# Regenerative Endodontic Procedures with scaffolds in immature teeth with apical periodontitis



Palma PJ<sup>1\*</sup>, Ramos JC<sup>1</sup>, Martins JB<sup>1</sup>, Viegas C<sup>2</sup>, Santos JM<sup>1</sup>

<sup>1</sup>Dentistry Department, Faculty of Medicine of Coimbra University, Coimbra, <sup>2</sup>Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal e-mail: ppalma@fmed.uc.pt

# Introduction

# Pulp revascularization procedure

# Regenerative Endodontics / Tissue Engineering

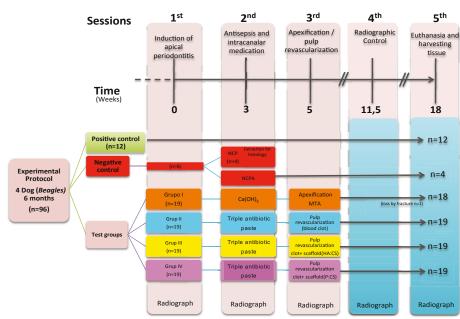
Aim: This work aimed to determine the regenerative potential of the immature dog teeth, through the employment of a new regenerative approach. To this end both the induction of a vital supporting structure as a blood clot, and the implantation of chitosan scaffolds within the root canal space were tested.

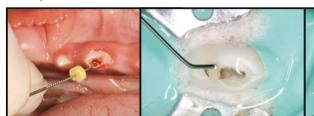
# Morphogenic and growth factors



# Methodology

Pulp necrosis and apical periodontitis were attained by infecting the immature teeth of 4 Beagle dogs, in a total of 96 root canals. Teeth were then divided into 4 test groups according to the treatment protocol: I - MTA apical plug; II - revascularization protocol; III - revascularization plus sodium hialuronate:chitosan scaffold; and IV - revascularization protocol plus pectin:chitosan scaffold. All revascularizations were performed following root canal disinfection with a triple antibiotic paste. Thirteen weeks post-treatment the animals were sacrificed and the jaw block harvested for processing and observation by light microscopy. Morphological and histomorphometric analysis of mineralized tissues in the canal space was carried out. Statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney U tests, with a level of significance of P < 0,05.





Canalar irrigation with 2.5% sodium hypochlorite. Pulp disruption to induce apical periodontitis.

antibiotic paste (metronidazole minocycline and ciprofloxacin).





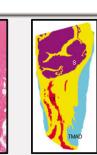
(HA:CS) and pectin:chitosan (P:CS)

Aspect of the pulp chamber after placing the MTA plug.

# Results



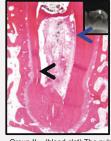




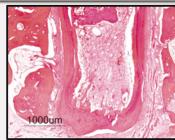
Group I apexification -

closed apex by the induction of an apical

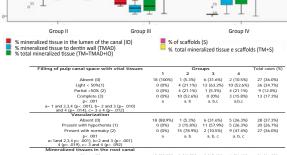
cementum bridge (B).







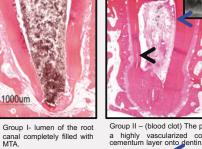




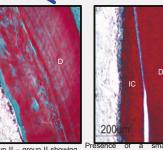
Vascularization					
Absent (0)	18 (88,9%)	1 (5.3%)	6 (31.6%)	5 (26.3%)	28 (37.3%)
Present with hyperhemia (1)	0 (0%)	3 (15.8%)	11 (57.9%)	5 (26.3%)	20 (26.7%)
Present with normalcy (2)	0 (0%)	15 (78.9%)	2 (10.5%)	9 (47.4%)	27 (36.0%)
p< .001	a	a, b	a, b, c	a, b, c	
a=1and 2,3,4 p< .001), b=2 and 3 (p< .001) 4 (p= .019), c= 3 and 4 (p= .092)					
Mineralized tissues in the root canal					
Absent (0)	18 (88,9%)	2 (10,5%)	9 (47,4%)	4 (21,1%)	31 (41,3%)
Present (1)	0 (0%)	17 (89,5%)	10 (52,6%)	15 (78,9%)	44 (58,7%)
p<0,001	a	a, b	a, b	a	
a=1 and 2,3 4 (p< .001), b=2 and 3 (p= .013)					
Mineralized tissues formed on root canal wall					
Absent (0)	6 (33.3%)	0 (0%)	4 (21.1%)	3 (15.8%)	13 (17.3%)
Present with increased thickness of the root wall (1)	3 (16.7%)	7(36.8%)	7 (36.8%)	9 (47.4%)	26 (34.7%)
Present with increased length of the root (2)	6 (33.3%)	1 (5.3%)	0 (0%)	0 (0%)	7 (9.3%)
Present with increased length and thickness of the root (3)	3 (16.7%)	11 (57.9%)	8 (42.1%)	7 (36.8%)	29 (38.7%)
p = .270					
Apical closure	•		-		
Absent (0)	3 (16.7%)	6 (31.6%)	7 (36.8%)	6 (31.6%)	22 (29.3%)
Partial (1)	9 (50%)	4 (21.1%)	7 (36.8%)	7 (36.8%)	27 (36%)
Complete (2)	6 (33.3%)	9 (47.4%)	5 (26.3%)	6 (31.6%)	26 (34.7%)
p = .681					

# Acknowledgments

Laboratory hard tissues of Medicine Faculty of Coimbra ,Department of Chemica Engineering, Coimbra University, Coimbra, Laboratory for Biostatistics and Medical Informatics, Faculty of Medicine of Coimbra University, Coimbra,

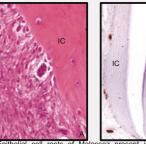


Group II – (blood clot) The pulp space showed the presence of



Group II - group II showing separating the intracanal cementum (IC) and the dentin (D). Continuity between this well as pre-dentin and an





tissue, nearby the intracanalar cellular cementum, in the apical portion of canalar space . A) HE. B) Positive  $\,$ immunocytochemistry staining

The lumen of the root canal completely filled with MTA with creation of an apical bridge between root canal walls in 83% of the samples in group I. A vital vascularized tissue was found in Groups II. III and IV. For these groups, apical closure happened in 66.7% of the treatments and root growth was detected more often as an increase in thickness (85.6%) than in length (45.6%), The greatest amount of mineralized tissue inside the canal were observed in group II (blood clot), with significant differences compared to group III (P = 0.006), and group IV (P = 0.025). Radiographic evaluation post-treatment revealed that the apical region was remineralized with reestablishment of a periodontal ligament space. Histological evaluation revealed that apical mineralization was composed of of cellular cementum and dentine, surrounded by periodontal ligament, containing epithelia rests of Malassez, exhibiting a similar composition to the walls of any ordinary root apex, including the presence of foramina.

# Conclusions

The addition of the scaffolds to the blood in regenerative procedures did not improve the formation of new mineralized tissues along the root canal walls and the histologic evidence of the regeneration of a pulp-dentin complex. Despite of the scaffolds used, REPs allowed the continued development of root walls with presence of bone-like tissue, cementum and periodontal ligament often observed into the canal space. In spite of the theoretical possibility of a more effective regeneration with different scaffolds (group III, IV), our results sustain that better results were found with the induction, formation and stabilization of a blood clot (group II). REP's should be considered as a potential option for the treatment of immature permanent teeth with irreversible pulpitis or pulp necrosis.