



## Diabetes and Stem Cells – A 10-year Perspective

a report by

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We are approaching the 10th anniversary of the isolation of human embryonic stem (huES) cells,<sup>1</sup> a seminal breakthrough that promptly germinated into one of the most prolific fields in recent scientific history. Concepts such as 'regenerative medicine' or 'stem cell therapies', so commonly used today, did not start to appear in the scientific literature until the late 1990s. Although stem cell transplantation had been in clinical use for several decades for blood-related disorders, the notion that totally plastic, indefinitely expandable cells could be used as building blocks for the *in vitro* regeneration of any tissue was nothing less than revolutionary. Until then, and despite reports of ES cells obtained from many species,<sup>2-4</sup> it is as though we had not envisaged applications for these singular cells other than to create animal models for human diseases, increase livestock output or improve the production of therapeutic proteins from transgenic animals. The feeling of unexpectedness that saluted the birth of this new field is reflected in the fact that 1998 marked the starting point not only of huES cell research, but also of a sudden interest in adult stem cells as an alternative source of tissues. After all, the procurement of ES cells from human and non-human primates had been hampered by technical difficulties up to that point, but the technology necessary to expand most adult stem cells was already in use a decade ago. Why had we not been pursuing the idea of using adult stem cells for medical purposes until Thomson and colleagues came up with the first embryonic stem cell lines? Be that as it may, a new field was born as the result of the confluence of disciplines as diverse as embryology, immunology, cell biology and transplantation surgery.

Ten years later, the promise of this new field is evidenced by the use of several types of adult stem cells in clinical trials for a variety of conditions, including Crohn's disease,<sup>5</sup> myocardial infarction<sup>6</sup> and graft-versus-host disease.<sup>7,8</sup> New applications of autologous bone marrow transplantation are currently being developed either to tackle autoimmunity<sup>9-11</sup> or to induce regeneration in diseases such as diabetes.<sup>12</sup> Since they are still experimental, it is too early to determine whether or not these therapies will eventually change the state of the art in treating these conditions. Also (in what represents a reversion of the usual 'bench to bedside' directionality), once these therapies prove safe and effective, we must investigate the mechanisms behind the potential action of the transplanted cells. Do they work by differentiating into the types of cells that were damaged, or – as suggested by preliminary evidence – merely by flooding the damaged tissues with trophic signals that aid in self-regeneration?

We can afford to answer these questions after the trials because, in the context of their proposed applications, most adult stem cells are relatively safe. This course of action is not possible with huES cells, and this is the only reason why they seem to lag behind their adult counterparts in terms of clinical applicability. The very same property that makes huES cells superior to other stem cells (i.e. their ability to be expanded indefinitely) is also a cause of concern because of the risk that some non-differentiated escapees

may give rise to teratomas *in vivo*. Some groups have approached this problem by screening the number of undifferentiated cells present in each transplantable preparation. Their reasoning is that if this number is below the threshold known to produce tumours in immunodeficient mice, these preparations should be considered safe for clinical use.<sup>13</sup> This method, however, is not foolproof. First, not even the best cell-sorting techniques can ensure a 100% depletion of a rare subset of cells in a population. Second, the above threshold has been determined empirically. In theory, even a single non-differentiated cell could potentially develop into a tumour. Finally, it does not take into account the risk of de-differentiation after transplantation.<sup>14</sup> This is why other groups have addressed this problem by integrating 'suicide genes' into ES cells. These elements sensitise ES cells to specific pro-drugs, which can be either added to the culture medium *in vitro* or administered to the recipient *in vivo*. The proteins encoded by these exogenous genes will react with the pro-drug and convert it into a toxic compound, which will subsequently kill the cell.<sup>15-19</sup> A drawback of this strategy is that if teratomas were to form *in vivo* due to de-differentiation of implanted cells, the use of the pro-drug would result in the destruction of the entire graft (see *Figure 1*). At any rate, the escape of undifferentiated cells is only one of the ways in which an embryonic stem cell may become teratogenic. Less attention has been paid to a much subtler risk: the accumulation of genomic instabilities as a result of long-term culture. Initial reports about the karyotypic stability of huES cells<sup>1,20-22</sup> have recently been revisited in view of the observation that the adaptation of these cells to prolonged *in vitro* culture does indeed favour the development of chromosomal aberrations.<sup>23</sup> The unequivocal similitude between *in vitro* proliferative adaptation and malignant transformation<sup>24</sup> warrants additional studies to assess the overall safety of huES cell-based therapies.

In view of the above, it is understandable that clinical trials with huES cells have been approached with much more caution than those based on the use of adult stem cells. If everything proceeds according to schedule, these first trials will take place before the end of 2007, and will look at the efficacy

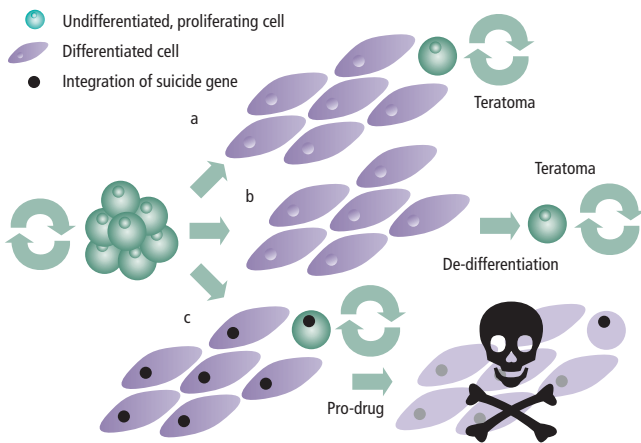


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**Figure 1: Undifferentiated Embryonic Stem Cells (left) Are Characterised by their Ability to Proliferate Indefinitely Under the Appropriate Conditions**



Upon differentiation and transplantation, a few undifferentiated escapes may retain their proliferative potential, causing a teratoma (a). These tumours may also be formed upon de-differentiation of differentiated cells (b). Strategies aimed at increasing the safety of ES cell-based therapies include the use of suicide genes, which bring about the self-destruction of the cell in the presence of a pro-drug (c). New lines of research focus on ways to activate these suicide elements only in the cells that remain undifferentiated, rather than in the entire graft.

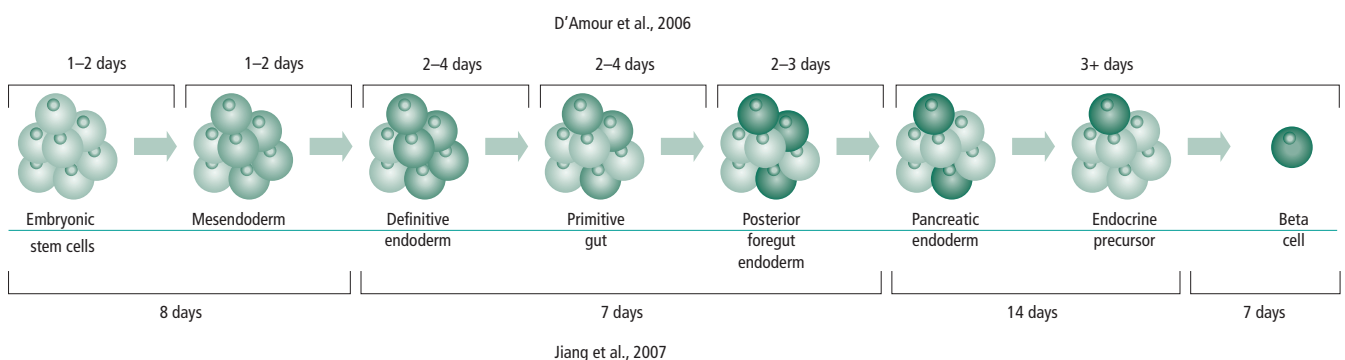
of huES-cell-derived oligodendrocytes to treat acute spinal cord injuries. A positive result will definitely help to soften the opposition of a still significant sector of the population to huES cell research. By the same token, a negative outcome would be likely to set the entire field back, perhaps irreversibly. Social pressure to develop cures, fuelled by some unrealistic promises about the time-frame and scope of these treatments, should not stand in the way of their cautious planning and implementation. The lessons that we have learned from failed gene therapy trials must remain fresh in our memory.

Despite obvious advances in this field, the experimental challenges remain the same as they were a decade ago. The most important one is the inability to mimic *in vitro* the intricate biochemical regulation of *in vivo* development. Insulin-producing  $\beta$  cells, which are the main subject of our review, are a perfect example of these limitations. These cells are destroyed by the immune system in type 1 diabetes, and therefore are prime candidates for cell replacement therapies. As islet transplantation from deceased donors was demonstrated to be safe and efficacious,<sup>25,26</sup> proof of principle was established that huES-cell-derived  $\beta$  cells could be used to effectively treat the disease. Decades of progress in the identification of the main molecular determinants of pancreatic specification gave shape to the idea that this process could be reproduced *in vitro* by simply providing huES cells with the appropriate combination of

extracellular signals. This has proved to be much more difficult than anticipated. Throughout pancreatic development, cells respond differentially to extracellular cues depending on their precise location, their interaction with surrounding tissues and time. Fine gradients of Nodal (for endoderm/gut endothelium specification), FGF and Shh (for pancreatic differentiation) and direct cell-to-cell interactions in the Notch pathway (for endocrine specification) are examples of the complex differentiation mechanisms that we are only now beginning to understand. The biochemical environment is just one of the levels of complexity with which *in vitro* differentiation protocols have to deal. Increasing lines of evidence point to physical variables as important determinants of development/cell specification. Among these, some of the most studied are mechanical forces,<sup>27,28</sup> pH and bioelectrical fields,<sup>2,9-31</sup> oxygenation levels<sup>32-35</sup> and the nature of the substrate/mode of culture.<sup>36-39</sup> The metabolic activity of the adult islet is highly dependent on the complex network of blood vessels that pervades it. In fact, although islets account for only 1–2% of the total number of cells of the pancreas, they use 25% of the pancreatic O<sub>2</sub> supply.<sup>40,41</sup> When removed from their *in vivo* environment, the islet microvascular network is destroyed and viability decreases dramatically.<sup>42</sup> In short, standard culture practice is not favourable for long-term islet survival and function.<sup>42</sup> How can we generate islets from stem cells if the physical conditions for islets to survive in the first place have not yet been optimised? The very same limitations that result in  $\beta$ -cell death *in vitro* may also prevent their efficient differentiation from immature progenitors.

In summary, 10 years of research has led us to the humbling realisation that mimicking pancreatic development *in vitro* is a much more formidable enterprise than previously thought. Today, even the best protocols for directed huES cell differentiation yield only a small percentage of  $\beta$  cells,<sup>43</sup> which in some cases are not even glucose-responsive<sup>44</sup> (see Figure 2). New trends to address this problem illustrate the need for interdisciplinary approaches in order to succeed at translating basic research into clinical therapies. One example is the cross-pollination between biology and physical sciences, which has led to the development of an entirely new discipline called tissue engineering. The notion that physical and molecular microenvironments are equally important in the evolution of pancreatic development is still a relatively new one, at least in the context of *in vitro* differentiation. We have shown, for instance, that molecular oxygen is a critical determinant of  $\beta$ -cell differentiation.<sup>45</sup> The design of novel culture devices to improve oxygen delivery (thus enhancing differentiation of stem cells into insulin-producing cells) is one example of the fruitful cross-pollination between molecular biology and biophysical disciplines (see Figure 3). As the molecular environment is only one part of the equation of islet differentiation, replacement is just the most visible facet of any future cure

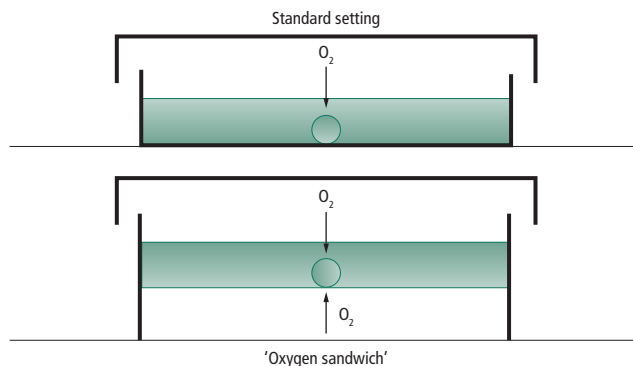
**Figure 2: Two of the Most Advanced Protocols for the Differentiation of huES Cells Are the Ones Described by D’Amour et al.<sup>44</sup> and Jiang et al.<sup>43</sup> Both Methods Aim at Recapitulating the Sequence of Developmental Milestones Seen During *In Vivo* Pancreatic Specification**



for type 1 diabetes. There is widespread consensus that the re-education of the immune system must be an essential component of any therapeutic approach. Even in a best-case scenario where the exogenously provided  $\beta$  cells were autologous (through therapeutic cloning or from adult stem cells obtained from the patient), this approach would be insufficient to prevent the recurrence of autoimmunity. In fact, there is evidence suggesting that the body attempts to regenerate its  $\beta$ -cell mass for decades after the diagnosis of the disease, but auto-reactive processes keep targeting these new cells as they appear.<sup>46</sup> On the other hand, immunological interventions alone might not be sufficient to completely restore  $\beta$ -cell function. A recent study designed to 'reset the clock' of the immune system to a point prior to the onset of the disease was partially successful in recently diagnosed patients, but did not work in individuals with long-standing diabetes.<sup>11</sup> This would be consistent with the hypothesis that the body cannot regenerate a functional  $\beta$ -cell mass after a threshold of destruction, a point of no return beyond which re-education of the immune system would have to be supplemented with a boost of exogenous  $\beta$  cells.

Despite what might be perceived as a slow pace in translating basic findings into effective therapies for type 1 diabetes, the last decade has been enormously productive in terms of framing the problem and shaping the overall direction of the field. Indeed, progress along this line of research has been steadfast, and the current state of the art suggests that huES cell-based trials, perhaps combined with immunological therapies, might be

**Figure 3: The 'Oxygen Sandwich' Principle<sup>45</sup>**



Based on the *in vitro* basallapical delivery of atmospheric oxygen to tissues (bottom), as opposed to the conventional culture system where air can reach the cells only by diffusion through the culture medium (top).

around the corner. As type 1 diabetes is a complex disease, it is reasonable to expect that a cure will come only from a multidisciplinary effort, which will almost certainly include a strong stem cell component. ■

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