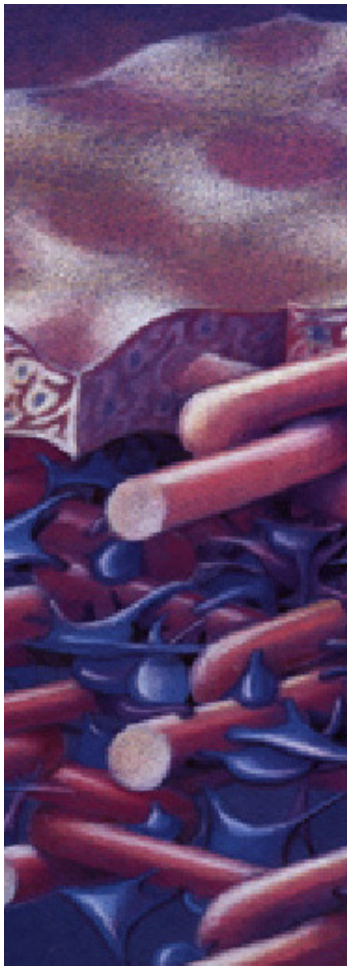

The implications of Stem Cells and Tissue Engineering in Cardiovascular Repair

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Heart Failure

- Chronic condition
- When the pumping ability of the heart fails to meet the body's demands
- Different from Myocardial Infarction (Heart Attack)
- Following a Myocardial Infarction, The injured myocardium is not replaced by new cardiovascular tissue, but instead by a collagen scar tissue (fibrotic tissue). As a result, the heart will be permanently damaged.
- The weakened heart can no longer pump the blood efficiently and this will cause fluid to build up in the lung and other body tissues.
- In order to makeup for its decreased pumping ability, the heart:

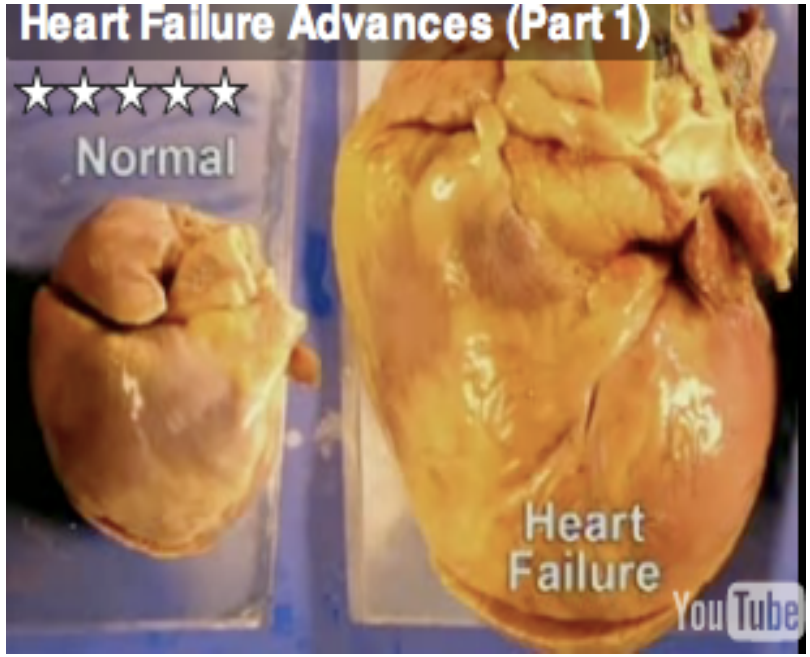
  - Enlarges, to pump the blood effectively

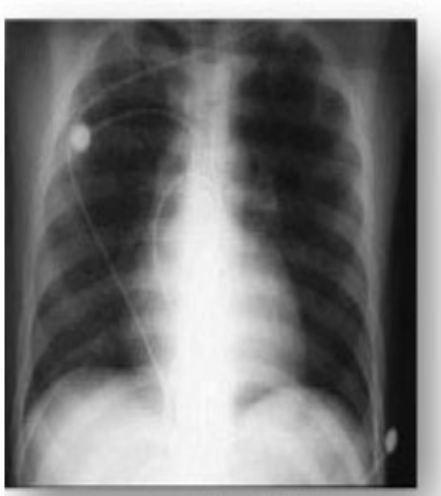
  - Develops more muscle mass

  - Pumps the blood at a faster rate to augment the ejection fraction

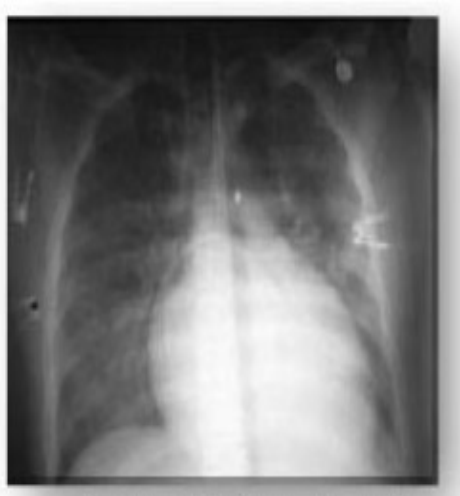
- This places an enormous amount of pressure on the heart
- Usually occurs above 65 years of age and leads to the death of 65% of patients within 5 years.

Normal Heart vs. Abnormally large heart (as a result of heart failure)





normal sized heart



abnormally large heart (cardiomegaly)

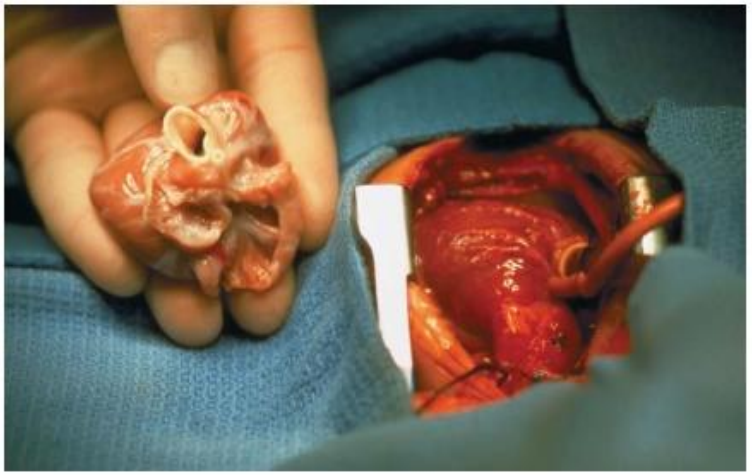
Treatment

- Drugs:
  - Angiotensin converting enzyme (ACE) inhibitors
  - Diuretics
  - Beta blockers
- Coronary Artery Bypass Surgery
- Valve Replacement
- Heart Transplant

Is heart transplant the cure?

- First curative treatment, but includes a number of disadvantages:

- Donor Shortage and thus organ shortage
- Transplant rejection by the body's immune system (acute or chronic)
- Immunosuppressant drugs given after the transplant increase risk of bacterial or fungal infections
- High cost



New Approaches

Replacement or injection of Cardiomyocytes

How could Cardiomyocytes be replaced?

- By obtaining Human Cardiac Progenitor Cells (CPC)

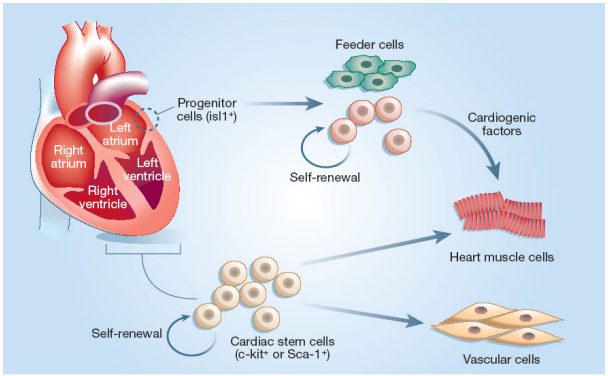
- 10th of February 2005, "The Medical News" Headline – "First evidence of cardiac progenitor cells, which are rare, specialized stem cells located in the newborn hearts of rats, mice and humans, was shown by researchers at the UCSD school of Medicine". Scientists first believed that these cells only existed in the tissue of fetal hearts but then discovered a small amount of CPCs in the atrium of a newborn's heart. These cardiac progenitor cells, also called ISL1+ cells (Insulin Gene Enhancer Protein ISL1+ coded by the ISL gene), are programmed to create cardiac muscle during fetal growth.

- Authors of this article Dr. Sylvia Evans and Dr. Alessandra Moretti explained that "the progenitor cells are able to become spontaneously beating cardiac muscle cells (cardiomyocytes) simply by exposure to neighboring heart cells.

- Scientists were also able to discover the possibility of "expanding a few cardiac progenitor cells found in a newborn's heart into millions of cells into lab culture dishes". Thus these cells could be harvested in order to repair damaged cardiac muscle tissue.

- Patient's heart tissue

- Human fetal hearts



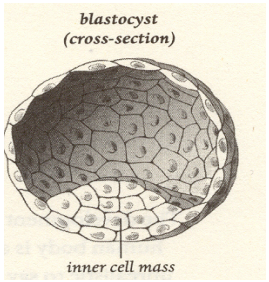
Or

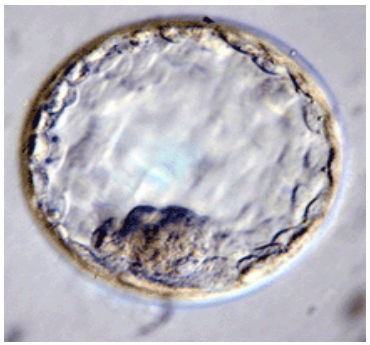
- ✓ By obtaining Human Embryonic Stem Cells (ESC)

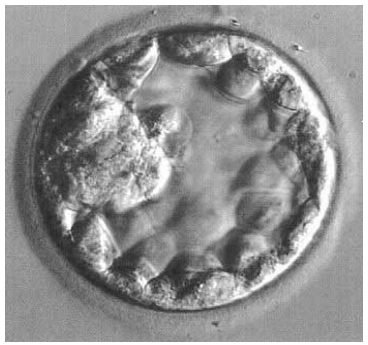
- Following the process of fertilization, a zygote (fertilized egg) is produced. Within 3 to 5 days, the zygote divides rapidly to form a condensed ball consisting of about 12 cells called a morula. After a period of about 5 to 7 days after fertilization, the cells continue to divide in order to give rise to an embryo composed of a hollow cluster of approximately 100 cells called a blastocyst. The blastocyst consists of an outer layer (the trophoblast) and an inner layer of about 30 cells, referred to as the "inner cell mass", which is the source of human embryonic stem cells.

- Called pluripotent because they have the ability to differentiate into any cell type.

- Can renew themselves indefinitely.







- Despite the cardiac progenitor cell's potential to replace cardiomyocytes, "a thorough understanding of how these cells function, how they might be expanded in culture and their true potential as a source for cell transplantation remains to be elucidated". Therefore, Human Embryonic Stem Cells become the appropriate option.

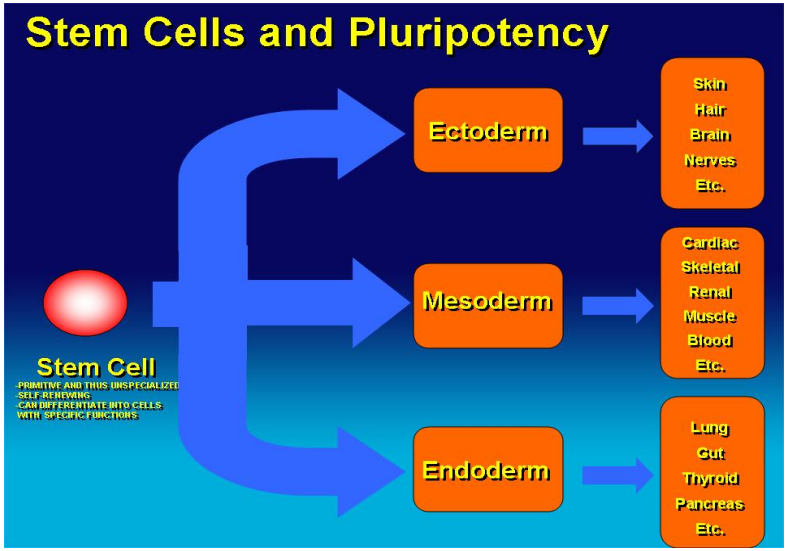
How do the Embryonic Stem Cells differentiate into Cardiomyocytes?

- "By cultivation in vitro as 3D aggregates called embryoid bodies, ES cells can differentiate into derivatives of all 3 primary germ layers" – *"Differentiation of Pluripotent Embryonic Stem Cells Into Cardiomyocytes" by Kenneth R. Boheler, Jaroslaw Czyz, David Tweedie, Huang-Tian Yang, Sergey V. Anisimov, Anna M. Wobus*

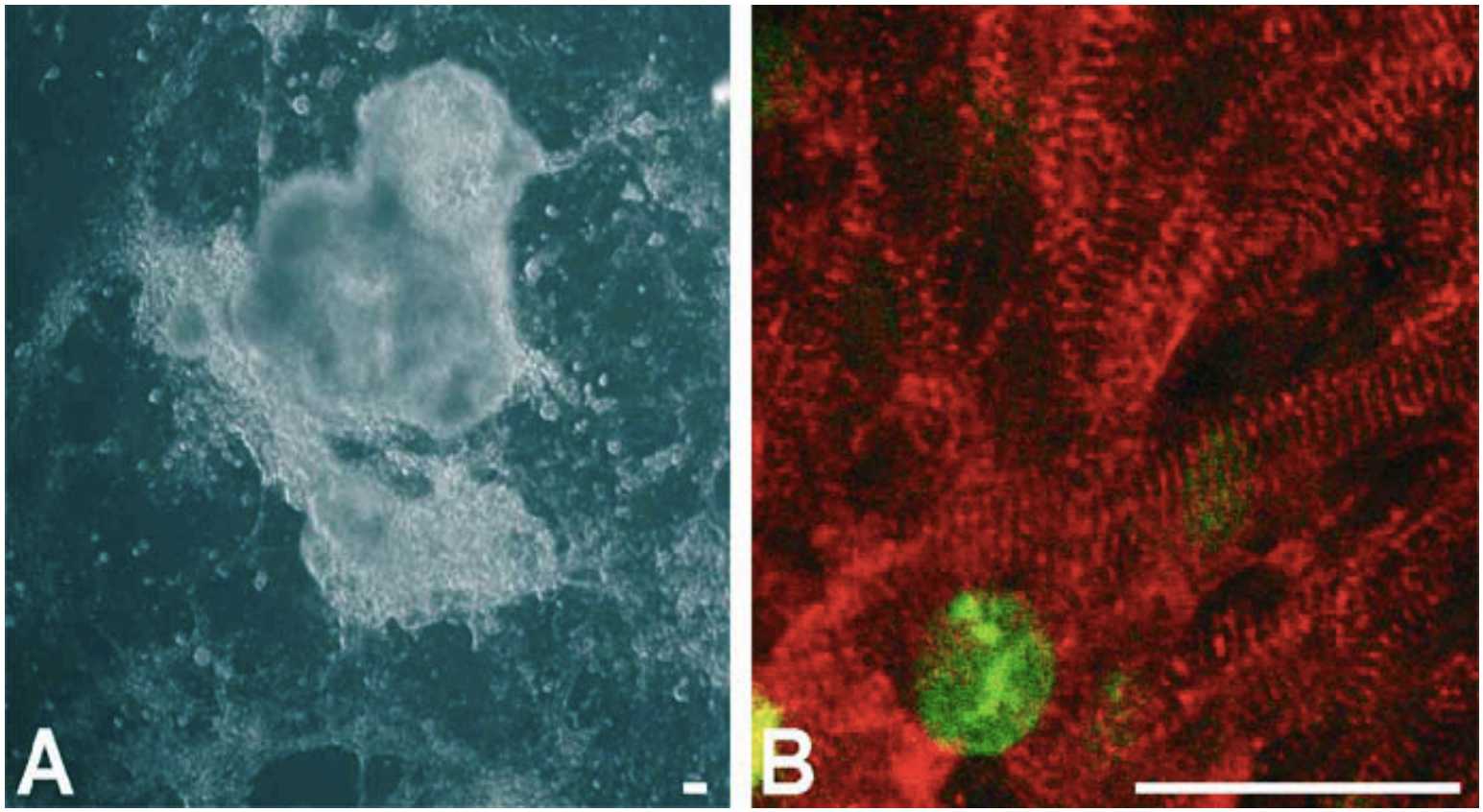
- Ectoderm route – to form nerve cells

- Endoderm route – to form pancreas, lung cells

✓ Mesoderm route – to form cardiomyocytes



HESC differentiating into Cardiomyocytes



Source: Cardiomyocytes from Human Embryonic Stem Cells – by R. Passier, C.Denning, C.Mummery
A: HESC differentiating
B: Beating areas (red), marker for proliferation (green)

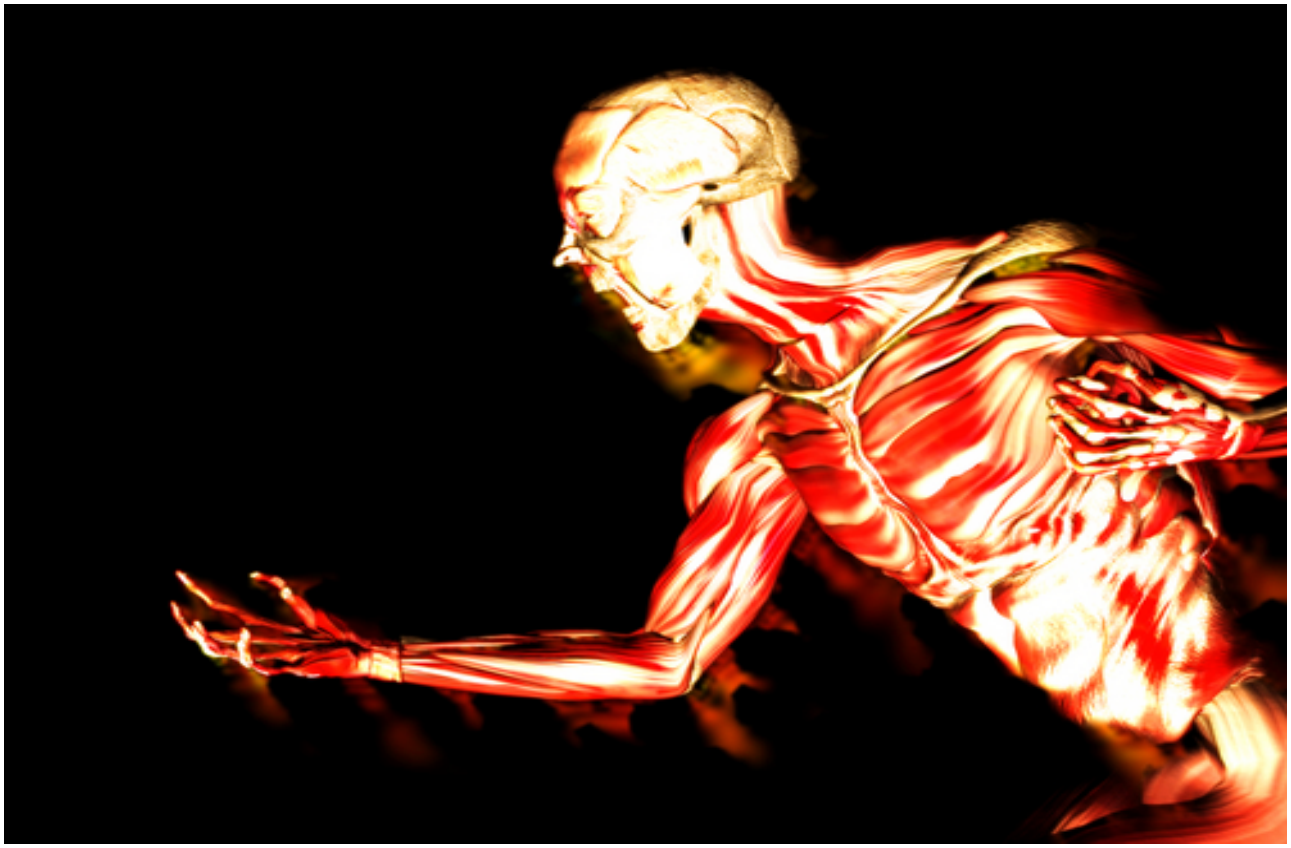
Procedure and Results

- The differentiated cells are then injected into a mouse's heart in the hope that they will align along the scarring tissue.
- This experiment will have positive and negative controls. The negative control will be the results obtained with non-cardiomyocytes and the positive control will consist of results obtained by the injected cardiomyocytes after a given period of time. The expected value will be the ejection fraction (the volume of blood pumped by the heart at each contraction). This will thus help scientists determine the effectiveness of the injected cardiomyocytes.
- In a certain study done by Dr. Christine Mummery at Harvard University, neither of the controls indicated significant changes after 2 days. However after 4 weeks it was observed that the heart with the injected HESC-derived cardiomyocytes was pumping out blood much more efficiently. The ejection fraction was thus very significant. On the other hand, as the controls were examined for a longer time, Dr. Mummery discovered that after approximately 12 weeks, the effect was gone. The ejection fraction was no longer significant, which meant that "despite putting in all these cardiomyocytes [we] were not able to cure the mouse. This means that we are also very far away from curing human beings" – Dr. Christine Mummery

Why the use of Stem Cell derived Cardiomyocytes fails

- According to the paper "Cardiomyocytes from Human Embryonic Stem Cells" by Dr. Catherine Mummery, R. Passier and C.Denning, "It is clear that HESCs can be differentiated towards cardiomyocytes that appropriately respond to different stimuli…However while maturation of the HESC-CMs does occur, during prolonged culture, currently these cells fail to attain the characteristics of adult cardiomyocytes.

- This therefore indicates that a new strategy is required in order to effectively replace the Cardiomyocytes. This new technique Dr. Mummery says, will combine "cardiomyocytes and tissue constructs to offer a solution".



Tissue Engineering

What is Tissue Engineering?

- A relatively new field involved in research that aims to produce human organs and tissue in order to replace damaged or diseased ones.

- Tissue engineers begin the process by constructing a scaffold or a framework that is usually in the shape of the organ to be replaced. The scaffold is created using biodegradable substances such as calcium, collagen and a specific polysaccharide called alginate. "Seeding" cells are then placed on the framework and the entire apparatus is then immersed in a nutrient rich medium to let the cells grow and divide. After a considerable period of time, the seeding cells form several layers on the scaffold and adopt the shape of the mold. The engineering of skin grafts, bone and teeth structures has been very effective in the recent years.

- In the early 1990's, a scandalous image of a mouse with a human ear growing on its back was revealed by Dr. Charles Vacanti, one of the pioneers of tissue engineering. The human ear had been produced using the above procedure, only the seeding cells that were employed to build layers on the ear-shaped scaffold were cow cartilage cells. The human ear was never transplanted onto a human being, firstly because the cartilage cells came from cows and would have been immediately rejected by the individual's immune system, and secondly because the ear excluded the inner structures responsible for detecting sound. However, this image alone indicated that tissue engineering was soon to become extremely successful.

Cells types

- Various types of cells may be used as the "seeding" cells for the scaffold. Each cell type has its advantages and disadvantages which affect the decision of the engineer. In the case of cardiovascular tissue engineering, these cells types include:

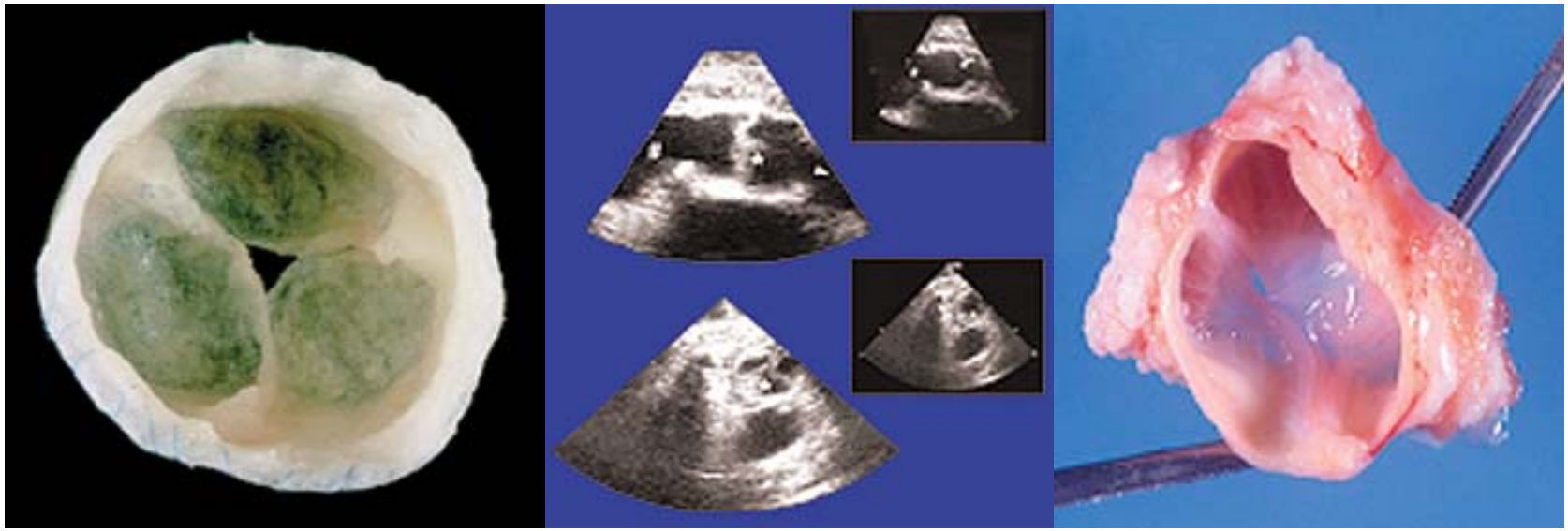
- Autologous: (obtained from the patient). These cells may be sparse or unavailable (especially in older patients) or impossible to use if the patient has a genetic disease. They may also have little ability to proliferate.

- Allogenic: Obtained from the body of a donor (of the same species. The risk of immunorejection and virus/pathogenic infection in this case will be high.

- Xenogenic: obtained from the body of a donor (different species). The risk of immunorejection and virus/pathogenic infection will be eve higher than in the case of Allogenic cells.

- Thus, the autologous cell source seems to be a much more convenient option (because the cells come from the patient's organism). The cells could be: "(1) differentiated cells from primary tissues (e.g., cardiomyocytes), (2) tissue-specific adult stem cells (e.g., hematopoietic or neural stem cells), (3) bone marrow stromal cells (e.g., mesenchymal progenitor cells), (4) bone marrow-derived, circulating stem cells and (5) pluripotent embryonic stem cells." – source: "Cardiovascular Tissue Engineering", by Elena Rabkin and Frederick J. Schoen

Scaffolds before and after implantation

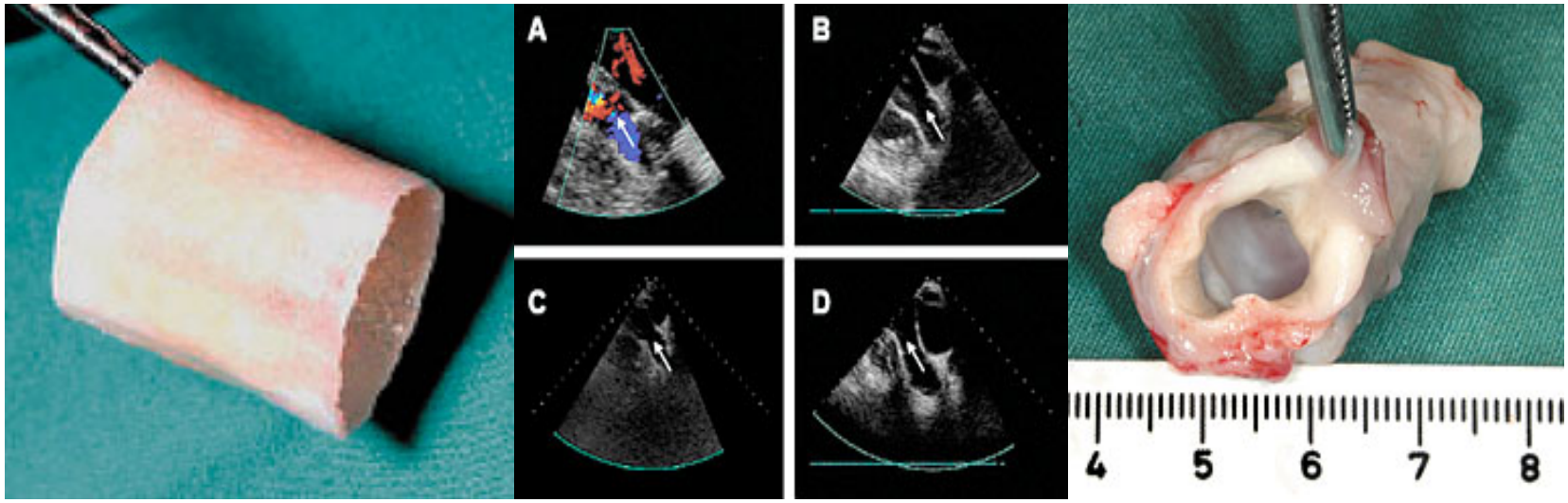


Pre-implantation

In vivo

Post-implantation

Source: Cardiovascular Regenerative Medicine (Tissue Engineering and Cell Transplantation) by Simon Philipp Hoerstrup



Post-implantation

In vivo

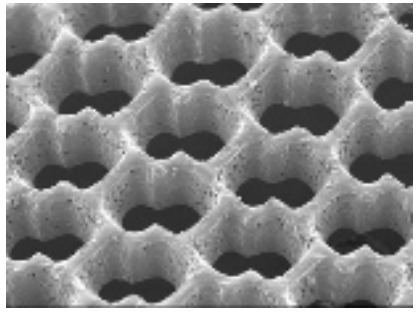
Post-implantation

Source: Cardiovascular Regenerative Medicine (Tissue Engineering and Cell Transplantation) by Simon Philipp Hoerstrup

The role of Tissue Engineering

- Towards the end of her speech at Harvard University, Dr. Mummery explains how the implanted HESC-derived cardiomyocytes differ from the existing heart cells: When observed under the microscope, "normal adult cardiomyocytes have what seem to be strips on them which are responsible for the contraction of these cells". The implanted cardiomyocytes (although surviving) lack these strips which means that they do not all contract in the same direction. Tissue engineering will hopefully find new techniques to make these cells align so they can collaboratively contract and pull as hard as they can.

- This is exactly what has been achieved recently. In an article published by Elizabeth Thomson on the 2nd of November 2008, engineers at MIT were able to design a new scaffold that would make the cardiomyocytes align: "*The accordion-like honeycomb scaffold, reported in the Nov. 2 online edition of Nature Materials, is the first to be explicitly designed to match the structural and mechanical properties of native heart tissue. As a result, it has several advantages over previous cardiac tissue engineering scaffolds.*" – Mending Broken Hearts with Tissue Engineering by Elizabeth Thomson

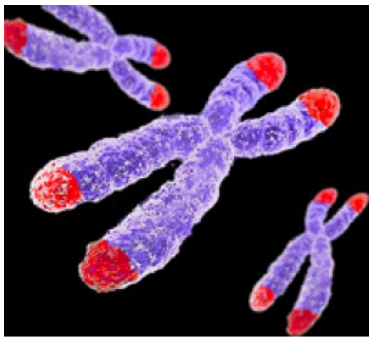


Importance of the Human Genome Project

- Tissue Engineering will benefit a great deal from the knowledge about genes discovered by the Human Genome Project. An example of such a gene a telomere, which have a significant impact on a cell's lifespan.

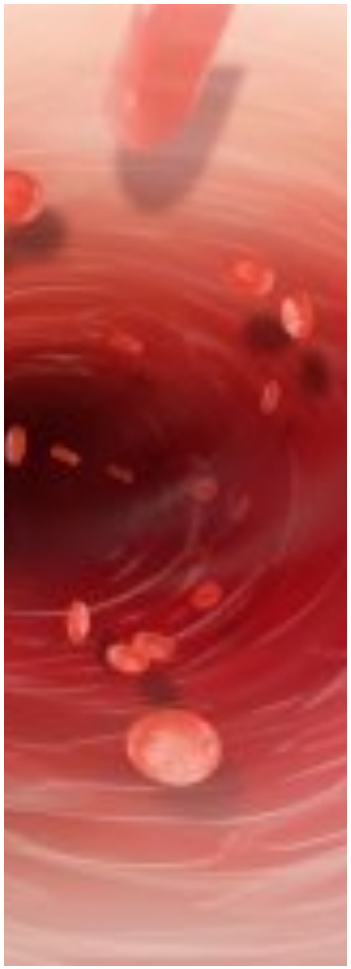
- Telomeres can be thought of as "caps" at the end of chromosomes. They consists of repeated sequences of the bases 5'-TTAGGG-3'. Each time a cells divides, telomere shortening occurs. The continued process of cell division and telomere shortening brings about ageing, or "senescence".

- Cells such as cancer cells possess an enzyme called telomerase which replaces the lost sequences of the telomere during each round of cell division. This enzyme thus helps cancer cells achieve immortality. Could telomerase genes be thus injected into human cells to help them achieve immortality? From a Tissue Engineering perspective, it would be extremely helpful to inject this gene in cultured human cells and allow them to produce immortal, normal human cells. This would even allow scientists to treat age-related diseases, (and possibly age – related cardiovascular disease), more efficiently.



Conclusion

- Thus, the combination of Stem Cell research, the novel field of Tissue Engineering and the Human Genome Project will enable scientists to develop more efficient techniques to cure not only heart failure, but a variety of other diseases. The use of Stem Cells in cardiovascular repair was successful to a certain extent, but thanks to Tissue Engineering, which combines "Stem Cell derived Cardiomyocytes and Tissue constructs" (Dr. Mummery), an effective manner of curing heart failure is hopefully on its way.



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