

Apoptosis: an introduction for the endodontist

P. G. Satchell¹, J. L. Gutmann² & D. E. Witherspoon³

¹Private Practice Limited to Endodontics, Houston, ²Private Practice Limited to Endodontics, Dallas, and ³Private Practice Limited to Endodontics, Plano, Texas, USA

Abstract

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Apoptosis plays an important role in many aspects of endodontics, yet there is a paucity of information in this regard in the endodontic literature. Apoptosis is a single deletion of scattered cells by fragmentation into membrane-bound particles that are phagocytosed by other cells. It is a key process in the embryological development of the tooth, periodontal ligament and supporting oral tissue in the progression of oral disease, bone resorption,

immunological response and inflammation, and in wound healing and certain pharmacological effects. The understanding of the ability of clinical materials to induce or inhibit apoptosis and the investigation of apoptosis as it relates to the pathogenesis of pulpal and periradicular pathology may eventually lead to new treatment approaches for the endodontist. The purpose of this review is to familiarize the clinical endodontist with current knowledge on apoptosis as it relates to the pulp and periradicular tissues.

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Introduction

Apoptosis is essentially cell suicide, and was first described in the *British Journal of Cancer* in 1972 (Kerr *et al.* 1972). According to these authors, at the appropriate time and under certain conditions, cells self-destruct without damaging adjacent cells. This process is occurring constantly and can happen to seemingly healthy cells. Whilst it is unique to animals and is studied classically in the nematode worm *Caenorhabditis elegans*, it has been increasingly studied *in vitro* and in higher organisms.

There has been an explosion in the number of articles on the subject of apoptosis. A conservative estimate places the number at nearly 45 000. Whilst apoptosis has been a topic of extensive research in the scientific community for some time, it has received little attention in the endodontic literature. The purpose of this article is to familiarise the endodontist with apoptosis and to discuss some of the potential implications of apoptosis to endodontics.

Why and how is apoptosis important to endodontics?

Apoptosis plays a ubiquitous role in the body. Amongst other things, apoptosis is a key process in oral development (Vaahtokari *et al.* 1996, Harada *et al.* 1998, Cerri *et al.* 2000), the progression of oral disease (Polverini & Nor 1999), periradicular lesion development (Onishi *et al.* 1997), resorption (Hughes & Boyce 1997), immunological response and inflammation (Onishi *et al.* 1997), wound healing (Fanning *et al.* 1999) and in certain pharmacological effects (Hughes *et al.* 1995). The understanding of the ability of clinical materials to selectively induce or inhibit apoptosis is leading to new treatment modalities (Bamford *et al.* 2000). An understanding of the mechanisms of apoptosis may lead to adjunctive treatments for pulpal and periradicular diseases through yet unexplored pathways.

What is the apoptotic process?

The entire process of apoptosis takes about 1 h from initiation (Figs 1 and 2). The initiating triggers are many and varied, and are grouped broadly as physiological or nonphysiological. These include, but are not limited to,

Correspondence: Dr James L. Gutmann, 1416 Spenwick Terrace, Dallas, Texas, TX 75204-5529, USA (Tel.: +1 214 827 5378; fax: +1 214 827 4848; e-mail: jlgutmann@earthlink.net).

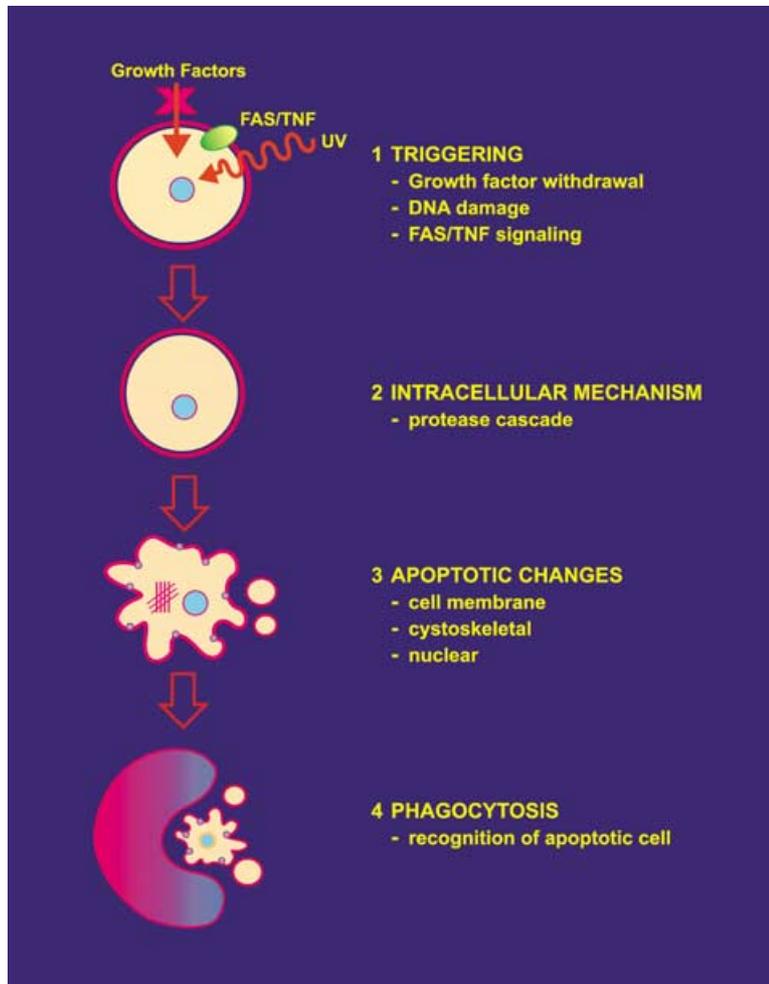


Figure 1 The major stages of apoptosis include the initiating trigger (1) that leads to the activation of the intracellular mechanism for the apoptotic process (2). Morphological changes of the cell (3) include cell membrane changes that signal phagocytic cell recognition and elimination (4) without promoting inflammation.

the following: Fas ligands (Fas), tumour necrosis factor (TNF), nerve growth factor (NGF), nitric oxide (NO), lipopolysaccharide (LPS), host immune reactions, kinins and glucocorticoids (McKenna *et al.* 1998). The best characterised apoptotic trigger is the Fas ligand, a member of the TNF super-family. The Fas receptor is a cell surface glycoprotein that mediates apoptotic signals from the cell surface into the cytoplasm (Yoshioka *et al.* 1996). When the Fas ligand binds to the Fas receptor on the cell membrane, the newly formed Fas complex is allowed to associate with intracellular proteins. The morphological changes of specific intracellular proteins induced by this complex result in the activation of other substances such as IL-1 β converting enzyme (ICE).

In this particular mechanism, and there are many, Procaspase (inactive form) is activated to the protease Caspase. An amplification cascade then ensues with Caspases activating other Caspases, eventually cleaving the host cell by acting on a variety of cell structures such

as the nuclear membrane (Nicholson & Thornberry 1997). The cell shrinks in the process and there is a loss of cell-cell junctions resulting in detachment from adjacent cells. The chromatin condenses, the cytoplasm 'blebs' (forms so-called 'pseudopods') and the cell breaks up into fragments known as 'apoptotic bodies'. Indirectly activated endonucleases lead to breakdown of the DNA (Kerr *et al.* 1972) into multiples of 180–200 base pair fragments (Cohen & Duke 1984). Finally, either macrophages or adjacent cells phagocytose the apoptotic bodies (Kerr *et al.* 1972) (Figs 1 and 2).

How does necrosis compare to apoptosis?

Cells usually die either by necrosis or apoptosis. The characteristics of apoptotic death are more clearly understood when compared to the characteristics of necrotic death (Table 1). Necrosis is a pathological death of cells resulting from irreversible damage that occurs

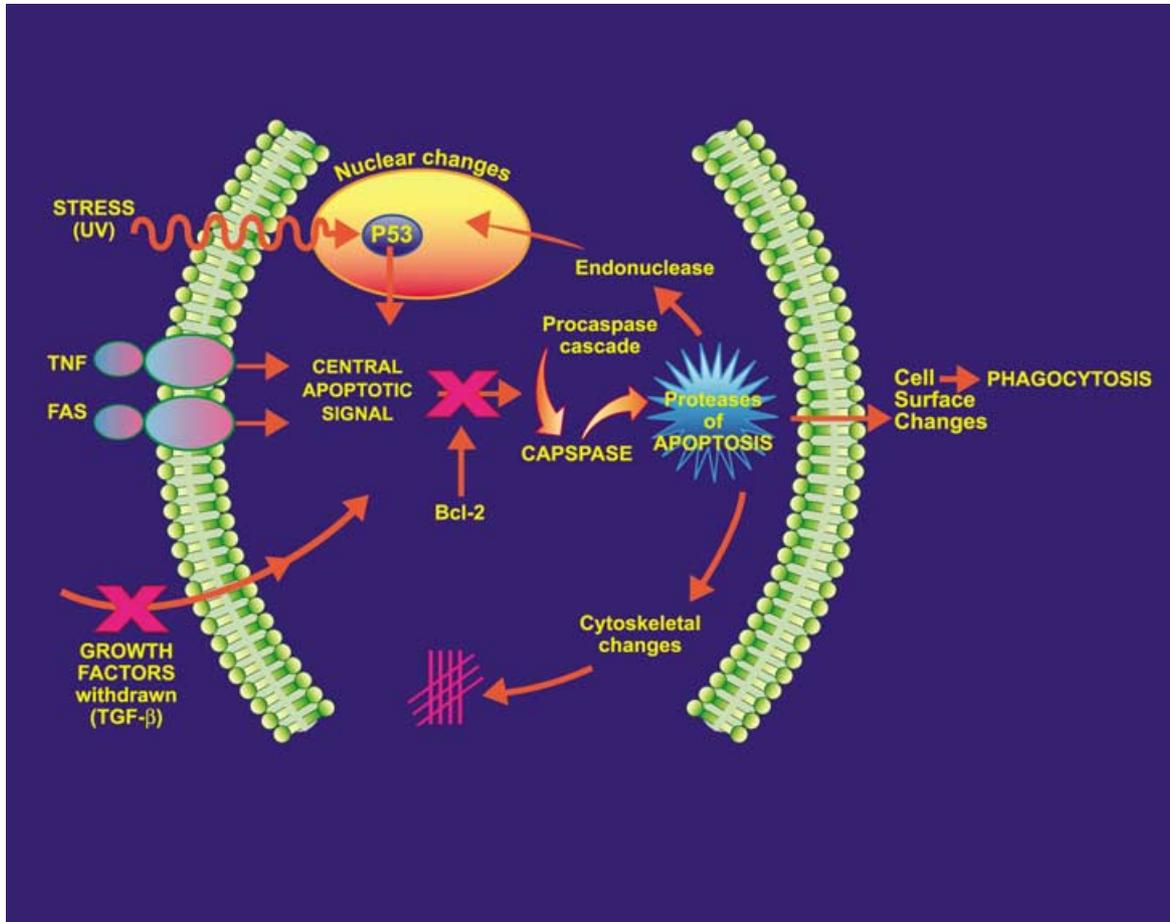


Figure 2 Various initiating triggers effect the activation of a central apoptotic signal. This central signal that can be blocked by bcl-2 leads to the activation of proteolytic enzymes via the caspase cascade. Those enzymes lead to morphological changes in the cell structures including the nucleus, cytoskeleton and membrane. Cell membrane changes include expression of proteins for recognition by phagocytic cells.

in the dental pulp and is a term commonly used in pulpal diagnosis. The earliest irreversible changes are mitochondrial, consisting of swelling and granular calcium

Table 1 Comparison of classic features of apoptosis (programmed cell death) and necrosis (pathologic cell death) (Polverini & Nor 1999)

Apoptosis	Necrosis
Controlled process	Uncontrolled process
Energy dependent (ATP required)	No energy required (passive)
Cells shrink (apoptotic bodies)	Cells swell (lysis)
No loss of membrane integrity	Membrane integrity lost
Noninflammatory	Inflammatory
No scarring occurs	Scarring
Individual or small cell groups	Large cell groups (organ segments)
Nuclear fragmentation	Nuclear dissolution
Physiological stimuli	Pathologic stimuli

deposits. After such changes, the outlines of individual cells are indistinct and affected cells may become merged, sometimes forming a focus of coarsely granular, amorphous or hyaline material (Stedman 1995). These features include cell swelling, membrane lysis and an inflammatory response (Wyllie *et al.* 1980), and are distinctly different from the features of apoptosis described above.

What role does apoptosis play in development?

Apoptosis plays an important role in all stages of life. Developing human branchial arches, embryonic tails and finger webbing cannot resorb before birth without organised programmed cell death. In mature organisms, homeostasis is maintained by balancing the continuous

mitosis and differentiation of cells with the apoptotic process (Kerr *et al.* 1972).

Apoptosis has multiple roles in tooth development from the beginning of tooth formation to the completion of root development. There is evidence of apoptosis in the reduction of cells of the stellate reticulum, at the initiation of enamel formation (Vaahtokari *et al.* 1996, Baratella *et al.* 1999) and in the stratum intermedium (Bronckers *et al.* 1996, Vaahtokari *et al.* 1996). This process also occurs during the transition stage between secretion and maturation of ameloblasts during enamel formation (Nishikawa & Sasaki 1995). After enamel formation, approximately 25% of the ameloblasts die and following enamel matrix maturation, another 25% undergo apoptosis (Joseph *et al.* 1994). Apoptosis has been shown to occur around the crowns of teeth during tooth eruption (Bronckers *et al.* 1996, Kaneko *et al.* 1997), playing a major role in the elimination of reduced ameloblasts located at cusps (Shibata *et al.* 1995). It is thought that the inhibition of apoptosis in reduced ameloblasts may occur after the elimination of the tooth organ, allowing for the establishment of the junctional epithelium (Shibata *et al.* 1995). Whilst the precise mechanisms for these events remain largely undiscovered, some work has shown that bcl-2, an apoptotic inhibitor, is involved in maintaining the viability of the enamel organ during tooth development (Slootweg & de Weger 1994).

Fibroblast-like cells of the periodontal ligament (PDL) exhibit apoptosis during tooth development (Cerri *et al.* 2000), and apoptotic cells, probably osteoclasts, are also found on the surfaces of developing alveolar bone (Vaahtokari *et al.* 1996). Apoptosis is also responsible for at least partial elimination of the cells of Hertwig's epithelial root sheath (HERS) after root formation is complete (Kaneko *et al.* 1999, Cerri *et al.* 2000). Furthermore, cell rests of Malassez, which can proliferate to produce periradicular cysts, may be partially as a result of incomplete apoptosis of HERS.

What role does apoptosis play in the dental pulp?

Apoptosis is a part of normal pulp homeostasis (Nishikawa & Sasaki 1999), occurring more in the occlusal (incisal) than in the apical portions of the pulp (Vermelin *et al.* 1996, Nishikawa & Sasaki 1999). Most apoptotic cells in normal pulp can be found at the periphery and are usually associated with the subodontoblastic region rather than with the odontoblastic layer (Vermelin *et al.* 1996, Piattelli *et al.* 2001). Odontoblasts seem to compensate for the reduction in pulp chamber volume as a result of physiological (secondary) dentine

formation by odontoblastic layering rather than by cell death. Apoptosis is more evident in odontoblasts after injury to odontoblastic processes as seen with cavity preparation (Goldberg *et al.* 1994, Bronckers *et al.* 1996, Kitamura *et al.* 2001). There is evidence that reparative (tertiary) dentine formation, in response to injury, is associated with a large decrease in the number of odontoblasts. Up to half of the pulp odontoblasts can be eliminated in only 4 years by this process (Franquin *et al.* 1998). Fibroblasts and vascular endothelial cells of the pulp proper also show evidence of apoptosis (Franquin *et al.* 1998). Although certain characteristics of apoptotic death are favourable to necrosis (i.e. an inflammatory response is not triggered), apoptosis does not lead to pulp recovery. This may be due in part to the elimination of regenerative cells by apoptosis (Goldberg *et al.* 1994). Ultimately, when apoptosis occurs, apoptotic bodies and debris are phagocytosed by major histocompatibility Class II (MHC II) positive and negative macrophages (Nishikawa & Sasaki 1999).

What role does apoptosis play in other oral tissues?

Apoptosis seems to be necessary for maintaining homeostasis within continually renewing tissues such as the oral mucosa and skin (Funato *et al.* 1999). Gingival tissue has a high cell turnover, and apoptosis has been demonstrated to occur in this tissue in 90% of the individuals tested (Yoshioka *et al.* 1996). Here, as in other parts of the body, apoptosis is essentially a counterbalance to mitosis. Unfortunately, the role of apoptosis in the differentiation of oral epithelial cells is not clear (Harada *et al.* 1998), and more work is required to fully describe the events associated with this process in normal oral tissues as a baseline to further study.

What role does apoptosis play in bone?

In the process of bone remodelling, some osteoblasts die via apoptotic mechanisms, whilst those remaining become embedded as osteocytes (Ihbe *et al.* 1998, Jilka *et al.* 1998). Many osteoclasts that lose their attachment to bone die by the process of apoptosis (Hughes & Boyce 1997). In this regard, a third term in addition to necrosis and apoptosis is oncosis, which describes cell death associated with slow ischaemia and cell swelling. This occurs to some osteoblasts during transition to osteocytes (Darzynkiewicz & Traganos 1998).

TNF- α has been shown to enhance osteoblast apoptosis and may contribute to bone loss associated with inflammation (Hill *et al.* 1997, Jilka *et al.* 1998, Tsuboi

et al. 1999). Neutrophils can also induce apoptosis of osteoblasts, demonstrating the relationship between inflammatory cells and bone resorption associated with inflammation (Kawakami *et al.* 1997). On the other hand, growth factors, cytokines and other bone-stimulating hormones can reduce apoptosis of osteoblasts during periods of bone resorption (Hill *et al.* 1997, Jilka *et al.* 1998).

Apoptosis occurs in periradicular tissue during bone remodelling associated with orthodontic tooth movement (Rana *et al.* 2001). Certain mediators may limit the resorptive process and aid in bone formation during remodelling (Hill *et al.* 1997). There is significant evidence that osteoblasts may be involved in the regulation of osteoclast apoptosis (Fuller *et al.* 1993; 1998, Greenfield *et al.* 1999). In fact, most of the mediators that stimulate osteoclast activity seem to act through osteoblasts (Greenfield *et al.* 1999). In general, however, factors that stimulate bone resorption inhibit osteoclast apoptosis and factors that inhibit bone resorption promote osteoclast apoptosis (Hughes & Boyce 1997).

Apoptosis of osteoclast precursors may be one way that the osteoclast cell population is controlled (Hughes & Boyce 1997), effectively reducing bone resorption (van't Hof & Ralston 1997). Furthermore, members of the TNF super-family inhibit osteoclast apoptosis (Fuller *et al.* 1998) and induce precursor maturation to mature functioning osteoclasts (Fuller *et al.* 1998), thereby contributing to bone loss.

In the presence of high extracellular calcium concentrations as a result of ongoing resorptive processes, osteoclast apoptosis is induced (Lorget *et al.* 2000). Similarly, oestrogen, glucocorticoids, bisphosphonates and TGF- β have been shown to stimulate apoptosis of osteoclasts (Greenfield *et al.* 1999).

What role does apoptosis play in immune cells?

Apoptosis is associated with the maintenance of immune cell homeostasis (Usherwood *et al.* 1999). Neutrophil production is balanced by apoptosis and clearance from tissues without inducing an inflammatory response (Onishi *et al.* 1997). Apoptosis also plays a critical role in eliminating harmful or injured cells from tissues. This suggests its participation in inflammatory processes and in the resolution of inflammatory reactions (Onishi *et al.* 1997). In periradicular lesions, apoptosis occurs predominantly in neutrophils (Takahashi *et al.* 1999), a process that may be defective in abscess formation as a result of the acidic environment (Onishi *et al.* 1997). This lack of clearance of apoptotic cells can present a persistent antigen, inducing an autoimmune

response (Onishi *et al.* 1997). Apoptosis of neutrophils is the major means of ending neutrophil-associated inflammation (Marshall & Watson 1997). Induction of this process therefore could be a way of minimizing inflammation that would occur as a result of neutrophil necrosis (Onishi *et al.* 1997), which is mediated and induced by certain environmental signals (Marshall & Watson 1997). There is also evidence that the failure of apoptotic mechanisms in neutrophils can contribute to pathogenesis of disease (Marshall & Watson 1997). Neutrophils in exudate show delayed apoptosis and loss of TNF- α , which is necessary for neutrophil survival in the extravascular fluid (Seely *et al.* 1998). In addition, neutrophils in diabetics do not undergo lipopolysaccharide (LPS)-induced inhibition of apoptosis. This may contribute to the increased susceptibility to infection observed in diabetic patients (Tennenberg *et al.* 1999). Calcium ions have been shown to affect neutrophil apoptosis (Onishi *et al.* 1997). The signalling mechanisms for other immune cells have been studied less extensively than those for neutrophils.

What is the role of apoptosis in oral disease?

Apoptosis is widely involved in disease mechanisms of the oral cavity. Oral diseases in which apoptosis plays a role include lichen planus (Dekker *et al.* 1997, Bloor *et al.* 1999), odontogenic keratocysts (Muraki *et al.* 1997), leucoplakia (Muraki *et al.* 1997), squamous cell carcinoma (Muraki *et al.* 1997, Kaur & Ralhan 2000, Ravindranath *et al.* 2000), oral lymphoma (Regezi *et al.* 1998), aphthous ulceration (Honma *et al.* 1985), erythema multiforme (Chrysomali *et al.* 1997), Sjögren's syndrome (Ishimaru *et al.* 2000) and mucocutaneous candidiasis (Heidenreich *et al.* 1996).

Can microorganisms induce or inhibit apoptosis?

Bacterial proteases have been implicated as inducers of host cell apoptosis (Wang *et al.* 1998). *Fusobacterium nucleatum* induces apoptosis in peripheral blood mononuclear cells and neutrophils (Jewett *et al.* 2000). This is significant because premature immune cell apoptosis may decrease host resistance and promote infection (Geatch *et al.* 1999).

Furthermore, apoptosis of both neutrophils and monocytes decreases in the presence of LPS (Mangan *et al.* 1993, Preshaw *et al.* 1999, Tennenberg *et al.* 1999), resulting in a heightened immune response (Hiroi *et al.* 1998). Another organism, *Prevotella intermedia*, does not induce apoptosis in cultured human osteoblastic

cells (Morimoto *et al.* 1999), whilst in *Streptococcus mutans*, lipoteichoic acid may be a factor in pulpitis as there is evidence that it causes apoptosis in human dental pulp cells of deciduous teeth (Wang *et al.* 2001). Generally, these interactions are complex and unpredictable as microorganisms may increase apoptosis in some host cells and decrease apoptosis in others.

Whilst bacteria and their by-products can induce pulpal inflammation, they do not always lead to pulp necrosis (Bergenholtz 2000). Bacterial proteases have been implicated as inducers of host cell apoptosis (Wang *et al.* 1998). Although certain characteristics of apoptotic death are favourable to necrosis, apoptosis does not lead to pulpal regeneration (Goldberg *et al.* 1994).

How is apoptosis involved in periodontal disease?

There is a considerable amount of research on the role of apoptosis in periodontal disease. Apoptosis occurs in cells of the periodontium as part of normal turnover and remodelling (Koulouri *et al.* 1999), and may be even more prevalent than necrosis in periodontal disease (Sorkin & Niederman 1998). It seems to play a role in age regulation of some immune cells and may be involved in the maintenance of local immune homeostasis in inflamed gingival tissue (Tonetti *et al.* 1998). There is also evidence that increased inflammation is associated with increased epithelial cell apoptosis in the periodontium of patients with periodontal disease (Carro *et al.* 1997).

Various microorganisms associated with periodontal diseases have been shown to generate different short-chain carboxylic acids as metabolic by-products, which can promote inflammation by inhibiting normal apoptosis of certain inflammatory cells (Niederman *et al.* 1997). For example, lactic acid and propionic acid commonly produced by oral microorganisms in periodontal diseases have been shown to inhibit apoptosis of various inflammatory cells (Niederman *et al.* 1997, Yamamoto *et al.* 1997, Sorkin & Niederman 1998). The resultant presence of increased numbers of inflammatory cells results in an elevated inflammatory state.

What is the relationship between gingival trauma, wound healing and apoptosis?

There is a strong evidence that trauma, including surgical trauma, inhibits immune cell apoptosis (Ertel *et al.* 1999, Fanning *et al.* 1999, Ogura *et al.* 1999, Nolan *et al.* 2000). Apoptosis occurs in cells at the advancing epithelial edge in wound healing (Brown *et al.* 1997). There is speculation that the signal for apoptosis and downregulation

of inflammation in a wound may in fact be derived from the epithelium because it appears concurrently with re-epithelialisation of the wound (Brown *et al.* 1997, Leonardi *et al.* 2001).

Granulation tissue fibroblasts (myofibroblasts) play a role in wound contraction. When granulation tissue evolves into a scar, myofibroblasts disappear, probably as a result of apoptosis. Myofibroblasts persist in excessive scarring conditions (Desmouliere 1995), possibly because of an inappropriate inhibition of apoptosis in these cells. Certain mediators may be potential stimulators of apoptosis in myofibroblasts after re-epithelialization in the palatal wound healing process (Funato *et al.* 1999).

What is the role of apoptosis in pharmacotherapeutics?

The principle therapeutic goal of apoptosis research is the understanding of how to induce or inhibit apoptosis in specific cells. This is probably the best approach for altering rates of apoptosis in target cells whilst avoiding systemic toxic effects that may be otherwise associated. Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and selective cyclooxygenase 2 (COX2) inhibitors induce apoptosis in certain cells via a caspase activation mechanism. Certain drug combinations may induce apoptosis by a mechanism involving the disruption of the cell cycle (Bamford *et al.* 2000).

The induction of osteoclast apoptosis for the treatment of bone resorption is another therapy that utilizes apoptotic mechanisms (Hughes & Boyce 1997). Antiresorptive drugs such as the bisphosphonate family have been shown to inhibit bone resorption by inducing osteoclast (Hughes *et al.* 1995, Rogers *et al.* 1996, Hiroi-Furuya *et al.* 1999) and macrophage apoptosis (Rogers *et al.* 1996). Bisphosphonates inhibit osteocyte and osteoblast apoptosis contributing to their antiresorptive effects (Plotkin *et al.* 1999). Tetracycline can also inhibit resorption by inducing osteoclast apoptosis (Cillari *et al.* 1998, Vernillo & Rifkin 1998). This is part of the process by which doxycycline downregulates the inflammatory process (Liu *et al.* 1999). There is some thought that tetracycline uses a unique mechanism to induce this selective apoptosis (Bettany & Wolowacz 1998); however, details of this mechanism have not been fully described.

Which dental materials have apoptotic effects?

An understanding of the difference between necrotic and apoptotic forms of cell death is important for understanding the pulp reaction to dental materials (Goldberg

et al. 1994). In cultured cells derived from the human PDL, evidence of apoptosis in response to different dental materials has been described (Adams et al. 1995) and may occur in damaged areas of the pulp because of certain toxic substances in those materials (Goldberg et al. 1994). In some cases, dental materials may lead to uncontrolled development of apoptosis within central pulp cells, but may leave the odontoblasts more or less intact (Goldberg et al. 1994).

More specifically, and related to endodontic treatment, *in vitro* apoptotic changes are documented in human PDL cells exposed to calcium hydroxide (Adams et al. 1995). Calcium hydroxide has antibacterial properties and has shown predictable healing and hard tissue formation when used in cavity preparations (Bergenholtz 2000). Calcium ions may also affect neutrophil apoptosis (Onishi et al. 1997). Unfortunately, neither the apoptotic effects of composite resin on the dental pulp nor the potential apoptotic effects of extruded root canal sealers on the cells that make up the supporting periradicular tissues have been fully documented. Therefore, the long-term effects of these treatment variables may or may not influence the ultimate treatment outcome and should not be overlooked as potential aetiological factors in failure.

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