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Review

Hypoxia-based strategies for regenerative dentistry—Views from the different dental fields

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ABSTRACT

The understanding of the cell biological processes underlying development and regeneration of oral tissues leads to novel regenerative approaches. Over the past years, knowledge on key roles of the hypoxia-based response has become more profound. Based on these findings, novel regenerative approaches for dentistry are emerging, which target cellular oxygen sensors. These approaches include hypoxia pre-conditioning and pharmacologically simulated hypoxia. The increase in studies on hypoxia and hypoxia-based strategies in regenerative dentistry highlights the growing attention to hypoxia's role in regeneration and its underlying biology, as well as its application in a therapeutic setting.

In this narrative review, we present the current knowledge on the role of hypoxia in oral tissues and review the proposed hypoxia-based approaches in different fields of dentistry, including endodontics, orthodontics, periodontics, and oral surgery.

1. Introduction

The development of regenerative strategies in the field of dentistry is inspired by the understanding of the cell biological processes underlying development and regeneration of oral tissues. A key factor in successful healing is the response to hypoxia. Therefore, over the last ten years, hypoxia-based strategies have been met with high interest which is highlighted by increasing numbers of publications in this field (Fig. 1). The idea started with local application of recombinant pro-angiogenic growth factors to stimulate regeneration via enhanced formation of blood vessels and was developed further to more sophisticated approaches which include transplanting hypoxia conditioned cells (Hadjipanayi & Schilling, 2013; Hsiao, Dilley, Dusting, & Lim, 2014; Hu et al., 2007). Since about 2010 cell-free methods have come into focus of research (Hadjipanayi & Schilling, 2013; Di Santo et al., 2009; Hadjipanayi et al., 2013).

Various pathologic dental conditions and treatment of these can lead to tissue damage caused by trauma, inflammation, or ischemic diseases, further leading to local hypoxic and catabolic conditions due to lacking vascularization in these areas (Niklas, Proff, Gosau, & Römer, 2013). Particularly in the early phase of healing, hypoxia signaling is of high relevance. Upon tissue damage and the establishment of a blood clot, reactions at the cell biological level are induced. Activated platelets lead to immigration of mesenchymal progenitor cells and endothelial cells into a hypoxic environment which can modulate the cellular response (Gruber et al., 2004; Gruber, Kandler, Agis, Fischer, & Watzek, 2008; Kandler, Fischer, Watzek, & Gruber, 2004). Cells are equipped with a molecular machinery that allows them to respond to these conditions. Different types of tissue can withstand different minimum oxygen levels, which is *e.g.* 1.3% in bone (Spencer et al., 2014; Werle, Chagastelles, Pranke, & Casagrande, 2016). The activation of this specific pathway also depends on the extent of hypoxia (Ehrismann et al., 2007; Werle et al., 2016).

A fundamental cellular requirement is the maintenance of high adenosine triphosphate (ATP) levels, as nearly all cellular processes are driven by hydrolysis of ATP, requiring oxygen (Hochachka, 1986). Under hypoxic conditions, cells adapt by increasing anaerobic glycolysis and decreasing energy-consuming processes (Boutilier, 2001). Cell activities with high ATP consumption such as protein, DNA, RNA synthesis are inhibited first (Buttgereit & Brand, 1995). If ATP production falls under the level of maintaining the cell's demands, cell necrosis is initiated by membrane depolarization, Ca^{2+} influx, and phospholipase and protease activation (Michiels, 2004). Furthermore, hypoxia may stimulate reactive oxygen species (ROS) release from mitochondria leading to a transcriptional and posttranslational response and to the formation of vascular endothelial growth factor (VEGF)

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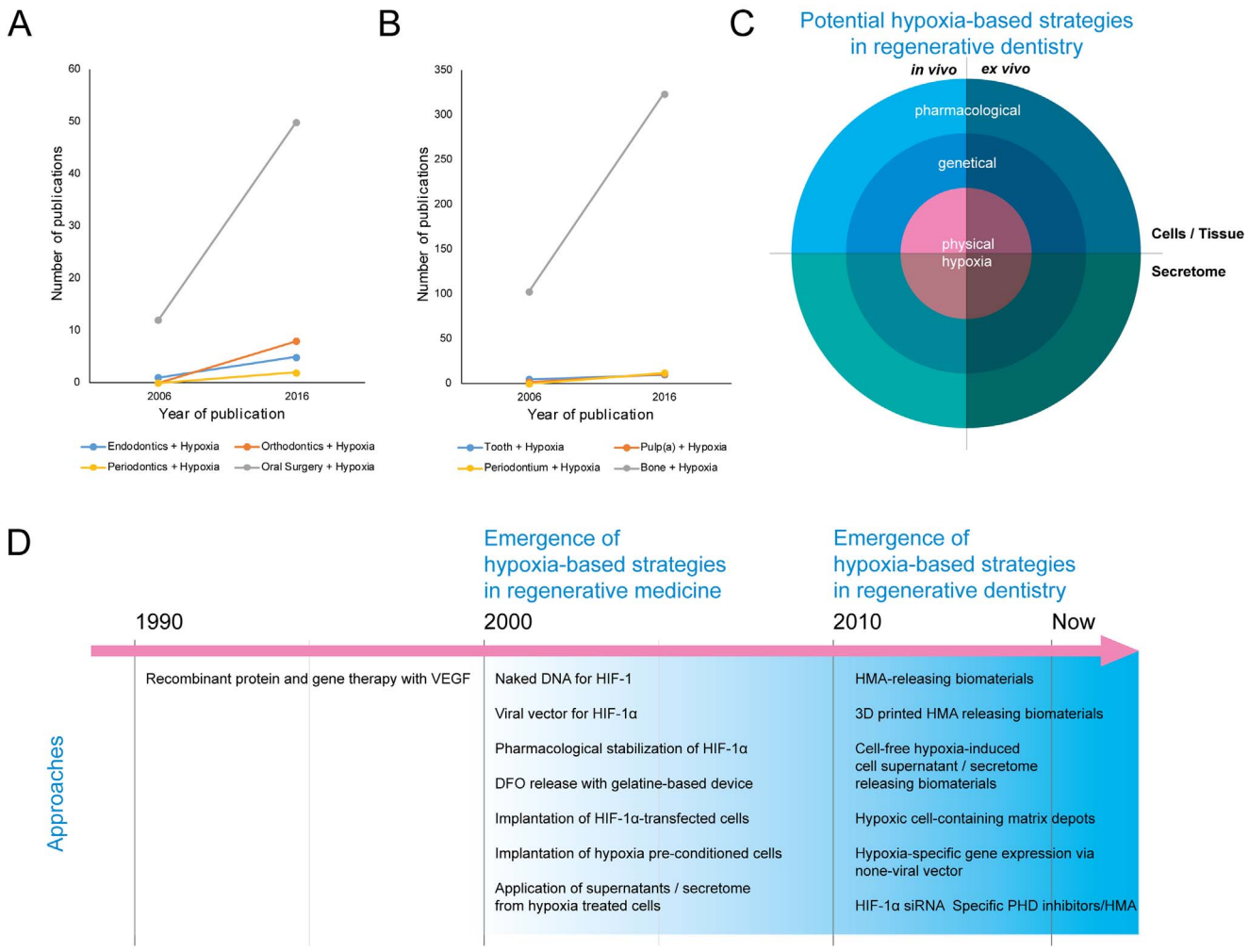

Fig. 1. Hypoxia and hypoxia-based strategies in dentistry.(A) Publication numbers on the basis of a PubMed (http://www.ncbi.nlm.nih.gov) search with the keywords hypoxia in combination with the dental specialties (Endodontics, Periodontics, Orthodontics, Oral Surgery). The numbers are given in total numbers as publications in the year 2006 and the year 2016 (date of research: 8 December 2016). (B) Publication numbers on the basis of a PubMed (http://www.ncbi.nlm.nih.gov) search with the keywords hypoxia in combination with dental key words (tooth; periodontium; pulp(a); bone). The numbers are given in total numbers as publications in the year 2006 and the year 2016 (date of research: 8 December 2016). (C) Different approaches of hypoxia-based strategies for tissue regeneration *in vivo* and *ex vivo/in vitro*. Hypoxia signaling can be induced pharmacologically, genetically, or physically. (D) Schematic diagram of the development of hypoxia-based regenerative strategies in general since 1990 showing key events in the emergence of hypoxia-based strategies. Adopted with modifications from (Hadjipanayi & Schilling, 2013).

(Burki & Tetenta, 2014; Giaccia, Simon, & Johnson, 2004).

Hypoxia has also shown to be associated with an inflammatory response. Some of the hydroxylases which are inhibited under hypoxic circumstances play a key role in inflammatory signaling pathways (Bartels, Grenz, & Eltzschig, 2013). In humans, hypoxic exposure for 60 min did not reveal an increase in inflammatory markers such as alveolar macrophages (AMs), clara cell specific protein (CC16), tumor necrosis factor (TNF)-α, interleukin (IL)-6, or high sensitivity CRP (hsCRP), but a rise in hypoxia inducible factor (HIF)-1α and VEGF levels (Burki & Tetenta, 2014).

The HIF family consists of HIF-1α, HIF-2α, HIF-3α, and HIF-1β subunits. HIF-1α and HIF-2α are closely related and regulate oxygen dependent gene transcription (Benita et al., 2009).

In the hypoxia signaling pathway HIF-1α plays a major role (Fig. 2). The amount of HIF-1α depends on the hypoxic state, whereas HIF-1β is hypoxia independent (Fraisl, Aragonés, & Carmeliet, 2009). Upon accumulation of HIF-1α it binds as transcription factor to the promotor region of target genes, such as *vegf*, *angiopoietin-2*, *angiopoietin-like 4,* or *platelet-derived growth factor-BB*. (Rabinowitz, 2013). In osteoblasts an increased expression of HIF-1α drives VEGF production and subsequently elevates angiogenesis, leading to higher vascularization and density of the bone (Rankin et al., 2012; Weng et al., 2014).

HIF-1α is constitutively produced but in conditions with normal oxygen levels, it is degraded immediately through hydroxylation by prolyl hydroxylases (PHD) or factor-inhibiting HIF (FIH)-1. The Hippel Lindau E3 ubiquitin ligase then binds to the hydroxylated HIF-1α complex and ubiquitinates it as a target for degradation by the proteasome (Fraisl et al., 2009). Under hypoxic conditions PHD are inhibited. Thus, the concentration of HIF-1α rises inside the cell.

Currently only little is known on the kinetics of oral tissue response. A study on murine retina investigated kinetics for the induction of the HIF-1α pathway after hypoxic conditions by measuring the downstream products such as VEGF and Erythropoietin (Epo). A peak in HIF-1α stabilization was detected 2 h after the onset of hypoxia, parallel to the expansion of hypoxia across the retina, which was measured by primonidazole labelling. Upregulation of the expression of VEGF and Epo followed closely (Mowat et al., 2010).

Like the labile HIF-1α, also HIF-2α is degraded in the presence of oxygen. Under hypoxic conditions HIF-2α is stabilized involving similar mechanisms as HIF-1α. HIF-2α, while having distinct functions, some functions are shared with HIF-1α including their role in angiogenesis. The role of HIF-3α is more complex. In addition to having distinct target genes, some HIF-3α variants have also overlapping target genes with HIF-1α. Other variants can inhibit HIF-1α − and HIF-2α related