Rehabilitative medicine – Progress and promise of regenerative medicine: from molecule to society

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"The 3rd annual conference - Ortho posturo gnosis – the knowledge to influence and control the disease" took place in Iasi during the 4Th of December, 2010, addressed to all of the doctors which contributed to this scope for therapy (neurologists cardiologists, rehabilitative in medicine, reumathologists, neurosurgeons, orthopedists etc).
The theme of this year was interesting and promising field for the future of the medicine: Rehabilitative medicine – progress and promise of regenerative medicine: from molecule to society.
We choose from the conference same specials lectures which were selected by the Scientific Committee.

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Progress and promise of Regenerative Medicine – Molecule to Society

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Introduction

On this auspicious occasion of celebration I take this opportunity to make a brief presentation on the theme of this conference - the knowledge to influence and control diseases – as it applies to my field of biotechnology. An intriguing question that we can ask is how can we use regenerative medicine to increase lifetimes while allowing us to age gracefully? In my presentation I will provide some interventions and try to cover the following topics: 1) the history of tissue and organ regeneration; 2) approaches to regenerative medicine; 3) current clinical trials underway; 4) current research projects in my laboratory and 5) some economic factors influencing our industry.

What exactly is regenerative medicine? The origins of regenerative medicine are based in Greek mythology and I am sure evidence can be found elsewhere in other civilizations. As the story goes, Zeus, who was the supreme ruler of the 12 gods on Mount Olympus, was upset that Prometheus gave the technology of making "fire" to the Greeks. To punish Prometheus, Zeus ordered him tied to a

rock and sent an eagle to eat his liver each day. Well Prometheus was able to survive the ordeal because his liver was able to regenerate itself daily. Fortunately today we still have that regenerative ability to some extent.

To begin it is necessary to define regenerative medicine as it has different meanings to different people. This is because the field has become populated with engineers, physicists, biopolymer/biochemists, molecular biologists, mathematicians, nanotechnologists and computer specialists bringing diverse tools such as finite element analysis and computer aided designs to create scaffolds and a host of bio-materials for use in regenerative medicine. So today we can define regenerative medicine as "understanding the body's healing process and speeding-up that process to a clinically acceptable time scale or outcome." This involves using stem cell therapy, regenerative factors, specific acellular scaffolds, therapeutic cloning and finally integration in-vivo of the organ or tissue to affect a regenerative outcome in a patient. Despite having some 100 trillion cells, the human body is very poor at repair as we seem to be in acute or chronic decline through disease, trauma or aging. The "Holy Grail" of regenerative medicine would be the duplication in humans the regenerative power of amphibians like the salamander which can regenerate an amputated limb in 70-days1.

The history of tissue and organ regeneration

As we survey the historical medical landscape we see interesting "tipping-points" occurring every 100 years or so that have transformed the way doctors treat patients and the resulting improvement in successful outcomes. The giants like Pasteur, Lister and Koch led a revolution in modern medicine. However, it took another 100 years before immunosuppressive drugs became available to doctors and then we saw a flurry of organ transplants between 1954 and 1967 – liver, lung, pancreas and heart. In the 90's we were fascinated with Wilmot's somatic cell nuclear transfer cloning of "Dolly." Bio-techniques have been developed to quantitatively decellularize complex organs such as heart, liver, and kidney. These acellular matrices can provide attractive scaffolds for repopulation with the recipient's own cells for tissue engineering as the extracellular matrix template contains appropriate three-dimensional architecture and regional-specific cues for cellular adhesion. In 2006 this work was pioneered by Atala2 whose tissue engineered bladder was successfully transplanted using the patient's own cells without antigenicity.

In 2008 Macchiarini3 and his multinational team at the University of Barcelona was the first to have successfully transplanted a trachea into a patient, with end-stage airway disease, without antigenicity. The patient presented with dysphonia and cough due to tuberculosis infiltration of the cervical trachea and entire left main bronchus. A CT scan showed a circumferential, near-occlusive 3 cm airway stenosis starting 2 cm subglottically, and a hypoplastic left main bronchus with expiratory collapse. Macchiarini removed cells and the major histocompatibility complex (MHC) antigens from a human donor trachea (cadaver), which was then readily colonized by epithelial cells and mesenchymal stem-cell-derived chondrocytes that had been cultured from

cells taken from the recipient bone marrow. This graft was then used to replace the recipient's left main bronchus. The patient is healthy after 4 months and had no anti-donor antibodies and is not on immunosuppressive drugs.

Approaches to regenerative medicine

As we step through the frontiers of the 21st century, we are all witnesses to the Cinderella story in the form of the induced pluripotent stem cells (iPSC's). The process developed by Yamanaka4 of re-programming adult somatic cells to derive (iPSC's) with the wand of transcription factors (Oct4, Sox2, Klf4, and c-Myc) and then differentiating them back to adult somatic cells. This is the most fascinating breakthrough in regenerative medicine since the structure of DNA was elucidated. Rossi5 further improved efficiencies by developing a non-integrating strategy for reprogramming cell fate based on administration of synthetic mRNA modified to overcome innate antiviral responses – no teratomas.

3.1 Intervention - Bioengineered tooth

Unlike other vertebrates, mammals can replace only their deciduous teeth. During mammalian evolution this regenerative capacity was lost6. The deterioration and loss of teeth that comes with old age affects more than smiles. Periodontal disease has been associated with increased risk for heart disease and might quicken the pace of aging. Poor nutrition after tooth loss could also cause problems. Etsuko7 led a tooth regeneration team that devised an ambitious plan that sought to use stem cell biology, engineering, and computational biology to replicate the developmental program for odontogenesis. They proposed a laboratory-grown tooth rudiment that would be capable of executing the complete program for odontogenesis when transplanted into a mouse host, recreating all of the dental tissues, periodontal ligament, cementum, and alveolar bone associated with the canonical tooth.

To this end epithelial and mesenchymal stem cells were placed within a collagen gel and cells were expanded (ex-vivo) for 7-days to obtain ~200,000 cells which were then transplanted with the correct orientation into a properly-sized bony hole in the upper first molar region of the alveolar bone. The results showed that the cusp tip of the bioengineered tooth was exposed into the oral cavity with full occlusion at 36.7± 5.5 days after transplantation. A more elegant solution would be to design a gene to allow a third set of teeth erupting at the age of 55. This would be an ingenious fix if we ignore the pain some of us experienced with the eruption of our molars.

3.2 Regenerating the articular surface of a synovial joint

Osteoarthritis is a debilitating disease that manifests as structural breakdown of cartilage and bone that affects over 80 million individuals in the USA alone8. To understand the nature of the problem the synovial joint consists of multiple tissues including articular cartilage, subchondral bone, hematopoietic marrow, and synovium making repair somewhat problematic. Current therapy requires arthritic joints to be replaced by total joint arthroplasty using metallic and synthetic materials which fail mainly because of aseptic loosening or infection induced by wear debris9. This is an interesting problem that can only be solved by biological regeneration. Although stem cell transplantation has been tried, the results

are mixed for a variety of reasons, with some patients finally having joint arthroplasty.

To solve this problem, Jeremy Mao10 and his team regenerated the articular surface of the synovial joint of a rabbit with a biological cue spatially embedded in an anatomically correct bioscaffold. The surface morphology of a rabbit proximal humeral joint (cadaver) was captured with laser scanning and reconstructed by computer-aided design. The team fabricated an anatomically correct bioscaffold using a composite of poly-e-caprolactone and hydroxyapatite. The entire articular surface of humeral condyles of skeletally mature rabbits was surgically excised and replaced with the bioscaffolds spatially infused with transforming growth factor ß3 (TGFß3)-adsorbed or TGFß3-free collagen hydrogel. Locomotion and weightbearing were observed in the test rabbits 3–4 weeks after surgery. At 4 months the entire articular surface of the synovial joint was regenerated without cell transplantation. Regeneration of complex tissues is probably by homing of endogenous cells, as exemplified by stratified avascular cartilage and vascularised bone.

Clinical Trials

The first approved U.S. clinical trial to use human embryonic stem cells to treat a disease has enrolled its first patient. Geron Corp., which is sponsoring the trial using stem cells to treat spinal cord injury, announced that the first patient was treated on October 8th 2010 at a hospital in Atlanta. The primary objective of this Phase I study is to assess the safety and tolerability of GRNOPC1 in patients with complete American Spinal Injury Association (ASIA) Impairment Scale grade A thoracic spinal cord injuries. Participants in the study must be newly injured and receive GRNOPC1 within 14 days of the injury. Geron will be testing Oligodendrocyte progenitor cells, precursors to some nervous system cells the company developed from one of the original human embryonic stem cell lines developed by James Thomson's lab at the University of Wisconsin, Madison. The goal is not to create new nerve fibers but to support those still intact by making the nerve insulator myelin. To prevent rejection, patients will take immune-suppressing drugs for about 60 days. Although the primary goal is to assess safety, Geron will be looking for hints that the cells had an effect—for example, improving bladder and bowel function, sensation, or mobility.

Advanced Cell Technology will conduct the second FDA approved clinical trial using embryonic stem cell–derived retinal pigment epithelial cells to treat Stargardt's Macular Dystrophy, a congenital eye disease. The company is also filing an application to try the treatment in age-related macular degeneration, a disease with similar characteristics that affects as many as 30 million people in the United States and Europe. Cell therapy seems promising for the disease for several reasons. First, the eye is an immune-privileged site, so researchers hope that patients won't have to take antirejection drugs after receiving the transplant. Second, because the retina can be observed at the single cell level it should be possible to follow the transplanted cells' behavior very precisely. The patients will receive up to 200,000 retinal pigment epithelium (RPE) cells that the company derived from hES cells, transplanted directly into the eye.

Our Laboratory

Our laboratory is specialized in the isolation, expansion and cryostorage of stem cells obtained from umbilical cord blood, Wharton's Jelly, deciduous teeth and adipose tissue. We are currently in the process of making therapeutic doses for sale. Our laboratory (450 m2) has clean rooms and is fully certified having ISO 14644-1/1999, ISO 14644-2/2000, ISO 14644-3/2005, ISO 9001:2008, FDA/GMP Annex1/2003 with FACT-NETCORD pending.

Although this is a hybrid commercial laboratory, we have embarked on a modest research project to regenerate the retina in patients suffering from trauma, disease and age-related loss of vision using autologous mesenchymal stem cells. This is a joint project with Prof. Fotiadiou at the University of Basel, Switzerland and local Greek ophthalmologists. Hopefully at our next meeting I would be able to report some results.

Economic Considerations

In the US it is generally believed that there are approximately 1.400 biotech laboratories with approximately 300 traded on Wall Street. These companies have produced over 200 approved therapies using stem cells which have been approved in the US, Europe and Australia. The geographical split of the biotech market in 2004 shows the US accounting for 59.6% of the global market, or $26.4bn, around 2.8 times the total combined sales in the top five European countries – France ($2.4 billion), Germany ($2.8 billion), Italy ($1.6 billion), Spain ($1.3 billion) and the UK ($1.2 billion). The rest of the world estimated at $5.5 billion. This is a significant difference considering that a similar number of products were available in both regions at any given time. The total worth of the Global biotech market in 2004 was $44.3 billion11.

I have performed a more comprehensive analysis of the global biotech market for 2009 and the results show an impressive gain of 122% in the 5-year period amounting to $98.7 billion – see Table 1.

TABLE 1
Global biotech market

Global Biotech Market	2009
Technology	2009 (US Mill $)
Stem cell transplant	9.250
Cord blood collection and Storage	1.800
Tissue engineering	25.500
Blood transfusion products	36.000
Cell-based gene therapy	9.000
Encapsulated cell therapy	2.700
Cell-based cancer vaccines	2.200
Xenotransplantation	2.700
Supporting technologies: Cell lines, cell culture, delivery devices	9.563
Total	98.713

In summary, I believe that in the future is bright for biotechnology companies allowing us to gain the knowledge to influence and control diseases. In 2011 we will see the cost for a personal genome analysis below $1.000 and results taking less than one week. This will open a new field that will allow nanotechnology to design robots to repair our DNA before the onset of debilitating diseases. Furthermore in the very near future, it will be possible for every person to have their own iPS cell lines,

prepared when they were still healthy, for future applications in clinical examination and/or therapy.

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