Regenerative Endodontic Procedure of Immature Permanent Teeth with Leukocyte and Platelet-rich Fibrin: A Multicenter Controlled Clinical Trial

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ABSTRACT

Introduction: The aim of this nonrandomized, multicenter controlled clinical trial was to evaluate the impact of leukocyte–platelet-rich fibrin (LPRF) on regenerative endodontic procedures (REPs) of immature permanent teeth in terms of periapical bone healing (PBH) and further root development (RD). **Methods:** Healthy patients between 6–25 years with an inflamed or necrotic immature permanent tooth were included and divided between the test (= REP + LPRF) and control (= REP-LPRF) group depending on their compliance and the clinical setting (university hospital or private practice). After receiving REP ± LPRF, the patients were recalled after 3, 6, 12, 24, and 36 months. At each recall session, the teeth were clinically and radiographically (by means of a periapical radiograph [PR]) evaluated. A cone-beam computed tomographic (CBCT) imaging was taken preoperatively and 2 and 3 years post-operatively. PBH and RD were quantitatively and qualitatively assessed. **Results:** Twenty-nine teeth with a necrotic pulp were included, from which 23 (9 test and 14 control) were analyzed. Three teeth in the test group had a flare-up reaction in the first year after REP. Except for 2 no shows, all the analyzed teeth survived up to 3 years after REP, and, in case of failure, apexification preserved them. Complete PBH was obtained in 91.3% and 87% of the cases based on PR qualitative and quantitative evaluation, respectively, with no significant difference between the groups with respect to the baseline. The PR quantitative change in RD at the last recall session with respect to the baseline was not significant (all P values $> .05$) in both groups. The qualitative assessment of the type of REP root healing was nonuniform. In the test group, 55.6% of the teeth presented no RD and no apical closure. Only 50% of the 14 teeth assessed with CBCT imaging presented complete PBH. Regarding volumetric measurements on RD 3 years after REP for the change with respect to the baseline in root hard tissue volume, mean root hard tissue thickness, and apical area, the control group performed significantly in favor of RD than the test group ($P = .03$, .003, and 0.05 respectively). For the volumetric change 3 years after REP with respect to the baseline in root length and maximum root hard tissue thickness, no significant difference ($P = .72$ and .4, respectively) was found between the groups. The correlation between the PR and CBCT variables assessing RD was weak (root lengthening) to very weak (root thickening). **Conclusions:** REP-LPRF seems to be a viable treatment option to obtain PBH and aid further RD of necrotic immature permanent teeth. Caution is needed when evaluating REP with PR. *(J Endod 2021;47:1729–1750.)*

KEY WORDS

Cone-beam computed tomography; dental infection; mesenchymal stem cells; outcome assessment

SIGNIFICANCE

A total pulp necrosis and presence of a periapical infection pre-operatively negatively impact cell homing-based REP in terms of pulp-dentin complex regeneration. Caution is needed when adding leukocyte-containing APC as a scaffold and interpreting the REP-outcome by PRs.

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Regenerative medicine is an emerging and exciting field. Regeneration of tissue rather than restoration offers an alternative perspective to endodontic treatment. A regenerative endodontic procedure (REP) is defined as a biologically based treatment of immature permanent teeth designed to replace damaged structures, including dentin and root structures as well as cells of the pulp-dentin complex[1]. Mesenchymal stem cells (MSCs) are attracted into the root canal by creating bleeding in the periapical region and/or chemotactic factors (cell homing principle) or by transplanting stem cells into the root canal (cell-based principle)[1–5]. These undifferentiated stem cells are able to differentiate into several different cell types when the specific growth factors and scaffold are added[1,4]. The initial differentiation occurs necessarily in a transitory scar matrix formed by fibrin and fibronectin. This is why fibrin is preferentially used as a support matrix for the transplantation of MSCs. Several studies have reported that a fibrin matrix is an optimal support to transplanted MSCs for obtaining pulp and osseous defect regeneration[6–11].

Among the natural scaffolds applied in REPs, autologous platelet-rich fibrin (PRF) is gaining more interest[12,13]. Platelet-rich preparations have been evaluated with encouraging results to accelerate tissue healing[14,15]. It is known that platelets contain high quantities of key growth factors, such as platelet-derived growth factor, transforming growth factor beta, and vascular endothelial growth factor, which are able to stimulate cell proliferation, matrix remodeling, and angiogenesis[16]. We can divide platelet-rich preparations into 2 groups: first-generation platelet-rich plasma (PRP) and second-generation PRF. PRP has been applied in some REP studies with promising results[10,13,17]. Even if PRP is easily injectable, its production demands the addition of bovine thrombin and a double spin in the centrifuge. Furthermore, the cytokines beneficial for wound healing are lost by PRP's loose matrix in less than 14 hours[18]. On the other hand, PRF and its leukocyte-rich derivate, leukocyte–platelet-rich fibrin (LPRF), present many advantages compared with PRP. It is centrifuged blood without any additives. In the absence of anticoagulants, platelet activation and fibrin polymerization are triggered immediately. Fibrinogen will first appear at the top of the tube, whereafter the circulating thrombin transforms soluble fibrinogen into insoluble strands of fibrin. Blood is separated into 3 components with the formation of a fibrin clot in the middle, acellular plasma at the top, and red blood cells at the bottom (Fig. 1)[19]. This clot acts as a matrix that contains most light blood components, such as platelets and leukocytes, as well as circulating molecules, such as growth factors and fibronectin. After compression of the LPRF clot, it can be easily used as a membrane (Fig. 1) but has to be cut into pieces in order to insert it with pluggers into a root canal. Additionally, LPRF acts as a reservoir that gradually releases growth factors over a period of 7–14 days[20,21].

LPRF has been appraised in the periodontal literature[22,23]. It has the potential advantage of creating a bioactive construct that stimulates the local environment for the differentiation and proliferation of MSCs and progenitor cells. Of interest for the regenerative endodontic field, PRF was demonstrated to stimulate cell proliferation and differentiation of dental pulp stem cells by up-regulating osteoprotegerin and alkaline phosphatase expression[9]. Furthermore, the osteo- and odontogenic differentiation of the stem cells of the apical papilla (SCAPs) is improved by PRF[24]. These PRF matrices have the potential advantage of creating a bioactive construct in which the provisional fibrin scaffold acts as a conductive matrix for the migration of cells involved in wound repair. The anti-inflammatory cytokines in leukocytes, such as interleukin (IL)-4 and vascular endothelial growth factor, aid this wound repair[19]. However, they contain proinflammatory cytokines as well, such as interleukin IL-1β, tumor necrosis factor alpha, and IL-6[19].

The European Society of Endodontology and the American Association of Endodontics have prescribed evidence-based treatment protocols to standardize REPs and to determine the utmost outcomes to be achieved by REPs[25,26]. A growing body of evidence reports successful treatment outcomes for REPs (without autologous platelet concentrates [APCs])[27–29]. Because of the previously mentioned favorable properties, APCs are being applied and appraised in REP. Nevertheless, these studies seem to have a questionable level of evidence[30]. Also, the impact of venipuncture on the compliance of young patients, the user-friendliness of LPRF application during a REP, and the potential adverse events when adding immunity modulating scaffolds to REPs have not been reported to our knowledge[30]. Hence, we have designed this nonrandomized, noncommercial, multicenter controlled clinical trial to test the hypothesis that the use of LPRF in REP of immature permanent teeth will accelerate periapical bone healing (PBH) and stimulate root development (RD).

METHODS

Trial Design, Registration, Randomization, and Blinding

This study was designed as a multicenter controlled clinical trial with the UZ KU Leuven (Leuven, Belgium) Medical Ethics Committee assigned as the central committee (registration number: S56810). The teeth were treated in the following centers in Belgium by N.M. (endodontist) or G.V.G. (pedoendodontist): UZ Leuven (University Hospitals Leuven, Leuven, Belgium), Megabite & co (Brussels and Herent, Belgium), and EndoVanGorp (Rotselaer, Belgium). The patients were divided between the test (REP + LPRF) and control (REP-LPRF) group depending on their compliance and the clinical setting. Randomization was not possible because of noncompliance of the youngest and/or most anxious patients during venipuncture (necessary for LPRF) in the pilot phase of the study. No centrifuge or nurse (for venipuncture) was available in the included private practices. Only the assessors (M.E., A.E.T.G., and P.L.) and the statistician were blinded regarding the patients' names and allocation of the patients to the groups. This study was conducted according to the principles of good clinical practice (International Council on Harmonization, 1996), which rely on the ethical principles of the Declaration of Helsinki. This trial is registered on ClinicalTrials.gov (ID: NCT02437708) where the protocol can be accessed.

Participants

Healthy patients between 6–25 years with an inflamed or necrotic immature permanent tooth were included. The eligibility criteria are provided in Table 1.

Interventions and Treatment Protocol

Patients in need of a REP were screened for possible inclusion in this study. The subjects were informed orally and in writing about the study. Written informed consent from the patient/patient's guardian was obtained before any examination was performed for the study. The screening procedure included the collection of demographic, clinical, and radiographic data. The teeth were clinically assessed for percussion, periodontal condition (mobility and pocket depth), discoloration, swelling of the mucosa, and sensitivity by means of carbon dioxide snow (CDS) and an electric pulp tester (EPT [Pulppen DP2000 Digital; Dental Electronic, Ballerup, Denmark]). A periapical radiograph (PR [Digora and Minray; Soredex, Tuusula, Finland])

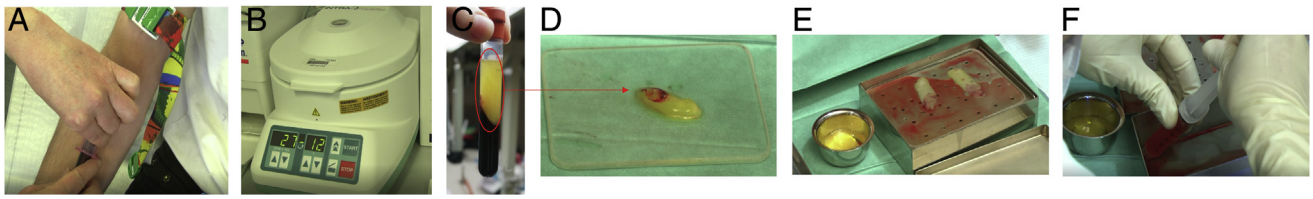

FIGURE 1 – The production of LPRF. (*A*) After venipuncture, 10 mL blood is collected in a sterile tube without any additives. (*B*) The tubes are centrifuged (Intra-Lock International, Boca Raton, FL) for 12 minutes at 2700 rpm. (*C*) Blood is separated into 3 components with the formation of a fibrin clot in the middle, acellular plasma at the top and red blood cells at the bottom. The (*D*) clot itself can be transformed into a membrane by means of pressure under a (*E*) sterile glass plate for 5 minutes. (*F*) The exudate of the pressed clot can be collected as well.

standardized by means of a silicone guide (Optosil Comfort Putty; Kulzer, Hanau, Germany) was taken preoperatively. Furthermore, a cone-beam computed tomographic (CBCT) image (Accuitomo; J Morita, Osaka, Japan) was taken as well if the device was available (not at the previously mentioned private practices). With subjects fulfilling all inclusion criteria and none of the exclusion criteria (Table 1), the treatment plan as well as the costs were discussed.

In the control group, REPs were performed in 2 sessions following the protocol described by Diogenes et al[4]. Briefly, local anesthesia with adrenaline was administered. After rubber dam isolation and access cavity preparation, the working length measurements were performed with PRs if no preoperative CBCT image was available. Afterward, the root canal was irrigated 1 mm short of the working length with 20 mL 1.5% sodium hypochlorite and subsequently with 20 mL saline. The root canal was dried with sterile paper points, and calcium hydroxide (UltraCal XS; Ultradent Products, Inc, South Jordan, UT) was injected into the root canal (1 mm short of the working length). The tooth was temporarily sealed by means of a sterile cotton pellet and glass ionomer cement. During the second session, local anesthesia without adrenaline was administered, and the tooth was isolated by means of a rubber dam. After rinsing with 30 mL EDTA 17% 1 mm short of the working length, a blood clot was triggered periapically. Subsequently, a Collaplug (Zimmer Biomet, Berlin, Germany) was placed on the blood clot, and Pure Portland Cement Med-PZ (MPC; Medcem, Weinfelden, Switzerland) was used instead of mineral trioxide aggregate (MTA) to prevent tooth crown discoloration[31]. The tooth was sealed by means of a glass ionomer lining and composite restoration.

In the test group, the treatment was performed similar to the control group, with the exception that LPRF was added as a scaffold during the second session. LPRF was collected and produced as described in Figure 1*A–F*. The second treatment session was commenced, as described by Diogenes et al[4], and in the meanwhile the blood samples were centrifuged. Fibrin clots were collected after centrifugation, and 2 of them were transformed into membranes after 5 minutes of pressure under a sterile glass plate (Fig. 1). The exudate of the pressed clots was used as a final rinse of the root canal. Afterward, a blood clot was triggered periapically into the root canal. The fibrin clots and membranes were inserted into the root canal with endodontic pluggers until 3 mm below the cementoenamel junction (CEJ). No Collaplug was applied below the MPC because the LPRF scaffold provided enough resilience to the MPC.

For both groups, the entire treatment was performed under microscopic magnification. Furthermore, in case of flare-up, the treatment provided at the first session was repeated, and a double antibiotic paste (metronidazole 500 mg and ciprofloxacin 200 mg, macrogel ointment, and propylene glycol) was injected in the root canal, as described by Diogenes et al[4]. However, the exact concentration of antibiotic paste was not measured. Furthermore, systemic antibiotics were prescribed as well, including amoxicillin or erythromycin (in case of penicillin allergy), and dosed depending on the patient's weight.

The patients were recalled at 3, 6, 12, 24, and 36 months. At each recall session, the same clinical assessments were performed as during the intake session, and 1 PR (with the customized silicone guide) was taken. A CBCT image was taken 2 and 3 years postoperatively if the patient and/or their guardian consented.

TABLE 1 - The Eligibility Criteria of the Controlled Clinical Trial

Inclusion	Exclusion
Provision of informed consent	Unlikely to be able to comply with the study procedures as judged by the principal investigator
Permanent immature teeth with weak root canal walls	Patients >25 years
Patients <25 years	Deciduous teeth
	Permanent immature teeth that can be treated by a conventional root canal treatment/apexification
	Known or suspected current malignancy
	History of chemotherapy within 5 years before the study
	History of radiation in the head and neck region
	History of other metabolic bone diseases
	A medical history that makes REP unfavorable
	Involvement in the planning and conduct of the study

REPs, regenerative endodontic procedure.

Outcomes

The primary outcome variable was the amount of PBH post-REP, which was quantitatively measured on PRs. The PRs were aligned with a dedicated tool created in MeVisLab (MeVis Research, Bremen, Germany) for image registration, and the periapical lesions were outlined and quantified with ImageJ software (National Institutes of Health, Bethesda, MD). Additionally, the full-scale and final periapical

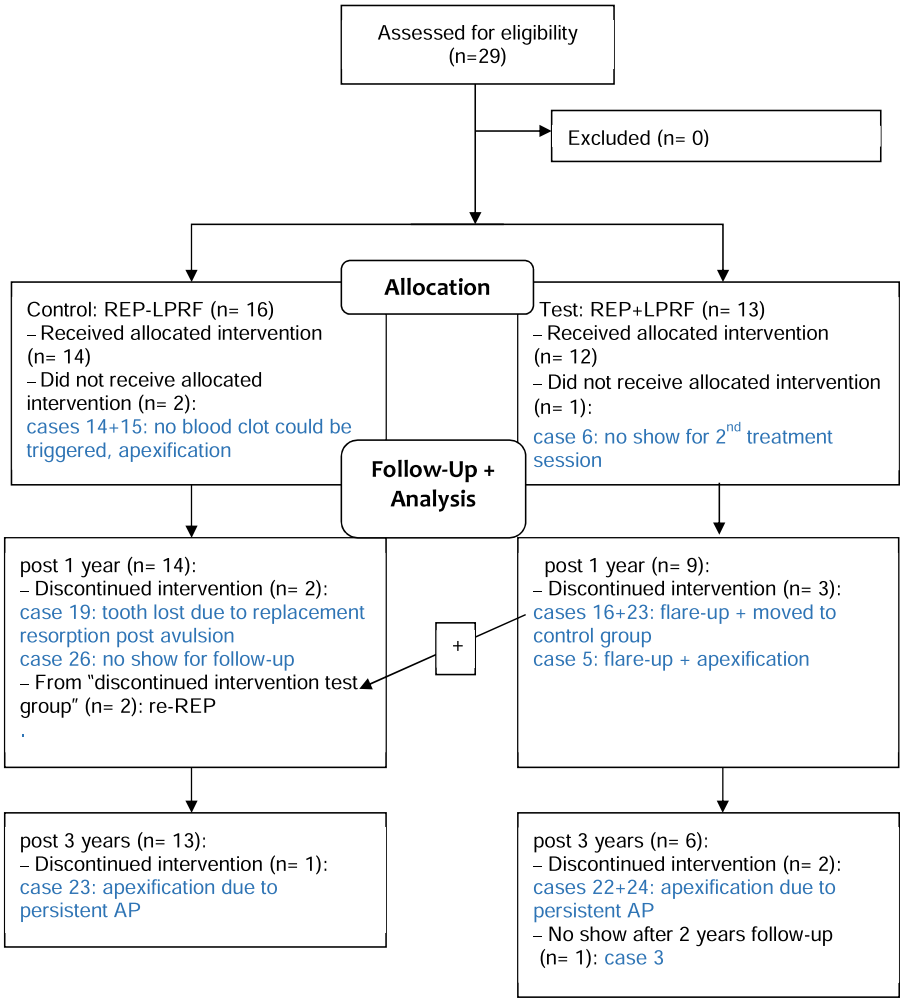

FIGURE 2 – A flow diagram of a multicenter controlled clinical trial in REPs with LPRF. The case numbers refer to the cases in Table 2. AP, apical periodontitis.

index (PAI) according to Kirkevang et al[32] were applied to assess PBH qualitatively.

The secondary outcome was the amount of RD post-REP based on 2-dimensional (2D) measurements on PRs. The change in root canal wall length (RL) and the radiographic root area (RRA) were measured with ImageJ software following the method described by Flake et al.[33] The RL was measured from the apical part of the MPC until the apex.

Qualitative assessment of the REP root healing was performed according to the 5 scores described by Chen et al.[34] Furthermore, in case these healing types or a combination of them were not enough, other types of healing were described as well.

Three blinded and independent assessors performed the radiographic measurements. The 2D measurements were performed by M.E. and A.T.G., and the 3-dimensional (3D) measurements were performed by M.E. and P.L.

3D Analysis of REP Outcomes

Based on the 3D measurements of the CBCT images taken 2 and 3 years post-REP, the change in RL, apical area (AA), root hard tissue volume (RHTV), and the maximum and mean root hard tissue thickness (RHTT) were measured.[27,35] CBCT scans were imported into MeVisLab. All teeth (pre- and postoperatively at the last follow-up) were then automatically segmented using a novel artificial intelligence–driven tooth segmentation tool[36] and largely based on the previously validated tool for 3D REP analysis.[37] During the segmentation, the AA at the most apical axial slice was measured, and the root canal space was subtracted from the segmented image of the tooth pre- and postoperatively. For each tooth, the segmented hard tissue preoperative image was then spatially aligned to the segmented postoperative image using the dentin-enamel and the cement-enamel junctions as alignment landmarks and the maximization of mutual information as a registration metric.[38] The 3D triangle-based surface of the hard tissue was reconstructed. The RHTV and its RL were calculated measuring from the CEJ to the apex pre- and postoperatively. Morphologic quantification was performed in 3matic (Materialise, Leuven, Belgium). The root hard tissue was analyzed to record the mean and maximum RHTT pre- and postoperatively and then expressed as a color-coded map showing the calculated minimal distances from the canal surface to the external root surface. The measurements were then normalized and expressed as the percentage of change related to the preoperative form.

Sample Size Calculations

As observed during the pilot phase of this study, the addition of venipuncture (to obtain LPRF) burdened the youngest patients, and some of them even refused further compliance. Thus, adding LPRF to the REP

TABLE 2 - Demographic Patient Data of the Controlled Clinical Trial

Case number	Sex	Age (y)	Tooth number	Etiology	Symptoms	Center treated	Practitioner	Follow-up (mo)	EPT and C post REP	Discoloration at last recall session
1	M	19	9	Trauma	Discoloration	UZL	N.M.	36	All −	Yellow
2	M	18	9	Caries	—	UZL	N.M.	38	3 mo: EPT+, C+	—
3	M	14	8	Trauma	Fistula	UZL	N.M.	24	2 y: EPT+, C−	—
4	F	7	9	Trauma	Fistula	UZL	N.M.	37	All −	Gray
5*	F	16	9	DI	Fistula	UZL	N.M.	NA	NA	NA
6*	F	15	9	Trauma	Fistula	UZL	N.M.	NA	NA	NA
7	M	13	9	Trauma	—	PP	N.M.	40	3 mo: C+, 1 y: all +	Gray
8	M	9	9	Trauma	Discoloration	PP	N.M.	34	All −	—
9	M	8	9	Trauma	—	PP	N.M.	34	3 y: EPT+	Gray
10	F	8	9	Trauma	Discoloration and abscess	PP	N.M.	33	All −	Yellow
11	F	11	9	Trauma	Abscess	PP	N.M.	34	2 y: C+, EPT−	—
12	F	7	8	Trauma	Abscess	PP	N.M.	38	All−	—
13	F	7	9	Trauma	Abscess	PP	N.M.	41	All−	Yellow
14*	F	7	8	Trauma	Abscess	PP	N.M.	NA	NA	NA
15*	M	7	9	Trauma	Fistula	PP	N.M.	NA	NA	NA
16	M	15	9	Trauma	Discoloration	UZL	N.M.	36	All−	Yellow
17	M	8	8	Trauma	—	UZL	N.M.	54	All−	Yellow
18	M	18	8	Trauma	—	UZL	N.M.	42	All−	—
19*	F	6	8	Trauma	Abscess	UZL	N.M.	7	All −	Yellow
20	F	11	21	DE	Abscess	UZL	G.V.G.	38	All −	—
21	M	8	9	Trauma	Fistula	UZL	N.M.	34	1 y: C+, EPT−	Yellow
22	F	8	8	Trauma	Abscess	UZL	N.M.	17	1 y: EPT+, C−	Brown
23	F	8	9	Trauma	Abscess	UZL	N.M.	17	1 y: EPT+, C−	Brown
24	M	12	7	DI	Fistula	UZL	N.M.	12	1 y: C+, EPT−	—
25	F	13	8	Trauma	Abscess	UZL	N.M.	37	All −	—
26*	F	10	8	Trauma	Abscess	UZL	N.M.	NA	NA	NA
27	M	8	8	Trauma	Abscess	UZL	N.M.	36	All −	Yellow-gray
28	M	8	8	Trauma	Fistula	UZL	N.M.	36	All −	Grey
29	F	8	9	Trauma	Percussion pain	UZL	G.V.G.	36	1 y: C+, EPT+	—

C, cold test; DE, dens envaginatus; DI, dens invaginatus; EPT, electric pulp tester; F, female; M, male; NA, not applicable; PP, private practice; UZL, University Hospital Leuven.
The pulpal status of all cases was necrosis. Cases 1–6, 21, 22, 24, 25, and 27 = test group; cases 7–20, 23, 26, 28, and 29 = control group.
*Dropout.

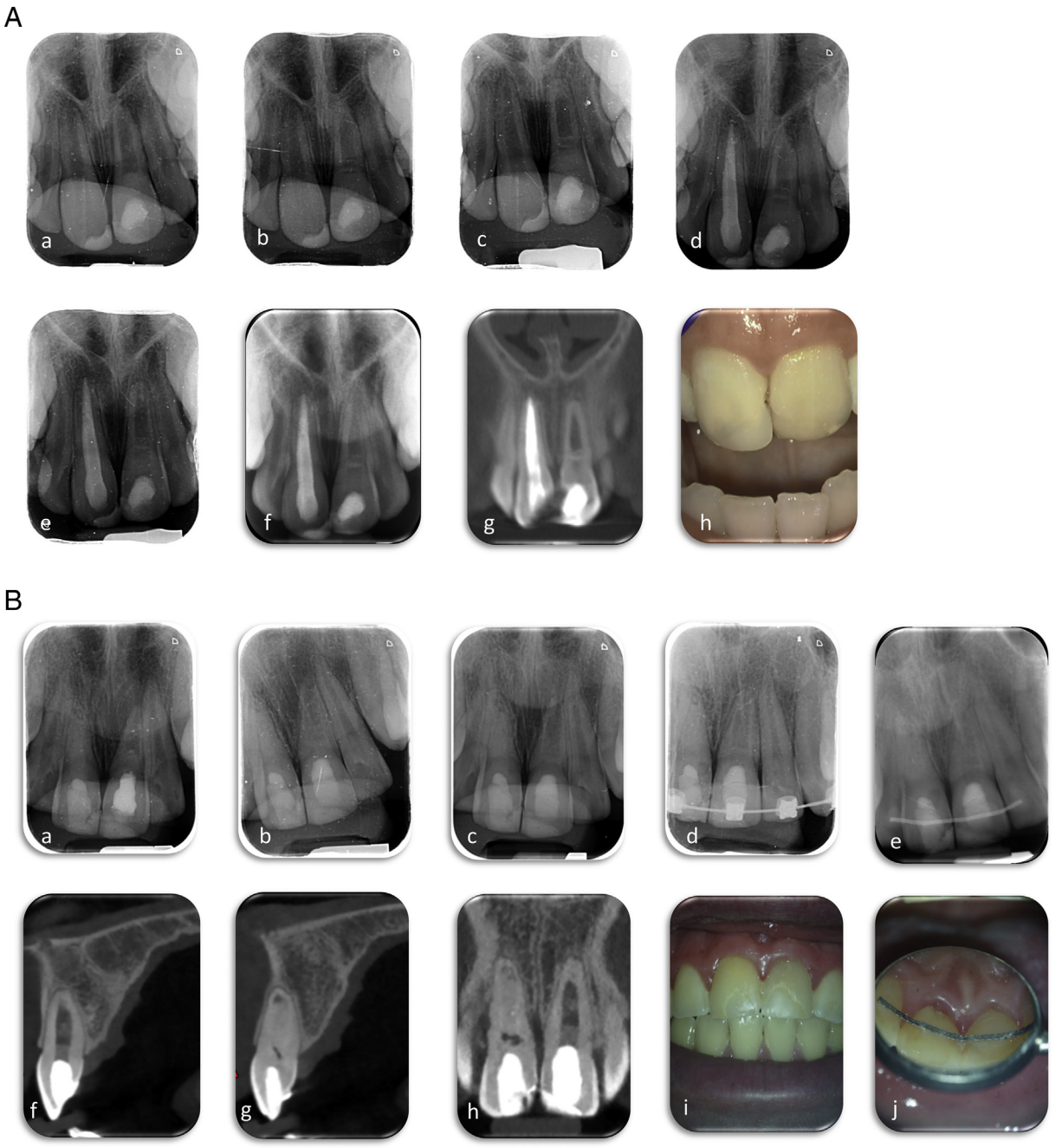

FIGURE 3 – Cases of the REP-LPRF group. Because of an enamel-dentin fracture of both central upper permanent incisors, an endodontic intervention was required. (*A*) Case number 8 of Table 2. The right upper central incisor received an apexification treatment with an apical MTA plug, resin backfill, and a fiber glass post. On the left upper central incisor, a REP was performed. PRs: (*a*) baseline and follow-up at (*b*) 3 months, (*c*) 6 months, (*d*) 12 months, (*e*) 24 months, and (*f*) 36 months. (*g*) CBCT image after 36 months. (*h*) The clinical image at 36 months. (*B*) Case number 7 of Table 2. The right upper central incisor received an apexification treatment with Biodentine (Septodont, Saint-Maur-des-Fossés, France) and the left upper central incisor a REP. PRs: (*a*) baseline and follow-up at (*b*) 3 months, (*c*) 6 months, (*d*) 1.5 years, and (*e*) 3 years. (*f–h*) CBCT images and (*i* and *j*) clinical images at 36 months; the right upper central incisor presented on root level regions of inflammatory resorption and ankylosis and coronally a palatal abfraction of the enamel due to sequelae of trauma and/or collateral damage due to orthodontic treatment. (*C*) Case number 20 of Table 2. REP of an infected immature premolar due to an evagination on the buccal cusp. (*I*) Clinical images (*a–g*) during the first REP session. (*a*) Jaw swelling, (*b*) arrow points at evagination, (*c* and *d*) necrotic pulp tissue below the evagination, (*e*) inflammatory bleeding, (*f*) after disinfection, (*g*) calcium hydroxide inside the root canal. (*h–n*) Clinical images during the second REP session. (*h*) Periapical blood is triggered after disinfection, (*i* and *j*) a Collaplug is placed on the blood clot, (*k*) Portland cement (Medcem) is placed on the Collaplug, (*l*) glass ionomer cement is placed on the Portland cement, (*m*) composite restoration. (*o* and *p*) Three years post-REP. (*II*) PRs: (*a*) baseline and follow-up at (*b*) 3 months, (*c*) 6 months, (*d*) 12 months, (*e*) 24 months, and (*f*) 36 months. (*III*) CBCT images (*a–d*) before treatment, (*e–g*) 2 years post-REP, and (*h–j*) 3 years post-REP. (*IV*) Volumetric part comparison analysis: (*a–c*) assessment of the root surface remodeling by superimposing the pre-REP images on post-REP. The distance between the pre- and post-3D surfaces is measured and expressed as a surface distance mask (= color-coded map on the 3D surface post-REP), showing where the root has changed (negative > positive values: blue > green > yellow > red). CBCT, cone beam computed tomography; m, months.

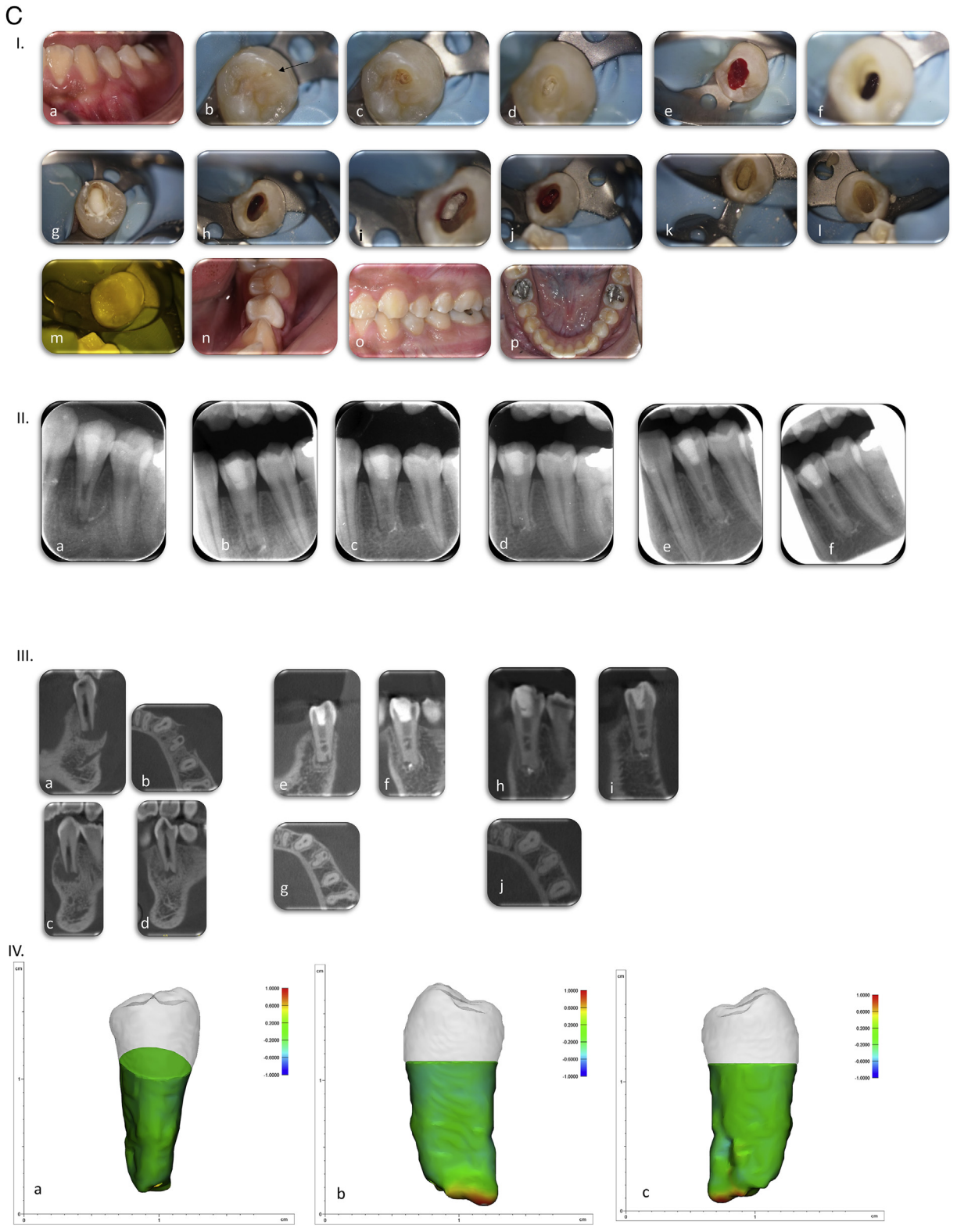

FIGURE 3 – Continued

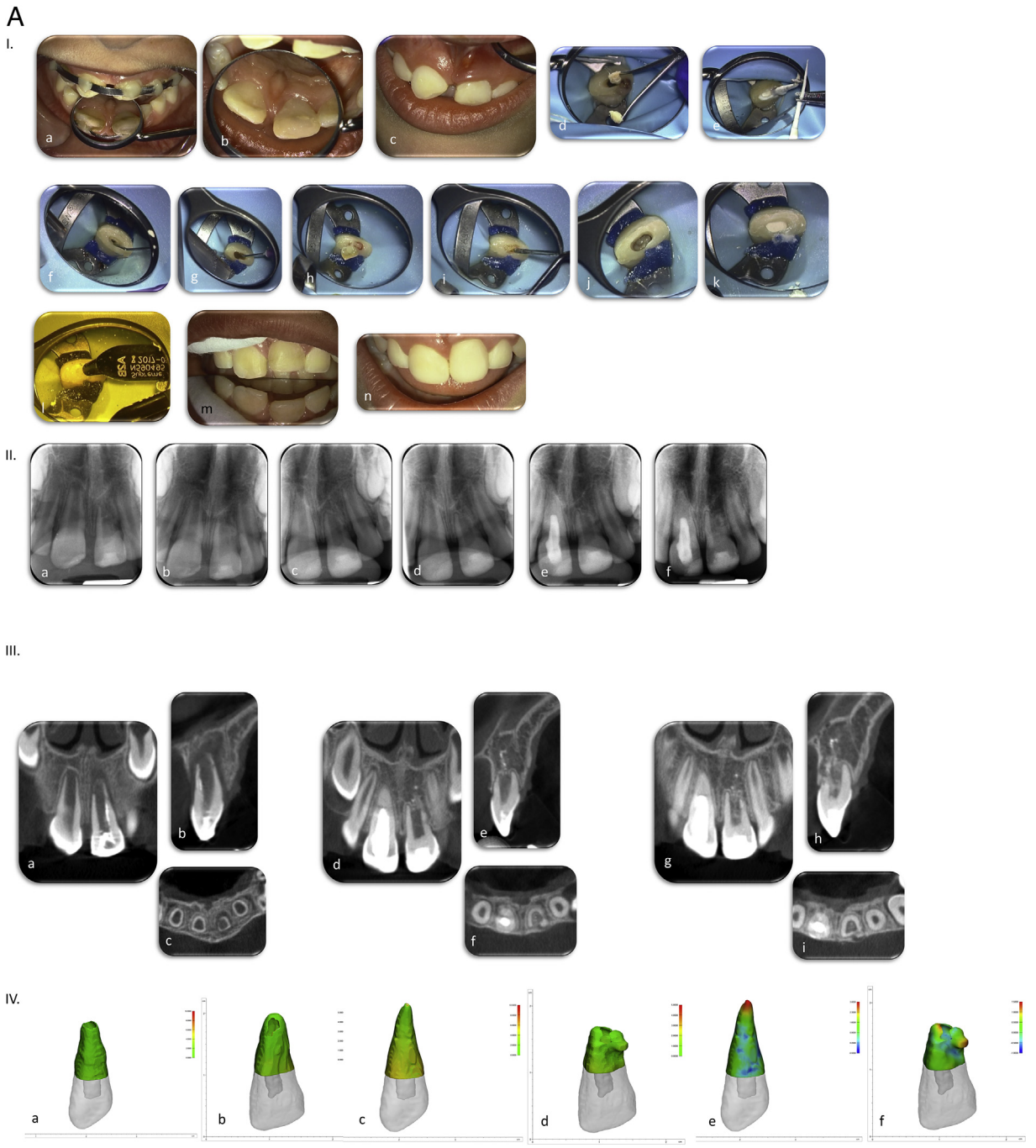

FIGURE 4 – Cases of the REP + LPRF group. (*A*) Case number 4 of Table 2. (*I*) Clinical images. (*a*) Enamel-dentin fracture of both upper central permanent incisors. The left upper central incisor was also intruded and presented alveolar bone loss. Both teeth were covered with calcium hydroxide, glass ionomer lining, and a composite restoration. A rigid splint was placed for 4 weeks. (*b* and *c*) After splint removal, the left upper central incisor presented a vestibular abscess and received a REP treatment. (*d* and *e*) The first REP session: the root canal was disinfected, and (*e*) calcium hydroxide was placed. (*f–h*) The second REP session: (*f*) after rinsing with EDTA, (*g*) the root canal was rinsed with LPRF exudate; (*i* and *j*) a periapical blood clot was triggered, and the root canal was filled with a LPRF clot. The LPRF was covered with (*k*) Portland cement (Medcem), glass ionomer lining, and (*l*) a composite restoration. (*m*) Three years post-REP: the left upper central incisor is ankylotic, and in infraocclusion, that was corrected by means of (*n*) a composite restoration. (*II*) PRs: (*a*) baseline and follow-up at (*b*) 3 months, (*c*) 6 months, (*d*) 12 months, (*e*) 24 months, and (*f*) 36 months. During the second year after trauma, the right upper central incisor presented pain symptoms and received an apexification treatment with an apical Portland cement plug, resin backfill, and a fiber glass post. REP was not performed on this tooth because at this stage the root was quite mature. (*III*) CBCT images (*a–c*) before treatment, (*d–f*) 2 years post-REP, (*g–i*) 3 years post-REP. (*IV*) Volumetric hard tissue thickness analysis; morphologic comparison between (*a* and *c*) the untreated tooth (tooth #8) and the (b and d) REP tooth (tooth #9). (*a* and *b*) Baseline and (*c* and *d*) 3 years later. Volumetric part comparison analysis of teeth (*e*) 8 and (*f*) 9; assessment of the root surface remodeling by superimposing the pre- on postimages. The distance between the pre- and post-3D surfaces is measured and expressed as a surface distance mask (= color-coded map on the 3D surface post-REP), showing where the root has changed (negative > positive values:
blue > green > yellow > red). (*B*) Case number 27 of Table 2. (*I*) Clinical images. (*a*) Symptomatic right upper central permanent incisor due to concussion and enamel-dentin fracture. A REP was performed. (*b* and *c*) Three years post-REP; the treated tooth is grayish discolored but asymptomatic. (*II*) PRs: (*a*) baseline and follow-up at (*b*) 3 months, (*c*)

protocol would be appreciated only if it would have a significant positive influence on the healing process. Based on a 2-sample pooled *t* test of a mean ratio with lognormal data, there were 9 teeth per group needed to have minimal 80% power to detect a doubling of the PBH (primary outcome) assuming a coefficient of variation equal to 0.5. This coefficient of variation was obtained from Nagy et al[39]. Taking into account possible dropouts, 20 teeth (10 per group) in total were included in the study. The 8 teeth included during the pilot phase of this study remained included. If the preset required number of 20 teeth was achieved in less than 2 years, the inclusion process in this study would not stop until September 2016. As such, the precision of the estimates for the primary and secondary end points was increased.

Statistical Methods

The assessment of the outcome variables was executed by a statistician (C.W.) under blinded conditions; namely, the patients' names and groups to which the teeth were allocated were encoded. Before analysis, percentage changes between the measurements at distinct time points and the measurement at baseline were calculated for every tooth individually. For assessing differences between treatments, the percentage changes with respect to baseline were subject to a linear mixed model with treatment and time as crossed fixed factors and patient and tooth nested in patient as random factors. Residual analysis by means of a normal quantile plot and a residual dot plot showed that the assumptions of normality of residuals were met. Only for 2D PBH, a log($\times$ + 40) transformation was needed. The value of 40 had to be added to render all data strictly positive. Differences between treatments were assessed by constructing the relevant contrasts based on the coefficients of the fixed effects of the linear mixed model and their variance-covariance matrix. *P* values for those contrasts were corrected for simultaneous hypothesis testing according to Sidak[40]. The relation between the measurements on 2D images and those on 3D images was assessed by means of calculating the simple linear regression line and inference about its slope and by means of the Spearman rank correlation coefficient.

RESULTS

Baseline Data, Recruitment, Numbers Analyzed, and Participant Flow

From September 2014 until September 2016, 29 teeth (from 27 patients) were recruited and assessed for eligibility (Table 1). The teeth were not randomized but allocated to a group (REP ± LPRF) depending on the location and the patient's compliance. More specifically, all teeth recruited in private practice were allocated to the REP-LPRF group (due to the lack of centrifuge and nurse), and all teeth recruited at the UZ Leuven were allocated to the REP + LPRF group unless the patient did not allow venipuncture. The number of teeth treated and analyzed over time are shown in a flow diagram (Fig. 2). The patients' baseline and demographic data are presented in Table 2. Fifteen male and 14 female patients were recruited, 6 and 5 of whom, respectively, were in the test group. The patients were between 6 and 19 years old. In 2 patients, 2 teeth were treated (cases 12 and 13 and cases 22 and 23). In the test group, the age range was 7–19 years; 64% were older than 10 years. In the control group, the age range was 6–18 years; 28% were older than 10 years. Regarding the type of teeth, 1 upper lateral incisor, 1 lower first premolar, and 27 upper central incisors were treated. Regarding the etiologic factor, 25 teeth received REP because of trauma, 2 because of an anatomic anomaly (dens invaginatus), and 1 because of caries. Regarding the pulpal status, all teeth were necrotic. Five teeth were symptomless, 1 was percussion sensitive, 4 were discolored, 8 presented a fistula, and 12 presented an abscess. Twenty teeth were treated at UZ Leuven and 9 at a private practice. N.M. treated 27 teeth, and G.V.G. treated 2. The follow-up period ranged between 7 and 54 months. Postoperatively, only 9 teeth revealed a positive reaction on CDS and/or EPT at 1 specific time point. Discoloration post-REP was observed in 14 cases (7 yellow, 4 gray, 1 yellow-gray, and 2 brown). However, from these cases, 3 were already discolored preoperatively.

Outcomes and Adverse Events

Outcomes and Estimation

Three cases from the REP-LPRF group are presented in Figure 3*A–C*, and 2 cases from the REP + LPRF group are presented in Figure 4*A* and *B*. For the 2D qualitative and quantitative analyses, the intra- and interobserver values for parametric data as well as for categoric data varied between 0.6 and 1 (moderate–almost perfect agreement). Therefore, the assessors performed the assessments only once.

Regarding the primary outcome, the quantitative and qualitative measurements on PRs of the periapical lesion area are reported in Table 3. From the 23 teeth analyzed, 9 were from the test group and 14 from the control group. It was noted that the mean periapical bone lesion at baseline in the test group (65.3 mm^2 ± 59 mm^2) was 3-fold that in the control group (20.8 mm^2 ± 36.6 mm^2). Furthermore, as shown in Table 3, in 9 teeth (1 test and 8 control), all periapical lesion areas on all time points were equal to 0. All teeth in the control group healed completely or remained unchanged (0 values on each time point). In the test group, 1 tooth (#24) presented bone loss 1 year post-REP, 2 teeth (#1 and 22) showed incomplete PBH, and 6 teeth showed complete PBH or remained unchanged (0 values on each time point). In total, 87% of all the cases presented complete PBH on the last recall time point when quantitatively assessed with PRs. Regarding the change in PBH in time with respect to the baseline (Fig. 5*A*), the following tendency is visible: most of the PBH occurred during the first year in both groups. However, no significant difference (*P* [3 months] = 1.00; *P* [6 months] = 1.00; *P* [1 year] = 0.53; *P* [2 years] = 0.9; and *P* [3 years] = 0.9) was found between both groups with respect to the baseline.

Qualitatively, 16 teeth (4 test and 12 control) presented a PAI (final) score of 1, and 5 cases (3 test and 2 control) presented a PAI (final) score of 2. This implies that 91.3% of the cases presented qualitatively successful PBH. In the test group, 2 cases had a PAI score of 3 and 1 a PAI score of 4.

Regarding the secondary outcome, the RL and RRA measurements of the treated teeth and their contralaterals are presented in Table 3. For some teeth (#1, 2,

6 months, (*d*) 12 months, (*e*) 24 months, and (*f*) 36 months. (*III*) CBCT images (*a–c*) before treatment, (*d–f*) 2 years post-REP, and (*g–i*) 3 years post-REP. (*e* and *h*) Periapically: calcium hydroxide puf. (*IV*) Volumetric hard tissue thickness analysis; morphologic comparison between the (*a* and *c*) REP tooth (tooth #8) and the (*b* and *d*) untreated contralateral tooth (tooth #9). (*a* and *b*) Pre-REP and (*c* and *d*) 3 years later. Volumetric part comparison analysis of teeth (*e*) 8 and (*f*) 9; assessment of the root surface remodeling by superimposing the pre- on postimages. The distance between the pre- and post-3D surfaces is measured and expressed as a surface distance mask (= color-coded map on the 3D surface post-REP), showing where the root has changed (negative > positive values: blue > green > yellow > red). CBCT, cone beam computed tomography; m, months.

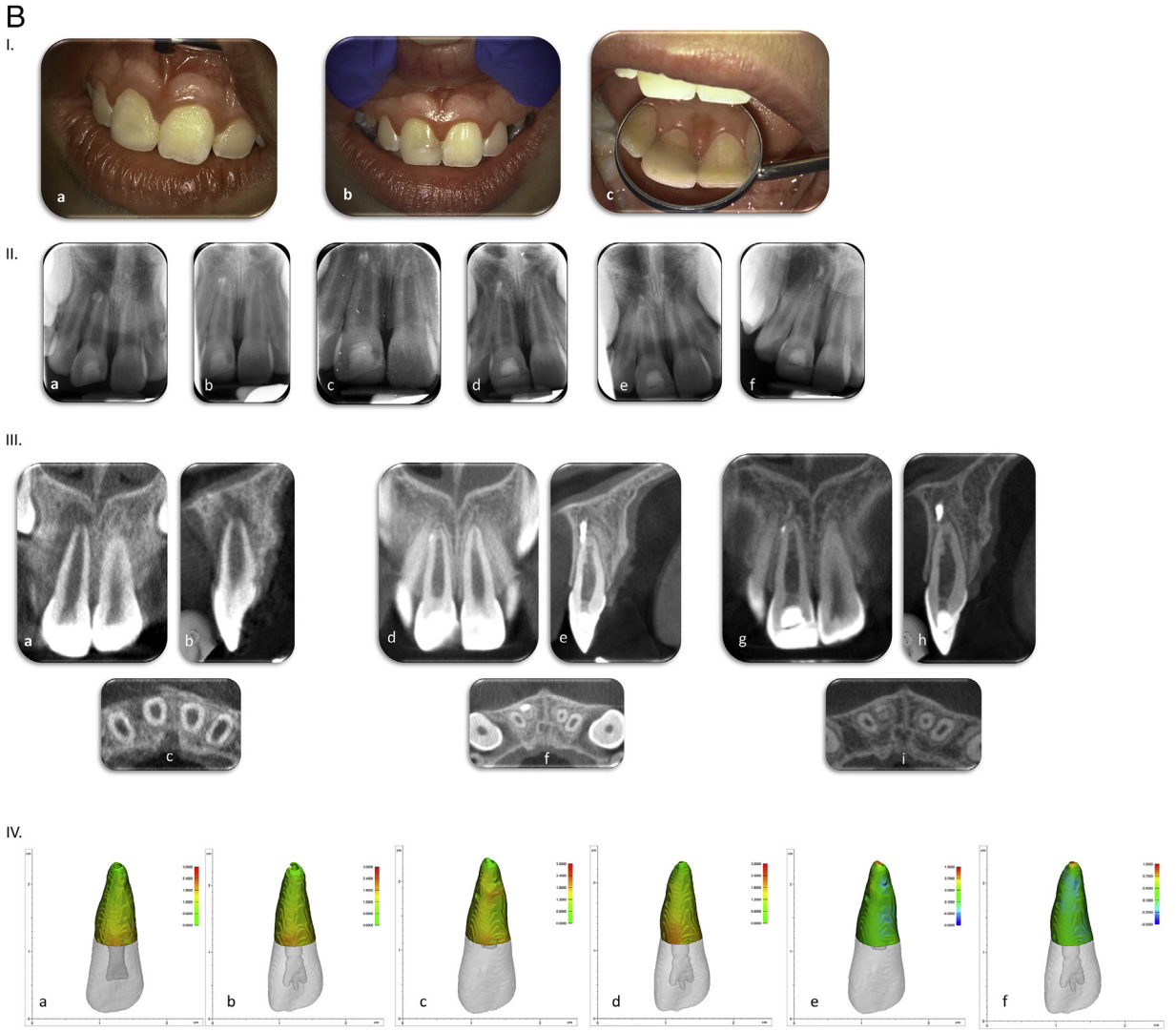

FIGURE 4 – Continued

3, 10, 11 and 18), the root dimensional changes (Δ in Table 3) scored negatively irrespective of the follow-up time, group, and type of measurement (RL or RRA). Regarding the RL (Fig. 5*B*), the following tendency is visible: from the 12-month recall onward (positive and negative), changes were more prominent than before that period. The RL change in time with respect to the baseline was not significant (P [3 months] = 1.00; P [6 months] = 0.5; P [1 year] = 0.9; P [2 years] = 1; and P [3 years] = 0.9) in both groups.

Regarding the RRA (Fig. 5*C*), a slow and steady change in time is visible. Similar to the RL, the RRA change in time with respect to the baseline was not significant (P [3 months] = 0.4; P [6 months] = 1; P [1 year] = 1; P [2 years] = 0.2; and P [3 years] = 0.4) in both groups.

The 2D qualitative assessment of the type of REP root healing is presented in Table 3. The 5 types described by Chen et al[34] occurred, but also a combination of these types was visible (cases 8, 9, 20, 28, and 29). Furthermore, the following types of healing were seen as well: no RD and no apical closure or ingrowth of hard tissue (cases 1–4, 12, 13, 22, and 23). The type of root healing was nonuniform in the control group; all of the previously mentioned root healing types occurred. Nevertheless, in the test group, 55.6% of the cases presented no RD and no apical closure (Table 3).

It is remarkable that in all cases and in all types of 2D measurements (PBH, PAI, RL, and RRL) fluctuations (alternating increasing and decreasing) are seen in time (Table 3).

Ancillary Analyses

The volumetric assessments are presented in Table 4. From 14 cases (8 test and 6 control), a CBCT image was taken at least 1 year post-REP. The periapical lesions 1–3 years post-REP were healed in 50% of the cases. Regarding the change in RHTV (Table 4) 3 years post-REP and with respect to the baseline (Fig. 6*A*), the control group presented significantly (P = .03) more RHTV than the test group. For the change in RL 3 years after REP (Table 4) and with respect to the baseline (Fig. 6*B*), there was no difference between both groups (P = .72). Regarding the change in AA 3 years post-REP (Table 4) and with respect to the baseline (Fig. 6*C*), there was a significant difference between both groups (P = .05). The same conclusions were drawn if the outlier (case 4) was omitted from the statistical calculations. For the change in

TABLE 3 - 2-Dimensional Quantitative and Qualitative Assessment of the Teeth (Case Numbers in Table 2) Treated by a Regenerative Endodontic Procedure (REP)

Case number	Time point PR after REP	Periapical lesion area (mm^2)	PAI and final score	Length from apical part MPC to apex (mm)	RRA (mm^2)	Type of REP healing
	Baseline	135.8	5	13.7	40.7	
	3 mo	133.5	5	13.9	37.7	
	6 mo	77.7	5	13.9	44.5	*
1	1 y	61.8	3	12.9	38.8	
	2 y	22.9	3	11.2	35.7	
	3 y	34.8	3	12.8	39.2	
	Δ	101 (74.4%)	2	−0.9 (−6.6%)	−1.5 (−3.7%)	
	Baseline	102.2	5	11.4	43.1	
	3 mo	0	2	12.2	48.2	
2	6 mo	14.6	3	13.8	46.4	*
	1 y	0	2	11.9	39.2	
	2 y	0	2	13.4	44.9	
	Δ	102.2 (100%)	3	2 (17.5%)	1.8 (4.2%)	
	Baseline	58.9	5	11	40.4	
	3 mo	17.4	3	10.5	38.4	
3	6 mo	0	2	11.7	39.5	1
	1 y	0	1	11.4	37.8	
	2 y	0	1	11.6	38.8	
	Δ	58.9 (100%)	4	0.6 (5.5%)	−1.6 (−4%)	
	Baseline	0	1	11.3	22.4	
	3 mo	0	1	11.1	22.5	
	6 mo	0	1	10.6	23.6	* + †
4	1 y	0	1	10.2	25.2	
	2 y	0	1	10	23.2	
	3 y	0	1	8.4	20.9	
	Δ	0	0	−2.6 (−23%)	−1.5 (−6.7%)	
	Baseline	0	2	8	39.9	
	3 mo	0	3	8.3	55.2	
7	6 mo	0	2	8.6	43.7	3
	1 y	0	1	10.1	40.6	
	2 y	0	1	10.6	39.6	
	Δ	0	1	2.6 (32.5%)	−0.3 (−4.3%)	
	Baseline	0	1	11.9	57.1	
	3 mo	0	1	11.1	51.7	
	6 mo	0	1	12.2	60	1 + 5
8	1 y	0	1	10.3	52.7	
	2 y	0	1	11.3	56	
	3 y	0	1	10.7	55.1	
	Δ	0	0	−1.2 (−10.1%)	−2 (−3.5%)	
	Baseline	0	1	9	60.1	
	3 mo	0	1	9.4	60	
	6 mo	0	1	9.2	61	
9	1 y	0	1	8.7	57.5	1 + 4
	2 y	0	1	9	56.8	
	3 y	0	1	9.9	63.4	
	Δ	0	0	0.9 (10%)	3.3 (5.5%)	
	Baseline	15.8	4	10.6	70	
	3 mo	0	1	11.2	70.6	
10	1 y	0	1	9.1	53.8	2
	2 y	0	1	9.7	59	
	3 y	0	1	10.4	60.9	
	Δ	15.8 (100%)	3	−0.2 (−9.4%)	−9.1 (−13%)	
	Baseline	65.4	5	13.3	100.1	
	3 mo	51.3	5	13.5	100.4	
11	6 mo	0	2	13.8	104.8	2
	2 y	0	2	13.2	103.1	
	3 y	0	1	12.4	93.5	
	Δ	65.4 (100%)	4	−0.9 (−6.8%)	−6.6 (−6.6%)	
	Baseline	0	3	10	55.5	
	3 mo	0	1	12.1	59	
12	6 mo	0	1	10.2	59.3	* + †

(*continued on next page*)

TABLE 3 - Continued

Case number	Time point PR after REP	Periapical lesion area (mm^2)	PAI and final score	Length from apical part MPC to apex (mm)	RRA (mm^2)	Type of REP healing
	1 y	0	1	9.8	59.7	
	3 y	0	1	9.6	61.8	
	Δ	0	2	−0.4 (−4%)	6.3 (11.4%)	
	Baseline	0	1	4.2	51.6	
	3 mo	0	1	4.4	59.6	
13	6 mo	0	1	4.1	58.7	* + †
	1 y	0	1	4.8	52.3	
	3 y	0	1	3.8	60.4	
	Δ	0	0	−0.4 (−9.5%)	8.6 (16.7%)	
	Baseline	119.4	5	6.6	64	
	3 mo	59.5	5	6.6	68.2	
	6 mo	56.1	5	6.4	63.4	
16	1 y	0	2	6.4	69.7	1
	2 y	0	1	5.5	64.2	
	3 y	0	1	5.4	77.1	
	Δ	119.4 (100%)	4	−1.2 (−18.2%)	13.1 (20.5%)	
	Baseline	0	1	10.3	72.5	
	3 mo	0	1	8.3	67	
17	6 mo	0	1	10	76.6	1
	1 y	0	1	11.4	86.8	
	2 y	0	1	10.1	80.4	
	Δ	0	0	−0.2 (−1.9%)	7.9 (11%)	
	Baseline	65.8	5	8.3	65.4	
	3 mo	54.7	5	8.1	66.5	
	6 mo	79	5	8.7	74	
18	1 y	9.7	3	8.4	68.7	3
	2 y	0	2	8.2	71.5	
	3 y	0	2	8.4	69	
	Δ	65.8 (100%)	3	0.1 (1.2%)	−3.6 (−5.5%)	
	Baseline	9.1	4	9.4	66.7	
	3 mo	9	4	9	77	
	6 mo	0	1	8.1	63.5	
20	1 y	2.2	3	7	66.5	2 + 5
	15 mo	2	3	7.3	65.1	
	27 mo	0	2	9.7	69.4	
	Δ	9.1 (100%)	2	0.3 (3.2%)	2.7 (4.1%)	
	Baseline	13.6	3 (final score: 4)	15.3	41.9	
	3 mo	0	2	15.4	43.9	
	6 mo	0	2	15.7	40.4	
21	1 y	0	2	16.3	51	3
	2 y	0	2	16	41.4	
	3 y	0	1	16.7	45.2	
	Δ	13.6 (100%)	2 (3)	1.4 (9.2%)	3.1 (7.4%)	
	Baseline	43.7	4	10.3	37.3	
	3 mo	0	2	9.8	34.3	
22	1 y	7.3	2	11.1	27.1	*
	18 mo	5.4	2	10.2	33.4	
	Δ	38.3 (87.6%)	2	−0.1 (−1%)	−3.9 (−10.5%)	
	Baseline	15.3	4	9.5	22.1	
	3 mo	0	2	9.3	39.5	
23	1 y	0	2	8.1	28.6	*
	18 mo	0	1	8.2	43.1	
	Δ	15.3 (100%)	3	−1.3 (−13.7%)	21 (95%)	
	Baseline	62.3	5	11.4	30.1	
	3 mo	68.4	5	10.9	31.7	
24	6 mo	3.6	3	10.3	38.5	1
	1 y	70.1	4	10.4	42.7	
	Δ	−7.8 (−12.5%)	1	−1 (−8.8%)	12.6 (41.9%)	
	Baseline	37.6	4	15.7	62.8	
	3 mo	17.5	3	16.2	58.8	

(*continued on next page*)

TABLE 3 - Continued

Case number	Time point PR after REP	Periapical lesion area (mm^2)	PAI and final score	Length from apical part MPC to apex (mm)	RRA (mm^2)	Type of REP healing
	6 mo	12.5	3	16.9	67	
25	1 y	12.7	2	18.3	68.1	*
	2 y	7.8	2	16.3	65.4	
	3 y	0	2	16.4	75.2	
	Δ	37.6 (100%)	2	0.7 (4.5%)	12.4 (19.8%)	
	Baseline	14.6	4	15.1	42.7	
	3 mo	0	1	15.4	47.7	
	6 mo	0	1	19.4	76.1	
27	1 y	0	1	15.6	43.7	2
	2 y	0	1	16	45	
	3 y	0	1	16.3	44.7	
	Δ	14.6 (100%)	3	1.2 (8%)	2 (4.7%)	
	Baseline	0	2	7.5	17.2	
	3 mo	0	1	8	46	
	6 mo	0	1	6.9	21.7	
28	1 y	0	1	8	19.6	1 + 2
	2 y	0	1	7.6	21.7	
	3 y	0	1	11.3	39.9	
	Δ	0	1	3.8 (50.7%)	22.7 (132%)	
	Baseline	0	1	10	34.2	
	3 mo	0	1	10.4	34.1	
	6 mo	0	1	11	40.4	
29	1 y	0	1	11.3	33.9	1 + 2
	18 mo	0	1	13	48.8	
	25 mo	0	1	13.2	48	
	Δ	0	0	3.2 (32%)	13.8 (40.4%)	

Δ, change between baseline and the last recall time point; MPC, Pure Portland Cement Med-PZ; PAI, periapical index; PR, periapical radiograph; REP, regenerative endodontic procedures; RRA, radiographic root area.
The periapical lesions were qualitatively assessed with the full-scale PAI scores and final PAI scores according to Kirkevang et al,[32] giving the same results for both, except for 1 case (case number 21, baseline). Full-scale PAI scores: 1 = normal periapical structures, 2 = small changes in bone structure, 3 = changes in bone structure with some mineral loss; 4 = apical periodontitis with well-defined radiolucent area, and 5 = severe apical periodontitis with exacerbating features. PAI final scores: 1 and 2 = success or healthy and 3–5 = failure or diseased. The change in RRA is also mentioned in percentage. Baseline indicates directly after REP. The 5 types of REP healing as described by Chen et al[34] are as follows: 1 = increased thickening of the root canal walls and continued root maturation, 2 = no significant continuation of root development with the root apex becoming blunt and closed, 3 = continued root development with the apical foramen remaining open, 4 = severe calcification (obliteration) of the root canal space; and 5 = a hard tissue barrier formed in the canal between the coronal MPC plug and the root apex.
*No root development and no apical closure.
†Ingrowth of hard tissue.

maximum RHTT 3 years after REP (Table 4) and with respect to the baseline (Fig. 6*D*), there was no difference between both groups ($P = .4$). Regarding the change in mean RHTT (Table 4) 3 years after REP and with respect to the baseline (Fig. 6*E*), the control group performed with a value of 76.8%, which was significantly ($P = .003$) different than the test group (−2.3%).

Additionally, the correlation between the 2D and 3D outcomes was investigated via linear regression. First, the change in RHTV (3D) was compared with the change in RRA (2D) with respect to the baseline (Fig. 7*A*). The slope value of 0.06 (95% confidence interval, −0.32 to 0.43; $P = .75$) and the Spearman rank correlation of −0.094 ($P = .74$) showed a very weak correlation between both variables. Second, the change in RL (3D) was compared with the change in RL (2D) with respect to the baseline (Fig. 7*B*). The slope value of 0.20 (95% confidence interval, −0.1 to 0.5; $P = .17$) and the Spearman rank correlation of 0.28 ($P = .34$) showed a weak correlation between both variables.

Adverse Events

Cases 5, 16, and 23 (Table 2) presented a flare-up reaction within the year after REP + LPRF (Fig. 8). Consequently, case 5 received an apexification treatment 6 months after REP + LPRF. Cases 16 and 23 were moved to the control group 4.5 months and 1 week after REP + LPRF, respectively (Fig. 2).

Case 19 presented aggressive replacement resorption due to sequelae of trauma (avulsion) and was extracted 5.5 months after REP. The immunohistologic analysis of this tooth was reported in Meschi et al[41].

Harms

Cases 10 and 17 (both in the control group and in 8-year-old patients) were performed under nitrous oxide sedation due to anxiety. This is also why case 17 was not added to the test group. Adding nitrous oxide sedation during an endodontic treatment is an extra burden for the patient (costs) and the endodontist (extra assistance and treatment time needed; requires adequate training, know-how, and equipment; and the nose coverage reduces the space to work in the mouth, especially in the upper front region).

In the REP + LPRF group, for 1 patient a vasovagal syncope occurred after venipuncture (case 1), and 4 patients between

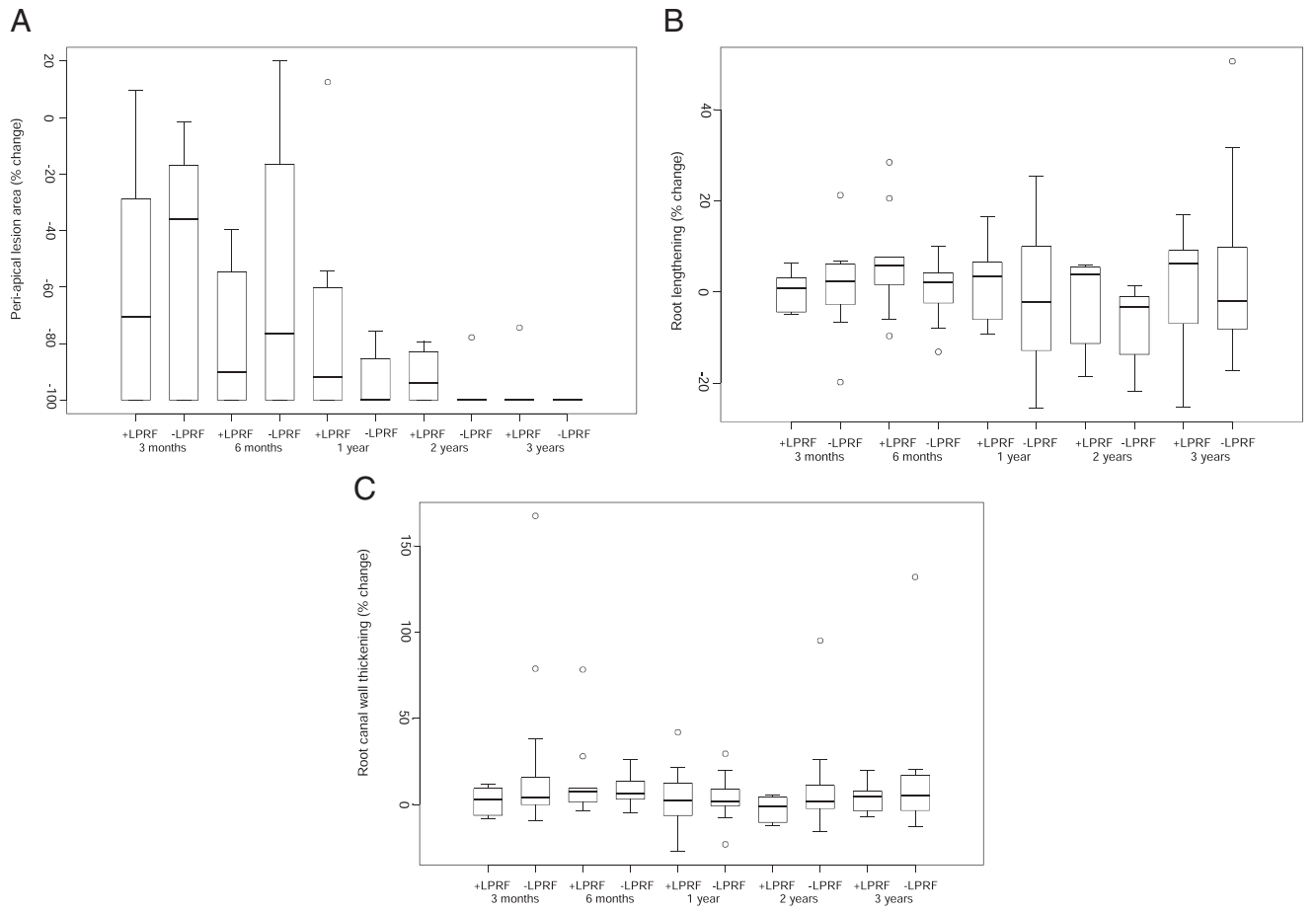

FIGURE 5 – Box plots of the 2D measurements as presented in Table 3. The change of the outcome variables in time with respect to the baseline. +LPRF, test group; −LPRF, control group. (*A*) The periapical lesion area. (*B*) Root lengthening. (*C*) RRA.

7 and 13 years old wept during venipuncture (cases 4, 22, 25, and 27).

DISCUSSION

The European Society of Endodontology and the American Association of Endodontics have prescribed success criteria for the position statement on revitalization procedures and the clinical considerations on REP, respectively[25,26]. In the current study, the impact of LPRF on these success criteria was investigated.

Generalizability

Regarding the primary outcome, successful PBH was obtained qualitatively (91.3%) and quantitatively (87%) in the majority of the cases. This is in accordance with other trials (at least case series) in which REP was applied with[11–13,42,43] or without APC[27–29]. However, the CBCT outcome regarding PBH of 14 cases is less promising (Table 4); half of the lesions did not heal within 1–3 years after REP irrespective of the application of LPRF.

Similarly, in another study based on CBCT analysis of REP-treated teeth, it was concluded that the presence of a periapical lesion before treatment negatively impacts the outcome.[44]

Regarding further RD based on PRs, no significant difference was found between the control and the test group. However, the change in RHTV 3 years after REP was more favorable for the control group in comparison with the test group. Most of the negative values in root dimensional changes in time, more specifically cases 2, 4, 22, and 25 (Table 4), were cases of the test group. Regarding the 2D RRA mentioned in other studies, a great variability exists. In a systematic review, a pooled rate for RD in randomized clinical trials (REP ± APC) of 79% was calculated, with rate variation between 21% and 100%[29]. Moreover, assuming that a 20% increase in RRA is a clinically significant change, then only 2 cases in the test group (cases 24 and 25) and 4 in the control group (cases 16, 23, 28, and 29) met this rule (Table 3)[45]. It has been reported that further RD in a REP is a slow process (ie, a 30% increase in RL in 3 years and 30% root canal wall thickening in 1 year).[46] However, this is not consistent with the current study; concerning RL, this would apply to only case 29 (control group).

In a retrospective case series on REPs, the untreated contralaterals were assessed to compare physiologic and post-REP root maturation[27]. This comparison was possible due to a baseline equivalency in age between all the patients (6–11 years); consequently, the contralaterals were not fully developed on baseline. However, in the current study, 35% of the patients were older than 11, and most of them were allocated to the test group.

The qualitative assessment of the type of REP root healing was overall not predictable and nonuniform, which is in line with other studies[27,34]. It should be emphasized that all cases had a baseline necrotic pulpal status (Table 2); hence, the chance to have an at least partially vital pulp tissue was low. Furthermore, before treatment, 20 of the 29 included teeth presented symptoms of an infection (abscess/fistula, Table 2), implying that the microbial load

TABLE 4 - Volumetric Measurements of Teeth Treated with a Regenerative Endodontic Procedure

Case number	Change (%) in root hard tissue volume	Change (%) in root length	Change (%) in apical area	Change (%) in maximum root hard tissue thickness	Change (%) in mean root hard tissue thickness	Complete healing periapical lesion 1–3 years post-REP
1	23.0	7.5	−38.1	2.9	22.2	No (3)
2	−15.9	0.0	0.0	2.0	−9.1	No (3)
3	11.6	1.9	0.0	11.7	8.3	Yes (2)
4	−27.7	−27.8	490.9	−30.8	−18.2	Yes (3)
16	28.1	0.9	−100	−1.4	158.3	Yes (3)
18	17.0	1.8	−36.8	5.1	38.5	Yes (3)
20	13.0	1.0	−66.7	9.9	58.3	Yes (3)
22	37.1	−9.4	−100.0	8.6	300.0	No (1)
23	52.6	1.2	−100.0	−9.4	172.7	No (1)
24	0.9	0.0	−47.1	2.0	7.7	No (1)
25	−7.8	4.8	−6.3	1.8	−6.3	No (3)
27	3.3	6.7	−40.0	−16.2	0.0	Yes (3)
28	38.8	2.0	−100.0	6.7	92.3	No (3)
29	31.2	4.6	−75.4	1.9	36.4	Yes (3)

For all measurements, the percentage of change was related to the preoperative measurement. The last column presents the periapical bone healing on the last cone-beam computed tomographic image taken post-REP (numbers in parentheses = 1, 2, or 3 years).

was high at baseline. Additionally, because there is a lack of mechanical debridement in REPs, an *in vitro* study reported the detrimental role of a residual biofilm on the release of transforming growth factor beta 1 after dentin conditioning[47]. Moreover, in REP cases with a persistent infection, longer periods of disinfection may clinically lead to success but histologically to repair rather than regeneration[48].

A systematic review and meta-analysis failed to show any evidence for a difference in outcomes between these etiologic factors[49]. However, a study published after that systematic review reported that the age-adjusted success percentage for REPs was the lowest with caries, higher with an anomaly, and the highest with trauma[28]. In the current study, most of the teeth (25/29) received REP because of trauma, only 1 tooth because of caries, and 3 others because of an anatomic anomaly (Table 2). Hence, it is not possible to draw any accurate conclusions on this basis.

Regarding pulp sensitivity, only one third of the cases reacted positively on CDS and/or EPT and only on 1 specific follow-up time point. It should be noted that despite the presence of a persistent apical periodontitis 1 year after REP + LPRF for cases 22, 23, and 24, a positive reaction was still registered on that time point (Table 2). Furthermore, if there were a partially vital pulp in REP-treated teeth, there is still the issue that the neuronal access to the coronal dentinal tubules is blocked by restorative material[50–52]. On the one hand, the latter questions the reliability of the clinical tests as well as the patient's response to such tests. On the other hand, possible explanations for the detection of cold sensibility could be the expression of some thermosensitive ion channels of dental afferent neurons and the release of adenosine triphosphate from pulp cells[53]. This inconsistency is also present in the scientific literature. Some previous studies with[12,54] and without[27,41,55] APC have reported a total lack of or no significant positive response on pulp sensitivity tests. However, other studies with[13,30] and without[28] APC reported significant positive reactions on CDS and/or EPT. Chrepa et al[28] showed that teeth with positive sensitivity responses were associated with greater values for RD than the teeth with negative responses, emphasizing the role of peripheral sensory neurons in hard tissue regulation[56]. However, in the current study, 55.6% of the test group reacted (on 1 time point) positively on CDS and/or EPT and 35.7% of the control group, even if the control group obtained significantly more RHTV.

MPC was used to avoid discoloration after REP. Nevertheless, postoperative discoloration was noted in both groups and in 14 teeth in total, most probably due to the presence of a blood and/or LPRF clot[57]. However, the discolorations were less severe than the ones with MTA because of the lack of bismuth oxide[31].

Limitations

Even if platelet concentrates are being appraised in the literature[22,23], some hurdles exist in their implementation in dental practice. In some countries, dentists are legally not allowed to perform venipuncture on patients. For instance, in Belgium, only physicians and nurses are allowed to perform venipuncture (Royal Resolution of 1/6/1934 on the regulation of exercising dentistry in Belgium). Furthermore, obtaining APC demands assistance, investment in a centrifuge, and the adequate equipment to manipulate the APC properly. Possible complications during or after venipuncture should be considered as well, such as hematoma at the venipuncture site, nerve injury, and vasovagal syncope[58,59]. Furthermore, the compliance of young and/or anxious patients might be reduced or lacking[59,60]. Caution is also needed for bleeding disorders[59]. Hence, the application of APC in dentistry is not risk free and demands investment in an adequate setting in terms of equipment and personnel.

Because of these hurdles, randomization was not possible in the current study. Hence, an inconsistency exists in the present study between the test and the control group regarding the age of the included patients. Due to anxiety for venipuncture and the lack of equipment and personnel to perform venipuncture in private practice, more patients younger than 10 years were treated in the control group (72%) than in the test group (36%). Furthermore, more cases in the control group (57%) had a 0 value for PBH on all follow-up time points compared with the test group (11%), and the mean baseline periapical lesion in the test group was 3 times larger than in the control group. Knowing that the pool of MSC reduces with age[61,62] and that infection control during REP and follow-up is directly proportional to the REP outcome[41,47,48], the control group was predestined to obtain more PBH and further RD than the test group. Hence, the inability to randomize has negatively impacted the LPRF group for all outcomes assessed.

Another drawback of the current study is the small sample size even if a sample size calculation and power analysis have been performed. It does not provide room for

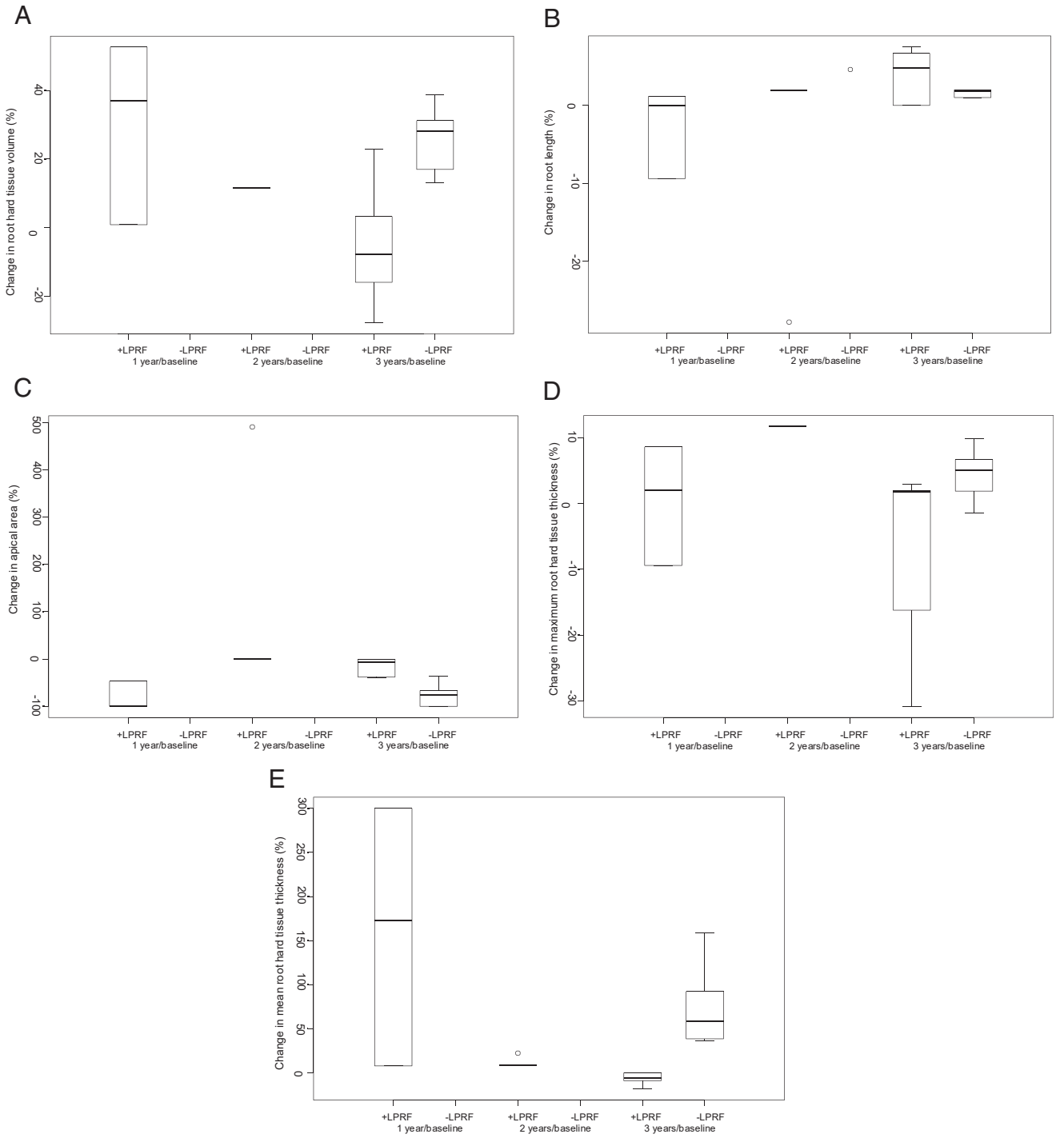

FIGURE 6 – Box plots of the 3D measurements as presented in Table 4. The change of the outcome variables in time with respect to the baseline. +LPRF, test group; −LPRF, control group. (*A*) The change in RHTV. (*B*) The change in root length. (*C*) The change in apical area. (*D*) The change in maximum RHTT. (*E*) The change in mean RHTT.

correlations between other factors involved in the treatment that might be of interest, such as the impact of the REP etiology or the operator (N.M. vs G.V.G.) on the outcome. However, it should be emphasized that the study was not designed for such evaluations because they might inflate a type I error.

The occurrence of the many 0 values in the PR assessment of PBH might be due to inaccuracy of the 2D radiographic assessment[27,37]. For instance, case 4 presents 0 values on all time points (Table 2). However, in Figure 4*A*, the preoperative CBCT image of case 4 presents a periapical lesion that is not visible on the baseline PR. Furthermore, the fluctuations in some root dimensional changes (Table 2) might be due to distortion in the PR as the patients and their changeable dentition grew out of the silicone guides during the 3 years of follow-up.

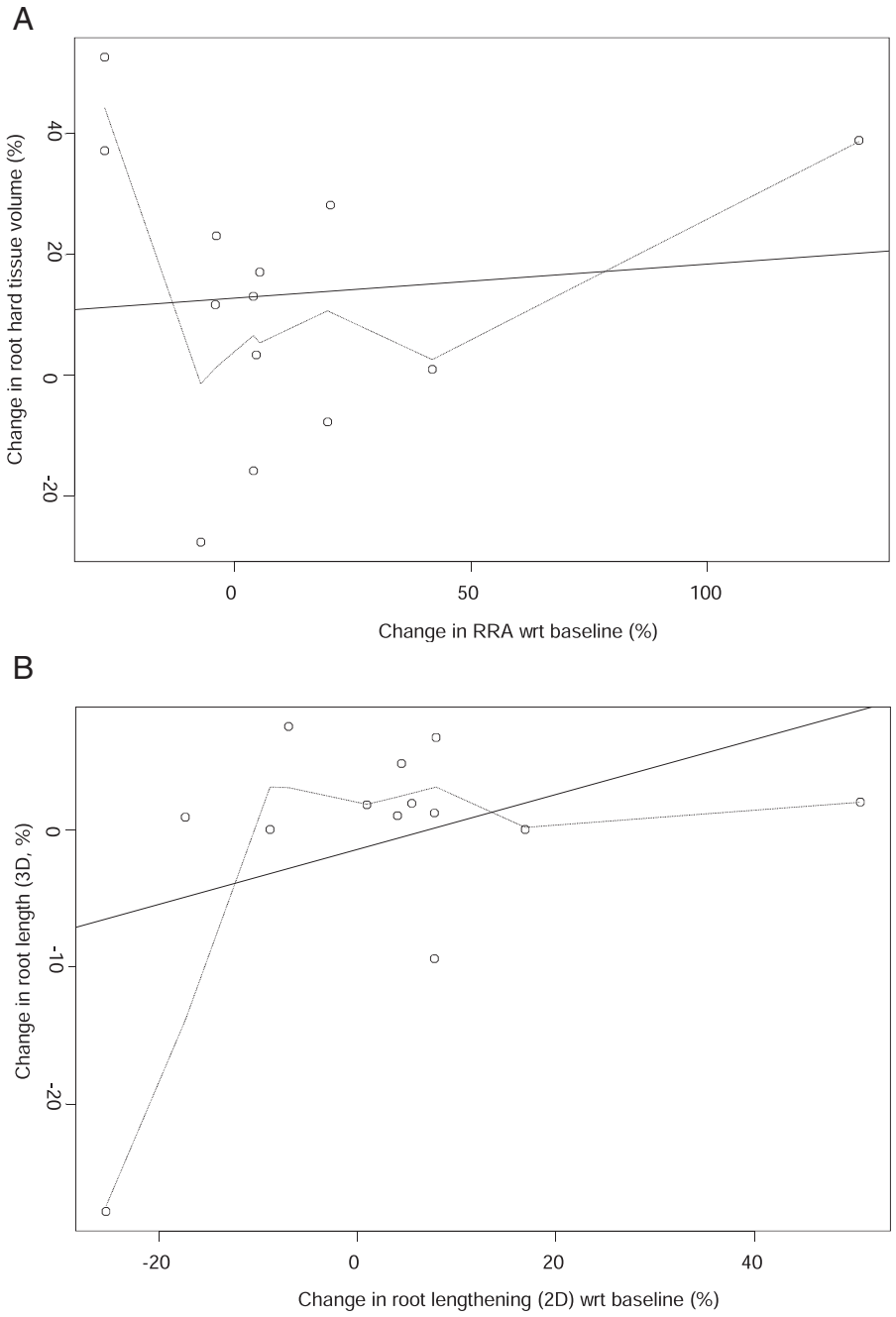

FIGURE 7 – Comparison of 2D with 3D measurements of all REP-treated teeth (+ and −LPRF) with respect to the baseline via linear regression. (*A*) The change in RHTV versus change in RRA. (*B*) The change in root lengthening 2-dimensionally versus 3-dimensionally.

A critical note is that the application of MPC might have introduced some bias in the PR RL measuring techniques because of the fact that the radiopacity of MPC is difficult to differentiate from root dentin[63,64]. First, the 2D RL is measured in the current study from the apical part of the MPC, but the 3D RL is measured from the CEJ. Second, if there would be a calcified bridge formed under the MPC due to the cemento- and osteoinductive property of calcium silicate cements[55,65], then the 2D RL measurement would be falsely smaller. Hence, this might explain why the correlation between the 2D and 3D RL (Fig. 8*B*) was weak.

Another critical note on the application of APC in general is that, even if healthy patients are included in a trial, the blood cell and growth factor content per patient are different and are furthermore age and sex related.[66–68] Consequently, this will introduce bias in the analyses. Furthermore, the flare-ups appeared only in the test group (Fig. 5) and amounted up to 25% of the included cases in that group. This high flare-up rate has not been reported yet in other trials concerning REPs with or without APC to our knowledge[6,10,12,13,42]. On the one hand, the proinflammatory cytokines in LPRF, such as IL-1β, tumor necrosis factor alpha, and IL-6,

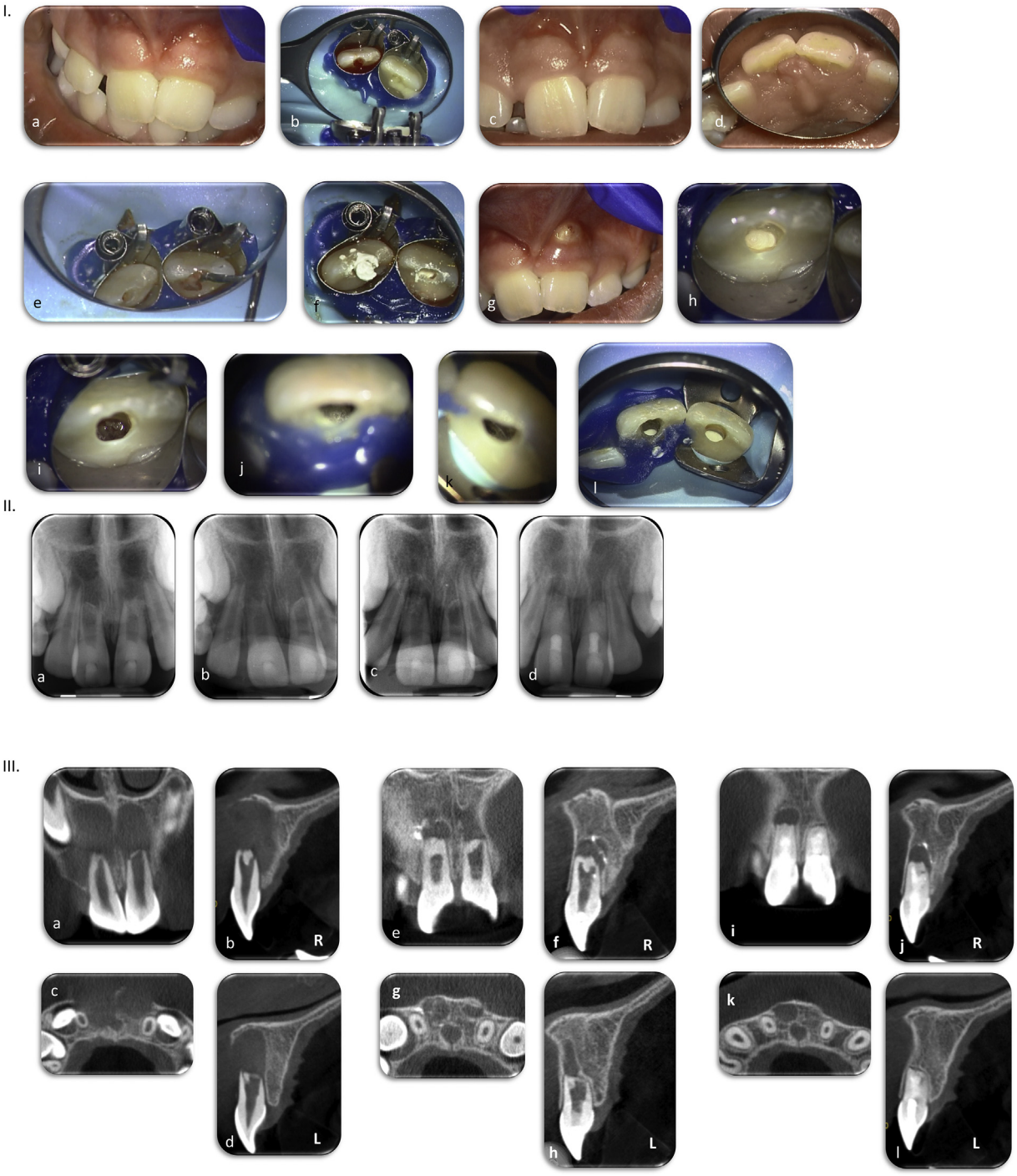

FIGURE 8 – Test group cases 22 and 23 of Table 2. (*I*) Clinical images. (*a*) Intruded right and left upper central permanent incisor presenting severe vestibular swelling. (*b*) An REP was performed. After preparation of the access cavity (by means of a ninja access), the right upper central incisor presented spontaneous inflammatory bleeding, and pus came out of the left incisor. (*c*–*f*) The second REP session. (*c* and *d*) The vestibular swelling and clinical symptoms disappeared. (*e*) LPRF was placed as a scaffold. (*f*) Portland cement (Medcem) was placed on LPRF to seal the root canal. (*g*–*i*) A week after the second REP session, the left upper central incisor presented (*g*) a vestibular abscess. A REP without LPRF was performed on this tooth. (*h*) After removal of the composite restoration and glass ionomer, the Portland cement was drilled out with a long neck bur. (*h*) Below the Portland cement the LPRF was intact. One year later, the patient presented without any symptoms. However, because there was not enough periapical bone healing obtained before orthodontic treatment, apexification was performed on both teeth. (*j* and *k*) After emptying both root canals, the apex of the (*j*) right and (*k*) left upper central incisor was visible through the microscope. (*l*) Portland cement was placed in both root canals. (*II*) Peri-apical radiographs. (*a*) Baseline, (*b*) 3 months post-REP for the right upper central incisor and baseline post flare-up for the left upper incisor, (*c*) 12 months for the left upper incisor and 18 months for the right incisor, and (*d*) postapexification of both teeth. (*III*) CBCT images. (*a*–*d*) Before, (*e*–*h*) 1 year post-REP, and (*i*–*l*) 1 year postapexification. CBCT, cone beam computed tomography; L, left upper central incisor; m, months; R, right upper central incisor.

might have induced the flare-up reaction[19]. On the other hand, the amount of LPRF applied might have played a role as well. Dohan Ehrenfest et al[69] showed that the proliferation of human jawbone MSC is promoted by PRF in a directly proportional and dose-dependent manner. However, this is not the case for the SCAPs. Bi et al[24] reported that the most optimal microenvironment to stimulate SCAPs for osteo/odontogenic differentiation was to apply only one eighth of a PRF clot. The bigger the clot, the more this effect was reduced. These *in vitro* studies do not tell if the findings are immediately translational to the clinical reality. However, the current findings and the fact that the mechanism of how PRF regulates the function of MSC is still unclear are a wake-up call to rationally apply LPRF in REPs. Additionally, another factor that might have influenced or impeded further RD in the test group is that the concentration of double antibiotic paste applied might have exceeded the prescribed 1–5 mg/mL[26], which could have been lethal for the SCAPs[70]. Hence, introduction of an *in vitro* analysis of a LPRF clot per included patient and an age-related cohort in the study design might partially shed some light on the previously mentioned issues.

Regarding the user-friendliness of the LPRF application as a scaffold in REP, a fibrin clot/membrane, even if cut into pieces, is difficult to introduce into a root canal. Hence, other APCs, such as injectable PRF for instance, might be easier to apply[71].

CONCLUSIONS

REP (without LPRF) seems to be a viable treatment option to obtain PBH, cure clinical symptoms, and aid further RD of necrotic immature permanent teeth. Nevertheless, all the analyzed teeth survived up to 3 years post-REP, and, in case of failure, apexification helped to preserve them. Caution is needed when evaluating REPs with PRs.

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