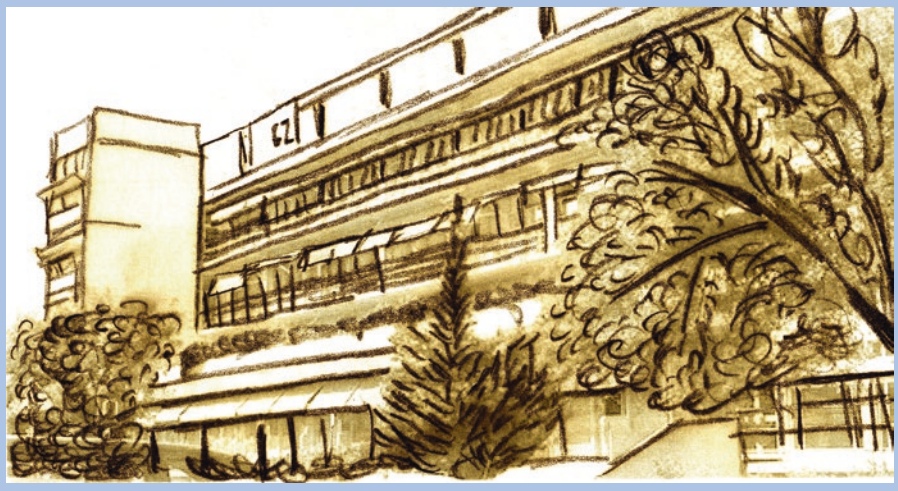
Introduction: A Decade Teaching Stem Cell Biology

Gabriela Rodrigues



Contents

G. Rodrigues, B. A. J. Roelen (eds.), *Concepts and Applications of Stem Cell Biology*, Learning Materials in Biosciences, https://doi.org/10.1007/978-3-030-43939-2_1

1

What Will You Learn in This Chapter?

This chapter is aimed at introducing the structure of this book, which is inspired by the Stem Cell Biology and Technology course running in the Faculty of Sciences of the University of Lisbon. Early mouse development is revisited as a basis for introducing the concept of pluripotent stem cells. A simple practical protocol is described in which the pluripotent differentiation potential of mouse embryonic stem cells is highlighted. Finally, students and readers are invited to navigate through some groundbreaking subjects that will be detailed in the following chapters, such as epigenetics, reprogramming, cloning, regenerative medicine and other innovative ideas spinning off the stem cell biology field.

1.1 Introduction

For more than a decade, the Stem Cell Biology and Technology (SCBT) course has been part of the Master's Programme in Evolutionary and Developmental Biology (MSc EDB) at the Faculdade de Ciências da Universidade de Lisboa/Faculty of Sciences of the University of Lisbon (FCUL), represented in the front page image of this chapter [1–3], and it was the driving force for composing this book. In the SCBT course, students learn the basics of stem cell biology, linking these concepts with the knowledge they already have on the developing embryo and navigating through subjects such as epigenetics, differentiation, plasticity and cell fate decisions. Other areas rooted in the stem cell field, such as regeneration, reprogramming, cloning and ageing, are discussed as well. Finally, applied and translational areas are introduced to the students, highlighting the biotechnological and biomedical use of stem cells at the forefront of modern stem cell biology.

The structure of this book follows the organisation of the course, in the sense that the first chapters are based on more fundamental science and, progressively, subsequent chapters describe more translational applications of stem cell biology. In harmony with the mammalian embryo developmental timeline, the book begins with preimplantation embryology (▶ Chap. 2), followed by the specification of germ cells and the generation of pluripotency (▶ Chap. 3). Because these early stages of development are particularly dynamic and several epigenetic landmarks are established in the embryo at that time, chapters approaching induced pluripotent stem cells (▶ Chap. 4), ageing (▶ Chap. 5) and cloning (▶ Chap. 6) follow. Then, ▶ Chaps. 7 (plants) and ▶ 8 (mouse) explain how stem cells contribute to the establishment and the morphogenesis of the body plan. In the subsequent four chapters, stem cells in specific tissues (skeletal muscle in ▶ Chap. 9, cardiac muscle in ▶ Chap. 10 and neuronal tissues in ▶ Chaps. 11 and 12) are described. The use of stem cells as disease models is discussed in ▶ Chaps. 11 and 12. The final chapter approaches translational aspects of stem cells, where mesenchymal stem cells and their secretome are presented as a potential biomedical tool to ameliorate wound healing (▶ Chap. 13).

1.2 The Embryo and the Stem Cell Concept

Fertilisation triggers the development of a new organism and, since that moment, a series of events occur in the embryo that will give rise to an organism with anatomy, physiology and behaviour that enables it to live and interact with its environment.

This complex process is not achieved in one single step, but instead through a series of events that progressively specify different types of cells, tissues and organ systems that eventually end up building a coherent organism.

The blastomeres resulting from the first rounds of cleavage in the early mammalian embryo are totipotent since these cells can give rise to the embryo itself and to extra-embryonic membranes, including the foetal contribution to the placenta. After several divisions, the first polarised cells appear in the embryo leading to lineage segregation. The blastocyst consists of two types of cells: the trophoblast cells, needed for implantation in placental mammals and which will contribute the foetal part of the placenta, and the inner cell mass (ICM) that holds the pluripotent cells that will generate the whole embryo and some extra-embryonic membranes as well. These pluripotent cells will differentiate to all the populations of cells that derive from the germ layers of the embryo: ectoderm, mesoderm and endoderm, in addition to the germ cells. The ICM cells can be retrieved from the blastocyst and cultivated to generate embryonic stem cell (ESC) lines (see ▶ Chap. 2 for more information; see ◘ Fig. 1.1a). Stem cells with a more limited developmental potential are continuously generated throughout embryonic and foetal development. In fact, these stem cells are of paramount importance for a properly organised and oriented organogenesis to take place in the right place at the right time. The ability of nascent stem cells to pattern the embryo is illustrated in plants (▶ Chap. 7), the vertebrate body (▶ Chap. 8), skeletal muscle (▶ Chap. 9), the heart (▶ Chap. 10) and the brain (▶ Chap. 11) throughout this book.

After birth, some cells remain in the adult body in specific organ micro-environments, which are still multipotent. These cells can give rise to a multi-lineage progeny but limited to the germ layer of origin.

This progression of differentiation has been beautifully represented by Waddington in the 1950s in an image where marbles (totipotent cells) roll downhill on an 'epigenetic landscape' and become progressively more differentiated, finally stably resting at the bottom of a valley. Each marble would then lie still in its own valley, unable to climb back the hill or jump to side valleys, therefore representing a terminally differentiated cell [4]. This representation of cell differentiation occurring in a unidirectional flow has been the dogma for many years but has now been replaced by a more plastic and accurate interpretation, greatly due to the contribution of the stem cell biology field.

The epigenetic mechanisms by which cells acquire their cell fate – or, by analogy, roll down Waddington's hills – fall mainly under two molecular mechanisms operating on their genetic material: DNA methylation and histone modification. In ▶ Chap. 2, students can learn more and understand thoroughly the processes that underlie fertilisation and lineage specification in the early embryo and in ▶ Chap. 6 the epigenetic mechanisms that condition cell lineage restriction are described, as well as the epigenetic regulation variability that occurs during cloning. The reversion of the differentiated state to a pluripotent state either by somatic cell nuclear transfer (▶ Chap. 6) or by direct reprogramming generating the so-called induced pluripotent stem (iPS) cells (▶ Chap. 4) not only defies classical biology concepts, but also opens avenues to the use of patient-specific cells in personalised medicine and custom-tailored drug development (see ▶ Chaps. 11 and 12). However, the cells that are used for cloning or reprogramming are usually aged somatic cells, and epigenetic constraints can hamper the reprogramming efficiency or compromise the differentiation capacity of these cells (see the discussion in ▶ Chap. 5).

Similarly to what happens during development in the embryo and later in postnatal life, stem cells grown in a Petri dish can exhibit several levels of developmental potency. However, one compulsory condition to be defined as a 'stem cell' is the ability to self-renew, i.e. upon dividing, the stem cell has to give rise to a cell identical to itself, thereby maintaining the original pool of stem cells. In addition, stem cells can give rise to cells with a more specialised fate. Therefore, although it is not a simple task, a putative definition of 'stem cell' can be stated as the following: a stem cell is a cell that has to be able to self-renew and give rise to at least one differentiated cell type.

1.3 Pluripotent Stem Cells

The history of stem cell biology can be traced back to the late nineteenth century when experiments with rabbit embryos were performed, as well as with mouse embryonal carcinoma cells [5, 6]. In the 1960s, Till & McCulloch, working on bone marrow transplantation in mice, paved the way for the conceptualisation of the idea of 'stemness' [7]. However, the first successful isolation of a mouse embryonic stem cell line occurred in 1981 [8, 9]. Only much later – due to ethical restrictions and technical difficulties – in 1998, Thomson and his team were able to derive a human embryonic stem cell line [10]. Ever since then, the field of stem cell biology has boosted exponentially and diversified in several branches of research.

During embryonic development, the inner cell mass of the mouse blastocyst eventually separates in two layers: the epiblast, which will give rise to the embryo, and the hypoblast, an extraembryonic sheet of cells (▶ Chap. 2). Similar to the ESCs, the cells of the epiblast are also pluripotent (they are the so-called epiblast stem cells – EpiSC), although epigenetically they are more limited in terms of pluripotency than ESCs. Therefore, EpiSCs are generally termed 'primed pluripotent stem cells' in comparison to ESCs which are characterised as more 'naïve' pluripotent cells. One of the differences between these two types of pluripotent stem cells is that while germ cells can be derived from naïve ESCs, it is quite difficult to differentiate them from primed EpiSCs (see ▶ Chap. 3).

Additionally, another line of pluripotent stem cells has been generated: these are the embryonal carcinoma cell lines and they derive from tumours called teratomas or teratocarcinomas, which generally arise in the gonads [5].

To evaluate if the cell lines that are generated are indeed pluripotent, they have to comply with several conditions tested in the laboratory. One of these experiments consists of injecting these cells into a mouse blastocyst. This so-called chimeric blastocyst can be implanted into the womb of a foster mother and the mice that are born can be tested for the presence of the injected cells in various organs, so as to ascertain that the tested cells contributed to the derivatives of the three germ layers and the germ cells. Alternatively, and especially in the case of human ESCs, with which the generation of chimeras is ethically and legally restrained, a different experiment can be undertaken. In this case, the pluripotent cells line can be injected into an immunocompromised mouse, and an ectopic teratoma is expected to form in the mouse. This teratoma can then be analysed for the presence of tissues representative of the three germ layers derivatives [5].

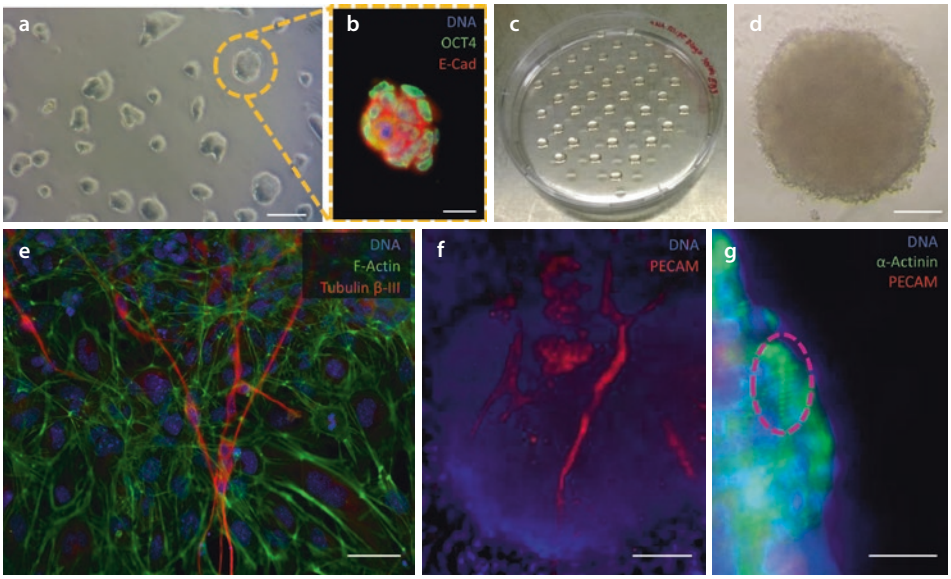


Fig. 1.1 Results of the practical exercise on the evaluation of ESCs differentiation potential: **a** Mouse ESCs growing on a gelatine-coated Petri dish for 2 days in a culture medium specific for the maintenance of pluripotency: small and round clumps of ESCs can be observed. **b** Immunocytochemical staining of ESCs obtained in **a** (blue: DNA, green: OCT4, red: E-cadherin); the stained ESCs shown is an example of the general staining obtained in the cultured cells (yellow circle in **a**); all the cells in the clumps display OCT4 expression, a marker of pluripotency; in addition, E-cadherin, an epithelial membranar adherent junctions' marker, is a hallmark of ESCs pluripotent cells as well. **c** 20 μl drops hanging upside-down on the lid of a 60 mm diameter Petri dish containing ESCs resuspended in a culture medium that no longer maintains pluripotency. **d** EB obtained after 4 days of incubating the cells in the hanging drops. **e–g** Immunocytochemical staining obtained from several experiments using cells grown as explants from the EBs obtained in D after an additional 2 days period upon plating the EBs in gelatine-coated Petri dishes growing in the same culture medium; in **e** (DNA in blue, F-actin in green and tubulin β3 in red), it is possible to observe cells' actin cytoskeleton in all cells (green) and tubulin β3 staining reveals that some cells differentiated towards the neural fate (red cells); in **f** (DNA in blue, PECAM in red), some 'endothelial-like cells' (red) are highlighted and these cells were able to form very rudimentary blood vessels; in **g** a few cells express a protein (α-Actinin, in green) present in the z-lines of cardiomyocytes' sarcomeres; the striped pattern of α-Actinin staining (see magenta circle) strongly suggests that this cell differentiated to the cardiomyocyte fate (DNA in blue and PECAM in red, the red marker does not show any specific staining). Scale bars: 50 μm in **a**, **d**, **e** and **f**; 20 μm in **b** and **g**. EB Embryoid Body, E-cad E-cadherin, ESC Embryonic Stem Cell, PECAM platelet–endothelial cell adhesion molecule, SCBT Stem Cell Biology and Technology. (All the images were obtained from experiments performed in different years by the students in the practical classes of the SCBT course)

1.3.1 Practical Exercise: Evaluation of ESCs Differentiation

The ability of pluripotent stem cells to differentiate into several types of cells can be tested in a number of ways. In the practical classes of the SCBT course, a simple protocol is undertaken by the students to learn about mouse embryonic stem cell biology. In a first step, students learn the basics of cell culture and practice several standard procedures with regular cell lines in the cell culture facility. Once trained, the students perform a simple protocol to maintain ESCs and to differentiate them in embryoid bodies (EBs). EBs are forced aggregations of dissociated ESCs in sus-

pension and they are intermediate structures, no longer pluripotent, which can be channelled to differentiate to several cell lineages. In this exercise, students are expected to acquire skills in ESCs culture, to analyse stem cell behaviour and also to train the capacity to troubleshoot problems and critically interpret the obtained results.

Briefly, a line of mouse ESCs is cultivated in a humidified cell culture incubator (37 °C, 5% CO_2) on gelatine-coated Petri dishes for 2 days growing in stem cell culture medium whose formula was developed to maintain ESCs' pluripotency (Fig. 1.1a). A subset of cells is transferred to a suspension in a different culture medium (that no longer maintains pluripotency) for the preparation of EBs in hanging drops (Fig. 1.1c). Drops of 20 µl of the cell suspension are deposited in Petri dish lids, turned upside-down and further cultured for 4 days to force aggregation and, therefore, the formation of EBs (Fig. 1.1d). The EBs are harvested and plated on gelatine-coated Petri dishes for an additional period of 2 days in the same culture medium. At the end of the incubation time, ESCs and EB cells are fixed and prepared for immunocytochemistry, using markers of pluripotency and differentiation. Students will evaluate the maintenance of the pluripotent state in the ESCs and the differentiation of the plated EB cells towards some cell fates under study. In this way, students will also have the opportunity to gain expertise in immunocytochemistry and imaging techniques. Because the culture medium used for the formation and cultivation of EBs is not specific for the differentiation of any particular type of cell, students will have to carefully analyse the results using a panel of markers and try to understand if any specific type of cell has appeared in the cell populations growing out of the plated EBs. Indeed, while ESCs consistently express pluripotent markers, such as OCT4 and NANOG (Fig. 1.1b; see ▶ Chap. 2), cells growing in the EBs assumed neuronal (Fig. 1.1e), endothelial (Fig. 1.1f), or cardiac (Fig. 1.1g) cell fates in different sets of experiments.

1.4 Adult Stem Cells

In the adult organism reservoirs of stem cells are present in almost every organ, generally in a quiescent state. These cells are usually multipotent, which means that their proliferative and multi-lineage differentiation capacities are limited compared to those of ESCs. Some adult stem cells, like the epithelial cells that constantly renew the epidermis or the gut lining in our bodies, are very active. On the contrary, some others, such as neural stem cells, are not easily activated. When skeletal muscle is injured, satellite cells, the stem cells of skeletal muscle, can become activated, fuse with the damaged muscle fibre and repair the local defect. Muscle satellite stem cells, and others, such as neural stem cells are approached in several chapters in this book (▶ Chaps. 9 and 11).

One of the best studied types of adult stem cells is the mesenchymal stem cells [11]. These cells have the potential to give rise to several kinds of cells, including condrogenic, osteogenic and adipogenic lineages. Mesenchymal stem cells can be harvested from the bone marrow, from fat tissue – even from liposuction material if collected under good medical practice – and from the umbilical cord matrix – the Wharton jelly. These cells have been largely studied and used due to their plasticity, safety and immu-

nomodulatory properties. In addition, the secretome produced by mesenchymal stem cells has been investigated in view of many applications (see ▶ Chap. 13).

1.5 Stem Cells and the Changing Paradigm of Cell Biology

Roughly a century has passed since the first experiments leading to the definition of the stem cell concept. From then up to our days, an enormous amount of research has been conducted, ideas have emerged and dogmas were broken. After the demonstration that cells could revert the epigenetic memory imposed during cell differentiation, through cloning experiments (▶ Chap. 6) and direct reprogramming using iPS technology (▶ Chaps. 4 and 5) [12], Waddington's landscape had necessarily to be redrawn. Indeed, an updated epigenetic landscape has been represented with marbles jumping up, down and sideways on the hills [13–15].

More than being a major breakthrough in cell biology and a new vision of cell differentiation and plasticity, the reprogramming of somatic cells has opened promising avenues for the use of human iPS cells in many biomedical applications [14]. Given the possibility of using patient-specific cells from skin biopsies, for instance, to differentiate tissues or even to generate organoids in vitro, diseases can now be studied under a completely new perspective. Organoids are 'miniature organs' totally produced in vitro from stem cells. Organoids can reproduce part of the architectural composition of the native organ, preserving some of its functionality as well. Therefore, organoids have been used as tools to study disease progression, serving also as platforms to test drug toxicity and efficacy. The 'minibrains' [16] are an extraordinary example of a model that has been used to understand the progression of a pathological condition, in this particular case the infection of brain neuro-progenitors with the Zika virus, otherwise impossible to study directly in human brains [17]. Another very elegant application of organoids has been the use of cystic fibrosis patient-derived gut organoids to test drugs that are potentially particularly suited for each one of the patients [18].

The field of stem cell biology has greatly benefited from tissue engineering technology [5]. Expertise on natural and synthetic biomaterials, along with the fast development of 3D printing equipment, has allowed this field to thrive enormously. The project of producing an artificial human heart is no longer an idea only possible coming out of a science fiction movie [19]. Bioreactor technology can now be used to expand cells efficiently in such a way that it is possible to harvest enough cells for efficient transplantation procedures. The scaling up of cells is not only important for the urgent need of cells for transplantation but has also fueled cutting-edge 'outside the box' ideas, such as the production of in vitro meat [20]. The possibility of producing a hamburger in a Petri dish was a very important proof-of-concept and it will, or not, be scaled-up for common use in the food industry, depending on commercial constraints and societal decisions.

The stem cell field, much like Waddington's marbles, has rolled down and climbed up many hills, in a way that was difficult to imagine few years ago. Our expectation is that the students of the SCBT course and the readers of this book learn more about the fascinating and ever-growing world of stem cell biology and open their spirits to what is yet to come in this field in the near future.

1

Take-Home Message

- Pluripotent cells can be retrieved from the ICM of mouse or human blastocysts and be used to derive ESC lines.
- Stem cells can self-renew and give rise to at least one differentiated cell type.
- Patient-specific pluripotent stem cells can be generated by cloning (somatic cell nuclear transfer) or direct reprogramming originating iPS cells.
- Adult stem cells are multipotent and, therefore, more restricted in their differentiation capacity compared to ESCs.
- The traditional epigenetic landscape imagined by Waddington in the 1950s, with a unidirectional flow of cell differentiation, has been reinterpreted in the light of new data as a more dynamic and multidirectional 'cellular flowchart'.
- The expertise to grow organoids in vitro, together with considerable improvements in the technology to produce iPS cells, has propelled drug toxicity and efficacy testing in personalised medicine.
- Teaching stem cell biology is fascinating and research in the field of stem cell biology will certainly lead to exciting new frontiers.

Acknowledgements I would like to express my deep gratitude to all the students of the SCBT course for their hard work, enthusiasm and friendly interaction. I also want to thank Bernard Roelen for his inspirational lectures and discussions in the course, and Susana Chuva de Sousa Lopes for her continued support with cells, antibodies, and for sharing technical skills. Finally, I want to address a word of recognition to my colleague Sólveig Thorsteinsdóttir for her constant support, to Marta Palma and Luís Marques for their long-lasting cheerful help in the cell culture and the microscopy facilities, and to Ana Bernardo for drawing beautiful illustrations for this book.

References

1. https://ciencias.ulisboa.pt/en.
2. http://bed.campus.ciencias.ulisboa.pt/.
3. Thorsteinsdóttir S, Rodrigues G, Crespo EG. History and present state of teaching and research in Developmental Biology in Portugal – a personal account. Int J Dev Biol. 2009;53:1235–43.
4. Waddington CH. The strategy of genes: a discussion of some aspects of theoretical biology. London: George Allen and Unwin; 1957.
5. Mummery C, van de Stolpe A, Roelen BAJ, Clevers H. Stem cells: scientific facts and fiction. Amsterdam: Academic Press/Elsevier; 2014.
6. Solter D. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research. Nat Rev Genet. 2006;7:319–27.
7. McCulloch EA, Till JE. Perspectives on the properties of stem cells. Nat Med. 2005;11(10):1026–8.
8. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;292(5819):154–6.
9. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A. 1981;78(12):7634–8.
10. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145–7.
11. Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell. 2011;9(1):11–5.
12. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861–72.

13. Hochedlinger K, Plath K. Epigenetic reprogramming and induced pluripotency. Development. 2009;136:509–23.
14. Takahashi K, Yamanaka S. Induced pluripotent stem cells in medicine and biology. Development. 2013;140:2457–61.
15. Takahashi K, Yamanaka S. A developmental framework for induced pluripotency. Development. 2015;142:3274–85.
16. Lancaster MA, Renner M, Martin C-A, et al. Cerebral organoids model human brain development and microcephaly. Nature. 2013;501(7467):373–9.
17. Qian X, Nguyen HN, Jacob F, Song H, Ming GL. Using brain organoids to understand Zika virus-induced microcephaly. Development. 2017;144(6):952–7.
18. Dekkers JF, Wiegerinck CL, de Jonge HR, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med. 2013;19(7):939–45.
19. Noor N, Shapira A, Edri R, Gal I, Wertheim L, Dvir T. 3D printing of personalized thick and perfusable cardiac patches and hearts. Adv Sci. 2019;6:1900344.
20. Post MJ. Cultured meat from stem cells: challenges and prospects. Meat Sci. 2012;92(3):297–301.