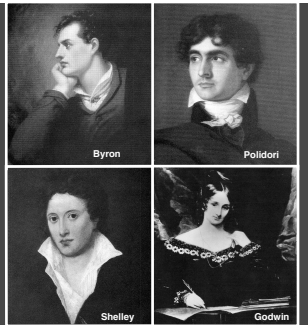
THE LEGACY OF FRANKENSTEIN: REGENERATIVE BIOLOGY AND MEDICINE

David L. Stocum
Department of Biology, IUPUI
and
Indiana University Center for Regenerative Biology and Medicine

Mount Tambora, Indonesia, April, 1815

VILLA DIODATA, LAKE GENEVA
JUNE, 1816



"FRANKENSTEIN, or A MODERN PROMETHEUS"

Mary Shelley, 1818

CENTRAL THEMES OF "FRANKENSTEIN"

- ASSEMBLY OF BODY PARTS
- RESURRECTION
- CONSEQUENCES OF SCIENTIFIC ACHIEVEMENTS WE DO NOT MORALLY KNOW HOW TO USE

These themes have followed us through the 20th Century into the 21st Century as we have developed the field of Regenerative Biology and Medicine, which seeks to restore the structure and function of injured or dysfunctional organs and tissues. This is the legacy of Frankenstein.

THEME 1: ASSEMBLY OF BODY PARTS IN THE 21st CENTURY

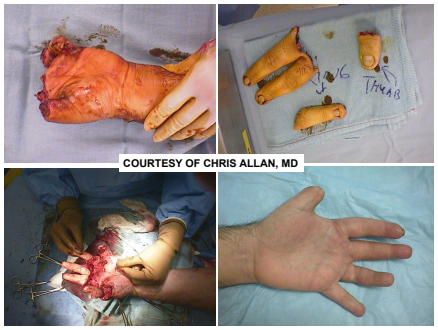
- ORGAN TRANSPLANTS
- CELL TRANSPLANTS
- BIOARTIFICIAL TISSUES

ORGAN TRANSPLANTS

- AUTOTRANSPLANT
- ALLOTRANSPLANT
- XENOTRANSPLANT

AUTOTRANSPLANT

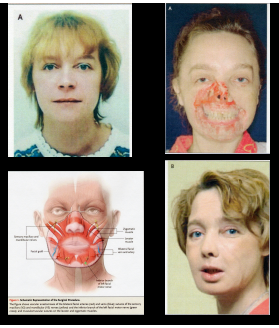
A TRANSPLANT WITHIN THE SAME INDIVIDUAL



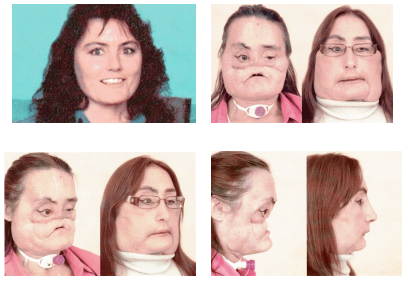
COURTESY OF CHRIS ALLAN, MD

ALLOTRANSPLANT

A TRANSPLANT BETWEEN GENETICALLY DIFFERENT INDIVIDUALS OF THE SAME SPECIES



FIRST NEAR TOTAL FACE TRANSPLANT PERFORMED AT CLEVELAND CLINIC BY A SURGICAL TEAM LED BY DR. MARIA SIEMIONOW



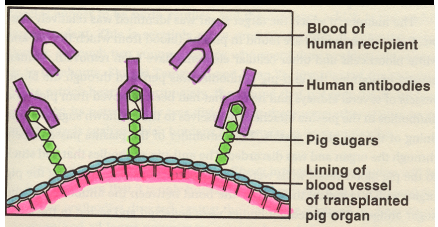
MAJOR DRAWBACKS OF ALLOTRANSPLANTS

- SIDE EFFECTS OF IMMUNOSUPPRESSION
- DONOR SHORTAGE

XENOTRANSPLANT

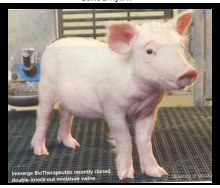
A TRANSPLANT FROM ONE SPECIES TO ANOTHER (E.G., PIG TO HUMAN)

MAJOR DRAWBACK: HYPERACUTE REJECTION



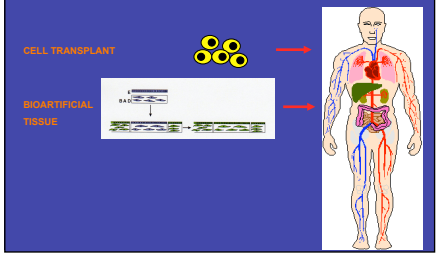
Production of
α1,3-Galactosyltransferase–
Deficient Pigs

Carol J. Phelps,[1] Chihiro Koike,[3,4] Todd D. Vaught,[1] Jeremy Boone,[1] Kevin D. Wells,[1] Shu-Hung Chen,[1] Suyapa Ball,[1] Susan M. Spacht,[3,4] Irina A. Polejaeva,[1] Jeff A. Monahan,[1] Pete M. Jobst,[1] Sugandha B. Sharma,[3,4] Ashley E. Lamborn,[1] Amy S. Garas,[1] Marilyn Moore,[2] Anthony J. Demetris,[3,5] William A. Rudert,[3,6] Rita Bottino,[3,6] Suzanne Bertera,[3,6] Massimo Trucco,[3,6] Thomas E. Starzl,[3,4] Yifan Dai,[1]* David L. Ayares[1]*

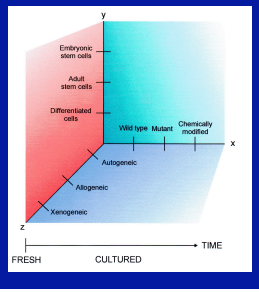


Ionnerge BioTherapeutics recently cloned double-knock-out miniature swine

RESTORING STRUCTURE AND FUNCTION THROUGH CELL TRANSPLANTS AND BIOARTIFICIAL TISSUES



MAJOR ISSUE: CELL SOURCES



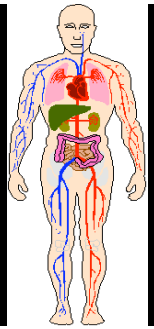
DESIRED CHARACTERISTICS OF CELLS TO BE USED FOR TRANSPLANTS

- UNLIMITED GROWTH POTENTIAL
- PLURIPOTENCY (UNIVERSAL DIFFERENTIATION)
- AUTOGENEIC (NO IMMUNOREJECTION)

FOCUS HAS BEEN PRIMARILY ON ADULT STEM CELLS

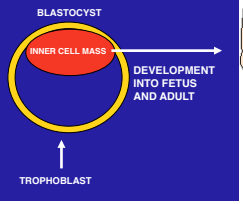
ADULT STEM CELLS

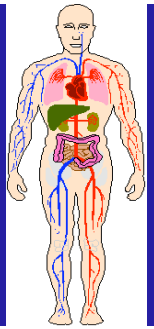
- Set aside in various tissues during development and maintained in an undifferentiated state
- Function as the body's maintenance staff: regenerate cells lost by normal wear and tear and to acute injury
- Many not easy to harvest
- Some lose potency when expanded
- Are not pluripotent

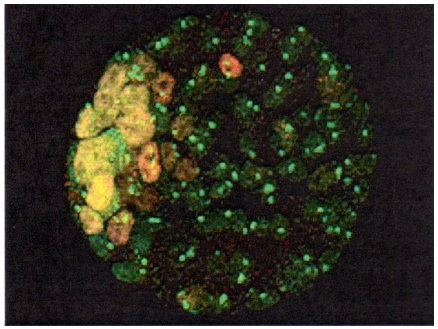


THE BLASTOCYST CONSISTS OF A TROPHOBLAST AND AN ICM.

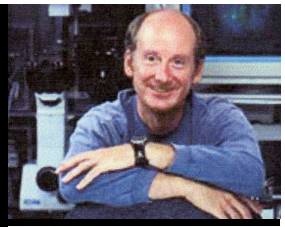
THE ICM CONTAINS 13-25 PLURIPOTENT CELLS THAT GIVE RISE TO THE ~220 CELL TYPES OF THE HUMAN BODY



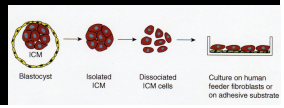


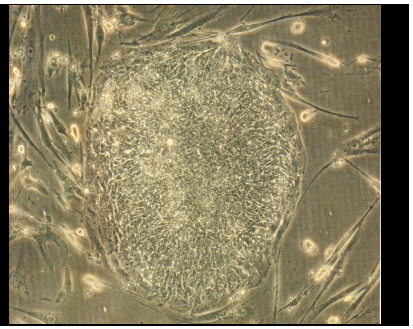


1998 JAMES THOMSON: HUMAN EMBRYONIC STEM CELLS

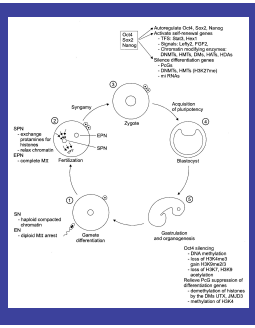


- UNLIMITED GROWTH
- SELF-RENEWING
- PLURIPOTENT





STOCUM AND ZUPANC (2009) DEV DYNAM 237:3648-3671

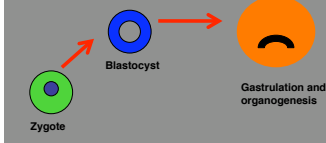


Pluripotency Genes are Active (Hypomethylated) Through Blastulation and are Then Silenced (Hypermethylated)

Hypomethylated
***Oct4, Sox2, Nanog* expression**
1. **Autoregulate own production**
2. **Activate self-renewal genes**

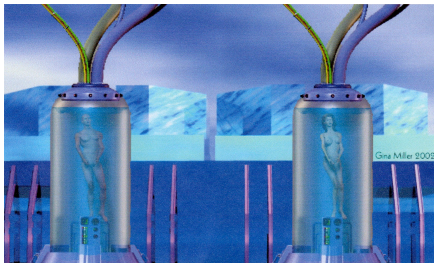
Hypermethylated
Differentiation genes silenced

Hypermethylated
Oct4, Sox 2, Nanog
Hypomethylated
Cell-specific fractions of differentiation genes

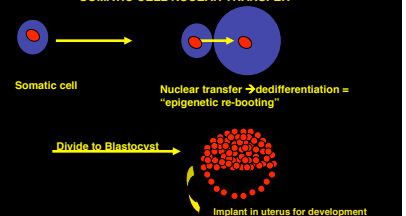


MAJOR DISADVANTAGE OF ESC DERIVATIVES: IMMUNOREJECTION

THEME 2: RESURRECTION

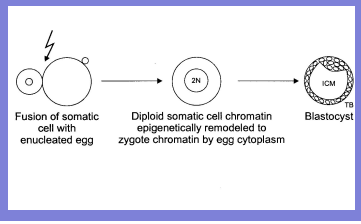


EMBRYOLOGICAL RESURRECTION: REPRODUCTIVE CLONING BY SOMATIC CELL NUCEAR TRANSFER



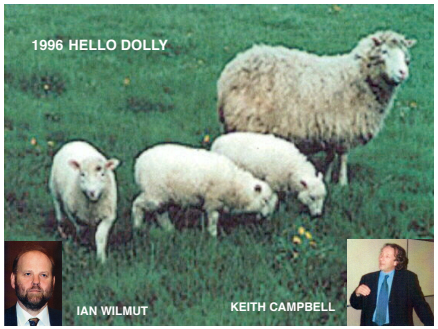
J. GURDON (1970): ALBINO NUCLEUS TO WILD-TYPE EGG

EGG CYTOPLASM CAN ERASE THE EPIGENETIC MARKS OF DIFFERENTIATED NUCLEI AND REIMPOSE THE EPIGENETIC MARKS OF THE ZYGOTE NUCLEUS

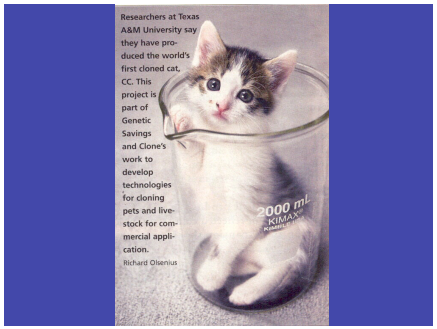


FEARS OF HUMAN CLONING

1980S: "DON'T WORRY—WE WILL NEVER BE ABLE TO CLONE MAMMALS"



RESURRECT YOUR PETS

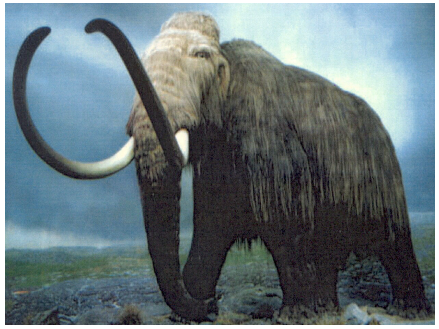


RODENT RESURRECTION

MOUSE FROZEN FOR 16 YRS: BRAIN CELLS USED AS NUCLEAR DONORS FOR SCNT

WAKAYAMA ET AL (2008) PNAS 105:17318-17322

MIGHT WE RESURRECT EXTINCT SPECIES?

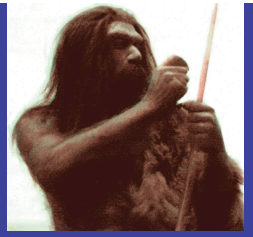


LET'S MAKE A MAMMOTH

Sequencing the nuclear genome of the extinct woolly mammoth

Webb Miller[1], Daniela I. Drautz[2], Aakrosh Ratan[1], Barbara Pusey[1], Ji Qi[1], Arthur M. Lesk[1], Lynn P. Tomsho[1], Michael D. Packard[1], Fangqing Zhao[1], Andrei Sher[2], Alexei Tikhonov[2], Brian Raney[3], Nick Patterson[3], Kerstin Lindblad-Toh[3], Eric S. Lander[3], James R. Knight[4], Gerard P. Irzyk[4], Karin M. Fredrikson[4], Timothy T. Harkins[4], Sharon Sheridan[4], Tom Pringle[4] & Stephan C. Schuster[4]

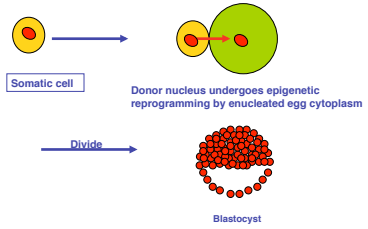
Nature: 456:387-390 (2008)



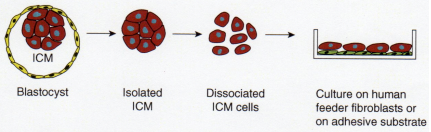
Neanderthal female mitochondrial genome sequenced (Green et al, 2008). First draft of nuclear genome completed on 38,000 yr. old bones (Paabo et al, unpublished)

TISSUE AND ORGAN RESURRECTION VIA SCNT (THERAPEUTIC CLONING): AUTOGENEIC CELLS FOR REGENERATIVE MEDICINE

STEP 1: MAKE A BLASTOCYST CLONE OF YOURSELF

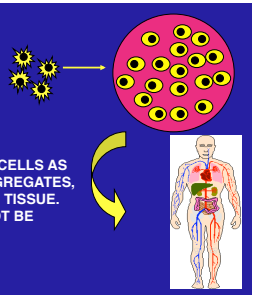


STEP 2: CREATE AN EMBRYONIC STEM CELL CULTURE



STEP 3: DIRECT THE DIFFERENTIATION OF ESCS TO THE DESIRED CELL TYPES

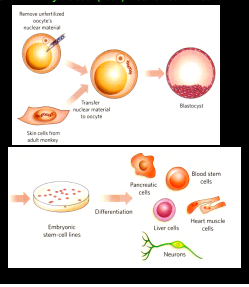
STEP 4: TRANSPLANT CELLS AS SUSPENSIONS OR AGGREGATES, OR IN A BIOARTIFICIAL TISSUE. THESE CELLS WILL NOT BE IMMUNOREJECTED



ESCs PRODUCED FROM SCNT DERIVED BLASTOCYSTS

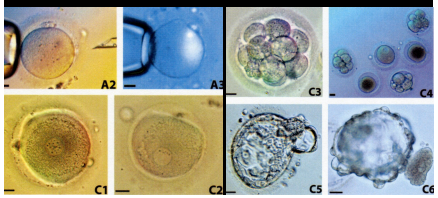
- MOUSE: *Munsie et al (2000) Curr Biol 10:989-992*
- MONKEY: *Byrne et al (2007) Nature 450:497-502*
- HUMAN BLASTOCYST HAS BEEN CLONED , BUT NO ESCs DERIVED: *French et al (2008) Stem Cells 26:485-493*

AUTOGENEIC MONKEY ESCS HAVE BEEN DERIVED BY SCNT Byrne et al (2007) Nature 450:497-502



AUTOGENEIC HUMAN BLASTOCYSTS HAVE BEEN CLONED BY SCNT

French et al (2008) Stem Cells 26:485-493



TECHNICAL HURDLES TO THE CREATION OF PATIENT-SPECIFIC ESCs BY SCNT

- LOGISTICS: *SHORTAGE OF HUMAN EGGS, INEFFICIENT*
- EXPENSIVE: *HUNDREDS OF THOUSANDS OF DOLLARS TO CREATE, TEST AND TRANSPLANT*

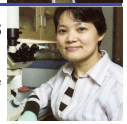
ESCs FROM ADULT SOMATIC CELLS

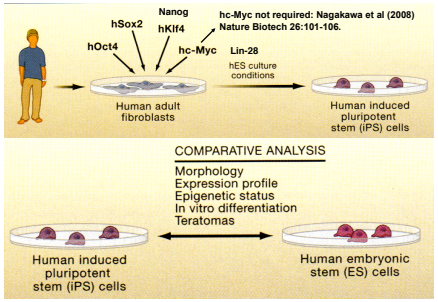
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[2] and Shinya Yamanaka[1,2,3,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
[3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA
*Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2007.11.019

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2,*] Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[3] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,3,*]

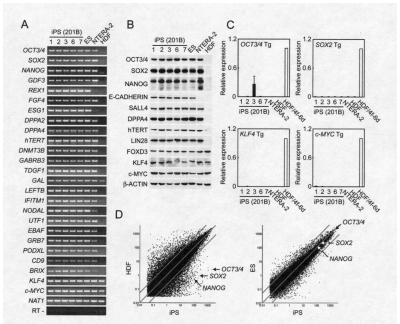


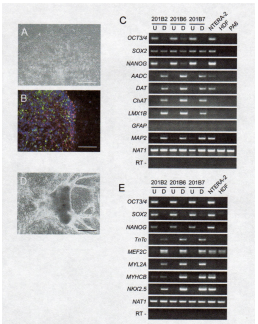


EVIDENCE THAT HUMAN iPS CELLS ARE SIMILAR TO HUMAN ESCS

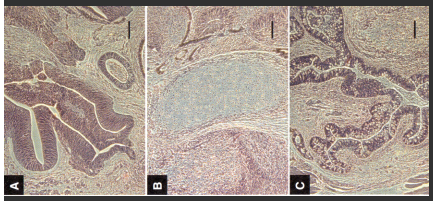
- GLOBAL GENE EXPRESSION PATTERNS ARE SIMILAR IN hiPS AND hES CELLS
- hES CELL-SPECIFIC PROMOTERS ARE ACTIVE IN hiPS CELLS AND THEY DISPLAY SIMILAR METHYLATION PATTERNS
- hiPS CELLS CAN BE DIRECTED TO DIFFERENTIATE INTO MULTIPLE CELL TYPES *IN VITRO*
- hiPS CELLS FORM TERATOMAS *IN VIVO*, WITH TISSUES REPRESENTING ALL THREE GERM LAYERS

CELLS	METHYLATION STATUS OF OCT 4, SOX2, REX1
HDF	HYPERMETHYLATED
hIPS	HYPOMETHYLATED





Directed Differentiation of hiPS Cells *in vivo*



DIRECTED DIFFERENTIATION OF hiPS CELLS *IN VITRO*

	GENES OFF	GENES ON
TO NEURONS	OCT3/4 NANOG SOX-2[a]	AADC DAT CHAT
TO CARDIO-MYOCYTES	OCT3/4 NANOG SOX-2	MYLC2A MYHCB NKX2.5

[a]Expression reduced but not eliminated

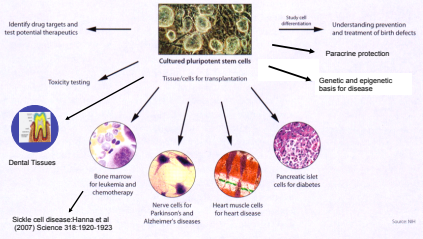
CURRENT iPSC RESEARCH: BASIC BIOLOGY

- UNDERSTANDING MECHANISMS OF INDUCTION
  - --ACTION OF TRANSCRIPTION FACTORS
  - --EPIGENETIC CHANGES
- SIMPLICITY AND EFFICIENCY OF INDUCTION
  - --RETROVIRAL VECTORS→ADENOVIRAL VECTORS→PLASMIDS→PROTEINS→ SYNTHETIC SMALL MOLECULES
  - --EFFICIENCY IMPROVED BY SMALL MOLECULE TREATMENT
- DIRECTED DIFFERENTIATION
  - --PROTOCOLS
  - --EFFICIENCY
  - --ROBUSTNESS
  - --LONGEVITY

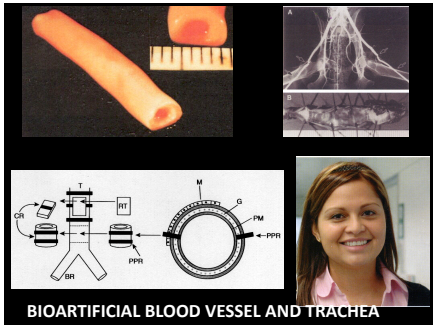
CURRENT iPSC RESEARCH: APPLIED BIOLOGY

- DRUG SCREENING
- TOXICITY TESTING
- DIRECTED DIFFERENTIATION OF iPSCs FOR CELL TRANSPLANT
  - --BLOOD CELLS TO ALLEVIATE SICKLE CELL DISEASE
  - --NEURONS TO ALLEVIATE PARKINSON'S DISEASE
- GENETICS AND EPIGENETICS OF DISEASE
  - --GENERATION OF PATIENT-SPECIFIC CELLS (NEURONS FROM iPSCs OF ALS AND SPINAL MUSCULAR ATROPHY PATIENTS)
- TIME OF PRODUCTION AND COST

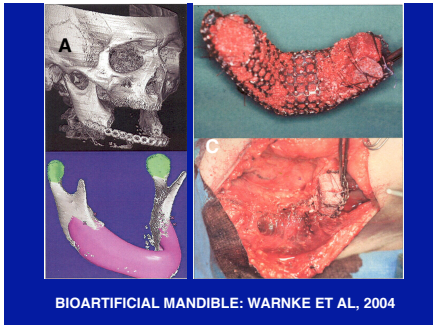
POTENTIAL USES OF hiPS CELLS



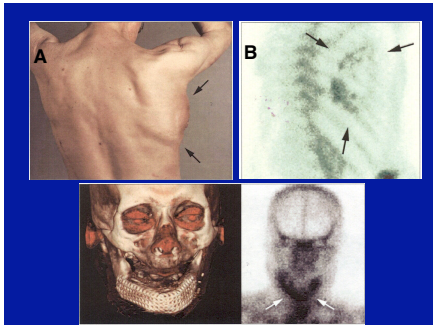
BIOARTIFICIAL TISSUES AND ORGANS



BIOARTIFICIAL BLOOD VESSEL AND TRACHEA

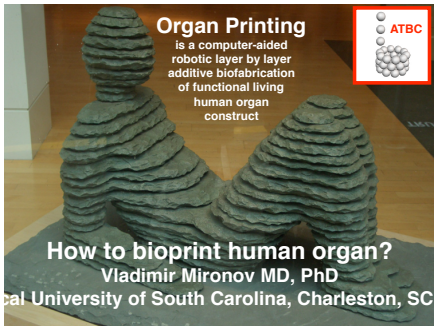


BIOARTIFICIAL MANDIBLE: WARNKE ET AL, 2004

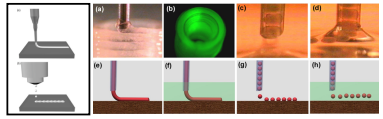


OTHER BIOARTIFICIAL TISSUES

- SKIN
- BONE
- EARS
- NOSES?
- ARTICULAR CARTILAGE?
- MUSCLE?
- NEURAL (RETINA, SPINAL CORD, BRAIN)?
- FACES?

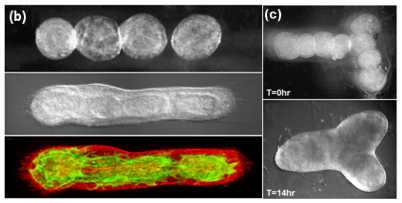


Microfluidics: Bioprinting



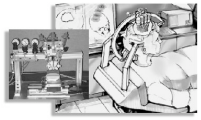
Continuous and digital (droplet) microfluidics-based bioprinting

Digital Bioprinting: Tissue Spheroids Fusion (Linear and Branched Segments of Vascular Tree)



Biomaterials, 2009

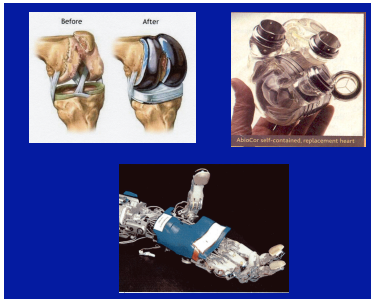
Bioprinting in situ (an emerging concept)



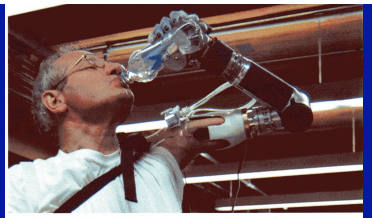
Lee Weiss and Phil Campbell of the Bone Tissue Engineering Center have developed a prototype printer, left, for fabrication of scaffolds with growth factors to produce bone. It is used only with lab animals. The illustration, right, envisions future application of the machine to a human patient's skull.

Clinical Bioprinter - Project "Michelangelo"?

BIONICS: JOINTS, LIMBS AND OTHER COMPLEX STRUCTURES



BIONIC DEVICES



The DEKA Arm

Affectionately dubbed "Luke" (after Luke Skywalker), the robotic arm is a DARPA funded project intended to restore functionality for individuals with upper extremity amputations. The project is still in development, please check back for updates.

NFN NMI Data

Lieutenant Commander
DOB: February 2, 2338
LOB: Omicron Theta (Kiron III)

THEME 3: MORAL CONCERNS REGARDING ESC RESEARCH

BIOETHICAL ARGUMENTS AGAINST HUMAN ESC RESEARCH

- THE FERTILIZED EGG IS A GENETICALLY DISTINCT POTENTIAL HUMAN LIFE. THEREFORE IT HAS THE MORAL RIGHT OF EXISTENCE, OR PERSONHOOD.
- UNLIKE AN ADULT, THE EMBRYO CANNOT CONSENT TO, OR REFUSE ANYTHING. THUS ITS MORAL RIGHT TO EXIST MUST BE PROTECTED BY SOCIETY
- DESTROYING THE EMBRYO IS ABORTION (MURDER)
- THIS RESEARCH OPENS THE WAY TO A TOTALLY UTILITARIAN AND DEHUMANIZING SOCIETY THAT DESTROYS THE DIGNITY OF HUMAN LIFE AND LEADS TO CRIMES AGAINST HUMANITY

BIOETHICAL CONCERN ABOUT SCNT HUMAN ESCs: *CREATING A POTENTIAL HUMAN LIFE AND THEN DESTROYING THAT LIFE FOR A SELFISH PURPOSE*

BIOETHICAL ARGUMENTS IN FAVOR OF RESEARCH ON HUMAN ESCS

- DESTRUCTION OF A NON-IMPLANTED EMBRYO IS NOT ABORTION
- MOST EXISTING BLASTOCYSTS WILL BE DESTROYED WITHOUT BENEFIT TO ANYONE BECAUSE IT IS IMPOSSIBLE TO FIND ENOUGH SURROGATE MOTHERS. THEIR DEVELOPMENTAL POTENTIAL FAILS FOR LACK OF ENABLEMENT
- PERSONHOOD IS DEPENDENT ON ATTAINING SENTIENCE
- THE MORAL CONFLICT BETWEEN RIGHTS OF SENTIENT SUFFERERS AND THEIR FAMILIES AND THE RIGHT OF THE BLASTOCYST TO EXIST SHOULD FAVOR THE FORMER

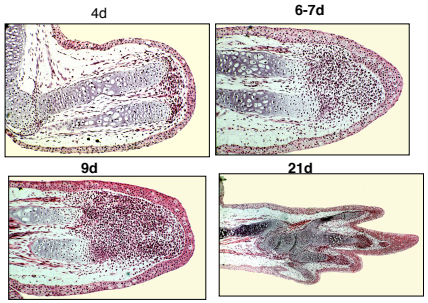
BIOETHICAL ISSUES WITH hiPS CELLS

- DERIVATION OF HUMAN GAMETES FOR USE IN *IN VITRO* FERTILIZATION PROCEDURES
- EASE OF DOING UNETHICAL EXPERIMENTS: HUMAN NEURAL CELL TRANSPLANTS TO NON-HUMAN PRIMATES

THE HOLY GRAIL: INDUCTION OF REGENERATION *IN SITU*

- MECHANISMS
  - --activation of resident adult stem cells
  - --dedifferentiation of mature cells to adult stem cells
  - --compensatory hyperplasia
  - --transdifferentiation
  - --inhibition of fibroblast proliferation (scarring)
- STIMULATORS/INHIBITORS
  - --molecular martinis
  - --regeneration templates (sis, pla, ceramics, etc)
  - --templates plus molecules

THE AXOLOTL: A URODELE SALAMANDER THAT HAS THE NATURAL ABILITY TO REPROGRAM SOMATIC CELLS FOR LIMB REGENERATION

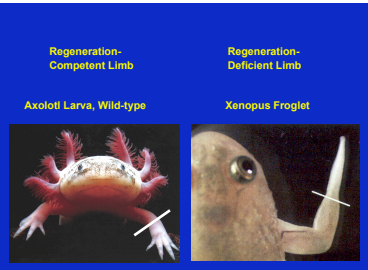


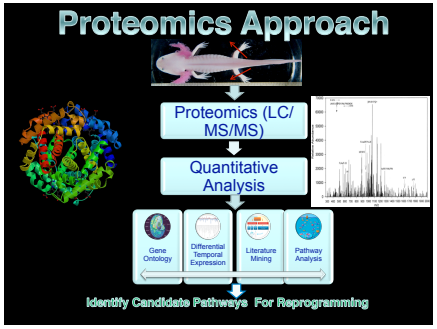
MECHANISMS OF BLASTEMA FORMATION

- HISTOLYSIS TO LIBERATE CELLS—ACID HYDROLASES, MMPS
- REPROGRAMMING OF LIBERATED CELLS (DEDIFFERENTIATION)—CHANGE IN EPIGENETIC MARKS TO AN ADULT STEM CELL STATE
- PROLIFERATION--GROWTH FACTORS FROM WOUND EPIDERMIS, NERVES
- PATTERNING: GROWTH FACTORS FROM WOUND EPIDERMIS, SHH, HOX GENES

AMPHIBIAN LIMB REGENERATION AS A SYSTEM TO IDENTIFY MOLECULES THAT CHARACTERIZE REGENERATION COMPETENCE AND DEFICIENCY

AXOLOTL VS. XENOPUS FROGLET





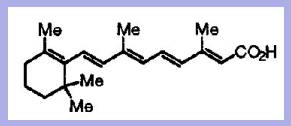
MAJOR FINDINGS

- 309 proteins identified with significant fold change, representing 10 biological process categories
- Blastema formation requires mechanisms to avoid apoptosis
  - --reduced metabolism
  - --differential regulation of pro and anti-apoptotic proteins
  - --initiation of an unfolded protein response
- Pre-mitotic cell cycle arrest is linked to maximum dedifferentiation through high levels of the centrosomal protein EVI5

CAN WE IDENTIFY SYNTHETIC SMALL MOLECULES AND SCAFFOLDS THAT INDUCE REGENERATION AT THE SITE OF INJURY?

- SCREEN SYNTHETIC SMALL MOLECULES (SSMs) ON CELLS USING LAB ON A CHIP (LOC) MICROFLUIDIC AND NANOFLUIDIC TECHNOLGY
- DEVELOP SIMILAR TECHNOLOGY TO SCREEN NATURAL AND BIOMIMETIC ECM OR ECM:SSM COMBINATIONS

A SMALL MOLECULE THAT REPROGRAMS BLASTEMA CELLS



ALL-TRANS RETINOIC ACID: PROMOTES DEDIFFERENTIATION OF MESODERMAL DERIVATIVES AND CHANGE IN POSITIONAL IDENTITY OF DEDIFFERENTIATED CELLS IN REGENERAING SALAMANDER LIMBS