Evaluation of Controlled Release Patterns of Ornidazole using Bio-degradable Polymers and Determination of its Antibacterial Activity

Mahesh Neurgaonkar, Shaila Veerappa Kothiwale*
Department of Periodontics, KLE Vishwanath Katti Institute of Dental Sciences, KAHER, Belgaum, Karnataka, INDIA.

ABSTRACT

Aim: Controlled release (CR) local drug delivery (LDD) devices are useful in periodontitis management since they facilitate prolonged maintenance of high antimicrobial concentrations in the periodontal pockets. The study aimed, to formulate and evaluate the in-vitro release pattern of Ornidazole (OZ) from three different biodegradable polymer films and to determine the antibacterial activity of released OZ on periodontal pathogens.

Materials and Methods: Four samples of 3 LDD systems were formulated by incorporating OZ into 3 different biodegradable polymer films, viz. hydroxypropylmethyl cellulose (Group I), chitosan (Group II) and hydroxypropyl cellulose (Group III). The release of OZ from these formulations was studied spectrophotometrically. The antibacterial activity of the released OZ on periodontal pathogens was evaluated by determining the inhibition zone (IZ) area. Data was statistically analyzed using ANOVA.

Results: OZ release and IZ of chitosan films were 6 days, HPMC 4 days and HPC films 3 days. In-vitro bio adhesion (5.50± 0.100 g), OZ concentration released and IZ area were greatest with chitosan films (p<0.05). HPMC films showed a significantly greater OZ release and IZ area compared to HPC films for the first 3 days (p<0.05).

Conclusion: Chitosan incorporated with OZ may be considered as a suitable bioabsorbable CR LDD system as an adjunct to mechanical periodontal therapy.

Key words: Chitosan, Local drug delivery, Hydroxypropylcellulose, Ornidazole, Periodontitis.

INTRODUCTION

Chronic periodontitis (CP) refers to an inflammatory disease of the periodontal tissues characterized by loss of periodontal attachment and eventually alveolar bone. It affects about 20-50% of the global population. Ever since the nature of bacterial etiology of CP has been understood, there have been many antimicrobial approaches to disease management. Local drug delivery (LDD) is one such approach that avoids the limitations of systemic drug administration like first pass metabolism, side effects, strain resistance, super infection, inadequate crevicular concentration and patient compliance. It also overcomes the limitations of topical agents like insufficient drug-microbial contact time. To achieve a positive effect on periodontal parameters, local application must fulfill 3 criteria: Reach the intended site of action; achieve therapeutic concentration; and last for a sufficient amount of time. Since the periodontal pocket is a defined site surrounded by tissues, it allows relatively easy access to insertion of LDD devices. However, it is naturally bathed with gingival crevicular fluid, which shows a higher flow rate in CP causing rapid evacuation of the drug from the periodontal pocket. Therefore, drug release rate should be higher in the initial stages to achieve an immediate therapeutic level of the drug in the periodontal pocket, while a moderate release profile is required...
subsequently to maintain this therapeutic level in the periodontal pocket.\(^9\)

Among the various antimicrobials used in periodontal therapy, Ornidazole (OZ) provides useful subgingival effects with a specific action against obligate anaerobes inhabiting the depths of periodontal pockets.\(^{10}\) Moreover, OZ requires a very low minimum inhibitory concentration (MIC) against periodontal pathogens (0.1-1 μg/mL).\(^{11}\) In order to maintain high concentrations of OZ in the periodontal pockets for a prolonged period of time, biodegradable controlled delivery systems are preferred since they follow zero order kinetics and do not demand a second visit for removal, thus avoiding disturbance of the healing site after therapy.\(^{12}\) This warrants development of simple, safe and reliable controlled-release LDD systems for periodontal treatment.

Hence, the present research aimed to formulate and evaluate the in-vitro release patterns of OZ from three different biodegradable polymer films (hydroxypropylmethyl cellulose, chitosan and hydroxypropyl cellulose) and to determine the antibacterial activity of released OZ on periodontal pathogens (Porphyromonas gingivalis, Fusobacterium nucleatum, Prevotella intermedia).

**MATERIALS AND METHODS**

This hospital-based, experimental, prospective study was conducted at a tertiary care hospital, after obtaining ethical clearance from the Institutional Ethics Board. The experimental design consisted of two parts - Part A (drug-release testing) and Part B (antibacterial testing).

**Part A** - This measured the in vitro release of OZ from three different LDD systems.

**LDD system preparation** - A total of 4 samples each of the 3 types of LDD films were prepared by incorporating OZ into 3 different bio-degradable polymer films, viz. hydroxypropylmethyl cellulose (HPMC)(Group I), chitosan (Group II) and hydroxypropyl cellulose (HPC) (Group III), by solvent casting technique.\(^{13-15}\) HPMC polymer (250 mg) was dissolved in 95% ethanol for Group I films, chitosan (250 mg) polymer was dissolved in 1% acetic acid (10 mL) for Group II films and HPC polymer (900 mg) was dissolved in 95% ethanol (10 mL) for Group III films, by stirring constantly on a magnetic stirrer for 1 hr. To each formulation, 1.6% glycerin and 1.35% propylene glycol were added as plasticizers and stirring was continued for 1 hr. OZ (250 mg) was dispersed in each solution by stirring for 30 sec to form a homogeneous solution. Each solution was poured into a clean petri dish placed on a horizontal plane. An inverted funnel was placed on the petri dish to control evaporation rate. The solvent was left to evaporate slowly at 37°C overnight. The films were then carefully removed from the petri dish, wrapped in aluminum foil and stored in a desiccator containing anhydrous calcium chloride (CaCl\(_2\)) to prevent contact with atmospheric air or any other contaminants. The dried OZ-polymer films were cut smaller pieces, 5 mm x 5 mm in size, with each film piece containing 1 mg of OZ.\(^{13-15,17}\)

**LDD system evaluation** - Each film piece was evaluated for the following:\(^{18-21}\)

(a) Film thickness - Determined using a calibrated Dial caliper after 4 folds.\(^{18}\)

(b) Content uniformity - One biodegradable polymer film containing OZ was dissolved in 10 mL of dichloromethane. This was extracted with two successive quantities each of 10 mL of isotonic phosphate buffered saline (iPBS) (pH-6.8) in a separating funnel. The aqueous phases were separated and suitably diluted and the absorbance was determined at 319 nm for OZ using UV spectrophotometer. The extract of polymer without drug was served as a blank.\(^{18}\)

(c) Weight uniformity - 10 patches were cut from different places of the same formulation and their individual weights were determined using the digital balance.\(^{19}\)

(d) Folding endurance - Assessed by determining the number of times a small strip of the film (2 cm x 2 cm) could be folded at the same place without breaking.\(^{20}\)

(e) Bio adhesion - This was tested using bovine cheek pouch as a model mucosal surface, that was excised and trimmed evenly from the sides. It was then washed in phosphate buffer (pH 6.8) and preserved in the same or used immediately. The two sides of the balance were equilibrated with a 5g weight on right hand side. The bovine cheek pouch excised and washed was tied tightly, with the mucosal side upwards, using a thread over the protrusion in the rubber block, which was covered with inert aluminum surface. The block was then lowered into the glass container, which was then filled with iPBS (pH 6.8) kept at 37°C±1°C, such that the buffer just reached the surface of mucosal membrane and kept it moist. This was then kept below the left hand set up of the balance. The film was then glued at the border adhering to an aluminum surface hanging on the left-hand side and the beam raised, with the 5g weight removed on the right pan side. This lowered the aluminum surface along with the film over the mucosa, with a weight of 5g. The balance was kept in this position for 8 min and then slowly water was added to the plastic container in the right pan by pipette. The addition of water was stopped as soon as the two surfaces detached. Weight of water was measured. The excess weight in the pan, i.e.
total weight minus 5 g was the force required to separate the film from the mucosa. This gave the bio adhesive strength of the formulation in grams (g).²³

**Drug-release testing** - Each of the OZ-polymer film (Groups I, II and III) was placed in a test tube containing 2 mL of iPBS solution. The tubes were then sealed and incubated at 37°C for 24 hr. After every 24 hr, 1 mL of iPBS solution was removed with a micropipette from the test tube and replaced with fresh 1 mL of iPBS solution. Using a double beam UV/visible spectrophotometer, the concentration of the drug released in iPBS solution from each film was determined by measuring the absorbance at 319 nm and calculated using the standard calibration curve. This procedure was continued over 7 consecutive days. Similarly, the test was performed 4 times for each group and the mean values were recorded.²²

**Standard calibration curve (SCC)** - For standard curve, PBS (pH 6.8) was used. In a 200 mL volumetric flask, 50 mL of 0.2 M potassium dihydrogen phosphate (1.36 g) was placed and 34.7 mL of 0.2M sodium hydroxide was added along with water to volume. An accurately weighed 100 mg of OZ was dissolved in 100 mL of PBS (pH 6.8) pH to get stock solution of 1000 µg/mL. From this solution, aliquot of 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5 mL was pipetted out and diluted to 25 mL in order to get a concentration range of 2.20 µg/mL. The absorbance was measured at 319 nm using UV spectrophotometer. The average of three readings was calculated. The SCC was obtained by plotting absorbance versus concentration in µg/mL (Figure 1). The average absorbance values for concentrations of 0 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL and 20 µg/mL were found to be 0, 0.263, 0.464, 0.677 and 0.89, respectively. With a correlation coefficient of 0.998 and regression value of Y = 0.0189, the SCC was found to be linear in the concentration range of 0-20 µg/mL (Beer's range) at 319 nm. The absorbance was taken to be linear in the concentration range of 0-20 µg/mL and regression value of Y = 0.0189, the SCC was found respectively. With a correlation coefficient of 0.9988, the SCC was calculated using the standard calibration curve. This procedure was continued over 7 consecutive days. Similarly, the test was performed 4 times for each group and the mean values were recorded.²²

**Statistical analysis**

Data was compiled and analyzed using statistical software Statistical Package for the Social Sciences (SPSS)
version 18.0 (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.) and Microsoft Excel. Measurements of continuous variables were expressed as mean ± standard deviation (SD) and those of categorical variables were presented in number (%) format (frequency tables). One-way ANOVA was used for multiple group comparisons, in order to assess the statistical significance of difference in the mean values between the three drug delivery groups up to the 3rd day of the release study. Unpaired student’s t-test was used to assess the statistical significance between the mean values for the respective variables on day 4 of the drug-release study and all variables in the antibacterial study. Post hoc Scheffe’s test was used to correct alpha for all simple (pairwise) comparisons of means between the groups as well as for complex comparisons of means involving contrasts of more than two means at a time. p-value ≤ 0.05 indicated statistical significance.

RESULTS

The study consisted of 4 samples each of HPMC, chitosan and HMC polymer films containing OZ (total 12 films) and perio-pathogens from 9 CP patients. The physicochemical properties of these films are summarized in Table 1. Film thickness ranged between 9.4 ± 0.3 µm to 9.8 ± 0.44 µm, while weight ranged between 4.13 ± 0.11 mg to 5.28 ± 0.32 mg with the % weight variation within the pharmacopoeial limits of ±7.5%. Both, film thickness and weight, were nearly uniform in all the three formulations. Folding endurance measured the ability of the film to withstand rupture, with all the three formulations maintaining integrity and showing no cracks even after > 200 folds. The drug (OZ) content of the films varied between 979.8186 µg to 995.6916 µg, with uniformity noted among the three formulations. The in-vitro bio adhesion to bovine cheek mucosa was seen to be greatest with Group II films (5.50 ± 0.100 g) followed by Group I and Group III films (1.8 ± 0.208 g).

Table 2 presents the inter-group comparison of OZ release pattern from the three LDD systems. Group II films consistently showed controlled release of OZ for the longest period (6 days), while Group I and Group III films released OZ for only 4 days and 3 days, respectively. All the LDD systems showed an initial burst release followed by a continuous decrease in drug concentration. The mean OZ release was greatest in Group I > Group II > Group III for the first 2 days, with the difference between the groups showing statistical significance (p<0.0001 for Scheffe’s multiple comparison test and ANOVA) and the values being consistently higher than the MIC for anaerobic periodontopathic bacteria. It was also significantly greater in Group II compared to Group I on the 4th day (p<0.0001 using two sample t-test). No OZ release was noted from any formulation on the 7th day.

Table 3 presents the inter-group comparison of the IZ area created by OZ released from the three LDD systems, reflecting their antibacterial properties. An IZ was seen for the longest period with Group II films (6 days) followed by Group I (4 days) and Group III films (3 days). All the LDD systems showed an initial wide IZ followed by a consistent marginal decrease in the IZ area. The mean IZ area was greatest in Group II > Group I > Group III for the first 2 days, with the difference between the groups showing statistical significance (p<0.05 using ANOVA and post hoc Scheffe’s test). On the 3rd day, the mean IZ area was significantly wider in Group I > Group III (p<0.05), but this difference was not statistically significant between Groups I and II (p=0.0787). IZ was also significantly wider in Group II compared to Group I on the 4th day (p<0.0001 using two sample t-test). No IZ was noted around any formulation on the 7th day.

DISCUSSION

LDD has proven to be a useful adjunct to mechanical periodontal therapy. The need for safe and reliable controlled-release LDD systems that ensures adequate antimicrobial release and action at the desired subgingival location motivated the designing of the present research. This study was conducted to formulate and evaluate the in-vitro release pattern of OZ from three different biodegradable polymer films (HPMC, chitosan and HPC) and to determine the antibacterial activity of released OZ on periodontal pathogens. The

### Table 1: Ornidazole-polymer film properties of the three local drug delivery systems.

<table>
<thead>
<tr>
<th>Local drug delivery system</th>
<th>Folding endurance</th>
<th>Thickness (in µm)</th>
<th>Weight (in mg)</th>
<th>Drug content (in µg)</th>
<th>In-vitro bio adhesion (in g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>&gt;200</td>
<td>9.8±0.44</td>
<td>4.54±0.58</td>
<td>996.6213</td>
<td>2.13 ± 0.152</td>
</tr>
<tr>
<td>Group II</td>
<td>&gt;200</td>
<td>9.6±0.54</td>
<td>4.13±0.11</td>
<td>995.6916</td>
<td>5.50 ± 0.100</td>
</tr>
<tr>
<td>Group III</td>
<td>&gt;200</td>
<td>9.4±0.3</td>
<td>5.28±0.32</td>
<td>979.8186</td>
<td>1.8 ± 0.208</td>
</tr>
</tbody>
</table>

Notes: Group I = Ornidazole + hydroxypropymethyl cellulose; Group II = Ornidazole + chitosan; Group III = Ornidazole + hydroxypropyp cellulose. Values in mean ± SD.
use of bioabsorbable polymers eliminated the need for a second visit for LDD removal, allowing undisturbed healing of the treated sites.12,16

Bio adhesion is another important property required in these LDD devices to enhance the residence time of the drug in the periodontal pocket. In concordance with the present study, Shukla et al. demonstrated that, out of six natural biodegradable polymers used for the formulation of OZ gel, Chitosan gel showed maximum in-vitro bioadhesive strength, greater than cellulose, xanthan gum, locust bean gum and starch.24 This may be due to the cationic nature of chitosan which ultimately improves the adhesive strength of the fabricated formulation.24-26

It has also been reported that chitosan, owing to its cationic nature, is capable of opening tight junctions in a cell membrane, thus acting as a permeation enhancer for hydrophilic drugs. Furthermore, increasing the charge density on the polymer led to higher permeability, as observed by the quaternation of the amine functionality on chitosan. Moreover, drugs dispersed in chitosan were found to be released at a constant rate, suggesting that it is a useful matrix for controlled release of drugs.25,26 These findings were reflected in the works of Shukla et al. who noted that chitosan was the best formulation in terms of cumulative percent drug release (79.23%) for 7 days, similar to the present study.24 It fulfilled many other requirements of a suitable LDD such as once a week delivery, easy fabrication, cost effectiveness, increased patient compliance and satisfactory IZ.24 Moreover, Mastiholimath et al. and Ahmed et al. found that chitosan, HPMC and HPC films showed drug release for 7 days, 4 days and 3 days, respectively, which closely mirrors the current research.13,16 The difference in release profile may be accounted for by the differences in the functional groups and the characteristics (particle size, water solubility, porosity) of these polymers, with an increase in the solid content of the polymers having a negative effect on drug release.13,16 The possible reason for the prolonged controlled drug release from chitosan could be due to increased cross linking of the drug with this polymer and smaller pore size.16,25,26 The possible reasons for rapid release of OZ from HPMC and HPC films could be due to hydrophilic nature of these polymers and the drug, which was superficially absorbed and rapidly leached out in 3-4 days and also due to the formation of more pores and channels in film matrix due to lower molecular weight of these polymers.22

### Table 2: Comparison of release pattern of Ornidazole from the three LDD systems.

<table>
<thead>
<tr>
<th>Days</th>
<th>LDD system</th>
<th>Mean release of Ornidazole</th>
<th>Inter-group comparison</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean±SD [in µg/mL]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Group I</td>
<td>53.7373% 8.89±0.10</td>
<td>Groups I &amp; II</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>37.955% 8.44±0.04</td>
<td>Groups I &amp; III</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>61.0787% 8.03 ± 0.05</td>
<td>Groups II &amp; III</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>2</td>
<td>Group I</td>
<td>74.9573% 7.31 ± 0.08</td>
<td>Groups I &amp; II</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>50.631% 6.76 ± 0.05</td>
<td>Groups I &amp; III</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>82.9198% 5.95 ± 0.05</td>
<td>Groups II &amp; III</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>3</td>
<td>Group I</td>
<td>91.040% 5.27 ± 0.08</td>
<td>Groups I &amp; II</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>60.1286% 5.88 ± 0.05</td>
<td>Groups I &amp; III</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>95.137% 3.55±0.06</td>
<td>Groups II &amp; III</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>4</td>
<td>Group I</td>
<td>96.6469% 2.73± 0.03</td>
<td>Groups I &amp; II</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>69.6019% 4.98± 0.06</td>
<td>Groups I &amp; III</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>0% 0</td>
<td>Groups II &amp; III</td>
<td>NC</td>
</tr>
<tr>
<td>5</td>
<td>Group I</td>
<td>87.192% 3.76 ± 0.04</td>
<td>Groups I &amp; II</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>0% 0</td>
<td>Groups I &amp; II</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>0% 0</td>
<td>Groups II &amp; III</td>
<td>NC</td>
</tr>
<tr>
<td>6</td>
<td>Group I</td>
<td>95.842% 1.79 ± 0.12</td>
<td>Groups I &amp; II</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>0% 0</td>
<td>Groups I &amp; III</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>0% 0</td>
<td>Groups II &amp; III</td>
<td>NC</td>
</tr>
<tr>
<td>7</td>
<td>Group I</td>
<td>0% 0</td>
<td>Groups I &amp; II</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td>0% 0</td>
<td>Groups I &amp; III</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>0% 0</td>
<td>Groups II &amp; III</td>
<td>NC</td>
</tr>
</tbody>
</table>

Abbreviations: *Significant at 5\% level of significance; LDD = Local drug delivery; NC: Not possible to compute; t = Two sample t-test. Notes: Group I = Ornidazole + hydroxypropylmethyl cellulose; Group II = Ornidazole + chitosan; Group III = Ornidazole + hydroxypropyl cellulose.
In line with the present research, Addy et al. and Ahmed et al. too, observed an initial burst release followed by a slow and sustained release, possibly due to rapid luting or elution of the soluble drug from the outer layers of the carrier material.16,22

The clinical plaque sampling used in the present study facilitated subjecting the prevalent periodontal pathogens to the study and eliminated the difficulty in obtaining standard strains. All the formulations released OZ in concentrations much above the MIC for entire study period (0.1 to 1 µg/ml), which is essential to be effective in the plaque biofilm, as suggested by Wust et al.23 The antibacterial action was greatest and longest with chitosan, in accordance with the findings of Parmar et al. (144-288 hr).24 Kumar and Ilango et al. also suggested that chitosan itself possesses antimicrobial properties, which could contribute to the results of this study.25,26

Hence, the present study has established that OZ-loaded chitosan is the most suitable controlled-release LDD system for periodontal therapy, followed by HPMC and HPC devices. This study paves way for further research exploring use of these LDD devices in medically compromised patients where surgical procedures are contraindicated, elimination of periodontopathic microbial flora thus minimizing chances of surgical intervention in diseased sites as well as their simplicity, safety and ease of fabrication in a clinical set up. However, this research has its limitations in being a single-center study with a limited sample size. These can be overcome by multicentric, long-term, prospective studies with a larger sample size.

**CONCLUSION**

Chitosan incorporated with OZ may be considered as a suitable bioabsorbable controlled-release LDD system for use as an adjunct to mechanical periodontal therapy.

**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**ABBREVIATIONS**

CP: Chronic periodontitis; LDD: Local drug delivery; OZ: Ornidazole; MIC: Minimum inhibitory concentration; HPC: Hydroxypropyl cellulose; HPMC: Hydroxypropylmethyl cellulose; iPBS: Isotonic phosphate buffered saline; SCC: Standard calibration curve; PPD: Pocket probing depth; IZ: Inhibition zones.

**REFERENCES**


**PICTORIAL ABSTRACT**

**SUMMARY**

The study aimed to formulate and evaluate the in-vitro release pattern of Ornidazole (OZ) from three different biodegradable polymer films and to determine the antibacterial activity of released OZ on periodontal pathogens. Four samples of 3 LDD systems were formulated by incorporating OZ into 3 different biodegradable polymer films, viz. hydroxypropylmethyl cellulose (Group I), chitosan (Group II) and hydroxypropyl cellulose (Group III). The release of OZ from these formulations was studied spectrophotometrically. The antibacterial activity of the released OZ on periodontal pathogens was evaluated by determining the inhibition zone (IZ) area. And based on the results it was found that chitosan incorporated with OZ may be considered as a suitable bioabsorbable CR LDD system as an adjunct to mechanical periodontal therapy.

Dr. Mahesh Neurogaonkar MDS, is an ex-postgraduate student of Department of Periodontics, KLE Vishwanath Katti Institute of Dental Sciences, Belgaum. He is currently a private practitioner in Pune, Maharastra.

**About Authors**

Dr. Shaila Kothiwale MDS, PhD is currently a Professor in the Department of Periodontics, KLE Vishwanath Katti Institute of Dental Sciences, Belgaum. She has published several international and national research papers and is also a contributing author for textbooks such as ‘Herbs in Periodontics’ and ‘Local drug delivery in periodontics’. The key interest of the author is local drug delivery of antibiotics, analgesics and herbs in periodontal therapy and periodontal tissue engineering where she has conducted research work using amnion-chorion membranes and bone grafts and has published and presented on international and national platforms as a guest speaker.