Science:

Therapeutic Cloning and Stem Cell Therapy

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Recent advances in biotechnology have been preparing the way for human human therapeutic cloning. Subsequently investigators and other advocates have strongly signaled their support.[1] As a result, the allocation of public funds has been recommended for human embryo experimentation, which is a prerequisite for therapeutic cloning. The primary goal of therapeutic cloning is to produce tissues and ultimately organs for replacement therapy.

Facets of this goal range from what is becoming immediate and realizable, for example, skin, blood, and neuronal cell replacement, to that which is more complex and distant, namely, constructing intact whole organs such as heart, liver, and kidneys. These new cloning technologies show great promise and are perceived to be admirable for several reasons: 1) There are an increasing number of individuals awaiting tissue and organ transplants; 2) Since stem cells and cloned tissues originate from the patient, they would be immunologically matched, eliminating the need for life-long immunosuppressive therapy; 3) Current cloning techniques do not necessarily require human donor eggs and fetal tissue for the construction of cloned tissues (via the reconstruction of cloned embryos), and thus alleviate some ethical concerns; 4) The ongoing discovery and characterization of adult stem cells afford an alternative approach to cellular replacement therapy, as opposed to nuclear transfer and cloning; 5) Finally, the ability to isolate and maintain stem cells in continuous culture offers a system for targeted gene therapy whereby a gene of interest may be deleted, inserted, or corrected.

Private investigators are proceeding at a deliberate pace, seeking the most workable way to make therapeutic cloning and stem cell replacement therapy a reality.[2] Though goals and claims are admirable, the means chosen to achieve them should

[1] Declaration in defense of cloning and the integrity of scientific research. *Free Inquiry* 17, no. 3.

[2] Human therapeutic cloning. *Nature Medicine* 5, no. 9 (Sept. 1999).

not bypass our moral understanding of life and life processes. In addition, the great appeal that certain technologies have should not lead us to disregard the investigation of other research alternatives.

In the following paragraphs, I will review, from a Catholic perspective, the dangers and possible concerns that these new therapies pose over alternatives, such as transplants from human adult and fetal cadavers, and from nonhuman species.

Review of Therapeutic Cloning

In theory, the procedure for therapeutic cloning is simple. It is based on the observation that each cell of the body carries within its nucleus a representative copy of a person's entire genetic makeup. Someone seeking tissue or organ replacement therapy would provide a tissue biopsy, for example, from the skin. From this biopsy a single adult cell with a viable nucleus would be fused with a nonfertilized egg (oocyte) whose nucleus has been removed. The recipient egg is more than an inert shell to house the donor nucleus, as it provides a rejuvenating environment for the genome contained within the nucleus of the skin cell. In part, the egg is responsible for prompting a dedifferentiation process whereby the genome becomes responsive to early embryonic developmental cues. In other words, the mature adult differentiated skin cell reverts back to an embryonic state.

Following fusion of the donor nucleus and recipient egg, cell machinery within the egg directs the earliest phases of embryonic development up until the activation of the embryo's own genome, mimicking the process that follows natural fertilization. At this point, the reconstructed embryo is a human clone, equivalent to a viable embryo with the potential to develop as a whole. Subsequently, a distinct blastocyst-like structure forms with typical embryonic morphology, including trophectoderm, blastocoel cavity, and inner cell mass. Embryonic stem cells extracted from the inner cell mass would then be persuaded with various hormones and growth factors toward the development of a desired cell line. Such a cell line, for example, nerve cells, might be used to treat a person suffering from Parkinson's disease.

Recently investigators have discovered that cow eggs function well as recipient hosts in the nuclear transfer process for various other species.[3] Indeed, some private investigators have succeeded in recreating human cloned embryos using enucleated cow eggs.[4] The advantage in using cow eggs is largely due to their ease of procurement (slaughter houses) and their low cost relative to the acquisition of purchased human eggs.

However, a potential problem arises in using animal eggs for human nuclear transfer. This is because the recipient egg contributes an extremely small number of genes that are not associated with the nucleus (the maternal mitochondrial DNA), but whose products are partly responsible for embryological development. As early development proceeds, the distribution of maternal mitochondrial DNA dominates, and by the time of blastocyst formation, donor mitochondrial DNA is eliminated.[5]

[3] *Biology of Reproduction* 60 (1999): 1496–1502.

[4] *Nature Medicine* 5, no. 9 (Sept. 1999): 975–8.

[5] *Journal of Reproduction Fertility* 116, no. 2 (July 1999): 253–9.

Though the effects of mitochondrial DNA products are not entirely understood, they most likely do not contribute to phenotypical traits. Nonetheless, possible risks resulting from DNA mixing between species should not be ignored.

In order to alleviate ethical concerns, it is commonly asserted that embryonic stem cells harvested from the reconstructed embryo (that is, from the inner cell mass) are no longer equivalent to an embryo in their developmental power because they are not totipotent (that is, capable of regenerating all the tissues of the developing fetus). Instead, they are pluripotent, and are thought to be incapable of developing into a whole viable organism if implanted within a uterus.

However, this claim is misleading. The difference between totipotency and pluripotency lies primarily in the cells' ability to produce extra embryonic membranes, that is, trophoblastic cells, which are the progenitors of placental tissue. Once removed from the blastocyst, inner cell mass cells are not totipotent. However, they are totipotent within a proper environmental context, for example, within a recipient oocyte cytoplasm, and possibly when co-cultured with appropriate feeder cells that mimick the rejuvenating environment of the oocyte.[6]

When cultured with appropriate feeder cells such as mouse embryonic fibroblasts, human inner cell mass cells aggregate into what has recently been termed embryoid bodies.[7] In an unstructured fashion, embryoid bodies mimic the embryo developmentally. To further demonstrate the plasticity or totipotent nature of embryoid bodies, investigators have transplanted these cellular aggregates into immune compromised (Severe Combined Immunodeficient or SCID) mice which lack the ability to reject foreign tissue grafts. These transplanted 'inner cell mass cells-embryoid bodies' spontaneously develop into a disorganized variety of tissues representing each of the principle embryonic germ layers (ectodermal, mesodermal, and endodermal tissues). Though not a cancer, an embryoid body is similar to teratocarcinoma tissue. Teratocarcinoma tissue is a cancerous product that is derived from primordial germ cells and that consists of a disorganized mass, often containing nervous tissue, bones, muscle, skin, hair, eyes, teeth, and the like.[8]

Because SCID mice lack the ability to reject foreign tissue grafts, they serve as attractive models for tissue culture and engineering. But domestic large animals serve as attractive models in the same manner. The advantage of using domestic large animals is that a greater and more workable mass of tissue could be harvested than is possible with mice. And, since the SCID defect naturally occurs in various large animal species, the expense of intentionally designing SCID defects in animal models would be eliminated.

Adult or Post Natal Stem Cells

Currently there are three sources of human tissue which have been demonstrated to give rise to pluripotent stem cell lines representing all three embryonic germ layers. These include stem cells derived from inner cell mass cells of the blastocyst

[6] *Biology of Reproduction* 62 (2000): 470–475.

[7] *Nature Biotechnology* 18 (April 2000): 399–404.

[8] *Science* 287, no. 5457 (25 Feb. 2000): 1418.

stage embryo, stem cells derived from primordial germ cells of the fetal testicle or ovary (progenitors to the oocyte and spermatogonia), and stem cells derived from embryonic teratoma tissues. In addition to the advantage of their pluripotent nature, these stem cells are capable of immortalization and of unlimited proliferation *in vitro,* and thus provide an unlimited source of cells that have the ability to differentiate into a wide range of tissues for transplant therapy.

But not all "stem cells" exhibit the same plasticity that these do. For example, some stem cells function to reconstitute particular defined cell types, such as bone marrow (hematopoietic) stem cells, whose function is to replenish blood cells during the life span of an individual. Similarly, stem (or progenitor) cells exist within the basal layer of the epidermis, and are responsible for the constant renewal of sloughing epidermal cells.

Currently, post natal stem cells (including neonatal stem cells from fetal cord blood) are being identified increasingly in many other tissues that have similar regenerating capacities. For example, pancreatic epithelial duct cells have been shown to be progenitors to other pancreatic cells. When isolated and cultured *in vitro* they can be coaxed into becoming mature insulin producing cells.[9]

Likewise progenitor cells from the adult brain have been identified. When transplanted these cells retain the ability to develop into new neurons even when transplanted into areas of the adult brain which normally do not regenerate neurons. Furthermore, it has been observed that adult brain progenitor cells can give rise to the development of blood cells, and that bone marrow stem cells can be stimulated to become brain cells (astrocytes and glia cells, and perhaps even neurons), or even mature adult liver cells.[10]

To date approximately twenty major stem cell types have been identified in mammals.[11] These observations are encouraging since they support the idea that post natal stem cells can provide an alternative to embryonic stem cells for tissue replacement therapy, though their potential for diversity is limited. Nonetheless, current clinical trials are becoming commonplace.

Regarding post natal stem cells, it should be noted that no single cell type or universal donor is likely to be useful as a cellular graft for all diseases. Apart from being restricted to particular tissue types, extensive genetic manipulation would be required to make adult stem cells universally immunologically compatible. The alternative option in procuring compatible tissues for transplantation therapy would be to reconstruct cloned embryos for the purposes of harvesting inner cell mass cells for directed development.

A further limitation of post natal stem cells, as opposed to embryonic stem cells, is that they often lose their ability to differentiate into a desired mature cell. For example, stem cells harvested from brain tissue lose the ability to produce the neu-

[9] *Proceedings of the National Academy of Sciences* 97 (July 5, 2000): 7999–8004.

[10] *Science* 287, no. 5457 (25 Feb. 2000): 1433–1438.

[11] *Nature* 406 (27 July, 2000): 361–364.

[12] *Cells Tissues Organs* 166 (2000): 1–5.

rotransmitter dopamine, which is desired in the treatment of Parkinson's disease. As a result, the perceived risk would be the development of cancer originating from transplanted stem cells that fail to complete the differentiation and maturation process. In spite of the optimism, it is clear that before stem cells may be utilized for therapeutic purposes, the mechanisms that control their birth, fate, and death will have to be better elucidated.

The Flexible Meaning of the Word "Stem Cell"

With regard to current commentaries and publications on stem cell therapy and therapeutic cloning, the question arises as to what actually constitutes a stem cell. In a general sense a stem cell is simply an undifferentiated or unspecialized cell that can renew itself and give rise to one or more specialized cells with specific functions.

Traditionally, this definition was restricted to germ line cells, oocytes, and spermatogonia that have the capacity to contribute to the entire organism. Stemness in this context is most general and basic, since as development proceeds from the fertilized oocyte cells become channeled into particular pathways of differentiation and their developmental potential or stemness becomes modified and limited.

In a less strict sense the term has long been applied to hematopoietic progenitor cells that have the capability of differentiating into, and repopulating, all dependent blood cell lineages. Yet now we have begun to see that the stemness or plasticity of a cell is not necessarily recognized by how a cell functions in its native dependent tissue within an individual, but rather is based on the ability to manipulate it in culture. In one respect this should not be surprising since blood obviously is not produced in the brain, neither is neuronal tissue produced in bone marrow. This has become dramatically more clear since it was demonstrated that a nucleus transplanted from an adult differentiated cell can contribute to all cells of an animal through nuclear transfer and cloning, as in Dolly the sheep.

For the purpose of ethical investigation it would seem meritorious that not only should the distinction between embryonic and post natal stem cells be made clear, but also that qualifications be made between post natal "stem cells" and subsequent laboratory manipulation. In accord with Catholic tradition, procuring embryonic stem cell tissue from either a naturally developed or reconstructed embryo is illicit. But when encouraging "adult stem cell" research, the water becomes morally murky if the definition includes those cells having the capability of being rejuvenated to a state that is equivalent to inner cell mass-*like* cells (in other words, into embryoid bodies).

From a Catholic ethical perspective, "adult stem cell" merits reevaluation, perhaps considering instead "progenitor cell." A progenitor cell typically refers to a cell that has a multi-potential versus a pluri-potential status, and whose fate is already committed toward a defined tissue or cell type such as progenitor ductal cells that have the potential to develop into mature insulin producing islet cells.

Otherwise a qualification should perhaps be recommended regarding the production of embryoid bodies, or like structures, from the "adult stem cell." Since inner cell mass cells formally give rise to the fetus during gestational development, the potential ability to produce inner cell mass-*like* cells from adult stem cells is a questionable practice.

Recent investigation has begun to elucidate the factors involved in the development of inner cell mass cells.[12] As a result producing "embryoid bodies" while bypassing the step of embryo reconstruction will become an option. Once developed, whether the embryoid body is to be expanded *in vitro* or within an animal system, another question arises. Namely, what precisely constitutes a human organism or even a species? Private investigators have transplanted human embryonic stem cells into animals. What is the status of these products, of the disorganized masses of tissues that develop?[13]

In addition, it should be noted that the application of stem cells for transplantation therapy assumes that it will be possible to grow up such cells on a large scale.[14] However, present systems fall short of this goal, since a lot is required for minimal results. For example, in a clinical trial for Parkinson's disease, fetal brain tissue was required from six aborted fetuses in order to treat a single individual.[15] Given the great strides that have been made in producing artificial systems using biodegradable matrices, or scaffolding, to grow up skin and other connective tissues, animal systems seem to afford an alternative approach.

From the perspective of Catholic ethics, it is illicit to produce a human cloned embryo for any purpose, let alone for the purpose of harvesting inner cell mass cells. Should the same concern exist if inner cell mass-*like* cells were to be produced while bypassing the step of actual embryo reconstruction, thus affording a source of pluripotent stem cells? From a Catholic ethical viewpoint, would it be acceptable to implant such cellular aggregates (that is, groups of cells that resemble and developmentally mimick the naturally growing embryo) into animals? Whether in tissue culture as embryoid bodies, or within an animal as a disorganized mass of tissue, these developing cells appear to be more than simply a mass of tissues. Internal developmental cues are still produced in what appears to be an attempt at becoming a living whole organism.

A certain repugnancy immediately becomes elicited when envisioning animals producing human teratoma like structures (fetal monsters) for tissue harvest or for any other purpose. Physiologically, these embryoid bodies are not a cancer. In philosophical terms, if they could be said to have their own internal principle of operation then they could be considered an organism in their own right.[16] This is a new and unique exploitation of the human organism. It may very well be considered to be a form of sacrilege. In the Catholic tradition sacrilege refers to the violation of something sacred by treating it irreverently. In the *Gospel of Life*, Pope John Paul II reiterated that human life, being a gift of God, is to be considered inviolable and sacred. What are the guidelines to be established?

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[13] *Science* 290 (1 Dec. 2000): 1674.

[14] *Nature Biotechnology* 18 (April 2000): 399–404.

[15] *Science* 287 (25 Feb. 2000): 1421.

[16] Dundon, S. J., Dept. Philosophy California State University Sacramento.