Endodontic Biology: Towards Regenerative Endodontics

Ove A. Peters, DMD MS PhD
Diplomate, American Board of Endodontics

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How I Got There...
Introduction

Physiology

Microbiology

Regeneration

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

Endodontics

Engineering  ⇄  Biology
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
Was Surgery Indicated?

- Diagnosis:
  - radicular cyst
  - significant morbidity

The Endodontic Disease

- Therapeutic concepts require a disease
  - endodontic therapy addresses prevention or resolution of periradicular inflammation
  - Orstavik 1999, Trope 2003

- Microbial etiology of periradicular pathosis
  - germ-free rats do not develop periapical lesions
  - teeth with lesions harbor bacteria in their canals
  - Kakehashi 1965, Sundqvist 1976

- Reduction of bacteria via endodontic therapy
  - chemo-mechanical preparation
  - Byström 1981

- Prognosis depends on antimicrobial efficacy
  - positive culture correlates with less healing
  - Sjögren 1997
Successful Disinfection

Pre-operative radiographs

Recalls 6 m 9 m

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

Introduction
Physiology
Microbiology
Regeneration

Whitworth 2000
Host Response

- Innate immunity
  - Recruitment of leukocytes into periapical spaces
  (lymphocyte/macrophage/dendritic cell interaction)

- PMN
- Macrophage

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

T-Cell Subclasses

- Lymphocyte differentiation based on surface markers

<table>
<thead>
<tr>
<th>Markers</th>
<th>Effects</th>
</tr>
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<tbody>
<tr>
<td>Th0</td>
<td>CD4+</td>
</tr>
<tr>
<td>Th1</td>
<td>CD4+ INF-γ, IL-2</td>
</tr>
<tr>
<td>Th2</td>
<td>CD4+ IL-4, 5, 10, 13</td>
</tr>
<tr>
<td>T17</td>
<td>CD4+ IL-17</td>
</tr>
<tr>
<td>Treg</td>
<td>CD4+, CD25+ Foxp3+, IL-10</td>
</tr>
<tr>
<td>Ts</td>
<td>CD8+</td>
</tr>
<tr>
<td>Tc</td>
<td>CD8+</td>
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Interactions between immune system and mesenchymal stem cells in dental pulp and periapical tissues

J. G. Leprince 1,2,3, B. D. Zeitlin 4, M. Tolar 5 & O. A. Peters 1

1Department of Endodontics, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA; 2School of Dentistry and Stomatology, Université catholique de Louvain, Brussels, 3CRIBIO (Center for Research and Engineering on Biomaterials), Brussels, Belgium; 4Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA; and 5Department of Orthodontics, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, USA

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.

Correspondence: Julian Gregoire Leprince, School of Dental Medicine and Stomatology, Université catholique de Louvain, Avenue Hippocrate, 10/5721, Brussels 1200, Belgium (e-mail: julian.leprince@uclouvain.be).

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

  Sussman 1993

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

  Margelos 1995

Apical Lesions & Implants
Normal Pulpal Histology

- Specific cellular arrangement in layers
  - stroma consists of fibroblasts, vessels, nerves

Blood Vessels

Nerve Fibers (Elephant)

Nair 1995
Weissengruber 2005
Pulpal Nerve Supply

- Sensory (Aδ fibers)
  - fast pain, sharp (small myelinated fibers)
  - reaction to cold under normal conditions
  - delivery of neuropeptides

- Sensory (c-fibers)
  - slow pain, dull burning (unmyelinated)
  - reaction to cold/warm (are sensitized by inflammation)

- Others
  - sympathetic (unmyelinated): control of blood flow, (+?)
  - Aβ (larger myelinated): function unclear, pain

Etiology of Pulpal Disease

- Caries
  - dental plaque: bacteria, acid and destruction of enamel and dentin

- Mechanical trauma
  - avulsion, luxation and concussion
  - disruption of blood supply

- Thermal injury
  - high-speed preparation without water
  - coagulation begins at app. 47°C

Loss of Blood Supply

- Sterile necrosis
  - local problems
  - surgical intervention
  - typically: avulsion
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 2mins 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**

![Graph showing MMP-9 Content](image)

**Pulp Testing Study**

- **Sensitivity / Specificity**
  - 3 symptomatic pulps were actually necrotic
  - better tests are needed to enhance outcomes

- **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.

**Anti-Inflammation**

- Suppression of cytokine signaling SOCS

![Diagram of anti-inflammation mechanisms](image)

*from Menezes 2008*
Pulpal Histology After Injury

- Response to injury: hard tissue deposition
  - contribution of various factors

TGFβ

- Localization
  - radicular dentin (accessible in RCT)
  - coronal dentin (accessible below carious lesion)

- 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
Introduction

Physiology

Microbiology

Regeneration

Immunocompetent Cells

PMNs
- are abundant in peripheral blood and react to stimuli by exiting blood vessels along chemotactic gradient
- kill bacteria at the spot

Lymphocytes (few)
- T-lymphocytes
- B-lymphocytes

Dendritic cells and their role
- accumulate, mature and migrate into regional lymph nodes to become interdigitating cells
- stimulate naive CD4+ T-lymphocytes to elicit a primary immune response

ECM Extracted From Dentin

Graham et al, Biomaterials 2006, 27:2865-2873
Acquired Immunity: Pulpitis

- Antigen is detected by DC and also innate immunity (TLRs).
- T-cells travel to target tissues to combat antigen.

Lymph node: DCs activate naive T-cells

Receptors

- Spatial pattern fit
  - signal molecules (neurotransmitters, interleukines)
  - self recognition (bacterial antigens, HLA)
  - organization of tissues (integrins, VCAM, ICAM)

LPS Pattern Recognition

- LPS-Binding protein
- CD14
- GPI Anchor
- TLR 4
- adapter proteins
- activation of NFκB
- transcription of target genes
Toll-like Receptors

- Evolutionary very old mechanism of innate immunity

<table>
<thead>
<tr>
<th>TLR2</th>
<th>peptidoglycan, lipoteichoic acid</th>
<th>Signal through NFκB, TRAF6</th>
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<tbody>
<tr>
<td>TLR4</td>
<td>LPS</td>
<td></td>
</tr>
<tr>
<td>TLR5</td>
<td>flagellin</td>
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</tr>
<tr>
<td>TLRs 6, 9, 11..</td>
<td>LTA, virus, CpG-DNA</td>
<td></td>
</tr>
</tbody>
</table>

Trigeminal nociceptors express TLR-4 and CD14: a mechanism for pain due to infection. J Dent Res, 85: 49-53

**Aim**
- to investigate if Gram- bacteria can directly activate nociceptors

**Methods**
- human trigeminal ganglia were collected max 7h post mortem; healthy and inflamed pulps were collected after splitting teeth directly after extraction
- rat trigeminal ganglia were frozen and prepared for immunohistochemistry along with the human preparations; double staining with fluorescent markers was performed to detect co-localization of TLR-4 & CD14 with TRPV1 & N52
- fluorescent staining was visualized in a confocal laser scanning microscope

- Double immunostaining for co-localization studies
  - inflamed human pulp
Cell Biology: Conclusions

- Disease mechanisms
  - there is much to be learned about basic disease mechanisms in development of periapical lesions, which may be transferrable to other diseases

- Role of pattern recognition
  - conserved structures like LPS and receptors recognizing it play very important roles in bone resorption
  - potential target for analgesics, anti-inflammatory drugs

- How about healing?
  - what we are really interested in is the deposition and not the resorption of bone
  - regenerative medicine/endodontics/DPSCs

Compromised Patients

- Innate vs. specific immune system
  - lesion induction depends on bacteria but equally on the potential for PNM to migrate (LAD-syndromes)
    - Kawashima 1999

- HIV+, AIDS patients
  - in oral surgery model, no healing problems
  - in endodontic outcomes, no healing problems
    - Quesnell 2005
    - Alley 2008

- Diabetes
  - healing may be delayed
  - evidence is not very strong at this point
    - Fouad 1997

Bisphosphonates

- Extremely frequently prescribed
  - cancer, osteoporosis
  - i.v. and oral applications, very safe in general

- Oral issues
  - ONJ, BRON: mandible preferred
  - bone necrosis after seemingly minimal trauma
  - periodontal bone loss is delayed

- Mechanism of action
  - interferes with angiogenesis
  - inhibits enzymes leading to osteoclast apoptosis
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

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!

E. faecalis?

S. Erlandsen, U Minn