

Evaluation of the Mucoadhesive Properties of PCCA Ellage™

Part 2: Ex Vivo Testing on Animal Vaginal Tissues

SUMMARY: The *ex vivo* testing on animal vaginal tissues, together with the bioadhesion testing (PCCA document 99836), evaluate the mucoadhesive properties of PCCA Ellage. A comparative study was conducted by testing simultaneously the well-established combination MucoLox™/VersaBase® Gel (50:50) and a reference OTC long-lasting vaginal moisturizer. It was concluded that PCCA Ellage, as well as MucoLox/VersaBase Gel (50:50), exhibit prolonged mucoadhesion to *ex vivo* animal vaginal tissues.

Introduction:

The vaginal mucosa offers a large surface area and rich blood supply making it a promising site for delivery of medication but it faces a multitude of challenges; in particular, the leakage potential of drugs due to the vaginal fluid that is continuously released [1].

A series of studies were conducted to evaluate the mucoadhesive properties of PCCA Ellage Anhydrous Vaginal. The *Part 1: Ex Vivo Bioadhesion Testing Using a Texture Analyzer* (PCCA document 99836) has demonstrated that PCCA Ellage has a good bioadhesion profile. On the other hand, the leakage test (PCCA document 99816) has shown that PCCA Ellage, and its corresponding PCCA formulas (13834 and 13845), have high retention potential *in vitro* (agar plates at 37°C).

The aim of this study was to build on the existing results by evaluating the mucoadhesive properties of PCCA Ellage using *ex vivo* animal vaginal tissues. A comparative study was conducted by testing simultaneously the well-established combination MucoLox/VersaBase Gel (50:50) and the over-the-counter (OTC) vaginal moisturizer of reference that claims to be long-lasting (up to 3 days).

Methodology:

Frozen pig vaginal tract (BioIVT, Westbury) was cut into small pieces with a square mucosal surface area of approximately 2x2 cm. The *ex vivo* vaginal tissues were washed with Hanks' Balanced Salt Solution (HBSS) and equilibrated at 37°C in HBSS prior to the experiment.

A Vaginal Fluid Simulant (VFS) was prepared to model the fluid produced in the human vagina by healthy, nonpregnant premenopausal women [2]. The *ex vivo* vaginal tissues were fixed on stainless steel using cyanoacrylate glue and were washed with the VFS. The fluorescent dye fluorescein sodium (Sigma-Aldrich, F6377) was dissolved in ethanol and a trace amount was added to the three products: PCCA Ellage, MucoLox/VersaBase Gel (50:50), and the OTC long-lasting vaginal moisturizer. Each fluorescently labeled product was directly applied on the top of the *ex vivo* vaginal tissues (50 µL/cm²) and it was spread evenly using a pellet pestle. A control test was prepared by applying PCCA Ellage (without the fluorescent dye) to the *ex vivo* vaginal tissues (50 µL/cm²), as displayed in Figure 1.

The fluorescently labelled and control tissues were rinsed intermittently in VFS by dipping the stainless steel up and down, three consecutive times, at the following pre-determined time points: 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min and 90 min. The labelled tissues were observed under UV light at 390 nm and images were acquired at the same lighting and angle using an iPhone camera.

This test was conducted at the PCCA R&D Laboratory in accordance to the *in vitro* studies by Song *et al.* and Cazorla-Luna *et al.* [3,4].



Figure 1. *Ex vivo* animal vaginal tissue exposed to PCCA Ellage (*t*= 0 min).

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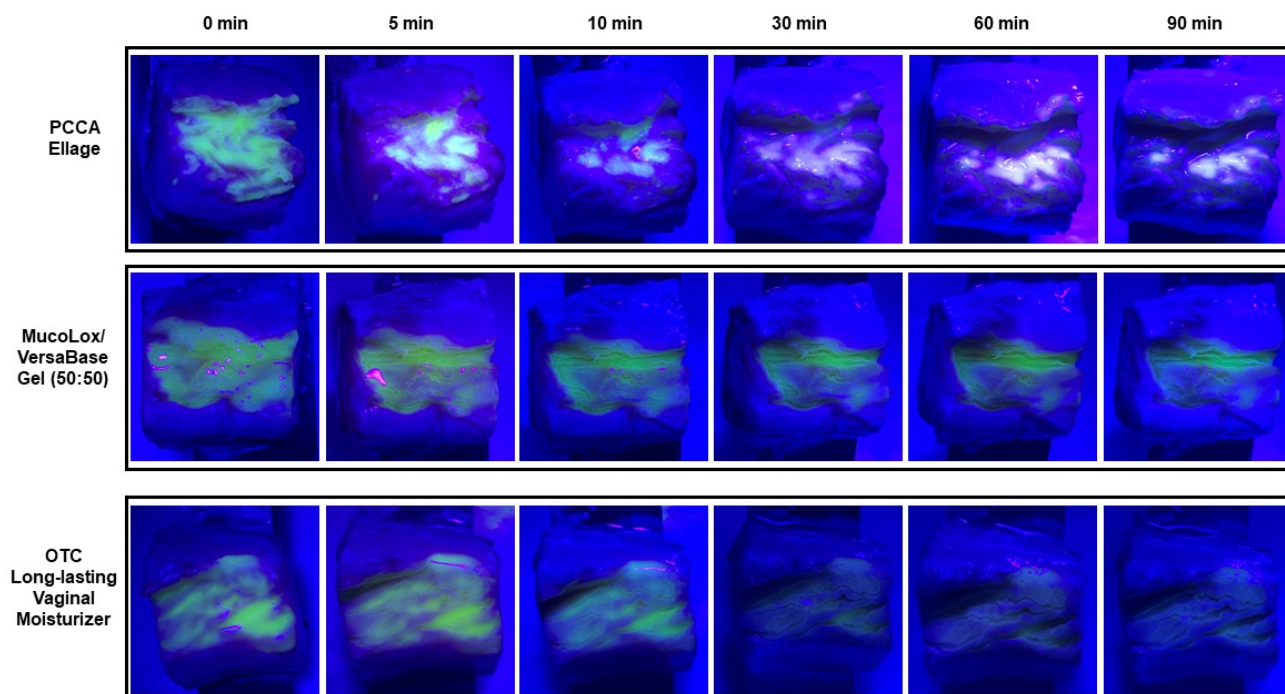


Figure 2. Ex vivo animal vaginal tissues exposed to 3 products (PCCA Ellage, MucoLox/VersaBase Gel (50:50) and an OTC Long-lasting Vaginal Moisturizer), dyed with fluorescein (green color) and analysed for a study period of 90 minutes.

Results & Discussion:

The testing products MucoLox/VersaBase Gel (50:50) and OTC are both clear, as opposed to PCCA Ellage which is white color. For this reason, the fluorescent dye was needed to visualize the mucoadhesion properties of the clear testing products. As shown in Figure 2, the MucoLox/VersaBase Gel (50:50) exhibited longer mucoadhesion at all time points in comparison to the OTC, which showed almost no green color by the end of the study.

The fluorescent dye was added to the three testing products in order to expose the ex vivo tissues to the same experimental conditions. However, since PCCA Ellage is an anhydrous vaginal base and the fluorescein sodium is water soluble, it was to be expected that the green color would fade with the intermittent rinsing in VFS. However, the white color shown in the control test (Figure 1) was clearly visible at all time points in Figure 2, which demonstrates that PCCA Ellage exhibits prolonged mucoadhesion to ex vivo vaginal tissues.

References:

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2. Owen, D.H., Katz, D.F. (1999) 'A vaginal fluid simulant'. *Contraception*, 59 (2), p. 91-5. DOI:10.1016/s0010-7824(99)00010-4.
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