Endodontic Biology: Towards Regenerative Endodontics

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How I Got There...
Goals

- To review contemporary endodontics
  - biologic foundational knowledge
  - clinical applications

- To provide a range of information
  - confirmatory elements
  - challenging parts, references

- To give my view of the evidence
  - context, literature and clinical strategies
  - where we may be going

Well We Will See....
Case: Sinus Tract (MB, 04)

Pre-operative clinical situation

After 2wks of Ca(OH)$_2$ dressing

Pre-operative radiographs

Working length

Completed treatment
**Chronic Perirad. Abscess**

**Pre-operative radiographs**

**Working length**

**4yr follow-up**

**Introduction**

**Physiology**

**Microbiology**

**Regeneration**

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**Was Surgery Indicated?**

- Diagnosis:
  - radicular cyst
  - significant morbidity

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The Endodontic Disease

- Therapeutic concepts require a disease
  - endodontic therapy addresses prevention or resolution of periradicular inflammation

- Microbial etiology of periradicular pathosis
  - germ-free rats do not develop periapical lesions
  - teeth with lesions harbor bacteria in their canals

- Reduction of bacteria via endodontic therapy
  - chemo-mechanical preparation

- Prognosis depends on antimicrobial efficacy
  - positive culture correlates with less healing

Successful Disinfection

- Yes, but...
  - healing depends on many factors
  - defining and detecting healing is even more complex

Immune Response

- Innate (unspecific) immunity
  - early and drastic response mounted
  - cellular, humoral components
  - developmentally old

- Acquired (specific) immunity
  - needs prior contact with antigen to be effective
  - once activated, very powerful
  - relatively young

- System with multiple interactions
  - significantly researched because of implications
  - new and powerful tools are available
  - will be significant in the near future: regeneration
Interactions between immune system and mesenchymal stem cells in dental pulp and periapical tissues

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Abstract

Leprince JG, Zeitlin BD, Tolar M, Peters OA. Interactions between immune system and mesenchymal stem cells in dental pulp and periapical tissues. International Endodontic Journal. The recent isolation and characterization of mesenchymal stem cells (MSCs) in dental tissues constitutes a major step forward in the development of new treatment strategies. MSCs are essential for dental pulp repair and the success of regenerative endodontic procedures. It is important to understand that immune cells and cytokines can affect stem cell function, which can impact their healing potential. On the other hand, stem cells are immunoprivileged and have the ability to modulate immune and inflammatory responses, which can be utilized to improve treatments outcome. This review addresses both aspects of this interaction and suggests that any change on both sides can tip the balance in favour of either persistence of inflammation or healing. Finally, the therapeutic relevance of the interaction between MSCs and immune system relative to current treatments is discussed, and future research and treatment perspectives are suggested.

Keywords: cytokines, growth factors, immune system, immunosuppression, inflammation, mesenchymal stem cells, periapical lesion, pulp capping.

Introduction

The recent isolation and characterization of mesenchymal stem cells (MSCs) in dental tissues constitutes a step forward in the development of alternate treatment strategies. MSCs are cells capable of self-renewal and differentiation in vitro and in vivo into several tissues of mesenchymal origin, for example bone, cartilage and adipose tissue (Deans & Moseley 2000, Dominici et al. 2006, Le Blanc 2006). These possibilities have generated hope and opened a range of new research and therapeutic perspectives (Zandstra & Nagy 2001). In addition to these characteristics, MSCs are also characterized by their adherence to plastic culture surfaces (Dominici et al. 2006). Typical for MSCs is the expression of specific surface antigens, for example CD29, CD73, CD90, CD105, in parallel with the absence of others, like CD34 or CD45 (Dominici et al. 2006).

Several types of dental MSCs have been described, i.e. dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from the periodontal ligament (PDLSCs), progenitor cells from the dental follicle (DFPCs) and stem cells from the apical papilla (SCAPs) (Huang et al. 2009, Rodriguez-Lozano et al. 2011). Figure 1 illustrates the characterization of cultured SCAPs, based on their surface markers.

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2011

- review
- MSCs, other stem cells are impacted by inflammation
- MSCs, other stem cells are immunomodulatory
Biology ~ Relevance

- Interrelation between oral disease and health
  - correlation between oral and cardiovascular health?
  - mechanistic treatment no longer feasible

- To treat disease appropriately
  - currently, endodontic therapy relates to canal disinfection
  - the microorganisms are well understood but not healing

- Future: regenerative endodontics
  - clearly, all involved agree that future therapy concepts have to involve regeneration as opposed to repair
  - for that to happen, mechanisms need to be understood

Implants

- Apical lesions have been described
  - typical periimplantitis is marginal but occasionally lesion around the implant apex appear

- Can implants affect adjacent teeth?
  - during insertion, a vital tooth may be damaged
  - a root-canal treated tooth may be infected during insertion, not clear that it occurs

  Margelos 1995

- Can teeth with lesions can affect implants?
  - apical lesion growth may encompass implant
  - implant may be “infected”

  Susman 1993

Apical Lesions & Implants

LC, 08; 1m follow-up, case courtesy A. Nattestad
Clinical Assessment of Pulpal Pathosis

Cold Testing
- Normal or momentary sensation
- Exaggerated, longer sensation
- No response

Pulp status
- Normal pulp, Reversible pulpitis
- Irrev. pulpitis, DD: dentin hypersensitivity, partial pulp necrosis
- Necrosis, mineralization, internal resorption, trauma, previously initiated Therapy

Material & Methods
- 31 patients participated; 19 had irreversible pulpitis based on clinical tests: lingering and spontaneous pain, no PARL; 12 had no symptoms but needed deep fillings replaced
- Dentinal fluid was picked up over 2 mins with a filter paper
- A sensitive fluorescent assay was used to detect MMP-9 content
- Non-parametric statistics were used

J Endod 2011, 37:1293-1295
MMP-9 Content

ng/mL

Pulpitis    Healthy

Clinical data
- vital pulp therapy is currently not very predictable
- may provide an alternative to pulp regeneration

Conclusion
- significant development potential
- multivariate methods needed: microarrays, etc.

Sensitivity / Specificity
- 3 symptomatic pulps were actually necrotic
- better tests are needed to enhance outcomes
**Experimental data**
- increases expression of genes associated with mineralization, cell differentiation

**Clinical conclusion**
- TGFβ is a relevant molecule in repair/regeneration
- liberation of sequestered TGFβ from dentin is important
Mixed Infection

bacteria

fungi

virus

Successful Decontamination
How Do Microbes Succeed

- They multiply and have well adapted mutations
  - bacteria can escape ecological pressure through many strategies, one of which is mutation

- They can persist in vegetative forms
  - dormant bacteria remain after systemic or low-dose antibiotics

- They can co-operate to escape host defence
  - biofilms allow bacteria to protect themselves

- They co-operate to increase virulence
  - LPS and peptidoglycans co-stimulate osteoclasts

Molander 2003, Figdor 2003
Dahle 1992
Byström et al 1981
Jiang 2003

Current Microbiology

- Using molecular methods
  - viability of the detected species not clear
  - finer methods will detect more species

- Sample acquisition
  - ideally, 100% yield
  - clinically difficult

- Conclusion
  - bacterial composition perhaps similar comparing primary endodontics to retreatment flora!

Hong et al 2013

E. faecalis?

S. Erlandsen, U Minn
Bacteria in Dentinal Tubules

Extraradicular Infection

- Consensus: infection is primarily inside canal
  - good evidence from SEM and TEM studies
  - culturing is prone to false positives
- Upside: treatment rationale
  - intraradicular infection is much more accessible
  - non-surgical retreatment has high success
- When is extraradicular infection present
  - acute abscess, chronic abscess
  - special bacteria: Actinomyces, Propionibacterium
- Anatomy: where does the canal end?
  - bacteria may lodge in apical canal parts, close to the "end" of the root canal

Sundqvist und klinisches Bild mit "guter" WF
Nair 2004
How Do Bacteria Attack?

- With virulence!
  - They come in VERY big numbers
  - They have several unpleasant abilities

- With strategy!
  - They arrange in biofilms

(movie from Singh et al., Nature 417, 2002)
Regenerative Endodontics

- What is it?
  - return of a vital response after nonvital response
  - in this context: reconstitution of functional pulp

- What are the goals and benefits
  - deposition of hard tissue for immature teeth
  - immunologically competent functional tissue

- What are the indications
  - currently: teeth with large apical foramina
  - research is underway to extend the spectrum

Stimulation vs. delivery

- removal of cells and tissues, recruitment of undifferentiated cell populations
Regenerative Endodontics

- Stimulation vs. delivery
  - autologous cells, apical papilla cells
  - stem cells from other sources

- Scaffold
  - collagen, gel etc.
  - fibrin matrix

- Crucial: Blood supply
  - VEGF and other key factors
  - transportation of bacteria/debris into periapical area

Clinical steps
- indication, prognosis
- anesthesia
- 1st appointment
  - irrigation
  - disinfectant paste
  - scaffold
- 2nd appointment
  - pulp space barrier
  - definitive filling

Preop

9m recall

Case by Dr. Patrick King
Considerations

- **Indication**
  - large apical diameter, young patients

- **Diagnosis**
  - pulp necrosis, sinus tracts

- **Expected outcomes**
  - favorable / unfavorable

- **Clinical steps**
  - disinfection, scaffold, definitive closure

**Clinical steps**
- indication, prognosis
- anesthesia
- 1st appointment
  - irrigation
  - disinfectant paste
  - scaffold
- 2nd appointment
  - pulp space barrier
  - definitive filling
Expected Outcomes

- Apical barrier
  - radiographically visible, apical stop for fill

Regenerative Endodontology

- Nature of the deposited hard tissue
  - strengthening effect not clear
  - hard and soft tissues are important

- Predictability
  - treatment outcome may depend strongly on conditions
  - no clear clinical recommendation for procedure

- Conclusion
  - vital pulp therapy may become attractive?
  - significant development potential

Immunohistological Characterization of Newly Formed Tissues after Regenerative Procedure in Immature Dog Teeth

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J Endod 2011, 37:1499-1503
Histologic Characterization of Engineered Tissues in the Canal Space of Closed-apex Teeth with Apical Periodontitis

Introduction:
The capacity of endodontic regenerative procedures combining an induced blood clot, platelet-rich plasma (PRP) gel, bone marrow aspirate (BMA) gel, and PRP/BMA gel to regenerate dental pulp in canine closed-apex necrotic teeth. Apical periodontitis was induced in 20 upper and lower premolars of 2 dogs. After biomechanical preparation, enlargement to a #60 file, and disinfection, the teeth were randomly assigned to 4 treatment groups: blood clot (BC), BC + PRP gel, BC + BMA gel, and BC + BMA/PRP gel. Negative controls were also included. After a 3-month follow-up period, the animals were killed. Histologic analysis showed the presence of newly formed vital tissues (connective, cement-like, and bone-like tissue) in 23 of the 32 treated roots across the treatment groups.

Results:
New vital tissues were formed and characterized as connective, cementum-like, or bone-like, but not as tissue ingrowth. Bone marrow aspirate (BMA) can be a straightforward, low-cost, and fast method with a low risk of infection (14, 15). Moreover, as the pulp diminishes with age, alternative sources used as an alternative source for dog pulp regeneration as aspirate (BMA) can be a straightforward, low-cost, and fast method with a low risk of infection (14, 15). Moreover, as the pulp diminishes with age, alternative sources used as an alternative source for dog pulp regeneration.

Conclusions:
Dog pulp regeneration has been achieved by using stem cell transplantation and the induction of a blood clot alone did not promote pulp regeneration in immature necrotic dog teeth (9). The most common ERP uses blood clots and cell transplantation. In a number of studies, the induction of a clot caused an increase in root length and thickness compared with that observed with the induction of a blood clot alone (10–12). The use of bone marrow mesenchymal stem cells (MSCs) has been complete restoration of a functional dentin-pulp complex cannot be achieved (3). Moreover, in a human subject, injection of PRP and blood clot (8) stimulated human periodontal ligament fibroblasts with the potential to form cementum- or bone-like tissue and fibrous connective tissue.

Human histology:
“The tissues formed in the canal of revitalized human tooth are similar to cementum- or bone-like tissue and fibrous connective tissue.”

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First Appointment

- Case selection
  - post space needed, allergies, compliance?
- Consent
  - mostly minors
  - discoloration of the crown possible: TAP, MTA
- Access
- Disinfection
  - no instrumentation
  - irrigation with 1.5% NaOCl
  - place Ca(OH)$_2$ or TAP/DAP
- Temporary filling

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Issues

- TAP/DAP
  - 1:1:1 minocyline, ciprofloxacinc, metronidazole
  - staining due to minocycline, remove or seal dentin
- Ca(OH)$_2$
  - recent data shows less cytotoxic compared to TAP
  - readily available, place with lentulo spiral
- Temporary filling
  - should avoid recontamination
  - *e.g.*, 4mm layer Cavit plus IRM
**Toxicity**
- TAP in the currently used way has high concentration
- this is toxic to stem cell, impacting proliferation and differentiation
- lower concentrations and Ca(OH)\(_2\) are preferred

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**Second Appointment**

- **Timing**
  - after 3-4 weeks, longer not advisable

- **Anesthesia**
  - no vasoconstrictor

- **Reaccess and irrigation**
  - EDTA rather than antimicrobials

- **Scaffold**
  - overinstrument to create bleeding, alt. collagen plug

- **Tissue barrier**
  - collagen app. 3mm below CEJ, MTA fill, alt. GIC

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**Effect of Irrigants on the Survival of Human Stem Cells of the Apical Papilla in a Platelet-rich Plasma Scaffold in Human Root Tips**

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- **Material & Methods**
  - SCAP were isolated from third molars and characterized
  - human root segments were subjected to irrigation with either 17% EDTA; 6% NaOCl/EDTA; EDTA/2%CHX or NaOCl/EDTA/NaOCl/alc/CHX
  - root segments were filled with SCAP and PRP, cultured for 21 days and then processed for immunohistochemistry
  - cell viability was determined
Follow-up

- Clinical
  - no pain or tissue swelling
  - between the two primary appointment and at 6m recall

- Radiographic
  - apical lesion resolves (6-12m)
  - root wall thickness increase (12-24m)
  - root lengthening, apical closure (variable)

- Discoloration
  - sometimes esthetically compromising

What to Expect:

- Public health impact
  - patient population currently small
  - extension to mature apices possible?

- Research impact
  - expect to broaden biological knowledge base
  - will establish molecular methods in the clinic

- Strategies
  - vital pulp therapy may become attractive and feasible
  - significant development potential
- MTA pulp caps
  - success rate varies from >90% to about 50%

- Immature root development
  - reasonable alternative to RCT
  - MTA material of choice

- Strategies
  - avoiding pulp exposure preferable
  - few well-done clinical studies
Conclusions

- Long-range: two pillars
  - vital pulp therapy
  - minimal invasive conventional endodontics

- Transition period
  - gradual R & D for both
  - special cases: define indications and techniques

- Cognitive framework
  - establish best practices, currently insufficient evidence
  - socioeconomics and access to care

- Elimination of microbes is key to success
  - presence of organisms linked to disease symptoms
  - both counts and virulence of colonies are important

- Efficacy of antimicrobial regime can vary
  - understanding chemical and biological interactions
  - assessing cases and their individual challenges

- Clinical strategies must vary
  - wide range of possibilities
  - technical and biological details

A Break?

False Summit, Mt. Tam