Evaluation of the Absorption of a Sublingual Semaglutide Compounded Formulation (SubMagna™ SL HMW) using the EpiGingival™ and EpiOral™ *In Vitro* Tissue Models

SUMMARY: GLP-1 agonists have been increasingly utilized in the treatment of type 2 diabetes and obesity. The semaglutide commercial oral tablets have extremely low absorption and an alternative sublingual compounded formulation is proposed: semaglutide in SubMagna SL HMW. The *in vitro* tissue models suggest that SubMagna SL HMW is able to deliver the peptide into and through human gingival and oral tissues.

Introduction:

There is a growing demand worldwide for glucagon-like peptide (GLP)-1 agonists, a class of medications utilized in the treatment of type 2 diabetes and obesity. Semaglutide, the active ingredient in the injectable medications Ozempic® and Wegovy® (Figure 1), is the most popular GLP-1 agonist and there are often shortages in the marketplace [1].

Many patients would prefer to avoid injections if possible, and there is an extremely low absorption of the oral tablets (less than 1% per the labeling for Rybelsus®). For these reasons, prescribers and patients may prefer a patent-pending compounded formulation of semaglutide for sublingual administration comprising Rybelsus tablets and SubMagna SL HMW [2]. SubMagna is an anhydrous, self-emulsifying drug delivery system intentionally developed to carry drugs of high molecular weight (HMW) in a sublingual route of administration. This innovative compounding base also benefits from mucoadhesive properties which increase the contact time of the drug in the sublingual space [3].

The purpose of this study was not to determine the appropriate sublingual dose of semaglutide but, instead, to evaluate the ability of the SubMagna to deliver the peptide into and through human gingival and oral tissues. This analysis is not a substitute for *in vivo* pharmacokinetic studies.

Figure 1. *Self-administration of semaglutide injection; stock illustration ID: 2403927641 (adapted from Caroline Ruda /Shutterstock.com).*

Methodology:

The EpiGingival and EpiOral tissues, manufactured by MatTek (Ashland, MA), were the models used to evaluate *in vitro* the absorption of the sublingual compounded formulation semaglutide 3 mg/mL in SubMagna SL HMW. Six tissues of each were incubated overnight at 37º C and 5% CO_2 for equilibration. The assay medium (Teer-Buffer-GLC buffer) was pre-warmed to 37° C and pipetted into 6-well plates. The tissues were transferred into the plates together with the assay medium. The semaglutide compounded formulation was then applied and, following 15 min of elapsed permeation time, the receptor media was collected for analysis. This procedure was repeated for 30 min of total elapsed permeation time.

The quantification of semaglutide was performed using the ELISA analysis, kit purchased from OriGene (Rockville, MD). The standards and test samples were loaded into the wells of the immunoplate. The antiserum was added, and the plate was incubated at room temperature for 1 hr. Following incubation, the rehydrated Bt-tracer was placed on each well and incubated for 2 hrs. After washing, Streptavidin-HRP was added to the plate and the color was then generated with TMB chromogenic solution. Absorbance was read at 450 nm following termination of enzymatic reaction, and the permeation flux of semaglutide was calculated.

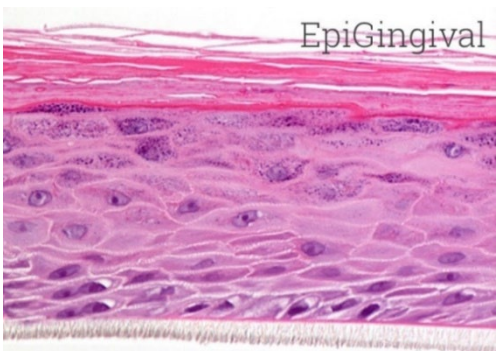

Figure 2. *Illustration of the EpiGingival™ tissue model (adapted from MatTek).*

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Results and Discussion:

MatTek's EpiGingival and EpiOral tissues consist of normal, human-derived oral epithelial cells which have been cultured to form multilayered, highly differentiated models of the human gingival and oral phenotypes. These tissue models exhibit *in vivo*-like morphological and growth characteristics, which are uniform and highly reproducible. As such, these models are commonly used for *in vitro* testing of transbuccal delivery of drugs [4-7]. In this study, the absorption of semaglutide into and through the EpiGingival and EpiOral tissues was detected as early as 15 minutes post-application of the sublingual compounded formulation. The permeation flux of the sublingual semaglutide is shown in Figure 3 for the gingival tissues and in Figure 4 for the oral tissues.

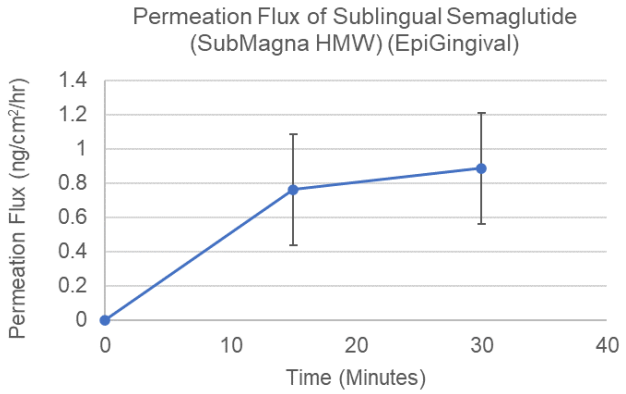

Figure 3. *Permeation flux of the sublingual semaglutide compounded formulation over time for 30 minutes.*

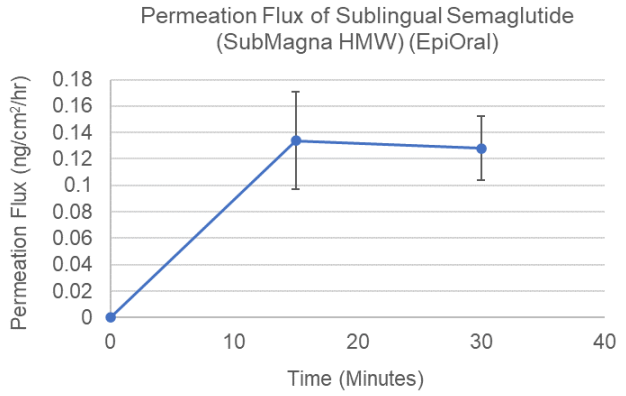

Figure 4. *Permeation flux of the sublingual semaglutide compounded formulation over time for 30 minutes.*

Conclusions:

The buccal mucosa is an attractive site to administer drugs, for either local or systemic delivery, because of its diminutive barrier properties, relatively neutral pH and limited enzymatic activity. Underneath the epithelium there is the mucosal tissue which includes blood and lymphatic vessels. When in the buccal region, drugs can be rapidly and directly absorbed into the systemic circulation by means of a venous drainage to the superior vena cava [8].

Considering that the semaglutide commercial oral tablets have extremely low absorption, the sublingual route of administration is a potentially interesting alternative. This *in vitro* study demonstrates that SubMagna SL HMW is able to deliver the peptide into and through human gingival and oral tissues.

References:

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Evaluation of SubMagna™ SL HMW Liposomal Formation Using Fluorescence Microscopy

SUMMARY: Green fluorescent protein (GFP) was used in this study to mimic the peptide semaglutide. When GFP is incorporated in SugMagna and the formulation is exposed to water, there is spontaneous formation of liposomes which is a favorable attribute for the delivery of medications.

Introduction:

Green fluorescent protein (GFP) is a protein that exhibits bright green fluorescence when exposed to blue light. It is commonly used in scientific research as a marker to visualize proteins.

Liposomes are lipid vesicles that can encapsulate drugs or other molecules, making them useful in drug delivery and research. When observing liposomal formation, GFP may incorporate into the liposomal membrane and/or encapsulate within the liposome. Using fluorescence microscopy, GFP is a valuable tool to track the localization and distribution of liposomes.

Methodology:

GFP (Abcam, Boston, MA) was used in this study to represent the peptide semaglutide. GFP was mixed with SubMagna™ SL HMW to make a final concentration of 0.1 mg/mL. The mixture was added to water to make a 1:1 dilution with gentle mixing to mimic administration and contact of the formulation with saliva. The distribution of GFP in SubMagna was observed under microscopy using blue light or white light at 40x magnification.

Results and Discussion:

When SubMagna is exposed to water, there is spontaneous formation of vesicles (liposomes), as displayed in Figure 1.

The white light evaluation shows the GFP inside the liposomes, but it is not evident because the images are colorless. On the other hand, the blue light evaluation shows clearly the fluorescent protein encapsulated inside liposomes and distributed on the membranes.

The spontaneous liposomal formation of SubMagna when in contact with water is a favorable attribute for the delivery of medications. It avoids the instability issue often associated with liposomes. Moreover, liposomes contain lipid bilayers composed of phospholipids and cholesterols, mimicking the structure of cell membranes. Thus, liposomes can fuse with cell membranes to release the drug instead of relying on endocytosis. This mechanism ensures rapid drug delivery, independent of drug molecular size, and reduces risk of drug degradation.

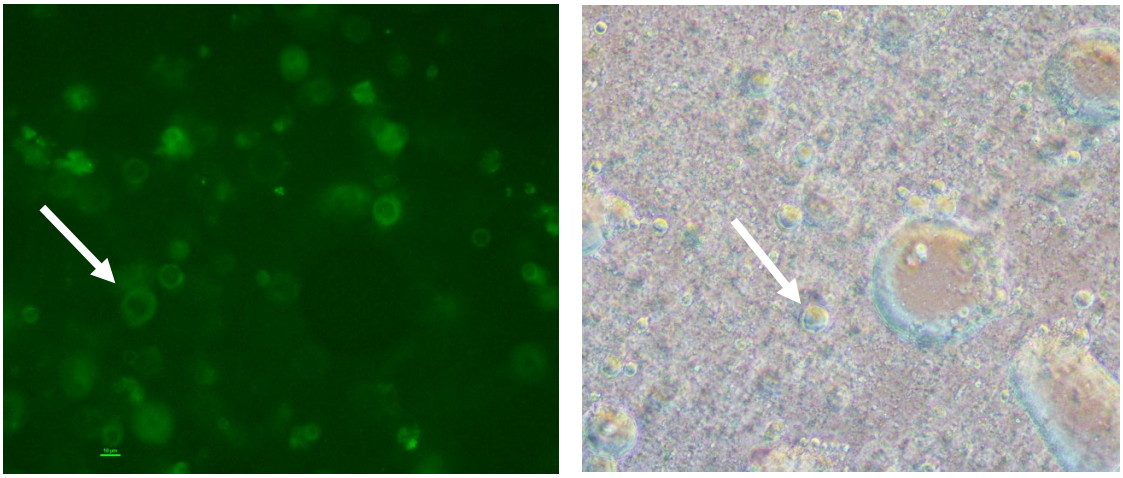

Figure 1. *Fluorescence microscopy: GFP 0.1 mg/mL in SubMagna using blue light (left) and white light (right), at 40x magnification; white arrows highlight selected liposomes.*