CONFIDENTIAL

EVALUATION SWINE TEETH AFTER USAGE OF AN ORAL MEDICAL DEVICE: A HISTOLOGICAL EVALUATION

Histology Final Report

Study No.: HMS-201-HIS

Test Facility

Pharmaseed Ltd.

Hamazmera St. # 9

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Date

July 4, 2016

Sponsor

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1. ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
GLP	Good Laboratory Practice
H&E	Hematoxylin & Eosin
OECD	Organization for Economic Co-Operation and Development
SOP	Standard Operating Procedure

2. STUDY INFORMATION

Study Title:	EVALUATION SWINE TEETH AFTER USAGE OF AN ORAL MEDICAL DEVICE: A HITOLOGICAL EVALUATION
Study Number:	HMS-201-HIS
Key Personnel	
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Histological evaluation:	
Samples arrival:	May 2016
Start evaluation:	6-6-2016
End evaluation:	19-6-2016
Sponsor:	Liora Levi (Ph.D.) Clinical & Regulatory Affairs Manager Home Skinovations Ltd. Cell +972-50-9260334 Email: lioral@silkn.com

3. AUTHORIZATION SIGNATURES

Histopathologist:

E. Loeb 4-7-16

Emmanuel Loeb, Vet. Path Specialist Date

Study QA:

July 4th, 2016

Noa Bischitz, MSc, CMQ/OE Date

4. SUMMARY

The objective of the study was to evaluate, histologically, a possible negative effect that might be a result of repeated usage of an oral medical device in swine teeth and gingiva. Teeth of three animals (10 teeth per animal) were fixated, decalcified and prepared in Pharmaseed and trimmed in proper cassettes (30 samples in total). In all 30 teeth samples no pathological changes were observed. Therefore, it can be concluded that the oral medical device was compatible and had no safety problem or other negative effect.

5. QUALITY ASSURANCE

The study quality assurance manager was informed by the Histopathologist when the study was initiated. The study quality assurance manager periodically inspected the study, ensuring adherence to Pharmaseed's SOPs and the study plan.

6. STUDY COMPLIANCE

Pharmaseed complies with OECD principles of Good Laboratory Practice ENV/MC/CHEM (98)17. This study is considered a non-GLP study. The study followed the study plan and Pharmaseed's SOPs.

7. DISTRIBUTION

Copies of study report will be distributed to:

1. Sponsor (electronic copy of the original)
2. Histopathology (original)
3. Study QA (electronic copy of the original)

8. BACKGROUND

Gingivitis is the most common form of periodontal disease, referring to an inflammation of the gum tissue and is mainly caused by poor or ineffective oral hygiene. With good oral hygiene gingivitis can be reversible; however, in some cases, it can progress into periodontitis, which can lead to the loosening and subsequent loss of teeth. In this study we introduce an innovative RF-utilizing toothbrush (Silk'n Toothbrush), intended for the treatment and prevention of gingivitis. We have conducted preliminary in-vitro studies and demonstrated a notably increased teeth cleaning efficacy of our device as compared to placebo non-RF treatments. Our hypothesis is that by facilitating a highly greater teeth cleaning efficiency, we can maintain a good oral hygiene on a daily basis and provide an effective way to treat and prevent gingivitis. Although RF energy is used for many years in different medical applications, to our knowledge, this is the first RF device intended for dental use. Therefore, prior to a clinical investigation, we first aim to verify the safety of our device in-vivo. The purpose of this study is to examine the safety of the device by testing whether the RF treatments have affected the teeth vitality, and whether the periodontal tissues and structures were harmed by the treatment.

9. STUDY OBJECTIVE

The purpose of the proposed study was to evaluate, histologically, a possible negative effect that might be a result of repeated usage of an oral medical device (Silk'n toothbrush) in the swine teeth and gingiva.

10. MATERIALS AND METHODS

10.1 Treatment procedure

Three young swine, 30-40 kg of weight, were allocated for this study. The swine oral cavity, gingiva, mucosa, and teeth were examined by the study veterinarian and shown to be in good condition prior to treatments. Study procedure included a treatment phase of 2 weeks, and a follow-up phase of additional 2 weeks. During the treatment phase, animal teeth were thoroughly brushed with Silk'n toothbrush for 10 minutes, twice a day, using a standard, commercially available toothpaste. During the follow-up phase the animals were examined periodically, documenting any potential change in their health status and behavior. A veterinarian examination of the oral cavity, gingiva, teeth and mucosa were conducted prior to scarification, and indicated that no apparent damage was inflicted by the treatment. The animals were then scarified and their mandibles and maxilla were taken out for histopathological examination.

10.2 Organ/Tissue Collection & Fixations

Premolar mandibular and maxillary teeth of 3 animals (10 teeth per animal) were fixated, decalcified and prepared in Pharmaseed and trimmed in proper cassettes (30 samples in total).

10.3 Slides Preparation

Embedded organs in paraffin were sectioned at approximately 5 µM thickness. Sectioning and slide preparation was performed by L.E.M Ltd. (Pinchas Sapir 7 St. Science Park, Ness-Ziona 70400, Israel). The slides were stained with Hematoxylin & Eosin (H&E) and subjected to histological evaluation.

10.4 Measurement method

Evaluation was made using microscope (Olympus BX43) and objectives at x10 - x40 magnifications.

11. DATA EVALUATION

11.1 Experimental design –

Three swine were allocated for this study, each went through 2 weeks treatment period followed by 2 weeks follow-up. Treatments included teeth brushing for 10 minutes, twice a day, using a standard commercially available toothpaste.

11.2 Histological evaluation

All slides were stained with H&E and were examined by one pathologist who was blinded to the treatment groups. For each animal, the following tooth structures were evaluated:

- Mucus membrane, including the squamous epithelium
- Bone pathology
- Collagen fibers increase
- Infiltration of inflammatory cells
- Teeth structures

The gingiva and oral mucosa were evaluated for possible pathological lesions (Necrosis, Hemorrhages, and additional pathological changes or remarks) after treatment with a medical device orally. This evaluation was a common semi-quantitative analysis.

Pathological score for possible lesions severity:

The grade of the teeth and surrounding tissue was held from 0 to 4.

0= There is no reported pathological change.

1= There is a very mild pathological change.

2= There is a mild pathological change.

3= There is a moderate pathological change.

4= There is a severe pathological change.

Once a pathological change was observed a full description of the lesion was added.

12. RESULTS

12.1 Histological findings

- No pathological changes were observed in all 30 teeth samples.
- No necrotic or hemorrhagic changes were noted due to the treatment in all mucose membranes, bone tissues, and teeth structures.
- Increase of mainly active macrophages was found in some tooth samples in the dental papilla. However, this change was related to the relative young age of the animals and not to the treatment.

Table 1: Estimation of gingival and tooth structures results

Animal ID	Sample	Pathological score	Necrosis	Hemorrhages	Additional remarks
P3706	R-man-a	0	-	-	-
P3706	R-man-b	0	-	-	-
P3706	R-man-c	0	-	-	-
P3706	R-max-d	0	-	-	-
P3706	R-max-e	0	-	-	-
P3706	L-man-a	0	-	-	-
P3706	L-man-b	0	-	-	-
P3706	L-man-c	0	-	-	-
P3706	L-max-d	0	-	-	-
P3706	L-max-e	0	-	-	-
P3707	R-man-a	0	-	-	-
P3707	R-man-b	0	-	-	-
P3707	R-man-c	0	-	-	-
P3707	R-max-d	0	-	-	-
P3707	R-max-e	0	-	-	-
P3707	L-man-a	0	-	-	-
P3707	L-man-b	0	-	-	-
P3707	L-man-c	0	-	-	-
P3707	L-max-d	0	-	-	-
P3707	L-max-e	0	-	-	-
P3708	R-man-a	0	-	-	-
P3708	R-man-b	0	-	-	-
P3708	R-man-c	0	-	-	-
P3708	R-max-d	0	-	-	-
P3708	R-max-e	0	-	-	-
P3708	L-man-a	0	-	-	-
P3708	L-man-b	0	-	-	-
P3708	L-man-c	0	-	-	-
P3708	L-max-d	0	-	-	-
P3708	L-max-e	0	-	-	-

13. CONCLUSION

No pathological changes were observed in all examined 30 tooth samples. Therefore, it can be concluded that the oral medical device was compatible and had no safety problem or other negative effect.

14. ARCHIVING

The final report and relevant correspondence will be retained in the Non-GLP document archive for a period of one year. At the end of that time period the Sponsor will be contacted and requested to receive the archived material, or to agree in writing to its destruction or to leave it in Pharmaseed archive for an annual fee. The processes of documents storage, retention and removal are conducted according to Pharmaseeds' SOP No. 312: GLP and Non-GLP Archive. Slides and paraffin blocks will be archived for a year.

15. REFERENCES

1. Sahng GK, Steven IR, Rest Dent Endo, Jun 2013 (http://dx.doi.org/10.5395/rde.2013.38.4.194).
2. Ao Min et al, PLOS one, Aug 2015 (DOI:10.1371/journal.pone.0137249).
3. Gopikrishna V et al, Int J Paed Dent, 2009;19, 3-5.
4. Struillou X et al, Open Dent J, 2010; 4, 37-47.
5. Grellmann AP, Orig Res Periodont, 2016;30, e68.