1 PMIG cells were seeded in parallel to other cells at second day. The opposite was also applied. Then, cells were examined under microscope at certain days and images were captured as in the previous experiment.

Another experiment was like that 7F2 cells were seeded into patterning material at first day and D1 PMIG cells were seeded at a 45 degree angle with the other cells. Then, cells were examined under microscope at certain days and images were captured.

Parallel patterning of two type cells were also observed. 7F2 cells were seeded into patterning material at first day and D1 PMIG cells were seeded as parallel to 7F2 cells at second day. The opposite was also applied. Then, cells were examined under microscope at certain days and images were captured. Finally, the analysis of patterning images of the cells was done by Image J Fiji software to determine pattern thickness and cell intensity.

2.4. Statistical Analysis

During the study, all experiments were repeated in triplicate. Thickness of patterns and cell intensity data were presented with the mean and standard deviation (mean ± SD).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Optimization Of Gd^{3+} Concentration

In this study, it was used a cell patterning system composed of several magnets and a cell patterning material (Figure 18.a). Magnets attached to each others created the magnetic force. Since magnetic susceptibility of paramagnetic liquid was higher than magnetic susceptibility of cells, cells escaped from high magnetic field and tended to low magnetic field and it caused forming of cell patterns (Figure 18.b,c). Patterning material that was constructed from PDMS kept cells inside as alive. It was nontoxic and biocompatible [110-112].

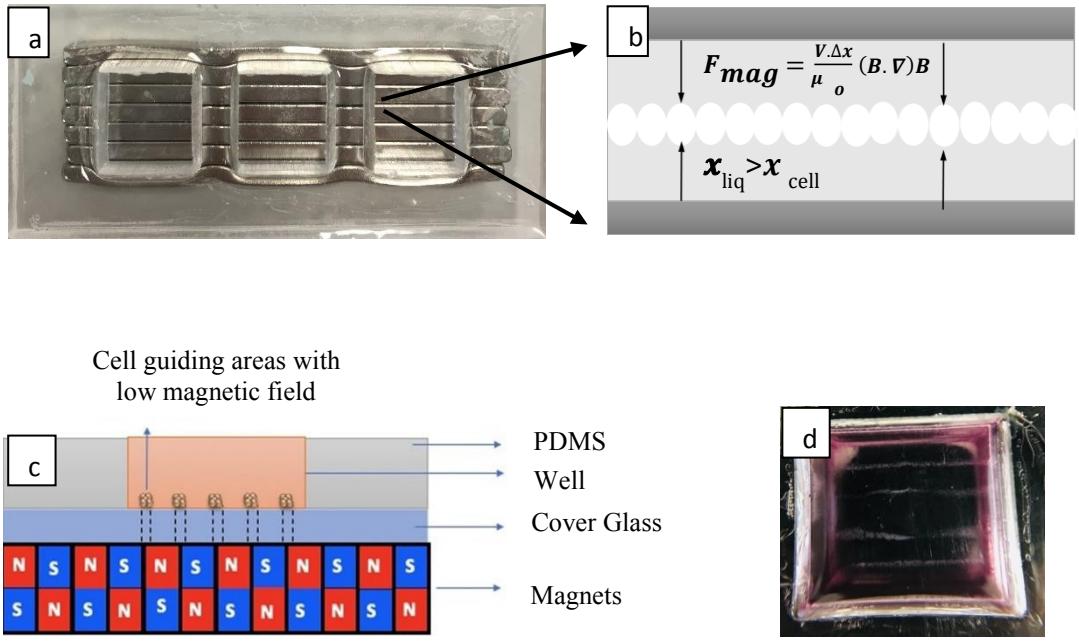


Figure 3.1. Cell patterning system a) Magnets and PDMS cell patterning material were used to construct cell patterning system. b) Representative image of positioning of cells via negative magnethophoresis. c) Left side view of patterning material. d) Photoimage of patterned cells.

In a previous study, Gadavist concentration that is equal and lower than 100 mM showed nontoxic effect [96]. Therefore, 0, 5, 10, 15, 20, 25, 50, 100 and 200 mM Gd^{3+} concentrations with 10^5/well cell number were used in this study. Any pattern was not observed when Gd^{3+} was not used (0 mM). Bigger than 5 mM Gd^{3+} concentration did not form exact cell patterns (Figure 19). However, they caused some cell levitation. Cells detached from surface after a while. The most proper and exact pattern formed when 5 mM Gd^{3+} concentration was used. Then, cell patterns were observed when 5 mM Gd^{3+} is used with $2.5x10^4$, $5x10^4$ and 10^5 cells/well for 3 hours, 24 hours and 48 hours.

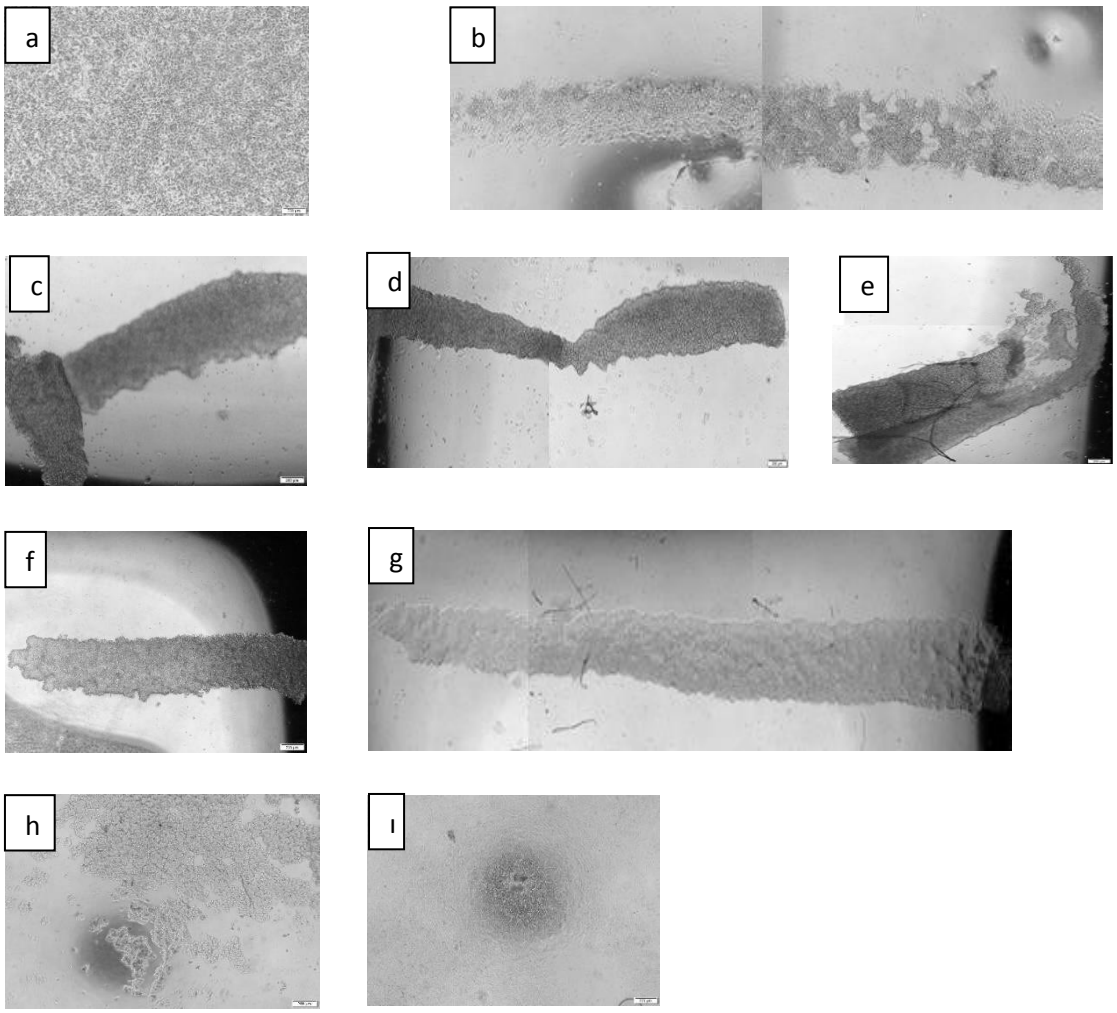


Figure 3.2. Effects of different Gd^{3+} concentrations after 3 hours. a) 0 mM Gd^{3+}, b) 5 mM Gd^{3+}, c) 10 mM Gd^{3+}, d) 15 mM Gd^{3+}, e) 20 mM Gd^{3+}, f) 25 mM Gd^{3+}, g) 50 mM Gd^{3+}, h) 100 mM Gd^{3+}, ı) 200 mM Gd^{3+}

3.2. Detection of Thickness of Cell Patterns

After 5 mM Gd^{3+} concentration was chosen as most appropriate for cell patterns, effects of different cell numbers and culture duration were observed. 5 mM Gd^{3+} is used with $2.5x10^4$, $5x10^4$ and 10^5 cells/well for 24 hours and 48 hours.

Firstly, 10^5 7F2 dsRed cells/well which were diluted in the medium contains 5 mM Gd^{3+} were seeded into each well and placed on magnets. After three hours, they were examined under microscope and images were taken (Figure 20.a). Then, pattern thicknesses were measured with Image J Fiji software. Thickness of patterns was from where the cells were intact. The thickness of all the patterns were almost measured close to each other (Figure 20.b). The average thickness of all patterns is 267.531 uM.

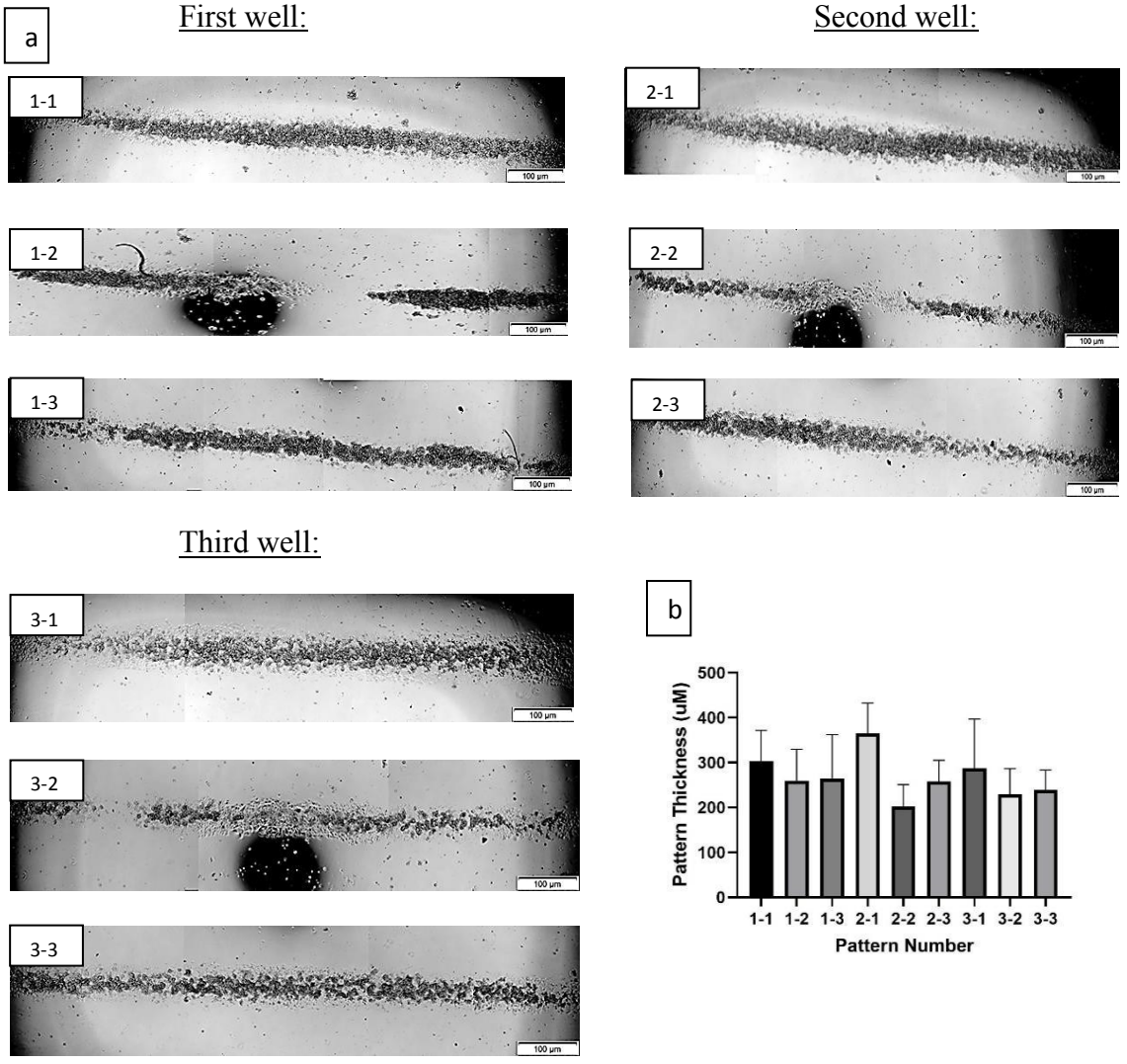
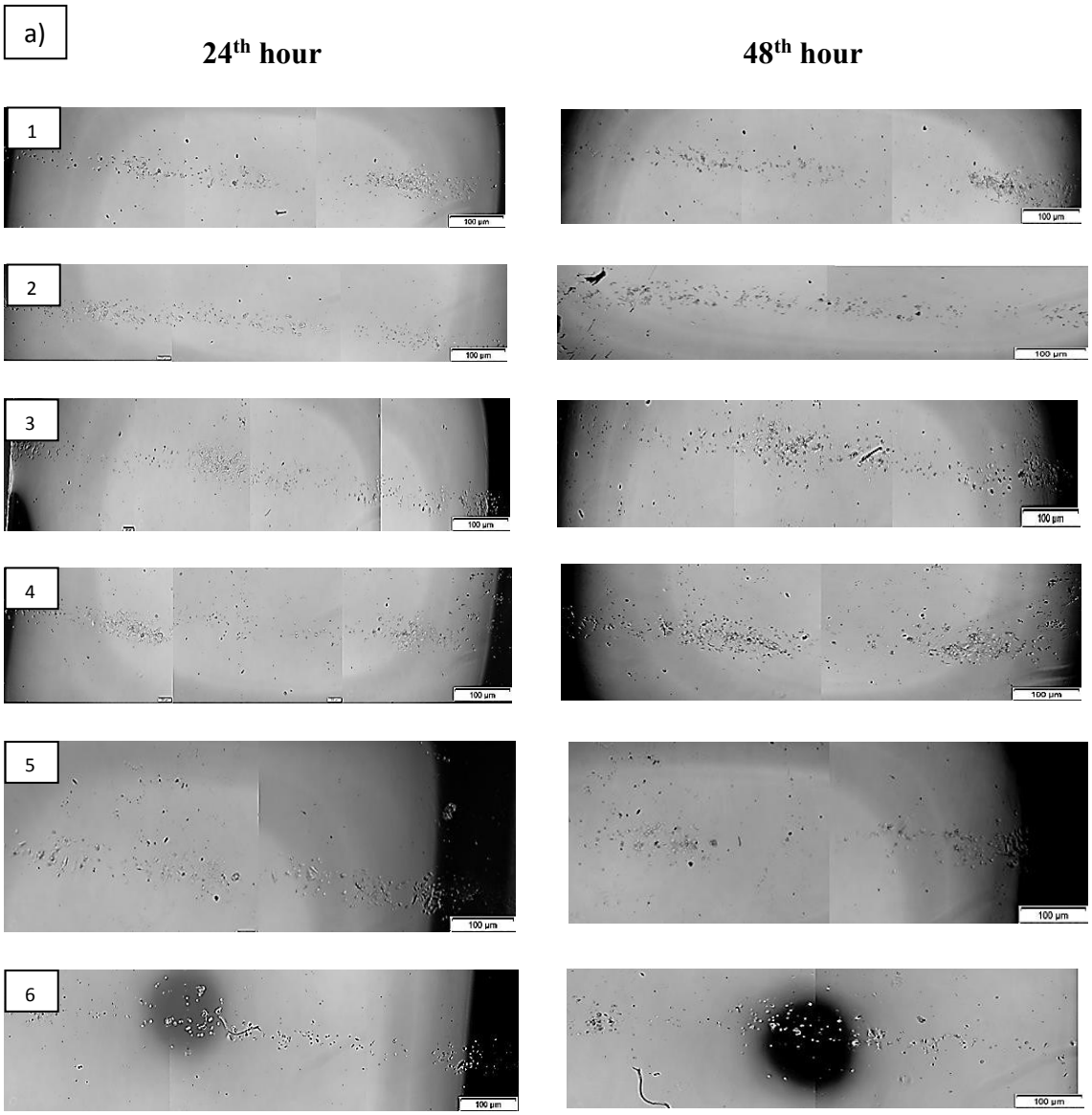


Figure 3.3. Patterned 5×10^5/ml 7F2 cells using 5 mM Gd3+. Images were taken 3 hours after they were seeded. a) Images of patterns from three wells. b) Pattern thickness from three wells.

Then, long term cell patterning was tried. For this purpose, $1.25x10^5$, $2.5x10^5$ and 5×10^5/ml 7F2 cells which were diluted in the medium contains 5 mM Gd^{3+} were seeded into each well and placed on magnets. Then, patterns were observed for five day48 hours. Magnets were not removed from the patterning material during 48 hours.

When 1.25×10^5 cells/ml were used, thin and scattered patterns occured. 24 hours and 48 hours after cells were seeded, images were taken (Figure 21.a). Thickness of cells were measured at both 24^{th} and 48^{th} hours. While thickness of some patterns increased, thickness of other patterns decreased (Figure 21.b). Reason of the decreasing of thickness of patterns was dispersal of cells to the around.



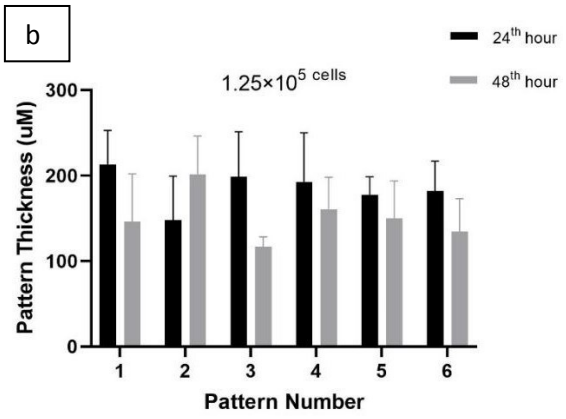
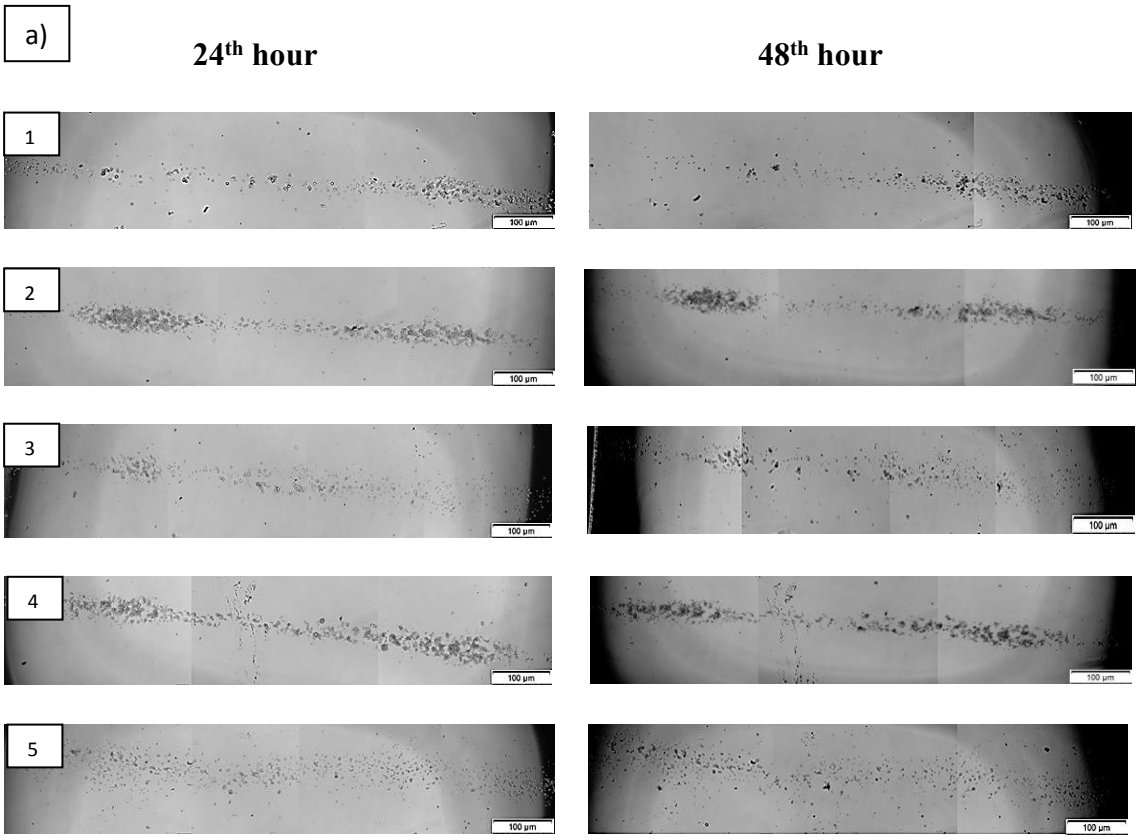


Figure 3.4. Patterning Images of 1.25×10^5 cells/ml a) Patterns at 24th and 48th hours b)Thickness of patterns

When 2.5×10^5 cells/ml were used, thicker patterns occured. 24 hours and 48 hours after cells were seeded, images were taken (Figure 22.a). Thickness of cells were measured at both 24th and 48th hours. While thickness of some patterns increased, thickness of other patterns decreased (Figure 22.b). Reason of the decreasing of thickness of patterns was dispersal of cells to the around.



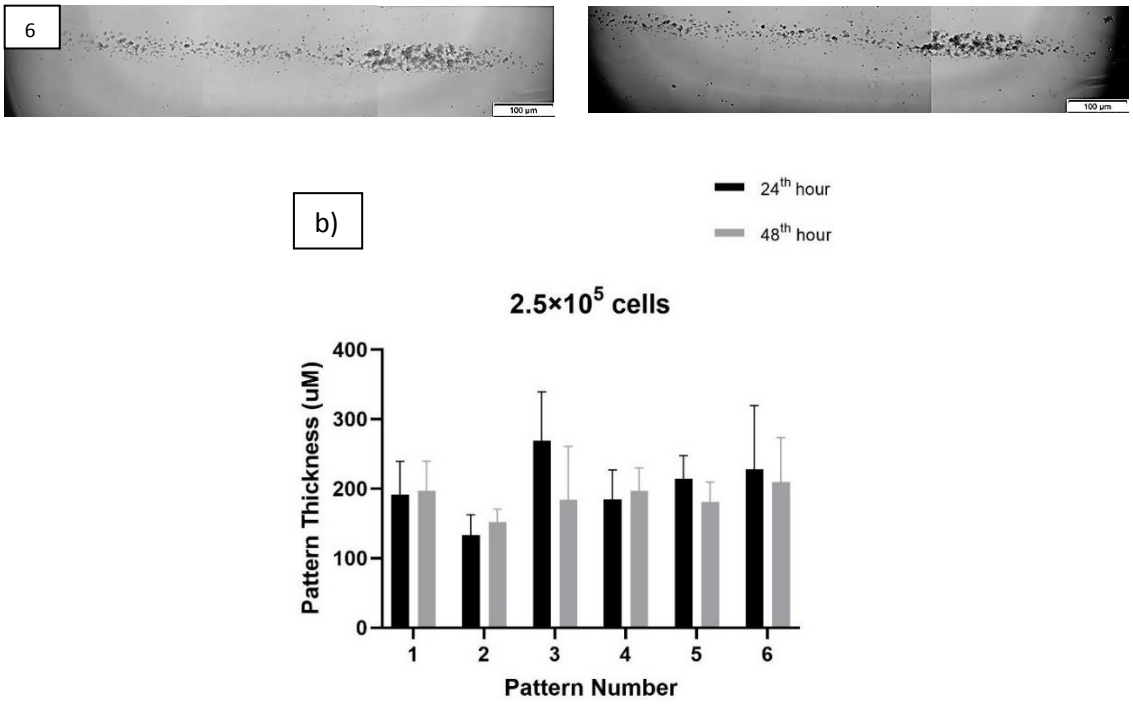
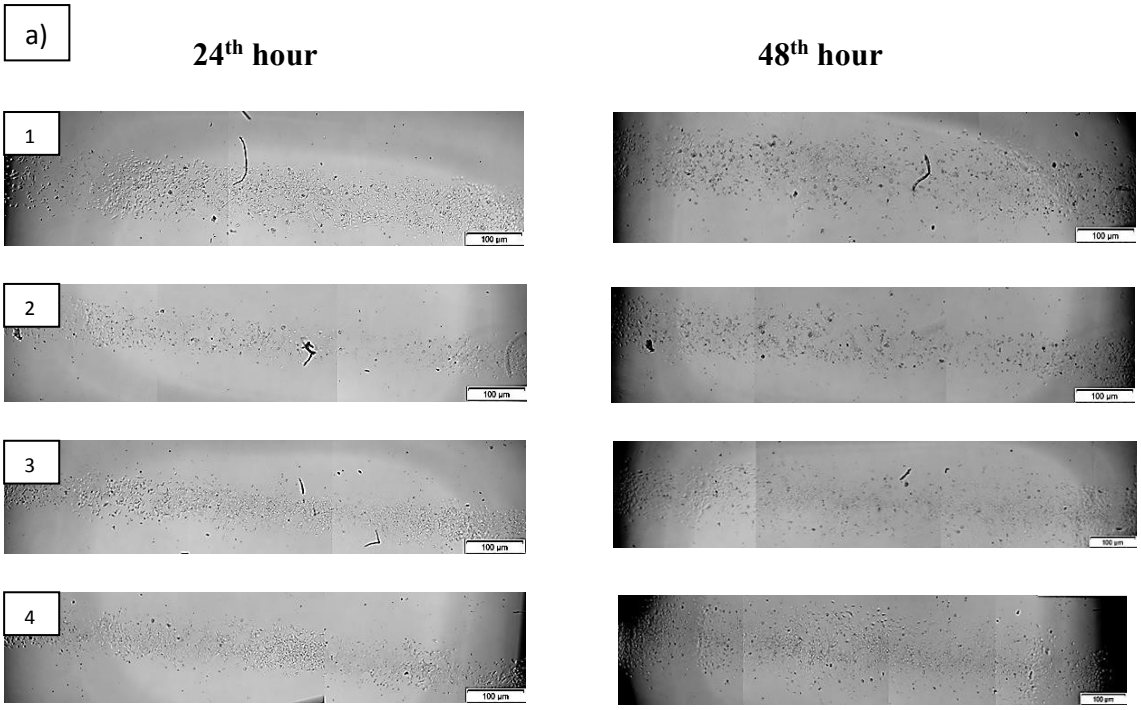


Figure 3.5. Patterning Images of 2.5×10^5 cells/ml a) Patterns at 24th and 48th hours b)Thickness of patterns

When 5×10^5 cells/ml were used, the thickest and the most proper patterns occured. 24 hours and 48 hours after cells were seeded, images were taken (Figure 23.a). Thickness of cells were measured at both 24th and 48th hours. Thickness of all patterns increased (Figure 23.b). It was thought that cells proliferated and spreaded.



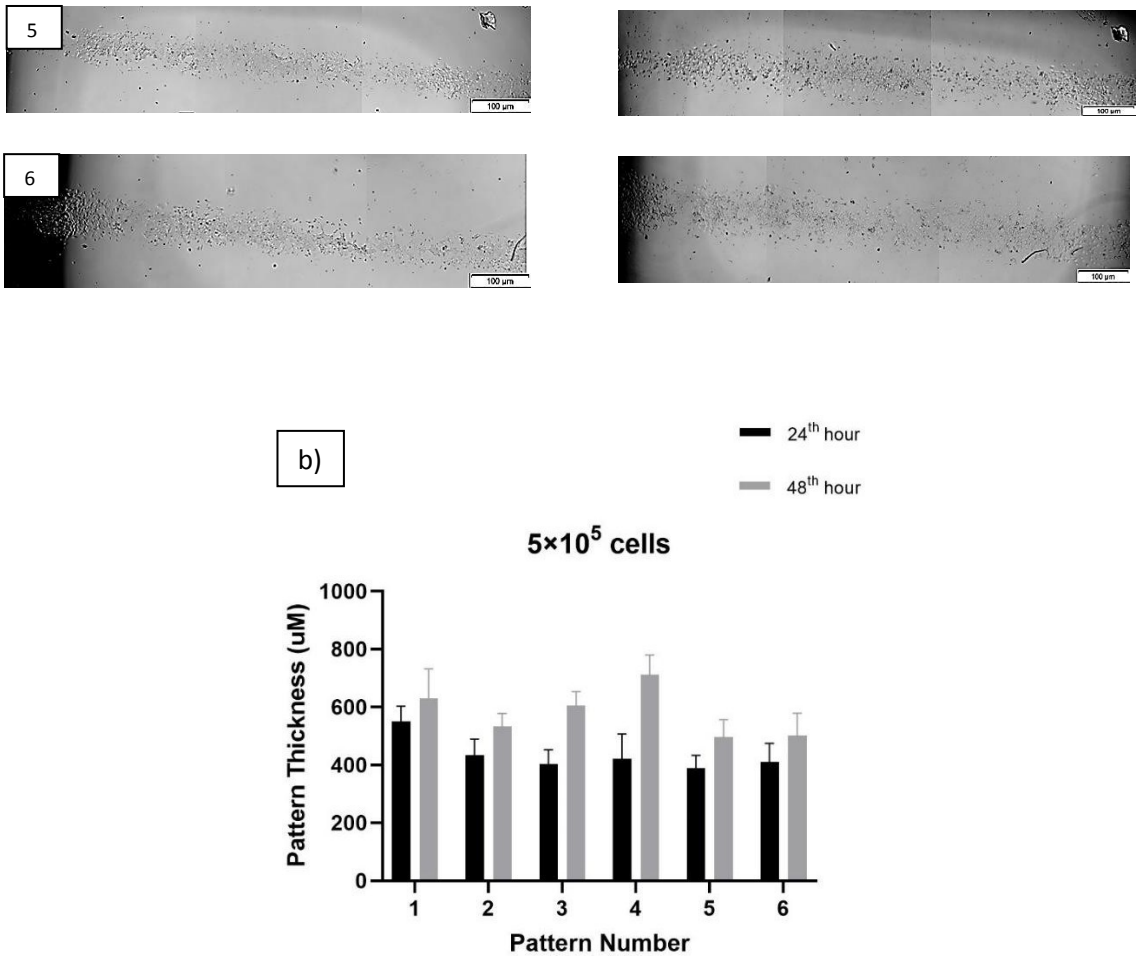
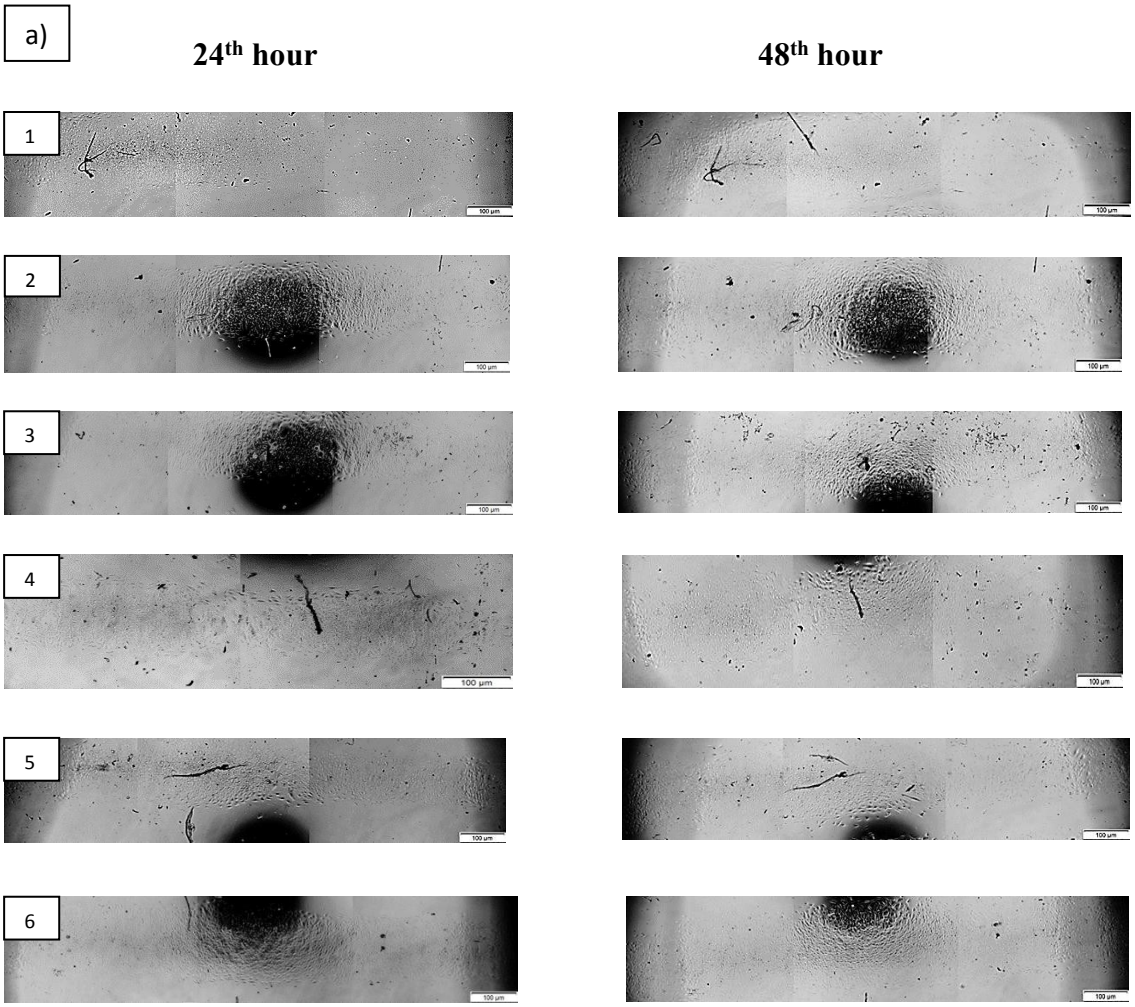


Figure 3.6. Patterning Images of 5×10^5 cells/ml a) Patterns at 24th and 48th hours b)Thickness of patterns

At another experiment, removing of magnets from patterning material was tried. 1.2.5x10^5, 2.5x10^5 and 5×10^5/ml 7F2 cells were seeded into patterning material at first day and magnets were removed after 24 hours. Images were taken at 24th and 48th hours.

When 1.25×10^5 cells/ml were used, disorganized patterns occured. 24 hours and 48 hours after cells were seeded, images were taken (Figure 24.a). Thickness of cells were measured at both 24th and 48th hours. Thickness of all patterns increased from 24th hours to 48th hours (Figure 24.b). While thickness of patterns increased a little bit and decreased in the experiment where magnets were not removed from patterning material, thickness of all patterns increased at this experiment. It is thought that cells proliferated and they spreaded more due to removal of magnetic force.



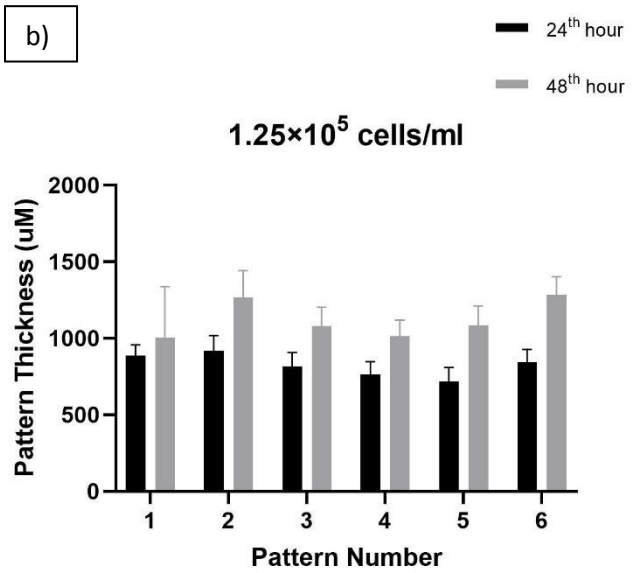
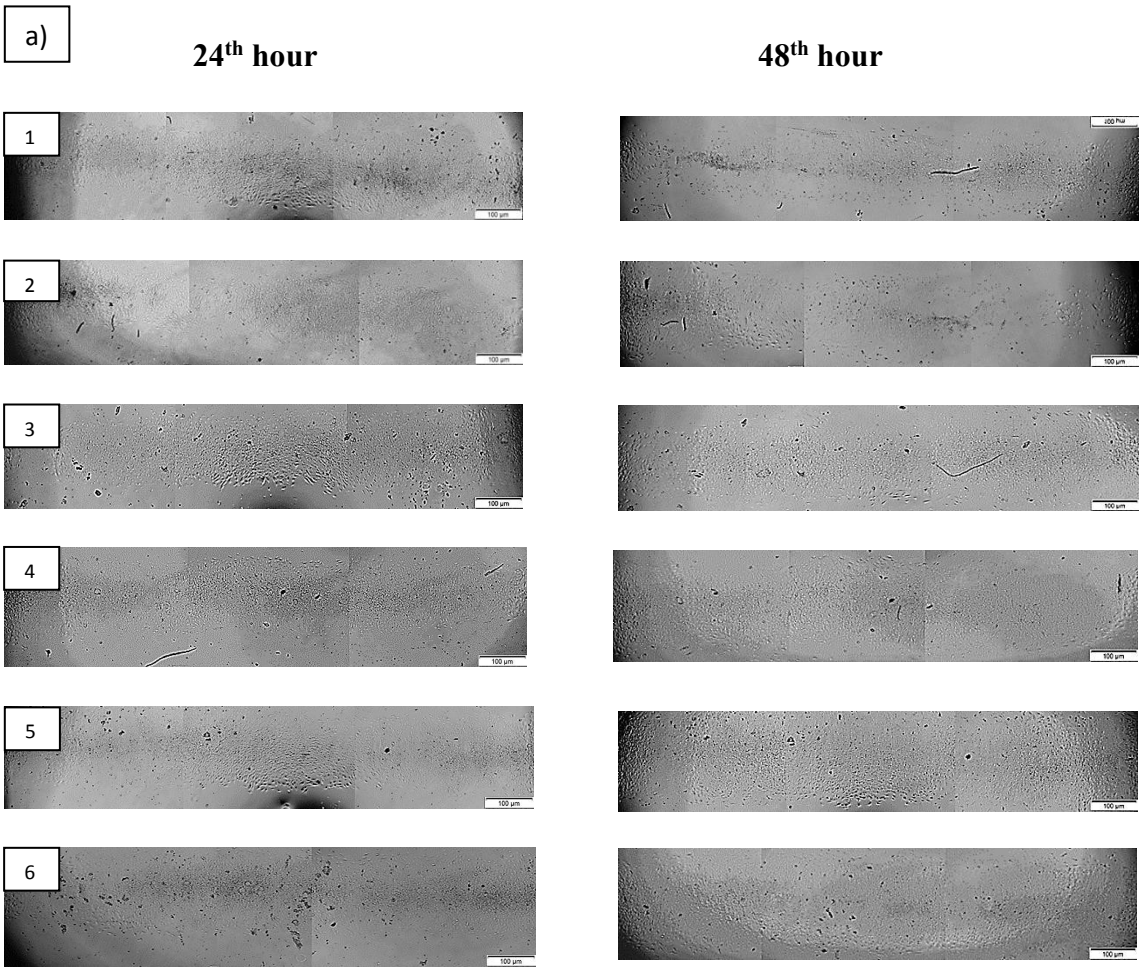


Figure 3.7. Patterning Images of 1.25×10^5 cells/ml when magnets were removed. a) Patterns at 24th and 48th hours. b) Thickness of patterns.

When 2.5×10^5 cells/ml were used, thickher and more intense patterns occured. 24 hours and 48 hours after cells were seeded, images were taken (Figure 25.a). Thickness of cells were measured at both 24th and 48th hours. Thickness of all patterns increased from 24th hours to 48th hours (Figure 25.b). While thickness of patterns increased a little bit and decreased in the experiment where magnets were not removed from patterning material, thickness of all patterns increased at this experiment. It is thought that cells proliferated and they spreaded more due to removal of magnetic force.



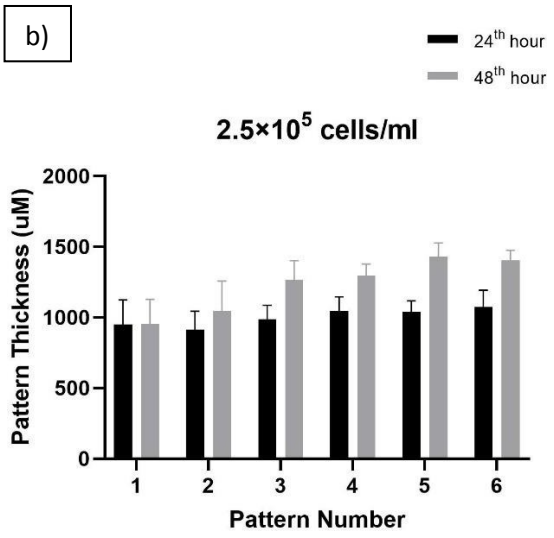
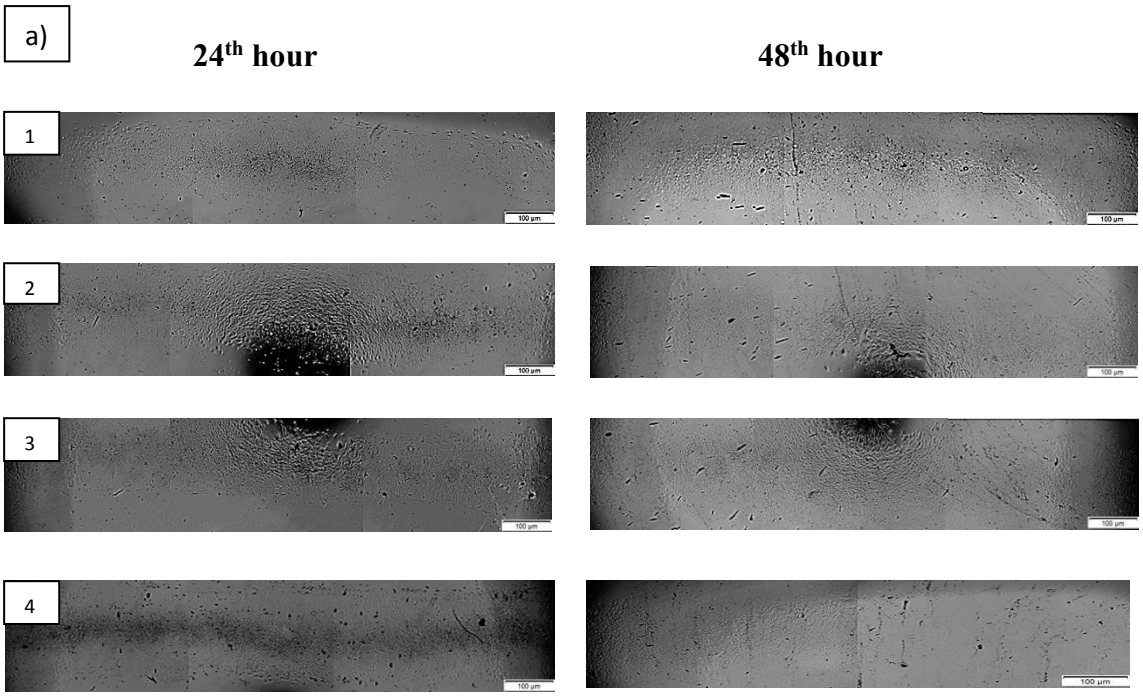


Figure 3.8. Patterning Images of 2.5×10^5 cells /ml when magnets were removed. a) Patterns at 24th and 48th hours. b) Thickness of patterns.

When 5×10^5 cells/ml were used, the thickhest and the most intense patterns occured. 24 hours and 48 hours after cells were seeded, images were taken (Figure 26.a). Thickness of cells were measured at both 24th and 48th hours. Thickness of all patterns increased from 24th hours to 48th hours (Figure 26.b). It is thought that cells proliferated and they spreaded more due to removal of magnetic force and high cell number.



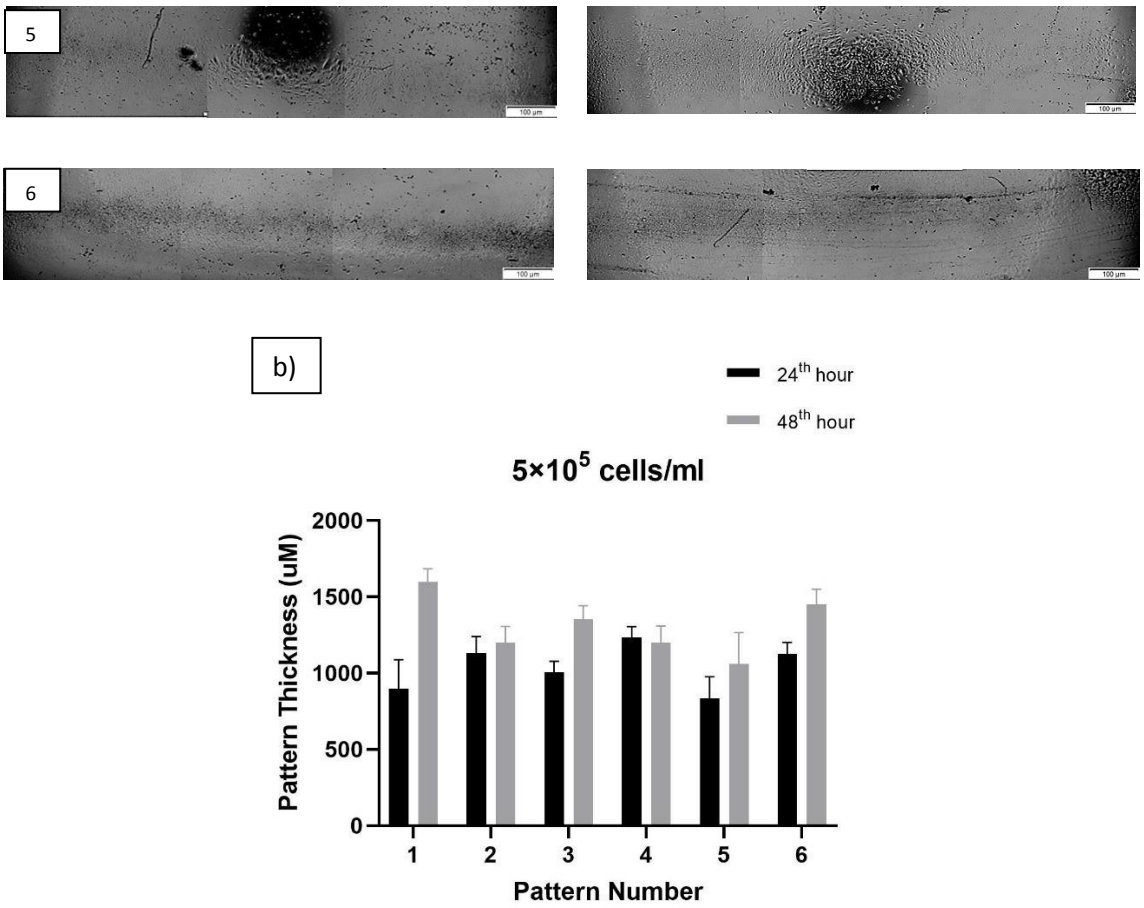


Figure 3.9. Patterning Images of 5×10^5 cells/ml when magnets were removed. a) Patterns at 24th and 48th hours. b) Thickness of patterns.

3.3. Detection of Cell Intensity of Cell Patterns

How the cells had a distribution in the pattern (cell intensity) was analyzed. Some cells showed uniform distribution and some cells showed non-uniform distribution. When 5×10^5 cells/ml was seeded into well which included 5 mM Gd^{3+} and cultured for 3 hours, it demonstrated that cells in the patterns showed uniform distribution (Figure 27).

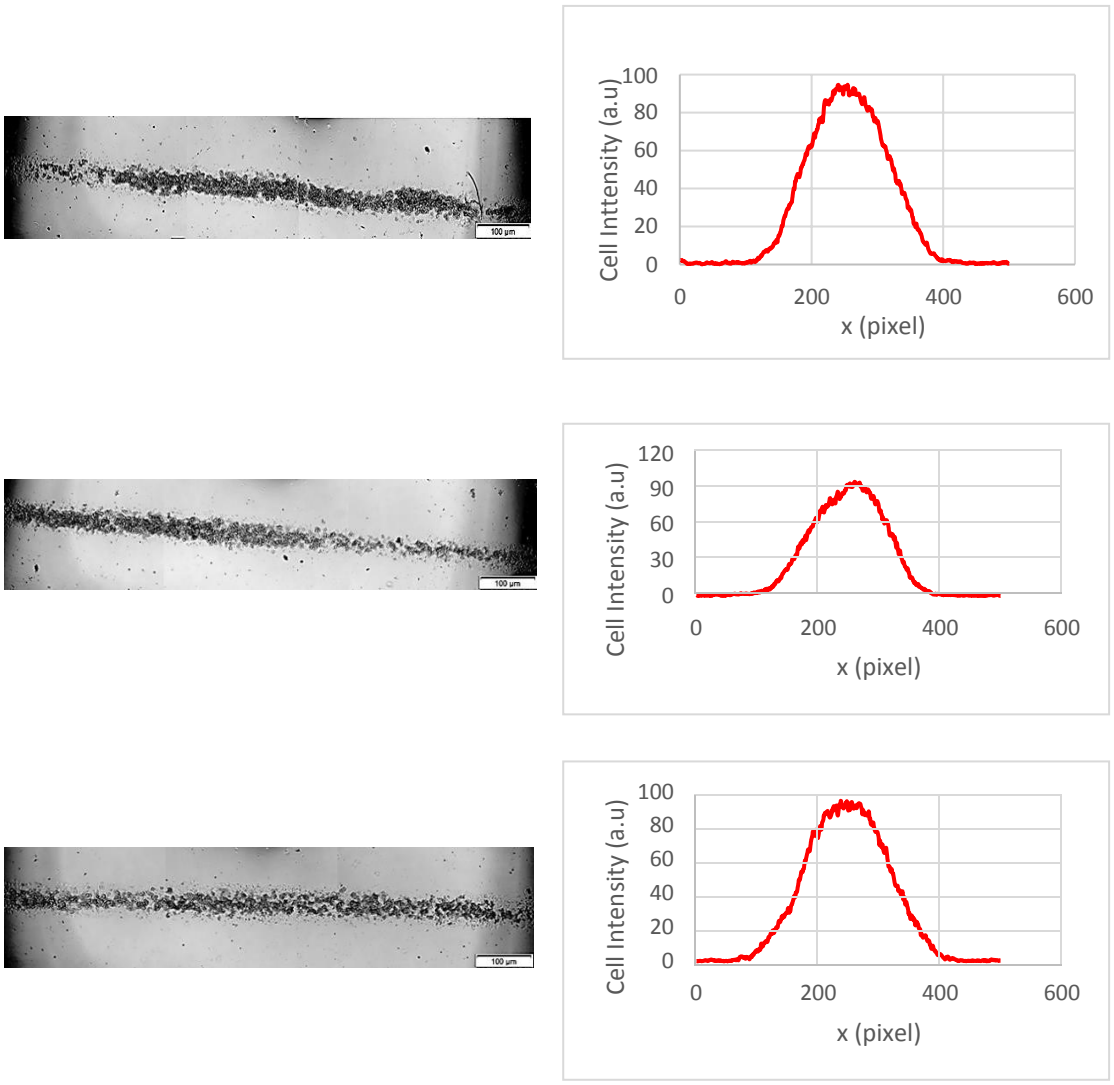
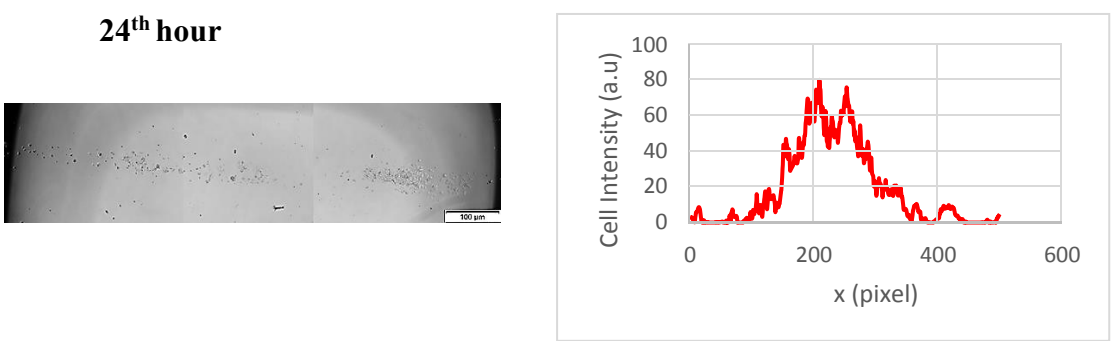
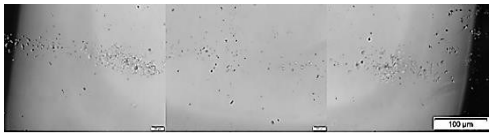


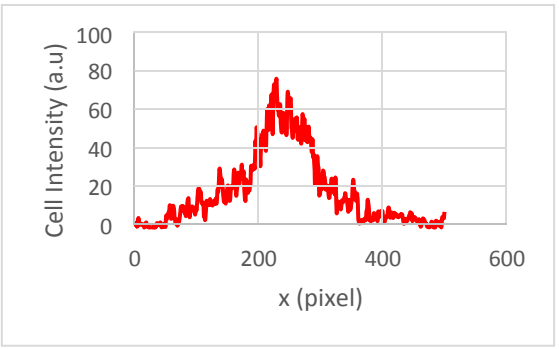
Figure 3.10. Cell Intensity of patterned 5×10^5/ml 7F2 cells with 5mM Gd^{3+} after 3 hours. Cells showed uniform distribution in the patterns.

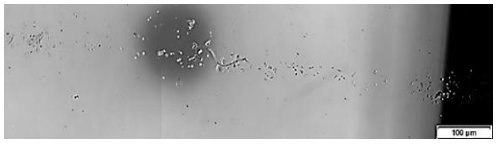
Cell intensity was also analyzed for long term culturing. When 1.25×10^5 cells/ml were used with 5 mM Gd^{3+}, cells showed non-uniform distribution in patterns at both 24^{th} and 48^{th} hours. Reason of this scattering of low amount of cells (Figure 28).

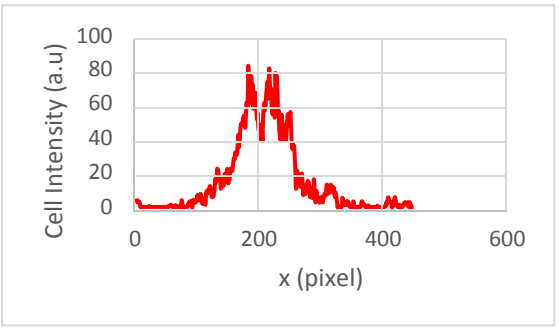
24^{th} hour



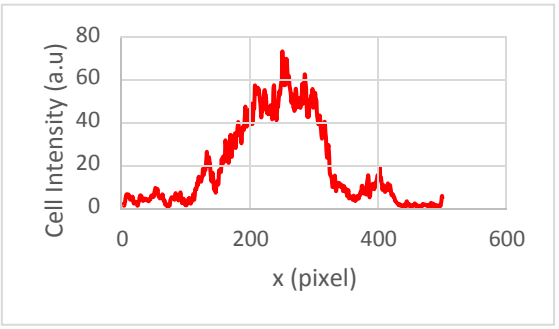


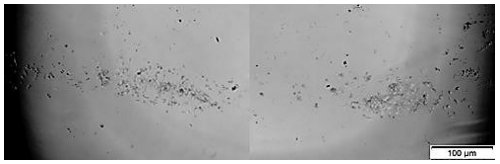

Cell Intensity (a.u)
100
80
60
40
20
0
0
200
400
600
x (pixel)

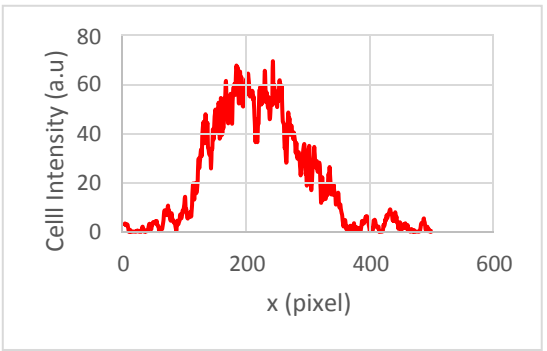


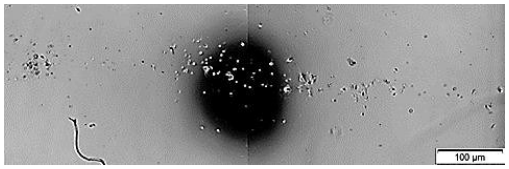

Cell Intensity (a.u)
100
80
60
40
20
0
0
200
400
600
x (pixel)

48th hour


Cell Intensity (a.u)
80
60
40
20
0
0
200
400
600
x (pixel)




Celll Intensity (a.u)
80
60
40
20
0
0
200
400
600
x (pixel)



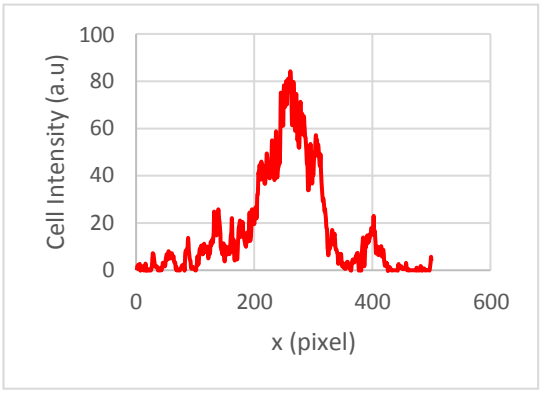
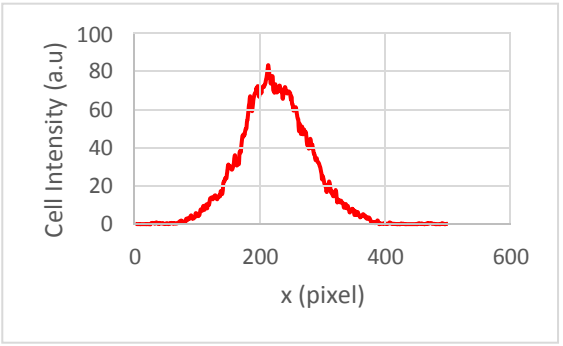
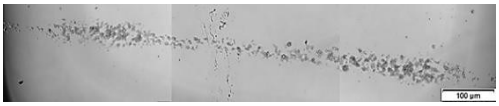


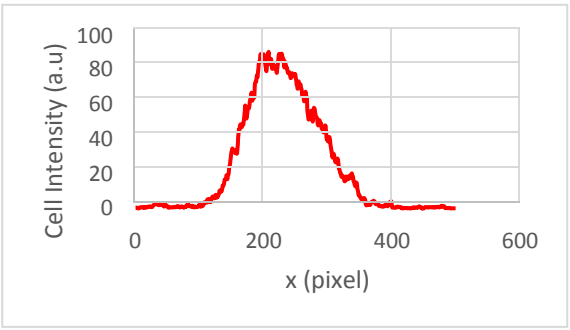
Figure 3.11.Cell Intensity of patterned 1.25×10^5/ml 7F2 cells with 5mM Gd^{3+} at 24th and 48th hours. Cells showed non-uniform distribution in the patterns.

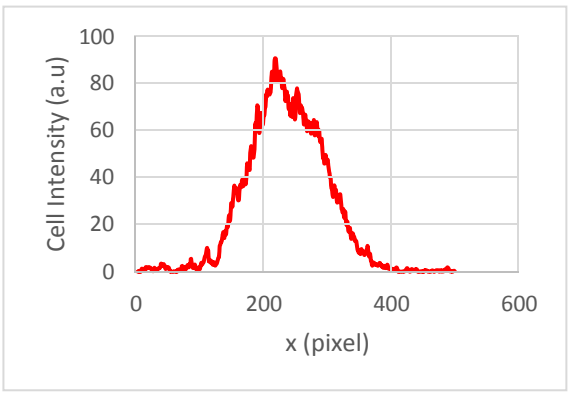
When 2.5×10^5 cells/ml were used with 5 mM Gd^{3+}, cells showed more uniform distribution in the patterns at both 24th and 48th hours. More cell concentration provided more uniform distributions (Figure 29).

24th hour

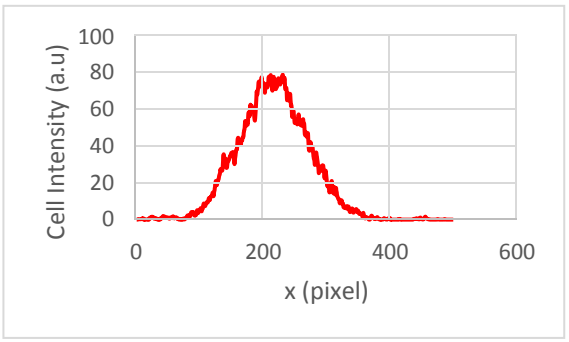


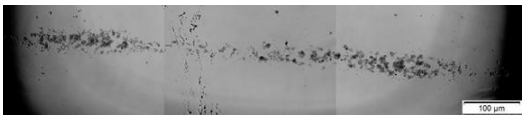


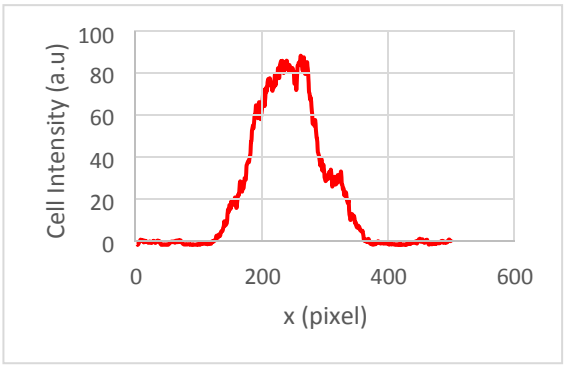




48th hour







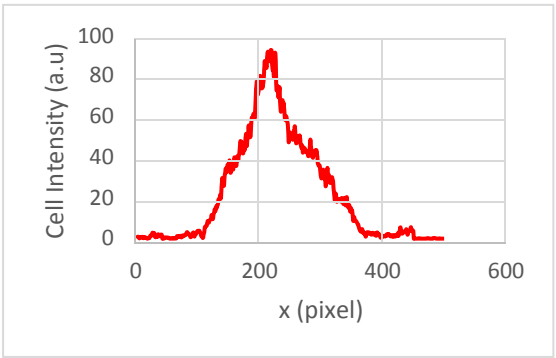
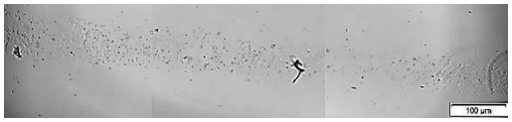
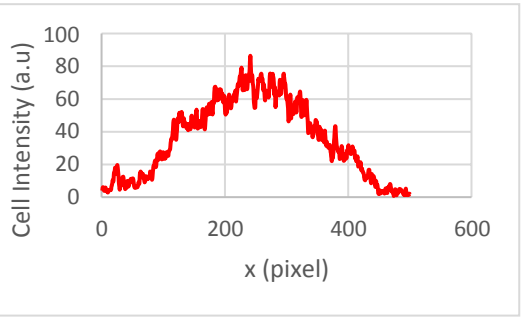


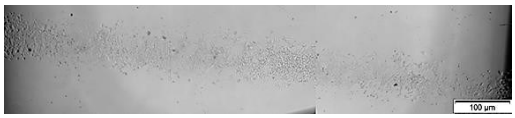
Figure 3.12.Cell Intensity of patterned 2.5×10^5/ml 7F2 cells with 5mM Gd^{3+} at 24th and 48th hours. Cells showed more uniform distribution in the patterns.

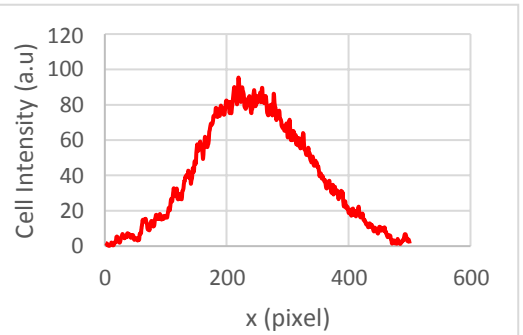
When 5×10^5 cells/ml were used with 5 mM Gd^{3+}, cells more uniform distribution in the patterns at both 24th and 48th hours. More cell concentration provided more uniform distributions (Figure 30).

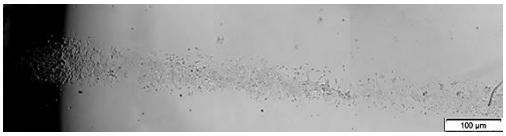
24th hour

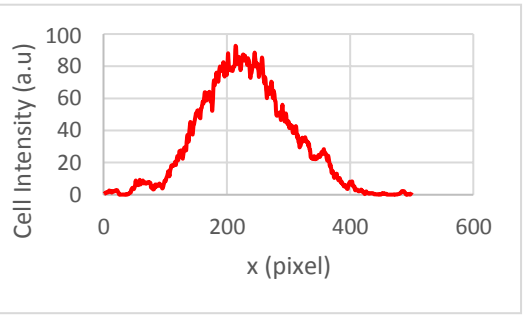




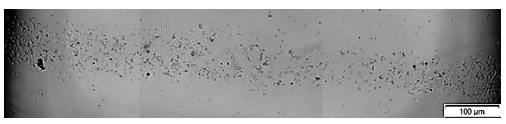


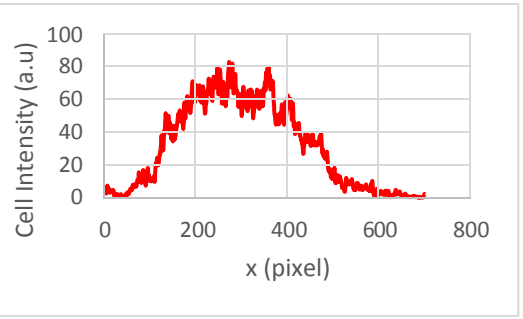


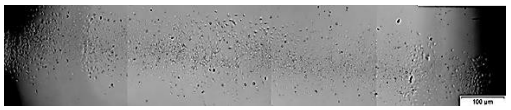


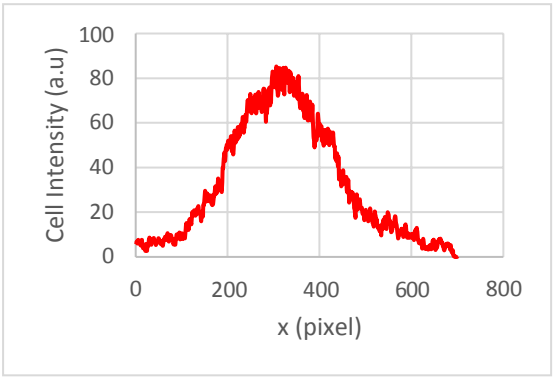


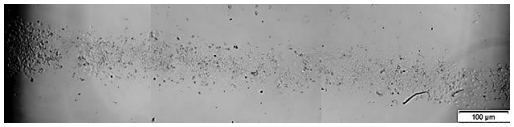
48th hour











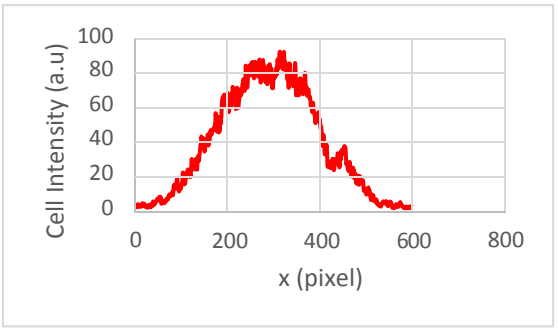
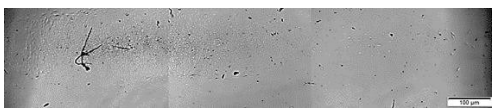
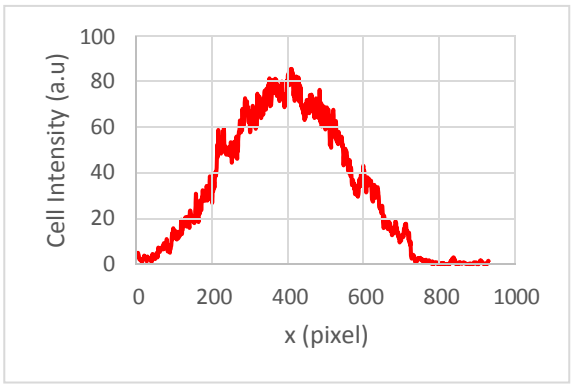


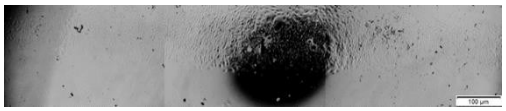
Figure 3.13. Cell Intensity of patterned 5×10^5/ml 7F2 cells with 5mM Gd^{3+} at 24^{th} and 48^{th} hours. Cells showed uniform distribution in the patterns.

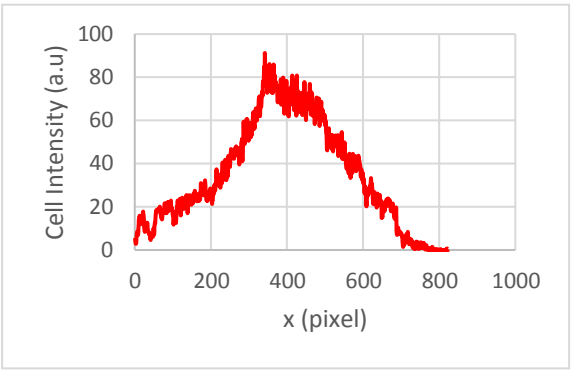
Cell intensity was also analyzed for the experiment that magnets were removed after 24 hours. When 1.25×10^5 cells/ml were used with 5 mM Gd^{3+}, cell showed both uniform and non-uniform distribution. After 48 hours, cells began to show non-uniform distribution due to proliferation and removal and magnetic force (Figure 31).

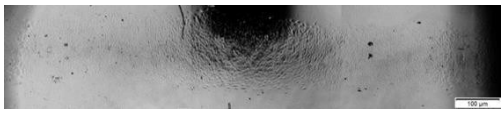
24^{th} hour

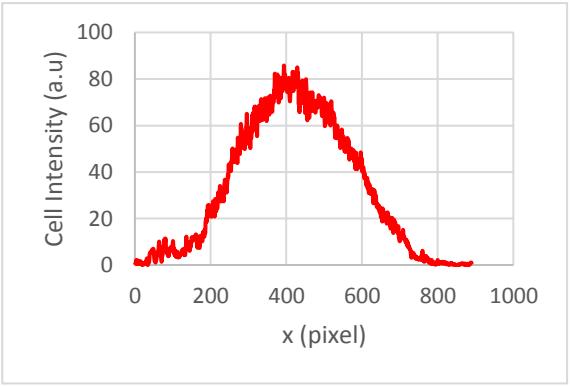




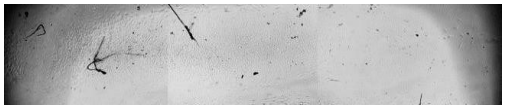


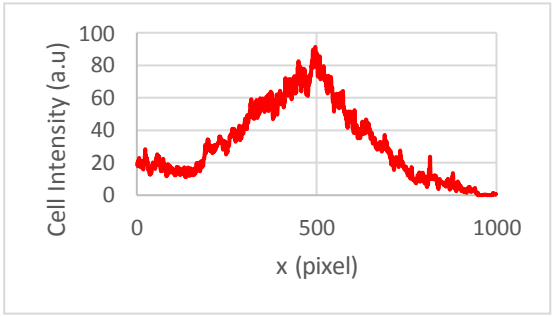

Cell Intensity (a.u)
100
80
60
40
20
0
0
200
400
600
800
1000
x (pixel)

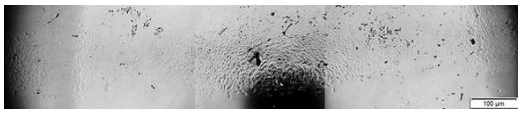


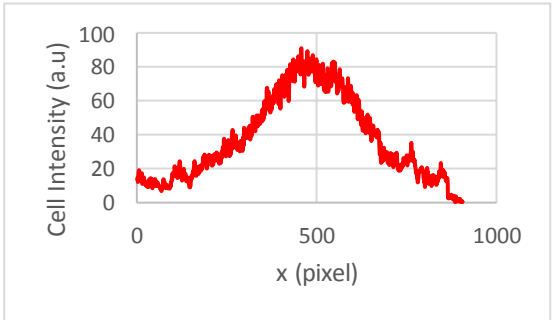

Cell Intensity (a.u)
100
80
60
40
20
0
0
200
400
600
800
1000
x (pixel)

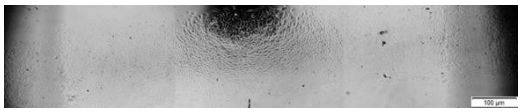
48th hour




Cell Intensity (a.u)
100
80
60
40
20
0
0
500
1000
x (pixel)




Cell Intensity (a.u)
100
80
60
40
20
0
0
500
1000
x (pixel)



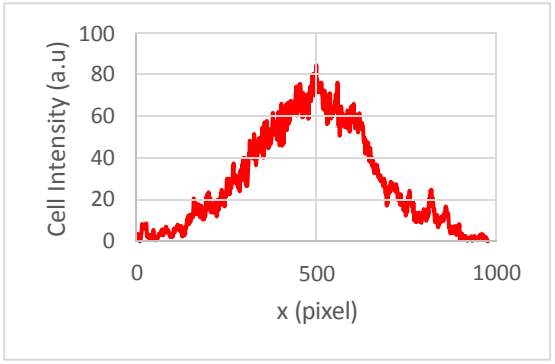
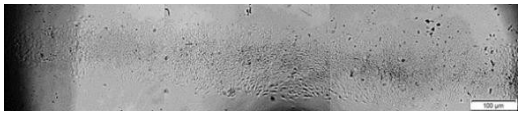
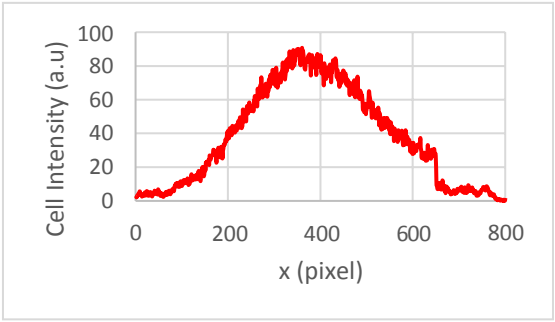


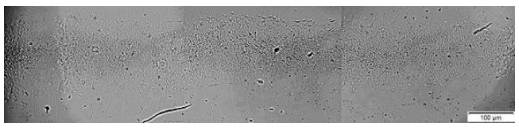
Figure 3.14.Cell Intensity of patterned 1.25×10^5/ml 7F2 cells with 5mM Gd^{3+} at 24th and 48th hours. Cells showed uniform distribution in the patterns.

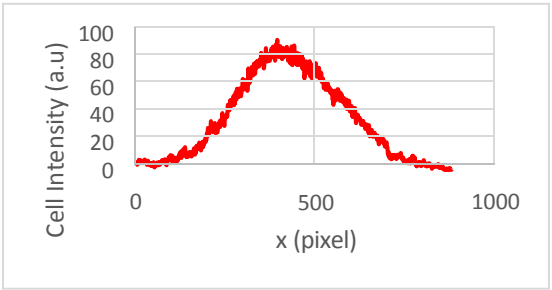
When 2.5×10^5 cells/ml were used with 5 mM Gd^{3+}, cells showed uniform distribution after 24 hour. Non-uniform distribution was also observed after 48 hours (Figure 32).

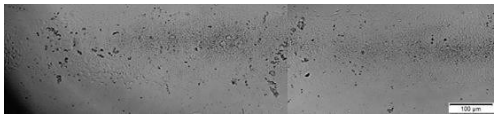
24th hour

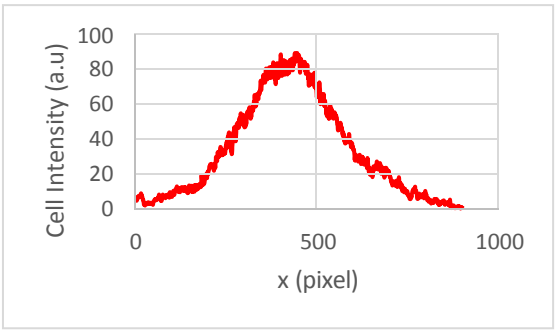




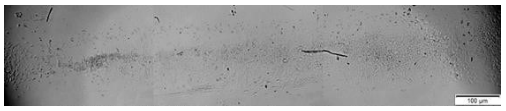


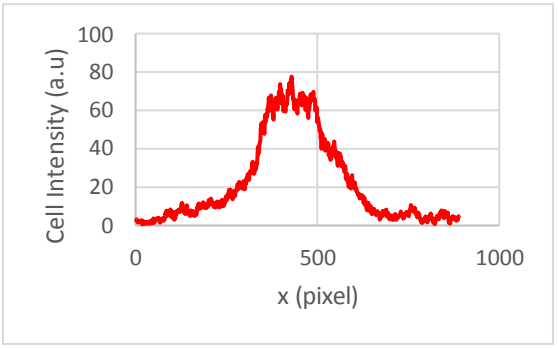


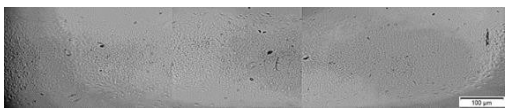


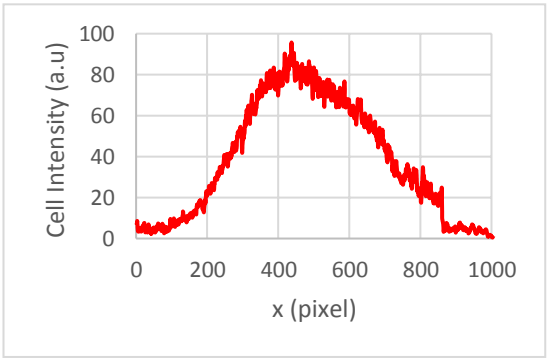


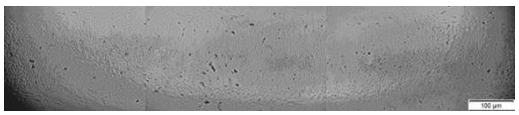
48th hour











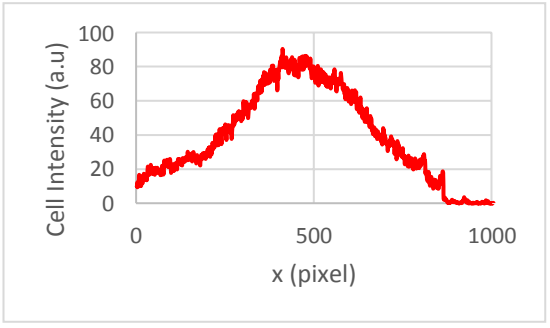
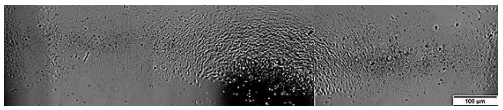
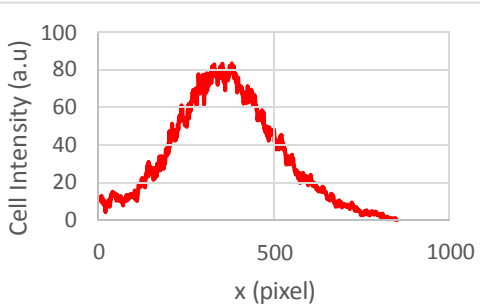


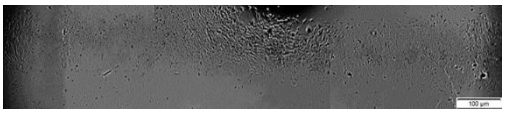
Figure 3.15.Cell Intensity of patterned 2.5×10^5/ml 7F2 cells with 5mM Gd^{3+} at 24th and 48th hours when magnets were removed after 24 hours. Cells showed uniform distribution in the patterns.

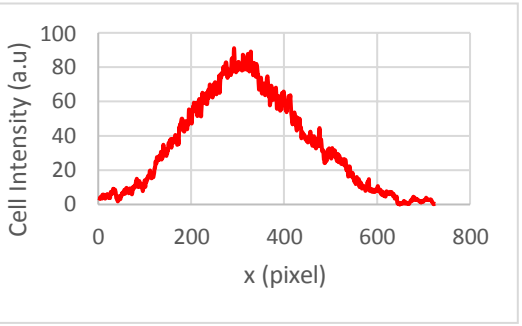
When 5×10^5 cells/ml were used with 5 mM Gd^{3+}, some cells showed uniform distribution after 24 hours but cells began to show non-uniform distribution after 48 hours due to cell proliferation of high amount of cells and removal of magnetic force (Figure 33).

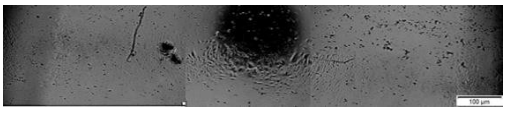
24th hour

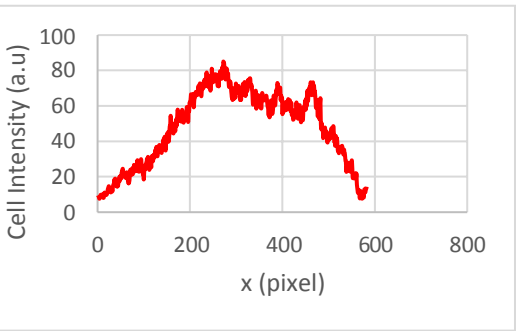












48th hour

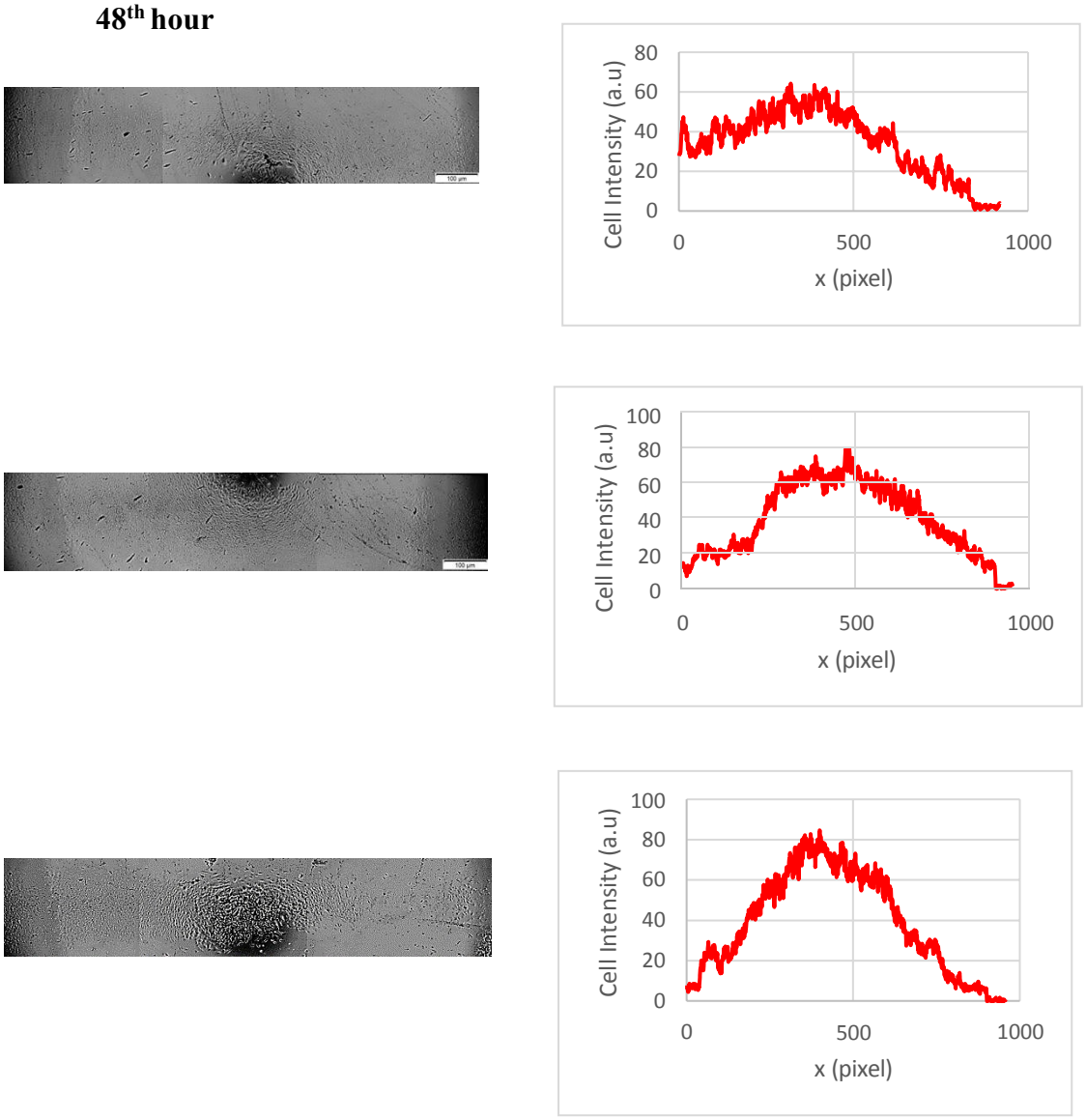


Figure 3.16. Cell Intensity of patterned 5×10^5/ml 7F2 cells with 5mM Gd^{3+} at 24th and 48th hours when magnets were removed after 24 hours. Cells showed uniform distribution in the patterns after 24 hours and showed uniform distribution after 48 hours.

3.4. Patterning of Two Type Cells in Different Shapes

Patterning of two type cells was observed. 7F2 dsRed and D1 PMIG cells were used. For this purpose, first type of cells were seeded (at day 0) and second type of cells were seeded by rotating the magnets ninety degrees (Figure 34) and forty five degrees (Figure 38) after 24 hours (at day 1). Another experiment was like that first type of cells were seeded and second type of cells were seeded parallel to the first type of cells. Finally, they were examined under microscope (at day 2).

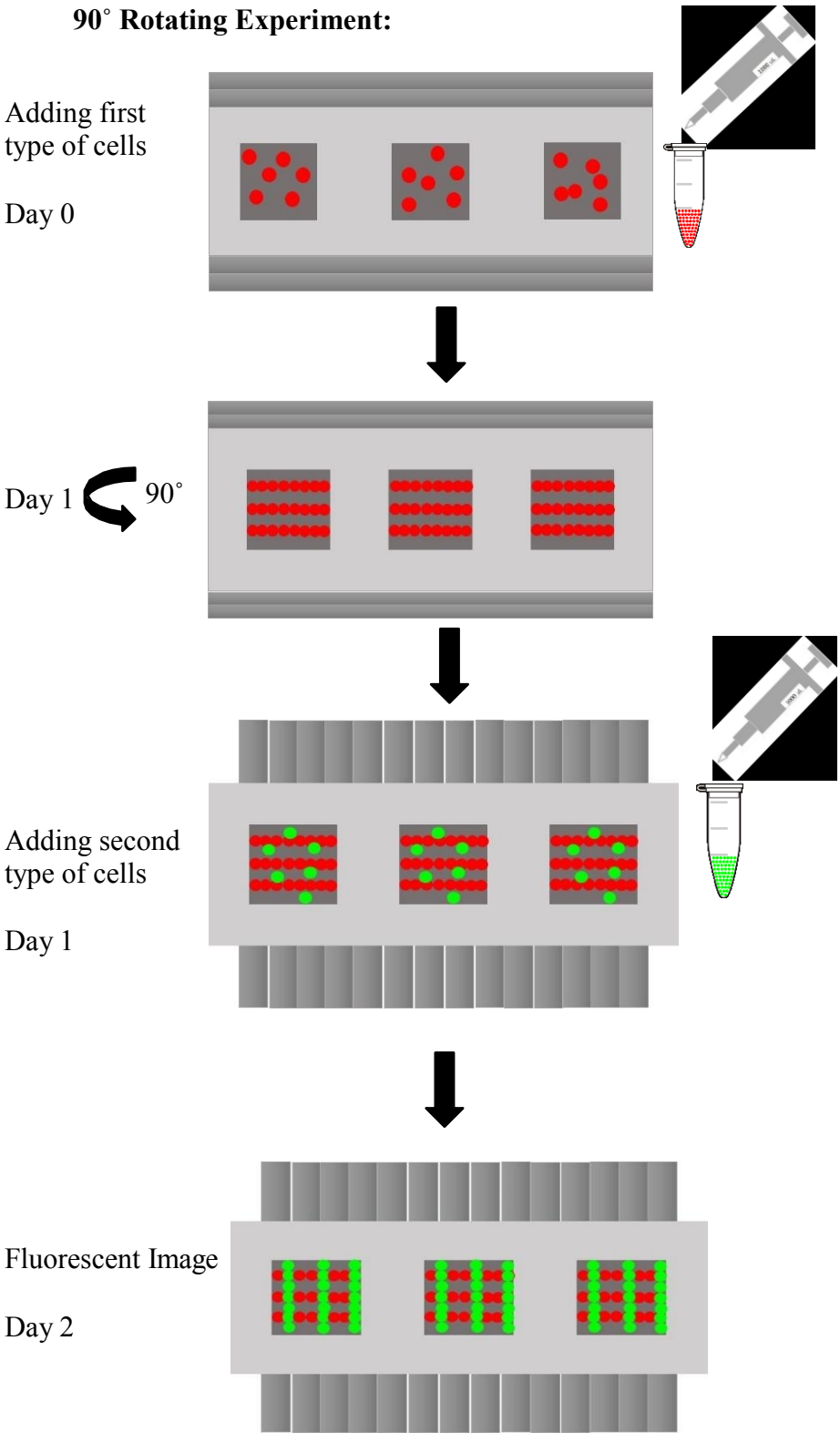


Figure 3.17. Schematic Illustration of 90˚ Rotation of Magnet

1.25×10^5/ml 7F2 dsRed cells were seeded and D1 PMIG cells were seeded by rotating magnets 90˚ after 24 hours (day 1). Cells were examined under microscope at day 2, 3 and 4. Cells proliferated and patterns began to break down at day 4 (Figure 35).

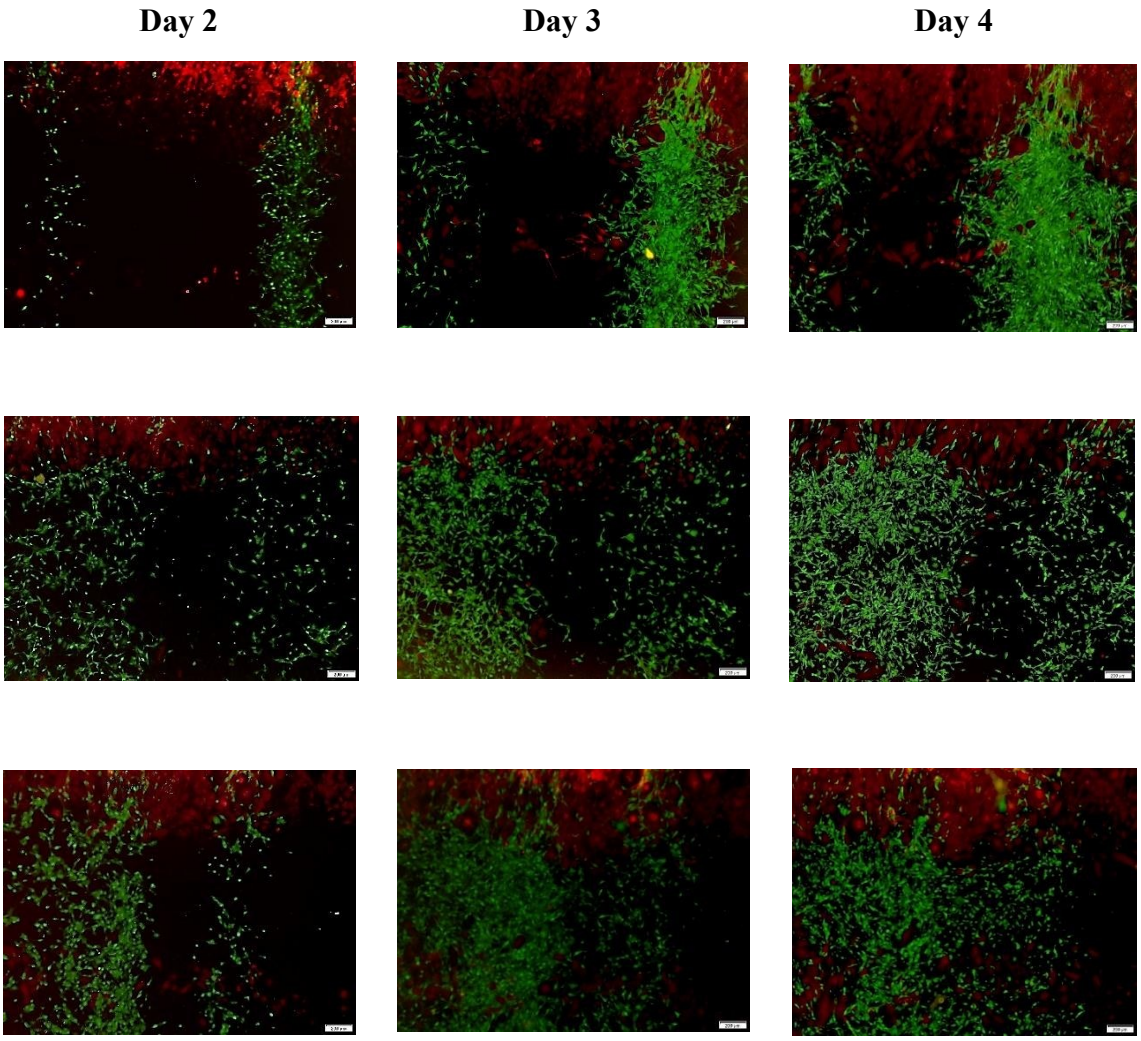


Figure 3.18. Patterning Images of 1.25×10^5/ml 7F2 dsRed cells and D1 PMIG cells together. Firstly, 7F2 dsRed cells were seeded and D1 PMIG cells were seeded after 24 hours by rotating magnets 90˚. Cells proliferated and patterns began to break down at day 4.

5×10^5/ml 7F2 dsRed cells were seeded and D1 PMIG cells were seeded by rotating magnets 90˚ after 24 hours (day 1). Cells were examined under microscope at day 2, 3 and 4. Cells proliferated and patterns began to break down at day 4 (Figure 36).

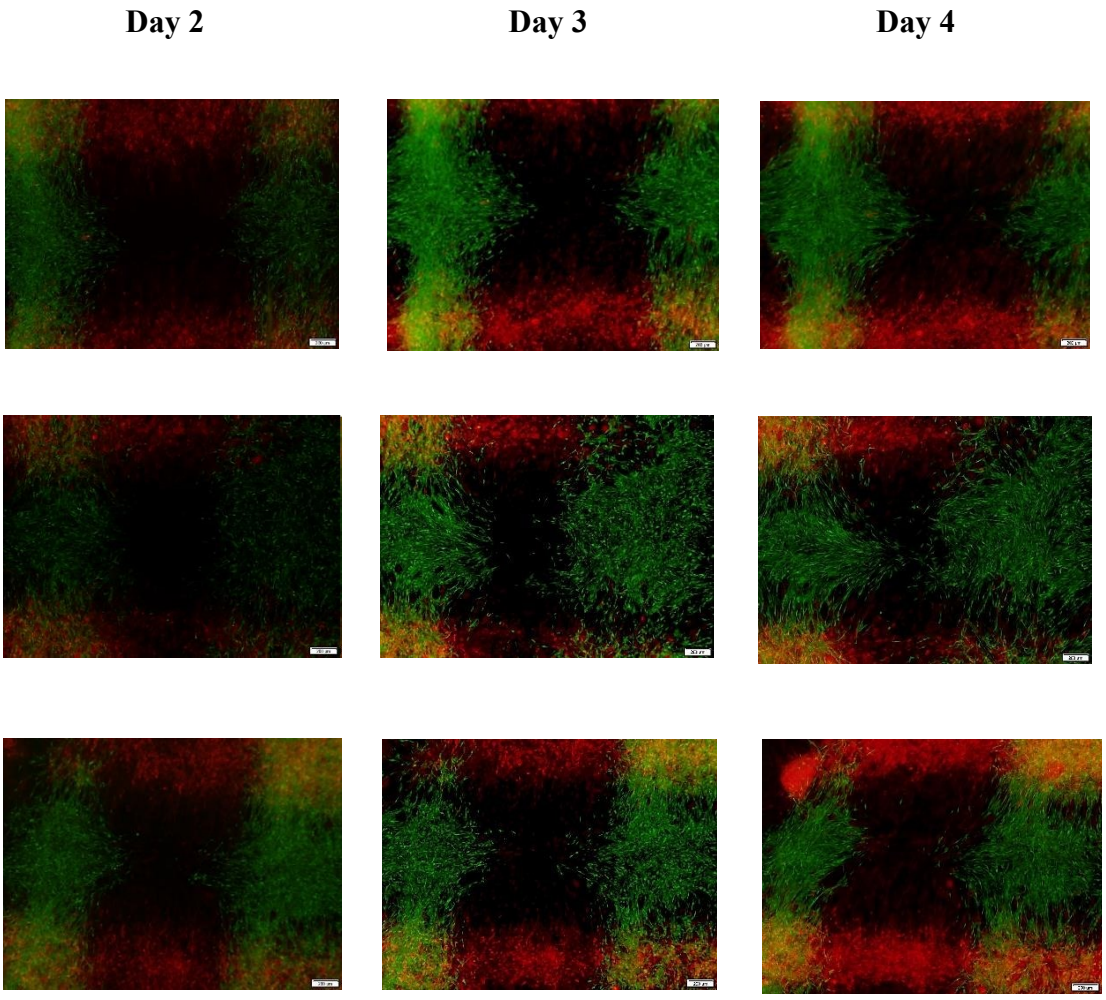


Figure 3.19.Patterning Images of 5×10^5/ml 7F2 dsRed cells and D1 PMIG cells together. Firstly, 7F2 dsRed cells were seeded and D1 PMIG cells were seeded after 24 hours by rotating magnets 90˚. Cells proliferated and patterns began to break down at day 4.

5×10^5/ml 7F2 dsRed cells were seeded and D1 PMIG cells were seeded by rotating magnets 90˚ after 24 hours (day 1) and the opposite was also done. Cells were examined under microscope at day 2 (Figure 37).

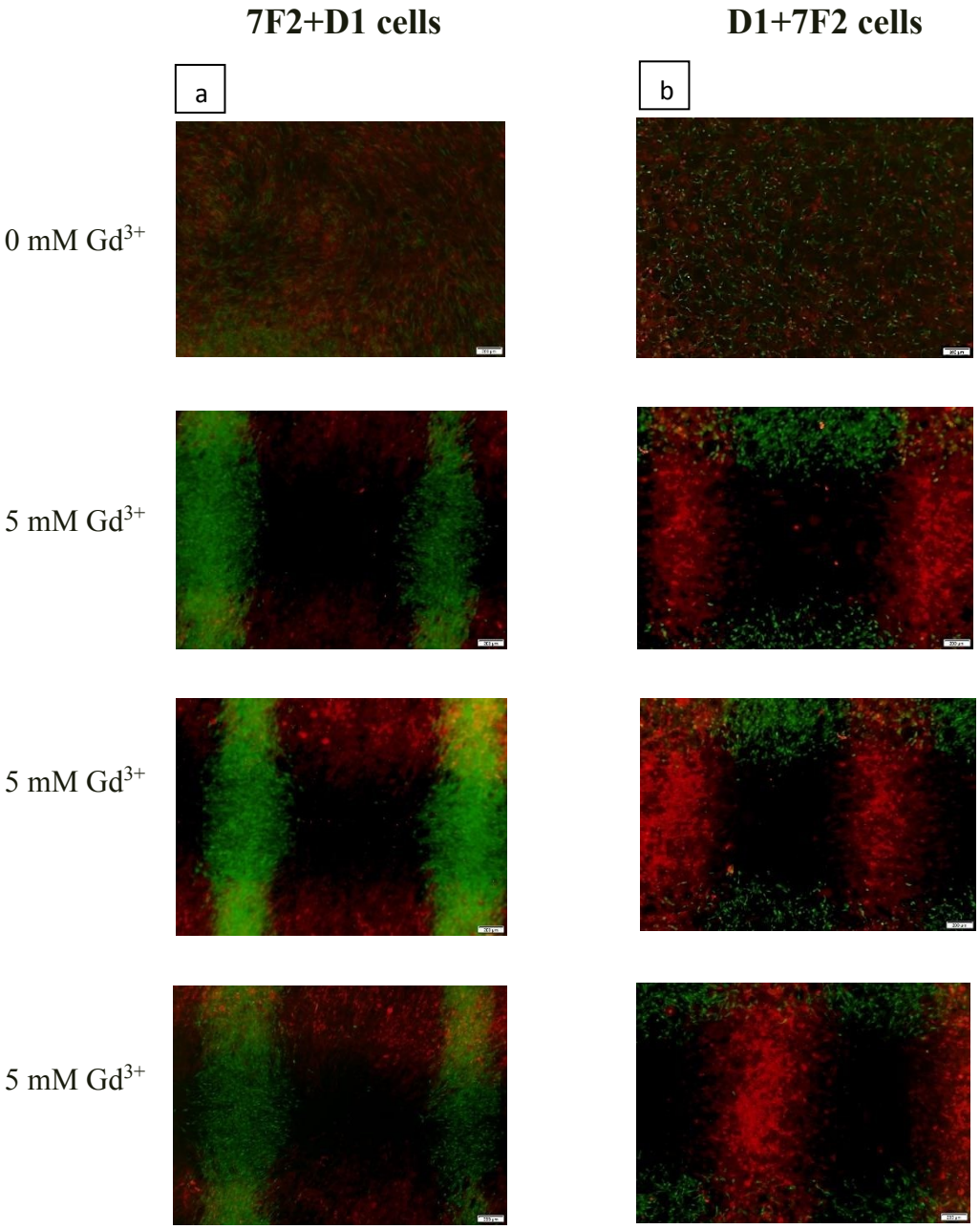


Figure 3.20. Experiment of 90˚ rotating of magnets. a) Firstly, 5×10^5/ml 7F2 dsRed cells were seeded and 5×10^5/ml D1 PMIG cells were seeded after 24 hours. b) Firstly, 5×10^5/ml D1 PMIG cells were seeded and 5×10^5/ml 7F2 dsRed cells were seeded after 24 hours. (7F2 dsRed cells: Red cells, D1 PMIG cells: Green cells)

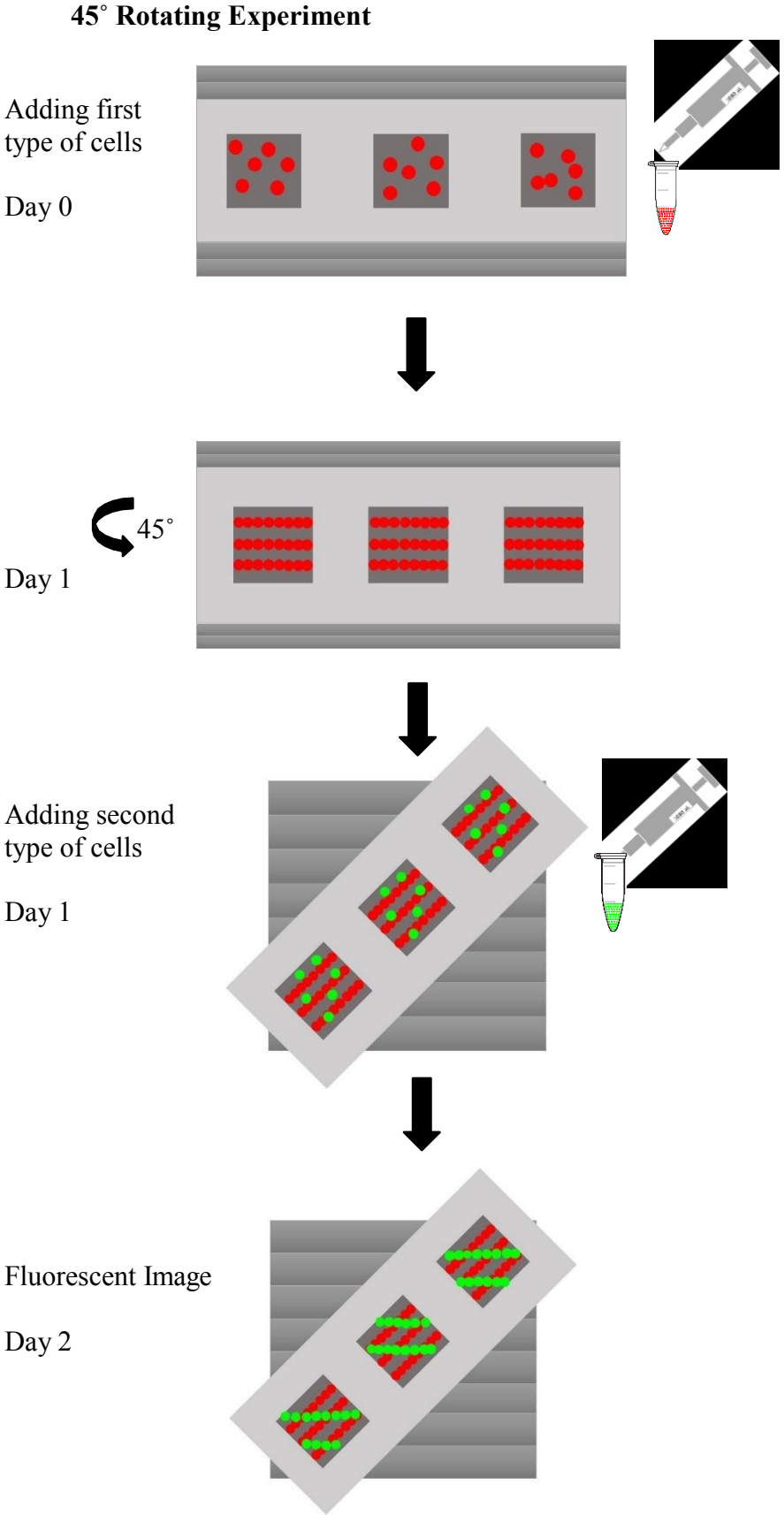


Figure 3.21. Schematic Illustration of 45˚ Rotation of Magnet

5×10^5/ml 7F2 dsRed cells were seeded and D1 PMIG cells were seeded by rotating magnets 45˚ after 24 hours (day 1) and the opposite was also done. Cells were examined under microscope at day 2 (Figure 39).

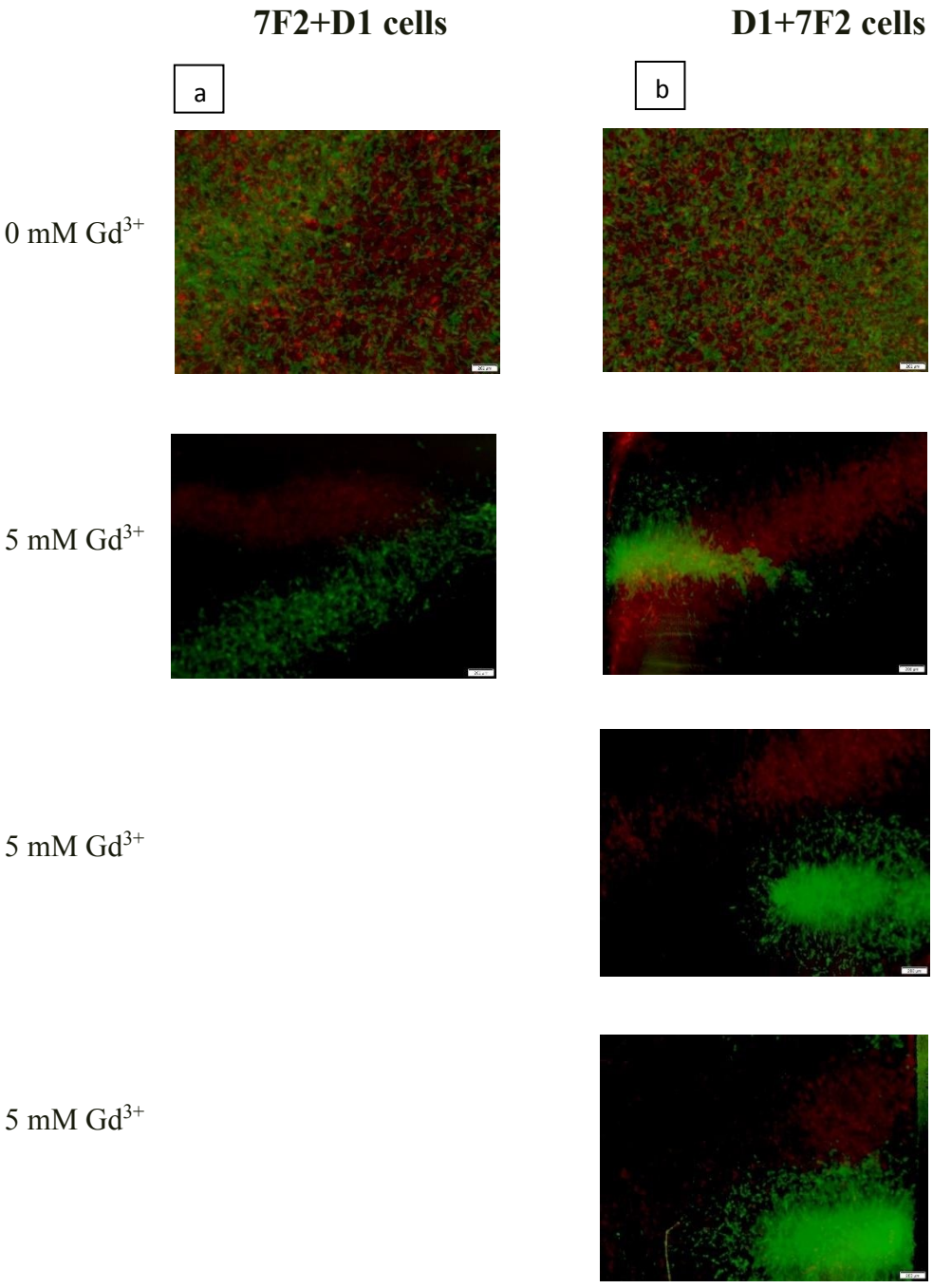


Figure 3.22. Experiment of 45˚ rotating of magnets. a) Firstly, 5×10^5/ml 7F2 dsRed cells were seeded and 5×10^5/ml D1 PMIG cells were seeded after 24 hours. b) Firstly, 5×10^5/ml D1 PMIG cells were seeded and 5×10^5/ml 7F2 dsRed cells were seeded after 24 hours.

Another experiment was like that first type of cells were seeded and second type of cells were seeded parallel to the first type of cells. In other words, 7F2 dsRed cells were seeded, D1 PMIG cells were seeded after 24 hours to be parallel to 7F2 dsRed cells. The opposite was also done (Figure 40).

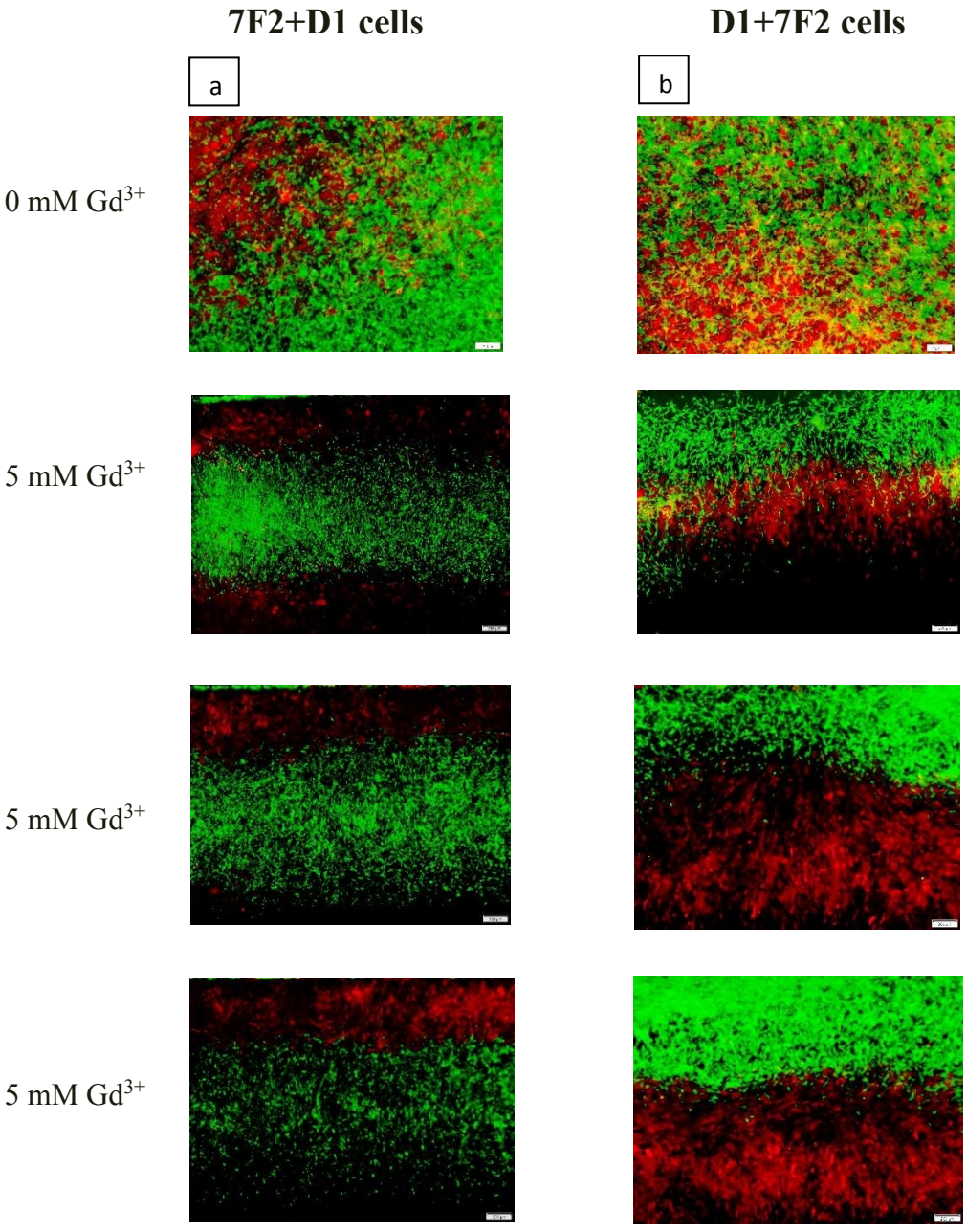


Figure 3.23. Parallel patterning of cells. a) Firstly, 5×10^5/ml 7F2 dsRed cells were seeded and 5×10^5/ml D1 PMIG cells were seeded after 24 hours. b) Firstly, 5×10^5/ml D1 PMIG cells were seeded and 5×10^5/ml 7F2 dsRed cells were seeded after 24 hours.

In this study, cells were cultured with 0, 5, 10, 15, 20, 25, 50, 100 and 200 mM Gd^{3+}. The most proper patterns occured when 5 mM Gd^{3+} was used. Then 5 mM Gd^{3+} was used in the other experiments (Figure 19).

Cells were initially cultured for three hours in the patterning material which was placed on magnets. Cells escaped from high magnetic field and they located in a place where there was low magnetic field via negative magnetophoresis (Figure 20). As positive magnetophoresis needs labeling of cells [98], however, cell patterns occured formed without labeling cells in a short time in this study [64,66].

Long term culturing of different amounts of cells was examined. When patterns were observed in the presence of magnetic force for 48 hours, thickness of some patterns increaesed and some decreased (Figure 21-23). However, thickness of all patterns increased when magnetic force removed after 24 hours (Figure 24-26).

Patterning of different type of cells in different shape was also examined. 7F2 dsRed and D1 PMIG cells were patterned at 90˚ with each other (Figure 35-37). They were examined up to fourth day. Patterns were maintained for long time [115,116]. 7F2 dsRed and D1 PMIG cells were patterned at 45 with each other (Figure 39). They were also patterned as parallel to each other (Figure 40). This showed cells can be patterned with desired shapes by controlling the magnetic field [64].

Cell intensity in the patterns was also analyzed. Most of the cells showed uniform distribution in the patterns after 3 and 24 hours. However, uniformity began to break down after 48 hours when magnetic force was removed (Figure 33).

CHAPTER 4

CONCLUSION

In this study, it was aimed to pattern cells using negative magnetophoresis without labeling them. 7F2 dsRed and D1 PMIG cells were used to form pattern with using appropriate paramagnetic agent. Patterning of cells for long time and patterning of two type cells in different shape were also examined. Additionally, how the cells had a distribution in the patterns was analyzed.

Patterning of cells was observed in a short time (three hours). When two type of cells were patterned together, they stayed as patterned for 48 hours. When magnetic force was removed after 24 hours, patterns began to break down after 48 hours. This shows that once cells were patterned, they maintained as patterned up to 48 hours. 7F2 dsRed and D1 PMIG cells were patterned at 45˚ and 90˚ angle with each other. These cells were also patterned as parallel to each other. This shows cells can be patterned in the desired shapes. Thus, tissues that similar to real tissues can be produced with this way.

In this study, cells were patterned without labeling of them in a short time. Patterned cells maintained for long time and different shaped patterns were formed. Cells continued to proliferate in the patterns in the presence of magnetic field. This system may be good alternative to conventional tissue construction methods with the advantages such as being rapid, label free and non-toxic.

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