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Ferdinando Mannello and Gaetana A. Tonti

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 **AlphaMed Press**

## Concise Review: No Breakthroughs for Human Mesenchymal and Embryonic Stem Cell Culture: Conditioned Medium, Feeder Layer, or Feeder-Free; Medium with Fetal Calf Serum, Human Serum, or Enriched Plasma; Serum-Free, Serum Replacement Nonconditioned Medium, or Ad Hoc Formula? All That Glitters Is Not Gold!

FERDINANDO MANNELLO, GAETANA A. TONTI

Institute of Histology and Laboratory Analysis, University "Carlo Bo," Urbino, Italy

**Key Words.** Mesenchymal stem cells • Embryonic stem cells • Fetal calf serum • Serum-free medium • Autologous human serum  
Heterologous human serum • Platelet-factor-rich supernatant

### ABSTRACT

The choice of an optimal strategy of stem cell culture is at the moment an impossible task, and the elaboration of a culture medium adapted to the production of embryonic and adult mesenchymal stem cells for the clinical application of cell therapy remains a crucial matter. To make an informed choice, it is crucial to not underestimate the theoretical health risk of using xenogenic compounds, to limit the immunological reactions once stem cells are transplanted, to not overestimate the controversial results obtained with human serum, plasma, and blood derivatives, as well as to carefully examine the pros and cons of serum-free and ad hoc formulation strategies; besides that, to also maintain multipotentiality, self-renewal, and transplantability. The

extent to which we are able to achieve effective cell therapies will depend on assimilating a rapidly developing base of scientific knowledge with the practical considerations of design, delivery, and host response. Although clinical studies have already started, many questions remain unsolved, and concomitantly even more evidence on suitable and safe off-the-shelf products (mainly xeno-free) for embryonic and mesenchymal stem cells is cropping up, even though there should be no rush to enter the clinical stage while the underlying basic research is still not so solid; this solely will lead to high-quality translational research, without making blunders stemming from the assumption that all that glitters is not gold. *STEM CELLS* 2007;25:1603–1609

Disclosure of potential conflicts of interest is found at the end of this article.

### INTRODUCTION

No question is of greater interest to the researchers and clinicians dealing with adult and embryonic stem cells than the identification of "optimal" conditions for the cell culture medium [1]. The development of techniques to grow in vitro human and murine embryonic pluripotent stem cells (hESC and mESC, respectively) and/or adult human and murine multipotent mesenchymal stromal stem cells (h and mMSC), and the deeper understanding of pathways of cell differentiation, have expanded the horizon of likely therapeutic uses. In general, the use of stem cells to create human tissues ex vivo for transplantation into patients with degenerated or injured tissues has attracted increased interest, inducing the Food and Drug Administration to set the rules of stem-cell-based therapies as health policies [2]. The level of concern about potential contamination and damage to cells/tissues depends on how (and how much) they have been handled and manipulated. When stem-cell-based products involve more than minimal manipulation (such as expansion or differentiation), the cells will probably be grown in in vitro culture, and the standard culture medium contains bovine serum. However, this common process,

which involves the use of nonhuman serum (often obtained from fetal calves), may be the source of possible contamination (e.g., infection from bovine disease by prion, virus, and zoonosis) or immune-reaction to foreign proteins (e.g., xenogenic bovine serum antigens), underlining the necessity for a more cautious evaluation of culture media. Moreover, to minimize the risk of transmitting infectious diseases from animals, hESC lines have been cultured in a conditioned medium obtained from human cells as feeders and human and recombinant serum components or serum-free conditions; hence, contrasting results have been widely obtained, and a heated debate has been opened on this topic (both in *STEM CELLS* and in other journals) [3–7]. On the basis of the point of view that scientists still have much to learn about determining the safety and efficacy of stem-cell-based products, we bear in mind that the more we know about the biology of self-renewal and differentiation of both ESC and MSC, the more readily the risks of inappropriate cell function can be assessed. We are following the debate on this field with great interest, because the clinical application of ESC and MSC in cell therapy needs the elaboration of more appropriate culture media and defined culture conditions [8], evaluating the beneficial and detrimental effects of feeder cell layer or feeder-free conditions, human allogeneic/autologous and fetal calf sera in

Correspondence: Ferdinando Mannello, Ph.D., Institute of Histology and Laboratory Analysis, Faculty of Sciences and Technologies, University of Urbino "Carlo Bo," Via O. Ubal dini 7, 61029 Urbino (PU), Italy. Telephone: +39-0722-351479; Fax: +39-0722-322370; e-mail: f.mannello@uniurb.it Received February 15, 2007; accepted for publication March 20, 2007; first published online in *STEM CELLS EXPRESS* March 29, 2007; available online without subscription through the open access option. ©AlphaMed Press 1066-5099/2007/\$30.00/doi: 10.1634/stemcells.2007-0127

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comparison with serum-free conditions, or a new formulation ad hoc in order to present an updated overview and to set the basis for an informed choice in translational stem cell research.

**FEEDER CELL LAYER OR CONDITIONED MEDIUM IN FEEDER-FREE CULTURE SYSTEMS: THE MEDIUM IS THE MESSAGE**

The hESC, the most pluripotent known stem cells, were derived with fetal mouse fibroblast as feeder cells [9] with a procedure similar to that used for derivation of mESC lines [10]. Several purified polypeptides (such as leukemia-inhibitory factor) are able to maintain the nondifferentiated growth of mESC [9] but not of hESC [11]. The hESC cell lines are isolated, and continue to be maintained, with human or mouse embryonic fibroblast as feeder layers or as a source of conditioned medium (or both) for propagation of the cells in the undifferentiated state [12, 13]. Exposure of these cells lines to live animal cells presents a risk of contamination with retroviruses and other pathogens or causes concern for infection with recognized or as yet unrecognized infectious agents that could be transmitted to the patients and the wider population [2, 14]. On the other hand, in conditioned medium from mouse embryonic fibroblast feeder layers, 136 unique protein species were identified, which included some that are known to participate in cell growth and differentiation, extracellular matrix formation, and remodelling, in addition to the unexpected but interesting finding of many nominally intracellular proteins [15]. These studies highlight the complexity of the environment provided by the feeder cells and some of the basic differences in the self-renewal mechanism and pluripotency between m- and hESC [16].

Although hESC lines have been derived by human feeder systems—with fetal human fibroblasts and postnatal human skin fibroblasts as feeder cells [17, 18] as well as by feeder-free systems [6, 16, 19]—these approaches generally require addition of feeder-conditioned medium (which can contain potential pathogens) or at a minimum require extensive screening of donor sources for derivation of potential pathogen-free hESC. Use of feeder layers also limits stem cell research design, since experimental data may result from a combined ESC and feeder cell response to various stimuli [8]. In this respect, some studies described the system for feeder-free and conditioned medium-free culture of hESC, even though these have been exposed to feeder cells during derivation [6, 16]. An additional study derived and established new hESC lines in completely feeder-layer-free and serum-free conditions using an extracellular matrix prepared from mouse embryonic fibroblasts [20]. The extrinsic factors necessary for maintaining hESC pluripotency and self-renewal are poorly understood, partly because of complex culture conditions that include both growth-inactivated feeder cells and serum, a cocktail of myriad proteins, and soluble factors. The use of such an extracellular matrix may eliminate the risk of transmitting animal pathogens to the hESC [20].

The extracellular matrix is a uniquely assembled three-dimensional molecular complex that varies in composition and diversity; it consists of basic components (such as fibronectin, collagens and other glycoproteins, hyaluronic acid, proteoglycans, and elastins), and it also arbors molecules such as hormones and growth factors (like transforming growth factor  $\beta$ 1, leukemia inhibitory factor, basic fibroblast growth factor, stem cell factor, fetal liver tyrosine kinase-3 ligand, thrombopoietin, ciliary neurotrophic factor, oncostatin M interleukin-6 [IL-6] family members, etc.). In particular, some of these factors (e.g., IL-6 and ciliary neurotrophic factor) have shown effects in maintaining the pluripotency of mESC [21, 22], whereas basic

fibroblast growth factor (alone or in combination with other factors) supports undifferentiated growth of hESC [23]. Interestingly, hESC cultured in nonconditioned medium supplemented with stem cell factor, fetal liver tyrosine kinase-3 ligand, thrombopoietin, and leukemia inhibitory factor but without basic fibroblast growth factor are not sufficient to maintain the growth of undifferentiated cells, which showed almost complete differentiation after 6 weeks in culture [16, 23]. This evidence emphasizes the hypothesis that autocrine and paracrine factors produced by h- and mESC are not adequate to support the growth of stem cells in long-term culture. However, all the bioactive components present in extracellular matrix may play a key role in mediating signals that recapitulate developmental processes in tissue-specific differentiation and morphogenesis of both h- and mESC, as well as regulate stem cell homing in experimental animal models and clinical transplantation protocols [24, 25].

The serum replacement with extracellular matrix prepared from mouse embryonic fibroblasts [20], with conditioned medium from mouse embryonic feeder [6, 19] and using several growth factors in the absence of conditioned medium [13, 23], contains animal proteins that raise the possible problem of immunogenicity of these xeno-components in these culture systems. In this respect, several studies have shown that hESC cultured on mouse feeder cells and with commercial serum replacement culture medium were reported to be contaminated by the xeno-carbohydrate *N*-glycolylneuraminic acid (Neu5Gc) [26]; this immunogenic nonhuman sialic acid can be potentially taken-up by hESC, making them potentially unfit for human therapy. Moreover, a recent study revealed that similar contamination occurred also in hMSC cultured in the presence of fetal bovine serum, suggesting that Neu5Gc is present in both glycoprotein and lipid-linked glycans [27]. Although major complications have not been reported in the clinical trials with hMSC exposed to fetal bovine serum, the immunogenic contamination may potentially be reflected in the viability and efficacy of the transplanted cells and, thus, bias the published results.

Several studies are in progress in order to define more safe culture conditions for stem cells; even though it is believed that the serum replacement medium should ideally be replaced by one containing only human proteins, hormones, and growth factors, the results obtained testing the effects of different culture media for hESC are actually contradictory. It has been reported that 1,417 genes were found to be differentially expressed when hESC cultured in serum-containing medium were compared with those cultured in serum-replacement medium, highlighting that, in serum-replacement, the hESC were maintained in an undifferentiated state, whereas differentiated phenotypes were induced when cells were cultured in serum-containing medium [28]. Although it has been described that human serum as matrix and medium conditioned by differentiated hESC reduces exposure of stem cells to animal ingredients and provides a safer direction toward a completely animal-free condition [29], more recent evidence demonstrates that any xeno-free culture medium was able to maintain the undifferentiated growth of hESC [30]; moreover, this comparative study reports that medium containing human serum was found to sustain undifferentiated hESC proliferation to some extent, even though inferior to the conventional serum-replacement medium [30]. Even though it has been stated that hESC for transplantation have to be cultured in conditions of Good Manufacturing Practice to guarantee the safety and quality of these cells [2], many studies are still needed to avoid immune-reactions in transplantation, to prevent chromosomal, epigenetic and genetic changes during h- and mESC culture [31, 32], and to finely control differentiation of these cells before clinical treatment.

## FETAL CALF SERUM: THE PROS AND CONS

Fetal bovine or calf serum (FBS and FCS, respectively) are the most widely used growth supplements for cell culture, primarily because of their high levels of growth stimulatory factors and low levels of growth inhibitory factors. Maintaining successful and consistent cell fermentations can be difficult, as FBS and FCS are complex natural products and may vary from lot to lot even from a single manufacturer; moreover, the quality and concentration of both bulk and specific proteins can affect cell growth. Quality control tools for FBS/FCS are relatively primitive and expensive given the complexity of the sample and the large amount of FBS/FCS used. Since 1975, a high degree of serum variability was found both within and between suppliers concerning chemical, protein, and endocrine parameters, suggesting that caution should be employed in the interpretation of results from experiments utilizing serum supplements without specific quantitation of possible interfering or modulating factors [33]. In addition, a recent time course study of FBS composition revealed that, in the fermentation medium, the amount of several extracellular matrix and structural proteins (which are indicators of cell growth) increased over time, and components supplied by the FBS addition (such as nutritional-related and cell-spreading-related proteins) decreased over time [34]. Such variability not only forces most laboratories to perform their own FCS/FBS screening and to use "preselected" lots thereafter, it also often makes it hard to compare the data received by the different investigators.

A variety of on-going clinical/research trials use hMSC and hESC in search of a treatment for various diseases. At the moment, most isolation and expansion protocols for clinical-scale production of stem cells use culture media frequently supplemented with FCS, which may contain potentially harmful xenogeneic compounds. In particular, bovine serum proteins may be internalized in stem cells stimulating immunogenicity [26, 35]; consequently, a host of potential problems can arise such as viral and prion transmission [2, 36] and/or immunological reactions due to the bovine protein attachment to cells in culture that act as antigenic substrates once transplanted [27]. For example, patients infused with lymphocytes cultured in medium supplemented with FCS develop arthus-like reactions [37]; cellular cardiomyoplasty using skeletal myoblasts cultivated with FCS causes malignant ventricular arrhythmias and sudden death in patients [38], even though intracoronary transplantation of FCS-cultivated MSC in infarcted human myocardium fails to provoke any ventricular arrhythmias [39, 40]; patients undergoing clinical gene therapy trial for adenosine deaminase deficiency, using autologous T cells grown in FCS-supplemented medium, develop unique IgG immunity to FCS proteins [41].

Other than these well known "side effects," the role of serum source and serum treatment (e.g., heat inactivation) on the behavior of cells has long been neglected. Although serum is chemically ill defined, a reduction of variability in qualitative and quantitative cell culture medium composition secondary to interbatch differences should be expected, leading to a more efficient level of quality control. Bovine serum contains a variety of polypeptides that may "attach" to cells, inducing significant metabolic/morphologic changes. For example, the use of FCS in soluble form in the culture medium or coated in polyvinyl substrates can significantly alter both cell behavior and morphological differentiation, regulating the potential of the central nervous system neural stem cells [42]; bone marrow derived mesenchymal progenitor cells show a different behavior

in culture performed in the presence of FCS with or without heat inactivation [43]; a significant antibody response to bovine lipoproteins is observed using autologous T cells grown in FCS-supplemented medium [41], linked to the observation that FCS contains a low density lipoprotein, which, in turn, binds to its human receptor [44]; adipocyte precursor cells are stimulated to differentiate when cultured in the presence of FCS that enhances the activity of glycerophosphate dehydrogenase (a sensitive adipose differentiation marker) [45]; phospholipids present in microvesicles found in FCS may compete with antibodies secreted by human cells, obscuring the detection of anticardiolipin antibody secreting clones [46];  $\beta$ 2-microglobulin present in high concentration in FCS can promote peptide binding to class I major histocompatibility molecules on cells cultured *in vitro*, significantly affecting its stability [47]; the long-term expansion and proliferation rate of adult neural progenitor cells is significantly reduced when cultured in FCS with N2 and without cytokines [48]; finally, FCS contains a high concentration of fetuin-A, a glycoprotein with a wide spectrum of biological functions on cell growth and differentiation [49, 50], and it may be released from authentic disulfide-bridged two-chain forms present in FCS-supplemented media through the proteolytic action of matrix metalloproteinases [51], key proteinases that have been involved in the regulation of MSC and ESC differentiation [52, 53]. All of this evidence supports the hypothesis that FCS is unsuitable for an effective expansion of stem cells to be used in cellular therapy, suggesting that cell culture medium composition should distance itself from animal products [54, 55].

On the other hand, it is fair to strike a blow for FCS; in fact, there are several published clinical trials using hMSC for the treatment of various disorders, all of which use FCS for supplementing culture medium, demonstrating so far that FCS doesn't cause any significant side effects [4, 56]. Moreover, FCS contains low-molecular weight bioactive factors (e.g., serofendic acid) with neuroprotective functions, playing a crucial role in attenuating cytotoxic consequences induced by necrotic and apoptotic signals (such as mitochondrial membrane depolarization and caspase-3 activation) mediated by neuronal glutamate receptors [57]. Finally, hMSC expanded in FCS-supplemented medium display comparable morphology, phenotype, and differentiation capacity to MSC expanded in medium supplemented with platelet lysate; moreover, hMSC in FCS are more efficient in suppressing alloantigen-induced lymphocyte subset proliferation, suggesting FCS-supplemented medium is more suitable for preventing/treating alloreactivity-related immune complication [58].

Although the quality of FCS has proved to strongly influence the large-scale production of ESC and MSC while maintaining their multilineage and immunosuppressive capacity, other culture parameters/conditions (e.g., basal medium, glucose concentration, stable glutamine, stem cell plating and passaging density, and plastic surface quality) affect the final outcome [1, 59, 60]. All of this evidence suggests the need to proceed with caution, because the *ex vivo* culture phase (with detrimental and beneficial contribution of FCS constituents) may induce subtle and unexpected modifications in the biology of stem cells when the cell products are released and infused into the patients.

## HUMAN SERUM AND BLOOD DERIVATIVES: A SUITABLE ALTERNATIVE?

In theory, using human blood derivatives should eliminate or reduce the risk of secondary effects due to FCS constituents.

Different considerations indicate that we should not rule out the possibility of using equally human serum or plasma, due to evident involvement of coagulation/fibrinolytic pathways. In fact, the necessity of using hESC as a source of tissue for cell therapy in regenerative medicine brings about its establishment and propagation according to Good Manufacturing Practice quality requirements [61], avoiding the cultures with animal substances in order to exclude the risk of infections and immunogenicity [2, 26, 36], and finally obtaining cells and tissues that are genetically and epigenetically normal. In particular, it has been demonstrated that hESC grown in matrix derived from human serum maintains all embryonic stem cell features after prolonged culture (including the developmental potential to differentiate into representative tissues of all three embryonic germ layers) and also unlimited and undifferentiated proliferative ability with the maintenance of normal karyotype [29]. On the contrary, hESC line was functionally adapted toward a decreased dependence of extracellular matrix for *in vitro* survival, revealing a paralleled karyotype change in all cells, maintaining the expression of peculiar hESC markers but showing a decreased pluripotency [31].

For what concerns MSC, there has been much controversy about what constitutes a suitable FCS supplement. Apart from human plasma, in which have been identified factors that might be important for the differentiation of adipocyte precursor cells (e.g., growth hormone and insulin) [45], several studies have focused the attention on the use of autologous serum. In this respect, several papers evidenced that autologous serum/plasma preserves the differentiation capacity, induces a greater cell amplification, and promotes motility of stem cells (mesenchymal and bone marrow derived stem cells), improving cellular and genetic therapies [3, 62–68]. However, it is currently accepted that the life span of hMSC is limited *in vitro*, due to replicative senescence and telomeres shortening [4]. Yet, the use of autologous/autogenic plasma/serum is limited by the amount of autologous supplements necessary to expand hMSC for clinical use and the variability of serum, especially for patients receiving prior chemotherapy [1, 56], even though the autologous strategy is a matter of debate [4]. Up until now, even though the biochemical difference between human plasma and serum is well known, surprisingly no data are available on the possible effects on hMSC cell features of autologous/autogenic plasma and serum.

In the literature, the results for allogeneic serum/plasma are instead contradictory. Some studies have been successful in isolating and expanding hMSC using heterologous serum [62, 69, 70], whereas others have observed very rapid senescence and growth arrests of hMSC [3, 67, 71]. Although human serum is described to not fully support growth of hMSC *in vitro* [72], several studies evidenced that hMSC cultivated in media supplemented with fresh frozen plasma and platelets, platelet-lysate, platelet-rich plasma, and platelet-derived growth factors showed vigorous proliferation and migration ability, high clonogenic efficiency, immunosuppressive activity, and capacity to differentiate toward the osteogenic, chondrogenic, and adipogenic lineage [58, 72–76]; this evidence suggests that the platelet-rich plasma may be a powerful and safe substitute for FCS in the development of tissue- and cellular-engineered products in clinical settings using hMSC [58, 77], leaving space for the hypothesis of a difference between serum and plasma. Of particular interest is the recent evidence that human AB heterologous serum, as well as thrombin-activated platelet-rich-plasma, provides significantly higher proliferative effects on adipose-tissue hMSC, retaining differentiation capacity and marker expression throughout long-term culture [77].

Curiously, in the search for the optimal culture condition, it was found that a mixture of minimal quantity of human AB

serum or human autologous plasma with a new serum substitute, containing vegetable-derived proteins, displayed growth and differentiation of hMSC comparable with that obtained with FCS or serum substitute containing animal-derived proteins, demonstrating that the mixture has synergistic effects on colony formation unit-F formation [70]. Moreover, the most primary phenotypes have been described even in poor serum-free culture media [4], and so it seems possible that low serum concentration, while limiting cell proliferation, may enable production of cells for therapeutic use.

### SERUM-FREE MEDIUM: FRIEND OR FOE?

Several studies characterized hESC cell lines derived from embryos under completely cell and serum-free conditions [6, 7, 14]; although this culture strategy allowed maintenance of normal karyotype and markers of pluripotency, retaining also the potential to form derivatives of all three embryonic germ layers both *in vitro* and in teratomas [7, 20, 78–81], other studies reported that the hESC cultured in xeno-free medium does not maintain the undifferentiated growth of undifferentiated stem cells, showing that the proliferation decreased substantially [30], probably linked to the loss of gap junctional intercellular communication [82]. Cultivation in serum-free conditions has demonstrated contradictory results, and no breakthroughs have yet been reached. In particular, some studies revealed that hMSC from marrow and umbilical cord blood were efficiently *ex vivo* expanded in serum-free medium [83, 84]. In particular, cord blood MSCs were expanded in Dulbecco's modified Eagle serum-free medium supplemented with high glucose, basic fibroblast growth factor, human albumin, hydrocortisone, and SITE (sodium selenite, bovine insulin, human transferrin, and ethanolamine); these cells preserved the differentiation potential into three mesenchymal lineages *in vitro* (including chondrocytes, adipocytes, and osteoblasts), suggesting the application of this culture strategy for cell-based therapy and biomedical research [83]. Interestingly, during the true serum deprivation culture condition (in  $\alpha$ -modified minimum essential medium without cytokines or other growth factor supplements), a mesenchymal stem cell subpopulation with early progenitor cell features was isolated; these cells showed longer telomeres with respect to control cells cultured in FCS-complete medium and enhanced expression of genes characteristics of embryonic cells [84].

On the other hand, additional studies demonstrated that serum-free media cannot promote hMSC growth without the addition of cytokines and/or growth factors [85, 86], possibly due to the fact that serum induces intracellular calcium oscillations, which are vital to stem cell proliferation and differentiation [87]. Moreover, mMSC were reported as sensitive to hypoxia and serum deprivation stimuli, suggesting that the survival-growth factor withdrawal and hypoxia cause apoptosis via the caspase-dependent manner in transplanted cells [88]. To further complicate the serum-free culture condition, other parameters of cell culture, in particular initial cellular density, basic medium, the presence of any cytokine, and the plastic quality/characteristics, are variables that may explain, at least to some extent, the inconsistencies among study results.

### FUTURE PERSPECTIVE

Stem cells are the functional elements of reparative medicine and tissue engineering, and the use of living cells as a therapy presents several challenges [2, 14]. These include identification

of a suitable source, development of adequate methods, and proof of safety and efficacy. We are now well aware that embryonic and adult stem or pluripotent cells offer an exciting potential source for a host of functional cell types. Their true potential will only be realized through continued effort to increase basic scientific understanding at all levels, the development of adequate methods to achieve a functional phenotype, and attention to safety issues associated with careful control of cell localization, proliferation, and differentiation [89].

Control of cell growth and phenotypic expression does not end in the culture vessel, but goes beyond to the patient, highlighting the dynamic characteristic of living cell therapy, as is the host response. The cells or tissue constructed in most cases will not behave as a whole-organ transplant. It is therefore important that we understand a cell or tissue therapy's ability to react and interact within the host, since clinical effectiveness has proven to be one of the most difficult milestones to achieve.

The living cell therapy through hESC and hMSC offers great potential to alter the human condition, encompassing alteration of the current biological state of a targeted tissue or organ, augmentation of depleted or lost function, or absolute functional tissue replacement. The extent to which we are able to achieve effective cell therapies will depend on assimilating a rapidly developing base of scientific knowledge with the practical considerations of design, delivery, and host response (excellently reviewed in [89]).

For these reasons, growing interest in STEM CELLS and other journals is currently emerging regarding the use of FCS, serum replacement, feeder layers, human autologous/heterologous serum/plasma supplemented media, and serum-free and conditioned medium for the large scale expansion of stem cells to be used in cellular therapy (although this use is of regulatory nature). Although we have not covered all aspects of this complicated area of human stem cell research, we have presented the state-of-art of more recent studies aimed to define optimal conditions of stem cell culture, to minimize the theoretical health risk of using xenogenic compounds, to limit the immu-

nological reactions once stem cells are transplanted, but maintaining multipotentiality, self-renewal, and transplantability.

Even though clinical studies have already started, many questions remain unsolved, and concomitantly even more evidence on suitable and safe off-the-shelf products (mainly xenofree) for ESC and MSC are cropping up. The growing studies that promote exciting alternatives as the optimal choice of stem cell culture medium may open the realm of the stem cell clinical research, even though there should be no rush to enter the clinical stage while the underlying basic research is not so solid; this only will lead to high-quality translational research, without blunders, because novel things that glitter are not all gold. The evaluation of the best stem cell culture optimal condition is at the moment an impossible task. However, we believe that FCS seems not anymore the best choice, whereas the strategy of serum-free supplemented by autologous and/or heterologous human serum, as well as the supplementation of blood derivatives (e.g., platelet-factor rich plasma), may represent a promising tool, opening new frontiers in stem cell culture condition, even though controversial results about hMSC osteogenic differentiation capacity remain [74, 75]. In the same way, studies directed to derive and establish hESC lines in completely feeder-layer free and serum-free conditions are crucial to solve one of the major problems associated with the use of hESC differentiated progeny in the treatment of human medical conditions, thus eliminating the risk of contamination with pathogenic agents capable of transmitting diseases to patients. All we can do is make an informed choice in order to continue the crucial translational research and trials of stem cell therapies [89] for treating irreversible pathological disorders not responding to conventional therapies.

### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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Ferdinando Mannello and Gaetana A. Tonti

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