Master in Cellular and Molecular Biology

Medical and Cancer Genetics course

MEDICAL GENETICS

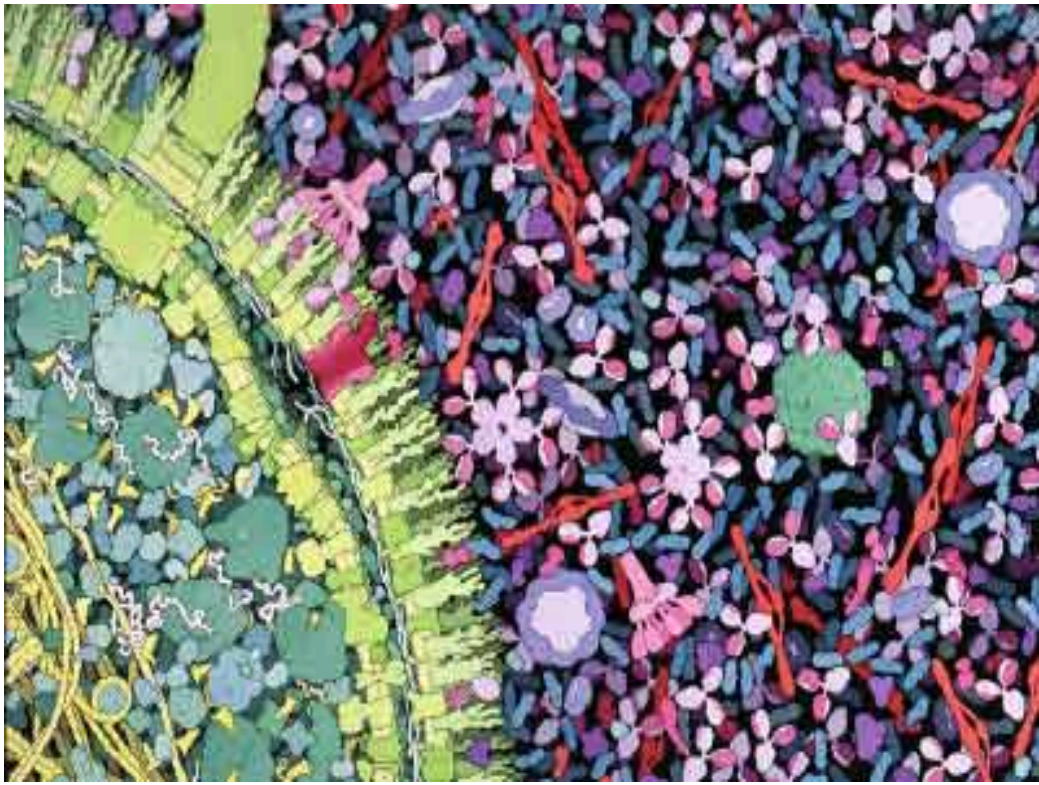
Teacher: Claudia Giachino

Lesson 6

Immunogenetics, transplants and regenerative medicine

Immunogenetics and medicine

- Introduction to immunogenetics
- Genetics of transplantation
- HLA
- Transplants
- Future perspectives



Immune Recognition by David Goodsell

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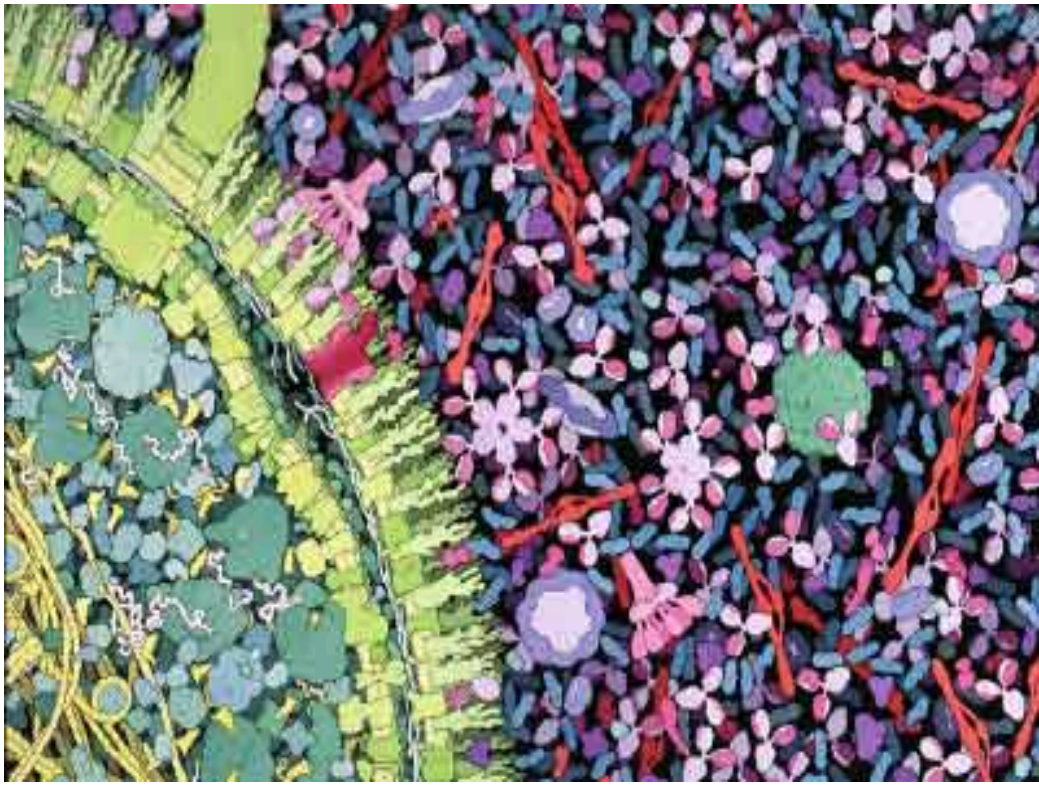


Immune Recognition by David Goodsell

Immunogenetics = immunology + genetics

- An overview of immunology
- An overview of genetics

What dangers have determined the evolution of the immune system?

The invasion of multicellular and complex organisms by:

- **MICROORGANISMS**
- **EXTRANEOUS GENES (nucleic acids)**
- **OWN CELLS MUTATED**
- **?***

Three defense strategies:

1. Shell
2. Innate immunity
3. Acquired (or adaptive) immunity

Shell:

Evoluzione di una barriera continua, elastica e resistente

Shell:

Evolution of a continuous, elastic and resistant barrier

Shell:

Efficacy depends on three mechanisms

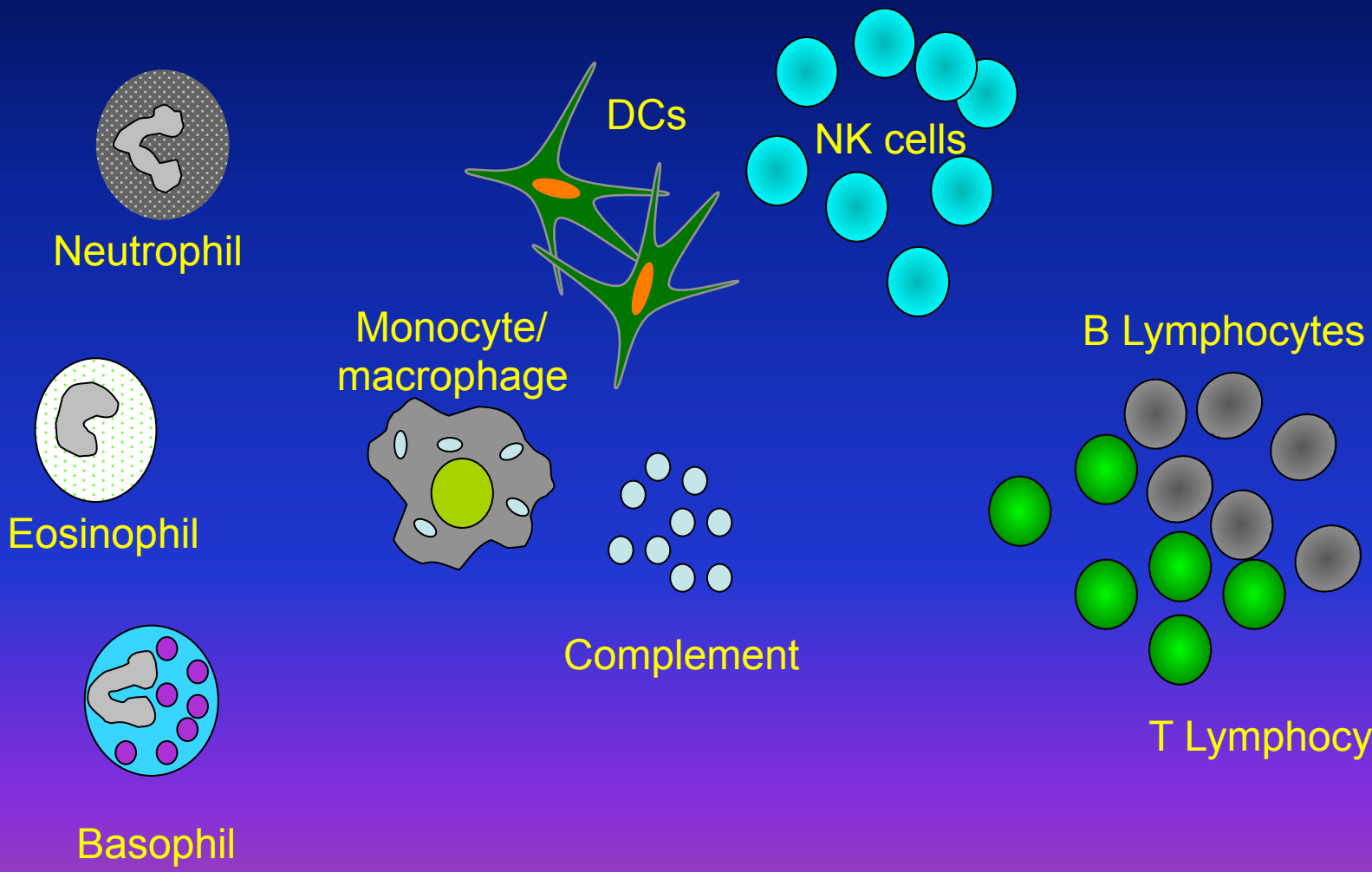
1. **Physical**
2. **Chemical**
3. **Biological**

Weaknesses:

1. The mucus membranes
   - of the gastrointestinal tract
   - of the respiratory system
   - of the genital apparatus

2. Skin
   - Trauma
   - Environmental conditions

INNATE AND ACQUIRED IMMUNITY

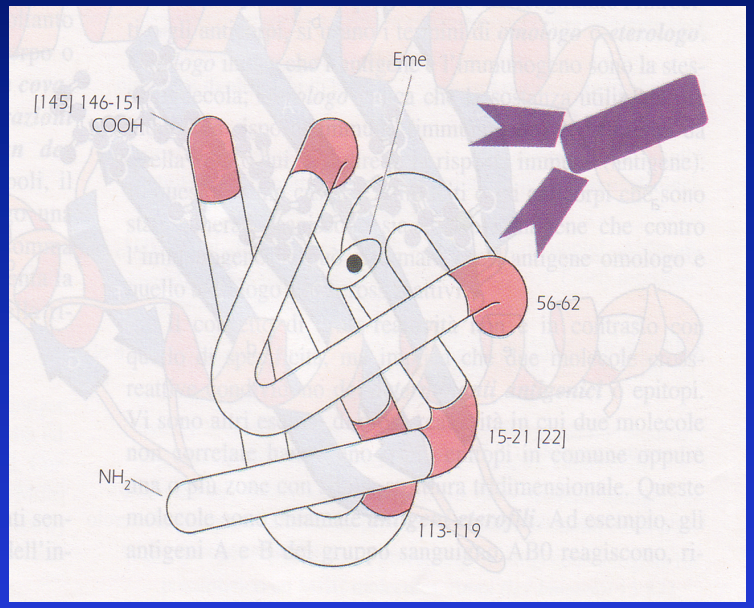


Features of acquired immune response

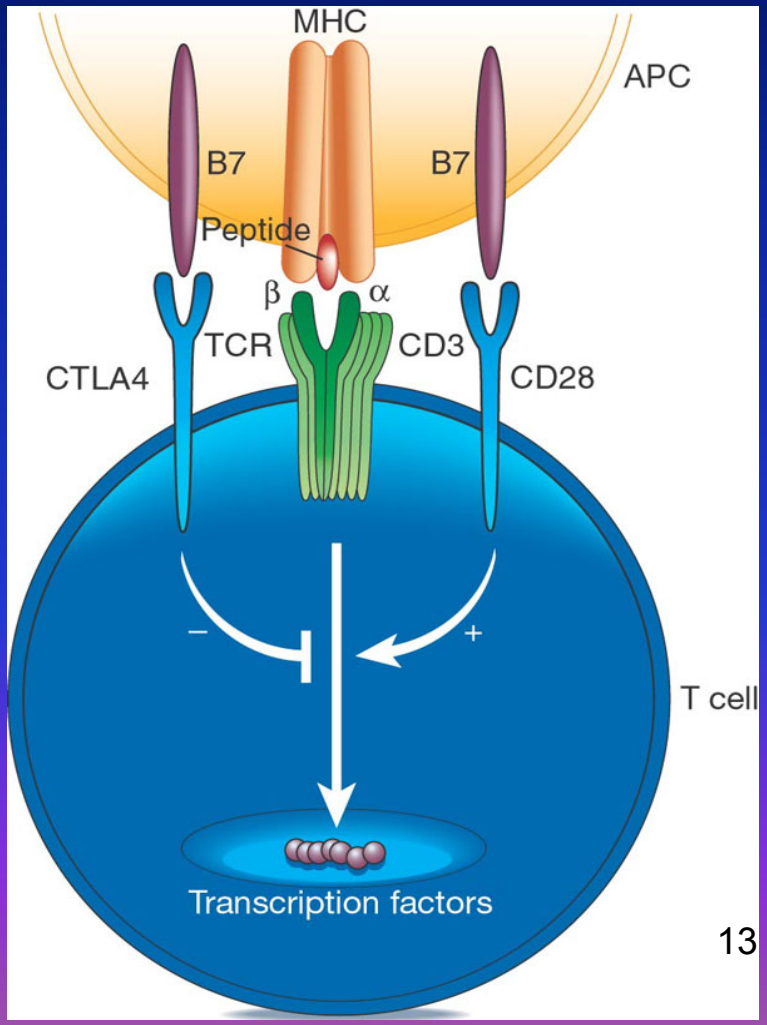
- Specificity (antigens, epitopes)
- Diversity (repertoire, over 10^{15})
- Discrimination between self and non-self
- Memory

Clonal selection theory, Jerne e Burnet, years ‘50

RECOGNITION OF ANTIGENS BY B LYMPHOCYTES



RECOGNITION OF ANTIGENS BY T LYMPHOCYTES

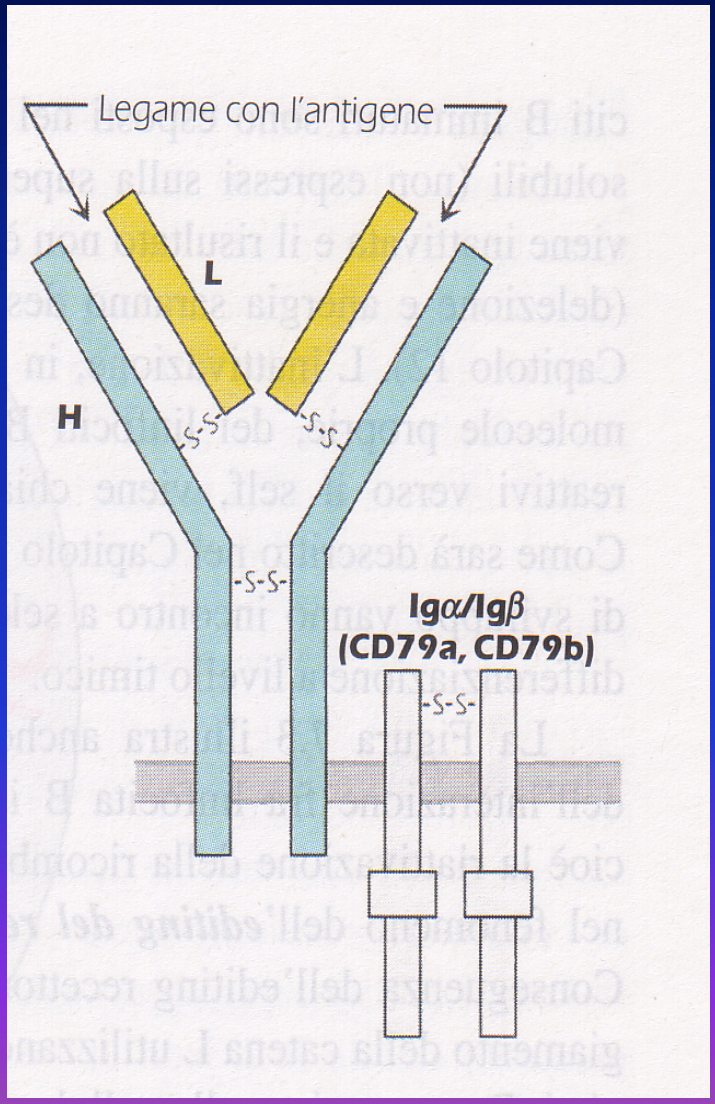


Tools with which to study the ontogeny of lymphocytes

- Analysis of cancer cell lines
- Bone marrow and fetal thymus cultures
- KO mice
- Immunodeficiencies

Discovery of the rearrangement

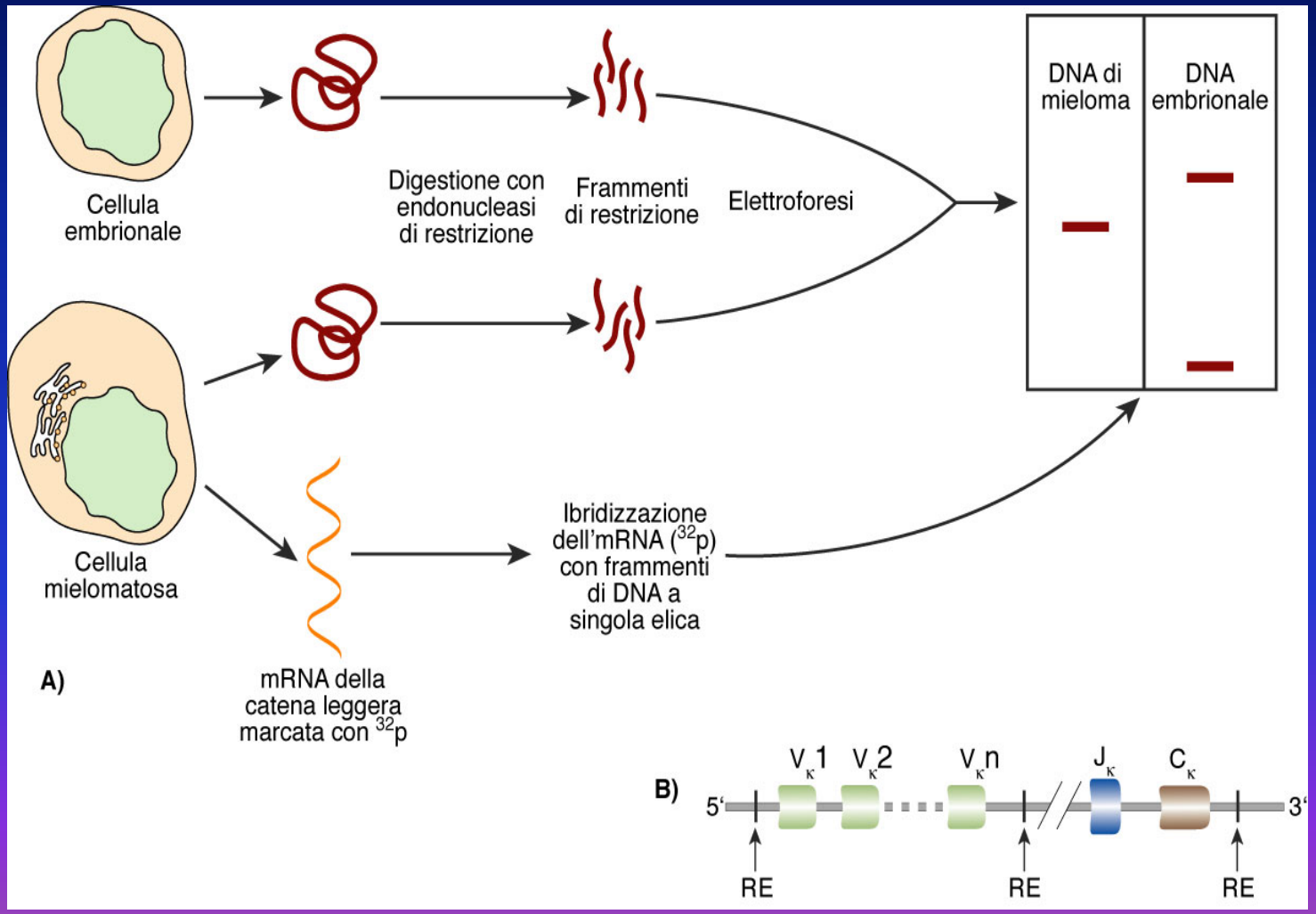
B cell receptor for antigen (BCR)



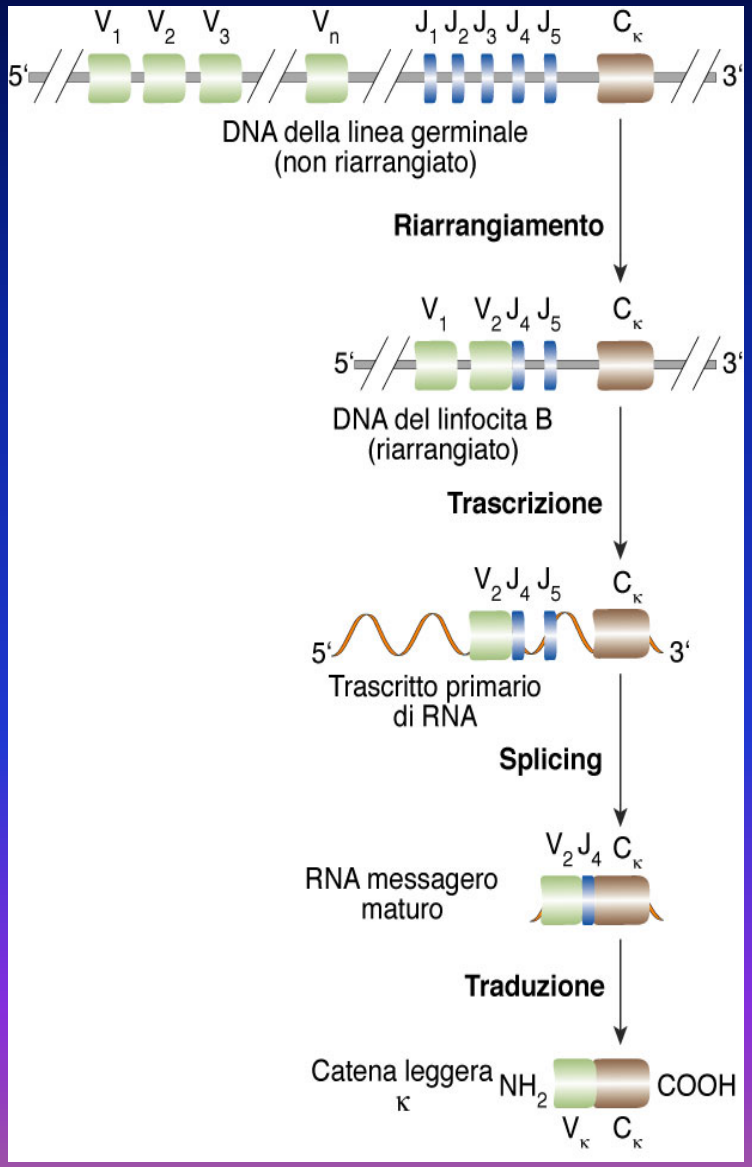
Discovery of the rearrangement of Ig genes

- Years ’50: diversified V regions, conserved C regions
- 1965, Dreyer and Bennett. Ig are encoded by two different genes
- 1976, Tonegawa. Formal proof through hybridization techniques

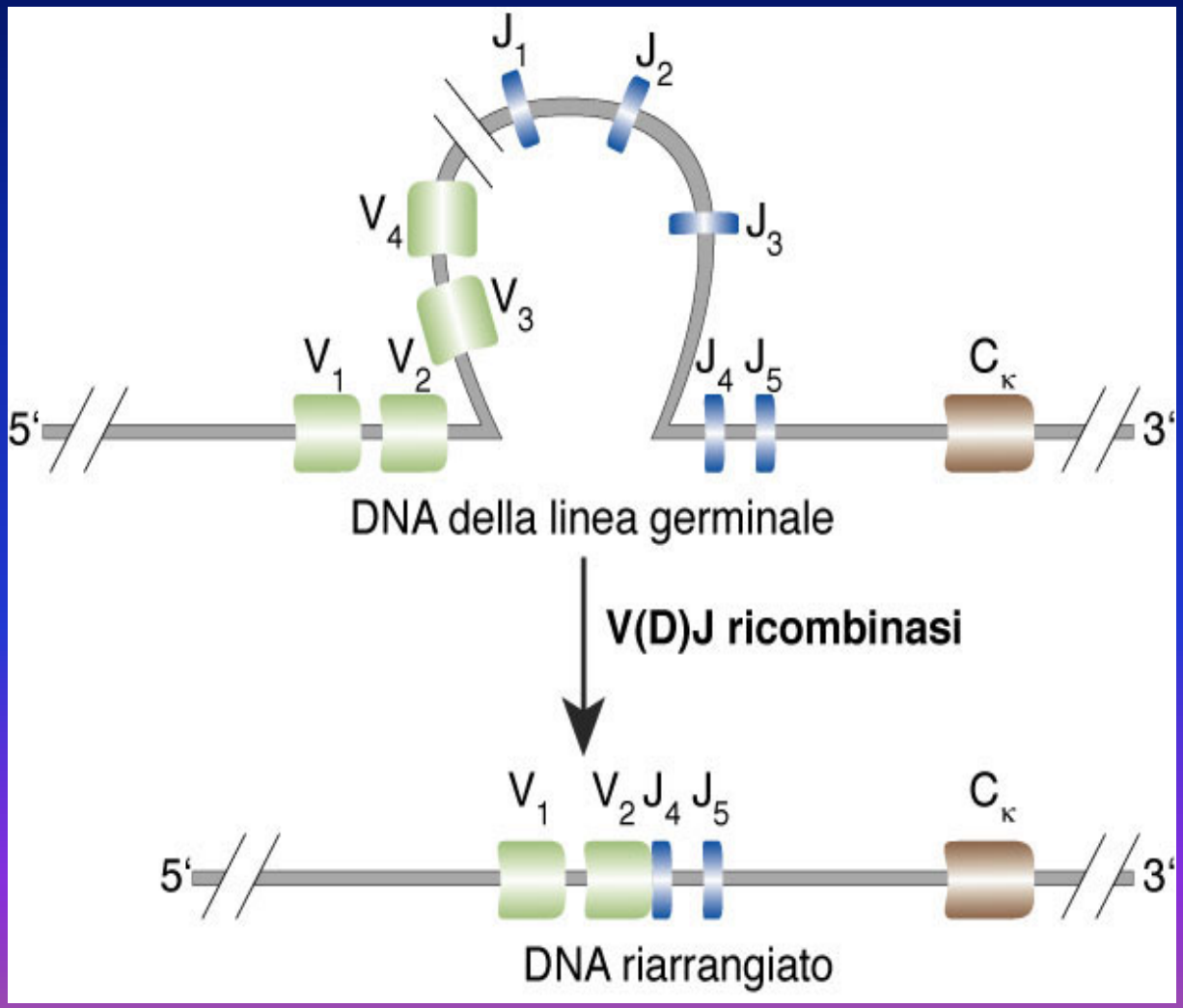
Tonegawa's experiment
(Nobel prize in 1987)



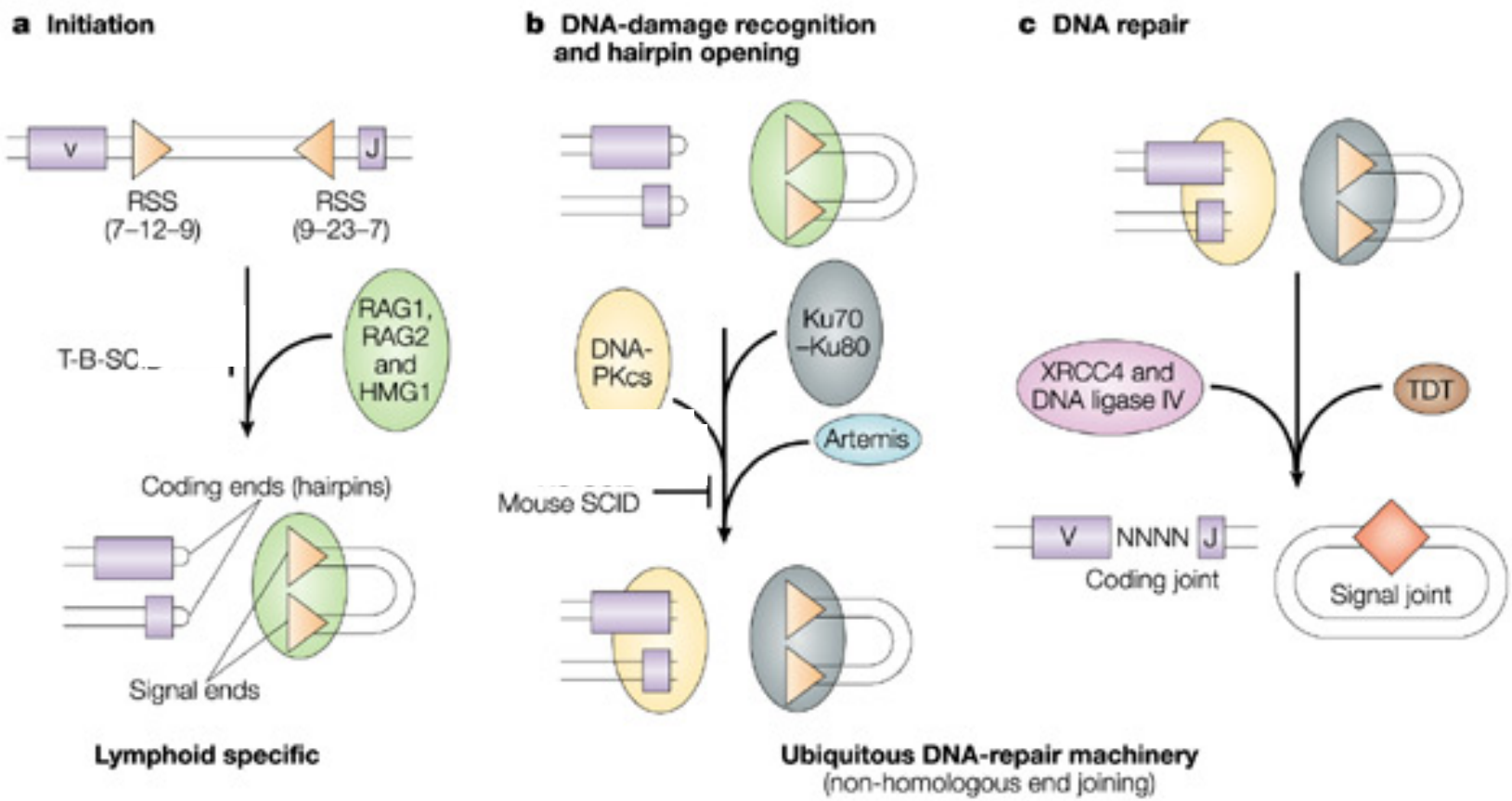
The genetic mechanisms implicated in the synthesis of an immunoglobulin



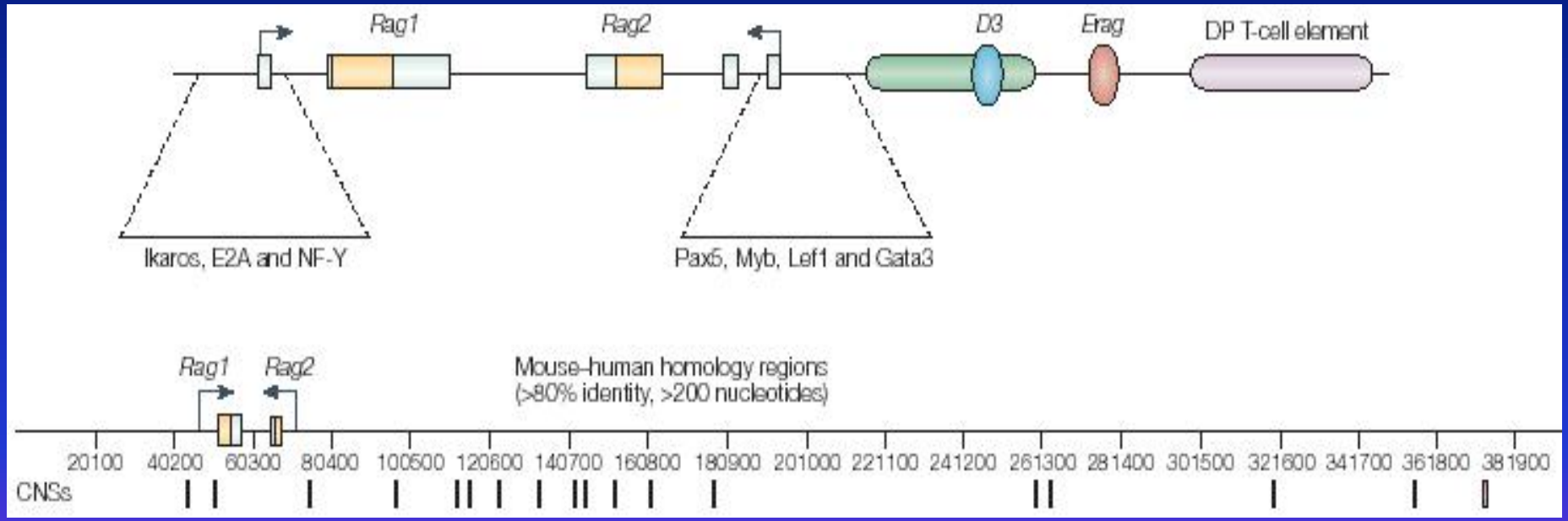
Rearrangement of the DNA encoding for an immunoglobulin



Mechanisms of rearrangement



Rag1 and Rag2 genes are both necessary and sufficient to promote the rearrangement



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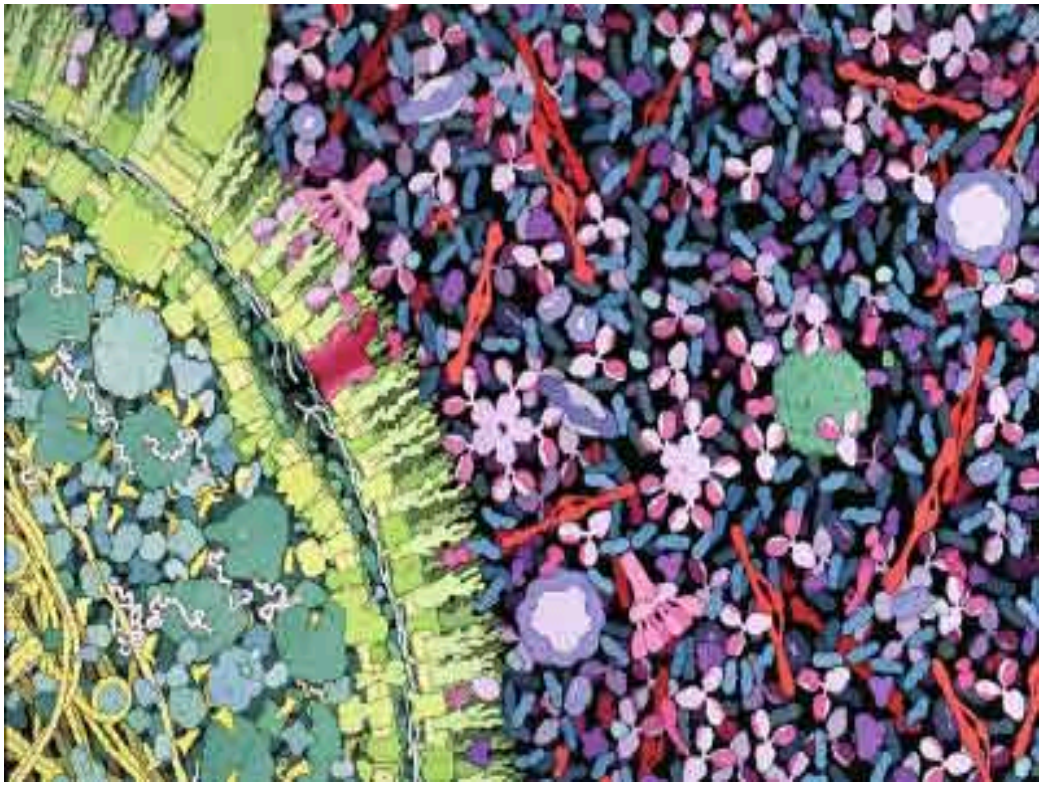


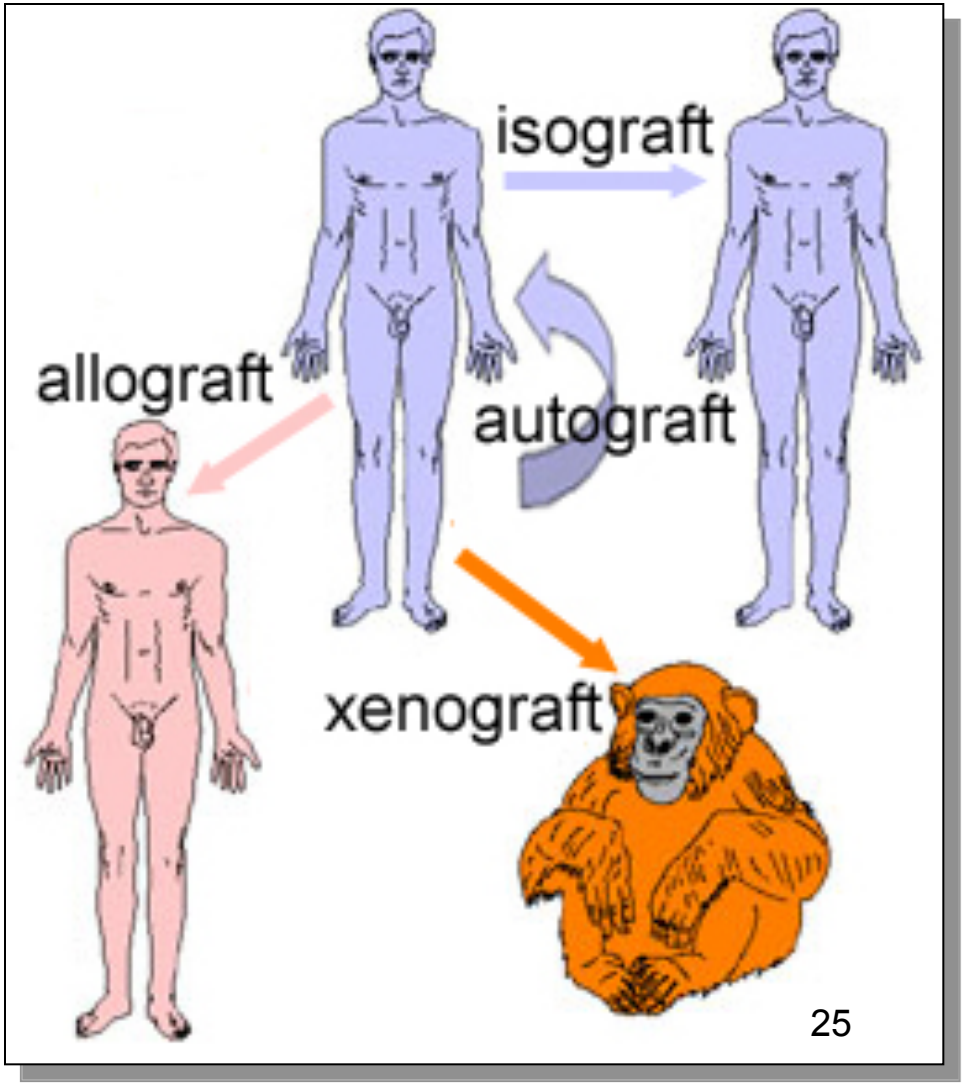
Immune Recognition by David Goodsell

GLOSSARY

- **Histocompatibility (transplantation) antigens:** Antigens on tissues and cells that determine their rejection when grafted between two genetically different individuals;
- **Major histocompatibility (MHC) antigens:** Histocompatibility antigens that cause a very strong immune response and are most important in rejection;
- **MHC complex:** Group of genes on a single chromosome encoding the MHC antigens;
- **HLA (human leukocyte antigens):** MHC antigens of man (first detected on leukocytes);
- **H-2 antigens:** MHC antigens of mouse;

Genetic barriers to transplantation

- autograft: in the same individual
- isograft: between genetically Identical individuals, *i.e.*, identical twins (inbred animals)
- allograft: between individuals of the same species
- xenograft: between individuals of different species

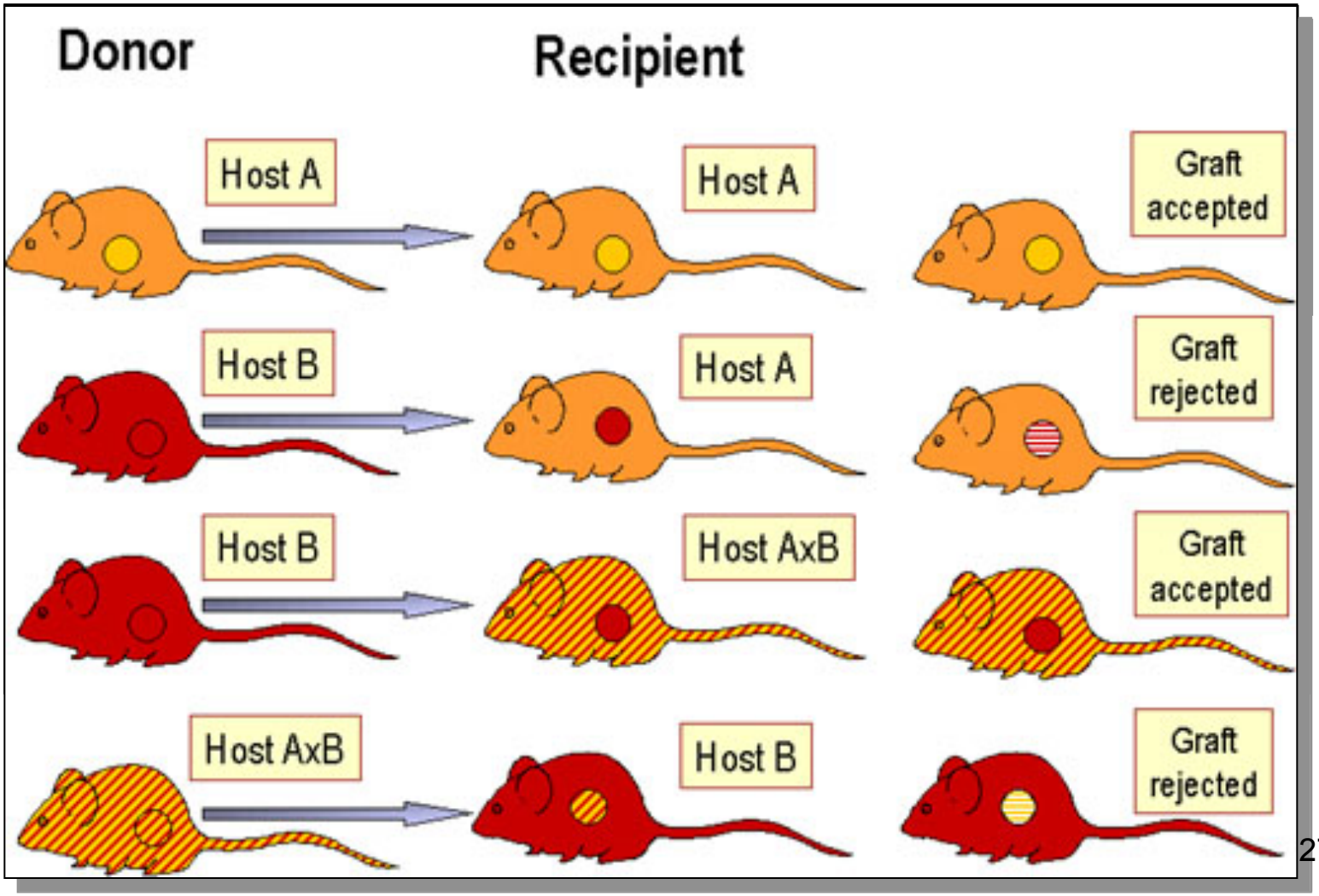


Xenograft: Grafts between members of different species (also known as heterologous, xenogeneic or hetero- grafts);

Allograft: Grafts between two members of the same species (also known as allogeneic or homo- graft);

Isograft: Grafts between members of the same species with identical genetic makeup (identical twins or inbred animals);

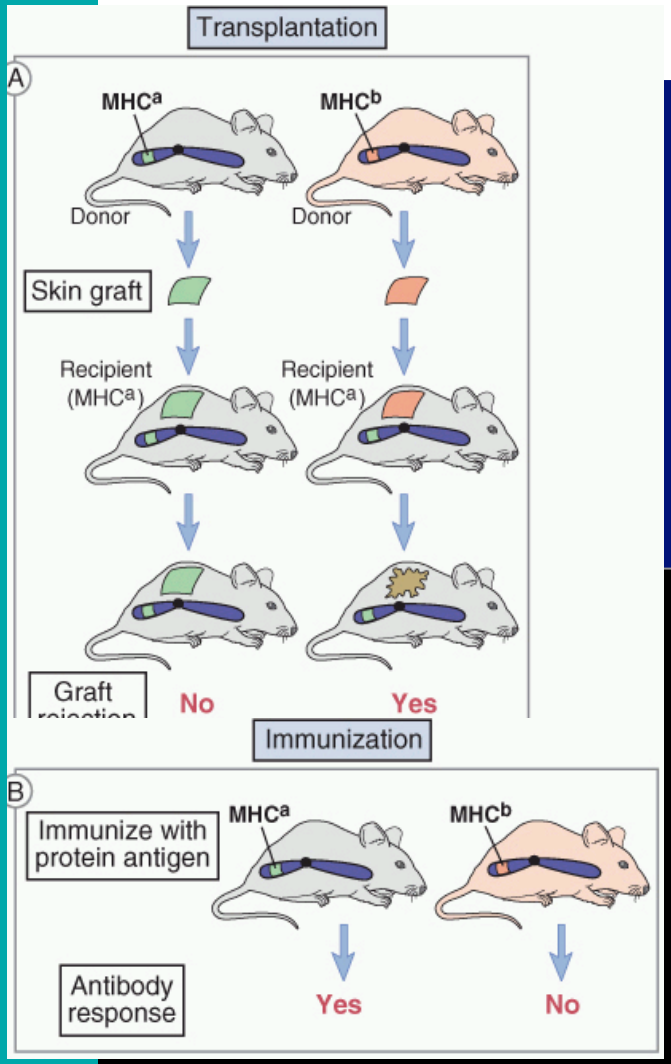
The laws of transplantation



LAWS OF TRANSPLANTATION:

- An immunocompetent host recognizes the foreign antigens on grafted tissues (cells), and mounts an immune response which results in rejection. On the other hand if an immunocompromised host is grafted with foreign immunocompetent lymphoid cells, the immunoreactive T-cells in the graft recognize the foreign antigens on the host tissue and cause their damage.

Discovery of Human MHC



- Recognition of a graft as self or foreign is an inherited trait
- histocompatibility genes: differences between self and foreign were attributed to their genetic polymorphisms
- Mouse study: identification of MHC locus

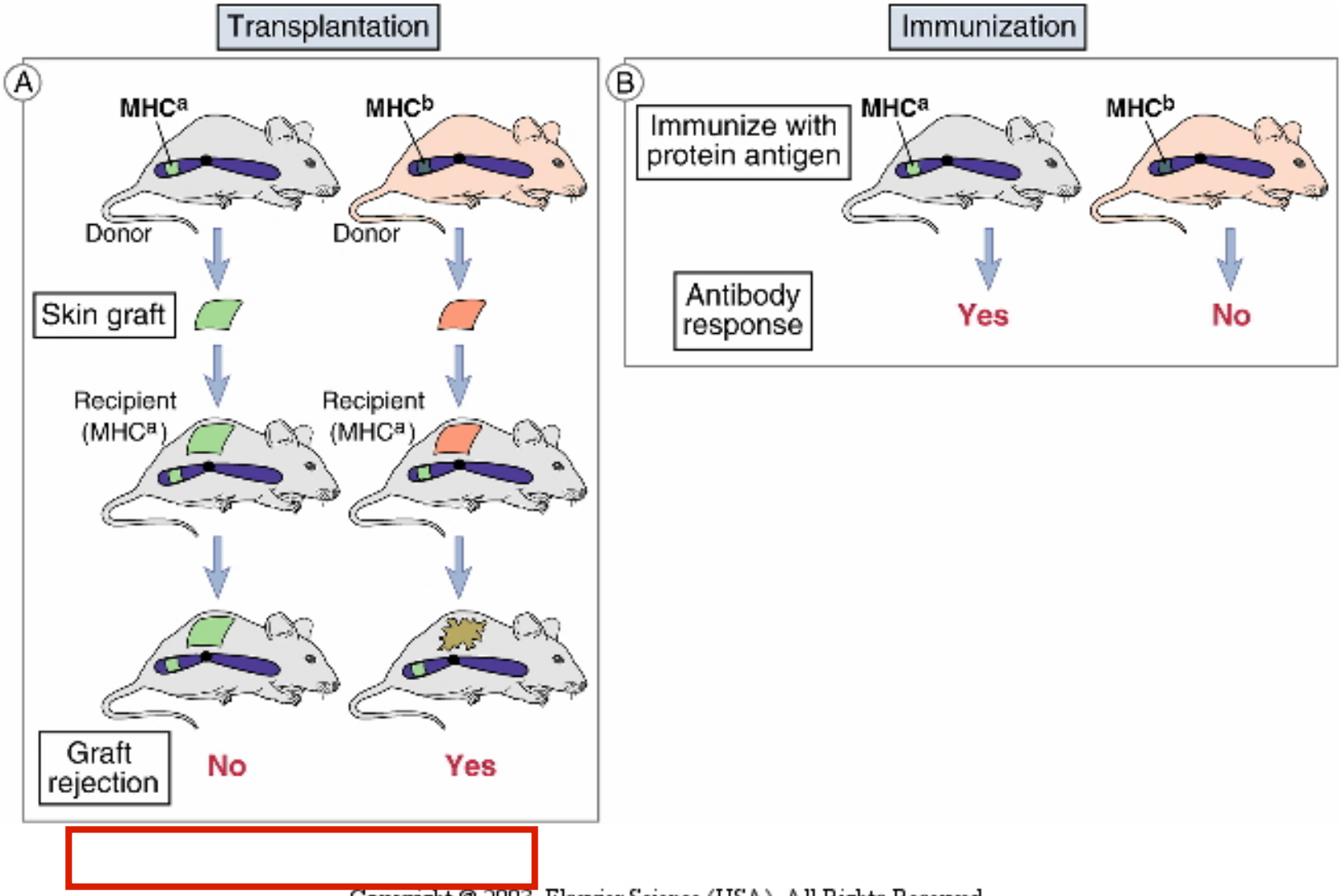
- Human MHC

  In study with transplanted patients discovered „human leukocyte antigens“ HLAs

  HLA-A, HLA-B, HLA-C (**class I MHC genes)**

  In study of mixed leukocyte reaction identified HLA-DR, HLA-DP, HLA-DQ (**class II MHC genes**

GENETIC MHC COMPLEX WAS FIRST IDENTIFIED AS GENETIC REGION THAT CONTROLLED THE TRANSPLANT REJECTION AMONG DIFFERENT INBRED STRAINS OF MICE (Snell, 1940)



We saw that in the mouse a genetic region (MHC) controls the cell-mediated rejection response.

And in humans?

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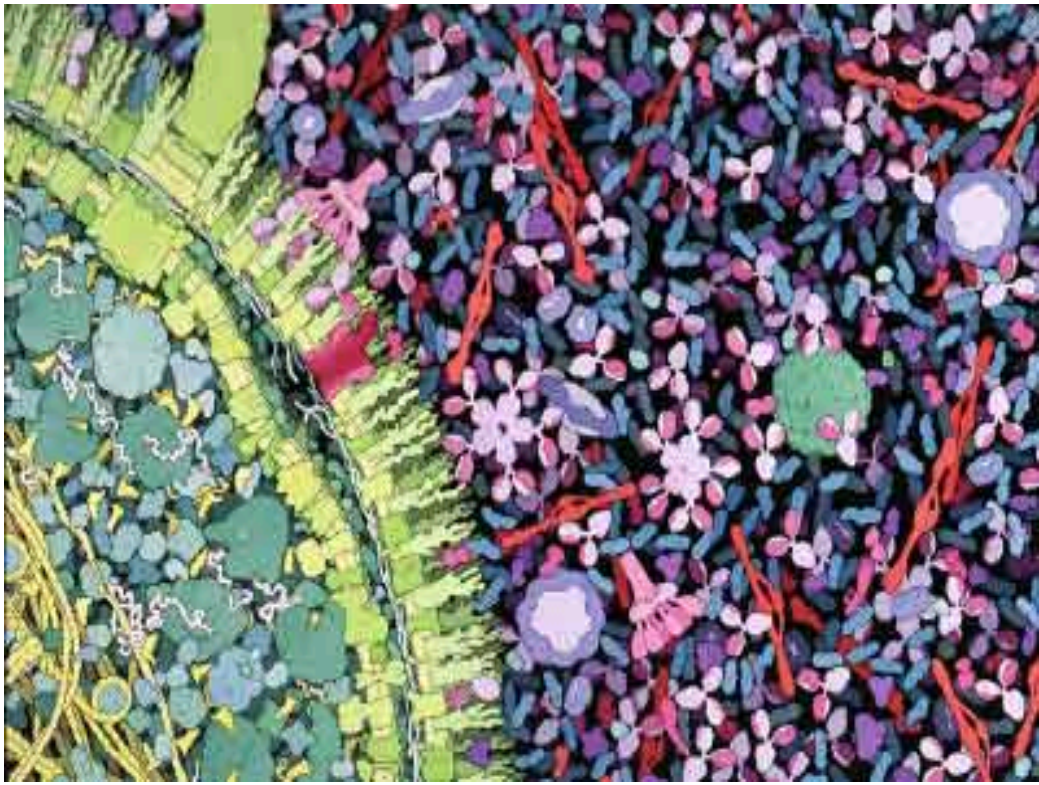
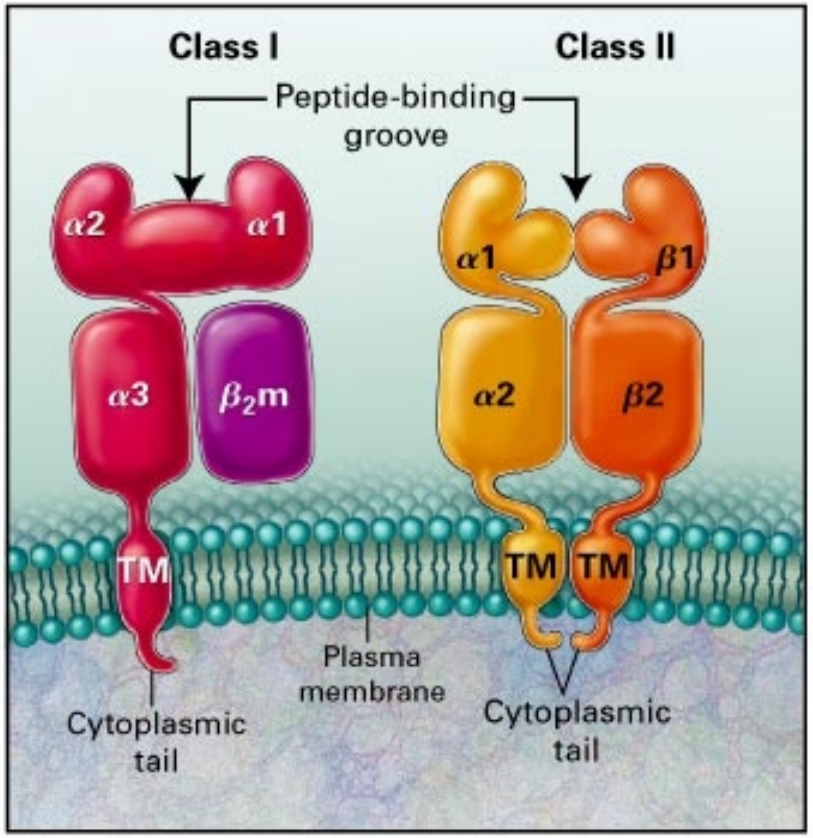
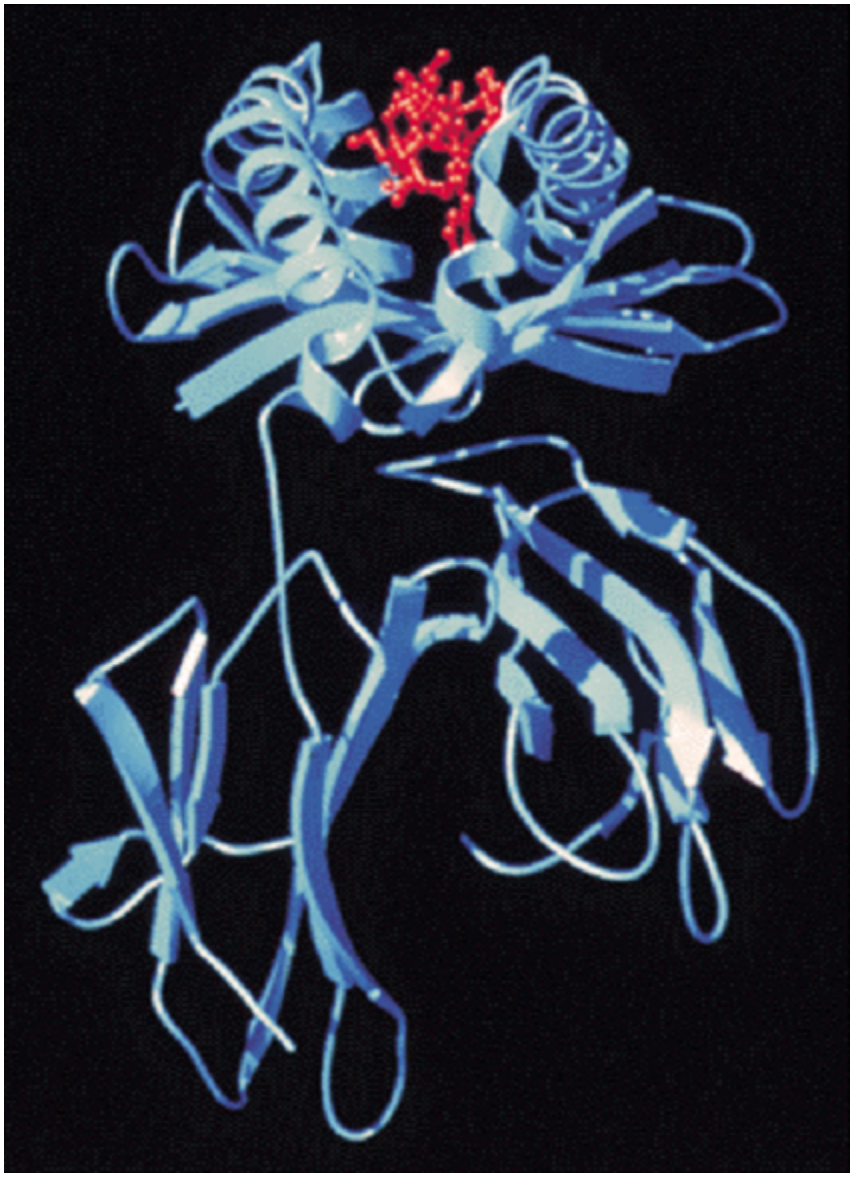


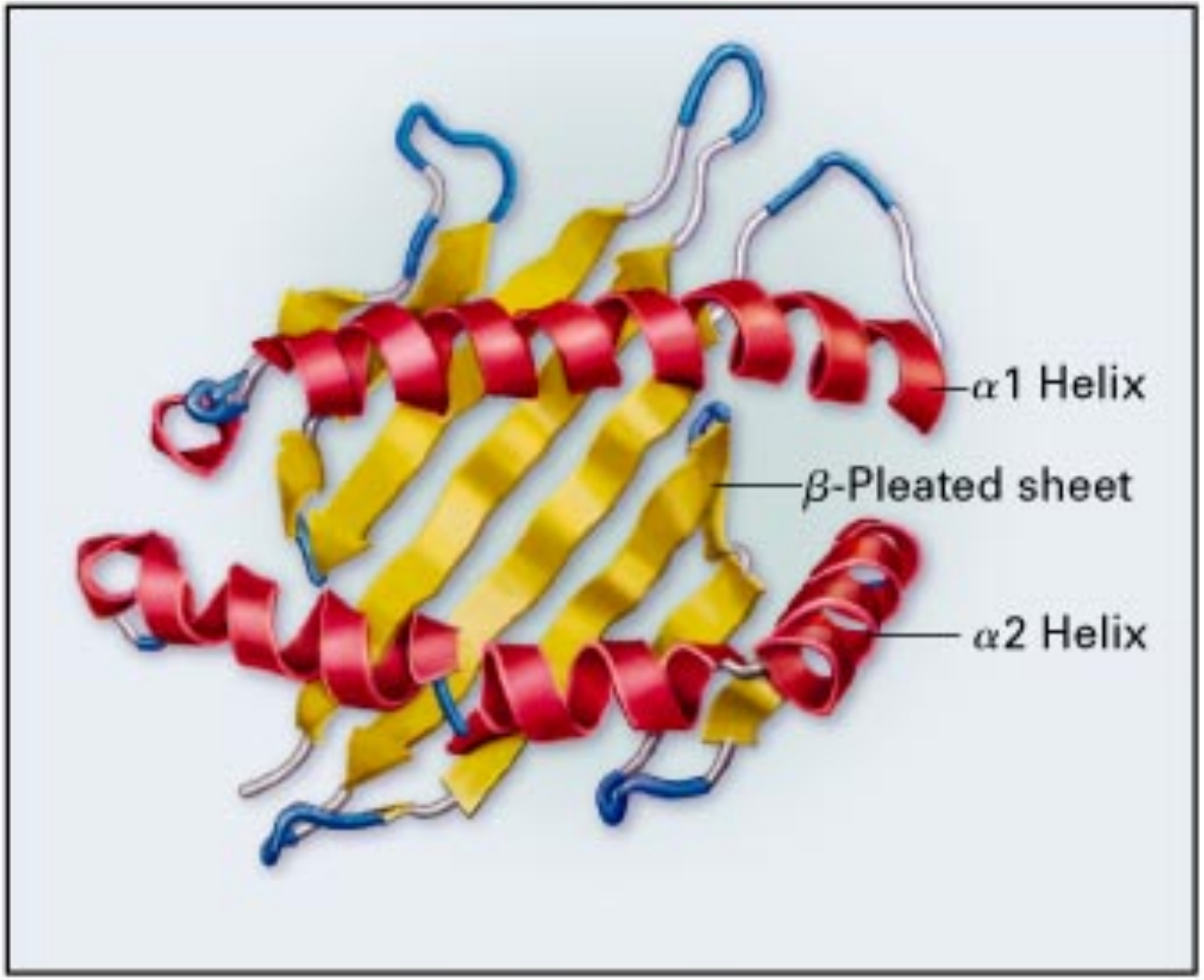
Immune Recognition by David Goodsell

The HLA system

- **HLA antigens: what are they?**



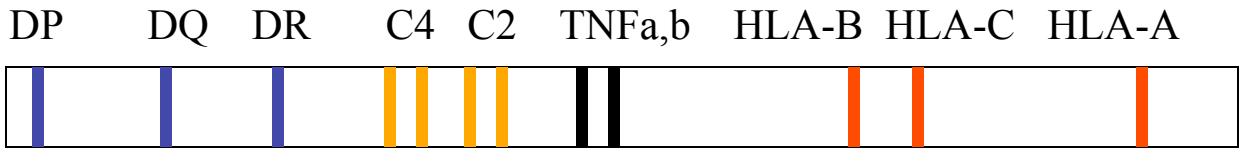


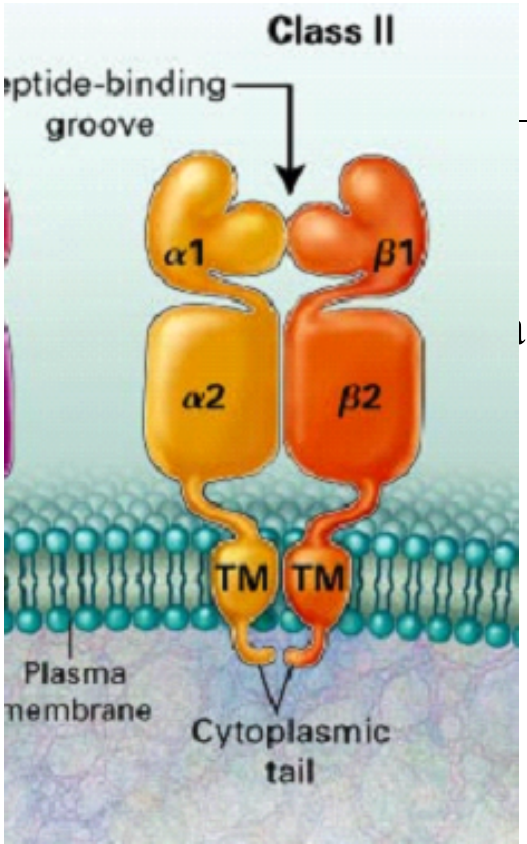

α1 Helix
β-Pleated sheet
α2 Helix

HLA map: 1993

6p21.3

4 Mbp about 50 genes





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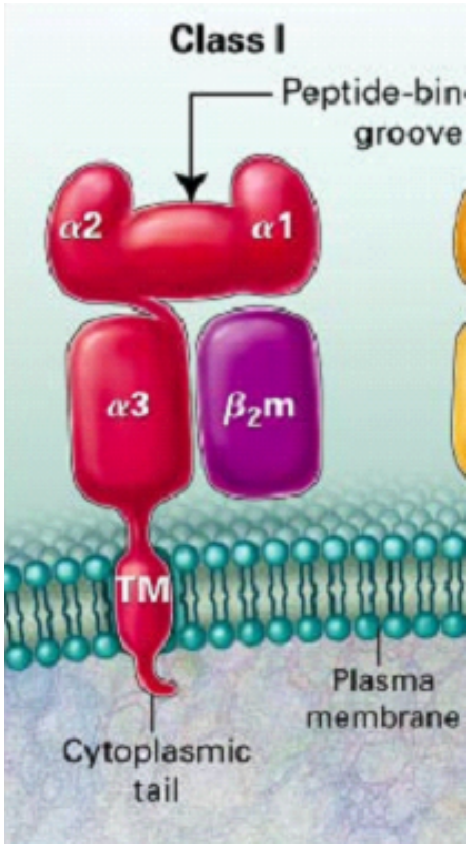
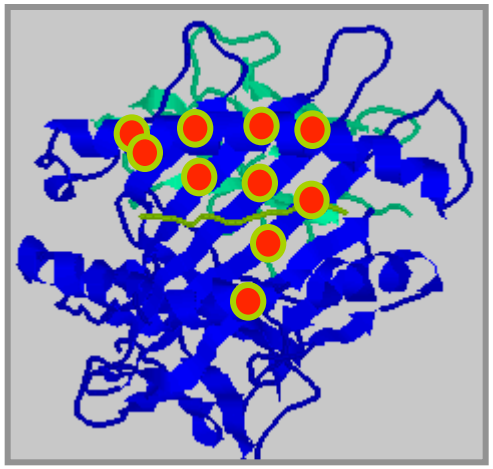
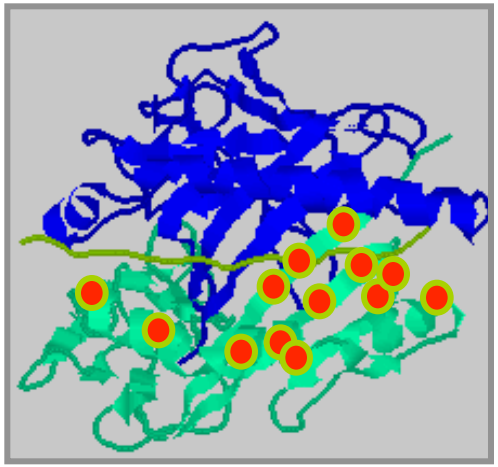


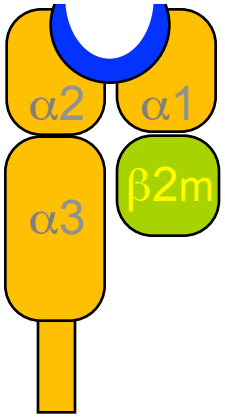
The HLA system is highly polymorphic

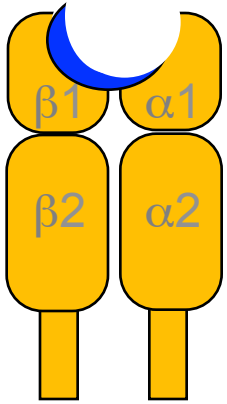
Allelic polymorphism is concentrated in the peptide antigen binding site





Class I

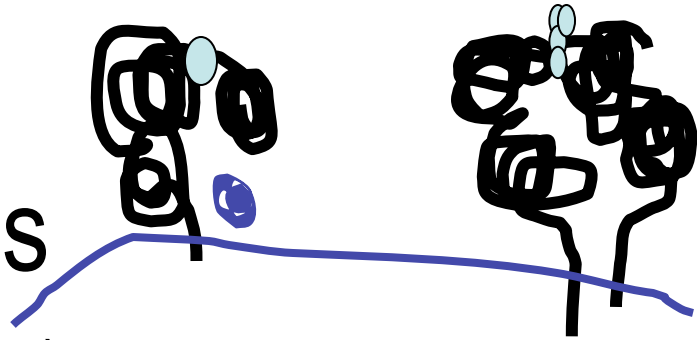




Class II
(HLA-DR)

Polymorphism in the MHC affects peptide antigen binding
Allelic variants may differ by 20 amino acids

structure of HLA molecules



- glycoproteins, heterodimers (two chains)
- Structure of HLA molecules of both classes enables antigen binding and contact with T cell receptors. Extracellulary located peptide binding cleft
- polymorphic (predominantly in the cleft).
- Nonpolymorphic part of the molecule contains binding sites for the T cell molecules CD4 and CD8

HLA – MHC: basic facts

- Two groupes of MHC genes:

  structurally and functionally distinct

  class I recognition by CD8+ T cells

  class II recognition by CD4+ T cells

- HLA molecules are responsible for the compatibility of the tissues of genetically different individuals and for the rejection of transplant

- MHC genes are codominantly expressed in each individual
- monozygotic twins have the same histocompatibility molecules on their cells
- MHC genes are the most polymorphic genes present in the genome!

  (Up to 250 alleles identified for some loci)

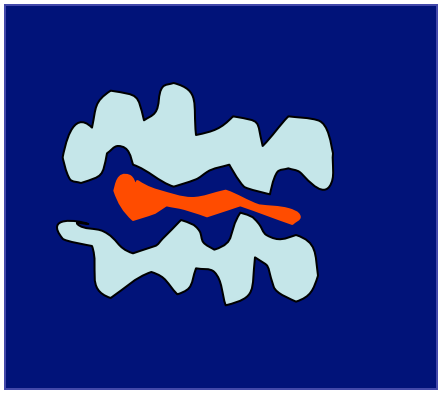
MHC expression

Class I

On all nucleated cells (no MHC on red blood cells, weak expression on cells in CNS)

Class II

Found on **antigen presenting cells**



HLA system function

MHC Class I pathway

protein

Figure by Eric A.J. Reits

MHC Class I pathway

Figure by Eric A.J. Reits

MHC Class I pathway

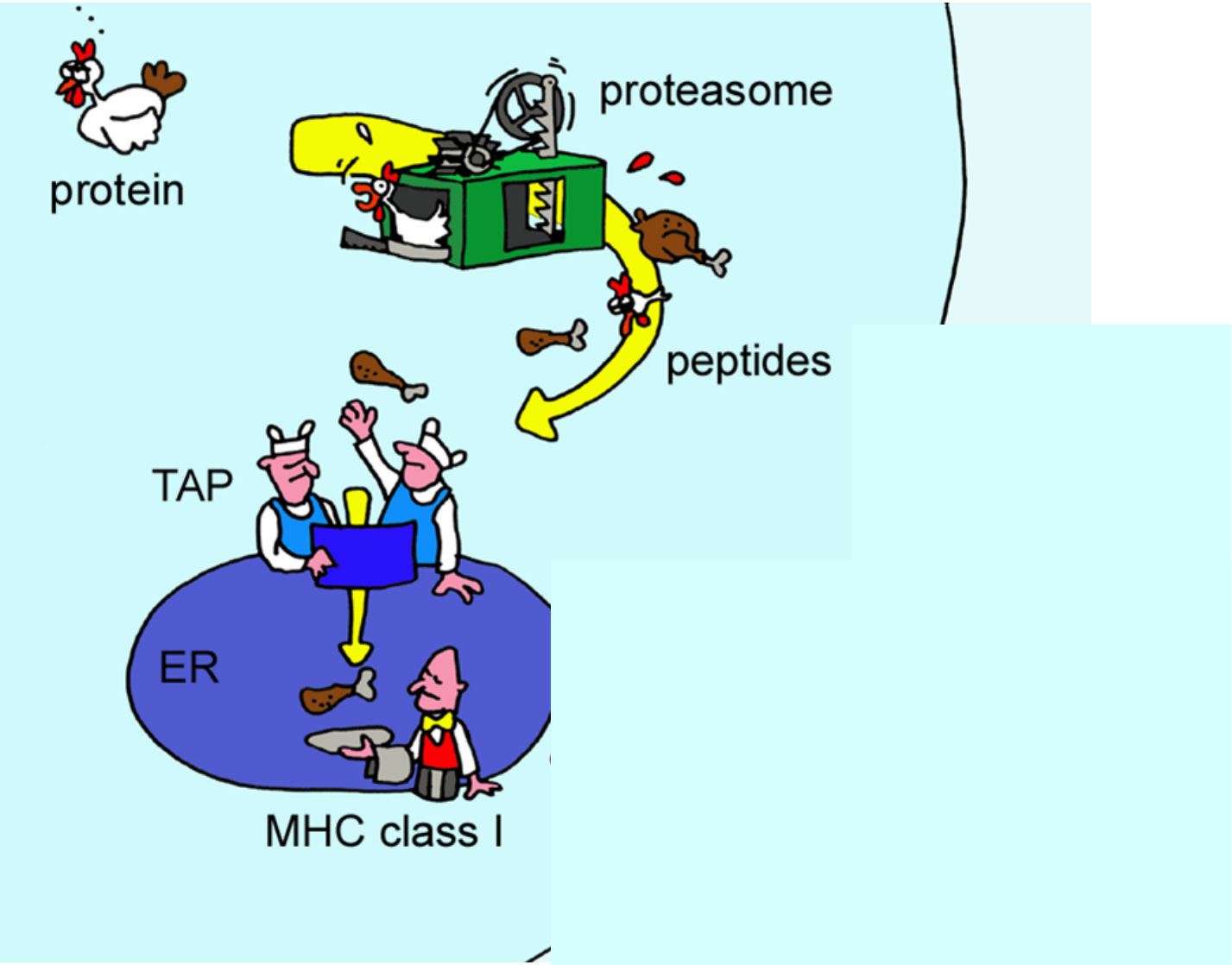


Figure by Eric A.J. Reits

MHC Class I pathway

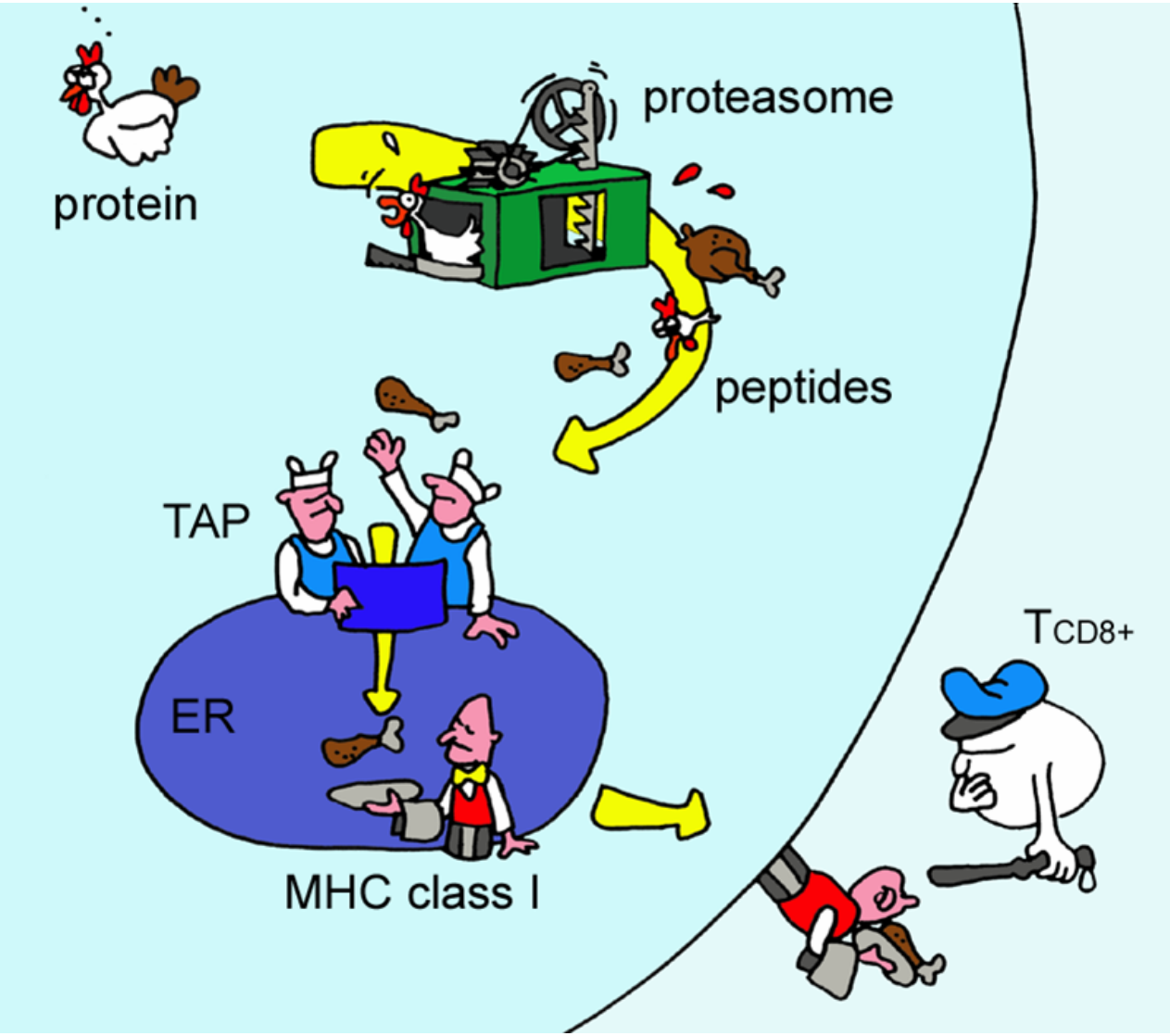
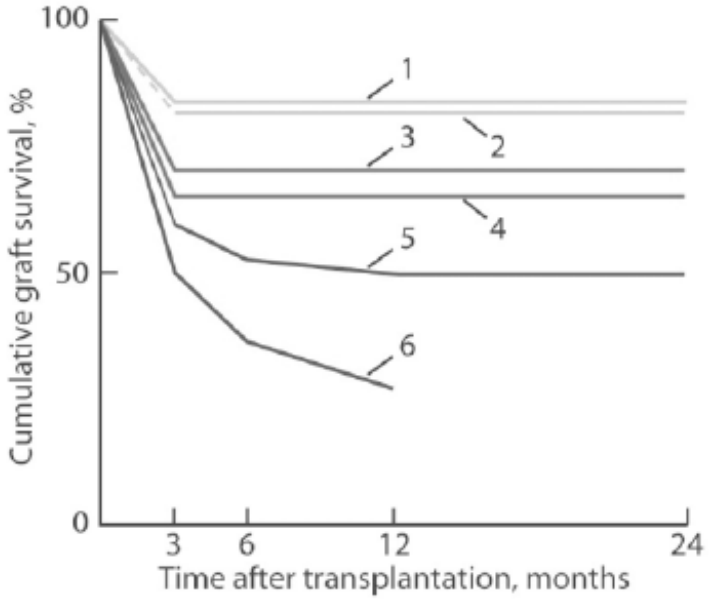


Figure by Eric A.J. Reits

MHC controls post-transplant survival



Curve no.	HLA mismatches (no.) Class I	Class II
1	0	0
2	1 or 2	0
3	3 or 4	0
4	0	1 or 2
5	1 or 2	1 or 2
6	3 or 4	1 or 2

- A graft is compatible only if there is a complete match at all MHC alleles, i.e. a two haplotype match for all MHC loci

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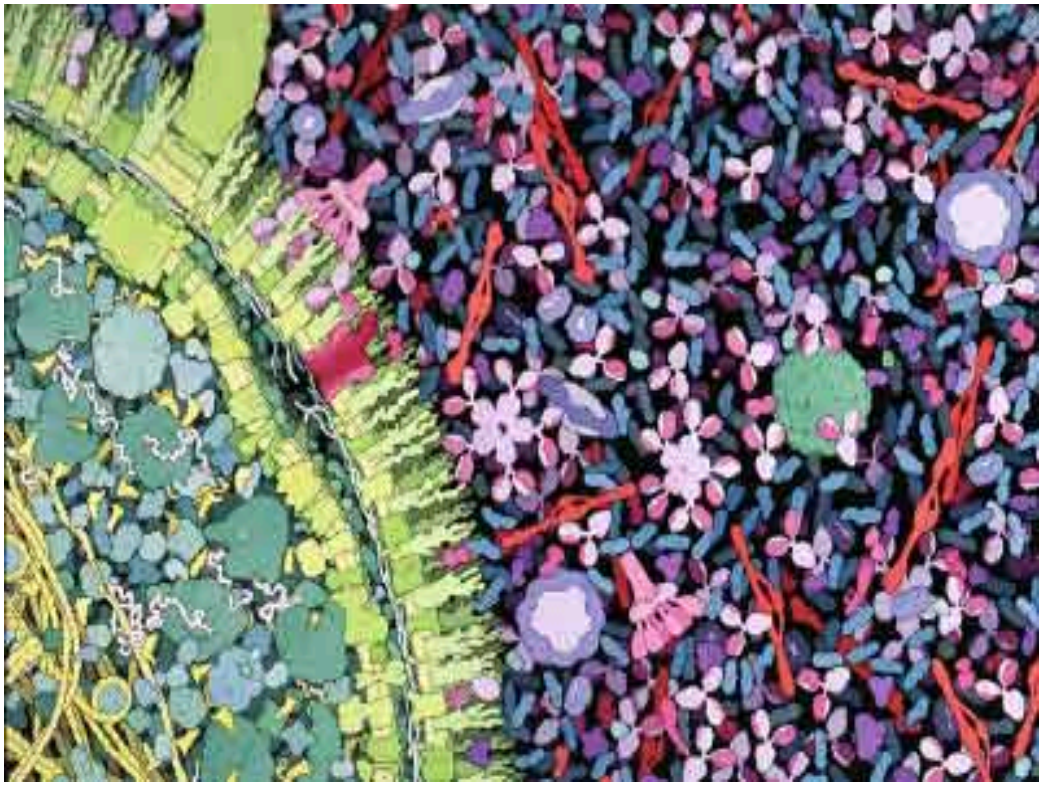
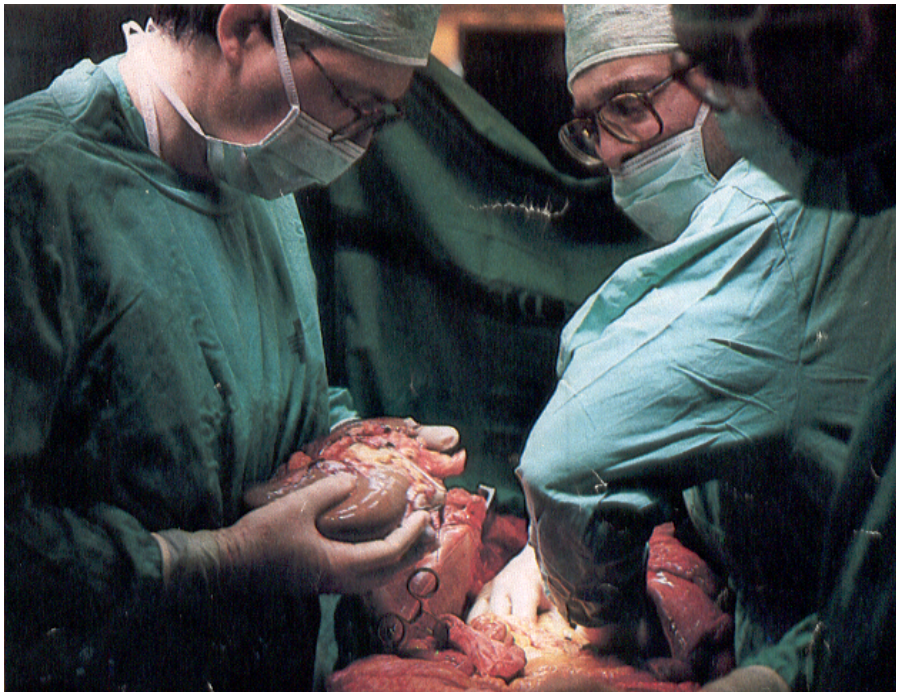


Immune Recognition by David Goodsell

MHC and Transplants

- Organ transplants
- Haemopoietic stem cell transplants
- Tissue transplants

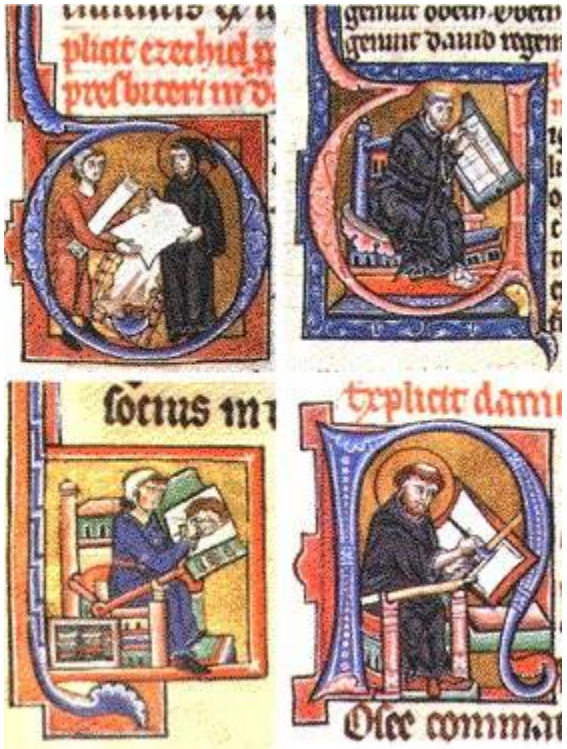


Is it still worth typing for HLA donor-recipient pairs?

- It is clear the effect of HLA-A, B, DR compatibility for all organ transplants, except the liver
- HLA-DQ and DP compatibility also have a benefit, at least in the kidney, and especially in the re-transplanted and in the immunized
- 10% improvement in 10 years for more compatible transplants than those less compatible
- It's still the parameter that determines the effectiveness of transplant

What criteria guide the choice of the most suitable candidate?

- Legislation principles
- Biologic principles
- Ethical principles



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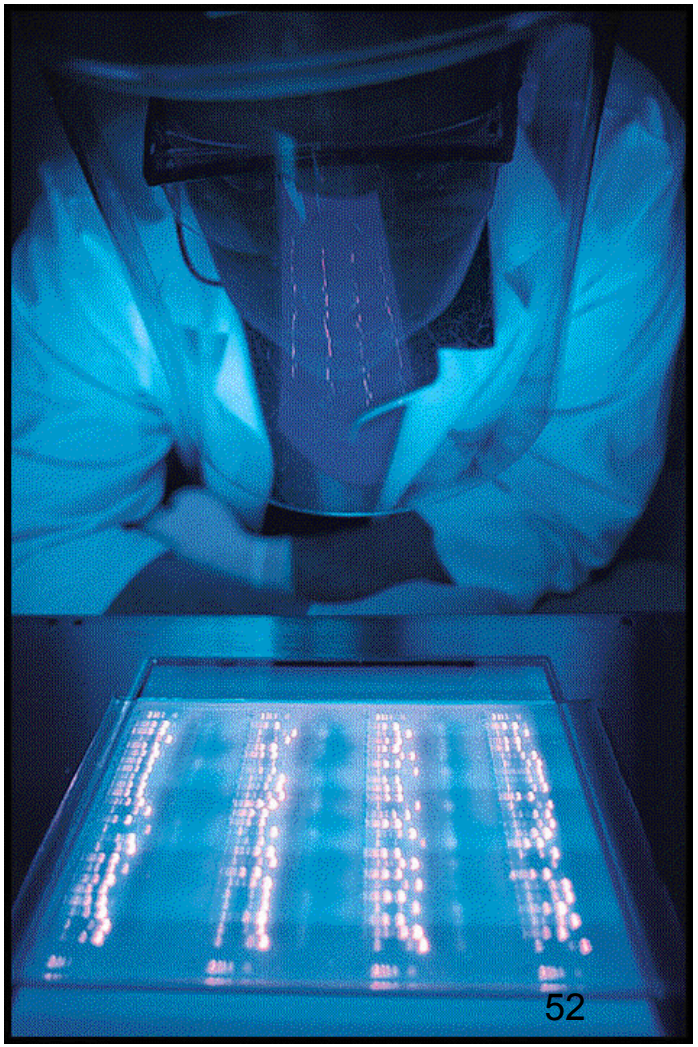
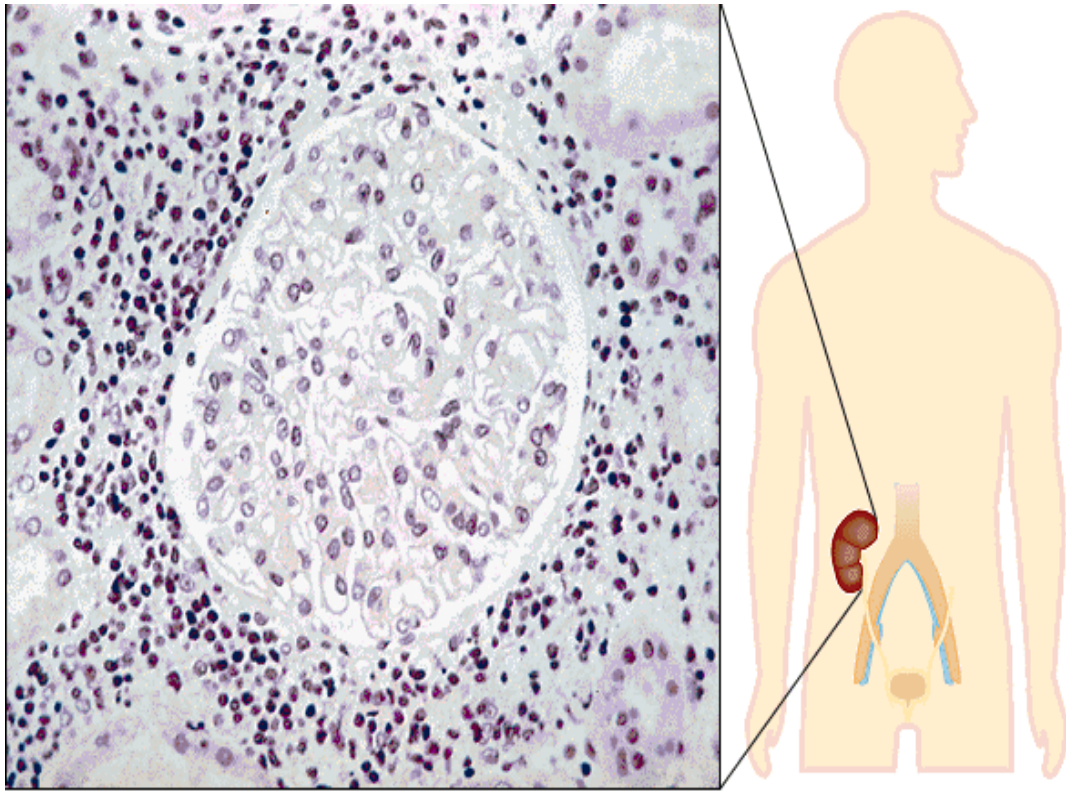


The biological laws of transplants

- ABO group
- HLA compatibility
- Absence of anti-tissue antibodies specific for the donor
- Age
- ….



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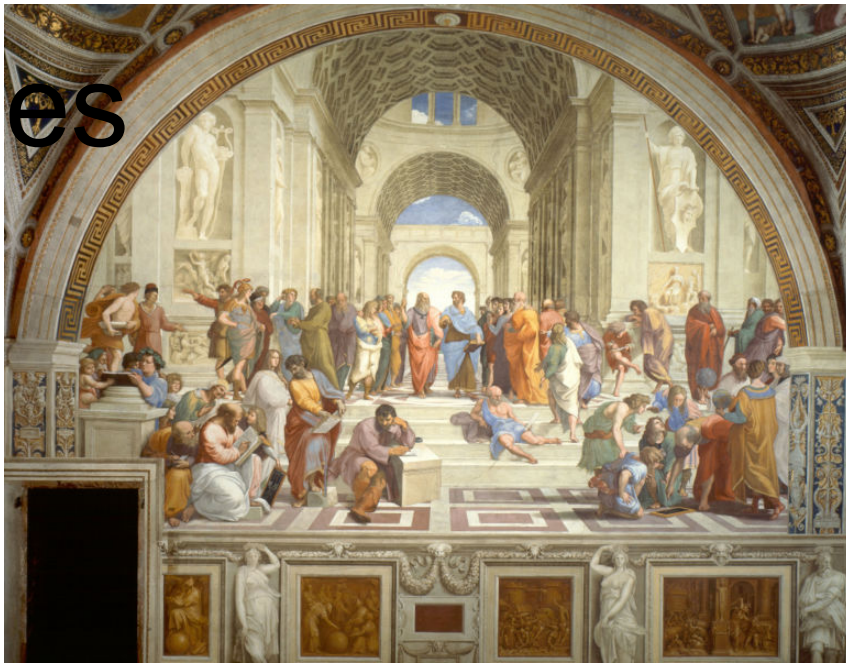


La scuola di Atene
Raffaello Sanzio
1512 affresco, 772 × ? cm
Città del Vaticano, Musei Vaticani

What principles we refer to:

- Valuing a limited resource

  Allocate organ to the most suitable candidate depending on the probability of transplantation success

- Justice

  Take into account the gain, in terms of predicted lifespan or life quality, transplant offers to that candidate

- Equity

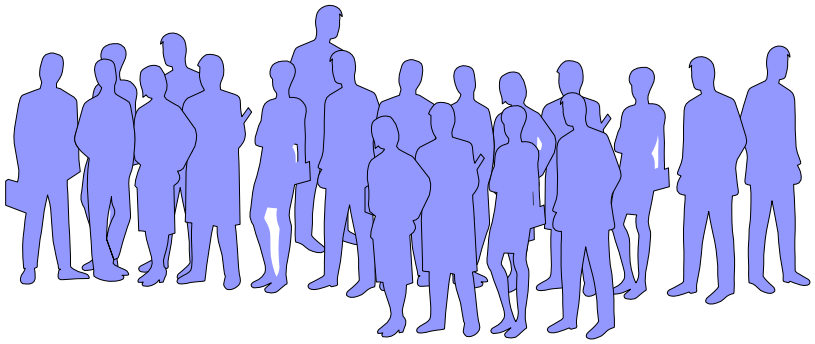
  Give all candidates equal opportunity of access to transplantation

Allocation criteria

- Heart
- Liver
- Lung

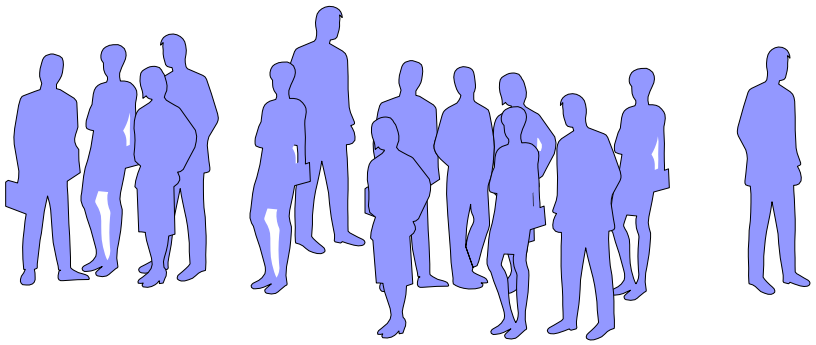
Allocation criteria

- Heart
- Liver
- Lung



Allocation criteria

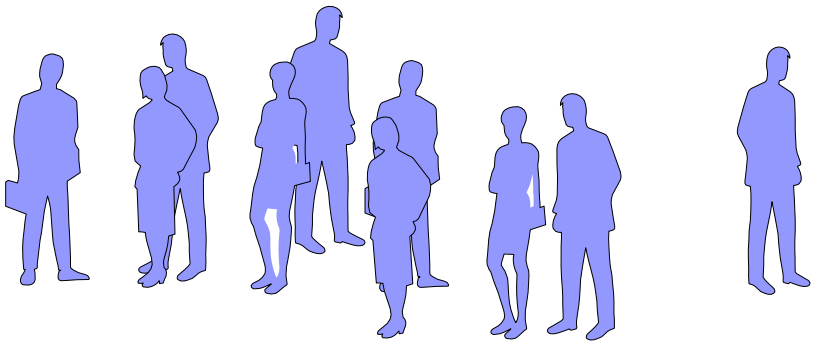
- Heart
- Liver
- Lung



AB0 compatibility

Allocation criteria

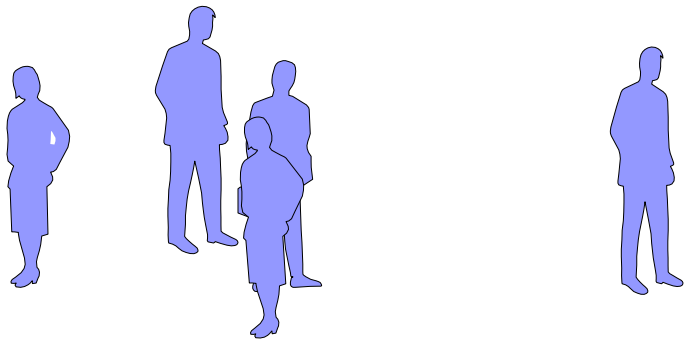
- Heart
- Liver
- Lung



AB0 compatibility
Body size

Allocation criteria

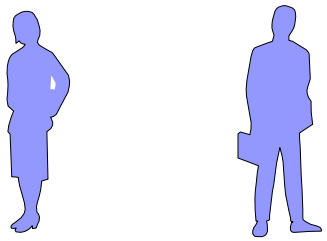
- Heart
- Liver
- Lung



AB0 compatibility
Body size
Severity of illness

Allocation criteria

- Heart
- Liver
- Lung



AB0 compatibility
Body size
Severity of illness
Seniority in list

Procedure of choice of transplant receiver

- Favour candidates for who:
  - can be predicted a high probability of transplantation success
  - can be predicted the lower likelyhhood of re-transplant

Procedure of choice of transplant receiver

- With a procedure that is
  - automatized
  - transparent
  - available for public consultation at the regional transplantation center

Numero Verde
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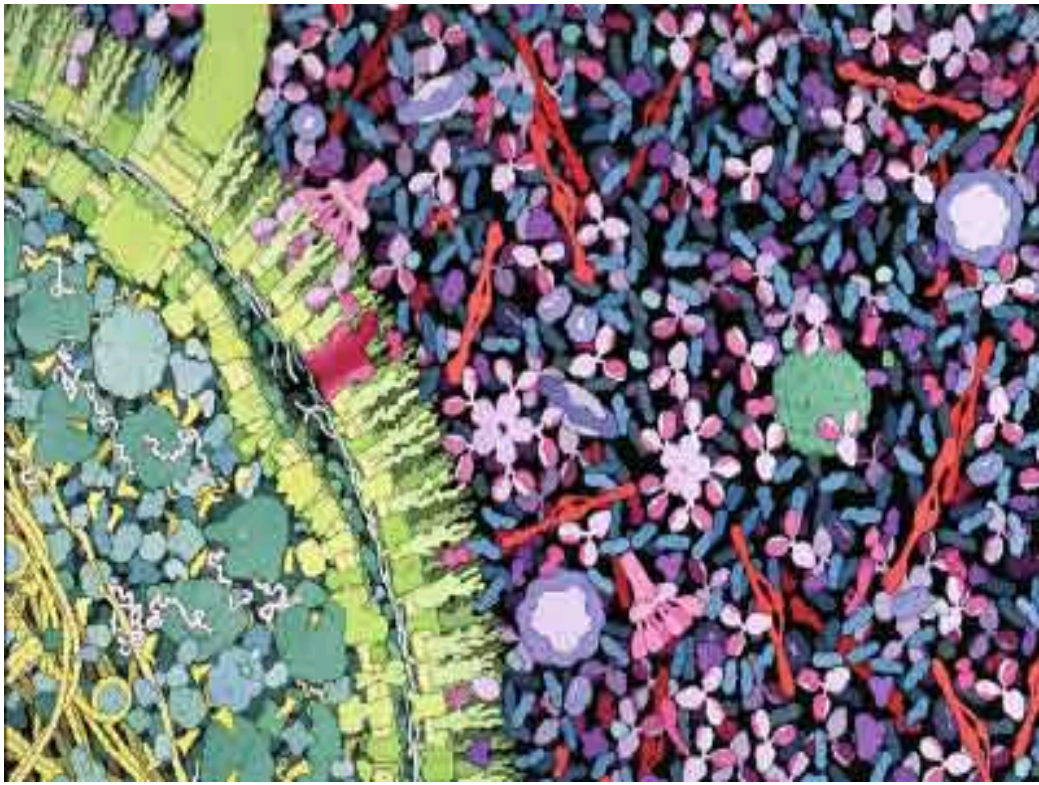
e Trapianto
REGIONE
PIEMONTE

Immunogenetics and medicine

- Introduction to immunogenetics
- Genetics of transplantation
- HLA
- Transplants
- Future perspectives



Immune Recognition by David Goodsell

Future perspectives: applications to public health

- Stem cells
- Tissue engineering
- New transplants

Future perspectives: applications to public health

- Stem cells
- Tissue engineering
- New transplants

◆ etymology:

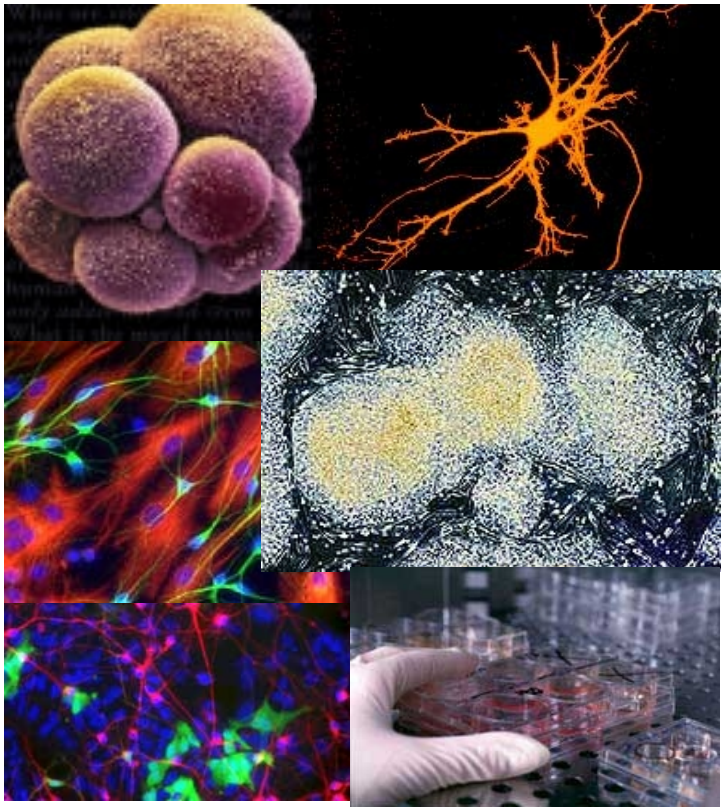
- *In english* “stem” = plant stalk.

- In italian the "thread of life" was the thread that was linked the fate of the men, which the Fates spun and cut at the appropriate time.

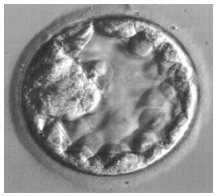
◆ definition:

- Cells with the capacity to self-renew indefinitely and to differentiate in at least one mature cell type.

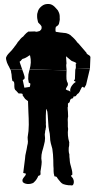
  You can distinguish: *unipotent, multipotent, totipotent.*



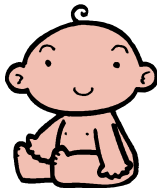
◆Where the stem cells are recoverable?:



- Embryo



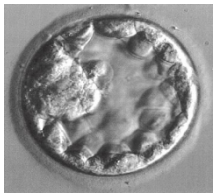
- Adult organism



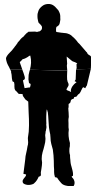
- Umbilical cord

- Non-physiological sources

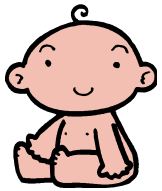
◆Where the stem cells are recoverable?:



- Embryo

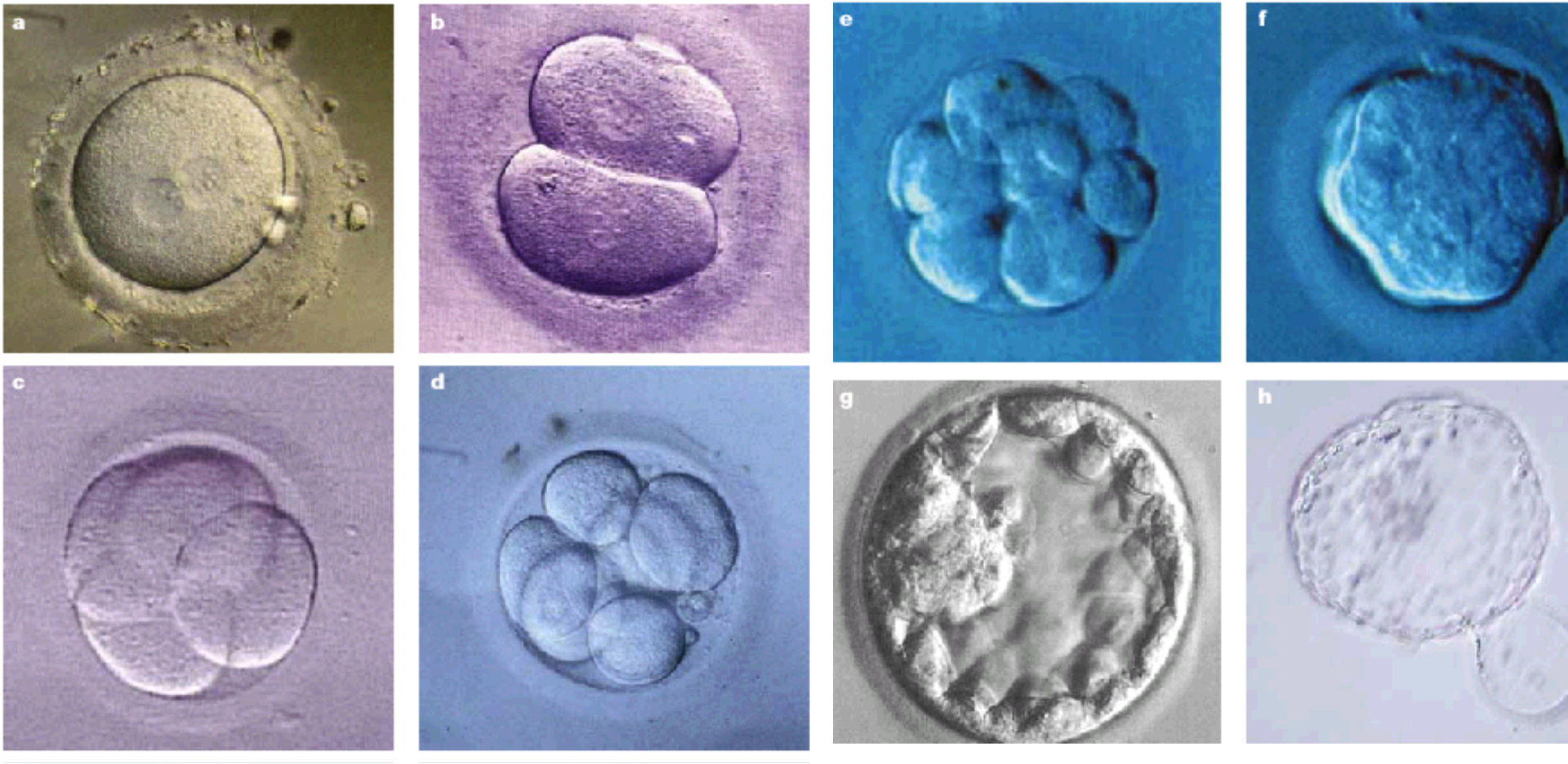


- Adult organism



- Umbilical cord

- Non-physiological sources


a
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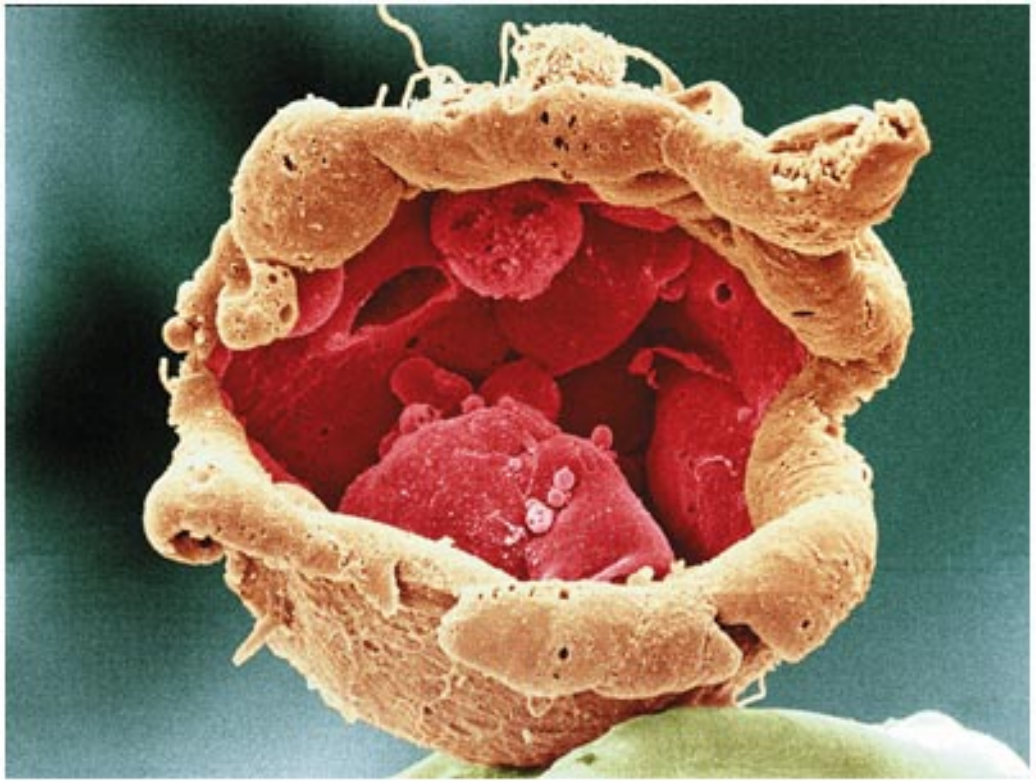
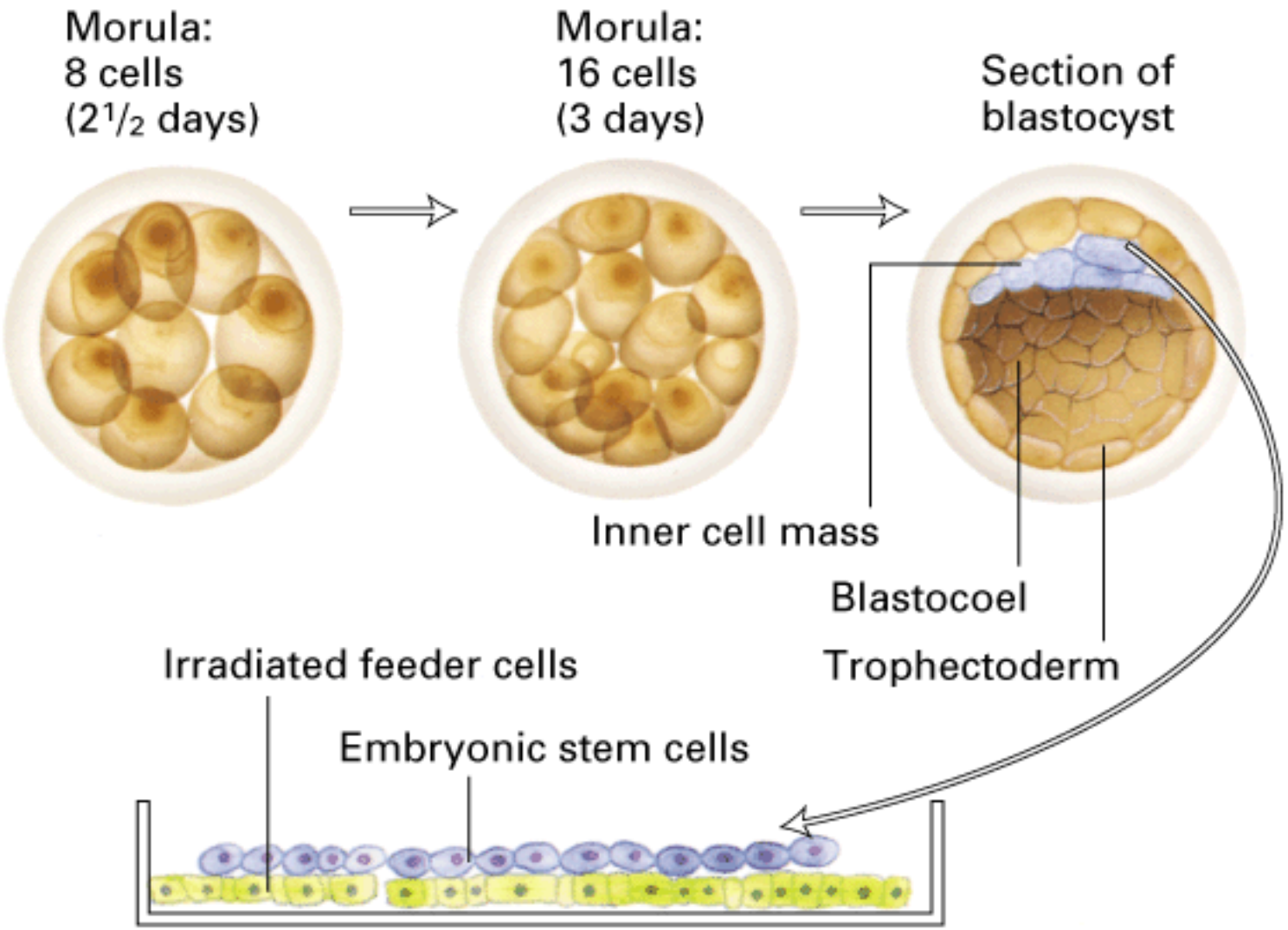
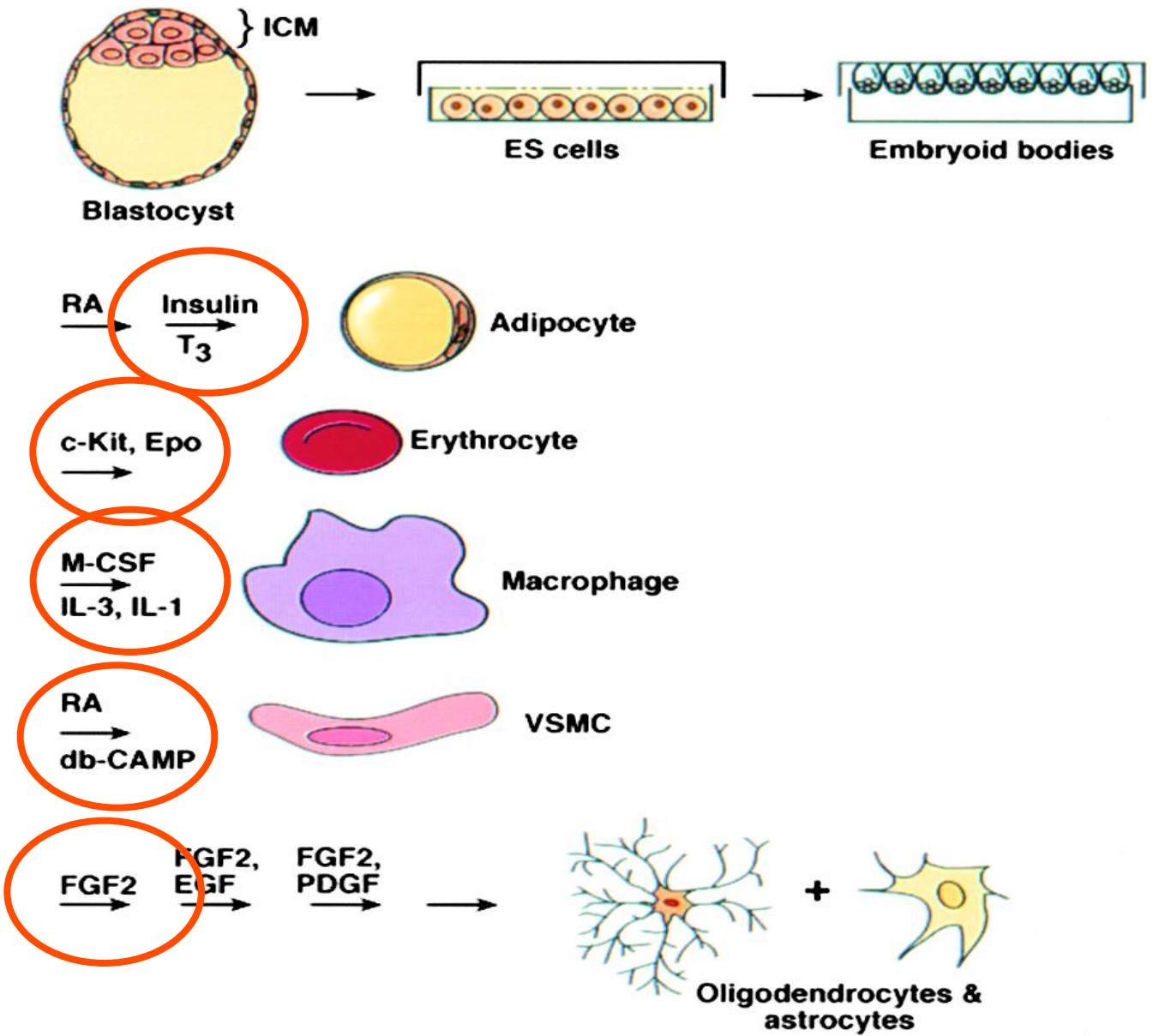


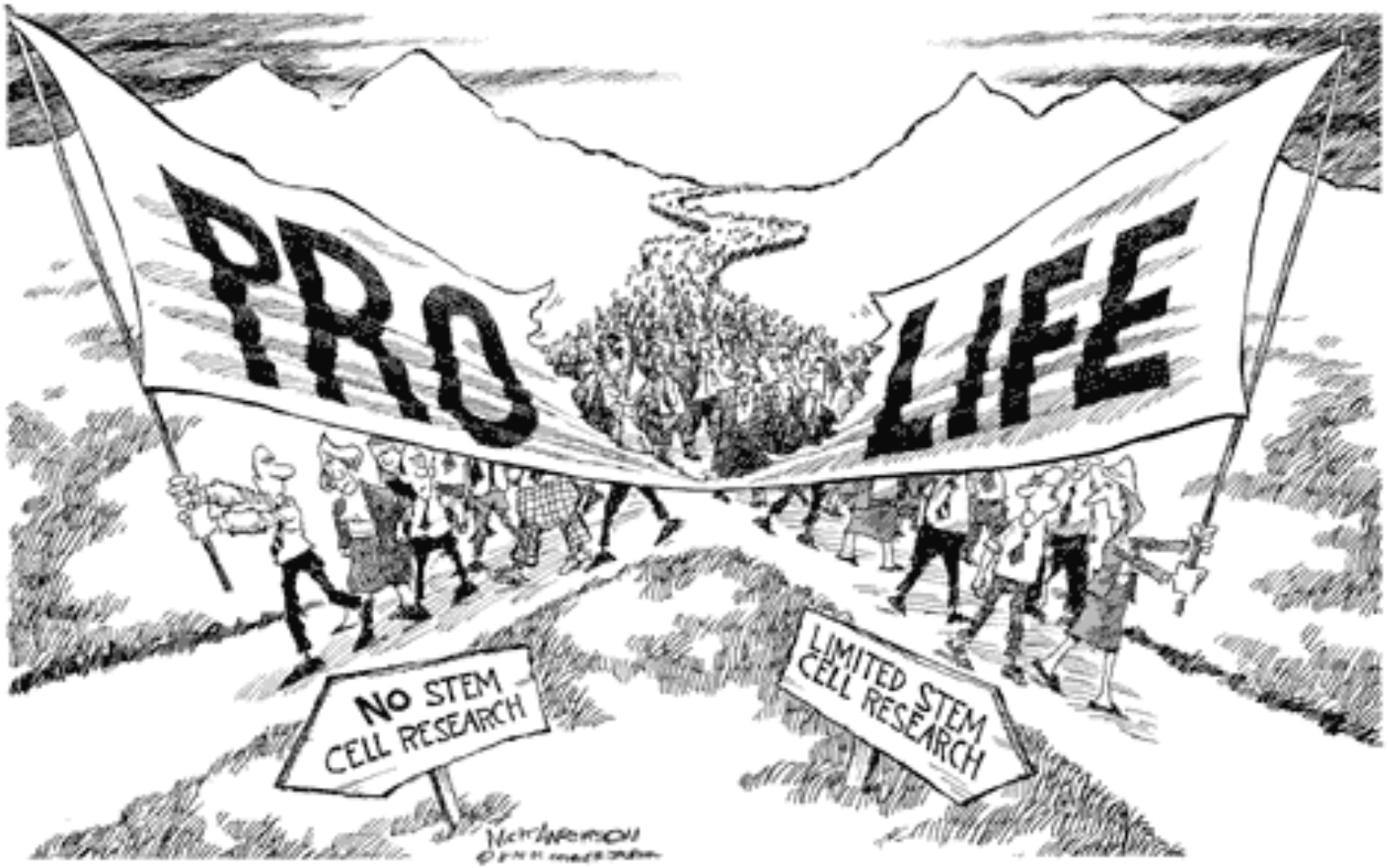
Generation of embryonic stem cells



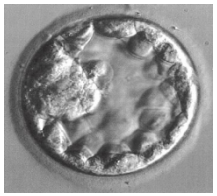
DIFFERENTIATION OF EMBRYONIC STEM CELLS



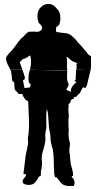
What problems?



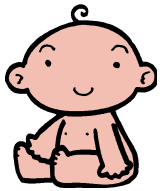
◆Where the stem cells are recoverable?:



- Embryo



- Adult organism



- Umbilical cord

- Non-physiological sources

Bone Marrow Stem Cells

Other Cell Types ??

Bone Cells

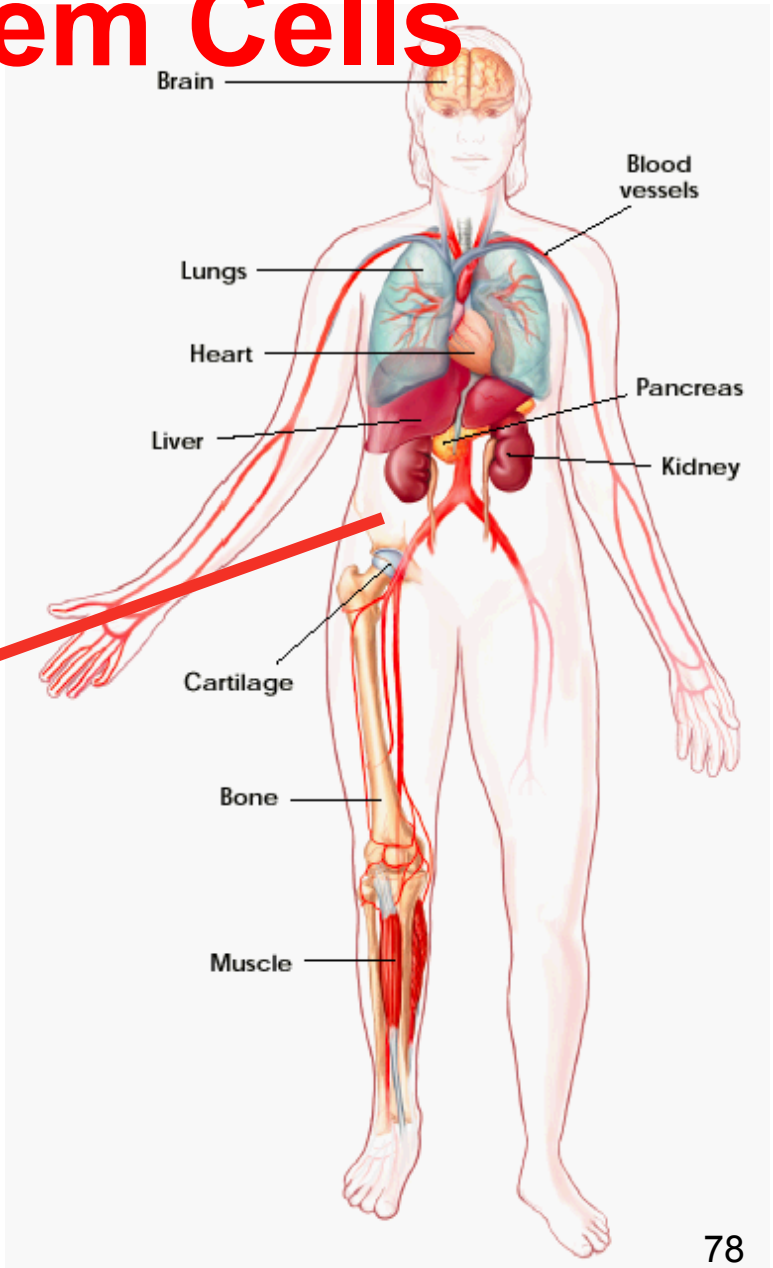
Mesenchimal stem cells

Bone Marrow Stem Cells

Hemopoietic stem cells

Other Cell Types ??

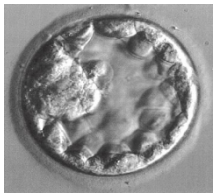
Blood Cells



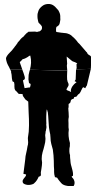
Dove troviamo le SC adulte?

- bone marrow
- peripheral blood
- digestive tract
- cornea
- liver
- brain
- epidermis
- pancreas
- retina
- skeletal muscle
- vasculature
- dental pulp
- heart

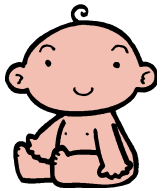
◆Where the stem cells are recoverable?:



- Embryo

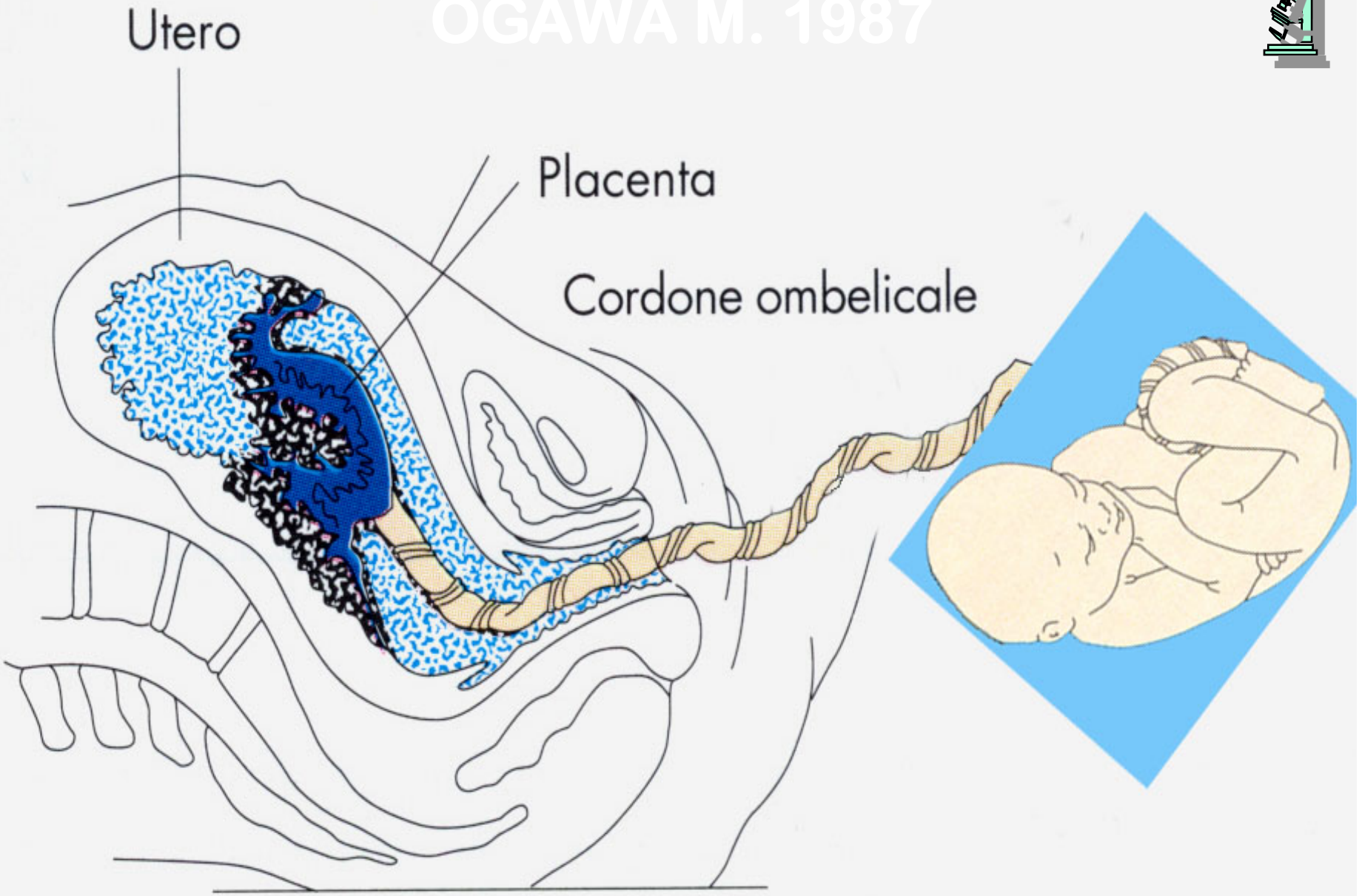


- Adult organism

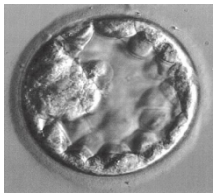


- Umbilical cord

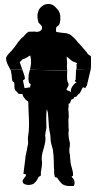
- Non-physiological sources


OGAWA M. 1987
Utero
Placenta
Cordone ombelicale

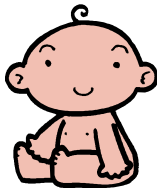
◆Where the stem cells are recoverable?:



- Embryo



- Adult organism



- Umbilical cord

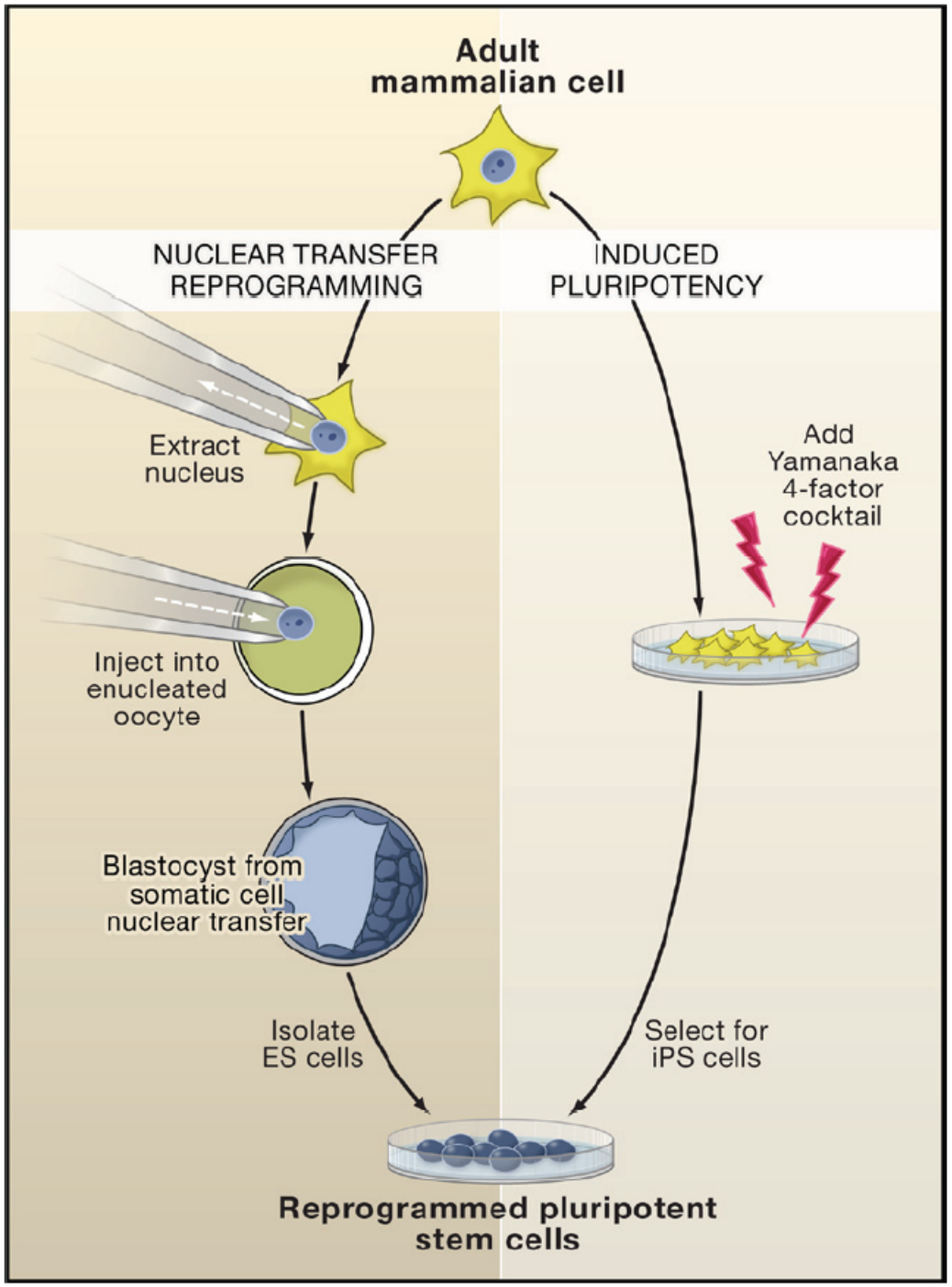
- Non-physiological sources

From differentiated cells

- Possibility to ***re-program*** their genome through different technologies.

Nuclear transfer

Nuclera transfer reprogramming involves injection of the nucleus of an adult cell into an enucleated oocyte. After a few days of development to the blastocyst stage, embryonic stem (ES) cells can be generated.

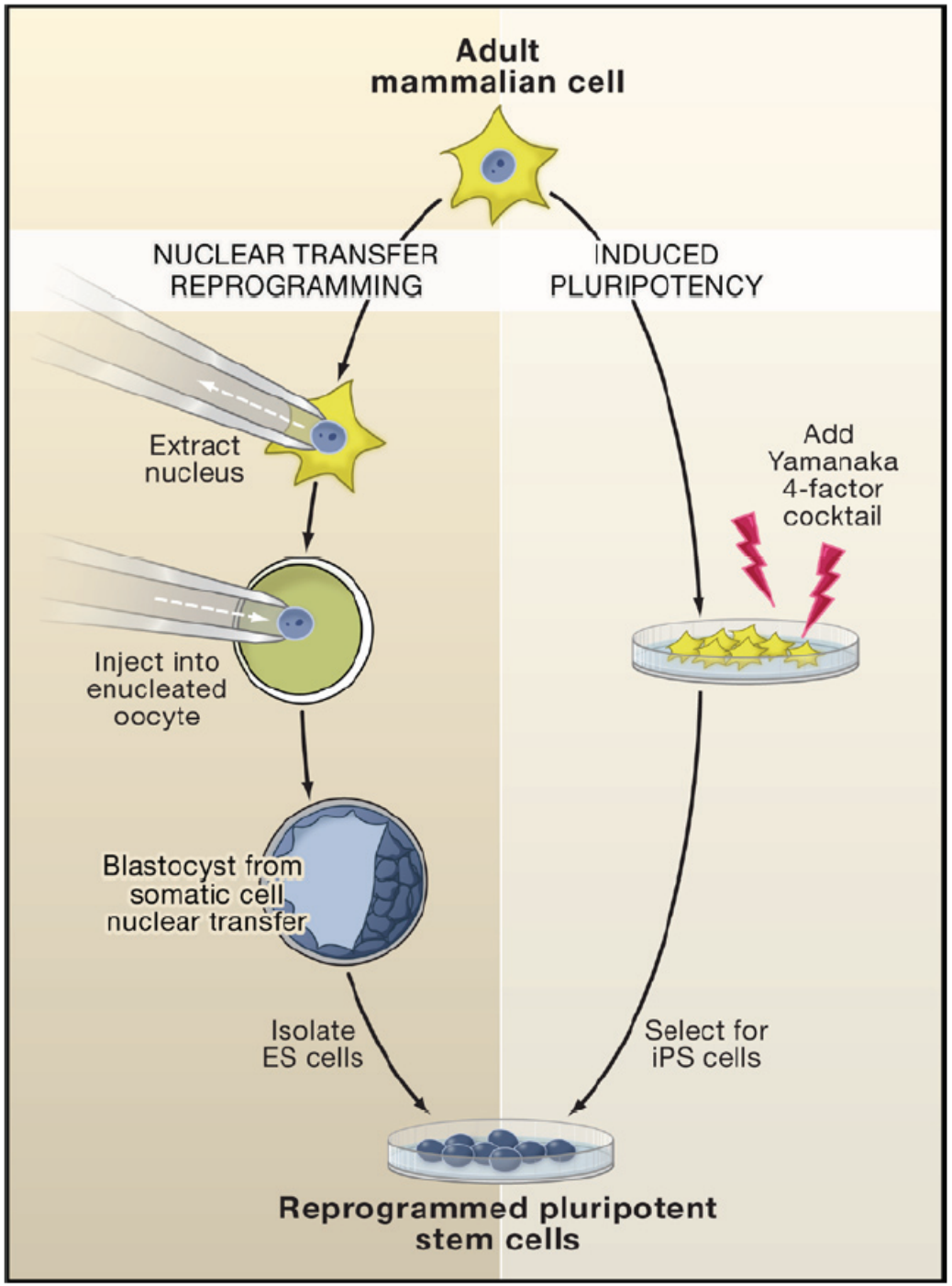


Induced pluripotent cells

During induced Pluripotency, the adult cell is directly reprogrammed to pluripotency by transfection of a set of genes encoding key transcription factors, followed bt careful selection and isolation of ES cell-like induced pluripotent stem (iPS) cells over a period of 2-3 weeks.

Both approaches have proved feasible in mice.
In humans, no nuclear transfer-derived stem cells have yet been reported; however, there are now numerous human iPS cells from normal and disease-bearing individuals available for study.

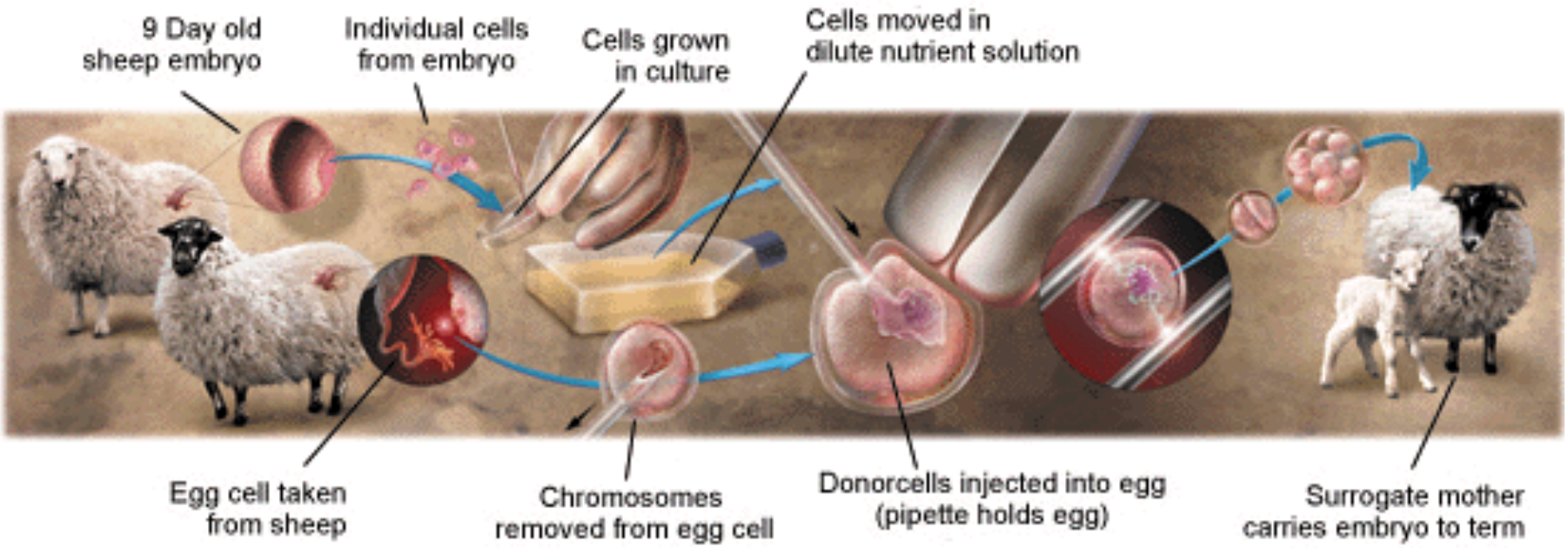
Nuclear transfer



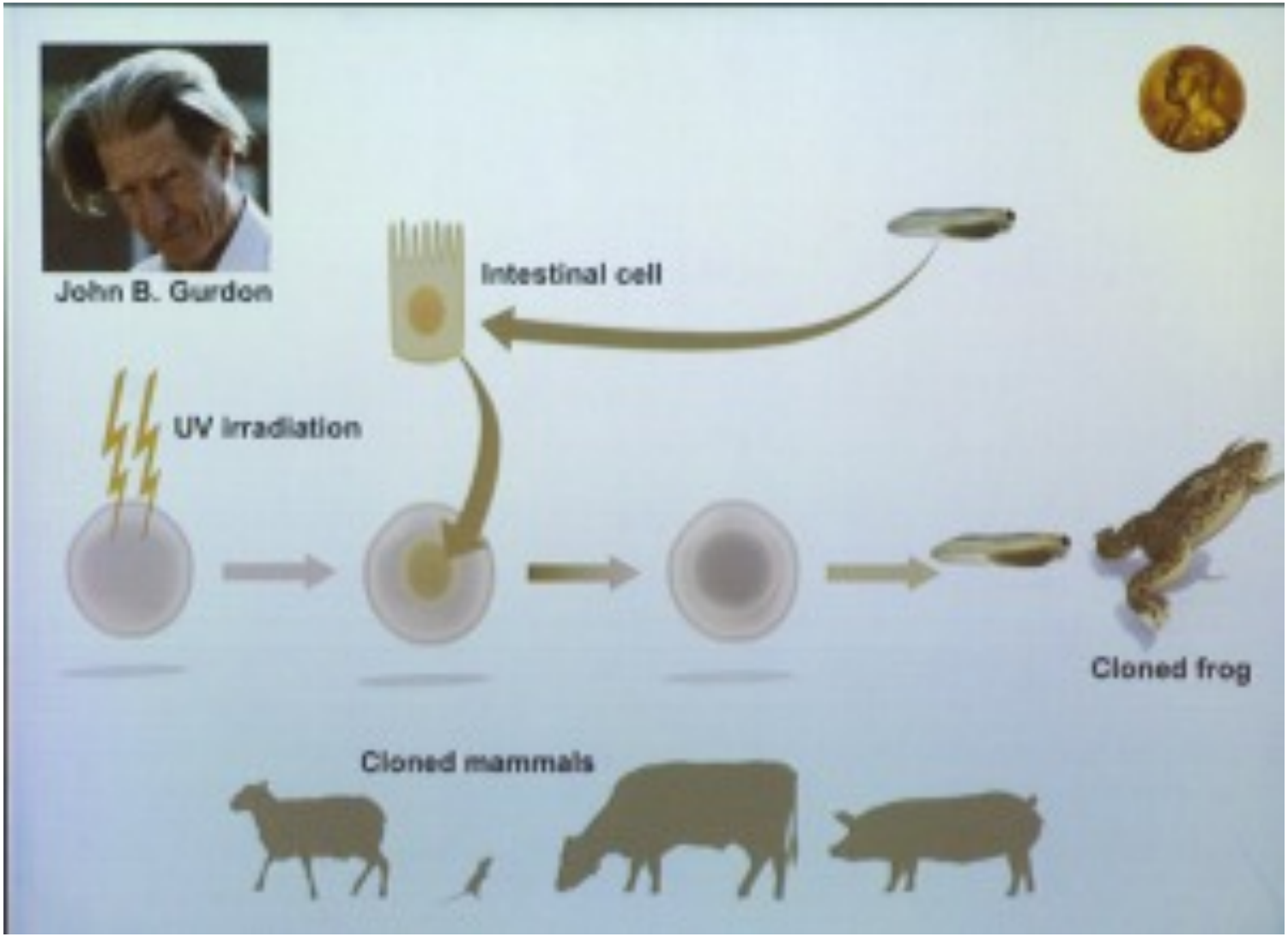
Induced pluripotent cells

Numerous organisms have been cloned

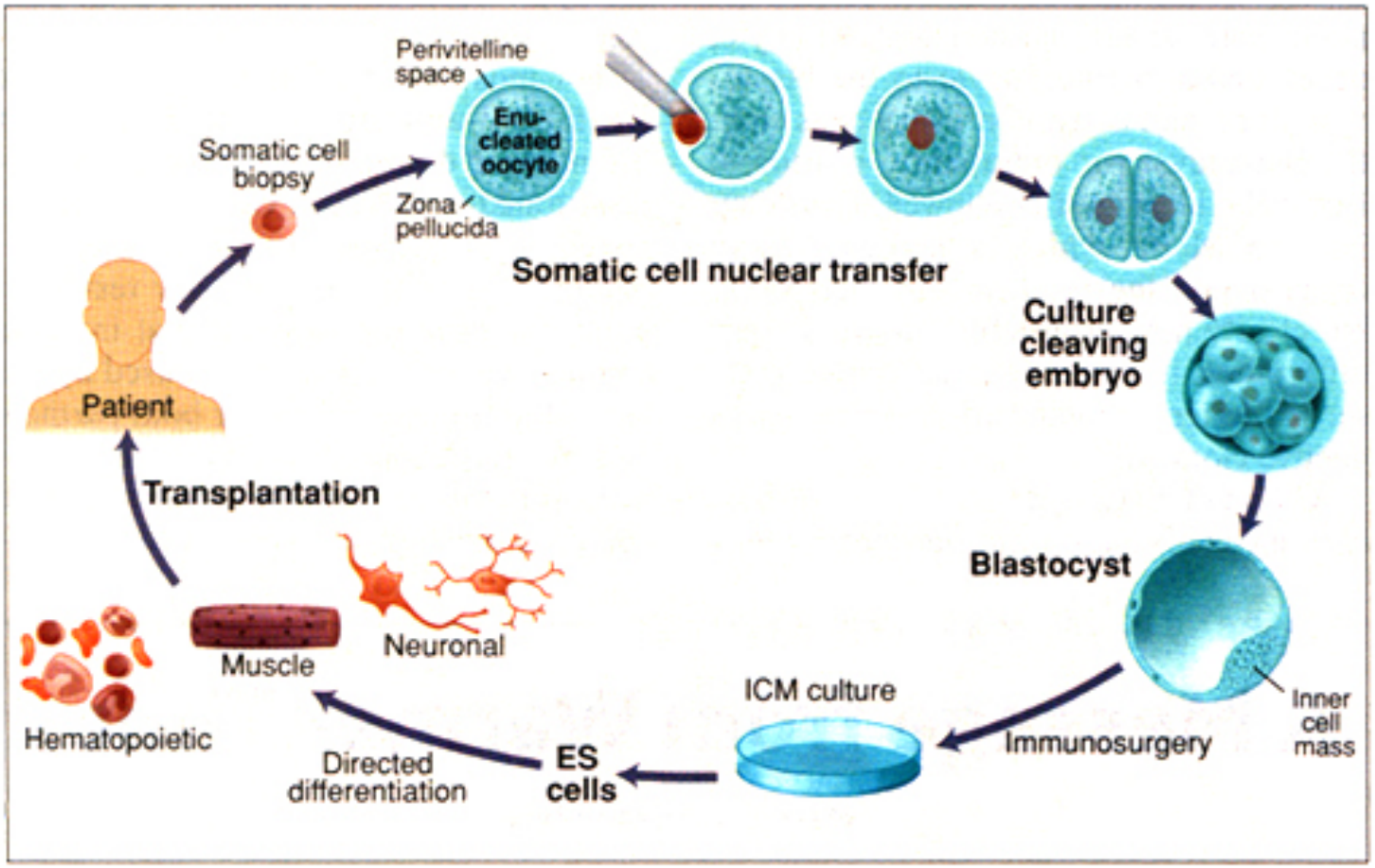
- ***From Dolly te sheep,***



- ***Dogs, cats, bulls, mice……***


John B. Gurdon
Intestinal cell
UV irradiation
Cloned frog
Cloned mammals

"Therapeutic" cloning: Derivation of embryonic stem cells from the patient



Solter and Gearhart, *Science* 1999

This Week

PAGE 1751
Dinosaurs' growth spurt

1752
Meeting of the minds on innovation

STEM CELLS

Korean University Will Investigate Cloning Paper

SEOUL AND TOKYO—Embattled Korean stem cell scientist Woo Suk Hwang and his university have bowed to pressure for an investigation into a growing list of questions about a landmark paper he and colleagues published in *Science* in June 2005 (17 June, p. 1777). On 12 December, Seoul National University (SNU), where Hwang works, announced it will conduct an investigation at the scientist's request. This follows a 7 December petition for an investigation from 30 SNU faculty members to university president Un Chan Chung. Prompted initially by anonymous allegations made on a public Web site about irregularities in the paper, scientists in Korea and elsewhere are calling for the paper's key DNA fingerprinting tests to be redone by an independent researcher.

Back to work. Cloning researcher Woo Suk Hwang returned to his lab on 12 December. He had been hospitalized for several days suffering from symptoms of stress and fatigue.

(As *Science* went to press, one of Hwang's co-authors, Gerald Schatten of the University of Pittsburgh in Pennsylvania, asked *Science* to remove his name from the paper.)

Meanwhile, stem cell researchers elsewhere are worried about the possible fallout. The lab's as-yet-unreplicated feat of creating human embryonic stem (ES) cell lines that match the DNA of patients inspired a global ramp-up in stem cell efforts. Such ES cell lines might one day provide replacement cells genetically matched to a patient suffering from Parkinson's disease or diabetes. Hwang's team not only showed that producing such ES cell lines was possible but also that it could be done efficiently, with relatively few donated oocytes per cell line. Alan Colman, head of Singapore-based ES Cell International and a member of the team that produced Dolly the sheep, the first cloned mammal, says, "I'd still like to believe this is a case of sloppy presentation but good science." If the results of the paper do not hold up, he says it could set the field back to a time when many thought the research "was too difficult and inefficient to pursue." It would also provide ammunition to opponents of the research, he says.

The latest revelations center on the DNA fingerprinting in the paper's supplementary online material first posted on 19 May 2005; the fingerprinting data purportedly show that the ES cells are genetically identical to the patients. There are also new allegations about another set of images in the online material that Hwang last week told editors at *Science* had been erroneously duplicated (*Science*, 9 December, p. 1595). All the scientific questions can apparently be traced to anonymous observations about the paper posted on an Internet message board hosted by the Biological Research Information Center (BRIC) (bric.postech.ac.kr). BRIC officials declined to comment, but a senior Korean scientist who has followed the postings agreed to discuss the issue provided he not be identified. (The Korean scientists contacted for this article requested anonymity because they fear a backlash against what are perceived to be attacks on Hwang, who has become a national icon. "This issue is now completely beyond the realm of science," one laments.)

The senior scientist says the message board writer, who claims to be a life science researcher, first pointed out the possibility of duplicated images early on 5 December Korea time. Hwang's e-mailed notice of problems with duplicate images arrived at *Science*'s editorial offices on 4 December at 11:29 p.m. Eastern Standard Time, which would have been 1:29 p.m. on 5 December in Korea, or several hours after the images were posted on the message board.

On 7 December, a critique of the DNA fingerprinting results appeared on the BRIC site. DNA fingerprinting shows a genetic match between two samples when peaks in the traces line up. But because the height and shapes of peaks are influenced by random factors, they should not be identical. The anonymous poster pointed out that the traces for several cell lines appear to be identical to the traces from the respective patients. In other cases, the background noise on the two traces looks very similar.

Alec Jeffreys, a genetic fingerprinting expert at the University of Leicester, U.K., said in an e-mail that "some of the traces do look unusually similar in peak shape and background noise." He declined to comment further without seeing the original data.

The anonymous poster also notes that Hwang's admission of duplicated images does not include other images that appear to have been duplicated.

The postings have elicited a flurry of responses. The consensus, says the senior scientist following the BRIC postings, seems to be that if Korean scientists don't take the lead in reviewing the paper, "the integrity of the Korean scientific community might be questioned by the world community."

Two of the 30 SNU professors who signed the petition asking for an investigation told *Science* the group first learned of the questions surrounding the paper from the BRIC discussion. One of the two professors contacted by *Science* says that they are not trying to discredit Hwang. "Dr. Hwang is a pioneer researcher in the field, and his studies should be pursued. We just see a serious need for a review."

The investigation comes amid a flurry of claims and counterclaims in the Korean media. On 10 December, a Korean news Web site called Pressian reported that it had seen a transcript from an unaired documentary by the Korean Munhwa Broadcasting Corp. MBC pulled the documentary, prepared for a weekly TV show called *PD Notebook*, in response to public outcry over allegations ▶

CHUNG SUNG-JUN/GETTY IMAGES

Writing a new ending for a story of scientific f

He was once hailed as a research pioneer, a credit to his country—South Korea, which treated him as a hero—and as *Scientific American*'s Research Leader of the Year for 2005. But now the career of Hwang Woo Suk has been moved on to the marginalia of history, where he joins a small but infamous group of scientists who have committed fraud.

In 2004, Hwang published a paper in *Science*, which reported the successful cloning, using 242 donor eggs, of a human embryo, from which he was able to derive stem cells. The next year, in *Nature*, Hwang announced the creation of Snuppy, an Afghan hound, the first cloned dog. But it was a May, 2005, *Science* report that catapulted him into the spotlight. In that paper, Hwang described the cloning, using only 185 eggs, of 11 separate stem-cell lines from the DNA of patients, an achievement thought to be the first step towards customised, genetically matched repair of injured tissues.

The first sign of trouble emerged when it was uncovered that, contrary to Hwang's previous assertions, as many as 1100 eggs may have been used for the 2005 paper. Furthermore, some of those used in the earlier paper came from research staff who worked in Hwang's laboratory (an ethical lapse) and some from paid donors (currently illegal in South Korea).

More revelations quickly followed. On Dec 29, a Seoul National University panel announced that Hwang could produce no data to support the findings reported in the May, 2005, paper. The same day, *Science* reported that it was "moving toward retraction" based on news reports. Investigation of the Snuppy paper is now underway at *Nature*. And *Scientific American* recanted the honour it had bestowed on him. Hwang claims that he is the victim of a conspiracy and that his technology will eventually be vindicated, but he has resigned his position and prosecutors are looking into the case.

A further troubling aspect of the scandal is the involvement of an American collaborator, Gerald Schatten, a professor at the University of Pittsburgh School of Medicine. He was the last named, so-called senior author on the 2005 *Science* paper, despite having little to do with the research. He is now also the target of a formal university investigation.

Perhaps no previous case of scientific fraud has been so intertwined with political, economic, and public-policy issues. South Korea was
research could legally be
on embryonic stem c
regulated. The South
results that would not o
also put the country on
poured money—about
laboratory. Another $
creation in October of a
cell lines would be churn
one of Hwang's co-aut
adviser to the President
As patient advocacy
world turned up the
restrictions on stem-cel
the encouragement of
work promised therapeu
conclusion *The Lancet* w
the people most in n
research are the ones wh
The real victims here a
that this story demands
currently being offered.
about what causes sc
limitations of peer revi
communities need to fi
future integrity and reput
Some critics will call f
bureaucracy to control
regulation would be enti
existing oversight proce
discover misconduct. The
quickly been shown to be fake is proof that the system of oversight currently in place has the power to self-correct when a transgression occurs. More oversight will only hamper vital patient-centred research efforts.

Instead, politicians and policy-makers will probably recall that bad cases make bad law. Nobody can legislate to prevent a breach of trust—Hwang's most serious error. Collectively, we must create a better, more collaborative, and more favourable public environment for this important research to take place. An over-reaction now to this deeply regrettable episode of fraud could have damaging long-term consequences for those whose interests matter most. ■ *The Lancet*

For Hwang's papers in *Science* and *Nature* see
Nature 2005; 438: 641
DOI:10.1038/438641a
Science 2005; 308: 1777–83
DOI: 10.1126/science.1112286
Science 2004; 303: 1669–74
DOI: 10.1126/science.1094515
For the previous *Lancet* editorial on stem-cell research see
Lancet 2005; 365: 1904
DOI:10.1016/S0140-6736(05)66634-2

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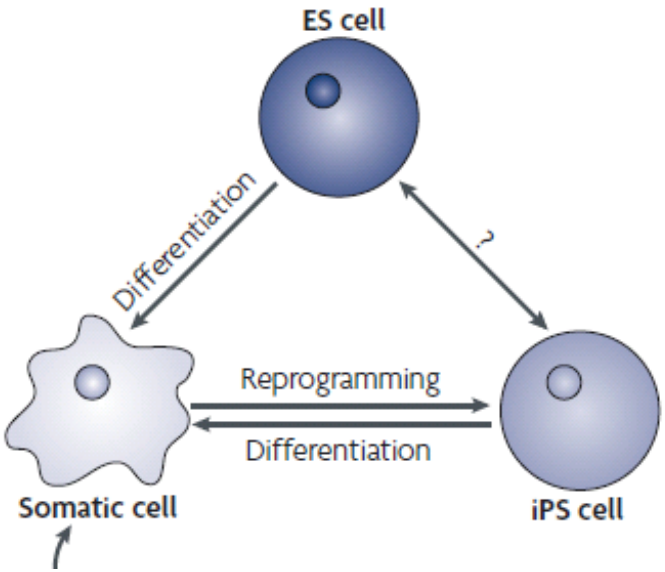
iPS cells

- Development is dictated by epigenetic episodes: as such, it can thus be reversed.
- The iPS (induced pluripotent stem) cells are somatic cells that reacquire embryonic stem cell features through the introduction of defined factors.



History of iPS cells

- The first published paper

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]

- *Cell, Agosto 2006*

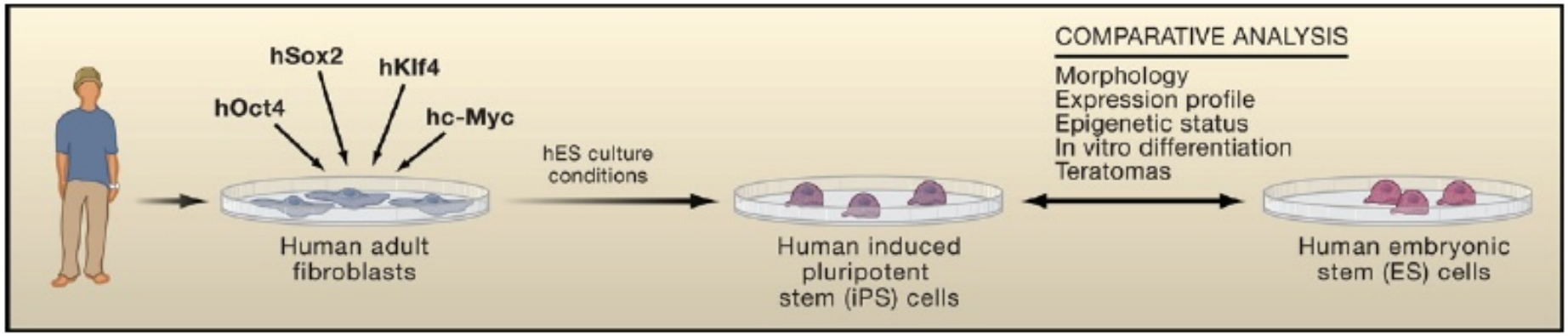
Please cite this article in press as: Takahashi et al., In
Factors, Cell (2007), doi:10.1016/j.cell.2007.11.019

Induction of Pluri
from Adult Huma
by Defined Facto

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohn
and Shinya Yamanaka[1,2,3,4,*]
[1]Department of Stem Cell Biology, Institute for Frontie
[2]CREST, Japan Science and Technology Agency, Kav
[3]Gladstone Institute of Cardiovascular Disease, San F
[4]Institute for Integrated Cell-Material Sciences, Kyoto
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

SUMMARY

Successful reprogramming of differentiated human somatic cells into a pluripotent state would allow creation of patient- and disease-specific stem cells. We previously reported generation of induced pluripotent stem (iPS) cells, capable of germline transmission, from mouse somatic cells by transduction of four defined transcription factors. Here, we demonstrate the generation of iPS cells from adult human dermal fibroblasts with the same four factors: Oct3/4, Sox2, Klf4, and c-Myc. Human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Furthermore, these cells could differentiate into cell types of the three germ layers in vitro and in teratomas. These findings demonstrate that iPS cells can be generated from adult human fibroblasts.


hSox2
hKlf4
hOct4
hc-Myc
hES culture conditions
COMPARATIVE ANALYSIS
Morphology
Expression profile
Epigenetic status
In vitro differentiation
Teratomas
Human adult fibroblasts
Human induced pluripotent stem (iPS) cells
Human embryonic stem (ES) cells

Cambio di rotta Dopo la scoperta di uno scienziato giapponese: i geni per far «ringiovanire» le cellule adulte

Il padre di Dolly: inutile la clonazione

Ian Wilmut: «Create staminali senza gli embrioni, scelgo questa via»

La scoperta dell'università di Kyoto consente di creare staminali da cellule adulte della pelle

Cellule staminali senza bisogno di embrioni. Riportando indietro l'«orologio biologico» di quelle adulte. Vi sarebbero riusciti ricercatori giapponesi e martedì prossimo il loro lavoro verrà pubblicato su una rivista scientifica. Ma c'è chi sa già tutto e anticipa la rivoluzione. E' il britannico Ian Wilmut, il «papà» della pecora Dolly: il primo mammifero clonato nel 1997 dalla cellula di un altro mammifero adulto. Wilmut, del *Roslin Institute* di Edimburgo, ha deciso di abbandonare la via della clonazione a scopo terapeutico (aveva il via libera del governo inglese per applicare sull'uomo la tecnica-Dolly) a favore della produzione di cellule staminali con la tecnica giapponese. E lo ha dichiarato al quotidiano *Daily Telegraph*. E' la grande rinuncia.

Lo scienziato che ha fatto radicalmente cambiare idea a

Zoo dei cloni

Pecora
Il primo animale clonato nella storia della scienza è una pecora, Dolly. L'anno è il 1997, esplodono le polemiche

Vitello
Nel 1999 Galileo è il primo animale maschio clonato da un adulto. Il suo «papà» è l'italiano Cesare Galli

Lo scienziato e la sua creatura Ian Wilmut abbraccia Dolly, primo mammifero clonato da una cellula di un altro mammifero adulto

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assume zero insolation during winter (polar night) throughout glacial–interglacial cycles. They themselves acknowledge this point, and suggest that other factors not accounted for in their approach may explain the discrepancy.

Nevertheless, we must now consider that the orbital-precession rhythm in Antarctic ice cores can partly be attributed to local conditions. In the same way that an ill-fitting piece of a jigsaw puzzle can be disconcerting, this pseudo-rhythm will be discomfiting to those who study palaeoclimate and climate dynamics. 'Is the signal I see really created by climate change?', is a question they will have to ask themselves. And they will need to take a hard look at the principles on which their data are founded. The relationship between the isotopes in water and air temperature, for instance, is based on geographical (spatial) observations only. But its temporal variability has not been confirmed at any ice-core drilling sites in inland Antarctica, even by observations on an annual timescale. Sometimes, in science as in life, it is necessary to pause in order to make progress. ■

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STEM CELLS

The dark side of induced pluripotency

Induced pluripotent stem cells have great therapeutic potential. But genomic and epigenomic analyses of these cells generated using current technology reveal abnormalities that may affect their safe use. SEE ARTICLES P.58, P.63 & P.68

MARTIN F. PERA

Induced pluripotent stem cells (iPSCs) are generated through the reprogramming of differentiated adult cells and can be coaxed to develop into a wide range of cell types. They therefore have far-reaching potential for use in research and in regenerative medicine. But the ultimate value of these cells as disease models or as sources for transplantation therapy will depend on the fidelity of their reprogramming to the pluripotent state, and on their maintenance of a normal genetic and epigenetic (involving aspects other than DNA sequence) status. Five recent surveys[1–5], including three in this issue[1–3], show that the reprogramming process and subsequent culture of iPSCs *in vitro* can induce genetic and epigenetic abnormalities in these cells. The studies raise concerns over the implications of such aberrations for future applications of iPSCs.

It has long been known[6] that, during cultivation *in vitro*, human embryonic stem cells (ESCs) can become aneuploid; that is, they acquire an abnormal number of chromosomes. The new papers have applied various state-of-the-art genomic technologies to assess in detail the occurrence and frequency of genetic and epigenetic defects in both human iPSCs and ESCs.

Hussein *et al.*[1] (page 58) studied copy number variation (CNV) across the genome during iPSC generation, whereas Gore and colleagues[2] (page 63) looked for point mutations in iPSCs using genome-wide sequencing of protein-coding regions. Lister *et al.*[3] (page 68) examined DNA methylation — an epigenetic mark — across the genomes of ESCs and iPSCs at the single-base level. These studies, along with other investigations into changes in chromosome numbers[4] and CNV[5] in the two kinds of stem cell, lead to the conclusion that reprogramming and subsequent expansion of iPSCs in culture can lead to the accumulation of diverse abnormalities at the chromosomal, subchromosomal and single-base levels. Specifically, three common themes, regarding the genetic and epigenetic stability of ESCs and iPSCs, emerge.

First, by several measures, iPSCs display more genetic and epigenetic abnormalities than do ESCs or fibroblasts — the cells from which they originated. Chromosomal abnormalities appear early during the culturing of iPSCs[5], a phenomenon not generally observed in ESCs. Also, the frequency of mutations in iPSCs is estimated to be ten times higher than in fibroblasts[2]. And there are greater numbers of novel CNVs (CNVs not found in the cell of origin or in human genomes of comparable background) in iPSCs than in ESCs[1,5]. Similarly, the epigenome of iPSCs features incomplete reprogramming (with cells retaining epigenetic marks of the cell of origin), aberrant methylation of CG dinucleotides, and abnormalities in non-CG methylation — an epigenetic feature seen only in pluripotent cells[3].

Second, the studies show that genetic abnormalities can arise at different stages of iPSC generation. Some lesions are inherited from the cell used for reprogramming. Gore *et al.*[2] employ a particularly sensitive approach to demonstrate that a subset of point mutations found in iPSC lines pre-existed in a small minority of fibroblasts used for reprogramming. Other lesions seem to arise early on in reprogramming, as mentioned previously. For example, Hussein *et al.*[1] found large numbers of new CNVs during early passages (subcultures) following reprogramming, but noted that subsequent growth *in vitro* seemingly selected against most of the changes, which implies that they are deleterious for the cells that bear them. The studies also report changes that apparently relate to long-term adaptation to cell culture. These include over-representation either of the short arm of chromosome 12 (12p) or of this entire chromosome[4,5], and of a subregion in the long arm of chromosome 20 (ref. 5). Both of these changes have been observed[6] in ESC lines, with an increased number of 12p being a hallmark of testicular germ-cell tumours — the malignant prototype of human pluripotent stem cells.

Third, several of the groups[2,4,5] report clues to the potential function of the genetic lesions that arise in ESCs and iPSCs. For example, regions prone to amplification, deletion or point mutation seem to be enriched in genes involved in cell-cycle regulation and cancer. Although the changes observed do not strongly implicate any particular gene functionally as a target for change during the amplification of iPSCs or during their adaptation to culture conditions, the frequent association of the affected genes with cancer gives cause for concern.

This highly significant body of data[1–5] provides a revealing, in-depth portrait of the status of the genome and the epigenome during cellular reprogramming. But it also leaves open some fairly challenging questions.

The studies provide little insight into the crucial question of what aspects of the reprogramming methods might predispose the cells to the accumulation of recurrent genetic or epigenetic lesions. Although recurrence of change in specific genomic regions across a number of cell lines strongly implies a selective process, in several studies the researchers noted that there was no obvious correlation between the extent of genetic damage in a

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ARTICLE

doi:10.1038/nature09798

Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells

Ryan Lister[1]*, Mattia Pelizzola[1]*, Yasuyuki S. Kida[2], R. David Hawkins[3], Joseph R. Nery[1], Gary Hon[3], Jessica Antosiewicz-Bourget[4,5], Ronan O'Malley[1], Rosa Castanon[1], Sarit Klugman[3], Michael Downes[2], Ruth Yu[2], Ron Stewart[4,5], Bing Ren[3,6], James A. Thomson[4,5,7,8], Ronald M. Evans[2] & Joseph R. Ecker[1]

Induced pluripotent stem cells (iPSCs) offer immense potential for regenerative medicine and studies of disease and development. Somatic cell reprogramming involves epigenomic reconfiguration, conferring iPSCs with characteristics similar to embryonic stem (ES) cells. However, it remains unknown how complete the reestablishment of ES-cell-like DNA methylation patterns is throughout the genome. Here we report the first whole-genome profiles of DNA methylation at single-base resolution in five human iPSC lines, along with methylomes of ES cells, somatic cells, and differentiated iPSCs and ES cells. iPSCs show significant reprogramming variability, including somatic memory and aberrant reprogramming of DNA methylation. iPSCs share megabase-scale differentially methylated regions proximal to centromeres and telomeres that display incomplete reprogramming of non-CG methylation, and differences in CG methylation and histone modifications. Lastly, differentiation of iPSCs into trophoblast cells revealed that errors in reprogramming CG methylation are transmitted at a high frequency, providing an iPSC reprogramming signature that is maintained after differentiation.

Generation of iPSCs from somatic cells offers tremendous potential for therapeutics, the study of disease states, and elucidation of developmental processes[1,2]. iPSC production techniques introduce active genes that are necessary for pluripotency, or their derivative RNA or protein products, into a somatic cell to induce pluripotent cellular properties that closely resemble those of ES cells[3–8]. Indeed, iPSCs have been used to produce viable and fertile adult mice, demonstrating their pluripotent potential to form all adult somatic and germline cell types[8,9].

The reprogramming process by which a somatic cell acquires pluripotent potential is not a genetic transformation, but an epigenomic one. A recent study reported minimal differences in chromatin structure and gene expression between human ES cells and iPSCs, indicating that ES cells and iPSCs are nearly identical cell types[10]. On the other hand, there are recent reports indicating epigenomic differences between ES cells and iPSCs[11–15] and alterations in the differentiation potential of iPSCs compared to ES cells[13,16,17]. Together, these findings indicate that fundamental differences between ES cells and iPSCs exist, prompting the question of how complete and variable the reestablishment of ES-cell-like DNA methylation patterns are throughout the entire genome.

Presumably, optimal reprogramming of somatic cells to a pluripotent state requires complete reversion of the somatic epigenome into an ES-cell-like state, but until now a comprehensive survey of the changes in such epigenetic marks in a variety of independent iPSC lines has not been reported. Accordingly, we have performed whole-genome profiling of the DNA methylomes of multiple human ES cell, iPSC and somatic progenitor lines, encompassing reprogramming performed in different laboratories, using different iPSC-inducing technologies and cells derived from distinct germ layers. We show that although on a global scale ES cell and iPSC methylomes are very similar, every iPSC line shows significant reprogramming variability compared to both ES cells and other iPSCs, including both somatic 'memory' and iPSC-specific differential DNA methylation. Further, all iPSC lines share numerous non-randomly distributed megabase-scale regions that are aberrantly methylated in the non-CG context, associated with alterations in CG methylation, histone modifications and gene expression. Lastly, we show that differentially methylated regions in iPSCs are transmitted to differentiated cells at a high frequency.

Globally similar ES cell and iPSC methylomes

To assess the degree to which a somatic cell DNA methylome is reprogrammed into an ES-cell-like state by induction of a pluripotent state, we generated whole-genome, single-base resolution DNA methylomes of a range of human cell types using the shotgun bisulphite-sequencing method, MethylC-Seq[18]. Our central focus was a high-efficiency, feeder-free reprogramming system[19], in which female adipose-derived stem cells (ADS) were reprogrammed into a pluripotent state by retroviral transformation with the *OCT4*, *SOX2*, *KLF4* and *MYC* genes (ADS-iPSCs), satisfying the criteria for pluripotency in human cells[20]. Additionally, we analysed the DNA methylome of adipocytes derived from the ADS cells (ADS-adipose) through adipogenic differentiation conditions. Further, to explore the variation between independent iPSC lines potentially due to stochastic reprogramming events, progenitor somatic cell type, reprogramming technique and laboratory-specific effects, we generated full DNA methylomes for four additional iPSC

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Somatic coding mutations in human induced pluripotent stem cells

Athurva Gore[1]*, Zhe Li[1]*, Ho-Lim Fung[1], Jessica E. Young[2], Suneet Agarwal[3], Jessica Antosiewicz-Bourget[4], Isabel Canto[2], Alessandra Giorgetti[5], Mason A. Israel[2], Evangelos Kiskinis[6], Je-Hyuk Lee[7], Yuin-Han Loh[3], Philip D. Manos[3], Nuria Montserrat[5], Athanasia D. Panopoulos[8], Sergio Ruiz[8], Melissa L. Wilbert[2], Junying Yu[4], Ewen F. Kirkness[9], Juan Carlos Izpisua Belmonte[5,8], Derrick J. Rossi[10], James A. Thomson[4], Kevin Eggan[6], George Q. Daley[3], Lawrence S. B. Goldstein[2] & Kun Zhang[1]

Defined transcription factors can induce epigenetic reprogramming of adult mammalian cells into induced pluripotent stem cells. Although DNA factors are integrated during some reprogramming methods, it is unknown whether the genome remains unchanged at the single nucleotide level. Here we show that 22 human induced pluripotent stem (hiPS) cell lines reprogrammed using five different methods each contained an average of five protein-coding point mutations in the regions sampled (an estimated six protein-coding point mutations per exome). The majority of these mutations were non-synonymous, nonsense or splice variants, and were enriched in genes mutated or having causative effects in cancers. At least half of these reprogramming-associated mutations pre-existed in fibroblast progenitors at low frequencies, whereas the rest occurred during or after reprogramming. Thus, hiPS cells acquire genetic modifications in addition to epigenetic modifications. Extensive genetic screening should become a standard procedure to ensure hiPS cell safety before clinical use.

Human induced pluripotent stem cells have the potential to revolutionize personalized medicine by allowing immunocompatible stem cell therapies to be developed[1,2]. However, questions remain about hiPS cell safety. For clinical use, hiPS cell lines must be reprogrammed from cultured adult cells, and could carry a mutational load due to normal *in vivo* somatic mutation. Furthermore, many hiPS cell reprogramming methods use oncogenes that may increase the mutation rate. Additionally, some hiPS cell lines have been observed to contain large-scale genomic rearrangements and abnormal karyotypes after reprogramming[3]. Recent studies also revealed that tumour suppressor genes, including those involved in DNA damage response, have an inhibitory effect on nuclear reprogramming[4–9]. These findings suggest that the process of reprogramming could lead to an elevated mutational load in hiPS cells.

To probe this issue, we sequenced the majority of the protein-coding exons (exomes) of 22 hiPS cell lines and the nine matched fibroblast lines from which they came (Table 1). These lines were reprogrammed in seven laboratories using three integrating methods (four-factor retroviral, four-factor lentiviral and three-factor retroviral) and two non-integrating methods (episomal vector and messenger RNA delivery into fibroblasts). All hiPS cell lines were extensively characterized for pluripotency and had normal karyotypes before DNA extraction (Supplementary Methods). Protein-coding regions in the genome were captured and sequenced from the genomic DNA of hiPS cell lines and their matched progenitor fibroblast lines using either padlock probes[10,11] or in-solution DNA or RNA baits[12,13]. We searched for single base changes, small insertions/deletions and alternative splicing variants, and identified 12,000–18,000 known and novel variants for each cell line that had sufficient coverage and consensus quality (Table 1).

hiPS cell lines contain a high level of mutational load

We identified sites that showed the gain of a new allele in each hiPS cell line relative to their corresponding matched progenitor fibroblast genome. A total of 124 mutations were validated with capillary sequencing (Fig. 1, Table 2 and Supplementary Fig. 1), which revealed that each mutation was fixed in heterozygous condition in the hiPS cell lines. No small insertions/deletions were detected. For three hiPS cell lines (CV-hiPS-B, CV-hiPS-F and PGP1-iPS), the donor's complete genome sequence obtained from whole blood is publicly available[14,15]; we used this information to further confirm that all 27 mutations in these lines were bona fide somatic mutations. Because 84% of the expected exomic variants[16] were captured at high depth and quality, the predicted load is approximately six coding mutations per hiPS cell genome (see Table 1 for details). The majority of mutations were mis-sense (83 of 124), nonsense (5 of 124) or splice variants (4 of 124). Fifty-three mis-sense mutations were predicted to alter protein function[17] (Supplementary Table 1). Fifty mutated genes were previously found to be mutated in some cancers[18,19]. For example, *ATM* is a well-characterized tumour suppressor gene found mutated in one hiPS cell line, and *NTRK1* and *NTRK3* (tyrosine kinase receptors) can cause cancers when mutated[20] and contained damaging mutations in three hiPS cell lines (CV-hiPS-F, iPS29e and FiPS4F-shpRB4.5) that were reprogrammed in three labs and came from different donors. Two kinase genes from the *NEK* family, which is related to cell division, were mutated in two independent hiPS cell lines. In addition to cancer-related genes, 14 of the 22 lines contained mutations in genes with known roles in human Mendelian disorders[21]. Three pairs of hiPS cell lines (iPS17a and iPS17b, dH1F-iPS8 and dH1F-iPS9, and CF-RiPS1.4 and CF-RiPS1.9) shared three, two and one mutation, respectively;

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Ryan Lister[1]*, Jessica Antosi Bing Ren[3,6], J

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[1]Genomic Analysis La Studies, La Jolla, Calif [5]Genome Centre of W Primate Research Cer *These authors contri

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Athurva Gore[1]*, Alessandra Gior Athanasia D. Pa Derrick J. Rossi

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[1]Department of Bioengi [2]Department of Cellular Hematology/Oncology, 53705, USA. [3]Center of University, Cambridge, USA. [4]The J. Craig Venter *These authors contribu

ARTICLE

doi:10.1038/nature09871

Copy number variation and selection during reprogramming to pluripotency

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The mechanisms underlying the low efficiency of reprogramming somatic cells into induced pluripotent stem (iPS) cells are poorly understood. There is a clear need to study whether the reprogramming process itself compromises genomic integrity and, through this, the efficiency of iPS cell establishment. Using a high-resolution single nucleotide polymorphism array, we compared copy number variations (CNVs) of different passages of human iPS cells with their fibroblast cell origins and with human embryonic stem (ES) cells. Here we show that significantly more CNVs are present in early-passage human iPS cells than intermediate passage human iPS cells, fibroblasts or human ES cells. Most CNVs are formed *de novo* and generate genetic mosaicism in early-passage human iPS cells. Most of these novel CNVs rendered the affected cells at a selective disadvantage. Remarkably, expansion of human iPS cells in culture selects rapidly against mutated cells, driving the lines towards a genetic state resembling human ES cells.

Reprogramming somatic cells to pluripotency can be achieved by forced expression of a defined set of factors[1,2]. Several methods have been developed for generating human iPS cells, such as retroviral transduction[1], DNA-transposition-based systems[3,4], transient plasmid delivery[5] and integration/plasmid-free systems[6,7]. To improve efficiency and in an effort to understand the process of reprogramming, several groups have demonstrated that modulating key components of the cell cycle, such as repression of the *Ink4a/Arf* locus or downregulation of the p53–p21 pathway, have marked positive effects on reprogramming efficiency[8–12]. However, p53 suppression can lead to increased levels of DNA damage and genomic instability. These findings suggest that the reprogramming process places a heavy burden on cellular integrity and highlight the importance of further exploring the nature of the DNA damage that is associated with the reprogramming process.

High CNV levels in early-passage human iPS cells

To determine whether reprogramming is associated with *de novo*-generated CNVs, we used the Affymetrix SNP array 6.0 to characterize 22 human iPS cell lines along with 17 human ES cell lines[13], as well as three parental and one unrelated fibroblast lines as controls (Supplementary Table 1). The human iPS cell lines were established either by retroviral[2] or *piggyBac*[3,4] gene delivery methods and confirmed as human iPS cells using established criteria[14] (Supplementary Figs 1–3 and Supplementary Table 2). Nine of the 22 human iPS cell lines were characterized at more than one passage to track CNVs during propagation.

The median number of CNVs in human iPS cell lines (109) was about twofold higher than in human ES cell lines (55) and fibroblasts (53) (Supplementary Fig. 4a and Supplementary Tables 3 and 4). We found that the majority of CNVs (52.4%) in human iPS cells were not present in either human ES cells or fibroblasts (Supplementary Fig. 4b). Interestingly, the number of CNVs negatively correlated with the passage number. This was surprising because fibroblasts and human ES cells showed no significant changes during intermediate length passaging (Supplementary Fig. 4c, d). Both the number and the total size of CNVs in human iPS cell lines decreased during propagation (Fig. 1a and Supplementary Fig. 4e). Neither the reprogramming factor delivery method, fibroblast source or viral integration sites nor the presence or absence of Myc during reprogramming (Fig. 1b, c and Supplementary Fig. 5) influenced these results. This trend was verified in an independent data set on human iPS cell lines derived from four adult skin fibroblasts (Supplementary Table 5), as well as within individual human iPS cell lines analysed at early and later passages (Fig. 1b–d). Our findings indicate that CNVs are generated during the reprogramming process.

Genetic mosaicism in human iPS cells

The decrease in CNVs during passaging could be explained either by DNA repair mechanisms or by mosaicism followed by selection. We propose that DNA repair may not be efficient enough to explain the rapid decrease in CNVs but, instead, that *de novo*-generated CNVs create mosaicism, which is followed by selection favouring less damaged cells during propagation. To obtain direct proof for mosaicism, we established new human iPS cell lines and tested these at very early passages (passage 2 and 3) for CNVs by using fluorescence *in situ* hybridization (FISH). We chose a probe that maps to a locus on chromosome 1 that, according to our single nucleotide polymorphism (SNP) array data, is frequently affected in human iPS cell lines (Fig. 2a). A control probe was selected from a chromosome 1 location that showed normal copy number (2) across all human iPS cell lines that were tested. During early passages, the test probe demonstrated a

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*These authors contributed equally to this work.

Pros and Cons to iPS cell technology

- Pros:
  - Cells would be genetically identical to patient or donor of skin cells (no immune rejection!)
  - Do not need to use an embryo
- Cons:
  - Cells would still have genetic defects
  - One of the pluripotency genes is a cancer gene
  - Viruses might insert genes in places we don't want them (causing mutations)

NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

Reprogrammed Cells Earn Biologists Top Honor

Life is a one-way tri
start as embryos and
versible developmen
death. It was natural
assume that this inexo
to all the cells that ma
and that a cell's diff
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Demonstrating t
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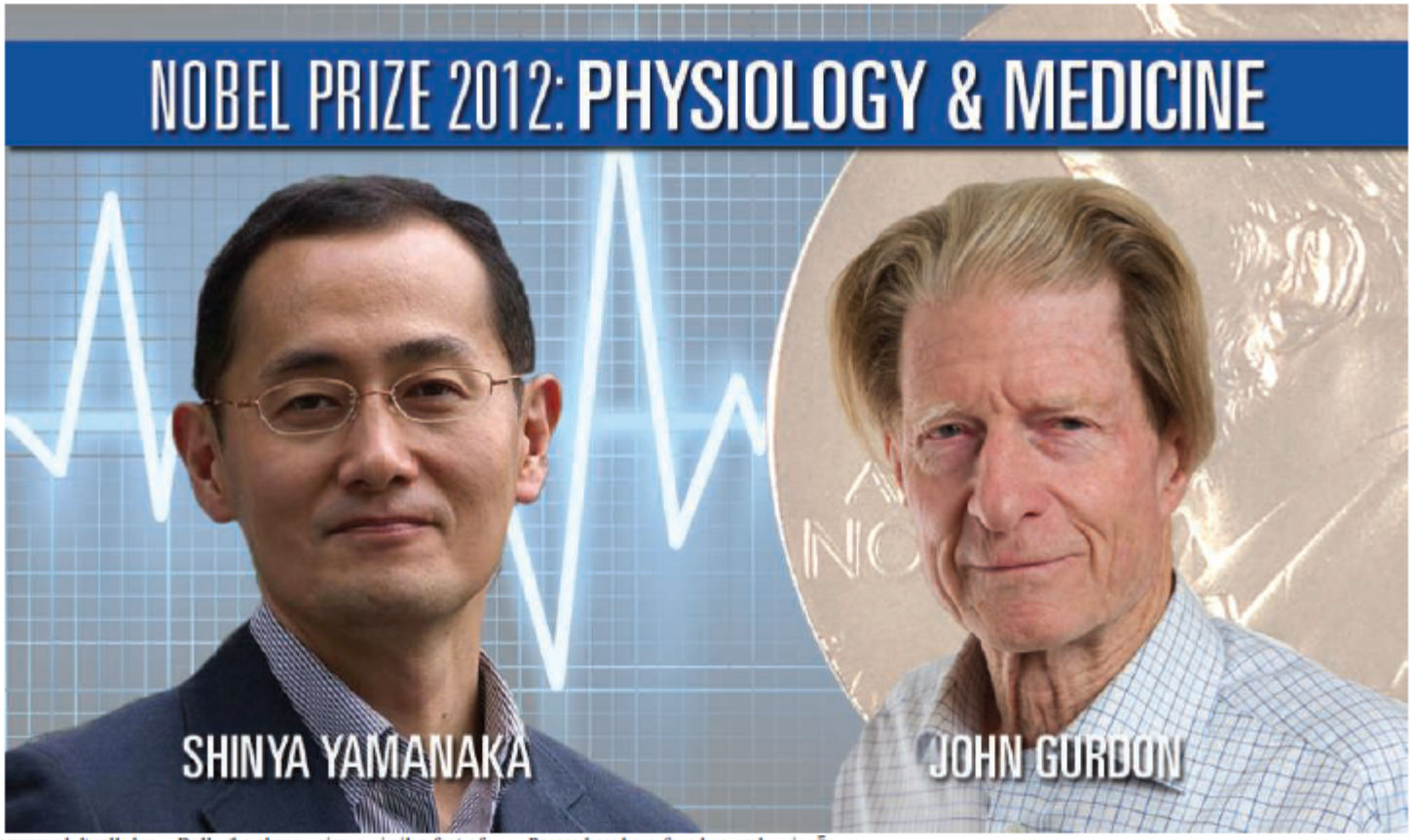

The ability to reprogram adult cells has made it possible for researchers to study diseases in new ways and raises the possibility of someday growing replacement tissues or even organs in the lab. Yamanaka's work, published just 6 years ago, "is completely transformative," says Austin Smith, a developmental biologist at the University of Cambridge. Although the Nobel Committee often waits decades before awarding a prize, in Yamanaka's case, Smith says, Dolly the sheep using a similar feat of nuclear transfer. That breakthrough demonstrated that mammalian cells could undergo the same transformation from mature to immature. The technique was extremely inefficient, however, resulting in many more failures than successes. And no one has been able to get the method to work in human cells.

A fundamental question dogged the field: Could researchers reconstitute in a lab dish an egg cell's ability to turn back the cellular clock? "What we all believed … was that it could be done but must be a very, very complicated process," Colman says. "Then Shinya comes along and showed only four factors could do it."

Researchers have found several variations on Yamanaka's original recipe for making iPS cells, which involved inserting extra copies of genes that are involved in cancer; the resulting cells were prone to forming tumors. It's now possible to reprogram adult cells to a stem cell state without making permanent genetic changes in them. Several groups are working on ways to reprogram mature cells—say, skin cells—directly into another mature cell type such as cardiac

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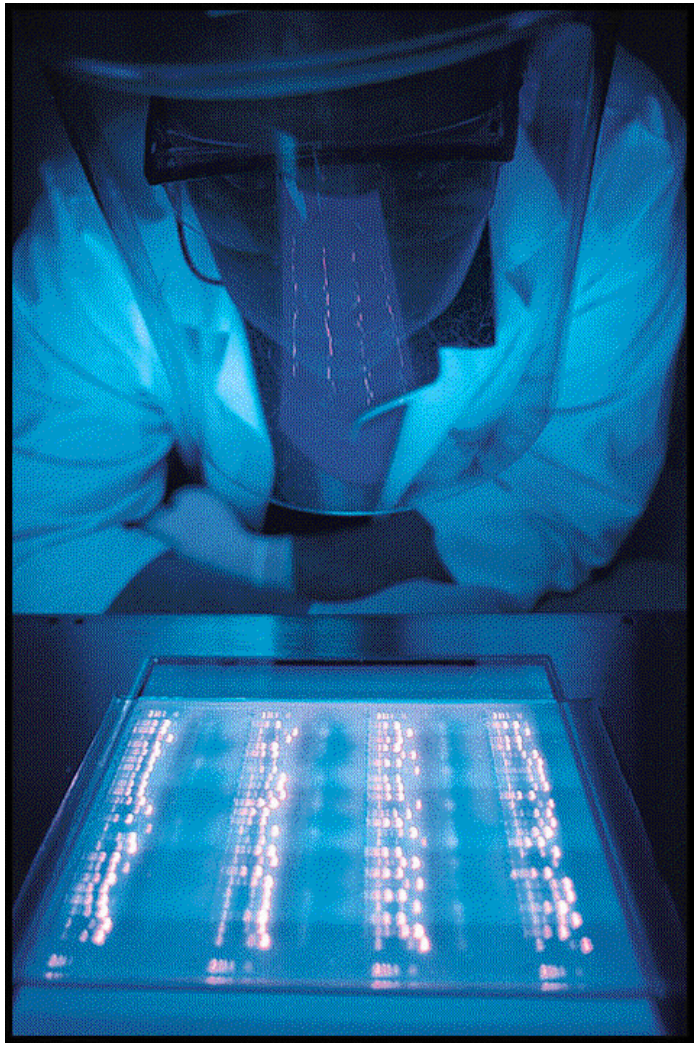
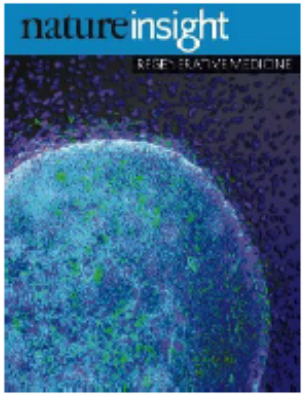
Hopes in Medicine with stem cells



- ***Consolidated therapies with adult stem cells***
- ***Tissue engineering***
- ***Experimentsl therapies with stem cells***
- ***Conclusions***

www.nature.com/nature Vol 453 | Issue no. 7193 | 15 May 2008

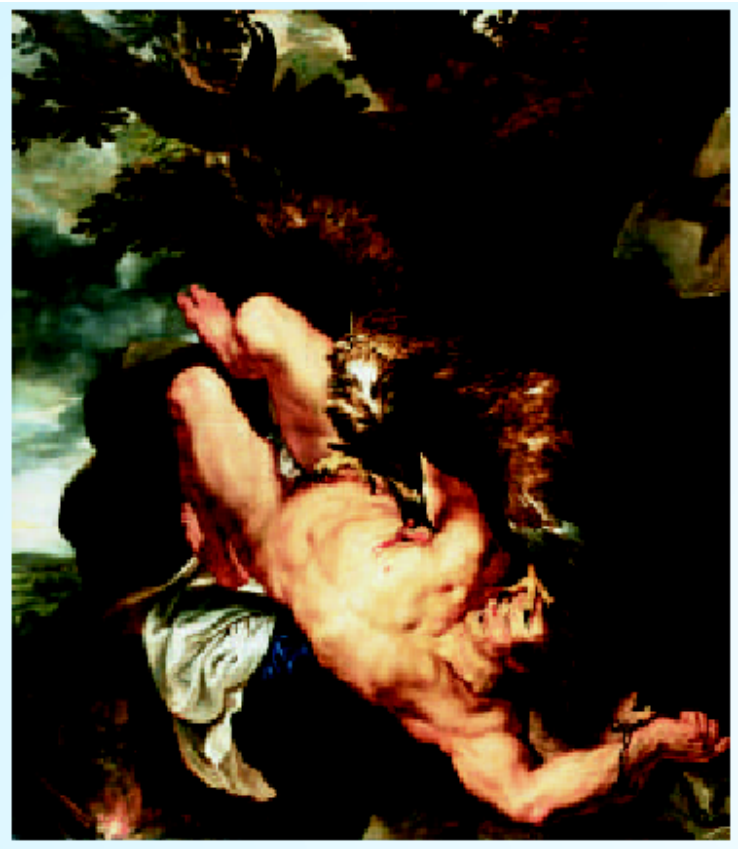


Cover illustration
A colony of human embryonic stem cells (light blue) growing on fibroblasts (dark blue). (Courtesy of A.Michalska and A. Trounson, Monash University (MISCL))

REGENERATIVE MEDICINE

Life is regenerative, by definition. But by and large, humans lack the regenerative capacity of creatures such as newts and hydra. Although some of our cells have the innate ability to replenish themselves — and, by doing so, to repair ageing and injured tissues and organs — most of the body's cells form the specialized cell type they are destined for and then go into lock down.

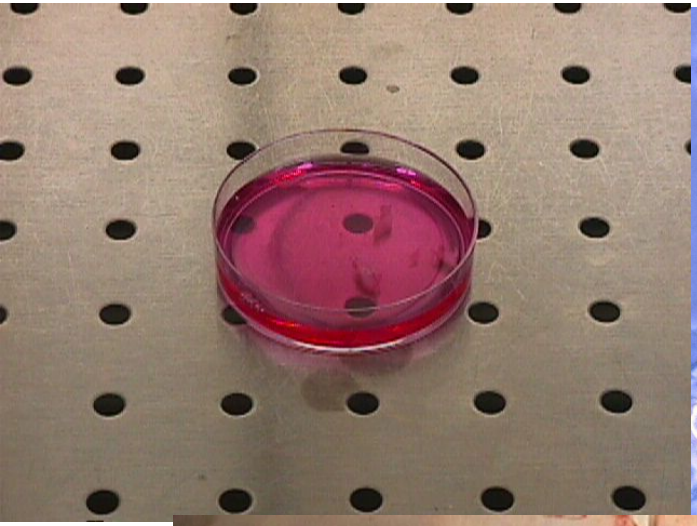
Hopes in Medicine with stem cells

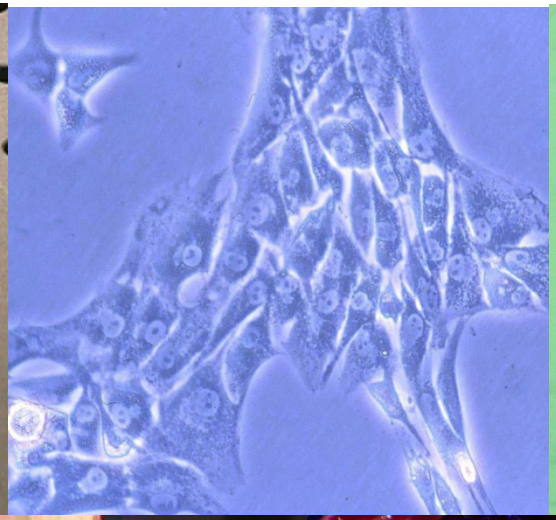


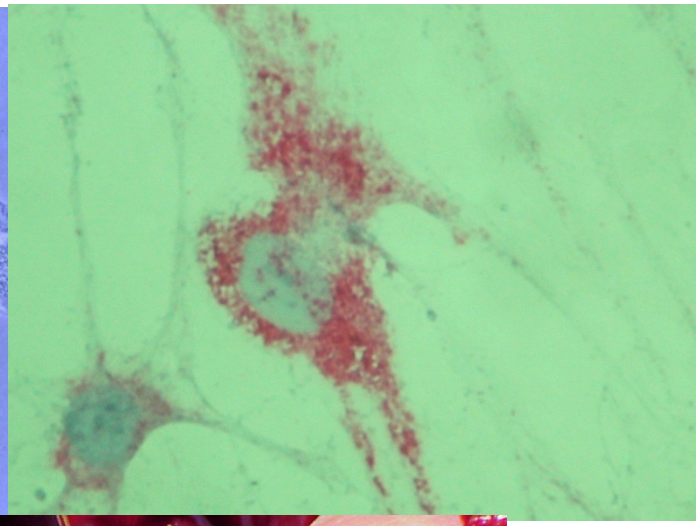
- ***Consolidated therapies with adult stem cells***
- ***Tissue engineering***
- ***Experimentsl therapies with stem cells***
- ***Conclusions***

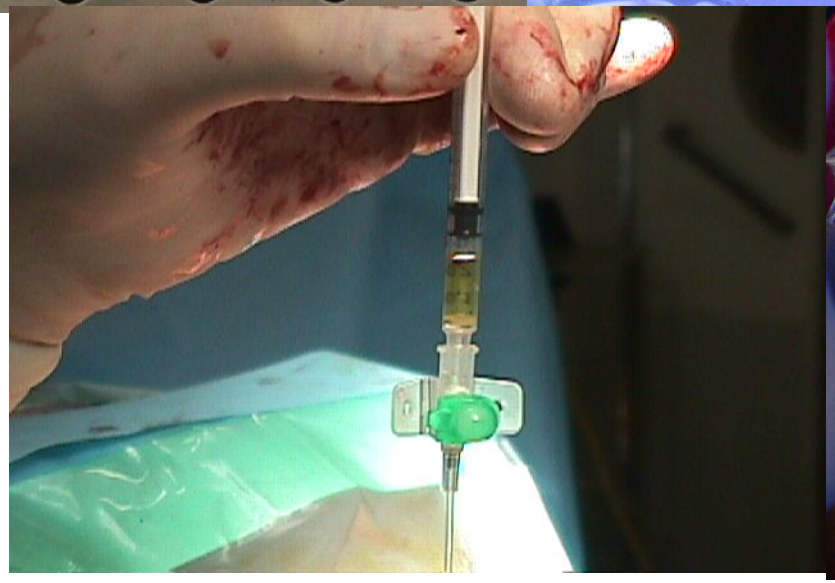
- hemopoietic stem cells, for the treatment of hematological diseases, some solid tumors, diseases of the hemopoietic and immune system.
- mesenchimal stem cells, for the prevention of Graft versus Host disease and regeneration of bone and cartilage.
- expanded in vitro skin, keratinocytes and fibroblasts for treatment of burns and skin diseases.
- limbar stem cells, for the treatment of corneal lesions.

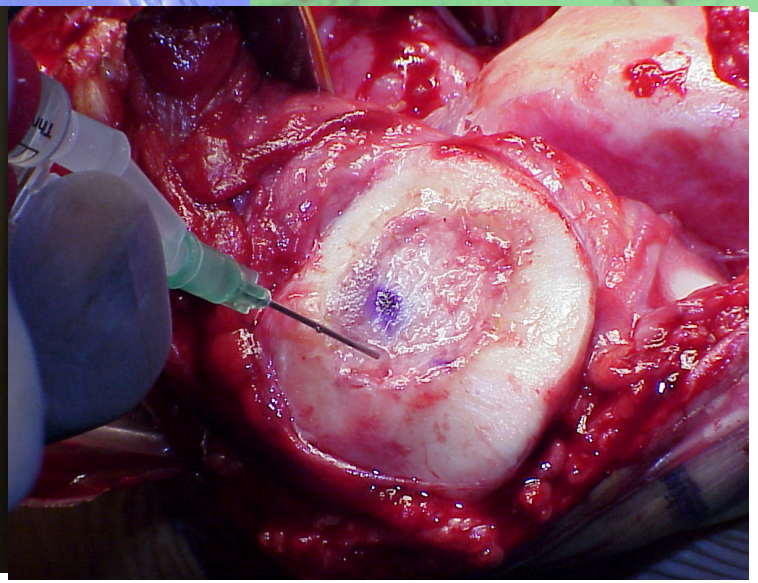
AUTOLOGOUS CHONDROCYTE TRANSPLANTATION

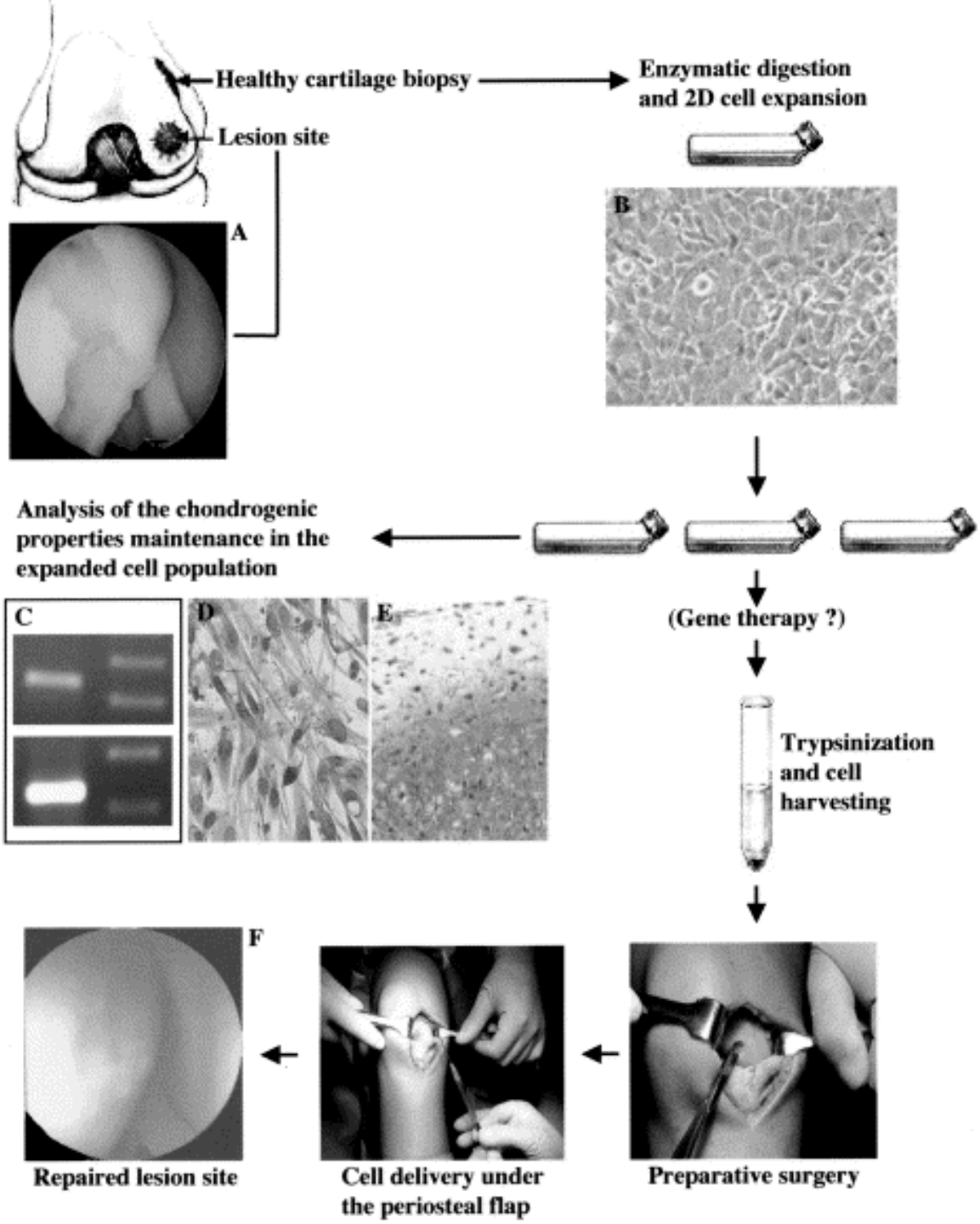


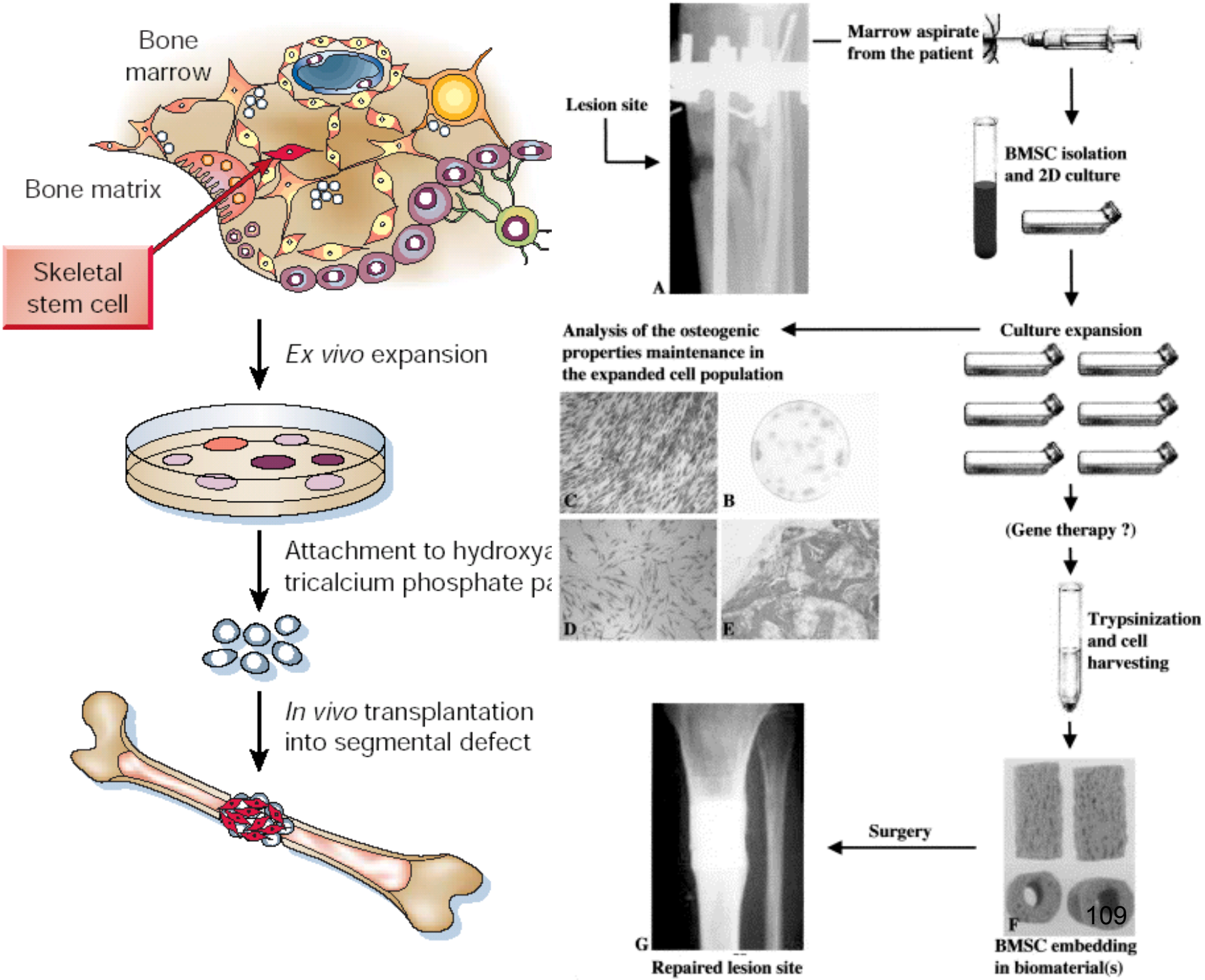









Healthy cartilage biopsy
Lesion site
A
Enzymatic digestion
and 2D cell expansion
B
Analysis of the chondrogenic
properties maintenance in the
expanded cell population
C
D
E
(Gene therapy ?)
Trypsinization
and cell
harvesting
F
Repaired lesion site
Cell delivery under
the periosteal flap
Preparative surgery


Bone marrow
Bone matrix
Skeletal stem cell
Ex vivo expansion
Attachment to hydroxya
tricalcium phosphate pa
In vivo transplantation
into segmental defect
Lesion site
A
Marrow aspirate from the patient
BMSC isolation and 2D culture
Culture expansion
Analysis of the osteogenic properties maintenance in the expanded cell population
C
B
D
E
(Gene therapy ?)
Trypsinization and cell harvesting
F
BMSC embedding in biomaterial(s)
Surgery
G
Repaired lesion site

REMOVAL OF SKIN FOR THE CULTIVATION OF KERATINOCYTES

- It is basically similar to a skin biopsy
- It is necessary to send to the laboratory a smallo fragment of full-thickness skin of the pationt (2 cm x 1 cm) taken from a healthy area, possibly hidden (prelevato da un'area sana, possibilmente nascosta (behind the ear region or groin region)
- Cultured keratinocytes will be ready after an average time of about three weeks.

- The keratinocytes cultured in vitro generate cohesive sheets of laminated epithelium that retain the characteristics of the epidermis
- Cultured autologous keratinocytes are widely used for the treatment of extensive burned areas and epidermal renewal

- **LIMITS:** infections, graft site preparation can cause problems (dermabrasion, necrectomia, ecc.)

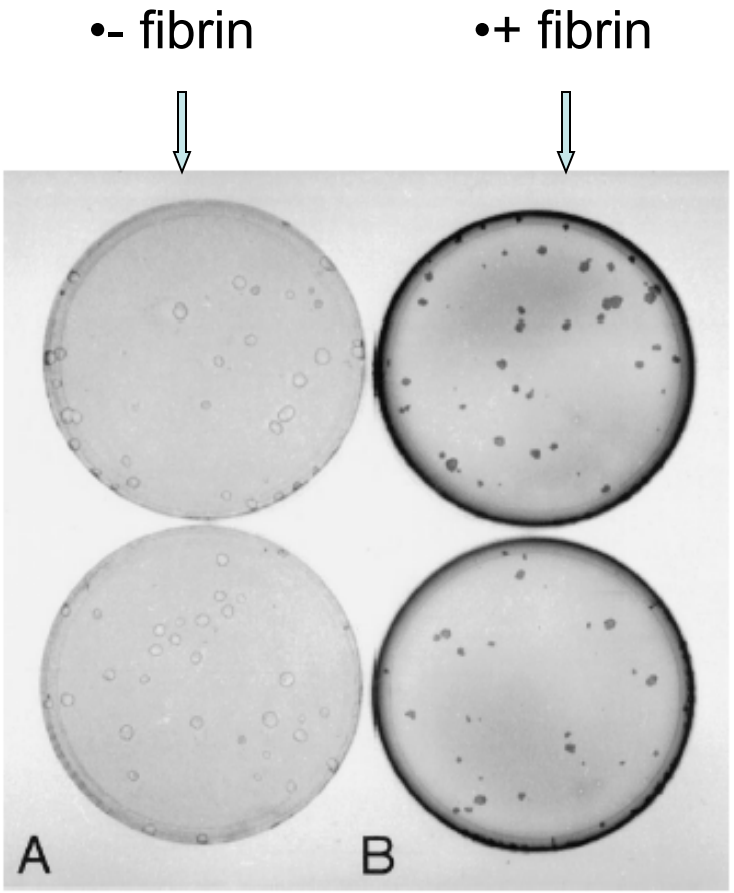


FIGURE 2. Colony-forming efficiency of human keratinocytes grown on a fibrin matrix. One hundred human keratinocytes (strain YF29, culture V) were plated onto 100-mm Petri dishes containing lethally irradiated 3T3 cells, in the absence (A) or presence (B) of a fibrin matrix. Cells were cultured for 12 days and were then fixed and stained with Rhodamine B (A) or Nile Blue (B). The presence of a fibrin matrix did not affect the clonogenicity of the keratinocytes. Similar numbers of colonies (54) were obtained with or without the fibrin matrix. A similar result was obtained with other strains of human keratinocyte. Colonies cultured on a fibrin matrix were less regular in shape and smaller than the control colonies.

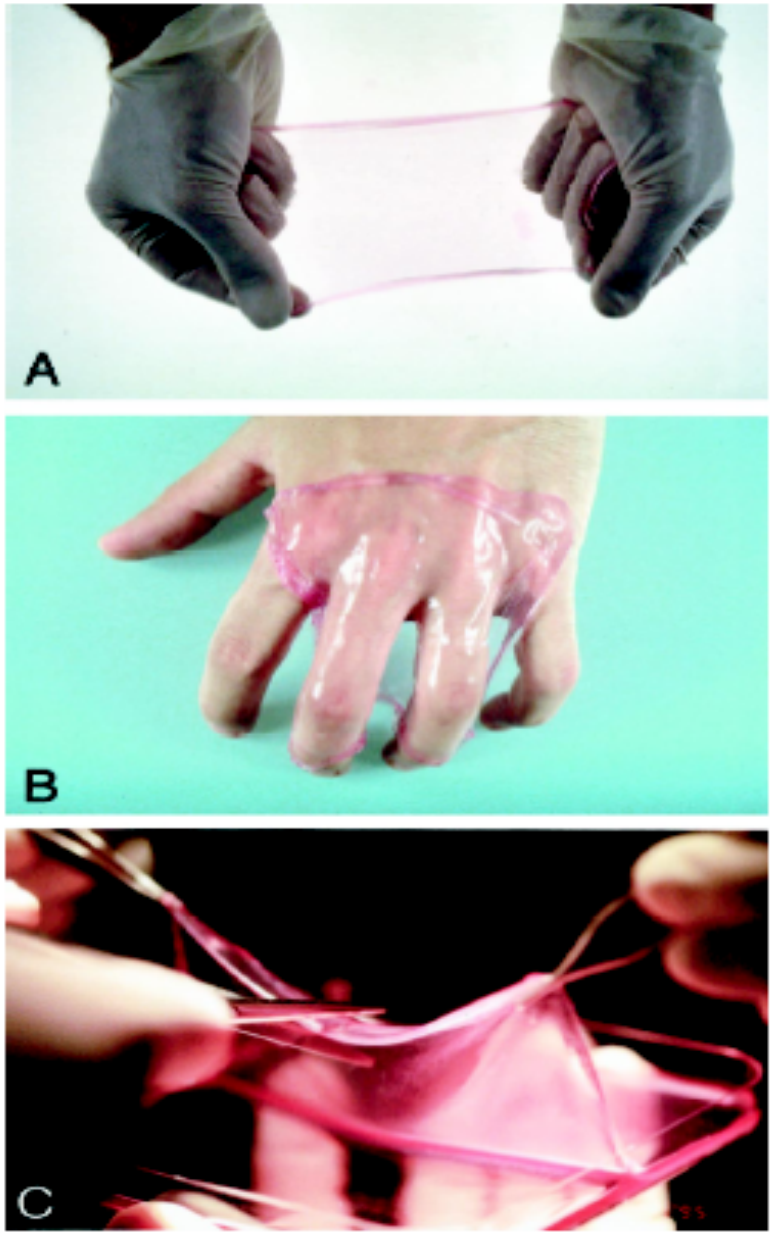
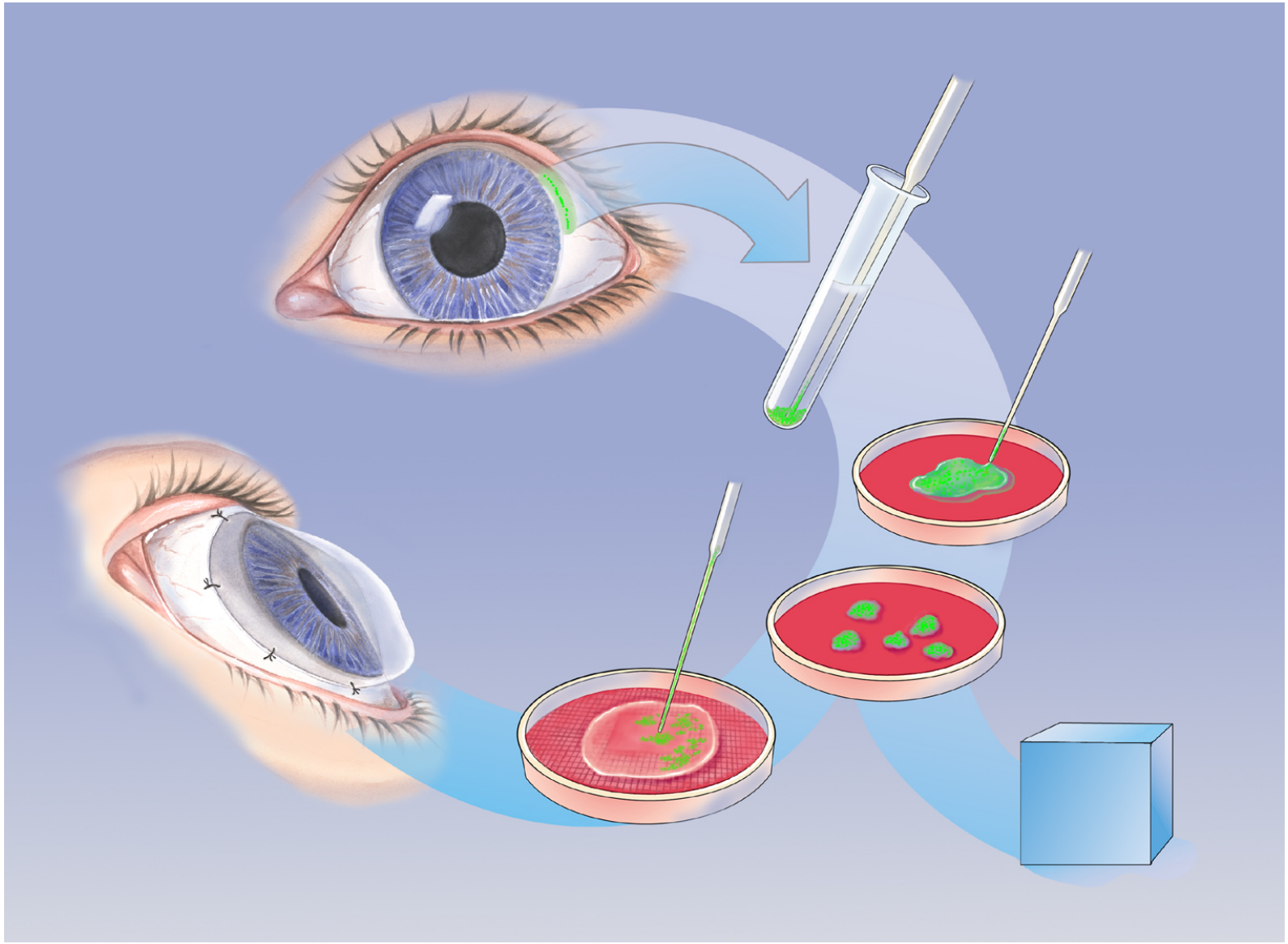
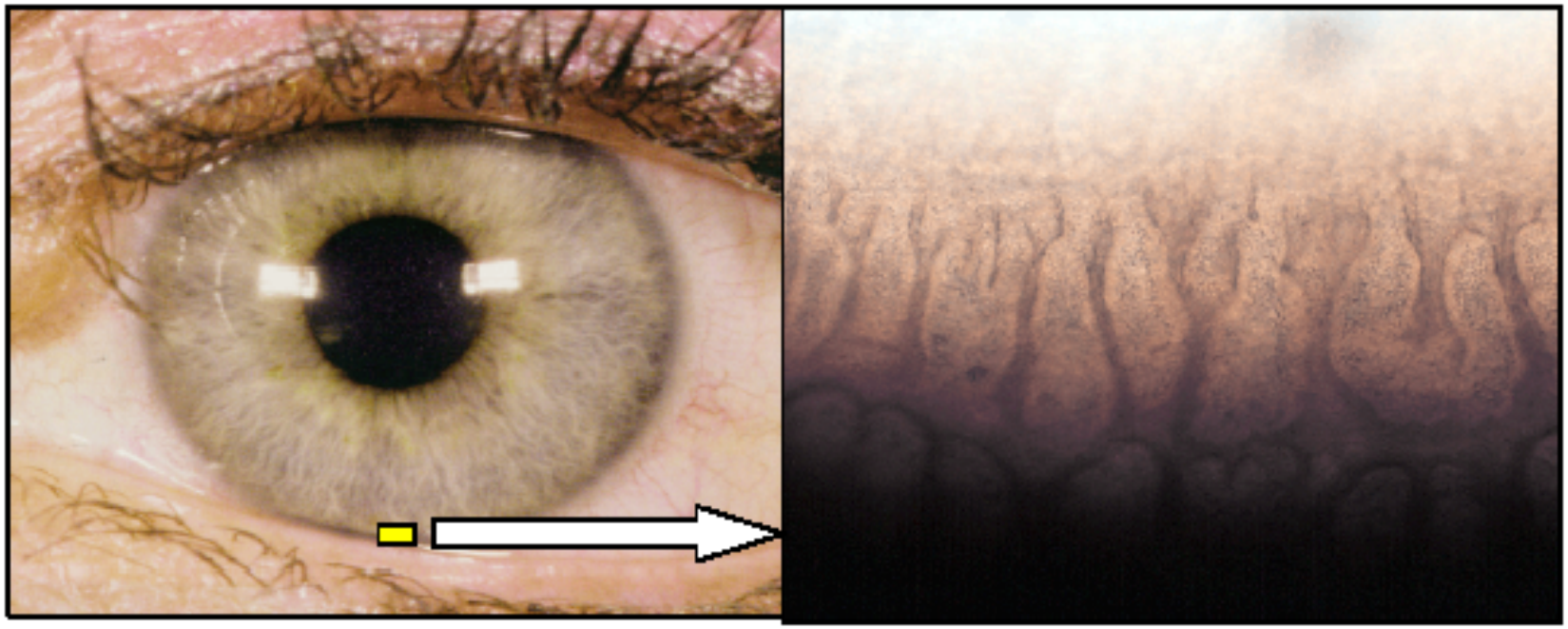


FIGURE 1. Appearance of a cultured epithelium grown on a fibrin matrix. A cultured epithelium grown on a fibrin matrix was detached from the culture dish using two thin forceps. Note its transparency, its molding properties and the ease of handling.

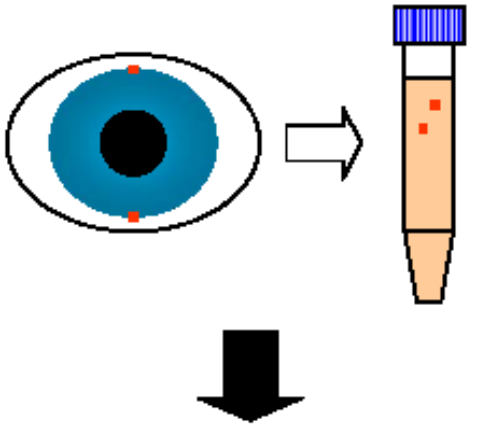


The Limbus…



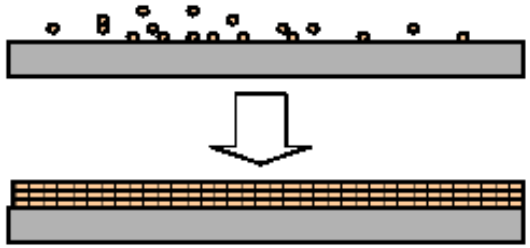
- Boundary between cornea and conjunctiva
- Defined by Palisades of Vogt
- Location of progenitor cells for corneal epithelium

1. Biopsy

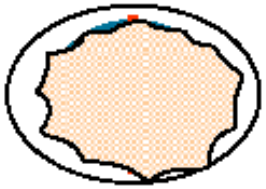


2. Culture expansion
(with FBS and 3T4 cells)

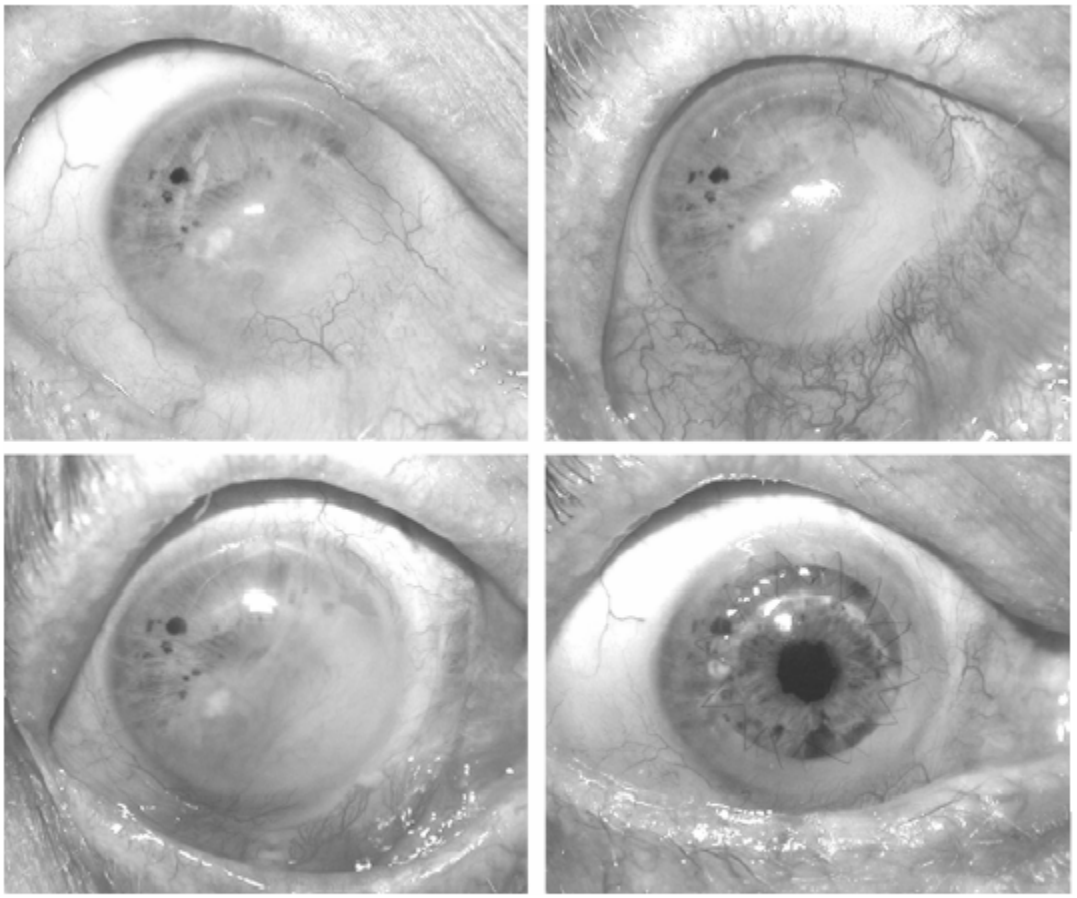
3. Seeding onto carrier
(amniotic membrane)



4. Grafting onto injured eye



Severe burn by alkali in a 75-year-old patient



Corneal regeneration following limbar stem cell transplantation

Hopes in Medicine with stem cells

- ***Consolidated therapies with adult stem cells***
- ***Tissue engineering***
- ***Experimentsl therapies with stem cells***
- ***Conclusions***

Growth and transplantation of a custom vascularised bone graft in a man

Lancet 2004; 364: 766–70

See Comment page 735

P H Warnke, I N G Springer, J Wiltfang, Y Acil, H Eufinger, M Wehmöller, P A J Russo, H Bolte, E Sherry, E Behrens, H Terheyden

Department of Oral and Maxillofacial Surgery, University of Kiel, Arnold-Heller-Str 16, 24105 Kiel, Germany (P H Warnke MD, I N G Springer MD, Prof J Wiltfang MD, Prof Y Acil PhD, E Behrens DMD, Prof H Terheyden MD); Department of Oral and Maxillofacial Surgery, Knappschaftskrankenhaus Recklinghausen, Recklinghausen, Germany (Prof H Eufinger MD, M Wehmöller PhD); Department of Rheumatology, Australian National University, Canberra, Australia (P A J Russo MD); Department of Diagnostic Radiology, University of Kiel, Kiel, Germany (H Bolte MD); and Department of Orthopaedic Surgery, University of Sydney, Penrith, Australia (E Sherry MD)

Correspondence to:

Summary

Background A major goal of research in bone transplantation is the ability to avoid creation of secondary bone defects. We aimed to repair an extended mandibular discontinuity defect by growth of a custom bone transplant inside the latissimus dorsi muscle of an adult male patient.

Methods Three-dimensional computed tomography (CT) scanning and computer-aided design techniques were used to produce an ideal virtual replacement for the mandibular defect. These data were used to create a titanium mesh cage that was filled with bone mineral blocks and infiltrated with 7 mg recombinant human bone morphogenetic protein 7 and 20 mL of the patient's bone marrow. Thus prepared, the transplant was implanted into the latissimus dorsi muscle and 7 weeks later transplanted as a free bone-muscle flap to repair the mandibular defect.

Findings In-vivo skeletal scintigraphy showed bone remodelling and mineralisation inside the mandibular transplant both before and after transplantation. CT provided radiological evidence of new bone formation. Postoperatively, the patient had an improved degree of mastication and was satisfied with the aesthetic outcome of the procedure.

Interpretation Heterotopic bone induction to form a mandibular replacement inside the latissimus dorsi muscle in a human being is possible. This technique allows for a lower operative burden compared with conventional techniques by avoiding creation of a secondary bone defect. It also provides a good three-dimensional outcome.

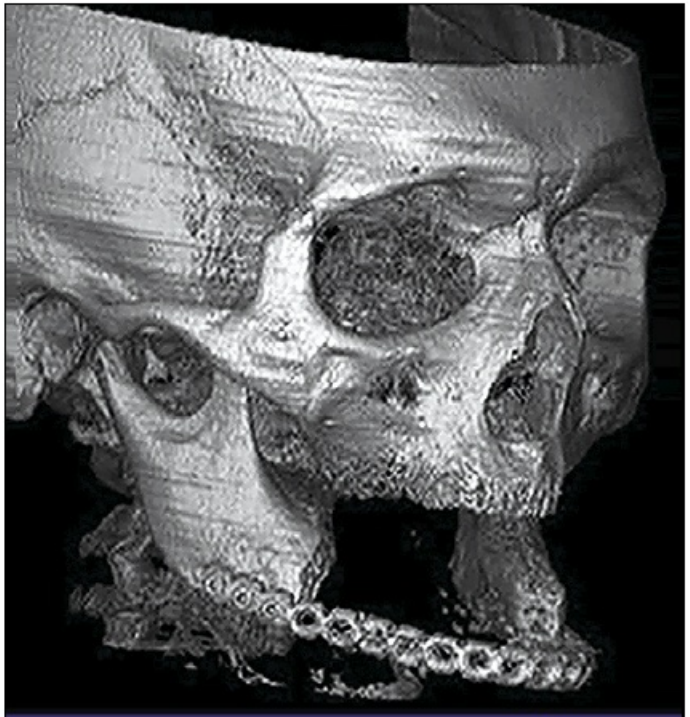


Figure 1: Three-dimensional CT scan of size defect (upper) and CAD plan of ideal mandibular transplant (lower)

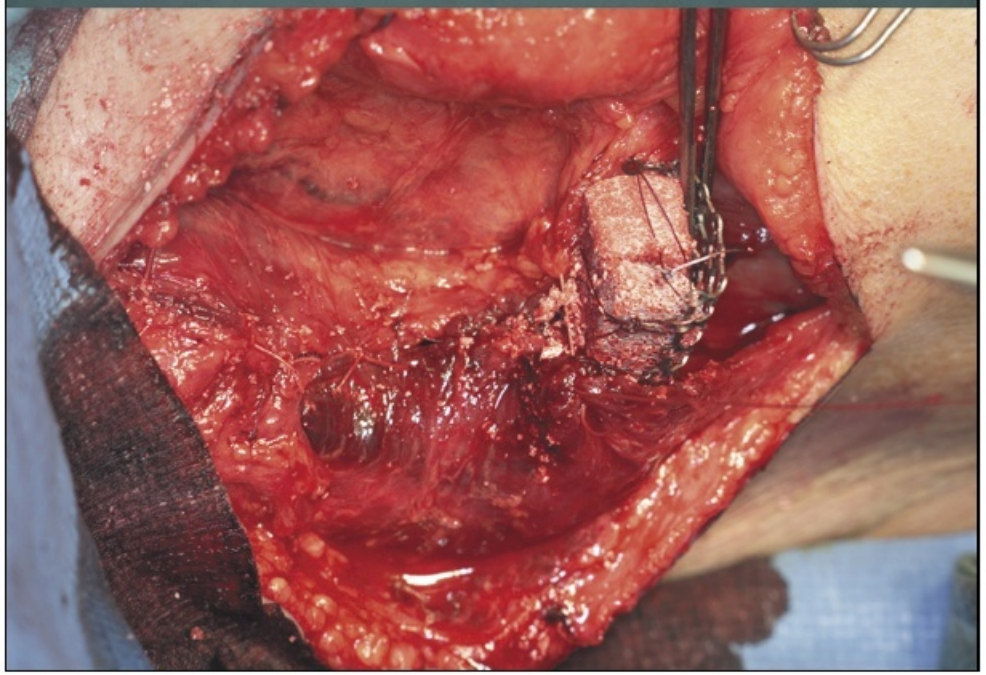


Figure 2: Titanium mesh cage filled with bone mineral blocks infiltrated with recombinant human BMP7 and bone-marrow mixture (upper) and implantation into right latissimus dorsi muscle (lower)

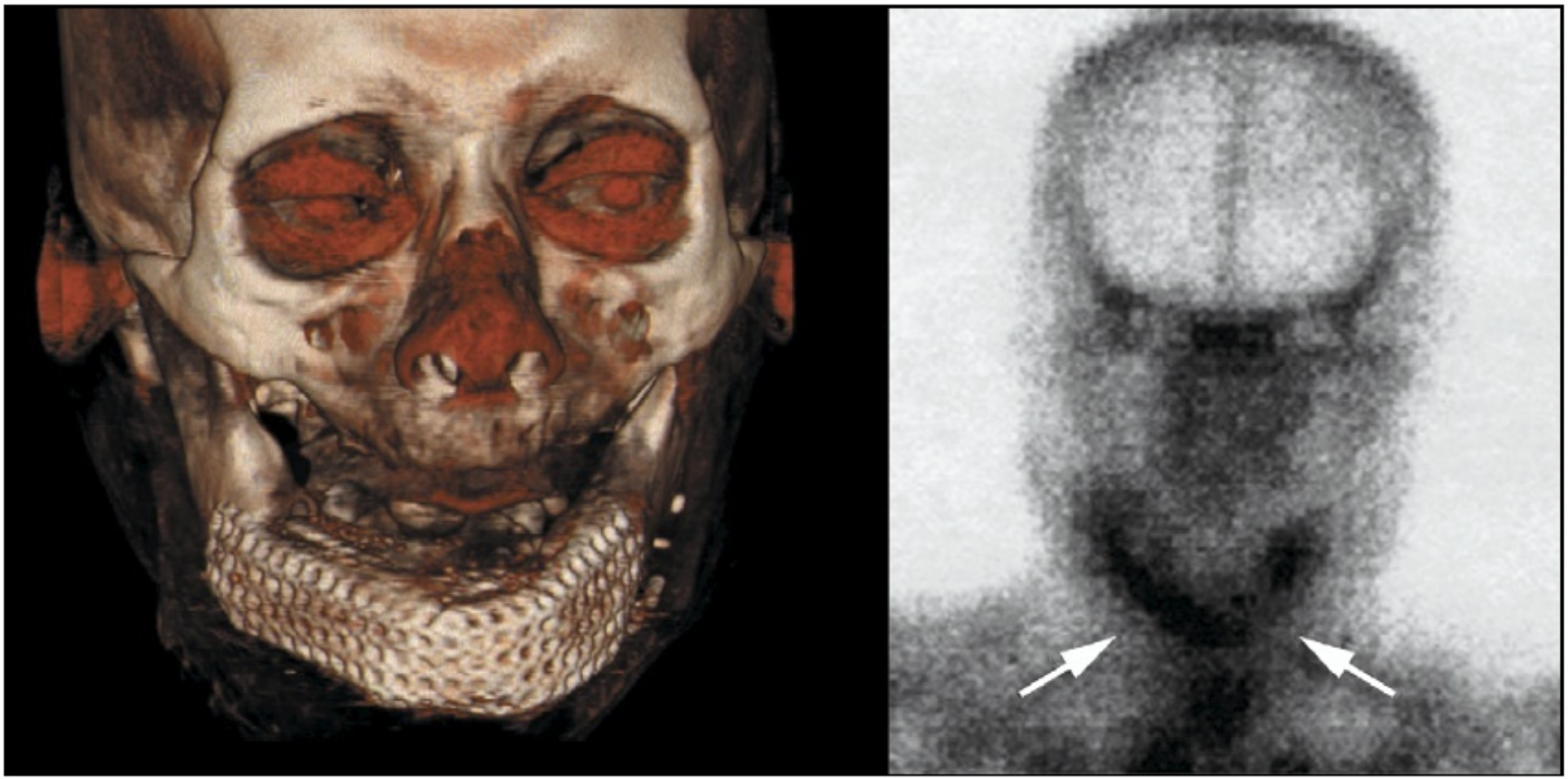


Figure 5: Three-dimensional CT scan (left) after transplantation of bone replacement with enhancement of soft tissue (red) and repeat skeletal scintigraphy (right) with tracer enhancement showing continued bone remodelling and mineralisation (arrows)

Tissue-engineered autologous bladders for patients needing cystoplasty

Anthony Atala, Stuart B Bauer, Shay Soker, James J Yoo, Alan B Retik

Summary

Background Patients with end-stage bladder disease can be treated with cystoplasty using gastrointestinal segments. The presence of such segments in the urinary tract has been associated with many complications. We explored an alternative approach using autologous engineered bladder tissues for reconstruction.

Methods Seven patients with myelomeningocele, aged 4–19 years, with high-pressure or poorly compliant bladders, were identified as candidates for cystoplasty. A bladder biopsy was obtained from each patient. Urothelial and muscle cells were grown in culture, and seeded on a biodegradable bladder-shaped scaffold made of collagen, or a composite of collagen and polyglycolic acid. About 7 weeks after the biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted either with or without an omental wrap. Serial urodynamics, cystograms, ultrasounds, bladder biopsies, and serum analyses were done.

Results Follow-up range was 22–61 months (mean 46 months). Post-operatively, the mean bladder leak point pressure decrease at capacity, and the volume and compliance increase was greatest in the composite engineered bladders with an omental wrap (56%, 1·58-fold, and 2·79-fold, respectively). Bowel function returned promptly after surgery. No metabolic consequences were noted, urinary calculi did not form, mucus production was normal, and renal function was preserved. The engineered bladder biopsies showed an adequate structural architecture and phenotype.

Conclusions Engineered bladder tissues, created with autologous cells seeded on collagen-polyglycolic acid scaffolds, and wrapped in omentum after implantation, can be used in patients who need cystoplasty.

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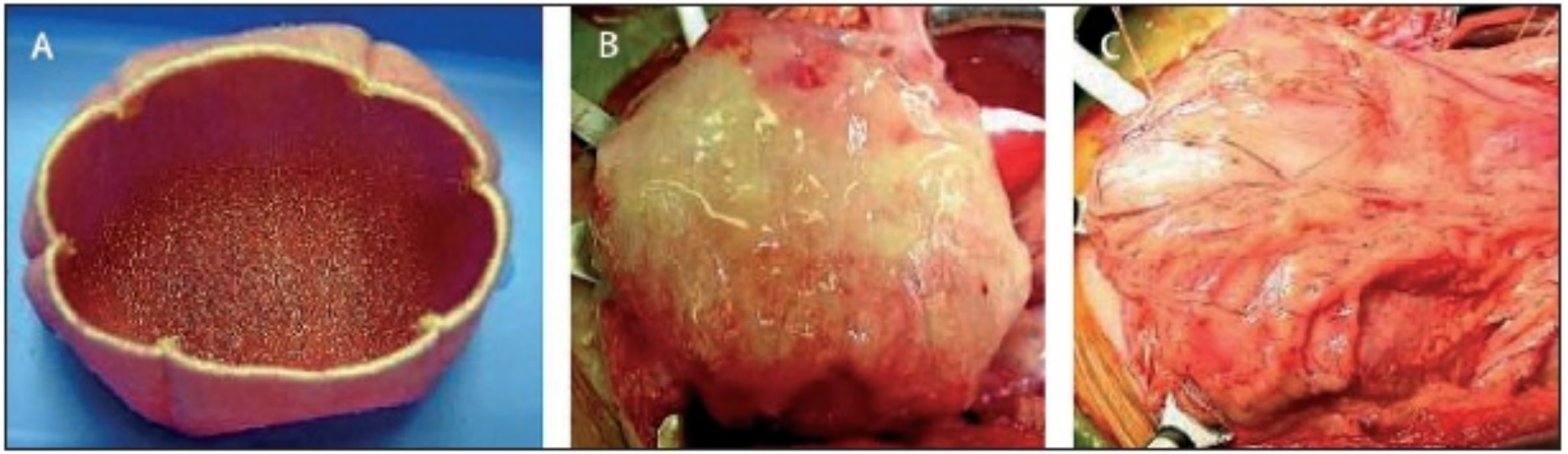


***Figure 1*: Construction of engineered bladder**

Scaffold seeded with cells (A) and engineered bladder anastamosed to native bladder with running 4–0 polyglycolic sutures (B). Implant covered with fibrin glue and omentum (C).

Trachea transplantation:

Example of adult stem cell-based tissue regeneration

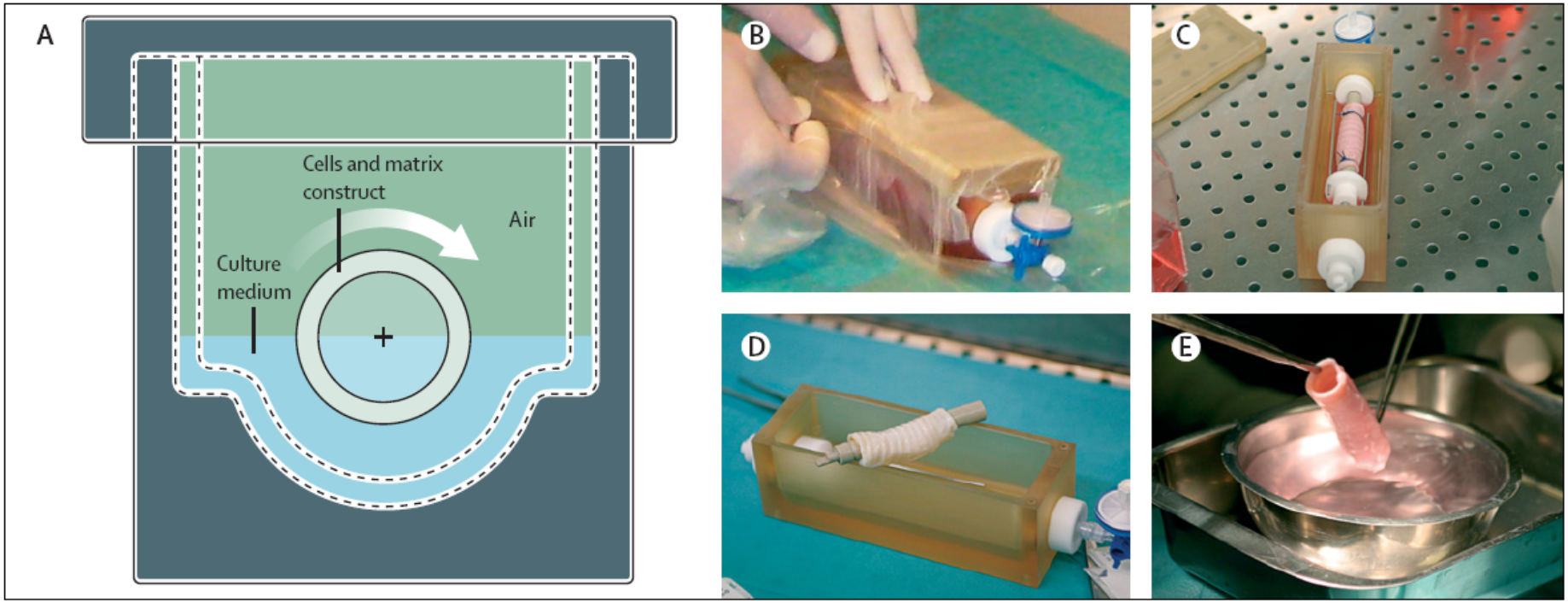


Figure 2: **Bioreactor developed for airway tissue engineering**
(A) Schematic lateral view, highlighting the rotation of the matrix around its longitudinal axis. The design has separate compartments for lumen and outer surface, and is regularly rotated through a motor to apply the sheer stress needed for growth, distribute nutrients and waste, and ensure even exposure to applied cells. (B) The sealed device. (C) Bioreactor with the graft in situ. (D) Bioreactor after removal of the graft. (E) The final graft immediately before surgical implantation.

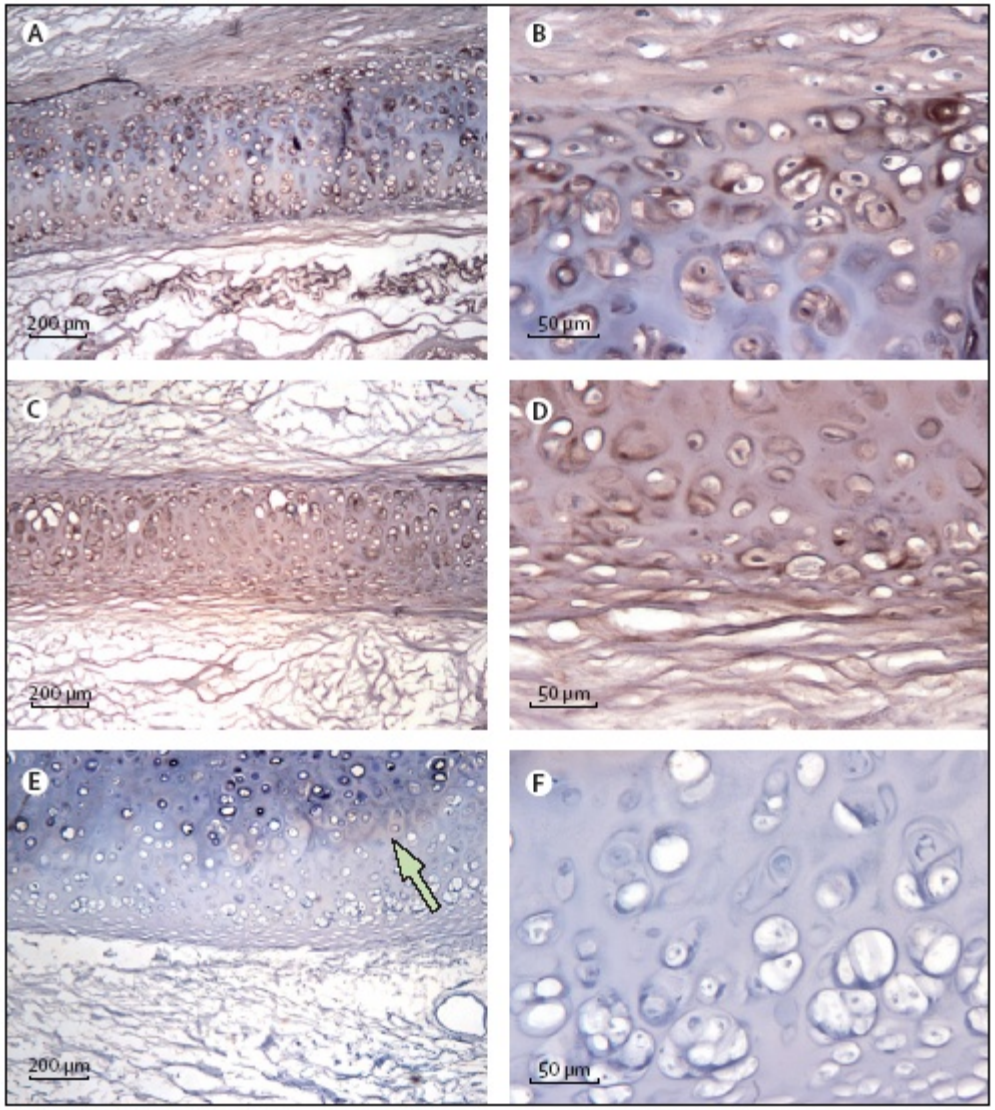


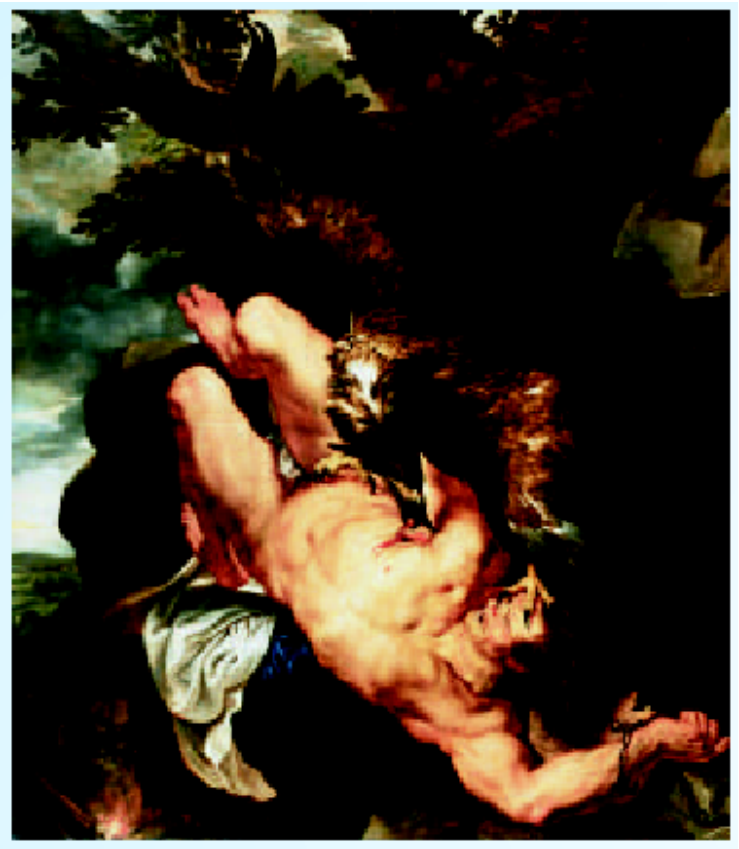
Figure 3: Immunohistochemistry of sections of graft wall at retrieval (A and B), and after 17 cycles (C and D) and 25 cycles (E and F) of detergent-enzymatic treatment

Brown staining represents MHC class I (A, C, and E) and II (B, E, and F). After 25 cycles (E and F), epithelial cells and glands completely disappeared, whereas only a few chondrocytes were detectable inside the cartilage rings, and even they were disrupted: most did not have a nucleus and their cell borders were indistinct. However, compared with native trachea (A and B), the treated tissues maintained their structural integrity. After 17 cycles (C and D), a diffuse immunoreactivity against both MHC class I and II antigens was still present and 25 cycles (E and F) were needed to remove nearly all HLA-positive cells from the tracheal matrices. At implantation, only a few small areas of cartilage were weakly positive for MHC class II (arrow) and no class I staining was visible.

REX FEATURES

Claudia Castillo, the recipient of a bioengineered human airway, the world's first transplanted organ grown from stem cells

Hopes in Medicine with stem cells



- ***Consolidated therapies with adult stem cells***
- ***Tissue engineering***
- ***Experimentsl therapies with stem cells***
- ***Conclusions***

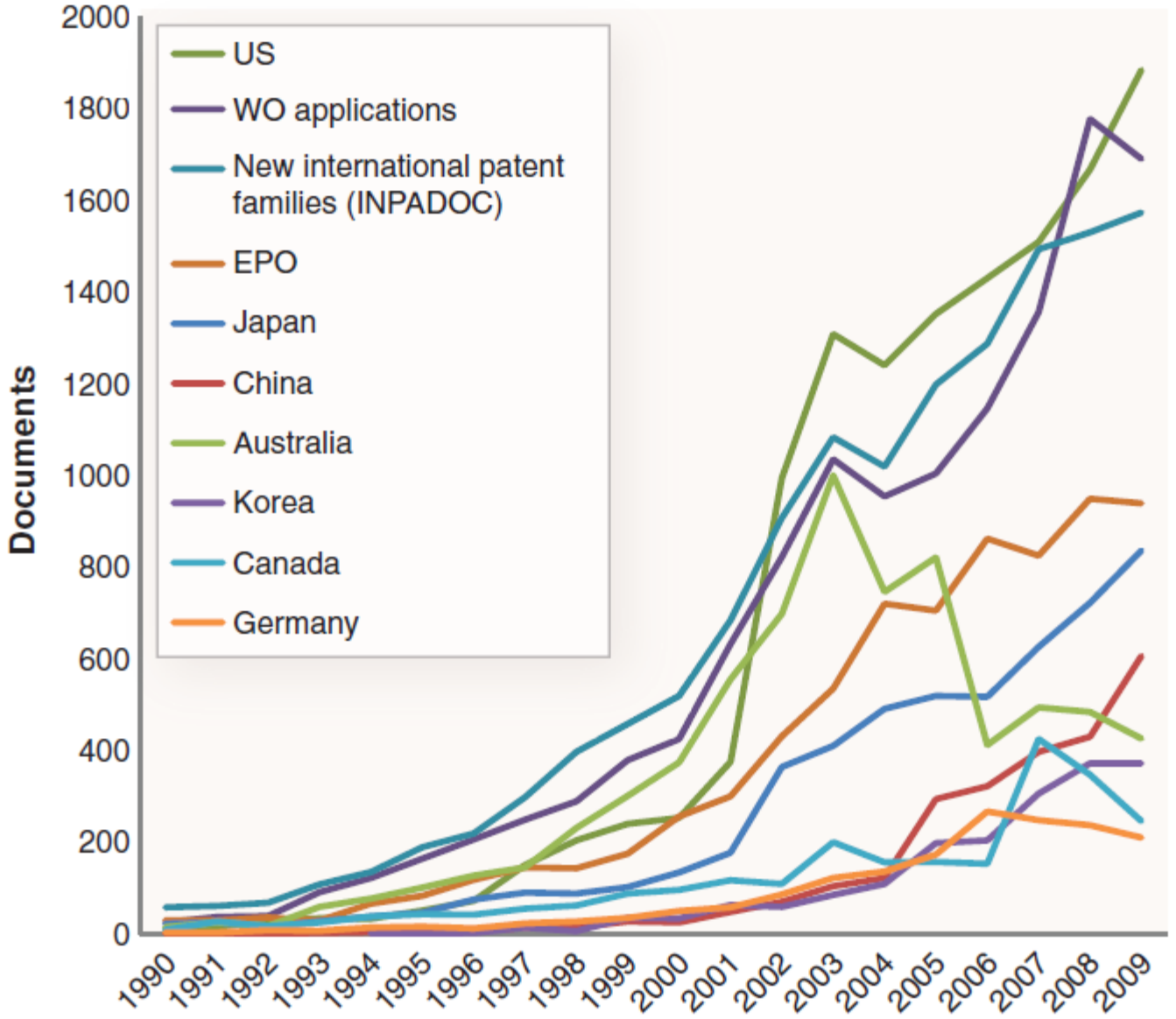


Fig. 2. Stem cell patents and patent applications published by various patent offices. Data source: Thomson Innovation (2010), queried using methods of Bergman and Graff (*13*).

Target Diseases for future Stem Cell Therapy

- Heart Disease: ES cells can be induced to form cardiac muscle cells that actually beat in culture
- Brain and Spinal Cord Injury: HSCs can also be transplanted into the brain where they are reprogrammed to generate neurons and glial cells
- Type 1 Diabetes: Insulin-producing structures similar to pancreatic islets have been generated from mouse ES cells in culture

What is the "unmet need" for CVD?

- Despite the enormous progresses in the treatment of coronary artery disease, it remains the most common cause of heart failure and the leading cause of death in the Western countries

- New translational therapeutic approaches based on personalized and regenerative medicine are needed

- In this study we fabricated bioartificial constructs mimicking anisotropic structure and mechanical properties of the myocardium

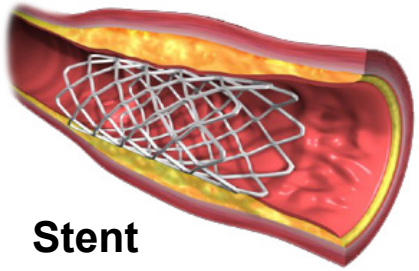
Claudia Giachino

What are the target populations for PPPM intervention?

- Patients with acute heart failure might benefit from such approach
- Patients with coronary artery disease

Aim of the project

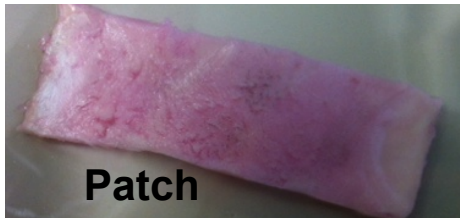
Making innovative therapeutic tools against myocardial ischemia, the main cause of acute heart disease



Stent

Opening coronary with innovative stents…

…myocardium implant of biomimetic and bioinductive scaffolds (made of polymeric bioartificial materials as PHBHV/gelatin and PLGA/gelatin)



Patch

Scaffold requirements

Scaffolds must:

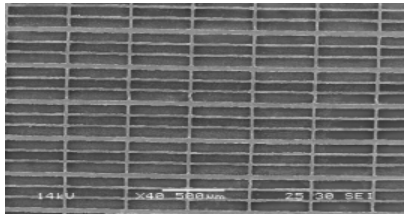
- ✓ Mimic myocardium structure and biomechanics
- ✓ Be biocompatible

Scaffolds must have:

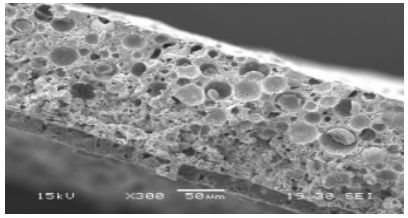
- ✓ A cardioinductive function on stem cells, to promote tissue regeneration
- ✓ A cardioprotective effect, to limit reperfusion injury
- ✓ A chemoattractive function, to recruit both resident and circulating stem cells

Mimic myocardium structure and biomechanics

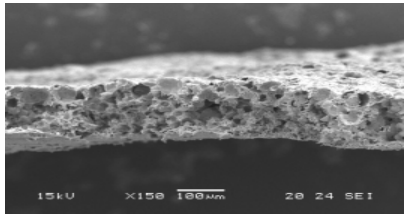
A



Microstructured scaffold surface

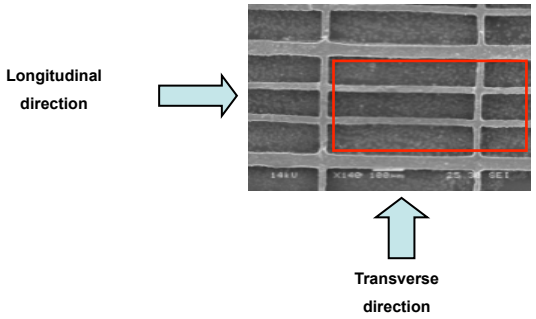


Microstructured scaffold section



Non-microstructured scaffold section

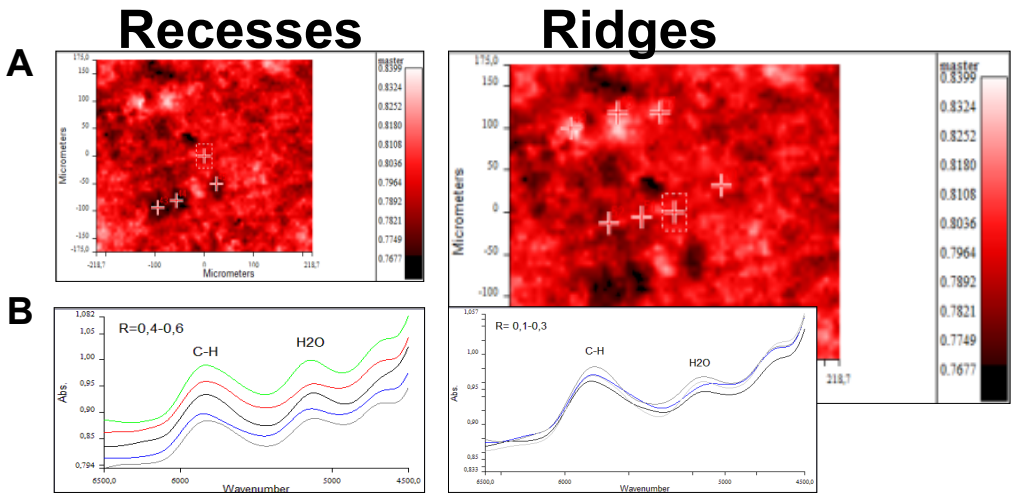
B



SEM images of microfabricated material surfaces showed the presence of rectangular recesses following the soft lithography model geometry and micro-mesopores distributed diffusely at the level of the entire structure due to chemical composition and solvent casting procedure. The macrocavities, considered as polymeric guides to induce alignment of cardiomyocytes have longitudinal and transversal dimension of 480 μm and 25 μm and a depth of 40 μm, and are separated among them by an array of longitudinal regular ridges of 60 μm and 25 μm and cross ridges of 30 μm.

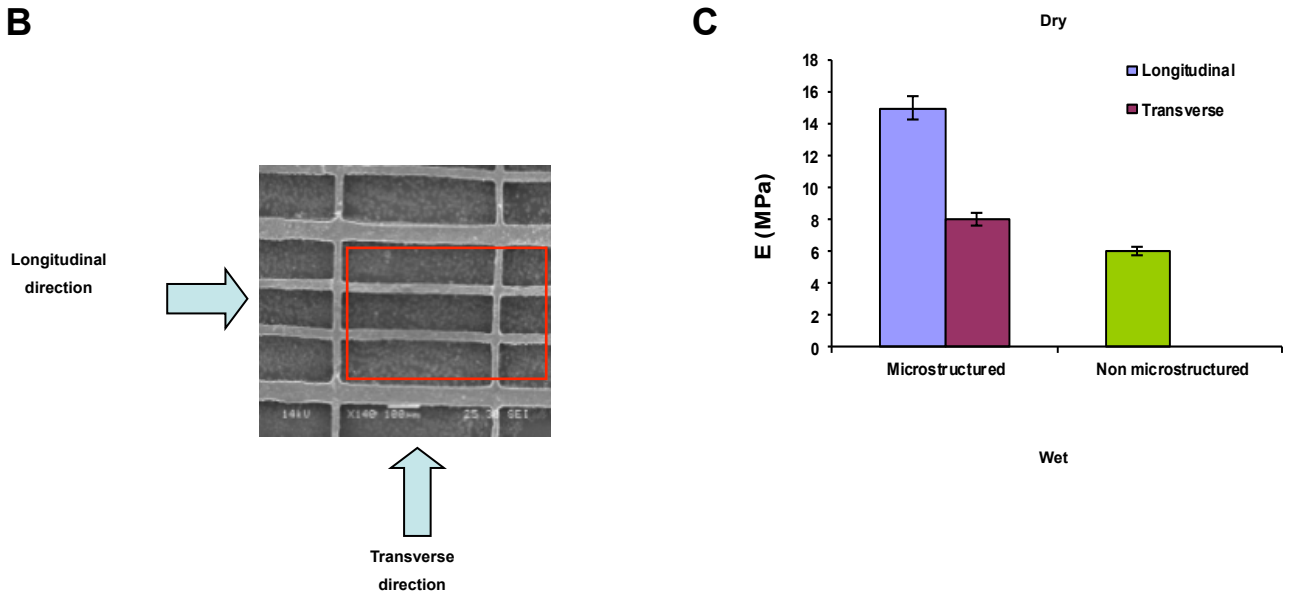
Mimic myocardium structure and biomechanics

Chemical imaging technique, a useful and innovative tool to investigate about hydrophilicity and molecular interactions between material components, suggested that these scaffolds exhibit hydrophilicity properties matching those of healthy myocardium sample.
Maps acquired in μATR mode



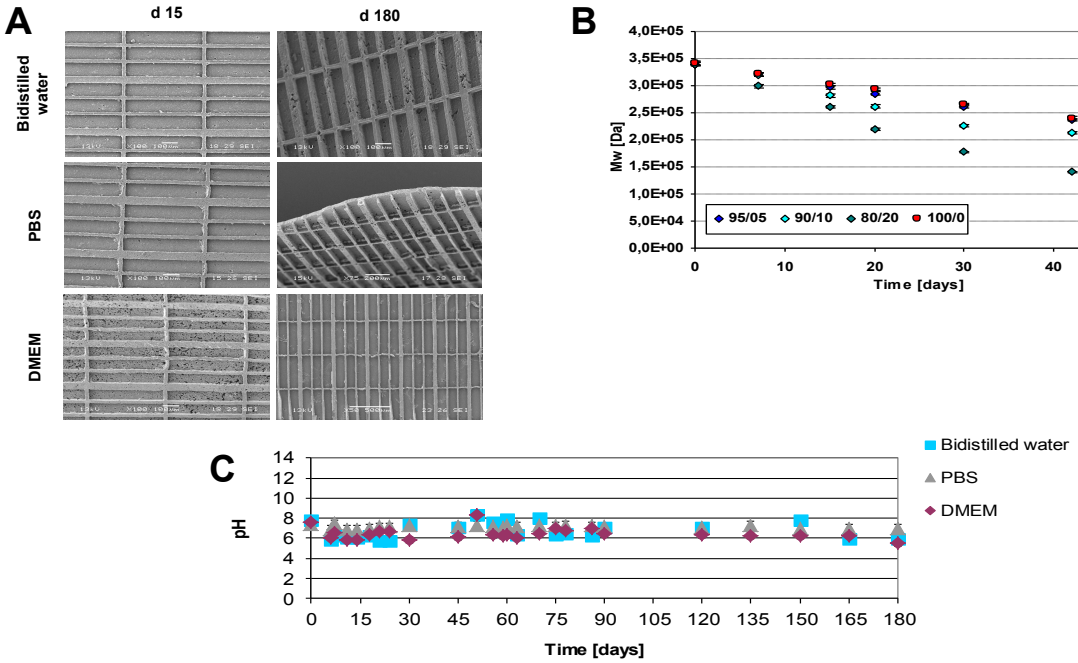
NIR maps showed that the amount of OH groups respect to CH groups was higher in recesses than in ridges indicating a specific anisotropic surface hydrophilicity of the materials that can be fundamental to explain the behavior of stem cells seeded onto these matrices.

Mimic myocardium structure and biomechanics



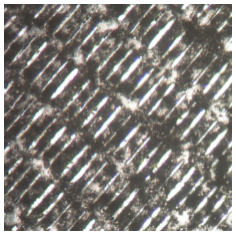
The evaluation of mechanical properties of the produced systems showed that storage modulus resulted in the order of MPa, in both non and microstructured samples, being these values similar to the healthy cardiac tissue. In addition, the anisotropic structure conferred by soft-lithography induced anisotropic mechanical properties with a higher storage modulus value in the longitudinal direction than that along the transversal direction.

Mimic myocardium structure and biomechanics

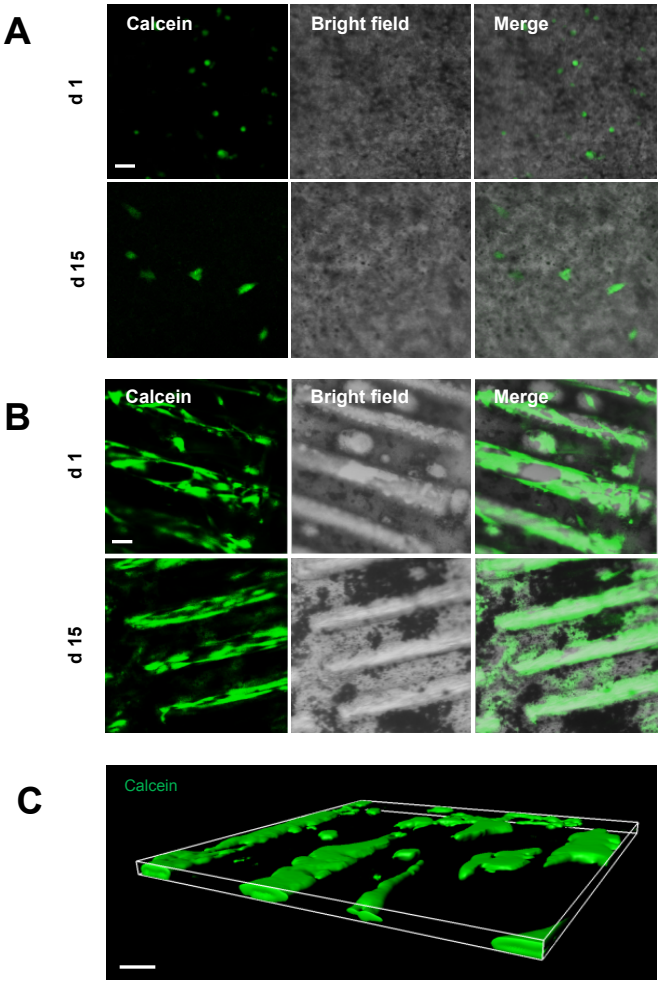


Biodegradation properties of the scaffolds was examined *in vitro* using different media and for prolonged times. Morphological analysis, pH trend of degradation solutions and GPC analysis confirmed a slow and gradual degradation of the scaffolds, the maintenance of a regular surface microstructure, an unaltered pH in the degradation media thus preventing possible inflammatory responses *in vivo*.

Biocompatibility



Viability and proliferation assays demonstrated that these constructs allow adhesion and growth of mesenchymal stem cells (MSCs) and cardiac resident non myocytic cells (NMCs). Immunofluorescence analysis demonstrated that stem cells cultured on these constructs adopt a distribution mimicking the three-dimensional cell alignment of myocardium.



Non-microstructured film

Microstructured scaffold

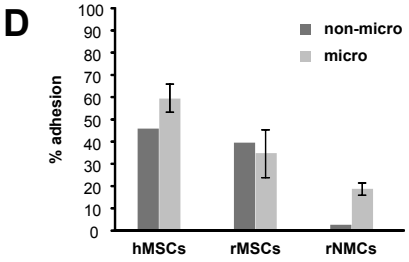
3D reconstruction
on microstructured scaffo

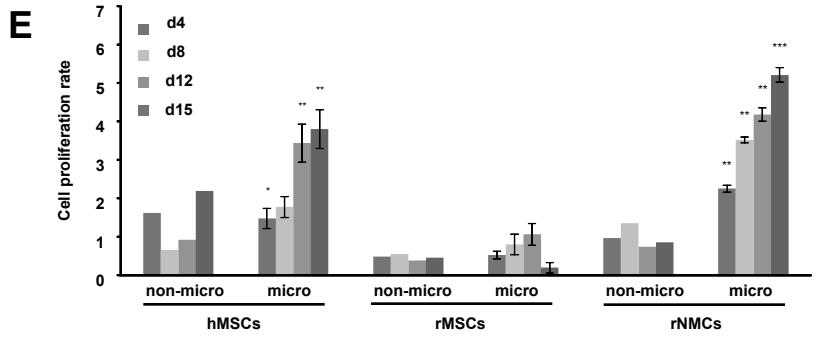
Biocompatibility

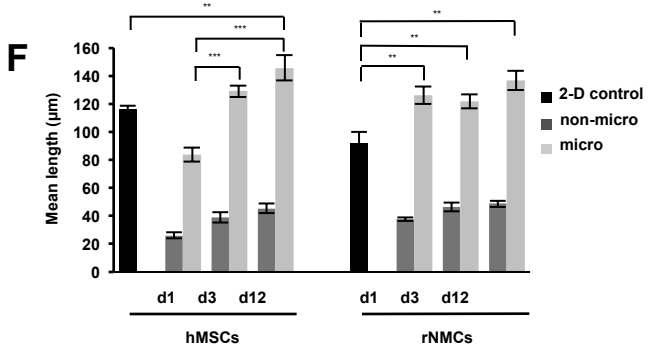
Cell adhesion

Cell proliferation

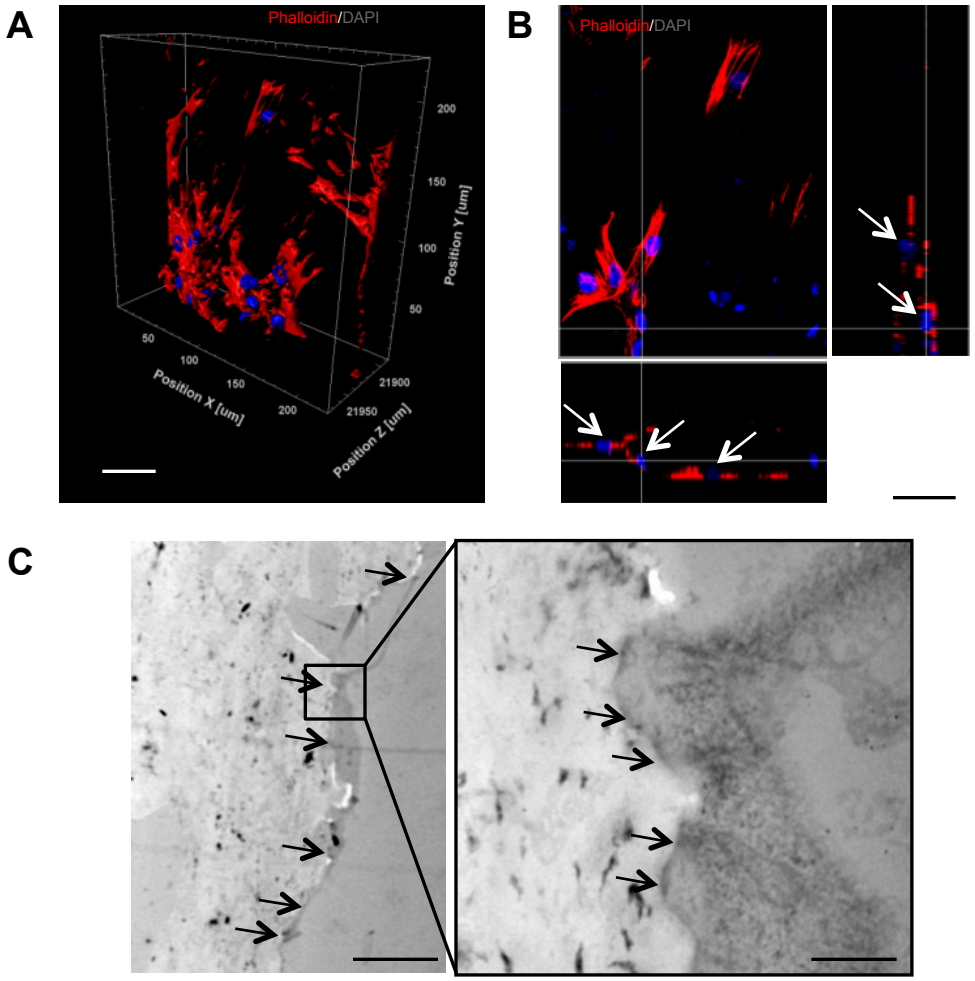
Cell elongation





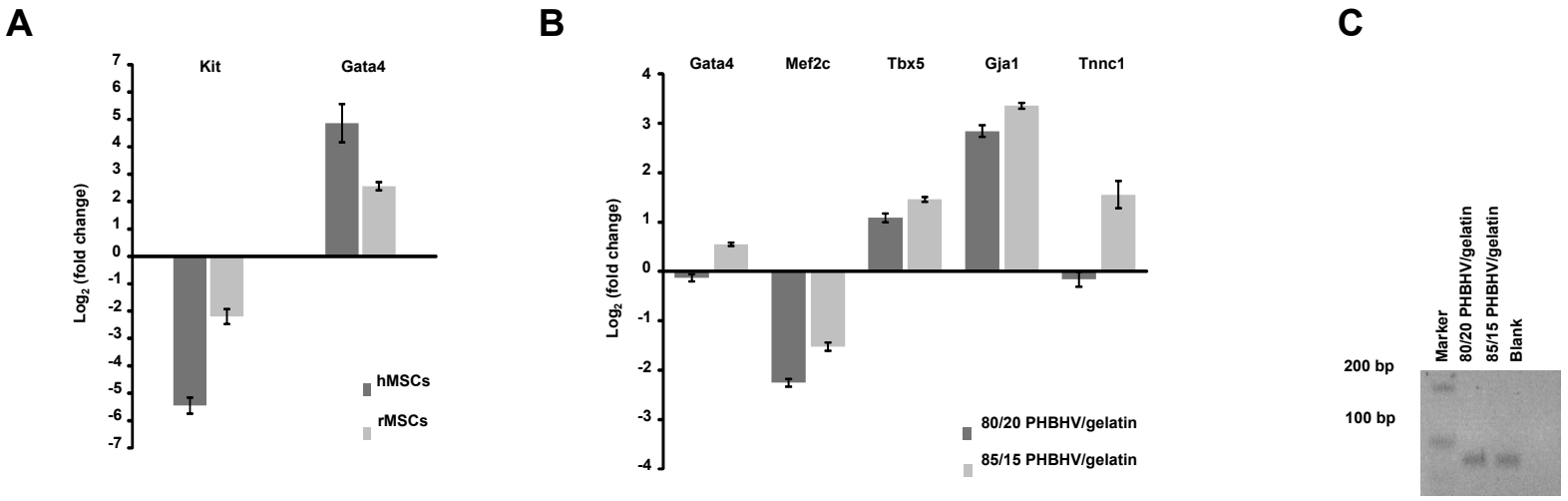


Biocompatibility



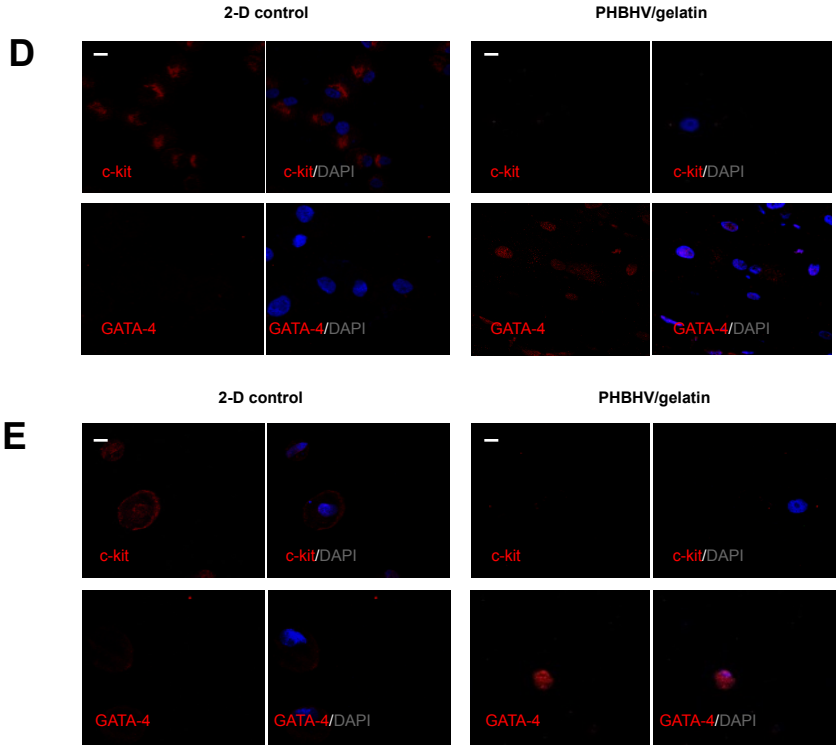
During scaffold colonization cells maintained an optimal cytoskeletal organization and they were able to colonize the entire construct thickness, growing in at least three cell layers as shown in the 3-D scaffold reconstruction. Transmission electron microscopy analysis allowed to appreciate electron-dense areas, known as focal adhesions or focal contacts, typically elongated, in which the plasma membrane runs parallel to the overlying matrix.

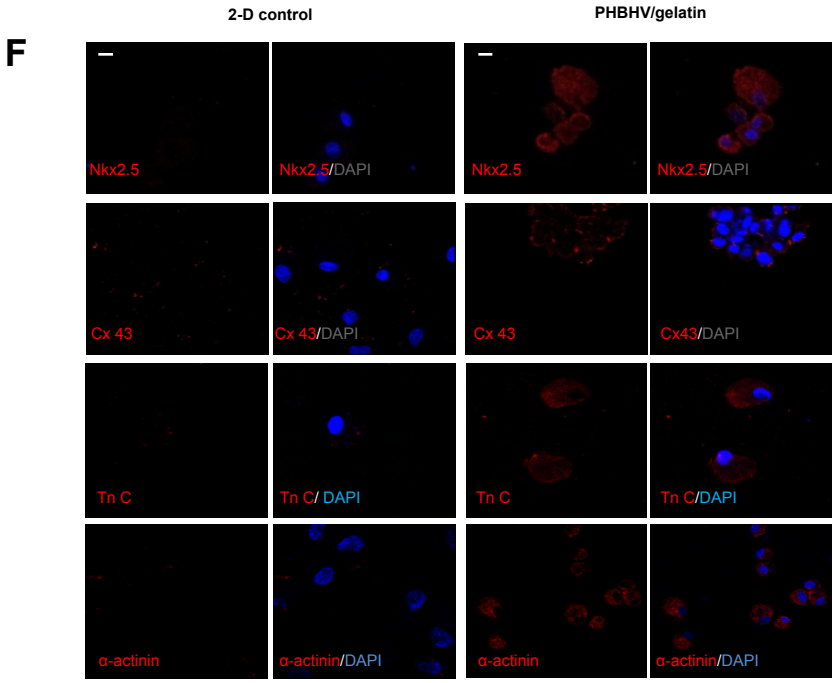
Cardioinductivity



Real-time qPCR analysis showed the ability of these construcst to direct initial MSC and NMC lineage specification towards cardiomyogenesis. Both MSCs and NMCs showed down-regulation of the stemness marker kit and up-regulation of the cardiac transcription factor GATA-4. Moreover NMCs acquired the expression of the transcription factor Tbx5, the GAP-junction gene Connexin 34, and the sarcomeric gene Troponin C.

Cardioinductivity



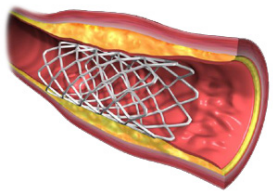


Immunofluorescence analysis confirmed down-regulation of c-kit and up-regulation of GATA-4 protein in MSCs. It also documented expression of the cardiac transcription factor Nkx2.5, the GAP-junction protein Connexin 43 as well as the sarcomeric proteins Troponin C and α-actinin in NMCs.

Cardioprotection

Ischemia/reperfusion injury

Despite timely reperfusion, nearly 10% of AMI subjects die during hospitalization, and 25% of survivors progress to develop chronic h… failure



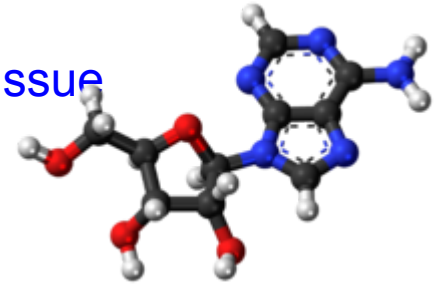
Reperfusion of ischemic myocardium can also cause cardiomyocyte deat… microvascular damage through a process referred to as myocardial ischemia/ reperfusion injury

These data suggest that despite progress in the treatment of AMI, a major scientific problem is the need for novel approaches to limit myocardial damage resulting from reperfusion injury

ADENOSIN: protection factor

Vasodilatory action and protection of myocardic tissue

Scaffolds were functionalized with adenosin that is released gradually after the implant



Cardioprotection

Ischemia induction:
left anterior descending coronary (LAD) binding

preparation anhestesia

Ischemia induction

Biopsy #

Biopsy #3

Surgical breach closure

Biopsy #1

Patch application

Reperfusion

Biopsy #4

Waking up

Patch application:
few minutes after LAD binding

Reperfusion:
after 50 minutes from LAD binding

Biopsies:

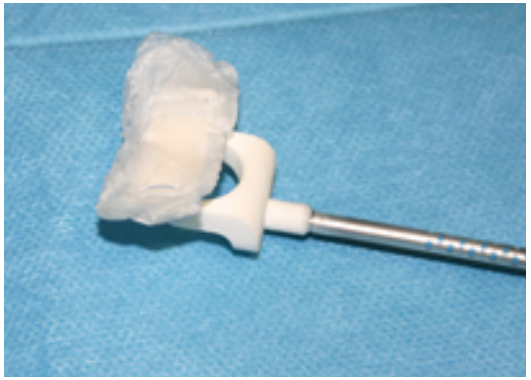
1. before LAD binding
2. 40 minutes after LAD binding
3. 10 minutes after reperfusion
4. 60 minutes after reperfusion

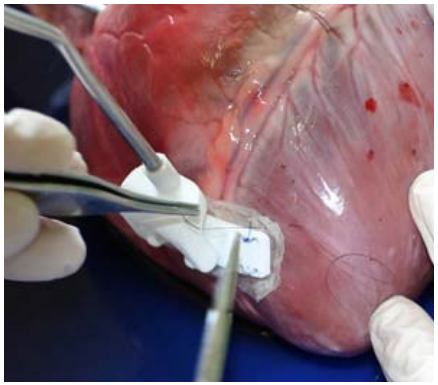
protein analysis of cardioprotective markers

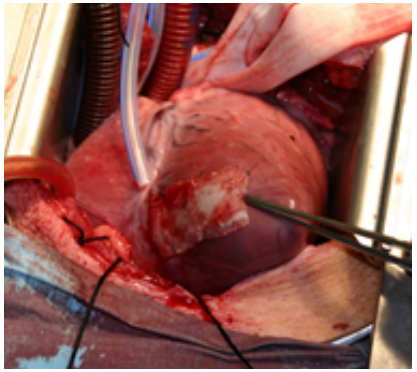
CARDIOPROTECTION

Myocardic implant

The patches are easily implanted and adhere perfectly on the epicardial surface



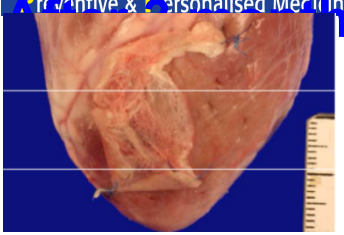


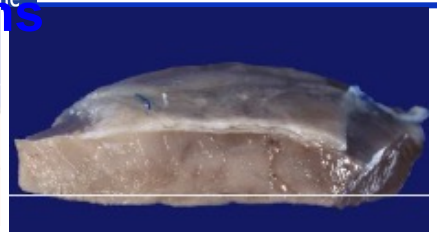


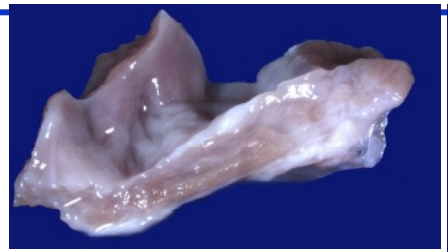
Use of a novel delivery system optimized by Sorin Group, a medical products group based in Italy, with significant operations in France, the United States, and Japan, specializing in cardiac devices.

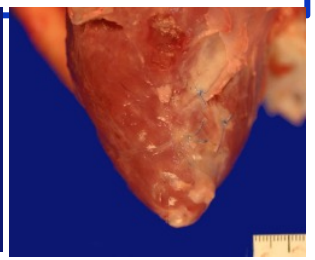
Myocardic implant

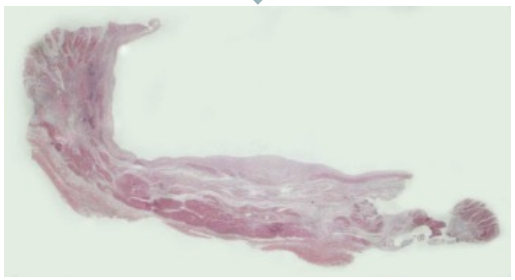
After 24 h

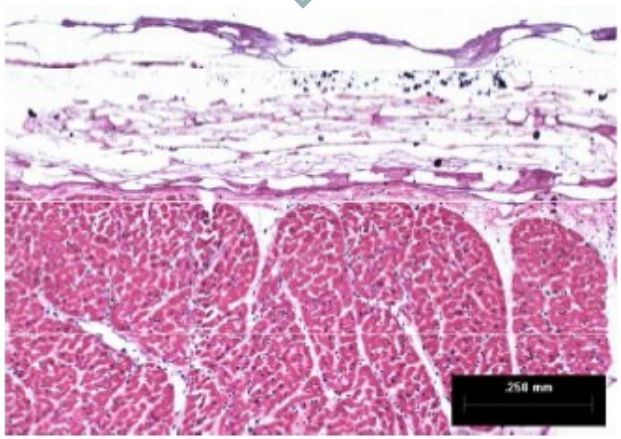


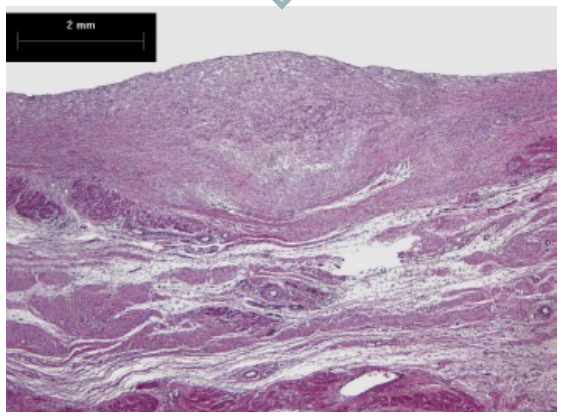








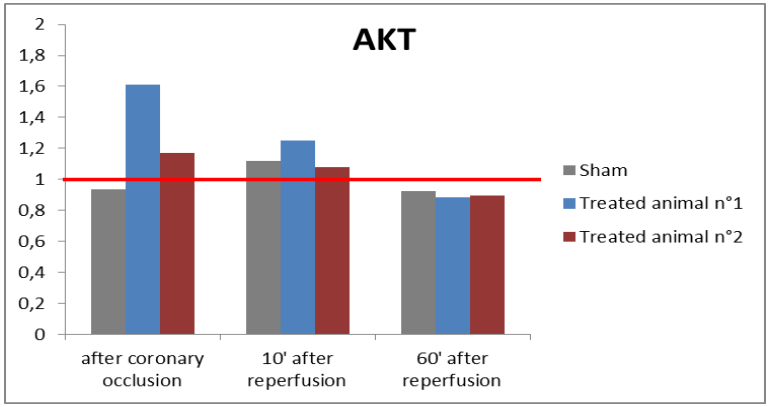


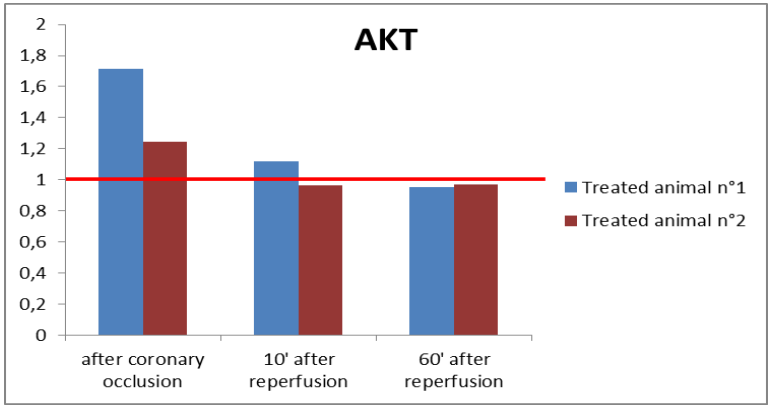


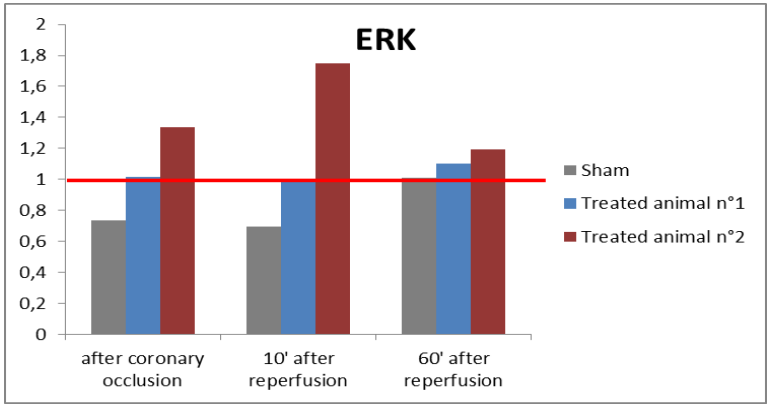
European Association for Predictive Preventive & Personalised Medicine

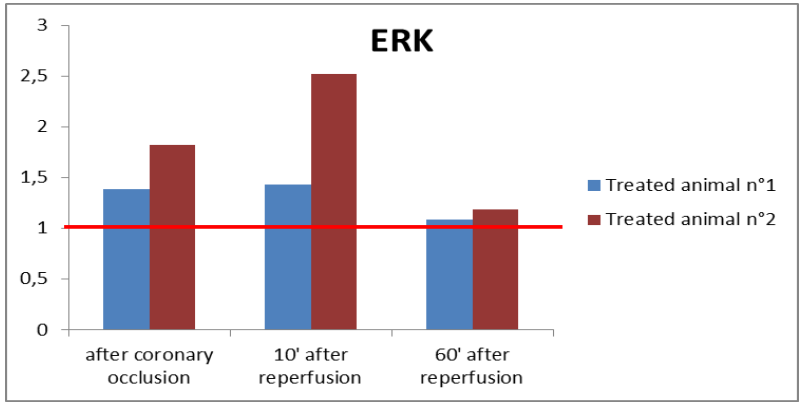
Cardioprotection

Analysis of reperfusion injury salvage kinase (RISK) pathway activation









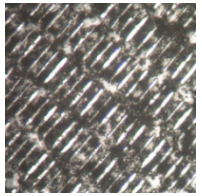
Major results

- Cardiac patches are cytocompatible and stem cells show a good adhesion and proliferation

- Cardiac patches induce stem cell commitment towards cardiac phenotype

⇩

The microstructure is important for cell orientation and differentiation



- Patches functionalised with adenosin reduce the hypoxic damage *in vitro*, suggesting a cardioprotective effect

- Cardiac patches are easily implanted *in vivo* and are completely adsorbed after 3 months

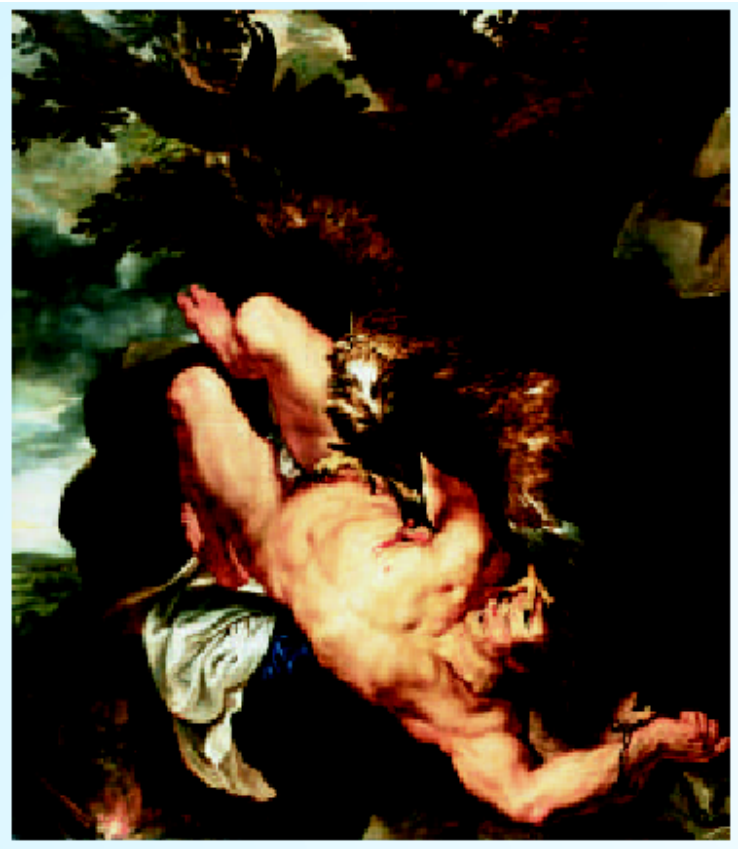
Next steps

- Functionalisation of cardiac patches with stem cell chemoattractants
- Conduction of a large *in vivo* study on the selected animal model

The guidance of stem cell cardiomyogenic differentiation by bioartificial scaffolds mimicking myocardium structure and biomechanics

Thanks for your attention!

Hopes in Medicine with stem cells



- ***Consolidated therapies with adult stem cells***
- ***Tissue engineering***
- ***Experimentsl therapies with stem cells***
- ***Conclusions***

The offers of stem cells

- The press reports many disputes for and against stem cell research.
- Because the media has an important role in forming the opinion on recent progress, the stem cell debate is often based on a lack of accurate information and objective.
- Even those who want to speculate on diseases and out of desperation and hope of those affected uses instrumentally mass media.

la Repubblica — Quotidiano — Data 21-07-2005 — Pagina 24 — Foglio 1 / 3

Staminali, nuove speranze per cuore e cervello

La ricerca corre, soprattutto in Asia. Quasi una scoperta al giorno

ELENA DUSI

ROMA — Scienziati australiani hanno annunciato ieri che le cellu-

LA STAMPA NOVARA VERBANO-CUSIO-OSSOLA — Quotidiano — Data 21-02-2007 — Pagina 63 — Foglio 1

BORGOMANERO. LA SFIDA

"In Cina per vincere la sclerosi"

Barbara, 26 anni e una figlia: le cellule staminali sono la mia s

MARCELLO GIORDANI
BORGOMANERO

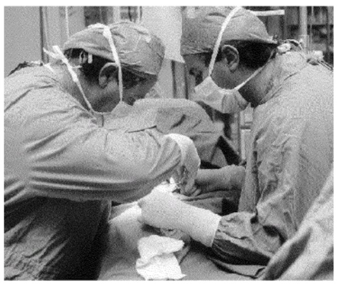
Costa novecento euro un viaggio low cost per la Cina e Barbara Boscolo ha acquistato il biglietto non per le vacanze dell'estate ma per un viaggio della speranza. Barbara ha 26 anni, un bambino di quattro anni, abita a Briga Novarese e fa la cassiera in un supermercato di Borgomanero. Da due anni e mezzo la sua vita è cambiata perchè è stata colpita da sclerosi multipla.

«Nell'estate del 2004 ho iniziato ad avvertire formicolio alle mani, un disagio sempre più forte, tanto che non riuscivo più a battere ai tasti della cassa; venne a mancare completamente il tatto, iniziarono i problemi alle gambe. Le risonanze magnetiche che mi vennero fatte - racconta Barbara Boscolo - non lasciavano dubbi: numerose lesioni nell'encefalo e nella colonna cervico-dorsale segnalavano la sclerosi multipla». Iniziano le cure, che però non danno risultati soddisfacenti, e «nel giugno 2005 ebbi un calo visivo con la cecità all'occhio destro e parzialmente a quello sinistro. Recuperai la vista dopo quindici giorni in seguito a una terapia cortisonica endovenosa. Dall'ottobre del 2005 sono in terapia immunomodulante, con un'iniezione al giorno di Glatiramer acetato, nel tentativo di arrestare il degenerare della malattia. Tutto questo non è curativo e non ti protegge con sicurezza da ricadute ma anzi compromette il funzionamento del sistema immunitario». Un quadro delicato e complesso da gestire ma Barbara Boscolo non si è arresa e ha iniziato, anche tramite Internet, a informarsi sul tipo di terapie esistenti al mondo per fronteggiare la sclerosi multipla: «Dopo centinaia di tentativi inutili, ho scoperto che in Cina è possibile effettuare il trapianto di cellule staminali, ottenuto dal cordone ombelicale di neonati sani e non prematuri, con madri sane».

Barbara prosegue: «Questo intervento arresta la malattia e fa regredire le lesioni preesistenti, come dimostrano le numerose testimoni[...] curati con succes[...] in Europa non vie[...]

Così Barbara [...] contatto con il Na[...] tal, a Shenzhen, [...] tri di distanza da[...] «Per poter usufru[...] cura occorrono 30[...] con lo stipendio [...] durissima. Così h[...] to, ho racimolato [...] centesimo, e per ri[...] cercato il bigliet[...] cost: l'ho trovato [...] lista di attesa in C[...] due anni ma son[...] avere l'intervento [...] sto: sarà il più be[...] della mia vita, pe[...] buone possibilità [...] la malattia e sconf[...]

Fra grinta e scienza
Barbara Boscolo ha cercato su Internet la meta per l'intervento



In Italia no. Per il trapianto Barbara andrà al Nan Shan Hospital, a Shenzhen

Cassiera in u[...] ha risparmiat[...] euro per l'int[...] che sarà il 15

la Repubblica — Quotidiano — Data 21-12-2004 — Pagina 1 — Foglio 1 / 3

REPORTAGE

Dal dottor Huang che cura con le cellule embrionali

dal nostro corrispondente
FEDERICO RAMPINI

PECHINO

SANITARIO dei Lavoratori, Xixiazhuang, Shijingshan District. La località più vicina è Badachu, che vuol dire le Otto Meraviglie, perché un tempo era un paesaggio di colline verdeggianti, pagode e monasteri buddisti. Oggi è una periferia squallida e sporca, tutta fabbriche e caseggiati popolari, nella grande area metropolitana di Pechino a un'ora e mezzo di auto dal centro della capitale.

SEGUE A PAGINA 15

MA PER molti occidentali è la Terra Promessa. Vengono qui in centinaia dall'America e dall'Europa: paralizzati da incidenti alla spina dorsale, paraplegici, malati di sclerosi multipla o del morbo di Lou Gehrig, affetti dal Parkinson o con emiplegie da ictus. La loro speranza si chiama Huang Hongyun, un neurochirurgo cinese che ha studiato negli Stati Uniti ma che solo qui a Pechino può applicare la sua terapia rivoluzionaria a base di iniezioni di cellule embrionali (da non confondersi con le cellule staminali). A 49 anni il professor Huang è una celebrità internazionale, anche se il suo metodo divide il mondo della scienza. L'ho incontrato in mezzo ai suoi pazienti e di questo posso testimoniare: non ha l'atteggiamento di un guru, non cerca di vendere illusioni.

Il primo impatto con l'ospedale non è rassicurante. Il cortile esterno e la facciata, i corridoi d'ingresso, i primi piani sono malandati e bui. L'aspetto è quello di una sanità povera, le condizioni d'igiene la-[...]

[...]vede un giardino di bambù, il personale è di una gentilezza irreprensibile, la pulizia regna e si vedono arrivare vassoi di gastronomia cinese che non assomigliano al rancio della mensa interna. I centralinisti (telefono 00.8610.51625950) hanno imparato l'inglese, e per forza.

L'elenco dei 90 pazienti ricoverati in questi giorni riproduce una mappa completa degli Stati Uniti. Vengono da New York, New Jersey, Michigan, Wisconsin, Texas, Florida, Nevada, California.

Dall'Europa ci sono due belgi. E c'è Vanessa Wessels: è nata in Sudafrica ma lo ha lasciato 25 anni fa, dal 1989 è cittadina italiana, vive a Loro Ciuffenna in provincia di Arezzo. Vanessa ha 40 anni, è una bella donna alta un metro e ottantacinque, bionda e con gli occhi verdi. Ha una figlia di 10 anni e un bambino di 8. È programmatrice informatica e inoltre disegna mobili. È una sportiva, ciclista e maratoneta. Oggi per parlarmi è costretta a farsi togliere il tubo dell'ossigeno, e durante la nostra conversazione deve assisterla un'amica che manualmente le preme la pancia per attivarle la respirazione. Questa amica venuta dall'Italia, Carla Perli, tira su lo schienale del suo lettino d'ospedale e la solleva di peso per metterla in posizione seduta.

È uno sforzo duro ma Vanessa vuole parlare perché altri possano avere speranza. «Nell'estate del 2003 ebbi i primi sintomi di indebolimento: una fatica intensa, non riuscivo più a puntare i piedi o a saltare alla corda. La diagnosi arrivò nel settembre 2003: ho la sclerosi laterale amiotrofica, la malattia di [...]

la Repubblica — Quotidiano — Data 03-12-2005 — Pagina 31 — Foglio 1 / 2

L'intervento sperimentale in una clinica di Rotterdam, in Olanda. Il direttore del centro: "Ma le reazioni variano da paziente a paziente"

"Curata con le staminali, ora cammino"

Iniezione di cellule su malata di sclerosi. Il governo inglese: troppi rischi, meglio astenersi

DAL NOSTRO CORRISPONDENTE
ENRICO FRANCESCHINI

[...] paese non sono autorizzati interventi del genere». Commenta la dottoressa Carol Cooper, esperta di medicina del Sun: «Le staminali sono cellule vive, che restano nel corpo per sempre e si moltiplicano. Ci sono rischi legati alla possibile trasmissione di virus e perfino alla crescita di tumori. È necessario continuare la ricerca, prima di considerare una cura di questo tipo come eventuale soluzione».

La legge sulle staminali varia da paese a paese, anche all'interno dell'Unione Europea. Le autorità britanniche consentono la ricerca in questo ambito, indagando sulla possibilità di curare forme di cancro, diabete, lesioni della spina dorsale, morbo di Parkinson e altre malattie. Ma la gran parte della sperimentazione avviene sugli animali e ci vorrà tempo prima che Londra permetta terapie sugli esseri umani. Nel frattempo medici e scienziati cercano di rispondere a una serie di dubbi: che genere di staminali funziona meglio per quale tipo di malattia? Come far crescere abbastanza staminali per usarle in una cura? Quale è il modo migliore di farle arrivare nel punto giusto del corpo? E per quanto tempo, una volta iniettate, continuano a lavorare?

CHIARAMONTI

Viaggio della speranza per guarire dalla sclerosi

Da circa dieci anni è su una carrozzina a causa della sclerosi multipla primaria progressiva e ora parte per la Cina per fare un trapianto di cellule staminali embrionali, intervento vietato in Italia e in gran parte dell'Europa. La storia è quella di Giovanni Pietro Sanna, 42 anni, nativo del Belgio ma con genitori originari di Nulvi e Chiaramonti. Una vita pressoché normale sino al 1991. Una laurea in lingue moderne, una moglie (Lucia) nativa di Chiaramonti, conosciuta in Belgio, ed uno splendido figlio di 14 anni, arrivato dopo una prima gravidanza sfortunata. Le vicende della vita li riportano entrambi in Sardegna a vivere proprio a Chiaramonti. Lui sfrutta il suo titolo di studio curando i rapporti con l'estero di una grossa impresa sassarese. Ma all'improvviso la situazione crolla. «Ho cominciato nel 1991 ad avere dolori ad un arto — dice Sanna — poi all'altro e piano piano al resto del corpo. Dopo qualche tempo ebbi l'esito dei vari esami effettuati: sclerosi multipla prima progressiva». Iniziò così un vero calvario, con una serie di visite effettuate presso l'Asl di Sassari, terapie a base di cortisone, interferone fino all'attuale cura fatta con Azatioprina. Ma la situazione di Giovanni Pietro Sanna è gradatamente peggiorata, sino a relegarlo sette anni fa su una carrozzina. Ma qualche tempo fa si è aperta una nuova possibilità: un viaggio della speranza presso un centro di Pechino specialista nel trattamento di malattie neurologiche e cardiologiche con le cellule staminali embrionali, intervento che nel nostro Paese è vietato dalla legge. La clinica è la Neuro Regeneration Repair, di recente agli onori della cronaca perché avrebbe dovuto accogliere Ambrogio Fogar. «Dovrò andare a Pechino a gennaio, ma per far questo devo avere a disposizione 25mila euro per il mio ricovero». Sono molti gli italiani che si sono già rivolti a questo centro con buoni risultati. La famiglia Sanna ha aperto un conto corrente presso il Monte dei Paschi di Siena e ora spera nel buon cuore di qualcuno.

PAOLO PASCA

IL NOTIZIARIO

AP

Spedizione in abbonamento postale, D. L. 353/2003
(conv. in L.27/02/2004 n.46) art. 2 comma 2 - CNS/AC ROMA
Autorizzazione Tribunale di Roma n. 136/85

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ASSOCIAZIONE PARAPLEGICI DI ROMA E DEL LAZIO - ONLUS
Aderente alla FAIP - Federazione Associazioni Italiane Paraplegici

Febbraio - Aprile 2008

DOPPIA MORALE

Pietro Vittorio Barbieri

Dopo Tangentopoli, esiste ancora il Paese dove regna la raccomandazione come pratica della relazione umana e la diffusa corruzione sociale come il voto di scambio, che rendono labile l'aspirazione ai diritti individuali, al rispetto della dignità di ognuno? Insomma, esiste ancora il sopruso di una persona su un'altra, la violenza che rende asserviti?

Alla prima domanda ogni cittadino del Bel Paese risponderebbe in modo affermativo senza mostrare alcun dubbio. La sensazione più comune è che i "capponi" di manzoniana memoria sono ancora la moneta di scambio per ottenere i propri diritti, e che i Renzo si rivolgano agli azzeccagarbugli con deferenza e sottomissione. Vengono degradati però a poco più di un malcostume inguaribile, un'infezione resistente ma tutto sommato non troppo dannosa.

Poi del resto la vulgata sostiene che la vera tutela dell'interesse della persona è nel clan familiare, il luogo essenziale della vita della comunità nazionale (vedi alla voce Family Day).

Non importa se questo comporta l'abbandono del proprio vicinato lasciando la propria immondizia per strada magari contribuendo come dipendente pubblico dell'azienda municipalizzata della raccolta dei rifiuti di un comune del napoletano, se all'Università di Bari impera il più bieco nepotismo, e se le assunzioni nel Consiglio Regionale della Calabria, nelle Asl e negli ospedali sono riservati a cittadini al di sopra di ogni sospetto però aderenti a clan ben identificabili. È solo malcostume nazionale definire morto un dirigente del sistema sanitario che ha assunto il ginecologo con più titoli, e non il candidato appartenente al clan di Ceppaloni al quale il manager Asl doveva la sua nomina.

In un clima auto assolutorio, tutto viene ascritto al fenomeno della casta come fosse altro rispetto ai noi plurale, alla comunità in cui viviamo. Noi in fondo viviamo

LA CINA DEI MIRACOLI

Aumenta la consapevolezza e la qualità della vita delle persone con lesioni midollari, ma a questo non corrisponde una diminuzione del ricorso, nella migliore delle ipotesi, a trattamenti sperimentali costosi e non validati. I ricercatori mettono in guardia sulle terapie promosse in alcune nazioni asiatiche

*Giuliano Giovinazzo**

Negli Stati Uniti sono sempre di più le persone con lesione midollare o Parkinson che si imbarcano alla volta della Cina per sottoporsi a dei trattamenti di cui il mondo scientifico occidentale sa molto poco; ipotecano le loro case, la loro comunità si dà da fare per inventarsi i più disparati modi per raccogliere i fondi necessari per il viaggio, e per sostenere la speranza di una cura miracolosa. Un discreto numero di questi turisti della medicina al suo rientro rivendica un minimo successo. Jim Savage ad esempio, un uomo tetraplegico di Houston, ha dichiarato di riuscire ad avere nuovamente la funzionalità del suo braccio destro. Penny Thomas, delle Hawaii, ha dichiarato che buona parte dei tremori dovuti al Parkinson sono spariti a seguito del trattamento in Cina. I genitori di Rylea Barlett, del Missouri, nata con un difetto ottico, dicono che ora riesce a vedere. Ma la documentazione è nella maggior parte dei casi lacunosa. Gli scienziati ed i medici occidentali sostengono che questi pazienti vengono usati come cavie, porcellini d'India in una paese che non sta facendo i necessariamente rigorosi test indispensabili a provare che un trattamento è sicuro ed effettivo. Notando la mancanza di evidenza, tre dottori occidentali hanno intrapreso uno studio che ha coinvolto sette pazienti con lesione midollare che avevano scelto di sottoporsi ad iniezioni di tessuto cerebrale fetale in un ospedale cinese. Dallo studio non è emerso "alcun utile miglioramento", anche se i pazienti erano convinti di stare meglio. Cinque addirittura hanno sviluppato complicazioni, quali la meningite.

Gli esperti occidentali hanno delle teorie riguardo il perché alcune persone credono di avere dei miglioramenti quando l'evidenza è davvero al lumicino. L'effetto placebo può essere un fattore. John Steeves, professore alla University of British Columbia, direttore di un gruppo internazionale di monitoraggio sui trattamenti delle lesioni al midollo spinale, ha un'altra teoria. Alcuni pazienti potrebbero essere influenzati dalla cifra che hanno pagato e dall'aiuto che hanno ricevuto da donatori o da chi li ha aiutati a racimolare il denaro.

"L'evidenza scientifica è davvero al lumicino"

"Inutile a dirsi, una volta tornati cosa andranno a riferire ai loro amici e vicini? Che non ha funzionato? – ha commentato Steeves. "Nessuno vuole sentire ciò". Insieme ad altri esperti Steeves ha redatto una guida per mettere sull'avviso le persone che stanno prendendo in considerazione questo tipo di trattamenti. I dottori occidentali scoraggiano i loro pazienti dal ricorrere a questi viaggi, notando come è impossibile misurare la sicurezza ed i benefici effettivi dei trattamenti, o quantomeno sapere cosa c'è nelle iniezioni che vengono profuse nei cervelli e nei midolli spinali. Ma per troppe persone quello verso la Cina è ormai il viaggio della Speranza. Savage, 44 anni, precedentemente citato, è un avvocato che si è procurato una grave lesione midollare durante una escursione in canoa 25 anni fa. Ha trascorso due mesi e mezzo a cavallo tra il 2006 ed il 2007 in un ospedale di Shenzhen, nel sud della Cina, per ricevere quello che gli è stato detto essere una serie di iniezioni di cellule staminali nel suo midollo, attraverso il sangue del cordone ombelicale.

"Molto poco è stato pubblicato su riviste scientifiche"

A seguito di queste iniezioni Savage ha dichiarato di essere in grado di muovere il suo braccio destro per la prima volta a seguito dell'incidente. Un video girato nell'ospedale sembra mostrare un leggero movimento. Ha inoltre riscontrato più forza nell'addome ed una maggiore sensibilità della sua pelle. Quanti stranieri come Savage stanno arrivando in Cina per questi trattamenti è un dato sconosciuto, e la Cina è solo uno dei paesi dove sono offerte queste tecniche.

Molti dottori cinesi non aspettano i risultati di test rigorosi prima di trattare i pazienti, ed offrono quello che dicono essere cellule staminali o altri trattamenti cellulari a tutti coloro in grado di pagare. Quello che si sa riguardo queste procedure arriva dai materiali presenti sui loro siti o dai pazienti che danno dettagliati resoconti dei loro viaggi. Molto poco è stato pubblicato su riviste scientifiche per una valutazione da parte degli altri medici.

L'uso di cellule staminali per alcuni trattamenti non è una cosa nuova. Per decenni dottori di tutto il mondo hanno usato cellule staminali adulte del sangue e del midollo osseo – più recentemente del sangue del cordone ombelicale – per trattare tumori del sangue come leucemia e linfoma, o malattie del sangue quali l'anemia falciforme. Gli scienziati hanno studiato se tali cellule staminali adulte o altre cellule come quelle della retina o del tessuto cerebrale fetale potrebbero essere utilizzate per sostituire le cellule perdute a causa di un trauma o di una malattia, e stanno cercando di capire se c'è un modo per stimolare le cellule staminali del proprio corpo a fare riparazioni.

Ma queste strategie sono ancora in fase di studio sugli animali nei laboratori, e c'è stato un numero davvero limitato di questi test sulle persone. Se alcune cliniche in Cina stanno usando le controverse cellule staminali embrionali – come sostengono dottori di altri paesi – non è chiaro.

(segue a pagina 6)

Una via di Pechino nei pressi della Città Proibita

GUIDARE A PECHINO

INCHIESTA

Rocco Vadalà, esperto del Centro per l'Autonomia di Roma sui temi della mobilità, ha fatto parte di una delegazione del Ministero degli Esteri recatasi in Cina per una serie di

ALL'INTERNO

NATURE|Vol 459|14 May 2009

Stem-cell therapy faces more scrutiny in China

But regulations remain unclear for companies that supply treatments.

BEIJING

The Chinese Ministry of Health has implemented regulations on the clinical application of cutting-edge therapies such as stem-cell injections.

Stem-cell scientists in China contacted by *Nature* hope that the rules may help to curtail a growing trade in unproven treatments that attract patients from around the world, risking their health and potentially damaging the reputation of stem-cell research.

The new regulations, which came into effect on 1 May, designate all forms of stem-cell therapy as 'category 3' medical technologies — those deemed "ethically problematic",

are likely to face fines or have their permit to practice medicine revoked, says Renzong Qiu, a bioethicist based at the Peking Union Medical College in Beijing.

"These regulations will make people understand that the Ministry of Health and many scientists in China are concerned about these unverified procedures," says Ching-Li Hu, a paediatrician and senior adviser to Shanghai Jiaotong University's medical school, and a member of the International Bioethics Committee of the United Nations Educational, Scientific and Cultural Organization.

Hu and Qiu are members of an expert panel that will deliver recommendations to the min-

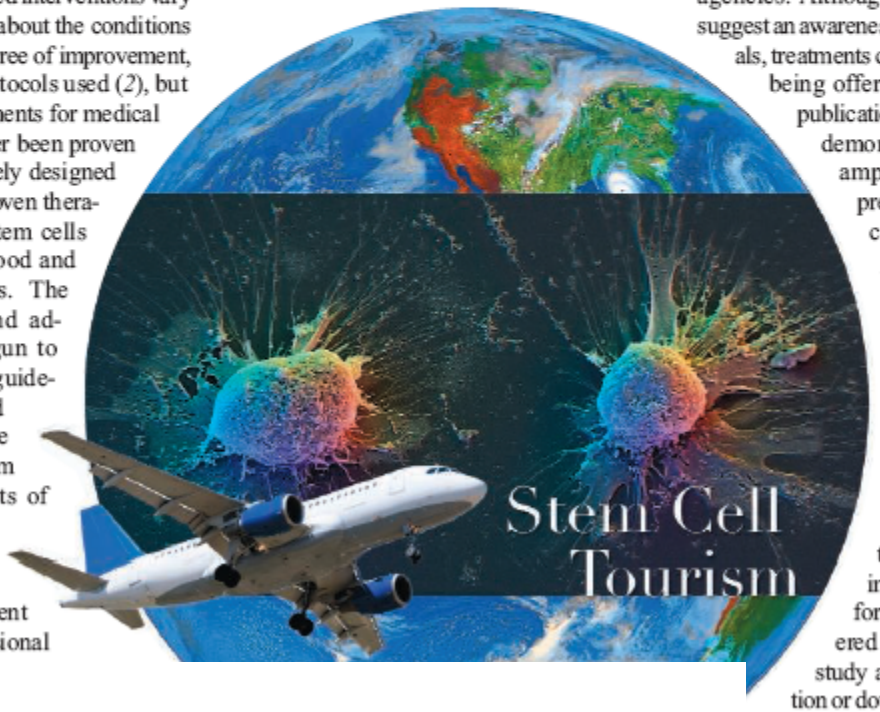
MEDICINE

Monitoring and Regulating Offshore Stem Cell Clinics

Unverified medical treatments based on stem cells are proliferating and need oversight.

Sorapop Kiatpongsan[1,2,3] and Douglas Sipp[4,5]*

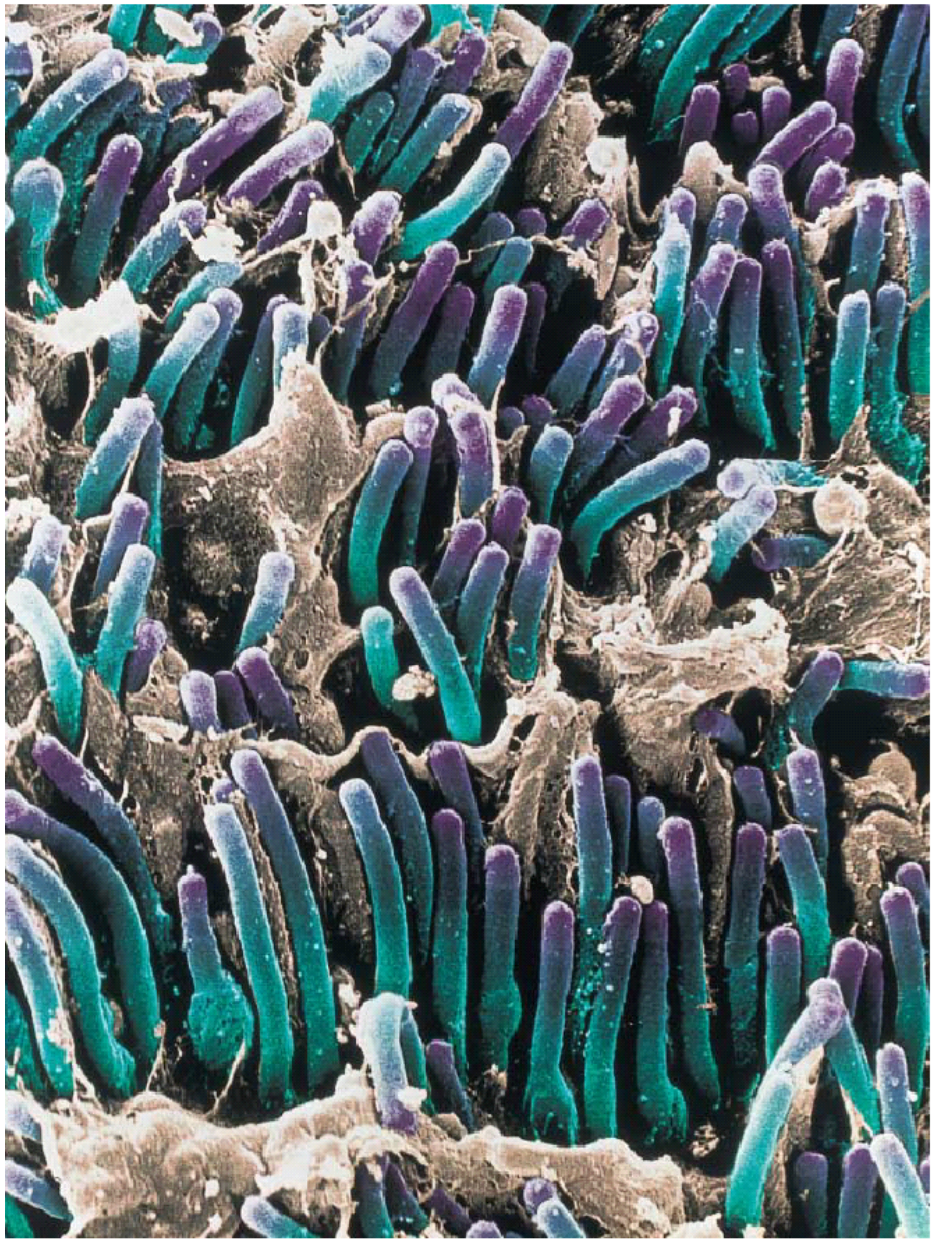
Traveling to another country in the hope of finding a stem cell–based treatment for a disease—"stem cell tourism"—has been the object of intense scrutiny in recent years, following reports of charlatanry, baseless claims, and adverse medical events (*1*). Providers of stem cell–based interventions vary widely in their assertions about the conditions that can be treated, the degree of improvement, and the cell types and protocols used (*2*), but there are many advertisements for medical procedures that have never been proven efficacious in appropriately designed clinical trials. To date, proven therapeutic applications for stem cells have been mainly for blood and immunological disorders. The scientific community and advocacy groups have begun to respond by formulating guidelines for physicians and scientists engaged in the clinical translation of stem cell research (*3*) and lists of questions for prospective patients to ask when considering an experimental stem cell treatment (*3*, *4*). Inaction and occasional that can legally be made by providers or to relegate them to operating outside of their borders. The possibility of operating extraterritorially has meant that unapproved treatments could be had by those willing to travel abroad, but in the great majority of instances, this has



(PLANE) ISTOCKPHOTO; (STEM CELLS) PETER ARNOLD, INC./ALAMY

Netherlands (*9*), and Ireland (*10*); others have been forced out of business (*11*) or prevented from opening by negative publicity (*12*, *13*).

Successful clinics that remain in business are sometimes supported by local medical associations, governments, and regulatory agencies. Although the company Web sites suggest an awareness of the need for clinical trials, treatments costing $20,000 or more are being offered in the absence of prior publication of peer-reviewed studies demonstrating efficacy. For example, TheraVitae has an impressive list of Thai physicians, including the current presidents of the Thai Heart Association and the Thai Atherosclerosis Society (*14*), and recognition from the Davos-based World Economic Forum as a 2006 Technology Pioneer (*15*). However, the peer-reviewed article listed by the company as "accreditation" for its therapeutic regime of adult stem cell therapy for heart disease was considered by the authors to be a safety study and did not use randomization or double-blind controls (*16*, *17*). Perhaps as important as the government and medical establishment links are marketing and patient recruitment stra-



Oxford Scientific Photo Library

Cellule sempre NUOVE

Riprogrammare le funzioni del genoma consente di indirizzare la differenziazione cellulare e schiude nuove opportunità terapeutiche

di Silvia Garagna, Carlo Alberto Redi e Maurizio Zuccotti

LA RETINA, IL TESSUTO SENSORIALE dell'occhio, conserva anche nell'adulto alcune cellule staminali totipotenti.

La biologia dello sviluppo vive oggi un periodo particolarmente felice. La genetica e la biologia cellulare hanno messo a disposizione degli embriologi un bagaglio di conoscenze e tecniche che permettono di affrontare, e molto probabilmente risolvere, quesiti che per decenni sono rimasti senza risposta. Tra questi, il più avvincente riguarda le modalità con cui avviene il processo di differenziamento cellulare durante l'embriogenesi. In particolare: quali sono i meccanismi e le molecole che inducono una cellula indifferenziata verso una via differenziativa piuttosto che un'altra? Il processo di differenziamento è terminale oppure la cellula può tornare allo stato iniziale di totipotenza?

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