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# *The Use of Simvastatin Mixed Nano-Hydroxyapatite Following Ethylenediaminetetraacetic Acid Root-Surface Etching for the Treatment of Periodontal Intrabony Defects - Randomized Clinical Trial*

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**ABSTRACT**

*Introduction: The main challenge usually facing clinicians in the use of any biomaterial for the treatment of periodontal defects is its long-term availability. Simvastatin has been reported as a promising biomaterial that could improve the outcome of the use of graft materials to treat intrabony defects. This study was designed to test clinical outcomes following the use of simvastatin gel and to determine if the use of nano-hydroxyapatite (NHA) graft and ethylenediaminetetraacetic acid (EDTA) root-surface etching as suggested options could improve drug availability clinical outcomes.*

*Methods: Thirty non-smoking patients with severe chronic periodontitis participated in this prospective, blinded clinical trial. Each person presented with one interproximal defect and was randomly assigned to one of the following groups according to treatment (10 patients each): G1, nanograft filling (NAH) of the defect following open flap debridement (OFD); G2, simvastatin mixed nanograft filling (NAH) of the defect following OFD; or G3 simvastatin mixed nanograft filling of the defect following OFD and EDTA root-surface etching for 2 minutes. Clinical follow-ups were scheduled at 6 and 9 months following the therapy.*

*Results: Analysis of variance (ANOVA) was used to compare means among the different groups. Tukey's test was used for pairwise comparisons among the groups if ANOVA was significant. At 9 months, G2 and G3 showed significantly higher pocket reduction and attachment gain compared with G1. G3 showed a statistically significantly higher pocket reduction and attachment gain compared with G2. At 9 months, G3 showed a statistically significantly higher pocket reduction and attachment gain compared with the 6-month observation period. Intrabony defect fill was found to be significantly improved in all studied groups when compared with baseline data at both observation periods. No statistically significant differences were found between G1 and G2 at the 6- and 9-month observation periods. At 6 and 9 months, G3 showed the highest reduction in the intrabony component depth compared with both G1 and G2.*

*Conclusions: Within the limits of the present study, we can conclude that NHA–EDTA root-surface treatment is a promising delivery regimen for improving simvastatin gel clinical outcomes, a finding that could be related to improved simvastatin availability in the defect area.*

*Clinical significance: clinical evaluation of the outcome of a nanosized HA-combined simvastatin gel following EDTA root-surface etching for the treatment of two- and three-wall intrabony periodontal defects.*

**Keywords**: *Ethylenediaminetetraacetic acid, Nanograft, Periodontal pockets, Periodontal regeneration, Simvastatin*

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## INTRODUCTION

As an inflammatory disease affecting the periodontium, periodontitis often leads to bone resorption, leading to alveolar bone resorption that may ultimately result in tooth loss. The main objective of periodontal therapy has

always been the restoration of the damaged periodontal tissues to their original nature and architecture. Several treatment options have been studied over time to achieve this objective.[1-3] The use of various forms of alloplastic materials for reconstructive or regenerative periodontal treatment has been shown to significantly improve the outcomes of periodontal therapy, as evidenced by improved probing depths and clinical attachment levels.[4] Conversely, it has been reported in many histological studies that the use of alloplastic materials resulted in no or only an unpredictable amount of periodontal regeneration.[5,6] Hydroxyapatite (HAp) [$Ca_{10}$ $(PO^4)^6(OH)_2$] is a biocompatible, bioactive material with low solubility in moist media due to its similarity to hard tissues of the human body.[7-9 ] HAp is the most osteogenic phase of calcium phosphate (CAP); however, its rate of resorption is low, making the material rigid and brittle.[10] The recent approach of using nanotechnology to overcome the limitations of calcium phosphate ceramics has allowed for improvement in their bioreactivity.[11]

The bioactivity of nanomaterials usually appears to be superior to that of the larger-particle-sized traditional materials, which could be largely attributed to their large surface-to-volume ratio and superior chemical/electronic additive effects.[12] The physical properties of nanoHA (NHA), including surface grain size, pore diameter, and surface wettability, could control protein interactions such as adsorption, configuration, and bioactivity better than conventional ceramic formulations.[13-15] Their close contact with native tissues, quick resorption, and high numbers of surface molecules provide additional advantages of such materials in comparison with macrosized materials.[16,17] Moreover, undisturbed osseous binding and total material remodeling could be observed, phenomena reported to be due to the osteoclastic resorption process during the remodeling of the newly developed bone tissue.[17]

Root surfaces affected by periodontitis become hypermineralized and contaminated with endotoxins as well as other toxic bacterial products.[18] Such surfaces do not favor cell migration and attachment, which are essential for periodontal healing.[19] Ethylenediaminetetraacetic acid (EDTA) has been found to be effective, like most low-pH etchants, in smear layer removal and has been found to be superior in the selective exposure of root-surface-associated collagen in both experimental and clinical studies.[10,20 ] Removal of the smear layer and exposure of a dentinal tubule by EDTA root-surface treatment has been reported to enhance β-tricalcium phosphate graft adhesion to root surfaces altered by periodontitis.[10]

Among the biomaterials reported to improve the outcomes of grafting periodontal defects are different statin preparations. Statins have been reported to increase the expression of bone morphogenetic protein-2 and angiogenesis[21], throwing light on a new paradigm in the field of periodontal treatment. Animal studies showed that simvastatin (SMV) applied locally has an anti-inflammatory effect that could help in periodontal regeneration.[22,23] Bone anabolic behavior of SMV and other members of the statin family has been attributed mostly to an up-regulation of BMP-2.[21] The reported significant anti-inflammatory and antioxidant properties of SMV are factors of great interest from a periodontal therapeutic standpoint.[24] In addition, the systemic administration of SMV has been reported to be associated with significant reduction in tooth loss in patients diagnosed with chronic periodontitis, over a seven-year period.[25]

Because of the improved surface-to-volume ratio of nanografts as a delivery material for simvastatin, and the reported improved graft retention over EDTA-biomodulated root surfaces,[26,27] the availability of simvastatin was hypothesized in this study to be enhanced, with subsequent positive effects on clinical outcomes. The aim of the present study was clinical evaluation of the outcome of a nanosized HA-combined simvastatin gel following EDTA root-surface etching for the treatment of two- and three-wall intrabony periodontal defects.

## MATERIALS AND METHODS

### Sample Selection and Assignment

Thirty non-smoking patients with severe chronic periodontitis[28] participated in this prospective, blinded clinical trial (Figure 1). The subjects were recruited consecutively from the list of patients seeking periodontal treatment in the private clinic of the author, between January 2016 and October 2016. The inclusion criteria were: 1) the absence of systemic diseases that could affect the outcome of therapy; 2) satisfactory compliance with plaque control instructions; 3) vitality of selected teeth, with score 0 mobility; 4) the presence of at least one tooth shared with two- or three-wall intrabony interproximal defects of anterior or premolar upper or lower teeth; 5) selected intrabony component (IBC) depth ranging from 3-5 mm as detected in diagnostic periapical radiographs; 6) selected pocket depth (PPD) ≥ 6 mm and clinical attachment level (CAL) ≥ 6 mm four weeks following initial therapy; 7) availability during follow-up and maintenance visits; 8) no periodontal treatment or systemic medication for the

preceding 6 months; 9) no smoking; and 10) no occlusal interference. Pregnant females were excluded from the study. The research protocol was explained to all patients, who agreed to participate and signed the appropriate informed consent.

Initial therapy of a thorough full-mouth scaling and root planning was performed in quadrants with both hand and ultrasonic instrumentation. Patients were recalled every four days for four weeks to receive mechanical plaque control instructions and re-evaluate home care performance. Supragingival plaque removal was performed if necessary. Four weeks following initial therapy, each patient was re-evaluated, and baseline data were recorded. Clinical periodontal evaluations of the selected sites were performed with plaque index (PI),[29] gingival index (GI),[30] probing depth (PD),[31] and clinical attachment level (CAL).[32] Patients were assigned randomly to one of the following 3 groups according to treatment (10 patients each): G1, nanograft filling (NAH) (see Table 1 for list of materials) of the defect following open flap debridement (OFD); G2, simvastatin mixed nanograft filling (NAH) of the defect following OFD; and G3 simvastatin mixed nanograft filling of the defect following OFD and EDTA root-surface etching for 2 minutes. Computer-assessed randomization was performed with a computer software package just before surgery. Simvastatin gel was prepared by the mixing of 5% hydroxypropyl methylcellulose gel (HMPC) at a concentration of 1.2% w/w.

**Surgical Procedure and Post-Operative Care**

The surgical treatment phase was performed only if the selected patient had a full-mouth dental plaque score of less than 1 and the selected site score of 0. A mucoperiosteal flap was elevated by intrasulcular incisions, and vertical releasing incisions were used whenever necessary. Complete debridement of all intrabony granulation tissue and root planning with both hand and ultrasonic instruments were performed. Periodontally exposed parts of the root surfaces were etched with pH-neutral 24% EDTA gel. The defects and the adjacent soft tissue were meticulously rinsed with sterile saline to remove any EDTA residue. NHA prepared by the chemical precipitation-hydrothermal synthetic method[33] was mixed thoroughly with distilled water or simvastatin gel. Simvastatin gel was prepared by the mixing of 5% hydroxypropyl methylcellulose gel (HMPC) at a concentration of 1.2% w/w. Mixed grafts were left for 5 minutes before being placed in the defects. For all groups, the mucoperiosteal flap was repositioned and sutured with a non-resorbable suture. The patients were instructed to rinse twice daily for 2 minutes with 0.12% chlorhexidine gluconate for 2 weeks. The sutures were removed 2 weeks after surgery. Clinical and radiographic measurements were reassessed at 6 and 9 months after therapy. All clinical measurements were recorded by one masked examiner (AD).

**Data Analysis**

The primary efficacy parameters for the study were the clinical soft-tissue parameters of CAL, PPD, GI, and PI alterations at 6 and 9 months after surgery. The secondary parameter was radiographic changes following the use of simvastatin-mixed NHA following EDTA root-surface etching. A power analysis was designed based upon the changes in clinical attachment level measurements obtained from the available literature, with an ($\alpha$) level of 0.05 (5%) and a ($\beta$) level of 0.20 (20%), i.e., power = 80%; the predicted minimum sample size (n) was a total of 27 cases, i.e., nine cases in each group. Sample size calculation was performed with G$^{\dagger}$POWER Version 3.1.9.2. For all groups, data were presented as means and standard deviations. Analysis of variance (ANOVA) was used to compare means among the different groups. Tukey's test was used for pairwise comparisons among the groups if ANOVA was significant. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with the Statistical Package for Scientific Studies 16.0® for Windows.

## RESULTS

**Study Population**

In total, 46 patients were assigned to participate in the study. During surgery, 15 patients were excluded due to unsuitable defect morphology. Seven cases were found with combined one- and two-wall defects (3 for G1 and 4 for G2), 4 cases were excluded with combined one- and three-wall defects (G2 and G3), and another 4 cases with one-wall defects (G2). One patient in G3 was lost to follow-up and was excluded from the study. In total, 29 participants (13 males and 16 females) who were 29 to 53 years of age at the time of baseline examination (mean age, 36.4 ± 5.2) completed the study. Study profile and disposition of patients are described in Table 2. No complications were reported during the healing period except for normal post-surgical mild swelling or pain. No unexpected adverse reactions to the materials used were reported.

**Clinical and Radiographic Outcomes**

The initial analysis of the data showed a homogeneous distribution of all parameters used, with no significant differences in all groups. At baseline, GI, PI, PPD, CAL, and IBC showed no statistically significant differences among groups, exhibiting homogeneity (Table 2). During the observational period, plaque index and gingival index were maintained under a score of 0.5 in all groups, with no significant differences during observation periods except for G3, where the gingival index at 6 months was found to be significantly lower than that of G1 and G2 (Table 3). After treatment, all groups showed significant reductions in pocket depth and gains in clinical attachment compared with baseline (Tables 3, 4). At 6 months, G2 and G3 simvastatin mixed nanograft filling without and with EDTA root-surface etching, respectively, showed statistically significantly higher pocket depth reduction and attachment gain compared with the G1 nanograft defect fill. No significant differences were found between G2 and G3 in spite of the increased numbers of recorded values of pocket reduction and attachment gain reported with G3. At 9 months, G2 and G3 still showed significantly higher pocket reduction and attachment gain compared with G1. G3 showed a statistically significantly higher pocket reduction and attachment gain compared with G2. No significant differences were reported for G1 and G2 during both observation periods. Conversely, at 9 months, G3 showed a statistically significantly higher pocket reduction and attachment gain compared with those at the 6-month observation period. Intrabony defect fill was found to be significantly improved in all 3 studied groups when compared with baseline data at both observation periods. Statistically significantly higher reduction was reported for G2 compared with G1 at the 6- and 9-month observation periods. At 6 and 9 months, G3 showed significantly higher reduction in the intrabony component compared with both G1 and G2.

Differences between baseline and 6 months and baseline and 9 months are shown in Table 5. In the G1 nanograft fill, the PPD reduction was 2.6 ± 1.23 mm, the CAL gain was 1.2 ± 1.13 mm, and the IBC reduction was 0.9 ± 0.34 mm (46.2%) at 6 months. In the G2 simvastatin – nanograft defect fill group, the PPD reduction was 3.2 ± 2.11 mm, the CAL gain was 2.0 ± 1.34 mm, and the IBC reduction was 1.7 ± 1.1 mm at 6 months. The G3 simvastatin-nanograft defect fill following EDTA root-surface etching showed PPD reduction of 4.1 ± 1.78 mm, a CAL gain of 2.2 ± 1.33 mm, and an IBC reduction of 2.8 ± 1.22 mm. Differences between G2 and G3 were statistically significant compared with those of G1 at 6- and 9-month time-points. Within the same group, no significant differences were found between the 2 time-points except for G3 PPD reduction and CAL gain, where more significant improvements were seen at 9 months compared with 6 months.

**DISCUSSION**

The delivery of local statins in periodontal therapy has been considered a reasonable approach to achieve high drug concentrations at the defect site and avoid the side-effects of systemic administration, such as liver toxicity, since higher doses of statin are required to counter the liver metabolism and express osteogenic function.[35] Owing to the reported effects of statins on enhancing bone formation, this study was directed toward improving local simvastatin availability in the defect area. Mixing simvastatin gel with NHA, with its high surface area, could improve the clinical outcomes of the drug. In the era of nanomedicine, nano-delivery systems could be loaded with small- and large-molecule drugs to promote the entrance and build-up of the nanomedicines in particular cells and tissues.[34] In addition, the use of EDTA gel for root-surface etching, with its suggested improvement of root-surface adsorption power, could be a significant regimen for improving the local availability of simvastatin. To our knowledge, this is the first study to evaluate EDTA gel combined with nanograft-carried simvastatin gel.

The simvastatin concentration used in this study was 1.2 mg/0.1 mL. According to Pradeep et al., 1.2 mg/0.1 mL of simvastatin on methylcellulose gel produced no complications or adverse effects when used locally in human periodontal pockets and furcation defects. A 1.2% concentration of simvastatin was used to produce a flowable gel with adequate viscosity which could not be achieved with lower concentrations.[36,37] In addition, a 1.2 mg/0.1 mL concentration of simvastatin was used to reduce the probability of post-operative swelling or inflammation, consistent with higher doses, yet preserving its osteogenic potential.[22] Methylcellulose gel, as a non-toxic, non-allergic, and non-irritating material, was used as a carrier for simvastatin.[36-39]

The results of the present study showed that, at 6 months, G2 and G3 simvastatin mixed nanograft filling, without and with EDTA root-surface etching, respectively, showed statistically significantly higher pocket depth reduction and attachment gain compared with G1 nanograft defect fill. In addition, G3 treated sites showed significantly lower gingival index scores when compared with G1 and G2 at 6 months. These findings could shed light on the

possibility of a more prolonged availability of simvastatin gel in G3, which could be attributed to EDTA root-surface etching. These significant soft-tissue improvements compared with non-simvastatin mixed NHA G1 could also be attributed to the anti-inflammatory effect of simvastatin. Statins were found to reduce inflammatory cytokine levels in GCF, including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α).[40,41] In addition, simvastatin was found to trigger vascular endothelial growth factor (VEGF) release.[42] These results were consistent with those of Pradeep et al,[36,38,39]who reported that simvastatin allowed for great reductions in the bleeding-on-probing index, probing depth, clinical attachment level, and radiographic bone fill. Nine months following therapy, the G3 EDTA-treated root-surface group showed a statistically significantly higher pocket reduction and attachment gain compared with non-treated G2. This finding suggests a more prolonged effect of simvastatin-blended NHA which could be induced by the EDTA root surface. Gamal et al.[43] reported that EDTA root-surface etching improved the mechanical impact of nanomaterials within the exposed dentinal tubules, improving the release patterns of any chemical agents carried. The increased finding of statistically significantly higher pocket reduction and attachment gain seen at 9 months in G3 treated sites compared with those at the 6-month observation period supports a prolonged effect of simvastatin in the EDTA-treated group. The limited outcome of the G1 NAH-treated group compared with that of G2 and G3 parallels the outcomes of a study performed by Horváth et al., who reported that nano-HA has limited potential to promote periodontal regeneration in human intrabony defects.[44]

The present results showed that, at 6 and 9 months, G3 showed the significantly highest reduction in the intrabony component compared with both G1 and G2, a finding that strongly supports the work of Pradeep et al,[37 ]who also reported significant bone fill in class II furcation involvement after 6 months and suggested a constructive role for simvastatin. Simvastatin was found to encourage osteogenesis by different mechanisms, including, most importantly, increasing the viability and differentiation of osteoblasts. It was suggested that simvastatin supported BMP-induced osteoblast differentiation. In a dose-dependent manner, simvastatin preserves osteoblasts from apoptosis through the TGF-β-Smad3 signaling pathway.[45] In addition, simvastatin regulates estrogen receptors (ER), which play a role in the inhibition of osteoclasts.[46,47]

## CONCLUSION

Within the limits of this study, we conclude that NHA–EDTA root-surface treatment is a promising delivery regimen for improving simvastatin gel availability, which could reflect positively on enhancing soft and hard tissues affected by periodontal destruction. The release pattern of simvastatin following the use of such a regimen needs to be evaluated.

**Figure 1.** Study profile and patient disposition.

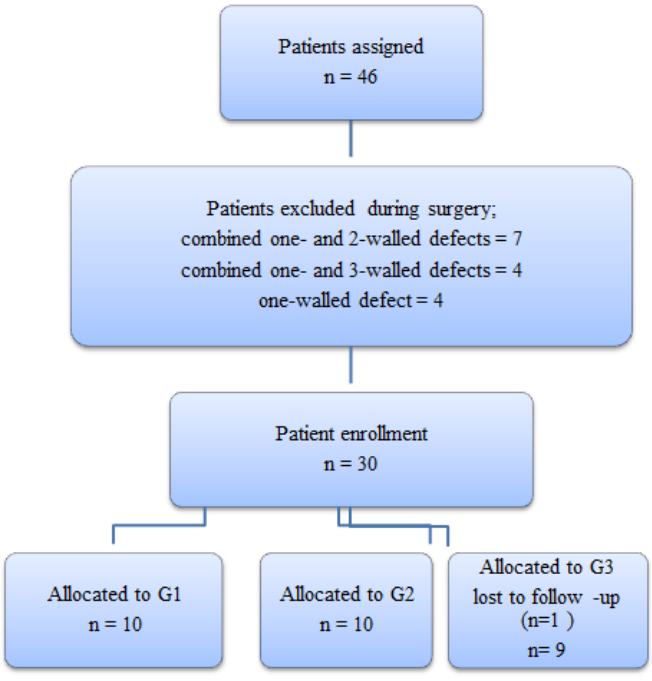

**Table 1.** Sources of materials used in the study (listed in order of appearance in the text)

| Material | Manufacturer information |
|---|---|
| Nanograft filling (NAH) | Bahgat Inc., 6-October City, Cairo, Egypt |
| Computer software package | GPower, Düsseldorf, Germany |
| Simvastatin gel | Global Napi Pharmaceuticals, Giza, Egypt |
| EDTA gel | PrefGel, Straumann, Basel, Switzerland |
| Non-resorbable suture | W.L. Gore & Associates Medical Products, Flagstaff, AZ, USA |
| Chlorhexidine gluconate | Hexigel, Napcofarm Laboratories, Cairo, Egypt |
| SPSS 16.0 | Chicago, IL, USA |

**Table 2.** Mean, standard deviation (SD) values, and results of one-way ANOVA and Tukey's test for comparison between baseline parameters in the four groups

| | G1 | | G2 | | G3 | | p-value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| CAL | 4.2 | 0.5 | 3.9 | 0.8 | 4.3 | 0.6 | < 0.231 |
| PD | 6.4 | 0.6 | 5.8 | 0.4 | 6.9 | 0.3 | < 0.122 |
| GI | 0.5 | 0.1 | 0.5 | 0.1 | 0.4 | 0.1 | < 0.134 |
| PI | 0.3 | 0.1 | 0.5 | 0.1 | 0.3 | 0.1 | < 0.211 |
| IBC | 3.7 | 0.4 | 3.8 | 0.2 | 4.2 | 0.3 | < 0.341 |

* Significantly different ($p<0.05$).

**Table 3.** Mean clinical parameters values (gingival index and plaque index) and results of Kruskal-Wallis tests for the three groups, initially and at 6 and 9 months after treatment

| Parameters | GI | | | PI | | |
|---|---|---|---|---|---|---|
| | Baseline | 6 M | 9 M | Baseline | 6 M | 9 M |
| G1 | 0.5 ± 0.1 | 0.3 ± 0.4 | 0.5 ± 0.3 | 0.3 ± 0.1 | 0.4 ± 0.3 | 0.4 ± 0.3 |
| G2 | 0.5 ± 0.1 | 0.4 ± 0.6 | 0.4 ± 0.5 | 0.5 ± 0.1 | 0.4 ± 0.5 | 0.3 ± 0.4 |
| G3 | 0.4 ± 0.1 | 0.1* ± 0.1 | 0.3 ± 0.3 | 0.2 ± 0.02 | 0.3 ± 0.4 | 0.3 ± 0.5 |
| p-value | 0.41 | 0.034 | 0.23 | 0.21 | 0.31 | 0.31 |

* Significantly different between group ($p<0.05$).

**Table 4.** Mean clinical parameters values (intrabony component, probing depth, and clinical attachment level) and results of Kruskal-Wallis test for the three groups, initially and at 6 and 9 months after treatment

| Parameters | IBC | | | PD | | | CAL | | |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 6 M | 9 M | Baseline | 6 M | 9 M | Baseline | 6 M | 9 M |
| G1 | 3.7 ± 0.4 | 2.8 ± 0.4 | 2.6 ± 0.5 | 6.4 ± 0.6 | 3.8 ± 0.4 | 3.4 ± 0.3 | 4.2 ± 0.5 | 3.0 ± 0.6 | 3.1 ± 0.4 |
| G2 | 3.8 ± 0.2 | 2.1 ± 0.6 | 1.8 ± 0.4 | 5.8 ± 0.4 | 2.6 ± 0.5 | 2.7 ± 0.2 | 3.9 ± 0.8 | 1.9 ± 0.4 | 1.6 ± 0.5 |
| G3 | 4.2 ± 0.3 | 1.4 ± 0.5 | 1.3 ± 0.3 | 6.9 ± 0.3 | 2.8 ± 0.3 | 2.1 ± 0.2 | 4.3 ± 0.6 | 2.1 ± 0.3 | 1.6 ± 0.2 |
| p-value | 0.46 | 0.032* | 0.022* | 0.41 | 0.035* | 0.022* | 0.23 | 0.036* | 0.033* |

**Table 5.** Variable changes (mm) between baseline and 6 months and baseline and 9 months

| Group | Baseline to 6 months | Baseline to 9 months |
|---|---|---|
| PD reduction | | |
| G1 | 2.6 ± 1.23 | 3.0 ± 1.67 |
| G2 | 3.2*± 2.11 | 3.1* ± 1.56 |
| G3 | 4.1*± 1.78 | 4.8*± 1.38 |
| CAL gain | | |
| G1 | 1.2 ± 1.13 | 1.1 ± 1.11 |
| G2 | 2.0* ± 1.34 | 1.9* ± 1.23 |
| G3 | 2.2* ± 1.33 | 2.7* ± 1.71 |
| IBC depth reduction | | |
| G1 | 0.9 ± 0.34 | 1.1 ± 0.56 |
| G2 | 1.7* ± 1.1 | 2.0* ± 1.23 |
| G3 | 2.8* ± 1.22 | 2.9* ± 1.34 |

* Significantly different ($p<0.05$) between groups.

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**Conflict of interest:**
The author reports no conflicts of interest.

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