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Next Generation: Cartilage Solution

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I. THE CARTILAGE, AS WE KNOW IT

A. Articular Cartilage Complex: Structural Specificity and Function
   - Chondrocytes 1-10%
   - Water 70-80%
   - Collagen 12-14%
     - Type II 10-12%
     - Type IX ~1%
     - Type XI ~1%
   - Proteoglycans 7-9%
     - Hyaluronan-Proteoglycan-Aggregate 6-8%
     - Other proteoglycans ~1%
   - Mineral <4%
   - Matrixproteins <1%

B. Biology of Articular Cartilage: A Very Limited Capacity of Healing
   - Cartilage is avascular
   - Chondrocytes have lost the ability to divide in vivo
   - Chondrocytes - embedded into the extracellular matrix, inhibited from migrating to the defect

C. Biology of Chondrocytes in Monolayer Culture
   - Chondrocyte proliferation - re-enter the cell-cycle by isolation and culture
   - Chondrocyte dedifferentiation - flattened fibroblast-like appearance; synthesis of collagen type I

D. Biology of Chondrocytes in 3D. Culture
   - Chondrocyte redifferentiation - rounded shape; restart of collagen type II expression

II. CARTILAGE TREATMENT TODAY

AUTOLOGOUS CHONDROCYTE IMPLANTATION
   - Indications and Application
     - Symptomatic, focal, full thickness cartilage lesion
     - Absence of significant arthritis
     - Young patients (15-50 years)
   - Treatment Goal
     - Restoration of the structural and biomechanical integrity of the articular surface to optimize function

A. 1st Generation: Peterson Periosteal Patch Technique
   - developed by Dr. Peterson (Sweden) in 1987
   - obtained FDA approval in August 1997
   - to date, > 15000 patients have been treated worldwide
   - used mainly for injuries of the knee joint
   - with documented good long-term results

   - Is still an actual procedure?
     - complex & invasive
     - difficulty in managing chondrocyte culture solution
     - “water-tight” periosteal suturing
B. 2nd Generation ACI with 3D Scaffold:

novel scaffold, no periosteal patch, optimised cell line

Requirements:

- Biodegradable Scaffolds to anchor, deliver and orient cells
- Bioactive factors to provide instructional cues
- Cells responsive to their environment

Surgical techniques for 2nd generation ACI:

- First Phase (diagnostic arthroscopy and graft harvest)
- Second Phase (graft implantation)
  - Arthrotomy approach
  - Mini arthrotomy approach for patellar lesions
  - Arthroscopic technique

Evaluation Of Outcome

- Non-invasive: Clinical, MRI, Ultrasound
- Invasive: Arthroscopy and Biopsy at 4, 12, 24 months

Clinical Studies: From 2000, more than 3500 patients treated with hyaluronan-based scaffold in Europe

Articular Cartilage Engineering with Hyalograph C. 3-year Clinical Results.
Conclusion: The positive clinical results obtained indicate that Hyalograft C is a safe and effective therapeutic option for the treatment of articular cartilage lesions

Conclusion: Biodegradable scaffolds seeded with autologous chondrocytes can be a viable treatment for chondral lesions

Advantage Of 2nd Generation ACI

- Biologic and Structural - enhanced cell proliferation, maturation
- Surgical - no need for periosteal tissue harvest; reduced implant related morbidity; reduced surgical time

Possible Complications:

- Scaffold detachment, Synovitis, Arthrofibrosis

How to Improve:

TiGenix ChondroCelect™: Better Quality and Higher Concentration of Chondrocytes

ChondroCelect-Score: molecular profile of stable cartilage forming cells

ChondroCelect™:
Reconstitution of Tissue Function Correlation ChondroCelect™ Score and Stable Cartilage
Stable phenotype, High proliferation, High ability of ECM synthesis

III. FUTURE DIRECTION: CARTILAGE DEFECTS TREATMENT

Stem Cells, Growth Factors, Biomaterials, One step surgery, Better techniques & instruments

Mesenchymal Stem Cell for Cartilage Defect Treatment

- Properties of MSC
  - Ability to self-replicate indefinitely
- Ability to become specialized cells

- **Adult MSC**
  - Derived from adult tissue
  - Involved in host tissue maintenance/repair
  - Multiple sources
    - Bone marrow, Adipose tissue, Peripheral blood, Brain, Skeletal muscle, Skin, Liver

- **Plasticity of Adult MSC**
  - Capable of forming cells other than host tissue
  - Example: bone marrow
    - Original belief: blood cells only (HSCs)
    - Further research: bone, cartilage, fat (MSCs)
    - Recent evidence: neural, muscle, heart

- **MSC Culture**
  - Cells Harvesting
    - Bone marrow, Peripheral blood, Adipose tissue
  - Cells Proliferation
    - Growth factors increases proliferation rates in MSC culture
  - Cells Differentiation
    - Osteogenesis
    - Adipogenesis
    - Chondrogenesis
      - Micromass culture or pellet culture system as a good model of chondrogenesis
      - Added growth factors with chondrogenic potential into culture medium
        - TGF, BMPs, FGF, PDGF, IGF, EGF

- **MSC Concentration**
  - Goal: one step surgery
    - Max. concentration: 4x
  - Starting from 30 ml of bone marrow aspiration, we can obtain 34,200 mesenchymal stem cells...
    - are those cells enough?

- **Osteogenic and Chondrogenic differentiation of 3DST derived from Human Adipose Tissue (MSC)**
  - Tissue Engineering without Scaffold
  - Synovial or Adipose Cell Therapy for Repair of Cartilage & Meniscus
    - (Norimasa Nakamura Osaka, Japan)

- **MSC-magnetic beads complex for articular cartilage repair**
  - The formation of the MSC-magnetic beads complexes (Anti CD44 antibody + CD44-MSC) ➔ The distribution of the complexes under EMF condition ➔ Chondrogenesis of the complexes under EMF condition
    - (Mitsuo Ochi Hiroshima, Japan)

- **Possible Advantages of 3rd Generation**
  - No need of arthrotomy
  - No need of harvesting the normal cartilage
  - Possibly no cell culture
  - Possibility to repeat MSC injection
  - Ability to use cytokine: growth factor simultaneously

- **MSC for Cartilage Defects Treatment:** Clinical Studies
  - Conclusion: MSCs were capable of regenerating a repair tissue for large chondral defects

**Gene Transfer for Cartilage Protection by Viral Vectors**
- Gene transfer of cytokines and cytokine-inhibitors
  - Cytokines: interleukin-4, interleukin-10 (potentially joint-protective molecules)
  - Cytokine-inhibitors: tumor necrosis factor-a, interleukin-1
- Gene transfer of intracellular signaling molecules
  - Inhibition of the transcriptional activator NFkB
- Gene transfer of inhibitors of matrix-degrading enzymes
  Overexpression of tissue inhibitor

**Other Possibilities for One Step Surgery**

- Articular Cartilage Defects Reconstruction by Plasma Rich Growth Factors
  - Increase in the total glycosaminoglycans synthesis and decrease in its degradation
  - Increase in the matrix collagen II content
  - Stimulation of the chondrogenesis increasing proliferation, differentiation and adhesion of the chondrocyte
  (Ramon C, et al. Clinica del Pilar, Barcelona Spain)

- Cartilage Defect Treated with Microfracture/BMP-7(OP-1)
  - Microfracture vs Microfracture plus BMP-7
    - Microfracture mostly fibrocartilage
    - Microfracture plus BMP-70% hyaline or hyaline like repair
  (Rodrigo JJ, et al Colorado, U.S.A.)

- GelrinCartilage™
  - Mechanism PEGylated fragments released, Chondroinductive
  - Advantages Low-cost, Off-the-shelf, Arthroscopic, Delivery
  - Disadvantages Early technology, Osteochondral model
  (Regentis Biomaterials LTD.)

- TrueFit Plug
  - Mechanism Osteoinductive, Chondroinductive
  - Advantages Low-cost, Off-the-shelf, Carrier
  - Disadvantages OC defect, Size limitations
  (OsteoBioligics, Inc. - Smith & Nephew USA)

- NeoCart
  Core Technology
  3-D Bovine collagen, Autologous chondrocytes, Expand/Seed, Hydrostatic bioreactor (6 wk), Implant bioadhesive (Histogenics Corporation)

- Cartilage repair with engineered scaffold-free ACI
  Scaffold-free 3D Synthetic Tissue (3DST) derived from Synovium
  - Advantages Low-cost, Off-the-shelf,
  - Disadvantages No clinical studies yet
  (Norimasa Nakamura Osaka, Japan)

- Autologous chondrocyte implantation without ex vivo cell expansion: CAIS/Griffin Project
  - Mechanism Local cloning, Matrix/Collagen II
  - Advantages Low-cost, Off-the-shelf, Arthroscopic
  - Disadvantages Size limitations, Hyaline-like, no clinical studies with long term follow up
  (DePuy Orthopaedics,Mitek Inc)

- “Culture-free” & “Scaffold-free” Technique
  - Articular Cartilage Healing by OP-1 Based Cocktail of Growth Factors
  - Advantage: Partial thickness cartilage lesions
  - Disadvantage: No clinical studies, high cost
  (Jelic m, University of Zagreb, Croatia)

**CONCLUSION**

- Human’s dream is to remain young and active forever
- Cartilage regeneration is the “challenge” for the next 20 years
- Understanding the “Chondropenia concept” and instruct patients
- Respect the “envelope” of function
- Surgery might not be the only solution
REFERENCES