Stem Cell Discovery, 2016, 6, 39-48
Published Online January 2016 in SciRes. http://www.scirp.org/journal/scd
http://dx.doi.org/10.4236/scd.2016.61004

Stem Cells: Daddy or Chips?

—An Up-to-Date Review on Ground-Breaking Discoveries in Stem Cell Research, with Special Attention to iPSC Applications in Osteoarthritis

Pierre Luc Pace[1], Renald Blundell[2]

[1]Faculty of Medicine and Surgery, University of Malta, Msida, Malta
[2]Department of Physiology and Biochemistry, University of Malta, Msida, Malta
Email: pierre.pace.12@um.edu.mt

Received 16 September 2015; accepted 18 January 2016; published 21 January 2016

Abstract

"***Stem Cells is what stem cells does***"

not Forrest Gump

In the present day Stem Cells are increasingly becoming popularized as the potential "ultimate" cure for the most challenging maladies… the "Daddy of medical intervention". Forefront SC research on human induced pluripotent stem cells (iPSCs) and other sub-disciplines, is quickly revolutionizing healthcare towards "Regenerative Medicine", as beautifully exemplified by the use of iPSCs in treating and possibly curing osteoarthritis, discussed at the end of this publication. This review documents and reflects on the most topical discoveries in SC research, and the challenges researchers in this field nowadays face. Major Findings: 1) In 2006 Yamanaka *et al.* generated the first iPSCs from *mouse* fibroblasts, using retroviral transmission of c-Myc, Oct3/4, Klf4 and SOX2 transcription factors. Later, they successfully generated iPSCs from *human* fibroblasts (2007). 2) Contemporary cultivation methods carry high risks of iPSC genome disruption, possibly leading to tumorigenesis, teratoma formation and reducing iPSC induction efficacy. 3) Many studies on preserving genome integrity and decreasing malignancy in iPSCs, suggest using valiproic acid and protecting tumour suppressor genes. 4) In many *malignant* tumours only a small minority of cells, called Cancer Stem Cells, metastasise and hyper-proliferate. 5) Not all mature cell sources yield the same [undifferentiated iPSCs: lineage-committed] ratio as others. Feb 2014: Obokata *et al.* claimed to have generated iPSCs by exposing mature cells to a 25 min, pH 5.7 bath. These iPSCs were termed "Stimulus-triggered Acquisition Pluripotency Cells" (STAP). However by July 2014 this study had been revoked, as the results could not be replicated. Conclusion: Stem cells have enormous potential to offer, especially iPSCs. Although currently not a viable treatment option on their own, for many daunting diseases they will definitely be at the core of multi-disciplined therapies within the near-future, including multi-factorial diseases like osteoarthritis.

How to cite this paper: Pace, P.L. and Blundell, R. (2016) Stem Cells: Daddy or Chips? *Stem Cell Discovery*, **6**, 39-48.
http://dx.doi.org/10.4236/scd.2016.61004

Keywords

Cancer Stem Cell, iPSC, Yamanaka, STAP, Arthritis

1. Introduction: What Are Stem Cells?

Stem cells are simply, complicated little things. In the last few years they have been causing many a hullabaloo and a to-do, than ever before. And rightly so.

Recent breakthroughs in stem cell technology have led to a better understanding of how they proliferate, grow, and then differentiate into functional tissues and organs. Yet more astonishingly is how much we've learned regarding how these natural, physiological processes can be conditioned to do the reverse, *i.e.*: to go from a highly specialized cell to a pluripotent stem cell.

Stem cells are every doctor's, and hence every patient's, hope for a far better future of standard care. This is because stem cell technology holds a possible cure for the most daunting and most heart-breaking of diseases, including cancer, auto-immune disease, neurological disorder, diabetes, cardiovascular disease and arthritis, to mention a mere few.

Stem cells differ from other cell types, being defined as:

"Clonogenic, self-renewing progenitor cells that have the ability to divide for an indefinite period and can give rise to one or more differentiated cell types" [1].

The uniqueness behind stem cells lies in **two** main capacities [2] [3]:

1) The capacity to **self-renewal**, *i.e.* proliferating into a line of cells which retain the same degree of potency as the original mother cell.

2) To give rise to a progeny with a more **specialized** function, where potency decreases from one generation to the next, as differentiation increases.

As the progeny from a single stem cell line grows and progresses from one generation to the next via mitosis, the cells of each generation become even more committed to a particular cell line of specialized cells, at the expense of becoming less able to differentiate into other cell types. These "transit amplifier cells" present in the intermediate stages, between the undifferentiated parent cells and the end-line specialized progeny, also exhibit a decreasing ability for self-renewal together with the increasing possibility for differentiation [4].

Selecting the specialty of the mature, end-lineage daughter cell basically depends on the *potency* of the original 1st generation stem cell, as well as which specific chemicals (growth factors, cytokines, hormones etc.) the cell line was exposed to during its journey to differentiation. Moreover some chemical components have to be present only at certain specific times, in order to contribute to specialization of a progeny [3].

This is brilliantly demonstrated by the near-magical complexity responsible for the precise interplay between countless growth factors and cytokines, all necessary for the growth and development of a foetus. And it all starts off from just a single cell. It must be noted that under different ambient conditions, which include culture medium, ECM composition and temperature, various cell types can be derived from each kind of stem cell both *in vitro* and *in vivo* [1].

1.1. Stem Cell Classification

Stem cells can be classified by **Origin**, thus belonging to one of the 3 classical groups:

1) **Embryonic Stem cells (ESCs)**, derived from the embryonic cell pool at any stage of development during gestation.

2) **Umbilical stem cells (USCs)**, sampled from cord blood

3) **Adult stem cells (ASCs)**, or more correctly *mature* stem cells, found in all post-natal humans

Now, recent paradigms are pushing on with the addition of two other, newly-discoveredclasses of origin, namely **Cancer stem cells** and "reprogrammed" adult stem cells or **Induced Pluripotent Stem Cells (iPSCs)**, which shall be discussed further ahead [1].

Stem cells are also classified by their **Degree of Potency**, determined by the number of diverse cell types a single parent cell can potentially give rise to. Different classes of stem cells possess different degrees of this so-called "developmental plasticity" [1]. The highest level of potency lies with the fertilized ovum (zygote),

which is thus called **totipotent**, since not only is iTable to give rise to *any* type of cell, but can develop into a separate, individual, multicellular organism: a foetus. Totipotency also belongs to early embryonic blastomeres, but potency decreases rapidly with each generation of cells. In fact between day 3, when the morula forms, and day 14 (in humans) the blastomeres are termed **pluripotent**. Pluripotent SCs, like totipotent ESCs, can still produce specialized cells from any one of the 3 embryonic germ layers: endoderm, mesoderm and ectoderm.

However a single pluripotent cell on its own cannot give rise to an individual, multi-cellular foetus. Such a wonderful capacity, as head-scratching in origin as the legendary "life spark" that blazed into all life on Earth, *only* belongs to *totipotent* ESCs. Post-morular ESCs and USCs are examples of similarly powerful, pluripotent SCs [1].

Totipotent and many pluripotent cells are also considered biologically immortal, capable of proliferating endlessly if nourished constantly, where the capacity to self-renewal diminishes little. This immortality is thanks to the high activity of telomerase and DNA repair mechanisms which keep the SCs' karyotype healthy and fully intact, throughout their actively reproductive life span [1].

After day 14 many embryonic cells are termed **multipotent**. They have now lost much of the capacity to self-renewal and only differentiate into a limited number of cell lines. For example, Hematopoietic stem cells (HSCs) mainly generate white blood cells, erythrocytes and platelets. Embryonic mesenchymal stem cells (MSCs) give rise to muscle, bone and ligamentous tissue. Although both HSCs and MSCs do quite often demonstrate pluripotency. HSCs and MSCs are present both in embryos and post-natal humans, and hence are termed Adult/mature/somatic Stem cells (ASCs) [1] [5].

ASCs also include the pluripotent-to-multipotent stem cells in the Gastro intestinal mucosal epithelium, skin epidermis, liver stem cells etc. [5].

Other stem cells include cancer stem cells (CSCs), which are the cause of malignant tumorigenesis. It has very recently been reported in numerous studies, that the "majority of cells within a population of cancer cells are non-replicating *i.e.* non-malignant" [1] [6]. It is only the small minority of CSCs that are actively tumorigenic and can colonize a secondary site, hence resulting in the metastatic spread of malignant cancers from one tissue to another.

1.2. The Biochemistry of Stem Cells

Apart from their highly reproductive nature and high telomerase activity, exhibiting varying degrees of potency, stem cells often require various cytokines and growth factors, as well as other specific conditions to promote proliferation of one cell line and not the other [7].

For example, progenitor HSCs give rise to various cell lineages in the bone marrow. However in order to specifically produce erythrocytes (RBCs) as opposed to white blood cells or platelets, HSCs must undergo 3 phases: **1) non-specific proliferation; 2) lineage specific proliferation; 3) maturation**. All 3 processes require interactions with various chemicals [7]:

1) Non-specific proliferation: (see **Figure 1**) HSCs normally remain "dormant" within the G_o phase of the cell cycle. To initiate proliferation, Stem Cell factor is required to (SCF) bind its specific SCF receptor (tyrosine kinase receptor) on the HSC. This ligand-receptor complex undergoes autophosphorylation.

This autophosphorylation then activates the Grb2/Sos adaptor complex and the PI3-kinase pathway, to stimulate the Ras/MAPk pathway. MAPk ultimately activates early response genes, which activate late response genes coding for cyclins and Cyclin-dependent kinases (CDKs). The end-result: HSC proliferation [7].

(Follow **Figure 2**) Lineage non-specific cytokines IL-3, IL-6, IL-9, Granulocyte-Colony Stimulating Factor and Granulocyte Macrophage-CSF, then stimulate the transformation of activated HSCs into **CFU-GEMM**[1] cells. These CFU-GEMMs have decreased self-renewal, yet later give rise to the more differentiated CFU-GM (Granulocyte/Macrophage), CFU-Megakaryocyte, Blast Forming Unit-Erythroid (**BFU-E**) colonies [7].

2) Lineage specific proliferation: Interaction with IL-11 and Insulin-like growth Factor (IGF-1) commits CFU-GEMMs to transform into BFU-Es, which can only generate RBCs. Other specific molecules are required to commit CFU-GEMMs to CFU-Meg or CFU-GM families, as shown in **Figure 2** [7].

3) Maturation: BFU-Es become **CFU-Es**, under control of erythropoietin (EPO), IGF-1, IL-9 and GM-CSF. EPO then stimulates the last steps of CFU-E maturation into fully functional, highly-specialized RBCs, which bear almost none of the self-renewal and potency of the original HSC [7].

[1]Colony Forming Unit—Granulocyte, Erythrocyte, Macrophage, Megakaryocyte.

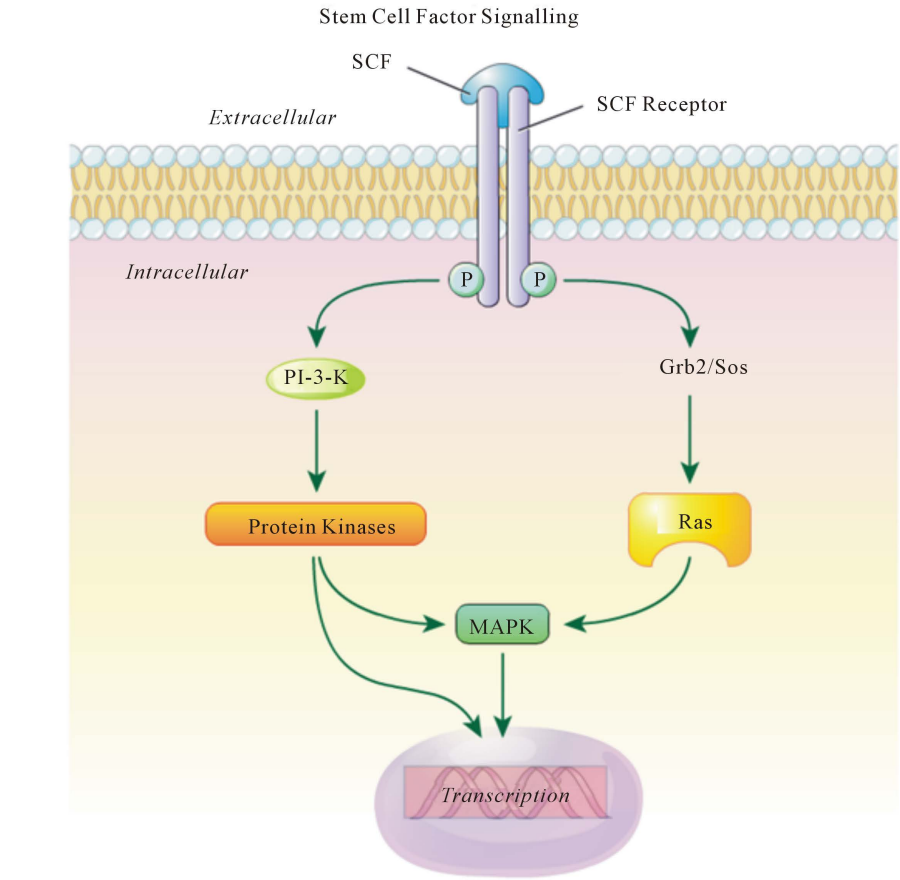

Figure 1. SCF/SCFr mediated chemicalpathway inducing non-specific HSC proliferation [7].

Apart from growth factors Stem cells may require other forms of conditioning. For example, proliferating HSCs must be "nursed" by stromal cells such as fibroblasts, osteoblasts and macrophages etc. which actually supply the necessary cytokines for development [7]. The bone marrow ECM also plays a vital role. Inactive HSCs adhere to the ECM via integrins and selectins expressed on the cell surface, which then detach to set the activated HSCs free to expand and differentiate.

In conclusion, understanding the interactions of SCs with various growth factors, ECM modulation and the use of helper-nurse cells where required, is thus clearly a major priority for developing novel Stem cell therapies, whatever the disease.

2. Before We Had iPSCs

In 1998, Thomson et al had become the first to extract pluripotent ESCs from human blastocysts, yet this had required the consequent termination of many human embryos [8] [9]. Klimanskaya (2006) however managed to extract human pluripotent ESCs from the Inner Cell Mass, without causing any significant harm to the embryo [10]. This thus may have proven a better alternative, as regards alleviating the many obvious ethical controversies in Thomson's and others' methods. Nevertheless, Klimanskaya's method is still a very invasive procedure carrying a number of risks. Hence it is certainly not the ideal way of extracting pluripotent human stem cells [1].

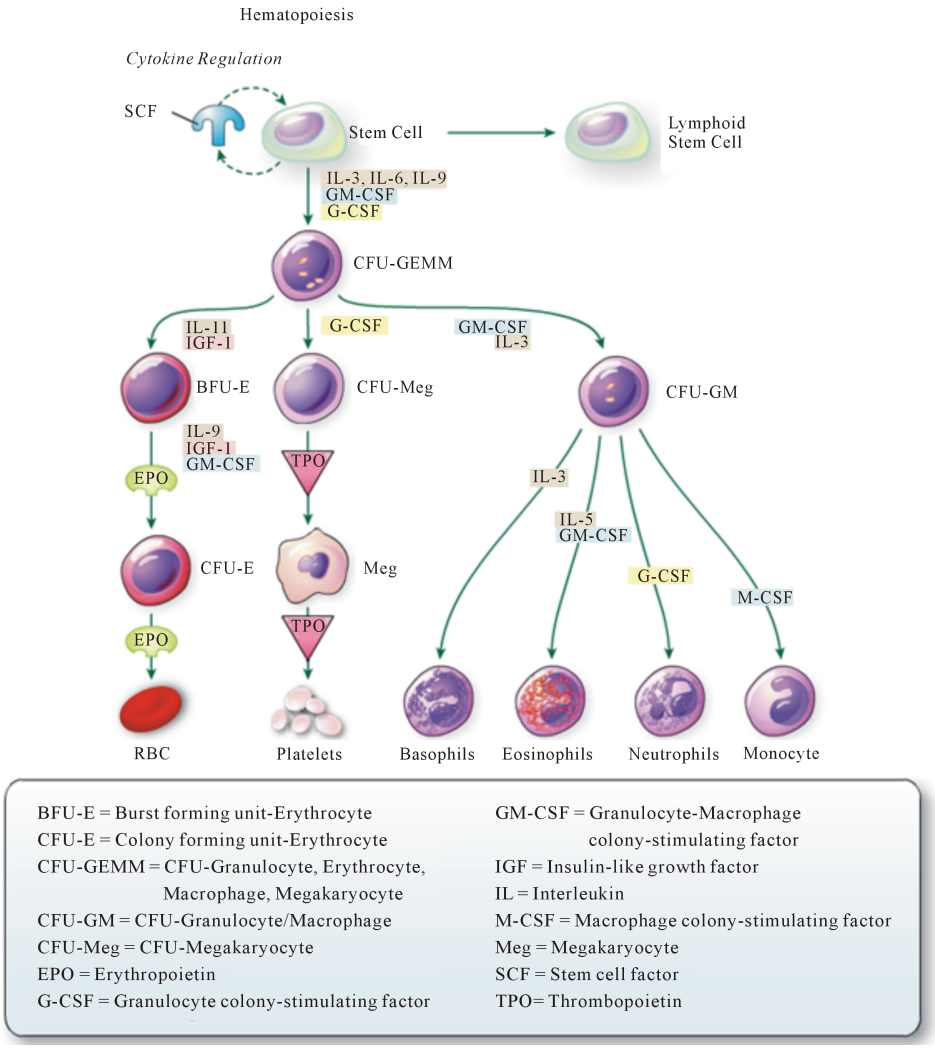

Figure 2. Haematopoietic pathway as controlled by interactions with lineage specific and non-lineage specific factors [7].

As regards USCs, harvesting umbilical cord blood simply requires a needle and a specially designed bag for collection. They can then be concentrated and stored in liquid nitrogen and still remain stable for years. When needed they are then "thawed out" [1]. The main advantage of USCs is that they do not present with the moral headaches tied with ESCs, as one is actually using a rapidly available stem cell source that does not put the donor's life at risk. In fact, as is well-known, the umbilical cord is usually discarded post-partum without a second thought.

Moreover, as regards patients requiring bone marrow transplants, USC transplants to HLA-matched siblings [11], relatives [12], as well as non HLA-matched recipients [13], markedly demonstrate superiorhost acceptance and reduced graft-versus-host disease, compared to conventional bone marrow transplants in similar donor-recipient scenarios. This hints that pluripotent USCs have a stronger immunological naiveté than the collectively less potent bone marrow cells and probably other lineages of similarly inferior potency, even when USCs are donated from one person to another who is not fully HLA-matched [14]. Could this mean that with finer tuning in USC transplantation, even the headaches of cautiously administering immuno-suppressive therapy in post-transplant patients, may one day become nothing more than a fading memory?

Despite these very plausible hypotheses, the current applications of USCs in treating disease are still limited. Although USCs may even at times match the pluripotency of ESCs, USCs do not always produce all cell types effectively. Rather, they frequently differentiate into the more-multipotent-than-pluripotent HSCs and MSCs,

which generate quite a limited number of specialized cells in total [1]. Furthermore cord blood samples have a fixed, small volume. "Cord blood" grafts yield only a mean total nucleated cell dosage (nucleated cells/kg of patient's mass) of less than circa 1/10th of the average bone marrow graft, making graft-host integration much slower than conventional transplants [15].

Therefore despite successful use in even curing haematological disease, including Fanconi's anaemia and breast cancer, USCs are not sufficiently effective in regenerating complex tissues like hyaline cartilage for example [1]. Therefore having said that, USCs are currently an unlikely treatment option for arthritis, a family of diseases marked by cartilage degeneration in synovial joints.

3. iPSCs: Their Discovery and the Possibilities they Promise

The issues currently causing greatest excitement in the world of stem cell research regard induced Pluripotent Stem Cells (iPSCs) [8]. In 2006, Dr Shinya Yamanaka and Takahashi became the first to stimulate iPSC formation *in vitro*, by using **retroviral transduction** of the 4 transcription factors c-Myc, Oct3/4, Klf 4 and SOX2 into mature rodent fibroblasts.

In vitro, these "reprogrammed" cells formed ESC-like aggregates, and when injected into mice they generated teratomas *in vivo*. Cells from all 3 embryonic layers were produced in either case, which provided strong evidence of the high degree of potency belonging to these iPSCs (refer to **Figure 3**).

Yamanaka then established *human* iPSCs in 2007 using the same 4 transcription factors, whilst in the same time period Thomson succeeded similarly with Oct3/4, Nanog, Lin28 and SOX2 [8]. Thus far human iPSCs have been derived from keratinocytes, skin fibroblasts and blood cells to mention a few. It is clear what potential iPSCs have to offer in treating ailments like myocardial infarcts, arthritis and neurological disease.

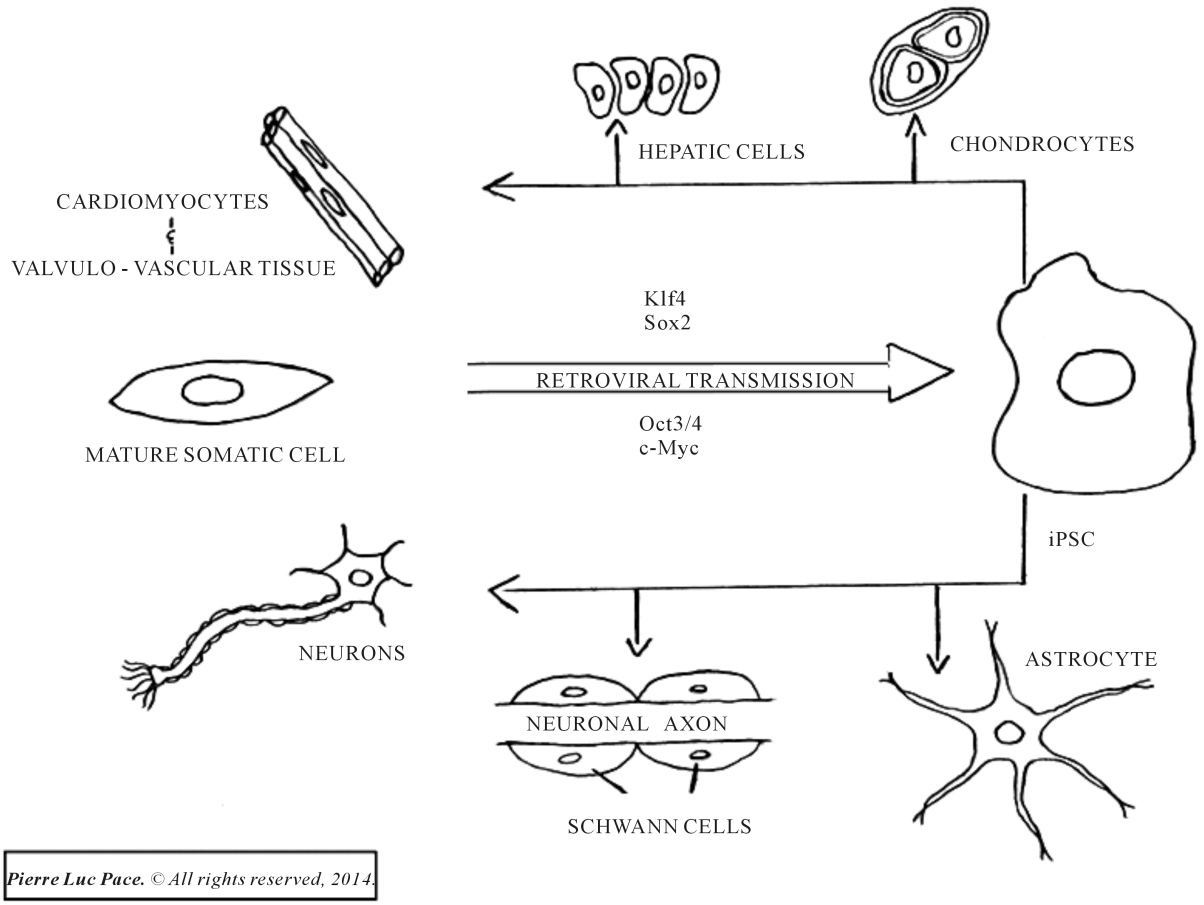

Figure 3. Illustrating the generation of induced Pluripotent Stem cells (iPSCs) from somatic cells by *in vitro* transfection of the 4 Transcription Factors Oct3/4, SOX2, Klf 4 and c-Myc. iPSCs are then able to differentiate into various lineages.

"Now one needn't worry much about finding HLA-matched donors, as required in bone marrow transplants or heterologous USC infusions. Neither do we need trouble with the ethical concerns of having to use human ESCs. They are no longer the only powerful pluripotent cell. Autologous iPSCs can now be made from virtually any mature cell from one's own body. Moreover, each patient would be his/her own SC donor, thus avoiding tedious HLA matching and immunological rejection reactions." [8]

However despite the "miracle" of iPSCs, Yamanaka himself admits that retroviral transgene invasion can disrupt the host cell genome, although much of transcription factor activity is silenced during reprogramming [8].

Some residual transcription factor hyperactivity may still persist: If oncogenic c-Myc remains expressed *after* induction of a pluripotent state, it can cause tumorigenesis in transplanted iPSC-derived cells. Okita *et al.* also reported tumours caused by c-Myc reactivation, together with a low reprogramming success rate of somatic cells into iPSCs [16]. Other studies reported epigenetic changes, which coupled with particular genomic errors, may even cause serious teratoma formation [17].

Reducing the risk of tumorigenesis and increasing induction efficiency, are challenges crucial to making iPSC therapy truly effective *and* safe [8]. Numerous approaches to preserving iPSC genome integrity are being investigated, including growth factors and chemicals like valiproic acid (histone deacetylase inhibitor). Suppression of TSGs like p53 may improve the induction efficiency, however may increase risk of tumorigenesis. One must also note that not all mature cells yield iPSCs equivalently. Miura *et al.* (2009) attempted neural regeneration in mice [8]. They found that with iPSCs derived from tail-tip fibroblasts, the secondary neurospheres had significantly yielded more *undifferentiated* iPSCs than mouse embryonic fibroblasts. Hence different tissues in humans may also yield various ratios of [Undifferentiated: Lineage-committed] iPSCs.

Yet overall the most unbelievable of all approaches to iPSC generation, is that of February 2014 when Haruko Obokata *et al.* claimed to have simply exposed differentiated T-cells to an acid bath of pH 5.7 for circa 25 mins, and iPSCs were produced [18].

This simple technique is termed 'Stimulus-triggered Acquisition of Pluripotency Cells' (STAP cells) "which requires neither nuclear transfer nor the introduction of transcription factors" [18]. The basis is that under certain forms of stress (Ex: physical stricture, toxoid exposure or mildly low pH), specialized cells can revert back to a pluripotent progenitor state.

However, just recently new doubts cropped up amongst researchers including Obokata herself, as attempts to replicate her results with the same method proved unsuccessful. Thoughts of re-writing the paper with more accurate results and photographs were to be implemented in March 2014 [19]. Yet, by July 2014 most STAP papers published by Obokata were revoked [20].

Despite this very short-lived paradigm shift and the disappointing, even sorrowful events that followed[2], further investigation into the matter is still of the utmost importance. A series of slip-ups should not falter our hope in digging deeper into whether STAP cells are indeed a reality waiting to be discovered, even if under a completely different set of laboratory conditions. It is in this reviewer's opinion: "Let's not be stupid by hesitating to try and try again".

4. iPSCs in Osteoarthritis: Direct SC Therapy & Our Tools for Further Study

Osteoarthritis (OA) is one of the most common musculoskeletal problems causing pain, disability, and a significant economic burden on the patient, his/her family, and society itself for that matter. As life expectancy increases in today's ageing population, the prevalence of OA will also increase. Increasing the number of highly invasive and highly expensive, joint replacement surgeries per year for end-stage, severe OA is not an option. Economic pressure on patients and government funds would have to rise disproportionately, since relatively little can be done to resolve these patients' disabling predicament [21].

Therefore the quest for more effective, less invasive treatment has become ever more crucial.

There is already growing evidence that bone marrow and adipose-derived Mesenchymal Stem Cells, aka bm-MSCs and ADSCs respectively, have a great role to play in cartilage repair stratagems, against the chondro-degenerative pathogenesis of OA. However, they do show decreased differentiation potential in elderly and/or obese individuals [22], which are the main groups vulnerable to OA, and which make the majority of OA cases. Yet if the autologous source is a problem, **allogeneic** MSC sources may sometimes be a safe alternative [23].

However, an autologous source is always optimal, and at times vital. IPSCs permit efficient **autologous** tis-

[2]Tragically, one of the scientists involved in Obokata'sSTAP team, YoshikiSasai, committedsuicide [20].

sue-engineering even for elderly and/or obese OA patients [22]. Moreover they are relatively easy to obtain since even fibroblasts from skin scrapings may be stimulated pluripotentally.

Still, the persistent challenge is to commit all the iPSCs in a single culture toward effective chondrogenesis, without resulting in teratoma formation [22]. As had been done by others with human embryonic SCs, Diekman et al initiated chondrogenesis in a simple fashion by short-term exposure of rodent iPSCs to BMP-4 (bone morphogenetic protein 4). About **10%** of the cells expressed hyaline-specific, Collagen Type II (Col2 gene) and aggrecan (Acan). These were marked by green fluorescent protein expression (GFP+ cells).

The small GFP+ population was then "**purified**" from GFP- cells by separating the two populations through flow cytometry.

After expansion both groups were cultured with six passages of TGF-β3. The successfully differentiated GFP+ culture yielded **larger** cartilage pellets, with a **higher concentration** of GAGs and hyaline type II collagen. Purification of GFP+ cells also helped *eliminate* unspecialized cells that could form teratomas, *only allowing chondrogenic cells* to develop [22].

However after passage 3, GFP+ cells became *de-differentiated* due to excessive expansion. In fact pellets became less homogenous and hyaline-specific since type II collagen decreased, whilst synthesis of ***fibrocartilage*** marker, collagen I increased.

In conclusion passage 2 GFP+ cells offered the best results, synthesizing cartilage similar to ***immature*** (almost embryonic) cartilaginous tissue, since collagen VI seemed scattered throughout the matrix, rather than peripherally zoned as in mature tissue. What is remarkable is that GFP+ cartilage had a **stronger elastic modulus** at the periphery than the centre, thus strongly resembling **native** hyaline cartilage which has similar zonal variations in mechanical function. Moreover when introduced into femoral pig FCLs, the iPSC derived cells showed good defect filling and integration with native cartilage [22].

If not directly used for in-vivo reconstruction of FCLs and OA, iPSC-engineered cartilage may be used as an important model for conducting new pathophysiological or pharmacological research on defect repair. This is an advantage over using animal models, which allow a limited number of tests each [22]. In fact, through in-vitro studies on the effects of chondrogenic factors on MSCs, the molecule Kartogenin was recently discovered. It promotes chondrocyte differentiation *and* chondroprotective pathways. When tested as a pharmaceutical, Kartogenin proved "efficacious in two OA animal models" [24].

Furthermore, using iPSC expansion, samples from animals or humans with increased genetic susceptibility or resistance to OA may be replicated multiple times for any number of desired studies.

So far iPSCs are painting a bright future for OA patients. However there is more to OA than one may want to imagine. As is so with many multi-factorial diseases, several studies emphasize OA as being at least partially heritable. Various twin and family studies estimate that OA has a 40% - 65% genetic factor [25]. iPSCs and MSC therapies alone may not be enough to cure OA completely. Gene modification therapy is clearly needed, and ideally should be tailor-made to the individual patient's particular alleles.

Combined therapy is probably the key. More accurate, cost-effective diagnosis of early cartilage lesions, and a standardized treatment protocol, involving (1) Intra-articular Platelet-Rich Plasma Injections (PRPIs), Autologous Conditioned Serum (ACS) or Autologous Protein Solution (APS) with (2) Stem Cells, ex: autologous iPSCs and MSCs, together with (3) Gene therapy [21]. That's where we are headed, although there's no clear-cut yellow, brick road to get there. But hopefully this gold-standard protocol might eventually one day, blow the need for tedious joint replacement to Betsy.

5. Conclusions

After this very brief overview of the latest advancements in Stem Cell research, it may be now easier to paint a clearer, more hopeful, yet more realistic picture of the future of Modern Medicine. Most tissues lack the ability to regenerate, be it the precious cartilage lining our arthritic joints, the transmural infarcts in our fragile hearts or the deep-seated glial scars in our brains.

And this is the promise of Stem Cells: that degenerative conditions like osteoarthritis, cardiovascular and neurological disease, assaulting our most vulnerable structures, may be forgotten… for good [1]. This may well mark one large step forward in the journey of modern medicine. And the best part, for many, is that thanks to Yamanaka and numerous other research teams, the vast potential of Pluripotent SCs can be exploited without sacrificing our precious moral standings, serving as the very bedrock for the beliefs of millions of people.

The question yet still remains: "Are SCs Daddy or chips?"

It is easy to imagine stem cells as the "Daddy of all cures and medical interventions". Still, we know so little about the true aetiologies and pathogeneses of multi-factorial diseases like cardiovascular disease, that saying that stem cells alone can replace conventional treatment is illogical. For now stem cells are more like the "chips" (fries) next to your quarter-pounder and salad; a side dish that can complement wonderfully to the whole meal, but still not the focus of the main course.

However the future is definitely looking brighter. It is impossible to imagine stem cells side-lined out of either standard care or complex treatment. It is in this reviewer's opinion that to combat multi-factorial disease, one needs multi-disciplined treatment. Stem cells would definitely be at the core of *combination therapy*, for example working together with gene therapy and surgical interventions, as is being researched regarding the treatment of OA. They simply won't be working alone.

"*Chips for now… but not for too long mind you lad*"

Acknowledgements

My sincerest gratitude goes to Dr Renald Blundell for being my mentor and for providing sound guidance throughout this project.

Further regards go to Dr Pierre Schembri-Wismayer and Dr Isabelle Borg for their very helpful advice.

I would also like to thank Dr Christopher Vidal, a leading researcher in genetic studies of the Maltese population, for giving me support in this field despite being retired from Medical Science.

Best thanks must also go to Stephanie Searle and Prof Israels for helping me obtain legal permission to use Figures 5-1 and 5-2 from "*Mechanisms in Hematology*", as **Figure 1** and **Figure 2** in this review. Both of these figures were reproduced with permission from Core Health Services Inc. © Core Health Services Inc. All rights reserved.

Above all I am grateful for my kind family for whom the title was inspired. My parents, Jacqueline and Edmund Pace for their love and scientific wit, as well as my sisters Nicole, Raquel and Gyllain who teach me so much, often without them realizing it.

For all the mentioned above and everything else, I thank God for His endless love despite my weaknesses, and for putting me on the right track in spite of my many detours.

References

[1] Blundell, R. (2013) Stem Cells Technology. LAP Lambert Academic Publishing, Saarbrücken.

[2] NIH Report (2001) The Human Embryonic Stem Cell and the Human Embryonic Germ Cell. Stem Cells: Scientific Progress and Future Research Directions, 11-21.

[3] Verfaillie, C.M. (2006) "Adult" Stem Cells: Tissue Specific or Not? In: Lanza, R., *et al.*, Eds., *Essentials of Stem Cell Biology*, Elsevier Inc., Oxford, UK, 233-239.

[4] Preston, S.L., *et al.* (2003) The New Stem Cell Biology: Something for Everyone. *Molecular Pathology*, **56**, 86-96. http://mp.bmj.com/cgi/content/full/56/2/86 http://dx.doi.org/10.1136/mp.56.2.86

[5] The National Institutes of Health Resource for Stem Cell Research (2006) What Are Adult Stem Cells? http://stemcells.nih.gov/info/basics/basics2.asp

[6] Wade, N. (2006) Stem Cells May Be Key to Cancer. http://www.nytimes.com/2006/02/21/health/21canc.html

[7] Israels, L.G., Israels, E.D. and Israels, S. (2002) Chapter 5—Haematopoiesis, Granulopoiesis, Red Cell Lifespan and Bilirubin Metabolism. In: *Mechanisms in Hematology*, 3rd Edition, Core Health Services. http://www.mechanismsinhematology.com/site/chapter.aspx?chapter=5

[8] Yoshida, Y. and Yamanaka, S. (2010) Recent Stem Cell Advances: Induced Pluripotent Stem Cells for Disease Modeling and Stem Cell-Based Regeneration. *Circulation*, **122**, 80-87. http://dx.doi.org/10.1161/CIRCULATIONAHA.109.881433

[9] Thomson, J. (1998) Embryonic Stem Cell Lines Derived from Human Blastocysts. *Nature*, **282**, 1145-1147. http://dx.doi.org/10.1126/science.282.5391.1145

[10] Klimanskaya, I., Chung, Y., Becker, S., Lu, S.J. and Lanza, R. (2006) Human Embryonic Stem Cell Lines Derived from Single Blastomeres. *Nature*, **444**, 481-485.

[11] Rocha, V., Wagner, J.E., Sobocinksi, K.A., Klein, J.P., Zhang, M.J., Horowitz, M.M. and Gluckman, E. (2000) Graft-

versus-Host Disease in Children Who Have Received a Cord-Blood or Bone Marrow Transplant from an HLA-Identical Sibling. *New England Journal of Medicine*, **342**, 1846-1854. http://dx.doi.org/10.1056/nejm200006223422501

[12] Gluckman, E., Rocha, V., Boyer-Chammard, A., Locatelli, F., Arcese, W., Pasquini, R., Ortesa, J., Souillet, G., Ferreira, E., Laporte, J.P., Fernandez, M. and Chastang, C. (1997) Outcome of Cord-Blood Transplantation from Related and Unrelated Donors. *New England Journal of Medicine*, **337**, 373-381. http://dx.doi.org/10.1056/nejm199708073370602

[13] Long, G.D., Laughlin, M., Madan, B., Kurtzberg, J., Gasparetto, C., Morris, A., Rizzieri, D., Smith, C., Vredenburgh, J., Halperin, E.C., Broadwater, G., Niedzwiecki, D. and Chao, N.J. (2003) Unrelated Umbilical Cord Blood Transplantation in Adult Patients. *Biology of Blood and Marrow Transplantation*, **9**, 772-780. http://dx.doi.org/10.1016/j.bbmt.2003.08.007

[14] Laughlin, M.J., Barker, J., Bambac, B., Koc, O.N., Rizzieri, D.A., Wagner, J.E., Gerson, S.L., Lazarus, H.M., Cairo, M., Stevens, C.E., Rubenstein, P. and Kurtzberg, J. (2001) Hematopoietic Engraftment and Survival in Adult Recipients of Umbilical-Cord Blood from Unrelated Donors. *New England Journal of Medicine*, **344**, 1815-1822. http://dx.doi.org/10.1056/NEJM200106143442402

[15] Rubinstein, P., Scaradavou, A., *et al*. (2010) National Cord Blood Program (NY Blood Centre). http://www.nationalcordbloodprogram.org

[16] Okita, K., Ichisaka, T. and Yamanaka, S. (2007) Generation of Germline-Competent Induced Pluripotent Stem Cells. *Nature*, **448**, 313-317. http://dx.doi.org/10.1038/nature05934

[17] Hussein, S.M.I., Elbaz, J. and Nagy, A.A. (2013) Genome Damage in Induced Pluripotent Stem Cells: Assessing the Mechanisms and Their Consequences. *BioEssays*, **35**, 152-162. http://dx.doi.org/10.1002/bies.201200114

[18] Obokata, H., Wakayama, T., Sasai, Y., *et al.* (2014) Stimulus-Triggered Fate Conversion of Somatic Cells into Pluripotency. *Nature*, **505**, 641-647. http://dx.doi.org/10.1038/nature12968

[19] Gallagher, J. (2014) Stem Cells: Scientist Asks for Research to Be Withdrawn. *BBC News*. http://www.bbc.com/news/health-26516458

[20] Knoepfler Lab Stem Cell Blog (2014) http://www.ipscell.com/stap-cell-timeline/.

[21] Demange, M.K., Sisto, M. and Rodeo, S. (2014) Future Trends for Unicompartmental Arthritis of the Knee: Injectables & Stem Cells. *Clinics in Sports Medicine*, **33**, 161-174. http://dx.doi.org/10.1016/j.csm.2013.06.006

[22] Diekman, B.O., Christoforou, N., Willard, V.P., *et al*. (2012) Cartilage Tissue Engineering Using Differentiated and Purified Induced Pluripotent Stem Cells. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 19172-19177. http://dx.doi.org/10.1073/pnas.1210422109

[23] Diekman, B.O. and Guilak, F. (2013) Stem Cell-Based Therapies for Osteoarthritis: Challenges and Opportunities. *Current Opinion in Rheumatology*, **25**, 119-126. http://dx.doi.org/10.1097/BOR.0b013e32835aa28d

[24] Johnson, K., Zhu, S., Tremblay, M.S., *et al.* (2012) A Stem Cell-Based Approach to Cartilage Repair. *Science*, **336**, 717-721. http://dx.doi.org/10.1126/science.1215157

[25] Panoutsopoulou, K. and Zeggini, E. (2013) Advances in Osteoarthritis Genetics. *Journal of Medical Genetics*, **50**, 715-724. http://dx.doi.org/10.1136/jmedgenet-2013-101754