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Reprogramming the Stem Cell Behavior by Shear Stress and Electric Field Stimulation: Lab-on-a-Chip Based Biomicrofluidics in Regenerative Medicine

Sharmistha Naskar[1,2,3,4] · Viswanathan Kumaran[2] · Bikramjit Basu[1,3,4]

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Abstract

The biophysical cues of endogenous origin, i.e., shear stress and electric field, are known to significantly modulate cell functionality, in vitro. While this has been relatively well investigated in conventional petri dish culture, it is important to validate such important phenomenon in physiologically simulated cellular microenvironment. In this perspective, this review critically discusses the importance of lab-on-a-chip (LOC)-based microfluidic devices to probe into this aspect to develop an insight towards the application in regenerative medicine. While reviewing several literature reports, an emphasis has been placed to unravel the intriguing aspects of shear and electric field modulated differentiation of stem cells in the biomicrofluidics devices. The potential application focusing the stem cell culture was emphasized in this article as the stem cells are the foundation of tissue regeneration. Several challenges in tissue regeneration and introduction of personalized medicine could be addressed through microfluidic technology. Culturing of organ-specific multiple cell types within lab-on-a-chip and biophysical stimulation mediated activations of intracellular signal transduction under gradient shear/electric field are highlighted in this review.

Lay Summary

Conceptually, regenerative medicine is considered as an emerging approach for treating traumatized, malfunctional anatomical parts of the patients with stem cells to establish normal functionality of the tissue. The regenerated tissue should preferably be the patients' autologous tissue, grown under artificially created in vivo physiological environment. Biomicrofluidic-based lab-on-a-chip technology enables to perform in vitro cell/tissue engineering under endogenous cues, like shear and electric field.

Therefore, this review discusses two aspects of regenerative medicine in terms of autologous transplantation of cells/tissues to improvise personalized regenerative medicine and to recreate an organ-specific tissue under the influence of biophysical stimulation in an attempt to improve the physiological functionality.

Keywords Regenerative medicine · Biomicrofluidics · Lab-on-a-chip · Stem cell · Shear stress · Electric field

Future work The use of biomicrofluidic-based lab-on-a-chip technology not only reduces the chance of unexpected deviations from the in vitro results, but also shows the promise to solve some of the innate problems like the innervation and angiogenesis. More quantitative assessment into the biophysical stimulation should be pursued in future.

✉ Bikramjit Basu
bikram@iisc.ac.in

[1] Centre for BioSystems Science and Engineering, Indian Institute of Science, Bangalore 560012, India

[2] Department of Chemical Engineering, Indian Institute of Science, Bangalore 560012, India

[3] Laboratory for Biomaterials, Materials Research Centre, Indian Institute of Science, Bangalore 560012, India

[4] Translational Centre on Biomaterials for Orthopaedic and Dental Applications, Materials Research Centre, Indian Institute of Science Bangalore, Bangalore, India

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Introduction

How can the biomicrofluidics be a part of regenerative medicine in fostering personalized treatment? The answer significantly helps to overcome the obstacles in advancing human healthcare in future which would have significant impact on implant-based treatments. The biological cellular system can be considered as a complex physicochemical reactor, which can undergo adaptive changes towards external biophysical dues. In order to understand the biological response of a cellular system towards different abiological, it is critical to develop a better insight into such influence in a system which can closely mimic in vitro physiological conditions. Microfluidic devices provide a unique platform to study many of the intracellular processes along with the cellular responses. A cornerstone of biomicrofluidic studies is based on cell culture techniques coined with cellular engineering, i.e., the ability to grow biological cells outside the living system within a tailored device capable of simulating physiological conditions. In comparison to unconventional micro-cultures, conventional macro-culture (Petri dish) approaches are often too naïve to provide realistic data. Biomicrofluidics offers an opportunity to bridge these two extremes using lab-on-a-chip concept, implementing an integrative approach of using biologically inspired structural designs, together to mimic the physiological microenvironment of cells, in vitro. The interdisciplinary concept of biomicrofluidics is continuously maturing, and this demands the development of novel strategies to realize accurate outcomes regarding cell-based studies.

On bringing the biomicrofluidics technology in the field of regenerative medicine, the prospect regarding the prognosis of diseases is expected to be increased as well as the distress of treatment-unresponsive degenerative diseases would decrease. Regenerative medicine while moving towards more personalized level needs explants of the patients to reduce the risk of the tissue implantation. Every human being, even if biologically related, genetically differs from others, which leads to the concept of "precision medicine." According to Schork et al., precision medicine initiatives require a different scheme of the clinical trials that focus on an individual not on averaging the population but taking into account the genetic variability and lifestyle for each patient [1].

The cells donated by the patient, such as stem cells isolated from human bone marrow or hemopoietic cells collected from the blood, can be used to regenerate the tissues. This approach can avoid unwanted immunogenic tissue rejection, like serious issues, related to implantation. The use of self-explants is considered to be the safest procedure in personalized regenerative medicine. The microfluidic engineering has brought a remedy for immunogenic reactions from the highly demanded personalized medicine point of view by introducing on-chip cell culture techniques. Vertical electric field stimulated neuronal cell growing on porous amorphous carbon electrodes show enhanced protrusion of neuronal processes [2]. The physiological parameters play an intrigued role for the cell to decide the molecular genetics. Biomaterial substrates are one of the recent development for influencing the cells biologically through application of a physical regulator [3–5]. Electric field is one of the potent physiological parameter which can drive the growth process of the cell. For example Ravikumar et al. proved that applying electric field onto conductive substrates can enhance myotube formation [6].

The discussion of the review is focused on the biomicrofluidics with a lab-on-a-chip perspective towards implementation in order to mimic the in vivo regulatory parameters, like shear stress and electric field. This also establishes the promise to improve the outcomes of regenerative therapies (Fig. 1a). In the next section, the potential applications of biomicrofluidics with the concept of regenerative medicine has been described. The "Application of Biomicrofluidics in Lab-on-a-Chip (LOC): a tool for regenerative medicine" section describes LOC as a special application of biomicrofluidics and the advantages of using LOC in regenerative medicine. The "Tripartite application of LOC and stem cell with a regenerative medicinal aspect" section precisely focuses stem cell culture using LOC. A special emphasis on the stem cells was given because they are the cornerstone of tissue regeneration. To make the stem cell microculture simulated like in vivo conditions, it is important to expose the cultured cells to the physiological cues. The two most effective cues of shear stress and electric field were chosen for discussion in the "Application of biomimetic physiological cues in regenerative medicine with LOC" section, along with their physiological relevance. At the closure, the review concludes with the future application of the biomicrofluidics in regenerative therapies.

Role of Biomicrofluidics in Regenerative Medicine

Regenerative medicine and cellular therapy are the newest fields of translational medicine. Regenerative medicine is the concept of newer medicine which consist of tissue instead of pharmaceutical chemicals to treat the traumatized, malfunctional of anatomical parts of a patient. Pharmaceutical drugs target the endogenous healing capacity of the body by enhancing it or by preventing infection and unwanted reactions. The regenerative medicine rather provides the damaged body part with externally well-grown healthy cells or tissues which function to restore or establish normal function. The stem cells are considered as the foundation of the regenerative medicine because of their potential to recreate differentiated tissue types. A critical issue affecting in vitro regeneration of tissue is the inability to create in vivo physiological environment which plays an important role in cell proliferation and differentiation. Apart from that, the

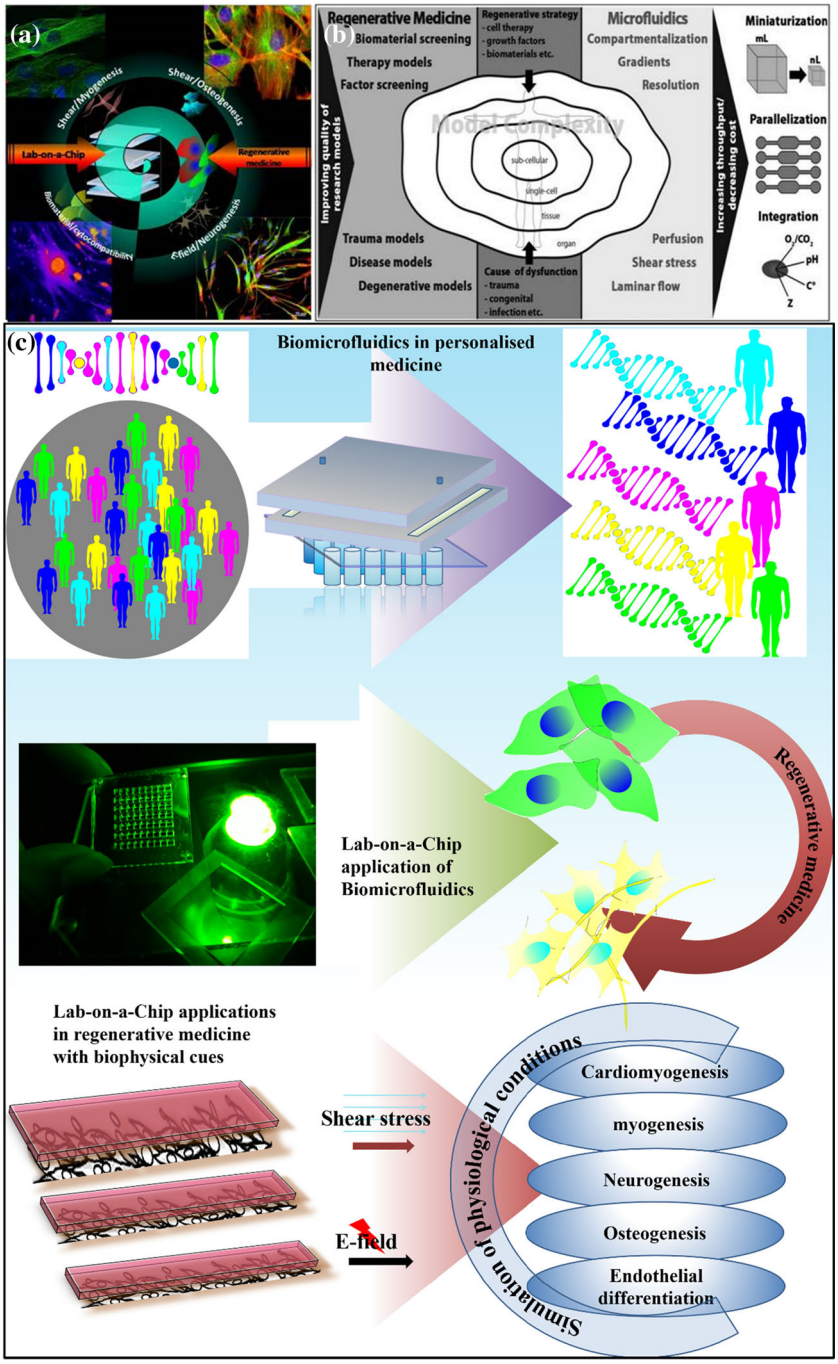

Fig. 1 The interdisciplinary approach of biomicrofluidics has significant potential in regenerative medicine in the context of the personalization of tissue regeneration. Application of biophysical cues in such tissue-regenerative approaches increases the success rate of regenerative therapies by decreasing the chances of tissue rejection. **a** The theme of the review paper with application of microfluidics towards regenerative medicine under the influence of biophysical cues (shear and electric field). **b** Schematic description of the microfluidic application on regenerative medicine [7]. **c** Schematic illustration to depict the overview of this review paper highlighting biomicrofluidics with a lab-on-a-chip perspective to mimic the in vivo regulatory parameters, like shear stress and electric field. This also established a potentiated promise to improve the outcome of regenerative therapies

regenerative tissue should preferably be the patients' own tissue to reduce the immunogenic reaction. Therefore, the two aspects of regenerative medicine perhaps could be summarized in terms of use of autologous transplantation of cells/tissues to improvise personalized regenerative medicine and recreating an organ-specific tissue/cells under the influence of physical regulators to improve the physiological functionality of the tissues.

Personalized regenerative medicine is based on modifying therapies and treatments according to the unique biochemical, genetic, and other characteristics and of an individual. In order to develop personalized regenerative medicine, it is crucial to have the molecular level of understanding of the genetic scenario of an individual patient, thereby its influence on the cell transformation. The biophysical regulators in vivo physiological could be multiple, like shear, electric field, surface charge, osmotic pressure etc. Among these regulators, the shear stress and electric field are the most prominent physiological parameters, which can determine the path of the cellular progression. It has already been proven that the biophysical cues are transduced intracellularly by the membrane proteins which flows the stimulus in the form of chemical signal to carry out genetic modifications. This motivates the cell to differentiate and to

form a new type of tissues during embryogenesis and functional tissues replacing the traumatized one in the adult body.

The tailored use of biomicrofluidic technology in terms of lab-on-a-chip devices. Lab-on-a-chip approaches, derived from the of microtechnology, has provided a unique and unrivaled contrivance towards using it in stem cell biology and its related fields of regenerative medicine. Several attempts were made to recreate the microenvironment in conventional Petri dish cultures which even if were able to bring the cells under the influence of the abiological cues but fails to generate reproducibility due to its inhomogeneous application on a huge population of cells. Therefore, cell and tissue engineering procedures as an application towards the personalized regenerative medicine have a great challenge to create the biophysically simulated environment to culture the cells. Therefore, the application of customized lab-on-a-chip is the justified approach in order to attain high accuracy in implementing stem cell regeneration. The LOC technology essentially mimics the abiological environment at the time of in vitro cell culture, especially for the stem cells which respond vastly to the applied biophysical stimuli. The tailored designs of LOC devices not only provides unprecedented physiological approaches with the biological range of shear and electric field but also provides an insight about the molecular mechanism of differentiation/proliferation/cytocompatibility responses of the stem cells or precursor cells to evaluate them genealogically and phenotypically. As an inference, it can be stated that the biomicrofluidics inspired LOC devices used for enkindling the stem cell/precursor cell/blood cells have been endowed with the virtue of mimicking the body-like condition which could minimize the risk of unwanted and unpredicted complicacy during the clinical trials. Thus, the regenerative medicine should be collaborated with the biomicrofluidics along with the biophysical cues to achieve the results up to the mark of satisfactory. The interdisciplinary application of biomicrofluidics in regenerative medicine demonstrates different facets of the complexity of the research. The final aim of the research on regenerative medicine with an association to biomicrofluidics is the improvement of the outcomes than the existing strategies, leading to the increased success rate of clinical applications. It is imperative for the regenerative medicine study to reach the targeted success rate, either by a detailed understanding of fundamental molecular mechanisms of cell regeneration or by studying the experimental biology aspect of tissue regeneration with scaffolds. The diversified outcome of the regenerative models depends on specific aspects like (i) cell/tissue/organ type, (ii) damaged tissue condition and cause, and (iii) regenerative strategy (Fig. 1b, c) [7]. Though the increasing demand for regenerative medicine has progressed using tissue engineering strategies, still, the success rate of translating the tissue engineering concept from the bench to bedside is very low. The possible reason for such failure is due to the inability of the animal experiments to produce reliable results. As a consequence, significant deviation from the expected results could be found during the clinical trials, which compels to reject the experimental approach at the end, putting all the efforts in vain. The root cause of the problem lies in the diversified results of in vitro studies. The in vitro studies, performed to reveal the tissue engineering concept, is experimented mainly using conventional Petri dish system in absence of biophysical cues. This leads to erroneous results as they are devoid of actual physiological consequences. This could be one of the reasons of failure of regenerative medicine in the pre-operative stages. The failure at post-operative stages has multiple reasons both due to infection and inflammation. The immunogenic rejection could be extremely severe, which is actually causing the risk of high mortality in the implant surgeries. The motivation behind the discussion in this section is to emphasize the precision and personalization of regenerative medicine achieved by using microfluidic technology.

Biomicrofluidics in Personalized Medicine: a Promise towards Successful Tissue Regeneration

The field of biomicrofluidics within the close embracement to the autologous cell regenerative medicine has developed the way to succeed hurdles of tissue implant related post-surgical complexities. The personalized medicine deals with differences between individuals and tailors the treatment for each patient using the conventional healthcare system. As true for all medicines, regenerative medicine also faces the challenge of how much must be customized and how much can be standardized. The microfluidic technology with LOC devices has enabled the tissue engineering to experience a customized approach towards the treatment of each patient with varying degrees of standardization. In case of regenerative medicine patients generally, it is possible to simulate the physiological regulating factors affecting the behavior of human cells, such as physiological shear or endogenous electric field. An example would be relevant to discuss the challenges and advantages of the personalization of regenerative medicine. In a recent report on Kalydeco usage, FDA (Food and Drug Administration, USA) published that the personalized medicine is not only limited to pharmaceutical therapy but also could be achieved with non-medical technologies like computer programming, artificial intelligence, sensors etc. [8]. In an intrigued review done by Contreras-Naranjo et al., the potential to analyze the exosomes and other extracellular materials as a part of the emerging liquid biopsy techniques was reported (Fig. 2) [10]. They also have shared their opinion on the immense potential of such unconventional diagnostics in personalized medicine.

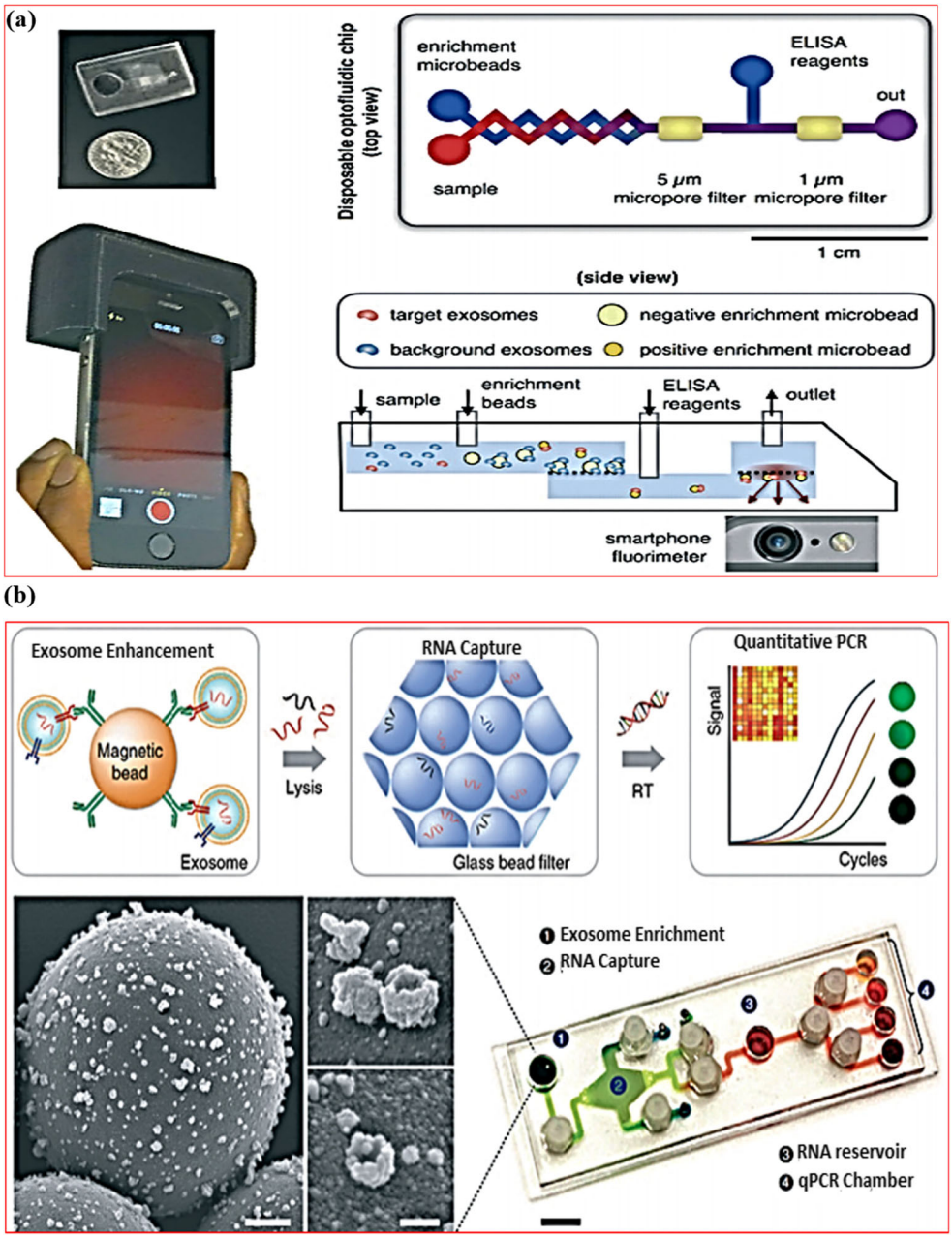

Fig. 2 Exosome analysis could pose a promise towards personalized medicine. **a** Smartphone-based microfluidic mobile exosome detector (μMED) for point-of-care (POC) analysis of exosomes in disease detection [9]. **b** Immunomagnetic exosomal RNA (iMER) chip for quantification of exosomal mRNA levels which revolves around microfluidic-directed personalized care [10, 11]

Biomicrofluidics in Regenerative Medicine: Cross-Disciplinary Regime Spanning Advantages of Precise Monitoring

In the realm of tissue engineering vis-à-vis to regenerative medicine, there are several challenges, which have enacted to get successful in vitro tissue regeneration. The intervention of biomicrofluidics into the unexplored or less explored area of regenerative medicine has allowed the naïve technology to penetrate deep into the biological basis of cell culture, and it finds solutions to overcome challenges of tissue culture.

Physiological Microenvironment of the Cell Niche of any cell external environment is responsible for cellular adaptation, leading to a change in phenotype and function. Zervantonakis et al. used microfluidic devices to study cell-to-cell interactions in controlled microenvironments reveals [12].

Soluble Gradients The biochemical gradient of growth factors rules the cell behavior. For example, the polarity developed during embryogenesis is due to the maintained gradient of the growth factors. Wu et al. made an equally interesting demonstration, where the use of biomicrofluidics has been implemented for studying the transport of particles and whole organisms [13].

Multi-Cell Type Culture While most conventional study in the field of tissue engineering involves the use of single type, the co-culture of multiple cell types is however ideal. Pirlo et al. have explored an interesting development in the context cellomics, where the technology has been used to carry out biological studies regarding hybrid technology which is relevant to understand functional neurology [14]. Taylor et al. have designed a hybrid cell culture microdevice to seed two different types of neuron and were able to observe for the behavior of those at the synaptic region [15].

Organ-On Chip Cells to organ formation in vitro is of a great challenge as proper function of the organ is the orchestral output of the multiple cell types. Srigunapalan et al. have used the microfluidic technologies for angiogenesis that is perhaps the first step towards creating synthetic tissues [16].

Application of Biomicrofluidics in Lab-on-a-Chip (LOC): a Tool for Regenerative Medicine

LOC is one of the special applications of biomicrofluidics, which directly deals with the in vitro microculture of the cells along with other acellular biochemical assays. In the current days, the LOC based on cell culture technique is getting involved more into the tissue regeneration, which not only assures the accuracy of the outcome but also can predict the in vivo consequences as well. In order to signify the need of LOC in regenerative medicine, we have focused our discussion on the association of LOC with biomicrofluidic technology and its implementation in regenerative medicine.

Bridge between Biomicrofluidics and LOC

The miniaturized microfluidic devices are capable of performing single to multiple tasks of any complexity. This can be considered as a replacement of spacious labs within a few square centimeters, which is termed as the "lab-on-a-chip" or "LOC." These LOC approaches of the cell culture system can bring more realistic data [17, 18]. It is worthwhile to mention that conventional approaches are often too naïve to provide realistic data due to their failure to simulate in vivo details. LOC offers an opportunity to bridge these two extremes giving a suitable justification to this interdisciplinary amalgamation. LOC improvises modeling of the physiological complexity of the human system in a simplistic manner by introducing multiple biophysical cues to the on-chip cell culture system. These enable the researchers to appreciate the critical factors of cellular behavior carefully and quantitatively. In addition, LOC decreases the use of animals in researches by using customized devices equipped with the provision to impart biophysical cues (electric field and shear stress) on the growing cells. This enables the cells to grow in the physiomimetic environment and helps to capture human-relevant data without invasive procedures. A number of researchers can adapt these LOC systems easily and cost-effectively to a variety of designs, facilitating resolution of high-throughput cell culture studies with physiological relevance. These strengths should allow LOC platforms to reveal critical parameters of regulating the human body-specific cells and provide insights, which will help in the translation during clinical trials. Along with the reduction in spatial occupancy, this emerging LOC technology enables experiments being run with a very small amount of sample and reagent, thus reducing procedural cost. Such devices have the ability to perform the assays accurately as they essentially have negligible or nil dead volume. The success of these devices lies in the fact that the functional components are serially arranged according to the experimental steps and hence they are devoid of any manual errors. Moreover, the results, obtained by this technique do not depend on the differences of input parameters due to manual variations. Thus, the technique has the capability of producing reproducible results—the most fundamental criterion for any new technique to be accepted by the scientific community.

Chronological Development of LOC

In the backdrop of the above discussion, LOC-based microfluidics technology gives an enormous opportunity to unfold new techniques of cellular characterization. The chronological evolution of biomicrofluidic techniques has its distant origin in developing microanalytical methods for gas-phase chromatography (GPC), high-pressure liquid chromatography (HPLC), and capillary electrophoresis (CE). These were designed in micrometer dimension that has revolutionized analytical experiments, which first brought the concept of LOC. That was obviously the first LOC used by S.C. Terry at Stanford University in the year of 1979.

The realization of a LOC or a "micrototal-analysis-system (μTAS)" has been recognized as a functionalized extrapolation of microelectromechanical-systems technology at the beginning of the 1980s. The miniaturization of the system has defeated the hurdles of running complex experimental procedures. These scale-down systems are suitable to perform the entire laboratory protocols performed with the advantages of a smaller size, reduced reaction/analysis time, with little amount of sample/reagent consumption; decreased risk of contamination; and increased sensitivity, reliability, and, most importantly, the capacity to run multiple processes sequentially or in parallel in an integrated system [19, 20]. The fabrication of LOC devices has advanced and is used in the regenerative medicine, from the year 2000 (Fig. Fig. 3). It has become an integral part of regenerative medicine, where the microfluidic engineering has met the tissue engineering for the benefit the medical science.

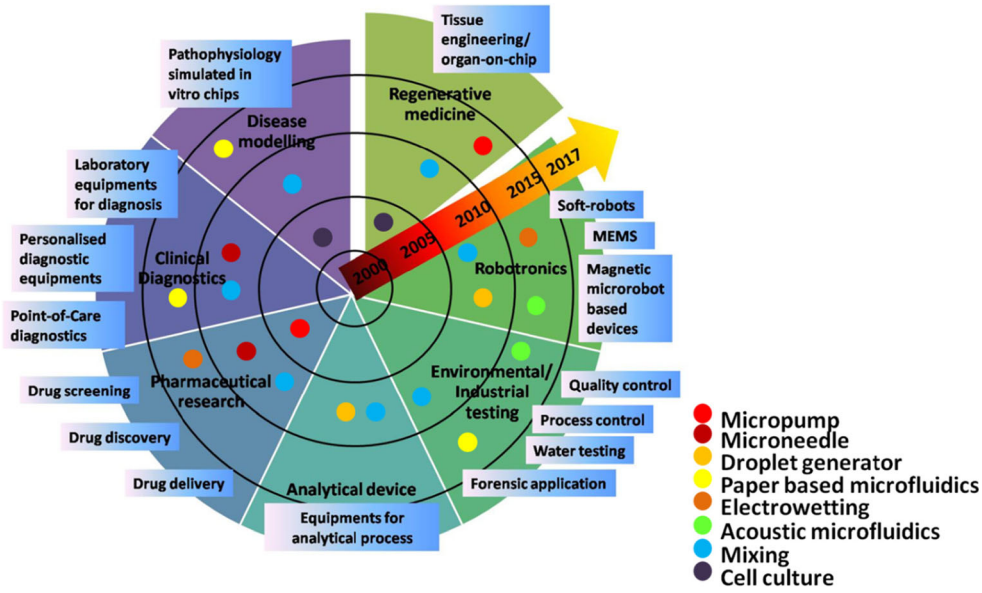

Fig. 3 Chronological advancement of LOC and application in regenerative medicine. The concentric circles show each era of LOC. It is worthwhile to mention that the applications of LOC devices are vast (e.g., robotics, pharmaceutical research etc. along with regenerative medicine) with some of which are depicted by pie slices. The cell culture is one of the initial techniques used with LOC which has been advanced with the implementation of several sophistications like paper-based microfluidics etc.

Summarizing, the comprehensible distinction of LOC with conventional Petri dish experiments infers a twofold conclusion: (a) microfluidic experiments closely mimic in vivo-like physiological environment and (b) continuous shear flow, electric field-like biophysical cue-induced cellular changes cannot be captured in Petri dish experiments. The focus on microfluidic technique-based LOC systems has therefore been to study the stem cells, which in the recent years is advanced by applying shear/electrical cues on living cells (Fig. 4) [21]. This review work is intended to shed light on the application of microfluidics in stem cell research with biophysical cues towards regenerative medicine.

LOC in Regenerative Medicine

The goal of regenerative medicine is to restore or to re-establish the physiological function of the damaged tissues or traumatized organs. The natural regeneration capacity in the human body is seen for very few tissues, which is not even lifelong. For example, blood and skin are such kind of tissue, which can be continually renewed. On the other hand, liver, bone, muscle, and blood vessels possess a limited capacity for self-repair [22]. The unavoidable biological fallacy is that in an adult body, fully matured nerve is incapable of self-restoration. After the organs experience loss-of-function due to the

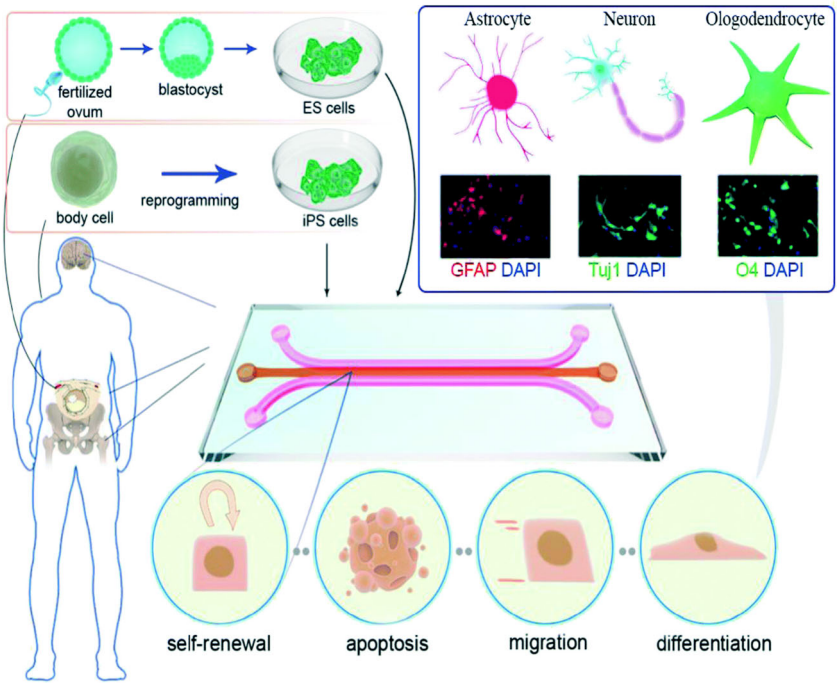

Fig. 4 Stem cell differentiation using LOC under the influence of biophysical cues, mimicking the physiological condition [21]. The basic technique of cellular regeneration starts with the isolation of body cells like gametes or somatic cells which are used for reproducing the stem cells. These cells, under multiple biophysical cues regenerate into target cell type (like glial and neuron cells) using microfluidic devices

damage of the constituent tissues, they should be replaced back with new functional tissue by own. The limited regenerative capacity of the adult tissues is not sufficiently capable to compete with the extent of damage after a traumatic injury or loss of organ due to disease. For example, the scar tissue which is formed to replace the wounded tissue is reported to be functionally impaired [23]. In order to bring back the gain-of-function of such organ, an artificial device or an explant organ is required. Here comes the need for regenerative therapy or tissue implant. However, despite advancements in the medical field, medical devices rarely last very long or have hardly same functional repertoire as the lost organ.

The evolution of regenerative medicine renders since it was first coined by William Haseltine [24]. As per Haseltine's prediction, the first phase is to simulate or copy the body's own repair mechanisms by mimicking the actions of growth factors. The second phase involves implanting tissues or organs grown outside the body after the necessary growth factors have been identified. The third phase will involve technologies that can rejuvenate old tissues, by resetting a cell's biological clock. The final phase will be to exploit the emerging science of nanotechnology.

The process of tissue repair involves a cascade of biological events under the stringent regulation of chemokines, evoked by the inflammatory reaction at the site of contusion [25, 26]. The restoration of physiological homeostasis would soon be targeted by the injured site with the amendment of biophysical and biochemical signals.

In order to meet this demand, not only the potential candidate cells are selected, but also the cells need to be evaluated for the desired performance at a reliable and up to a satisfactory mark. Since the technology of microfluidics has already been validated for high throughput performances, it is possible to provide new paths to accelerate research and development in the field of regenerative medicine using the biomicrofluidics. In addition, the microfluidic system offers other advantages, such as the ability to create in vivo microenvironments and to ensure the accurate results. In view of complexities of organs or tissues, the next big challenge for the biomicrofluidics is to recreate the required functional design to achieve proper organ function. The question, therefore, arises whether it is possible to integrate complex components in a microfluidic system without compromising the experimental reliability.

Tripartite Application of LOC and Stem Cell with a Regenerative Medicinal Aspect

The human body has its innate capability of regeneration, which is attained through the endogenous stem cell system. These cells are found almost in all type of tissues. Regenerative medicine, being the science of restoration of function of the organs/tissues, uses stem cell technology vastly. On the backdrop of such application of stem cell, it is justified to discuss the advancement of LOC in sector stem cell culture.

Stem Cell Stimulation Using LOC System: a Key Ingredient of Regenerative Medicine

Stem cells are multipotent cells, which are sensitive to various physiological and/or environmental cues. Also, it has been a challenge to get targeted differentiation of stem cells. Hence, the investigation of the stem cell differentiation under abiological/biological stimulations in the desired pathway is of great interest for the benefit of regenerative medicine. In conventional petri-dish culture, it is difficult to regulate the concentration of the soluble factors precisely, or near to impossible to apply physical stimulation on the stem cells, keeping the reproducibility unhampered. On the other hand, LOC-based microfluidic chips provide a manageable platform to stimulate cells not only by biochemical cues but also by structural cues, mechanical stress, and electromagnetic forces. For example, Yang et al. recapitulated in vivo-like paracrine signaling of hMScs in 3D ECMs within a microfluidic platform to enhance functional neuronal differentiation of hNScs (Fig. 5) [27]. Genetically engineered hMScs, which overexpressed glial cell-derived neurotrophic factor, were co-cultured with hNScs, leading to reduced glial differentiation of hNScs and enhanced differentiation into neuronal cells, including dopaminergic neurons. Besides, with defined geometries and controlled perfusion flow rates, microfluidic chips provide an in vitro cell culture platform that allows precise mimicking of the shear stress in the physiological environment. Kim et al. reported the increased osteogenic differentiation of hMScs within an osmotic pump-driven microfluidic chip that generates constant and extremely low shear stress [28]. The low shear stress stimulation significantly induced TAZ (transcriptional coactivator with PDZ-binding motif) nuclear localization and transcriptional activity, thereby facilitating osteogenic differentiation. Shi et al. constructed a microfluidic cell culture platform that integrated stretchable PDA-coated parafilm supporting stem cell adhesion and proliferation [29]. Adipose-derived MSCs, which were cultured on the PDA-coated parafilm with grooved micropatterns, exhibited significantly higher osteogenic commitment in response to mechanical and spatial cues, compared to the cells without stretching. Occasionally, multiple stimuli were applied for stem cell differentiation in microfluidic devices. For instance, Winer et al. combined chemical stimulation and shear stress to promote stem cell differentiation [30]. Human placenta-derived multipotent stem cells were successfully cultured on a microfluidic platform and induced to differentiate into neuronal cells by 1-methyl-3-isobutylxanthine stimulation. During this process, different shear forces were applied by adjusting the flow rate of 1-methyl-3-isobutylxanthine solution and was found to accelerate the

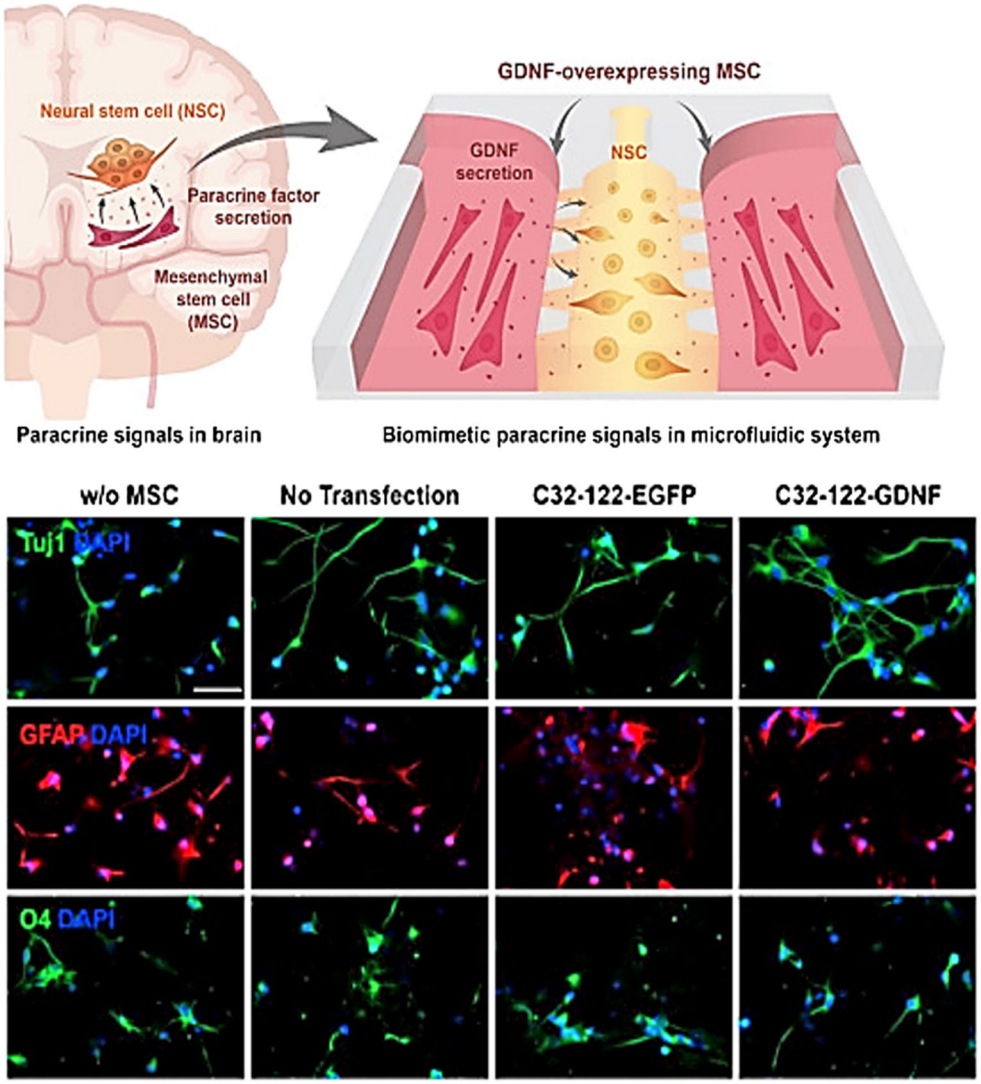

Fig. 5 Use of microfluidic technology in stem cell differentiation. **a** hNSCs are captured in the central channel to effectuate the paracrine effect of the hMSc. **b** The GDNF transfected hMSc could mediate a better paracrine effect onto the hNSc [27]

placenta-derived multipotent stem cell differentiation into neural cells [31].

Advantages of LOC in Adapting Biomimetic Physiological Cues

The introduction of LOC-based biomicrofluidics integrated with the provision of applying biophysical cues like shear and electric field has opened a new era in biological investigation. It has enabled us to understand the fundamental and sophisticated aspects of unexplored phenomena occurring at the subcellular level of living cells. The investigation of neural injury at a molecular level using LOC technology has provided an opportunity to reveal fundamental facts at single cell level [32]. Moreover, the neighboring cells obscure individual cellular functionality. Microfluidic-based microculture devices have scaled down the cell population to a few hundred cells or even at a single cell level. It enables to monitor single cell response in the presence of micro-environmental cues. Some groups have already reported that the thermodynamic, kinetic, and mechanical characteristics of cell locomotion (protrusion, attachment, and translocation) can be better understood with experiments performed at single cell resolution [33, 34]. On the contrary, conventional macroscopic cell cultures typically contain 10^4–10^7 cells. Huge cell numbers should be avoided, because biological assays often end up giving an average result from the participating cells [35].

Several investigators have reported the correlation of cellular behavior with the amount of available nutrient media. Stephan et al. mentioned the influence of growth media on the cell survival [36]. The amount of media provided to each cell is a crucial parameter for simulating in vivo conditions. It cannot be achieved in conventional cell cultures. The kind of cellular environment, provided in conventional cultures with a large amount of nutrients and culture fluid, is not a realistic representation of in vivo conditions.

Cellular co-cultures can be conducted on the same chip, which minimizes the need for multiple experimental setups.

Those have provided a manipulative measure to unveil many underlying phenomena of cell physiology. Tehranirokh et al. mentioned that high throughput cell culture system is not possible with an abundant amount of media (Fig. 6) [41]. This not only dilutes the chemical factors coming from the treated cells but also obscures the effect of minimally concentrated growth factors, which can potentially result in falsified outcomes [41].

Microfluidics has diagnostic applications by enabling isolation of specific diseased cells. It is possible to diagnose oral cancer cells with the help of microfluidic devices [42]. As reported by several researchers, circulating cancerous cells can be isolated and trapped using optical tweezers or antibody-antigen based microfluidic methods [42–45]. Normal cells are also trapped in order to study single-cell mechanisms by exploiting antigen-antibody specificity [46]. Some electrode-based devices also enable a single cell to get trapped for impedance measurements to determine the cell status [47]. Cell migration along with deformation, while passing through narrow channels, can be monitored using microfluidic technology, as a measure of cellular functionality [48]. Disease-specific proteins also can be detected using biosensor-based microfluidic devices. As an example, alpha-fetoprotein, which is considered as a marker of many pathological conditions, can be detected by exploiting such technologies [49]. Even detection of pathogen-based diseases like malaria is achievable on the basis of the deformation recovery-dependent internal viscosity of the RBCs [50]. Single cell analysis is one of the techniques to characterize cell types with perceptible accuracy, because of unmasked output. This is achieved by isolating a single cell from the cell population by trapping the cell onto electrodes and by directly studying the gene expression [51]. Subnuclear biomolecules, like DNA as well as siRNA, mRNA can also be detected quantitatively. It helps us to reveal cellular epigenetics. [52]

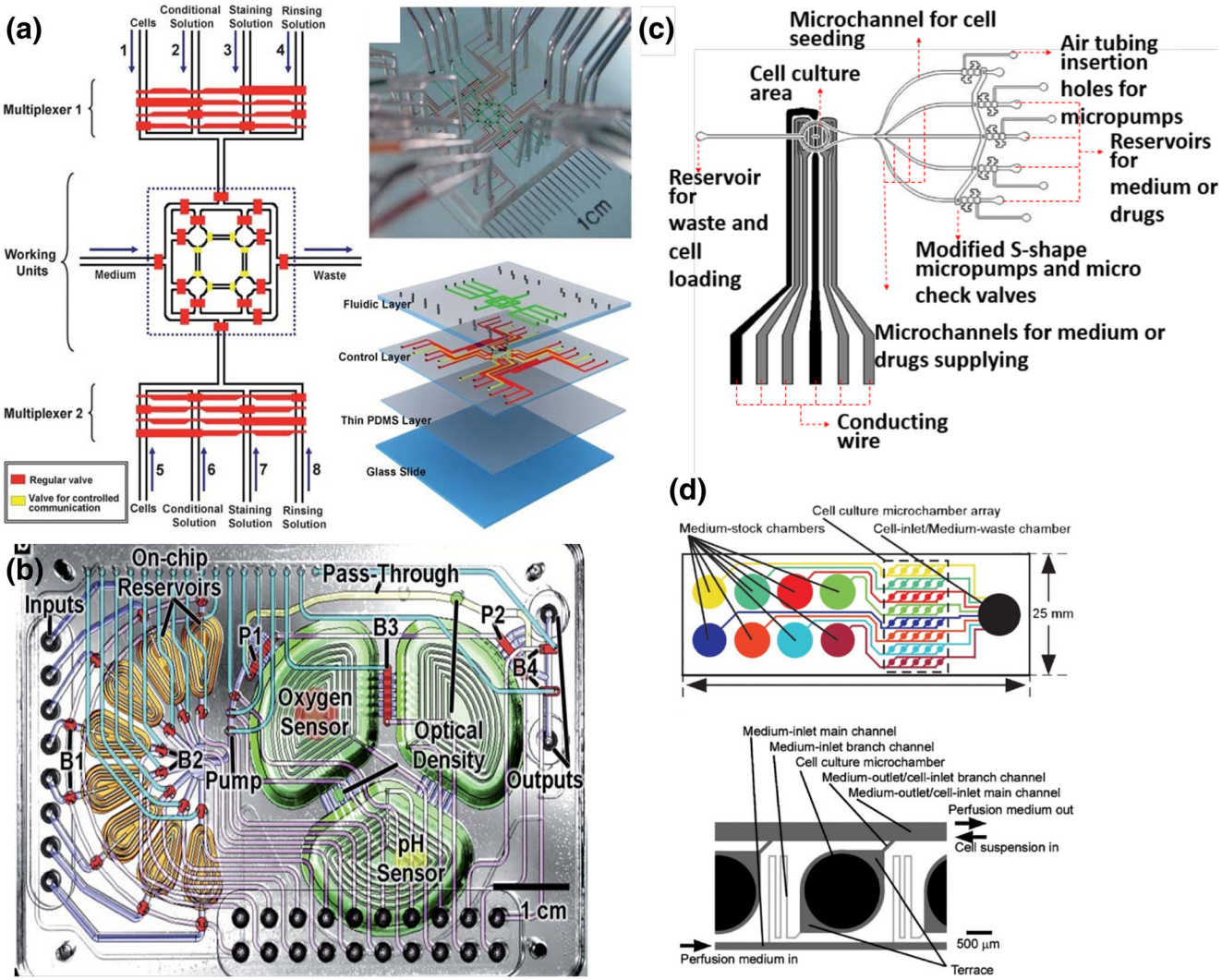

Fig. 6 Integration and multiplexing. **a** Four-layer integrated microfluidic system for studying cell-microenvironmental interactions with four culture chambers [37]. **b** An automated and continuous cell culture device [38]. **c** Illustration of a platform comprising modules for cell culture and temperature control [39]. **d** Microchamber array and microchannel networks of the perfusion culture chip [40, 41]

It has been well documented that cellular migration, proliferation, differentiation, and tissue development are all influenced by the presence of biophysical enhancement, but how these external cues are translated into activated intracellular signaling cascades remains poorly understood. Fortunately, technological improvements in artificial microdevice fabrication have provided the researchers with the tools to test cellular interactions within controlled, biophysical stimuli. We focus especially on integrative approaches that aim to characterize the properties of specific biophysical cues, like shear stress and electric field. Such approaches create a biomimetic environment to elucidate specific cellular response to the individual biophysical cues presented within the LOC device (Fig. 7). These types of comprehensive studies are essential for understanding the impact of extracellular abiological source of stimulus, which can affect at even exceedingly minimal amount. The in vivo physiological homeostasis is essentially maintained by such regulatory factors without which it results in clinical conditions. The cue could be given in the form of electromagnetic stimulus, optical stimulus with laser flash, shear stress following fluid flow, among which shear stress and electric field have an endogenous origin. These have seen to affect the stem cells starting from the embryonic stage up to the adult body in every aspect of physiological as well as pathological conditions. Parate et al. demonstrated that pulsed electromagnetic fields have the capability to recruit calcium-signaling cascades directed towards chondrogenesis. The application of brief (10 min), low-intensity (2 mT) exposure to 6-ms bursts of magnetic pulses, at 15 Hz, only once is optimal for the onset of chondrogenesis. This is mostly mediated downstream by TRP (transient receptor potential) channels [53]. Dong et al. analyzed the effect of shear on stem cell and have found that shear stress significantly increases the expression of endothelial cell markers, such as platelet-endothelial cell adhesion molecule-1 (PECAM-1), VE-cadherin, and CD34, at both the mRNA and protein levels [54]. The undeniable fact of mechanical cue given by the stem biomaterial reveals that the substrate stiffness can direct the stem cell behavior from neurogenesis to osteogenesis [55]. The compliant materials encourage more of neurogenesis (50 to 400 Pa), whereas stiffer facilitates of adipogenesis (800 to 2000 Pa), myogenesis (~ 12 MPa), and osteogenesis (> 20 MPa) [56]. Human adipose-derived stem cells (hADSCs) could be differentiated to smooth muscle lineages, when the stem cells were exposed to a 636-nm diode laser at a power of 5 J/cm^2. The expression of smooth muscle cell markers, β1-integrin and Thy-1, and SMC markers, smooth muscle alpha-actin (SM-αa), desmin, smooth muscle myosin heavy chain (SM-MHC), and smoothelin were found to have elevated post-exposure of the laser [57]. Interestingly, the sound wave could differentiate the hMScs into neural cells by application of sound wave (1 kHz, 81 dB) for 7 days. The cells started to express the neural makers along with Pyk2 and CREB phosphorylation, which could be the upstream effectors for the differentiation process [58].

Fig. 7 Schematic illustration showing various biological aspects involved in cellular differentiation on lab-on-a-chip device. Image showing the impact of the biophysical cues on stem cells through the involvement of the subcellular component. The cues should be optimized as they cause a detrimental effect on the shear/electric field sensitive cells

The outcome of biophysical cues on the stem cell is diverse. The non-canonical regenerative research could be modulated using all these cues which have a huge potential to differentiate the stem cell into stubborn lineages. In this review article, we decided to discuss the detailed effect of shear and the electric field as those also have an endogenous origin.

Application of Biomimetic Physiological Cues in Regenerative Medicine with LOC

The newer advancement regarding the stem cell usage in regenerative medicine has focused on implementation of physiological cues to simulate the body conditions. Shear stress and electric field are most potential biophysical cues to have high efficacy towards directing stem cell behavior with endogenous origin. In this section, both the physiological cues (shear and electric field) were discussed in light of stem cell differentiation using microfluidic and also their usefulness in regenerative medicine.

Shear Stress

The integration of perfusion flow parameters with confinement of the cells, which are constrained by the scaffold architecture of the microchannels, can potentially change the

cellular functionality (Fig. 8). Added to this, shear flow and gradient of the soluble factors also act as critical parameters. This would lead to specific cellular decisions like cell differentiation, apoptotic pre-programming, rate of proliferation etc. All these cell fate processes create a spatiotemporal profile in the microenvironment within the microchannel. Static microscale cell cultures have succeeded in enabling novel experiments to take advantage of controlling cell-cell and cell-matrix interactions, using micropatterning technologies [59–61].

In biomicrofluidics, the shear flow can be realized under fluid flow in customized microchannels. For example, laminar flow profile is maintained inside the rectangular microfluidic culture chambers because of the low Reynolds number (Re) of the flow. The Reynolds number is estimated by the following equation to determine the flow regime inside the channels:

$$Re = \frac{\overline{v} \times d \times \rho}{\mu} \tag{1}$$

$$\overline{v} = \frac{Q}{WH} \tag{2}$$

where $\overline{v}$ is the flow velocity in the channel, W is the width of the channel, H is the height, ρ is the density of the cell media (997 kg/m^3) [62], and μ is the dynamic viscosity of the cell (0.001 Pa s). The fluid flow-induced shear stress on the bottom wall can be approximated by [63]

$$\tau_{wall} = 12\frac{\mu.Q}{\mathrm{W.H}^2} \tag{3}$$

where τ_{wall} is the wall shear stress, μ is the fluid viscosity, Q is the volumetric flow rate, W denotes the channel width, and H represents the channel height. In a recent study from the authors' group, applying the same equation, the shear, experienced by the cells growing on the biomaterials using pitted microfluidic culture device, was determined.

Endogenous Shear Stress: In Vivo Regulator of Cellular Functionality

All the cells within a physiological tissue are subjected to the shear stress, resulting from the flow of the body fluids. The biophysical cue in the form of shear gets translated into downstream intracellular signals, which modify the cellular functions like, e.g., proliferation, plithotaxis, membrane permeability, and cytoskeleton remodeling. These form a vicious cycle in between the gene expression and various protein expressions to execute the effect of the shear stimulus. The ECM uses multiple sensing mechanisms to detect changes in mechanical forces, leading to the activation of signaling networks, which then passes down inside the nuclei to trigger epigenetic gene modification.

Role of Endogenous Shear Stress in Embryogenesis In addition to signaling molecules, shear also plays a pivotal role in the development of embryos during the cardiogenic phases of gestation. The first functional organic system developed in vertebrate embryos is the cardiovascular system, which is initially regulated from the genetic solicitation. The chorionic fluid seems to model arterial and venous gene expression before the formation of blood [64, 65]. Early expression of arterial/venous markers is genetically regulated, but early vascular endothelial markers are expressed by shear stress [64]. The velocity of blood flow exerts laminar flow shear stress in developing embryos in between 0 and 5.5 dynes/cm^2 at the period of 8.5 to10.5 days [66]. The left to right chirality of embryogenesis is also an outcome of shear flow of the extra-embryonic fluid [67, 68]. The forces exerted on the wall are expected to activate intracellular signaling pathways, inducing alterations in gene expression patterns that are at the basis of differentiation steps. As a consequence, abnormal flow patterns and the origin of congenital malformations are closely interlinked. The same holds for disease processes, such as plaque formation after birth and during aging [69]. Groenendijk et al. have described the genes, *klf-2*, *et-1*, and *nos-3*, for shear-based cellular modifications. Interestingly, KLF-2 and NOS-3 have been found to have increased expression in the narrow parts of the cardiovascular system, like the cardiac inflow tract, the atrioventricular canal, outflow tract. They have also intensely appeared in the early stages of the aortic sac and the pharyngeal arch artery formation. The ET-1 is negatively regulated by the shear cues [70]. More interestingly, Boselli et al. investigated that oscillatory flow in the atrioventricular canal, which is responsible for its functional structure. The myocardial

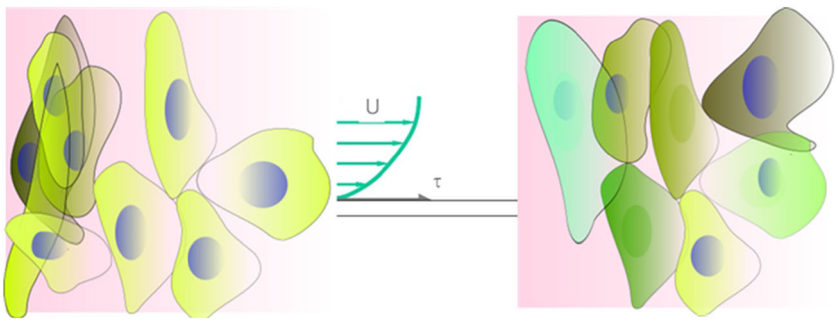

Fig. 8 Shear stress effectively alters the cell behavior. It regulates the cellular functionality by modifying the cell growth pattern, cellular morphology, nuclear shape, and differentiation

contraction and the shear pattern generated through the blood flow provide essential mechanical cues for the developmental morphogenesis of the cardiac system. The specific shear stress patterns, which are established by the flowing of blood, direct anisotropic tissue convergence. This leads to the formation of constriction and inflation of the atrioventricular canal (Fig. 9) [71]. Hence, the importance of shear in maintaining physiological condition could be easily apprehended.

Endogenous Shear Stress as an Essential Cue for Adult Normal Physiology The physiological shear is essential for maintaining the normal functionality of the bodily organs. The shear has effective control on several in vivo phenomena like intima cell proliferation rate, upregulation of angiogenic genes, morphology of the cells etc. Microvascular models were used to recapitulate the effect of the endogenous shear onto the directly shear exposing cell of the body which are the endothelial cells of the blood vessel lining. Li et al. have intrigued experimented the molecular mediator as a cause for the shear sensitivity of the cells. The Piezo1 ion channel responds to the shear stress and brings out the efficacy of this biophysical cue (Fig. 10) [72]. The alignment of the cells with effective cell shape is an essential phenotypic manifestation of the endogenous shear.

Exogenous Shear Stress: In Vitro Regulator for Cellular Decision

The stem cell differentiation could be regulated by applying mechanical forces. The cellular/molecular method of converting the mechanical force into the biological response is mediated by the interplay between integrin and FLK-1 signaling [73]. According to Liu et al., several intracellular signaling molecules are recruited in the mechanotransduction of hMScs, such as MAPK, NO/cGMP/PKG, and Ca^{2+} [74]. The same authors have claimed ERK1/2 to be the central molecule for accomplishing this mechanism. These together help to determine the cell fate.

Shear-Induced Osteogenesis Many researchers have reported that subtle shear, exerted by the dynamic fluid environment, plays a pivotal role to direct the cells towards proliferation,

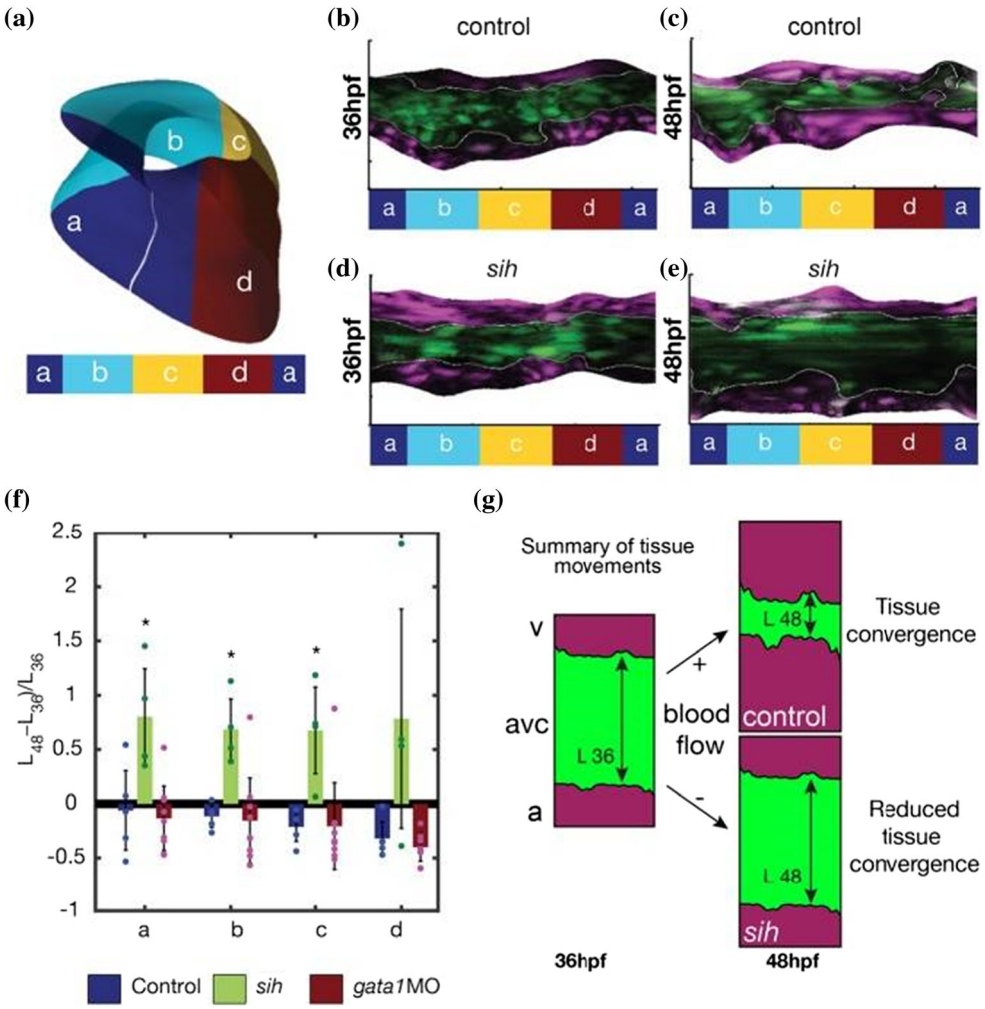

Fig. 9 Cardiovascular system development of the embryo is dependent on the shear exerted by the blood flow. **a** The atrioventricular canal folds to form the different structures of the heart. **b**–**e** At a different gestational time point of 36 and 48 h post-fertilization, the unfolded atrioventricular canal shows assigned part of the different structures. The sih or the silent heart without blood flow shows lesser tissue migration than the control at 48 hpf. **f** The convergence index is positive for the sih form of embryo which indicates the canal width has been increased instead of the converging. **g** Summary showing the less atrial and ventricular tissue convergence without the blood flow at 48 hpf [71]

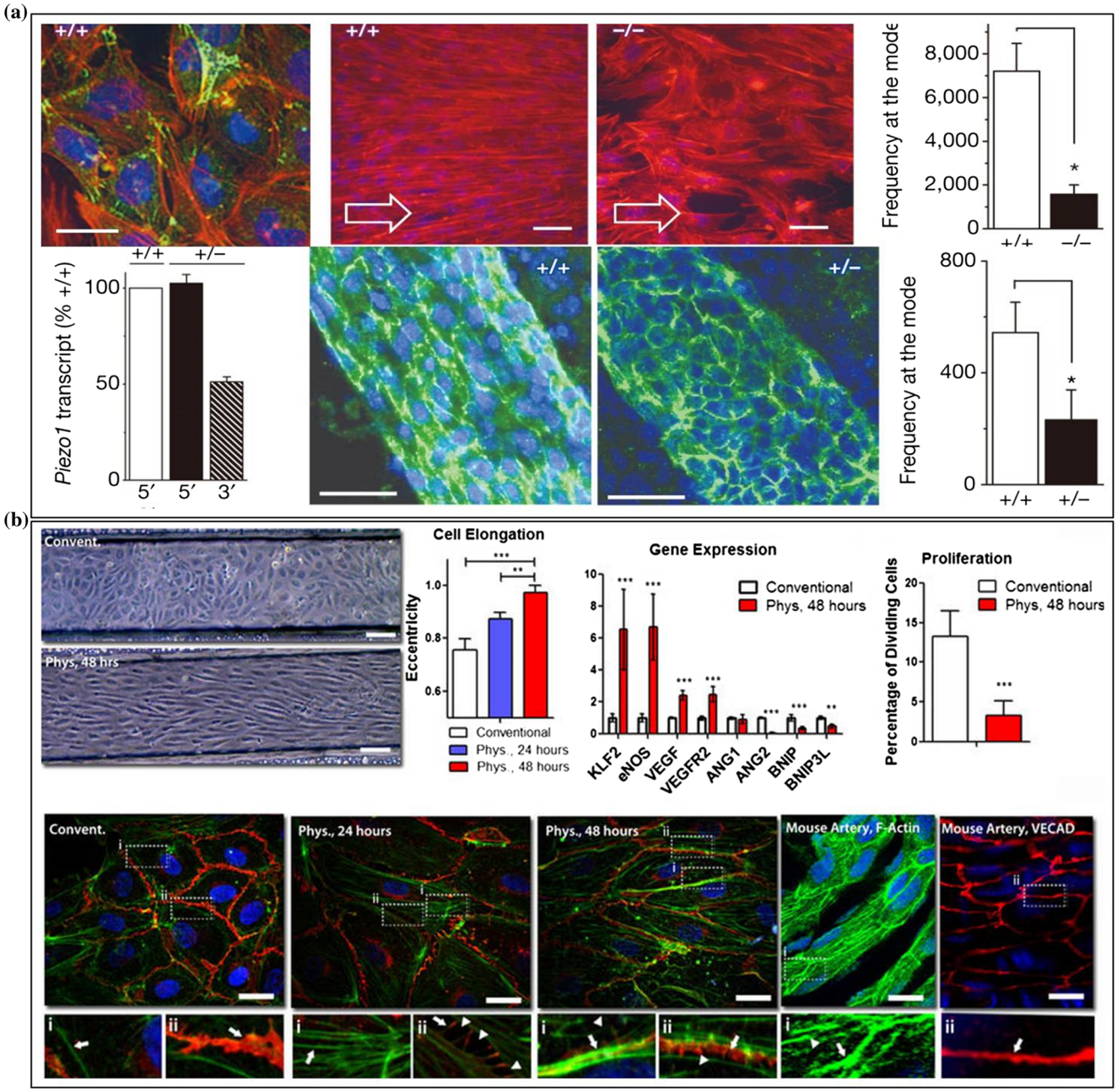

Fig. 10 In case of the adult body, the endothelial cell morphology is controlled by the shear exerted by the blood flow. **a** The molecular mechanism is involved with expression of a particular protein known as PIEZO1. It helps in the alignment of the endothelial cells. The wild-type (+/+) and control with normal expression of the protein dictates an aligned morphology whereas the null mutant (−/−) fails to do so. Even the hydride allele show disturbances in the cellular alignment. **b** A flow induces aligned pattern of the cells with increased elongated cell morphology, increased expression of some of the targeted genes. The endothelial lining of mouse artery depicts the cellular adaptation to physiological shear on the basis of actin organization at 24 and 48 h of shear stimulation: the lower panel first image shows (i) cortical actin of control and (ii) continuous adherens junctions of control without shear; the second image shows (i) stress fibers and (ii) membrane protrusion after 24 h stimulation, and the third and fourth image shows (i) cortical actin rim formation after 48 h of shear application, with (ii) and (ii) reestablished adherens junctions (indicated by arrows in the respective images). The last image of the lower panel describes the expression of endothelial CAD [72]

differentiation, formation of functional structures, and release of chemical factors [75, 76]. For example, Yourek et al. investigated that shear stimulation (0.9 Pa shear stress continuously for 3 days) in osteogenic media is sufficient to induce osteogenesis of hMSc [77]. Liu et al. reported that intermittent fluid shear stress (IFSS) acts as a potent and physiologically relevant cue to differentiate the hMScs towards osteogenesis, through transient receptor potential melastatin 7 (TRPM7)-Osterix signaling pathway [78]. Moreover, TAZ (transcriptional coactivator with PDZ-binding motif) and YAP (Yes-

associated protein) were recently characterized as the signaling mediators of mechanotransduction [79, 80]. To perform the transcriptional regulation, TAZ and YAP interact with several transcription factors, like Runx2, which mediates the osteogenic differentiation [81].

Kim et al. have used a channel 4 mm wide, 10 mm long, and 200 μm high as the central microchip, which was connected to a 1-ml microtip. This has served as the reservoir for the media to flow inside the channel. The outlet has been connected to the pump to ensure the flow of media through the channel. In this process, 80% of the cells were subjected to 0.012–0.015 Pa shear, which could be correlated to the in vivo interstitial flow. The shear increases the localization of the TAZ/YAP in the nuclear region, which drives hMScs towards osteogenesis (Fig. 11) [28].

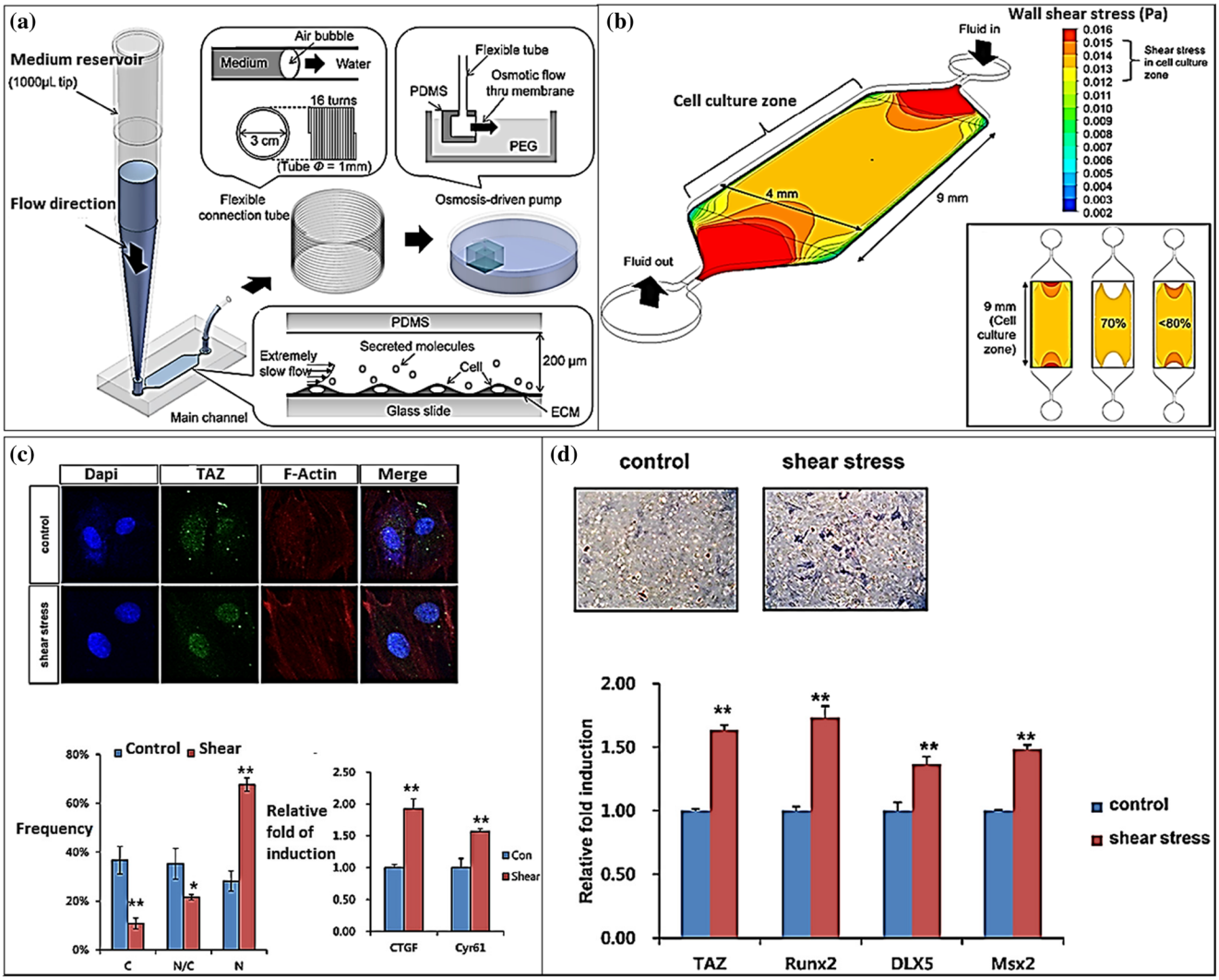

Fig. 11 TAZ is one of the intracellular molecule which deals with the tranduction mechanism to convert the mechanical cue into biological signal. **a** The microfluidic system composed of a 1-ml microtip which was used as an inlet reservoir. The both tapered channel where cells were cultured was exposed to shear flow, using a coiled tube and an osmotic pump dipped into a Petri dish containing 0.1 M PEG solution. **b** The coiled tube had a large capacity that served as an outlet reservoir providing clean water to the osmotic pump. An air bubble moved through the tube to prevent the mixing of medium and water. **c** The osmotic flow was generated at the membrane window by the concentration difference between water and PEG solution. **d** Cells in the main microchannel (200-μm height) were exposed to an extremely slow interstitial level of flow. **b** The rectangular area (9 mm × 4 mm) of the main microchannel served as the cell culture zone. The solution through finite element method reveals that 80% of the area has an acceptable uniformity. **c** MSCs were loaded into the microfluidic chip, and osmotic pressure-driven shear stress was applied to the cell residing inside the chip. Stationary cultures were used as controls. After 48 h of culture under ~ 2 Pa of shear, the cells were fixed and analyzed for TAZ using immunocytochemistry. It has been observed that shear increases nuclear localization of TAZ. **d** As a marker of osteogenesis, the alkaline phosphatase activity has been monitored which has been elevated by application of shear stress with respect to the control cells which were incubated in the osteogenic differentiation media without shear stress. The relative expression levels of TAZ, Runx2, DLX5, and Msx2 were determined by qRT-PCR after normalization to the GAPDH level. $^{**}p < 0.01$, *t* test [28]

Shear-Induced Endothelial Differentiation Mechanical stimulation in the form of shear is an essential regulator of tissue homeostasis. It is indispensable for the normal function of connective tissues. A recent study by Yuan et al. has focused on applying shear stimulation on the stem cells. They have successfully differentiated the stem cell into endothelial cells [82]. The western blot analysis has confirmed the expression of von Willebrand factor (vWF), VE-cadherin, and CD31-like endothelial markers when the cells were exposed to a shear stress of 2 Pa for 5 consecutive days [82]. The microfluidic setup used by the investigators was composed of a flow chamber developed by sandwiching a silicone gasket between an input/output unit and a cell-seeded culture dish. They concluded that the differentiation in the path of endothelial cell type was due to upregulated expression of vascular endothelial growth factor (VEGF). The authors have claimed that the stimulation of VEGF secretion by the cells recruits the stem cell more into endothelial differentiation, which could lead towards successful regenerative vascular therapy (Fig. 12).

Electric Field

The cell membrane and cytoplasm have specific electrical properties, which can influence cell functionality. Each cell has a characteristic potential difference across the membrane, denoted as transmembrane potential. Also, the transmembrane potential in the absence of any perturbation is termed as resting membrane potential. In comparison to the intracellular side, the extracellular surface is positive due to the presence of an asymmetric ionic distribution of three major ions; Na+, K+, and leaky ions (L). Moreover, the negatively charged proteins, which are present inside the cell, contribute to the

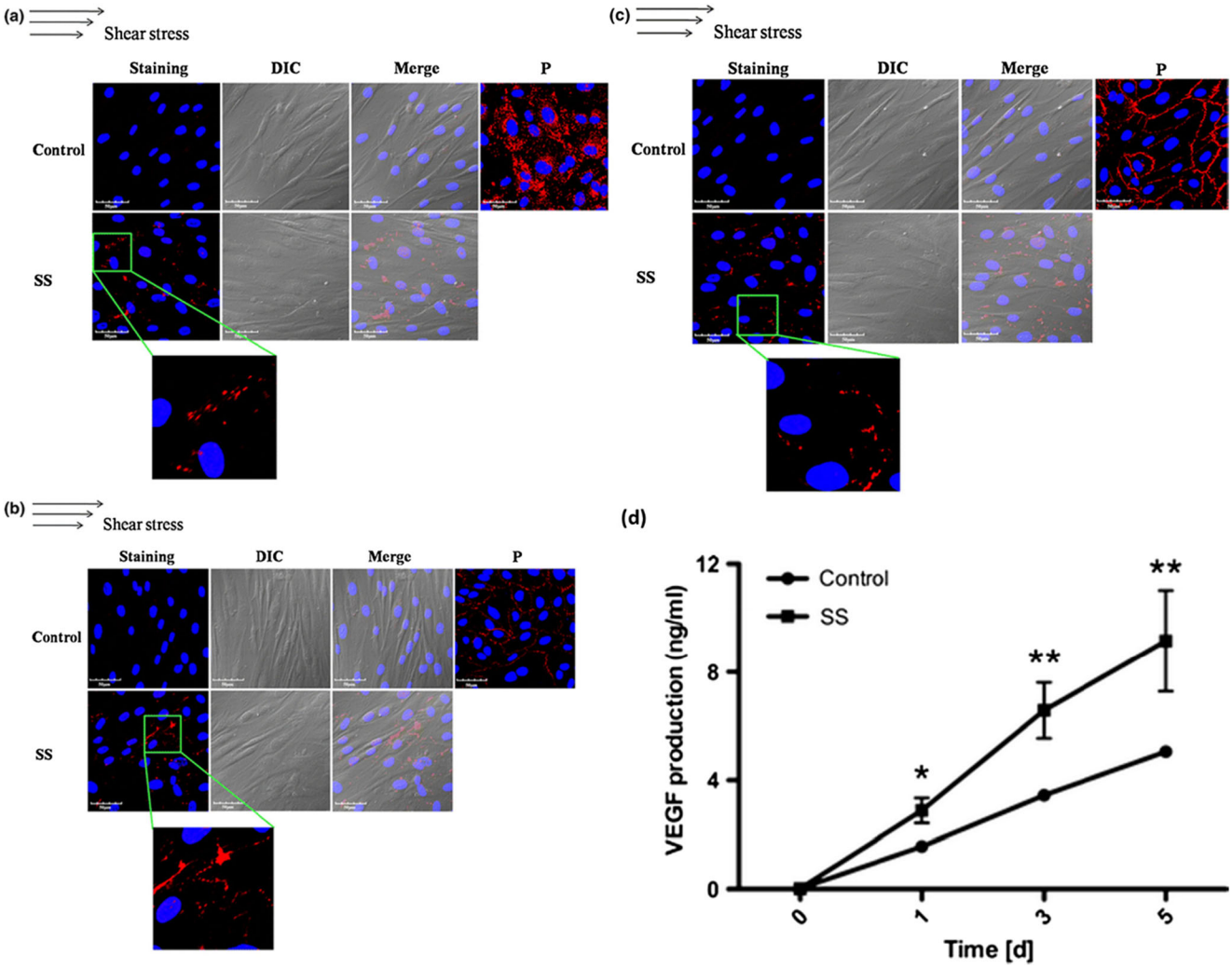

Fig. 12 The applied shear on the stem cells growing in a microfluidic setup show increased expression of endothelial markers. The range of 0.2–2 Pa of shear has efficiently brought out the endothelial differentiation of the stem cells. The wide range of shear resembles venous to arterial physiological shear. **a** vWF expression is increased for the cells growing under shear. **b** Another shear marker VE-cadherin was also found to have increased expression. **c** Similarly, the CD31 was high in the cell exposed to shear stress. **d** The reason behind the endothelial marker expression is due to elevated secretion VEGF by the shear stimulated cell than that of the control [82]

negativity of the inner surface (Gibbs-Donnan effect), but they do not contribute to the electrical circuitry of the cell, because they cannot move across the membrane. The membrane is composed of phospholipid bilayer (major component) along the ion channels of high specificity towards a particular ion. According to Hodgkin-Huxley model (Fig. 13), the cell can be constructed as resistor-capacitor circuit (RC circuit) using the basic electric circuitry components, like voltage source, resistance, and capacitance. The cell membrane is considered as the capacitor, which holds the charges. The membrane potential difference provides the input voltage with ion channels as resistors. The ion permeability of the membrane for sodium, potassium, and other ions is taken into account in terms of a sodium, potassium, and leakage ion channel conductance. This is the source of endogenous electric field.

The external electric field is used in various biomedical applications, both in invasive and non-invasive manner. For example, the application of low-intensity direct electric fields has been experimentally used in the clinics to treat a number of brain disorders, predominantly using transcranial direct current stimulation approaches [83]. Low-intensity, intermediate-frequency (100–300 kHz) alternating electric fields, delivered by means of insulated electrodes, were found to have a profound inhibitory effect on the growth rate of a variety of human and rodent cell lines [84]. Some researchers have reported that applied steady electric fields within a range of 0.1 to 10 V/cm were found to have marked effects on the neurite growth of single isolated Xenopus neurons in culture [85]. An extracellular electric field can alter the migration, can shape the morphology, and can influence the growth of a number of cell types [86].

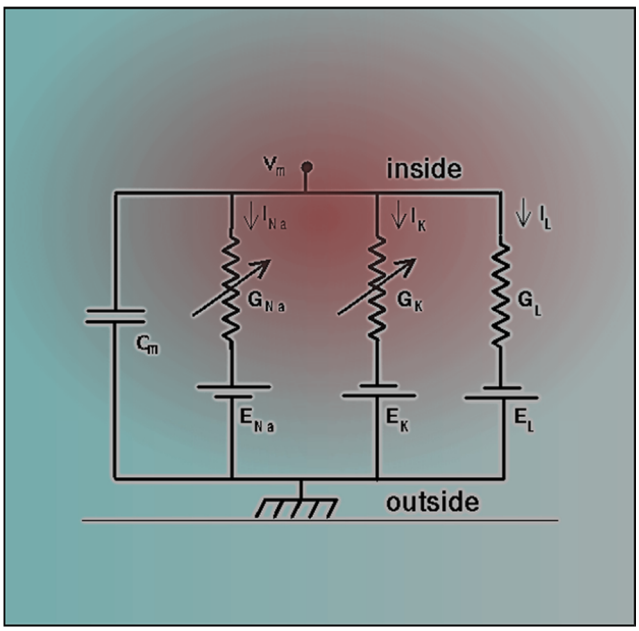

Fig. 13 The Hodgkin-Huxley model describes the origin of the potential difference in the cells which is the source of endogenous electric field. V_{mem} is the transmembrane voltage difference, which is usually cell specific. For example, V_{mem} is − 65 mV for muscle, − 30 mV for endothelial cell, and − 90 mV for cardiac muscle cell. C_{mem} is the capacitance of the cell membrane. I is the flow of current due to flow of sodium ions, potassium ions and other leaky ions (I_{Na}, I_K, I_L). G stands for the conductance for the abovementioned three specific ions. E is the potential difference created because of ion distribution difference for these three ions

In the backdrop of the above discussion, the stem cell functionality could be monitored under the influence of electric application of electric field. For example, Thrivikraman et al. have demonstrated that the differentiation potential could be enhanced by applying an electric field of 2 V/mm [87]. The cells tend to get differentiated in neural lineages when they were grown on conductive substrates. Nanoactuators could be implemented to drive the stem cell towards neurogenic and cardiogenic profile by changing the application pattern of the pulses [88]. Jain et al. have observed N2A cell differentiation towards neural maturation after applying the electric field [2]. The electric field, being such a potent biophysical cue with the endogenous origin, should be explored to reveal its efficacy towards the living cellular systems. On creating a biomimetic environment using LOC based biomicrofluidic devices, the non-canonical culture system would provide advantages to regenerative medicine approaches.

Endogenous Electric Field: In Vivo Regulator of Cellular Functionality

Role of the Endogenous Electric Field in Embryogenesis The embryos maintain ionic currents within themselves, which plays the role as the source of internal electric fields. While measuring the electric field in the chick embryos, it was found to be an amount of ~ 20 mV/mm. Two- to 4-day-old embryos were chosen for such study, and it was measured near the posterior intestinal portal. The electric field is imperative for the development of tail structures as it was observed that decreasing the magnitude results in abnormal tail development. The similar magnitude of the electric field has been detected for axolotl at the rostral-caudal area. Any perturbation of this electric field during neurulation causes developmental anomalies. However, the gastrulation process was determined to be insensitive to such experimental modifications. The development-specific embryonic left-right chirality in frog and chick embryos is a consequence of the voltage difference between blastomeres at the initial stages of development. This field was measured for the chick embryo to have ~ 10–20 mV/mm across the primitive streak [89].

Hotary et al. explored that the ionic currents leaving the posterior intestinal portal were found to be significantly less in phenotypically abnormal mutants than in wild-type and phenotypically normal embryos [90]. They have used conductive and non-conductive implants to the shunt, the current, and thereof perturbing the current. As the current flow is the source of the field, it has been found that the electric field

was lowered about 30% for the conductive implants, which has resulted in the tail region anomaly [90].

Endogenous Electric Field as an Essential Cue for Adult Normal Physiology Wounds could be demarked as disruption of epithelial layers, traumatizing the visceral mass of an organ in the body. Wounds and damaged tissues were detected with endogenous electric fields which play potential roles in tissue repair and regeneration. The intact epithelia maintain a steady potential difference across themselves, which initiates an altered injury current at the region of the wound. The generated electric field has been measured to be in multiple experimental cases as ~ 40–200 mV/mm [91]. The keratinocytes along with the epithelia are capable to detect electric fields of this altered magnitude and start to respond in site-directed galvanotaxis. The growth factors induced intracellular kinase activity along with Ca^{2+} influx, which leads to active several cellular signaling pathways. Various cell type uses diverse signaling strategies. For example, protein kinase C-mediated pathway is required by neural crest cells and cAMP-dependent protein kinase is used by keratinocytes while MAPK is required by corneal epithelial cells. Experimental evidences indicate 25% slower wound healing when the electric field was removed. Moreover, inspired from the experimental confirmation of in vivo occurrence of the endogenous electric field, in recent days, clinical trials were performed deploying electric fields to stimulate healing rates of the wounds. The outcome came as expected with an elevated rate of 50% higher wound healing from as low as 13% [91].

Marselli et al. described the rigorous process of electric field generation and maintenance by the cells. The intact epithelial layer of cuboidal cell restricts the free ion flow due to the presence of several types of cell-to-cell junction apparatus. This motivates the formation of a physiological electric field of 50 mV/mm. The rupture of the cell layer due to wound forms short-circuiting and alters the electric field. Similarly, they have explored the inside story of endogenous electric field versus electric field at the wounds in neurons, which was found to have deviation from the physiological field due to low-resistance circuit formation in case of injury (Fig. 14) [92].

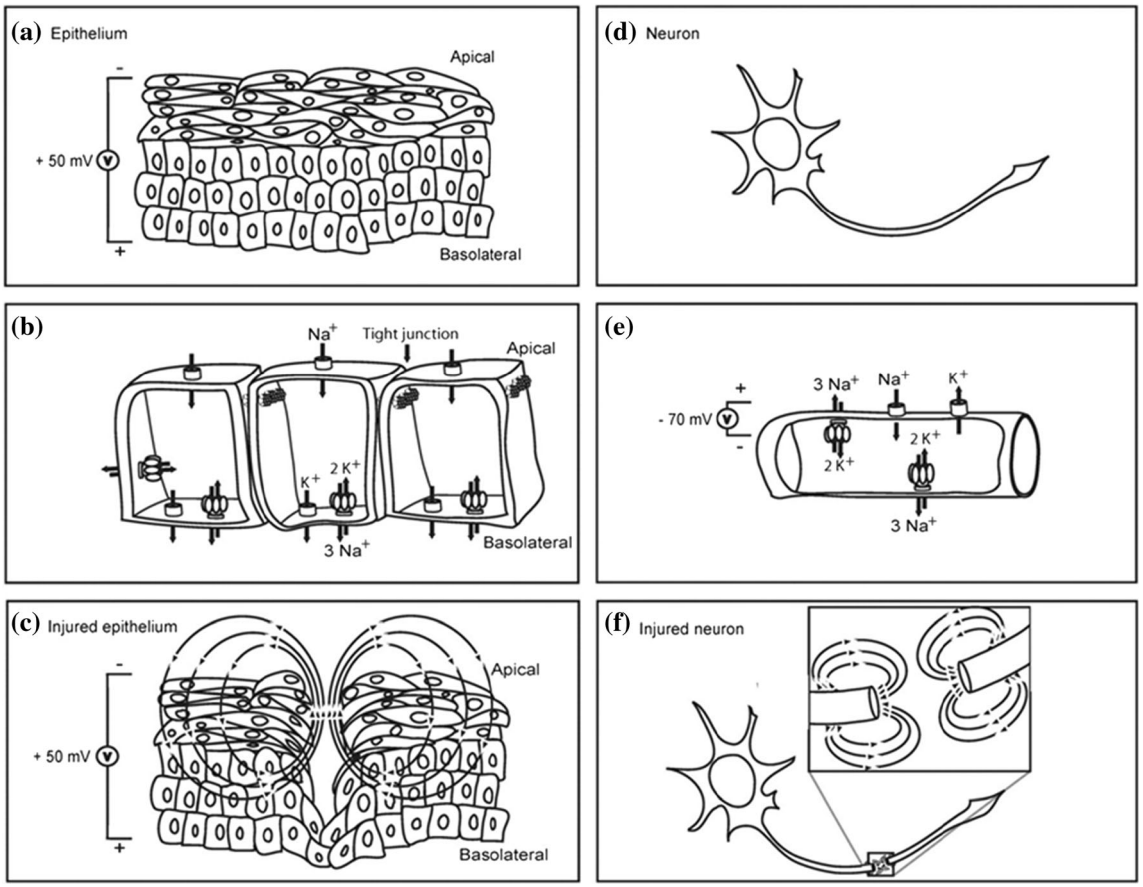

Fig. 14 Source of endogenous electric field and disturbances during wound formation. (**a**) Physiologically, 50 mV potential difference is maintained by the intact non-keratinocyte epithelium. **b** The adherens junction control restricted flow of ions in normal conditions with physiological exchange of sodium and potassium ions by Na-K-ATPase. **c** Injured epithelial layers form electrical shunts which disturb the local field. **d**–**e** Intact neuron maintains the electric field by exchange of Na^+ and K^+ ion flow. **f** Injured axon causes low-resistance circuit which lower the electric field strength [92]

Exogenous Electric Field: In Vitro Regulator for Cellular Decision

Nanoactuation by Electric Field Exposure can Drive Neurogenesis/Cardiomyogenesis Thivikraman et al. elucidated nanomechanical intervention on stem cells through internalized nanoelectroactuators [88]. The same authors stated that electroactuation has been found to be an effective strategy to define stem cell differentiation which is applicable for tissue engineering and regenerative medicine. This study explains that the physical force exerted by the electroactive gold nanoparticles (GNPs) has a strong influence on the regulation of human mesenchymal stem cell (hMSc) commitment, towards a specific lineage. The combination of intracellular and extracellular GNP as a nanomanipulator is designed to bring out neurogenic/cardiomyogenic differentiation in hMSc under electric field stimulation (Fig. 15). For example, h-MScs were treated with intermittent physiologically relevant direct current or pulsed electric field stimulation, respectively, to mimic the in vitro microenvironment of development and maintenance of neural and cardiac tissue. Upon exposure to a regular intermittent cycle of DC electric field stimulation, the majority of GNP-driven hMScs were characterized with elongated filamentous protrusions with several neural-like structural branching points.

These morphological changes corroborate well with higher levels of mRNA expression of certain neuronal markers. Concomitantly, pulsatile electric field has induced morphological changes through cardiomyogenic pathways, transforming them into tubular phenotype associated with upregulation of specific cardiac markers. The observed effects were seen to be significantly supported by intracellular calcium. Hence, such dual-mode stimulation protocol has enabled the differentiation of the DC-EF stricken cells into neural-like cells and PEF-treated cells into cardiomyocyte-like cells through the effective intervention of the GNP nanoactuation. It has been concluded by the authors that such a multipotential approach can be a novel approach to deal with the tissue wastage during cerebral injury or cardiac failure [88].

Electrical Stimulation of Carbon Nanotubes Leads to Cardiomyogenesis Poor regeneration and repair capability of the cardiomyocytes has led the cardiac regenerative medicine at the forefront of biomedical engineering. Mooney et al. successfully addressed this limitation by using electroactive carbon nanotubes (CNTs), which they

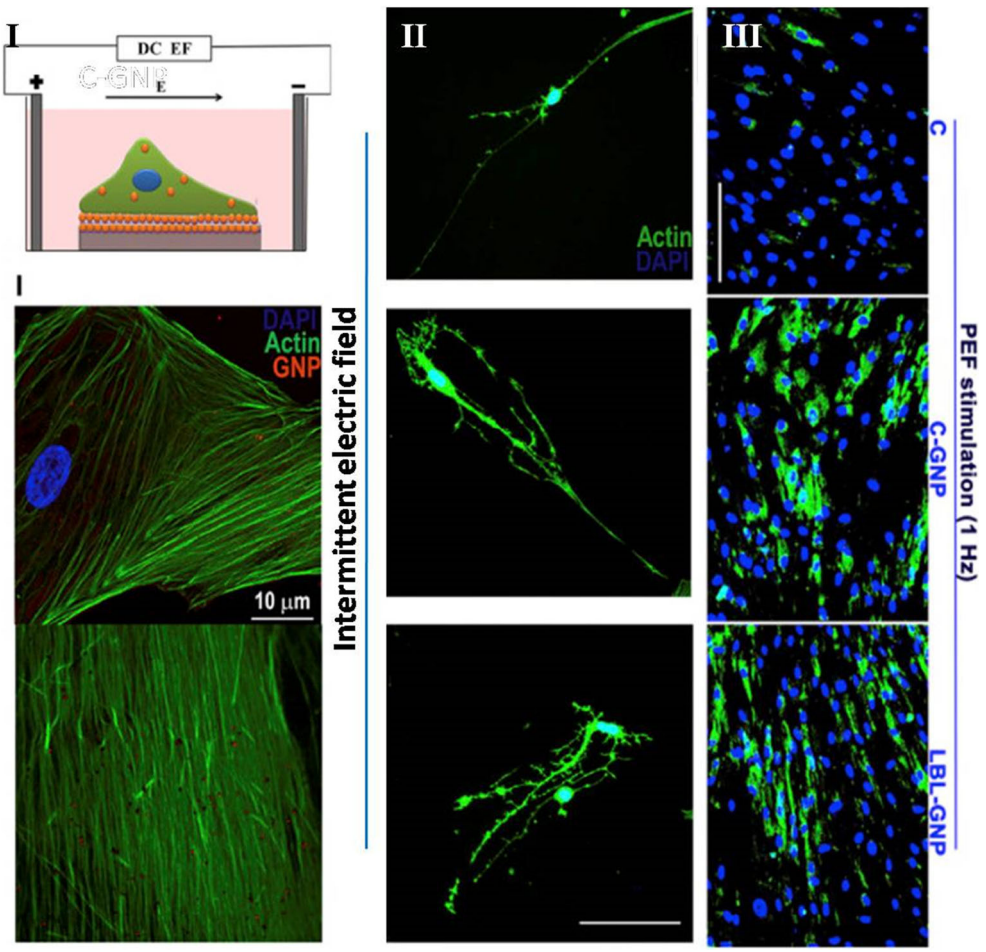

Fig. 15 Electric field-induced nanoactuation leads stem cells towards differentiation. (I) GNPs as an internal conduit for the manipulation of hMSCs by external electric fields. Confocal images showing the internalization of the GNP embedded inside the actin filaments. (II) The intermittent electric field stimulation found to be neurogenic over the hMScs. The representative images depict the filamentous process formation of the hMScs resembling neural-like feature. (III) Immunofluorescence staining revealing the expression of cardiac Troponin I in hMSCs grown pulsatile electric field stimulation of 100 mV/cm 1 Hz in cycles of 15 min/day. Cell nuclei were stained with Hoechst 33,258. Scale bar 100 μm [88]

have shown to deploy the mesenchymal stem cell (MSC) differentiated through cardiomyocytic lineage (Fig. 16) [93]. They have implemented two approaches of exposing MSCs to the medium containing CNT and seeding the same cell type onto CNT-polylactic acid composite scaffolds. The researchers have applied the electric field stimulation using an electrophysiological bioreactor. The applied electrical stimulation has been seen to effectively reorient the cells perpendicular to the direction of the current and adopted an elongated morphology. The gene-level expression was determined through qPCR, which was observed with an elevation of specific cardiac markers, like cardiac myosin heavy chain (CMHC) significantly in both the cases. The western blot analysis was carried out to determine protein expression of cardioprogenitor cell-specific markers like Nkx2.5, GATA-4, cardiac troponin t (CTT), and connexin43 (C43). All of them were upregulated, as confirmed by the immunofluorescent staining. C43 staining has revealed the efficient cross-talk of the cells which is the signature of cell-to-cell simulation after electrical stimulation. The authors claimed that the outcome demonstrates an exemplary paradigm for electrical

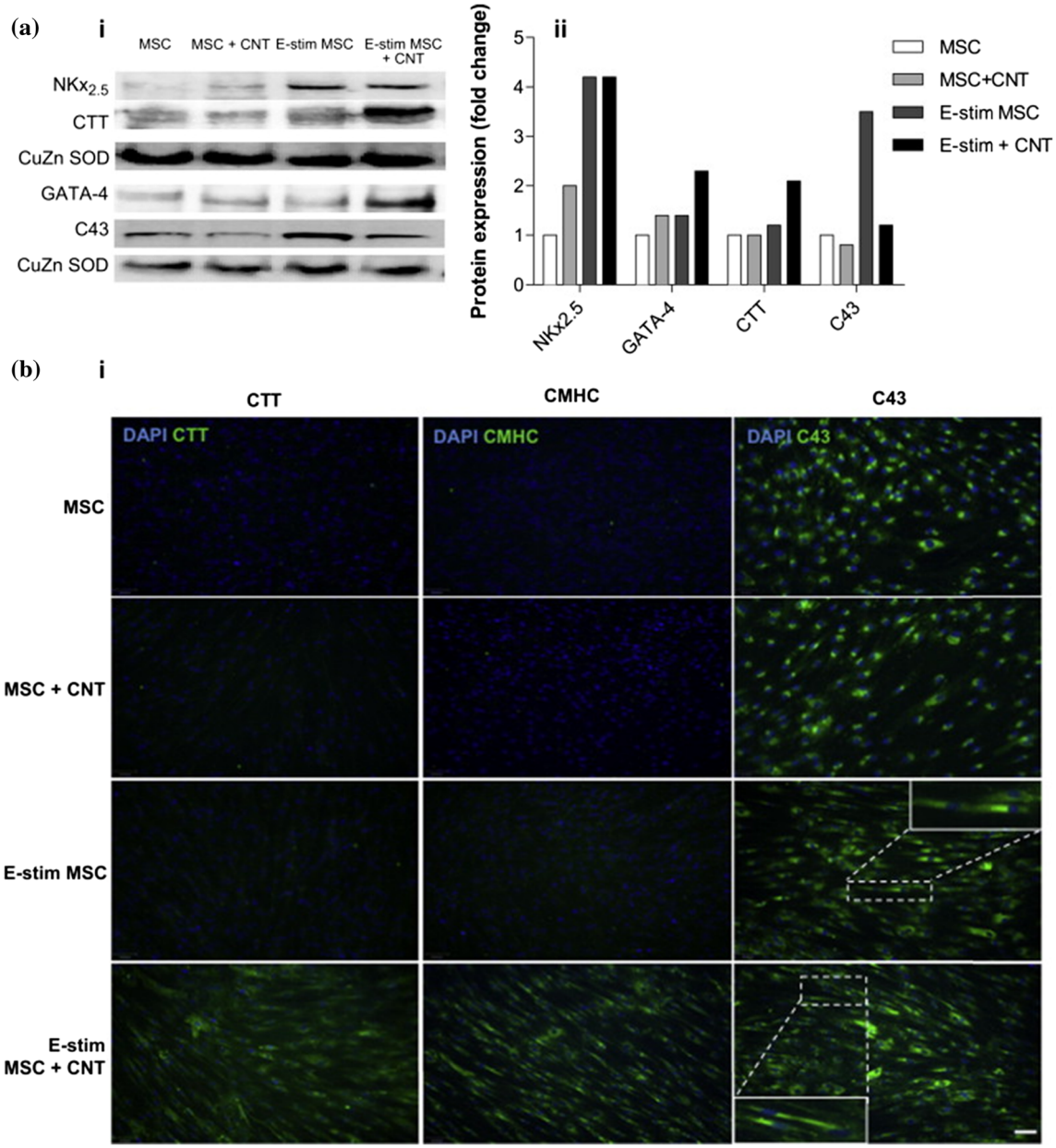

Fig. 16 MSc seeded on carbon nanotube with electric stimulus drive the cells towards cardiomyocytes. **a** Cardiac marker expression reveals the cardiomyogenesis (i) quantification of the marker expression by western blot through densitometric analysis. CuZn SOD used to normalization. **b** Immunofluorescence staining for cardiac troponin T, cardiac myosin heavy chain, and connexin43; MSC cultured in carbon nanotube containing media and scaffold have upregulated markers [93]

cues at nanoscale to recreate cardiomimetic microenvironment. This strategy has been conferred with the potency to be translated to other regenerative applications of electrically sensitive tissues [93].

Electrical Stimulus Drives the Cells into Osteoinductive Path Zhang et al. reported that galvanotaxis and osteogenic differentiation of human adipose-derived mesenchymal stem cells upon electrical stimulation (Fig. 17) [94]. In their experiments, cells, while growing on electroconductive PCL-PPy scaffold, were exposed to electric stimuli of 200 μA direct current for 4 h per day. They have proven the consecutive roles played by the voltage-gated ion channels (Ca^{2+}v, Na^{+}v, K^{+}v, and Cl^{-}v) in the differentiation process. The authors deduced the results from these experiments that electrical stimulation has regulated the migratory and differentiative behavior with application of electrical stimulation. Electric field has been applied to cells in LOCs to stimulate electrically sensitive (stem cells) or stimulated cells (nerve or muscle cells) and to modifying the cellular expression/functionality. Other than modulating cellular behavior by applying electric field, the same can be even used for delivering drugs, proteins, and nucleic acids into cells for cellular manipulation. Disease prognosis with electric field has also been seen to be effective. Oncogenic cells are found to have altered functionality under the influence of electric field. In contrast to several advantages, microscale electric field distribution, however, deviates from this estimation, due to the highly non-linear nature of the distribution pattern [94].

LOC Platform for Shear and Electric Field Applications: a Success Tool for Regenerative Medicine Applications

LOC Devices for Shear Stress Application

Lu et al. has intervened the cell adhesion and related cell mechanics using suitable microfluidic devices. They have manipulated the geometry and surface chemistry of the microdevices to vary the shear force (Fig. 18) [95]. The time-lapse video-microscopy has revealed the dynamics of cell detachment under different flow conditions. They demonstrated the assessment of cell adhesion to fibronectin-coated substrates as a function of the shear stress or fibronectin concentration in microchannels. Furthermore, a combined perfusion-shear device is designed to maintain cell viability for long-term culture as well as to introduce exogenous reagents for biochemical studies of cell adhesion regulation. Well corroborated with the pre-existing work in this field, it has been observed that fibroblasts have reduced adhesion strength in response to epidermal growth factor stimulation.

The designed two short-term devices were fabricated with PDMS by casting it onto a silicon wafer procured by UV lithography. The two types of devices have specifications that those could accommodate different surface chemistries or cell types (multi-sample) and the multishear device, capable of generating different shear stresses. The continuous flow of the medium was used to provide the cells with the necessary nutrient supply. It was observed that the cell adhesion is not only dependent on the substrate-to-cell interaction but also on the soluble growth factors. The treatment of epidermal growth factor (EDGF) had loosening effect on the cells. The treated one got removed with a high amount of shear of 640 Pa applied for 3 min, whereas the control could withstand the shear up to 9 min. This device could be useful as an analytical chip for the upstream attachment of cell culture system [95].

Shear Stress Stimulation to Modulate Stem Cell Using LOC It is important to discuss the relevance of stem cell functionality modulation to understand the possible advancement regarding regenerative medicine. Geun-Chung et al. have described a gradient-generating microfluidic platform for optimizing proliferation and differentiation of neural stem cells (NSCs) in culture under shear (Fig. 19) [96]. They have successfully created the platform of a concentration gradient of growth factors under continuous flow to target the cell for neural differentiation. The rationale for using the continuous flow is to minimize autocrine and paracrine signaling, which will give unbiased outcome solely from the shear of the growth factor containing media. Human NSCs (hNSCs) were maintained under the culture condition for more than 1 week in the microfluidic device while constantly exposed to a continuous

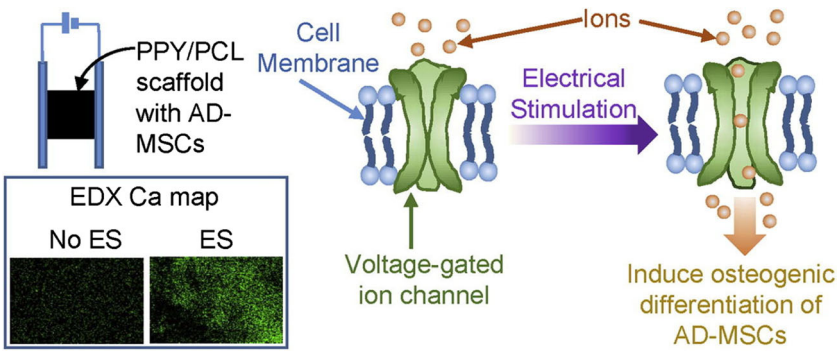

Fig. 17 Human adipose derived-mesenchymal stem cells growing on PPY/PCL scaffold shows increased galvanotaxis when exposed to 200 μA direct current for 4 h per day together with enhanced osteogenesis [94]

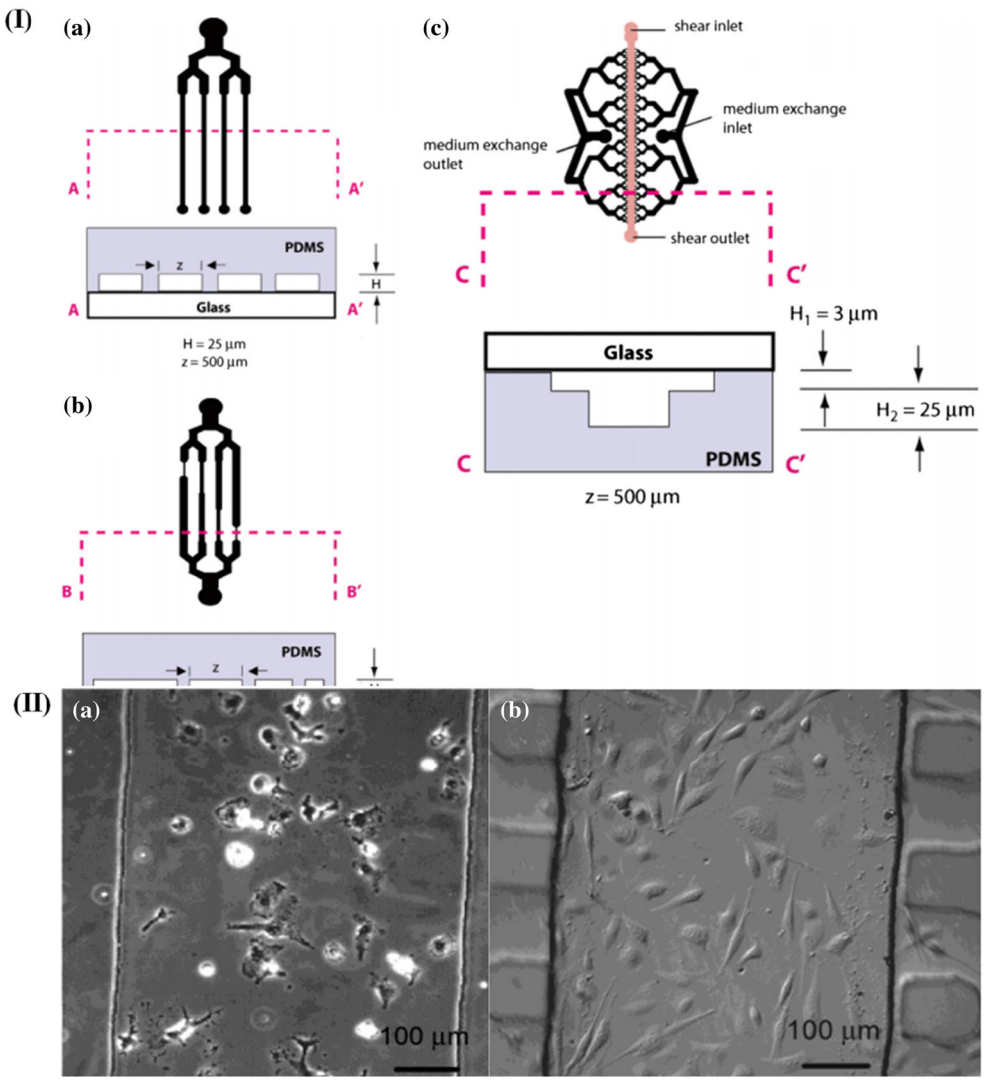

Fig. 18 (I) **a**, **b** Short-term multisampling device for cell culture where a range of shear could be accommodated. **c** Long-term cell culture device for determining adhesion assay incorporated with uniform nutrient supply. (II) **a** The image shows dead cells due to not replenishing the culture media for 12 h. **b** Medium perfusion in continuous mode keep the cells viable and healthy even after 15 h of culture [95]

gradient of a growth factor mixture containing epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), and platelet-derived growth factor (PDGF). The stem cells were differentiated into astrocytes, which were monitored by time-lapse microscopy and immunocytochemistry.

LOC Devices for Electric Field Application

The electric field is an obligatory component of some microfluidic systems, like Micro-Total Analysis System (μ-TAS) and Biomedical Microelectromechanical System (Bio-MEMS) [97, 98]. Lab-on-a-chip (LOC) devices also incorporate the use of the electric field in non-mandate fashion with application-specific manner. For example, electrical dissolution on a portable LOC device can be fabricated for point-of-care (POC) diagnostic purposes. Wei et al. actively developed POC devices that perform DNA detection in a few seconds. This will have application-oriented benefits for criminal investigations and forensic procedures [99].

Uniform or non-uniformly distributed of the electric fields can be effective for regulating biological phenomena, like cell lysis, cell membrane electroporation, electrophoretic separation, nucleic acid detection and separation, and electro-osmotic flow generation for electrokinetic microfluidic pumps.

In the pursuit to develop microdevices for electrical stimulation study, the placement of the electrodes could be either in contact or in a non-contact fashion with the microchannel. The contact mode should ensure the design of the electrodes, wherein they are kept in touch with the complete cross-section at both the terminals of the microchannel. In non-contact mode, the electrodes are placed along both sides of the microchannel covering the entire length. The microchannel contains cell culture media or PBS, which are strong electrolytic solutions. According to the existing literature, the relative permittivity is used as ca. 80 and conductivity (σ_{ch}) is ca. 1.5 S/m, respectively [100]. For a long-term application on live cells, a low-intensity field in the non-contact mode is preferable in order to prevent the electrolysis.

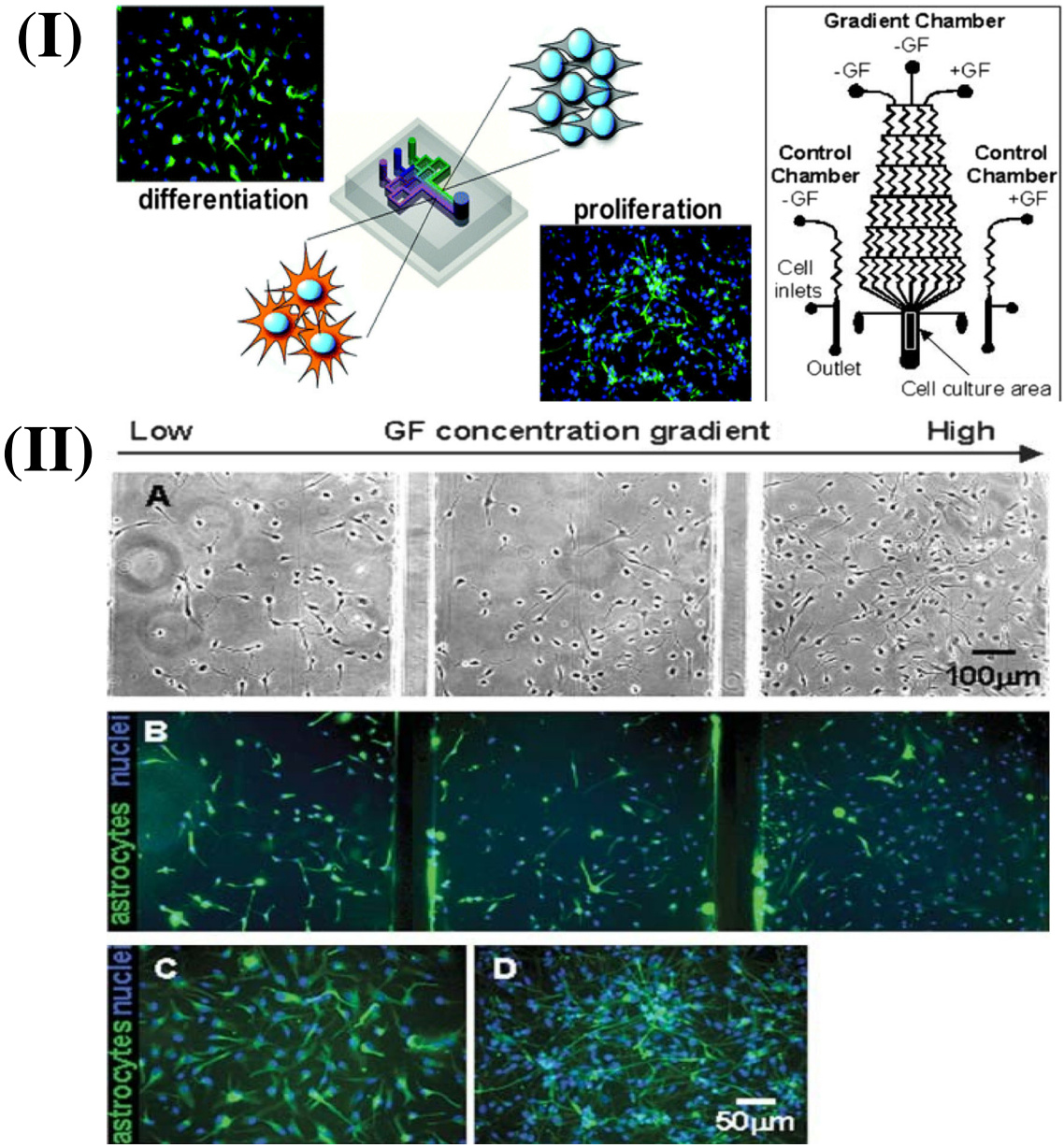

Fig. 19 (I) Shear flow enhances stem cell differentiation towards neurogenesis by generating the growth factor gradient. (II) **a** Conventional mixing based gradient generator. **b** Two branches of microchannels flow inside the gradient generator and mixing generates the growth factor gradient. **c** Static diffusion generator for stem cell shear stress experiments [96]

The electric field, developed inside the microchannel is due to the stimulatory voltage, which is applied either lengthwise or across the width of the channel. In an ideal condition, this develops a homogenous electric field across the microchannel. For this ideal model of the microchannel, the electric flux generated inside the microchannel can be approximated as uniform. The electric field can lead to temperature increase inside a microchannel, which is media-specific, depending on the effective surface area, conductive heat transfer coefficient, and thermal resistance. A microchannel consists of a minimum of two access holes, which enable fluidic connection with the reservoirs.

LOC Platform for Electric Field Applied Stem Cell Differentiation Study Pavesi et al. conducted stem cell culture under the influence of electrical stimulus [101]. In this work, they have described the design and fabrication of a microscale cell stimulator capable of simultaneously providing mechanical, electrical, and biochemical stimulation. The outcome of the experimentation in such regulated environment was extracted in terms of detailed morphological and gene-expression analysis of the cells. They have used the values of the electric field of 5 V/cm, which is claimed to be in the range of physiological values. The device has inbuilt electrodes, which remain embedded in a three-layered PDMS chip.

Lithography was performed to get the silicon molds onto which the conductive PDMS + CNT mixture was poured to get an electrocompatible surface for the cells. The fabricated microfluidic device is suitable for immunofluorescence procedures and confocal imaging. They have observed almost the similar effect of mechanical and electrical stimulus on the cells. The application of electromechanical stimulation induced morphological changes along with actin-based cytoskeletal remodeling has helped the cells to orient in the direction perpendicular to the applied electrophysical and biomechanical strain (Fig. 20). As an extension of the quantitative data analysis of the electric field stricken cells, they were harvested from the device to perform gene expression analyses, using qRT-PCR. The experiment has successfully demonstrated the ability of the mechano-electrical cues to trigger subcellular changes in cell morphology, cytoskeletal fiber orientation, and gene expression changes. The authors have claimed that this novel bioengineering approach can be applied to the field of cardiac-regenerative medicine [101, 102].

Au et al. reported the application of electric field to achieve cardiomyocytic differentiation of stem cells [103]. The motivation of their work has been triggered by developing a multicue intrigued microfluidic system. They have reported a microfabricated system, which could deliver electrical cues along with topographical stimulation on a single chip. The on-chip cell culture system was created by hot embossing of

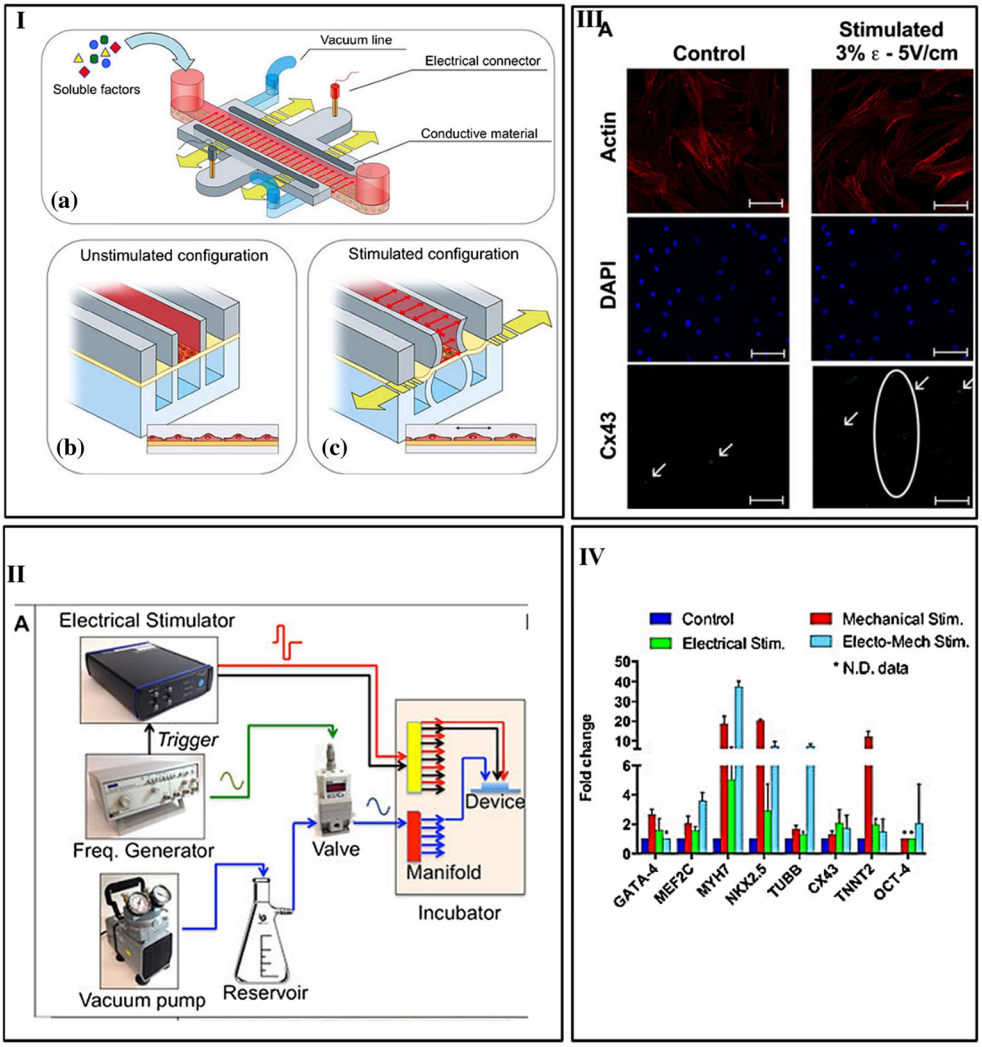

Fig. 20 (I) **a** Schematic of the device plan showing the central channel (in red) containing cell media. This supply nutrients and soluble factors for the growth of the cultured cells. The pneumatic channels (light blue) are utilized to stretch the PDMS membrane (yellow arrows) to deliver a mechanical stimulus to the adherent cells. The electrical layer contains two conductive regions composed of a mixture of CNTs and PDMS (in light gray). The uniform electric field is created by the conductive layer composed of a mixture of CNTs and PDMS (light gray). This is connected to the stimulator through two external gold-coated connectors (in red and black). **b**, **c** The stimulated conformation with the reference of unstimulated one. (II) schematic of the control system. An alternating vacuum is maintained with the help of an electronic valve to increase the efficacy of the electrical stimulator. (III) The immunocytochemically stained actin, nuclei, and connexin43 expression show elevation in the maker expression for the electromechanically stimulated devices at 3% strain and 5 V/cm electrical stimulation. Scale bar is 100 μm. (IV) Histogram plot depicting the fold changes in the expression of the targeted maker genes. GAPDH was kept as the housekeeping gene [101]

polystyrene to introduce microgrooves and microridges of precise depth, width, and periodicity. The culture floor consisted of grooves and ridges of 0.5 μm width with a periodicity of 1 μm, and the other pattern consists of 3-μm-wide grooves with ridges of 1 μm wide having 4-μm period. The depth of the microgrooves was kept at 400 nm. The electrodeposited gold electrodes were built 1 cm apart in order to have the microgrooves either oriented parallel or perpendicular in between the electrodes. This is designed to enable the cells to have synergistic interaction with respect to both topographical and electrical cues. They have cultured cardiomyocytes of neonatal rat origin, which were cultured on the patterned microgrooved substrates for 7 days under simultaneous electric field. The cells exhibited an elongated and aligned well-developed contractile apparatus. This serves as an evidence for maturation of the precursor of cardiac muscles into myocardial units. The same has been revealed by sarcomeric α-actinin staining [103].

The authors reported that the pattern with 1 μm periodicity was able to obtain increased protein expression compared to 4 μm periodicity. They have affirmed that simultaneous application of both electrical pulses and topographical cues resulted in the formation of gap junctions to the cell-cell end junctions. The elongated cardiomyocytes were observed on the parallelly aligned microgrooves and along the electric field direction (Fig. 21). [103] Due to the ability to independently control field stimulation parameter (biochemical and topographical) cues on each chip, the developed LOC system was found to be useful for maturation of stem cell-derived cardiomyocytes.

Summary

Microfluidics has been developed to mimic in vivo-like cellular microenvironments. The optical transparency and protocol automation for the different cell types together with their screening and monitoring could be performed using the biomicrofluidic system. The LOC system not only reduces space by conducting stem cell differentiation-like complex cell culture experiments but also mimics physiology to improve the stem cell response with higher efficacy. In recent years, the use of the microfluidic platform for stem cell research has drawn wider attention. A systematic review of cell culture targeting stem cell differentiation was focused in this article under the influence of shear and electric field. These two stimuli have been chosen as they have endogenous origin. Apart from these, it is worthwhile to put forward the regulatory nature of magnetic field on the behavior of the cells. Boda et al. followed the trend of change in differentiation responses on application of magnetic field on stem cell [104]. They claimed that the application of magnetic field leading to magnetization of the substrate forces the stem cell to accept the osteogenic path. We have developed different designs for LOC devices, which were capable of directing the cells into differential cues. The main focus was the cellular differentiation study under shear and electric field along with some explore intervention of the material properties towards redirecting the cells. It is very well known that due to the presence of endogenous existence of both of these two cues, they intimately affect the cellular decision on top of the genetic pre-program. Therefore, the development of "smart" platforms with reproducible application of the well-controlled shear and electric field has provided the ample scope of exploring such concept. By new organizational approaches, these can be enlightened with tailored devices and intelligently formulated protocols.

Shear stress is critically important in regulating the normal physiology as well as the pathobiology and dysfunction of the tissue. For example, through complex molecular mechanisms, shear is responsible for promoting the atherogenesis. Can it be modeled with the disease-on-chip concept to visualize closely the governing factors and stepwise progression of the disease?

The use of growth-arrested feeder cells is a well-established protocol to promote cell proliferation, particularly for the low-seeding density inocula with stubborn cells to grow (e.g., human embryonic stem cell, needing the fibroblast support). Essentially, the feeder cells consist of an undividable layer of cells, which provides paracrine support to another cell to proliferate. It is slightly different from the cocultured system because in the former, only one cell type is allowed to grow. The secretory growth factors by the feeder layer are not the only effector parameters to promote the proliferation of targeted cells, but also the feeder cells probably harness multiple other modes of supporting the cells. Can the feeder cell concept be explored by creating a biomimetic microfluidic niche during stubborn cultures and differentiation of the various shear and electric field-sensitive stem cell?

Microfluidic devices enable novel means of emulating neurodegenerative disease pathophysiology in vitro. These organ-on-a-chip systems can potentially reduce animal testing and substitute (or augment) simple 2D culture systems. Reconstituting critical features of neurodegenerative diseases in a biomimetic system using microfluidics can thereby accelerate drug discovery and improve our understanding of the mechanisms of several currently incurable diseases. Can an on-chip neurohomologous device be capable to describe neurodegenerative diseases of the central nervous system and the peripheral nervous system and thereby formulate the pharmacokinetics and pharmacodynamics?

In conclusion, an improved understanding of the stem-cell and regenerative biology, as well as a better control of stem-cell fate with exogenous application of endogenous origin physical stimuli, is likely to produce treatments for many devastating diseases and injuries. Biomicrofluidic approaches are

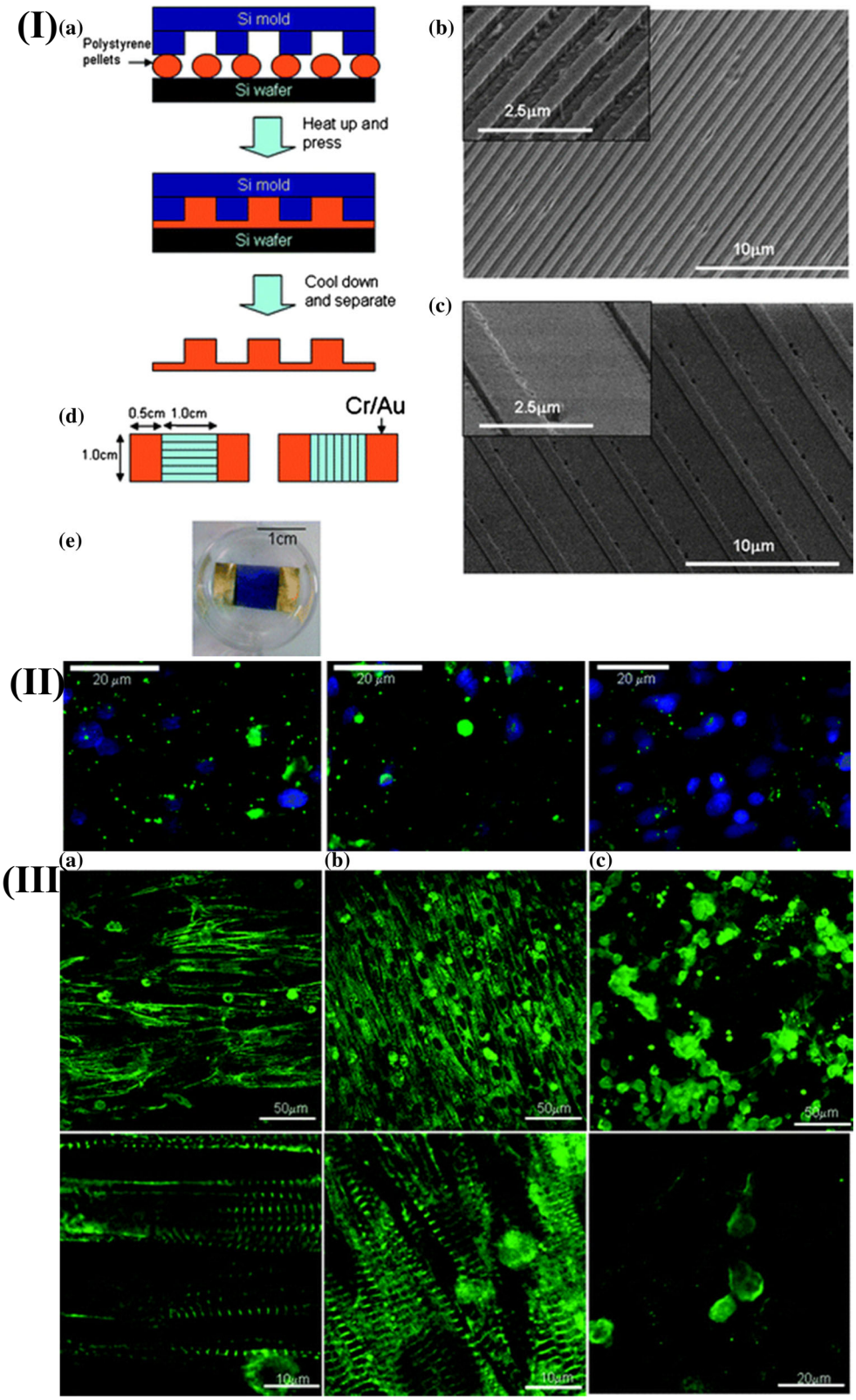

Fig. 21 (I) Patterned polystyrene surfaces with microgrooves were used for culturing cells. Hot embossing procedure was used for patterning. **a** Schematics showing the stepwise hot-embossing process. Scanning electron micrograph of the microgrooved surface with **b**, **c** two different patterns were designed with dimensions of 1 μm period, 0.5 μm groove width, and 400 nm groove height and 4 μm period, 3 μm groove width, 400 nm groove height. **d** The orientation of the grooves are both vertical and horizontal in reference to the electrodes. **e** The cell culture area with the differentiated cardiomyocytes between electrodes (blue) is visible with Giemsa staining. (II) Immunocytochemically stained cells for Connexin-43 (green) along with nuclei (blue) which are counterstained with DAPI. (III) Sarcomeric-α-actinin immunostaining of the cardiomyocytes cultivated on both type of patterns. **a** 4 μm period. **b** 1 μm period. **c** Controls without patterned surfaces [103]. Gomez-Sjoberg et al. have built an automated PDMS-based cell culture chip with 96 microfluidic culture chamber that can be controlled individually, each containing a volume of only 60 nl. Individual addressability confers to develop a combinatorial diversity within a small experimental space. Variation in culture environment as a function of time and chamber location has enacted to recreate the experimental parameter diversity in a single setup. The events recorded in each chamber are imaged with time-lapse microscopy. This is used to deduce the transient stimulation on the cell proliferation, osteogenic differentiation, and motility of human primary mesenchymal stem cells [103]

starting to play an increasingly important role in this advancing field. Though this review does not include all the physical cues as a means of modulating cell functionality, it offers a valuable glimpse into less explored stem cell manipulation via electrical and shear cues. The knowledge on the stem cell behavior encompasses an application-oriented scientific

research on regenerative medicine and thereby could contribute towards societal benefits.

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References

1. Schork NJ. Personalized medicine: time for one-person trials. Nature. 2015;520:609–11.
2. Jain S, Sharma A, Basu B. Vertical electric field stimulated neural cell functionality on porous amorphous carbon electrodes. Biomaterials. 2013;34:9252–63.
3. Basu B. Biomaterials Science and Tissue engineering: principles and methods: Cambridge University Press; 2017.
4. Basu B. Biomaterials for musculoskeletal regeneration: concepts: Springer; 2016.
5. Basu B, Katti DS, Kumar A. Advanced biomaterials: fundamentals, processing, and applications: John Wiley & Sons; 2010.
6. Ravikumar K, Kar GP, Bose S, Basu B. Synergistic effect of polymorphism, substrate conductivity and electric field stimulation towards enhancing muscle cell growth in vitro. RSC Adv. 2016;6:10837–45.
7. Harink B, Le Gac S, Truckenmuller R, van Blitterswijk C, Habibovic P. Regeneration-on-a-chip? The perspectives on use of microfluidics in regenerative medicine. Lab Chip. 2013;13:3512–28.
8. Food U, Administration D. Paving the way for personalized medicine: FDA's role in a new era of medical product development. Silver Spring, MD: US Food and Drug Administration 2013.
9. Ko J, Hemphill MA, Gabrieli D, Wu L, Yelleswarapu V, Lawrence G, et al. Smartphone-enabled optofluidic exosome diagnostic for concussion recovery. Sci Rep. 2016;6:31215.
10. Contreras-Naranjo JC, Wu H-J, Ugaz VM. Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine. Lab Chip. 2017;17:3558–77.
11. Shao H, Chung J, Lee K, Balaj L, Min C, Carter BS, et al. Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. Nat Commun. 2015;6:6999.
12. Zervantonakis IK, Kothapalli CR, Chung S, Sudo R, Kamm RD. Microfluidic devices for studying heterotypic cell-cell interactions and tissue specimen cultures under controlled microenvironments. Biomicrofluidics. 2011;5:013406.
13. Wu W-I, Selvaganapathy PR, Ching CY. Transport of particles and microorganisms in microfluidic channels using rectified ac electro-osmotic flow. Biomicrofluidics. 2011;5:013407.
14. Pirlo R, Sweeney A, Ringeisen B, Kindy M, Gao B. Biochip/laser cell deposition system to assess polarized axonal growth from single neurons and neuron/glia pairs in microchannels with novel asymmetrical geometries. Biomicrofluidics. 2011;5:013408.
15. Taylor AM, Dieterich DC, Ito HT, Kim SA, Schuman EM. Microfluidic local perfusion chambers for the visualization and manipulation of synapses. Neuron. 2010;66:57–68.
16. Srigunapalan S, Lam C, Wheeler AR, Simmons CA. A microfluidic membrane device to mimic critical components of the vascular microenvironment. Biomicrofluidics. 2011;5:013409.
17. Daw R, Finkelstein J. Lab on a chip. Nature. 2006;442:367.
18. Haeberle S, Zengerle R. Microfluidic platforms for lab-on-a-chip applications. Lab Chip. 2007;7:1094–110.
19. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu H, Schueller OJ, et al. Fabrication of microfluidic systems in poly(dimethylsiloxane). Electrophoresis. 2000;21:27–40.
20. Park TH, Shuler ML. Integration of cell culture and microfabrication technology. Biotechnol Prog. 2003;19:243–53.
21. Karimi M, Bahrami S, Mirshekari H, Basri SMM, Nik AB, Aref AR, et al. Microfluidic systems for stem cell-based neural tissue engineering. Lab Chip. 2016;16:2551–71.
22. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;453:314–21.
23. Atala A, Irvine DJ, Moses M, Shaunak S. Wound healing versus regeneration: role of the tissue environment in regenerative medicine. MRS Bull / Materials Research Society 2010;35:https://doi.org/10.1557/mrs2010.528, 597, 606.
24. Petit-Zeman S. Regenerative medicine. Nat Biotech. 2001;19:201–6.
25. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. J Investig Dermatol. 2007;127:514–25.
26. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev. 2003;83:835–70.
27. Yang K, Park HJ, Han S, Lee J, Ko E, Kim J, et al. Recapitulation of in vivo-like paracrine signals of human mesenchymal stem cells for functional neuronal differentiation of human neural stem cells in a 3D microfluidic system. Biomaterials. 2015;63:177–88.
28. Kim KM, Choi YJ, Hwang J-H, Kim AR, Cho HJ, Hwang ES, et al. Shear stress induced by an interstitial level of slow flow increases the osteogenic differentiation of mesenchymal stem cells through TAZ activation. PLoS One. 2014;9:e92427.
29. Shi X, Li L, Ostrovidov S, Shu Y, Khademhosseini A, Wu H. Stretchable and micropatterned membrane for osteogenic differentation of stem cells. ACS Appl Mater Interfaces. 2014;6:11915–23.
30. Winer JP, Janmey PA, McCormick ME, Funaki M. Bone marrow-derived human mesenchymal stem cells become quiescent on soft substrates but remain responsive to chemical or mechanical stimuli. Tissue Eng A. 2008;15:147–54.
31. Xu L-C, Bauer J, Siedlecki CA, Group ACftHaBIR. Proteins, platelets, and blood coagulation at biomaterial interfaces. Colloids and surfaces B, Biointerfaces. 2014;124:49–68.
32. Siddique R, Thakor N. Investigation of nerve injury through microfluidic devices. J R Soc Interface. 2014;11:20130676.
33. Shah P, Zhu X, Chen C, Hu Y, Li CZ. Lab-on-chip device for single cell trapping and analysis. Biomed Microdevices. 2014;16:35–41.
34. Pang L, Liu W, Tian C, Xu J, Li T, Chen S-W, et al. Construction of single-cell arrays and assay of cell drug resistance in an integrated microfluidic platform. Lab Chip. 2016;16:4612–20.
35. Halldorsson S, Lucumi E, Gómez-Sjöberg R, Fleming RMT. Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. Biosens Bioelectron. 2015;63:218–31.
36. Stephan S, Johnson WE, Roberts S. The influence of nutrient supply and cell density on the growth and survival of intervertebral disc cells in 3D culture. Eur Cells Mater. 2011;22:97–108.

37. Liu W, Li L, Wang X, Ren L, Wang X, Wang J, et al. An integrated microfluidic system for studying cell-microenvironmental interactions versatilely and dynamically. Lab Chip. 2010;10:1717–24.
38. Lee KS, Boccazzi P, Sinskey AJ, Ram RJ. Microfluidic chemostat and turbidostat with flow rate, oxygen, and temperature control for dynamic continuous culture. Lab Chip. 2011;11:1730–9.
39. Hsieh C-C, Huang S-B, Wu P-C, Shieh D-B, Lee G-B. A microfluidic cell culture platform for real-time cellular imaging. Biomed Microdevices. 2009;11:903–13.
40. Sugiura S, Ji E, Kikuchi K, Sumaru K, Kanamori T. Pressure-driven perfusion culture microchamber array for a parallel drug cytotoxicity assay. Biotechnol Bioeng. 2008;100:1156–65.
41. Tehranirokh M, Kouzani AZ, Francis PS, Kanwar JR. Microfluidic devices for cell cultivation and proliferation. Biomicrofluidics. 2013;7:051502.
42. Remmerbach TW, Wottawah F, Dietrich J, Lincoln B, Wittekind C, Guck J. Oral cancer diagnosis by mechanical phenotyping. Cancer Res. 2009;69:1728–32.
43. Du Z, Colls N, Cheng KH, Vaughn MW, Gollahon L. Microfluidic-based diagnostics for cervical cancer cells. Biosens Bioelectron. 2006;21:1991–5.
44. Kokoris M, Nabavi M, Lancaster C, Clemmens J, Maloney P, Capadanno J, et al. Rare cancer cell analyzer for whole blood applications: automated nucleic acid purification in a microfluidic disposable card. Methods (San Diego, Calif). 2005;37:114–9.
45. Chaw KC, Manimaran M, Tay FE, Swaminathan S. A quantitative observation and imaging of single tumor cell migration and deformation using a multi-gap microfluidic device representing the blood vessel. Microvasc Res. 2006;72:153–60.
46. Siyan W, Feng Y, Lichuan Z, Jiarui W, Yingyan W, Li J, et al. Application of microfluidic gradient chip in the analysis of lung cancer chemotherapy resistance. J Pharm Biomed Anal. 2009;49:806–10.
47. Maeng JH, Lee BC, Ko YJ, Cho W, Ahn Y, Cho NG, et al. A novel microfluidic biosensor based on an electrical detection system for alpha-fetoprotein. Biosens Bioelectron. 2008;23:1319–25.
48. Huang T, Jia CP, Jun Y, Sun WJ, Wang WT, Zhang HL, et al. Highly sensitive enumeration of circulating tumor cells in lung cancer patients using a size-based filtration microfluidic chip. Biosens Bioelectron. 2014;51:213–8.
49. Hong J-L, Lan K-C, Jang L-S. Electrical characteristics analysis of various cancer cells using a microfluidic device based on single-cell impedance measurement. Sensors Actuators B Chem. 2012;173:927–34.
50. Shelby JP, White J, Ganesan K, Rathod PK, Chiu DT. A microfluidic model for single-cell capillary obstruction by plasmodium falciparum-infected erythrocytes. Proc Natl Acad Sci U S A 2003;100:14618–14622.
51. Marcus JS, Anderson WF, Quake SR. Microfluidic single-cell mRNA isolation and analysis. Anal Chem. 2006;78:3084–9.
52. Toriello NM, Douglas ES, Thaitrong N, Hsiao SC, Francis MB, Bertozzi CR, et al. Integrated microfluidic bioprocessor for single-cell gene expression analysis. Proc Natl Acad Sci. 2008;105:20173–8.
53. Parate D, Franco-Obregón A, Fröhlich J, Beyer C, Abbas AA, Kamarul T, et al. Enhancement of mesenchymal stem cell chondrogenesis with short-term low intensity pulsed electromagnetic fields. Sci Rep. 2017;7:9421.
54. Dong JD, Gu YQ, Li CM, Wang CR, Feng ZG, Qiu RX, et al. Response of mesenchymal stem cells to shear stress in tissue-engineered vascular grafts. Acta Pharmacol Sin. 2009;30:530–6.
55. Han F, Zhu C, Guo Q, Yang H, Li B. Cellular modulation by the elasticity of biomaterials. J Mater Chem B. 2016;4:9–26.
56. Halder G, Dupont S, Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. 2012;13:591.
57. de Villiers JA, Houreld NN, Abrahamse H. Influence of low intensity laser irradiation on isolated human adipose derived stem cells over 72 hours and their differentiation potential into smooth muscle cells using retinoic acid. Stem Cell Rev. 2011;7:869–82.
58. Choi Y, Park JE, Jeong JS, Park JK, Kim J, Jeon S. Sound waves induce neural differentiation of human bone marrow-derived mesenchymal stem cells via ryanodine receptor-induced calcium release and Pyk2 activation. Appl Biochem Biotechnol. 2016;180:682–94.
59. Mehling M, Tay S. Microfluidic cell culture. Curr Opin Biotechnol. 2014;25:95–102.
60. Gómez-Sjöberg R, Leyrat AA, Pirone DM, Chen CS, Quake SR. Versatile, fully automated, microfluidic cell culture system. Anal Chem. 2007;79:8557–63.
61. Regehr KJ, Domenech M, Koepsel JT, Carver KC, Ellison-Zelski SJ, Murphy WL, et al. Biological implications of polydimethylsiloxane-based microfluidic cell culture. Lab Chip. 2009;9:2132–9.
62. Dardik A, Chen L, Frattini J, Asada H, Aziz F, Kudo FA, et al. Differential effects of orbital and laminar shear stress on endothelial cells. J Vasc Surg. 2005;41:869–80.
63. Chin L, Yu J, Fu Y, Yu T, Liu A, Luo K. Production of reactive oxygen species in endothelial cells under different pulsatile shear stresses and glucose concentrations. Lab Chip. 2011;11:1856–63.
64. Jones EA, le Noble F, Eichmann A. What determines blood vessel structure? Genetic prespecification vs. hemodynamics. Physiology. 2006;21:388–95.
65. Wang HU, Chen Z-F, Anderson DJ. Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell. 1998;93:741–53.
66. Ema M, Rossant J. Cell fate decisions in early blood vessel formation. Trends in cardiovascular medicine 2003;13:254–9.
67. McGrath J, Brueckner M. Cilia are at the heart of vertebrate left–right asymmetry. Curr Opin Genet Dev. 2003;13:385–92.
68. Levin M. Left–right asymmetry in embryonic development: a comprehensive review. Mech Dev. 2005;122:3–25.
69. Poelmann RE, Gittenberger-de Groot AC, Hierck BP. The development of the heart and microcirculation: role of shear stress. Med Biol Eng Comput. 2008;46:479–84.
70. Groenendijk BC, Hierck BP, Gittenberger-De Groot AC, Poelmann RE. Development-related changes in the expression of shear stress responsive genes KLF-2, ET-1, and NOS-3 in the developing cardiovascular system of chicken embryos. Dev Dyn. 2004;230:57–68.
71. Boselli F, Steed E, Freund JB, Vermot J. Anisotropic shear stress patterns predict the orientation of convergent tissue movements in the embryonic heart. Development. 2017;144:4322–7.
72. Li J, Hou B, Tumova S, Muraki K, Bruns A, Ludlow MJ, et al. Piezo1 integration of vascular architecture with physiological force. Nature. 2014;515:279–82.
73. Wang Y, Miao H, Li S, Chen K-D, Li Y-S, Yuan S, et al. Interplay between integrins and FLK-1 in shear stress-induced signaling. Am J Physiol-Cell Physiol. 2002;283:C1540–C7.
74. Liu L, Yuan W, Wang J. Mechanisms for osteogenic differentiation of human mesenchymal stem cells induced by fluid shear stress. Biomech Model Mechanobiol. 2010;9:659–70.
75. Alshihabi SN, Chang YS, Frangos JA, Tarbell JM. Shear stress-induced release of PGE 2 and PGI 2 by vascular smooth muscle cells. Biochem Biophys Res Commun. 1996;224:808–14.
76. Naskar S, Kumaran V, Basu B. On the origin of shear stress induced myogenesis using PMMA based lab-on-chip. ACS Biomater Sci Eng. 2017;3:1154–71.
77. Yourek G, McCormick SM, Mao JJ, Reilly GC. Shear stress induces osteogenic differentiation of human mesenchymal stem cells. Regen Med. 2010;5:713–24.
78. Liu Y-S, Liu Y-A, Huang C-J, Yen M-H, Tseng C-T, Chien S, et al. Mechanosensitive TRPM7 mediates shear stress and modulates

osteogenic differentiation of mesenchymal stromal cells through Osterix pathway2015.
79. Halder G, Dupont S, Piccolo S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. Nat Rev Mol Cell Biol. 2012;13:591–600.
80. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011;474:179–83.
81. Hong J-H, Hwang ES, McManus MT, Amsterdam A, Tian Y, Kalmukova R, et al. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. Science. 2005;309:1074–8.
82. Yuan L, Sakamoto N, Song G, Sato M. High-level shear stress stimulates endothelial differentiation and VEGF secretion by human mesenchymal stem cells. Cell Mol Bioeng. 2013;6:220–9.
83. Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F. Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist. 2010;16:285–307.
84. Berdichevsky Y, Sabolek H, Levine JB, Staley KJ, Yarmush ML. Microfluidics and multielectrode array-compatible organotypic slice culture method. J Neurosci Methods. 2009;178:59–64.
85. Patel N, Poo M-M. Orientation of neurite growth by extracellular electric fields. J Neurosci. 1982;2:483–96.
86. Giaever I, Keese CR. Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture. IEEE Trans Biomed Eng. 1986;BME-33:242–7.
87. Thrivikraman G, Madras G, Basu B. Intermittent electrical stimuli for guidance of human mesenchymal stem cell lineage commitment towards neural-like cells on electroconductive substrates. Biomaterials. 2014;35:6219–35.
88. Thrivikraman G, Madras G, Basu B. Electrically driven intracellular and extracellular nanomanipulators evoke neurogenic/cardiomyogenic differentiation in human mesenchymal stem cells. Biomaterials. 2016;77:26–43.
89. Nuccitelli R. Endogenous electric fields in embryos during development, regeneration and wound healing. Radiat Prot Dosim. 2003;106:375–83.
90. Hotary KB, Robinson KR. Evidence of a role for endogenous electrical fields in chick embryo development. Development. 1992;114:985–96.
91. Nuccitelli R. A role for endogenous electric fields in wound healing. Curr Top Dev Biol. 2003;58:1–26.
92. Messerli MA, Graham DM. Extracellular electrical fields direct wound healing and regeneration. Biol Bull. 2011;221:79–92.
93. Mooney E, Mackle JN, Blond DJP, O'Cearbhaill E, Shaw G, Blau WJ, et al. The electrical stimulation of carbon nanotubes to provide a cardiomimetic cue to MSCs. Biomaterials. 2012;33:6132–9.
94. Zhang J, Li M, Kang E-T, Neoh KG. Electrical stimulation of adipose-derived mesenchymal stem cells in conductive scaffolds and the roles of voltage-gated ion channels. Acta Biomater. 2016;32:46–56.
95. Lu H, Koo LY, Wang WM, Lauffenburger DA, Griffith LG, Jensen KF. Microfluidic shear devices for quantitative analysis of cell adhesion. Anal Chem. 2004;76:5257–64.
96. Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, Monuki ES, et al. Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. Lab Chip. 2005;5:401–6.
97. West J, Becker M, Tombrink S, Manz A. Micro total analysis systems: latest achievements. Anal Chem. 2008;80:4403–19.
98. Manz A, Fettinger JC, Verpoorte E, Lüdi H, Widmer H, Harrison D. Micromachining of monocrystalline silicon and glass for chemical analysis systems a look into next century's technology or just a fashionable craze? TrAC Trends Anal Chem. 1991;10:144–9.
99. Wei F, Lillehoj PB, Ho C-M. DNA diagnostics: nanotechnology-enhanced electrochemical detection of nucleic acids. Pediatr Res. 2010;67:458–68.
100. Mittal N, Rosenthal A, Voldman J. nDEP microwells for single-cell patterning in physiological media. Lab Chip. 2007;7:1146–53.
101. Pavesi A, Adriani G, Rasponi M, Zervantonakis IK, Fiore GB, Kamm RD. Controlled electromechanical cell stimulation on-a-chip. Sci Rep. 2015;5:11800.
102. Pavesi A, Soncini M, Zamperone A, Pietronave S, Medico E, Redaelli A, et al. Electrical conditioning of adipose-derived stem cells in a multi-chamber culture platform. Biotechnol Bioeng. 2014;111:1452–63.
103. Heidi AHT, Cui B, Chu ZE, Veres T, Radisic M. Cell culture chips for simultaneous application of topographical and electrical cues enhance phenotype of cardiomyocytes. Lab Chip. 2009;9:564–75.
104. Boda SK, Thrivikraman G, Basu B. Magnetic field assisted stem cell differentiation–role of substrate magnetization in osteogenesis. J Mater Chem B. 2015;3:3150–68.