Principles of Periodontal Tissue Engineering

☺ Introduction ..

- Tissue engineering in general is : **trails to replace lost tissues**.
- All what we are doing in dentistry is a part of tissue engineering.
- Medicine (mainly the surgical part of medicine) and all the dentistry is about surgery.
- Class I and class II cavity preparations are surgical intervention because we are cutting in tissues, once we cut into this tissues particularly enamel and dentine that can't be replaced, that is why scientists came up with different materials to replace them, and these materials that we always use should be biocompatible and inert.
- Study after 2005 → Talked about effects of dental restorations from a pure biological point of view (cytotoxity and changes that happens after replacement of the restoration in the patient mouth, like : Color changes & release of some materials).
- Other techniques rather than restorations : bone grafts to replace soft tissues, implants, implants & bone grafts.
- We have different types of restorative materials, different types of dental implants and different types of bone grafts.
- Moreover all the surgical procedures have the same purpose which is : **replacement of missing tissues to restore function**.
- All of the following techniques are difficult extensive and aggressive surgeries associated with a certain morbidity rate which vary according to the patient and the procedure : bone grafts, membrane use, implants, parietal bone graft, symphysis block bone graft, sinus lifting and distraction osteogenesis.
- All these procedures and the restorative materials that we talked about don't provide you with the ideal required results to obtain the regeneration you want.

- Published article in 2014 → there was a very naive belief that materials were typically inert and it has been rightly suggested that is a misleading interpretation, as it became clear that **materials could indeed change physically and chemically following implantation** (and we all know that every single material used in dentistry get some changes once it is implanted in the human body).
  If the material release anything that means that it undergoes some chemical changes at least, this chemical changes will lead later on to certain mechanical changes that will make the material unable to full fit its original function.
  And it's the same for implants and bone grafting materials not only the restorative materials.

☺ Paradigm shift ..

- In 2006 they came with something called paradigm shift → which shift the medicine in general (surgical medicine in specific) **from a point of view that is looking to replace the tissues to a point of view which is looking to regenerate them.**
- Most of our work until now depends on putting a prosthesis to replace some part that is lost or that is missing. In this shift we are trying to go from a method of thinking where we are using synthetic materials some of them are non degradable to replace the tissues into a more biologically driven point of view.
- The most sophisticated form in biology is the human being where we must know applied physics, applied chemistry, organic chemistry, organic physics and mathematics in order to understand it.
- We are trying to find biological solutions to our biological problems.
- We are shifting or evolving from mechanical (eg. Surgical) to biological solutions.
- It is the result of the development in all of the science fields : engineering (new generations of microscope), biology (to understand DNA & cell functions) and chemistry(to understand nature of molecules) and all other sciences.
- There is a convergence between clinical dentistry and medicine (nowadays we can't take dentistry and separate it from medicine), human genetics, development and molecular biology, biotechnology, bioengineering and bioformatics.
- In 1950 and 1960 they tried to replace the lost tissue in many manners with many different materials, because there was a thought that the material is inert and they will not do any harm.
- Later on, in 1960 and 1970 they started to understand how the body deal with these materials and they found that there are some drawbacks for some materials.
- For example in hip replacement, the hip is replaced with prosthetic articulation and every 10-15 years it should be changed, and every time the prosthesis was changed they should remove some bone from hip and femur. After 2-3 times orthopedic shouldn't change the prosthesis or remove bone anymore, because it is difficult to compensate the lost bone and they use many cements and materials that affect tissues and function.
- More recently there has been a distinct and concentrated effort in the design and the use of both natural and degradable scaffolds and advanced biological consideration of the materials.
- All the works on the materials in medicine in general and even in dentistry in specific even the new generations that are still under experimentations, they are trying to respect these principles.

☺ Biotechnology ..

- Biotechnologies : **are the sum of methods and techniques using , as tools , living organisms or their parts**.
- They are used in different domains, they started with food production , agriculture , pharmacology and then they went to medicine.
- They started with food production and agriculture because it easier to deal with.
- Genetically modified products opened the way for modifications of biological components to enhance the response of the organism (animal or human), and try to replace the lost tissues in a biological way.
- Genetically modified products can bring up some modifications or mutations later on in consumers.
- The aim of biotechnology in medicine is : tissue regeneration.
- **Nude mouse** : it's an immunocompromised mouse, a virtual model to study some aspects of human cells in vivo without studying it on human beings. They took cartilage cells from human ear and put them on a scaffold like the human ear shape, they injected them subcutaneously in the back of the mouse. And what they've got is a mouse with human ear on his back.
- The idea of regeneration came from nature.

☺ Examples of regeneration:

1. **Beast (Monster with seven heads)** : if one of them is cut, it will grow back and the monster won't die unless the seven heads are cut at the same time (so the people have thought of the idea of regeneration and its possibility long time ago).
2. **Hydra** : a microscopic organism that lives in water, if you cut one side the other one will form the rest, one cell can form the whole animal (star fish is also the same).
3. **Salamander** : if you cut the tail or any of the limbs, it will reform completely within 2 weeks.
4. **Sharks** : have sets of teeth (continuous developing teeth), if any tooth is lost another one will form (havethe ability to form teeth from cells).
5. **Mouse** : has continuous erupting teeth (incisors) because of continuous deposition of enamel and cementum (which is different in human beings in which the external and internal enamel sheath is lost once the roots start forming but here in mouse they are still exist). Mouse has a high wearing rate, but the incisor length won't decrease. This is one of the ideal animal model to study the enamel and cementum biology.

All these are examples of living organisms that have capacity to regenerate.

☺ What is regeneration ?

- It is not to have the perfect body.
- It is not to have an endless life or prevent death.
- It is all about to be able with the help of God to provide people with a long and healthy life, and if it is possible to do that for everybody.
- Regeneration in dentistry : It is the restoration of the morphology and the function of damaged or lost tissues, in away similar to that occurring during development.

☺ principles of regeneration ..

- Comparison between development and regeneration : there are steps shared between development and regeneration controlled by the same factors. The difference is in the initial steps of both.
- in both of them : the progenitor cells once I get them, they are developed by the same factors (migration, adhesion, proliferation and differentiation factors) in development and regeneration to provide me with the differentiate cells that will be able to reproduce the tissues.
- **In development** ..
  Cellular condensation → spatial reorganization of the cells (depends on space and time) → progenitor cells → differentiated cells.
- **In regeneration** ..
  Blood clot formation → inflammatory reaction → granulation tissue → recruitment of stem cells → progenitor cells → differentiated cells.
- All scientists try to work on the **initial stages** to try to make it very similar to what occurs in development.
- In surgery one of the most important principles is to keep the blood clot as thin as possible (to keep the blood clot in its least possible volume).
  As the clot size increase scarring will increase.
  so in plastic surgery or skin transplant after burn, they use (مشدّات) to prevent swelling, edema, clot or anything that could change the volume.

☺ Components of regeneration ..

1. Stem cells
2. Regeneration space (space is filled with scaffolds)
3. Signaling molecules

☺ Tissue engineering ..

- One of the methods **to get regeneration** is tissue engineering.
- All our works including class III composite restoration are primitive form of tissue engineering.
- Bone grafts is a little more elaborated form of tissue engineering than the composite filling.

- **General definition** (from 1993 till now) : interdisciplinary domain involving biological sciences and principles of engineering aiming to develop biological substitutes in order to restore, maintain and ameliorate tissue function and morphology.
- **More accurate definition** : it is an approach that utilizes specific biodegradable synthetic or natural scaffolds as well as advanced molecular techniques in order to replace tissue function
- To engineer a functional biological structure, cells must be instructed to differentiate and receive positional cues (signals), and to synthesize the appropriate extracellular matrix molecules in the overall shape and dimensions of the diseased or missing tissues or organs
- It is the method that we can use to reach tissue regeneration
- **Componeentes of tissue engineering** (It's the same triad) :
  1. stem cells (progenitor cells)
  2. 3D scaffold
  3. signaling molecules
- signaling molecules can be carried on the scaffold in some specific molecular techniques.

☺ Periodontal tissue engineering ..

- we talked about it in the previous lecture, new techniques and materials of guided tissue regeneration is available like (methods of tissue engineering) :
  1. Emdogain (enamel matrix derivative)
  2. PRP growth factor (platelet rich plasma)
  3. GEM 21S (one of the platelet derivative growth factor)
- **Components of periodontal tissue engineering** :
  1. **stem cells (progenitor cells)**
  2. **3D scaffold**
  3. **signaling molecules**

  All at enough **time** and **appropriate environment**.

☺ Signaling molecules ..

- **Types of signaling molecules** :
  1. rhPDGF (recombinant human platelet-derived growth factor)
  2. BMP (bone morphogenetic protein)
  3. FGF-2 (fibroblast growth factor 2)
  4. EMD (enamel matrix derivative)
  5. PRP (platlet rich plasma)
- Each signaling molecule has different effects on cells function.
  The same molecule can do stimulation and inhibition to the same cell in different times.
  Knowledge of signaling molecules are still in experimental steps.

- **Why can't we use signaling molecules alone ?**
  1. short biological half life (insulin-like growth factor has the longest half life which is 6 hours).
  2. problems in receptor-binding properties.
  3. stability of carrier system (growth factor should be carried on a specific carrier and not all of the carriers are stable).
  4. cell adhesion (cells like osteoblasts can't function unless they adhere or attach to a surface).

☺ 3D scaffolds ..

- **Types of scaffold :**
  1. autogenous graft
  2. allogenous graft
  3. xenogenous graft
  4. synthetic (plastic) materials
- **Role of scaffold :**
  1. provide physical support
  2. work as a barrier to restrict cell migration in a selective manner (allow the entrance of some cells and prevent other cells)
  3. work as a scaffold that enable cell migration , adhesion , proliferation and then differentiation
  4. serve as time-release mechanism for signaling molecules
- In new generations they are trying to carry signaling molecules on the scaffold.
- **Why can't we use 3D scaffold alone ?**
  1. fibrous inclusion
  2. problems with resorption (if resorption occurs it will be through inflammatory reaction)
  3. cell adhesion
  4. porosity (the material should be porous enough to allow vascularization and at the same time it should be rigid enough to withstand stresses and stimuli. And as we increase the porosity of the material its strength will decrease)
  5. oxygen passage (all available materials don't allow oxygen passage for more than 200 microns. Stem cells can live in hypoxia up to 7 days)
  6. vascularization

☺ Stem cells ..

- **We have different types of stem cells** :
  1. pluripotent (give all the cells except the placenta or embryo supporting tissues)
  2. totipotent (give all cells including placenta)
  3. multipotent (give a big number of cell types, like mesenchyme)
  4. unipotent (give only one type of cells, like keratinocyte stem cells or epidermal stem cells)
  5. inducible pluripotent (like fibroblasts through the activation of certain genes)
- **Mesenchymal cells** :
  1. they are undifferentiated cells
  2. they have a high proliferation rate over long time
  3. they can differentiate into different cell types
  4. asymmetrical mitosis (which gives two cells one is like the mother cell and the other is one is more differentiated)
- Mesenchymal stem cells can give us different tissues like : bone, cartilage, muscle, bone marrow and fat tissues.
- Stem cells are covered by different factors and differentiation process is very complex.
- **Types of stem cells** :
  1. bone marrow stem cells (BMSC)
  2. epithelial stem cells (ESC) (not mesenchymal)
  3. dental pulp stem cells (DPSC) (ecto mesenchymal)
  4. stem cells from exfoliated deciduous teeth (SHED)
  5. periodontal ligament stem cells (PDLSC)

☺ Dental stem cells ..

- The idea of dental stem cells came from tooth development
- **Gronths** is the first who talked about dental stem cells and pulp stem cells in 2000
- The idea of dental stem cells came from **reparative and reactionary dentine formation** under stimulus like pulp capping . Dentine is formed again this means there is pulp stem cells which can differentiate into odontoblast
- Gronths took pulp tissue, cut it under microscope, put it in tissue culture dish, then in scaffold (gel), and implanted it subcutaneously in a nude mouse, after several weeks dental like structure has formed (dentine , cells that resemble odontoblast even in its spatial organization and pulp-like tissue in the centre)
- Odontoblasts don't proliferate so don't regenerate but it can produce some types of tissues

- They studied dental stem cells under microscope using certain techniques, these stem cells like any other stem cells contain markers (alkaline phosphatase, dentine sialoprotein and osteocalcin). Dental stem cells can give osteoblasts and odontoblasts
- **Dental stem cells experiments** :
  1. Dental pulp stem cells (DPSCs) .. 2000
  2. Stem cells from exfoliated deciduous teeth (SHED) .. 2003
  3. Periodontal ligament stem cells (PDLSCs) .. 2004
  4. Stem cells from apical papilla (SCAP) .. 2006 , 2008
  5. Dental follicle precursor cells (DFPCs) .. 2005
- **characteristics of dental stem cells** :
  1. Higher proliferation rate than mesenchymal stem cells under the same conditions ..
     Cyclin-dependent Kinase – 6
  2. Expression of certain factors (STRO-1, VCAM-1, α-sma) ..
     a. Heterogenous population (stem cells at different stages of differentiation and it is better for regeneration)
     b. Perivascular niche (their location around blood vessels)
  5. High plasticity (they can give different type of tissues and cells)
     a. Osteoblasts
     b. Chondroblasts
     c. Adipocytes
     d. and there is a new reports in 2012, 2013 and 2014 said that they were able to produce neurons from dental pulp stem cells in vitro. And they did some studies on animals to reestablish some neurological function in mice using dental pulpstem cells by injecting it in the injured spine and after injection some of the lost function will return to normal.

☺ **Periodontal tissue engineering ..**

- collecting teeth cells (stem cells) from patient → porous polymer scaffold → in vitro cell culture → in vivo implantation → total tooth regeneration
- In 2005 , they put a periodontal ligament stem cells sheet and on a defect , after a period of time they check the defect and they found a functional periodontal ligament.

☺ **Conclusion ..**

- Tissue engineering is an interdisciplinary approach
- We have **2 types of tissue engineering** :
  1. **Ex vivo tissue engineering** ..
     We take a biopsy (cells) → isolation → culture → proliferation → we add scaffold with some signaling molecules → organization → development → implantation
     Or we can take a biopsy → inject it into animal → then return it into the human being
  2. **In vivo tissue engineering** ..
     I can inject some stem cells with signaling molecules and the process will take place inside the human being
- We can use tissue engineering in **dentine repair and in tooth tissue engineering** (to create a whole tooth) , this was done by Paul Sharpe who was able to make a tooth to erupt out of only 2 simple layers of cells mesenchymal and epithelial, he prepared these cells and put them in a certain scaffold then planted them inside the mouse's mouth in the diastima between incisors and molars , he only gave them the initial signal which is fibroblasts from epithelial to mesenchymal and the tooth erupted and was functional with a full pulp chamber and a full root length
- We can use it in tissue engeneering in general to produce :
  1. bone
  2. cartilage
  3. nerve
  4. muscles
- in 2004 , a patient had a trauma or cancer and as a sequence his mandible was removed. They did multiple CTs; a 3D scaffold was constructed and a mesh formed from titanium. They put on this mesh stem cells taken from bone marrow. Then they cultured these cells on mesh in the lab for a period of time then they culture all of these under pectoralis muscle to obtain good environment and no antigenicity .Then they culture these in the mandible. This patient lived for 3 years and died by heart attack, his death was with no relation of this procedure.

☺ **Challenges ..**

- **Biological challenges :**
  1. Growth factors (some of them have 2 different actions on the same cell at different times)
  2. signaling pathways
  3. root development (one of the most difficult development mechanisims in the human body)
- **Technical challenges :**
  1. Culture conditions
  2. Xenogenic products
  3. Timing
  4. Ideal scaffold
  5. Delivery system
- **Clinical challenges :**
  1. Immunogenic rejection
  2. Oncogenic properties
  3. Functional integration

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