Concise Review: Stem Cell Microenvironment on a Chip: Current Technologies for Tissue Engineering and Stem Cell Biology

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ABSTRACT

Stem cells have huge potential in many therapeutic areas. With conventional cell culture methods, however, it is difficult to achieve in vivo-like microenvironments in which a number of well-controlled stimuli are provided for growing highly sensitive stem cells. In contrast, microtechnology-based platforms offer advantages of high precision, controllability, scalability, and reproducibility, enabling imitation of the complex physiological context of in vivo. This capability may fill the gap between the present knowledge about stem cells and that required for clinical stem cell-based therapies. We reviewed the various types of microplatforms on which stem cell microenvironments are mimicked. We have assigned the various microplatforms to four categories based on their practical uses to assist stem cell biologists in using them for research. In particular, many examples are given of microplatforms used for the production of embryoid bodies and aggregates of stem cells in vitro. We also categorized microplatforms based on the types of factors controlling the behaviors of stem cells. Finally, we outline possible future directions for microplatform-based stem cell research, such as research leading to the production of well-defined environments for stem cells to be used in scaled-up systems or organs-on-a-chip, the regulation of induced pluripotent stem cells, and the study of the genetic states of stem cells on microplatforms.

SIGNIFICANCE

Stem cells are highly sensitive to a variety of physicochemical cues, and their fate can be easily altered by a slight change of environment; therefore, systematic analysis and discrimination of the extracellular signals and intracellular pathways controlling the fate of cells and experimental realization of sensitive and controllable niche environments are critical. This review introduces diverse microplatforms to provide in vitro stem cell niches. Microplatforms could control microenvironments around cells and have recently attracted much attention in biology including stem cell research. These microplatforms and the future directions of stem cell microenvironment are described.

INTRODUCTION

Since their discovery by Ernest McCulloch and James Till in 1963 [1], stem cells have been regarded as promising candidates for tissue engineering [2–5], organ regeneration [6–8], cell-based diagnosis [9, 10], and disease models [11–14]. Although stem cells are obtained from various sources such as embryoids (embryonic stem cells [ESCs]), bone marrow (mesenchymal stem cells [MSCs]), and, in some cases, adult cells (induced pluripotent stem cells [iPSCs]) for in vitro use, stem cells in vivo are established in “niches”—specific anatomic locations that regulate how stem cells participate in tissue generation, maintenance, and repair. The niche constitutes a basic unit of tissue physiology, integrating signals that mediate the balanced responses of stem cells and the needs of the organism [15]. This microenvironment preserves stem cells from physiological stimuli and protects the host from overproliferation of stem cells.

Because stem cells are highly sensitive to the physicochemical microenvironment, gaining an understanding of the interplay between stem cells and their microenvironments may be essential for advancing stem cell research and applications. Many investigations have attempted to replicate in vivo microenvironments with in vitro systems [16–20], but achieving such in vivo-like microenvironments in conventional cell culture procedures has faced significant obstacles. For regenerative cell therapies, for example, it is unclear whether stem cells maintain their original phenotype when cultured and grown on dishes.
and then implanted back into the patient for therapy [21]. Such changes in phenotype can occur on conventional cell culture dishes because stem cells are exposed to imprecise spatial and temporal control of the mechanical and physical cell microenvironments, unlike the highly controlled conditions in vivo [22], and stem cell fate can be easily altered by a slight change in the environment. This is a big barrier to the practical use of stem cells because we are unable to anticipate their fate precisely. New culture platforms that realize in vivo-like microenvironments make it possible to use stem cells more practically; discrimination of the extracellular signals and control of intracellular pathways for the fate of cells are required. Recent progress in micro- and nanofabrication and microfluidic technologies has enabled modulation of the soluble and insoluble cues of the stem cell microenvironment in a manner closer to that in vivo, which is shown in various examples [23–30]. An example is microplatforms for gradient generation: neural progenitor cells on chemical gradient-generated microplatforms experience chemical gradient similar to that of sonic hedgehog, and bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) play on neuronal identities along the dorsoventral and anterior-posterior axes during the early development of the vertebrate nervous system [31]. Microtechnology-based platforms in which multiple soluble and insoluble factors can be controlled simultaneously over space and time with high precision and that address the above-mentioned issues are being developed and thus are suitable for stem cell research. This has been shown in many studies using stem cells on a chip in drug discovery [32–34], stem cell therapy [35], regenerative medicine [3, 36], and genetic disorder treatment [37].

We reviewed the diverse microplatforms on which stem cell microenvironments are mimicked and the applications for these microplatforms in stem cell research, which has focused mainly on resolving remaining problems (i.e., controlling and understanding the behaviors of stem cells under particular circumstances). This review consists of four sections. In the first section, the microplatforms currently used by and potentially useful to stem cell researchers are described and categorized into four types: cell sorters, deliverers of molecules at micropillar volume, gradient generators, and microwell arrays. The second section introduces the microplatforms used to produce three-dimensional (3D) embryoid bodies (EBs), which are 3D aggregates of pluripotent stem cells. The 3D spheroid culture enables the nonadherent and scalable culture of EBs in suspension and is useful for producing large numbers of cells with diverse lineages for potential clinical applications. Third, microplatforms allowing soluble and physical factors for the regulation of stem cell behaviors such as adhesion, self-renewal, and differentiation are introduced and discussed with various examples. Finally, we describe potential future directions of stem cell research and further applications of microplatforms serving as stem cell microenvironments.

**Microplatforms for Delivering Biomolecules to Cells in a Controllable Way**

A major problem of stem cell research was the difficulty of observing the effects of biomolecules on stem cell behavior. To overcome this difficulty, various microplatform-based molecule delivery systems have been used (Fig. 1Bi), and these systems usually have isolated spaces on the platforms for concentrated targeting of biomolecules. Faley et al. fabricated platforms having array structures designed for trapping individual cells [33]. Single hematopoietic stem cells (HSCs) derived from chronic myeloid leukemia (CML) patients were captured by such cell-trapping structures and were exposed to dasatinib, a tyrosine kinase inhibitor approved for the treatment of CML. The authors observed that CML cells exposed to dasatinib, compared with normal cell populations, showed no difference on staining with propidium iodide and annexin V (stains for analyzing apoptosis) but lost their motility and could not migrate out of the trap. These observations indicate that the CML cells displayed resistance to the apoptotic effects of dasatinib because of additional activated survival pathways in these leukemic cells; these observations also indicate that dasatinib is a potent inhibitor of Abl/Src kinase, which plays a role in actin organization and enhancing cell motility. The trapping structure of this microplatform makes it possible to determine the effect of the drug specifically on stem cells without these cells being mixed with other factors. Wang et al. [32] and Cosson and Lutolf [38] also developed structure-specific microplatforms for toxic material delivery to stem cells. Formaldehyde, a well-known irritant, was successfully delivered to a chamber in which live human nasal epithelial stem/progenitor cells were seeded for an in vitro test of toxicity of gaseous formaldehyde [32]. In addition, delivery of retinoic acid (RA) showed a position- and concentration-dependent effect on EB size and Sox1-expression of EBs [38] (supplemental online Fig. 1A1). These microplatforms are regarded as excellent tools for screening and determining the effect of the dose and timing of specific molecules on stem cell fate.

Microfluidic techniques are excellent methods for delivering biomolecules and drugs in controllable ways because, in microfluidics, laminar flow is more dominant, which means that the streams of fluids have their own layer to flow in without mixing with neighbors. Villa-Diaz et al. [39] and Kawada et al. [40] used this phenomenon to deliver the molecules to the stem cells.
fabricated microchannels and introduced solutions into each inlet while solution contained factors for the reaction they tried to induce. Stem cells cultured on channels were then exposed to the introduced solutions directly [39] and indirectly [40].

**Microplatforms for Gradient Generation**

In interpreting the behavior of stem cells, gradients are of great importance because they are essential for the regulation of fundamental cell processes such as cell migration and tissue formation during early development [29]. Chemical gradients, for example, control tissue development during morphogenesis; cells in the surface ectoderm are turned into neural crest cells by chemicals in the microenvironment called *morphogen*, including BMPs, Wnt, and FGFs. Morphogen gradients are formed by diffusion, linear degradation, nonlinear degradation, and cell lineage transport [50]. Because gene expression in a cell is influenced by the local concentration of morphogens, the morphogen gradient is the main feature controlling the spatial pattern of embryos, which means that embryonic stem cells exposed to different concentrations of morphogens have different fates [51, 52].

Microplatforms are convenient tools for re-creating in vivo-like microenvironments of gradients of biochemical stimuli because it is relatively easy to establish gradients in a small space comparable to the in vivo microstructure of cellular environments (Fig. 1Bii). Jeon’s group provided an excellent description of this in reports of various microplatforms, such as ladder chambers and micromixers, to establish chemical gradients; they used these microplatforms to analyze the behaviors of cells such as cancer cells [53], neutrophils [54] and stem cells [41]. Chung et al.

### Table 1. Categorization of microplatforms used in stem cell research

| Platform type | Stem cell type | Remarks | Usable points for stem cell research | Reference |
|---------------|----------------|---------|-------------------------------------|-----------|
| Delivery      | CML stem/progenitor cell | Trapping single cells | Analysis on hundreds of nonadherent single cell in parallel | [33]       |
| hNESPCs       | Mimicking the human upper airway, gaseous formaldehyde toxicity via airway delivery | | Mimicking the 3D environment of stem cells | [32]       |
| mESC          | Biomolecule delivery through a gel layer, spatiotemporal control of neuronal commitment | | Spatiotemporal control of mESC via gradient delivery | [38]       |
| hESC          | Individual hESC colonies in dynamic or static condition, spatiotemporal delivery | | Maintenance of self-renewal property on microfluidic culture | [39]       |
| miPSC         | Spatiotemporally nonuniform culture on microplatform | | Control stem cell fate by delivery pattern switching | [40]       |
| Gradient generator | hNSC         | Christmas tree-like channels for generation of growth factor concentration gradients | Study of stem cell behavior to chemical gradient | [41]       |
| Primary HSPC  | Micro mixer, gradient hydrogel, overlapping patterns in 3D construct | | Study of stem cell behavior to ECM stimulation gradient | [42]       |
| NPC           | Osmosis-driven flow, continuous cytokine gradient, precise flow rate control even in nanoliter scale | | Study of stem cell behavior to chemical gradient | [31]       |
| L929          | Simultaneous chemical and mechanical gradients | | Study of stem cell behavior to mechanical gradient | [43]       |
| C2C12         | Oxygen concentration generation | | Study of stem cell behavior to oxygen gradient | [44]       |
| Microarrays   | MSC, NSC      | Modular stiffness(shear moduli) of 1-50kPa, Functionalized with combinations of proteins of interest | Probing the effect of key environmental factors on the fate (e.g., cell to cell interaction, substrate stiffness) | [45]       |
|              | MSC, NSC      | 32 different combinations of fiber ECM molecules | Probing the effect of key environmental factors on the fate (e.g., cell-cell interaction, substrate stiffness) | [46]       |
|              | H09, H13, Primary rat cardiac myocyte, C2C12 | Microbioreactor array, perfused with culture medium | Spatiotemporal investigation on factors for stem cell fate | [47]       |
|              | mESC          | Concave microwell, size-tunable EBs | Formation of uniform-sized EBs on static culture | [48]       |
|              | mESC          | Microfluidic array containing concave microwells, size-tunable EBs | Formation of uniform-sized EBs on dynamic culture | [49]       |

Abbreviations: 3D, three-dimensional; CML, chronic myeloid leukemia; EB, embryoid body; ECM, extracellular matrix; ESC, embryonic stem cell; h, human; hNESPCs, human nasal epithelial stem/progenitor cells; HSPC, hematopoietic stem cell and progenitor cell; iPSC, induced pluripotent stem cell; m, mouse; MSC, mesenchymal stem cell; NPC, neural progenitor cell; NSC, neural stem cell.
The ECM microarray patterned with 32 different combinations of 5 concentrations to optimize these functions.

Microarrays

Microarrays are widely used microplatforms for high-throughput screening in biological studies including stem cell research [55–58] (Fig. 1Bii). A unique property of microarrays is that researchers can conduct parallel experiments with tunable conditions on each array. Microarrays may be categorized as static and dynamic (Table 1).

Static microarrays contain many wells and display no flow. The surface of such microarrays is tunable with various kinds of molecules, which would affect the cells cultured; some detailed effects of the microarray surface on the behaviors of cells will be described later in this review. Gobaa et al. used robotic technology to functionalize the individual microwells with combinations of proteins [45]. This method allowed each well of the microplatform to have its own level of shear moduli of 1–50 kPa.

Because oxygen gradients in tissues can sharply define boundaries of metabolically active cells and areas of hypoxia, owing to the low solubility of oxygen in aqueous media [29], on-chip generation of oxygen gradients could be a great tool to use for stem cell research. Mehta et al. realized that zones could be formed in physiological tissue by creating a concentration gradient of oxygen on microfluidic bioreactors fabricated from gas-permeable polydimethylsiloxane (PDMS) [44]. C2C12 cells were cultured on the platform and used to generate an oxygen gradient and decrease perfusion velocities. This platform, with its oxygen concentration gradient, has wide potential for applications in stem cell research because oxygen has a profound influence on cell signaling involved in the survival, growth, and differentiation of cells, and different types of cells require different oxygen concentrations to optimize these functions.
Many researchers have applied methods to induce formation of EBs that use suspension culture and hanging drop culture [67, 72–75], but these conventional methods are beset by problems such as size heterogeneity of the EBs and poor scale-up for mass production. To allow cells to agglomerate in solution and form aggregates in suspension cultures, ESCs are cultured on nonadhesive culture dishes. The suspension culture method has the advantage of being scalable, but there are wide variations in sizes. The hanging drop method has the benefit of the ability to control EB size. Because the whole process involved in making hanging drops is laborious, this method is limited to studies with relatively few EBs. Takayama’s group has developed and commercialized hanging drop culture systems [76]. With this method, however, it is difficult to change media without disrupting the EBs, and mass production of EBs is too difficult because most of the process has to be carried out manually, which also makes this method difficult to scale up. To address the limits of these conventional methods to form EBs, other novel microtechnology-based methods efficiently control EB formation [48, 68, 69, 76–99] (Table 2).

Static methods are one of the two main types of methods used to control EBs. Many of the static methods use well-defined microwells to form EBs and to control their average size [48, 68, 83, 84, 94]. Physical restrictions formed by the microwell are known to affect the microenvironment of the EB, the degree of cell-cell interactions, and the extent of ECM deposition. In this type of culture, EBs grow until restricted by features of the microwell geometry such as its size and shape. Khademhosseini et al. presented a method to culture hESCs with controlled cluster sizes, for the maintenance and subsequent differentiation of these cells, using PDMS cylindrical microwells made by conventional soft lithography [84]. They demonstrated the possibility of culturing hESCs homogeneously while maintaining their undifferentiated state, as confirmed by the expression of the stem cell markers Oct-4 and alkaline phosphatase (ALP). Choi et al. reported static concave arrays able to control the average size of EBs [48]. Using deflection of a thin PDMS membrane, they fabricated concave molds with diverse sizes in a simple and cost-effective way. They successfully produced diverse sizes of EBs, which were differentiated to neural and cardiac cells. Moon et al. were able to regulate the size of EBs formed from single hESCs using concave microwells [101]. Defined numbers of single hESCs were forced to aggregate and generate uniformly sized EBs with high fidelity. They discovered that differentiation of H9- and CHA15-hESCs was affected by EB size in both the absence and presence of growth factors and demonstrated that concave microwells could be used to screen different EB sizes and growth factor concentrations to optimize differentiation for each hESC line. Due to the geometric effect, most of the cells aggregated well in a concave structure having a certain curvature, and their retrieval from the well was shown to be simple, which facilitated the preparation of the EBs without damage. With the concave well, homogeneous spheroids produced by diverse cell types including hepatocytes, cancer cells, neurons, and pancreatic cells have been successfully created, and this technology could be extensively applied in 3D tissue generation and organ regeneration [3, 102]. Choi et al. developed size-controllable networked neurospheres as a 3D neuronal tissue model using concave microwells, and this array of wells was commercialized (StemFIT 3D; Microfit, Gyeonggi-do, Korea, http://www.microfit.kr) [103]. They generated networks of homogeneous neurospheroids using
well-defined concave microwells and used this 3D neural tissue model to analyze the amyloid β effect. Hydrogels can be used as materials to fabricate microwells due to their biocompatibility and amenable fabrication. Karp et al. [84, 85] and Tekin et al. [95] used poly(ethylene glycol) (PEG) microwells to prevent cell adhesion because microwells fabricated from photocrosslinked PEG have cell-repulsive and inert properties. Ozawa et al. used an alginate gel microwell array fabricated on patterned indium-tin-oxide electrodes to make EBs [83]. This method has the advantage of there being no requirement to remove the molds, and the thickness of the hydrogel can be handled simply by changing the deposition time. Likewise, AggreWell plates (StemCell Technologies, Vancouver, Canada, http://www.stemcell.com) containing microwells, which can be used to aggregate stem cells into EBs, have been commonly used for static methods [100, 104].

In addition to the static techniques, dynamic techniques can also be used for controlling EB formation [82, 86, 90, 92, 96, 105]. Microfluidics is a major feature of the dynamic methods that is not used in the static methods. Microfluidic systems that use the flow in a chip have many advantages: the EB size can be controlled easily, and the fabrication process of the microfluidic chip is simple. In addition, they enable the researchers to study the EB under various changes of the microenvironment. Torisawa et al. developed a microfluidic device for straightforward and synchronous formation of uniform-sized EBs [88]. In this chip, the size of EBs can be controlled by changing the cross-sectional size of microchannels or by changing their geometry. EBs can also be extracted from microchannels without damage and used for further differentiation studies. Jeong et al. fabricated a microfluidic chip integrating concave microwells using the meniscus induced by the surface tension of a liquid PDMS prepolymer [90]. With this chip, mass production of EBs is possible without loss of cells and laborious intervention to refresh culture media. The EBs can be retrieved just by flipping over the chip and tapping a few times. Kang et al. developed a PDMS microfluidic chip containing concave microwells and a flat cell culture chamber [86] (Fig. 2Bii). This microfluidic chip could produce uniform-sized EBs and control EB replating without pipette-based manual cell retrieval. The advantage of this platform is that EBs were produced in concave microwells in which the influence of uniform shear stress could be sustained and replating of EBs was controlled simultaneously without pipette-based manual cell retrieval. By using this microsystem, differentiation and proliferation of multiple EBs could be studied simultaneously. Park et al. also fabricated a 3D in vitro
| Type        | Stem cell type | Materials                  | Characteristics of the platform | Method                      | Advantages of the platform                          | Reference |
|------------|----------------|----------------------------|--------------------------------|-----------------------------|------------------------------------------------------|-----------|
| Static     | hESCs          | Matrigel                   | Matrigel-patterned microwell  | Micromolding               | Uniform-sized EB formation                           | [68]      |
|            | mESCs          | PDMS                       | Microfabricated adhesive stencils | Micropatterning micromolding | Uniform-sized EB formation                           | [69]      |
|            | hESCs          | PDMS, Matrigel             | Matrigel-coated microwell     | Micromolding               | Homogeneous and controllable EB formation            | [79]      |
|            | hESCs          | PDMS                       | Square microwell              | Micromolding               | Uniform EB formation                                 | [81]      |
|            | mESCs          | Alginate gel               | Alginate gel microwell on a patterned ITO electrode | Electrodeposition | Successful EB formation                           | [83]      |
|            | hESCs coculturing with MEFs | PDMS                       | Fibroblast-layered microwell  | Micromolding               | Uniform EB formation                                 | [84]      |
|            | mESCs          | PEG                        | Cell-repellant PEG microstructure | Micromolding               | Uniform-sized EB formation                           | [85]      |
|            | hESCs          | PDMS                       | PEG microwell                 | Micromolding               | Uniform EB formation                                 | [92]      |
|            | mESCs          | PDMS                       | Matrigel-coated cuboidal microwell | Micromolding               | Uniform EB formation high throughput               | [93]      |
|            | HepG2          | PEG                        | Stimuli-responsive microwells | Micromolding               | Controllable size of EBs                              | [94]      |
|            | mESCs          | PDMS                       | Meniscus-induced concave microwell | Meniscus                   | Uniform EB formation mass production                | [95]      |
|            | mESCs          | PDMS                       | Combination of forced aggregation formation and rotary orbital suspension culture | Micromolding | Controllable size of EBs                              | [96]      |
|            | mESCs          | PDMS                       | Honeycomb microwells using a laser-jet printer | Micromolding | Uniform-sized EB formation                           | [98]      |
| Dynamic    | mESCs          | PDMS                       | Concave microwell             | Micromolding               | Controllable size of EBs                              | [99]      |
|            | mECC           | PDMS                       | EB-trapping microwell         | Micromolding               | Uniform-sized EB formation                           | [82]      |
|            | mESCs          | PDMS                       | 3 × 3 Multilayer              | Micromolding               | Uniform-sized EB formation EB reploting to be controlled | [86]      |
|            | mESCs          | PDMS                       | Semiporous polycarbonate membrane | Micromolding               | Straightforward uniform-sized EB formation           | [88]      |
|            | mESCs          | PDMS                       | Meniscus                      | Meniscus-induced           | Mass production of uniform-sized EB Without loss of cells | [89]      |
|            | mESCs          | PDMS                       | EB-trapping microchannel      | Micromolding               | EB patterning                                        | [91]      |
|            | iPSC ESC       | PDMS                       | Shallow trapping cup          | Micromolding               | enable to induce differentiation and keep the pluripotent and self-renewing state of the cells | [105]      |

Abbreviations: EB, embryoid body; ESC, embryonic stem cell; h, human; iPSC, induced pluripotent stem cell; ITO, indium tin oxide; m, mouse; MEF, mouse embryonic fibroblast; PDMS, polydimethylsiloxane; PEG, poly(ethylene glycol).
brain model from neuronal progenitor cells using a microfluidic chip and evaluated the effects of flow and amyloid β, the presence of which as plaques in the brain is one of the main features associated with Alzheimer’s disease, and demonstrated the possibility of using the system as a drug- or therapy-testing tool [49].

Another interesting application of a microwell system is the production of 3D microtissues cocultured with adult stem cells and cells from organs. No et al. generated uniform-sized hepatocyte spheroids using concave microwells [102]. By coculturing primary human hepatocytes and human adipose-derived stem cells (ADSCs) in concave microwell arrays, these authors generated human hepatocyte spheroids with high viability and improved hepatic function. Takayama’s group has developed a microprinting technology that uses immiscible aqueous solutions of two biopolymers for creating heterocellular stem cell niches to guide embryonic stem cell fate [106]. This printing technique uses a polymeric aqueous two-phase system composed of PEG and dextran (DEX) as the phase-forming polymers. The technique allows autonomous dispensing of the DEX phase containing target biomaterial onto a monolayer of cells maintained in the PEG phase. Using this system, they created freestanding printed mESC colonies on the two types of supporting feeder cells: PA6 stromal cells and mouse embryonic fibroblasts (MEFs). By coculturing, Takayama’s group demonstrated that mESC colonies on MEF support cell clusters that remained undifferentiated, whereas mESC colonies on PA6 support cell clusters that showed neuronal differentiation. Although studies of these 3D coculture models are at the early stages, these models are likely to be used to simulate adult stem cells in organ tissues and be broadly used for the study of adult stem cell behavior and function without the use of ECM when they are implanted into the mouse. From our experience, well-defined spheroids of human tonsil-derived mesenchymal stem cells using a microplatform and implanted the spheroids into a mouse with ovariectomy. We observed improved regeneration of ovary function when we implanted the spheroids into the mouse with parathyroidectomy without any ECM-based encapsulation. At 1 month after implantation, we observed improved parathyroid function. We also prepared spheroids of human placenta-derived mesenchymal stem cells using a microplatform and implanted them into mouse with ovariectomy. We observed the regeneration of ovary function when we implanted the spheroids into the mouse. From our experience, well-defined spheroids of mesenchymal stem cells could maintain their viability and function without the use of ECM when they are implanted for longer than 1 month, and we expect that the microplatform-based 3D spheroid technology will be applied popularly in organ regeneration.

These 3D stem cell spheroids could be used for the regeneration of organ functions. Jun et al. produced islets by coculturing primary islet cells with ADSCs using a microplatform (StemFIT 3D) with concave wells and transplanted the spheroids into a mouse model of diabetes. The cocultured ADSCs played a critical role in enhancing the function of the islet, and the glucose level of the diabetic mouse was maintained stably. Recently, we and our colleagues performed two interesting organ-regeneration experiments using 3D stem cell spheroids (unpublished data). We prepared spheroids of human tonsil-derived mesenchymal stem cells using a microplatform and implanted the spheroids into the mouse with parathyroidectomy without any ECM-based encapsulation. At 1 month after implantation, we observed improved parathyroid function. We also prepared spheroids of human placenta-derived mesenchymal stem cells using a microplatform and implanted them into mouse with ovariectomy. We observed the regeneration of ovary function when we implanted the spheroids into the mouse. From our experience, well-defined spheroids of mesenchymal stem cells could maintain their viability and function without the use of ECM when they are implanted for longer than 1 month, and we expect that the microplatform-based 3D spheroid technology will be applied popularly in organ regeneration.

### Soluble Factors

Soluble molecules are the most frequently used factors to direct the fate of stem cells. As noted, BMPs, Wnt, and FGFs transform surface ectoderm into neural crest cells; EGF, FGF2, and PDGF induce proliferation; and FGF4 is used for neuroectodermal specification of mESCs. However, there remain many soluble factors with functions that are unknown, and whether factors other than FGF4, for example, are also involved in determining the fate of mESCs is unknown.

Many kinds of microplatforms are being exploited to elucidate the effects of such molecules on stem cells. Blagovic et al. developed a multiplex microfluidic platform to isolate the effects of FGF4 on fate specification of mESCs [116] (Fig. 3Ai). This platform consisted of fluidic and control layers and separated chambers to continuously remove cell-secreted factors, which, in turn, could be used to manipulate signaling from soluble factors such as FGF4. It was shown that both FGF4-dependent and -independent pathways were used when mESCs were committed to neuroectodermal specification driven by autocrine/paracrine signaling. Ju et al. also designed a platform with the cell culture chamber separated from the seeding channel. This platform was used to perform perfusion of hepatic differentiation medium to observe the differentiation of MSCs into hepatic cells [121]. A culture medium consisting of hepatocyte growth factor, EGF, and other factors was perfused toward the cell culture chamber connected to the seeding channel. The results indicated that those cells cultured on the microplatform had the ability to take up lipoprotein just like cells cultured on plates but showed more positive Dil-Ac-LDL signals, indicating that perfusion of hepatic differentiation

### Use of Microplatforms to Control the Behaviors of Stem Cells

Controlling the function and behavior of stem cells, such as attachment, migration, self-renewal, and differentiation, is important, particularly when dealing with therapeutic applications of these cells. Because many stem cells have the potential to form tumors when implanted into the body [21, 107, 108], this type of study could produce a huge advance for regenerative medicine research and even for future medical practices to control the function and behavior of such implanted stem cells. Knowing that the milieu that regulates the pattern of behavior of stem cells could be chemicals that indigenous cells secrete or physical effects such as contact with other cells and the extracellular matrix, further research should be carried out to determine the specific effects of the various factors on the behavior of the stem cells. In this regard, microplatforms are a powerful tool because of their enhanced analytical performance, including high sensitivity and reproducibility [109]. Using them, researchers can apply various environments on the microscale to stem cells controlled by a wide variety of manipulated variables. Regulation of the microenvironments that stem cells encounter is a prerequisite for researchers to clearly investigate the relationship between the manipulated and dependent variables and to quantify the effects of changes in the conditions on the stem cells when using microplatforms. Microplatforms containing, for example, peptides with certain amino acid sequence patterns [110], ECM [46, 111, 112] and various biomaterials [113–115] have been developed for the enhancement of stem cell research by making it possible to predict the path taken by the stem cells. Stem cells seeded on these platforms sense each pattern on platforms, and researchers have even observed induced responses in approximately 1,700 cases simultaneously. In this section, we will review the current microplatform-based approaches to control the behaviors of stem cells, emphasizing the types of factors, including soluble factors, physical factors, and others.
Figure 3. Schematics of microplatforms for stem cell control. (A): Soluble factors are applied to stem cells as fate determinants in various ways, for example, through valve control [116] (A(i)), porous membrane [40] (A(ii)), gradient generation [31] (A(iii)), or trapping cells [82] (A(iv)). (B): Physical factors applied as fate determinants include mechanical stimuli such as stiffness [117] (B(i)), shear stress [43] (B(ii)), and electrical signals [118] (B(iii)) and can be controllers according to an equivalent electrical circuit. (C): Other factors applied as fate determinants include temperature [119] (C(i)), genes [120] (C(ii)), and ECM [46] (C(iii)). Images used by permission. Abbreviations: BMP4, bone morphogenetic protein 4; CPEe1, constant phase element for electrode impedance; CPEm, constant phase element for impedance of cell membrane; ECM, extracellular matrix; FGF8, fibroblast growth factor 8; Re, extracellular resistance; Rs, spreading resistance; Shh, sonic hedgehog.
medium could direct differentiation of MSCs into hepatic cells. This platform can thus be used to show simple relationships between the perfused media and the differentiated state of the stem cells.

Applying laminar flow in microscale channels is an excellent strategy to investigate the functions of soluble factors on stem cell behavior. Kawada et al. presented a membrane-based microfluidic device designed to form spatiotemporally nonuniform culture environments for stem cells [40] (Fig. 3Aii). In their work, ESCs were cultured on a porous membrane that divided the channels into upper and lower layers and that, in turn, exposed the soluble factors to different flow patterns. Because the flow rate was approximately 0.4–4 μl/minute, the diffusion of the soluble factors was maintained in a laminar flow over a long enough period of time. The authors made flow patterns containing RA and leukemia inhibitory factor to determine whether the miPSCs expressed Nanog, a transcription factor required for self-renewal of ESCs. They showed that 12 hours of induction by a low concentration of RA was not sufficient to suppress Nanog; 72 hours was required instead. Cell lineage commitment of miPSCs was reported to be determined by a time-dependent switching of the delivery pattern, which was easily controlled by changing the rate of the applied laminar flow. At the same time, use of this microplatform led to new in vitro experimental procedures for stem cells by providing spatial heterogeneity in the surroundings of these cells, which could be of relevance to the detailed analyses of the characteristics of stem cell differentiation in time and space. An extremely slow rate of flow was used to expose stem cells to the gradient of soluble factors, as discussed earlier [31, 122]. Using a simple osmotic pump, the concentration gradient was generated with a slow flow rate, and stem cells took in biochemical factors in different concentrations and experienced different fates (Fig. 3Aiii). Using soluble factors, platforms were also used to induce differentiation of embryoid bodies. Kim et al. proposed a microplatform for on-chip differentiation of embryoid bodies [82] (Fig. 3Aiv). After adjusting the average size of embryoid bodies by changing the duration and rate of the flow, RA was applied to induce differentiation of EBs into a neuronal lineage. RA-treated cells in EBs showed a relatively intense response to the neuron-specific antibody TuJ1 and appeared as mature neuronal cells with long neurites. This microsystem holds promise for use in an on-chip method to induce and monitor differentiation of EBs.

**Physical Factors**

The field of stem cell mechanobiology recently garnered attention for its potential to explain why the adhesion, motility, and differentiated state of cells are influenced by mechanical stimuli such as stiffness and shear stress [117, 123] (Fig. 3B, 3Bi). Because cells are the components of viscoelastic tissues, mechanical forces provide a crucial set of signals that can alter the structures of cells and regulate their functions, resulting in a change of their behavior. Adhesions of cells, for example, are attained through focal adhesions and are increased in number according to the modulus of the matrix, causing alterations in transmembrane adhesion receptors and the actin-myosin cytoskeleton and finally in the mechanotransduction processes of the cells; however, it is difficult to realize and modulate mechanical factors on conventional culture dish. In contrast, microplatforms can provide in vivo-like physical microenvironments, such as stiffness of substrate and shear stress generated by fluid flow, and other types of physical microenvironments, such as electricity and pressure. It means that microplatforms can widen the range of stem cell research by allowing researchers to investigate the role of mechanical factors. In this section, we describe the consequences of stem cells being exposed to various types of physical cues on a chip.

In general, cells on stiffer substrates have stiffer, more organized cytoskeletons and more stable focal adhesions [124, 125]. Gilbert et al. used a bioengineered microplatform with substrates to recapitulate key biophysical and biochemical microenvironmental features and cultured muscle stem cells (MuSCs) on this platform to regulate their fate [126]. Cultured MuSCs were then implanted into mice and showed properties that depended on the elasticity of the culture plate; soft substrates with moduli of 12 kPa were observed to be better for self-renewal of MuSCs and muscle regeneration. These results show that recapitulating physiological tissue rigidity in a platform holds promise for the development of cell-based therapies for muscle diseases. Similarly, variable moduli interpenetrating polymer networks (vmIPNs), which are synthetic interfacial hydrogel systems, were developed to analyze the effects of material moduli on adult NSCs [127]. The spread of NSCs and the induction of their self-renewal and differentiation were blocked on vmIPNs with moduli of approximately 10 Pa. In contrast, NSCs were most likely to express the neuronal marker β-tubulin III on the vmIPNs, with moduli of about 500 Pa, and differentiate into glial cells with moduli of approximately 1,000–10,000 Pa. Modifying substrate stiffness, which changes the interaction between cells and substrates, is one method for controlling the fate of stem cells. Cell-cell interactions can also be exploited to control stem cells. Rodríguez et al. and Luo et al. reported on microplatforms designed to regulate cell adhesion and multicellular organization by creating different adhesive [128] and density [114] conditions in regions in which stem cells are found. These platforms enable spatial and temporal control over the path and direction of migration and thus recapitulate coordinated multicellular migration and organization in vitro.

Shear stress induced by fluid flow upregulates gene expression of MAP kinase 8 (MAP3K8) and interleukin-1β, which may be a mediator of intracellular mechanotransduction of certain types of cell behaviors [129]. Lee et al. developed an integrated microfluidic culture device to control the cell docking and shear stress profiles [130]. This chip was designed with an air control layer and a fluidic layer with a 4 × 4 micropillar array. By exploiting microvalves, this platform can be actuated for cell seeding and medium perfusion, which make it easy to control microenvironmental conditions such as shear stress, with a flow rate ranging from 1 to 10 μl/minute. The authors immunostained seeded embryonic stem cells with platelet endothelial cell adhesion molecule (PECAM; an endothelial marker), and smooth muscle actin (a muscle cell marker). They found that most of these cells were PECAM positive, which showed that the ESCs differentiated into endothelial cells under the uniform cell docking and shear stress conditions. Kim et al. also investigated the osteogenic differentiation of MSCs according to the applied shear stress through transcriptional coactivator with PDZ-binding motif (TAZ) activation using a microplatform with an osmosis-driven pump [131] but using a different order of magnitude of flow when it was induced by an interstitial level of slow flow. As a transcriptional modulator of MSCs, TAZ interacts with many transcription factors including Runx2, TEFs, TTF-1/Nkx2.1, Tbx5, Pax3, Smad2/3 complexes, and MYOD [132–138]. Kim and colleagues analyzed the effect of shear stress by investigating target genes of TAZs such as CTGF and Cyr61 with activation by TAZ. The interstitial level of shear stress caused
Table 3. Microplatforms exploiting various factors as a stem cell fate determinants

| Factor | Stem cell type | Characteristics of platform | Remarks | Reference |
|--------|----------------|-----------------------------|---------|-----------|
| Soluble | mESC | 2 parts; fluidic layer and valve layer | Downregulated autocrine/paracrine signaling, sufficiency of extracellular factors, neuroectodermal commitment | [124] |
|         | hMSC | 3 parts; cell culture chamber, perfused channel, cell seeding channel | Perfused hepatic differentiation medium and differentiated cells | [125] |
|         | iPSC | 2 layers divided by a porous membrane | Spatiotemporally nonuniform culture on microplatform | [40] |
|         | NPC | Osmotic pump with polyurethane tube | Osmosis-driven flow, continuous cytokine gradient, precise flow rate control even in nanoliter scale | [31] |
|         | hMSC | Osmotic pump with glass tube | Osmosis-driven flow, continuous cytokine gradient, precise flow rate control even in nanoliter scale | [107] |
|         | hNSC | Christmas tree-like microchannels for micromixing | Study of stem cell behavior to chemical gradient | [41] |
| Physical | MuSC (Muscle stem cell) | PEG hydrogels with tunable mechanical property | Substrate elasticity as a regulator of MuSC fate (cell-substrate interaction), recapitulating physiological tissue rigidity | [131] |
|         | NSC | Variable moduli interpenetrating polymer networks | Cell-substrate interaction | [132] |
|         | hMSC | Micropatterning 3 distinct regions | Effect of cell-substrate interaction on cell-cell interaction | [133] |
|         | ESC | Integrated device; air control channel, fluidic channel with 4 × 4 micropillar arrays | Control of uniform cell docking and shear stress profile, differentiation into endothelial cells | [135] |
|         | hMSC | Osmotic pump with polyurethane tube | Interstitial level of shear stress, activation of TAZ target genes, osteogenic differentiation | [136] |
|         | mMSC | Microfluidic perfusion device embedded with aligned nanofibers | Regulating fibrochondrogenic differentiation of MSCs | [143] |
|         | ARPCs | Porous polycarbonate membrane sandwiched between two PDMS layers with infusion pump | Mimicking the 3D environment of stem cells | [144] |
|         | hMSC | Multifunctional organic bioelectronics | rGO for adhesion and alignment of hMSCs, osteogenic differentiation | [146] |
|         | Rat MSC | Laser patterning | In vitro cardiac muscle model with multiple muscle fibers, on-chip analyses of electrical conductivities of different cell types | [12] |
|         | hMSC | Pneumatic actuator with a flexible diaphragm consisting of an air chamber and cell chamber | Compressive pressure to hMSC for inducing osteogenesis | [147] |
TAZ to become localized in the nucleus and induced osteogenic differentiation of MSCs. At the same time, TAZ-depleted cells showed no osteogenic differentiation under the same shear stress condition used with TAZ. Using this platform, the authors were able to conclude that TAZ was an important mediator in osteoblast differentiation of MSCs.

Biomimetic microplatforms were also developed and used the above-mentioned physical factors to control the behavior of the cells. Zhong et al. developed a microfluidic chamber embedded with aligned nanofibers with various angles, a biomimetic nanotopographical microenvironment capable of integrating information about location and flow stimuli [139]. With these aligned fibers, this platform could direct the flow at different angles. The MSCs were cultured on this platform to investigate the effects of the microfluidic and nanotopographical environment on the morphology of the cell and on fibrochondrogenesis. The involvement of the RhoA/ROCK pathway and Yes-associated protein (YAP)/TAZ were also investigated. The RhoA/ROCK pathway is required for fibrochondrogenic differentiation, whereas YAP/TAZ knockdown significantly upregulated chondrocyte-specific markers. Perpendicular flow at a rate of 1 dyne/cm² caused MSCs to be more chondrocyte-like, with an elongated and flattened cell shape and a more rounded nuclear shape. This platform was used to show that the flow direction perpendicular to the aligned nanofibers was conducive to fibrochondrogenesis of MSCs. Sciancalepore et al. presented a microdevice that mimics the proximal tubule in the kidney [140]. The multipotent tubular adult renal stem/progenitor cells (ARPcs) were seeded in this chip and were subjected to fluid shear stress to induce cell polarization. ARPCs exposed to fluid shear stress expressed the aquaporin-2 transporter and localized them at the apical region while localizing Na⁺K⁺ATPase pump at the basolateral portion. Regarding the functions of the cells, recoveries of urea and creatinine of 20 ± 5% and 13 ± 5%, respectively, were obtained.

Electrical factors have also been used to stimulate stem cells. Whether an electrode-based chip with electrical impedance spectroscopy can be used to characterize the growth of hMSCs on electrodes was investigated using finite element method simulation [118]. Cell-cell and cell-substrate gaps were modeled as equivalent electrical circuits to investigate the electrical characteristics of the cell layers (Fig. 3Biii). This paper showed that the electrode-based cell chip combined with microfluidic systems increased the efficiency of the treatment of the cells. Because it was reported that electrical field stimulation could induce cardiogenesis of hESCs even in the EB form [141], Hsiao et al. tried to apply electrical stimulation to hMSCs for on-chip manipulation on multifunctional graphene and poly(3,4-ethylenedioxythiophene) (PEDOT) microelectrode arrays [142]. This platform was composed of reduced graphene oxide, for promoting the adhesion and alignment of cells and accelerating osteogenic differentiation, and dexamethasone21-phosphate disodium salt-loaded PEDOT for electroactive release of drugs. Under electrical modulation, the osteogenic differentiation of hMSCs was upregulated.

Table 3. (Cont’d)

| Factor     | Stem cell type | Characteristics of platform                                      | Remarks                                                                 | Reference |
|------------|----------------|-----------------------------------------------------------------|------------------------------------------------------------------------|-----------|
| Temperature| Drosophila embryo | Embryo located at the middle of channel | Temperature step, variation in the Bicoid morphogen gradient,            | [148, 149] |
| Oxygen     | Human endothelial progenitor, hESC-derived smooth muscle cell | PMMA enclosure, oxygen chamber, PDMS wall, culture channel, glass bottom of the device | Dynamics of dissolved oxygen and shear stress in vasculature            | [150]     |
| Peptide    | hADSC          | Self-assembling peptide nanofiber hydrogels                     | Functionalized self-assembling peptide nanofiber hydrogels on a chip, mimicking stem cell niche to control hADSC behavior in vitro | [19]      |
| ECM        | hMSC, HSPC     | Monolayer of immobilized fibronectin                            | Reliable anchorage of native cell-secreted ECM                           | [82]      |
|            | C2C12          | Nanoliter-scale analysis of biomaterials                        | Rapid, Simultaneous characterization of >1,700 hESC-material interactions and characterization of their interactions with cells | [82]      |
|            | Primary HSPC   | Micromixing platform                                            | Study of stem cell behavior to ECM stimulation gradient                 | [42]      |

Abbreviations: 3D, three-dimensional; ADSC, adipose-derived stem cell; ARPC, adult renal stem/progenitor cell; ECM, extracellular matrix; ESC, embryonic stem cell; h, human; HSPC, hematopoietic stem cell and progenitor cell; m, mouse; MSC, mesenchymal stem cell; MuSC, muscle stem cell; NPC, neural progenitor cell; NSC, neural stem cell; PDMS, polydimethylsiloxane; PMMA, polymethyl methacrylate; PEG, poly(ethylene glycol); rGO, reduced graphene oxide.
Other types of physical factors have also been exploited. Sim et al., for example, used compressive pressure as a stimulator to induce differentiation of hMSCs into specific cell types [143]. They used a pneumatic actuator with a flexible diaphragm consisting of an air chamber and cell chambers and applied various magnitudes of cyclic compressive stimulation to hMSCs while the ALP activity of stimulated hMSCs was monitored. It was concluded that cyclic pressure enhanced the osteogenesis of hMSCs, whereas mechanical stimulation of approximately 5 kPa would be the most appropriate pressure.

**Figure 4.** Future trends in stem cell research. More in vivo-like microenvironments would be established. (A): Building blocks such as cell sheet (Ai), microtissue (Aii), and encapsulated microtissue (Aiii) can create three-dimensional environments in which stem cells are located. (B): Organ-on-a-chip techniques can mimic actual in vivo states: brain-on-a-chip (Bi), lung-on-a-chip (Bii), heart-on-a-chip (Biii), liver-on-a-chip (Biv), kidney-on-a-chip (Bv), gut-on-a-chip (Bvi).

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**Other Stimuli**

Types of stimuli other than soluble and physical factors have also been used on chip to control the behaviors of stem cells. In a remarkable example, Lucchetta et al. placed an embryo in a microplatform and introduced a flow with different temperature steps to the anterior and posterior halves of the embryo [119, 144] (Fig. 3Ci). It was shown that temperature is also a significant factor in early development process of the embryo of Drosophila melanogaster, which can affect the variation in the bicoid morphogen gradient. Oxygen is also an important factor influencing stem cells. Dissolved oxygen has more functions than just keeping cells alive, as discussed in the section, “Microplatforms for Stem Cell Research,” and microbioreactors developed by Mehta et al. [44] and Abaci et al. [145] are valuable tools. These experiments have helped determine the lower and upper dimension limits in microbioreactors, which can be widely used in culturing stem cells. Table 3 shows examples of other determinants of stem cell fate.

**CONCLUSION**

As discussed, microplatforms are great tools to manage stem cell research and will advance stem cell engineering regarding the establishment of cell-based microenvironments using various niche stromal cells, as shown by Torisawa et al., who developed bone marrow-on-a-chip that permits culture of living marrow with a functional hematopoietic niche on a microplatform by engineering new bone in vivo [20]. In vivo, however, stem cells are routinely placed in complex three-dimensional extracellular environments, whereas most developed microplatforms provide stem cells with only two-dimensional environments. Because cells in three dimensions display different patterns of behaviors [146, 147], ECM topologies [148], polarity [149, 150], expression of receptors [151], and responses to drugs [152] than do cells in two dimensions, stimuli such as adhesiveness and mechanical and soluble cues that are delivered to cells on a microplatform can yield results that are dissimilar in many respects from those shown in vivo. Consequently, constructing 3D blocks from cell sheets or size-tunable (encapsulated) microtissues is crucial for mimicking natural physiological environments (Fig. 4Ai–4Aiii). Furthermore, such stem cell aggregates fabricated by the proposed bottom-up method demonstrate better performance in differentiation including higher production of target cells and more time, labor, and cost savings than do conventional methods.

In general, for the ESC culture, a feeder layer or other surface treatment is required to maintain pluripotency; however, in the case of a concave well, EBs were observed to be formed within 1 day, and pluripotency was maintained well without a feeder layer, which is a great advantage. We expect that the use of arrays of concave wells with nanopatterned surfaces or chemically treated surfaces will promote the use of cell cultures free of feeder layers.

An issue that is especially important for the use of stem cells for organ regeneration, cell therapy, and tissue engineering is the production of large enough quantities of target cells. Such production is challenging to carry out in a controlled manner and in a well-defined environment when using microplatforms, and scale-up of the system is needed. The advantage of a microfluidic system is that it is possible to establish parallel processes on a single platform because multiple microchannels can be compactly integrated in a small chip. Continued progress in the fields of integration technology and peripheral devices including valves and
pumping systems should help overcome several obstacles in producing large numbers of well-defined stem cells and target cells. Due to the marked rapid advances in microfabrication technologies, several types of microplatforms have been developed to engineer 3D microtissues including vascular structure, to assemble microtissues into small organs or tissues with the help of diverse methods such as 3D printing, and to encapsulate cells and microtissues with biocompatible materials to protect them from immune attack. Once target cells are successfully prepared, therapy and organ regeneration using stem cells prepared in a well-defined stem cell microenvironment should show improved effectiveness.

Another important application of well-defined stem cells is organ-on-a-chip, which is a high-end technique to mimic in vivo environments as much as possible [140, 153, 154] (Fig. 4Biv–4Bvi). Advanced microfluidic technologies enable the establishment of an organ model on a microfluidic chip, and by networking organ models, multiple organs mimicking a human organ system can be created on a single platform. Organ-on-a-chip technology is likely to replace single animal models and be widely used for drug and toxicity screening. For screening with human-mimicking models, use of human-cell-based chips is a prerequisite; however, it is difficult to obtain human primary cells, although just a small number of cells is required. In this case, specific organ cells differentiated from stem cells could be excellent substitutes for human primary cells, and the various microplatforms proposed for use in stem cell research should be significant help. The study of iPSCs is another important research topic of stem cell microenvironments on a chip, but few microplatforms are suitable for iPSCs. In iPSC studies, gene delivery with high efficiency and without using virus is very important. Gene transfection is one of the important applications of microfluidic chips, and diverse chips for well-regulated and efficient gene delivery have been reported [120, 155, 156]. Although there has been little related research, we expect that a highly improved virus-free gene delivery chip will be developed soon and will accelerate the progress of iPSC research. The study of the genetic states of stem cells on microplatforms is another important research topic. Genes are conclusive indicators for states of cells, and we have to do additional work if it is needed to verify genetic state of stem cells on a microplatform. Additional work on stem cells can be another stimuli to stem cells, and it can produce different results from that of intact stem cells on microplatforms. Consequently, many types of microplatforms that contain systems for epigenetic identification of stem cells will be developed.

Conclusively, microplatforms can control multiple soluble and insoluble factors simultaneously over space and time with high precision, a critical feature to provide well-defined microenvironments for stem cells. Consequently, we expect that diverse microplatforms will be used extensively in stem cell research and other applications in the future.

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AUTHOR CONTRIBUTIONS
D.P. and J.L.: collection and/or assembly of data, manuscript writing; J.Y.P.: data interpretation, manuscript editing; S.-H.L.: concept design of paper, manuscript writing and editing, final approval of manuscript.

DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
The authors indicated no potential conflicts of interest.

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