122. Characterization of single- and multi-species Bacterial Vaginosis (BV)-associated biofilms in an ex vivo 3D human vaginal epithelium model

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Background. The polymicrobial biofilm present on the vaginal epithelium during bacterial vaginosis (BV) plays an important role in the progress of this infection. Several studies have demonstrated that the BV biofilm contains mainly *Gardnerella* spp. and in a minor part *Fannyhessea vaginae*. However, this biofilm is often populated by many other facultative or strict anaerobes, but very little is known about their role in vaginal colonization. Thus, a suitable *ex vivo* vaginal colonization model, to address the interactions between vaginal pathogens and the host, is crucial.

Objectives. Due to the ethical and practical difficulties to study BV biofilms in vivo, most studies have been performed in vitro. Herein we aimed to determine the feasibility of using a commercially available 3D *ex vivo* human vaginal model (EPISKIN) to study BV-associated multi-species biofilm formation.

Methods. Commercially obtained reconstituted human vaginal epithelium (RHVE) inserts were placed in 24-well plates and infected with either a *G. vaginalis* suspension or a suspension composed of six BV-associated species. RHVE was then analysed using PNA-FISH, qPCR and Periodic-Acid-Schiff and Hematoxylin and Eosin staining's. As a control, this experiment was also conducted in 24 well-plates without the RHVE inserts.

Results. Both *G. vaginalis* alone or the polymicrobial consortia bacteria were able to colonize the RHVE, leading to the formation of a biofilm, that had a distinct bacterial composition, in comparison with the no-RHVE control. Furthermore, the polymicrobial biofilm formed had significantly higher biomass than *G. vaginalis* mono-species biofilm.

Conclusions & Significance: To our knowledge, to date, this study was the first to assess the bacterial colonization of BV-associated species in an *ex vivo* 3D vaginal epithelium model. Our findings strengthen the idea that when co-incubated the vaginal pathogens, they interact, leading to the formation of a dense biofilm, which might be associated with BV failure treatment.

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