Review

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Lab-on-a-chip technologies for stem cell analysis

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The combination of microfabrication-based technologies with cell biology has laid the foundation for the development of advanced *in vitro* diagnostic systems capable of analyzing cell cultures under physiologically relevant conditions. In the present review, we address recent lab-on-a-chip developments for stem cell analysis. We highlight in particular the tangible advantages of microfluidic devices to overcome most of the challenges associated with stem cell identification, expansion and differentiation, with the greatest advantage being that lab-on-a-chip technology allows for the precise regulation of culturing conditions, while simultaneously monitoring relevant parameters using embedded sensory systems. State-of-the-art lab-on-a-chip platforms for *in vitro* assessment of stem cell cultures are presented and their potential future applications discussed.

Need for advanced technology in stem cell research

In 2010 the global stem cell (see Glossary) market was estimated at $21.5 billion and is projected to reach $63.8 billion by 2015 [1], thus outlining the importance of stem cell technology for medical therapeutics, drug development, and a variety of health-care applications including toxicological studies, disease modeling, and cell replacement therapies. Stem cells are defined as cells capable of continued self-renewal through replication and of becoming precursor cells of specific tissue types. In other words, stem cells can offer a consistent supply of physiologically relevant cells from validated pathogen-free sources that differentiate into mature somatic cells *in vivo* and *in vitro*. Stem cells have been successfully used to replace cells lost due to degenerative disease and are known to assist in the repair of damaged tissue. Examples of stem cell application range from bone healing, repair of suspensory ligament tears, and nerve injury, as well as inflammatory relieve during arthritis and immune suppression in the context of graft rejection [2].

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An important aspect of stem cell research is the availability of well-characterized and validated pluripotent stem cells comparable to those of renowned cell banks. As a consequence, the major challenges associated with culturing stem cells *in vitro* are: controlled expansion while maintaining a homogeneous culture of undifferentiated cells, and the ability to control and direct stem cell differentiation reliably. To assess the generation of fully functional and specific cell types derived from stem cells, a variety of cell-based assays are routinely used in stem cell research. The drawback of using conventional cell assays, however, is their limited reproducibility, reliability, and robustness that can lead to experimental inconsistencies, and difficulties in cell culture propagation and stem cell differentiation. Additionally, the majority of available bioassays rely on endpoint detection methods based on optical labels, thus providing a limited view on dynamic cellular mechanisms.

Lab-on-a-chip technology could deliver the next generation of cell analysis tools capable of inexpensively testing large numbers of single cells or small numbers of cell

Glossary

Brain-derived neurotrophic factor (BDNF): an enhancer of neural stem cell chemotaxis.
CXC motif chemokine 12 (CXCL12): a chemokine protein important for stem cell trafficking.
Dielectrophoresis (DEP): sensing or manipulation of cells by application of a nonuniform electric field.
Electrical cell-substrate impedance sensing (ECIS): a powerful noninvasive and label-free electroanalytical cell measurement technique; registered trademark of Applied BioPhysics Inc.
Extracellular matrix (ECM): noncellular, structural, and biochemical components of a tissue.
Field-effect transistor (FET): a sensor technology that is sensitive to electrostatic potential changes at the biointerface.
Lab-on-a-chip: miniaturized system to perform one or several laboratory tasks within a single device.
Microelectrode array (MEA): multiple electrodes used for potentiometric and conductometric sensing.
Micro-electro-mechanical systems (MEMS): miniaturized systems including electrical and mechanical components.
Mesenchymal stem cell (MSC): a multipotent stromal cell type.
Polydimethylsiloxane (PDMS): a soft polymer widely used for microfluidics fabrication.
RGD (l-arginine, glycine, and l-aspartic acid tripeptide): important cell adhesion motif.
Stem cell: cell type with self-renewal and differentiation potential.
Surface plasmon resonance (SPR): an optical sensing technique with sensitivity towards surface changes.

Table 1. LOC aspects for SC analysis[a]

Features	Importance for SC LOC	Existing solutions	Refs	Trends
Material	Controlled oxygen supply; defined shear forces; mechanical properties; sensor integration	PDMS devices	[13,14]	More task-oriented selection of material; towards hybrid-systems
Trapping, selection, sorting	Identification, separation and positioning of desired cell types within the LOC system	Geometric traps; gravitational field-flow fractionation; DEP; optical tweezers; encapsulation and emulsion droplets	[15–25]	Further combination with other LOC elements (e.g., sensors, actuators, 3D cultures)
Parallelization	Systematic screening of stimuli (e.g., soluble chemicals, gas, ECM) requires parallel testing	Six, eight, and 64 integrated microchambers	[26–28]	Highly integrated and automated LOC using micropumps and valves
Chemical gradients/soluble factors	Generation of stable and reproducible gradients is essential to identify dose-response curves	Growth factors; chemokines	[29–31]	Systematic screening of dose-response relationships
Surface properties	Physicochemical surface properties are of key importance for physiologically relevant SC behavior	2D and 3D systems; hydrogels; ECM variations; microtopography	[33–37]	Advanced 3D (co)-culture systems with improved physiological relevance
Cell patterning	Separation of cultivation and fluid handling/ analysis areas within a LOC device; spatially defined co-culture models to investigate SC signaling	Micropillar array; two-layer microfluidic; laser direct-write technology	[40–42]	Precisely controlled cocultures and defined cell positioning within 3D cultures
Shear stress	Defined fluid mechanical stress enables differentiation; low shear stress is required for stemness maintenance	Adjustable flow profiles; integrated low shear culture chambers	[38,77–83]	Systematic and standardized screening of shear stress responses
Mechanical and electrical actuators	Stimulation for guided and improved differentiation	Mechanical stretching; atomic force deformation; electronic actuators	[43–51,53,54]	Combination with chemical and topographical stimuli
Sensors	Continuous and label-free monitoring of dynamic SC responses; control of SC potency	Microscopy; SPR; voltammetry; potentiometry; impedance spectroscopy; DEP; FET	[56–58,60,61,63–67,69]	Integrated sensors with increased sensitivity/ selectivity; multiplexed analysis

[a]LOC, lab-on-a-chip; SC, stem cell.

populations under controlled and reproducible measurement conditions [3]. Microfluidic systems are vital for stem cell analysis because it is the only technology capable of providing spatial and temporal control over cell growth and stimuli by combining surfaces that mimic complex biochemistries and geometries of the extracellular matrix (ECM) with microfluidic channels that regulate the transport of fluid and soluble factors. An overview of lab-on-a-chip features and their importance for stem cell analysis is provided in Table 1.

Lab-on-a-chip materials and fabrication methods

The following section presents a short overview of methods and materials commonly used to design and fabricate lab-on-a-chip systems for stem cell analysis. Fabrication methods to build lab-on-a-chip systems are based on micro-electro-mechanical systems (MEMS) technology and include soft lithography, hot embossing, injection molding, laser micromachining, and photolithography. A common feature of lab-on-a-chip devices is that they consist of a network of microfluidic channels and contain integrated sensory systems. Of particular importance is the proper choice of biocompatible materials and biointerfaces because cellular functions are strongly influenced by the microenvironment [4,5].

An important feature of lab-on-a-chip technology is that it can integrate a variety of materials of various properties [6], which is a major advantage over existing plastic cell culture substrates. Material properties exploited include defined elasticity [7], stiffness [8], micro- and nanotopography [9], as well as surface patterns (e.g., proteins [10]). Additionally, the integration of mechanical functionalities such as microvalves, micropumps, and actuators based on deformable materials such as polydimethylsiloxane (PDMS), fluorocarbon, cyclic olefin polymer, and hydrogels allows the application of defined shear force conditions and the seamless addition of bioreagents. Generally, materials for lab-on-a-chip systems can range from inorganic substrates such as silicon, glass, and ceramic to polymers including elastomers, thermoplastics, hydrogels, and paper compositions. This means that gas-tight substrates in combination with deaerated media can be used to control