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Stem cell culture using cell-derived substrates

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Abstract

There have been great efforts to develop cell culture systems to regulate stem cell functions. Development of cell culture substrates is one of the important approaches for stem cell culture because substrates influence stem cell functions such as attachment, proliferation, self-renewal, and induction of differentiation. Stem cells are surrounded by their specific microenvironments *in vivo*, composed of cells, cytokines, and an extracellular matrix (ECM), which may dynamically change and affect cellular activities accordingly. To mimic such microenvironments, cell culture substrates can be prepared by coating bioactive proteins such as ECM proteins and signaling molecules as ligands for cell surface receptors. Compared with protein-coated substrates, cell- and cell-formed ECM-derived substrates have shown great progress and attracted significant attention as functional and prospective biomaterials for stem cell culture and regenerative medicine. In this review, we summarize the latest progress of these new substrates derived from cells and cell-formed ECMs.

1. Introduction

Stem cells are important cell sources for regenerative medicine [1]. There have been great efforts to develop cell culture systems to regulate stem cell functions. Development of cell culture substrates is an important approach for stem cell culture because substrates influence stem cell functions such as attachment, proliferation, self-renewal, and induction of differentiation [2, 3]. Although there are many reports of culture substrates composed of synthetic polymers and proteins, it is difficult to fully control the functions of stem cells.

One attractive strategy for the development of substrates to control stem cell functions is mimicking cellular microenvironments, because stem cells are surrounded by their specific microenvironments *in vivo*. Microenvironments are composed of cells, cytokines, and an extracellular matrix (ECM). As shown in Figure 1, stems cells interact with their microenvironments, triggering activation of various signals for proliferation, self-renewal, and differentiation. For example, hematopoietic stem cells interact with several cells in their stem cell niche to maintain their stemness [4]. Neural stem cells are surrounded by ECM structures called “fractones” to maintain their stemness [5]. When stem cells differentiate, the composition of the ECM surrounding the cells is changed to provide appropriate differentiation signals to differentiating cells according to their developmental states [6, 7].

To provide such biomimetic microenvironments to maintain the undifferentiated state of stem cells, cell- and cell-formed ECM-derived substrates have been developed for stem cell culture (Figure 2). Substrates with a feeder cell layer presenting signaling molecules on the feeder cell surface have been used to culture embryonic stem (ES) cells and induced pluripotent stem (iPS) cells [8, 9]. In addition to the use of feeder cells, coating of bioactive proteins such as ECM proteins and certain signaling molecules on substrates has been widely applied to construct biomimetic microenvironments for stem cell culture [10-16]. The coating method is very convenient and the composition of coated bioactive proteins is controllable. However, it is difficult to reconstitute *in vivo* microenvironments with synthetic polymers and single/multiple proteins by conventional chemical and physical methods because of the complexity of the ECM composition and the ligands

presented by cells. Therefore, cell-formed ECM-derived substrates have been developed to more closely mimic cellular microenvironments *in vivo*. In this review, the progress of these new substrates derived from cells and cell-formed ECMs for stem cell culture are summarized together with the future prospects.

2. Cell-derived substrates

Although numerous approaches have been evaluated for feeder-free culture of stem cells [17-20], co-culture is sometimes used for stem cell culture because it significantly improves understanding of what actually constitutes a stem cell niche [21]. As shown in Figure 3, ES and iPS cells are generally maintained on a feeder cell layer *in vitro* [8, 9]. Mouse-derived cells are the most frequently used feeders to maintain the pluripotency of human ES and iPS cells. Human-derived feeder cells are also used for human ES/iPS culture. However, in some cases, these cells have proven to be unsuitable for stem cell maintenance [22, 23]. Feeder cells typically need to be growth inactivated prior to use as feeders to prevent their proliferation and contamination of the stem cell culture [24]. Growth inactivation is commonly performed by treatment with mitomycin-C or X-ray irradiation [20]. These steps for feeder cell preparation require significant time and effort [25]. To reduce these steps, chemically cross-linked feeder cells have been used culture substrates for stem cell culture. Such chemical fixation techniques are gradually being recognized as convenient methods by researchers [26-28].

2.1 Chemically cross-linked cells

Chemically cross-linked cells have been used in fundamental cell biology to reveal the mechanisms by which membrane-associated factors or proteins affect cells [29-32]. Higashiyama *et al.* employed chemically cross-linked donor cells to evaluate the biological effects of membrane-anchored epidermal growth factor (EGF) on acceptor cells [29]. By co-culturing donor cells expressing pro-heparin-binding epidermal growth factor (pro HB-EGF/DTR) with acceptor cells expressing the EGF receptor, they demonstrated that pro-HB-EGF/DTR stimulated cell growth

in a juxtacrine manner [29]. The mechanism of cell stimulation by immobilized growth factors has been delineated in studies of intercellular regulation by chemically cross-linked cells that express growth factors or cytokines. For example, Stein *et al.* demonstrated the biological activity of immobilized colony-stimulating factor-1 (CSF-1) expressed on the surface of fibroblasts [30]. After chemical fixation of the fibroblast monolayer, the soluble form of CSF-1 was undetectable in the culture medium. Furthermore, the inability of CSF-1-dependent cells to form colonies on physically scraped areas of tissue culture coverslips suggested that direct cell-cell contact was required for cell stimulation [23]. Similarly, Yaeger *et al.* investigated the keratinocyte growth-promoting activity of chemically cross-linked fibroblast feeder cells on normal human keratinocytes, and found that glutaraldehyde-fixed fibroblast feeder cells promoted keratinocyte growth [31]. This growth-promoting effect required contact between fixed fibroblasts and keratinocytes. Moreover, the feeder activity was highly enriched in the plasma membrane fraction of fibroblasts. Therefore, at least some of the fibroblast feeder activity involved a keratinocyte growth-promoting factor that was immobilized on the fibroblast surface. In contrast, direct contact with chemically cross-linked stroma inhibits both the proliferation and survival of long-term culture-initiating cells [32].

Collectively, these studies demonstrate that chemically cross-linked cells present membrane-associated factors or proteins that affect cells in a juxtacrine manner. Thereafter, this method has been applied to stem cell culture.

2.2. Maintenance of the undifferentiated state of stem cells

Chemically cross-linked feeder cells have been used to maintain the undifferentiated state of stem cells. Lai and Ma demonstrated the feasibility of a glutaraldehyde cross-linked amniotic membrane as a niche to expand and transplant limbal epithelial progenitor cells [33]. Roy and Verfaillie used fixed stromal cells as feeders to culture cord blood-derived stem cells. The fixed feeder cells presented immobilized growth factors that affected cellular functions [34]. Meissner and colleagues immobilized human stromal cells in porous glass carriers in a fixed-bed reactor and co-cultured human hematopoietic progenitor cells for several weeks [35]. After inoculating

mononuclear cells derived from umbilical cord blood or peripheral blood stem cells into this fixed-bed reactor, the expansion of early progenitor cells increased by up to four-fold and that of later progenitor cells increased by up to seven-fold [35].

Ito *et al.* applied chemically cross-linked human stromal cells to support *ex vivo* expansion of human cord blood hematopoietic progenitor cells [36,37]. In addition, they demonstrated the utility of chemically cross-linked nurse cells as feeders to maintain ES cells in an undifferentiated state [38]. In the previous study, mouse and monkey ES cells were grown on chemically fixed mouse embryonic fibroblasts (MEFs) and human amniotic epithelial (HAE) cells, respectively. MEFs were fixed by incubation in glutaraldehyde or formaldehyde solutions. HAE cells were immortalized by transfection with human telomerase reverse transcriptase and chemically fixed with the same reagents. When mouse and monkey ES cells were cultured on these chemically fixed cells, the mouse ES cells proliferated and expressed pluripotency markers including alkaline phosphatase, stage-specific embryonic antigen (SSEA)-1 and -4, and octamer binding protein 3/4 (Oct-3/4). Furthermore, freeze-drying HAE cells and MEFs did not change their ability to support undifferentiated growth of ES cells. Conveniently, the chemically fixed cells could be used repeatedly to culture ES cells [38].

As shown in Figure 4, mouse embryonic fibroblasts (MEFs) cross-linked with formaldehyde (FA-MEFs) or glutaraldehyde (GA-MEFs) have also been used to maintain the pluripotency of mouse iPS cells [39]. Chemically fixed MEF feeders maintain both the pluripotency and undifferentiated state of mouse iPS cells and can be re-used several times without a change in their function [39]. Currently, efforts are underway to culture human iPS cells on chemically fixed autologous feeder cells (unpublished).

2.3. Regulation of stem cell differentiation

Feeder cells can also be used to induce differentiation of ES and iPS cells [40]. Chemically cross-linked cells can also induce differentiation of stem cells. The effect of cross-linked cells on differentiation of human ES cells was investigated by Vazin *et al.* [40]. Dopaminergic neurons can

be differentiated from human ES cells by co-culture with the mouse PA6 stromal cell line. This type of feeder cell-induced differentiation is termed stromal-derived inducing activity (SDIA). Because the exact mechanism of SDIA is unknown, various studies have suggested that SDIA involves either a fixation-resistant component located on the PA6 cell surface or factors secreted into the medium by PA6 cells. To further explore the exact mechanism, the number of differentiated tyrosine hydroxylase (TH)+ cells was reduced by only three-fold by fixation or irradiation of PA6 feeder cells, whereas mitomycin C treatment of PA6 feeder cells reduced the number of TH+ cells by 32%. The neural-inducing effect of PA6 cells, as monitored by β-III-tubulin expression, was minimally affected by mitomycin C treatment or fixation but was reduced to 50% by irradiation. Medium conditioned by PA6 cells was ineffective for inducing differentiation of TH+ cells when used alone. However, such conditioned medium combined with heparin and/or fixed PA6 cells induced TH+ cell differentiation, although less effectively than that of PA6 cell co-culture. Therefore, it was concluded that PA6 cell surface activity is required for neural differentiation of human ES cells, while secreted factors are required for the specific dopaminergic neuron-inducing effect [40].

Recently, Lee *et al.* reported a technique to transfer the complex membrane surface of glutaraldehyde-fixed human blood mononucleated cells to a polydimethylsiloxane (PDMS) substrate using an intermediate poly(vinyl alcohol) film as a transporter system, and demonstrated the bioactivity of the transferred membranes [41]. PDMS display of cell membranes is indistinguishable from fixed mononuclear blood cells in terms of morphology and biological activity to support human leukemic cell adhesion [41]. This observation further implies that, instead of live feeders, chemically cross-linked cells with their quintessential membrane proteins and growth factors immobilized on their surfaces can be used to culture stem cells *in vitro*.

3. Cell-formed ECM-derived substrates

Similar to chemically cross-linked, cell-derived substrates, cell-formed ECM is used as a substrate after removal of the cellular components or decellularization treatment. Such decellularized ECMs are important for tissue engineering because they retain the complex ECM composition to

present essential information to the cells. There have been many efforts to apply decellularized ECMs for the reconstruction of tissues such as the heart [42], lung [43,44], bladder [45,46], tendons [47], and liver [8,49]. Other applications of decellularized ECMs are well reviewed elsewhere [50,51]. Generally, a decellularized ECM is obtained from tissues by removal of the cellular components. Tissue-derived decellularized ECMs are used in many formats such as three-dimensional architectures [43,44,46,49], patch-type [47], coating-type [52], and injectable gels [45,53]. Thus, tissue-derived decellularized ECMs are useful tools for tissue engineering and regenerative medicine. However, it remains difficult to apply a tissue-derived ECM in large-scale cell culture because of the limited sources of tissue-derived ECMs. Moreover, it is difficult to obtain the correct ECM surrounding stem cells by decellularization of whole or minced tissues because the ECM surrounding stem cells is located in a limited space. Cells cultured *in vitro* secrete ECM proteins and deposit them beneath the cells. Similar to decellularized ECMs derived from tissues, these deposited ECM proteins can be used as cell culture substrates after decellularization [54]. In this section, we reviewed cell-derived ECMs to both maintain the undifferentiated state and enhance the differentiation induction of stem cells.

3.1. Preparation methods for decellularized ECMs

There are review articles that summarize the preparation methods for a decellularized ECM and highlight the dependence of ECM components and structures on such methods [55,56]. Therefore, the preparation methods should be carefully chosen to meet specific purposes. Moreover, a decellularized ECM is often treated with chemical cross-linking agents to stabilize its structure and composition. For chemical cross-linking of the ECM, it is commonly treated with glutaraldehyde, carbodiimide, or photo-oxidizing agents. As an alternative to chemical cross-linking reagents, natural products have been used to cross-link decellularized ECMs. Jiang *et al.* employed genipin, a naturally occurring cross-linker derived from the fruit of the gardenia plant (*Gardenia jasminoides*) [57]. They prepared decellularized fresh rat spinal cords by cross-linking them with genipin. Compared with a decellularized spinal cord ECM cross-linked by glutaraldehyde, the

genipin-cross-linked ECM demonstrated good biocompatibility while maintaining similar structural stability and mechanical properties to those of glutaraldehyde-cross-linked ECM.

Quercetin, a naturally occurring polyphenolic flavonol, has also been used as a natural cross-linker. Zhai *et al.* applied quercetin to cross-link porcine heart valve ECM and evaluated its mechanical properties, stability, anti-calcification effect, and cytocompatibility [58]. Their results showed that the tensile strength of a quercetin-cross-linked ECM was higher than that of glutaraldehyde-cross-linked ECM. After cross-linking with quercetin, there was also a clear increase in the thermal denaturation temperature of the ECM. The quercetin-cross-linked ECM could be stored in a buffer solution for at least 30 days without any loss of tensile strength and elasticity. Cell culture experiments showed no inhibition of the proliferation of vascular endothelial cells. Furthermore, *in vitro* anti-calcification experiments showed that quercetin cross-linking could protect the ECM from deposition of minerals in a simulated body fluid.

In contrast to the improvement of the stability and mechanical properties of cross-linked decellularized ECMs, cross-linking may induce a host immune response and decrease the biocompatibility of the cross-linked ECM. Cross-linking may also influence the function of cross-linked ECM. Therefore, to prepare a decellularized ECM, the need for cross-linking and selection of an appropriate cross-linker should be considered in addition to the decellularization method.

3.2. Maintenance of the undifferentiated state of stem cells

Because the ECM has been reported to play important roles in maintenance of the undifferentiated state of stem cells [59,60], decellularized ECMs have been used for feeder cell-free culture of stem cells. Klimanskaya *et al.* prepared a decellularized ECM derived from MEFs to maintain human ES cells in serum-free medium [61]. The human ES cells cultured on the decellularized ECM maintained a normal karyotype and expression of stem cell markers such as OCT-4, SSEA-3, SSEA-4, tumor-rejection antigen (TRA)-1-60, TRA-1-81, and alkaline phosphatase. The ES cells retained the potential to differentiate into all three embryonic germ layers in both *in*

vitro culture and *in vivo* teratoma formation, even after more than 6 months of culture. Moreover, this decellularized ECM can be used to generate new human ES cell lines, which is similar to the conventional feeder cell-based method.

In addition to the maintenance of ES cells, decellularized ECMs have been reported as a substrate to maintain other stem cells. Prewitz *et al.* prepared a decellularized ECM derived from mesenchymal stem cells (MSCs) undergoing osteogenesis and applied the ECM to *in vitro* hematopoietic stem and progenitor cell (HSPC) culture [62]. The cultured human peripheral blood $CD34^+$ HSPCs showed clustering and attached to the decellularized ECM. In addition, the HSPCs showed high proliferation on the decellularized ECM compared with that on conventional and fibronectin-coated culture plates. Transplantation of the HSPCs into mice demonstrated that the HSPCs engrafted and differentiated into $CD3^+$ T cells, $CD19^+$ B cells, and $CD33^+$ myeloid hematopoietic cells. These results indicated that the decellularized ECM was applicable to *in vitro* expansion of HSPCs while maintaining their undifferentiated states.

MSCs are a promising cell source for regenerative medicine of cartilage, bone, and adipose tissues because they can differentiate into chondrocytes, osteoblasts, and adipocytes. However, these differentiation properties decrease during culture [63]. This limitation of cultured MSCs is a major barrier for their application to tissue regeneration [64]. Chen and colleagues attempted to prevent the decline in the differentiation abilities of MSCs by culturing them on a decellularized ECM derived from MSCs. The MSCs proliferated on this decellularized ECM and maintained their differentiation properties, even up to passage 10 in culture. It is also generally accepted that MSCs gradually lose their differentiation properties during aging. The differentiation properties of aged MSCs can be recovered by culturing them on a decellularized ECM derived from MSCs isolated from young MSCs [64].

Spontaneous differentiation of MSCs can be prevented on a decellularized ECM derived from MSCs by suppression of bone morphogenetic protein (BMP) signaling [65]. In addition, the osteogenesis and adipogenesis of MSCs are suppressed on such decellularized ECMs, even in differentiation induction media [66]. On a decellularized ECM derived from MSCs, Wnt/β-catenin

signaling, which inhibits osteogenesis and adipogenesis, is strongly activated by Wnt ligands bound to the chondroitin sulfate chains in the decellularized ECM [67]. It is likely that the decellularized ECM derived from MSCs possesses the ability to maintain the undifferentiated state of MSCs. Therefore, such decellularized ECMs may be useful substrates to culture stem cells in an undifferentiated state.

3.3. Regulation of stem cell differentiation

ECM components also play important roles in stem cell differentiation [2, 3]. Decellularized ECMs can be readily adapted to study ECM environments with complex compositions by culturing different cell types. Therefore, decellularized ECMs have been studied as substrates for stem cell differentiation.

An artificial basement membrane prepared by decellularization has been applied to differentiate ES and iPS cells into pancreatic β cells [68]. The artificial basement membrane was prepared from a culture of HEK293 cells that stably expressed laminin-511. The HEK293 cells were cultured for deposition of basement membrane components, and then the cells were removed from the substrate to prepare the artificial basement membrane. Subsequently, mouse ES or iPS cells were seeded on the basement membrane, and the cells were sequentially differentiated into definitive endoderm, pancreatic progenitor cells, and then insulin-secreting pancreatic β cells.

Similar to ES/iPS cell differentiation culture, a decellularized ECM can be applied to differentiation of MSCs. Cheng *et al.* prepared a decellularized ECM derived from chondrocytes cultured in collagen microspheres. The chondrogenesis of MSCs was well supported in these decellularized microspheres [69]. Choi *et al.* prepared a decellularized ECM derived from chondrocytes in pellet culture [70]. MSCs were cultured in this three-dimensional scaffold that facilitated chondrogenesis compared with that in polyglycolic acid scaffolds.

Decellularized ECMs can be prepared by culture of developmentally mature cells. However, the composition of the ECM surrounding differentiating cells is altered according to their developmental stages [6, 7]. ECMs surrounding cells that are developmentally immature may facilitate stem cell

differentiation. Substrates mimicking the ECM surrounding developmentally immature cells can be prepared by culture of differentiating cells *in vitro* and decellularization techniques. Hoshiba *et al.* reported a decellularized ECM mimicking the *in vivo* ECM surrounding MSCs undergoing osteogenesis or adipogenesis at each developmental stage (Figure 5) [71-73]. The MSCs were cultured *in vitro* under osteogenic or adipogenic conditions to obtain cells at different developmental stages. After obtaining cells expressing marker genes of the expected developmental stages, the cellular components were removed from the substrates to obtain decellularized ECMs. The ECMs derived from cells undergoing osteogenesis or adipogenesis are called "stepwise osteogenesis-mimicking matrices" and "stepwise adipogenesis-mimicking matrices", respectively.

Such matrixes support the attachment and proliferation of MSCs. The osteogenesis of MSCs is promoted on a substrate mimicking the ECM surrounding cells at the early stage of osteogenesis (osteogenic early stage matrices) [66]. Conversely, the adipogenesis of MSCs is promoted on a substrate mimicking the ECM surrounding the cells at the early stage of adipogenesis (adipogenic early stage matrices) [72]. This regulation of MSC differentiation is mediated by regulation of transcription factor expression. When MSCs undergo osteogenesis, osteogenic early-stage matrices activate BMP signaling to express *RUNX2*, a bone-related transcription factor [66]. Moreover, osteogenic early-stage matrices activate Wnt/β-catenin signaling through Wnt ligands bound to chondroitin sulfate chains [67]. The Wnt/β-catenin signaling suppresses *PPARG* expression, which induces osteogenesis of MSCs on osteogenic early-stage matrices. Transcription factor regulation also promotes adipogenesis on adipogenic early stage matrices. The expression of *RUNX2*, a transcription factor that facilitates osteogenesis and inhibits adipogenesis, is suppressed by adipogenic early-stage matrices. In addition to *RUNX2* suppression, *TAZ* and *MSX2*, which act as suppressors of adipogenesis, are suppressed on adipogenic early-stage matrices. These results suggest that tissue- and stage-specific ECMs can control the differentiation of MSCs into specific cell types [71]. Decellularized ECMs prepared by culture of differentiating cells is an effective approach to present tissue- and stage-specific ECMs to stem cells to regulate their differentiation. Moreover, stepwise tissue development-mimicking matrices may be useful as *in vitro* models to

investigate ECM-cell interactions and elucidate the roles of the ECM in tissue development.

Decellularized ECMs can be easily applied as three-dimensional scaffolds. Lu *et al.* prepared decellularized ECM scaffolds from MSCs, chondrocytes, and fibroblasts by culturing the cells in a poly (lactic-*co*-glycolic acid) (PLGA) scaffold [73]. After formation of the cell-derived ECMs, the PLGA scaffold and cellular components were removed from the constructs to obtain three-dimensional decellularized ECMs that can be applied to MSC culture. Similar to these decellularized ECMs, a decellularized ECM derived from MSC-derived osteoblasts has been prepared on a titanium mesh [74]. Three-dimensional scaffolds can be prepared by cell culture in certain scaffolds as molds and subsequently applied to tissue regeneration. Therefore, cell-derived ECMs and scaffolds can mimic the ECM microenvironment *in vivo* and are useful to control stem cell functions for applications in regenerative medicine (Figure 6).

4. Conclusions and outlook

Regulation of stem cell functions is an important issue in regenerative medicine. Mimicking the *in vivo* extracellular microenvironments surrounding stem cells is an effective approach to regulate stem cell functions by preparation of functional substrates. Although protein coating and immobilization are useful tools to create such microenvironments [75,76], cell- and ECM-derived substrates show significant advantages. On substrates derived from chemically cross-linked cells or cell-formed ECMs, stem cells can either maintain their undifferentiated state or differentiate into a specific lineage. These methods can be further expanded to prepare any desirable ECM and scaffold by co-culturing two or more cell types. Therefore, the preparation of substrates using cultured cells to mimic *in vivo* extracellular microenvironments surrounding stem cells is beneficial for stem cell research and tissue regeneration.

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Figure legends

Figure 1 The three main interactions between cells and their microenvironments for regulation of cell functions.

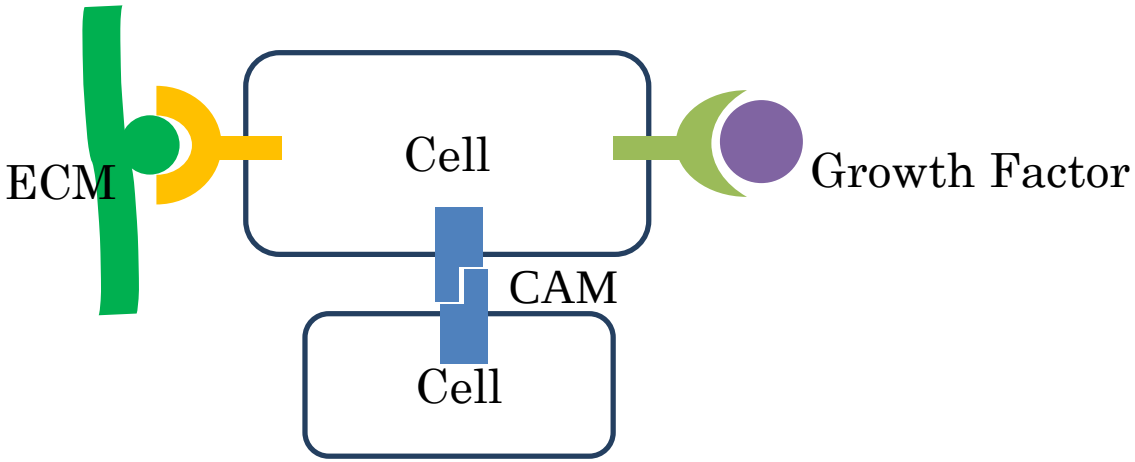
Figure 2 Stem cell cultures on cell- or extracellular matrix-derived substrates.

Figure 3 Culture of embryonic stem or induced pluripotent stem cells on feeder cells.

Figure 4 Morphology and Nanog-green fluorescent protein (GFP) expression in mouse-induced pluripotent stem cells cultured on mitomycin C-treated mouse embryonic fibroblasts (MEFs), chemically fixed MEFs, and gelatin-coated substrates (reproduced with permission from [36]). MMC-MEFs, GA-MEFs, and FA-MEFs refer to mitomycin C-, glutaraldehyde-, and formaldehyde-treated mouse embryonic fibroblasts, respectively.

Figure 5 Schematic for the preparation of stepwise tissue development-mimicking matrices. Stem cells are cultured in an undifferentiated state to prepare matrices mimicking the ECM surrounding stem cells. The stem cells are cultured under differentiation conditions until the cells reach each developmental stage. Thereafter, the cellular components are selectively removed from the substrates to obtain cell-formed ECMs called “stepwise tissue development-mimicking matrices”.

Figure 6 Application of cell-formed ECMs in stem cell research and regenerative medicine. Cell-formed ECMs can be prepared as cell culture substrates in two- and three-dimensional forms by decellularization treatment.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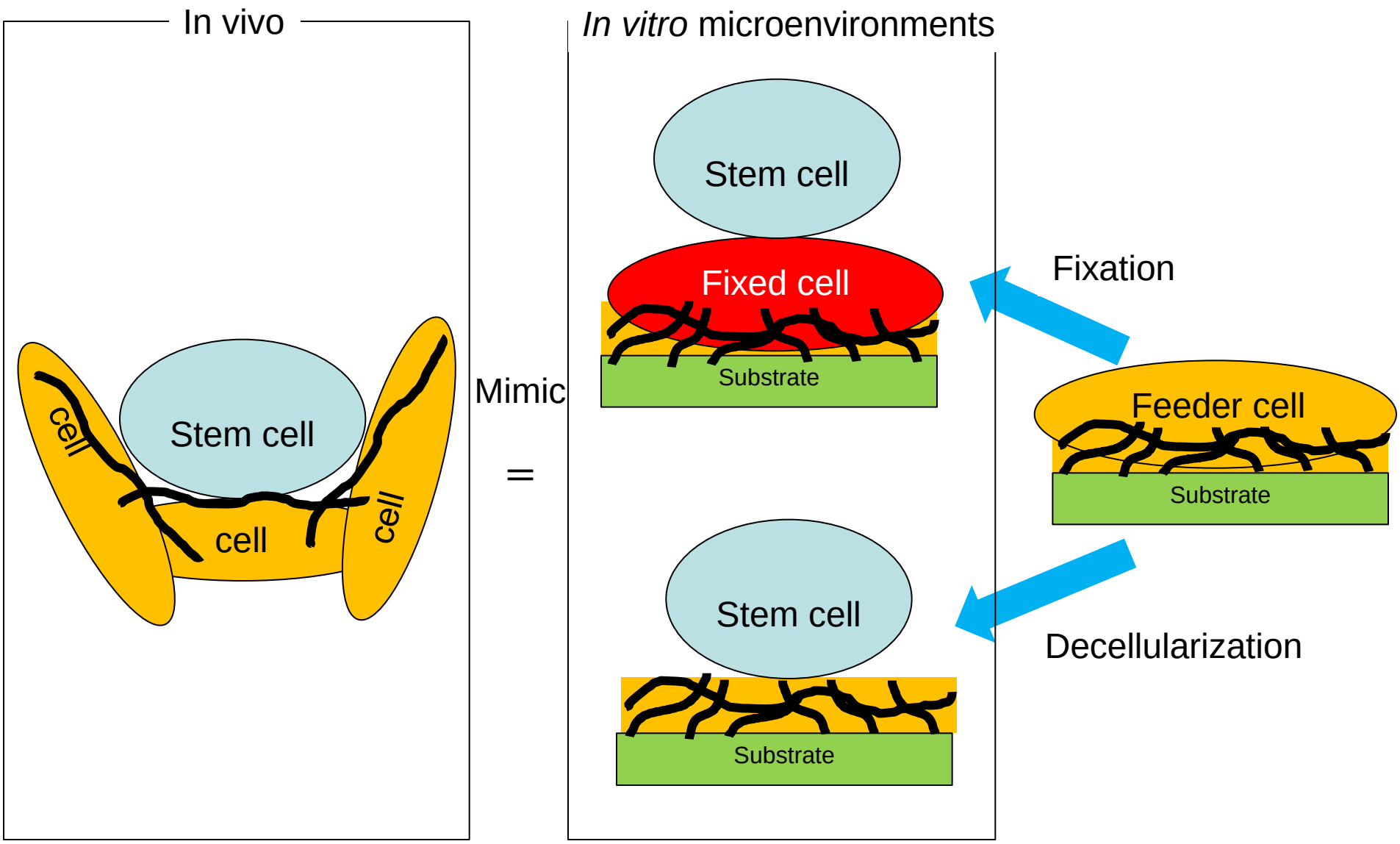
Cell
Growth Factor
CAM
Cell


In vivo
Stem cell
cell
cell
cell
Mimic
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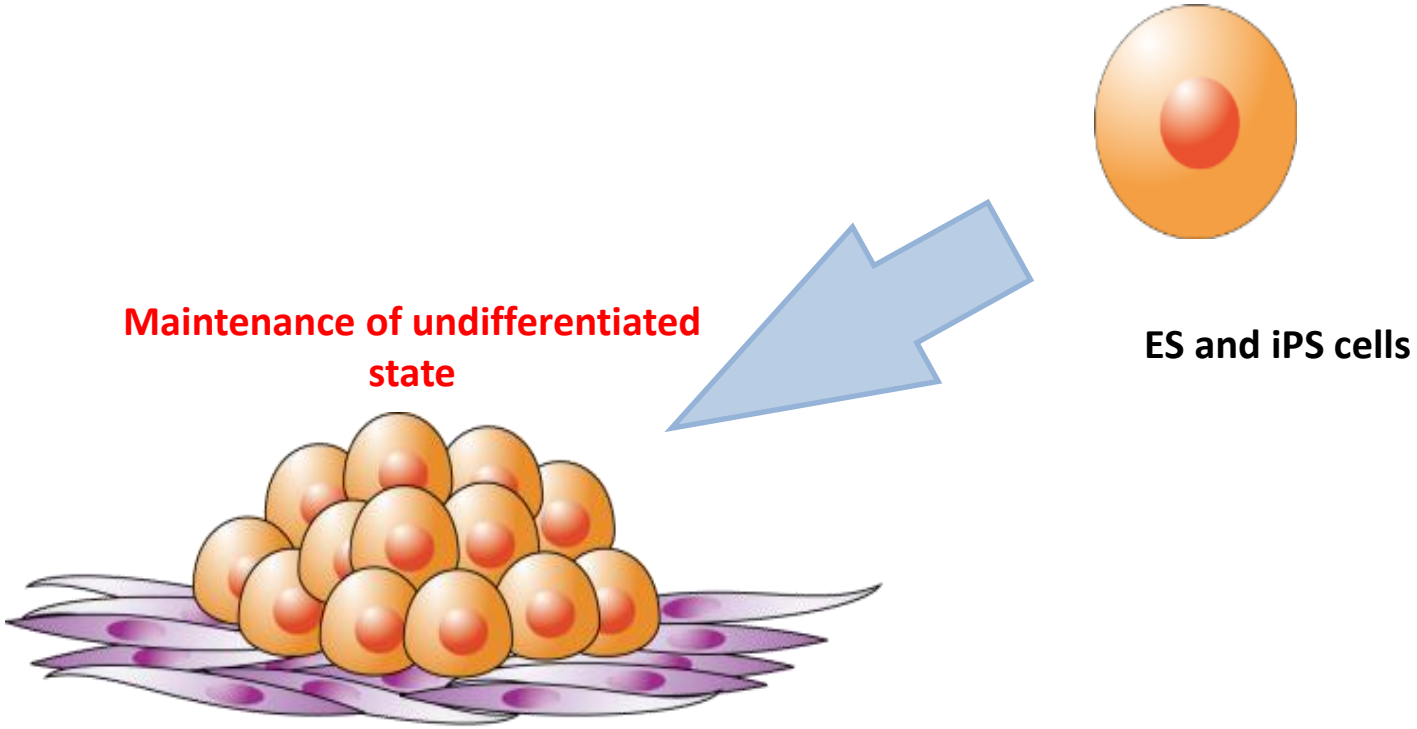
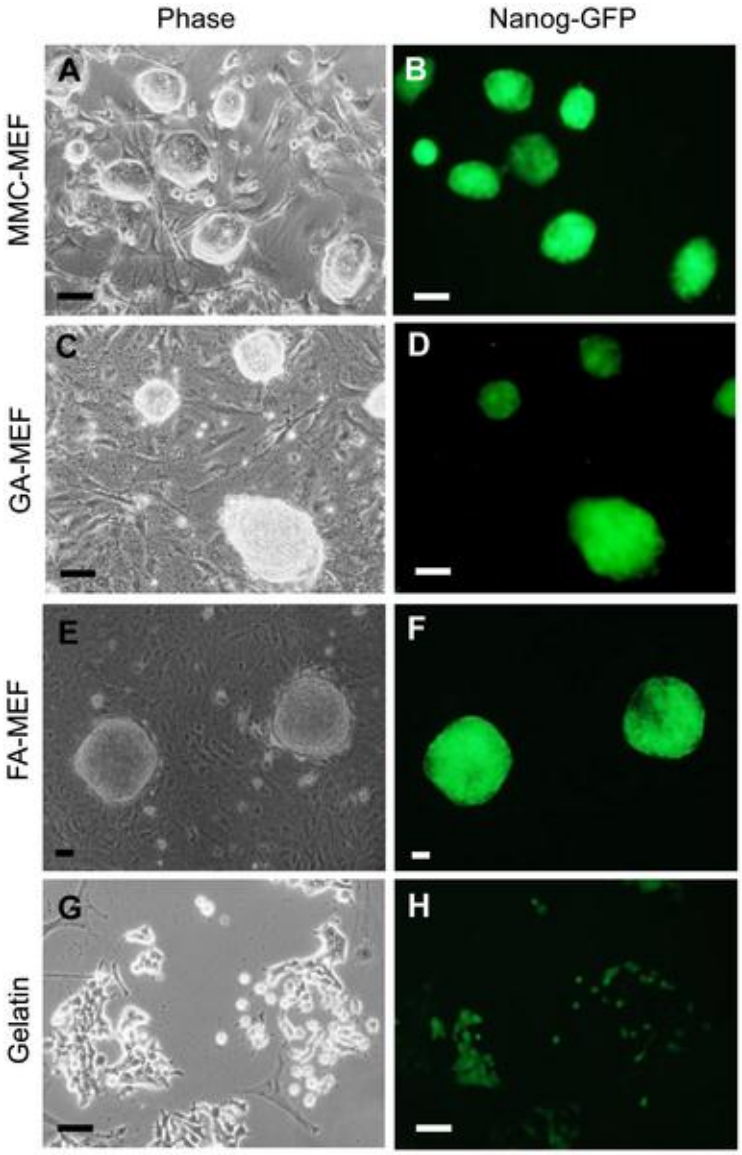
In vitro microenvironments
Stem cell
Fixed cell
Substrate
Fixation
Feeder cell
Substrate
Stem cell
Substrate
Decellularization


Maintenance of undifferentiated state
ES and iPS cells
Feeder cell


Phase
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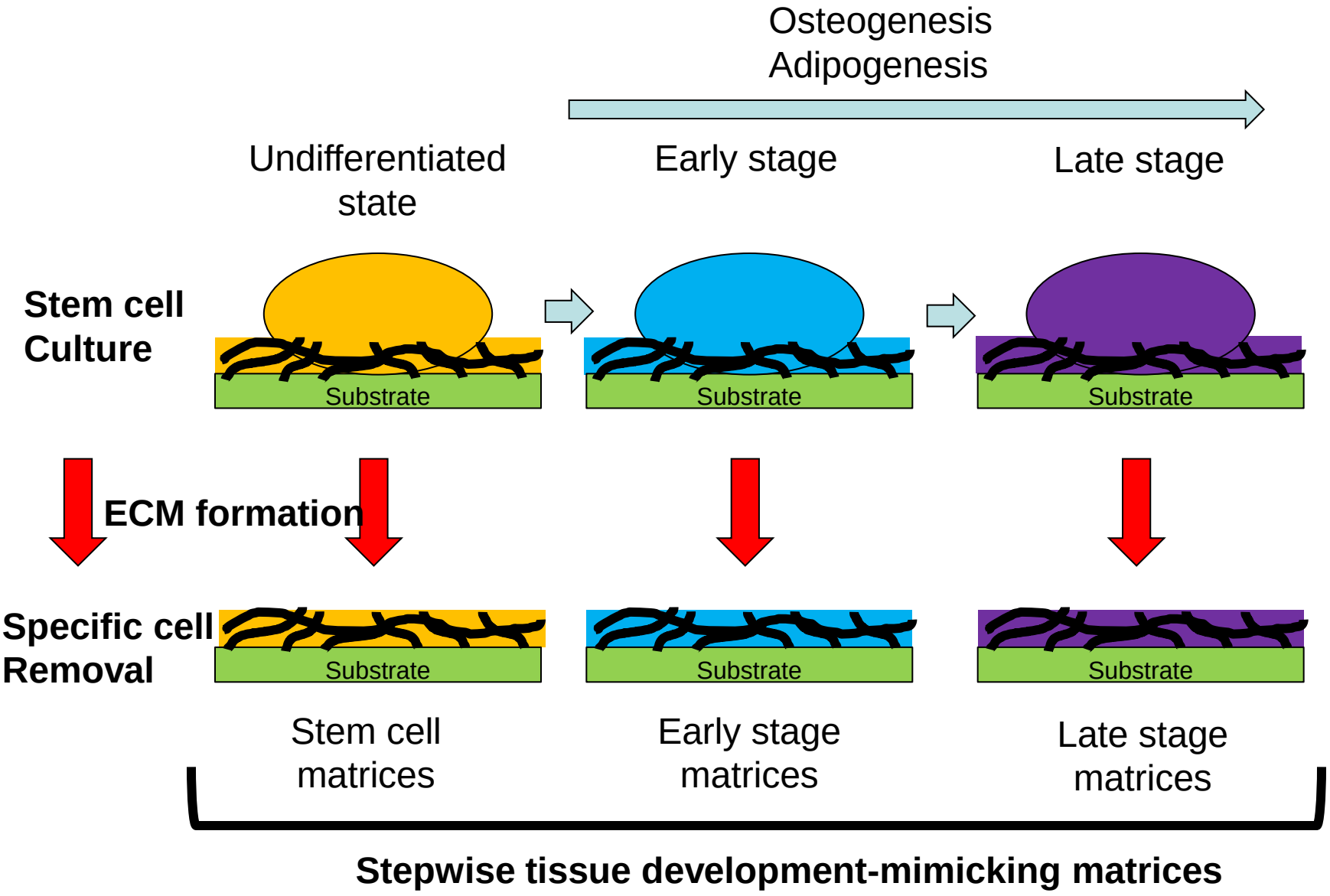
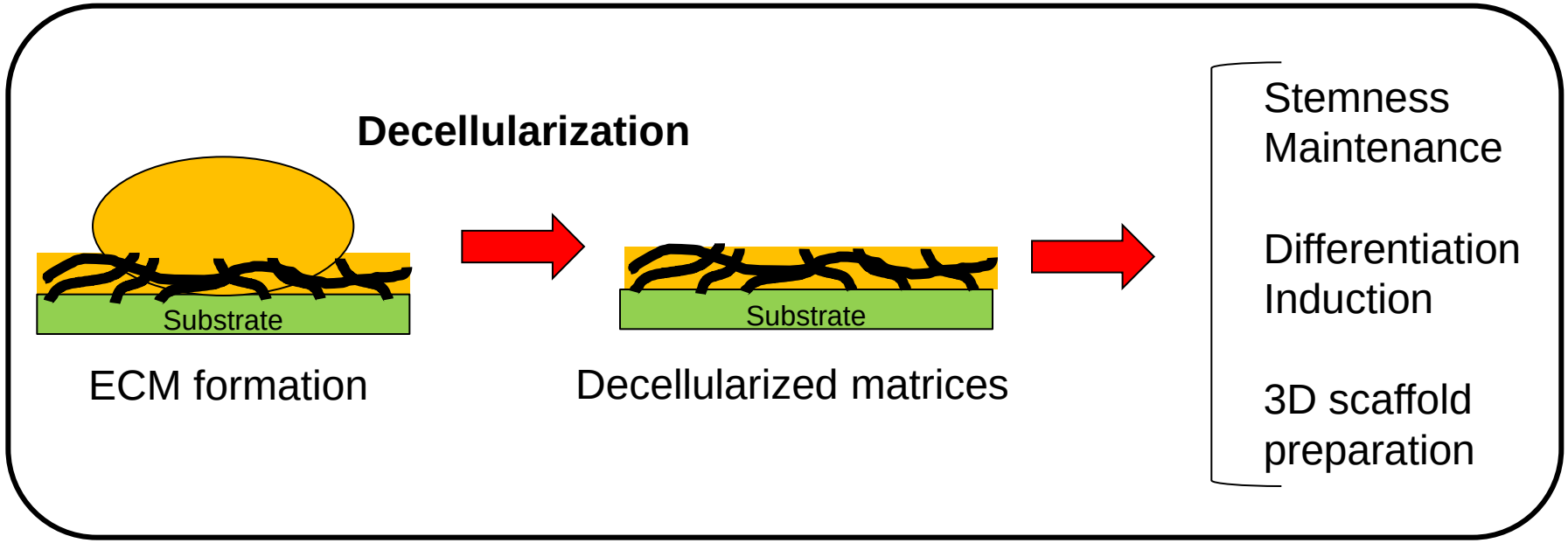
GA-MEF
FA-MEF
Gelatin
A
B
C
D
E
F
G
H


Osteogenesis
Adipogenesis
Undifferentiated state
Early stage
Late stage
Stem cell Culture
Substrate
Substrate
Substrate
ECM formation
Specific cell Removal
Substrate
Substrate
Substrate
Stem cell matrices
Early stage matrices
Late stage matrices
Stepwise tissue development-mimicking matrices



Application for stem cell engineering

- Regenerative medicine
- Pharmaceutical science
- Stem cell biology