Optimizing stem cell culture.

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Abstract.

Stem cells always balance between self-renewal and differentiation. Hence, stem cell culture parameters are critical and need to be continuously refined according to progress in our stem cell biology understanding and the latest technological developments. This led to the progressive replacement of ill-defined additives such as serum or feeder cell layers by recombinant cytokines or growth factors. Another example is the control of the oxygen pressure. For many years cell cultures have been done under atmospheric oxygen pressure which is much higher than the one experienced by stem cells in vivo. A consequence of cell metabolism is that cell culture conditions are constantly changing. Therefore, the development of high sensitive monitoring processes and control algorithms is required for ensuring cell culture medium homeostasis. Stem cells also sense the physical constraints of

their microenvironment. Rigidity, stiffness and geometry of the culture substrate influence stem cell fate. Hence, nanotopography is probably as important as medium formulation in the optimization of stem cell culture conditions. Recent advances include the development of synthetic bioinformative substrates designed at the micro- and nanoscale level. On going research in many different fields including stem cell biology, nanotechnology, and bioengineering suggest that our current way to culture cells in Petri dish or flasks will soon be outdated as flying across the Atlantic Ocean in the Lindbergh's plane.

Key words: STEM CELL; CELL CULTURE; OXYGEN; NANOTOPOGRAPHY

There is increasing interest in optimizing stem cell culture, not only because cell culture is widely used in basic research for studying stem cell biology, but also owing to the potential therapeutic applications of cultured stem cells. Defining universal optimal conditions for stem cell cultures is not easily achievable. Stem cell culture conditions must be refined according to the stem cell type, e.g: cell culture requirements for embryonic stem cells (ES) or for different kinds of adult stem cells may not be the same. Further, stem cell cultures may have different purposes, e.g. basic research or production of cells for cell therapies. Hence, cell culture parameters need to be refined according to the final purpose of the stem cell culture. Studying stem cell biology at the single-cell may need the optimization of microfluidic chips, whereas production of therapeutic stem cells in large-scale bioreactors may require different optimal settings. Likewise, stem cell culture conditions optimized for improving dopaminergic differentiation for the treatment of Parkinson's disease will not be similar to those used to maintain stem cell self-renewal and pluripotency. Nevertheless, a number of general considerations emerge regarding the attributes of a stem cell culture.

Stem cell culture condition must allow the establishment and the maintenance of phenotypically well defined and karyotipycally stable cells. The condition must maintain self-renewal and pluri/multipotency potentials. Cell cultures conditions need to be standardized and use well defined matrices and media. A notable difficulty with stem cell cultures is that stem cells are highly plastic. Changing their state is an inherent part of their biology. In vivo, adult stem cells reside in well-defined locations named stem cell niches. The stem cell niche is a three dimensional informative structure directing adult stem cell self-renewal and differentiation. [Peerani et al., 2010; Voog et al., 2010]. It provides the balance between inhibiting signals required for stem cell quiescence, and proliferation/differentiation-promoting cues required for tissue renewal and injury repair. This homeostatic function of the stem cell niche must be viewed as a dynamic structure. Hence, one major challenge of stem cell culture is to identify and to reproduce or mimic *in vitro* some of the cues provided *in vivo* by the stem cell microenvironment.

Stem cells in culture.

Nowadays three types of stem cells are commonly expanded in culture. Embryonic stem cells (ES), adult-stem cells, and induced pluripotent stem cells (iPS). Embryonic stem cells are pluripotent stem cells derived from the inner cell mass of the blastocyst. Under appropriate conditions they are able to self-renew indefinitely although they may develop specific karyotipic abnormalities during passage in culture. Tissue-specific stem cells are multipotent cells found in differentiated tissues. In culture, these adult-stem cells may have limited self-renewal. This limited self-renewing potential of adult stem cells in culture, as well as the cytogenetic changes observed with highly passaged ES cells, suggests that current stem cell culture conditions need to be optimized. This point is of major concern since, as discussed

below, culture conditions can significantly influence stem cell fate. A third type of stem cells named induced pluripotent stem (iPS) cells is now also cultured. These cells are somatic cells which have been reprogrammed back to a pluripotent stem cell state by introducing few genes such as the SOKM (Sox2, Oct4, Klf4 and c-Myc) or SOLN (Sox2, Oct4, Lin28, Nanog) combinations. According to their various origins, it can be argued that defining an optimal stem cell culture condition common for all the cultured stem cell types is not achievable. However, the current challenges with stem cell culture conditions are roughly the same for all the stem cell types. *In vivo*, adult stem cells reside in a highly specialized three dimensional microenvironment called the stem cell niche. Reproducing this complex and dynamic micro-environment in culture is not possible, either for technical reasons or the mechanisms that govern the stem cell fate in vivo are not fully understood. *In vitro*, cultured cells are subjected to an environment whose main components are the medium, the atmosphere, the substrate and the cell-cell interactions. Each of these components participates to a complex network of signaling pathways culminating in the determination of the stem cell fate. This will be discussed in the following paragraphs. The time is over that cell culture conditions were empirically determined. Research on the effects of the medium, the atmosphere, the substrate and the cell-cell interactions is an actively growing field of investigation with tremendous applications both for our understanding of stem cell biology and regenerative medicine.

The medium: more than a feeder.

In the beginning of animal cell culture, one of the first technical challenges was to determine what the components of an optimal cell culture medium should be. Composition of culture media were determined empirically and selected on their ability to provide large quantities

of cells for biochemical investigations and for the large scale production of virus for vaccine fabrication. The fact that cell culture medium was also named “cell growth medium” illustrates this point. It rapidly becomes clear that culture medium influences cell fate and acts not only as a feeder but also as an instructor. This point is particularly relevant for stem cells in culture which always balance between self-renewal or cell differentiation. Therefore, devising fully defined media able to maintain stemness, or alternatively to drive differentiation towards well-defined phenotypes, is a point of major concern for stem cell culture. Because stem cells are diverse, a universal optimal stem cell culture medium does not exist, and distinct stem cell types may require different culture conditions. Human embryonic stem cells (hESC) were first cultured on a feeder layer of embryonic fibroblasts in medium containing serum. These cell culture conditions are rather ill-defined. Serum components are not fully characterized and there is a lot of variability between batches of serum. Likewise, the mechanisms by which fibroblastic feeder layer provides a microenvironment essential for stem cell maintenance are far to be fully characterized. Moreover, cell feeders and animal supplements are not suitable for safety concerns when stem cells are cultured for therapeutic purpose. Therefore optimization of stem cell cultures required the development of well defined synthetic media supplemented with recombinant growth factors or cytokines. Nowadays, most stem cell culture use defined serum-free media containing various additive or growth factors. For example, one of the major soluble factors added to culture medium for regulating stem cell self-renewal is bFGF. This growth factor supplements the medium used to culture undifferentiated hESC, iPS or neural stem cells. Growth factor requirement may be specie specific. LIF supports the expansion of mouse but not human ESCs. Other factors uses in stem cell cultures include for example members of the BMP family which can either synergize with LIF to support mouse ES self-renewal by

inducing Id1 through Smad activation [Ying et al., 2003], or promote hES differentiation [Xu et al., 2002]. Nodal, activin A and TGF-beta are also used to maintain hESC undifferentiated state by inhibiting BMP signaling [Rao et al., 2005]. The fact that specific molecules such as retinoic acid, ascorbic acid, hormones (glucocorticoids…), DNA demethylating agents (5-aza-cytidine), or intracellular cAMP elevating agents (IBMX) can be added to culture media to trigger stem cell differentiation towards well defined pathways [Ding et al., 2004] suggests that, conversely, novel molecules capable to expand stem cells in an undifferentiated state is probably a fruitful area of research. For example, Y-27632, a ROCK inhibitor, permits survival of dissociated hES [Watanabe et al., 2007]. Likewise, SC1, a small molecule acting by dual inhibition of RasGAP and ERK1, can maintain the self-renewal of mES in the absence of feeder cells and exogenous factors [Chen et al., 2006]. Also pharmacological inhibition of GSK3 signaling has been shown to maintain mouse and human ES pluripotency [Sato et al., 2004]. More importantly, a cocktail of three small-molecules inhibitors CHIR99021, SU5402 and PD 184352 targeting glycogen synthase kinase-3, FGF receptor tyrosine kinases and the ERK cascade respectively, enable the self-renewal of mES in combination with albumin, transferring and insulin [Ying et al., 2008]. This suggests that mES can replicate constitutively *in vitro* without growth factor or cytokine. This point is of paramount importance for stem cell culture. Further, autocrine or even paracrine loops exist in stem cell culture and considerably affect stem cell fate. Using small-molecules inhibitors is an attractive way to neutralize these unavoidable and intractable variations. Another critical point to consider is that cell culture medium is dynamic and rapidly changing due to the release or consumption of numerous metabolites. Hence, culture of stem cells in completely defined conditions is not easily achievable in the static medium commonly found in cell culture flasks or dishes. Continuous perfusion of the culture with fresh medium can be a solution [King et al., 2007].

In bioreactors, stem cells have been expanded in stirred vessels or on perfused scaffolds with pH and oxygen monitoring. This culture process has been shown beneficial both in term of stem cell expansion and differentiation potential compared to conventional static cell culture conditions [King et al., 2007]. However, at least two points need to be addressed. Stirred and perfused culture disrupt the autocrine or paracrine loops that may occur in static cultures, and they can generate hydrodynamic shear stress that needs to be carefully evaluated and controlled. The future of stem cell culture is highly dependent on the development of sensor technology for monitoring and controlling culture media parameters. Hopefully, stem cell culture can benefit of the considerable amount of work performed by bioengineers for monitoring complex biotechnological processes ranging from fermentation to the production of monoclonal antibodies. Biosensors, chemosensors and optical sensors are developed for the on-line monitoring of an increasingly number of parameters, such as pH, oxygen, glucose, lactate, ammonia, hypoxanthine, amino acids, dopamine, [Becker et al., 2007]. Coupling these sensors to a controller to maintain concentrations of critical metabolites and growth factors in an optimal range is surely required for adequate and reproducible stem cell culture conditions.

The atmosphere: climate change for stem cell culture, low oxygen tension is forecast.

Cells are usually passaged under a laminar flow hood and maintained in incubators which are under atmospheric partial oxygen pressure (pO2). These conditions are usually defined as "normoxic" by cell culturists. Atmospheric pO2 is around 150 mm Hg (21% O2), whereas in vivo the physiological pO2 ranges between 50 and 5 mm Hg (7%-0.7%). The equilibration of the culture medium with atmospheric pO2 challenges the cells to a pO2 far above the value found *in vivo*. In other words, in cell culture the term "normoxia" does not refer to a physiological standard but to a *cultural* norm. *In vivo*, the pericelluar pO2 value for a cell in a

given tissue depends on several parameters, such as: O2 diffusion, O2 consumption, and the distance to the nearest capillary. Notably pO2 experienced *in vivo* by blastocyst in the non-vascularized uterine fluid can be as low as 11 mm Hg (1.5% O2) in the monkey. This suggests that *in vivo* ES cells can experience very low oxygen concentrations. The pO2 found in adult stem cell niches is variable. For example, quiescent hematopoietic stem cells are found either in bone marrow niches with negligible blood perfusion or in proliferative vascular niches. However, pO2 experienced by stem cells are always below atmospheric pO2 and the beneficial effects of lowering the pO2 of culture media to more physiologically relevant pO2 has been repeatedly demonstrated for almost all stem cell types [for reviews see Csete 2005; Wion et al., 2009]. For example, differentiation of hES is markedly reduced under hypoxia [Ezashi et al., 2005]. Numerous studies also demonstrated a more efficient bone marrow mesenchymal stem cell expansion at 2% O2. Conversely, low oxygen tension (2% O2) reduces proliferation of mES in the presence of LIF [Fernandes et al., 2010]. This paradoxical observation can be explained by the fact that the hypoxia-inducible transcription factor HIF-1α inhibits the LIF-STAT3 pathway [Jeong et al., 2007]. This underlines again the combinatorial complexity of the interactions existing between cell culture parameters, and points the importance of precisely monitor the pericellular pO2 for any stem cell culture. Nowadays, all culture steps can be performed under a controlled pO2 gas phase in hypoxia workstations. This point is critical. Culturing cells under low oxygen but changing cell culture medium in a hood under atmospheric oxygen pressure must be avoided, as it submits cells to oxygen fluctuations. For example, shifting a cell culture medium from atmospheric pO2 (20% O2) to an incubator with a gas phase at 2% O2 is not sufficient to ensure an immediate corresponding pO2 levels at the bottom of the culture medium. Depending on the depth of the medium, equilibration of the medium at the bottom of the plate with the gas phase can

take several hours if the medium is not stirred [Westfall et al., 2008; Fernandes et al., 2010]. This point is critical if we consider that the half-life of a critical oxygen-regulated transcription factor such as HIF-1α can be less than 15 minutes at 20% O2. Moreover, in non-perfused or non stirred cell cultures, the pericellular pO2 may differ considerably from the value monitored in the gas phase or in the medium depending on the cell density and the cell-type specific rate of O2 consumption [Pettersen et al., 2005]. Only pO2 measurement at the cellular level is relevant, as confluent cell cultures may experience a pericellular hypoxia even when they are cultured under atmospheric pO2 [Pettersen et al., 2005]. In this regard it can be suggested that one of the functions of the cell feeder layers used to expand some stem cell cultures is also to ensure a low pO2 micro-environment. The problem of measuring and maintaining a constant well-defined pericellular pO2 can be achieved by perfusing cell monolayers. However, when stem cells are cultured as spheroids it is virtually impossible to impose a uniform and controlled pericellular pO2 to all the cells. Notwithstanding these limitations, the cultivation of stem cells at a controlled dissolved oxygen partial pressure lower than the atmospheric should be the norm.

Intracellular sensing. The two-photon microscopy approach.

A major challenge in cell culture is the control and the maintenance of well-defined cell culture conditions. This requires the on-line monitoring of critical parameters such as pH and pO2. These parameters are currently measured in the culture medium. This approach has several severe limitations since the value obtained is not always representative of the value experienced by cells. As previously discussed, the pO2 measured in a cell culture medium may not reflect the pericellular pO2 experienced by cells in spheroids or when cultures are confluent. Hence, the development of non-invasive methods capable to measure critical

parameters in 3D structures at the cellular level is necessary. A promising approach for intracellular sensing is the use of fluorophores. These can be incorporated into the cytoplasm without changing cell functions. Alternatively, intrinsic cellular fluorophores like NAD(P)H, flavoproteins and lipofusin may be used to monitor non-invasively changes in the cell redox state as well as oxidative stress under different culture conditions [Rice et al., 2010]. In this regard, monitoring the redox status of stem cell is highly relevant since redox regulation mediates embryonic stem cell fate [Yanes et al., 2010]. The fluorescence signals of these endogenous fluorophores, however, are weak and often high (cell toxic) laser power is required for proper detection. Moreover, they are indirectly related to the oxygen tension. Nevertheless, both strategies combining both exogenous and endogenous fluorophores, should be explored to validate 'pO_2 sensing' under controlled stem cell culture conditions. This approach could benefit of recent development of new cell imaging techniques like two-photon microscopy (2PM) [Helmchen et al., 2005]. Two-photon has improved the imaging depth by approximately a factor 8 in comparison to confocal fluorescence microscopy. This point is critical for imaging cells in 3D environments such as spheroids or cells embedded in bioengineered scaffolds. 2PM uses a spatial and temporal compression (pulsed laser) of (near) infrared photons in the focal plane of the objective for two-photon excitation. This decreases out of focus photodamage and facilitates deep optical sectioning or multiplane acquisition in living 3D cell cultures tissues. The imaging depth of a confocal fluorescence microscope can be subsequently increased using a bundle of optical fibers [Snedeker et al., 2009]. Fiber optic based 2PM is less obvious, but new optical fiber technologies might enable this in the nearest future. More flexible imaging configuration will be available soon for deep intracellular pO_2 sensing in tissues and bioreactors. Note that existing optical fibre technologies for pO_2 measurements confine pO_2 sensitive fluorophores at the tip of the

fibre, thus the fluorophores are not in the cells [Wen et al., 2008]. Most common fluorophores for intracellular pO_2 sensing using one-photon excitation techniques are ruthenium (fluorescence quenching) [Sud et al., 2009] or palladium-porphyrin complexes (phosphorescence quenching) [Dunphy et al., 2002]. Ruthenium complexes can passively diffuse in to the cell [Puckett et al., 2008], whereas Pd-porphyrin complexes need vehicles like pluronic micelles for transmembrane transport. Fluorophores for pO_2 sensing using two-photon (non-linear) microscopy are not commercially available, but are currently in development. Previous complexes may be chemically modified into two-photon-enhanced oxygen sensors [Lebedev et al., 2008]. The fluorescence or phosphorence life time of these sensors is known to decrease at the presence of O_2. The oxygen dependence of the fluorophore lifetime (τ) can be described by the Stern–Volmer relationship [Dunphy et al., 2002]: $\tau 0/\tau = 1 + kq \cdot \tau 0 \cdot [O_2]$, where τ_0 is the fluorophore lifetime in the absence of oxygen and is decided by the characters of photosensitizer, k_q is the fluorescence quenching constant. Fluorescence (and phosphorence) life time imaging is compatible with conventional laser scanning imaging techniques after reducing the laser pulse frequency. Thus, design and optimization of optical imaging techniques and adequate fluorophores for intracellular sensing of pO_2 or other relevant physiological parameters in 3D stem cell culture is undoubtedly of great promise in optimizing stem cell culture conditions.

Cell-matrix associated signals: every soil does not bear the same fruit.

In vivo, cell-matrix and cell-cell interactions play critical roles in controlling stem cell apoptosis, quiescence, self-renewal, and differentiation [Czyz et al., 2001; Discher et al., 2009; Reilly et al., 2010]. One of the most investigated process by which cell matrix determine stem cell fate is probably the integrin signaling pathway. Integrins is a large family

of transmembrane adhesion proteins which interact with ECM proteins such as collagen, fibronectin, vitronectin and laminin. Integrins behave like classical signaling receptors by transmitting information into cells by “outside-in” signaling [Shattil et al., 2010]. In addition, intracellular signals can also activate integrin binding to ECM through “inside-out” signaling [Shattil et al., 2010]. Interactions of integrins with their cognate ECM ligands lead to intracellular signals controlling critical cell functions such as polarity, migration, gene expression, survival, differentiation and proliferation. This explains the importance of cell-matrix interactions in stem cell culture. For example, Matrigel, a basement membrane containing laminin and collagen plays a critical role in the long term maintenance of pluripotent hESC. However, Matrigel is of animal origin, it contains growth factors and its contents are not rigorously defined and subjected to batch-to-batch variability. Replacing the ECM provided by feeder cells or Matrigel with rigorously defined molecules capable to provide cues for stem cell self-renewal or differentiation is a major concern for stem cell culture optimization. Fibronectin or laminin have been widely used as coating substrate, and human recombinant laminin-511 is a valuable option for the long-term self-renewal of hES in a xeno-free and feeder-free system [Rodin et al., 2010]. Likewise, natural 3D porous scaffolds made for example from chitosan and alginate complex can support feeder-free self-renewal of hES [Li et al., 2010]. On the other hand, an increasingly number of synthetic surfaces for long-term stem cell self-renewal or differentiation are currently developed [see for examples Melkoumian et al., 2010; Villa-Diaz et al., 2010]. The future is in synthetic matrix presenting at their surface immobilized signaling proteins [Alberti et al., 2008]. For example, biologically active peptides derived from active domains of ECM have been recently successfully coupled with acrylate surfaces to support the self-renewal and differentiation of hES [Alberti et al., 2008; Pompe et al., 2010; Melkoumian et al., 2010].

Presentation of growth factors to their corresponding cell receptors is another critical function of ECM in vivo. Recent advances in stem cell culture conditions include the design of matrix where growth factors such as bFGF, SCF, LIF, VEGF are immobilized in an active conformation [Alberti et al., 2008; Dellatore et al., 2008; Pompe et al., 2010]. Nevertheless it is important to have in mind that cells in culture synthesize their own ECM. Thus ECM in cell culture is dynamic, and self-synthesized ECM can considerably modify the initial properties of the synthetic substrate used to culture cells, especially at later time points. Stem cell fate is also influenced by ECM physical characteristics such as elasticity and stiffness, and geometry [Discher et al., 2009; Reilly et al., 2010; Peerani et al., 2010]. These findings are not unexpected. In vivo, ECM elasticity is tissue specific and developmentally regulated [Reilly et al., 2010], and cell shape and cytoskeletal tension are involved in the determination of stem cell fate [McBeath et al., 2004]. Force and geometrical sensing are transduced in biochemical signals by mechanotransduction systems that in turns activate mechanoresponsive pathways [Vogel et al., 2006; Discher et al., 2009]. The importance of elasticity-directed transduction pathways is now taken into account and elasticity and stiffness are critical parameters integrated in the design of synthetic cell culture surfaces [Discher et al., 2009; von den Mark et al., 2010; Yim et al., 2010]. Likewise, geometrical cues capable to induce stem cell differentiation in the absence of other differentiation-inducing agents are now considered and can be provided by cell culture surface through nano-structures with controlled dimensions and alignment [Lee et al., 2010]. This nanotopographic approach is a promising tool for designing “cell-instructive” substrate capable to control stem cell self-renewal and differentiation (see below). By combining all these different parameters it will soon be possible to design the nanoscale topography of bio-informative substrates capable to control stem cell fate and elicit standardized specific cell response.

Micro-Nano-technology: moving the two-dimensional Petri dish to a tridimensional bioreactor mimicking the stem cell niche.

Micro-Nanotechnologies, through the integration of microfluidic, multifunctional devices and nano-materials, open new opportunities to move the old cell culture dish to an integrated bioreactor, mimicking more closely the human body complexity. One development has been to mimic the in vivo vasculature implementing fluid flow and cell perfusion cell culture devices using microfluidic systems [Gomez-Sjoberg et al., 2007; van Noort et al., 2009]. Such devices have been termed "Lab-on-a Chip", and integrate reaction chambers, sensors, and fluid control on one chip. Lab-on-a-chips are powerful tools to control the soluble and mechanical parameters of the cell culture environment. Miniaturization of cell culture platforms allows cell culture to be monitored in real-time to observe cellular behavior at the scale found in living systems with high-resolution imaging modalities. In microperfusion systems, the effect of shear stress on cell growth, migration, and differentiation could be studied by applying different flow rates, such as maintaining a constant soluble microenvironment and having a large surface area-to-volume ratio which is found in bio- logical systems. Cells have micrometer dimensions, but they evolve in vivo in close contact with the extra- cellular matrix (ECM), which size is in the nanometer range. Nano-technologies provide the possibility to produce surfaces, structures and materials with nanoscale features mimicking microenvironment of cells, modulating cell adhesion, cell mobility and cell differentiation. Cell response is affected by the physicochemical parameters of the biomaterial surface, such as surface energy, surface charges or chemical composition. Topography is one of the most crucial physical cues for cells. Microtopography and nanotopography can modulate cell behavior including adhesion, proliferation and

differentiation. Several methods for topographical and chemical surface modification have been developed including polymer demixing, chemical etching and colloidal lithography as well as soft-lithography to obtain organized pattern and regular geometries. Nanostructured surfaces influence the organization of integrins in the cellular membrane, and the concomitant activation of intracellular signaling cascades and guidance of stem cell behavior [Yim et al., 2010]. All these techniques can be associated to different nanomaterials. Nanostructures are of particular interest because they have the advantageous feature of a high surface-to-volume ratio, their suitability for high-density functionalization, their high diffusive capacities and unconventional mechanical properties. Nanoscale-engineered substrates and scaffolds have been designed to create biomimetic cellular environments [Lutolf et al., 2005; ; Ferreira et al., 2008; Discher et al., 2009; Lee et al., 2010]. Stem cells cultured on nanofiber scaffolds exhibit different morphologies, viabilities, and migrations from cells cultured on conventional substrates [Silva et al., 2004; Gelain, 2008; Feirreira et al., 2008]. Similarly, carbon nanotubes have demonstrating a strong impact on cell adhesion and differentiation depending of the diameters of the fiber [Ferreira et al., 2008]. Nanocale-engenireed substrates offer great potential for stem cell applications, but systematic studies are needed to define the best design modulating conformation, surface chemistry, conductive properties, length and diameter of the nanomaterials. Clearly, nanostructured synthetic matrices look to be the next generation scaffolds, opening powerful tools for a more relevant in vitro micro-environment reconstitution. The global perspective is the implementation of a multifunctional cell culture bioreactor, including perfusion system, biosensor and nanofiber scaffolds mimicking perfectly the different microenvironment modulating stem cell function.

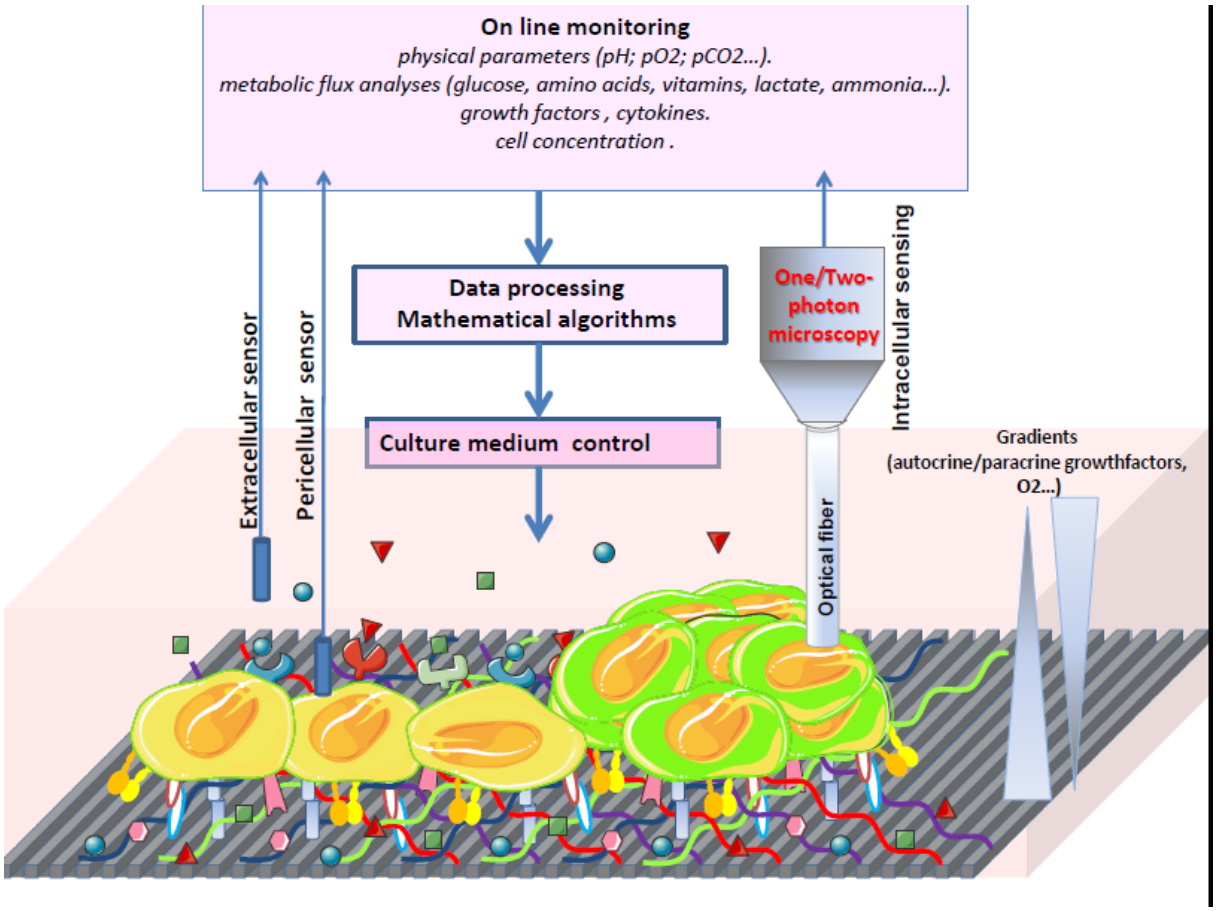
Concluding remarks.

For many years it has been assumed that mammalian cells could be grown in vitro in Petri dish or flask just as bacterial cells do, as long as temperature and adequate growth medium were provided. This situation progressively evolved when it appears that culture condition dramatically modifies the cell biology of cultured cells. Cell culture conditions do not act only to support cell growth but are now considered as "instructive". Optimization of stem cell cultures already benefit of the development of non invasive accurate sensors for the on-line recording of critical parameters such as pH, pO2, and metabolites [Kirouac et al., 2008]. Sensing the variations of critical molecules, enzyme activity and physical parameters (pH, pO2) at the extracellular and intracellular levels is achievable with nanoparticules [Ferreira L 2009] and bi-photon analysis. Mathematical algorithms are also needed for anticipating culture variations and ensuring medium homeostasis. Undoubtedly important breakthroughs are expected from the development of bioinformative materials [Lutolf et al., 2005]. Fully defined synthetic surfaces for culture vessels do not only provide a physical support but also biological cues for cell growth and differentiation. Consequently, nanotopography is becoming one of the most exciting fields of investigation for cell culturist. The future is probably in the development of 3D porous modular extracellular matrices in which biomimetic materials will be assembled according to the final purpose of the stem cell culture, e.g. stem cell expansion or control of cell differentiation towards clinically relevant cell phenotypes. Importantly, most of the technological breakthroughs issued from stem cell culture engineering, such as the design of nanosensors or of bioinformative substrate capable to influence cell fate, have direct clinical applications independently of their use in stem cell therapies. For example, self assembly peptide nanofiber scaffold can be delivered to living tissues [Silva et al., 2004; Feirreira et al., 2008] and has interests in the therapy of

degenerative or proliferative diseases by their own. On the other hand, advances in the technology of accurate nanosensors and calibration algorithms currently developed for monitoring stem cell culture parameters will generate devices capable of a real-time monitoring of an increasing number of parameters in human. Just like implantable sensors are capable of monitoring tissue glucose concentration by wireless telemetry, these systems will form the basic platform for future generations of products allowing the real-time monitoring of critical biological parameters in patients as well as in asymptomatic individuals, a major goal for prevention. Thus, the outgrowth of bioengineering research in its quest to optimize stem cell culture is likely to have much broader clinical repercussions going well beyond stem cell therapies.

Acknowledgments.

We apologize to those colleagues whose work could not be referenced due to space constraints. Work in the laboratory is supported by the Ligue contre le Cancer.

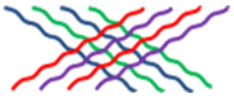


Legend to Figure

Optimizing stem cell culture environment.

Culture medium composition in non-perfused culture is continually changing as a consequence of cell metabolism. These changes may not be entirely measurable or understood. In non-stirred/non-perfused cultures, metabolites gradients can occur. Consequently, extracellular or pericellular values may differ. For example oxygen partial-pressure may be lower at the pericellular level than in the bulk of the medium. Conversely, concentrations of autocrine growth factors may be higher at the pericellular level than in the bulk of the medium. Sensors (chemo- and fluorescent sensors, spectroscopic analysis, in situ microscopy, affinity sensors….) monitor various analytes in cell culture at extracellular, pericellular or intracellular levels. Data processing and mathematical algorithms predict the evolution of cell culture and ensure the feed back control of medium composition. Another

major contributor to stem cell fate in culture is cell culture substrate through its physical properties, structure and geometry. These properties can be modulated by immobilizing ligands such as extracellular matrix proteins or growth factors.

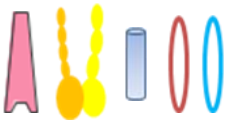


extracellular matrix

Nanosacle engineered bioinformative surface with immobilized ligands

Growth factors, cytokines.

Growtn factors receptors .



Transmembrane proteins (FGF-R, Integrins; Notch….)

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