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Cell interactions under controlled of surface substrate

Simzar Hosseinzadeh[1, 2*], Zeinab Zarei[3], Sara Esna-ashari[1], Masoud Soleimani[4, 2]

1- Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2- Stem cell technology research center, Tehran, Iran
3- Department of Applied cell sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
4- Hematology Department, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran

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Abstract: The interactions between cells and substrates are critical for biological processes as intercellular signaling, proliferation and differentiation into tissue or organ formation. The improvement of controlling the cell behavior relevant to another or substrate surface leads the more precise regenerative approaches of tissue engineering. While the presence of extra cellular matrix (ECM) components triggers into cell attachment, a specified chemical group deposited on the substrate surface is able to hamper cell adhesion and can change cell fate to death. Currently, benefiting from nanostructured surfaces has progressed the spatial arrangement of cells with nanometer level resolution. Also, the value of surface roughness as the presence of unique biomolecules or taking the advantage of a specified pattern governs the cell cycle strongly. Herein, we summarized the studies which were focused on the examination of cell fate relative to surface properties. In total, cell activity is directly influenced by surface modifications those try to provide a more biocompatible as well as biologic environment.

Keywords: cell interaction, substrate surface, nanotopography, fibronectin, stiffness

Introduction *Tissue engineering is* an interdisciplinary field employing a combination of *cells, the engineered material (scaffolds) alone or with bioreactor technology* [1]. Commonly utilized biomaterials by their dynamic structure as well as their composition determine the cell fate through cell-matrix interactions. *In a normal functioning engineered tissue*, the biocompatible material is a required property for preventing any unacceptable effect [2]. The most basic objective of biocompatibility is promoting specific cell fates from cell attachment until reaching a stable differentiated phenotype [3]. As we know, proteins like collagen and elastin with diameter ranging 10-300 nm generate the fibrillar framework of extra cellular matrix (ECM). In addition, as well as ECM proteins, glycosaminoglycans also provide the cell binding sites [4]. Thus, for a successful clinical trail along with commercial issues, the improvement of scaffold should consider the size, shape and mechanical properties beside intricate relationships with host tissues. On the other hand, the dynamic balance between cells and a three dimensional environment is a tremendous phenomena during cell growth and

*Corresponding Authors: Simzar hosseinzadeh
Tehran, Iran. Tel: +982188991118; Fax: +982188991117, s_hosseinzadeh@razi.tums.ac.ir

terminal differentiation [3]. Totally, tissue engineering needed to be promoted as a self-healing therapy for human tissues [2] that is obtained by serial biological events triggered in response to the surrounding matrix by cellular attachment. These interactions mediating cell signaling networks are influenced by various structural parameters and surface chemistry. Additionally, the biomimetic surfaces with nanopatterns and defined functional groups enhance the cellular spreading as well as more growth, differentiation and motility. In this review, the criteria which are considerably required for tissue engineering approaches are discussed in detail and the results highlighted the importance of chemical and physical properties of substrates for determining the seeded cells' fate. Totally, the cell responses are strictly dependent to combined effect of cell type and surface character that force to employ the specified substrates on the basis of tissue types.

The cell adhesion and cell cycle

During development, the embryo receives the applied forces from the surrounding environment which determines the cells' morphogenesis and differentiation fate [5] (Fig 1A). The tissue development is guided by modulation of gene expression under the signal transduction. Thus, the cell cycle events as proliferation, maturation, migration and apoptosis are adjusted by the transmitted from ECM which is able to influence internal cellular components [6].

This cellular dynamic behavior is considerably affected by the alteration in conformation of multi-molecular compartments as a function of applied force on focal adhesions. Then, their new folding would lead to a new binding activity. In principle, the applied force per molecule was calculated to be 1pN that is lower than the measured force of an individual focal adhesion [7]. The regulation of cell shape and growth by adhering proteins similar to fibronectin (FN) involves with RGD sequence. The attachment of cells with this peptide alters the function of transmembrane proteins or ion channels and starts the regulation of cell growth. The induction of intracellular signaling pathways depends on the transmembrane and cytoskeletal components [8].

Cell adhesion and proliferation behavior have been also studied on RGD and chemical modified spider silk by Wohlrab et al. They showed that genetically modified RGD silk film can improve the attachment and proliferation of BALB/3T3 mouse fibroblasts similar or slightly better than silk protein chemically modified with the cyclic RGD peptide [9]. In this regard, focal adhesions size can determine the speed of cell migration independent of their surface density. One study approved the lateral spacing of RGD peptide could change the organization of actin cytoskeleton of human mesenchymal stem cells (hMSCs). The corresponding changes were strongly attributed to focal adhesion formation. Vinculin immunolocalization was used here to detect matrix adhesions on both spaced peptides inclusive the 34-nm or 44-nm and 50-nm or 62-nm. Measurement of vinculin complex length determined that hMSCs cultured on 34-nm-spaced peptides had a significantly higher proportion of vinculin complexes with a length of 10 um or longer, indicative of fully mature FAs than hMSCs on 62-nm-spaced peptides. The result was converse for nascent focal complexes of less than 5 mm in length related to hMSCs on 62-nm- than 34-nm-spaced RGD [10] (Fig 1B). The results were in consistent with other reports that MEF speed increases with focal adhesion size with a threshold value of 0.7 and beyond this, the cell speed declines [11]. A

detailed examination was performed to identify the cell responses to ECM. It approved that each examined cell type possessed the specified interactions with a unique component of ECM as Fibroblasts best responded to fibronectin, followed by chondrocytes to collagen I and the other cell types to laminin [12]. The successful cell linkages with substrate happen in the presence of a transition level of collagen density at ≤ 160 um^{-2}. Thus, the increasing or lowering of collagen density more or less than 160 um^{-2}, has suppressive effect on surface adhesiveness. As Fig1C shows at different density of collagen, the applied force on the surface would be changed, i.e. at the high collagen density, the surface force becomes 1.0 pN which is lower than the required force for trapping an integrin segment [13].

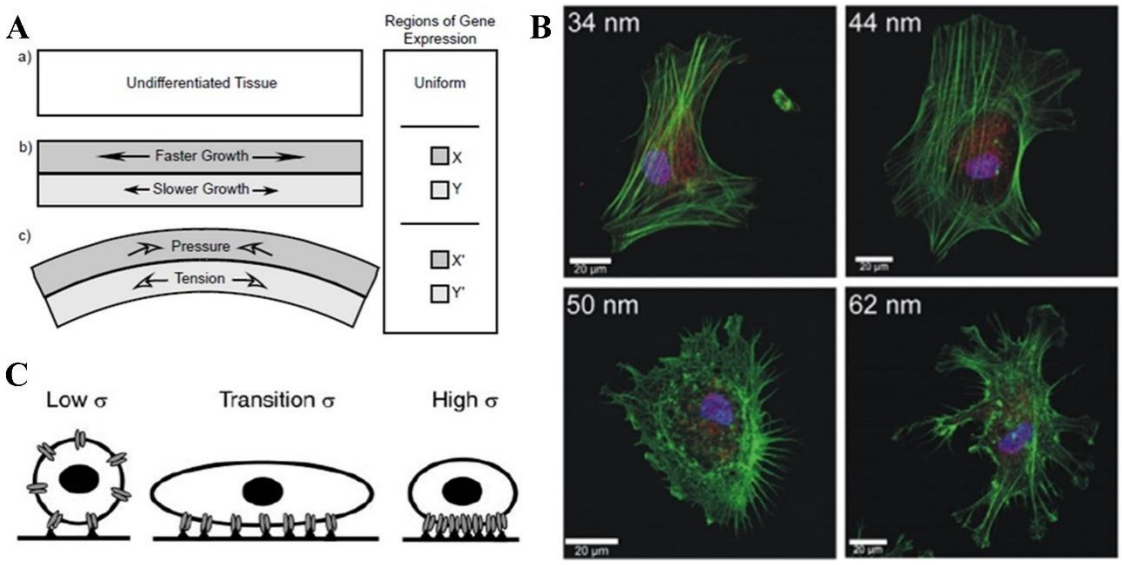

Fig 1. (A) Different layers of embryo are resulted on the basis of applied force from other type of cells. During development, the different cell layers grow at different rates that generate time-varying stresses throughout the cells and tissues [5], (B) ThehMSCs presented different morphologies depending upon the spacing of the RGD domains from well-spread cells on 34-nm-spaced RGD domains, to cells extending multiple filopodia on the domains with 62-nm-spaced RGD. The organization of the actin (green) cytoskeleton on the 34-nm- and 44-nm-spaced peptide domains was larger and well-defined stress fibres compared to the 50-nm-spaced and particularly the 62-nm- spaced peptide domains. Immunostaining for focal adhesion kinase (FAK) (red) also showed lower expression in hMSCs with lateral RGD domain spacing of 50 nm and above [10], (C) All integrins on the cells are bound to the substrate (a) and further spreading is possible when the additional areas of substrate allow cell binding (b). When the density of the sites on substrate is increased beyond the transition point, this saturation of integrin receptors results the lesser degree of cell spreading (c) [13].

Ronning et al cultured bovine primary muscle cell on various surfaces coated either with glycosaminoglycans (GAGs), single protein coatings (laminin or collagen), combined protein coating (ECL) or complex ECM surface coating (ECL+GAGs) to investigate cell proliferation and differentiation features. The muscle cells that cultured on ECL and ECL+GAGs clearly showed highest proliferation and differentiation rate compared to cells cultured on single-protein coatings. Moreover, the composite modification with a mixture of both glycosaminoglycans (GAGs) and fibrous proteins could improve muscle cell proliferation as well as myogenesis [14].

In the following of biomimicry, three dimensional collagen gels efficiently mimic the connective tissue for fibroblasts. thus, collagen gels are beneficial for simulating the tumor and cellular interactions with environment [13]. Three dimensional structure of collagen is considered as mechanically stressed gel that cells were relaxed and unloaded by detaching of cell loaded gels from the plate's surface [15]. The 3-dimensional culture of cells is the same as *in vivo* model in terms of the balance of cellular force and the resistance from involved substrates. Therefore, the alteration of surface molecular architecture can subsequently change the outcome of this balance and makes different biochemical events. This is because the thermodynamic and kinetic properties of cellular attachment are changed during cell growth cycle. Accordingly, the response of neurotransmitter to mechanical tension was applied across integrins by the release of calcium. By studying the site of applied force, it has been resulted that the FAC adhesions are enough rapid components of membrane for transduction of signals. Thereupon, the changes in ECM mechanics affect FAC position inside the cellular membrane and these alterations lead to transformation of cytoskeletal structure of cell (Fig 2A). The direction and magnitude of applied force alter FACs position as well as the intracellular components by the transfer of tensions from integrins [6]. In this regard, acrylamide substrate has the benefit to produce surfaces with various levels of elastic moduli. The extended cell morphology and attachment caused the higher expression of α5integrin on stiff surfaces as a function of more reactive crosslinking sites. Thus, the strong adhesion is generated by the higher contractile force of cytoskeleton that binds to integrins as a ligand [16].

It is also confirmed that by restriction of cells on the smaller adhesive areas (Fig 2B), the cell cycle is led to apoptosis and gradually DNA synthesis is reduced. In contrast, the separation of these regions in 3-5 nm in diameter as the size of FAC, the cells are flatten and start to proliferate in the response of better extension [6].

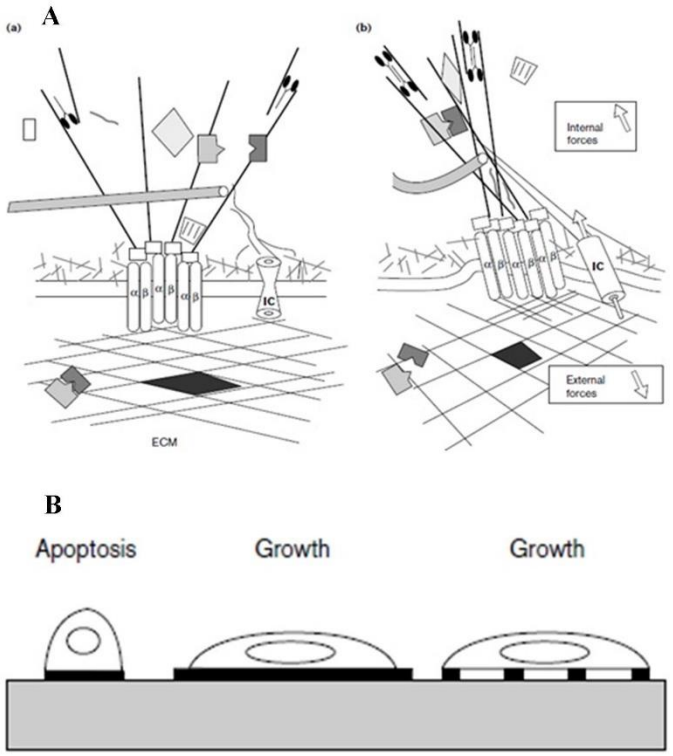

Fig 2. (A) The biochemical qualification of FAC adhesion controls the structure of cytoskeleton components. Altering the orientation of corresponding molecular elements (FAC) changes the position of regulatory components as kinases and phosphatases on substrates. The biochemical events are occurred and mechanically distort the FAC positions and cytoskeleton structure that changes the cell growth, (B) When cell spreading was progressively limited by plating on smaller and smaller adhesive islands, DNA synthesis was reduced that can switch on a death (apoptosis) program (a). By breaking up this small adhesive island into many smaller islands by non-adhesive regions (b) or increasing the cell attachment area up to enough value of cell interactions (c), the cells start to spread out and flatten (cell growth) [6].

The total generated force by separated focal adherent regions is assumed as a sufficient force in compared with the force which is generated by a larger area. By studying of cardiac myocytes, it is clarified that the direction of focal adhesions' force is determined by the direction of pattered elastic dots on the surface (Fig 3A). Since the forces of myocytes (~70 nN) unlike to fibroblasts (~20 nN) are higher, so the area with vinculin adhesions should be larger too. The generated force of fibroblast attachment is adhesions in the presence of 2,3-butanedione monoxime (BDM) as an inhibitor of actomyosin contractions. The relaxation time of focal adhesions' forces increased and the number of focal attachments are reduced simultaneously [7]. For more accurate bio-mimicry, in a study, the tissue-engineered teeth were assessed under the condition of shear stress. The size of new tissue and gene expression as a result of shear stress evidenced that cell differentiation strongly can be influenced by mechanical tensions. Thus, the up-regulation of alkaline phosphatase (ALP) activity of cell-polymer construct under shear stress exposed the modulation of differentiation (Fig 3B) [17]. In addition, by applying shear stress, cells are induced to reorient in the direction of flow and the cell behavior tends to be perpendicular to the direction of stretching [18].

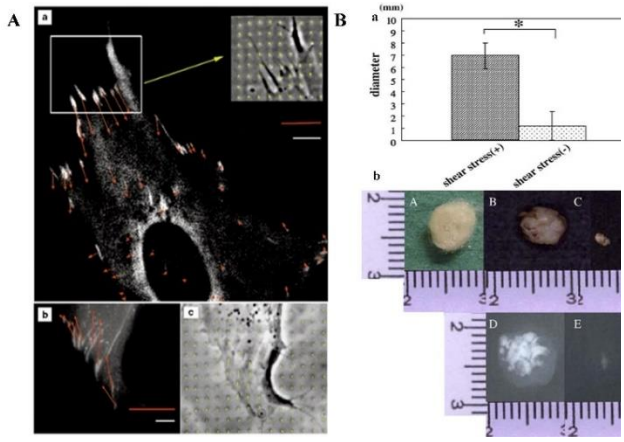

Fig 3. (A) A human foreskin fibroblast expressing GFP-vinculin which places at FAC on a patterned elastomeric surface exposed the forces (red arrows) extracted from the displacement of the dots (green arrows).White scale bars represent 4 um; red scale bars represent 30 nN [7], (B) The tissue group with applied shear stress provided the higher diameter of resulted tissue (a) and the X-ray photography confirmed the reduced calcification value with control group in the absence of shear stress (b) [17].

Surface stiffness into cell adhesion

The cell behavior changed when cells cultured on stiff or soft region. RGD motifs act with the size of 9 to 12 nm as the adhering molecule to ECM and cells. The surface coverage with a sufficient density of RGD is crucial for cell differentiation as well as spreading and the inter-spacing of 58 nm of RGD is perfectly able to make the cell adhesion [19]. In a study, the rate of cell migration was investigated on substrate rigidity by a gradient of collagen (Fig 4A). The less displacement of the receptor-ligand complex made larger tension on rough portions. Thus cells had received higher power for activation of tyrosine phosphorylation which leads to migrate strongly. Furthermore the cells were cultured on soft substrate, started to move over a long distance to land on a substrate with the stronger receptor-ligand complex in stiff portion.

Taken on, the attachment on stiff surface provides more force on embedded environment and the ongoing more calcium influx makes stronger interactions (Fig 4B). Overall, the surface stiffness is postulated to guide the cellular movements because of enhancing the myosin activation [20].

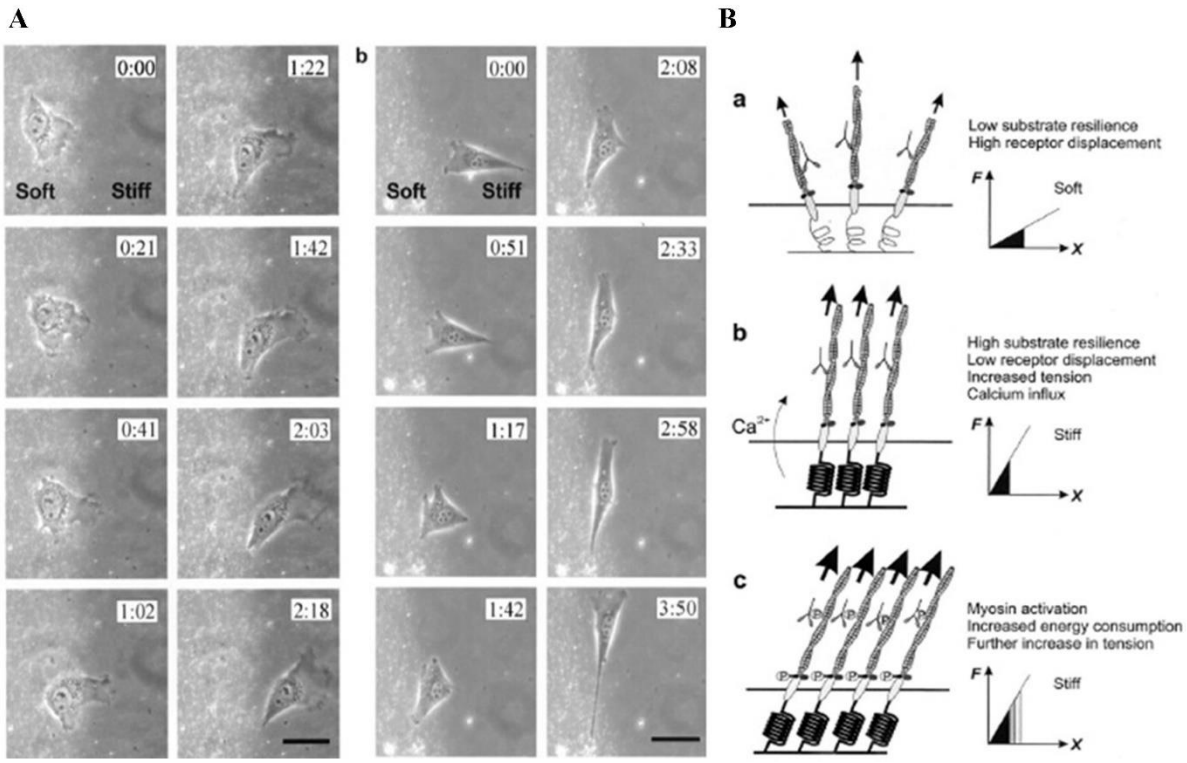

Fig 4. (A) When the leading part of cells encountered the substrate with higher degree of rigidity, the cell protrusion expanded until the whole cell volume inclusive trailing end passed through the boundary. The 25% increase was occurred for overall spreading area of cells when cell crossed from the soft to stiff side (a). In contrast, when cells approached from stiff side, the protrusion stopped at the leading side and the trailing end retracted. The protrusion continued laterally along the boundary of rigidity and reoriented cell shape to move away from the boundary. Finally, cells turned back toward the stiff side (b), Bar, 40 mm, (B) On the soft substrate, the receptor ligands are mobile and the tension at the anchorage side is weak. On the basis of energy input (black area under the force-displacement graph), the ligand complex can move a long distance (a). On the stiff type of substrate, a higher degree of cell tension makes lower the receptor ligands displacement and an influx of extracellular calcium through the stress activated channels is resulted (b). The higher level of calcium causes the phosphorylation of myosin which leads to an increased energy consumption (gray areas under the force-displacement graph). Consequently a stronger value of tension was produced (c) [20].

A study by Huang proved the spreading of endothelial cells by the pattern management of adhesive sites. If the cells had been were restricted on small islands (30 um2), the cell extension would be more appropriate and able to lunch the proliferation cycle by entry into S phase. Thus the cell extension ability as a defined morphology is noticeably resulted as a function of surface stiffness compared to more ECM binding [21] (Fig 5A). In another study, PDMS substrates of variable stiffness were fabricated by Evans et al and cell attachment, proliferation and differentiation of embryonic stem cells were assessed. Cell attachment was not influenced by the stiffness but cell growth increased in response to stiffer substrate. This team demonstrated that stiffness may direct cell differentiation to the osteogenic fate via upregulation of osteopontin genes [22]. Eroshenko et al studied embryonic stem cells count, attachment and differentiation on PDMS substrates with different stiffness. The data showed increasing Cell proliferation on the stiffer surface and by the passage of time cell fate directed to mesodermal (IGF2 expression) and endodermal (AFP expression) differentiation [23]. In another study Tam et al

assessed the behavior of hMSCs and normal human dermal fibroblasts (NHDFs) cultured on the prepared PCL scaffolds with different stiffness. The results showed that hMSCs could significantly spread on stiffer PCL when compared with normal fibroblast but the differentiation state of the cells was not affected by surface stiffness [24]. In the presence of hydrophobic surface, the adsorption or capture of proteins rapidly happens and as a result supports cell attachment. The interaction between proteins and a hydrophobic substrate is performed by specific linkages against a hydrophilic surface [18]. On the whole, there is a close and inverse relationship between the roughness and hydrophobisity magnitude [25] and the major difference between 2D and 3D substrates is the amount of stiffness which determines the force of cell contraction[26]. During the interactions of cell and a surface, the generation of forces by myosin and actin within the structure of cytoskeleton make a movement as 5 nm with an average force of 3-4 pN. On the other hand, the more ligand density eventuate higher traction forces between cells and surface. Accordingly, the higher degree of substrate stiffness implies larger focal adhesions and effectively cell migration strength should be confined by increasing traction force [18]. In another study by Genes, the impact of substrate stiffness on chondrocyte adhesion was evaluated by the calcium or barium crosslinker gradient. On the basis of this study, crosslinkers make a surface with more stiffness by modulus measurement. more stiffness is not applicably the reason of absolute attachments, but linearly decreases the time needed for adhesion [27].

In a study by Grover et al, collagen or gelatin-based films were crosslinked with carbodiimide and the roughness of the scaffolds were investigated. Cell adherence, spreading and reactivity were assessed using myoblastic C2C12 and C2C12-a2+ cell lines on these films. The result of this study showed that crosslinking can increase stiffness of collagen and reduce significantly roughness of the film. Finally crosslinking altered the physical properties of films which results in reduction of number of available cell binding sites and cell reactivity was dramatically decreased [28].

The cell attachment using nanoscale patterns

ECM environment is full of micro and nanotopographical cues which guide cellular behavior through the life steps. Thus using various nanoscale topographies on surface become a tool for inducing different proliferation and differentiation response.

Scaffolds could be reinforced with some bioactive molecules as well as culture surface [29]. By reducing fiber diameter which fabricates the structure of scaffolds, a higher ratio of scaffold surface is exposed to cell for attachment [30]. The most substantial difference between a nanotextured surface and a flat substrate is their surface energy. At first, the decreased in air content on a nanotopographical surface causes lower contact angle which is stated by Cassie–Baxter equation. Moreover, the chemical state of surface atoms on the nano and non-modified structures are distinct from each other due to higher surface energy of nanoarchitectured one [31]. A nanotopographical structure with 13 nm islands is significantly able to induce more cell extension [18]. By electrospining method, scaffolds with various diameters and direction can be fabricated. In a study PLGA was electrospun into matrices with the range of fiber diameter in 150–225, 200–300, 250–467 and 500–900 nm. Through cell seeding, the effect of these scaffolds diameter was investigated on the expression of collagen type I, type

III and elastin that highlights the considerable role of fiber diameters on the expression level of ECM structural proteins. For tissue engineering field, the wound healing process strongly depends to the composition of the ECM. Thus, for the successful establishment of new tissue, not only an appropriate cell extension is important, but also having suitable mechanical properties in the ECM is serious. Alternatively, scaffolds like PLGA provide the extended qualities by alterating the surface area [32] (Fig 5B).

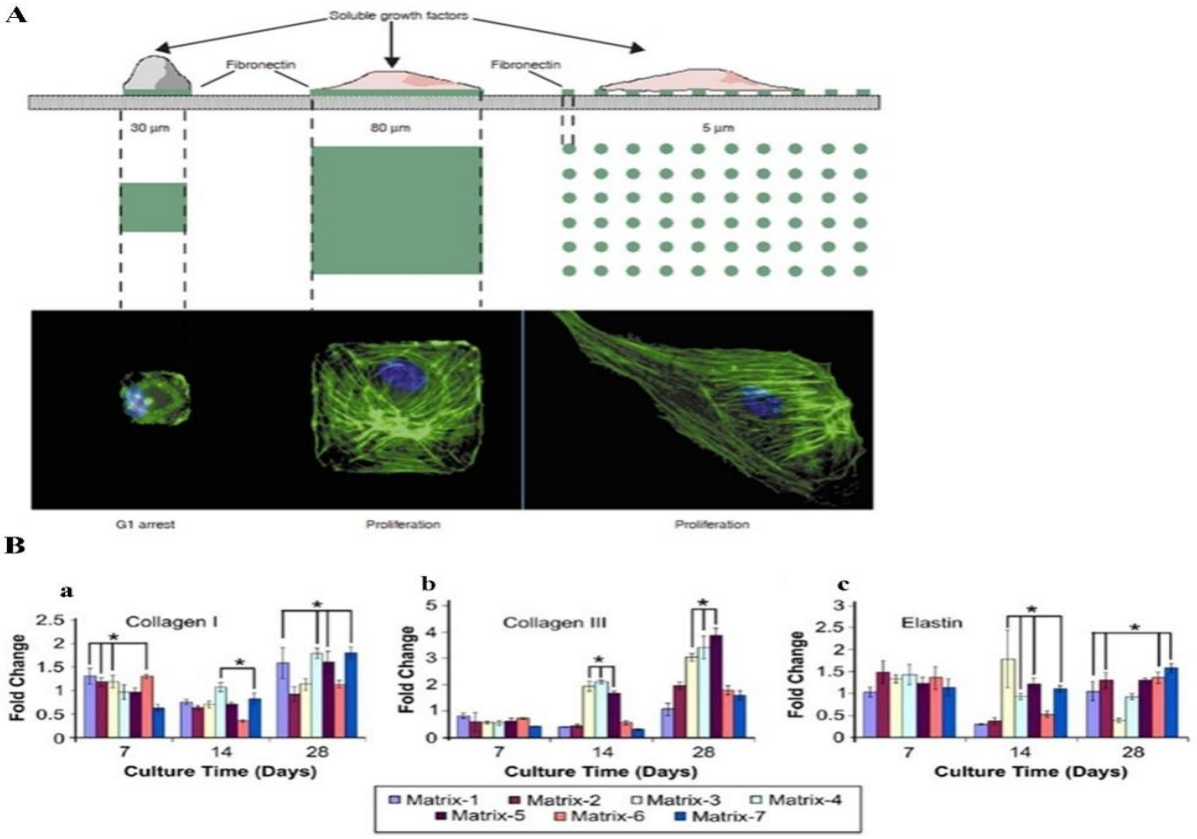

Fig 5. (A) A different pattern of ECM molecules governs cell behavior independently from surface chemistry and density of ECM molecules. The spreading of human endothelial cells was restricted by plating them on small adhesive islands and they were arrested at G1 phase (a). When the cells were cultured on a substrate that provides the enough surface area for cell spreading with or without pattern of small fibronectin-coated adhesive islands, the cells enter S phase in the presence of mitogens highlighting the control of cell shape is carried out independently of the total cell–ECM contact area. The actin microfilaments (green) spread and fully reorganize their cytoskeleton when cultured on many small adhesive dots (b-c), even though the total area of ECM directly bound by the cell is identical (a and c) [21], (B) Real Time PCR determined the fiber diameter dependency of quantitative expressed value of collagen I, collagen III and elastin by cultured human skin fibroblasts on electrospun scaffolds. The matrixes which are named from 1 to 7, respectively had fiber diameter as: 150-225, 200-300, 250-247, 500-900, 600-1200, 2500-3000 and 3250-6000 nm [32].

The micrographing of a surface with typical mean heights of 13 nm, diameters of 263 nm and center to center spacing of 527 nm by AFM, presents the cell extension with proper morphological property [33] (Fig 6A).

Februaryet al described the influence of surface nanotopography on bone healing. They analyzed the response of bone mesenchymal stem cells and osteoblasts on the surface which was made by Ca and P impregnation. ALP as a specific marker of bone differentiation showed significantly higher expression due to induction of nanotopographical properties of surface. The surface chemistry of Ca and P on this nanotextured substrate could be another factor for stronger differentiation [34]. As discussed above, the electrospun nanofibers provide high surface area and porosity for seeded cells which need the appropriate interactions [35].

The nanopatterned surface is able to afford the cell requirement for perfect adhesion and differentiation. By electro-spin technologies, the order and diameter of fibers could be controlled for various polymers [36]. As previously mentioned, cell behaviors including adhesion, proliferation, migration and differentiation rely on the favorable interactions and as well as biocompatibility of scaffolds. On the other hand, scaffolds with aligned directed fibers can be prepared by electrospining process. For myotube differentiation, it is necessary that orientation of cells is in along the same longitudinal axis for the more powerful contraction. Skeletal muscle cells are matured by formation of multinuclear cells as a result of cell fusion and the gene expression of cultured cells on aligned and random scaffold confirm this outcome [35] (Fig 6B).

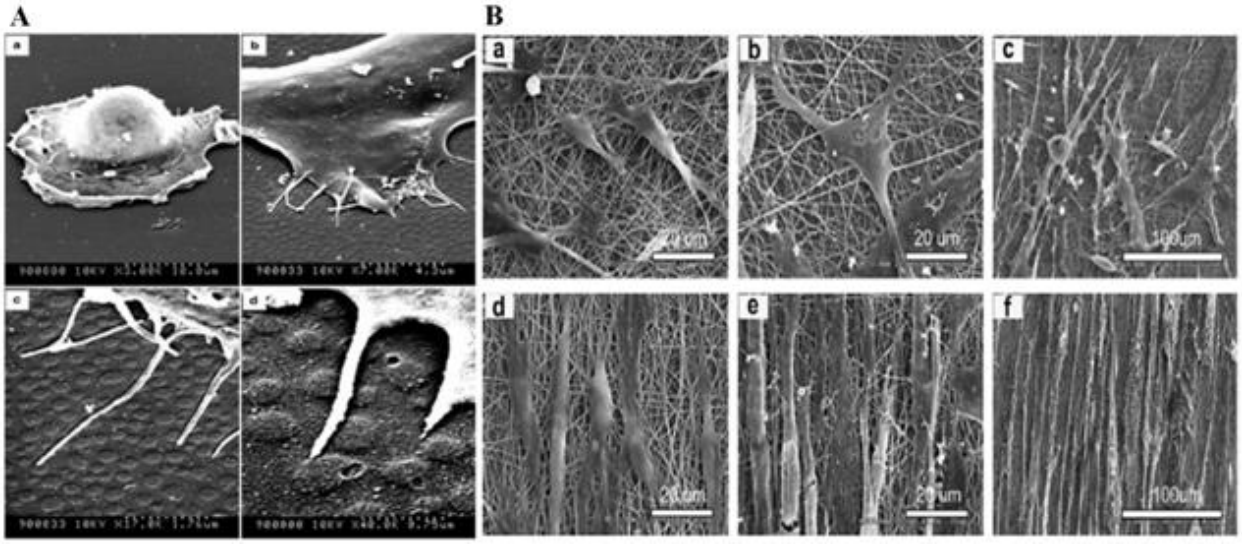

Fig 6. (A) The spreading cells with lamellapodia suggested cell movement on the planner surface (a-b) while cells cultured on the nano-islands were considerably more spread with many filopodia (c-d) [33], (B) The random orientation of fibers induced hSkMCs an irregular cellular orientation (a-c at 1, 3 and 7 days respectively) that was in contrast to the formation of myotubes on uni-directionally structure of fibers (d-f at 1, 3 and 7 days respectively) [35].

In another study by Scopelliti and Bongiorno TiO_x substrate is made by supersonic cluster beam deposition (SCBD) with a surface roughness ranging from 15 nm to 30 nm. They suggested that nanoscale morphology provide more available area as the nucleation site of proteins and increases K_d [37]. In a study by Berry, the microtopography architecture on quartz was used as cavities with pit diameter of 7, 15 and 25 um and pit distance of 20 and 40 for determination of fibroblast behavior. Among the whole 2D curved groups, the pit having the size of 7:20 showed the highest proliferation rate, migration speed and more entering cells. In the following of biomimicry, the structured of 7 um pitted substrate has considered in the mesh size of collagen from 14 to 5 um [38]. Nanotopography regulates hESC behaviors, including cell morphology, adhesion, proliferation, clonal expansion, and self-renewality. In a study Chen et al demonstrated that topological sensing of hESCs might regulate mechanosensory integrin-mediated cell matrix adhesion. The underlying result also showed cellular responses to nanotopography were dependent on cell type [39]. The integrin-activated focal adhesion kinase (FAK) was influenced using surface nanotopography as aligned stress fiber and upregulation of neurogenic and myogenic markers by hMSCs were observed on nanogratings with 250 nm line width on polydimethylsiloxane [40]. A study approved the osteogenic differentiation of MSC ,with a preference towards nanopillars with diameter of 50 nm [41].

Additionally, OCT-1 osteoblast-like cell behavior was analyzed on both pit and islands on PLLA and PS surfaces in comparison with non-islands patterned PLLA. This study showed that the cell stretching on pit or islands-patterned substrate by 2.2 and 0.45 um made the cultured cells have more contact angle and cell height. On the other hand, SEM studies proved that cells extended pseudopods which inserted into the patterned islands with more proliferative potency [42]. A study by Zhang established that unpatterned direction of microchannels influence cell orientation to be disordered, in contrast to the parallel alignment of microchannels due to contact guidance. In a similar way, the microchannels confinement could affect cell behavior and the cells are seeded in narrow microchannels, their orientation is same on patterned and unpatterned surfaces. Taken on, they concluded that threshold value for microchannels width was between 120 and 270 um [43]. The more cell attachment and differentiation on nanoscaled texture could be due to the role of nanoparticles curvature on folding of adsorbed proteins i.e. albumin and fibrinogen [44]. Hence, it could be born in mind that the behavior of some other proteins such as fibronectine, collagen and elastin may alter in contact with nanotopography. Since cell behavior is dependent to the type of topological surfaces, the focal adhesion signaling and cytoskeletal contractibility changes by alternating actin and myosin assembly.

The chemical properties of surface and cell adhesion

As all cell functions are occurred in molecular level between cell surface and substrates, the chemical nature of the surface is introduced as a powerful tool for regulation of cell functions. In this manner, some techniques similar to molecular self-assembly [45-46], plasma surface modification [47-50], laser irradiation [51], photochemical surface modification [52-53] and lithographic techniques [54-55] try to modify the surface chemical properties to afford the requirements of cell orientation. Kristin et al evaluated the self-assembled monolayers of alkyl thiols for modulation

of cell attachment and spreading. The prepared hydrophilic surface with carboxyl-terminated groups provided the contact angle of less than 20° and the presence of hydrophilic functional group support cell attachment by reducing contact angle [56]. Besides, by studying of extended filopodia on the aminated substrate, it was concluded that the stronger adhesion could be resulted by hydrophilic groups [57] (Fig 7A). In this manner, Luca et al treated PCL films with KOH, NaOH (hydrolysis) and hexamethylenediamine (HMD)/ 2-propanol (amniolysis) solutions to increase hydrophilicity of the surface. Schwann cells (SCs) that were harvested from sciatic nerves of the adult Sprague–Dawley rats, seeded on scaffolds. This experiment showed that SCs attachment and proliferation significantly increased on treated PCL films. So chemical treatment of PCL films can improve hydrophilicity and biocompatibility [58]. Also, the substrates are made by the etching of silicon wafers using Ag-nanoparticles and followed by oxidation with SiO_x. The surfaces were modified by amino group (NH2), fluorine (F) and their surface treatment with oxygen plasma and seeded with Chinese hamster ovary (CHO) cells.

By scanning electronic microscopy (SEM), the more extended morphology of cells on amino groups represented tendency to the more hydrophilic chemistry for adhesion. In addition, contact angle decreased with oxidized nanosponge. This study elucidated extended actin filaments across the center of cells on hydrophilic substrate but in contrast aggregation of actin bundles in the peripheral edge [25].

Chieh et al studied the effect of gold surface modified with four different functional groups (–CH3, –NH2, –COOH and –OH) on adipose- derived stromal cells (ADSCs). The cells showed the varying morphology on substrates with the corresponding functional groups (Fig 7B): the flat morphology on the surfaces modified with –OH- or –NH2, filopodia on the –COOH-modified surface, a spindle-like shape and filopodia at the leading edge, and on the –CH3-modified surface with highly hydrophobic characteristics rounded morphology were observed. Since the cells have low adherent site into –CH3-modified surface, the highest migration speed observed on this surface. After 7 days of incubation, metabolic activity of the ADSCs followed the trend: –COOH>–NH2>–OH>–CH3 [59].

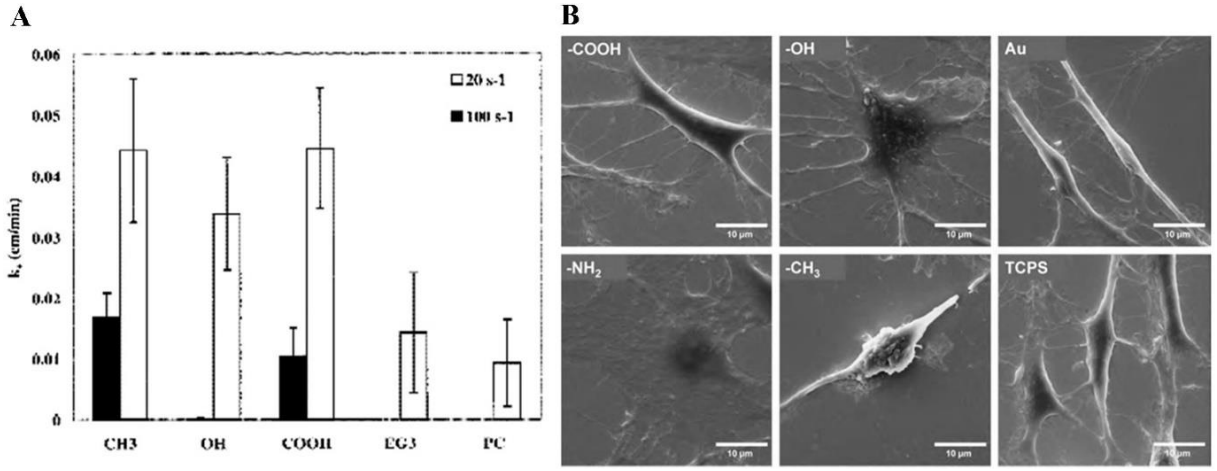

Fig 7. (A) The polymorphonuclear leukocytes (PMNs) exposed the adhesion dependency on the monolayer's terminal functionality. Adhesion was higher on the hydrophobic CH(3) surface and the polar COOH monolayer. Also, attachment was decreased with increasing shear rate, exhibiting a three-fold decrease between 20 and 100 s(-1) [57], (B) The ADSCs exhibited flat morphology and filopodia formation around the cell bodies on the surfaces with immobilized tail group of –OH- or –NH2. The –COOH-modified surface led cells to have a spindle-like shape and filopodia at the leading edge which was comparable with cells on the pristine gold and TCPS controls. In contrast, on the –CH3-modified surface with highly hydrophobic characteristics, cells showed a more rounded morphology with a smaller contact area [59].

The assessment of the aminated PES along with various combinations of the early acting cytokines exposed that the positive charge of amine groups on PES substrate promotes the cell adhesion. In this case, engagement of the surface bound amine groups with CD34 antigens can activate the signaling pathways into proliferative fate [60]. In another study, the surface charge of poly-L-lactide (PL) scaffold was modified to negative and positive regions by cerium oxide nanoparticles (CNPs). The hMSCs and osteoblast-like cells (MG63) were seeded to study the valence state of metal ions on cell function. The related results confirmed that surfaces with positive charge reduced cell adhesion and proliferation. However, the negative regions provided a hydrophobic surface and promoted cell proliferation [61].

In another study, Yu et al modified Au self-assembly monolayers with -SH,-CH3,-COOH and -OH groups and studied the adhesion and proliferation of cancer cells. HEPG2 exhibited different morphology on each surface: On -CH3 surface spherical and on -OH, -SH and -COOH modified surfaces spindle polygonal morphology with better cell adhesion. They reported the different value of surface hydrophobicity after chemical modification with the following order: -OH≈-COOH>-SH≫-CH3 and the surface which was modified with CH3caused HEPG2 cells death [62].

The different number of side chain of CH2 groups was used for increasing the hydrophobicity by Ayala et al. There was no alteration on charge and the value of stiff. The seeded cells on C1 hydrogels had less sticky behavior because of random and rather fast movements. In contrast, cells on C5 hydrogels had slowly moved in a more precise manner. Thus by influence shear-flow, more force was needed for the detachment of cells cultured on C5 hydrogels (for C1 group 4.3±0.8 nN and for C5 was 20.1±3.2 nN). On the other hand, more osteogenic differentiation happened on C5 hydrogels by higher expression of ALP, collagen type 1, calcium deposition and osteocalcin than other type of hydrogels. Their analysis was repeated for myoblasts and the results coordinated with osteogenic type. The parallel alignment of actin within the cytoskeleton was other reason for the optimum number of CH2 groups in C5 hydrogels. If the usage of C5 had been increased, therefore, the rate of adsorbed proteins would be higher. But the higher adsorbed proteins will not result in more cell attachments due to steric inhibition or conformational changes. Thus, for better interaction between cells and surface, the balance of hydrophobicity and hydrophilisity is an emergent requirement [63].

A study demonstrated that by laser irradiation new C=O containing chemical groups on PS surfaces were made. Therefore, the enhanced wet ability could be proved by contact-angle measurements. But under the exposure of laser at 30 and 45°, cells aligned along the direction of chemical groups. Furthermore, the cell orientation are influenced not only by surface hydrophilisity, but also the implied contact angle of surface chemical groups has the most significant impact [64]. Surface charge is a substantial factor to be described that enable to change the cell fate. With higher degree of surface charge density, the modulation of more cell attachment is expected as well

as the adsorption of proteins. thus, if the charge type, positive or negative, are changed by different functional groups, the spreading and differentiation rate of cell will have another specific pattern [65].

Another group modified chitosan layers with graphene oxide (GO) and studied topographic properties, antibacterial activity and the effect of this material on cell growth. GO- chitosan layers in the low concentration of GO improved human MSC proliferation but by increasing the amount concentration of GO, cell growth was inhibited [66].

By chemical treatments, surface endures a severe degradation that result more roughness [57]. Additionally, chemical groups as COOH carboxylic SAM are negatively charged and so hydrophilic for the cell attachment [67]. Thus the modification of surface with OH-terminated SAMs can function as well as the cell adhesion on phosphorylcholine substrate [67].

Conclusion

According to below table (Table 1), most studies have been focused on the relationship between surface properties and cell differentiation.

Table 1: Summery of reports related to surface properties.

Cell type	Surface material	Surface pattern	Cell function	Ref
MSCs	poly(ethylene glycol) (PEG) hydrogel	nanopattern	cell differentiation	[68]
murine osteoblast precursor cells	bioactive hydrogel (HAX-PolyP) scaffold	Immobilized inorganic polyphosphate (PolyP)	up-regulation of osteogenic marker genes	[69]
ESC-derived NSCs (hNSC H9 cells)	heparan sulfate proteoglycans (HSPGs)	protein	neuronal and astrocyte differentiation	[70]
MSCs and human osteoblasts (OB)	nanopillars in SiO2	nanopattern	cell adhesion, proliferation and differentiation.	[41]
neural stem/progenitor	GRGDS modified gellan gum (GG-GRGDS)	Protein+ polysaccharide	adhesion and proliferation	[71]
MSCs	collagen alone (C), aminated collagen (AC) and aminated collagen with GAGs (ACG)	protein	differentiation	[72]
NIH 3T3 fibroblasts	silica nanoneedles	nanopattern	adhesion and maintaining cell viability	[73]
MSCs	.Poly(propylene fumarate)	3D printing	enrichment and differentiation	[74]
MSCs	Osteoblast Extracellular matrix	protein	Osteogenic differentiation	[75]
MSCs	microcarriers	Cultispher-S, Cytodex-3, chitosan	Actin organization and differentiation	[76]
MSCs	PCL nanofibers with BFP-1 peptides	protein	Osteogenic differentiation	[77]
MSCs	collagen–glycosaminoglycan scaffolds	Protein+ polysaccharide	Cell differentiation	[78]
MSCs	poly(epsiloncaprolactone) scaffolds		fibrosis and biomineralization	[79]
MSCs	3D PCL nanofiber scaffold	nanopattern	adhesion, migration, proliferation and Osteogenic differentiation	[80]

Besides, surface of employed substrates are improved by candidate proteins for higher biomimicry. It had been approved that the anchorage of focal adhesions to cytoskeleton, stimulates the signaling pathways up to nuclear components that trigger a particular cell activity including growth, proliferation and apoptosis. It could be concluded that a nanopatterned surface might function as effective as a protein with receptor ligands for cell adhesion. Also, the combined behavior of corresponding proteins and surface orchestrate with nanosized arrays elucidated rather efficiency. It is in consistent with previous studies that well established that cell fate is affected by the combination of surface, cells and growth factors. The cell adaptation into a special environment is followed by changes of morphology, gene expression and cell cycle. Thus the surface modification is applied in terms of scale, physical properties and the kind of material. Bioengineering field is strikingly improving various branches of the bio-mimicking. Numerous observations evidenced the significance of cell spreading and appropriate attachment as well as surface topography. The cell behavior during cell cycle is introduced as contact angle and guidance by the adhesion potency and the extension of filopodia relative to the surface topography. On the whole, the lower contact angle resulted in the stronger compatibility and hydrophilicity of a surface which should be proposed as an ideal surface for cell culturing. The exposed surface for cell attachment is markedly increased by the higher surface area to volume ratio as an outcome of nanotechnology. Similarly, surface modification by the use of protein adsorption and chemical treatment plays the desirable biological mimicry with nanometer scale. Thus, the management of cell behavior has been interested by researchers and experienced the actual improvements recently. The patterned surfaces which are resulted by engineering methods render to specify the accessible area for cell focal adhesion sites. Typically, the controlled interactions between integrin and FAC molecules play a considerable role in folding and special architectural of membrane proteins and finally cell signaling. Although, not only surface properties act obviously on cell cycle, but also the ultimate fate depends on the presence of other unique parameters like growth or differentiating agents and cell healing. It is well known that the cell attachment specifies the cell activity as an exclusive approach of surface modification studies. Surface modification using chemical methods or topographical management compared to non-modified surface improves the intrinsic properties of a surface which yield a convenience of the biocompatible environment. Thus the quality of attractive forces between cell and surface express mainly subsequent biocompatibility and similarity of produced substrate to biologic milieu.

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