Review

Strategies for directing the differentiation of stem cells into the cardiomyogenic lineage in vitro

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Abstract

Most studies on stem cell transplantation therapy on myocardially infarcted animal models and phase-I human clinical trials have focused on the use of undifferentiated stem cells. There is a strong possibility that some degree of cardiomyogenic differentiation of stem cells in vitro prior to transplantation would result in higher engraftment efficiency, as well as enhanced myocardial regeneration and recovery of heart function. Additionally, this may also alleviate the probability of spontaneous differentiation of stem cells into undesired lineages and reduces the risk of teratoma formation, in the case of embryonic stem cells. The development of efficient protocols for directing the cardiomyogenic differentiation of stem cells in vitro will also provide a useful model for molecular studies and genetic manipulation. This review therefore critically examines the various techniques that could possibly be used to direct and control the cardiomyogenic differentiation of stem cells in vitro.

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1. Introduction

Ischemic heart disease is one of the leading causes of morbidity and mortality in the developed world [1]. Current treatment modalities are far from adequate. Routine revascularization techniques such as angioplasty and thrombolytic agents can relieve the cause of infarction but are unable to alleviate the irreversible myocardial injury that results from infarction [2]. Removal of damaged heart tissues by ventriculoplasty results in the loss of a substantial portion of nondiseased myocardium, and hence is a treatment option suitable only for severe dilated cardiomyopathy [3,4]. For pharmacologically intractable failing hearts, allogenic transplant is the treatment of choice. However, the disparity between the number of donors and recipients severely limits this approach [5]. Other treatment modalities that are currently being developed, such as xenotransplantation [6] and artificial mechanical heart [7], have not yet advanced significantly to impact on the clinical management of myocardial infarction. At present, the most promising treatment option that is appearing on the horizon is stem cell transplantation therapy for the repair of damaged myocardium. This is based on the principle of augmenting the inadequate intrinsic repair mechanisms within the diseased heart and could utilize either embryonic stem (ES) cells or adult stem cells derived from a variety of sources (i.e., mesenchymal stem cells, hematopoietic stem cells and skeletal satellite myoblasts). Orlic et al. [8] provide a comprehensive review of the different types of stem cells that could potentially be used for myocardial regeneration.

Proof-of-Concept studies with myocardially infarcted animal models have reported encouraging but limited success in regenerating damaged myocardium through stem cell
transplantation therapy [9]. A major limitation is that the majority of these studies have focused on the transplantation of undifferentiated stem cells, so that cardiomyogenic differentiation would be expected to take place in vivo within the transplant recipient. Because of the tendency of undifferentiated stem cells to spontaneously differentiate into multiple lineages when transplanted in vivo [10,11], it is likely that only a small fraction of the transplanted stem cells would differentiate into the cardiomyogenic lineage. This could in turn reduce the clinical efficacy of stem cell transplantation therapy for myocardial regeneration. There is a strong likelihood that both the engraftment efficiency of the transplanted stem cells, as well as the clinical efficacy of treatment may be improved, if undifferentiated stem cells were “directed” to some degree towards the cardiomyogenic lineage in vitro, prior to transplantation.

Another major consideration would be the safety aspect of using undifferentiated stem cells for transplantation therapy. In the case of embryonic stem cells, these have the potential to form teratomas upon transplantation in vivo [12]. Undifferentiated adult stem cells lack the ability to form teratomas but may differentiate into an undesired tissue type at the site of transplantation. In a recent study [13], the transplantation of undifferentiated mesenchymal stem cells into damaged cardiac muscles resulted in the differentiation of some of these cells into fibroblastic scar tissue, which could in turn impair recovery of heart function after myocardial infarction.

Moreover, the data obtained from transplantation studies involving undifferentiated stem cells is somewhat limited in scope and usefulness. It is extremely difficult to elucidate the molecular mechanisms and signaling pathways that regulate cardiomyogenic differentiation of stem cells in vivo within live animal models. Efficient protocols for directing cardiomyogenic differentiation of stem cells in vitro will therefore provide a model that is much more amenable to molecular characterization and genetic manipulation. In this review, we will critically examine the various techniques that could possibly be employed to achieve this objective.

2. Development of defined culture milieu for directing cardiomyogenic differentiation of stem cells in vitro

For clinical applications of stem cell transplantation therapy, it is imperative that in vitro culture protocols should be devoid of animal or human products, to avoid potential contamination with pathogens. The avoidance of products of animal or human origin would also reduce variability in the culture milieu and provide a more stringent level of quality control. Moreover, supplemented animal or human proteins may adhere onto the surface of cultured stem cells, which could possibly enhance their antigenicity after transplantation. Hence, the ideal culture milieu for promoting the cardiomyogenic differentiation of stem cells in vitro should be chemically defined, and either be serum-free or utilize synthetic serum replacements [14,15], with the possible supplementation of specific recombinant cytokines and growth factors (Table 1), if so required.

The major problem with culturing stem cells under serum-free conditions is that cells generally tend to have a lower mitotic index, become apoptotic, and display poor adhesion, in the absence of serum [14]. The absence of serum is detrimental to primary cultures of cardiomyocytes [16,17]. There is a rapid loss of cell adhesion and differentiated morphology with prolonged in vitro culture under serum-free conditions [16]. Additionally, it has also been reported that the presence of serum enhances mesodermal commitment to the cardiomyogenic lineage within embryoid bodies (personal communication with Dr. Gordon Keller, University of Ulm, Germany).

To prevent transmission of pathogens, the patient’s own serum could be utilized for the in vitro culture of stem cells. However, for clinical applications, there are a number of reasons that would make it preferable to eliminate serum from the in vitro culture milieu. First and foremost, the composition of serum is poorly defined, with a considerable degree of interbatch variation, even when obtained from the

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**Table 1**

Effects of various cytokines/growth factors/synthetic chemicals on the cardiomyogenic differentiation of stem/progenitor cells of embryonic, fetal and adult origin

<table>
<thead>
<tr>
<th>Cytokines/ growth factors/ synthetic chemicals</th>
<th>Key references on cardiomyogenic differentiation</th>
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<tbody>
<tr>
<td>TGF-β1 family (TGF-β1, BMP-2 and BMP-4)</td>
<td>Behfar et al. [23], Schuhleiss et al. [22]</td>
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<tr>
<td>Insulin-like growth factor I (IGF-I)</td>
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<td>Fibroblast growth factor (FGF)</td>
<td>Sachinidis et al. [24]</td>
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<td>Oxytocin</td>
<td>Paquin et al. [28]</td>
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<td>Erythropoietin</td>
<td>Wu et al. [29]</td>
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<td>5-azacytidine</td>
<td>Xu et al. [30]</td>
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**References**

[14] Takahashi et al. [37]
[15] Wobus et al. [36], Zandstra et al. [38]
[16] Ventura and Maioli [39], McBurney et al. [40], Skerjanc et al. [41]
same patient or manufacturer. This would impede good quality control in the laboratory. Serum is also not completely physiological because it is essentially a pathological fluid formed in response to blood clotting. Additionally, it may also contain uncharacterized growth and differentiation factors, which may result in the uncontrolled spontaneous differentiation of stem cells into divergent multiple lineages, other than the cardiomyogenic lineage.

A step towards serum-free culture conditions is the development of chemically defined synthetic serum substitutes. At present, there are a number of such commercially available synthetic serum substitutes [14,15]. Most notable of these is Knockout serum replacement (KSR), which was specifically developed for the maintenance of ES cells in an undifferentiated state within in vitro culture [15]. The exact chemical composition of KSR is not available because it is protected by patent laws. However, it is completely devoid of any undefined growth factors or differentiation-promoting factors [15]. This would be extremely useful for achieving the controlled differentiation of stem cells into the cardiomyogenic lineage in vitro. In a recent study, Sachindis et al. [18] developed a defined serum replacement medium (SRM) for differentiating ES cells into the cardiomyogenic lineage. There was reported to be a sixfold increase in the expression of cardiac-specific myosin heavy chain alpha and beta with the use of SRM, as compared to conventional fetal calf serum (FCS) supplemented medium.

The use of exogenous cytokines and growth factors (Table 1) is another step forward in the development of a defined culture milieu for directing the cardiomyogenic differentiation of stem cells. In the case of ES cells, they have the ability to spontaneously differentiate into the cardiomyogenic lineage in vitro, even in the absence of cytokines and growth factors [19–21]. This is manifested by the development of spontaneously contracting regions within embryoid bodies [19–21]. Hence, a possible strategy for ES cell transplantation therapy for myocardial regeneration would be to initially allow spontaneous differentiation of ES cells into multiple lineages (including the cardiomyogenic lineage) in vitro, in the absence of cytokines and growth factors. This would be followed by selective purification and expansion of the cardiomyogenic lineage isolated from embryoid bodies. However, this is a relatively inefficient and haphazard process. Kehat et al. [21] reported that spontaneously contracting regions appeared in only 8.1% of embryoid bodies cultured in the absence of exogenous cytokines and growth factors. It is therefore preferable to use exogenous cytokines and growth factors to enhance the cardiomyogenic differentiation of ES cells. With regard to adult stem cells, there is no evidence that these have the ability to spontaneously differentiate into the cardiomyogenic lineage in vitro, in the absence of stimulation by exogenous cytokines and growth factors. It is therefore much more technically challenging to direct adult stem cells into the cardiomyogenic lineage, as compared to ES cells.

Of particular interest for cardiomyogenic differentiation are members of the transforming growth factor-beta one (TGF-β1) family. These include TGF-β1 itself, as well as bone morphogenetic proteins 2 and 4 (BMP-2 and BMP-4) [22–24]. Behfar et al. [23] reported that priming of ES cells with TGF-β1 and BMP-2 enhanced cardiomyogenic differentiation, resulting in increased contractile regions within embryoid bodies together with increased myofibrillogenesis. In the same study, it was also shown that TGF-β1/BMP-2 stimulation enhanced the expression of a cardiac-specific promoter tagged to a fluorescent protein and promoted synchronous beating of the differentiated ES cells cocultured with postmitotic adult cardiomyocytes. Schultheiss et al. [22] reported that administration of BMP-2 and BMP-4 to explant cultures induced cardiac differentiation in medial mesoderm, a tissue that is not normally cardiomyogenic. Besides the TGF-β1 family, insulin-like growth factor 1 [24–26], platelet-derived growth factor [18], fibroblast growth factor [27], oxytocin [28] and erythropoietin [29] have also been implicated in cardiomyogenic differentiation.

In addition to protein-based cytokines and growth factors, a number of synthetic chemical compounds (Table 1) have also been shown to promote cardiomyogenic differentiation in vitro [30–41]. Synthetic chemicals tend to be less labile, with a longer active half-life in solution, compared to protein-based cytokines and growth factors. This is advantageous for prolonged in vitro culture over several days or even weeks. Moreover, unlike proteins that has to be synthesized in living organisms and subjected to complex posttranslational modifications (i.e., glycosylation, peptide splicing, conformational folding), synthetic chemical compounds are manufactured by chemical reactions in the laboratory, and hence are more structurally and chemically defined compared to proteins.

Among the synthetic chemicals that are known to promote cardiomyogenic differentiation in vitro are 5-azacytidine, ascorbic acid, retinoic acid, dimethyl sulfoxide (DMSO) and dynorphin B (Table 1). 5-Azacytidine is a synthetic nucleoside that is commonly used as an inhibitor of DNA methylation. It is a potent inducer of cardiomyogenic differentiation in both embryonic [30] and adult stem cells, in particular bone marrow-derived mesenchymal stem cells [31–35]. Both ascorbic acid (vitamin C) and retinoic acid, a derivative of vitamin A, have been shown to promote cardiomyogenic differentiation of ES cells [36–38]. Dimethyl sulfoxide is a commonly used cryoprotectant but has also been shown to induce cardiomyogenic differentiation in both ES cells [39] and embryonal carcinoma cells [40,41]. Additionally, the cardiomyogenic differentiation of embryonal carcinoma cells has also been shown to be stimulated by dynorphin B, which is a naturally occurring κ-opoid [39].

3. Use of extracellular matrix

In situ, the functional myocardium is composed of cardiomyocytes embedded within a supporting extracellular
matrix (ECM) that serves to maintain the structural integrity of the tissue. This is primarily composed of high molecular weight molecules that include collagen, elastin, laminin, fibronectin and a diverse array of proteoglycans and glycoproteins. In addition to its structural role, the ECM plays a physiological role by influencing the immediate microenvironment of the cardiomyocytes. Histological studies have reported extensive remodeling of the ECM of the myocardium during growth and development [42,43], as well as upon ischemic injury during myocardial infarction [44–46].

Hence, the introduction of appropriate extracellular matrix molecules within in vitro culture would certainly enhance the directed differentiation of stem cells into the cardiomyogenic lineage. With commonly used ECM supplements in cell culture, such as collagen, laminin and fibronectin, there was reported to be enhanced myofibrillogenesis, spontaneous contractile activity and differentiated morphology of neonatal cardiomyocytes cultured in vitro [47–49]. It is likely that such supplemented ECM molecules would also be beneficial for the cardiomyogenic differentiation of stem cells, although there are as yet no reported studies. Other ECM molecules that were shown to play an important role in cardiomyogenic differentiation in histological studies include Syndecan-4 [50] Tenascin C [42,46] and hyaluronic acid [51,52]. However, the effects of these molecules on the in vitro culture of primary cardiomyocytes, as well as the cardiomyogenic differentiation of stem cells remain to be investigated.

Besides purified ECM molecules, heterogenous mixtures of ECM components can also be used to promote cardiomyogenic differentiation in vitro. Implantation of porcine urinary bladder matrix (UBM) within the damaged myocardium in vivo was reported to induce regeneration through the recruitment of differentiating cardiomyocytes [53]. However, its effect on cardiomyogenic differentiation in vitro has not yet been investigated. Cardiogel is a heterogeneous preparation of extracellular matrix derived from in vitro cultured fibroblasts [49,54]. It has been reported to be superior to both purified laminin and fibronectin for the maintenance of the differentiated morphology of primary cardiomyocytes within in vitro culture [49,54]. The cell size, contractile activity and mitochondrial content were enhanced in the presence of cardiogel [49]. However, its effects on the cardiomyogenic differentiation of stem cell have not yet been studied.

A novel approach to cell culture could utilize 3-D matrices. Embryonic cardiomyocytes reconstituted within a 3-D collagen matrix produced a coherently contracting 3-D model heart tissue that allowed measurement of isometric contractile forces [55]. Besides collagen, alginate scaffolds have also been reported to provide a conducive environment for 3-D culture of cardiomyocytes [56,57]. Upon implantation into the infarcted myocardium, biografts composed of cardiomyocytes embedded within alginate stimulated intense neovascularization, as well as attenuated left ventricular dilation and failure in experimental rats [56]. It would indeed be interesting to investigate the use of such novel 3-D culture systems to promote the cardiomyogenic differentiation of stem cells.

A major challenge in the in vitro culture of cardiomyocytes for tissue engineering applications is the need for cell detachment by enzymatic methods. This would disrupt electrical communication through gap junctions, which results in contractile pulse synchrony of confluent layers of cardiomyocytes. This problem could be circumvented by the use of a biodegradable temperature-sensitive synthetic polymer surface for cell culture. In the study of Shimizu et al. [58,59], confluent sheets of neonatal cardiomyocytes were detached from the synthetic polymer surface simply by lowering the temperature. When detached sheets of cardiomyocytes were overlaid, they pulsed synchronously and displayed long-term survival upon transplantation [58,59]. This technology may be applied to stem cells that are differentiating into the cardiomyogenic lineage.

At present, there is rapid progress in the development of novel culture systems that involve the utilization of ECM molecules to promote the cardiomyogenic differentiation of stem cells. It is anticipated that more novel types of artificial matrices would be developed in the near future for the controlled differentiation of stem cells into the cardiomyogenic lineage.

4. Coculture and cell-conditioned media

Another strategy to direct the cardiomyogenic differentiation of stem cells would be to coculture the stem cells with a different cell population. Differentiation of ES cells into the cardiomyogenic lineage was enhanced by coculture with visceral endoderm-like cells [60,61]. With adult stem cells, a number of studies have also reported transdifferentiation into the cardiomyogenic lineage with coculture. Primary cardiomyocytes harvested from neonatal rats induced cardiomyogenic differentiation of endothelial progenitor cells [62,63] and the putative myogenic progenitors of skeletal muscle [64]. Using a commercially available source of human cardiomyocytes for coculture, Rangappa et al. [35] achieved cardiomyogenic differentiation of bone marrow derived human mesenchymal stem cells.

A deficiency of these coculture studies is that the culture systems do not faithfully replicate the physiological environment of ischemic injury during myocardial infarction. Hence, there could be a lack of signals for the recruitment and cardiomyogenic differentiation of stem cells. It is possible that ischemic injury could be simulated in vitro by subjecting primary cultures of cardiomyocytes to oxygen starvation, or alternatively by exposure to free radicals and reactive oxygen species. Such a simulated in vitro culture system would be extremely useful for the molecular characterization of signaling pathways that are involved in the
recruitment and differentiation of stems cells into the cardiomyogenic lineage, which occurs naturally in vivo upon ischemic injury during myocardial infarction.

The main advantage of coculture systems is that this allows intimate contact between different cell types, which may lead to a more efficient transduction of molecular signals that induce cardiomyogenic differentiation. The surface receptors of cocultured cells come into direct physical contact, and the autocrine and paracrine factors secreted by one cell type readily interact with the other cell type. In fact, coculture studies by Rangappa et al. [35] and Badorff et al. [63] demonstrated that intimate cell-to-cell contact is necessary for initiating cardiomyogenic differentiation because parallel control studies with conditioned media failed to elicit similar results.

More recently, there is evidence that intimate physical contact may lead to fusion of different cell types in vivo, resulting in the formation of heterokaryons [65,66]. In fact, cell fusion phenomenon has been used to explain the ability of adult stem cells to transdifferentiate into cell types that are radically different from their tissue of origin, when transplanted in vivo [67,68]. Nevertheless, there is as yet no evidence that the observed cardiomyogenic differentiation of stem cells in coculture studies is the result of cell fusion [63].

Coculture of two or more distinct cell populations also carries a strong risk of transmission of pathogens, in particular viruses. This would constitute a major obstacle to the clinical application of coculture for stem cell differentiation. In the clinical situation, it would be virtually impossible to promote cardiomyogenic differentiation of stem cells through coculture with an autogenic cell source because the myocardium of the prospective transplant patient is already diseased. To date, studies on the use of coculture to promote stem cell differentiation into the cardiomyogenic lineage have either utilized cells from animal sources (rat neonatal cardiomyocytes [62,63], murine visceral endoderm [61]) or a commercially available source of human cardiomyocytes [35]. It remains unclear as to whether legislation would permit the utilization of such animal and human materials for clinical therapy. An alternative would be to utilize ES cell-derived cardiomyocytes for coculture with adult stem cells because scalable production of ES cell-derived cardiomyocytes have already been reported [38].

Another major shortcoming of coculture is the difficulty in the separation of cocultured cell populations. The highest degree of purity upon separation could be achieved by fluorescence-activated cell sorting (FACS) [69]. However, FACS is skill-intensive and requires expensive instrumentation. Magnetic affinity cell sorting (MACS) [70] is much cheaper compared to FACS, but the degree of purity upon separation is much lower. Separation of cocultured cell populations also entails detachment of individual cells from each other. This has the disadvantage of disrupting gap junction mediated electrical coupling between differentiating cardiomyocytes, which is manifested by synchronous contractile pulsing of confluent cell layers.

The problem of separating distinct cell populations, as well as the potential problem of cell fusion, may be overcome by keeping cocultured cell populations physically separated through the use of commercially available Transwell inserts [71] or alternatively by switching to using filtered cell-conditioned media instead. But as mentioned earlier, intimate cell-to-cell contact is absolutely essential for initiating the cardiomyogenic differentiation of cocultured stem cells [35,63,64]. Contrary results were however reported by the study of Bader et al. [72], which showed that culture media conditioned by parietal endoderm cells isolated from embryoid bodies could stimulate the cardiomyogenic differentiation of ES cells. Nevertheless, it is unlikely that the parietal endoderm-conditioned media would have similar effects on adult stem cells.

5. Free radicals and reactive oxygen species

Exogenous free radicals and reactive oxygen species (ROS) may also be used to direct the cardiomyogenic differentiation of stem cells in vitro. Indeed, there is much evidence in the scientific literatures that have implicated ROS as important intracellular messengers during cell growth and differentiation [73]. Stimulation of cardiomyocytes with cytokines and growth factors that confer hypertrophic growth, have been shown to elicit the intracellular generation of ROS [74]. Additionally, the large amounts of free radicals and ROS that are generated during myocardial infarction [75], which play a major role in ischemic injury, may somehow also be involved in the signaling and activation of the intrinsic repair mechanisms of the damaged myocardium. This is thought to involve the recruitment and subsequent differentiation of the body’s latent pool of stem cells into the cardiomyogenic lineage, so as to regenerate the failing heart.

Investigations by Sauer et al. [76,77] reported that cardiomyogenic differentiation of ES cells within embryoid bodies was enhanced in the presence of exogenous hydrogen peroxide. On the other hand, incubation with free radical scavengers and antioxidants had an inhibitory effect on cardiomyogenic differentiation. Further investigation implicated an NADPH oxidase-like enzyme in ROS generation during embryoid body formation. It was therefore concluded that ROS had an important role in early cardiac development. The free radical nitric oxide (NO) has also been implicated in cardiomyogenesis. During embryonic development, expression of two different isoforms of nitric oxide synthase (iNOS and eNOS) was detected in atrial and ventricular cardiomyocytes [78]. In the same study, it was also reported that NOS-inhibitors led to a pronounced delay of the differentiation of ES cell-derived cardiac precursors.

Hence, it is clear from these relatively few number of studies that free radicals and ROS play an important role in
cardiomyogenesis. Further research needs to be carried out on the use of exogenous free radicals and ROS to direct stem cell differentiation into the cardiomyogenic lineage in vitro.

6. Physical stimuli

A possible avenue for cardiomyogenic differentiation that has remained less explored is the application of physical stimuli, i.e., in the form of electrical pulses, mechanical forces and heat treatment. Indeed, there is much evidence in the literature to show that such physical stimuli could play an important role in cardiomyogenesis.

The functional myocardium is subjected to constant electrical stimulation in situ. Rhythmic action potentials generated by the sinoatrial node are traversed to the myocardium via conducting myofibers (Purkinje fibers) [79]. Hence, it is likely that electrical stimulation could play an important role in cardiomyogenic differentiation in vivo. Indeed, electrical stimulation has been reported to be beneficial to primary cardiomyocytes cultured in vitro [80,81]. In the study of Sauer et al. [77], electrical pulse stimulation was reported to enhance cardiomyogenic differentiation of ES cells within embryoid bodies. In the same study, it was also shown that the stimulatory effect of electrical pulses on cardiomyogenesis was transduced through intracellular generation of ROS because the presence of free radical scavengers inhibited cardiomyogenic differentiation.

Mechanical stimuli have been reported to have profound effects on primary cardiomyocytes, although its effects on the cardiomyogenic differentiation of stem cells have not yet been investigated. Application of mechanical stimuli can either be in the form of passive mechanical stretch loading or active contractile stretch stimulation. With neonatal cardiomyocytes, passive mechanical stretch loading has been reported to upregulate myosin heavy chain expression and induced cardiomyocyte organization into parallel arrays of rod-shaped cells [82]. Adult cardiomyocytes were however weakly stimulated by passive mechanical load [83]. Wada et al. [84] reported that for adult cardiomyocytes, contractile mechanical stretching provided a much stronger stimulus for protein synthesis, as compared to passive mechanical load. With neonatal cardiomyocytes, contractile mechanical stimulation induced secretion of growth promoting factors [85], as well as upregulation of connexin-43 [86] and myosin heavy chain [87]. Both of these are markers of cardiomyocyte maturity. Kada et al. [88] reported that embryonic rat cardiomyocytes subjected to cyclical stretching resulted in parallel orientation of cardiomyocytes and their intracellular myofibrils. When cyclical stretch stimulation was prolonged, myofibrils that orientated perpendicular to the stretch direction emerged. To date, the pathway by which mechanical stimuli is transduced into a biochemical signal that initiate cardiomyogenic differentiation, has not yet been elucidated. Nevertheless, Schluter and Piper [89] have provided a hypothetical mechanism, in which mechanical forces are transmitted through costameres [90] that are coupled to integrin signaling molecules [91].

In addition to passive mechanical load and active contractile stretching, another form of mechanical stimuli would be simulated microgravity conditions for cell culture. This can be achieved within a NASA-designed high-aspect ratio vessel (HARV) bioreactor [92], which provides a low-shear

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**Table 2**

Various techniques and approaches to direct stem cells into the cardiomyogenic lineage in vitro. Relevant data on neonatal/adult cardiomyocytes are also included.

<table>
<thead>
<tr>
<th>Key references on cardiomyogenic differentiation</th>
<th>Serum-free/defined culture milieu</th>
<th>Extracellular matrix</th>
<th>Coculture/conditioned media</th>
<th>Free radicals/reactive oxygen species</th>
<th>Electrical stimulation</th>
<th>Mechanical stimulation</th>
<th>Elevated temperatures/heat shock</th>
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<tr>
<td>Sachinidis et al. [18]</td>
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<td>Mummery et al. [60,61],</td>
<td>Sauer et al. [76,77]</td>
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<td>Rangappa et al. [35], Cordorelli et al. [62],</td>
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<td>Badorff et al. [63], Iijima et al. [64]</td>
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environment for cardiomyocyte culture on microcarrier beads in static suspension. The 3-D organization of cardiomyocytes that was achieved within such a novel in vitro culture system, was reported to be superior to the 2-D organization of cardiomyocytes that is usually obtained under standard gravity conditions.

Another form of physical stimulus is heat shock by temporary exposure to elevated temperatures. Expression of TGF-β, a potent inducer of cardiomyogenic differentiation, was upregulated in rat cardiac cells that were subjected to hyperthermia [93]. At present, there are as yet no reported studies on the use of heat shock to induce cardiomyogenic differentiation of stem cells. Nevertheless, heat shock prior to transplantation was reported to enhance the survival of neonatal cardiomyocytes [94] and skeletal myoblast [95] that were grafted onto the myocardium of live animal models.

At present, there is a paucity of information on the use of physical stimuli to induce the cardiomyogenic differentiation of stem cells. Hopefully, more investigations will be conducted in this potentially interesting area of research in the near future.

7. Concluding remarks

Despite the large number of studies that have recently been carried out on the cardiomyogenic differentiation of stem cells in vitro, this particular area of research is still in its relative infancy. Cardiomyogenic differentiation may be further enhanced, if the various techniques that have so far been discussed are used in combination, rather than exclusively by themselves. In the natural milieu, the cardiomyogenic differentiation of stem cells probably involves multiple signaling pathways. This may be mimicked in vitro by using a combination of these various techniques (Table 2) to achieve a synergistic effect on the differentiation of stem cells into the cardiomyogenic lineage.

It must however be kept in mind that for clinical applications, it is imperative to develop well-defined and efficient in vitro protocols for the cardiomyogenic differentiation of stem cells, that would utilize chemically defined culture media supplemented with recombinant cytokines and growth factors (Table 1). This will then provide the stringent levels of safety and quality control that would make the clinical applications of stem cell transplantation therapy realizable. Hopefully, this would be achieved in the near future.

References


