Article

Regeneration of Intrabony Defects with Nano Hydroxyapatite Graft, Derived from Eggshell along with Periosteum as Barrier Membrane under Magnification—An Interventional Study

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Abstract: Intrabony defects can be treated by various approaches. Use of GTR along with bone grafts is said to enhance the outcome. The periosteum has been claimed to increase the regeneration. The egg-shell-derived nano hydroxyapatite (EnHA) has shown a scope as alloplastic graft. Thus, the following study was undertaken to combine the periosteal pedicle along with EnHA for the treatment of intrabony defects under magnification to achieve optimal bone regeneration. A total of 21 patients, having intrabony defects with ≥6 mm probing depth (PD) and two or three wall defects as detected on CBCT, satisfying inclusion criteria were enrolled. The sites were randomly allocated as Group A, B and C ($n = 7$). The following parameters, defect density and defect fill in CBCT (at baseline and 6 months), PPD, RAL, Plaque index (PI), Gingival index (GI) and Gingival Bleeding Index (GBI) were recorded at baseline, 1, 3 and 6 months. $p < 0.05$ is considered as statistically significant. Bone density and bone fill values were found to be much higher in pedicle with EnHA and EnHA alone group and the values showed statistically significant results. The current clinical research showed that periosteal pedicle along with EnHA and EnHA as stand-alone therapy gave superior results compared to OFD alone, which is an innovative and feasible treatment option.

Keywords: bone density; eggshell-derived nano hydroxyapatite; intrabony defects; microsurgery; periodontal regeneration; periosteal membrane

1. Introduction

A major goal of periodontal therapy continues to be regeneration of attachment structures of teeth, including new bone, periodontal ligament (PDL), and cementum, which have been destroyed by periodontal diseases or trauma [1]. Periodontal regeneration in intrabony defects, has various approaches including open flap debridement (OFD), bone grafts, guided tissue regeneration (GTR), biologic mediators such as enamel matrix derivatives (EMD) and combination of barrier membranes with grafts [2].

The periosteum (rich in osteoprogenitor cells) has acquired popularity in the recent times as an alternative to other barrier membranes [2,3]. The advantages of using an

autogenous periosteal membrane are that it requires only one surgical procedure, minimizes any untoward tissue responses during healing and has the potential for stimulating new bone formation [4].

Several alloplastic graft materials have been used in the past. With the emerging technological advances, even an eggshell can be used as a graft by processing it into nano hydroxyapatite particles [5]. The Eggshell-derived nano-hydroxyapatite (EnHA) meets the required criteria and properties of an ideal graft substitute with its inherent porosity and osteotransconductivity [6,7]. The human eggshell is a ubiquitous source of hydroxyapatite, and nanotechnology has made it possible to elicit a veritable biomaterial in the form of Eggshell-derived nano Hydroxyapatite (EnHA).

Microsurgery enhances our surgical skills and improves the overall results. The main advantages are: a small surgical site, minimal tissue trauma and a better surgical outcome [8]. Major principles of microsurgery include improvement of motor skills, an emphasis on passive wound closure with exact primary apposition of the wound edge and the application of microsurgical instrumentation and suturing to reduce tissue trauma.

Hydroxyapatite is used for sinus grafting, socket preservation, grafting of bony defects, ridge augmentations, as a bone graft expander when combined with autogenous bone, and so on. Eggshell-derived hydroxyapatite seems to be promising graft material with excellent properties for grafting with viable various production techniques. Lack of disease transfer risks, biocompatibility and ease of use makes EHA a viable choice as regenerative material. Eggshell-derived hydroxyapatite is hydrophilic in nature, absorbed by body fluids and blood, so that handling becomes easy for placing it in the surgical site [7].

The present study is a pioneer in all its aspects. To our knowledge, to date none of the studies have used Eggshell-derived nano Hydroxyapatite (EnHA) for regeneration of intrabony defects and have quantified it with CBCT. Using a periosteal pedicle with EnHA in an intrabony defect and comparing it to EnHA, OFD using a precise technology such as CBCT for bone density is a path finder for future studies.

Thus, the aim of the following study was to evaluate the regeneration of intrabony defects by the confluence of periosteal membrane and eggshell-derived nano hydroxyapatite graft (EnHA) with a more precise technique microsurgery.

2. Materials and Methods

2.1. Study Design

The present study is a prospective, unicentre comparative randomized clinical trial to evaluate the effectiveness of a combination of periosteal pedicle as a barrier and EnHA as a bone graft for regeneration of intrabony defects and EnHA solely as a regenerative material for intrabony defects. The study protocol was reviewed and approved by the institutional review board of Krishnadevaraya College of Dental Sciences and Hospital Ethical Committee (REF: Ethical comm/031/2019-20). The study was conducted in full accordance with the declared ethical principles (World Medical Association Declaration of Helsinki, version VI, 2002).

2.2. Source of Data

Patients visiting the outpatient Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore were enrolled for the study based on the inclusion and exclusion criteria. The enrolled subjects were informed verbally and a signed informed consent was obtained.

2.3. Method of Collection of Data

The selected intrabony defects were allocated to one of the three experimental groups using a computer-generated randomisation (random allocation software) as:

Group A (*n* = 7)—EnHA graft and periosteal pedicle as barrier membrane.
Group B (*n* = 7)—Only EnHA as graft.
Group C (*n* = 7)—An open flap debridement procedure only.

Patients between 25 to 55 years age with chronic periodontitis having intrabony defects $\geq$ 6 mm probing depth (PD), 2 or 3 wall defects detected using CBCT, gingival biotype >1.5 mm and systemically healthy compliant subjects who had not received any periodontal treatment in the past six months were included.

Teeth with pulpal/periapical involvement or having poor prognosis, smokers, pregnant/lactating females and patients with known systemic diseases/conditions precluding any elective surgery were excluded from the study.

The parameters recorded were defect density and defect fill in CBCT [9,10] (at baseline and 6 months), Pocket probing depth (PPD), Relative attachment level (RAL), Plaque index (PI) [11], Gingival index (GI) [12], Gingival Bleeding Index (GBI) [13] at baseline, 1, 3 and 6 months.

Bone fill is calculated using CBCT in the following manner:

Cone beam computed tomography (CBCT) was used for radiographic analysis at base line and at 6 month follow-up. CBCT with slice thickness of 0.2 mm, exposure of 90 kV, 10 mA with a scan time of 0.018 s /slice was used. Using the software, points were marked on the cementoenamel junction (CEJ), base of the defect (BD) and alveolar crest (AC) and the defect depth was measured using (CEJ to BD)–(CEJ to AC) (Figure 1).
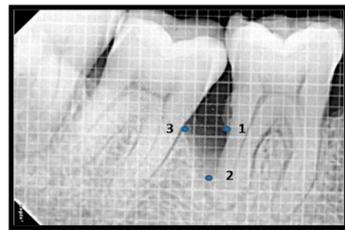

Figure 1. Pictorial representation of points considered during intrabony defect depth (IBD) measurement. 1 = Alvelolar bone crest (AC); 2 = Intrabony defect base (BD); 3 = Bone crest at defect width(BC).

Intra-bony defect (IBD) measurements were taken using the following:

- Distance from CEJ to the base of the defect (CEJ–BD).
- Distance from the CEJ to the most coronal extension of alveolar bone crest (CEJ-AC).

The intrabony component of the defects was measured as (CEJ-BD)–(CEJ-AC).

Angle was defined as the angle between lines 1 to 2 and 2 to 3 (Refer Figure 1).

Bone fill is not the same as regeneration and can be defined as the restoration of tissue in a treated periodontal alveolar defect. In the present study, the bone fill is calculated by taking two measurements, namely,

(a). Distance from CEJ–BD (base of the defect).
(b). Distance from CEJ–AC (most coronal extent of alveolar crest).

Thus, the intrabony component of defect is measured as (CEJ-BD)–(CEJ-AC) (Refer Table 2). Therefore, the bone fill can be measured using these two parameters, i.e., (CEJ-BD)–(CEJ-AC) change from baseline to 6 months.

Bone density is measured using CBCT-HU:

The term "bone quality", however, is not so simple to define. There is no clear consensus on the definition of bone quality; however, in general, it encompasses multiple aspects of bone physiology, the degree of mineralization, the morphology and type of trabecular pattern.

When evaluating or classifying the density of the bone, Grey Values GVs obtained from 3D CT images are quantified as Hounsfield units.

2.4. Surgical Procedure

Under magnification (loupes-3$\times$ magnification), following Local Anesthesia (2% lignocaine with 1:80000 adrenaline), intracrevicular incisions were placed extending to a

minimum of two teeth mesially and one tooth distally to the tooth being treated. Then, a full thickness mucoperiosteal flap was raised and vertical releasing incisions were used if necessary for better access. The defect degranulation and root planning was carried out thoroughly using manual and ultrasonic instruments followed by saline irrigation.

In group A, (see Figure 2) the full thickness mucoperiosteal flap was elevated, which was extended apically to expose sufficient amount of periosteum. Periosteal membrane was separated from this flap and released with one vertical incision mesially and one horizontal incision apically. Posteriorly, the periosteum remained attached to the mucoperiosteal flap for adequate blood supply. Then, the graft material was packed into the defect and periosteal membrane was turned over to cover this defect completely. It was sutured with a synthetic 5-0 bioabsorbable vicryl suture and the mucoperiosteal flap was sutured with 3-0 silk suture material and a periodontal dressing was given.
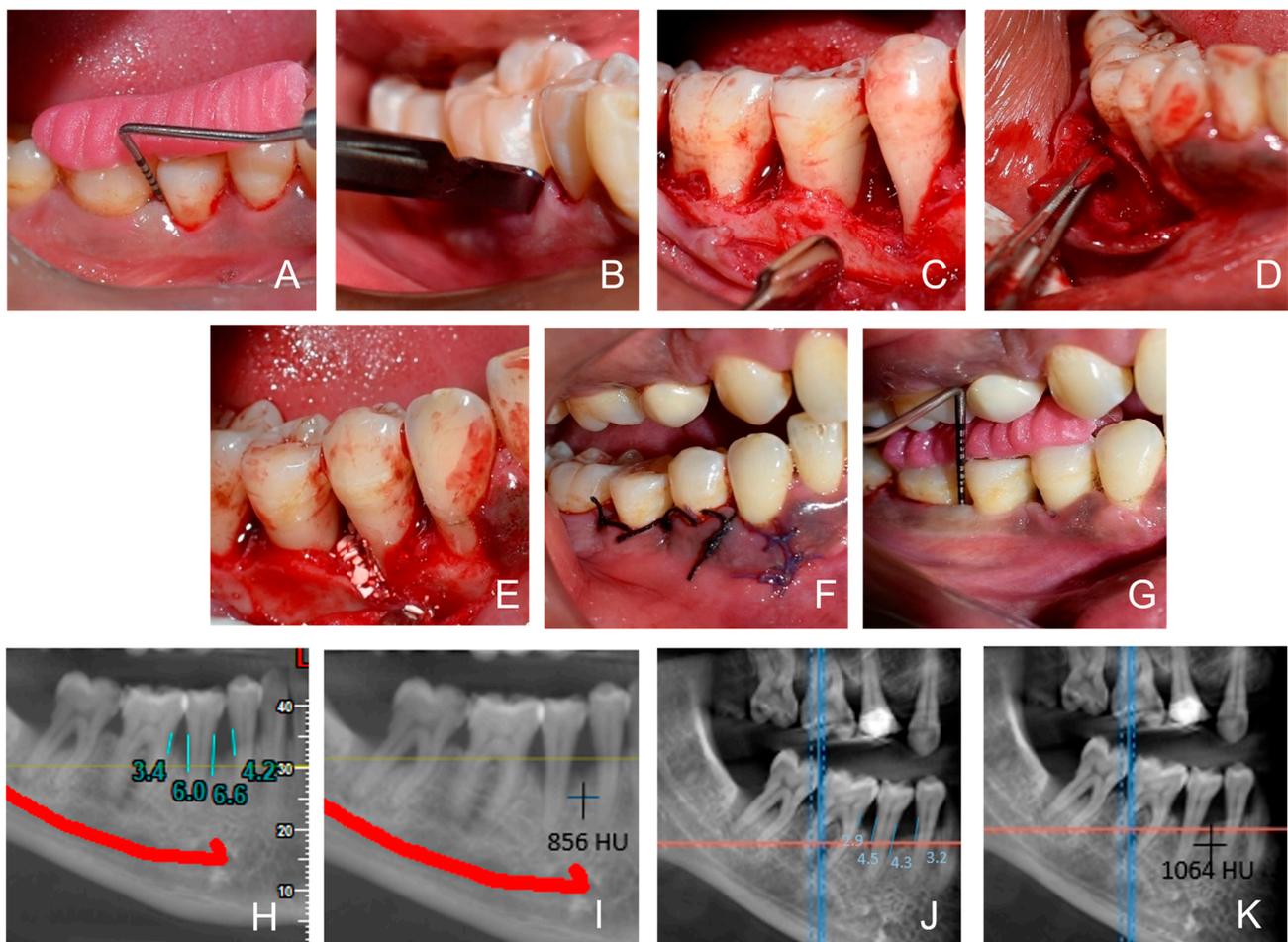

Figure 2. Showing surgical photographs of study group A (**A**)–pre op measurements, (**B**)–incision, (**C**)–reflection and debridement, (**D**)–separation of periosteum from mucoperiosteal flap, (**E**)–placement of eggshell graft into the defect, (**F**)–suturin, (**G**)–6 month follow up, (**H**)–pre op CBCT showing defect characteristics, (**I**)–pre op CBCT showing density, (**J**)–post op CBCT showing bone fill, (**K**)–post op CBCT showing density change.

In Group B, EnHA graft material was placed into the defect before suturing the mucoperiosteal flap.

In Group C, just open flap debridement was carried out.

Post-operative medications were non-steroidal anti-inflammatory drug (Tab Ibuprofen 400 mg thrice daily for three days) and Antibiotic (Cap Amoxicillin 500 mg thrice daily for three days) after meals. If patients were allergic to penicillin, they were given Cap

Clindamycin 300 mg three times daily for three days. Post-surgery ice pack application every 20 min per hour and abstinence from hot food and drinks was advised for next three hours. A soft diet for one week and mouth rinse (15 mL chlorhexidine 0.12%) twice a day for one minute duration for two weeks was prescribed. After two weeks, periodontal pack and sutures were removed.

2.5. Statistical Analysis

2.5.1. Sample Size

Cochran's Formula for Sample Size $\tilde{n} = zstd^2 \times p \times (1-p)/e^2$
Here, $z = 1.96$ for 5% Level of Significance,
$p = 0.05$,
and precision $e = 0.1$ (10%)
Power of Test: 80%
$n_0 = 1.96 \times 1.96 \times 0.05 \times 0.95/(0.1 \times 0.1) \approx 19$

2.5.2. Beta Correction Factor (Relativity Parentage, r_0)

Therefore, $r_0 = 10\%$
By using Cochran's Formula (With Beta CF),
Sample size is approximated to $\tilde{n} = n_0 \times Increase\ r_0 = 19 \times 10\%\ Increase = 20.9 \approx 21$
Estimated sample size would be 21 or more.

The results for each parameter (numbers and percentages) for discrete data and mean and standard deviation for continuous data are presented in Table and Figure. Normality assumption of data was tested using the Shapiro–Wilk test. The null hypothesis for this test is that the data are normally distributed. The Prob < W value listed in the output is the *p*-value. If the chosen alpha level is 0.05 and the *p*-value is less than 0.05, then the null hypothesis that the data are normally distributed is rejected. If the *p*-value is greater than 0.05, then the null hypothesis has not been rejected. If Normality assumption is not met, then comparison between the groups were carried out by Nonparametric test.

3. Results

In the present study a total of 21 patients, 17 males and 4 females were included in the study. The plaque and gingival index scores are elaborated below (Refer Table 1).

Table 1. Showing mean values of demographic characteristics and PI, GI and GBI at baseline and 6 months in control, group A and group B.

		Control	**Group A**	**Group B**
AGE		39.7	37.6	33.3
GENDER		M = 6, F = 1	M = 5, F = 2	M = 6, F = 1
PLAQUE INDEX (PI)	BASELINE	1.6	1.63	1.51
	6 Months	0.94	0.67	0.80
GINGIVAL INDEX (GI)	BASELINE	1.57	2.06	1.57
	6 Months	1.11	0.94	1.03
GINGIVAL BLEEDING INDEX (GBI)	BASELINE	0.93	1.0	1.0
	6 Months	0.43	0.31	0.57

The change from Baseline to 6 months AC-BD (Bone Fill) mean values were 0.71 ± 0.460 mm in control group, 2.06 ± 0.744 mm in Test group A and 1.77 ± 1.250 mm in Test group B with 'P' value (0.026), which is statistically significant. Comparison between the groups showed a mean difference of -1.34 between control group and Test group A, comparison between control group and Test group B showed a mean difference of -1.06,

comparison between Test group A and B showed a mean difference of 0.29. None of these values were statistically significant. (Table 2, Figure 3)

Table 2. Intergroup and intragroup comparison of Mean values of PPD, RAL, radiographic characteristics including Bone fill and Bone Density according to CBCT in Hounsfield unit (HU) between Control, Group A and Group B at Baseline 1, 3 and 6 months.

Outcomes	Time				
	Baseline	1 Month	3 Months	6 Months	Difference (Baseline-6 Mon)
PPD in mm					
CONTROL	7.57	6.0	4.86	3.71	3.86
GROUP A	9.86	8.0	6.29	4.83	5.0
GROUP B	9.43	7.29	5.43	4.0	5.43
P^a	0.032	0.11	0.185	0.09	0.09
RAL in mm					
CONTROL	7.57	6.0	4.86	3.71	3.86
GROUP A	10.14	8.29	6.57	5.14	5.0
GROUP B	9.71	7.57	5.71	4.29	5.43
P^a	0.01	0.05	0.11	0.10	0.09
CEJ-BD in mm					
CONTROL	6.54	-	-	5.09	1.46
GROUP A	7.5	-	-	4.39	3.11
GROUP B	7.73	-	-	5.01	2.71
P^a	0.15			0.32	0.01 *
CEJ-AC in mm					
CONTROL	3.91	-	-	3.17	0.74
GROUP A	3.7	-	-	2.64	1.06
GROUP B	4.01	-	-	3.07	0.94
P^a	0.62			0.15	0.25
BONE FILL in mm					
CONTROL	2.63	-	-	1.91	0.71
GROUP A	3.8	-	-	1.74	2.06
GROUP B	3.71	-	-	1.94	1.77
P^a	0.03			0.87	0.02 *
DENSITY in HU					
CONTROL	760.86	-	-	853.14	92.3
GROUP A	924.43	-	-	1144.29	219.9
GROUP B	865.86	-	-	1065.57	199.7
P^a	0.007 *			<0.001 **	<0.001 **

PPD—probing pocket depth, RAL—relative attachment level; CBCT measurements: CEJ-BD—distance from cementoenamel junction to base of the defect; CEJ-AC—distance from cementoenamel junction to alveolar crest; P^a—*p* value intergroup comparison; *—statistically significant; **—highly statistically significant; CBCT taken at baseline and 6 months.

The mean difference of bone density change from baseline to 6 months in control group was 92.3 ± 22.12 HU, 219.9 ± 51.50 HU in Test Group A and 199.7 ± 44.10 HU in Test Group B which was statistically significant ($p < 0.001$). The comparison between the groups showed a mean difference of -163.57 at baseline and -291.14 at 6 months between control group and Test group A, comparison between control group and Test group B showed a mean difference of -105.00 at baseline and -212.43 at 6 months, comparison between Test group A and B showed a mean difference of 58.57 at baseline and 78.71 at 6 months. (Table 2, Figure 4)

The test group A and B showed a greater reduction in PPD as compared to the control sites at all recall visits, with a mean difference between the visits being 1.57 ± 0.5 mm, 2.71 ± 0.4 mm, 3.86 ± 1.4 mm in control group 1.86 ± 0.6 mm, 3.57 ± 0.9 mm, 5.0 ± 0.8 mm in group A and 2.14 ± 0.6 mm, 4.0 ± 1.2 mm, 5.4 ± 1.5 mm in group B from baseline to 1 month, baseline to 3 months, baseline to 6 months, respectively, which were not statistically significant. RAL also showed similar results compared to control group. (Table 2)
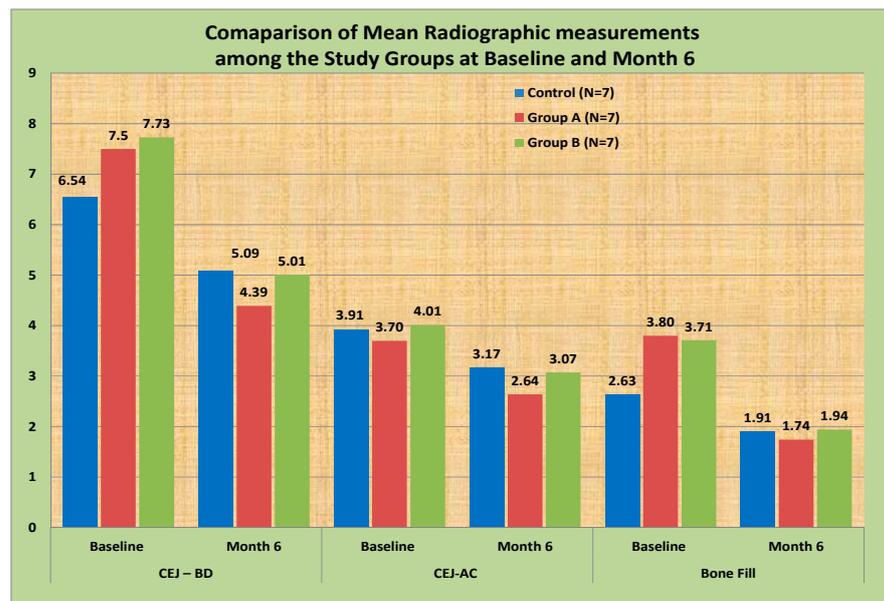
Figure 3. Comparison of Mean Radiographic measurements among the study groups at baseline and 6 months.
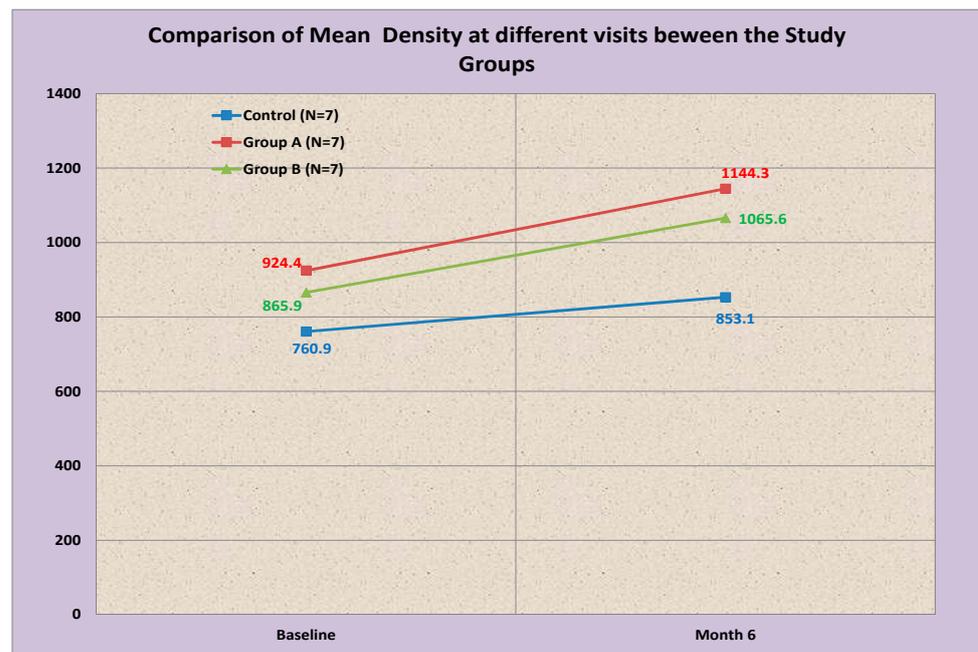

Figure 4. Comparison of Mean Density at different visits between the study groups.

4. Discussion

The enigma of periodontal regeneration is yet to unravel itself. For a long time, there has been an ongoing attempt to regenerate the components of the periodontium [14,15]. Although radiographic bone fill is achieved routinely, the ultimate goal of regenerative therapy is to obtain new cementum, periodontal ligament and alveolar bone as a total composite unit [16,17].

The human eggshell is a ubiquitous source of hydroxyapatite, and nanotechnology has made it possible to elicit a veritable biomaterial in the form of Eggshell-derived nano Hydroxyapatite (EnHA). Under SEM analysis (scanning electron microscopy) the individual hydroxyapatite crystals were spherical/semi-spherical with a porosity of 1.87–4.81 μm and particle size 200–700 μm [18–20]. The MTT assay for cell viability and proliferation

portrayed that the graft sample was amendable for the growth of osteoblasts and fibroblasts, the key players for bone regeneration.

The periosteum is an elixir of osteoprogenitor cells and is the inherent component of the mucoperiosteal flap [21]. In the last decade, microsurgery has found its way to the periodontal armamentarium and has now assumed the key spot for enhanced surgical results [22,23]. Thus, in the present study, a combination of periosteal membrane and EnHA is used for the regeneration of intrabony defects under surgical loupes for accentuated outcome.

The superiority of results of the pedicle group (group A) could be attributed to the intrinsic property of the periosteum. As we know, the periosteum has two component layers, an outer fibrous and inner cambial. The cambial layer contains undifferentiated mesenchymal cells and osteoprogenitor cells which help in bone formation (Squier CA 1990). Under ideal situations, the periosteal cells have been said to secrete extracellular matrix and form a membranous structure (Mizuno H 2006) [24].

When evaluating or classifying the density of the bone, Grey Values (GVs) obtained from 3D CT images are quantified as Hounsfield units. This implies that for every image voxel, a Hounsfield unit value based on the material inside that voxel needs to be determined during image reconstruction. Hounsfield units are defined as linear transformations of measured X-ray attenuation coefficients of a material with reference to water. Hounsfield units can be calculated for any material using the formula:

$$HU_{material} = 1000 \times (\mu_{material} - \mu_{water}) / \mu_{water}$$

where in $\mu_{material}$ and μ_{water} are the linear attenuation coefficients for the material and water, respectively. The Hounsfield unit scale is based on two fixed values, which are 0 HU for water and -1000 HU for air ($\mu_{air} = 0$). Materials or tissues that absorb more X-rays, such as bone, have a higher Hounsfield unit value. Although Hounsfield unit values are not absolute measurements of material density, they can be used for clinical purposes to quantify bone material density (BMD) [25,26]

Kaya et al. investigated bony changes in periapical lesions before and 2 years after treatment on 16 patients. They found a significant increase in GVs after treatment, using GVs in a relative way rather than trying to calculate actual bone density. Pre- and post-treatment comparison of CBCT GV is feasible when scanning patients under the same exposure conditions [27]

At baseline, bone density values for control group was 760.86 ± 110.99 HU, 924.43 ± 69.33 HU in test group A and 865.86 ± 70.90 HU in test group B. At 6 months, in control group was 853.14 ± 110.89 HU, 1144.29 ± 82.64 HU in test group A and 1065.57 ± 60.48 HU in test group B. There was a definitive increase in bone density in all the groups from baseline to 6 months with the highest in the periosteal pedicle group (group A—219.86 HU). Even the eggshell group (group B) did show a good gain of bone density, i.e., 119.71 HU. There was a clear-cut difference between OFD and the other test groups, i.e., 92.28 HU (OFD) vs 219.86 HU (group A), 199.71 HU (group B). Therefore, these intergroup differences were statistically significant. The superiority of results in the pedicle group (group A) could probably be attributable to the undifferentiated mesenchymal cells of periosteum [28]

The present study is a pioneer in all its aspects. To date, none of the studies have used Eggshell-derived nano Hydroxyapatite (EnHA) for regeneration of intrabony defects and quantified it with CBCT also. Using a periosteal pedicle with EnHA in an intrabony defect and comparing it to EnHA, OFD for bone density is a pathfinder for future studies.

A study by Kattimani et al. [29] to assess the effect of EnHA on bone healing after surgical removal of mandibular third molars bilaterally, the values at baseline and 6 months were 117.21 HU and 117.13 HU in control group, i.e., without EnHA, and in test group, i.e., with EnHA, it was 116.88 HU and 120.42 HU. The bone density gain was higher in the present study group B (EnHA) compared to the study by Kattimani et al.

Bone fill is not the same as regeneration and can be defined as the restoration of tissue in a treated periodontal alveolar defect. The mean bone fill for control group was

0.71 ± 0.46 mm, 2.06 ± 0.74 mm for group A and 1.77 ± 1.25 mm for group B. It can be appreciated that group A (pedicle group) has the highest bone fill followed by group B (eggshell group) with the difference being statistically significant.

Saimbi et al. in 2014 [4] compared OFD to periosteal pedicle as GTR membrane. Here, the defect fill was 1.40 ± 0.16 mm for test group and 0.90 ± 0.18 mm in control (OFD) group. The current study had higher values due to usage of combination therapy of periosteal pedicle and EnHA, which could be due to osteoinductivity of nanocrystalline HA.

A study by Kwan et al. [2] comparing periosteal pedicle with open flap debridement for regeneration of intrabony defects showed a mean defect fill of 0.58 ± 0.51 mm in control group and 2.42 ± 0.3 mm in test group at 6 months. These values coincide with the results of the current study.

A study by Paolantonio et al. [4] showed a defect fill of 1.7 ± 0.5 mm in control group (OFD), 2.3 ± 0.1 mm in GTR group (collagen membrane) and 3.1 ± 0.5 mm in aCPRT group (periosteal membrane and autogenous bone chips). The aCPRT group, when compared to OFD, showed statistically significant differences. The difference in values did not reach the same levels in the present study compared to the study by Paolantonio et al. due to the 'Gold Standard' quality of the autogenous bone chips. EnHA is still an alloplast and not an autograft, thereby the lower bone fill.

The PPD at the baseline was 7.57 mm (control), 9.86 mm (group A) and 9.43 mm (group B). It reduced by 1.57 mm, 1.86 mm and 2.14 mm at the first month and 2.71 mm, 3.57 mm and 4 mm at 3 months in the respective groups. The best reduction of PPD was seen at 6 months and was found to be 3.86 mm (control), 5 mm (pedicle group) and 5.43 (EnHA group).

In a study by Paolantonio et al. [30], PPD reduction of 4.4 ± 0.1 mm in aCPRT group, 5.3 ± 0.2 mm in GTR group and 3 ± 0.1 mm in OFD group. These findings correlate with our study findings. In the study by Kwan et al. [2], mean reduction of PPD from baseline to 6 months was 1.58 ± 0.49 mm in control group (OFD) and 2.67 ± 0.40 mm in the test group (periosteal pedicle) for the treatment of intrabony. The PPD reduction is higher in present clinical trial.

The mean CAL gain from baseline to 6 months in control group was 3.86 ± 1.46 mm, 5.0 ± 0.81 in test group A (periosteal pedicle and EnHA) and 5.43 ± 1.51 mm in group B (EnHA). In the study by Paolantonio et al. [26], the mean CAL gain was 3.2 ± 0.3 mm in aCPRT group (periosteal pedicle and autogenous bone chips), 3.2 ± 0.3 mm in GTR group and 1.6 ± 0.1 mm in OFD group. These values were lesser compared to the CAL gain in the current clinical trial, which may be due to the longer follow and baseline recession. In a preliminary case series by Jayashree et al. [6], eggshell-derived cement was used for treatment of intrabony defects, and here the CAL gain was 4.2 ± 0.3 mm at 6 months. This CAL gain was comparable to the present study though the values were marginally greater in our trial.

The plaque and gingival index scores showed a moderate reduction at 6 months. Both the control and test groups did not show much difference. (Refer Table 1) This denotes that the treatment outcomes were not affected by confounding factors such as oral hygiene.

At the end of 6 months of study, all the groups showed a positive outcome. None of the cases reported any adverse events. Upon observation, the periosteal pedicle group (group A) showed a mean bone density of 1144.29 ± 82.64 HU and EnHA (group B) showed 1065.57 ± 60.48 HU. Thus, the regeneration of bone radiographically could be called a success.

Though all the groups showed some increase in bone fill, group A and group B showed a better result (group A—2.06 ± 0.74 mm and group B—1.77 ± 1.25 mm mean bone defect fill at 6 months), which was statistically significant.

The limitations of this study were the inability to use sophisticated equipment for graft particle size standardization and a smaller sample size due to the prevailing COVID situation. Within the limitations of the present study, periosteal pedicle grafting is an ingenious technique and the eggshell-derived nano hydroxyapatite uses an indigenous

extraction technology and when combined they seem to give a supra-additive effect for periodontal regeneration.

5. Conclusions

In conclusion, it can be safely stated that the periosteal membrane as a GTR barrier along with eggshell-derived nano hydroxyapatite or the singular use of eggshell-derived nano hydroxyapatite provide substantial proof for bone regeneration both radiographically and clinically. This amalgamation of periosteal pedicle and EnHA for the treatment of intrabony defects shows a solid promise in the field of regeneration research.

References

1. Nevins, M.; Camelo, M.; Nevins, M.L.; Schenk, R.K.; Lynch, S.E. Periodontal Regeneration in Humans Using Recombinant Human Platelet- Derived Growth Factor-BB (rhPDGF-BB) and Allogenic Bone. *J. Periodontol.* **2003**, *74*, 1282–1292. [CrossRef] [PubMed]
2. Kwan, S.K.; Leković, V.; Camargo, P.M.; Klokkevold, P.R.; Kenney, E.B.; Nedic, M.; Dimitrijevic, B. The Use of Autogenous Periosteal Grafts as Barriers for the Treatment of Intrabony Defects in Humans. *J. Periodontol.* **1998**, *69*, 1203–1209. [CrossRef] [PubMed]
3. Gamal, A.Y.; Mailhot, J.M. A novel marginal periosteal pedicle graft as an autogenous guided tissue membrane for the treatment of intrabony periodontal defects. *J. Int. Acad. Periodontol.* **2008**, *10*, 106–117. [PubMed]
4. Gautam, A.; Khan, M.; Nandlal; Saimbi, C. Periosteum as a barrier membrane in the treatment of intrabony defect: A new technique. *J. Indian Soc. Periodontol.* **2014**, *18*, 331–335. [CrossRef] [PubMed]
5. Lee, S.-W.; Balázsi, C.; Balázsi, K.; Seo, N.-H.; Kim, H.S.; Kim, C.-H.; Kim, S.-G. Comparative Study of hydroxyapatite prepared from seashells and eggshells as a bone graft material. *Tissue Eng. Regen. Med.* **2014**, *11*, 113–120. [CrossRef]
6. Jayasree, R.; Velkumar, J.; Kumar, T.S.S. Egg shell derived apatite cement for the treatment of angular periodontal defects: A preliminary clinical and radiographic assessment. *Dent. Oral Craniofacial Res.* **2017**, *4*, 1–4. [CrossRef]
7. Kattimani, V.S.; Chakravarthi, P.S.; Kanumuru, N.R.; Subbarao, V.V.; Sidharthan, A.; Kumar, T.S.; Prasad, L.K. Eggshell derived hydroxyapatite as bone graft substitute in the healing of maxillary cystic bone defects: A preliminary report. *J. Int. Oral Health JIOH* **2014**, *6*, 15. [PubMed]
8. Kang, K.K.; Grover, D.; Goel, V.; Kaushal, S.; Kaur, G. Periodontal Microsurgery and Microsurgical Instrumentation: A Review. *Dent. J. Adv. Stud.* **2016**, *4*, 74–80. [CrossRef]
9. Vandenberghe, B.; Jacobs, R.; Yang, J. Detection of periodontal bone loss using digital intraoral and cone beam computed tomography images: Anin vitroassessment of bony and/or infrabony defects. *Dentomaxillofacial Radiol.* **2008**, *37*, 252–260. [CrossRef]
10. Fleiner, J.; Hannig, C.; Schulze, D.; Stricker, A.; Jacobs, R. Digital method for quantification of circumferential periodontal bone level using cone beam CT. *Clin. Oral Investig.* **2013**, *17*, 389–396. [CrossRef]
11. Silness, J.; Löe, H. Periodontal Disease in Pregnancy II. Correlation Between Oral Hygiene and Periodontal Condition. *Acta Odontol. Scand.* **1964**, *22*, 121–135. [CrossRef] [PubMed]
12. Löe, H.; Silness, J. Periodontal Disease in Pregnancy I. Prevalence and Severity. *Acta Odontol. Scand.* **1963**, *21*, 533–551. [CrossRef] [PubMed]
13. Newbrun, E. Indices to Measure Gingival Bleeding. *J. Periodontol.* **1996**, *67*, 555–561. [CrossRef] [PubMed]

14. Kao, R.T.; Nares, S.; Reynolds, M.A. Periodontal Regeneration—Intrabony Defects: A Systematic Review from the AAP Regeneration Workshop. *J. Periodontol.* **2015**, *86*, S77–S104. [CrossRef] [PubMed]

15. Cortellini, P.; Tonetti, M.S. Clinical concepts for regenerative therapy in intrabony defects. *Periodontology 2000* **2015**, *68*, 282–307. [CrossRef]

16. Sculean, A. Current clinical concepts in regenerative periodontal therapy. *Perio Insight* **2017**, *5*, 1–5.

17. Nibali, L.; Sultan, D.; Arena, C.; Pelekos, G.; Lin, G.; Tonetti, M. Periodontal infrabony defects: Systematic review of healing by defect morphology following regenerative surgery. *J. Clin. Periodontol.* **2021**, *48*, 101–114. [CrossRef]

18. Sanz, M.; Dahlin, C.; Apatzidou, D.; Artzi, Z.; Bozic, D.; Calciolari, E.; De Bruyn, H.; Dommisch, H.; Donos, N.; Eickholz, P.; et al. Biomaterials and regenerative technologies used in bone regeneration in the craniomaxillofacial region: Consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration. *J. Clin. Periodontol.* **2019**, *46*, 82–91. [CrossRef]

19. Jangid, M.R.; Rakhewar, P.S.; Nayyar, A.S.; Cholepatil, A.; Chhabra, P. Bone grafts in periodontal regeneration: Factors impacting treatment outcome. *Int. J. Curr. Res. Med. Sci.* **2016**, *2*, 20–24.

20. Yasmin, A.R.; Kalyani, D.; Chennai, A.U. Naturally derived porous hydroxyapatite/polymer biocomposite of cuttlebone and eggshell for dental and orthopedic applications. *Int. J. Res. Appl. Sci. Eng. Technol.* **2015**, *3*, 471–477.

21. Squier, C.A.; Ghoneim, S.; Kremenak, C.R. Ultrastructure of the periosteum from membrane bone. *J. Anat.* **1990**, *171*, 233. [CrossRef]

22. Perumal, M.P.B.; Ramegowda, A.D.; Lingaraju, A.J.; Raja, J.J. Comparison of microsurgical and conventional open flap debridement: A randomized controlled trial. *J. Indian Soc. Periodontol.* **2015**, *19*, 406–410. [CrossRef] [PubMed]

23. Burkhardt, R.; Lang, N.P. Coverage of localized gingival recessions: Comparison of micro-and microsurgical techniques. *J. Clin. Periodontol.* **2005**, *32*, 287–293. [CrossRef] [PubMed]

24. Mizuno, H.; Hata, K.-I.; Kojima, K.; Bonassar, L.J.; Vacanti, C.A.; Ueda, M. A Novel Approach to Regenerating Periodontal Tissue by Grafting Autologous Cultured Periosteum. *Tissue Eng.* **2006**, *12*, 1227–1335. [CrossRef]

25. Pauwels, R.; Jacobs, R.; Singer, S.R.; Mupparapu, M. CBCT-based bone quality assessment: Are Hounsfield units applicable? *Dentomaxillofacial Radiol.* **2015**, *44*, 20140238. [CrossRef] [PubMed]

26. Lindh, C.; Petersson, A.; Rohlin, M. Assessment of the trabecular pattern before endosseous implant treatment: Diagnostic outcome of periapical radiography in the mandible. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **1996**, *82*, 335–343. [CrossRef] [PubMed]

27. Kaya, S.; Yavuz, İ.; Uysal, İ.; Akkuş, Z. Measuring bone density in healing periapical lesions by 472 using cone beam computed tomography: A clinical investigation. *J. Endod.* **2012**, *38*, 28–31. [CrossRef]

28. Rauch, F.; Travers, R.; Glorieux, F.H. Intracortical remodeling during human bone development—A histomorphometric study. *Bone* **2007**, *40*, 274–280. [CrossRef]

29. Kattimani, V.; Lingamaneni, K.P.; Yalamanchili, S.; Mupparapu, M. Use of eggshell-derived nano-hydroxyapatite as novel bone graft substitute–A randomized controlled clinical study. *J. Biomater. Appl.* **2019**, *34*, 597–614. [CrossRef]

30. Paolantonio, M.; Femminella, B.; Coppolino, E.; Sammartino, G.; D'Arcangelo, C.; Perfetti, G.; Perinetti, G. Autogenous Periosteal Barrier Membranes and Bone Grafts in the Treatment of Periodontal Intrabony Defects of Single-Rooted Teeth: A 12-Month Reentry Randomized Controlled Clinical Trial. *J. Periodontol.* **2010**, *81*, 1587–1595. [CrossRef]