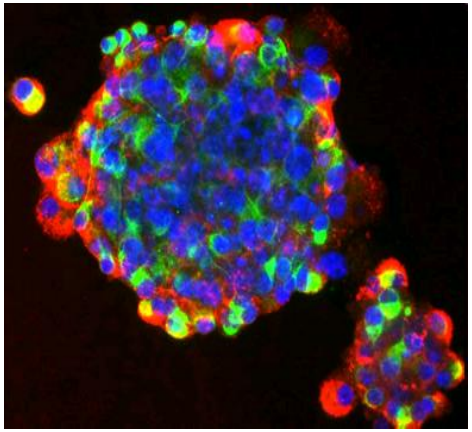
Stem cells

(Basic concepts)



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Stem cells

- defined as having the capacity to self renew, to proliferate extensively, and to differentiate into 1 or more cell/tissue types
- The ability to differentiate is known as “potency.” Potency is classified as uni-, multi-, pluri-, and toti-potency, depending on the number of different tissue types the stem cell can produce
- Stem cells are often classified, based on tissue origin, potency and possible surface markers.

Types of Stem Cells (origin)

Embryonic stem cells

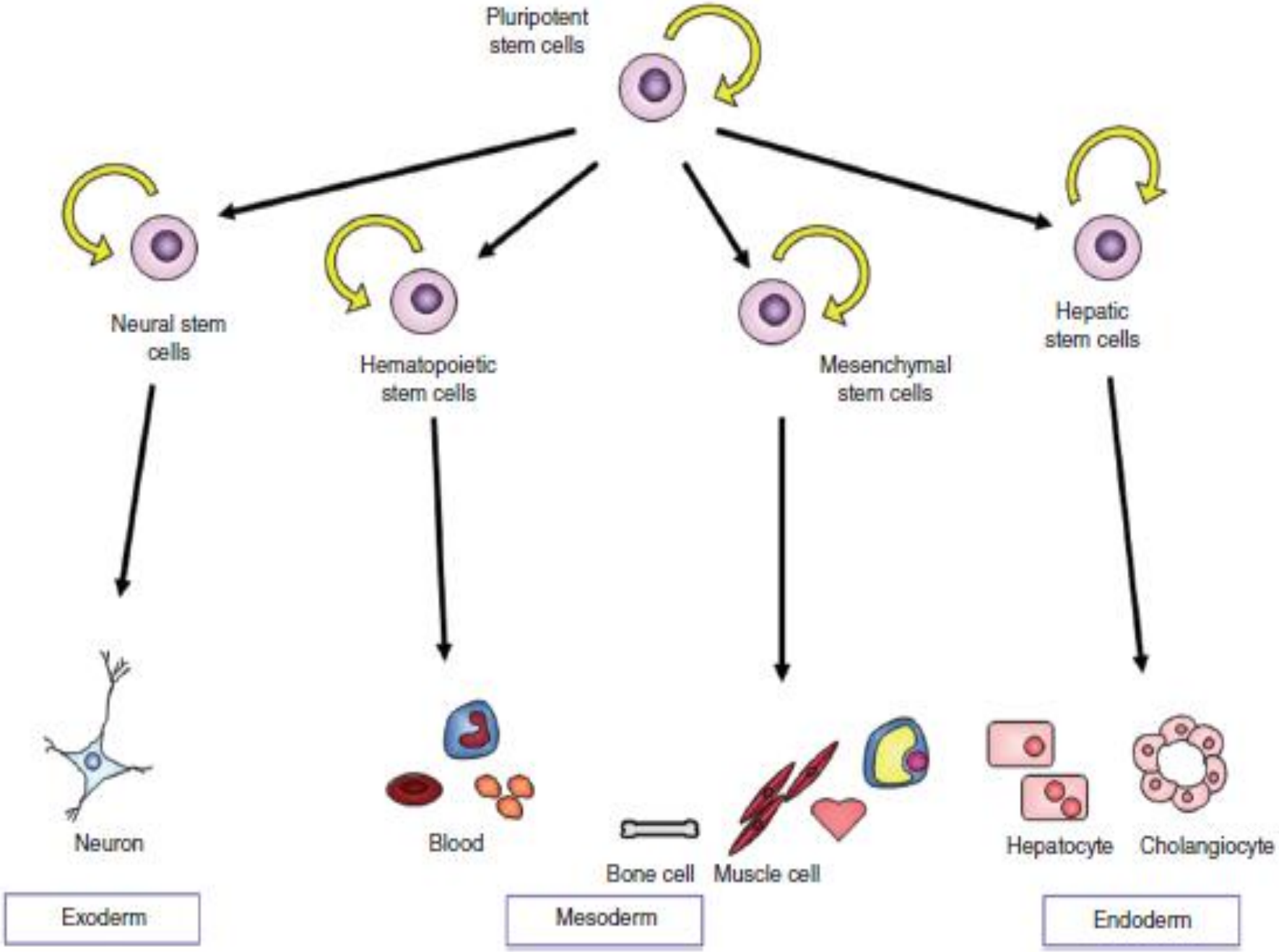
Fetal stem cells

Adult stem cells

- Hematopoietic stem cell, HSC
- mesenchymal stem cell, MSC
- neural stem cell, NSC
- hepatic stem cell

Neonatal stem cells

derived from the umbilical cord tissue or blood


Pluripotent stem cells
Neural stem cells
Hematopoietic stem cells
Mesenchymal stem cells
Hepatic stem cells
Neuron
Blood
Bone cell
Muscle cell
Hepatocyte
Cholangiocyte
Exoderm
Mesoderm
Endoderm

Types of Stem Cells (potency)

Stem cell type	Description	Examples
Totipotent	capable of generating any cell in the body, including those of the extra-embryonic tissues and the fetal tissues of the placental unit	the zygote and cells from the very early embryonic stages
Pluripotent	can give rise to all the 3 germ layers, in principle the whole organism	Embryonic stem cells
Multipotent	capable of giving rise to cell lineages of 2 different germ layers	Fetal tissue, cord blood, and adult stem cells
Unipotent	responsible for the ongoing renewal within a specific organ / tissue	residing stem cells of certain adult tissue (skin, liver, intestine)

Surface Markers

- Molecular markers in stem cells have been analyzed by florescence –activated cell sorting (FACS)
- Although functions have yet to be ascertained for many of these early markers, their unique expression pattern and timing provide a useful tool to identify & isolate stem cells
- The characteristics of stemness can be difficult to prove due to the lack of specific molecular or genetic markers of stem cell properties; therefore, the definition of stemness is currently, to a large degree, hypothetical

Surface Markers contd..

Embryonic Stem Cells

- **Oct-4(**DNA-binding protein that activates gene transcription via a *cis*-element containing octamer motif) and **SSEAs** (Stage Specific Embryonic Antigens)

Hematopoietic Stem Cells

- cluster of differentiation (CD)14, CD34, & CD45

Mesenchymal stem cells

-CD73,CD90, and CD105

Hepatic stem cells

-Major markers include EpCAM, E-cadherin, CD133,CD29

Neural stem cells

Nestin, PSA-NCAM (Polysialic acid-neural cell adhesion molecule), p75 Neurotrophin R (NTR)

Ethical Issues in Stem Cell Research

- Stem cell research offers great promise for understanding basic mechanisms of human development and differentiation, as well as the hope for new treatments for diseases such as diabetes, spinal cord injury, Parkinson's , and myocardial infarction .
- However, human stem cell (hSC) research also raises sharp ethical and political controversies.
- The derivation of pluripotent stem cell lines from oocytes and embryos is fraught with disputes regarding the onset of human personhood and human reproduction.
- Several other methods of deriving stem cells raise fewer ethical concerns.

Ethical issues at different phases of stem cell research

Phase of research	Ethical issues
Donation of biological materials	Informed and voluntary consent
Research with hESCs	Destruction of embryos Creation of embryos specifically for research purposes 1. Payment to oocyte donors 2. Medical risks of oocyte retrieval 3. Protecting reproductive interests of women in infertility treatment
Use of stem cell lines derived at another institution	Conflicting legal and ethical standards
Stem cell clinical trials	Risks and benefits of experimental intervention Informed consent

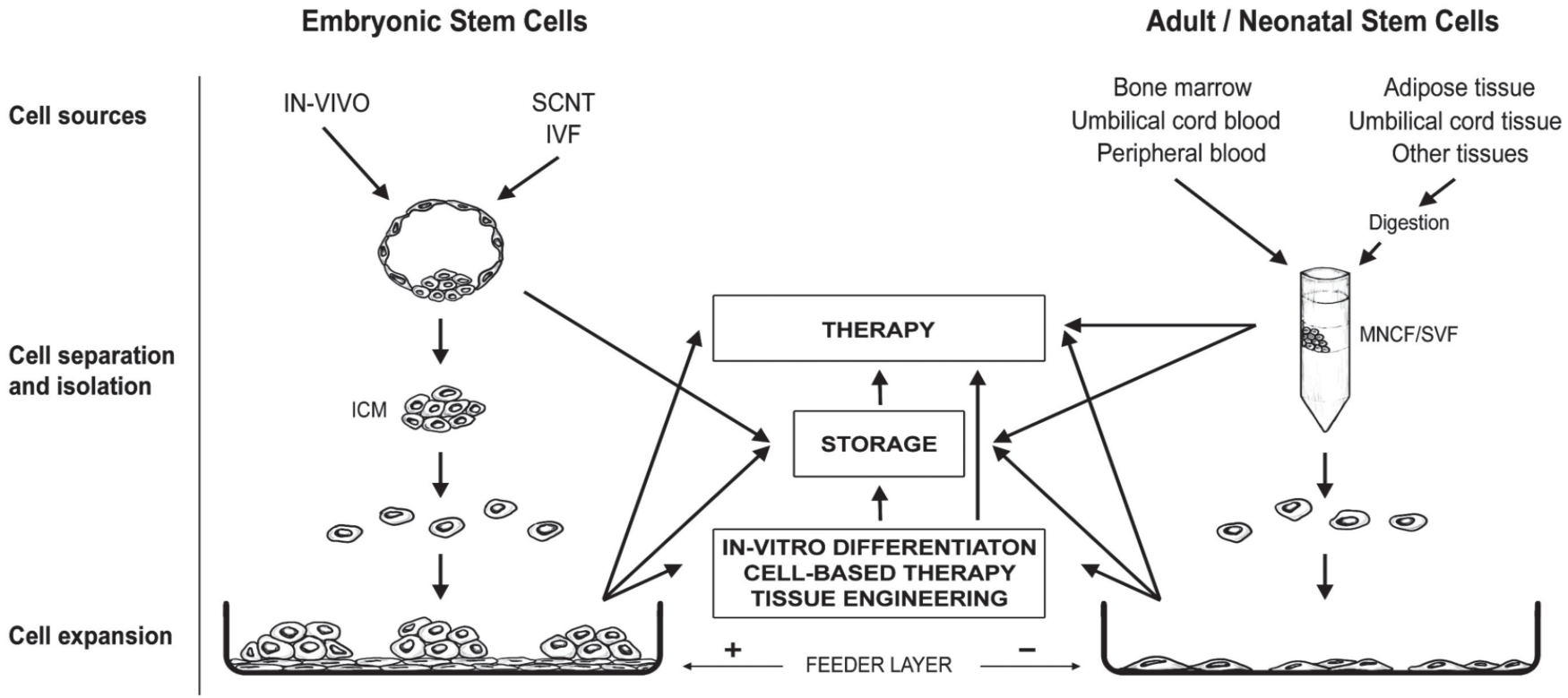
Stem cell sources & isolation

- **Embryonic stem cells** most commonly originate and isolated from the ICM of the blastocyst. hESC have also been derived from single blastomeres - then cultured in tissue dishes containing a mitotically inactivated feeder cell layer.
- **Fetal, adult, & neonatal stem cells** are isolated by separating out the mononuclear cell fraction from the tissue of interest.

With the appropriate culture medium, colonies of adherent cells are noted within days to weeks

Induced pluripotent stem (iPS) cells

- Most recently, mouse skin fibroblasts have been ‘induced’ to become pluripotent stem cells with similar potency as embryonic stem cells.
- These cells were produced by transfecting skin fibroblast with the genes coding for Oct 3/4, Sox 2, c-Myc, Klf4 under embryonic stem cell culture conditions and have been named induced pluripotent stem (iPS) cells
- Development of human iPS accelerates the research of stem cell biology, leading to regenerative medicine
- also as a tool to study the mechanisms of human diseases & assess efficacies and side effects of newly developed drugs
- However iPS have several problem to resolves, one of them is tumorigenesis



Stem cells can be derived from embryonic, fetal, neonatal, and adult tissues.
- Blastocysts can be produced *in vivo* after natural or artificial insemination or *in vitro* by somatic cell nuclear transfer (SCNT) or by *in vitro* fertilization (IVF) techniques.
-Adult and neonatal stem are isolated from the mononuclear cell fraction (MNCF) or stromal-vascular fraction (SFC) depending on the cell source.

Homing, engraftment, and niche

Treatment of various hereditary diseases relies on these 3 concepts:

- Homing describes the observation that some stem cells seed in preferred tissues after iv injection
- Engraftment - ability of stem cells to invade and be incorporated into tissues & organs
- “niche” refers to the local environment of the stem cells, which has crucial regulatory functions due to largely unknown factors, to maintain stemness and promote differentiation.

Allogenic hematopoietic stem cells from either bone marrow or umbilical cord blood are injected iv to restore normal myeloid function, erythroid function, or both. The injected stem cells home to the bone marrow and engraft, the niche then guides the stem cells towards hematopoietic cell lineages

Immunogenicity (alogenic SCs)

- Immune rejection, especially in graft versus host disease, is a valid concern when **allogenic cells** rather than **autologous cells** (from the same individual) are used
- Autologous stem cells might not always be feasible, due to donor site morbidity, in cases of hereditary diseases.
- Allogenic, hematopoietic bone marrow-derived stem cells generally need to be tissue-matched to the recipient , carry the risks of any allogenic grafts of transmitting infectious and noninfectious disease

- Patients suffering from therapy-resistant graft-versus host disease have been treated successfully with allogenic bone marrow-derived mesenchymal stem cells.
- Based on these encouraging results, it is now speculated that bone marrow derived mesenchymal stem cells may be used as immunotherapy for inflammatory bowel disease and other inflammatory diseases

Autologous SCs

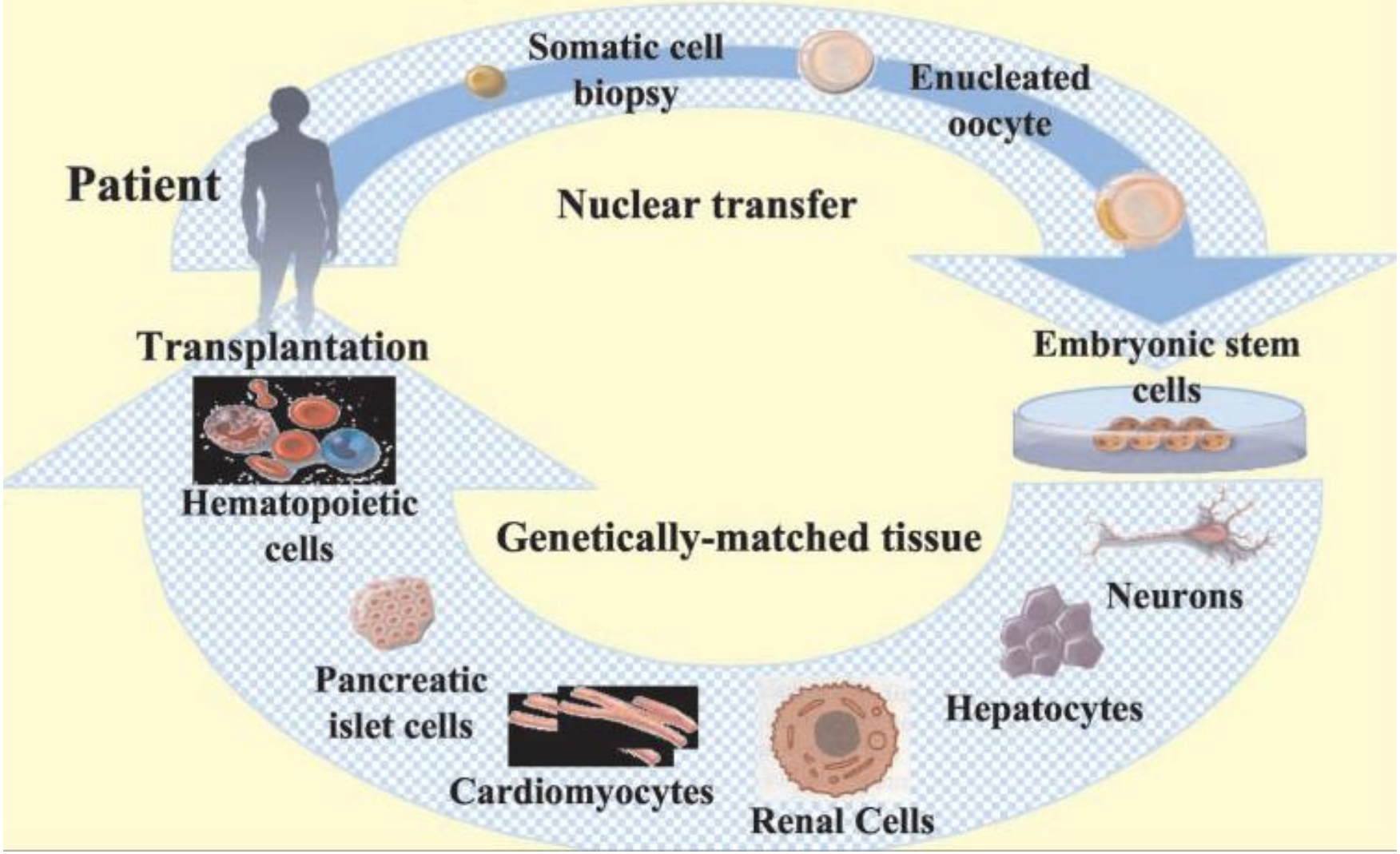
Currently, technologies might circumvent the problem of immune rejection altogether by creating autologous ESCs.

- The more established technique is somatic cell nuclear transfer. Isolation of embryonic stem cells from a cloned blastocyst reconstructed from a patient's somatic cell would be regarded as nearly autologous in nature, thereby reducing the risk of immune rejection
- The other option involves taking a biopsy of 1 or several cells from the ICM of a blastocyst without destroying the blastocyst.
- A third option to overcome immune rejection may be available with the use of iPS cells , which hold enormous potential for advancing our knowledge of basic stem cell biology and, long-term, may offer a very potent autologous stem cell source for clinical use

Tissue engineering

- A wide range of biomaterials (natural or synthetic) have been developed
- Combining stem cells with biomaterial scaffolds provides a promising strategy for engineering tissues and cellular delivery and could potentially be used as replacements for diseased or damaged tissues
- In combination with stem cells, these scaffolds have been evaluated for their suitability as potential replacements for bone, cartilage, nerve, liver and vasculature

Therapeutic Cloning Strategies



Diagnostic use of stem cells

The application of stem cells for individual (patient-specific) diagnostic, prognostic, and pharmacological purposes has very interesting prospectives

- The notion is that the individual genome determines the secretion of biological factors, surface markers, and tissue integrative properties of stem cells. All these factors together determine the overall homeostasis of the cell and have been referred to as the "**biological set point**"
- Molecular or genetic markers in mesenchymal stem cells will allow early prediction of who will suffer later in life from mesenchymal tissue dysfunctions, such as osteoporosis and osteoarthritis

- pharmacological use could be to isolate eg. MSC from a patient suffering from hepatitis and differentiate into hepatocytes *in vitro*. Then drugs (antibiotics, anti-inflammatories, etc.) could be tested in vitro for their pharmacological effects on tissue with that particular patient's biological set point.

This might optimize drug efficacy and reduce the risk of adverse effects by enabling the development of individual dosing regimes to replace the current use of general dosing regimes

Safety considerations

- The safety, both short-term and particularly long-term, of stem cell technologies is largely unknown
- Safety concerns to consider include, but are not restricted to
  - aberrant cell development
  - tissue or vehicle contamination with infectious agents of foreign biological and nonbiological substances used in the laboratory during processing of the stem cells
- Transmission of infectious diseases is of special concern if allogenous cells are being used