Critical Reviews in Biotechnology

http://informahealthcare.com/bty
ISSN: 0738-8551 (print), 1549-7801 (electronic)

Crit Rev Biotechnol, Early Online: 1–12
© 2014 Informa Healthcare USA, Inc. DOI: 10.3109/07388551.2014.922917

informa
healthcare

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

REVIEW ARTICLE

In vitro spatially organizing the differentiation in individual multicellular stem cell aggregates

Hao Qi[1,2,3], Guoyou Huang[1,2], Yu Long Han[1,2], Wang Lin[1,2], Xiujun Li[4], Shuqi Wang[5], Tian Jian Lu[2], and Feng Xu[1,2]

[1]MOE Key laboratory of Biomedical Information Engineering, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, People's Republic of China, [2]Bioinspired Engineering and Biomechanics Center, Xi'an Jiaotong University, Xi'an, People's Republic of China, [3]Department of Medical Genome Sciences, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Chiba, Japan, [4]Department of Chemistry, University of Texas at El Paso, El Paso, TX, USA, and [5]Brigham Women's Hospital, Harvard Medical School, Boston, MA, USA

Abstract

With significant potential as a robust source to produce specific somatic cells for regenerative medicine, stem cells have attracted increasing attention from both academia and government. *In vivo*, stem cell differentiation is a process under complicated regulations to precisely build tissue with unique spatial structures. Since multicellular spheroidal aggregates of stem cells, commonly called as embryoid bodies (EBs), are considered to be capable of recapitulating the events in early stage of embryonic development, a variety of methods have been developed to form EBs *in vitro* for studying differentiation of embryonic stem cells. The regulation of stem cell differentiation is crucial in directing stem cells to build tissue with the correct spatial architecture for specific functions. However, stem cells within the three-dimensional multicellular aggregates undergo differentiation in a less unpredictable and spatially controlled manner *in vitro* than *in vivo*. Recently, various microengineering technologies have been developed to manipulate stem cells *in vitro* in a spatially controlled manner. Herein, we take the spotlight on these technologies and researches that bring us the new potential for manipulation of stem cells for specific purposes.

Keywords

Embryoid body, embryonic stem cells, microfabrication, spatially controlled differentiation

History

Received 15 August 2013
Revised 22 December 2013
Accepted 21 February 2014
Published online 15 July 2014

Introduction

Mammals need to develop its whole body from a single cell, the fertilized egg. The building process is highly programmed and essential decisions are made during embryonic development (Bielinska et al., 1999; Lewis & Tam, 2006; Wells & Melton, 1999). In very early stages, the embryo develops a polarized structure with proximal–distal and anterior–posterior axes (Schier & Talbot, 2005; Peter & Davidson, 2009; Figure 1A). From studies of mouse embryonic development, the spatial polarity starts from the gastrulation stage with spatial patterns of differentiated specific cells for subsequent specific tissue development through mechanisms, such as gradient distribution of specific growth factors (Beddington & Robertson, 1998; Tam & Beddington, 1992; Tam & Behringer, 1997) and specific genetic circuits (Baker et al., 2008; Izraeli et al., 1999; Lenhart et al., 2011, 2013; Veerkamp et al., 2013). To achieve the development that rigorously follows the blueprint previously recorded in its genome, embryonic stem cells (ESCs) need to undergo a complicated differentiation process under strict spatio-temporal regulation with coordinating biochemical and physical cues (Arnold & Robertson, 2009; Pfister et al., 2007; Tam & Loebel, 2007).

So far, aggregation of multicellular ESCs [usually termed as embryoid bodies (EBs)] is the most popular method to induce spontaneous differentiation of ESCs *in vitro*. EBs *in vitro* differentiate into all three germ layers recapitulating early stages of embryogenesis *in vivo* (Bratt-Leal et al., 2009; Itskovitz-Eldor et al., 2000) and provide a nature-mimicking microenvironment for ESCs to achieve lineage-specific differentiation (Koike et al., 2007; Kurosawa, 2007). Various technologies have been developed to form EBs with widespread applications in both stem cell differentiation and the specific somatic cell generation of biomedical applications (Bratt-Leal et al., 2009; Carpenedo et al., 2007). However, there exist several challenges that have hindered the applications of EBs in clinical and tissue engineering fields: (i) the auto-formation of EBs from individual ESCs is inefficient, e.g. single hESCs isolated using enzyme cannot aggregate and form EBs (Burridge et al., 2007; Reubinoff et al., 2000); (ii) heterogeneous size/shape distribution of EBs results in the variation of differentiated lineages (Singla et al., 2007), e.g. ESCs-derived cardiogenesis and neurogenesis development (Choi et al., 2010b). Therefore, forming and

Address for correspondence: Hao Qi, MOE Key laboratory of Biomedical Information Engineering, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, People's Republic of China. Tel: +860 2982665937. E-mail: qhiroshi@mgs.k.u-tokyo.ac.jp; Lin Wang, E-mail: wanglin0527@126.com

culturing EBs with specified homogeneous features for clinical applications remain a big challenge (Carpenedo et al., 2009; Karlsson et al., 2008).

In addition, ESCs undergoes a wide range of differentiation processes in EBs, and many essential events in the early embryonic development can be recapitulated in the absence of extrinsic stimulations (Choi et al., 2005; Novik et al., 2006; Zambon & Barker, 2010). However, in contrast to the differentiation process of stem cells *in vivo*, stem cells in the format of EBs usually undergo a relatively random differentiation process (Figure 1B). For instance, the spheroidal layer of primitive endoderm (PE) cells uniformly forms on the exterior surface of embryonic body after aggregation (Maurer et al., 2008); however, the distinct patterns of differentiated cells (e.g. the highly ordered structure in the three germ layers) as those formed during *in vivo* embryonic development are not observed following further culture (Clark et al., 2004; Itskovitz-Eldor et al., 2000; Pekkanen-Mattila et al., 2010). Therefore, significant efforts are needed to regulate stem cell differentiation in a spatially controlled manner.

With recent advances in micro and nano technologies, various novel methods have been developed to engineer stem cell microenvironment, such as microfluidics, microgel encapsulation and three-dimensional (3D) cell printing, and to explore the possibility of regulating stem cell differentiation in a spatially controlled manner. In this article, we review recent achievements of *in vitro* technologies to form EBs and technologies to manipulate stem cells with spatial regulation for controlling differentiation. Since most *in vitro* approaches use multicellular stem cell aggregates, EBs, as the subjects, in this article, we firstly review recent *in vitro* technologies to form EBs and, secondly, summary the novel strategies to manipulate stem cells with spatial regulation for controlling differentiation.

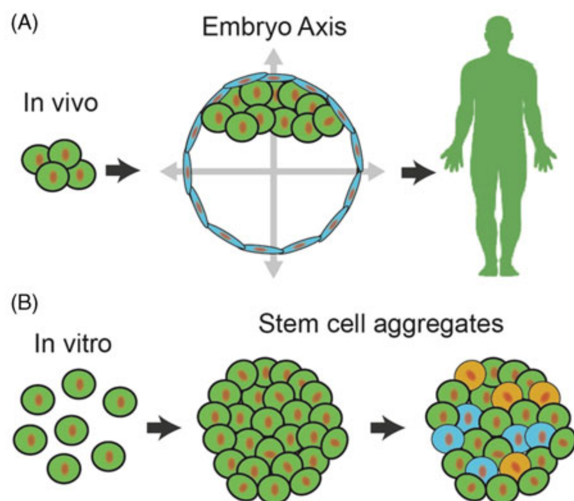

Figure 1. Stem cells differentiation process *in vivo* and *in vitro*. (A) Representation of the process embryonic stem cells development. (B) Representation of the differentiation of embryonic stem cells within the multicellular aggregates.

Microengineering methods for formation of EBs

Various methods have been developed to assemble ESCs for EB formation through promoting natural cell–cell adhesion interaction or artificially forcing cells to aggregate up (Kurosawa, 2007). These methods include traditional methods, e.g. hanging up (Boo et al., 2002; Segev et al., 2005), suspension-based methods (Abilez et al., 2006; Itskovitz-Eldor et al., 2000; Niebruegge et al., 2008; Ng et al., 2005), and more recently developed methods such as microwell-based methods (Choi et al., 2010b; Hwang et al., 2009; Karp et al., 2007; Khademhosseini et al., 2006; Lee et al., 2010; Moeller et al., 2008; Mohr et al., 2006, 2010), surface patterning (Bauwens et al., 2008; De Bank et al., 2003, 2007; Gothard et al., 2009; Lee et al., 2009; Park et al., 2007; Peerani et al., 2007; Ungrin et al., 2008) and microchannel compartments (Torisawa et al., 2007). We have compared these methods in terms of their effects on stem cell differentiation or potential application capability, including the EB size, size uniformity, throughput, easiness and biocompatibility (Table 1). For example, it has been demonstrated that the physical size of EBs is a crucial parameter that controls cell lineage-specific differentiation (Hwang et al., 2009). Interestingly, EBs with a size over 500 μm in diameter have been found to give improved mesoderm and endoderm differentiation while EBs, with a diameter of 100–500 μm, favor ectoderm differentiation (Park et al., 2007; Peerani et al., 2007). EBs with smaller size are less likely to form a contracting structure in which cardiomyocytes are enriched, while EBs with larger size have fewer cardiomyocytes (Mohr et al., 2010). It has been found that cardiomyocyte differentiation appears to be the most efficient in EBs with a size of ∼250 μm (Burridge et al., 2007; Sasaki et al., 2009). Here, we introduce the microengineering methods for forming EBs *in vitro*.

Traditional methods

The most simple and widely used method for EB formation is possibly the hanging up method, in which ESCs in hanging drops are assembled into multicellular spherical aggregates by

Table 1. Different methods for EB formation.

	Suspension	Hanging up	Surface patterning	Microwell	Cell printing
Size range	250–400 μm (Carpenedo et al., 2007)	250–300 μm (Kurosawa et al., 2003) 500–625 μm (Carpenedo et al., 2007)	200–1200 μm (Lee et al., 2009)	100–700 μm (Kurosawa et al., 2003; Jeong et al., 2013)	50–400 μm (Huang et al., 2011)
Size homogeneity	Poor	Good	Good	Good	Good
Shape	Irregularly	Spheroid	Colonies	Spheroid	Spheroid
Throughput capability	High	Low	High	High	High

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

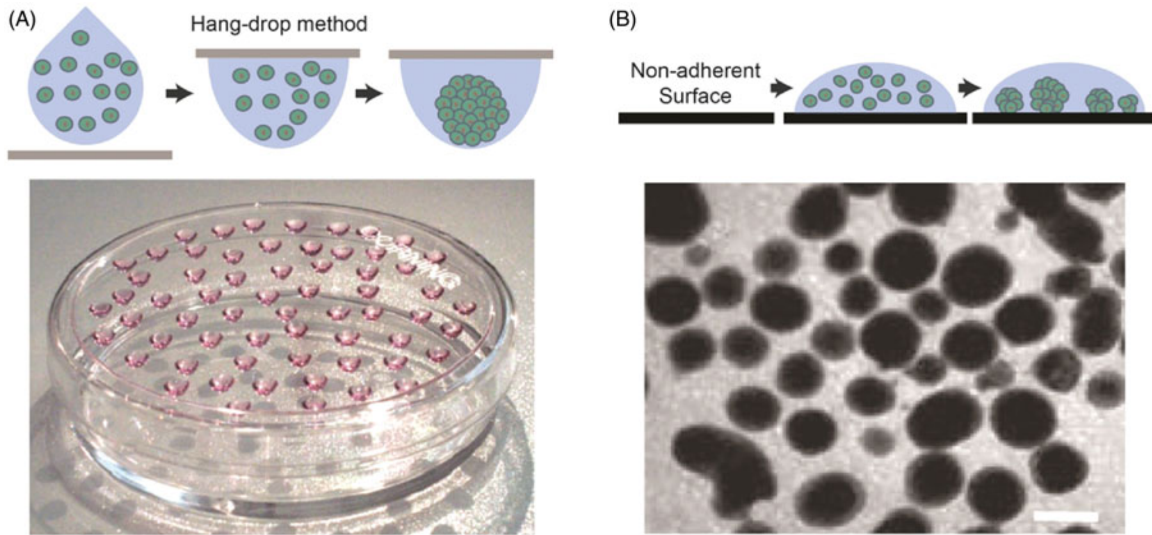

Figure 2. Engineered methods for EB formation. (A) (Top) Schematic representation for hang-drop culture for EB formation. (Bottom) EBs derived from mouse ESCs formed using the hang drop method (Seiler & Spielmann, 2011). (B) (Top) EB formation in a suspension culture system with a non-adherent surface. (Bottom) EBs formed by culturing human ESCs on hydrophobic surface (Valamehr et al., 2008). Scale bar is 250 µm.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

the force of gravity (Seiler & Spielmann, 2011; Figure 2A). This method has the advantage of easily regulating EB size through controlling the droplet size and initial seeding cell density. However, there are several limitations associated with this method. For instance, it is challenging to change culture media without disrupting the formed EBs and it is labor-intensive for large-scale EB culturing, since this method is generally performed manually. Another approach is a suspension method that has been commonly used to culture ESCs to form EBs without anti-differentiation factors (Kurosawa, 2007). In this method, ESCs placed on non-adherent tissue culture dishes can ''clump together'' in solution and form spherical aggregates (Figure 2B). Although suspension cultures are scalable, it is difficult to protect the formed EBs from collision and further aggregation due to the dynamic environment (Dang et al., 2002), which results in a wide heterogeneous distribution of EB sizes (Rohani et al., 2008; Singla et al., 2007; Youn et al., 2006).

Recently, researchers have used a pitched-blade turbine to generate an axial flow to address the above limitations (Yirme et al., 2008). However, stirring will induce hydrodynamic forces, which may negatively affect the proliferation, viability and aggregation process of ESCs (Schroeder et al., 2005). Surfaces made from hydrophobic material such as PDMS have been demonstrated to significantly improve the homogeneity of EB size (Huang et al., 2010; Valamehr et al., 2008; Yang et al., 2007). However, non-adherent surfaces yielded cell aggregates with highly irregular geometry (Yang et al., 2007). It was reported that the variation in EB size resulted in the heterogeneity of stem cell differentiation (Hwang et al., 2009) due to the difference in microenvironments within the individual EBs with various geometric properties (Hwang et al., 2009; Mohr et al., 2006). Therefore, it is still challenging to produce EBs with controlled features (e.g. shape and size) at high throughput for highly reproducible, efficient, scalable and specified homogeneous differentiation in clinical applications (Carpenedo et al., 2007; Gothard et al., 2009; Kurosawa, 2007; Mohr et al., 2010).

Microwell-based methods

In microwell-based methods, the homogeneity of EB size and shape can be controlled through physically restricting the growth of EBs using microwell arrays with various aspect ratios and sizes made from biocompatible and non-adherent materials [e.g. PEG (Hwang et al., 2009; Karp et al., 2007) and PDMS (Lee et al., 2010); Figure 3]. The contour of EBs can be controlled closely to a spherical shape using concave microwells (Choi et al., 2010b). Centrifugation can also be applied to assist in forming EBs with homogeneous size in microwell arrays. Besides the control of EB shapes and sizes, the benefits of microwell-based methods also include the high-throughput capability and media change allowance under mild conditions. However, this method is challenged by its complicated fabrication process for specific microwells, limited scalability and automation capability when centrifugation is applied (Kim et al., 2007). Besides, methods based on forced aggregation (e.g. rotary mass suspension and microwell centrifugation) may impose physical stress on EBs, thus disrupting the signaling between cells (Mohr et al., 2010). In general, EBs generated in cylinder-shaped microwells are disk-shaped while EBs from suspension cultures are spherical (Karp et al., 2007). Interestingly, the expression of crucial gene markers (e.g. SSEA-1 and AFP) has been observed to be different in EBs with various shapes or sizes (Karp et al., 2007; Figure 3C–H). It has been presumed that the shape or size of EBs may affect the internal mechanical forces and thus lead to the difference in phenotypes (Nelson et al., 2005; Zhang et al., 2012). Recently, an approach that exploited high throughput and controllability of cell printing to form controllable and uniform-sized EBs has been developed (Xu et al., 2011c). In this method, hanging-drop culture was utilized to assist the formation of EBs at the early stage before transferred to well plate for long-term culture. This method may provide an effective tool to generate optimized EBs for regenerative medicine and drug screening applications.

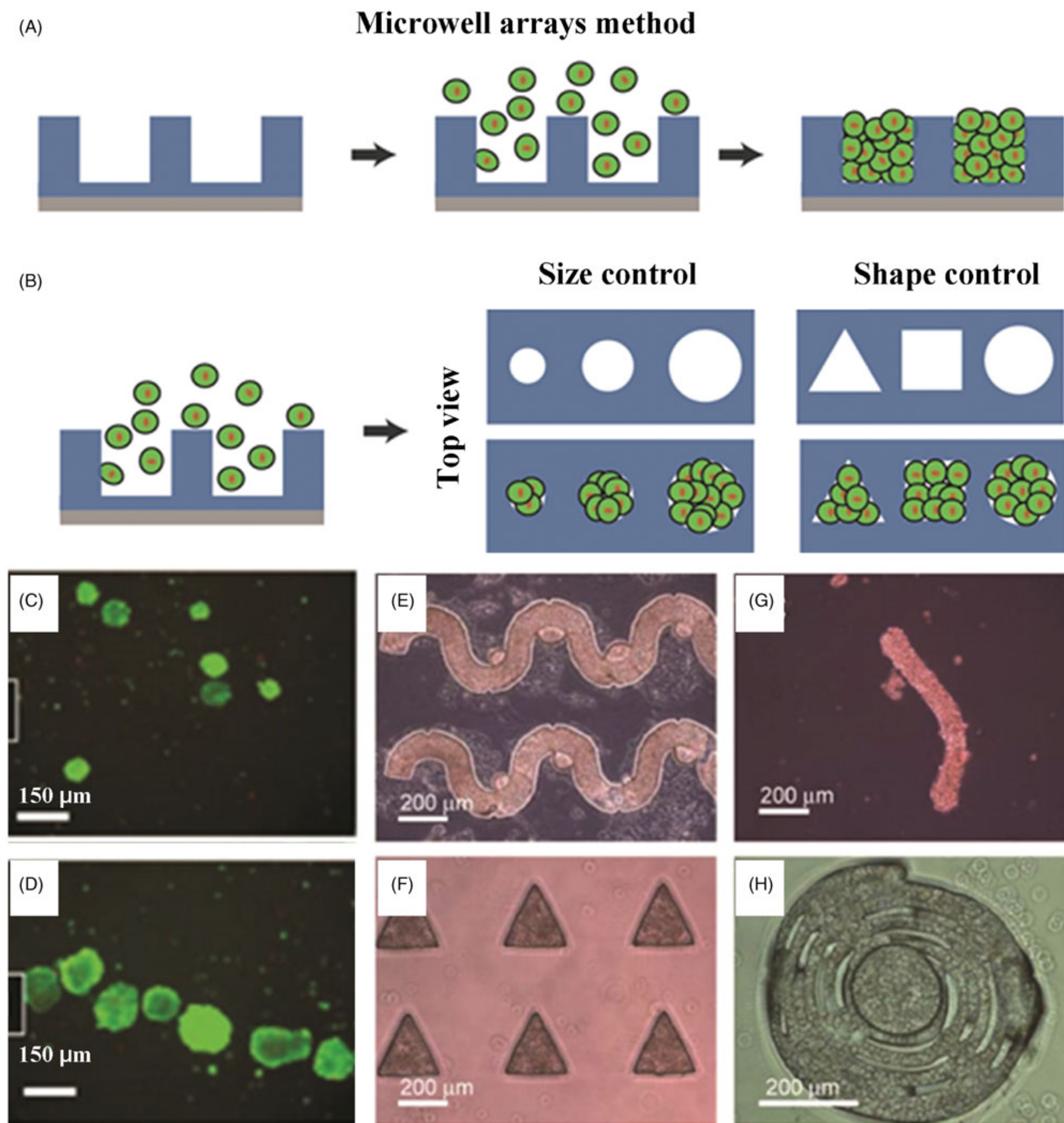

Figure 3. EBs formation in engineered microwells. (A) Schematic representation for EB formation in microwell arrays. (B) Schematic representation for the formation of EBs with controlled size and shape control in engineered microwells (Karp et al., 2007). (C, D). Various size EBs harvested from microwells. (E, H). PEG microwells fabricated with designed shape (Karp et al., 2007).

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

Surface patterning methods

Surface patterning methods (e.g. microcontact printing, adhesive stencils) employ microfabricated chemical or physical isolated patterns on substrate to promote EB formation. For example, substrates with different sizes (200–800 µm in diameter) as prepared using microcontact-printing can improve the EB formation with a uniform size and affect the differentiation through controlling the EB size (Bauwens et al., 2008; Peerani et al., 2007). However, microcontact-printing techniques can only control the EB size at the initial stage, which may become heterogeneous with culture time. In contrast, microfabricated adhesive stencils and microtextured surfaces composed of square–pyramidal pits in a silicon wafer (Ungrin et al., 2008) have been proved to be able to aggregate ESCs with a wide range of diameters (100–500 µm; Park et al., 2007). The technical challenge of this method is the requirement for expensive equipment and its complex fabrication process. In addition, the crosstalk between neighboring EBs in surface patterning methods may limit their applications for further controlled stem cell differentiation and drug screening applications.

There have also been efforts to modify cell surfaces to promote EB formation by enhancing cell–cell interactions. For example, mild oxidation of sialic acid residues on the cell

surface of ESCs with sodium periodate has been used to generate non-native reactive aldehyde groups, which are then biotinylated (De Bank et al., 2003). The surface-modified ESCs can then be rapidly aggregated with biotin–avidin binding in a controlled density-dependent manner upon avidin supplementation. Using this cell surface modification method, improved aggregation of ESCs has been achieved and larger, denser and more stable EBs have been formed as compared to traditional methods, with no significant decrease in viability (Gothard et al., 2009). However, significant core necrosis were observed in extended culture. Accelerated aggregation through engineered methods may circumvent this problem by reducing the EB formation time.

Compared with traditional methods (e.g. hanging-drop culture), microwell-based methods and surface patterning methods either increase the self-renewal capability of ESCs or improve the formation efficiency of EBs with highly maintained differentiation capability for the development of three primary germ layers. However, further kinetic studies of ESC aggregation process during EB formation are needed to identify the key events that crucially influence the ESC differentiation, through well-defined and controlled experiments.

Methods for spatial regulation of stem cell differentiation

Although the underlying mechanism of how the physical size affects the differentiation of EBs remains unclear, it is well known that spatial organization is a crucial aspect of embryonic development, which is however poorly achieved in *in vitro* applications developed thus far. To manipulate the behavior of stem cells in a spatial controlled fashion, it is necessary to learn from nature through mimicking the way by which spatial organization is achieved *in vivo*. However, organizing multiple types of cells to form desired spatial pattern is a highly dynamic and coordinated process. Especially, for spatial regulation of stem cell differentiation many crucial parameters must be considered, e.g. spatial distribution pattern of growth factors, architecture of specific extracellular microenvironment (niche for stem cells) and its asymmetric physical and biochemical properties. Significant efforts have been made to explore the biological mechanism underlying the spatial regulation of stem cell differentiation. Recently, the versatile microfabrication technology developed in semi-conduction industry extends the capability of constructing artificial structures with high resolution of close to a single cell level for mimicking native cell microenvironment. By recruiting new technologies, novel applications have been developed to manipulate stem cells in a spatially controlled manner for desired purposes (Table 2). However, there are still many challenges to be addressed for these *in vitro* applications, including fabrication resolution, throughput capability and manufacture easiness, etc.

Micro-patterned biocompatible hydrogel

Biocompatible hydrogel can be made from pure synthetic chemical polymers such as poly(ethylene glycol) (PEG) or engineered biomolecules such as polypeptide identified in the extracellular matrix (ECMs). All of them have been proven useful in biomedical and tissue engineering applications. Basically, hydrogels provide 3D space for stem cell growth and its physical and biochemical features can be easily controlled through chemical modification or decoration with functional biomolecules (DeForest & Anseth, 2011; Kloxin et al., 2009; Zhang et al., 2013). For instance, a hyluornic acid (HA)-based hydrogel can maintain the full differentiation capacity of human ESCs in an undifferentiated state for a long term (Gerecht et al., 2007). PEG-based hydrogels can be fabricated with physical properties including shape and architecture at a resolution of microscale for manipulating cells and functionalized with biodegradable and cell adherent peptide to support stem cell culture in a tunable manner (Azagarsamy & Anseth, 2013; DeForest & Anseth, 2011; Lutolf et al., 2003).

Moreover, utilizing microfabrication technology developed in semiconductor manufacturing, micro-patterned hydrogel has shown great potential for generating spatial controllability in stem cell differentiation, by mimicking the architecture of extracellular matrix, and has demonstrated its capability to transfer the patterning of its physical or biochemical properties to guide stem cells differentiation (Figure 4). For instance, Qi et al. (2010) reported a novel hydrogel for stem cell culture with spatial patterned differentiation. In this study, a simple strategy using photolithography was developed to fabricate hybrid hydrogel structures with asymmetrical biochemical features. This was achieved by fabricating two different hydrogel cubes, which are adjacent but independent from each other with a clear interface formed between them (Figure 4C). Biochemically inert PEG and biochemically active gelatin were used to fabricate the two gel cubes, respectively. As a simple model, this hybrid microgel can be used to mimic the asymmetrical architecture observed in a stem cell niche to induce spatially controlled differentiation. To prove this hypothesis, an individual embryonic body, derived from mouse embryonic stem cells, was embedded inside the hybrid hydrogel in the middle of the two different hydrogel cubes. Being exposed to distinct microenvironments at different sides, distinct differentiation of stem cells was observed at different locations on the same embryonic body, particularly, strong endothelial cells were identified only on the half exposed to gelatin. This study demonstrates that specific differentiation of stem cells could be spatially

Table 2. Comparison of the spatial controllability on stem cell differentiation.

	Patterned 3D hydrogel	Engineered Bioreactor	Microfluidic device	3D printing
Growth factors delivery	No advantage	Dynamic spatial control	Dynamic spatial control	No advantage
ECMs controllability	Spatially tunable	Tunable	No advantage	Spatially tunable
Mechanical Stimulus	Spatially tunable	Tunable	Tunable	No advantage
Structure complexity	Complex structure	No advantage	No advantage	Highly complex 3D structure

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

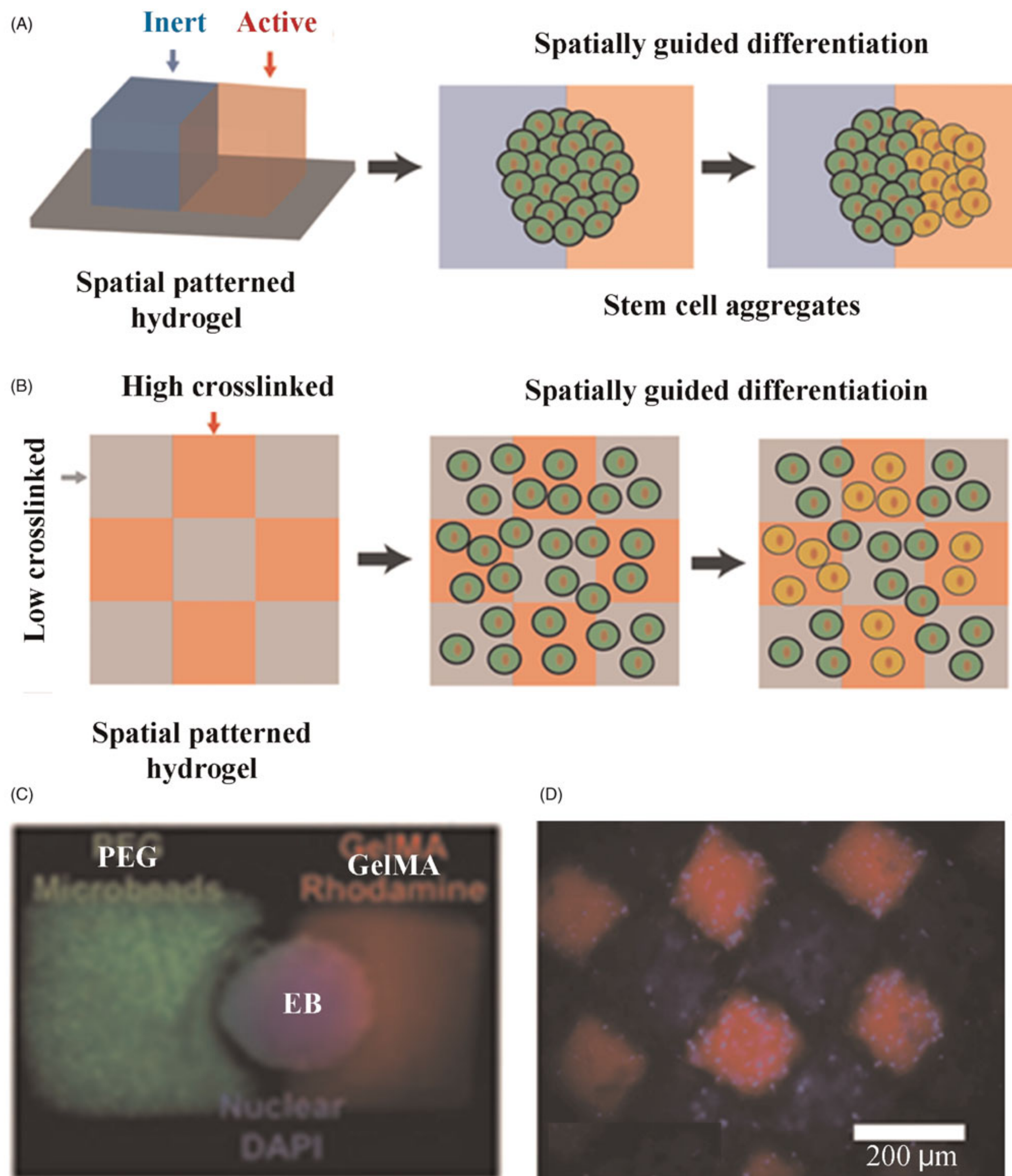

Figure 4. Micro-Patterned biocompatible hydrogel for spatial organization of stem cells. (A). micro-patterned hybrid microgel fabricated from two distinct material transfers its asymmetrical biochemical property to stem cells. (B). Biocompatible hydrogel transfers the pattern of its spatial physical features to the differentiation pattern of stem cells encapsulated inside. (C) Individual EBs was encapsulated in PEG/Gelatin microgel (Qi et al., 2010). (D) Hydrogel with patterned physical properties induced asymmetrical Osteocalcin differentiation (Trkov et al., 2010).

patterned on a single embryonic body through controlling the geometrical designs of the engineered hybrid hydrogel.

In another study, a novel approach to generate spatial regulation of stem cell differentiation was achieved by spatially controlling the physical properties in a 3D alginate hydrogel through controlling the degree of cross-linking of the hydrogel (Trkov et al., 2010; Figure 4D). When human adipose-derived stem cells were cultured inside the hydrogel, it was found that the micropattern size, the space between high cross-linked area and low cross-linked area, influenced the proliferation rate of stem cells. More interestingly, the extent of osteogenic and chondrogentic differentiation of stem cells inside the patterned hydrogel also depends on micro-pattern size. These results demonstrate that stem cell

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

differentiation can be spatially regulated through patterning the physical properties with a specific geometrical design.

Hydrogel is also attractive material for fabrication of microscale particles (Han et al., 2013a; Xu et al., 2011a,d). Complicated structures can be built through bottom-up approaches by which multiple hydrogel particles as building blocks can be self-assembled together in a spatially controlled manner. Previous studies demonstrated that hydrophilic/hydrophobic interaction (Du et al., 2008) or specific hybridization of DNA (Zhao et al., 2011) tethered on surface of hydrogel particles can drive self-assembly of cell-laden hydrogel particles to tissue-like structure in a spatially controlled manner. However, only simple structures have been built so far and the assembly efficiency and fabrication resolution restrict this technology for large-scale applications.

Engineered bioreactors

Stem cells are susceptible to both biophysical and biochemical changes of the environment around them. Since most of the stem cell differentiation mechanisms remain unclear, it is highly possible that even a slight variation in culture/experiment conditions will result in unpredictable changes in stem cell behaviors. Therefore, strict controllability is required for handling stem cells. In comparison with traditional cell culture systems, engineered bioreactors provide more dynamic control capabilities. It is capable to perform a strict and dynamic control on the exchange of nutrients and mechanical stimuli in engineered bioreactors (Grayson et al., 2010). Recently, bioreactors with specific designs have been successfully applied in the culture of many different types of stem cells for various biomedical purposes (van der Sanden et al., 2010).

Particularly, a dynamic bioreactor is designed for bone tissue engineering. Taking advantage of mass transport of nutrients and diffusion capability of designed bioreactor, *in vitro* culture of a large bone graft was improved successfully (Ishaug et al., 1997; Martin et al., 1999; Zhang et al., 2010). In addition, the mechano-transduction signaling pathways essential for spatially controlled bone morphogenesis can be triggered using dynamic media flow to enhance specific osteogenesis and mineralization (Chen et al., 2004; Gomes et al., 2003; Rauch et al., 2000). Moreover, engineered bioreactors have also been developed with advantages in regulation of cell propagation and differentiation for culture of various types of stem cells, like embryonic stem cells (Cormier et al., 2006; Fernandes-Platzgummer et al., 2011; Krawetz et al., 2010), mesenchymal stem cells (Zhao & Ma, 2005), hematopoietic stem cells (Choi et al., 2010a), neural stem cells (Kallos et al., 1999, 2003) and pluripotent stem cells (Azarin & Palecek, 2010; Kehoe et al., 2010).

Microfluidic devices

Microfluidics is a technology with miniaturization of cell culture geometrically to a small scale, typically from couples to hundreds of microns. Allowing manipulation of cells at the scale similar to that of living systems, microfluidics has attracted increasing interest with widespread applications in biomedical fields (Huang et al., 2011; Shin et al., 2012). With advances in mimicking the vasculature in cell microenvironment, microfluidics can be considered as excellent perfusion of stem cell culture systems (Huang et al., 2012, 2013; van Noort et al., 2009). It has been reported that cells in microfluidics devices exhibit better metabolic activities in comparison to conventional cultures (Lee et al., 2007; Ong et al., 2008; Pampaloni et al., 2007; Toh et al., 2007). Hence, the flexibility in controlling the soluble and mechanical parameters in the cell microenvironment makes microfluidics a powerful platform to achieve novel temporal and spatial regulation on stem cell differentiation.

Fung et al. (2009) developed a microfluidic system capable of delivering soluble growth factors in a spatially controlled fashion (Figure 5A). This device was built on a basic Y-channel device with two culturing media input and one main output, fully taking the advances of parallel laminar features at low Reynolds number and high Peclet number where two fluid streams flow in parallel without causing turbulence (Whitesides, 2006). When specific soluble growth factors were included only in one of the two culture media inputs, a flow with spatially asymmetrical distribution of the growth factors formed in the main channel. Specifically, the growth factors were restricted to flow into the half area of the main channel. To test the capability of spatial regulation of the stem cell differentiation, an individual EB derived from mouse ESCs was embedded in the middle of the main channel and sretinoic acid, a vitamin A-derived, non-peptidic and lipophilic chemical specific for neural differentiation was only supplemented in one of the two media inputs. Upon culturing, the two opposite halves of the embryonic body were exposed to different media at the same time. After a couple of days, expression of specific neural genes was clearly detected in the half of EB exposed to media with retinoic acid (Figure 5B; Fung et al., 2009). Therefore, specific neural differentiation was induced in a spatial controlled fashion on an individual EB.

Another study performed by Barkefors et al. (2009) demonstrated that soluble growth factor gradient distribution was achieved in specifically designed microfluidic devices. In particular, two parallel flow channels were connected through a culture chamber. When keeping the same flow speed in the two flow channels, no detectable flow will cross the culture chamber between the two parallel channels. When specific growth factors were supplemented with one of the two flows, concentration gradient formed in the culture chamber by diffusion of factors between the two parallel flow channels. Subsequently, an individual EB was embedded in the culture chamber within a VEGF gradient. After days of culture, stronger angiogenic sproutings were observed in the half side exposed to high concentration of VEGF than the opposite half side. More interestingly, besides the soluble growth factors, a gradient of a physical feature (e.g. temperature) was also generated between two laminar flows, one warm medium flow and one cold medium flow. The temperature gradient can be used as a powerful tool to study the spatial anterior-posterior formation in the *Drosophila* embryo (Figure 5C and D; Barkefors et al., 2009).

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

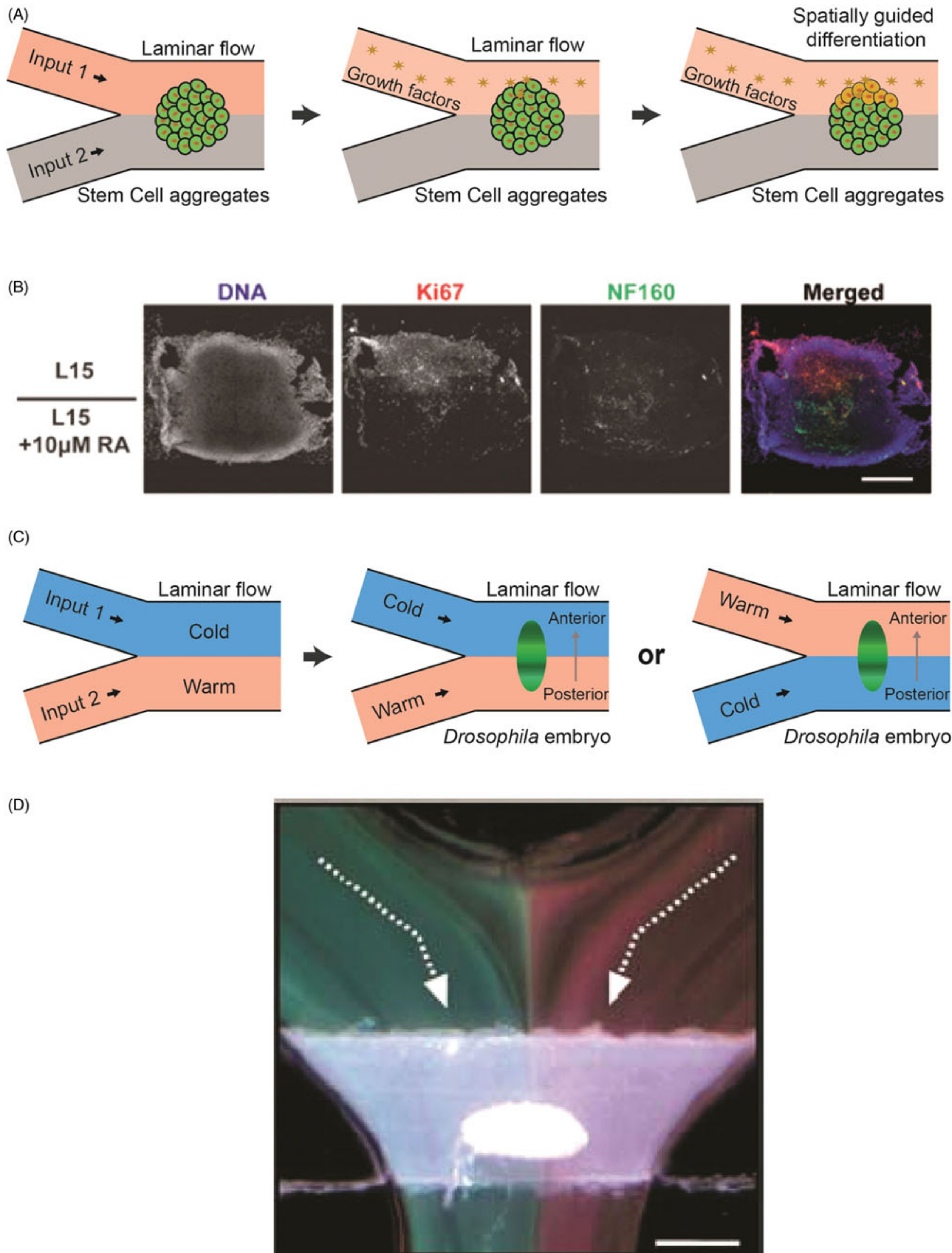

Figure 5. Microfluidic device for spatial organization of stem cell differentiation. (A) Schematic representation for growth factors gradient was formed in a Y-channel microfluidic device. Specific differentiation was spatially induced on part of embryonic body. (B) Neural differentiation on half of mouse EB induced by spatially controlled supplementary of retinoic acid (RA) in L15 medium in a microfluidic device. Spatially patterned expression of neural specific gene NF160 was detected in comparison with uniformly expressed gene Ki67 (Fung et al., 2009). Scale bar is 200 μm. (C) Schematic representation for temperature gradient was formed between warm and cold laminar flows in a microfluidic device for studying spatial patterning formation in *Drosophila* embryo development. (D) A *Drosophila melanogaster* embryo was cultured in a microfluidic device with spatially controlled temperature distribution (Barkefors et al., 2009). Scale bar is 400 μm.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

3D bioprinting

Recently, growing interest has been focused on bioprinting technology for exploring the possibility of generation of customized tissues in the laboratory. With a simple concept, several bioprinting systems have been developed, such as acoustic (Demirci & Montesano, 2007), inkjet (Boland et al., 2006), valve-based (Ceyhan et al., 2012; Moon et al., 2010; Xu et al., 2010, 2011b,e) laser printing (Gaebel et al., 2011; Guillotin et al., 2010), and more recently simple biopen (Han et al., 2014), by which specific cells wrapped within droplets of culture medium or biocompatible polymer solution are deposited on a receiving substrate by a computer controlled printer. Using these cell-laden droplets as a building block, 3D structures with complicated architecture can be printed out quickly in a programmable fashion (Figure 6). Through printing multiple types of specific cells, tissue-like structures can be generated for biomedical applications and regenerative medicine. Recently, a bioprinting system has been developed to print human ESCs droplets with desired cell numbers in a controllable manner for spheroid aggregate formation (Faulkner-Jones et al., 2013). This system provided a new powerful tool to perform stem cell research in a high-throughput manner. Moreover, a human skin-like structure was built by printing human skin cells (fibroblasts/keratinocytes) and human mesenchymal stem cells (hMSCs) together in a spatially controlled manner (Koch et al., 2010). More recently, Villar et al. (2013) reported an elegant and exciting bioprinting system, where thousands of droplets with picoliter volumes were printed and joined together by single lipid bilayers generating a cohesive material. Its elegant capability was demonstrated by fabricating numerous complicated 3D structures in a completely software-defined manner. Taken together, 3D bioprinting technology holds great promise to address the challenge for regeneration of the complicated structure observed in the real tissues.

Conclusion and future prospective

Building functional tissues in the laboratory for replacement of deficient organs in human body motives scientists and bioengineers to make great efforts, for a long time. With the possibility to generate any type of somatic cells, stem cells open the door to address this great challenge. Furthermore, a milestone study achieved by Yanamaka and coworkers in Japan in 2008 by developing the method for the generation of induced pluripotent stem cells (iPSCs) from an adult somatic cell paved the way to bring stem cells closer to practical medical applications. However, being capable of obtaining stem cell sources in a commercial and practical way is just the first step for this long journey. There are still many questions remaining about how to use stem cells to cure specific diseases. Particularly, it is challenging to tame stem cells to differentiate into desired functional somatic cells *in vitro*. The most rational approach is to recapitulate the *in vivo* process mimicking what happens in the human body. Theoretically, all the regulation mechanism information related to stem cell differentiation is encoded within the genome. Although a tremendous amount of human genome sequence information has been obtained in the past few decades, we are still far from understanding how stem cells function and build functional tissue with a spatially complicated architecture. Poverty of knowledge will be a huge obstacle for manipulating stem cells for desired purposes. Thus, as reviewed in this article, many efforts have been made to manipulate stem cells using *in vitro* systems in a spatially controlled way. A technology that can mimic or reconstruct the spatially patterned biophysical and biochemical cues of niches is crucial for building tissues with desired functions. Ultimately, *in vitro* technologies that can manipulate stem cells in a dynamic manner can potentially address the challenge of generating organs from ''scratch'' in the laboratory.

Besides experimental study, computation, which has been proven a powerful aid in biological research (e.g. system biology, bioinformatics and biomolecular engineering), may also help. For instance, computational modeling has recently been explored to investigate the mechanism by which stem cell differentiation is regulated by association of multiplex soluble signal factors. White et al. (2013) established a rule-based model to compute and explain the temporal and spatial patterns observed in stem cell differentiation within an individual EB. In particular, expression of Oct4, a key factor related to pluripotent capability of stem cell, was monitored using a confocal microscope and the spatial pattern in EBs was analyzed (Figure 7). Based on the number of differentiation, non-differentiation and transition-patterns over time, the spatial patterns were classified into six specific groups. Furthermore, basic modeling rules, including random, positive feedback and competing feedback, were configured

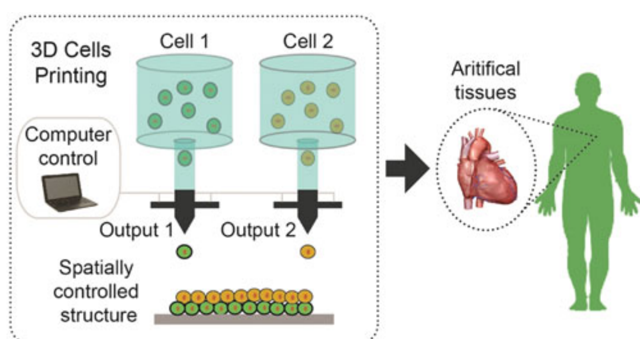

Figure 6. 3D cell Printing. Tissue-like structure with complicated architecture can be built from printing multiple cell-laden droplets together for regenerative medicine.

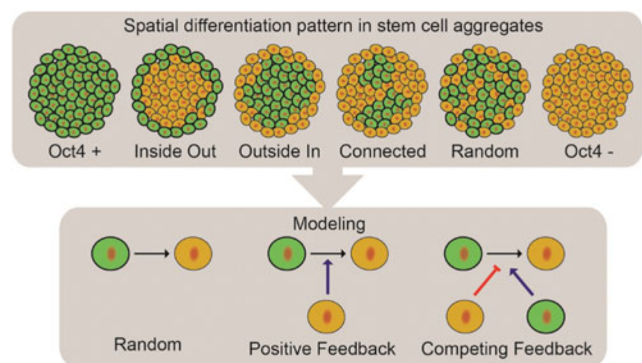

Figure 7. Computational modeling of spatial pattern in stem cell differentiation. Spatial Patterns of Oct4-cells formed in embryonic bodies were analyzed and simple model was developed to explain and predict its formation in multicellular aggregates.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

to model the emergent various spatial patterns associated with Oct4 expression. This study provided insight on possibility of predicting the emergent spatial patterns of differentiation among multicellular stem cells with the utility of computational modeling. However, for more complicated phenomena relevant to stem cell fate transition, more efforts such as collection of large sets of gene expression data and dynamic analysis were required.

Declaration of interest

The authors report no declarations of interest. This study was financially supported by the National Natural Science Foundation of China (11372243), the Major International Joint Research Program of China (11120101002), the National 111 Project of China (B06024), Key Program for International S&T Cooperation Projects of Shaanxi (2013KW33-01), National Key Scientific Apparatus Development of Special Item (2013YQ190467), and International Science & Technology Cooperation Program of China (2013DFG02930). FX was also partially supported by the China Young 1000-Talent Program and Shaanxi 100-Talent Program.

References

Abilez O, Benharash P, Mehrotra M, et al. (2006). A novel culture system shows that stem cells can be grown in 3D and under physiologic pulsatile conditions for tissue engineering of vascular grafts. J Surg Res, 132, 170–8.

Arnold SJ, Robertson EJ. (2009). Making a commitment: cell lineage allocation and axis patterning in the early mouse embryo. Nature Rev Mol Cell Biol, 10, 91–103.

Azagarsamy MA, Anseth KS. (2013). Bioorthogonal click chemistry: an indispensable tool to create multifaceted cell culture scaffolds. ACS Macro Lett, 2, 5–9.

Azarin SM, Palecek SP. (2010). Development of scalable culture systems for human embryonic stem cells. Biochem Eng J, 48, 378.

Baker K, Holtzman NG, Burdine RD. (2008). Direct and indirect roles for Nodal signaling in two axis conversions during asymmetric morphogenesis of the zebrafish heart. Proc Natl Acad Sci USA, 105, 13924–9.

Barkefors I, Thorslund S, Nikolajeff F, Kreuger J. (2009). A fluidic device to study directional angiogenesis in complex tissue and organ culture models. Lab Chip, 9, 529–35.

Bauwens CL, Peerani R, Niebruegge S, et al. (2008). Control of human embryonic stem cell colony and aggregate size heterogeneity influences differentiation trajectories. Stem Cells, 26, 2300–10.

Beddington RS, Robertson EJ. (1998). Anterior patterning in mouse. Trends Genet, 14, 277–84.

Bielinska M, Narita N, Wilson DB. (1999). Distinct roles for visceral endoderm during embryonic mouse development. Int J Dev Biol, 43, 183–205.

Boland T, Xu T, Damon B, Cui X. (2006). Application of inkjet printing to tissue engineering. Biotechnol J, 1, 910–7.

Boo JS, Yamada Y, Okazaki Y, et al. (2002). Tissue-engineered bone using mesenchymal stem cells and a biodegradable scaffold. J Craniofacial Surg, 13, 231–9.

Bratt-Leal AM, Carpenedo RL, Mcdevitt TC. (2009). Engineering the embryoid body microenvironment to direct embryonic stem cell differentiation. Biotechnol Prog, 25, 43–51.

Burridge PW, Anderson D, Priddle H, et al. (2007). Improved human embryonic stem cell embryoid body homogeneity and cardiomyocyte differentiation from a novel V-96 plate aggregation system highlights interline variability. Stem Cells, 25, 929–38.

Carpenedo RL, Bratt-Leal AM, Marklein RA, et al. (2009). Homogeneous and organized differentiation within embryoid bodies induced by microsphere-mediated delivery of small molecules. Biomaterials, 30, 2507–15.

Carpenedo RL, Sargent CY, Mcdevitt TC. (2007). Rotary suspension culture enhances the efficiency, yield, and homogeneity of embryoid body differentiation. Stem Cells, 25, 2224–34.

Ceyhan E, Xu F, Gurkan UA, et al. (2012). Prediction and control of number of cells in microdroplets by stochastic modeling. Lab on a Chip, 12, 4884–93.

Chen D, Zhao M, Mundy GR. (2004). Bone morphogenetic proteins. Growth Factors, 22, 233–41.

Choi D, Lee HJ, Jee S, et al. (2005). In vitro differentiation of mouse embryonic stem cells: enrichment of endodermal cells in the embryoid body. Stem Cells, 23, 817–27.

Choi YS, Noh SE, Lim SM, Kim DI. (2010a). Optimization of ex vivo hematopoietic stem cell expansion in intermittent dynamic cultures. Biotechnol Lett, 32, 1969–75.

Choi YY, Chung BG, Lee DH, et al. (2010b). Controlled-size embryoid body formation in concave microwell arrays. Biomaterials, 31, 4296–303.

Clark AT, Bodnar MS, Fox M, et al. (2004). Spontaneous differentiation of germ cells from human embryonic stem cells in vitro. Hum Mol Genet, 13, 727–39.

Cormier JT, Zur Nieden NI, Rancourt DE, Kallos MS. (2006). Expansion of undifferentiated murine embryonic stem cells as aggregates in suspension culture bioreactors. Tissue Eng, 12, 3233–45.

Dang SM, Kyba M, Perlingeiro R, et al. (2002). Efficiency of embryoid body formation and hematopoietic development from embryonic stem cells in different culture systems. Biotechnol Bioeng, 78, 442–53.

De Bank PA, Hou Q, Warner RM, et al. (2007). Accelerated formation of multicellular 3-D structures by cell-to-cell cross-linking. Biotechnol Bioeng, 97, 1617–25.

De Bank PA, Kellam B, Kendall DA, Shakesheff KM. (2003). Surface engineering of living myoblasts via selective periodate oxidation. Biotechnol Bioeng, 81, 800–8.

Deforest CA, Anseth KS. (2011). Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions. Nat Chem, 3, 925–31.

Demirci U, Montesano G. (2007). Single cell epitaxy by acoustic picolitre droplets. Lab Chip, 7, 1139–45.

Du Y, Lo E, Ali S, Khademhosseini A. (2008). Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs. Proc Natl Acad Sci USA, 105, 9522–7.

Faulkner-Jones A, Greenhough S, King JA, et al. (2013). Development of a valve-based cell printer for the formation of human embryonic stem cell spheroid aggregates. Biofabrication, 5, 015013.

Fernandes-Platzgummer A, Diogo MM, Baptista RP, et al. (2011). Scale-up of mouse embryonic stem cell expansion in stirred bioreactors. Biotechnol Prog, 27, 1421–32.

Fung WT, Beyzavi A, Abgrall P, et al. (2009). Microfluidic platform for controlling the differentiation of embryoid bodies. Lab Chip, 9, 2591–5.

Gaebel R, Ma N, Liu J, et al. (2011). Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. Biomaterials, 32, 9218–30.

Gerecht S, Burdick JA, Ferreira LS, et al. (2007). Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proc Natl Acad Sci USA, 104, 11298–303.

Gomes ME, Sikavitsas VI, Behravesh E, et al. (2003). Effect of flow perfusion on the osteogenic differentiation of bone marrow stromal cells cultured on starch-based three-dimensional scaffolds. J Biomed Mater Res A, 67, 87–95.

Gothard D, Roberts SJ, Shakesheff KM, Buttery LD. (2009). Controlled embryoid body formation via surface modification and avidin-biotin cross-linking. Cytotechnology, 61, 135–44.

Grayson WL, Bhumiratana S, Grace Chao PH, et al. (2010). Spatial regulation of human mesenchymal stem cell differentiation in engineered osteochondral constructs: effects of pre-differentiation, soluble factors and medium perfusion. Osteoarthritis Cartilage, 18, 714–23.

Guillotin B, Souquet A, Catros S, et al. (2010). Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials, 31, 7250–6.

Han YL, Yang Y, Liu S, et al. (2013). Directed self-assembly of microscale hydrogels by electrostatic interaction. Biofabrication, 5, 035004.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.

Han YL, Hu J, Genin GM, et al. (2014). BioPen: direct writing of functional materials at the point of care. Scientific Reports, 4, 4872 doi:10.1038/srep04872.

Huang C-C, Liao C-K, Yang M-J, et al. (2010). A strategy for fabrication of a three-dimensional tissue construct containing uniformly distributed embryoid body-derived cells as a cardiac patch. Biomaterials, 31, 6218–27.

Huang G, Wang S, He X, et al. (2013). Helical spring template fabrication of cell-laden microfluidic hydrogels for tissue engineering. Biotechnol Bioeng, 110, 980–9.

Huang G, Zhang X, Xiao Z, et al. (2012). Cell-encapsulating microfluidic hydrogels with enhanced mechanical stability. Soft Matter, 8, 10687–94.

Huang GY, Zhou LH, Zhang QC, et al. (2011). Microfluidic hydrogels for tissue engineering. Biofabrication, 3, 012001.

Hwang YS, Chung BG, Ortmann D, et al. (2009). Microwell-mediated control of embryoid body size regulates embryonic stem cell fate via differential expression of WNT5a and WNT11. Proc Natl Acad Sci USA, 106, 16978–83.

Ishaug SL, Crane GM, Miller MJ, et al. (1997). Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. J Biomed Mater Res, 36, 17–28.

Itskovitz-Eldor J, Schuldiner M, Karsenti D, et al. (2000). Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med, 6, 88–95.

Izraeli S, Lowe LA, Bertness VL, et al. (1999). The SIL gene is required for mouse embryonic axial development and left-right specification. Nature, 399, 691–4.

Jeong GS, Song JH, Kang AR, et al. (2013). Surface tension-mediated, concave-microwell arrays for large-scale, simultaneous production of homogeneously sized embryoid bodies. Adv Healthcare Mater, 2, 119–25.

Kallos MS, Behie LA, Vescovi AL. (1999). Extended serial passaging of mammalian neural stem cells in suspension bioreactors. Biotechnol Bioeng, 65, 589–99.

Kallos MS, Sen A, Behie LA. (2003). Large-scale expansion of mammalian neural stem cells: a review. Med Biol Eng Comput, 41, 271–82.

Karlsson KR, Cowley S, Martinez FO, et al. (2008). Homogeneous monocytes and macrophages from human embryonic stem cells following coculture-free differentiation in M-CSF and IL-3. Exp Hematol, 36, 1167–75.

Karp JM, Yeh J, Eng G, et al. (2007). Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. Lab Chip, 7, 786–94.

Kehoe DE, Jing D, Lock LT, Tzanakakis ES. (2010). Scalable stirred-suspension bioreactor culture of human pluripotent stem cells. Tissue Eng Part A, 16, 405–21.

Khademhosseini A, Ferreira L, Blumling III J, et al. (2006). Co-culture of human embryonic stem cells with murine embryonic fibroblasts on microwell-patterned substrates. Biomaterials, 27, 5968–77.

Kim C, Lee IH, Lee K, et al. (2007). Multi-well chip for forming a uniform embryoid body in a tiny droplet with mouse embryonic stem cells. Biosci Biotechnol Biochem, 71, 2985–91.

Kloxin AM, Kasko AM, Salinas CN, Anseth KS. (2009). Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science, 324, 59–63.

Koch L, Kuhn S, Sorg H, et al. (2010). Laser printing of skin cells and human stem cells. Tissue Eng Part C Methods, 16, 847–54.

Koike M, Sakaki S, Amano Y, Kurosawa H. (2007). Characterization of embryoid bodies of mouse embryonic stem cells formed under various culture conditions and estimation of differentiation status of such bodies. J Biosci Bioeng, 104, 294–9.

Krawetz R, Taiani JT, Liu S, et al. (2010). Large-scale expansion of pluripotent human embryonic stem cells in stirred-suspension bioreactors. Tissue Eng Part C Methods, 16, 573–82.

Kurosawa H, Imamura T, Koike M, et al. (2003). A simple method for forming embryoid body from mouse embryonic stem cells. J Biosci Bioeng, 96, 409–11.

Kurosawa H. (2007). Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. J Biosci Bioeng, 103, 389–98.

Lee LH, Peerani R, Ungrin M, et al. (2009). Micropatterning of human embryonic stem cells dissects the mesoderm and endoderm lineages. Stem Cell Res, 2, 155–62.

Lee PJ, Hung PJ, Lee LP. (2007). An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. Biotechnol Bioeng, 97, 1340–6.

Lee WG, Ortmann D, Hancock MJ, et al. (2010). A hollow sphere soft lithography approach for long-term hanging drop methods. Tissue Eng Part C Methods, 16, 249–59.

Lenhart KF, Holtzman NG, Williams JR, Burdine RD. (2013). Integration of nodal and BMP signals in the heart requires FoxH1 to create left-right differences in cell migration rates that direct cardiac asymmetry. PLoS Genet, 9, e1003109.

Lenhart KF, Lin SY, Titus TA, et al. (2011). Two additional midline barriers function with midline lefty1 expression to maintain asymmetric Nodal signaling during left-right axis specification in zebrafish. Development, 138, 4405–10.

Lewis SL, Tam PP. (2006). Definitive endoderm of the mouse embryo: formation, cell fates, and morphogenetic function. Dev Dyn, 235, 2315–29.

Lutolf MP, Lauer-Fields JL, Schmoekel HG, et al. (2003). Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. Proc Natl Acad Sci USA, 100, 5413–8.

Martin I, Obradovic B, Freed LE, Vunjak-Novakovic G. (1999). Method for quantitative analysis of glycosaminoglycan distribution in cultured natural and engineered cartilage. Ann Biomed Eng, 27, 656–62.

Maurer J, Nelson B, Cecena G, et al. (2008). Contrasting expression of keratins in mouse and human embryonic stem cells. PLoS One, 3, e3451.

Moeller HC, Mian MK, Shrivastava S, et al. (2008). A microwell array system for stem cell culture. Biomaterials, 29, 752–63.

Mohr JC, De Pablo JJ, Palecek SP. (2006). 3-D microwell culture of human embryonic stem cells. Biomaterials, 27, 6032–42.

Mohr JC, Zhang J, Azarin SM, et al. (2010). The microwell control of embryoid body size in order to regulate cardiac differentiation of human embryonic stem cells. Biomaterials, 31, 1885–93.

Moon S, Hasan SK, Song YS, et al. (2010). Layer by layer three-dimensional tissue epitaxy by cell-laden hydrogel droplets. Tissue Eng Part C Methods, 16, 157–66.

Nelson CM, Jean RP, Tan JL, et al. (2005). Emergent patterns of growth controlled by multicellular form and mechanics. Proc Natl Acad Sci USA, 102, 11594–9.

Ng ES, Davis RP, Azzola L, et al. (2005). Forced aggregation of defined numbers of human embryonic stem cells into embryoid bodies fosters robust, reproducible hematopoietic differentiation. Blood, 106, 1601–3.

Niebruegge S, Nehring A, Baer H, et al. (2008). Cardiomyocyte production in mass suspension culture: embryonic stem cells as a source for great amounts of functional cardiomyocytes. Tissue Eng A, 14, 1591–602.

Novik EI, Maguire TJ, Orlova K, et al. (2006). Embryoid body-mediated differentiation of mouse embryonic stem cells along a hepatocyte lineage: insights from gene expression profiles. Tissue Eng, 12, 1515–25.

Ong SM, Zhang C, Toh YC, et al. (2008). A gel-free 3D microfluidic cell culture system. Biomaterials, 29, 3237–44.

Pampaloni F, Reynaud EG, Stelzer EH. (2007). The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol, 8, 839–45.

Park J, Cho CH, Parashurama N, et al. (2007). Microfabrication-based modulation of embryonic stem cell differentiation. Lab Chip, 7, 1018–28.

Peerani R, Rao BM, Bauwens C, et al. (2007). Niche-mediated control of human embryonic stem cell self-renewal and differentiation. EMBO J, 26, 4744–55.

Pekkanen-Mattila M, Pelto-Huikko M, Kujala V, et al. (2010). Spatial and temporal expression pattern of germ layer markers during human embryonic stem cell differentiation in embryoid bodies. Histochem Cell Biol, 133, 595–606.

Peter IS, Davidson EH. (2009). Genomic control of patterning. Int J Dev Biol, 53, 707–16.

Pfister S, Steiner KA, Tam PP. (2007). Gene expression pattern and progression of embryogenesis in the immediate post-implantation period of mouse development. Gene Expr Patterns, 7, 558–73.

Qi H, Du Y, Wang L, et al. (2010). Patterned differentiation of individual embryoid bodies in spatially organized 3D hybrid microgels. Adv Mater, 22, 5276–81.

Rauch F, Lauzier D, Croteau S, et al. (2000). Temporal and spatial expression of bone morphogenetic protein-2, -4, and -7 during distraction osteogenesis in rabbits. Bone, 27, 453–9.

Reubinoff BE, Pera MF, Fong CY, et al. (2000). Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol, 18, 399–404.

Rohani L, Karbalaie K, Vahdati A, et al. (2008). Embryonic stem cell sphere: a controlled method for production of mouse embryonic stem cell aggregates for differentiation. Int J Artif Organs, 31, 258–65.

Sasaki D, Shimizu T, Masuda S, et al. (2009). Mass preparation of size-controlled mouse embryonic stem cell aggregates and induction of cardiac differentiation by cell patterning method. Biomaterials, 30, 4384–9.

Schier AF, Talbot WS. (2005). Molecular genetics of axis formation in zebrafish. Ann Rev Genet, 39, 561–613.

Schroeder M, Niebruegge S, Werner A, et al. (2005). Differentiation and lineage selection of mouse embryonic stem cells in a stirred bench scale bioreactor with automated process control. Biotechnol Bioeng, 92, 920–33.

Segev H, Kenyagin-Karsenti D, Fishman B, et al. (2005). Molecular analysis of cardiomyocytes derived from human embryonic stem cells. Dev Growth Differ, 47, 295–306.

Seiler AE, Spielmann H. (2011). The validated embryonic stem cell test to predict embryotoxicity in vitro. Nat Protoc, 6, 961–78.

Shin Y, Han S, Jeon JS, et al. (2012). Microfluidic assay for simultaneous culture of multiple cell types on surfaces or within hydrogels. Nat Protoc, 7, 1247–59.

Singla DK, Jayaraman S, Zhang J, Kamp TJ. (2007). Cardiomyocyte derivation from human embryonic stem cells. In: Master JR, Palsson BO, Thomson JA, eds. Human cell culture, 6. Embryonic stem cells. New York: Springer-Verlag.

Tam PP, Beddington RS. (1992). Establishment and organization of germ layers in the gastrulating mouse embryo. Ciba Foundation Symp, 165, 27–41; discussion 42–9.

Tam PP, Behringer RR. (1997). Mouse gastrulation: the formation of a mammalian body plan. Mech Dev, 68, 3–25.

Tam PP, Loebel DA. (2007). Gene function in mouse embryogenesis: get set for gastrulation. Nat Rev Genet, 8, 368–81.

Toh YC, Zhang C, Zhang J, et al. (2007). A novel 3D mammalian cell perfusion-culture system in microfluidic channels. Lab Chip, 7, 302–9.

Torisawa YS, Chueh BH, Huh D, et al. (2007). Efficient formation of uniform-sized embryoid bodies using a compartmentalized micro-channel device. Lab Chip, 7, 770–6.

Trkov S, Eng G, Di Liddo R, et al. (2010). Micropatterned three-dimensional hydrogel system to study human endothelial-mesenchymal stem cell interactions. J Tissue Eng Regen Med, 4, 205–15.

Ungrin MD, Joshi C, Nica A, et al. (2008). Reproducible, ultra high-throughput formation of multicellular organization from single cell suspension-derived human embryonic stem cell aggregates. PLoS One, 3, e1565.

Valamehr B, Jonas SJ, Polleux J, et al. (2008). Hydrophobic surfaces for enhanced differentiation of embryonic stem cell-derived embryoid bodies. Proc Natl Acad Sci USA, 105, 14459–64.

Van Der Sanden B, Dhobb M, Berger F, Wion D. (2010). Optimizing stem cell culture. J Cell Biochem, 111, 801–7.

Van Noort D, Ong SM, Zhang C, et al. (2009). Stem cells in microfluidics. Biotechnol Prog, 25, 52–60.

Veerkamp J, Rudolph F, Cseresnyes Z, et al. (2013). Unilateral dampening of Bmp activity by nodal generates cardiac left-right asymmetry. Dev Cell, 24, 660–7.

Villar G, Graham AD, Bayley H. (2013). A tissue-like printed material. Science, 340, 48–52.

Wells JM, Melton DA. (1999). Vertebrate endoderm development. Ann Rev Cell Dev Biol, 15, 393–410.

White DE, Kinney MA, Mcdevitt TC, Kemp ML. (2013). Spatial pattern dynamics of 3D stem cell loss of pluripotency via rules-based computational modeling. PLoS Comput Biol, 9, e1002952.

Whitesides GM. (2006). The origins and the future of microfluidics. Nature, 442, 368–73.

Xu F, Finley TD, Turkaydin M, et al. (2011a). The assembly of cell-encapsulating microscale hydrogels using acoustic waves. Biomaterials, 32, 7847–55.

Xu F, Moon S, Emre A, et al. (2010). A droplet-based building block approach for bladder smooth muscle cell (SMC) proliferation. Biofabrication, 2, 014105.

Xu F, Sridharan B, Durmus NG, et al. (2011b). Living bacterial sacrificial porogens to engineer decellularized porous scaffolds. PLoS One, 6, e19344.

Xu F, Sridharan B, Wang SQ, et al. (2011c). Embryonic stem cell bioprinting for uniform and controlled size embryoid body formation. Biomicrofluidics, 5, 22207.

Xu F, Wu C-AM, Rengarajan V, Finley TD, et al. (2011d). Three-dimensional magnetic assembly of microscale hydrogels. Adv Mater, 23, 4254–60.

Xu F, Wu J, Wang S, et al. (2011e). Microengineering methods for cell-based microarrays and high-throughput drug-screening applications. Biofabrication, 3, 034101.

Yang MJ, Chen CH, Lin PJ, et al. (2007). Novel method of forming human embryoid bodies in a polystyrene dish surface-coated with a temperature-responsive methylcellulose hydrogel. Biomacromolecules, 8, 2746–52.

Yirme G, Amit M, Laevsky I, et al. (2008). Establishing a dynamic process for the formation, propagation, and differentiation of human embryoid bodies. Stem cells and development, 17, 1227–41.

Youn BS, Sen A, Behie LA, et al. (2006). Scale-up of breast cancer stem cell aggregate cultures to suspension bioreactors. Biotechnol Prog, 22, 801–10.

Zambon AC, Barker CS. (2010). Microarray analysis of embryonic stem cells and differentiated embryoid bodies. Methods Mol Biol, 632, 45–61.

Zhang R, Mjoseng HK, Hoeve MA, et al. (2013). A thermoresponsive and chemically defined hydrogel for long-term culture of human embryonic stem cells. Nat Commun, 4, 1335.

Zhang W, Wang S, Lin M, et al. (2012). Advances in experimental approaches for investigating cell aggregate mechanics. Acta Mech Solida Sin, 25, 473–82.

Zhang ZY, Teoh SH, Teo EY, et al. (2010). A comparison of bioreactors for culture of fetal mesenchymal stem cells for bone tissue engineering. Biomaterials, 31, 8684–95.

Zhao F, Ma T. (2005). Perfusion bioreactor system for human mesenchymal stem cell tissue engineering: dynamic cell seeding and construct development. Biotechnol Bioeng, 91, 482–93.

Zhao Z, Liu Y, Yan H. (2011). Organizing DNA origami tiles into larger structures using preformed scaffold frames. Nano Lett, 11, 2997–3002.

Critical Reviews in Biotechnology Downloaded from informahealthcare.com by Kansas State University Libraries on 07/19/14
For personal use only.