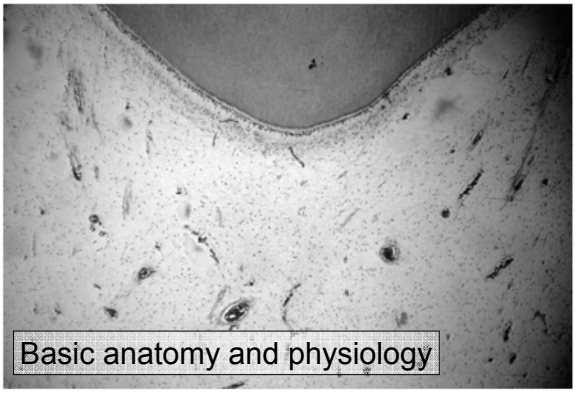
Pulp responses

Dag Ørstavik
UiO Core Curriculum II
Oral Biology
2009
www.uio-endo.no



Types of Dentin

The formation of primary dentin continues until the tooth becomes functional (Linde & Goldberg 1993) or until the root apex is closed (Torneck 1994). Thereafter dentin formation proceeds as secondary dentinogenesis, which continues at a slower rate than the primary dentinogenesis during the life-time of the individual.

- **Dentin**
  - Primary physiologic dentin
    - Mantle dentin
    - Circumpulpal dentin and predentin
    - Peritubular dentin
    - Intertubular dentin
  - Secondary physiologic dentin
  - Tertiary dentin or reparative dentin or reactionary dentin and/or irregular secondary dentin

The outer layer of primary dentin, which is synthesised at the onset of dentinogenesis, is called mantle dentin. Mantle dentin is slightly less mineralized than other layers of the primary dentin *i.e.* circumpulpal dentin.

Calcospherites – globular and interglobular dentin

http://herkules.oulu.fi/isbn9514270355/html/i259726.html
Mjør & Heyeraas 2008

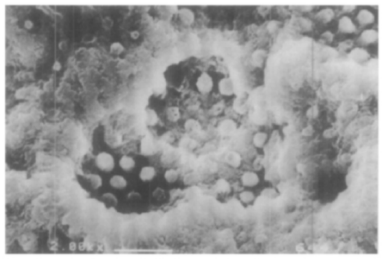


Fig. 3. Replica of calcospherites on surface. Note semi-globular concavities with several small projections and compare these morphologic features with Fig. 13, **A.** (Original magnification, ×2000.)

Wakabayashi et al 1992

Tertiary dentin (reactionary or reparative or irregular secondary dentin) is the outcome of odontoblastic response to irritation occurring mainly during secondary dentinogenesis and is caused by dental abrasion, attrition, cavity preparation, erosion or dental caries (Torneck 1994). Lesot *et al.* (1993) defines

reactionary dentin to be the result of irritation of post-mitotic odontoblasts,

whereas

reparative dentin is formed by odontoblasts or odontoblast-like cells which differentiate from pulp cells after the cell death of primary odontoblasts
(Magloire *et al.* 1992, Magloire *et al.* 1996).

http://herkules.oulu.fi/isbn9514270355/html/i259726.html

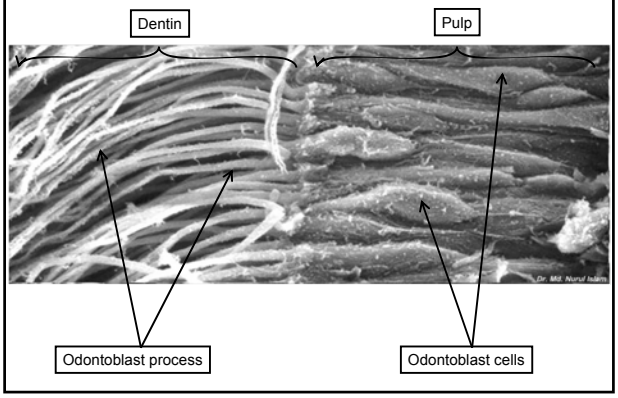
Continued intratubular mineralization of dentin occurs as an age change and may result in complete obturation of the tubules .. This process may be accelerated by external stimuli of various types, including certain restorative materials.

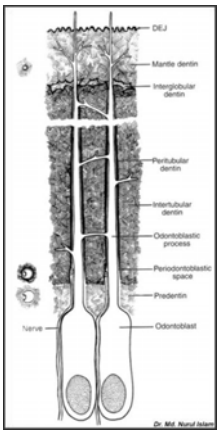
Another type of intratubular mineralization includes precipitation of mineral salts within the tubules, for example, as found in the “transparent zone” of dentin subjacent to a slowly progressing caries lesion.

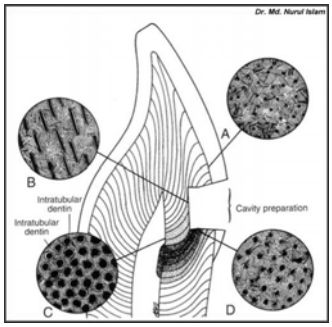
Both types of intratubular remineralizations are collectively referred to as *sclerotic dentin.*

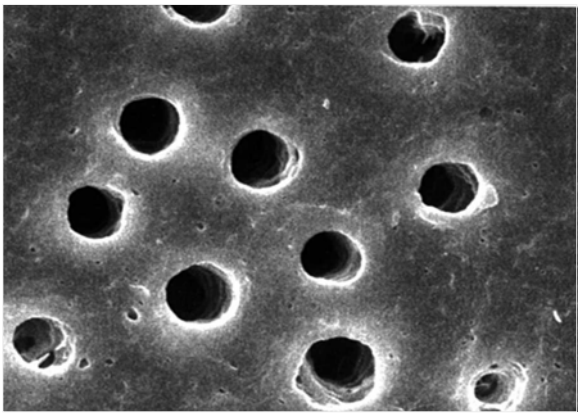
Mjør & Heyeraas in Essential Endodontology, 2008

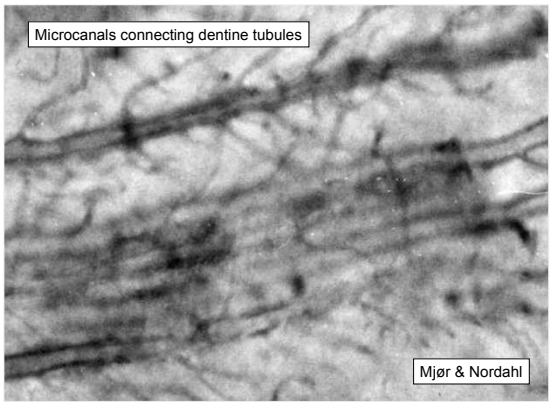
Odontoblasts and process

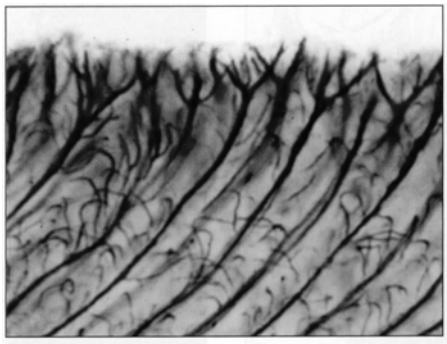












1.7 High-power view of lateral communication between dentinal tubules

Stock et al.

Dentin penetration: to and from the pulp

'the three (mechanims of protection by dentin) described:

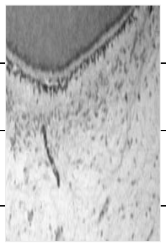
1) diffusion limitation;
2) limited wetness for hydrolysis; and
3) buffering by dentinal hydroxyapatite,

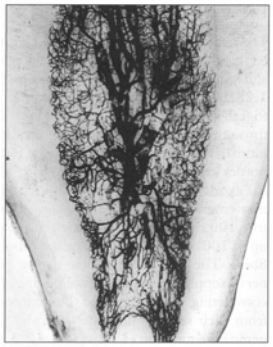
appear to allow the relatively safe use of a wide range of tooth restorative materials'

Influence of dentine on the pulpward release of eugenol or acids from restorative materials. *Hume WR*, J Oral Rehabil 1994;**21**(4):469-73

1) Microbial pathways in tubules
2) Antigenic diffusion in all directions

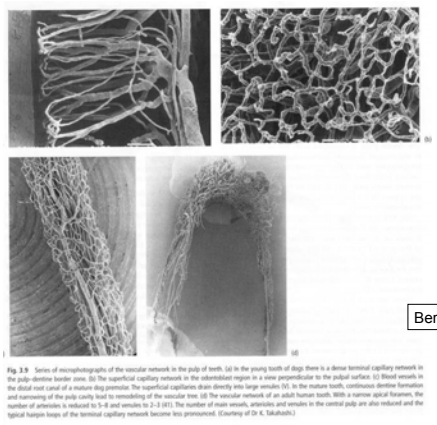
Microscopic Zones in Pulp

Zones-from outer to inner zone	Description
Odontoblastic layer 	Lines the outer pulpal wall and consists of the cell bodies of odontoblast. Secondary dentin may form in this area from the apposition of odontoblast.
Cell-free zone	Fewer cells than odontoblastic layer. Nerve and capillary plexus located here
Cell-rich zone	Increased density of cells as compared to cell-free zone and also a more extensive vascular system
Pulpal-core	Located in the center of the pulp chamber, which has many cells and an extensice vascular supply, similar to cell-rich zone



1.20 High-power view of network of blood vessels in the pulp (courtesy of Prof. I Kramer)

Stock et al.

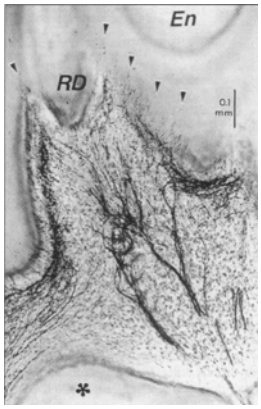


Bergenholtz et al.

A B

Castellucci

Fig. 7.1. **A**. Blood vessels in a healthy, adult pulp. **B**. Detail (courtesy of Dr. N. Perrini).



CGRP nerve fibers branching peripherally and into dentin, but avoiding reactionary dentin. (Byers et al 1990)

Stock et al.

ABSTRACT

Recent findings have indicated that immune responses are subjected to modulation by the sympathetic nervous system (SNS). Moreover, the findings show that the SNS inhibits the production of pro-inflammatory cytokines, while stimulating the production of anti-inflammatory cytokines. The present review is an attempt to summarize the current results on how the SNS affects inflammation in dental tissues. In dental tissues, it has been found that the SNS is significant for recruitment of inflammatory cells such as CD 43+ granulocytes. Sympathetic nerves appear to have an inhibitory effect on osteoclasts, odontoclasts, and on IL-1α production. The SNS stimulates reparative dentin production, since reparative dentin formation was reduced after sympathectomy. Sprouting of sympathetic nerve fibers occurs in chronically inflamed dental pulp, and neural imbalance caused by unilateral sympathectomy recruits immunoglobulin-producing cells to the dental pulp. In conclusion, this article presents evidence in support of interactions between the sympathetic nervous system and dental inflammation.

Haug & Heyeraas 2006

ABSTRACT

Recent findings have indicated that immune responses are subjected to modulation by the sympathetic nervous system (SNS). Moreover, the findings show that the SNS inhibits the production of pro-inflammatory cytokines, while stimulating the production of anti-inflammatory cytokines. The present review is an attempt to summarize the current results on how the SNS affects inflammation in dental tissues. In dental tissues, it has been found that the SNS is significant for recruitment of inflammatory cells such as CD 43+ granulocytes. Sympathetic nerves appear to have an inhibitory effect on osteoclasts, odontoclasts, and on IL-1α production. The SNS stimulates reparative dentin production, since reparative dentin formation was reduced after sympathectomy. Sprouting of sympathetic nerve fibers occurs in chronically inflamed dental pulp, and neural imbalance caused by unilateral sympathectomy recruits immunoglobulin-producing cells to the dental pulp. In conclusion, this article presents evidence in support of interactions between the sympathetic nervous system and dental inflammation.

CD43 is a cell surface-**associated mucin** that is abundantly expressed by most **leukocytes**, and that appears to function as a negative regulator of cell surface interactions, providing a repulsive barrier around cells. [1995]

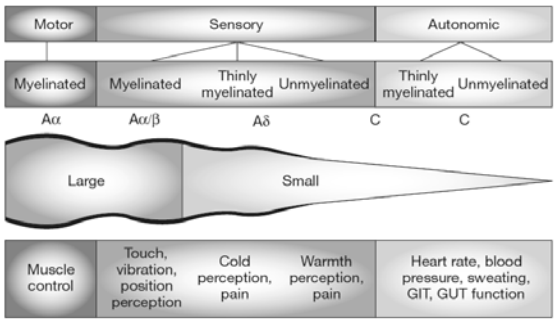
IL-1α and IL-1β

Both IL-1α and IL-1β are produced by macrophages, monocytes and dendritic cells. They form an important part of the inflammatory response of the body against infection. These cytokines increase the expression of adhesion factors on endothelial cells to enable transmigration of leukocytes, the cells that fight pathogens, to sites of infection and re-set the hypothalamus thermoregulatory center, leading to an increased body temperature which expresses itself as fever. IL-1 is therefore called an endogenous pyrogen. The increased body temperature helps the body's immune system to fight infection. IL-1 is also important in the regulation of hematopoiesis. IL-1β production in peripheral tissue has also been associated with hyperalgesia (increased sensitivity to pain) associated with fever.[6]

IL-1α and IL-1β

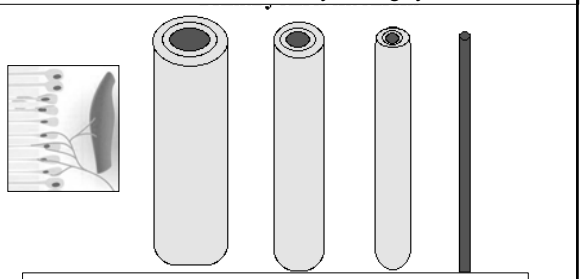
For the most part, these two forms of IL-1 bind to the same cellular receptor. This receptor is composed of two related, but non-identical, subunits that transmit intracellular signals via a pathway that is mostly shared with certain other receptors. These include the Toll family of innate immune receptors and the receptor for IL-18. IL-1α …. is produced by many cell types but is only secreted by monocytes and macrophages.

Aδ- og C-fibrenes funksjon



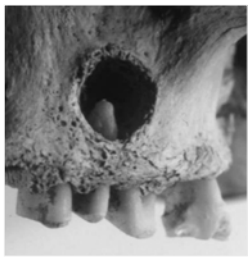
Vinik A et al, *Nature Clinical Practice*, 2006

Perifere nervefibre – tykke og tynne

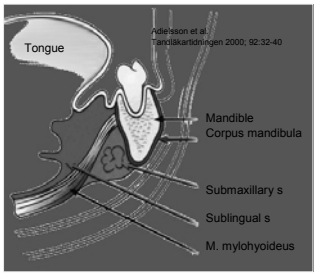


Axon type	Aα	Aβ	Aδ	C
Diameter (μm)	13-20	6-12	1-5	0.2-1.5
Hastighet (m/s)	80-120	35-75	5-35	0.5-2.0
Forekomst		1	:	4

PC from K. Ørstavik 2007



Pulp protection is prevention of apical periodontitis and spread of oral infection

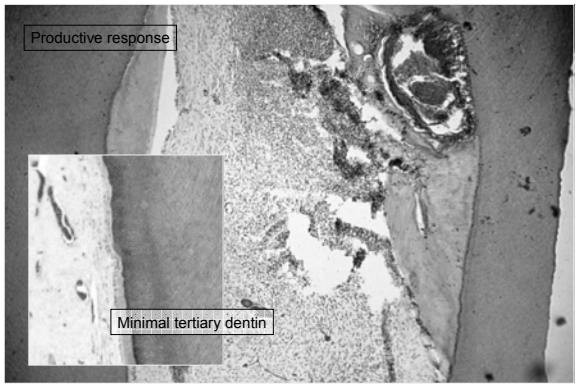


Responses of the Pulp

- Productive
- Nervous
- Cellular
- Vascular
- Inflammatory
- Degenerative
- (Molecular mechanisms)

Normal and pathological responses

- Normal:
  - Secondary and reactionary dentin formation
  - Pain reactions
- Pathological:
  - Tertiary dentin formation
  - Acute inflammation & pain
  - Chronic inflammation & pain
  - (Productive response)



Int. J. Dev. Biol. 39: 273-280 (1995)

Reactionary dentinogenesis

ANTHONY J. SMITH[1]*, NICOLA CASSIDY[1], HELEN PERRY[1], CATHERINE BÈGUE-KIRN[2], JEAN-VICTOR RUCH[2] and HERVÉ LESOT[2]

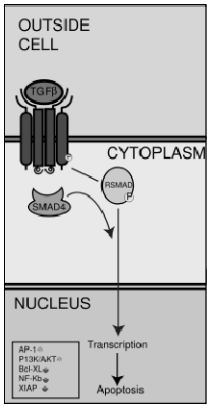
[1]Oral Biology, School of Dentistry, University of Birmingham, Birmingham, United Kingdom and [2]Institut de Biologie Médicale, Faculté de Médecine, Strasbourg, France

ABSTRACT Reactionary dentinogenesis is the secretion of a tertiary dentine matrix by surviving odontoblast cells in response to an appropriate stimulus. Whilst this stimulus may be exogenous in nature, it may also be from endogenous tissue components released from the matrix during pathological processes. Implantation of isolated dentine extracellular matrix components in unexposed cavities of ferret teeth led to stimulation of underlying odontoblasts and a response of reactionary dentinogenesis. Affinity chromatography of the active components prior to implantation and assay for growth factors indicated that this material contained significant amounts of TGF-ß$_1$, a growth factor previously shown to influence odontoblast differentiation and secretory behavior. Reactionary dentinogenesis during dental caries probably results from solubilization of growth factors, TGF-ß in particular, from the dentine matrix which then are responsible for initiating the stimulatory effect on the odontoblasts. Compositional differences in tertiary dentine matrices beneath carious lesions in human teeth have also been shown indicating modulation of odontoblast secretion during reactionary and reparative dentinogenesis.

KEY WORDS: *dentinogenesis, extracellular matrix, odontoblasts, growth factors, dental caries*

Reactionary dentinogenesis during dental caries may result from the solubilization of growth factors, ***transforming growth factor-beta (TGF-beta),*** from the dentin matrix which initiate the stimulation of odontoblasts (Smith *et al.* 1995, Sloan *et al.* 2000a). It has been demonstrated that TGF-beta 1 and beta 3 can stimulate secretion of extracellular matrix by odontoblasts, are mitogenic to pulp cells, and that TGF-beta 3 may have inductive effects on pulpal cells (Sloan & Smith 1999). Recent studies show that dentin and bone matrix contain various ***angiogenic growth factors*** (Roberts-Clark & Smith 2000), ***bone morphogenic proteins*** (Sloan *et al.* 2000b), ***bone sialoproteins and osteopontin*** (Qin *et al.* 2001), which may be beneficial to the reparative response of the dentin-pulp complex.

beta-defensin-2
macrophage inflammatory protein-3alpha



Transforming growth factor beta (TGF beta) is a biological protein. …. TGF beta controls proliferation, differentiation, and other functions in most cell types. It can also act as a negative autocrine growth factor.

Shiba H, Mouri Y, Komatsuzawa H, Ouhara K, Takeda K, Sugai M, Kinane DF, Kurihara H.

Macrophage inflammatory protein-3alpha and beta-defensin-2 stimulate dentin sialophosphoprotein gene expression in human pulp cells.
[ie, including odontoblasts]

Biochem Biophys Res Commun. 2003 Jul 11;306(4):867-71

Macrophage Inflammatory Proteins (MIP) belong to the family of chemotactic cytokines known as chemokines. In humans, there are two major forms, MIP-1α and MIP-1β that are now officially named CCL3 and CCL4 respectively. Both are major factors produced by macrophages after they are stimulated with ***bacterial endotoxins.[***1] They activate human granulocytes (neutrophils, eosinophils and basophils) which can ***lead to acute neutrophilic inflammation***. They also induce the synthesis and release of other pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6 and TNF-α from fibroblasts and macrophages. The genes for CCL3 and CCL4 are both located on human chromosome 17.[2]

Wikipedia

Shiba H, Mouri Y, Komatsuzawa H, Ouhara K, Takeda K, Sugai M, Kinane DF, Kurihara H.

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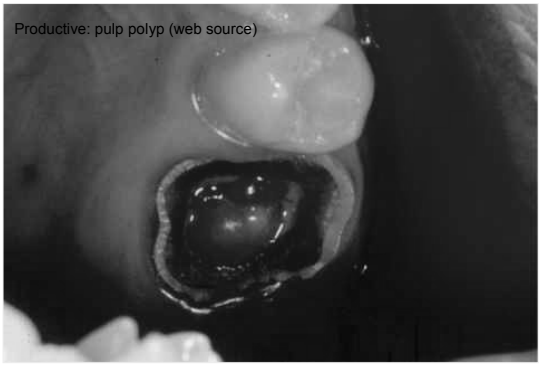
Defensins are small (29-51 residue) cysteine-rich cationic proteins found in both vertebrates and invertebrates. They are active against bacteria, fungi and enveloped viruses. They consist of 28-42 amino acids including six to eight conserved cysteine residues. Cells of the immune system contain these peptides to assist in killing phagocytized bacteria, for example in neutrophil granulocytes and almost all epithelial cells. Most defensins function by penetrating the microbial's cell membrane by way of electrical attraction, and once embedded, forming a pore in the membrane which allows efflux.

Wikipedia



Productive: pulp polyp (web source)

Dentin (hyper)sensitivity

- Pain elicitation
- Differential character
- Mechanisms
- Treatment

Nervous response

The hydrodynamic theory

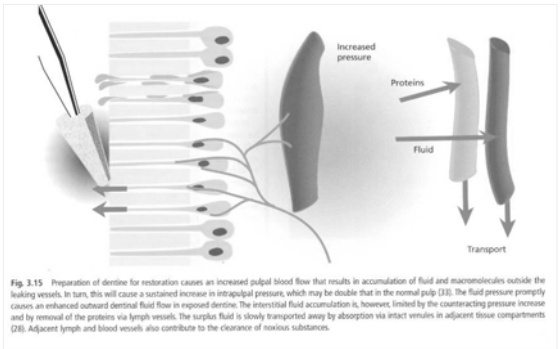


Fig. 3.15 Preparation of dentine for restoration causes an increased pulpal blood flow that results in accumulation of fluid and macromolecules outside the leaking vessels. In turn, this will cause a sustained increase in intrapulpal pressure, which may be double that in the normal pulp (33). The fluid pressure promptly causes an enhanced outward dentinal fluid flow in exposed dentine. The interstitial fluid accumulation is, however, limited by the counteracting pressure increase and by removal of the proteins via lymph vessels. The surplus fluid is slowly transported away by absorption via intact venules in adjacent tissue compartments (28). Adjacent lymph and blood vessels also contribute to the clearance of noxious substances.

Bergenholtz et al.

Table 3. Treatment Options

1. Desensitize the nerve
    a. potassium nitrate
2. Cover the dentinal tubules
    a. periodontal surgery/grafting
    b. composite/glass ionomer restoration
    c. crown placement
    d. plug (sclerose) the dentinal tubules
        1. ions/salts
            a. stannous fluoride
            b. sodium fluoride/stannous fluoride combination
            c. potassium oxalate
            d. ferrous oxide
            e. strontium chloride
            f. in combination with an adhesive
        2. precipitates - proteins/amino acids
            a. glutaraldehyde
        3. resins
            a. dentin sealers
            b. methyl methacrylate

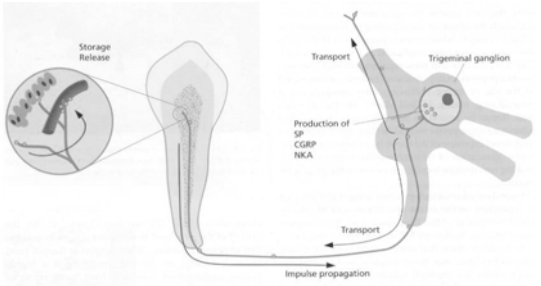


Fig. 3.8 A large portion of the sensory fibers, including C-fibers and some A-delta fibers, contain vasoactive neuropeptides such as calcitonin gene-related peptide (CGRP), substance P (SP) and neurokinin A (NKA) (83). The neuropeptides are produced in the trigeminal cell bodies and are transported via axonal flow to the nerve terminals in the pulp, where they are stored. In addition to their effect on pulpal blood flow and vessel permeability, SP and CGRP exert stimulatory effects on the growth of pulpal cells, such as fibroblasts (79), and repair odontoblasts. They are also active in the recruitment of immunocompetent cells in responses to bacterial infection.

Vasoactivity by nervous stimulation

Bergenholtz et al.

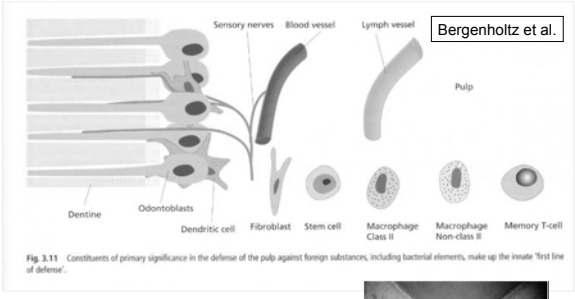
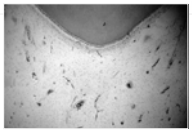
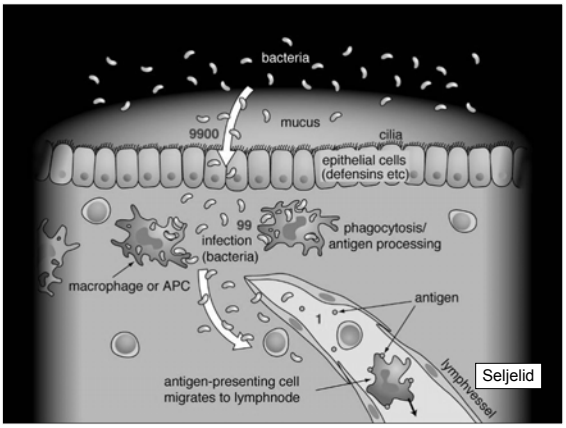


Fig. 3.11 Constituents of primary significance in the defense of the pulp against foreign substances, including bacterial elements, make up the innate 'first line of defense'.

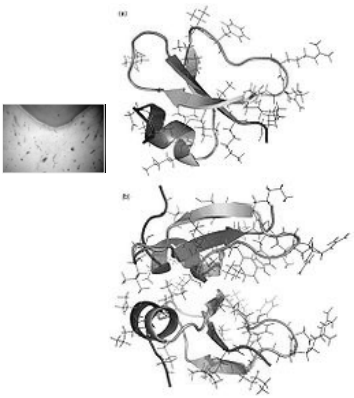
Bergenholtz et al.

Important cellular and vascular components in pulp defense reactions





Seljelid



Wikipedia

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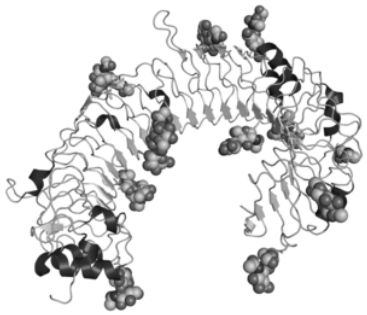
Wikipedia

Toll-like receptors (**TLRs**) are a class of proteins that play a key role in the innate immune system. They are single membrane-spanning non-catalytic receptors that recognize structurally conserved molecules derived from microbes. Once these microbes have breached physical barriers such as the skin or intestinal tract mucosa, they are recognized by TLRs which activates immune cell responses.

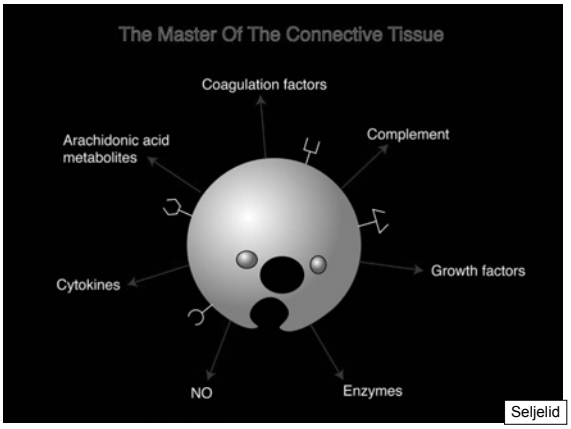
Wikipedia

In all, thousands of genes are activated by TLR signaling, and collectively, the TLRs constitutes one of the most pleiotropic yet tightly regulated gateways for gene modulation.



Macrophages
&
dendritic cells

Wikipedia



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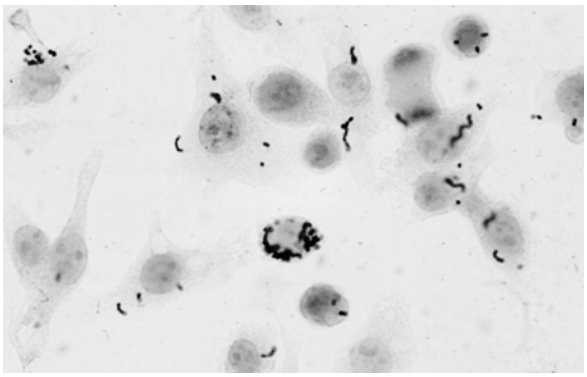


Macrophages processing *Enterococcus faecalis in vitro*

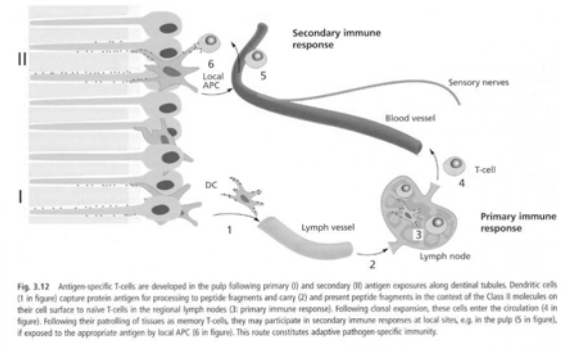
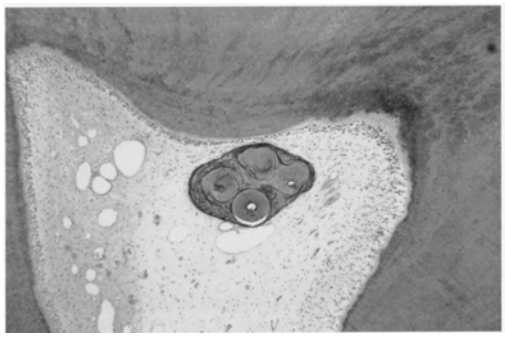


Fig. 3.12 Antigen-specific T-cells are developed in the pulp following primary (I) and secondary (II) antigen exposures along dentinal tubules. Dendritic cells (1 in figure) capture protein antigen for processing to peptide fragments and carry (2) and present peptide fragments in the context of the Class II molecules on their cell surface to naive T-cells in the regional lymph nodes (3: primary immune response). Following clonal expansion, these cells enter the circulation (4 in figure). Following their patrolling of tissues as memory T-cells, they may participate in secondary immune responses at local sites, e.g. in the pulp (5 in figure), if exposed to the appropriate antigen by local APC (6 in figure). This route constitutes adaptive pathogen-specific immunity.

Secondary response

Bergenholtz et al.



Degenerative processes: pulp stones and calcifications

Castellucci

Normal and pathological stimuli

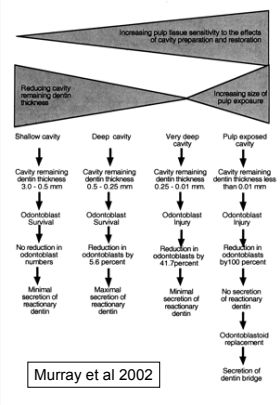
- Age and use, normal wear
- Pathological:
  - ***Attrition*** (*"normal" tooth on tooth*: the act of wearing or grinding down by friction), erosion (to eat into or away by slow destruction of substance (*chemical*: as by acid, infection, or cancer)), ***abrasion*** (*pathological mechanical*: a wearing, grinding, or rubbing away by friction), gingival recession
  - Caries and infection
  - Mechanical: orthodontics
  - Mechanical: preparation
  - Chemicals
  - "micro-leakage"; "nano-leakage"

Normal and pathological stimuli

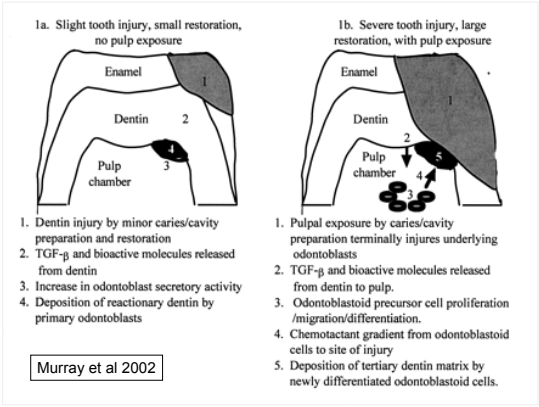
- Age and use, normal wear
- Pathological:
  - Attrition, erosion, abrasion, recession
  - Caries and infection
  - Mechanical: orthodontics (EGF released following orthodontic force application plays a part in the ***angiogenic*** response of the pulp; SP stimulates the production of PGE2 and RANKL and promoted bone resorption, and may be involved in ***pulpal inflammation and root resorption*** during orthodontic tooth movement)
  - Mechanical: preparation
  - Chemicals: medicaments, dental materials' components
  - "micro-leakage"; "nano-leakage"

Normal and pathological stimuli

- Age and use, normal wear
- Pathological:
  - http://crobm.iadrjournals.org/cgi/content/full/13/6/509
- ANALYSIS OF PULPAL REACTIONS TO RESTORATIVE PROCEDURES, MATERIALS, PULP CAPPING, AND FUTURE THERAPIES. Peter E. Murray*, L. Jack Windsor, Thomas W. Smyth, Abeer A. Hafez, Charles F. Cox



Murray et al 2002



Murray et al 2002

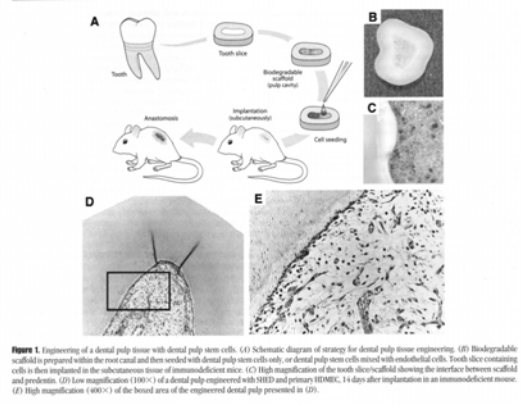


Figure 1. Engineering of a dental pulp tissue with dental pulp stem cells. (A) Schematic diagram of strategy for dental pulp tissue engineering. (B) Biodegradable scaffold is prepared within the root canal and then seeded with dental pulp stem cells only, or dental pulp stem cells mixed with endothelial cells. Tooth slice containing cells is then implanted in the subcutaneous tissue of immunodeficient mice. (C) High magnification of the tooth slice/scaffold showing the interface between scaffold and predentin. (D) Low magnification (100×) of a dental pulp engineered with SHED and primary HDMEC, 14 days after implantation in an immunodeficient mouse. (E) High magnification (400×) of the boxed area of the engineered dental pulp presented in (D).

Cordeiro et al 2008

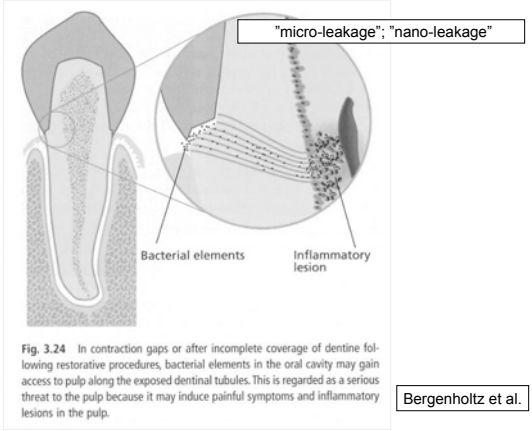
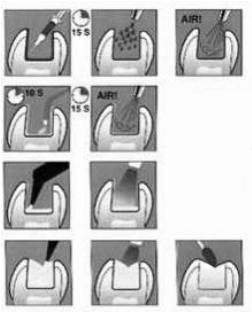


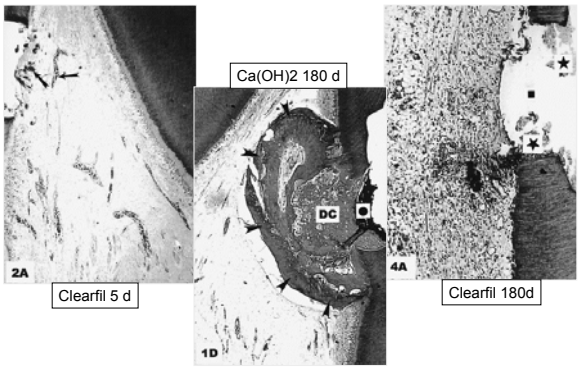
Fig. 3.24 In contraction gaps or after incomplete coverage of dentine following restorative procedures, bacterial elements in the oral cavity may gain access to pulp along the exposed dentinal tubules. This is regarded as a serious threat to the pulp because it may induce painful symptoms and inflammatory lesions in the pulp.

Bergenholtz et al.



Total etch issues:

pulp damage or complete control?



Response of human pulps capped with a self-etching adhesive system. C. A. de Souza Costa, A. B. Lopes do Nascimento, H. M. Teixeira and U. F. Fontana. Dental Materials 2001