Periodontal response to a tricalcium silicate material or resin composite placed in close contact to the supracrestal tissue attachment: a histomorphometric comparative study

Introduction

Restorative treatments in the biological width is an increasingly frequent challenge in daily clinical practice. Management of deep caries, resorptions and perforations are common in a day-to-day practice. The interrelationship between periodontics and restorative dentistry is present at many fronts, including location of restorative margins, restorative contours, final polishing and response of the gingival tissues to restorative preparations (Padbury 2003, Santamaría 2013). The biologic width is a commonly used clinical term defined as the gingival attachment along the root surface, from the most coronal portion of the epithelium attachment to the most apical portion of the connective attachment (Ingber 1977). Recently, in the "New classification of periodontal and peri-implant diseases and conditions" published in 2018 was recommended the use of *supracrestal tissue attachment* instead of biologic width (Jepsen 2018). The data and meta-analysis available nowadays show us that the variable dimensions of these supracrestal attached tissues ranged between 2.15 to 2.30 mm (Schmidt 2013) regardless the association with other factors such as tooth type, surface, phenotype, loss of attachment and presence of restorations, which make almost impossible to clearly define a "fixed" dimension (Ercoli 2018).

It is commonly accepted that this biologic width must be respected when restorative procedures are done, otherwise it could lead to an inflammatory response of the periodontium. From a clinical point of view, this reaction leads to localized gingivitis or periodontitis, including bleeding, swelling, suppuration, loss of attachment and periodontal pockets (Padbury 2003). Furthermore, even when the restoration is placed intracrevicularly, gingival inflammation and loss of clinical attachment have been observed (Newcomb 1974), but most of these results are obtained from research carried out with prosthetic components. Moreover, there are very little scientific data about the reaction of the supracrestal tissues to other materials, and most of the studies available are on materials that are no longer in use (Carnevale 1983, Schätzle 2001).

Therefore, the correct selection of the materials for this kind of treatments will be essential. There are several ones that have been used throughout history: gold, ceramics, resin-modified glass ionomers and composite resins (Sarfati 2018). Gold restorations are no longer used for aesthetics reasons and ceramics restorations have a different problem because of the accumulation of plaque in the gap. Resin modified glass ionomer, despite its recognized subgingival biocompatibility (Dragoo 1997), has shown poor mechanical properties, weakness of its long-term adhesion on tooth surface, and the aesthetic problem (almost 50% of the restorations presented a color change after 2 years) (Santamaria et al. 2014). Composite resins seem to be the gold standard today, given the high quality of their adhesion and aesthetics (compared to glass ionomer materials) (Sarfati 2018) and they are also reported to be well tolerated subgingivally (Martins 2007).

On the other hand, tricalcium silicate materials are used for root canal filling, restorative procedures such as vital pulp therapy, regenerative endodontics, apexification, perforation repair, root-endo filling, retrograde obturation in apical surgery and more recently as a sealer (Torabinejad 2018, Parirokh 2018, Trope 2015,

Jafari 2017). These materials have shown biological properties such as bioactivity (Moinzadeh 2016, Walsh 2018, Malkondu 2014) and biomineralization potential (Güven 2013). Biodentine (Septodont, St-Maur-des-Fosses, France) is a bioactive calcium silicate-based restorative material. It´s employed as a dentine substitute and can be used as a repair material for endodontic lesions, because of its short setting time, good seal, high compressive strength, biomineralizing properties and tissue compatibility (Leiendecker et al. 2012). Although it is a tricalcium silicate-based material (similar to MTA), the absence of aluminate on its composition reduces the working time and avoids potential health risks (Camilleri 2013). Additionally, it has low cytotoxicity (Zhou et al. 2013) and low tooth discoloration (Marconyak et al. 2016).

However, we do not have data nowadays about the response of the periodontium when these materials are found in the biological width. For that reason, the objective of this study is to analyze the behavior of the supracrestal tissue attachment when a tricalcium silicate material (Biodentine) or composite margins restoration are placed to 0.5mm and 1.5mm from the bone crest.

Material and methods

The Ethical Committee of the Rof Codina Foundation (Lugo, Spain) approved the study as a randomized controlled trial with intra-subject control for the comparison of four treatment procedures (01/17/LU-001). The animals were housed in the Animal Experimentation Service of the Rof Codina Foundation (Cebiovet, Lugo, Spain) and the surgical procedures were carried out from April to July 2018. All the experiments were performed according to Spanish and European regulations regarding the care and use of research animals, in full compliance with the ARRIVE guidelines (Kilkenny, Browne, Cuthill, Emerson & Altman, 2010).

Animals

Eight adult female Mongrel Hound type dogs with a weight of 19-22 kg (mean 20.5 kg) and mean age 16.5 months were used for this study (Marshall Bioresources, North Rose, NY, USA). None of the animals had pathology or systemic symptoms or clinical signs of gingival inflammation or periodontal disease at baseline.

During the experiment the dogs were fed with moistened granulated food in individual bowls and free supply of water. They were kept in group kennels with controlled temperature, natural light and access to outdoors. Dogs were monitored by a veterinarian accredited in laboratory animal science. The procedures started after a quarantine period of 3 weeks.

Study design and randomization.

Experimental sites were randomly assigned to one of the four treatment options according to a computer-generated randomization list. Allocation treatment was concealed by sealed envelopes until the surgical procedure.

The lower canines of the 8 dogs were used to evaluate the histomorphometric response of the supracrestal periodontal tissues to the placement of tricalcium silicate-based cement (biodentine, Septodont, St-Maurdes-Fossés, France) or composite (CeramX Universal, Dentsply, Konstanz, Germany) in class V cavities located at two different distances from the bone crest (0.5mm or 1.5mm).

Surgical and restorative procedures

The study was carried out in a single surgical phase under sterile conditions in an animal operating room and under general anesthesia induced by propofol (3–5 mg / kg / iv, Propovet, Abbott Laboratories, Kent, UK) and maintained on a concentration of 2.5-4% of isoflurane (Isoba-vet, Schering-Plough, Madrid, Spain). Animals were previously premedicated with medetomidine (20 mcg / kg / im., Domtor, Esteve, Barcelona, Spain) and the pain was controlled with morphine (0.4 mg / kg / im, Morphine Braun 2%; B. Braun Medical, Barcelona, Spain). Throughout the procedure the animals were monitored controlling electrocardiography, pulsioxymetry, capnography and non-invasive blood pressure.

After infiltrating local anesthesia in the area to be treated, a notch was made at the level of the gingival margin using a high-speed round diamond bur with a diameter of 1 mm (No. 1012, KG Sorensen) under saline irrigation sterile (Fig. XX). Then, an intrasulcular incision was made, which was extended mesially and distally, in order to raise a full thickness flap and expose the bone crest (Fig. XX). The Class V cavities of 3 mm (mesio-distal) x 2mm (palatal vestibule) x 2mm (incised-gingival) were prepared (Fig. XX). The apical part of the cavity was positioned to 0.5 mm or 1.5 mm from the bone crest. These cavities were then restored using calcium silicate-based cement (Biodentine, Septodont, St-Maurdes-Fossés, France) in the test group and composite resin (CeramX Universal, Dentsply, Konstanz, Germany) in the control group. After this, the flaps were repositioned and a primary wound closure was achieved with 5.0 resorbable suture (Coated VICRYL^{TM} Rapide; Ethicon US, LLC 2014).

At the end of the procedure atipamezol (50 mcg/kg/i.m., Esteve, Barcelona, Spain) was administered to revert the effect of the Medetomidine. Post-operative pain was controlled with morphine (0.2 mg / kg / im / 6 h, Morphine Braun 2%; B. Braun Medical, Barcelona, Spain) and meloxicam was administered as anti-inflammatory and analgesic treatment (0.2 mg / kg / im / SID, Metacam; Boehringer Ingelheim, Barcelona, Spain) for 5 days. Cefovecin (8 mg/kg/s.c./SID, Convenia, Zoetis, Gerona, Spain) was administered intraoperatively as antibiotic.

The animals were included in a plaque control program consisting in dental cleaning three times a week with gauzes wetted in 0.12% chlorhexidine oral rinse (during the first 2 weeks). After that toothbrushing was carried out 3 times a week with a chlorhexidine 0.2% gel.

Retrieval of samples and histological preparation

After 12 weeks of healing the dogs were euthanized with an overdose of intravenous injection of sodium pentobarbital (40–60 mg/kg/i.v.; Dolethal, Vetoquinol, France) previously sedated with medetomidine (30 mcg/kg/i.m., Esteve, Barcelona,

Spain). The lower jaws were dissected and the blocks containing the experimental samples were fixed in 4% neutral formality solution for 48 hours.

Blocks containing the tooth and soft tissues surrounding it were dehydrated and infiltrated with different graded mixtures of ethanol and glycolmetacrylate (Technovit 7200 VLC, Kulzer, Werheim, Germany) following a method previously published (Donath & Breuner, 1982). The samples were then polymerized and heated at 37ªC for 24 h to assure the complete polymerization.

A central 200 microns thickness section in a buccolingual direction of the restored area was obtained using a band saw and mechanically micropulished (Exakt Apparatebau, Norderstedt, Germany) until obtain samples with a thickness of around 20 microns. Slides were stained using Levai-Laczkó method (Laczkó and Levai, 1975).

Histomorphometrical analysis

For image acquisition a motorized stage light microscopy and a digital camera connected to a PC-based image capture system (BX51, DP71, Olympus, Tokyo, Japan) were used. Linear measurements were then obtained by a masked examiner using a PC-based image analysis program (Cell-sens 1.13, Olympus, Tokyo, Japan).

The following landmarks were identified (Fig. XX):

(1) Bone loose, distance between the bone crest and the previously position of the cavities (BL). Measured distance between the bone crest and the apical margin of the restoration and then subtracting the previous distance from the bone to the cavity.

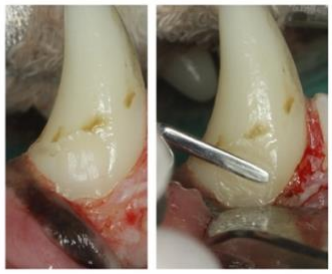
(2) STA = supracrestal tissue attachment

(3) REC = recession

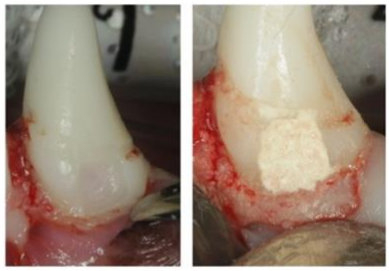
(4) Supracrestal connective tissue height, distance between the bone crest and the apical position of the junctional epithelium (CTh)

(5) Epithelium height, distance between the gingival margin and the apical portion of the junctional epithelium (Eh).

Composite 1.5



Biodentine 0.5



Biodentine 1.5

Statistical analysis

Descriptive statistics are expressed as means ± standard deviations. The normal distribution of the variables was examined with the Shapiro-Wilk test. The unit of analysis was the dog. The differences in histologycal parameters within the same group (composite or biodentine) at different localization were analyzed with paired T-Test. Histological parameters between groups were compared with T-Test. P values <0.05 were considered statistically significant.

Results

The 8 animals healed without any complication and all the specimens were available for the analyses

Histological description of the samples.

In all the specimens the junction epithelium was stablished apical to the tooth preparations. However, in the biodentine group, part of this material was embedded into the epithelium of the majority of the samples. It seems as if the junction epithelium was stronger attached to the biodentine. In the connective tissue we couldn´t appreciate any difference between groups with a normal distribution and attachment of the collagen fibers. No inflation could be observed.

Histometric analysis

Intergroup comparisons (biodentine vs composite)

A shorter distance to the bone (0.5 cavity), implies greater apical periodontal migration regardless of the material used. Table 2 showed the values of histological parameters after 12 weeks of treatment. There were no statistically significant differences comparing different groups biodentine and composite ($p > 0.05$).

In the biodentine groups the distance to bone loose and the supracrestal connective tissue height were greater than in the composite groups, while the supracrestal tissue attachment, the recession and the epithelium height were less.

Table 2. Histological parameters after 12 weeks of treatment from each group (mean ± standard deviation).

Groups	Histological parameters				
	BL	STA	REC	CTH	EP
Composite					
at 0.5mm	0.86 ± 0.30	5.23 ± 0.72	1.99 ± 0.93	1.13 ± 0.28	4.09 ± 0.83
at 1.5mm	0.48 ± 0.33	5.48 ± 0.51	3.41 ± 1.26	1.06 ± 0.23	4.42 ± 0.64
Biodentine					
at 0.5mm	1.15 ± 0.28	5.15 ± 0.86	1.87 ± 0.59	1.34 ± 0.16	3.81 ± 0.91
at 1.5mm	0.84 ± 0.29	5.10 ± 0.90	2.16 ± 0.65	1.48 ± 0.34	3.62 ± 1.05

BL = bone loose STA = supracrestal tissue attachment; REC = recession; CTH = supracrestal connective tissue height; EP = epithelium height.

Intragroup comparisons (0.5 vs 1.5)

Regarding intragroup comparisons, a statistically significant difference was observed in bone loose (BL), 0.37mm (p=0.016, 95% CI= 0.13 - 0.61) in composite group. Other intragroup comparisons showed not statistical differences (Table 3).

Table 3. Differences (mean ± standard deviation) of histological parameters after 100 days of treatment in intragroup comparisons.

Material			Composite	Biodentine
Histological parameters	BL	Mean ± SD	0.37 ± 0.15	0.31 ± 0.25
		p	0.016	0.089
		95% CI	0.13 - 0.61	-0.09 - 0.70
	STA	Mean ± SD	-0.25 ± 0.32	0.05 ± 0.41
		p	0.205	0.819
		95% CI	-0.76 - 0.25	-0.60 - 0.70
	REC	Mean ± SD	-1.43 ± 1.52	-0.30 ± 0.24
		p	0.156	0.094
		95% CI	-3.84 - 0.98	-0.68 - 0.09
	CTH	Mean ± SD	0.07 ± 0-07	-0.15 ± 0.24
		p	0.165	0.318
		95% CI	-0.05 - 0.18	-0.53 - 0.24
	EP	Mean ± SD	-0.32 ± 0.34	0.20 ± 0.54
		p	0.159	0.523
		95% CI	-0.87 - 0.23	-0.67 - 1.06

BL = distance to bone level; STA = supracrestal tissue attachment; REC = recession; CTH = supracrestal connective tissue height; EP = epithelium height. SD = standard deviation; 95% CI = Confidence interval at 95%.

Discussion

The employment of a restorative margin in the subgingival area has been related with inflammation and bone resorption in the periodontium (Parma-Benfenati 1985, 1986, Günay 2000, Padbury 2003, Schätzle 2001). Some studies have proposed that chemical or physical properties of restorative materials, such as roughness and acidic by-products, may factor into these observations (Nassar 1995, Wearhaug 1956). But it is important to observe that all these recommendations were made in a time when restorative material did not possess adhesive properties, leading to the frequent observation of bacteria in the limits between restoration and tooth (Svanberg 1990).

Composite group

Direct adhesive composite well-refined subgingival restorations signify an important clinical resource in dental practice for treatment of various conditions. Subgingival restorations are increasingly applied to treat no-carious cervical lesions (NCCLs), often in combination with root coverage procedures. Root coverage is facilitated by the restoration of the emergence profile of the clinical crown to provide a stable, convex and smooth surface for the positioning of the surgical flap (Zucchelli 2011). Furthermore, composite is routinely used for treatment of large class II, V, III and IV (Bertoldi 2019); most frequent and complex are large second-class restorations with deep cervical margins. The subgingival position of the margin makes isolation and adhesion particularly difficult in some cases (Veneziani 2010). Cavities of wide dimensions, often with the need for cuspal coverage an indirect restoration it´s necessary to restore the tooth properly (Veneziani 2010). To be able to take the impressions correctly, as well as to isolate and cement the indirect restorations it´s necessary to make a deep margin elevation or coronal margin relocation as an alternative of surgical crown lengthening. This conservative technique was introduced by Dietschi and Spreafico in 1998 and it is performed under rubber dam isolation following the placement of a matrix and then to place a base of composite resin to coronally move proximal margins underneath future indirect bond restorations (Dietschi 1998, Magne 2012). None of this two papers, shows how you can introduce in the biological width and when it´s necessary to make surgical crown lengthening. But, Veneziani, classified three different clinical situations based on technical-operating and biological parameters. Only in Grade 1, when rubber dam can be correctly placed in the sulcus sufficiently to show the cervical margin, the coronal relocation of the margin could be carried out. In the other two clinical situations, surgical exposition of the margin (Grade 2) or surgical lengthening of the clinical crown (Grade 3) is necessary in order to allow for correct isolation of the operating field (Veneziani 2010).

More recently, a randomized clinical trial compared the clinical results of crown lengthening and DME in posterior teeth. At 180 days, clinical attachment loss was obviously higher in the surgery group, but were similar in both groups, suggesting that DME was well tolerated by the periodontium. The problem of this report is that they didn't quantified the distance of the clean marginal level and the bone crest. In our study we made two Class V cavity, at 0.5mm and 1.5mm of the bone crest. In 0.5mm group an apical bone migration of 1mm was observed, however in group 1.5 the bone remained at the same level. This could be explained because connective tissue is not capable of joining the composite, according to the study by Martins and Santamaria (Martins 2007, Santamaria 2013). In the histology, it´s clear that no connective attachment is present with the composite, but few signs of inflammation was observed for 0.5 group and no signs of inflammation was observed for 1.5 group. No gap was observed and therefore it may be a reason that helps the correct functioning of the composite with the periodontal tissue. According to Safarti (Safarti 2018), we observed a different biologic width when we introduced composite in subgingival area; mainly composted of a long junctional epithelium and a slight connective attachment below the material. This situation seems healthy and well tolerated by the organism. In other words, we can say that we can do a direct restoration or a deep margin elevation at 1.5mm from the bone crest with a composite in a predictable way (always with a good adaptation and polishing of the composite) and without inflammatory problems in most cases, because we preserve the space for the connective attachment. On the other hand,

if the distance is lower, a surgical crown lengthening with osteotomy is indicated to give this space to the connective tissue. Furthermore, it´s really difficult to do a good restorative procedure in the apical millimeter of the biological space, where the adhesion is compromised.

Biodentine group

Iatrogenic perforations or external/internal resorptions may cause a communication between the tooth and the periodontium (Tirone 2018). Treat and seal these communications as soon as possible will be of high importance to improve the prognosis of the tooth. The location of a perforation or a resorption is probably the most important factor affecting treatment prognosis. Thus, the critical zone in terms of prognosis is the level of the crestal bone and the epithelial attachment (Estrela 2018). Close proximity to the gingival sulcus can lead a contamination with bacteria from the oral cavity. If the lesion is apically, the nearness to the epithelial attachment is critical and apical migration of the epithelium to the perforation/resorption site will create a periodontal defect. But crestal root lesions, are most susceptible for an epithelial migration and rapid pocket defect, for that reason they have the lowest success rate or repair (Fuss 1996). Silva (Silva 2019) studied the tissue response to MTA and biodentine after furcation perforations in dogs. They observed that both materials were biocompatible with a formation of mineralized tissue and partial reinsertion of periodontal ligament fibers in the newly formed mineralized tissue, but rather there was only partial and sparse fiber reinsertion. For that reason, ideally, in the biological width we need a material that allows the insertion of connective tissue on its surface. In our study, we made two boxes (as with composites) at 0.5 and 1.5mm of the bone crest and our results were completely opposite to Silva. In the two levels of the biological width, we didn´t observe any ligament fibers reinserted, we observed inflammation and migration of the bone crest apically.

Comparison of two groups

When we compare the two groups, no statistically significant differences were observed in bone loss between the composite and biodentine group (at 0.5 and 1.5mm). The trend is greater in biodentine as well as inflammatory infiltrate that can be observed in histology (fig. X). Bioactive ability observed in other areas does not appear in the biological space. This could be explained because the connective tissue couldn´t reinsert to the material, then the bone migrated apically, and a new biological width was made. Moreover, as we can see in the histologic images, biodentine is more roughness than composite and this can cause this inflammation (Waerhaug 1956). Furthermore, bacteria from the sulcus can contaminate the restoration and induce the inflammation. For this reason, it seems that Biodentine is not a suitable material to introduce into the biological width. The most favorable response by the periodontium to the composite could be related to the excellent adhesion observed in the histology of the dentin with this material, as well as the careful polishing carried out before closure (Blank 1979, Gomes 2005). Therefore, according to the literature, the space for the insertion of connective tissue must be preserved (Guezzi 2019). If this supracrestal tissue insertion is violated, inflammation and loss of periodontal support tissue will occur, accompanied by apical displacement of the junctional epithelium and insertion of the supracrestal tissue (Jepsen 2018).

The objective of this study was to observe the behavior of composite and Biodentine in the biological width to check if a periodontal reinsertion on these materials was formed and if the level of the bone crest was maintained. After histological analysis, it was observed that there was no periodontal reattachment and the bone crest goes down with two materials. Due to all this, in these types of lesions, it is recommended to perform composite restorations at the subgingival level whenever the distance to the bone crest is at least 1.5mm. If not, the indication would be to perform an surgical crown lengthening with osteotomy until you obtain this distance. If the esthetic zone was affected, the indication would be to compensate for this loss of tissue with a connective tissue graft.

Padbury A Jr,Eber R,Wang HL. Interactions between the gingiva and the margin of restorations. J Clin Periodontol 2003;30:379-85.

Santamaria MP, Suaid FF, Carvalho MD, Nociti FH Jr, Casati MZ, Sallum AW, Sallum EA. Healing patterns after subgingival placement of a resin-modified glass-ionomer restoration: a histometric study in dogs. Int J Periodontics Restorative dent 2013;33:679-87.

Ingber, J. S., Rose, L. F. & Coslet, J. G. (1977) The “biologic width” – a concept in periodon- tics and restorative dentistry. Alpha Omegan 70, 62–65.

Schmidt JC, Sahrmann P, Weiger R, Schmidlin PR, Walter C. Biologic width dimensions – a systematic review. J Clin Periodontol 2013;40:493-504.

Newcomb GM. The relationship between the location of subgingival crown margins and gingival inflammation. J Periodontol 1974;45:151-154.

Carnevale G, Sterrantino SF, Di Febo G. Soft and hard ttissue wound healing follow ing tooth preparation to the alveolar crest. Int J Periodontics Restorative Dent 1983;3:36-53.

Schätzle M, Land NP, Anerud A, Boysen H, Bürgin W, Löe H. The influence of margins of restorations of the perio- dontal tissues over 26 years. J Clin Periodontol 2001;28:57-64.

Sarfati A, tirlet G. Deep margin elevation versus Crown lengthening: biologic with revisited. Int J Esthet Dent 2018;13:334-356.

Dragoo, M. R. (1997) Resin-ionomer and hybrid- ionomer cements: part II, human clinical and histologic wound healing responses in specific periodontal lesions. International Journal of Periodontics & Restorative Dentistry 17, 75–87.

Santamaria, M. P., Mathias, I. F., Dias, S. B., Jardini, M. A. N., Junior, M. S. & Sallum, E. A. (2014) Esthetic evaluation of different approaches to treat gingival recession associ- ated with non-carious cervical lesion treatment: a 2-year follow-up. American Journal of Den- tistry 27, 220–224.

Martins TM , Bosco AF, Nóbrega FJ, Nagata MJ, Garcia VG, Fucini SE.Periodontal

tissue response to coverage of root cavities restored with resin materials: a histomorphometric study in dogs. J Periodontol 2007;78:1075-82.

Torabinejad M, Parirokh M, Dummer PM. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview-part II: other clinical applications and complications. Int Endod J 2018;51:284–317.

Parirokh M, Torabinejad M, Dummer PM. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview-part I: vital pulp therapy. Int Endod J 2018;51:177– 205.

Trope M, Bunes A, Debelian J. Root filling materials and techniques: bioceramic a new hope? Endod Topics 2015;32:86–96.

Jafari F, Jafari S, Etesamnia P. Genotoxicity, bioactivity and clinical properties of calcium silicate based sealers: a literature review. Iran Endod J 2017;12:407–13.

Moinzadeh AT, Aznar Portoles C, Schembri Wismayer P, et al. Bioactivity potential of EndoSequence BC RRM putty. J Endod 2016;42:615–21.

Walsh RM, He J, Schweitzer J, et al. Bioactive endodontic materials for everyday use: a review. Gen Dent 2018;66:48–51.

Malkondu Ö, Karapinar Kazandağ M, Kazazoğlu E. A review on biodentine, a contemporary dentine replacement and repair material. Biomed Res Int. 2014; 2014:16095

Güven EP, Tas¸li PN, Yalvac ME, et al. In vitro comparison of induction capacity and biomineralization ability of mineral trioxide aggregate and a bioceramic root canal sealer. Int Endod J 2013;46:1173–82.

Leiendecker AP, Qi YP, Sawyer AN, et al. (2012) Effects of calcium silicate-based materials on collagen matrix integrity of mineralized dentin. Journal of Endodontics 38, 829– 33.

Camilleri J (2013) Investigation of Biodentine as dentine replacement material. Journal of Dentistry 41, 600–10.

Zhou HM, Shen Y, Wang ZJ, et al. (2013) In vitro cytotoxic- ity evaluation of a novel root repair material. Journal of Endodontics 39, 478–83.

Marconyak LJ Jr, Kirkpatrick TC, Roberts HW, et al. (2016) A comparison of coronal tooth discoloration elicited by various endodontic reparative materials. Journal of Endodontics 42, 470–73.

Parma-Benfenati S, Fugazzotto PA, Ru- ben MP. The effect of restorative margins on the postsurgical development and nature of the periodontium. Part I. Int J Periodontics Restorative Dent 1985; 5:30–51.

Parma-Benfenati S, Fugazzotto PA, Fer- reira PM, Ruben MP, Kramer GM. The effect of restorative margins on the postsurgical development and nature of the periodontium. Part II. Anatomical considerations. Int J Periodontics Restor- ative Dent 1986;6:64–75.

Günay H, Seeger A, Tschernitschek H, Geurtsen W. Placement of the prepa- ration line and periodontal health—a prospective 2-year clinical study. Int J Periodontics Restorative Dent 2000;20:1 71–181.

Padbury A Jr, Eber R, Wang HL. Interac- tions between the gingiva and the margin of restorations. J Clin Periodontol 2003; 30:379–385.

Schätzle M, Land NP, Anerud A, Boy- sen H, Bürgin W, Löe H. The influence of margins of restorations of the peri- odontal tissues over 26 years. J Clin Periodontol 2001;28:57–64.

Nassar U, Meyer AE, Ogle RE, Baier RE. The effect of restorative and prosthetic materials on dental plaque. Periodontol 2000 1995;8:114–124.

Waerhaug J. Effect of rough surfaces upon gingival tissues. J Dent Res 1956;35:323-325

Zucchelli G, Gori G, Mele M, Stefanini M, Mazzotti C, Marzadori M, Montebugnoli L, De Sanctis M (2011) Non-carious cervical lesions associated with gingival recessions: a decision-making pro- cess. J Periodontol 81:1713–1724

Bertoldi C, Monari E, Cortellini P, Generali L, Lucchi A, Spinato S, Zaffe D. Clinical and histological reaction of periodontal tissues to subgingival resin composite restorations. Clin Oral Invest 2020;24:1001-1011.

Veneziani M. Adhesive restorations in the posterior area with subgingival cervical margins: new classification and differentiated treatment approach. Eur J Esthet Dent 2010;5:50-76..

Dietschi D, Spreafico R. Current clinical concepts for adhesive cementation of tooth-colored posterior restorations. Pract Periodontics Aesthet Dent 1998;10:47–54 quiz 56.

Magne P, Spreafico RC. Deep margin elevation: a paradigm shift. Am J Esthet Dent 2012;2:86-96.

Martins TM , Bosco AF, Nóbrega FJ, Nagata MJ, Garcia VG, Fucini SE.Periodontal tissue response to coverage of root cavities restored with resin materials: a histomorphometric study in dogs. J Periodontol 2007;78:1075-82.

Santamaría 2013

Tirone F, Salzano S, Piattelli A, Perrotti V, Iezzi G. Response of periodontium to MTA and Biodentine: a pilot histological study on humans. Aust Dent J 2018;63:231-241.

Estrela L, Decurcio D, Rossi-Fedele G, Silva JA, Guedes OA, Borges AH. Root perforations: a review of diagnosis, prognosis and materials. Braz Oral Res 2018;32:133-146.

Fuss Z, Trope M. Root perforations: classification and treatment choices based on prognostic factors. Endod Dent Traumatol 1996;12:255-64.

Silva RAB, Borges ATN, Hergandez-Gatón P, de Queiroz AM, Arzate H, Romualdo PC, Nelson-Filho P, Silva LAB. Histopathological, histoenzymological, immunohistochemical and immunofluorescence analysis of tissue response to sealing materials after furcation perforation. Int Endod J 2019;10:1489-1500.

Blank LW, Cafesse RG, Charbeneau GT. The gingival response to well-finished composite resin restorations. J Prosthet Dent 1979;42:626-632.

Gomes SC, Miranda LA, Soares I, Oppermann RV. Clinical and histologic evaluation of the periodontal response to restorative procedures in the dog. Int J periodontics Restorative Dent 2005;25:39-47.

Guezzi C, Brambilla G, Conti A, Dosoli R, Ceroni F, Ferrantino L. Cervical margin recolocation: case series and new classification system. Int J Esthet Dent 2019;14:272-284.