Research Strategy for Human Organ fabrication-Pig as In vivo Bioreactor-

Eiji Kobayashi, MD, PhD
Department of Organ Fabrication,
Keio University School of Medicine, Japan
Declaration of Istanbul

(Istanbul, 30th April – 3rd May 2008)

152 professionals from 78 countries

(Lancet 2008)
NHK Special 2009

Human Body “Production” —The impact of Regenerative Medicine—

Eiji Kobayashi, MD
Jichi Medical University
Green Fluorescence Protein

Osamu Shimonura
Novel Prize in Chemistry 2008
Benefits in bio-imaging system using ‘fluorescence’

Aequorea victoria

Granulocyte 98,2%
Lymphocyte 77,0%


Lung; 25 min after LPS injection

Transplanted lung immediately after re-perfusion
(Enomoto A, et al. Microsurgery 2007)
Differentiation from the fetal tissue

Neurosphere

Cerebral infarction

Migration

Promising future for the transgenic rat in transplantation research.
Doorschodt BM, Teubner A, Kobayashi E, Tolba RH.

The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves

The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves
International Collaboration using Photonics Rats

Germany
EK Geissler (Regensburg)
G Nikkhah (Freiburg)
R Tolba (Bonn)

Singapore
DW Hutmacher
S Cool (Bioporis)

Canada
A Keating (Toronto Univ)

U.S.A
John Critser (Missouri Univ)
SS Thorgeirsson (NCI)
P Leone (RWJ Med Sch)

Australia
GA Bishop (Sydney)
Clinical Microsurgery

1986  A-V shunt operation

2000  Hepatic artery reconstruction in liver transplantation

Experimental Microsurgery

1992  1st. ISEM  Rome, Italy

1994  2nd. ISEM  Kanazawa, Japan

1996  3rd. ISEM  Wuerzburg, Germany

1998  4th. ISEM  London/Ontario, Canada

2000  5th. ISEM  Catania, Italy

2002  6th. ISEM  San Diego, USA

2004  7th. ISEM  Debrecen, Hungary

2006  8th. ISEM  Montreal, Canada

2008  9th. ISEM  Shanghai, People's Republic of China

2010  10th. ISEM  Sao Paulo, Brazil

2012  11th. ISEM  Timisoara, Romania

2014  12th. ISEM  Kyoto, Japan

2016  13th. ISEM  Tianjin, China

2018  14th. ISEM  Debrecen, Hungary
Defining Standards in Experimental Microsurgical Training: Recommendations of the European Society for Surgical Research (ESSR) and the International Society for Experimental Microsurgery (ISEM)

Materials and Methods

Photinus pyralis  Firefly Tg Rat

Luminescence

Luciferin + Luciferase

蛻ラット
Light Emission of Luciferase Transgenic Rat

Chase the transplanted hepatocytes

Hepatectomized

Normal

(Hakamata Y, et al Transplantation 2006)
Bone Marrow-Derived Mesenchymal Stem Cells Ameliorate Hepatic Ischemia Reperfusion Injury in a Rat Model

In Vivo Bioimaging Analysis of Stromal Vascular Fraction-Assisted Fat Grafting: The Interaction and Mutualism and Cells and Grafted Fat

(Zhou SB, et al. Transplantation 2014)
In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels

Hypothermic temperature effects on organ survival and restoration

Transplantation of engineered chimeric liver with autologous hepatocytes and xenobiotic scaffold

Prohibited Medicine

MAKING PIG ORGANS SAFE
Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft

Reprogramming

John Bertrand Gurdon
Novel Prize in Physiology or Medicine 2012

Distraction of Nuclei by UV irradiation

unfertilized egg
Injection
Intestinal Cell

Mulberry Real Embryo

Tadpoles

Somatic Cell nuclear transplantation

( Gurdon JB, J Embryo Exp Morphol 1962)

Cloned Frog
Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer

Genetically technology for pigs
Production of `Colored` Pigs

(Kawarasaki T, et al. 2009)

Islets Hepatocytes

Bone Marrow Cells

MSC

GFP Pig

Kusabira-Orange Pig

adipogenesis

(Matsunari H, et al. 2009)
In the heat of argument, Pig as In vivo bioreactor for human organs


A theory of blastcyst complementation generating human organ in vivo in lacking organ cloned pig
<table>
<thead>
<tr>
<th><strong>Experimental Medicine</strong></th>
<th><strong>Transplantation Immunology</strong></th>
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<tbody>
<tr>
<td><strong>Dr. Claude Bernard</strong></td>
<td><strong>Sir Peter Medawer</strong></td>
</tr>
<tr>
<td><strong>Birth (Country)</strong></td>
<td><strong>1915 (Brazil)</strong></td>
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<tr>
<td>1813 (France)</td>
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<tr>
<td><strong>University</strong></td>
<td><strong>Oxford University</strong></td>
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<td>Universite de Paris</td>
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<tr>
<td><strong>Professor(Age)</strong></td>
<td><strong>Birmingham (32)</strong></td>
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<tr>
<td>Sorbonne (41)</td>
<td>Novel Award (45)</td>
</tr>
<tr>
<td><strong>Death(Age)</strong></td>
<td><strong>1987 (72)</strong></td>
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<td>1878 (65)</td>
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</table>
IMMUNOGENETIC CONSEQUENCES OF VASCULAR ANASTOMOSES BETWEEN BOVINE TWINS

Almost thirty years have passed since Lillie\textsuperscript{3} used the demonstrated union of the circulatory systems of twin bovine embryos of opposite sex to explain, on an endocrine basis, the frequent reproductive abnormalities of the female twin. Since the appearance of Lillie's paper, the freemartin, as the modified female is called, has become an important example of the effects of hormones on sex-differentiation and sexual development in mammals.\textsuperscript{3} Consequences other than endocrinological of nature's experiment in parabiosis have, however, received little attention.

Estimates of the frequency of identical as compared with fraternal twinning indicate that the former is relatively rare in cattle.\textsuperscript{4} Tests for inherited cellular antigens in the bloods of more than eighty pairs of bovine twins show, however, that in the majority of these pairs the twins have identical blood types. Identity of blood types between full sibs not twins is infrequent, as might be expected from the large number of different, genetically controlled antigens\textsuperscript{5} (now approximately 40) identified in the tests. If, therefore, the frequent identity of blood types in twin pairs can be explained neither as the result of monozygotic twinning nor as chance identity between fraternal twins, nor as the sum of these two factors, it is evident that some mechanism is operating to produce frequent phenotypic identity of blood types in genetically dissimilar twins. The vascular anastomosis between bovine twins, known to be a common occurrence,\textsuperscript{6} provides an explanation.
Immunological Effects of Experimental Embryonal Parabiosis

According to Burnet and Fenner\(^1\) and also Lopashov and Stroyeva\(^2\), the inability to react against autologous antigens by the formation of antibodies develops during fetal life (when the embryo is not yet able to produce antibodies), by the action of the antigens of the embryo's own tissues on the reticulo-endothelial system. According to Burnet and Fenner, a similar inability to form antibodies can be provoked by even a foreign antigen entering the reticulo-endothelial system during this stage. The experiments of Burnet, Stone and Edney\(^3\), in which living influenza virus \(A\), bacterial virus \(C\ 16\) and human erythrocytes were introduced into chick embryos, did not confirm their hypothesis. In agreement with the theory are, however, the findings of Owen\(^4\) in respect of bovine twins. In the case of twins this phenomenon is due to placental anastomosis, that is, to natural embryonal parabiosis.

<table>
<thead>
<tr>
<th>Table 1. Titres of Immune Agglutinins against Chick Erythrocytes in Ducks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parabionts</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>2*</td>
</tr>
<tr>
<td>4*</td>
</tr>
<tr>
<td>8*</td>
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<tr>
<td>4, 4</td>
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<tr>
<td>4</td>
</tr>
<tr>
<td>64†</td>
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</tbody>
</table>

* Animal immunized by erythrocytes of embryonal parabiotic partner.  
† Parabiont in which the exchange of blood was excluded by agglutination test.  
‡ Exchange of blood not unequivocally demonstrated.

All parabionts or their partners were tested by agglutination immediately after hatching for the exchange of erythrocytes.

For earlier material, in which embryonic erythrocyte exchange was not tested, see Frenzi et al. (ref. 8).

<table>
<thead>
<tr>
<th>Table 2. Titres of Natural (in Parentheses) and Immune Agglutinins against Duck Erythrocytes in Chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parabionts</td>
</tr>
<tr>
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</tr>
<tr>
<td>64 ((16)), 256 ((32))</td>
</tr>
<tr>
<td>128 ((2))</td>
</tr>
<tr>
<td>256 ((8))*, 256 ((1))</td>
</tr>
<tr>
<td>512 ((4)†)</td>
</tr>
<tr>
<td>128 ((2))</td>
</tr>
</tbody>
</table>

* Represents an animal immunized by the erythrocytes of its embryonal partner.  
† The skin of the duck partner was transplanted on the fifth day after hatching, the transplant surviving sixteen days.

All parabionts have been tested by agglutination immediately after hatching with respect to the exchange of erythrocytes.

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\(^3\) Burnet, F. M., Stone, R. H., and Edney, J. M., Nature 175, 1515 (1955).  
'ACTIVELY ACQUIRED TOLERANCE' OF FOREIGN CELLS

By Dr. R. E. BILLINGHAM*, L. BRENT and Prof. P. B. MEDAWAR, F.R.S.

Department of Zoology, University College, University of London

Experiments with Mice

A single experiment will be described in moderate detail: the recipients were mice of CBA strain, the donors of A strain. The data for transplantations between normal mice of these strains are as follows. The median survival time of A-line skin grafts transplanted to normal CBA adults (regardless of differences of sex, or of age within the interval 6 weeks–6 months) is 11.0 ± 0.3 days'. In reacting against such a graft, the host enters a state of heightened resistance; a second graft transplanted up to sixty days after the transplantation of the first survives for less than six days, and immunity is still strong, though it has weakened perceptibly, after four months. Heightened resistance may be passively transferred to a normal CBA adult by the intraperitoneal implantation of pieces of lymph node excised from a CBA adult which has been actively immunized against A-line skin*.

In the experiment to be described (Exp. 78), a CBA female in the 15–16th day of pregnancy by a CBA male was anaesthetized with 'Nembutal', and its body wall exposed by a median ventral incision of the skin. The skin was mobilized but not reflected, and particular care was taken not to damage the mammary vessels. By manipulation of the abdomen with dampened gauzes, six fetuses were brought into view through the body wall. Each was injected intra-embryonically with 0.01 ml. of a suspension of adult tissue cells through a very fine hypodermic needle passing successively through the body wall, uterine wall, and fetal membranes. (The inoculum itself, consisting of a suspension in Ringer's solution of small organized tissue clumps, isolated cells, and cell debris, had been prepared by the prolonged chopping with scissors of testes, kidney and splenic tissue from an adult male A-line mouse.) After injection of the fetuses, the skin was closed with interrupted sutures.

Preliminary Experiments with Chickens

Donors and recipients in these experiments were of Rhode Island Red and White Leghorn breeds, respectively. Skin transplanted from two weeks old Rhode Island Red chicks to White Leghorn recipients of the same age, using Cannon and Longmire's method', is completely destroyed within ten days of grafting, to the accompaniment of an inflammatory reaction of conspicuous violence.

The embryonic chick is particularly well suited to experiments which make use of cellular inoculation, because the intravenous route is so easily accessible. Using methods demonstrated to us by Dr. C. Kaplan, whose help has been of the greatest value, we have obtained successful results by transfusing 0.2 ml. unmodified whole blood from an 11–12 day old embryonic Rhode Island Red donor into a choioallantoic vein of a White Leghorn embryo of the same age. Fourteen days after hatching, a test-graft of skin was transplanted to the recipient from its original donor. In seven such trials, five grafts showed prolongation of survival; of these, three succumbed within fifty days to the accompaniment of very much subdued inflammatory changes, and two still survive, with normal growth of red feathers, to the present time (125 days).
Bone Marrow and Lymphoid Cell Injection of the Pig Foetus resulting in Transplantation Tolerance or Immunity, and Immunoglobulin Production

It is known that the pig is capable at birth of immune responses to some antigens (phage\(^1,2\); animal viruses\(^3-5\); toxoids\(^6\); and homografts\(^7,8\)). The finding that thymectomy in neonatal pigs is without effect on homograft rejection\(^8\) also suggests that the pig, unlike many laboratory rodents\(^9,10\) acquires the faculty of graft rejection before birth. Information on immune responses of pig foetuses to injected antigens has not been found in the literature. This communication outlines such research.

Large white pig foetuses at 60, 80 or 104 days of gestation were injected intraperitoneally through the uterine wall at laparotomy of the dam with an allogeneic white cell suspension taken either from blood and biopsied lymph nodes \((6 \times 10^8 \text{ nucleated cells/kg of body weight})\) or from tibial bone marrow \((13.5 \times 10^8 \text{ nucleated cells/kg of body weight})\). Pig lymph contains very few lymphocytes\(^11\), and so this rich source of lymphocytes in other species could be used for postnatal immunisation of a carbon-Rh* mother as well as for antigenic challenge of the litter with killed 'Ampicillin' and thymusless piglets from the litter to the hyperimmune state.

Fig. 1. Photomicrographs of biopsies from homografts on piglets treated at 60 days gestation with: A, control conventional antigens; B and C, bone marrow cell suspension from the skin graft donor \((7-5)\) (arrow denotes junction of graft on left and normal skin on right). A, Conventional antigen treated piglet 8 days after grafting. Primary homograft rejection. B, Bone marrow cell treated male piglet 60 days after grafting. Tolerant. C, Bone marrow cell treated female piglet 80 days after grafting. Tolerant.
Human Stem/Progenitor Cells

Drug inducing Apoptosis of Target Pig Organ

(E Kobayashi)
Thank you for ‘your attention’
for ‘Experimental Animals’