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Application of Apoptosis in Interdisciplinary Dentistry and Beyond (A Review)

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ABSTRACT

Apoptosis has become a major research area in the biomedical sciences. As there are more than 13,000 papers published annually on the topic, it is impossible to keep track on all developments in the area. The individual aspects of molecular control of apoptosis are well reviewed, but more general, introductory recent reviews into the field are lacking. In multicellular organisms, cells that are no longer needed or are a threat to the organism are destroyed by a tightly regulated cell suicide process known as programmed cell death, or apoptosis. The latter plays an important role in development of dental and oral tissues and also in the regulation and maintenance of cell populations in such tissues upon physiological and pathological conditions. The understanding of the ability of clinical materials to induce or inhibit apoptosis and the investigation of apoptosis as it relates to the pathology of the tissues may eventually lead to new treatment approaches for dental specialists. Hence, this review aims to give a brief overview of apoptosis for the dentistry field.

Key words: Apoptosis, molecular control, Interdisciplinary**,** dentistry field**.**

Introduction

Apoptosis, as a programmed cell death (PCD) is essential for normal cell mechanism. The word "Apoptosis" derives from Greek language "απόπτωσις" and means trees shedding their leaves in autumn, which describes the "dropping off" or "falling off" of petals from flowers, or leaves from trees. This language imaginarily described the cell death triggered by physiological and pathological stimulation. The apoptosis term was first described in1972 by John Foxton Ross Kerr group (Kerr *et al.,* 1972). They used apoptosis word to describe the tissue cell death. The above is the beginning of apoptosis researches and this period is the apoptosis formation; the second period about apoptosis is the biochemical level and apoptosis cell morphological changes researches. The purpose of this article is to familiarize the different specialties in the dental field with current knowledge on apoptosis as it relates to dental and oral tissues and to discuss some of potential implications of apoptosis to dentistry.

Apoptosis Term

Already since the mid-nineteenth century, many observations have indicated that cell death plays a considerable role during physiological processes of multicellular organisms, particularly during embryogenesis and metamorphosis (Lockshin and Zakeri, 2001). The term programmed cell death was introduced in 1964, proposing that cell death during development is not of accidental nature but follows a sequence of controlled steps leading to locally and temporally defined self-destruction. Eventually, the term apoptosis had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced in a publication by Kerr *et al.,* (1972). Apoptosis is of Greek origin, having the meaning "falling off or dropping off", in analogy to leaves falling off trees or petals dropping off flowers. This analogy emphasizes that the death of living matter is an integral and necessary part of the life cycle of organisms. The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues upon physiological and pathological conditions. It should be stressed that apoptosis is a well-defined and possibly the most frequent form of programmed cell death, but that other, non-apoptotic types of cell death also might be of biological significance (Leist and Jaattela, 2001).

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The significance of apoptosis

The development and maintenance of multicellular biological systems depends on a sophisticated interplay between the cells forming the organism, it sometimes even seems to involve an altruistic behavior of individual cells in favor of the organism as a whole. During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues (Meier *et al.,* 2000). A particularly instructive example for the implication of programmed cell death in animal development is the formation of free and independent digits by massive cell death in the interdigital mesenchymal tissue (Zuzarte-Luis and Hurle, 2002). Other examples are the development of the brain, during which half of the neurons that are initially created will die in later stages when the adult brain is formed (Hutchins and Barger, 1998). Also cells of an adult organism constantly undergo physiological cell death which must be balanced with proliferation in order to maintain homeostasis in terms of constant cell numbers. The majority of the developing lymphocytes die either during genetic rearrangement events in the formation of the antigen receptor, during negative selection or in the periphery, thereby tightly controlling the pool of highly efficient and functional but not self-reactive immune cells and at the same time keeping lymphocyte numbers relatively constant(Rathmell and Thompson, 2002).

Taken together, apoptotic processes are of widespread biological significance, being involved in e.g. development, differentiation, proliferation/homoeostasis, regulation and function of the immune system and in the removal of defect and therefore harmful cells. Thus, dysfunction or dysregulation of the apoptotic program is implicated in a variety of pathological conditions. Defects in apoptosis can result in cancer, autoimmune diseases and spreading of viral infections, while neurodegenerative disorders, AIDS and ischemic diseases are caused or enhanced by excessive apoptosis (Fadeel *et al.,* 1999).

Pathways

Apoptosis can be triggered in a cell through either the extrinsic pathway or the intrinsic pathway. The extrinsic pathway is initiated through the stimulation of the transmembrane death receptors, such as the Fas receptors, located on the cell membrane. In contrast, the intrinsic pathway is initiated through the release of signal factors by mitochondria within the cell (Fig 1).

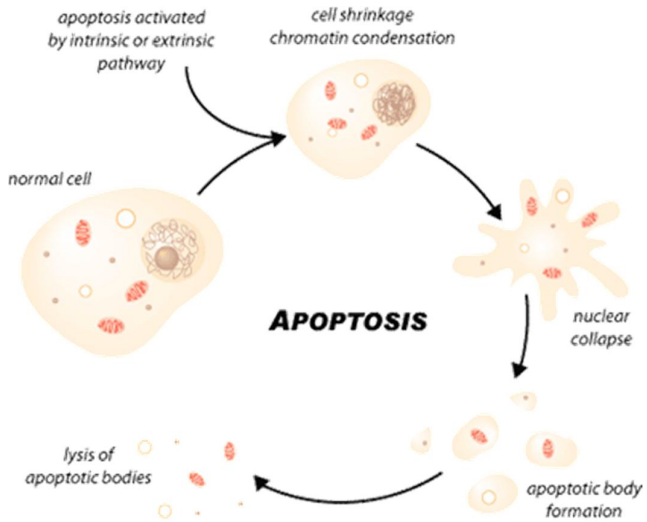

Fig. 1: Apoptosis – the programmed death of a cell.

The Extrinsic Pathway:

In the extrinsic pathway, signal molecules known as ligands, which are released by other cells, bind to transmembrane death receptors on the target cell to induce apoptosis. For example, the immune system's natural killer cells possess the Fas ligand (FasL) on their surface (Csipo *et al.,* 1998). The binding of the FasL to Fas receptors (a death receptor) on the target cell will trigger multiple receptors to aggregate together on the surface of the target cell. The aggregation of these receptors recruits an adaptor protein known as Fas-associated death domain protein (FADD) on the cytoplasmic side of the receptors. FADD, in turn, recruits caspase-8, an initiator protein, to form the death-inducing signal complex (DISC). Capase 8 can also facilitate the release of cytochrome c in the intrinsic pathway (Adrain *et al.,* 2002).

The Intrinsic Pathway:

The intrinsic pathway is triggered by cellular stress, specifically mitochondrial stress caused by factors such as DNA damage and heat shock (Adrain *et al.,* 2002). Upon receiving the stress signal, the proapoptotic proteins in the cytoplasm, BAX and BID, bind to the outer membrane of the mitochondria to signal the release of the internal content. However, the signal of BAX and BID is not enough to trigger a full release. BAK, another proapoptotic protein that resides within the mitochondria, is also needed to fully promote the release of cytochrome c and the intramembrane content from the mitochondria (Hague and Paraskeva, 2004). (Fig2).

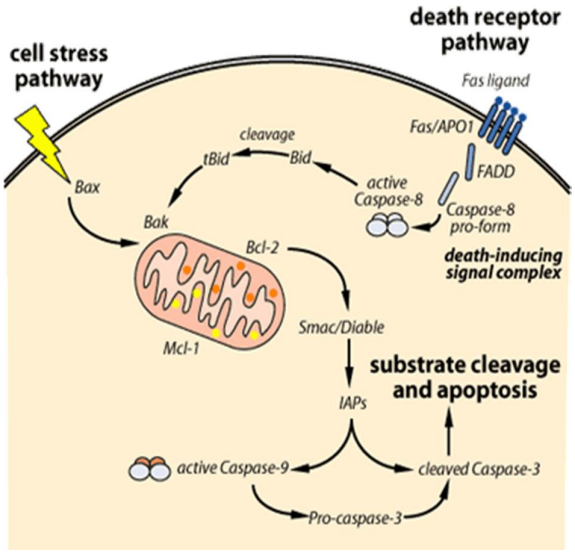

Fig. 2: The intrinsic and extrinsic pathways leading to apoptosis.

Histogensis of Apoptosis

Apoptosis is associated with a distinct set of biochemical and physical changes involving the cytoplasm, nucleus and plasma membrane. Early in apoptosis, the cells round up, losing contact with their neighbors, and shrink. In the cytoplasm, the endoplasmic reticulum dilates and the cisternae swell to form vesicles and vacuoles. In the nucleus, chromatin condenses and aggregates into dense compact masses, and is fragmented inter nucleosomally by endonucleases, which can be analysed by the typical ‘‘DNA ladder’’ formation in apoptosis, for which DNA (either total or cytosolic) is extracted from the cells and separated in an agarose gel.(Johnson *et al.,* 1996). The nucleus becomes convoluted and buds off into several fragments, which are encapsulated within the forming apoptotic bodies. In the plasma membrane, cell junctions are disintegrated, whereby the plasma membrane becomes active and convoluted, eventually blebbing. The cell breaks up in a florid manner leading to the ‘‘falling away’’ of several membrane spheres containing the ‘‘packaged’’ cellular contents identified as apoptotic bodies of various sizes. (Kerr et *al.,* 1994).

Morphological features of apoptosis and necrosis

Apoptotic cells can be recognized by stereotypical morphological changes: the cell shrinks, shows deformation and loses contact to its neighboring cells. Its chromatin condenses and marginates at the nuclear membrane, the plasma membrane is blebbing or budding, and finally the cell is fragmented into compact membrane-enclosed structures, called 'apoptotic bodies' which contain cytosol, the condensed chromatin, and organelles (Fig3). The apoptotic bodies are engulfed by macrophages and thus are removed from the tissue without causing an inflammatory response (Saraste and Pulkki, 2002). Apoptosis is in contrast to the necrotic mode of cell-death in which case the cells suffer a major insult, resulting in a loss of membrane integrity, swelling and disrupture of the cells. During necrosis, the cellular contents are released uncontrolled into the cell's environment which results in damage of surrounding cells and a strong inflammatory response in the corresponding tissue (Leist and Jaattela, 2001).

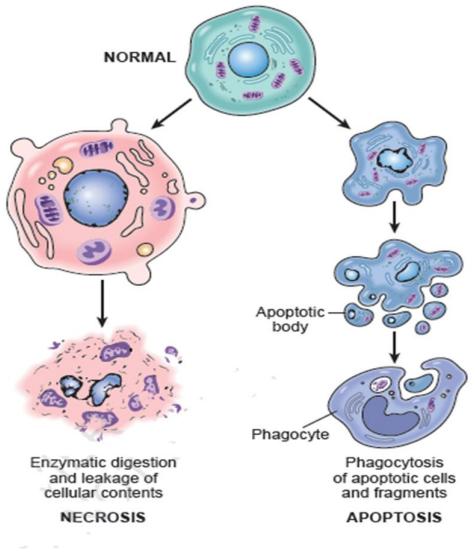

Fig.3: Hallmarks of the apoptotic and necrotic cell death process.

Apoptosis and proliferation

Apoptosis can result from perturbations of the cell cycle. Signaling molecules cover a continuum from proliferation to death. Many genes involved in cell cycle regulation are also involved in regulation of apoptosis (e.g., c-myc, c-fos, c-jun, p53, many kinases and phosphatases (Salvesen *et al.,* 2002).Thus, signals that promote proliferation can also promote apoptosis. If apoptosis is blocked by survival signals, increase in cell numbers occurs, which can manifest in cancer (Jarpe *et al.,* 1998). However, many neurons undergo PCD as post mitotic neurons—in these, factor deprivation appears to be the signal for apoptosis(Jarpe *et al.,* 1998). T cell receptor signaling leads to proliferation and—with some delay—to AICD(Ba¨hr *et al.,* 2000). Often the meaning of a signal has to be specified by a second signal, for example c-myc plus bcl-2 leads to proliferation, c-myc plus p53 leads to apoptosis (Sharma, 2000) p53 are a key element in apoptosis induction in cells in response to DNA damage. p53 is inhibited by MDM2 (murine double minute 2), an ubiquitin ligase that targets p53 for destruction by the proteasome. MDM2 is inactivated by binding to ARF (alternative reading frame). Cellular stress, including that induced by chemotherapy or irradiation, activates p53 either directly, by inhibition of MDM2 (including cleavage by caspases), or indirectly by activation of ARF (Evan and Vousden , 2001). ARF can also be induced by proliferative oncogenes such as RAS. Active p53 trans activates pro-apoptotic genes—including Bax, Noxa, CD95 and DR4—to promote apoptosis. p53 can also move directly to the mitochondria where it exerts proapoptotic activity (Guo and Hay,1991; Harbour and Dean,2000). Akt is a major kinase involved in ant-apoptotic signaling, Akt knockout mice show enhanced of spontaneous apoptosis (Moll and Zaika, 2001). One important substrate of Akt is the BH3-only protein Bad, which upon phosphorylation binds to 14-3-3 proteins. These sequester it to the cytoplasm, preventing it from translocating to the mitochondrion where it exerts its pro-apoptotic action. Furthermore, Akt phosphorylates forehead transcription factors, which, again by binding to 14-3-3 proteins, become excluded from the nucleus and cannot initiate transcription of proapoptotic genes like FasL and the BH3 only protein Bim. Through phosphorylation of the cAMP-response element binding protein (CREB) and activation of NF-kB, Akt can also initiate the transcription of ant apoptotic genes like Bcl-2 and IAP(Guo and Hay,1991; Harbour and Dean,2000). Phagocytosis of apoptotic bodies In vivo, apoptotic bodies are rapidly cleared away by phagocytosis, either by professional phagocytotic cells or simply by cells in the direct vicinity of the apoptotic bodies. Phosphatidylserine (PS) receptor is involved in phagocytosis of apoptotic cells (Moll and Zaika,2001). Usually, PS is maintained at the inner layer of the plasma membrane (PM) by the action of an ATP dependent PS flippase. This flippase is inactivated by caspases, and a scramblase is activated, leading to a redistribution of PS to the outer leaflet of the PM (Fig. (Chen, 2001) PSexternalisation is widely used to analyse apoptosis infects analysis due to PS binding to annexin V(Huang and Oliff, 2001). A range of cell surface molecules (e.g., thrombospondin 1 and its receptor, CD36, involved in recognition of the apoptotic bodies) and intracellular molecules (e.g., the DOCK180 [180 kDa protein downstream of CRK]/CRKII/RAC complex, involved in signaling in the engulfing cell) has been identified as being involved in the phagocytosis of mammalian apoptotic bodies and many of them are homologues of the C. elegans genes involved in dead cell removal in the worm(Huang and Oliff, 2001; Nicholson and Anderson,2002). Phagocytosis of dead cells appears to have a different meaning beyond that of waste disposal depending on the form of death that the cell underwent. Whereas phagocytosis of oncotic corpses

by dendritic cells leads to inflammation and immune response, phagocytosis of apoptotic bodies results in the release of anti-inflammatory cytokines and immune tolerance (Messmer , 2000).

Apoptosis and diseases

If the cell death and proliferation go to imbalance, many diseases will happen. Such as: some acute pathologies (stroke, heart attack, liver failure); cancer; neurodegenerative syndromes; diabetes and so on. Due to its no lethal effect to the body, Apoptosis play a fundamental role in organism development and tissue homeostasis, while if apoptosis was not under controlled, a variety of diseases will occur (Taylor et *al.,* 2004).

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 organized 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) and in the stratum intermedium . This process also occurs during the transition stage between secretion and maturation of ameloblasts during enamel formation (Nishikawa and 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, playing a major role in the elimination of reduced ameloblasts located at cusps (Shibata *et al.,* 1995). Fibroblast-like cells of the periodontal ligament (PDL) exhibit apoptosis during tooth development (Cerri *et al.,* 2000).

What role does apoptosis play in 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.,* 1996), 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 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, odontogenic keratocysts,leucoplakia, squamous cell carcinoma and aphthous ulceration (Dekker *et al.,* 1997).

Which dental materials have apoptotic effects?

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, more specifically, and related to endodontic treatment, *in vitro* apoptotic changes are documented in human periodontal ligament cells exposed to calcium hydroxide. 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. 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. (Onishi *et al.,* 1997).

What role does apoptosis play in the dental pulp?

Apoptosis is a part of normal pulp homeostasis, occurring more in the occlusal (incisal) than in the apical portions of the pulp ,most apoptotic cells in normal pulp can be found at the periphery and are usually associated with the sub odontoblastic region rather than with the odontoblastic layer, 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(Piattelli *et al.,* 2001).

Role of primary and secondary induction of apoptosis in odontoblasts after cavity preparation

Wound healing of dental pulp after cavity preparation involves two processes: the cell-death process of damaged odontoblasts and adjacent pulp cells, and the cell-differentiation process for reparative dentinogenesis by surviving odontoblasts or odontoblast-like cells. Dentinogenesis has been well-studied, and reparative dentinogenesis is known to be similar to primary dentinogenesis with regard to expression of genes and

proteins, including collagen, bone morphogenetic proteins and proto-oncogenes, in both surviving odontoblasts and odontoblast-like cells (Mitsiadis *et al.,* 1999).

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 remodeling, and may be even more prevalent than necrosis in periodontal disease.

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 There is also evidence that increased inflammation is associated with increased epithelial cell apoptosis in the periodontium of patients with periodontal disease(Koulouri *et al.,* 1999).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).

What role does apoptosis play in bone?

In the process of bone remodeling, some osteoblasts die via apoptotic mechanisms, whilst those remaining become embedded as osteocytes, many osteoclasts that lose their attachment to bone die by the process of apoptosis, 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 and Traganos , 1998).

Apoptosis occurs in per-radicular tissue during bone remodeling associated with orthodontic tooth movement Certain mediators may limit the resorptive process and aid in bone formation during remodeling, there is significant evidence that osteoblasts may be involved in the regulation of osteoclast apoptosis, in fact, most of the mediators that stimulate osteoclast activity seem to act through osteoblasts (Rana *et al.,* 2001). In general, however, factors that stimulate bone resorption inhibit osteoclast apoptosis and factors that inhibit bone resorption promote osteoclast apoptosis), apoptosis of osteoclast precursors may be one way that the osteoclast cell population is controlled), effectively reducing bone resorption, in the presence of high extracellular calcium concentrations as a result of ongoing resorptive processes, osteoclast apoptosis is induced (Lorget *et al.,* 2000).

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 ,apoptosis occurs in cells at the advancing epithelial edge in wound healing ,there is speculation that the signal for apoptosis and down regulation of inflammation in a wound may in fact be derived from the epithelium because it appears concurrently with re-epithelialization of the wound (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, 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 role does apoptosis play in immune cells?

Apoptosis is associated with the maintenance of immune cell homeostasis. Neutrophil production is balanced by apoptosis and clearance from tissues without inducing an inflammatory response. Apoptosis also plays a critical role in eliminating harmful or injured cells from tissues (Usherwood *et al.,* 1999).

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