

Introduction to Cell Culture

What is Cell Culture?

Cell culture refers to the removal of cells from an animal, and their subsequent growth in a favorable artificial environment

The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established

Acquiring Cell Lines

- You may establish your own culture from primary cells, or you may choose to buy established cell cultures from commercial or non-profit suppliers (i.e., cell banks);
- Reputable suppliers provide high quality cell lines that are carefully tested for their integrity and to ensure that the culture is free from contaminants;
- It can be advise against borrowing cultures from other laboratories because they carry a high risk of contamination. Regardless of their source, make sure that all new cell lines are tested for mycoplasma contamination before you begin to use them.

Cell Cultures/ Lines

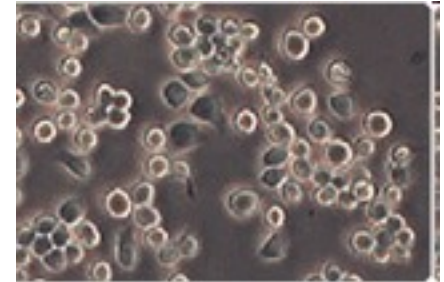
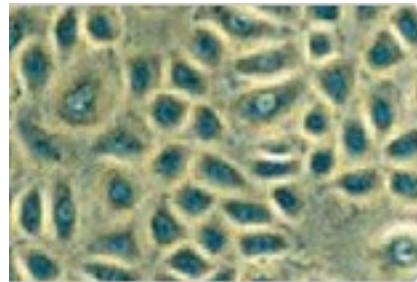
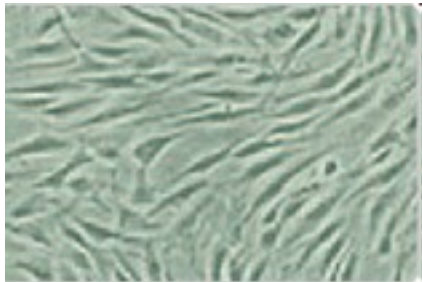
- Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach confluence). At this stage, the cells have to be subcultured (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth;
- After the first subculture, the primary culture becomes known as a cell line or subclone. Cell lines derived from primary cultures have a limited life span (i.e., they are finite; see below), and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population;
- If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cloned cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line.

<http://www.invitrogen.com/>

Mammalian Cell Morphology

Most mammalian cells in culture can be divided into three basic categories based on their morphology:

- 1) Fibroblastic (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate;
- 2) Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches;
- 3) Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface.



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Mammalian Cell Morphology

In addition to the basic categories listed above, certain cells display morphological characteristics specific to their specialized role in host.

Neuronal cells exist in different shapes and sizes, but they can roughly be divided into two basic morphological categories, type I with long axons used to move signals over long distances and type II without axons. A typical neuron projects cellular extensions with many branches from the cell body, which is referred to as a dendritic tree.

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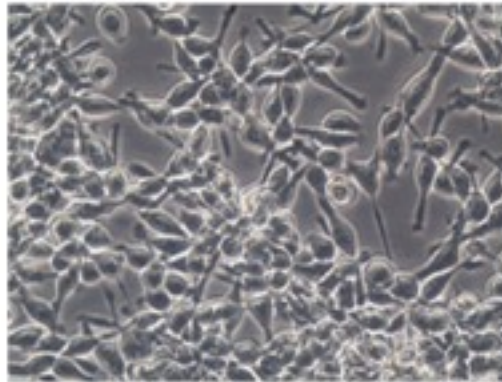
Mammalian Cell Morphology

Human Embryonic Kidney 293 cells, also often referred to as HEK 293, 293 cells, or less precisely as HEK cells are a specific cell line originally derived from human embryonic kidney cells grown in tissue culture.

1



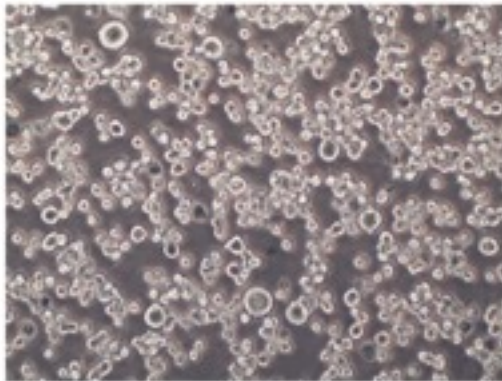
A



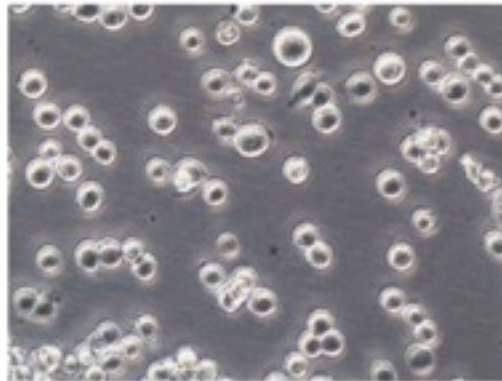
B

The phase contrast images below show the morphology of healthy 293 cells in adherent culture at 80% confluency (1) and in suspension culture (2)

2



A



B

<http://www.invitrogen.com/>

Finite vs Continuous Cell Line

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these cell lines are known as finite.

However, some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.

<http://www.invitrogen.com/>

Stem Cells

- Embryonic Stem Cells
- Induced Pluripotent Stem Cells
- Hematopoietic Stem Cells
- Mesenchymal Stem Cells
- Neural Stem Cells & Glial Precursor Cells

Selecting the Appropriate Cell Line

Consider the following criteria for selecting the appropriate cell line for your experiments:

- **Species:** Non-human and non-primate cell lines usually have fewer biosafety restrictions, but ultimately your experiments will dictate whether to use species-specific cultures or not;
- **Functional characteristics:** What is the purpose of your experiments? For example, liver- and kidney-derived cell lines may be more suitable for toxicity testing;
- **Finite or continuous:** While choosing from finite cell lines may give you more options to express the correct functions, continuous cell lines are often easier to clone and maintain;
- **Normal or transformed:** Transformed cell lines usually have an increased growth rate and higher plating efficiency, are continuous, and require less serum in media, but they have undergone a permanent change in their phenotype through a genetic transformation;

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Selecting the Appropriate Cell Line

Consider the following criteria for selecting the appropriate cell line for your experiments:

- **Growth conditions & characteristics:** What are your requirements with respect to growth rate, saturation density, cloning efficiency, and the ability to grow in suspension? For example, to express a recombinant protein in high yields, you might want to choose a cell line with a fast growth rate and an ability to grow in suspension.
- **Other criteria:** If you are using a finite cell line, are there sufficient stocks available? Is the cell line well-characterized, or do you have to perform the validation yourself? If you are using an abnormal cell line, do you have an equivalent normal cell line that you can use as a control? Is the cell line stable? If not, how easy it is to clone it and generate sufficient frozen stocks for your experiments?

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| Cell Line | Cell Type | Growth or Attachment Factor | Suggested Conc. (ng/ml) | Medium | Serum Suppl. |
|------------------|---------------------------|------------------------------------|--------------------------------|-------------------------------|---------------------|
| MCF7 | Human mammary carcinoma | EGF(a) | 100 | DMEM | 2% FBS |
| HF | Human foreskin fibroblast | EGF(a) | 2 | DMEM | 10% FBS |
| -- | Primary hepatocyte | EGF(a) | 10 | DMEM | 10% FBS |
| WB | Rat hepatic epithelium | EGF(a) | 10 | Richter's MEM | 10% FBS |
| HeLa | Human cervical carcinoma | EGF(a) | 2.5 x10 ⁴ | DMEM | 10% FBS |
| A431 | Epidermal carcinoma | EGF(a) | 2.5 x 10 ⁴ | DMEM | 10% FBS |
| -- | Primary human fibroblast | EGF(a) | 1x10 ³ | MEM Earle's | 10% FBS |

| Cell Line | Cell Type | Growth or Attachment Factor | Suggested Conc. (ng/ml) | Medium | Serum Suppl. |
|------------------|-------------------------|------------------------------------|--------------------------------|---------------------------|---------------------|
| MKN-7 | Human adenocarcinoma | EGF(a) | 50 | DMEM | 8% FBS |
| BALB/c 3T3 | Mouse fibroblast | EGF(a) | 50 | DMEM/F-12 | -- |
| TM-4 | Mouse testes epithelium | EGF(a) | 3 | DMEM/F-12 | -- |
| NRK-49F | Rat fibroblast | EGF(a) | 50 | DMEM/F-12 | -- |
| GH3 | Rat neuroblastoma | bFGF | 1 | F-12 | -- |
| BHK-21 | Baby hamster kidney | bFGF | 3 | DMEM/F-12 | 5% Calf |
| MKN-7 | Human adenocarcinoma | EGF(a) | 50 | DMEM | 8% FBS |

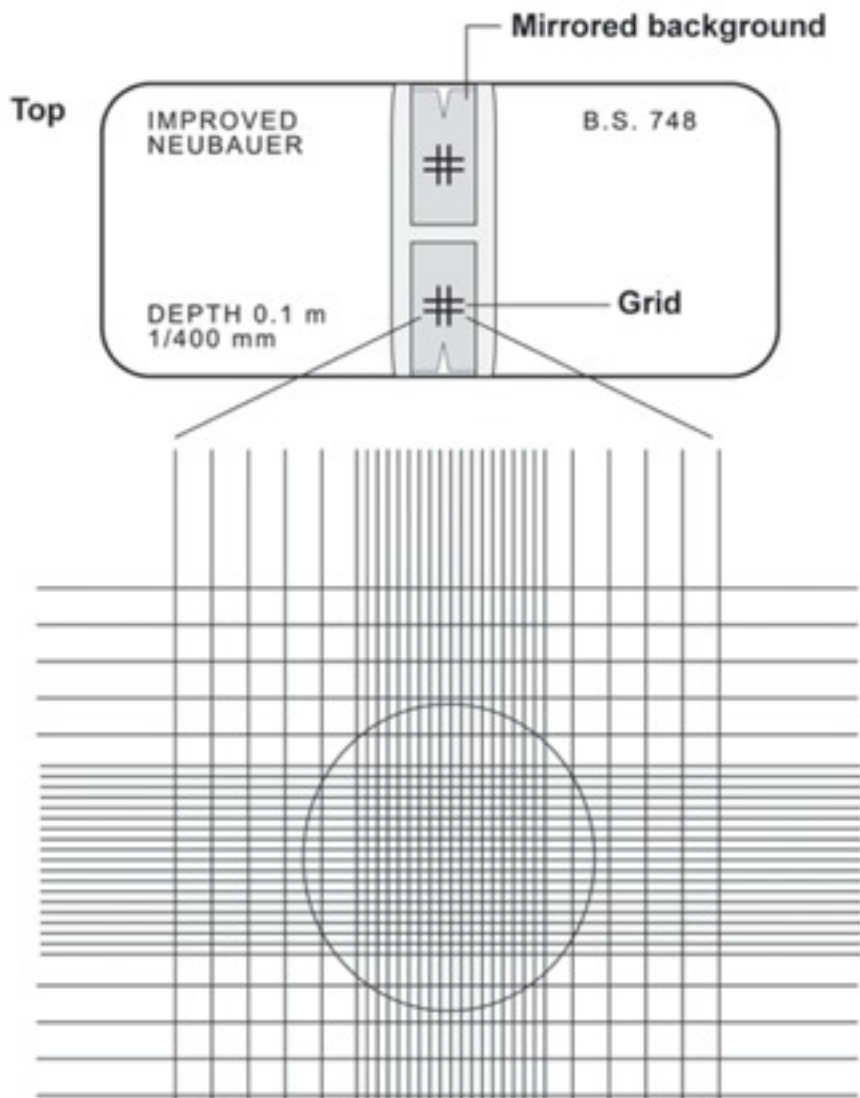
Culture Conditions

Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing the following:

- a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals)
- growth factors (FBS; defined media supplements)
- Hormones (extra)
- gases (O₂, CO₂)
- a regulated physico-chemical environment (pH, osmotic pressure, temperature)

Most cells are anchorage-dependent and must be cultured while attached to a solid or semi-solid substrate (adherent or monolayer culture), while others can be grown floating in the culture medium (suspension culture).

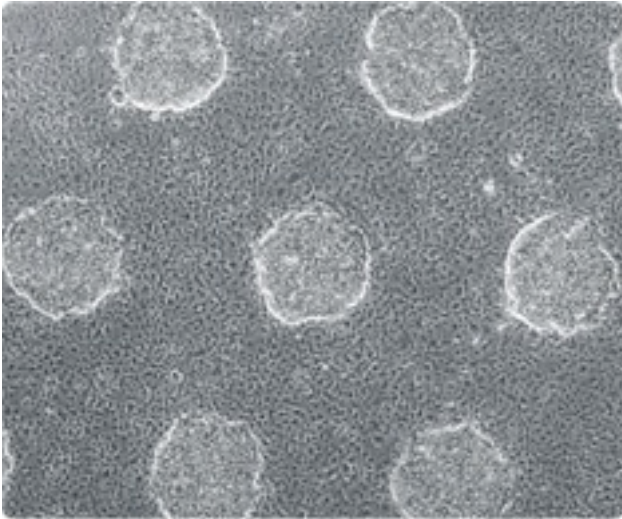
Counting Cells in a Hemacytometer



- Clean the chamber and cover slip with alcohol. Dry and fix the coverslip in position.
- Harvest the cells. Add 10 μL of the cells to the hemacytometer. Do not overflow.
- Place the chamber in the inverted microscope under a 10X objective. Use phase contrast to distinguish the cells.
- Count the cells in the large, central gridded square (1 mm^2). The gridded square is circled in the graphic below. Multiply by 10^4 to estimate the number of cells per mL. Prepare duplicate samples and average the count.

<http://www.invitrogen.com/>

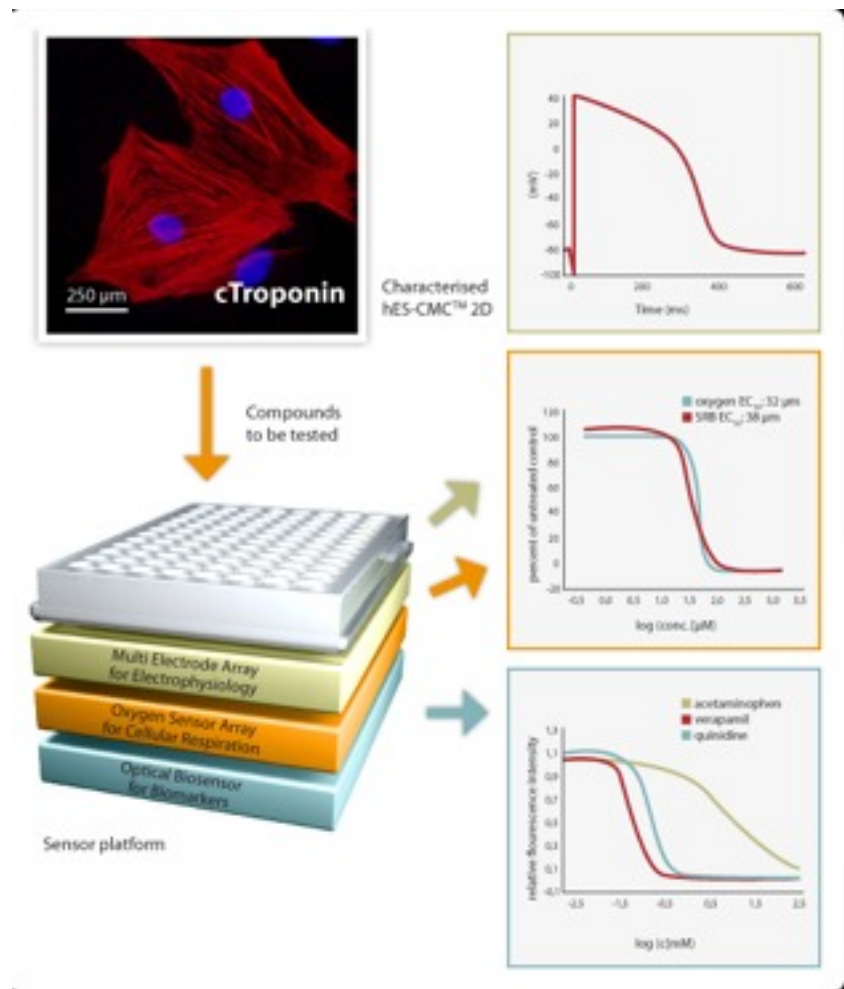
HepatoPac



- **HepatoPac Platform**
Hepregen is developing a unique, bioengineered microliver platform as a highly functional model of the liver *in vivo*.
- Microfabrication and tissue engineering technologies have been combined to create a precise, organized and miniaturized human liver model.
- Micropatterned industry-standard multiwell plates contain tiny colonies of organized liver cells surrounded by supportive stroma.

<http://www.hepregen.com/technology.html>

Human cells for screening



hES-CMC™ 2D are hES cell-derived cardiomyocyte clusters prepared as monolayers in a 96-well plate format.

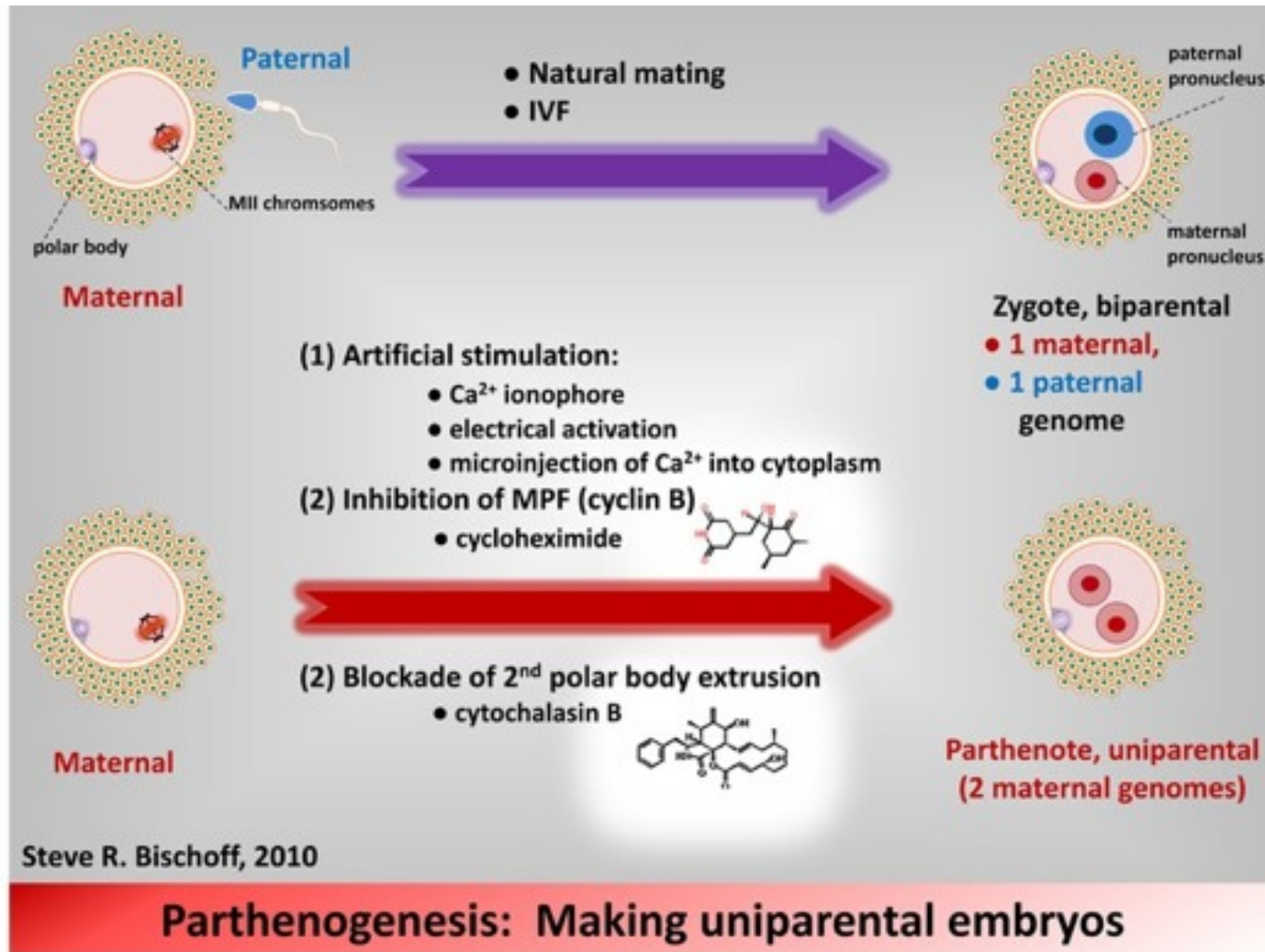
Advantages:

- Human cells
- Homogeneous source
- Native environment of multiple ion channels
- High toxicological relevance
- Genetically unmodified
- Unlimited supply
- Fresh and ready to use

Figure 1: Schematic illustration of an in vitro testing platform for hES-CMC™ 2D (adapted from Mandenius et al, 2011)

<http://www.cellartis.com/>

Use of an electrical or chemical stimulus can produce the beginning of the process of parthenogenesis



hpSC

- When used for transplant-based stem cell therapies, stem cells are likely to face the same HLA matching issues that limit solid organ allogeneic transplants and lead to immune rejection. The risk of rejection is proportional to the degree of disparity between donor and recipient cell-surface antigen-presenting proteins.
- Normally donor tissue is screened for antigens in order to determine the degree of histocompatibility with the recipient at the major histocompatibility complex (MHC). The human leukocyte antigen (HLA) system is the term used for the human MHC and represents antigens important for transplantation. Matching donor and recipient tissue for HLA antigens greatly increases the likelihood of transplant survival.
- Parthenogenetic activation of human oocytes may be one way to produce histocompatible/HLA matched cells for cell-based therapy.
- HLA homozygous hpSC, which may be histocompatible with significant segments of the human population

Stem cells

| | hpSCs | Embryonic | iPS | Adult SCs |
|---|-----------|-------------|-------------|-------------|
| Pluripotency (Suitable for any cell therapy) | Yes | Yes | Yes | No |
| Proliferation (Ease of expansion in culture) | Strong | Strong | Strong | Weak |
| Ethics (No use or destruction of viable human embryos) | Superior | Deficient | Superior | Superior |
| Manipulation (Restructuring of gene expression patterns) | Minimal | Minimal | Substantial | Minimal |
| Universal stem cells (Specific lines applicable to large population segments) | Practical | Impractical | Impractical | Impractical |

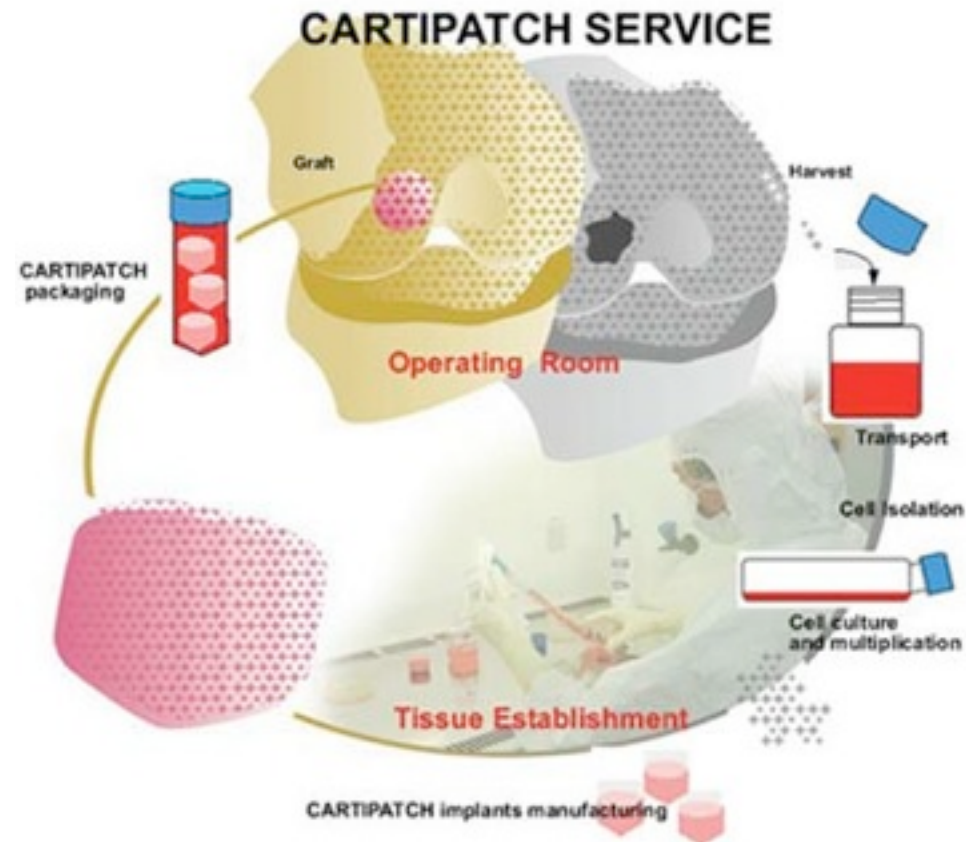
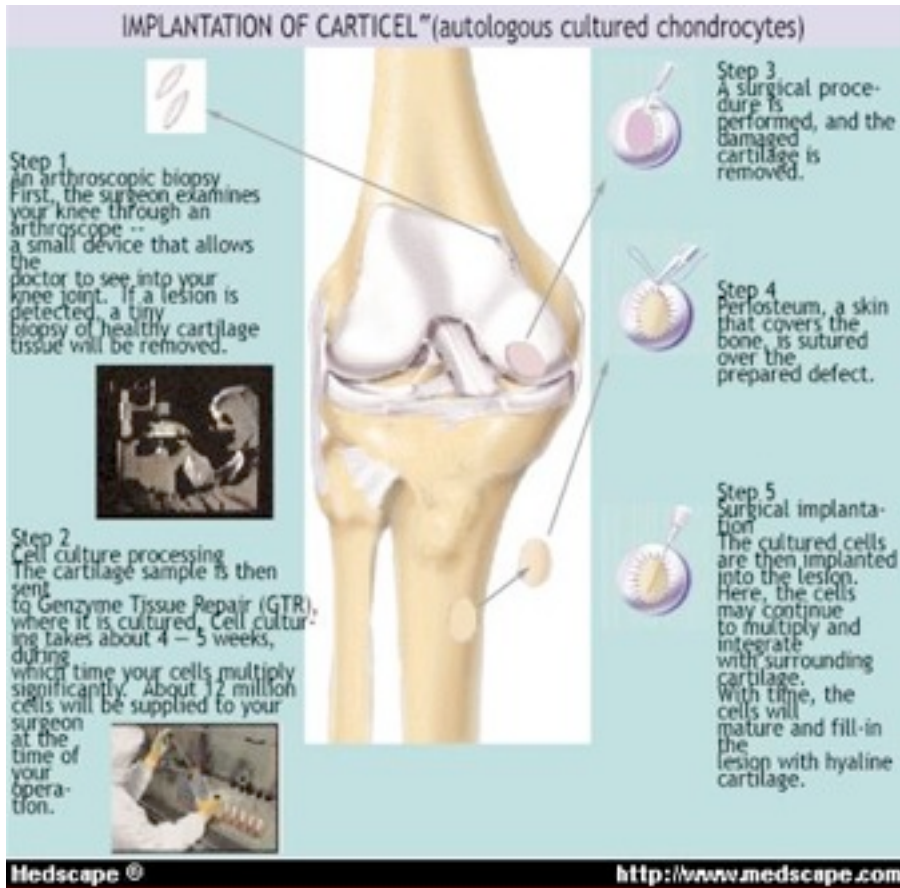
Cell based medicinal products

- substantial manipulation: during the manufacturing process the cell or tissue have been manipulated so that their biological characteristics, physiological functions or structural properties have been modified to achieve their intended function. Example of substantial manipulation: cell selection and expansion, genetic modified cells, etc.

It should be stressed that the substantial manipulation of the cell and tissue is viewed in light of its link to the effect exerted by the product.

- non-homologous use: the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor. A relevant example is represented by autologous bone-marrow derived cells which are minimally manipulated (e.g. bone-marrow aspirate) injected in the patient's heart for regeneration of the myocardium.

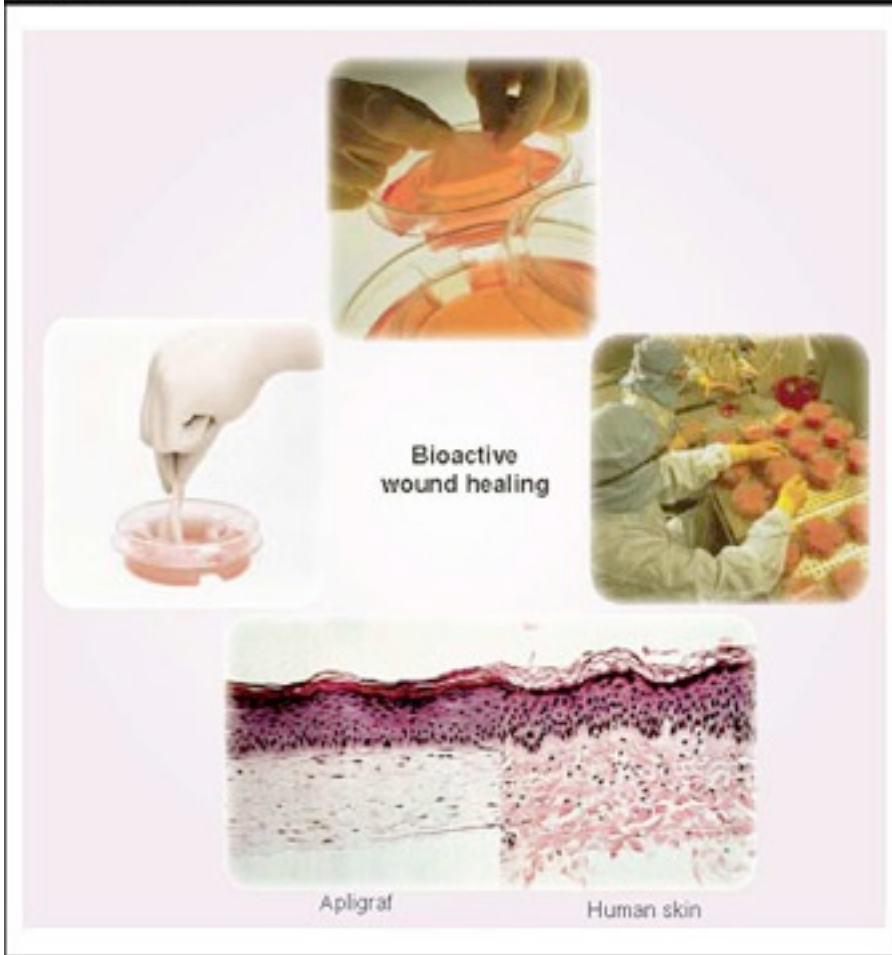
Cell based medicinal products



c. 15.000 patients

Tissue engineered medicinal products

Figure 2. Bioactive wound healing.

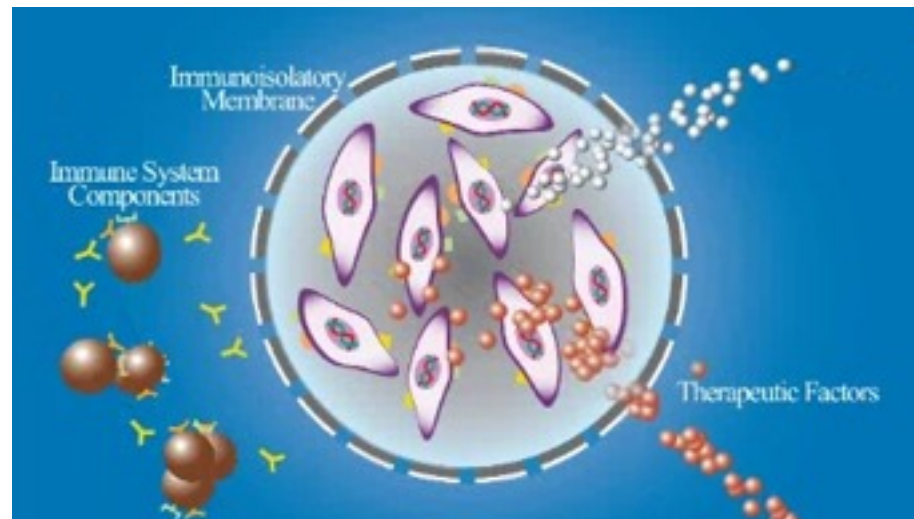
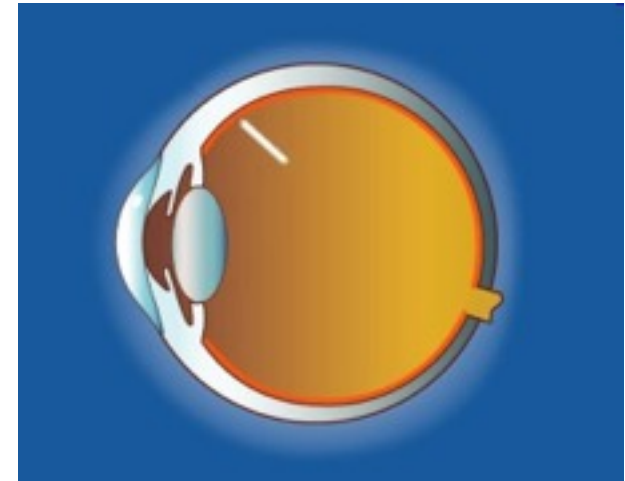
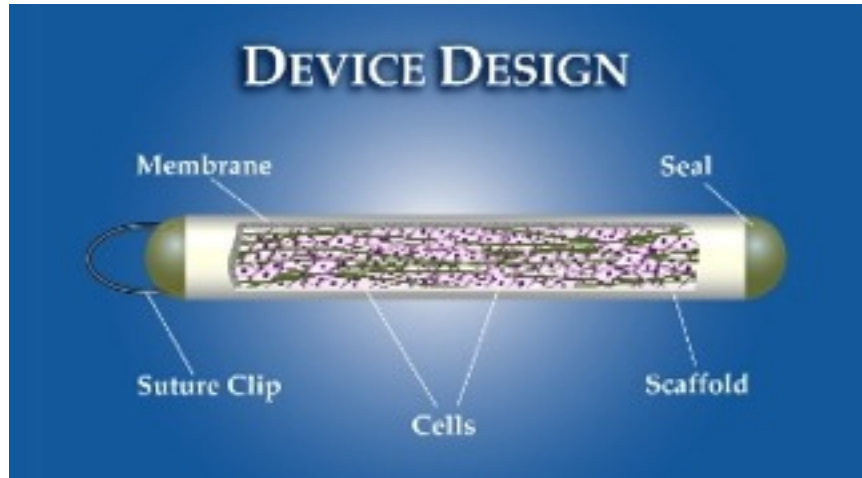


In vitro skin



Neurotech

Encapsulated Cell Technology



Neurotech

| | PRECLINICAL | PHASE 1 | PHASE 2 | PHASE 3 |
|------------------|-------------|---------|---------|---------|
| NT-501 | | | | |
| Dry AMD/GA | | | | |
| RP (early stage) | | | | |
| RP (late stage) | | | | |
| NT-503 | | | | |
| Wet AMD | | | | |

Age-related macular degeneration (AMD) is the main cause of blindness in elderly people in Western countries with a prevalence of 30% in individuals over seventy years of age. The degeneration of the macula (the central portion of the retina) causes loss of vision in the center of the visual field.

Retinitis Pigmentosa (RP) causes the progressive degeneration of rod and cone photoreceptor cells in the retina, which over time diminishes night and peripheral vision and eventually leads to blindness.

Neurotech cell cultures

Neurotech's product, NT-501, consists of encapsulated human cells genetically modified to secrete ciliary neurotrophic factor (CNTF). CNTF is a growth factor capable of rescuing dying photoreceptors and protecting them from degeneration. NT-501 is designed to continually deliver a low, safe and therapeutic dose of CNTF into the back of the eye.

Cell based medicinal products

Cell-based MPs EudraCT Clinical trial applications (3Q 2005 – 4Q 2009)

| | 3Q / 2005 | 3Q / 2006 | 3Q / 2007 | 3Q / 2008 | 4Q / |
|--|----------------|----------------|------------------|------------------|-------------------|
| 2009 | | | | | |
| Cancer immunotherapy 70 103 | | 3 | 23 | 45 | |
| Cardio-vascular therapies | 4 | 17 | 31 | 44 | 49 |
| Skin/liver/eye/diabetes/intestine/bone TE | 5 | 12 | 28 | 48 | 74 |
| Neurological | 1 | 4 | 5 | 6 | 7 |
| Lymphohistiocystosis (HLH) | - | 1 | 1 | 1 | 1 |
| AIDS | - | 1 | 1 | 1 | 1 |
| Infertility | - | 1 | 1 | 1 | 1 |
| <hr/> | | | | | |
| Products (trials) | 13 (25) | 59 (73) | 112 (132) | 171 (213) | 236 (329) |
| Data from Dr. E.Flory/Eudra CT | | | | | 246 (2010) |

Cell based medicinal products

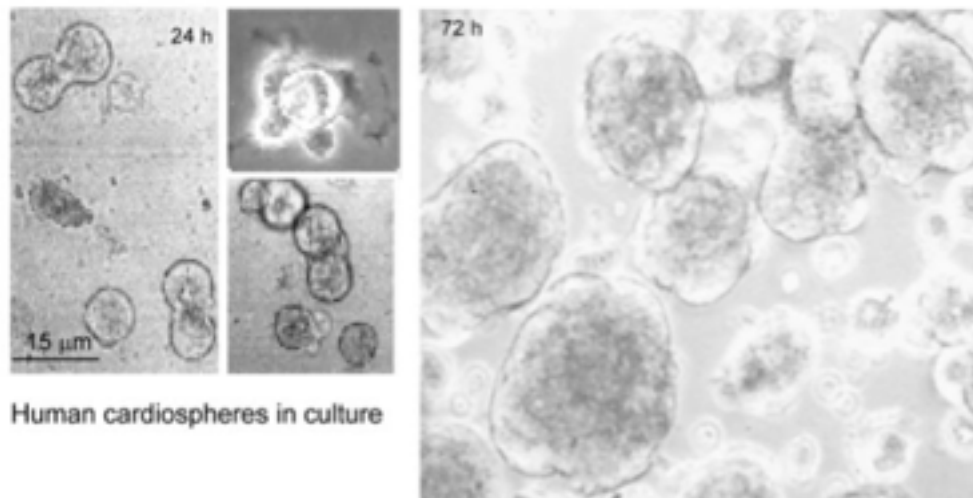
| Sun | Mon | Tues | Wed | Thurs | Fri | Sat |
|-----|-----|---|-----|-------|---------------------------------------|-----|
| 1 | 9 | 10 | 11 | 12 | 13 | 14 |
| 15 | 16 | 17 Cycle 1 Leukapheresis Procedure | 18 | 19 | 20 Cycle 1 PROVENGE infusion | 21 |
| 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| 25 | 30 | 1 Cycle 2 Leukapheresis Procedure | 2 | 3 | 4 Cycle 2 PROVENGE infusion | 5 |
| 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 13 | 14 | 15 Cycle 3 Leukapheresis Procedure | 16 | 17 | 18 Cycle 3 PROVENGE infusion | 19 |

■ Month 1 ■ Month 2

<http://www.provenge.com/dosing-administration.aspx>

The Lancet, 14th February 2012; R.R. Makkar et al: Intracoronary cardiosphere-derived cells (CDC) for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial.

Patients treated with CDCs showed reductions in scar mass ($p=0.001$), increases in viable heart mass ($p=0.01$) and regional contractility ($p=0.02$), and regional systolic wall thickening ($p=0.015$).



Circulation Research 2004, 95:911-921.
Isolation and Expansion of Adult Cardiac
Stem Cells From Human and Murine Heart.
E. Messina et al.

R&D around CADUCEUS

1. J Cell Mol Med. 2012 Jan 6.
Dose-Dependent Functional Benefit of [Human Cardiosphere Transplantation in Mice with Acute Myocardial Infarction](#).
Shen D, Cheng K, Marbán E.
2. Circulation. 2012 Jan 3.
Safety and efficacy of [allogeneic cell therapy](#) in infarcted rats transplanted with mismatched cardiosphere-derived cells.
Malliaras K, Li TS, Luthringer D, Terrovitis J, Cheng K, Chakravarty T, Galang G, Zhang Y, Schoenhoff F, Van Eyk J, Marbán L, Marbán E.
3. J Am Coll Cardiol. 2011 Jan 25;57(4):455-65.
Intramyocardial injection of autologous cardiospheres or cardiosphere-derived cells preserves function and minimizes adverse ventricular remodeling in [pigs with heart failure post-myocardial infarction](#).
Lee ST, White AJ, Matsushita S, Malliaras K, Steenbergen C, Zhang Y, Li TS, Terrovitis J, Yee K, Simsir S, Makkar R, Marbán E.

Physiological role of cells in circulation?

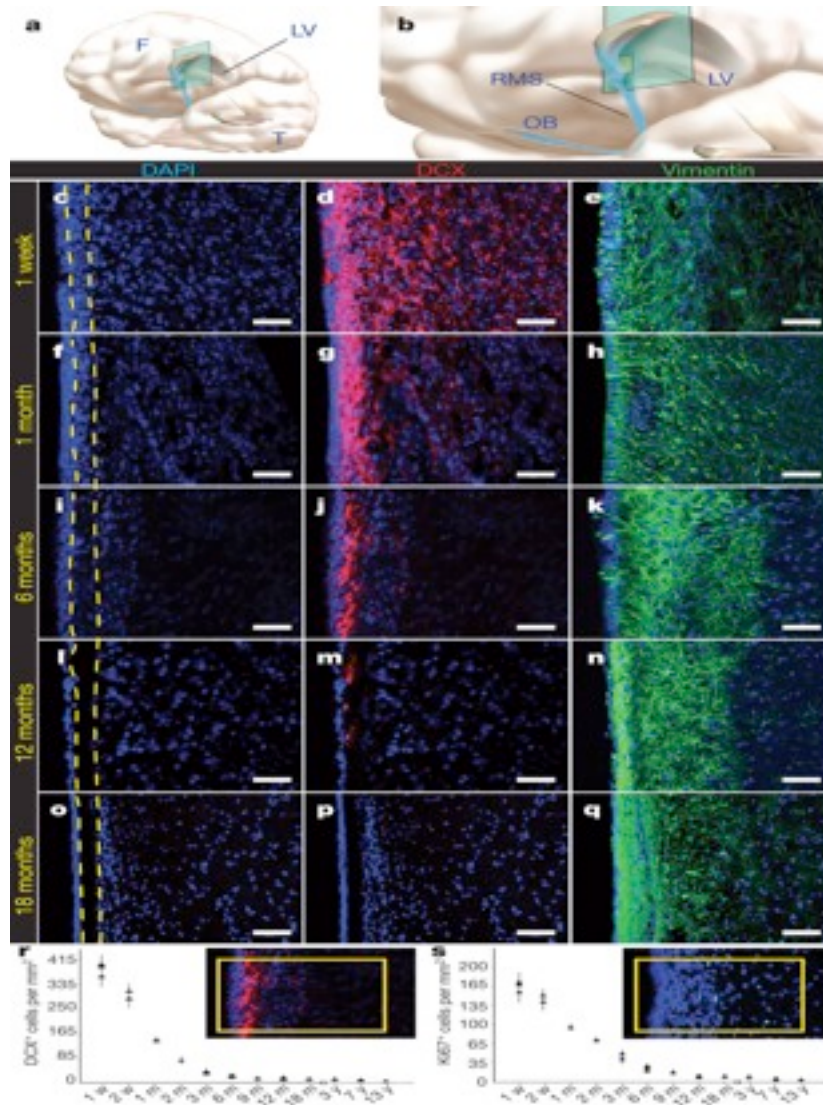
J Cell Mol Med. 2011 Aug;15(8):1726-36. Circulating stem cell vary with NYHA stage in heart failure patients. Fortini C, et al.

We have investigated the blood levels of subclasses of stem cells (SCs) [mesenchymal stem cells (MSCs), haematopoietic stem cells (HSCs), endothelial progenitor cells/circulating endothelial cells (EPCs/CECs) and tissue-committed stem cells (TCSCs)] in heart failure (HF) patients at different stage of pathology and correlated it with plasmatic levels of proangiogenic cytokines.

Corridors of migrating neurons in the human brain and their decline during infancy

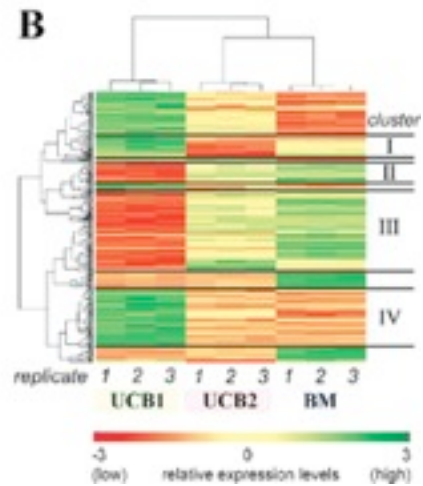
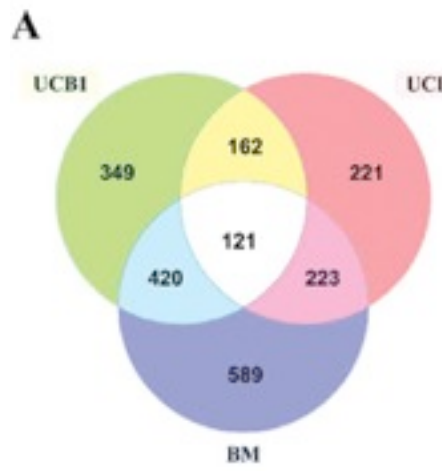
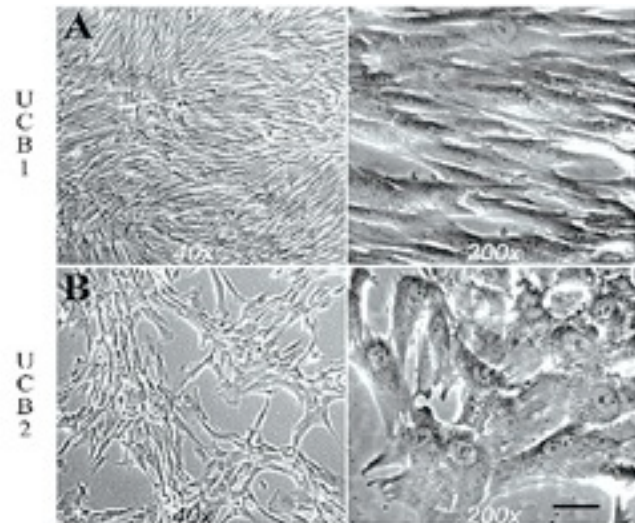
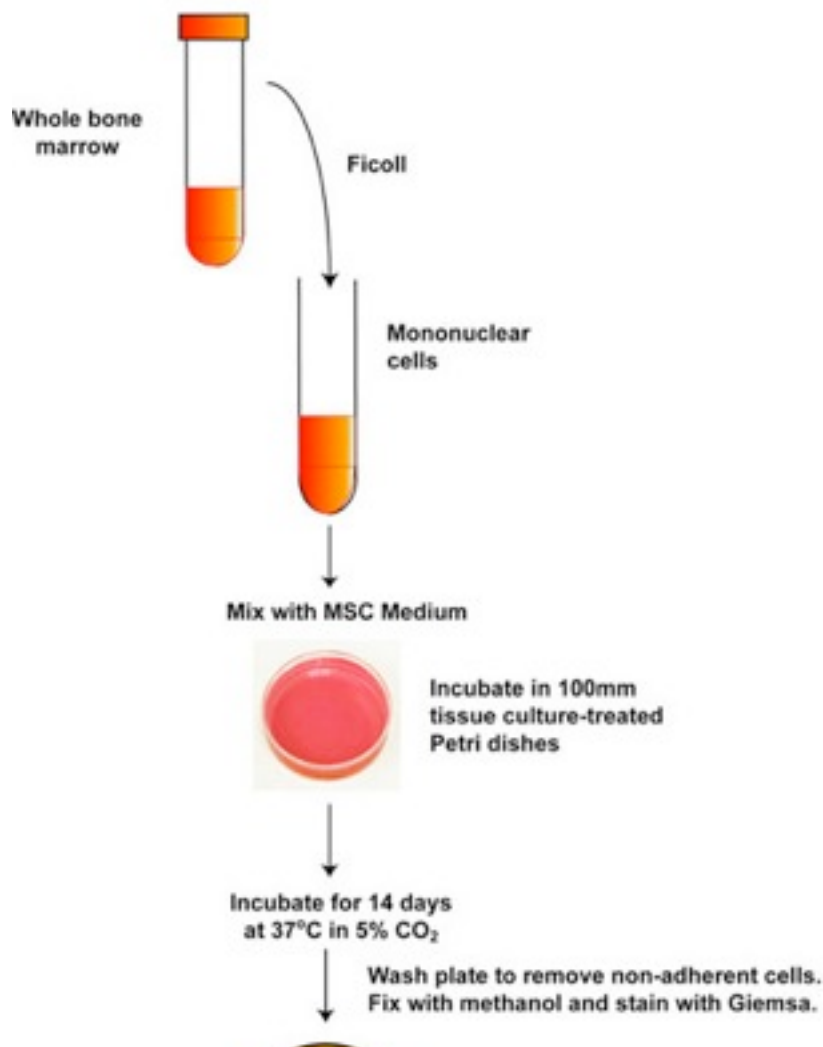
N Sanai *et al.* *Nature* (Published online 28 September 2011) doi:10.1038/nature10487

“... we describe a major migratory pathway that targets the prefrontal cortex in humans. “

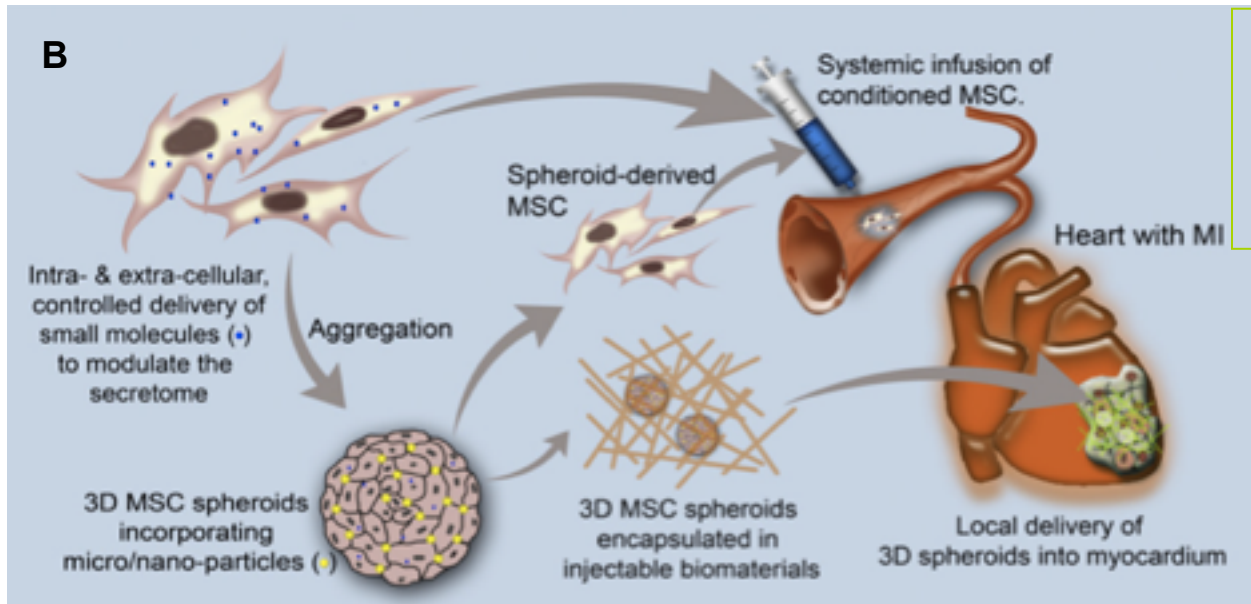
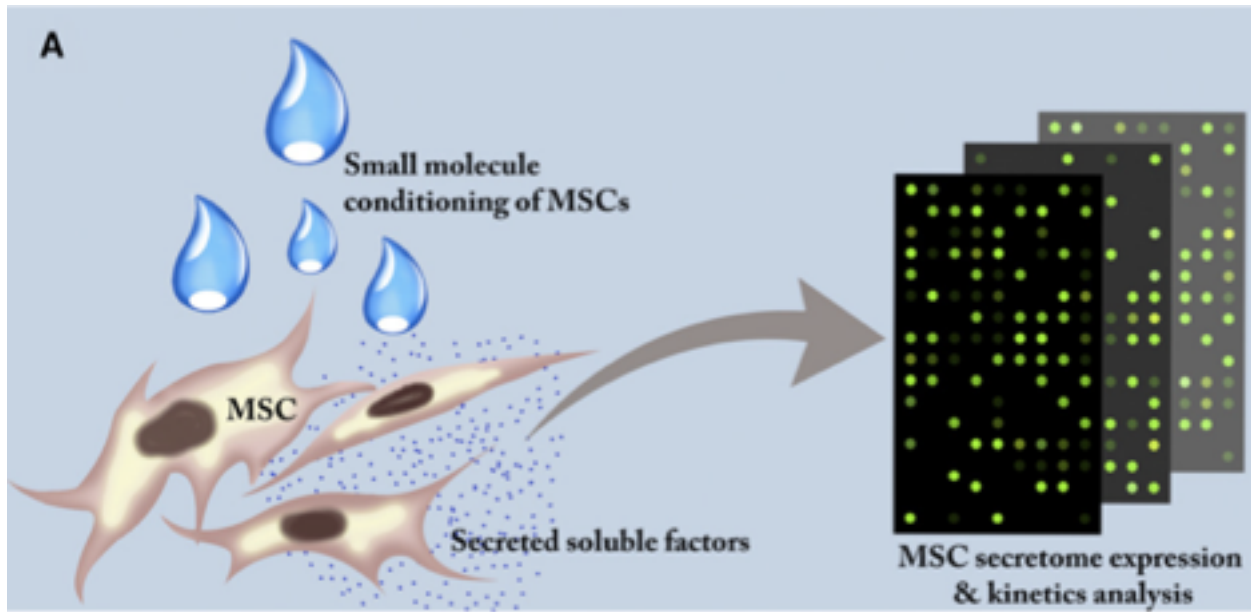


nature

MSC



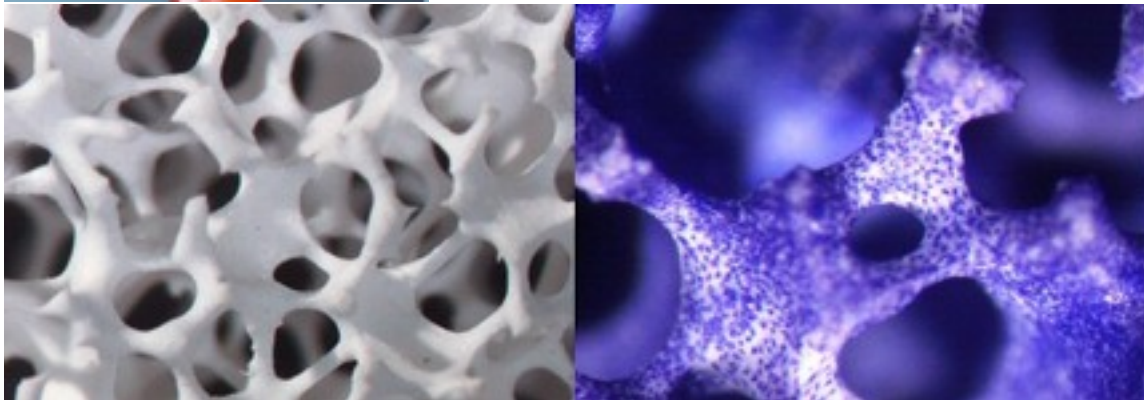
MSC



Harnessing the Mesenchymal Stem Cell Secretome for the Treatment of Cardiovascular Disease
Cell Stem Cell 10, March 2, 2012

ZellWerk GmbH

Zellwerk's Z® RP cell- and tissue culturing systems establish a unique **technological platform for fast and controlled expansion of adherent cells.** **Complying with GLP, GCP, GTP and GMP standards** Z® RP culturing systems enable users to grow cells in very high densities, inducing tissue-like organisation of cells embedded in their own extracellular matrix.

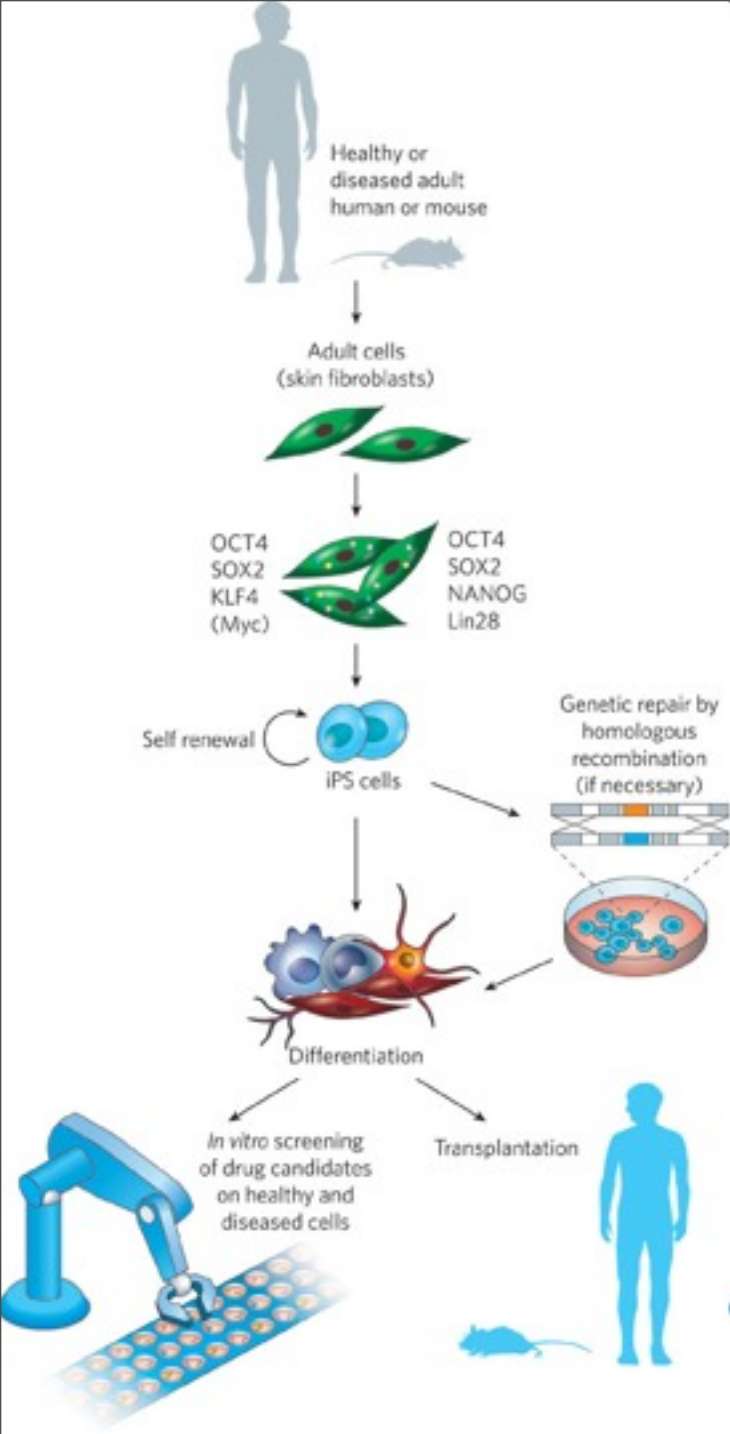


Avi: 1c



iPS

Avi: 2B, 2



| Year | Group | Strategy | Contribution |
|------|---------------------|----------------------------|--|
| 2006 | Yamanaka et al. | First to demonstrate | iPS cells were first generated using retroviruses and the four key pluripotency genes; failed to produce viable chimera. |
| 2007 | Yamanaka et al. | Different Selection Method | iPS cells were generated again using retroviruses, but this time produced viable chimera (they used different selection methods). |
| 2007 | Thomson et al. | Vector | iPS cells were generated again using lentiviruses, and again produced viable chimera. |
| 2008 | Melton et al. | Small Compound Mimicking | Using HDAC inhibitor valproic acid compensates for C-Myc. |
| 2008 | Ding et al. | Small Compound Mimicking | Inhibit HMT with BIX-01294 mimics the effects of Sox2, significantly increases reprogramming efficiency. |
| 2008 | Hochedlinger et al. | Vector | The group used an adenovirus to avoid the danger of creating tumors; however, this led to lower efficiency. |
| 2008 | Yamakana et al. | Vector | The group demonstrated reprogramming with no virus (they instead used a plasmid) |
| 2009 | Ding et al. | Proteins | Used recombinant proteins ; proteins added to cells via arginine anchors was sufficient to induce pluripotency. |
| 2009 | Freed et al. | Vector | Adenoviral gene delivery reprogrammed human fibroblasts to iPS cells. |
| 2009 | Blelloch et al. | RNA | Embryonic stem-cell specific microRNAs prompted iPS reprogramming. |
| 2011 | Morrissey et al. | RNA | Demonstrated another method using microRNA that improved the efficiency of reprogramming to a rate similar to that demonstrated by Ding. |