

Influence of Irrigants and Intracanal Medicaments on the Vitality of Stem Cells from Apical Papilla - An Overview

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Abstract: *Regenerative endodontics includes a therapy with new populations of mesenchymal stem cells, which are present in the apical papilla of teeth with incomplete root development, called stem cells from apical papilla. The preservation of these cells in the treatment of immature teeth may allow the continuation of root development to its final form and the closure of its apex. The choice of irrigants and intracanal medications in regenerative endodontics must be made taking into consideration their impact on the vitality of SCAP, in addition to their bactericidal/static effect.*

Keywords: immature teeth, regenerative endodontics, stem cells from apical papilla

1. Introduction

The endodontic treatment of immature permanent teeth with necrotic pulp is both a clinical problem - a challenge for the clinician, as well as a public health problem. Regardless of ability to apply the apexfixation procedure, the long-term prognosis for these teeth is questionable due to the small and incompletely formed root walls [1]. Regenerative endodontics has become a real alternative for the treatment of such teeth. It also includes a regenerative therapy with new populations of mesenchymal stem cells, which are present in the apical papilla of teeth with incomplete root development [1,2]. They are called Stem cells from apical papilla (SCAP). Although they exhibit similar characteristics to those of the dental pulp stem cells (DPSCs) discovered much earlier, they have a different appearance, which is assessed by methods of histology, immunohistochemistry, cell and molecular analysis [2]. Evidence is accumulating to support the hypothesis that SCAP are likely sources of primary odontoblasts, which are responsible for the formation of root dentin, while DPSCs are a possible source of replacement/spare odontoblasts [2]. The preservation of these stem cells in the treatment of immature teeth may allow the continuation of root development to its final form and the closure of its apex [2].

In 'Late bell' stage, when the formation of the dental crown has started, the dental papilla (papilla dentis) gradually changes its position by moving apically to the dental pulp. The first odontoblasts appear, the dentinogenesis is initiated and the formation of the root begins, alongside the formation of a double-layered epithelial vagina called 'Hertwig's epithelial root sheath' [3]. It is assumed that these epithelial cells play an extremely important role in the formation of the tooth root, although the exact nature of this process is not fully understood [4]. Among the various functions attributed to these cells are the inducer function and the regulator function of root formation - dentinogenesis, cementogenesis and the formation of the periodontium, the determination of size, shape and number of roots [3,5,6]. It is also believed

that they secrete chemotactic proteins in the basal membrane that guide the migration of pre-cementoblasts [4,7] or they themselves may undergo epithelial-mesenchymal transformation and become cementoblasts [8].

The histological characteristics of the connection between the pulp and the apical papilla have not yet been well-described, but it has been shown that the latter has a unique supply of mesenchymal stem cells [7]. Due to its apical localization, this tissue is also important because of its collateral circulation, which allows it to survive the pulp necrosis [2].

The apical papilla and its intrinsic stem cells (SCAP) were first described in 2006. This papilla is a rich reservoir of undifferentiated mesenchymal stem cells with great potential for proliferation and odontogenic differentiation [2,9,10]. The proximity of the apical papilla to the apex of the tooth and its natural seamless connectivity with the root canal area makes this rich source of stem cells easily accessible for regenerative endodontic procedures [9].

The overall root development of permanent teeth ends about three years after their eruption. For most teeth this process occurs during childhood and adolescence. It is a time during which the developing tooth can be affected by various factors that can lead to pulp degeneration and infection of the root zone. Such factors include carious lesions, traumatic injuries, development abnormalities of the teeth [11].

Unlike teeth with complete root development, pulp necrosis in immature teeth does not exclude the presence of residual pulp progenitor cells in the apical third of the root. In those teeth, because of their anatomical and physiological characteristics, accomplishing the objectives of conventional root canal treatment is difficult [12]. Mechanical cleaning is almost impossible, and the removal of necrotic debris from this wide canal is hindered. Even if that happens, the root remains short, with thin walls and prone to fracture. Some of the technological difficulties can be overcome by conducting apexfixation with calcium hydroxide or an apical barrier

Volume 6 Issue 5, May 2017

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with MTA and bio-ceramic material, but the risks of future fractures and tooth mobility remain due to the poor crown/root ratio [13,14,15].

With the development of the knowledge of the biology of the dentin-pulp complex and the potential of the progenitor/stem cells it is now possible to introduce better alternatives for the treatment of immature permanent teeth. Procedures attempting to preserve the residual pulp and mesenchymal stem cells from the apical papilla may lead to revascularization and completion of the root maturation [16]. These types of procedures form the regenerative endodontics.

Regenerative endodontics can be defined as biologically based procedure, the goal of which is the replacement of damaged structures including dentin, the dental root and cells of the dentin-pulp complex [17]. Several types of adult stem cells are used in a special scaffold placed in the root-canal system of the tooth. With the addition of growth factors, external or by the dentin and/or residual pulp, a tissue similar to that of the pulp is formed [18]. Stem cells from the pulp of permanent teeth (SCPPT), as well as stem cells from apical papilla (SCAP) can differentiate to cells, similar to odontoblasts with the potential to migrate, mineralize and form a dentin-like three-dimensional structure. SCAP however have a higher proliferative capacity than SCPPT [18]. Moreover, even in clinically proven necrosis of the pulp with periapical pathology and fistula, some SCAP may still be vital [19,2]. With good disinfection of the root canal system and appropriate stimulation of cells from the apical papilla a continuation of root development can be achieved [19].

In regenerative endodontics the protocol of disinfection of immature teeth with necrotic pulp includes (1) minimal or no instrumentation (2) irrigation (3) intracanal medication with antimicrobial agents [19]. It is relied on the chemical cleaning, the removal of necrotic debris and the use of intracanal medicaments to achieve disinfection and resolve the infection [9,20,21]. However, the chemical agents which are used in regenerative procedures should be selected not only based on their bactericidal/bacteriostatic properties, but also on their ability to ensure the survival and preservation of the proliferative capacity of the patient's stem cells [11]. This approach is substantially different from the classic endodontic clinical protocol for the treatment of root canals. [9] The most commonly used irrigants are sodium hypochlorite (NaOCl), Ethylenediaminetetraacetic acid (EDTA) and chlorhexidine [22].

2. Sodium hypochlorite

The in vitro survival of SCAP in a model of a root canal, irrigated previously with various combinations of widely used chemical agents, has been studied [23]. Using 6% NaOCl has a detrimental effect on these cells, while high concentrations of hypochlorite make the differentiation of stem cells in the dental pulp into odontoblast-like cells rather difficult, both in vitro and in vivo [24]. Therefore, irrigation with NaOCl in its maximum clinically used concentration significantly reduces the survival of stem cells and leads to the loss of their ability to differentiate into odontoblast-like

cells. Another study has tested the effects of lower concentrations of hypochlorite on SCAP and found that 1, 5% NaOCl has minimal effect on their survival and differentiation [25]. This confirms the findings from other studies that the irrigation with 6% NaOCl has a negative effect, while 17% EDTA is favorable in terms of survival and differentiation of stem cells kept in contact with the conditioned dentin surface [23].

3. Chlorhexidine

Chlorhexidine is a synthetic cationic bisguanide, consisting of two symmetrical 4-chlorophenyl rings and two biguanide groups linked with a central hexamethylene chain [26]. It is used because of its effectiveness against gram-positive and gram-negative bacteria, as well as fungi, and for its substantivity and relatively low toxicity [27]. The effect is due to the interaction between the molecules with a positive charge and the negative charge of the phosphate groups of the microbial cell wall, thereby changing the cellular osmotic balance [28]. The antimicrobial effect of 2% chlorhexidine is similar to that of 5.25% NaOCl, both in the form of a solution or as a gel [29]. It is suggested as an alternative to hypochlorite, particularly for teeth with an open apex or a documented allergy to bleaching solutions [30]. Its use in regenerative procedures, however, is questionable, since irrigation protocols with its participation have detrimental effect on the vitality of the cells [23].

4. EDTA

Conditioning of the dentin with 17% EDTA provides greater survival of SCAP. The use of EDTA after using 6% NaOCl reduces its detrimental effect on the vitality of the cells [23]. Important growth factors are included in the dentin matrix during the dentinogenesis. Some of them, like the transforming growth factor beta-1 and the vascular endothelial growth factor (VEGF) have a stimulating effect for the proliferation and differentiation of stem cells [31,32]. EDTA dissolves these growth factors from the dentin, thus increasing their bioavailability. It would therefore be useful for the clinician to use the inductive properties of the normally present in dentin morphogens and growth factors [33].

5. Intracanal Medications

The most widely used medicament in regenerative endodontics is a mixture of ciprofloxacin, metronidazole and minocycline, which is called a triple antibiotic paste. It is highly effective against the most common bacteria in infected root canals. [34] Other medications used include a combination of metronidazole and ciprofloxacin (dual antibiotic paste), a combination of metronidazole, ciprofloxacin, and cefaclor (modified antibiotic paste) and calcium hydroxide [35,36,37]. Out of these, only Ca(OH)₂, regardless of its concentration, promotes cell survival [38]. Clinically used concentrations of the triple, the double or the modified antibiotic paste lead to less than 20% of vitality of the cells. In a concentration of 1 mg/ml 33%-56% of the cells survived and further water dilution leads to 100% survival. Most studies use the antibiotic combination with

the consistency of a thick paste, which corresponds to a concentration of about 1000 mg/ml. Therefore such a mixture is supersaturated in terms of the concentration of each antibiotic, which would have a detrimental effect on the vitality of the cells [38].

6. Conclusion

Regenerative endodontics offers new opportunities for the treatment of teeth with incomplete root development and necrotic pulp. However, the choice of irrigants and intracanal medications must be made taking into consideration their impact on the vitality of SCAP, in addition to their bactericidal/static effect.

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