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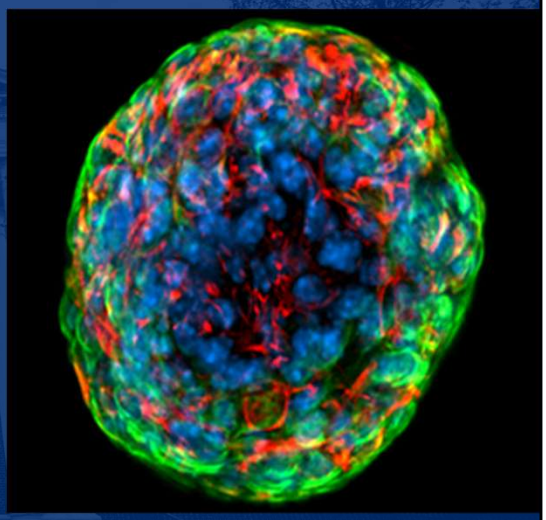
Cell and Tissue Engineering

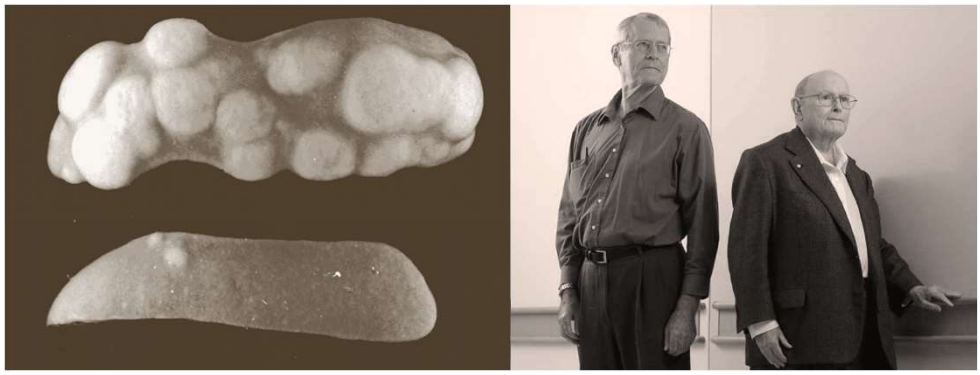
Stem Cell History and Regulation



Stemcell.ny.gov

Timeline – Proving Existence of Stem Cells

1961
James Till & Ernest McCulloch



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Thompson et al. Science 1998; Stemcellnetwork.ca

The history of stem cells begins with two Canadian scientists.

James Till and Ernest McCulloch publish accidental findings in the journal “Radiation Research” proving the existence of stem cells in the early 1960s.

In their work, they injected bone marrow into an irradiated mouse, this resulted in nodules in the spleen – like these here – the nodules generated multiple blood cell types, and underwent self-renewal.

We know what that means (teratoma!), but at the time Till and McCulloch did not. It took over a year before they saw the real potential of their discovery. That first paper in the journal Radiation Research went largely **unnoticed** by the scientific community, in fact it wasn’t until their follow-up paper in the journal Nature in 1963 that researchers became excited.

For the first time researchers showed that a single cell could form a colony of different cell types.

Timeline – First Bone Marrow Transplant

1968

First bone marrow transplant performed

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Mbbnet.umn.edu

A few short years later research, building on the findings of Till and McCulloh, led to the first successful bone marrow transplant.

This breakthrough treated two siblings with severe combined immunodeficiency. (SCID)

Timeline – America Debates Fetal Tissue Research

1973

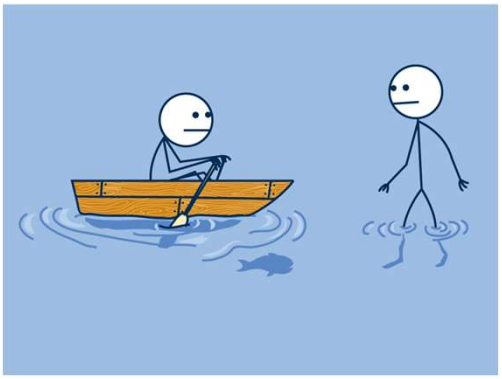
Roe v. Wade

1974

Congress bans all federally funded fetal tissue research

1975

Ethics advisory board established



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The next milestone in the road for stem cell research came in 1974, just a year after the verdict of Roe vs. Wade. The Supreme Court decision on abortion ruled 7 to 2 that a right to privacy under the 14th amendment extended to a woman's right to have an abortion.

This decision is what got Americans to begin discussing how to conduct ethical research on human **fetal** tissues – something of critical importance to the use of embryonic stem cells because at this time they had yet to be cultured.

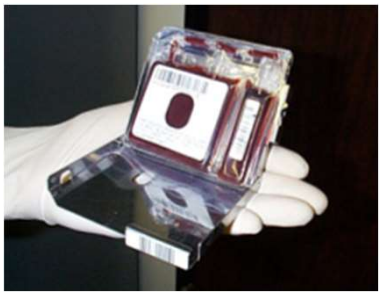
In fact it was so important that, immediately following, a ban was put on federally funded fetal tissue research until guidelines could be set for the protection of human subjects in research

Then in 1975 an ethics advisory board was established with was charged with creating guidelines for fetal and tissue research that originated from abortions

Timeline – Cord Blood, Mice, Primates

1978

Stem cells discovered in human cord blood



1981

First *in vitro* stem cell line developed from mice

1995

First embryonic stem cell line derived from a primate

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Nationalcordbloodprogram.org; Biovaultfamily.com

Through the legislative turmoil science continued – there was the discovery of stem cells in **human cord blood**, you can see what a typical cord blood collection looks like – small enough to fit in the palm of one hand. These are now stored at high volume facilities like these with huge cryo-storage units.

After cord blood stem cells came embryonic stem cell lines derived from mice and primates, like the marmoset example we saw earlier.

Timeline – Dicky-Wicker Amendment

1996

Dicky-Wicker Amendment

(1) The creation of a human embryo or embryos for research purposes

(2) Research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero

"incompatible with human dignity and the protection of human life." – *United Nations*

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Massacademy.wordpress.com

Then in 1996 an important piece of legislation passed – the Dicky-Wicker Ammendment.

This bill prohibited the use of federal funds for (1) **creating** human embryos for research purposes and (2) conducting research in which an embryo is **destroyed**, **discarded** or subjected to **risk** of injury or death

At the international level, the US voted in favor of a ban on embryo-based research while the UK, Belgium, and China voted against it.

Timeline – Cloned Lamb

1997

Leukemia origin found in hematopoietic stem cells indicating possible proof of cancer stem cells

Cloned lamb from stem cells

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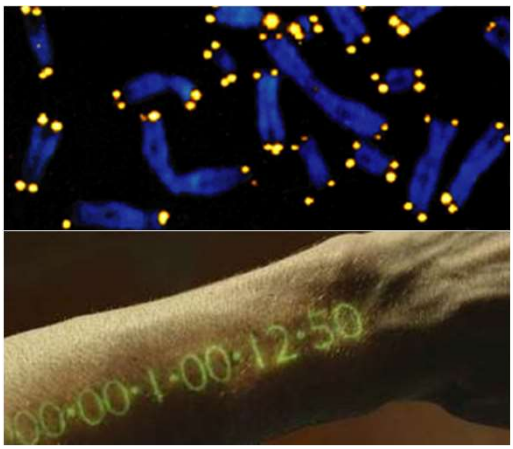
Usatoday30.useatoday.com

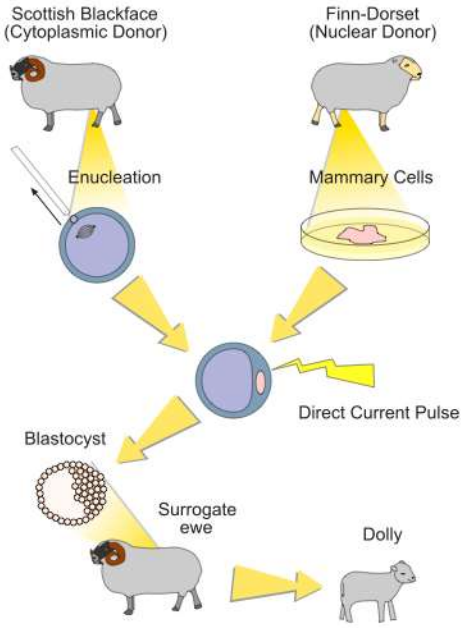
Within the year a few big events occurred – first the emergence of evidence for **cancer** stem cells. A potential origin for leukemia was located in hematopoietic stem cells – this is a topic we don't have time to cover in this course but I encourage you to look into on your own. Cancer stem cells have become a very hot topic since 2015.

Second, **reproductive cloning** of mammals was happening – and here is a picture of the famous cloned sheep **dolly**.

How was Dolly made?

Somatic Cell Nuclear Transfer (SCNT)





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Wikimedia Commons; Brusimm.com; Hodes et al, 2002

Let's back up for a second and peak at the **methods** used to make Dolly – we'll need this information to understand some of the upcoming legislation.

So how exactly did researchers make dolly….

They used a technique called **somatic cell nuclear transfer** where nuclear material is **isolated** from a **somatic** cell from one animal and put into an **enucleated oocyte**.

Electricity is used to stimulate growth of that oocyte which grows into a blastocyte that is then placed in a surrogate animal.

Only 1-2% of transfer result in live birth and only 20% of those appear normal. Dolly, cloned by Scottish researchers using this method lived just 7 years as opposed to 11-12 normal life expectancy.

Although unsettled, some of the reasons for this early death include **shortened telomere length** in the DNA from the donor sheet – The parts of the chromosome labeled in yellow in the upper left. So the embryo was made with DNA that had already starting running its clock.

Timeline – Cloning Prohibition Act

1997

Leukemia origin found in hematopoietic stem cells indicating possible proof of cancer stem cells

Cloned lamb from stem cells

Cloning Prohibition Act – *failed!*

…Severely delay progress in the development of very important therapeutic treatments of major public health diseases - AAAS

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Usatoday30.useatoday.com

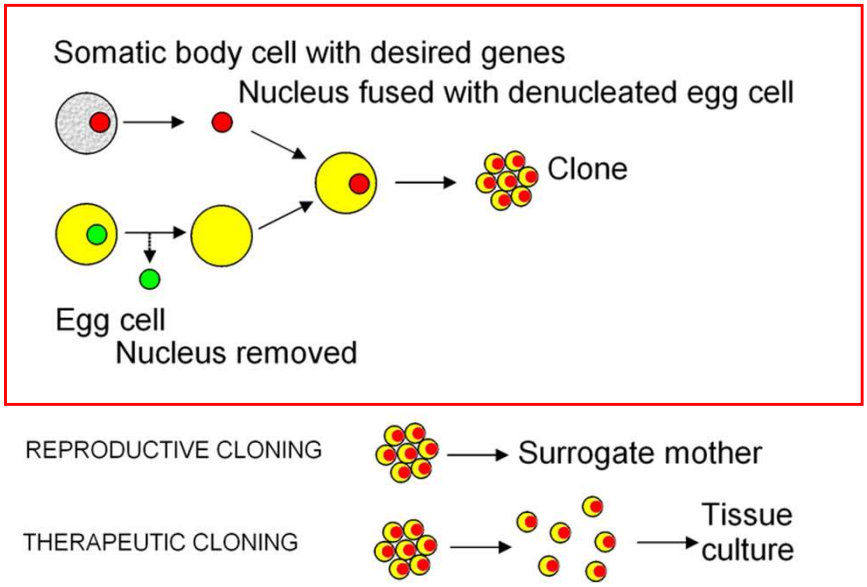
With the rapid pace of stem cell advancement which led discoveries like somatic cell nuclear transfer and reproductive cloning the US president, Bill Clinton, wanted to slow things down. President Clinton proposed **a 5 year moratorium** on federally and privately **funded human cloning research**. The intention was to let the public and scientific community digest what what happening. What stem cells were capable of, what reproductive cloning really meant for society.

This Cloning prohibition act failed – here is one quote from the American Association for the Advancement of Sciences

This ban would severely delay progress in the development of very important therapeutic treatments of major public health disease.

Scientist groups like AAAS lobbied heavily that the ban would jeopardize life saving research. And these lobbyists won.

Reproductive vs. Therapeutic Cloning



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Wikimedia commons

So why was the Clinton administration so cautious??

With Dolly in the picture, the door to tissue engineering applications became wide open – there was now a pathway for both **reproductive** cloning and **therapeutic** cloning.

You see the same somatic cell nuclear transfer method here in the red box – what happens with the cloned cell is what separates the two techniques on the bottom.

In reproductive cloning you go on to **build an organism**. To date more than 18 mammals have been cloned this way including horse, cattle, swine, goats, dogs, and of course mice. These process has been commercialized, for example ponies and dogs. Reproductive cloning has not progressed to humans, for ethical and technical reasons.

In therapeutic cloning the embryonic cells are used in vitro for other therapeutic applications, such as developing a cell source to use in building other tissues.

https://www.statnews.com/2020/02/21/human-reproductive-cloning-curious-incident-of-the-dog-in-the-night-time/

Why Use Reproductive Cloning?

Cloning for a better food supply
2008 FDA risk assessment
Concludes that…

"Meat and milk from cow, pig, and goat clones, and the offspring of any clones, are as safe as food we eat everyday"

Cloning for research purposes
Reproducible
Reliable
Invariant over time and space

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http://jaxmice.jax.org/genetichealth/index.html; Fda.gov

Based on our first lecture we know that there are a number of reasons we would want to clone therapeutically – including creating autologous stem cell lines for treating disease and building new tissues.

So let's talk about some of the reasons why we might want to reproductively clone.

There are a number of non-medical reasons – like bringing back endangered species and creating a better food supply. Very recently we saw the first cloned meat enter the food chain. This was backed by the FDAs 2008 risk assessment which stated that quote:

"Meat and milk from cow, pig, and goat clones, and the offspring of any clones, are as safe as food we eat everyday"

In the realm of medical research however cloned animals for research can improve the reliability and reproducibility in studies.

These two things don't sound terribly scary…

Timeline - Reproductive Cloning Fears

1998 **South Korean claims 4-cell clone**

2004 **Woo-Suk Hwang, National University South Korea publish 20 cloned human embryos in *Science***
Retracted in 2006

Despite being exposed as a fraud, Dr. Kwang Woo-Suk continues to claim cloning human stem cells is possible

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Theinterim.com

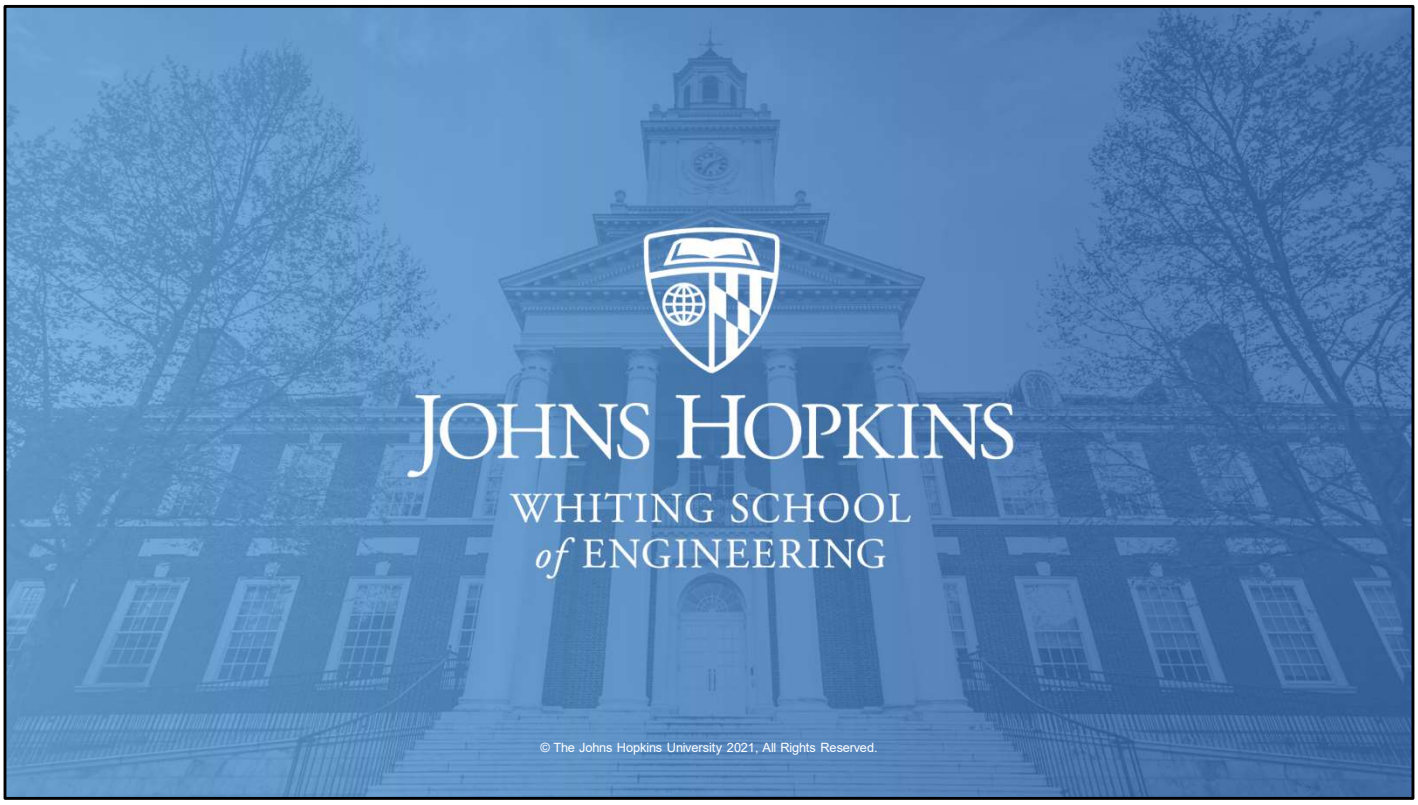
However, the real fears were with human reproductive cloning.

The year after Dolly in 1998 – a South Korean scientist claimed to have done just that. Cloned a human and interrupted development when it became a 4-cell embryo.

False claims of this nature have continued through the years and came to a fever pitch in 2004 with a publication in Science – on 20 cloned human embryos. After independent scientific review **found no proof and t**he article retracted it in 2006

One reason this is so difficult in humans are **Spindle proteins**. These are located close to chromosomes so removal of the nucleus from the donor egg often removes them and blocks cell division

Further, there is the **imprinting process** – two copies of every gene (father and mother) pass to the offspring. Some genes are only run by the maternal or paternal gene. However, if both copies are running (due to the SCNT process) the embryo is at risk for severe consequences.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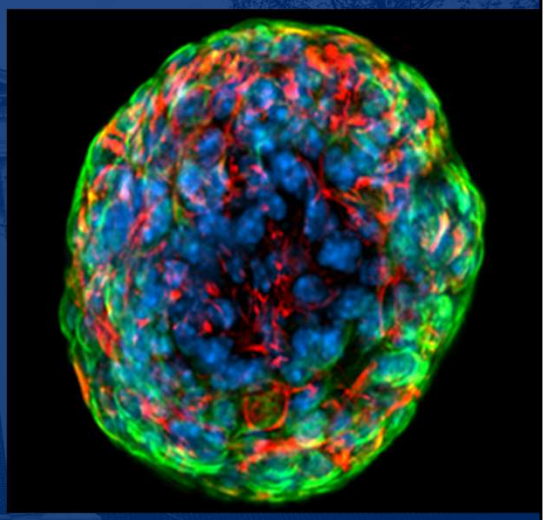
Cell and Tissue Engineering

Stem Cell History and Regulation (cont.)



Stemcell.ny.gov

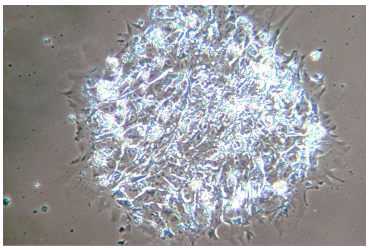
Timeline – Embryonic Stem Cells

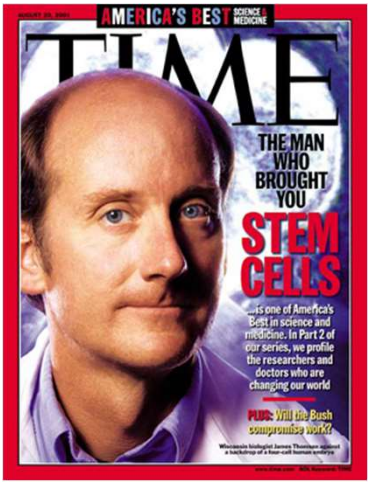
1998

James Thomson isolates human embryonic stem cells

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones





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Achievement.org; http://www.news.wisc.edu/newsphotos/thomson.html

With the pressure on federal funding for stem cell and cloning research and false advertising going on -

a researcher named James Thomson from University of Wisconsin was busy isolating the first human embryonic stem cells. Dr. Thompson showed that these embryonic derived cells had the capacity to develop into any of the **220 different tissues** of the human body. This image on the bottom shows you one of the **original human embryonic stem cell colonies cultured by Dr. Thomson.**

Timeline - US Stem Cell Legislation

1999

NIH Guidelines for Research Using Human Pluripotent Stem Cells

- **only embryos "created for the purpose of fertility treatment"**
- **"in excess of the clinical need of individuals seeking such treatment"**
- **Informed consent**

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Huffingtonpost.com

There we were in the late nineties – the age of human embryonic stem cell research

It was only a decade or so ago the NIH proposed guidelines for funding research on ESCs. Following the failure of the President Clinton's ban, the NIH was ready to see what the next presidency would do.

They set up guidelines that federal funds for embryonic stem cell research could only be done on embryos created for fertility treatments and **known to be in excess of clinical need** may be used and, importantly that the donors must also give informed consent.

Timeline – Limited US hESC Research

2001

President Bush permits federal funding for hESC research only on cells from embryos that have already been destroyed

21 →16 approved hESC lines

"the life and death decision has already been made"

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AP Photo, CNN

The NIH waited to fund proposals until the new president took office.

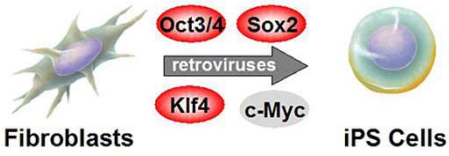
And in 2001 under President Bush there was not a categorical ban, but instead a limit to the **source** of stem cells that could be funded by the government. Only **21 lines** are originally approved although only **16** were found to be **derived ethically and remained eligible for funding** . No embryonic stem cell lines created after 2001 are permitted for federal funding. Note that this does not prohibit **private funding** of research.

He is quoted in saying
*"As I thought through this issue I kept returning to two fundamental questions. First, are these frozen embryos human life and therefore something **precious to be protected**? And second, if they're going to be destroyed anyway, shouldn't they be used for a **greater good**, for research that has the potential to save and improve other lives? "*

Timeline – Induced Pluripotent Stem Cells

2007

Yamanaka and Thomson independently derive iPS cells



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Wrtl.org

It was during that time that President Bush called for work on alternate sources for stem cells. Two groups independently derived **induced pluripotent stem cells**. Both created with human dermal fibroblasts. Yes, this is the same Dr. Thompson we saw before.

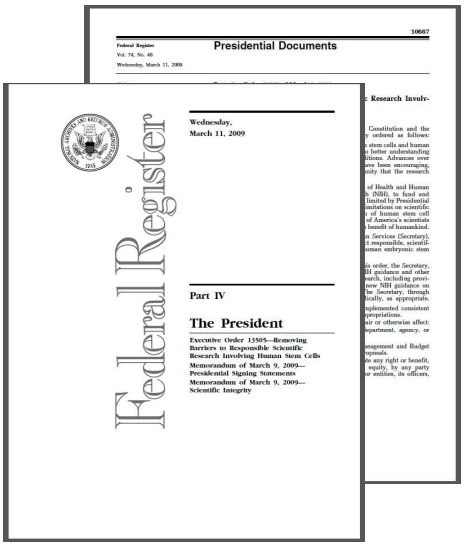
Next we have President Obama in 2009 and the reversal of President Bush's executive order.

Removing Barriers to Responsible Scientific Research Involving Human Stem Cells

2009 – Obama issued an executive order nullifying Bush's policy

"[NIH] may support and conduct responsible, scientifically worthy stem cell research including human embryonic stem cell research, to the extent permitted by law."

"That the potential [hESC research] offers is great, and with proper guidelines and strict oversight, the perils can be avoided." Obama, Signing the Executive Oder, 3/09/2009



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http://www.whitehouse.gov, 10/29/11; http://stemcells.nih.gov/policy, 10/22/11

This opened the door to excess embryos created for fertility purposes after 2001 – so now researchers are not limited to what was created prior to 2001.

Now we're back to embryos created from IVF only, but more than what was available during the Bush administrations

Just to reiterate these embryos were not created for research purposes only – they are consented excess from IVF.

Current NIH guidelines

- **No funding for the derivation of hESCs**
- **Funding of research using hESCs**
  - **derived from embryos created using IVF for reproductive purposes (not research purposes or SCNT)**
  - **no longer needed for IVF purposes**
  - **obtained from donors with informed consent**
    - **donors are not paid and would not received financial or any other benefit from commercial development generated from the donation**
  - **if the research has scientific merit**
- **hESCs must be listed in the NIH Registry**
- **Does not require that the IVF physician be different from the hESC researcher**

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http://stemcells.nih.gov/policy/2009guidelines.htm,

So where to we stand today… let's look at the current NIH guidelines – they aren't so different from what was laid out in 1999

NIH received 50,000 comments from patient advocacy groups, scientists, scientific societies, academic institutions, medical organizations, religious organizations, private citizens, congress. Using those comments they devised their current guidelines.

The NIH does not fund derivation of hESC lines

It will fund research on lines, however with the stipulations we say earlier.

All lines must be registered with the NIH. One note of interest here is that the physician that performs the IVF can be the same researcher that conducts hESC research. Many view this as a conflict of interest.

Today there are just over 400 registered hESC lines

https://grants.nih.gov/stem_cells/registry/current.htm

Timeline - Clinical Trials in Stem Cell Research

2010

Geron initiates first clinical trial of hESC-based therapy

Advanced Stem Cell Therapy (ASCT) is FDA approved for hESC therapy for degenerative eye disease

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http://www.aisquared.com/blog/2013/08/looking-back-my-one-year-participation-in-a-stem-cell-clinical-trial/; http://www.geron.com; http://www.advancedcell.com/

This bring us to the clinical trials we discussed earlier -- Geron – using stem cells to treat spinal cord injuries.

And advanced stem cell with trials using stem cell-derived retinal cells to treat degenerative eye disease.

Map of National Policy on Public Funding for Research

Map reflects

- Permissive
- Flexible
- Restrictive OR no established policy

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http://mbbnet.umn.edu/scmap.html,

The views on stem cell research across the globe do vary. This map indicates which countries are most permissive for public funding of stem cell research in dark brown –

You can see here counties like Australia, China, India, Japan, South Korea, the UK, and Israel. These countries fund various ESC derivation techniques including somatic cell nuclear transfer.

Flexible countries find derivation from fertility clinic donors only, limited again to embryos no longer needed for reproduction. This is the policy in counties like Brazil, Canada, France and Iran.

The restrictive countries range from outright prohibition of human embryo research to research permitted with only pre-established ESC lines.

NIH funding of stem cell research

Research/Disease Areas (Dollars in millions and rounded)	Stem Cell Research	Stem Cell Research - Embryonic - Human	Stem Cell Research - Embryonic - Non-Human	Stem Cell Research - Induced Pluripotent Stem Cell	Stem Cell Research - Induced Pluripotent Stem Cell - Human	Stem Cell Research - Induced Pluripotent Stem Cell - Non-Human	Stem Cell Research - Nonembryonic - Human	Stem Cell Research - Nonembryonic - Non-Human	Stem Cell Research - Umbilical Cord Blood/ Placenta	Stem Cell Research - Umbilical Cord Blood/ Placenta - Human	Stem Cell Research - Umbilical Cord Blood/ Placenta - Non-Human
2008	$938	$88	$150	+	+	+	$297	$497	$46	$38	$9
2009	$1,044	$120	$148	+	+	+	$339	$550	$49	$42	$10
2009 ARRA	$187	$23	$29	+	+	+	$58	$88	$10	$9	$1
2010	$1,099	$126	$175	+	+	+	$341	$570	$42	$40	$5
2010 ARRA	$187	$40	$20	+	+	+	$74	$74	$8	$7	$1
2011	$1,179	$123	$165	+	+	+	$395	$620	$41	$36	$10
2012	$1,374	$146	$164	$206	$175	$48	$504	$653	$47	$43	$8
2013	$1,273	$146	$154	$228	$199	$43	$431	$613	$40	$35	$7
2014	$1,391	$166	$150	$313	$280	$49	$443	$627	$34	$28	$7
2015	$1,429	$180	$159	$324	$282	$61	$445	$632	$35	$32	$6
2016	$1,516	$206	$146	$374	$335	$56	$457	$652	$42	$33	$10
2017	$1,646	$252	$129	$421	$382	$59	$484	$704	$40	$35	$6
2018	$1,824	$278	$130	$507	$468	$68	$518	$758	$39	$36	$4
2019	$2,014	$306	$140	$607	$563	$74	$569	$781	$38	$36	$2
2020	$2,105	$309	$141	$657	$613	$73	$608	$830	$35	$31	$5
2021 Estimated	$2,150	$317	$144	$672	$627	$75	$620	$846	$36	$31	$5
2022 Estimated	$2,229	$329	$149	$697	$651	$77	$642	$880	$37	$32	$6

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http://stemcells.nih.gov/research/funding/funding.htm

SO what does a flexible policy on stem cell funding look like, in terms of in dollars and cents in the US.

Here are the numbers for NIH funding of stem cell research in 2021, These numbers show how funding for non-embryonic stem cell and iPSC research exceeds human embryonic research.

There was a 4x increase in funding for human embryonic stem cell between 2008 and today.

State Stem Cell Funding

STATE	Initial Funding	
California	622M	2004
Connecticut	10M	
Illinois	15M	
Indiana		
Maryland	15M	2006
Massachusetts	1M	
New Jersey	23M	2004 -First to appropriate state funds for embryonic stem cell research
New York	100M	
Ohio		First to appropriate funds for adult stem cell research
Washington	28M	
Wisconsin	1M	
Virginia		

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http://www.ncsl.org/default.aspx?tabid=14413

There are additional funds at the sate level, some of which I've put in a table here. For those interested in Maryland we have a stem cell research fund put into effect by the MD stem cell research act. It started with just 15mill in and has given out approximately 80million dollars to over 200 grants.

CA has the most money available and this is in part due to bonds that they sell each year to fund the Californinia Institute for Regenerative Medicine (CIRM).

Private Stem Cell Funding

Private philanthropists

- **25M to USC**
- **16M to UC-San Fran**
- **75M to UC-Davis**
- **100M to JHU (Bloomberg)**

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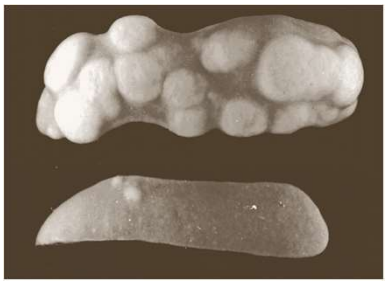
Positivelyparkinsons.blogspot.com; Greatercp.jdrf.org

Private funding is also available through funds and private philanthropists – some donations I've listed here. You might recall that JHU received 100M from NY's Michael Bloomberg. The Michael j fox foundation, the starr foundation, and the juvenile diabetes research foundation are other major sources.

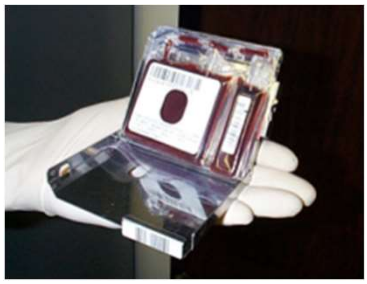
Review of Stem Cell Regulation

The discovery of stem cells



The Dicky-Wicker Amendment

The discovery of cord blood stem cells



Bush's Executive Order
The discovery of iPS cells
Obama's removal of the order

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We began with the discovery of stem cells by till and mcculloh in the early 60s – it is interesting that the first stem cell found was really the adult or somatic stem cell.

Then in the late **seventies cord blood – stem** cells were found

After that the dicky wicker amendment which still stands today regulating the use of embryos in research

A year later dolly was born and we had disagreement in back to back presidencies. President Bush limiting the embryonic stem cell research to embryos that had already been created and obama opening the door back up again.

Now that we come to the end of this module you should feel versed in stem cell facts and history. From here we'll move forward to look at current tissue engineering therapies which incorporate all of the pieces we've discussed up to now – cell selection, biomaterials, chemical gradients and mechanical factors.