REGENERATIVE MEDICINE ROADMAP 2.0

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Real-time cellular interrogation technology

- Fluorescent microscopy for detection of amiloidoogenic protein states
- Alternating current scanning electrochemical microscopy (AC-SECM) for real-time observation of living cells
- Tissue microarrays: real-time monitoring of expression of tumor supressor genes (p16, p53, RB)
- Microfluidic platforms for real-time imaging
  - Single-cell analysis of intracellular compounds and changes in intracellular compounds
  - Monitoring of host-pathogen interactions and cellular signaling

Monitoring of SC implants in the recipient organ

- Real-time tracking of migration, proliferation and fate of stem cells implanted in the myocardium
  - Magnetic resonance imaging scan
  - Optic microscopy
  - Through the expression of a labelled reporter gene (Na/I symporter, D-luciferin, etc.)
  - Positron emission tomography and/or single photon emission tomography
  - Non-radioactive labeling for detection

Diagnostic devices for targeting biomarkers indicative of cellular disorders

- Targeting of cancer-specific cellswith Tetrahymena group I intron ribozyme
  - Replacement of the cancer-specific transcript with a new (non-cancer) transcript
  - Selective delivery of a cytotoxin gene into a cancer cell
- Nanoparticles for detection of a target and drug delivery
  - Superparamagnetic iron oxide nanoparticles for detection of cancer cells, viruses, pathogenic bacteria, etc.
  - Gold and silver nanoparticles in detection of a specific DNA sequence, bacterium, virus, antibody, etc.

Micro-electromechanical system (Bio-MEMS) devices for studying cultivated microtubules in bioartificial muscle engineering

Development of biomarkers of aging, viability, health and pathologies

ORGANIZATIONAL ISSUES

- "Scale up"
- Technology commercialization
- Legal issues
- Reimburcements

SCIENTIFIC AND TECHNOLOGICAL ASPECTS

- Diagnostic platforms
- Data bases
- CELL-BASED TECHNIQUES
- Creation of technological platform
- Restoration of regenerative potential
- Therapeutic cloning
- Biobanking
- Mathematical modeling
- Cell therapy
- Tissue engineering

Preservation of regional SC and tissues

Stem cell preservation
- SC from umbilical blood
- SC from the placental complex
- Dental pulp SC
- Bone marrow SC
- Hair follicle SC
- Endometrium mesenchymal cells
- Other types of SC (more invasive collection procedures)

Tissue preservation
- Cornea
- Heart valves
- Skin, etc.

Methods of collection, processing, and testing

Cryopreservation and thawing

THERAPEUTIC CLONING

Reproductive technologies and animal cloning
- Oocyte nuclear and cytoplasm transfer
- Development of cell resources for therapeutic cloning
  - Oocyte cryopreservation (oocyte bank, immature oocytes)
  - Human amniotic fluid SC

Study of iPSC as a unique subtype of pluripotent cell
- Morphological criteria of iPSC isolation
- Gene expression signature and epigenetic remodeling of iPSC
  - Optimal combination of genetic factors for generation of therapeutically safe iPSC
  - Increasing reprogramming efficiency (e.g. using keratinocytes for iPSC generation, vitamin C)

Reprogramming methods
- Using hESC (somatic cell nuclear transfer, fusion with hESC)
- Without using hESC
  - Necessary and sufficient factors to generate iPSC
    - Using small molecules
    - Using genes or gene products
      - Reprogramming factors-delivery systems:
        - Non-integrating virus vectors,
        - Polycistronic nonviral vectors,
        - Adding recombinant reprogramming proteins, etc.

Therapeutic applications of iPSC
- Generation of patient-specific (disease-corrected) iPSC
- iPSC-based vaccination
- Infertility treatment
- Treatment of degenerative diseases:
  - Parkinson disease,
  - ALS etc.
- Treatment of genetic disorders:
  - Sickle cell anaemia,
  - Fanconi anaemia,
  - Hemophilia type A,
  - Duchenne muscular dystrophy
- Obtaining a range of different cell types from iPSC:
  - Motoneurons
  - Cardiomyocytes
  - Smooth muscle cells
  - Endothelial cells
  - Hematopoietic cells,
  - Primordial germ cells
- iPSC-based cellular and animal models of diseases:
  - Sickle cell anaemia
  - Familial dysautonomia
  - Spinal muscular atrophy
  - Down's syndrome
  - Gaucher disease
  - Muscular dystrophies
  - Parkinson's disease
  - Huntington's disease
  - Diabetes mellitus type I etc.

TISSUE ENGINEERING METHODS DEVELOPMENT

Bionics Tissue Engineering (BITE)

Intrabody rapid SC activation for formating tissue or organ

- Increase the number of available SC both in situ and in peripheral circulation (recruitment factors)
  - Granulocyte colony-stimulating factor (G-CSF)
- Activation of a permissive situation of wound healing (Permissive factors)
  - Trauma cytokines release control, such as IL-6, IL-1β and TNF-α
- Control of SC shifting towards a commitment (Commitment factors)
  - TGF-β and other factors to control in vivo differentiation towards cartilage rings
- Remodelling enhancement, inflammation reduction and SC activation to propagate and to protect against ischemia (Boosting factors)
  - Erythropoietin (Epo) as an "enhancing" factor in regeneration process

Development of graft acceptance and functioning methods
- Nerve development in the artificial organ
- Vascularization

Development of preculturing and transportation methods
- Cell sheets
- Incapsulated cells
- Cytospheres
- Cells on microcarrier's, etc.

Bioprinting

3D bioprinters development

Rapid SC activation for tissue or organ formation

- Generation of 2D- and 3D-homocellular cell aggregates:
  - Sheets and tubes
  - Cubes and rings
  - Rods and branching rods
  - Embryoid bodies
- Formation of 3D-heterocellular cell aggregates:
  - Simple models of artificial skin and neural tubes
  - Simplified bone marrow
  - Tissue-engineered vascular grafts
- Generation of three-dimentional vascularized microtissue and tissue (microvascular networks):
  - 3D-vascularized cardiac muscle, etc.
- Formation of intermediate diameter branching tubes and large tubes (macrovessels)
- Development of three-dimentional vascularized organ

CHOICE OF CELLS FOR USE IN TISSUE ENGINEERING

Study of SC properties of different origin
- Xenogenic
- Allogeneih
- Autologous

Use of stem cells of various stage specificity
- ESC
- Adult SC
- Neonatal SC
- iPSC

SCAFFOLD CREATION FOT TISSUE ENGINEERING

Decellularization

- Morphological evaluation of cytoarchitectonic organization of grafts using lazer-induced fluorescence
- Vessel decellularization with minimal damage to the extracellular matrices
- Rapid diagnostic technique of obtained grafts using laser radiation

Biomaterials

Natural biomaterials

NON-ORGANIC
- Coral
- Hydroxyapatite
- 3-calcium phosphate

ORGANIC
- Chitosan
- Silk
- Agar
- Hyaluronic acid
- Collagen
  - Bone, skin, coernea regeneration, filling in stroma defects, etc.
- Fibrin
  - Effects of fibrinogen- and thrombin based fibrin gels for neural tissue engineering applications
  - Optimization of photocrosslinking of fibrin gels for greater stiffness
  - Fibrin scaffolds with sustained release of endothelial groth factor
  - Addressing the issue of using xenogenic stem cell in fibrin gels
  - Hybrid stable porous network of fibrinogen, fibronectin and laminine

Synthetic biomaterials

CLASSIFICATION
- Biodegradable
  - Polyanhydrides
    - Sebacic acid
    - Carboxyphenoxypran, etc.
  - Aliphatic polyesters
    - Polylactate
    - Polyglucolate
    - Polylysine, etc.
- Non-biodegradable
  - Polyacrilates
    - Polyethylene acrilate, etc.
  - Polyesters
    - Polyethylen oxide, etc.
  - Polysiloxanes
    - Silicon glass, etc.
  - Polyethylene and its derevatives
    - Polypropylene
    - Polystyrene
    - Polyethylene
    - Polytetrafluoroethylene, etc.

GOALS
- Stypage of bone cavities
- In situ arterial regeneration
- Adipose tissue formation (adipose-derived hydrogels)
- Cartilage tissue engineering (self-assembled rosette nanotube/hydrogel composites)
- Bone repair (dioposide ($CaMgSi_2O_6$) powders and dense ceramics)
- Control of SC behavior and fate (electrospun nanofibrous scaffolds with biomimetic nanostructure and growth factors)
- Bone engineering (hydroxyapatite particles/poly(ester-urethane)
- Neural progenitor cell differentiation (Modifying the polymeric sugar hyaluronic acid with varying numbers of photocrosslinkable methacrylate groups)
- Nutrient supply and waste product removal (porous ε-caprolactone membranes)

STRUCTURE
- Hydrogels (porous, non-porous)
- Scaffolds of natural biological shape
- 3D structures (net, film, etc.)

PROBLEMS
- Construction of porous scaffolds (biodegradable microspheres with linking components from organic acid motifs and gelatin with polytrimethylene carbonate)
- Reduction of toxicity during biodegradation
- Better distribution of oxygen and nutrients

Biological membranes
- Tissue engineered scaffolds based on natural bacterial membanes

Biomaterials quality protocols development using the criteria:
- No toxicity when biodegraded
- Minimal inflammation
- Biocompatibility with host tissue
- Stiffness and stability at the site of implantation
- No rough encapsulation or rejection of endoprosthesis
- Shape and size preservation of reconstructed organ

RESTORATION OF REGENERATIVE CAPACITY

STUDY OF EXOGENOUS FACTORS ON REGENERATIVE POTENTIAL OF CELLS

- Investigation of dedifferentiation and transdifferentiation mechanisms *in vitro* and *in vivo*
- Impact on cellular microenvironment of SC niche to increase the repair potential of SC
  - Protection of cells from oxidative stress
  - Attraction of stem cells to the site of reparation
  - Stimulation of differentiation

Mechanisms of maintaining the regenerative potential of SC populations through:

- Trophic factors
- Mechanistic impacts on SC
- Secretion of cytokines and chemokines
  - Temporal patterns of neural growth factor stimulation
- Changes in basal membrane and extracellular matrix parameters
- Changes in intercellular contact parameters
  - Microvesicule-vectored transfer of genetic material between SC
- Interactions with capillary endothelium
  - Platelet-derived adhesin SDF-1 in differentiation of CD34+ into progenitor endothelial cells
- Secretion of non-protein substances
  - Effects of nitric oxide (NO) on intracellular regeneration processes

Biologically active compounds to stimulate SC

- Regeneration processes in muscle tissue:
  - Muscle-derived SC, mediated by the niche microenvironment
  - Non-muscle-derived SC
  - stimulatory and inhibitory growth factors
- Proangiogenic effects of exogenous erythropoietin
  Study of erythropoietin receptors on macrophages in fibrin-unduced wound healing
- Influence of various compounds on homing of grafted cells
  - Cytokines
  - Growth factors
  - Extracellular factors
  - Receptor expression

Changes in physical parameters of intercellular environment

- Various oxygen tension values in tissue for modulation of regeneration processes
- Influence of Ca ions on osteoblast differentiation for bone regeneration
- Influence of external electrical field on cytoceleton and membrain mechanics

CHANGES IN EXTRACELLULAR MATRIX AND ACTIVITY OF FUNCTIONAL CELLS

UV-induced connective tissue aging

Strategies for prevention and prophylaxis of photoaging
- Use of anti-inflammatory compounds (cyclooxygenase inhibitors, inhibitors of cytokine generation)
- Anti-oxidant treatment
- Inhibition of matrix metalloproteases (MMP) activity with:
  - neutrophil elastase inhibitors
  - retinoids
  - natural and synthetic inhibitors

Dissection of mechanisms of photoinduced aging:
- Functions and features of MMP
- Mechanisms of photoinduction and activation of MMP
- Specific and efficent MMP inhibitors

Non-enzymatically glycated products
- Detection and quantification of non-enzymatically glycated proteins (AGE-products) of the extracellular matrix
- Study of mechanisms of formation and regulation of AGE- products
- Expression levels of a soluble form of the AGE- products receptor as a biomarker of chronic inflammatory diseases (vascular atherosclerosis, diabetes, renal injury)

Prevention of deposition of AGE-proteins and removal of intermolecular protein-protein cross-links
- Generation of new glucose-lowering agents
- Search for efficient inhibitors (pyridoxine, pyridoxamine, aminoguanidine, 2,3-diamino phenazon, etc.)
- Development of synthetic drugs (2-phenylthiozoline, phenylsetam and its derivatives) and discovery of natural compounds
  - N-acetylcarnosine and histidyl hydrazide as promising therapeutics for treatment of senile cataracts and diabetic retinopathy

Beta-amyloid depositions

Mechanisms of beta-amyloid deposits formation
- Molecular mechanisms of the induction and progression of sporadic Alzheimer's disease
- Mechanisms of the increase in levels of metal ions in brain and other tissues and their role information of beta-amyloid deposits

Prevention of beta-amyloid deposition

Metal-chelators
- D-penicilamine-containing nanoparticles in treatment of Alzheimer's disease, Parkinson's disease, and other central nervous system disorders
- Lipophilic chelating agent DP-109 for prevention of new amyloid aggregates formation and for solubilization of preexisting amyloid depots
- Clioquinol in treatment of Alzheimer's disease

New agents to destabilize and remove preexisting amyloid deposits
- Protollin
- «Beta-sheet breakers»

Therapeutics preventing interaction between amyloid deposition precursors
- Inhibitors of serum amyloid P component
- Natural compounds (wine-related polyphenols, tannic acid, curcumin, nicotine)
- Inhibitors of the interaction between beta-amyloid fibers and beta-amyloid pathological chaperons (ApoE, glucosaminglycans, etc.)

GENETIC AND EPIGENETIC LEVEL

Regulation of transcription in tissue regeneration, also covalent modification of DNA and topological chromosome reorganization for selective manipulation of gene expression

Genes governing regeneration of SC:
- Notch signaling
- Directing proliferation (e.g.,Ptzf)
- IGF gene family encoding insulin-like growth factors
- Hox gene family encoding transcription factors
- Encoding MAP kinases
- Responsible for cytoskeleton structure (e.g. lamin A)
- STAT gene family encoding transcription activators
- 053/021 gene axis

CELL THERAPY

DEFECTED CELL REMOVAL

Identification of cancer stem cells markers in various types of tumors
- Population homogenity
- Molecular mechanisms of cancer stem cell population stability
- Signaling mechanisms of self renewal and tumorigenesis

Reduction of the number of anergic killer T-cells

Reduction of the amount of macrophages in visceral adipose tissues

Markers of senescent cells

Possible markers of defected cells to remove:
- Telomerase reactivation,
- Decreased levels of repair,
- Increase in oxidative DNA damage and inactivation of tumor supression

Methods of cell removal

Target therapy
- Cells as carriers of therapeutic agents
  - Agents:
    - Antibodies
    - Genes
    - Fluorochromes for photodynamic therapy
    - Pharmaceuticals

Apoptosis activation
- Activation of apoptosis though signaling pathways
- Direct activation of proapoptotic proteins, "death receptors" and suppression of the apoptosis inhibitors activity

Suicide gene therapy

Elimination of cells based on activation of angiogenesis

Immune response stimulation
- Therapeutic humanized monoclonal antibodies
- Study of the mechanisms of tumor cell sustainability
- Identification of antigens of unwanted cells
- Adaptive T-cell therapy

CELL INTRODUCTION

Stem cell biomarkers
- Specific markers of cellular surface
- Gene expression profiles in SC
- Epigenetic pattern features

Biomarker data base creation

In vitro culturing

New strategies in cell acquisition
- Search for new sources in the body
- New methods of SC selection out of cell mass

Optimal microenvironment for maintaining cell potency

Optimal microenvironment for cultivating (niche)
- Impact of adhesion molecules and extracellular matrix proteins on the aging rate of SC (P-selectin)

SC differentiation and proliferation control
- Via signalling pathways (JAK/STAT, MT-MMP, P19 ARF, etc.)
- SC proliferation control
- Epigenetic control
- Usage of targeted siRNA

Growth factors control
- Organo-specific growth factors (LGF, EGF, FGF, HGF, IGF, VEGF, BMDF, Epo, GCSF, GMCSF, etc.)

Growth factor receptor expression regulation
- NfκB transcription factor
- Sox
- Pax
- etc.

Use of SC in repair of organ-tissue functions lost during aging

Therapy of diseases caused by locomotor system aging
- Bone tissue and articular cartilage recovery using autologous MSC
- MSC isolation from adipose tissue

Therapy of diseases associated with loss of myocardial function and tissue blood supply
- Reactivation of myocardial SC
- Cardiomyocyte precursors isolation from hESC or iPSC

Cell and gene therapies (CD133, VEGF, FGF) for ischemia treatment:
- Lower limb atherosclerosis
- Diabetic foot
- Myocardial ischemia and others

Treatment of neurodegenerative disorders
- Parkinson's desease
- Alzheimer's desease
- Multiocular sclerosis
- ALS

Studies of integration features for all types of specialized cells using animal tissues

hESC differentiation potential

Into insulin-producing beta cells

Creation of well-characterized ESC lines in cell banks

Into hematopoetic or differentiated blood cells

Therapy applications:
- Differentiated hESC - for repair of the CNS integrity (multiocular sclerosis, trauma)
- Dopaminergic neurons - for transplantation to patients with Parkinson's disease
- hESC differentiation into neuroepithelium - for therapy of eye diseases
- Cells of vascular endothelium or their precursors - for treatment of vascular network pathologies

etc.