Evolution of gradient concept for the application of regenerative medicine

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Abstract

It has been recognized that tissue engineering and regenerative medicine (TERM) offers the next generation technology for whole organ and tissue transplantation for diseased, failed or malfunctioned organs. Biomaterials might be the one of the important factors to apply the complete bio-organ using TERM techniques. Around 30 years ago, the primitive concept of "Gradient Surface" had been introduced to improve the biocompatibility for biomaterials. However, the gradient concept of surface property is changing now numerous kinds of physicochemical properties very recently. In this review, the importance of the concept of gradient, the historical evolution of experimental methodology for the manufacturing of gradient surface during last over ~30 years in my research group and finally perspective as enabling technologies for the TERM area are summarized.

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1. Introduction

It has been recognized that tissue engineering and regenerative medicine (TERM) offers the next generation technology for whole organ and tissue transplantation for diseased, failed or malfunctioned organs [1]. Millions of patients are suffered by end-stage organ failure or tissue loss annually. Recent advances in TERM using stem cell science and technology have shown a great potential for the clinical trial and research of next generations medications. Science and technology of regenerative medicine might change the paradigm of the nature of medicine [1–4].

Very recently, four stem cell products have been launched in Korean market after the approval from Korea Food and Drug Administration including world first autologous bone marrow derived stem cell (BMSC) for the treatment of myocardial infarction on 2012, world first autologous adipose derived stem cell (ADSC) for the treatment of Crohn's disease, world first allogenic umbilical cord derived stem cell (UBMSC) for the treatment of chondyle defect on 2012 and BMSC for the treatment of amyotrophic lateral sclerosis (ALS) on 2014 [4]. Similarly, several tissue engineered products introduced in market as artificial skin using biomaterials scaffold and autologous or allogenic keratinocyte. Also over one–two hundred clinical trial phase I–III with broad range of medical area are in progress through the world. Even though this step might be an infant step for the development of TERM compare with conventional medication treatment, they show good sign for the future for the application of stem cell and TERM therapy [1].

To accomplish a successful treatment or therapy by regenerative medicinal technique, triad components such as (i) cells which are harvested and dissociated from the donor tissue including nerve, liver, pancreas, cartilage and bone as well as embryonic stem, adult stem or precursor cell, (ii) biomaterials as scaffold substrates which cells are attached and cultured resulting in the implantation at the desired site of the functioning tissue and (iii) growth factors (cytokines) which are promoting and/or preventing cell adhesion, proliferation, migration and differentiation by up-regulating or down-regulating the synthesis of protein, growth factors and receptors must be needed [5,6].

Among these triad components, biomaterials might be the one of the important factors to apply the complete bio-organ using TERM techniques. It has been widely recognized that the behavior of the adhesion and proliferation of anchorage-dependent cells and tissue on scaffold biomaterials depend on the surface characteristics such as wettability (hydrophilicity/hydrophobicity or surface free energy), chemistry, charge, roughness, and rigidity. In order to improve the biocompatibility, we have been carried out the surface modification including the fabrication of gradient surface [7–37].

In this review, the importance of the concept of gradient, the historical evolution of experimental methodology for the manufacturing of gradient surface during last over ∼30 years in my research group and finally perspective as enabling technologies for the TERM area are summarized. Readers can find many review and feature articles reported elsewhere.

2. The important of the concept of gradient

The meaning of gradient includes some properties changed gradually with physical, chemical, concentrational and so on of one, two or three dimension. One of the most significant examples very recently is that the embryonic development follows chemical gradient as vitamin E and/or pH concentration [9]. Lander calls this kind of gradient for developmental biology it as "Morphogen Gradient" [38]. Also whole our human body keeps gradients in cellular–extracellular architecture to satisfy spatially diverse functional needs.

Fig. 1 shows the schematic diagram of the phagocytosis action of white blood cell toward foreign body. Once some inflammation has been occurred at the injury site, the cytokine related with inflammation is produced. Patrolled neutrophils start to locomote at speeds of 200–300 μm/h along the gradient of cytokine concentration (chemogradient) toward source of infection through the extracellular matrix. Sometimes it called
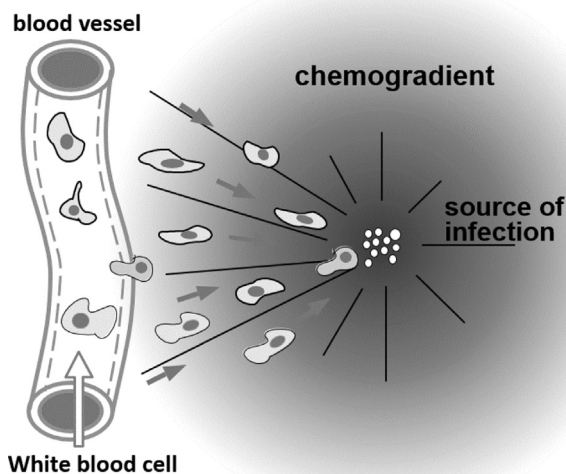

Fig. 1. Schematic diagram of the phagocytosis action of white blood cell toward foreign body.

"taxis" which means the cause cell migration toward various physical and chemical stimuli. Singh et al. [9] largely divided by (1) chemical stimulus includes chemotaxis and heptotaxis and (2) physical stimulus includes durotaxis/mechnotaxis, gavanotaxis, phototaxis, geotaxis and tensotaxis. The harmonization of controlled gradient physicochemical properties might be improved the cellular power as the tool of the tissue engineered and regenerative medicinal products.

Meanwhile, we could find firstly the concept of "Gradient" for the surface treatment around 30 years ago in the respect with the application of biomaterials. We had to need the surface modification of biomaterials to improve the biocompatibility, especially, cell-compatibility. One important problem in studies using different kinds of polymer surfaces is that the surfaces are heterogeneous both chemically and physically (different surface chemistry, charge, roughness, rigidity, crystallinity, etc.), which may result in considerable variation in experimental results [12]. Another methodological problem is that the evaluation of the behavior of biological species on a polymer surface is often tedious because a large number of samples must be prepared to cover the range of a desired variable, such as wettability, with the experiment must be carried out separately for each sample. Thus, there is the strong possibility of methodological error [15].

Many recent studies have been focused on the preparation of surfaces with a continuously varying chemical composition along one dimension. In such so-called "gradient surfaces", the gradually varying chemical composition on the surface produces gradients in wettability, thickness, dielectric constant, or other physicochemical properties. Substrates thus formed find uses in applications, including selective adsorption, gradient templating, controlled motion of liquid droplets, formation of patterns as templates for further processing (e.g., surface-initiated polymerization) and particle sorting, among other things [7]. In addition to their broad range of applications, such gradient surfaces are of particular interest for basic studies of the interactions between biological species and surfaces since the effect of a selected property, such as the wettability, can be examined in a single experiment on one surface. Some groups have reported the preparation of wettability gradient surfaces and their uses in studying interaction phenomena of biological species, including proteins, cells, and enzymes [18,19].

Nowadays, these gradient concepts have been expanded and broaden from surface property to so many physicochemical properties areas such as (1) directionality (orthogonal, radial and directional), (2) length scale (continuous and discrete), (3) types (chemical and physical), (4) functionality, (5) time dependency (static and dynamic) and (6) dimensionality (1, 2, and 3D) [8].

3. The historical evolution of manufacturing of gradient surface

3.1. Gradient surface by corona discharge treatment

Around the middle of 1980s, very few researchers had been started to introduce the concept of "Gradient Surface" as a tool to modify the surface of biomaterials to improve the

biocompatibility, that is, the change of wettability chemistry, charge, roughness, rigidity and so on. At firstly, they had been started to reduce the number of samples and the methodological error. Around 1985, Lee and Andrade [15] had been tried to design the prototype of gradient surface using continuous immersion of NaOH solution of poly(ethylene terephthalate) (PET). At almost same time, Elwing et al. [14] had been demonstrated a wettability gradient method by adsorption techniques used chlorosilane solution. In 1989, Pitt [13] had been tried to make a gradient surface using plasma treatment and then Lee et al. [18] had been successfully tested the possibility to fabricate gradient surface by corona discharge treatment with 5×5 cm^2 size on the low density polyethylene (LDPE) surface mainly. Corona discharge method revealed essentially the same effectiveness as the low vacuum plasma method, but it is more convenient and simple to apply under atmospheric conditions.

Fig. 2 shows the schematic diagram of preparing wettability gradients on biomaterial surfaces using a corona discharge treatment with radio frequency (RF) [18]. This RF corona discharge apparatus consists of (i) a knife-type electrode on the sample moving continuously increasing power and (ii) the sample bed is translated relative to the electrode. Thus, the surface is oxidized gradually along the sample length, resulting in a wettability gradient with selected functional groups as shown in Fig. 3. We divided by three regions as (i) hydrophobic region of ~90° of water contact angle as 0.5 cm, (ii) moderate hydrophilic region of 50–55° of water contact angle as 2.5 cm, and (iii) hydrophilic region of ~30° of water contact angle as 4.5 cm. Figs. 4 and 5 shows the surface analysis by FTIR–ATR and ESCA, respectively [18]. We could postulate the oxidation process depicted as shown Fig. 6 [18–20].

Corona and plasma discharge treatment on the LDPE surface may produce carbon radicals from a hydrocarbon backbone, followed by the formation of unstable hydroperoxides through rapid binding with oxygen in the reactor chamber as shown in Fig. 7 [21]. The unstable hydroperoxides are easily decomposed to produce various oxygen-based polar functionalities (hydroxyl group, ester, ketone, aldehyde, carboxylic acid, carboxylic ester, etc.) by reaction with additional oxygen in the reactor chamber or air. It seems that the oxygen-based functional groups produced on the polymer surface increase with increasing corona and plasma exposure time, resulting in the formation of a wettability gradient on the surface [16–22].
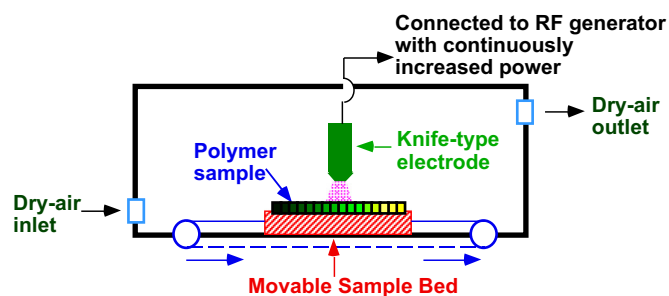

Fig. 2. Schematic diagram showing corona discharge treatment apparatus for the the preparation of wettability gradient surfaces.
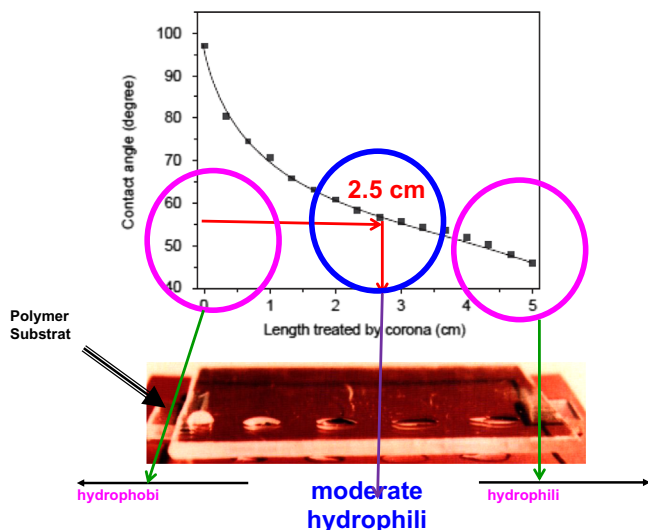
Fig. 3. Water contact angle of gradient LDPE surface. We divided by three regions as (a) hydrophilic region as 5 cm, 30°, (b) moderate hydrophilic region as 2.5 cm, 55° and (c) hydrophobic region as 0.5 cm, 90°.
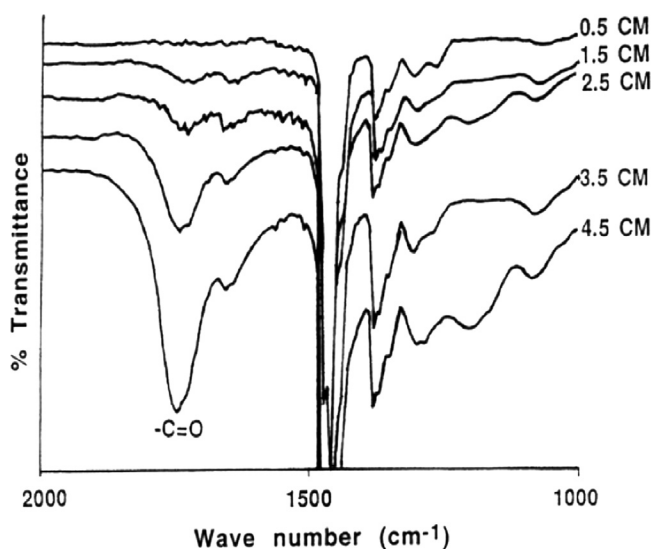
Fig. 4. FTIR–ATR spectra of a corona-treated LDPE surface with wettability gradient.

Fig. 5. ESCA carbon 1S core level spectra of a corona-treated PE surface with wettability gradient.
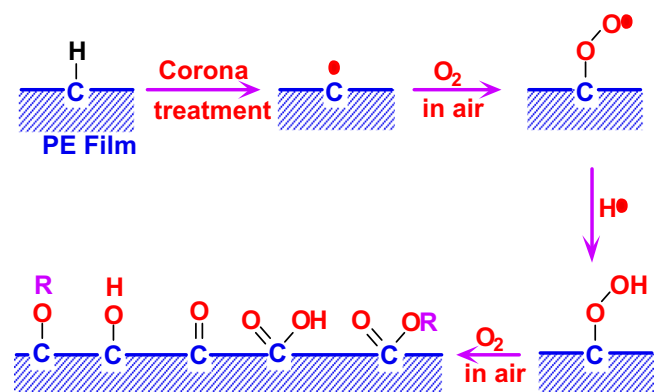
Fig. 6. Possible mechanism for the formation of oxygen-based functionalities on LDPE surface by corona discharge treatment.

We have more analyzed the chemical composition of the wettability gradient using atomic forced microscopy (AFM) [23], time-of-flight secondary ion mass spectroscopy (TOF-SIMS) [24,26] and so on.

3.2. Cell and blood compatibility on gradient surface

The wettability gradient surfaces prepared were used to investigate the adhesion behavior of platelets in the absence and presence of plasma proteins in terms of the surface hydrophilicity/hydrophobicity of polymeric materials [35]. The platelets adhered to the wettability gradient surfaces along the sample length were counted and examined by scanning electron microscopy (SEM). It was observed that the platelet adhesion in the absence of plasma proteins increased gradually

as the surface wettability increased along the sample length. The platelets adhered to the hydrophilic positions of the gradient surface also were more activated than on the more hydrophobic ones. However, platelet adhesion in the presence of plasma proteins decreased gradually with the increasing surface wettability; the platelets adhered to the surface also were more activated on the hydrophobic positions of the gradient surface. This result is closely related to plasma protein adsorption on the surface. Plasma protein adsorption on the wettability gradient surface increased with the increasing
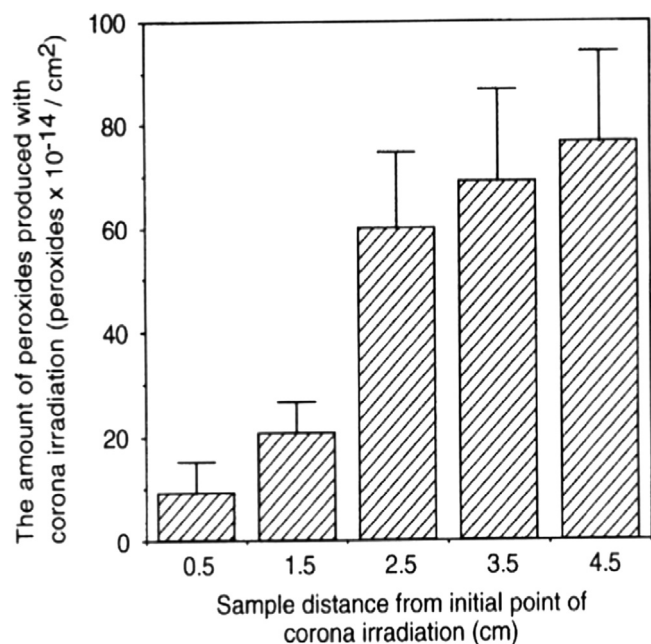
Fig. 7. The amount of peroxide produced by the corona treatment with wettability gradient.

surface wettability. More plasma protein adsorption on the hydrophilic positions of the gradient surface caused less platelet adhesion, probably due to platelet adhesion inhibiting proteins, such as high-molecular-weight kininogen, which preferably adsorbs onto the surface by the so-called Vroman effect. It seems that both the presence of plasma proteins and surface wettability play important roles for platelet adhesion and activation.

The wettability gradient surfaces were used to investigate the interaction of different types of cells (Chinese hamster ovary, fibroblast, and endothelial cells) as well as serum proteins in terms of the surface hydrophilicity/hydrophobicity of LDPE and poly(lactide-co-glycolide) (PLGA) films as shown in Fig. 8 [29,30]. The cells adhered and grown on the gradient surface along the sample length were counted and observed by SEM. It was observed that the cells were adhered, spread, and grown more onto the positions with moderate hydrophilicity of the wettability gradient surface than onto the more hydrophobic or hydrophilic positions. The maximum adhesion and growth of the cells appeared at around water contact angles of 55°, regardless of the cell types used. This result seems closely related to the serum protein adsorption on the surfaces; the serum proteins were also adsorbed more onto the positions with moderate hydrophilicity of the wettability gradient surface as shown in Fig. 9.

The adhesive strength of endothelial cells (ECs) attached on polymer surfaces with different hydrophilicity was investigated using wettability gradient LDPE surfaces [31]. The EC attached wettability gradient surfaces were mounted on parallel plate flow chambers in a flow system prepared for cell adhesiveness test. Three different shear stresses (150, 200, and 250 dyne/cm^2) were applied to the flow chambers and each shear stress was maintained for 120 min to investigate the
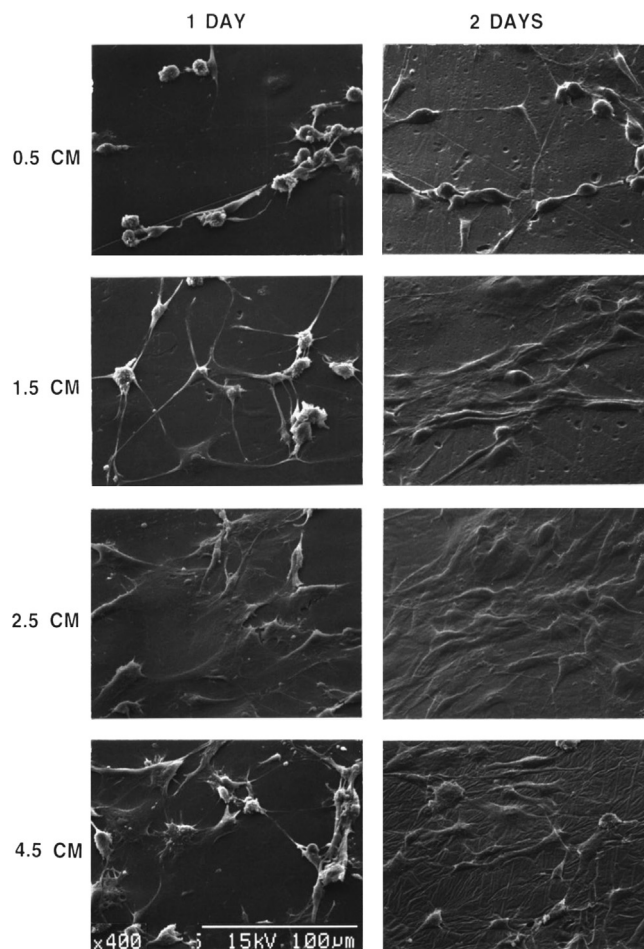

Fig. 8. SEM microphotograph of fibroblast cells attached onto PLGA surfaces with wettability chemogradient 1 and 2 days culture (original magnification; × 400).
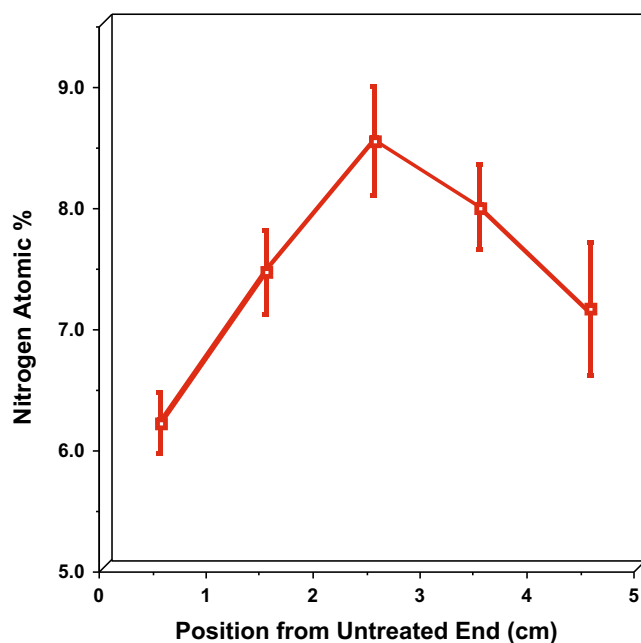

Fig. 9. Fetal bovine serum protein adsorption on the PLGA surfaces with wettability chemogradient.

effect of shear stress and surface hydrophilicity on the EC adhesion strength. It was observed that the ECs were adhered more onto the positions with moderate hydrophilicity of the wettability gradient surface than onto the more hydrophobic or hydrophilic positions. The maximum adhesion of the cells appeared at around water contact angles of 55°. The EC adhesion strength was higher on the hydrophilic positions than on the hydrophobic ones. However, the maximum adhesion strength of the cells also appeared at around water contact angles of 55°. More than 90% of the adhered cells remained on that position after applying the shear stress, 250 dyne/cm^2 for 2 h, whereas the cells were completely detached on the hydrophobic position (water contact angle, about 86°) within 10 min after applying the same shear stress. It seems that surface hydrophilicity plays a very important role for cell adhesion strength.

Induction and growth of neurites from the rat pheochromo-cytoma (PC-12) cells attached on the wettability gradient LDPE surfaces polymer surfaces were investigated [36]. Neurites were investigated for number and length of neurites in terms of surface wettability. It was observed that neurite formation of PC-12 cells was increased more onto the positions with moderate hydrophilicity of the wettability gradient surface than onto the more hydrophobic or hydro-philic positions. From those results, it could be assumed that initial adhesion of PC-12 cells was caused by more calf serum protein than nerve growth factor (NGF), whereas the neurite formation of PC-12 cells was caused by more NGF than CS protein. It follows from what has been said thus far that PC-12 cells are a differentiated neuronal phenotype with a long neurite at around the position 2.5 cm (water contact angle of about 55°). We have demonstrated that surface wettability plays an important role for neurite formation on the polymer surfaces for axon regeneration.

To determine the rate of proliferation on polymer surfaces with different wettability, the behavior of cell growth for NIH/3T3 fibroblast cells attached on the polymer surfaces with different hydrophilicity was investigated using wettability gra-dient LDPE as shown in Fig. 10 [34]. They were investigated for the number of grown cells from 24 to 60 h in terms of surface wettability. From the slope of cell number on PE gradient surface versus culture time, the proliferation rates (number of cell/cm^2 h) were calculated. It was observed that the proliferation rate was increased more on positions with moderate hydrophilicity of the wettability gradient surface than on the more hydrophobic or hydrophilic positions, i.e., 1111 (number of cell/cm^2 h) of 55° of water contact angle at the 2.5 cm position ($P < 0.05$). We confirmed that surface wett-ability plays an important role in cell adhesion, spreading, and proliferation on the biomaterial surfaces.

3.3. Functional group modified gradient surface

We have synthesized ω-methacryloyloxyalkyl phosphorylcho-line (MAPC) polymers as new blood-compatible materials, with attention to the surface structure of the biomembrane and investigated their blood compatibility [21,22]. The blood
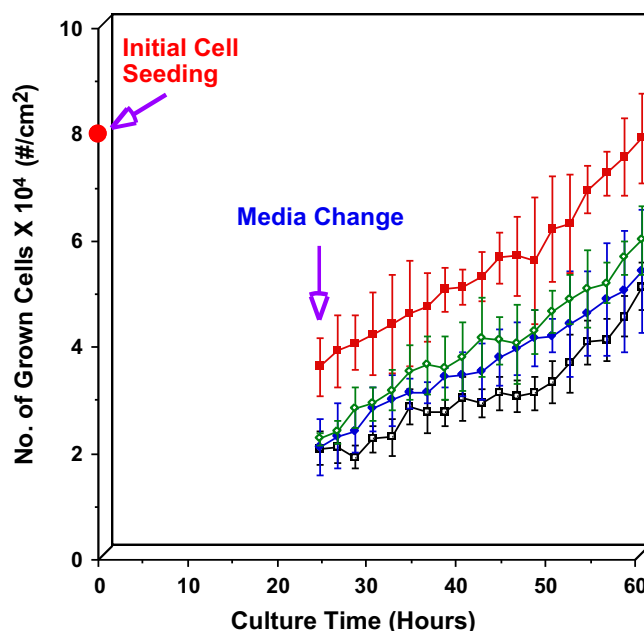
Fig. 10. Cell proliferation (migration) rate with different wettability on LDPE surface.

compatibility observed on the MAPC polymers is due to their strong affinity to phospholipids. When the blood comes in contact with the MAPC polymer, phospholipids in the plasma preferentially adsorb on the surface, compared with the plasma proteins or cells. The adsorbed phospholipids construct a biomembrane-like structure on the MAPC polymer surface. The MAPC polymers then have an excellent blood compatibility. We prepared a gradient poly(MAPC)-grafted polyethylene (PE) surface using a corona discharge treatment method to clarify the effect of the chemical structure of the MAPC unit on the blood compatibility of the MAPC polymers. The surface composition of MAPC and the hydrophilicity on the poly(MAPC)-grafted PE surface were determined by ESCA analysis and contact angle measurement with water, respectively. The phosphorus/carbon (P/C) ratio determined by the XPS analysis increased, but the water contact angle decreased with increasing corona irradiation energy. These results indicated that the surface density of the MAPC unit was increased. More than 2.5 cm from the starting point of the corona irradiation, the P/C ratio and water contact angle of the surface achieved a constant level. Thus, the surface was completely covered with the grafted poly(MAPC) chain. The effect of the methylene chain length of the MAPC unit on surface properties was also observed. The phospholipid polar group of the MAPC unit was effectively exposed on the surface as the chain length became longer. Moreover, the hydrophobicity of the surface was increased with the increase in the methylene chain length of the MAPC unit. The number of platelets adhering to the poly(MAPC)-grafted PE surface was reduced from the same point where the P/C ratio became constant.

We prepared comb-like PEO gradient surfaces by graft copolymerization of PEO-monomethacrylate macromers (PEO-MA) on PE surfaces. [19] The peroxides produced on the corona-treated PE surface act as initiators for graft

copolymerization. Macromers with 1, 5, and 10 PEO repeat units were used. The comb-like PEO gradient surfaces thus produced were characterized by water contact angles, FTIR–ATR and ESCA. All these measurements indicated that the PEO chains are grafted on the PE surface with gradually increasing density along the sample length by the corona discharge treatment with gradually increasing power along the gradient. The amount of grafted PEO-MA increased as the PEO chain length in the PEO-MA decreased owing to the steric interference of longer PEO side chains in the PEO-MA macromers.

Gradient surfaces with ionizable functional groups (carboxylic acid and sulfonate groups and amine groups) by graft copolymerization of acrylic acid (AA), sodium p-styrene sulfonate (NaSS), and N,N′-dimethyl aminopropyl acrylamide (DMAPAA) on a LDPE surface after corona discharge treatment have been prepared [29–33]. The peroxides produced on the corona-treated PE surface act as initiators for the graft copolymerization. The resulting gradient surfaces with ionic groups were characterized by measurement of water contact angles, ESCA, and FTIR–ATR. All these measurements indicated that the ionic groups were grafted on the PE surfaces with gradually increasing density along the gradient. In addition, we prepared an amide-group-grafted (PE-CONH$_2$) surface by reaction with amine after –COCl substitution of the –COOH group already grafted on the PE surface. PE-COOH and PENaSS are negatively charged and PE-CONH$_2$, PECH2NH2 and PE-DMAPAA are positively charged in phosphate-buffered saline or plasma at pH 7.3–7.4. However, the wettability of the surfaces grafted with ionic groups is not sensitive to the type of functional group grafted.

Also we carried out chemical modification of surfaces using PEI with different molecular weights [26]. A biotinylated gradient PE surface was prepared by the reaction of PEI and biotin. And then, a gradient biotinylated PE surface in a solution of avidin-coated quantum dots (QD)s to give a QDs-bound gradient PE surface have been prepared and analyzed [26,27].

4. Gradient concept expands from surface to physicochemical properties

Based on the concept of "Gradient Surface" for the application of biomaterials, the tool of gradient has been started to spread to another various physicochemical properties to whole research areas from late 1990s–early 2000s. In the following sections, a novel concept of gradients has been summarized in the advent of new surface treatment methods and analyzing methods. For the novel chemically and physically treatment methods are reported on the several review papers.

4.1. Micropattern gradient

Lui et al. [39] have been demonstrated a gradient micropattern immobilization technique using a photomask (a kind of lithographical methods) to investigate by microscopic observation the effect of the surface concentration of an immobilized

thermo-responsive polymer. Poly(N-isopropylacrylamide-co-acrylic acid) was chosen as the thermo-responsive polymer, and was conjugated with 4-azidoaniline to form a photo-reactive thermo-responsive polymer (PIA-Az). The PIA-Az was coated onto a polystyrene plate, and immobilized using UV irradiation in the presence of a gradient micropattern photomask. The behaviors of cell attachment have been investigated on the gradient micropatterned thermo-responsive polymer.

Julthongpiput et al. [40] have been presented a novel fabrication method for a new type of micropattern surface that exhibits a gradient in chemical contrast between the pattern domains. Design elements in the specimen allow chemical contrast in the micropattern to be related to well-established surface characterization data. These gradient specimens represent a reference tool for calibrating image contrast in chemically sensitive scanning probe microscopy techniques and a platform for the high-throughput analysis of polymer thin film behavior.

Choi et al. [41] have been demonstrated the micropattern array with gradient size (µPAGS) plastic surfaces fabricated by PDMS mold-based hot embossing technique for investigation of cell-surface interaction. The behavior of cell responses with adipose-derived stem cells (ADSC) have been carried out on the replicated PS surface with the height of 1.4 µm to investigate in response to the gradient micropattern array with gradient size. Cellular experiment results showed that the micropillar-arrayed surface improved cell proliferation as compared with the gradient microwell-arrayed surface. They could also estimate the ranges of pattern sizes having the desired effects on the cellular behaviors as a tool for gradient patterns.

Cantini et al. [42] have been proposed a novel methodology to obtain a series of biomimetic substrates with a hierarchical rough topography at the micro and nanoscale that span the entire range of wettability, from the superhydrophobic to the superhydrophilic regime, through an Ar–plasma treatment at increasing durations. Moreover, they have been employed the same approach to produce a superhydrophobic-to-superhydrophilic surface gradient along centimeter-length scale distances within the same sample. On a gradient surface cells are in fact exposed to a range of continuously changing stimuli that foster cell migration and detain the differentiation process.

Kim et al. [43] have been developed a simple and biocompatible method of patterning proteins on a wettability gradient surface by thermo-transfer printing. The wettability gradient is produced on a PDMS-modified glass substrate through the temperature gradient during thermo-transfer printing. The hydrophilicity on the PDMS-modified surface is revealed to gradually increase along the direction of the temperature gradient from a low to a high temperature region. Based on the wettability gradient, the gradual change in the adsorption and immobilization of proteins is achieved in a microfluidic cell with the PDMS-modified surface.

4.2. Heterogeneous gradient surface using homopolymers and diblock copolymers

Tsai et al. [44] have demonstrated the gradient heterogeneous surface topographies were prepared using thin films of mixtures of

homopolymers and diblock copolymers to vary the lateral size scale of heterogeneities from the microscopic to nanoscopic. Dewetting, phase separation, and cell adhesion have been demonstrated the utility of these surfaces having gradient heterogeneous topographies. By tuning the lateral size scale of the heterogeneities, surface patterns can be engineered to meet a specific function. It has been confirmed Gradient surfaces offer a straightforward method to optimize various length scales of heterogeneity.

4.3. Tethered PEO surface gradient

Mougin et al. [45] have demonstrated a gradient in the coverage of a surface-immobilized PEO layer is constructed to interrogate cell adhesion on a solid surface. Variation of surface coverage is achieved by controlled transport of a reactive PEG precursor from a point source through a hydrated gel. Immobilization of PEG is achieved by covalent attachment of the PEG molecule via direct coupling chemistry to a cystamine self-assembled monolayer on gold. This represents a simple method for creating spatial gradients in surface chemistry that does not require special instrumentation or microfabrication procedures. The kinetics of cell adhesion are quantified as a function of the thickness of the PEG layer.

Morgenthaler et al. [46] have demonstrated PLL-g-PEG coverage gradients were prepared during an initial, controlled immersion and characterized with variable angle spectroscopic ellipsometry and ESCA. Gradients with a linear change in thickness and coverage were generated by the use of an immersion program based on an exponential function. Gradients were combined with a patterning technique to generate individually addressable spots on a gradient surface.

Menzies et al. [47] have been report a one-step method for the fabrication of PEG-like chemical gradients as the gradient films toward primary amine groups in a graft copolymer of poly(L-lysine) and PEG (PLL-g-PEG copolymer). The gradient coating technique developed allows for the efficient and high-throughput study of biomaterial gradient coating interactions.

Pei et al. [48] have demonstrated gradients with a linear change in coverage of the polycationic polymer PLL-g-PEG were prepared on titanium dioxide surfaces by a controlled dipping process. The use of surface-gradient samples demonstrated the importance for protein adsorption of PEG conformation, the amount of exposed titanium dioxide surface area (and its distribution), and the structure and chemistry of the proteins involved.

4.4. A topography/chemical composition gradient

Zhang et al. [49] have prepared of a topography/chemical composition gradient PS surface, i.e., an orthogonal gradient surface, to investigate the relationship between surface wettability and surface structure and chemical composition. The prepared surface shows a one-dimensional gradient in wettability in the x, y, and diagonal directions, including hydrophobic to hydrophilic, superhydrophobic to hydrophobic, superhydrophobic to superhydrophilic gradients, and so forth.

These one-dimensional gradients have different gradient values, gradient range, and contact angle hysteresis, which lie on both the surface roughness and the surface compositions.

Gradients in surface nanotopography were prepared to study platelet adhesion and activation by adsorbing gold nanoparticles on smooth gold substrates using diffusion technique [50]. These results demonstrate that parameters such as ratio between size and inter-particle distance can be more relevant for cell response than wettability on nanostructured surfaces as well as gradient method might be the powerful method to study in biomedical area.

4.5. Stimuli-responsive polymer gradient

A poly(N-isopropylacrylamide) (PNIPAAm) gradient covalently anchored on a silicon substrate with a linear variation of thickness was fabricated by Li et al. [51] The work thus develops a method to fabricate the stable gradient surface with better quality control, and clarifies in a facile manner the appropriate thickness of the PNIPAAm brushes in terms of cell adhesion and detachment.

Ionov et al. [52] have demonstrated mixed polyelectrolyte brushes with a composition gradient were used as a platform for fabrication of stimuli-responsive command surfaces to control the generation of concentration gradients of adsorbed protein molecules. Protein adsorption and the direction of the adsorption gradient were tuned and also turned off and on or reversed by tuning the proton concentration in the pH range 4.0–8.6.

A PNIPAAm-b-poly(acrylic acid)-g-RGD (PNIPAAm-b-PAA-g-RGD) gradient surface was prepared by Li et al. [53]. In vitro culture of HepG2 cells showed that immobilization of the RGD peptide could accelerate cell attachment, while the thermoresponsive layer beneath could effectively release the cells by simply lowering temperature. Thus, the PNIPAAm-b-PAA-g-RGD gradient surface, combining the thermal response with cell affinity properties, can well regulate the cell adhesion and detachment, which may thus be useful for investigation of cell–substrate interactions with a smaller number of samples.

Tai et al. [54] have been attempted a pH-control of the protein resistance of thin hydrogel gradient films. The hydrogel gradients are composed of cationic poly(2-aminoethyl methacrylate hydrochloride) (PAEMA), and anionic poly(2-carboxyethyl acrylate) (PCEA) layers, which are fabricated by self-initiated photografting and photopolymerization. The pH-controlled protein adsorption and desorption was monitored in real-time by imaging surface plasmon resonance, while the corresponding redistribution of surface charge was confirmed by direct force measurements.

Won et al. [55] have been demonstrated a cell surface engineering to enhance mesenchymal stem cell migration toward an SDF-1 gradient. They have confirmed the improved migration of MSCs toward an SDF-1 gradient.

4.6. Microscale control gradient

A collagen gradient was constructed to interrogate cell adhesion on a poly(L-lactide) (PLLA) membrane surface [56]. Immobilization of collagen onto the gradient surfaces was performed by glutaraldehyde (GA) coupling to form the collagen gradients. The attachment and spreading behaviors of the chondrocytes were dependent on the surface collagen density. These results indicate a surface on which the variation of collagen gradient strongly modifies the biological response of chondrocytes.

Competitive adsorption of three human plasma proteins: albumin (HSA), fibrinogen (Fgn), and immunoglobulin G (IgG) from their ternary solution mixtures onto a sulfhydryl-to-sulfonate gradient surface was investigated by Ding et al. [57]. By fitting the experimental data to a simple model of competitive protein adsorption, the affinity of each protein to the surface at the gradient center position was ranked as: Fgn > HSA≫IgG. Competitive exchange of adsorbed proteins was related to the magnitude of desorption rate constants. Such competitive adsorption of the three major human plasma proteins illustrates the complex dynamics of blood proteins–biomaterials interactions.

4.7. Superhydrophobic gradient surfaces

Hollow microspheres of poly(ethyl α-cyanoacrylate) were prepared via vapor phase polymerization using micro-water-droplets as template and initiator [58]. Surfaces consisting of gradual decrease of roughness along the length direction were obtained, which presented gradient wetting property varied from superhydrophobic to hydrophobic. The superhydrophobic or gradient wetting surfaces could be a potential applications in biomedical field because of the biocompatibility of poly(ethyl α-cyanoacrylate).

4.8. Immobilized concentration gradients

Lagunas et al. [59] have been attempted to design a universal chemical gradient platforms using poly(methyl methacrylate) based on the biotin–streptavidin interaction for biological applications. Preliminary cell adhesion tests were also carried out by seeding NIH/3T3 fibroblast cell and mesencephalic cells on glass slides printed with concentration profiles of collagen and polylysine, respectively.

Oh et al. [60] have been demonstrated the growth factor gradients in three dimensional porous matrix by centrifugation and surface immobilization. Polycaprolactone (PCL)/Pluronic F127 cylindrical scaffolds with gradually increasing growth factor concentrations were fabricated by the centrifugation of fibril-like PCLs and the subsequent fibril surface immobilization of growth factors. The 3D porous scaffold with a concentration gradient of growth factors may become a useful tool for basic studies, including *in vitro* investigations of 3D chemotaxis/haptotaxis for the control of specific biological process. It may also be applied as a tissue engineering scaffolding system for a variety of tissues/organs requiring the spatial regulation of growth factors for effective regeneration.

Vozzi et al. [61] have been designed a novel method to produce immobilized biomolecular concentration gradients to study cell activities. A concentration gradient was thus obtained using a simple silane-based chemical reaction. To validate the method, image analysis was performed on glass slides printed with FITC–collagen and FITC–polylysine concentration gradients. Cell adhesion tests were also carried out by seeding NIH/3T3 fibroblast cells and mesencephalic cells on glass slides printed with concentration profiles of collagen and polylysine, respectively.

Leeet al. [62] have been demonstrated a simple method for the generation of multicomponent gradient surfaces on self-assembled monolayers (SAMs) on gold in a precise and predictable manner. Cell adhesion was investigated on RGD/PHSRN peptide/peptide gradient surfaces. Peptide PHSRN was found to synergistically enhance cell adhesion at the position where these two ligands are presented in equal amounts, while these peptide ligands were competitively involved in cell adhesion at other positions. This gradient strategy may be further expandable to develop of functional gradient surfaces of various molecules and materials, such as DNA, proteins, growth factors, and nanoparticles, and could therefore be useful in many fields of research and practical applications.

Lui et al. [63] have been demonstrated the immobilization of heparin/polylysine nanoparticles on dopamine-coated surface to create a heparin density gradient for selective direction of platelet and vascular cells behavior. Results suggested that gradient surface provides a potential tool to construct a suitable platform on a stent surface for selective direction of vascular cell behavior with low side effects.

4.9. Gradient doping

Ishiguro et al. [64] have been reported a novel electrochemical doping method for conducting polymer films based on bipolar electrochemistry. The electrochemical doping of conducting polymers such as poly(3-methylthiophene) (PMT), poly(3,4-ethylene-dioxythiophene) (PEDOT), and poly(aniline) (PANI) on a bipolar electrode having a potential gradient on its surface successfully created gradually doped materials. It can be expected a possibility of the application of the growth of neuron.

4.10. Control of stem cell fate by gradient tools

Harding et al. [65] have shown that plasma polymer gradients can reveal the subtle influence of surface chemistry on embryonic stem cell behavior and probe the mechanisms by which this occurs. A strong correlation between surface chemistry and cell attachment, colony size and retention of stem cell markers has been observed. Cell adhesion and colony formation showed striking differences on gradients with different plasma polymer deposition times.

Lagunas et al. [66] have been studied the correlation between cell adhesion and cell-adhesive on Arg-Gly-Asp (RGD) gradient

surfaces created on PMMA substrates by continuous hydrolysis and were then grafted with biotin–PEG–RGD molecules. The authors observed by AFM nonlinear dependence of cell adhesion on RGD gradient surfaces with different surface densities.

Zhuet al. [67] have been tried to control over the gradient differentiation of rat BMSCs on a PCL membrane with surface-immobilized alendronate (Aln) gradient. On the Aln-grafted gradient surface, the BMSCs showed gradient osteogenic differentiation as a function of membrane position in terms of cell morphology, alkaline phosphatase activity, calcium deposition, and the expression of osteogenesis marker proteins including collagen type I (COL I), Runt-related transcription factor 2 (Runx2), and osteocalcin (OCN).

Faia-Torres et al. [68] have been demonstrated a differential regulation of osteogenic differentiation of stem cells on surface roughness gradients. Surface roughness gradients of average roughness (Ra) have been fabricated with varying from the sub-micron to the micrometer range ($\sim$0.5–4.7 μm), and mean distance between peaks (RSm) gradually varying from $\sim$14 μm to 33 μm. The stem cell modulation by specific PCL roughness gradient surfaces may be the potential for creating effective solutions for orthopedic applications featuring a clinically relevant biodegradable material.

Ahn et al. [69] have been demonstrated that the proliferation of hADSCs on hydrophilic and rough gradient surfaces by RFGD corona discharge treatment was also higher than that on hydrophobic and smooth surfaces. Furthermore, integrin beta 1 gene expression, an indicator of attachment, and heat shock protein 70 gene expression were high on hydrophobic and smooth surfaces. These results indicate that the cellular behavior of hADSCs on gradient surface depends on surface properties, wettability and roughness.

A complementary density gradient of poly(3-dimethyl-methacryloyloxyethyl ammonium propane sulfonate) (PDMAPS, a zwitterionic polymer with antifouling property) and KHIFSDDSSE peptide (KHI, derived from neural cell adhesion molecule NCAM which mediates cell-cell adhesion) was fabricated by Ren et al. [70] The success of the complementary gradient relies on the appropriate interplay between the PDMAPS brushes and the cell-specific ligands, enabling the selective guidance of SCs migration.

Yu et al. [71] have been prepared a gelatin density gradient on PCL membrane and its influence on adhesion and migration of endothelial cells. The resulted gelatin density gradient ranged from 0.49 to 1.57 μg/cm^2 on the PCL membrane. The endothelial cells showed preferred orientation and directional migration toward the gradient direction with enhanced gelatin density at proper position.

Faia-Torres et al. [72] have been attempted to regulate a human mesenchymal stem cell osteogenesis by specific gradient surface density of fibronectin. The functional analysis of the gradient revealed that the lower FN-density elicited stronger osteogenic expression and higher cytoskeleton spreading, hallmarks of the stem cell commitment to the osteoblastic lineage.

Wang et al. [73] have been demonstrated the screening rat mesenchymal stem cell attachment and differentiation on surface chemistries using plasma polymer gradients. This study showed a simple but powerful approach to the formation of plasma polymer based gradients, and demonstrates that MSC behavior can be influenced by small changes in surface chemistry.

4.11. Summary

In the beginning stage, the gradient concept had been started from just surface modification. As a many results, gradient surface could provide a quick single experiment route to optimize surface characteristics without any trial and error, tedious and laborious procedure. This tool is convenient and time saving. It can be very valuable to analyze the functional biological variables in a single experiment at the same time and on the same surface. It must be simpler than conventional methods since we prepared lot of samples with different experimental conditions. From the ending of 1990s to the beginning of 2000s, the modification methods has been developed, expanded and combined with surface modification from semiconductor manufacturing, other vacuum technology, polymerization methods and so on leading to numerous effective physicochemical gradient surface.

Furthermore, many works using the gradient tools have been tested and confirmed to control of the stem cell fate as proliferation, differentiation, transdifferentiation, direct conversion, and so on. In the advent of precisely tailored biodegradable polymers, stimuli responsive polymers, smart hydrogel polymers, drug delivery techniques and newly functional polymers, the application of the gradient concepts may be tested and used to TERMS area.

5. Concluding remarks and future prospects

The main purpose of this feature article is (1) the introduction of historical and evolutional aspect of gradient surface and (2) the changes of the concept of gradient from only surface property to various physicochemical properties. The beginning of the first idea for gradient was looks like a small primitive prototype apparatus as a toy with limited number of researchers, however, very recently the tremendous manufacturing technology as over $\sim$100 kinds of fabricated methods have been introduced, developed, evolved and commercialized by much more laboratories.

As mentioned earlier, scaffold biomaterials might be one of most important components among triad components since it provides the anchorage site of cells and then stimulate the excretion of extra cellular matrix. Also surface property of scaffolds may be one of the critical properties to control the adhesiveness and growth of cells. In the respect of view for the surface properties, we can apply the surface gradients to find the optimum points of cellular activity for the scaffold biomaterials.

Possible gradient types of the application for the TERM area [9] might be (1) porosity and pore size gradient, (2) porosity and stiffness gradient, (3) crosslinking density, (4) materials composition gradient of metal, ceramic and polymeric materials, (5) oxidation degree gradient and so on. Also another chemical signal concentration might be the important gradient tool as (1) CAM/peptide gradient, (2) growth factor gradient,

(3) protein gradient, (4) soluble factor gradient, (5) controlled release for soluble factor gradient and so on.

Even though the above gradient as the porosity/pore size gradient, substrate stiffness gradient, chemical signal gradient and so on for the 3-D scaffold do not dealt in this review, these kinds of 3-D gradient properties also may be the important roles for the formation of organoids and bioorgan as well as the differentiation, transdifferentiation, direct conversion of adult stem cell, embryonic stem cell and iPS.

In order to accomplish to mimic to the gradient environment of human body, the integration of the several technologies must be needed. In the respect of the integration and convergence for multi-technology, multidisciplinary researches also have been tried massively. For example, the controlled release of the cytokine (such as vitamin E and/or pH) with gradient concentration in one scaffold can mimic in the first stage of the fertilized egg environment. Also gradient 3-D structure like osteochondral tissue can make easily using multidisciplinary cooperative works. Another possible application area will be cellular recognition, cellular separation, cellular motility, molecular sensing, diagnostic, and precisely gene and protein/peptide delivery.

References

[1] G. Khang, T. Shimizu, A manual for differentiation of bone marrow-derived stem cells to specific cell types, Manuals in Biomedical Research (Series editor: K.W. Ng), vol. 8, World Scientific, Singapore, 2014, pp. 1–7.

[2] Y. Tabata, Biomaterial technology for tissue engineering applications, J. R. Soc. Interface 6 (3) (2009) S311–S324.

[3] R.M. Nerem, Tissue engineering: from basic biology to cell-based applications, in: S. Li, N. L'Heureux, J. Elisseett (Eds.), Stem Cell and Tissue Engineering, World Scientific, Singapore, 2011, pp. 1–11.

[4] E.Y. Kim, et al., Recent advances in tissue-engineered corneal regeneration, Inflamm. Regen. 34 (1) (2014) 4–14.

[5] R. Langer, J.P. Vacanti, Tissue engineering, Science 260 (1993) 920–926.

[6] A. Atala, R. Lanza, J.A. Thompson, R.M. Nerem, Hybrid, composite, and complex biomaterials for scaffolds, in: Elsevier (Ed.), Principles of Regenerative Medicine, Academic Press, New York, 2008, pp. 636–655.

[7] M.S. Kim, G. Khang, H.B. Lee, Gradient polymer surfaces for biomedical applications, Prog. Polym. Sci. 33 (2008) 138–164.

[8] J. Genzer, R.R. Bhat, Surface-bound soft matter gradient, Langmuir 24 (2008) 2294–2317.

[9] M. Singh, C. Berkland, M.S. Detamore, Strategies and applications for incorporationg physical and chemical signal gradients in tissue engineering, Tissue Eng. Part B 14 (2008) 341–366.

[10] H.H. Ahn, I.W. Lee, H.B. Lee, M.S. Kim, Cellular behavior of human adipose-derived stem cells on wettable gradient polyethylene surfaces, Int. J. Mol. Sci. 15 (2) (2014) 2075–2086.

[11] J.H. Lee, H.B. Lee, Platelet adhesion onto wettability gradient surfaces in the absence and presence of plasma proteins, J. Appl. Polym. Sci. 92 (2004) 599–606.

[12] H.B. Lee, M.S. Kim, Y.H. Cho, G. Khang, Preparation of gradient polymer surface and their pluripotent biomedical applications, Polymer 29 (2005) 423–432.

[13] W.G. Pitt, Fabrication of a continuous wettability gradient by radio frequency plasma discharge, J. Colloid Interface Sci. 133 (1989) 223–227.

[14] J. Elwing, S. Welin, A. Askendahl, U. Nilsson, I. Lundstrom, A wettability gradient method for studies of macromolecular interactions at the liquid/solid interface, J. Colloid Interface Sci. 119 (1987) 647–649.

[15] H.B. Lee, J.D. Andrade, Cell adhesion on gradient surfaces, in: Proceedings of the Transactions of the Third World Biomaterials Congress, 1988, p. 43.

[16] J.H. Lee, J.W. Park, H.B. Lee, Characterization of hydroxyl group gradient surfaces prepared by water vapor plasma treatment, Polymer 14 (1990) 646–652.

[17] J.H. Lee, J.W. Park, H.B. Lee, Cell adhesion and growth on polymer surfaces with hydroxyl groups prepared by water vapor plasma treatment, Biomaterials 12 (1991) 443–448.

[18] J.H. Lee, H.G. Kimg, G. Khang, H.B. Lee, M.S. Jhon, Characterization of wettability gradient surfaces prepared by corona discharge treatment, J. Colloid Interface Sci. 151 (1992) 563–570.

[19] J.H. Lee, H.B. Lee, A wettability gradient as a tool to study protein adsorption and cell adhesion on polymer surfaces, J. Biomater. Sci. Polym. Ed. 4 (1993) 467–481.

[20] J.H. Lee, H.W. Kim, P.K. Park, H.B. Lee, Preparation and characterization of functional group gradient surfaces, J. Polym. Sci. A: Polym. Chem. 32 (1994) 1569–1579.

[21] Y. Iwasaki, K. Ishihara, N. Nakabayashi, G. Khang, J.H. Jeon, J.W. Lee, et al., Platelet adhesion on the gradient surfaces grafted with phospholipid polymer, J. Biomater. Sci. Polym. Ed. 9 (1998) 801–816.

[22] Y. Iwasaki, S. Sawada, N. Nakabayashi, G. Khang, H.B. Lee, K. Ishihara, The effect of the chemical structure of the phospholipid polymer on fibronectin adsorption and fibroblast adhesion on the gradient phospholipid surface, Biomaterials 20 (1999) 2185–2191.

[23] M.S. Kim, K.S. Seo, G. Khang, H.B. Lee, First preparation of biotinylated gradient polyethylene surface to bind photoactive caged streptavidin, Langmuir 21 (2005) 4066–4070.

[24] M.S. Kim, K.S. Seo, G. Khang, H.B. Lee, Preparation of a gradient biotinylated polyethylene surface to bind streptavidin-FITC, Bioconjug. Chem. 16 (2005) 245–249.

[25] J.H. Lee, G. Khang, J.W. Lee, H.B. Lee, Interaction of different types of cells on polymer surfaces with wettability gradient, J. Colloid Interface Sci. 205 (1998) 323–330.

[26] M.S. Kim, Y.H. Cho, S.Y. Lee, G. Khang, T.G. Lee, D.W. Moon, et al., Preparation and characterization of amine modified gradient polyethylene surfaces, Chem. Lett. 35 (2006) 728–729.

[27] S.Y. Lee, J.Y. Youn, Y.H. Cho, M.S. Kim, G. Khang, H.B. Lee, Preparation of quantum dot modified gradient surface, Colloids Surf. A (2007)http://dx.doi.org/10.1016/j.colsurfa.2007.04.158.

[28] G. Khang, J.W. Lee, J.H. Jeon, J.H. Lee, H.B. Lee, Interaction of fibroblasts on microgrooved polyethylene surfaces with wettability gradient, Biomater. Res. 1 (1997) 1–6.

[29] J.H. Lee, G. Khang, J.W. Lee, H.B. Lee, Interactions of proteins and cells on functional group gradient surfaces, Macromol. Symp. 118 (1997) 571–576.

[30] G. Khang, S.J. Lee, J.H. Lee, Y.S. Kim, H.B. Lee, Interaction of fibroblast cells on poly(lactide-co-glycolide) surfaces with wettability chemogradient, Bio-Med. Mater. Eng. 9 (1999) 179–187.

[31] J.H. Lee, S.J. Lee, G. Khang, H.B. Lee, The effect of fluid shear stress on endothelial cell adhesiveness to polymer surfaces with wettability gradient, J. Colloid Interface Sci. 230 (2000) 84–90.

[32] G. Khang, J.H. Lee, I. Lee, J.M. Rhee, H.B. Lee, Interaction of different types of cells on poly(ʟ-lactide-co-glycolide) surface with wettability chemogradient, Korea Polym. J. 8 (2000) 276–284.

[33] J.S. Choi, S.J. Lee, G. Khang, I. Lee, H.B. Lee, Interactions of schwann cell onto chloric acid treated PLGA surface with different wettability, Biomater. Res. 6 (2002) 17–24.

[34] C.W. Choee, S.J. Lee, Y.M. Lee, J.M. Rhee, H.B. Lee, G. Khang, The proliferation rate of fibroblast cell onto PE surface with different wettability, J. Appl. Polym. Sci. 92 (2004) 599–606.

[35] J.H. Lee, H.B. Lee, Platelet adhesion onto wettability gradient surfaces in the absence and presence of plasma proteins, J. Biomed. Mater. Res. 41 (1998) 304–311.

[36] S.J. Lee, G. Khang, Y.M. Lee, H.B. Lee, The effect of surface wettability on induction and growth of neuritis from the PC-12 cell on polymer surface, J. Colloid Interface Sci. 259 (2003) 228–235.

[37] M.S. Kim, Y.N. Shin, M.H. Cho, S.H. Kim, S.K. Kim, Y.H. Cho, G. Khang, I. Lee, H.B. Lee, Adhesion behavior of human bone marrow stromal cells on a differentially wettable polymer surface, Tissue Eng. (2007)http://dx.doi.org/10.1089/ten.2006.0062.

[38] A.D. Lander, Morphous unbound: reimaging the morphogen gradient, Cell 128 (2007) 245–256.

[39] H. Liu, Y. Ito, Gradient micropattern immobilization of a thermo-responsive polymer to investigate its effect on cell behavior, J. Biomed. Mater. Res. A 67 (4) (2003) 1424–1429.

[40] D. Julthongpiput, M.J. Fasolka, W. Zhang, T. Nguyen, E.J. Amis, Gradient chemical micro patterns: A reference substrate for surface nanometrology, Nano Lett. (2005) 1535–1540.

[41] M.J. Choi, J.Y. Park, K.J. Cha, J.W. Rhie, D.W. Cho, D.S. Kim, Micropattern arry with gradient size (μPAGE) plastic surfaces fabricated by PDMS (polydimethylsiloxane) mold-based hoy embossing technique for investigation of cell-surface interaction, Biofabrication 4 (4) (2012) 045006.

[42] M. Cantini, M. Sousa, D. Moratal, J.F. Mano, M. Salmerón-Sánchez, Non-monotonic cell differentiation pattern on extreme wettability gradients, Biomater. Sci. 1 (2013) 202.

[43] S. Kim, Y.S. Ryu, J.H. Suh, C.M. Keum, Y. Sohn, S.D. Lee, Biocompatible patterning of proteins on wettability gradient surface by thermo-transfer printing, J. Nanosci. Nanotechnol. 14 (8) (2014) 6069–6071.

[44] I.Y. Tsai, M. Kimura, T.P. Russell, Fabrication of a gradient hetero-geneous surface using homopolymers and diblock copolymers, Langmuir 20 (14) (2004) 5952–5957.

[45] K. Mougin, A.S. Ham, M.B. Lawrence, E.J. Fernandez, A.C. Hillier, Construction of a tethered poly(ethylene glycol) surface gradient for studies of cell adhesion kinetics, Langmuir 21 (15) (2005) 4809–4812.

[46] S. Morgenthaler, C. Zink, B. Städler, J. Vörös, S. Lee, N.D. Spencer, S. G., Tosatti Poly(L-lysine)-grafted poly(ethylene glycol)-based surface-chemical gradients. Preparation, characterization, and first applications, Biointerphases 1 (4) (2006) 156–165.

[47] D.J. Menzies, B. Cowie, C. Fong, J.S. Forsythe, T.R. Gengenbach, K. M. Mclean, L. Puskar, M. Textor, L. Thomsen, M. Tobin, B.W. Muir, One-step method for generating PEG-like polymer gradients: chemical characterization and analysis of protein interactions, Lagmuir 26 (17) (2010) 13987–13994.

[48] J. Pei, H. Hall, N.D. Spencer, The role of plasma proteins in cell adhesion to PEG surface-density-gradient-modified titanium oxide, Biomaterials 32 (34) (2011) 8968–8978.

[49] J. Zhang, Y. Han, A topography/chemical composition gradient poly-styrene surface: Toward the investigation of the relationship between surface wettability and surface structure and chemical composition, Langmuir 24 (3) (2008) 796–801.

[50] M. Hulander, A. Lundgren, L. Faxälv, T.L. Lindahl, A. Palmquist, M. Berglin, H. Elwing, Gradients in surface nanotopography used to study platelet adhesion and activation, Colloids Surf. B Biointerfaces 110 (2013) 261–269.

[51] L. Li, Y. Zhu, B. Li, C. Gao, Fabrication of thermoresponsive polymer gradients for study of cell adhesion and detachment, Langmuir 24 (23) (2008) 13632–13639.

[52] L. Ionov, N. Houbenov, A. Sidorenko, M. Stamm, S. Minko, Stimuli-responsive command polymer surface for generation of protein gradients, Biointerphases 4 (2) (2009) (FA45–49).

[53] L. Li, J. Wu, C. Gao, Gradient immobilization of a cell adhesion RGD peptide on thermal responsive surface for regulating cell adhesion and detachment, Colloids Surf. B Biointerfaces 85 (1) (2011) 12–18.

[54] F.I. Tai, O. Sterner, O. Andesson, T. Ekblad, T. Edrth, pH-control of the protein resistance of thin hydrogel gradient films, Soft Matter 10 (32) (2014) 5955–5964.

[55] Y.W. Won, A.N. Patel, D.A. Bull, Cell surface engineering to enhance mesenchymal stem cell migration toward an SDF-1 gradient, Biomaterials 35 (21) (2014) 5627–5635.

[56] H. Tan, L. Wan, J. Wu, C. Gao, Microscale control over collagen gradient on poly(L-lactide) membrane surface for manipulating chondrocyte distribution, Colloids Surf. B Biointerfaces 67 (2) (2008) 210–215.

[57] Y.X. Ding, V. Hlady, Competitive adsorption of three human plasma proteins onto sulfhydryl-to-sulfonate gradient surface, Croat. Chem. Acta 84 (2) (2011) 193–202.

[58] X. Li, H. Dai, S. Tan, X. Zhang, H. Liu, Y. Wang, N. Zhao, J. Xu, Facile preparation of poly(ethyl alpha-cyanoacrylate) superhydrophobic and gradient wetting surfaces, J. Colloid Interface Sci. 340 (1) (2009) 93–97.

[59] A. Lagunas, J. Comelles, E. Martinez, J. Samitier, Universal chemical gradient platforms using poly(methyl methacrylate) based on the biotin-streptavidin interaction for biological applications, Langmuir 26 (17) (2010) 14154–14161.

[60] S.H. OH, T.H. Kim, J.H. Lee, Creating growth factor gradients in three dimensional porous matrix by centrifugation and surface immobilization, Biomaterials 32 (32) (2011) 8254–8260.

[61] G. Vozzi, T. Lenzi, F. Montemurro, C. Pardini, F. Vaglini, A. Ahluwalia, A novel method to produce immobilized biomolecular concentration gradients to study cell activities: design and modeling, Mol. Biotechnol. 50 (2) (2012) 99–107.

[62] J. Lee, I. Choi, W.S. Yeo, Preparation of gradient surfaces by using simple chemical reaction and investigation of cell adhesion on a two-component gradient, Chem. Eur. J 19 (2013) 5609–5616.

[63] T. Liu, Y. Liu, Y. Chen, S. Liu, M.F. Maitz, X. Wang, K. Zhang, J. Wang, Y. Wang, J. Chen, N. Huang, Immobilization of heparin/poly-(L)-lysine nanoparticles on dopamine-coated surface to create a heparin density gradient for selective direction of platelet and vascular cells behavior, Acta Biomater. 10 (5) (2014) 1940–1954.

[64] Y. Ishiguro, S. Inagi, T. Fuchigami, Gradient doping of conducting polymer films by means of bipolar electrochemistry, Langmuir 27 (11) (2011) 7158–7162.

[65] F.J. Harding, L.R. Clements, R.D. Short, H. Thissen, N.H. Voelcker, Assessing embryonic stem cell response to surface chemistry using plasma polymer gradients, Acta Biomater. 8 (5) (2012) 1739–1748.

[66] A. Lagunas, J. Comelles, E. Martinez, E. Parts-Alfonso, G.A. Acosta, F. Albericio, J. Samitier, Cell adhesion and focal contact formation on linear RGD molecular gradients: study of non-linear concentration dependence effects, Nanomedicine 8 (4) (2012) 432–439.

[67] Y. Zhu, Z. Mac, C. Gao, Control over the gradient differentiation of rat BMSCs on a PCL membrane with surface-immobilized alendronate gradient, Biomacromolecules 14 (2) (2013) 342–349.

[68] A.B. Faia-Torres, S. Guimond-Lischer, M. Rottmar, M. Charnley, T. Goren, K. Maniura-Weber, N.D. Spencer, R.L. Reis, M. Textor, N. M. Neves, Differential regulation of osteogenic differentiation of stem cells on surface roughness gradients, Biomaterials 35 (33) (2014) 9023–9032.

[69] H.H. Ahn, I.W. Lee, H.B. Lee, M.S. Kim, Cellular behavior of human adipose-derived stem cells on wettable gradient polyethylene surfaces, Int. J. Mol. Sci. 15 (2) (2014) 2075–2086.

[70] T. Ren, S. Yu, Z. Mao, C. Gao, A complementary density gradient of zwitterionic polymer brushes and NCAM peptides for selectively controlling directional migration of schwann cells, Biomaterials 56 (2014) 58–67.

[71] S. Yu, Z. Mao, C. Gao, Preparation of gelatin density gradient on poly(ε-caprolactone) membrane and its influence on adhesion and migration of endothelial cells, J. Colloid Interface Sci. 451 (2015) 177–183.

[72] A.B. Faia-Torres, T. Goren, T.O. Ihalainen, S. Guimond-Lischer, M. Charnley, M. Rottmar, K. Maniura-Weber, N.D. Spencer, R. L. Reis, M. Textor, N.M. Neves, Regulation of human mesenchymal stem cell osteogenesis by specific surface density of fibronectin: a gradient study, ACS Appl. Mater. Interfaces 7 (4) (2015) 2367–2375.

[73] P.Y. Wang, L.R. Clements, H. Thissen, W.B. Tsai, N.H. Voelcker, Screening rat mesenchymal stem cell attachment and differentiation on surface chemistries using plasma polymer gradients, Acta Biomater. 11 (2015) 58–67.