livelihoods through marine fishing in the conservation of marine ecosystems, it was also evident that alternatives need to be formulated to minimize the growing pressure on these ecosystems. Providing incentives for prudent extraction of biological resources, sea farming and sea ranching were some of the suggestions made by the speakers. The participants also expressed concern on the lack of infrastructure and incentives in India to pursue specialized research in marine biology. It was felt that India should revitalize its 200-year tradition of marine biodiversity inventorying. The symposium concluded with drafting of a road map for action based on the above conclusions.

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RESEARCH NEWS

Tailor-made stem cells

Jyoti Bhojwani

Stem cells are the most primitive or unspecialized cells that have an extraordinary ability to grow rapidly and give rise to more specialized cells such as beating heart cells, skin cells displaying ciliary movements or pancreatic cells producing insulin. What is remarkable about them though, is that these rare categories of cells, which represent 'pure blank states' inside our body, have the capacity to divide and grow practically indefinitely over time. In nature, they reside in an early developing embryo or as discrete cells in adult organ systems, which undergo continuous replenishment. These systems include: the blood-forming system/bone marrow (hematopoietic system), pancreas, brain cells, muscle cells, skin and intestinal cells, etc. (Figure 1). Embryonic stem cells have the potential to become any cell or tissue of a human body like bone, skin or blood (referred to as 'pluripotency') and thus can replace any damaged tissue, a characteristic feature of these cells, described as 'plasticity'. In contrast, adult stem cells, which can be harvested from adults, are generally used to create specific cell types, like muscle tissue from muscle stem cells and so forth. They basically have the tendency to generate cell/tissue types from which they originate. However, their potential to demonstrate 'plasticity' has also been realized with recent experimentation and this allows researchers to use them for tissue repair in sites other than the organs from which they initially originate.

The embryonic stem cells (ESCs) are typically derived from a clump of cells known as 'inner cell mass' growing inside a very early developing embryo (3–5 days old, 100-celled, blastocyst stage). Studies in animal models and humans indicate that these cells could be potentially utilized with great versatility, to re-populate a damaged tissue, e.g. a heart after a stroke or damage, a spinal cord injury or brain damage, upon their successful isolation from the donor and engraftment into the host.

The human embryonic stem cells (hESCs) have the property of developing into any kind of cell/tissue in cultured conditions (*in vitro*) as well, which inspired researchers to employ them for cell-based therapies. However, the most challenging part is to grow these cells in a reliably directed fashion to become specific cell/tissue types. This is because these cells are often unstable, producing most unexpected results as they may divide prolifically to develop into cancerous growths. Therefore, they not only require strict and streamlined programming for their deliberate differentiation into specifically desired cell/tissue types, but also a well-coordinated signalling system comprising genetic factors and their microenvironment (called 'niche'). hESCs also cause immune rejection when transplanted into people. Cells used may be destroyed fast enough unless they are protected, which can perhaps be clinically accomplished by giving medication to suppress immune response in the host/patient undergoing treatment.

Despite the painstaking procedural protocols involved, potential of embryonic and adult stem cells has been realized in the field of biomedical research, and more precisely in regenerative or repairative medicine. Stem cells thus hold enormous promise for curing various human diseases like neurodegeneration, diabetes, cardiac disorders and infertility. These cells that basically represent 'blank' or 'quiescent' states in the body, also serve as elegant models for *in vitro* drug screening, understanding early embryonic development, cell–cell communication, differentiation, tissue remodelling and tissue engineering.

On the other hand, derivation of pure stem cells lines from adult and embryo, somatic cell nuclear transfer (SCNT) technology, cloning, lineage-specific differentiation and tissue regeneration have been some of the major advancements in this field, especially in the past one decade or so.

It was in February 2004, that a team of researchers at Seoul University, South Korea, led by Woo-Suk Hwang, reported their first exciting study[1] showing that cloning could be used to create human embryos that lived long enough in a laboratory culture plate to efficiently harvest stem cells from them. This major advance in stem-cell research immediately spurred a medical revolution, since it intended to help physicians produce cells and tissues 'tailored' and designed to perfectly match a patient's genetic identity[2], that could in turn be skillfully deployed to treat several human disorders.

Although reports and data are no more valid now, due to retraction, I am providing details of these so-called ground-breaking discoveries[1,2] here, to give a background of this study published in *Science* in 2004 and 2005. In their 2004 paper, the team reportedly collected 242 eggs from 16 volunteer women donors. The investigators then removed each egg's chromosomes. To replace this DNA (genetic material), they fused the egg with another cell (somatic in origin; somatic cells are cells other

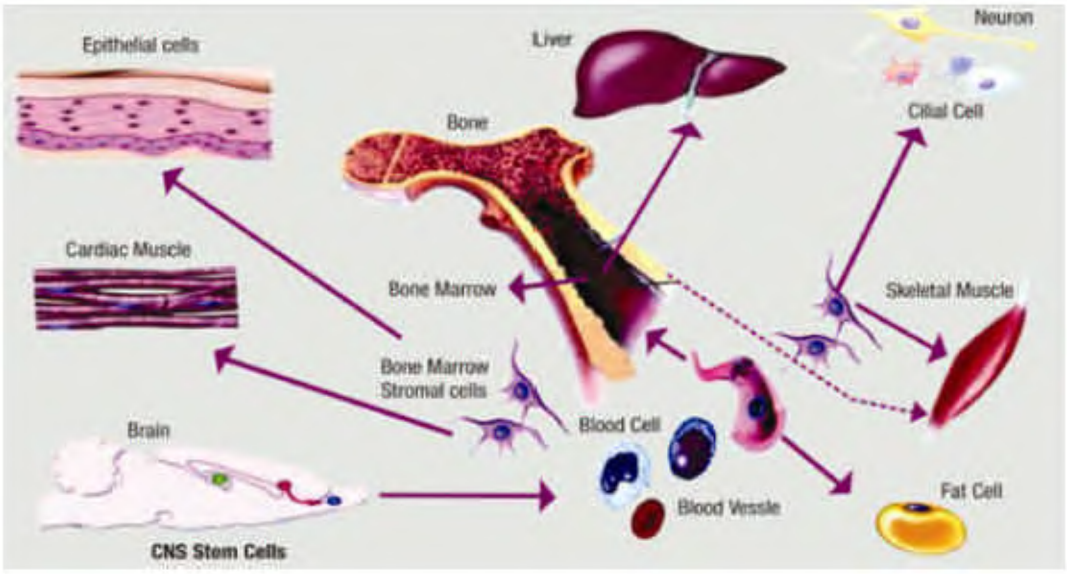

Figure 1. Enormous potential of stem cells to generate a wide variety of cell/tissue types.

than sperms or eggs) from the same woman, using a familiar SCNT technology, pioneered by creators of Dolly[3]. That is, these scientists used cumulus cells (as donor somatic cells), which normally surround the egg in an ovary of the female. Next, the eggs were treated with chemicals/electricity in culture plates to induce their division artificially, as if, fertilized by sperms in natural conditions. Eggs were then coaxed to grow to the blastocyst (3–5 days old) stage, which are round balls, each of the size of a sand grain. Under optimal conditions maintained in the laboratory, 25% of these eggs attained blastocyst stage. For example, they just settled for a two-hour delay between fusion of a cumulus cell with the egg and chemically/electrically activating the fused product. At the blastocyst stage, scientists harvested the interior (inner cell mass) of their cloned blastocyst embryo and discarded the rest of it. From one of their 30 experimental embryos, they claimed to obtain one stem cell line of long-lived cells that resembled embryonic stem cells in appearance and displayed characteristic cell-surface proteins in expression. The harvested cells were also shown to exhibit hallmark features of stem cells in shape (dome-shaped) and function ('teratoma' – three germlayer formation) upon engraftment into the host body. When grown in laboratory dishes with proper supplement nutrient media, feeder layers as sticky supports and the required genetic factors, etc. these cells were also shown to faithfully multiply and produce various cell types, such as bone, skin, muscles, etc.

In yet another dream project to derive patient-specific hESCs, Hwang and his colleagues undertook the challenging task of cloning special stem cells that would not be rejected by patients after transplantation. As responsible as the thought looked, this research team further announced the generation of 11 such patient-specific stem-cell lines. The cell lines were subsequently planned to be tested for engraftments on animal models prior to treating patients in clinics. These special lines were supposedly established using a similar scheme as above, but this time the only difference was the type of somatic cells used for SCNT. The enucleated egg cells were fused with somatic cells of skin biopsies from a variety of diseased patients (Figure 2 *a*). Resultant blastocysts thus recovered were described to be specifically cloned embryos, with genetic identities similar to those of the patients themselves. Among somatic cell donors reported were patients aged 2–56, suffering from juvenile diabetes, spinal cord injuries and a genetic immune deficiency (called congenital hypo-gamma-globulinaemia). In all, 18 volunteer females donated 185 eggs, out of which 125 came from women under the age of 30 years. Using these resources and priming embryo cells with genetic material from people with problems, 11 stable embryonic stem cell lines were believed to be established (Figure 2 *b*) from 31 artificially engineered cell constructs created by nuclear transfer. An average of 17 eggs were reportedly used for generating each cell line.

It all seemed undoubtedly remarkable that within just a year after these investigators managed to clone the human embryo, they claimed to have created the first batch of embryonic stem cell lines that genetically matched the patient's immune system. Unfortunately though, in an announcement that was much feared by the stem cell biologists all over the world, both of these reports were fully discredited and declared to be fraudulent on 10 January 2006, by an investigative panel at SNU and the reports were retracted (for reference see, the statement released by *Science*; http://www.sciencemag.org/sciext/hwang2005/science_statement.pdf).

While the entire scientific community continues to suffer a tremendous setback due to this, most of the stem cell biologists also feel that we are back to where we were several years ago when the first cloning experiment was initiated. Since 1998, when human embryonic cells were first isolated, researchers had hoped to clone and grow these cells into rejection-free transplant tissues for patients suffering from extensive tissue damage and diseases like Parkinson's, diabetes or even leukemia. With the falsified data revealed below, I mention the major problems related to Hwang's research, which brought about the fall of the 'rockstar' scientist.

A dramatic claim that Hwang's team had cloned a human embryo and extracted stem cells from it[1], heralding a stunning prospect of human cloning and the promise of using the stem cell therapy to treat incurable diseases[2], came

crashing down when Hwang publicly took responsibility for the major loopholes and ethical misdoings related to this work in December 2005 (http://www.nature.com/news/2005/051219/pf/051219-3_pf.html). Initially, the news that some 242 eggs used in the experiments, were donated by paid donors from a medical clinic and his subordinate scientists, cast a grave ethical shadow over his research. Also the number of eggs utilized for experiments and reported was inaccurate, i.e. there was much larger procurement of eggs (numbering 2061 eggs from 129 women) by Hwang's laboratory to conduct these experiments. Hwang was also accused of outright fabrication of the results reported in his papers. It was found that the 'patient-specific stem cells' created by his team turned out to be fake, although he repeatedly hinted at some long-planned conspiracy against him. What was worse was that the images of 11 different stem cell lines that accompanied the original article[2], describing a genuine creation of stem-cell lines, were in fact duplicates (http://www.climateaudit.org/?p=476) and also fabricated in the sense that only two of the stem cells lines were documented to make them look like 11.

Further, DNA samples from acclaimed stem-cell lines, tested by the panel, also did not match those of the donors, casting serious doubt over the validity of created stem-cell lines in the 2005 report. It now appears that the first cloned human stem-cell line reported in 2004 by Hwang was also produced from a different donor by fusing an egg still retaining its nucleus with a cellular body produced during egg maturation (called the 'polar body'). This initiates a process called 'parthenogenesis', where the egg can develop without being fertilized. Cloning, by contrast, uses a process called SCNT (Figure 2 *a*), which involves enucleation of donor egg (harvested from a hormonally stimulated female), transfer of nucleus from a somatic cell and triggering of divisions in the egg. Had the 11 stem-cell lines been real, they would have been 'a proof of principle' that the therapies specifically tailored to individual patients avoiding 'immune rejection' could one day be created!

It is a pity how this scandal unfolded, but the media accounted for an important role of young Korean scientists who worked together to help unearth the whole scandal. However, the questions remain; as to whether such a setback in scientific research will ever be overlooked or help rekindle the betrayed trust of people in a high profile and already controversial field of stem-cell research? Also, whether Hwang should be given a second chance to prove his source technology, as he has been claiming to create new stem cell lines within six months, if provided with appropriate amount of eggs and that, 'Snuppy', his cloned dog[4] is not an imposter (Figure 3). Given that 'Snuppy' is a legitimate clone, can the scientific community give Hwang another fresh chance to even develop a 'stem cell model' in animals in order to better understand human diseases (such as cancer, diabetes and joint deterioration), which was the main motive of these projects to begin with? Views on these issues are still divided.

Whatever the outcome, the fact remains that we are back to square one in terms of cloning human cells and that we have no evidence whatsoever that 'patient-specific' lines were created by nuclear transfer. Although this is described to be the biggest fraud in scientific history in many years, independent attempts to produce personalized/customized stem-cell lines would certainly be triggered as a key step towards making stem cell medically useful.

Hwang's debacle is a sharp setback for therapeutic cloning. The use of stem-cell therapy will now face a number of hurdles, as the relevant research has been stalled due to ongoing criminal investigation under high scrutiny, both at SNU, South Korea and University of Pittsburgh, USA, from where the senior author Gerald Schatten hails. Stem-cell researchers worldwide have also been dismayed and confused

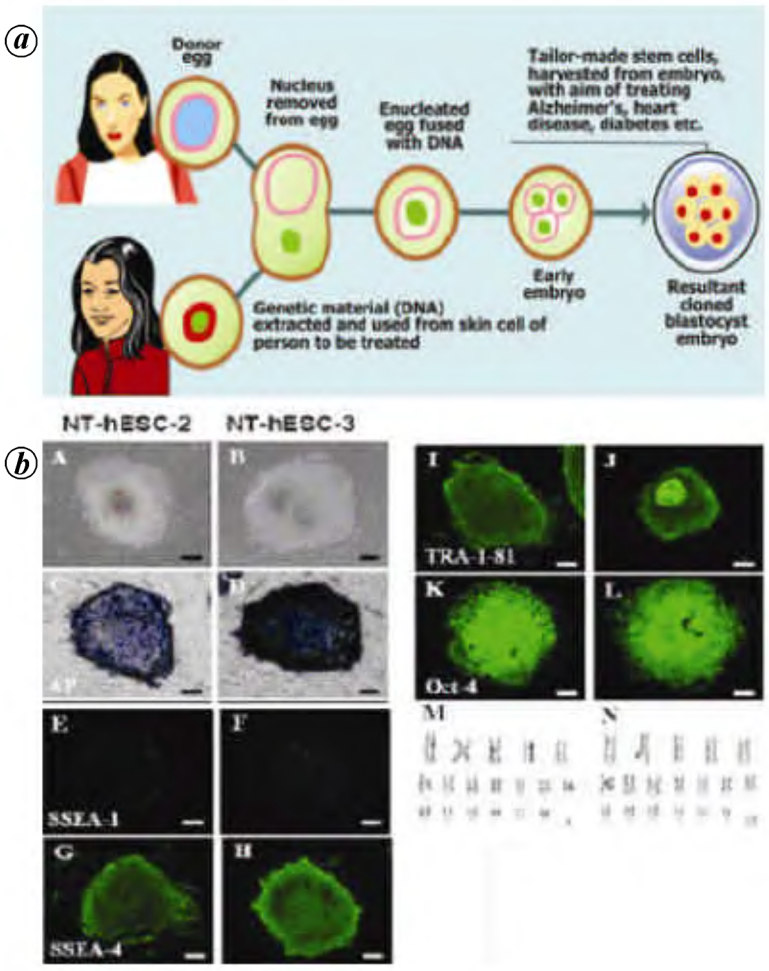

Figure 2. ***a***, Somatic Cell Nuclear Transfer (SCNT) technology reported by Hwang *et al.*[2] to generate 'Tailor-made stem cells' for potential clinical applications. ***b***, Hwang *et al.*[2] claimed to establish patient-specific stem-cell lines by SCNT. Shown here are the male-specific NT-hESC line (left column) and the female-specific NT-hESC line (right column), displaying cell surface protein/pluripotency markers like alkaline phosphatase (AP), SSEA-1, 4, TRA-1-81 and Oct-4. These lines were later proven to be 'fakes' and found to be produced by parthenogenesis rather than SCNT.

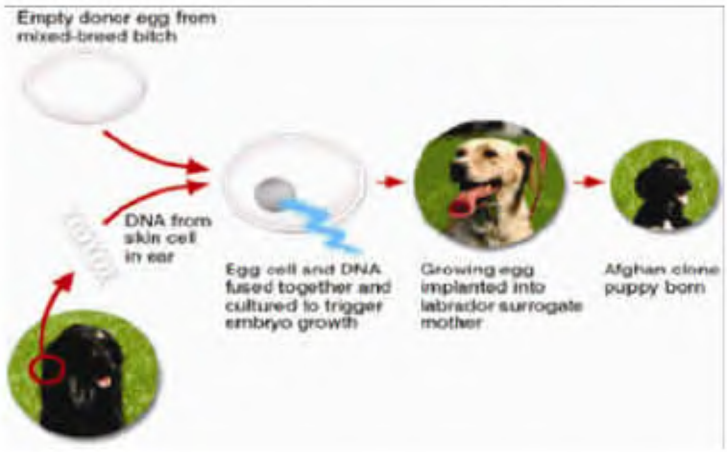

Figure 3. The first cloned canine 'Snuppy' under question has been declared as the real clone of an Afghan hound. http://news.nationalgeographic.com/news/2006/01/0111_060111_hwang.html. *Picture source: http://news.bbc.co.uk/2/hi/science/nature/4742453.stm.

over an abrupt end to this high-profile collaboration between Hwang and Schatten that resulted in two landmark papers in *Science*[2] and *Nature*[4], last year. The idea of being able to use cells from one's own body to replace cells in different parts or to cure degenerative diseases is innately appealing. However, this scandal has delivered a devastating blow to the stem-cell researchers around the world, as unacceptable practices in science can destroy an entire research enterprise!

Although the ability to make use of underlying technology in developing research models and incredible therapies is promising, there are clearly some critical lessons to be learnt from this story. Also, it will be surely important to know how close we are to clinical or nuclear transfer work and production of cloned stem cells lines. This project was also ambitious in thought and was anticipated to bear great biomedical importance for studies on disease and development, leading to advancement of clinical manipulations in stem-cell transplantation. Moreover, since this study did not claim to make cloned-babies (i.e. reproductive cloning), but rather emphasized on utilizing test-tube embryos that are either donated or discarded/aborted by women, it looked like a conscientious effort towards serving humanity in general. However, the harsh reality remains that there is a lot of groundwork to do now and under strict ethical constraints.

The most graceful way towards closure of this issue, however, would be to independently and genuinely attempt the derivation of 'customized' lines, which hold the key to successful transplantation without rejection in patients. Many hope and predict that such cells would not only shed light on understanding heritable diseases, but also offer promising options for new therapies.

A major objection raised against such studies though, is the destruction of human embryos to generate stem-cell lines. Creation of such embryo-like 'biological artifacts' of stem cells has been dogging the bioethics councils and pro-life campaigners all over the world. This would certainly not allow stem-cell researchers to follow their protocols without guilt. Scientific research on genetic manipulation of human cells, ova, sperms and human beings at embryonic stage, has resulted in creations for which no human language has words. Human/animal hybrids called 'chimeras', for example, in Greek mean 'wild', impossible scheme or unreal conception. However, the appreciation gained by animal models employed for these studies and prospective stem-cell lines that could be potentially utilized as 'tissue repair machinery' in patients, genuinely justifies the very purpose of therapeutic cloning fostering responsible research. Thus, the creation of the much craved 'tailor-made' hESCs will not only be helpful in repairing damage but also in understanding of a variety of complex human diseases, which remained elusive until now. Continued research on adult stem cells, on the other hand, provides invaluable tools for 'therapeutic targeting' of complicated diseases like Alzheimer's involving severe brain damage and also cancer. Now with embryonic stem-cell meltdown resulting from the Hwang scandal, adult stem cells are expected to gain more popularity. Adult stem-cell proponents have argued that these stem cells could be utilized by bypassing the immune rejection issue altogether. The scope is enormous; adult stem cells could also provide a less controversial alternative to embryonic stem cells to allow 'reconstruction without deconstruction' in our bodies.

Finally, our sharp mindfulness and caution on analysing and accepting scientific findings which appear 'too good to be true', will certainly take us far.

1. Hwang, W. S. *et al.*, *Science*, 2004, **303**, 1669–1674, http://www.sciencemag.org/cgi/reprint/303/5664/1669.pdf
2. Hwang, W. S. *et al.*, *Science*, 2005, **308**, 1777–1783, http://www.sciencemag.org/cgi/reprint/308/5729/1777.pdf
3. Wilmut, I. *et al.*, *Nature*, 1997, **385**, 810–813.
4. Lee, B. C. *et al.*, *Nature*, 2005, **436**, 7051, 641.
5. http://www.sciencemag.org/cgi/content/full/311/5757/36b

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