

Supporting information

Alignment of digital periapical radiographs using the TurboReg plug-in

All periapical digital radiographs were aligned using TurboReg plug-in on ImageJ (<http://bigwww.epfl.ch/thevenaz/turboreg/>). The x-ray with the best angulation was set as "Target" and the other x-rays were set as "Source". For each case, different options were tried and that of best results was used. For case #2, the best alignments were obtained using "Rigid body-Accurate-Manual" options. A brown reference line was set to connect between Distal canine cemento-enamel junction (CEJ) and Mesial CEJ of first molar. A circle reference had the same diameter and a green point was set at the apex of the first molar palatal root. For case #1, the best alignments were obtained using "Bilinear-Accurate-Manual" options. The reference points were; Mesio-incisal line angle of #10, apex of #10, Disto-incisal line angle of #8 and apex of #8. When Disto-incisal line angle of #8 was not visible the junction at the CEJ was used.

Cone beam computed tomography (CBCT)

For case#2, a CBCT was obtained pre-operatively and at 12 months follow-up. It was used to measure working length, confirm the presence of the second Mesio-buccal canal and its configuration and to compare the results of the regenerative treatment on the maturation of roots. CBCT images were acquired using a J.Morita R100 cone beam 3D imaging system (JMORITA MFG. CORP. Kyoto Japan) using the following parameters; field of view (FOV) of 100mm x H 80mm; 0.260 mm isometric voxel size; 90kVp, 8 mA and an exposure time of 9.4 seconds. In the pre-operative CBCT, all roots showed periapical radiolucencies and the apices were immature.. The two mesial canals were separated all over the root length and had two separate apical foramina. After 12 months, all roots showed maturation, no apical radiolucency and narrowing of canal space. However, no root lengthening was observed.

Measurement of bone mineral density using ImageJ software for case #2

Bone mineral density measurements using periapical radiographs were performed only

for case#1 as CBCT evaluation was not possible. Additionally, evidence of CBCT complete healing in case#2 was sufficient and so no further analysis was required.

Before bone density measurements, all x-rays for case#1 were standardized using MIPAV software (Medical Image Processing, Analysis, and Visualization, v7.3.0, US National Institutes of Health- Center for Information Technology). At first, they were "Histogram equalized" from Algorithms> Histogram tools> Histogram equalization> Regional adaptive. After that, they were "Histogram matched" to the last follow-up radiograph from Algorithms> Histogram tools> Histogram matching. The equalized last follow-up X-ray was selected as target.

The bone changes in periapical radiographs were measured by the Plot profile feature and occlusal radiographs were measured by "Interactive 3D Surface Plotw" feature in ImageJ software (National Institutes of Health, v1.50i; Java 1.8.0_77 [64-bit]).

Change in bone density in periapical radiographs (plot profile)

A rectangle was drawn on the pre-operative X-ray using "Rectangle tool". The rectangle was just apical to the open apex of the maxillary left central incisor and the right border touched the mesial aspect of the left lateral incisor. The left border was just at the mesial border of the bone defect. The rectangle was added to ROI manager by pressing "t" and the dimensions were copied from Edit> selection> specify. The same rectangle's position and dimensions were drawn on the last follow-up radiograph from Edit> selection> specify using the width and coordinates options. To make the comparison possible, the minimum Y-axis values were set at 0 and the maximum at 250. This was done from Edit> option> Plot> check fixed Y-axis. The plot values of the radiographs were saved and transferred to an Excel sheet to be plotted as a single graph

3D interactive surface plotting

The pre-treatment occlusal radiograph was histogram matched to the 9-months occlusal radiograph using MIPAV software. The radiographs were subtracted using ImageJ. Process> Image calculator> "subtraction" was selected from the "operation list". The 9-

month radiograph was set as "Image 1" and Pre-treatment radiograph was set as "Image 2". The region of interest was loaded from ROI manger as outlined from the 9-month radiograph. The plot was drawn using Plug-in> 3D> 3D Interactive Surface Plotting. From the first list at the top of the window, "filled" was selected instead of "lines". From the second list "Spectrum LUT" instead of "original" was selected.

Original Xrays (Without aligning using Turboereg)

Figure 2

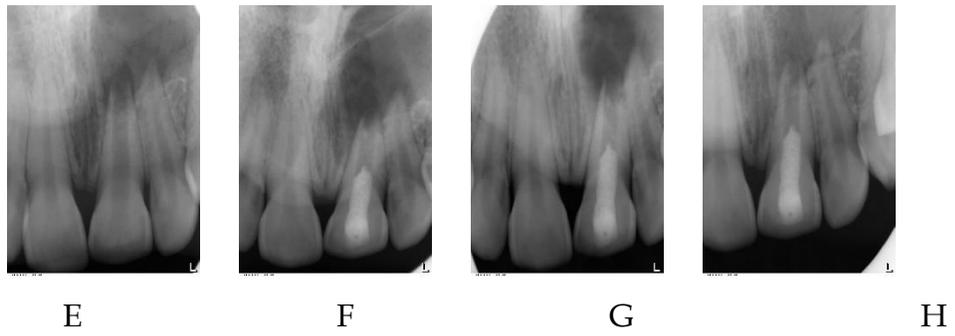


Figure 4

