



Use of simulated vaginal and menstrual fluids to model *in vivo* discolouration of silicone elastomer vaginal rings

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ABSTRACT

Vaginal rings releasing antiretrovirals – either alone or in combination with contraceptive progestins – are being developed for prevention of human immunodeficiency virus (HIV) transmission *via* vaginal sex. Following Phase I trials, significant discolouration was observed on the surface of investigational silicone elastomer antiretroviral-contraceptive matrix-type vaginal rings containing either 25 mg dapivirine or 200 mg dapivirine plus levonorgestrel. In this study, potential causes of the discolouration have been assessed *in vitro* using simulated vaginal and menstrual fluids (SVF and SMF, respectively) to model *in vivo* exposure. The fluid compositions also included hydrogen peroxide (H₂O₂), hydrogen peroxide plus a copper intrauterine device (IUD), or synthetic dyes (representing personal care and household cleaning products). No discolouration was observed for rings exposed to SVF + hydrogen peroxide (with or without an IUD). However, the SVF + dye compositions showed significant ring discolouration, with staining patterns similar to those observed with rings that had been exposed to highly-coloured personal care and household cleaning products during clinical trial use. Exposure of rings to SMF compositions invariably caused yellow surface discolouration, dark spotting and markings, similar to the staining patterns observed following clinical use. The darker marks on the ring surface were identified as blood debris derived from the SMF. The study indicates that surface discolouration of rings *in vivo* can be attributed to exposure to menstrual fluid or highly coloured personal care or household cleaning products. Discolouration of the rings was not associated with any specific safety risks for the user, though severe discolouration could potentially impact acceptability and adherence.

1. Introduction

The concept and first prototypes of drug-releasing vaginal rings were first described in the late 1960s (Dziuk and Cook, 1966; Mishell et al., 1970; Mishell and Lumkin, 1970). Since then, seven steroid-releasing contraceptive rings (Estring®, Femring®, NuvaRing®, Progering®, Fertiring®, Ornibel® and Annovera™) and various generic products have reached market (Algorta et al., 2017; Archer et al., 2019), and a raft of new experimental drugs and devices are currently at various stages of preclinical and clinical development (Vincent et al., 2018; Keller et al., 2019; Boyd et al., 2014; Verstraete et al., 2017; Fetherston et al., 2014; McBride et al., 2019; Zhao et al., 2017; Clark et al., 2014; Baum et al., 2015; Welsh et al., 2019; Brache et al., 2012; Weiss et al., 2019). Many of the advances in ring design and technology during the last twenty years have been driven by concerted global efforts to develop new

antiretroviral-releasing ring products for preventing sexual transmission of HIV infection in women (Vincent et al., 2018; Keller et al., 2019; Baum et al., 2015; Kiser et al., 2012; Woolfson et al., 2006; Malcolm et al., 2005; Baeten et al., 2020; Malcolm et al., 2016; Malcolm et al., 2014; van der Straten et al., 2016; Malcolm et al., 2010; Spence et al., 2015; Malcolm et al., 2012; Thurman et al., 2013). A matrix-type silicone elastomer vaginal ring providing sustained release of the antiretroviral drug dapivirine (DPV) – a potent non-nucleoside reverse transcriptase inhibitor – was developed by the International Partnership for Microbicides (IPM) to offer women a self-initiated, long-acting HIV prevention option (Devlin et al., 2013; Nel et al., 2009; Nel et al., 2016a; Baeten et al., 2016; McCoy et al., 2017; Nel et al., 2014; Chen et al., 2019; Bunge et al., 2020). In two Phase III safety and efficacy trials, the 25 mg DPV ring was shown to reduce the risk of HIV acquisition in women compared to a placebo ring (Nel et al., 2016a; Baeten et al.,

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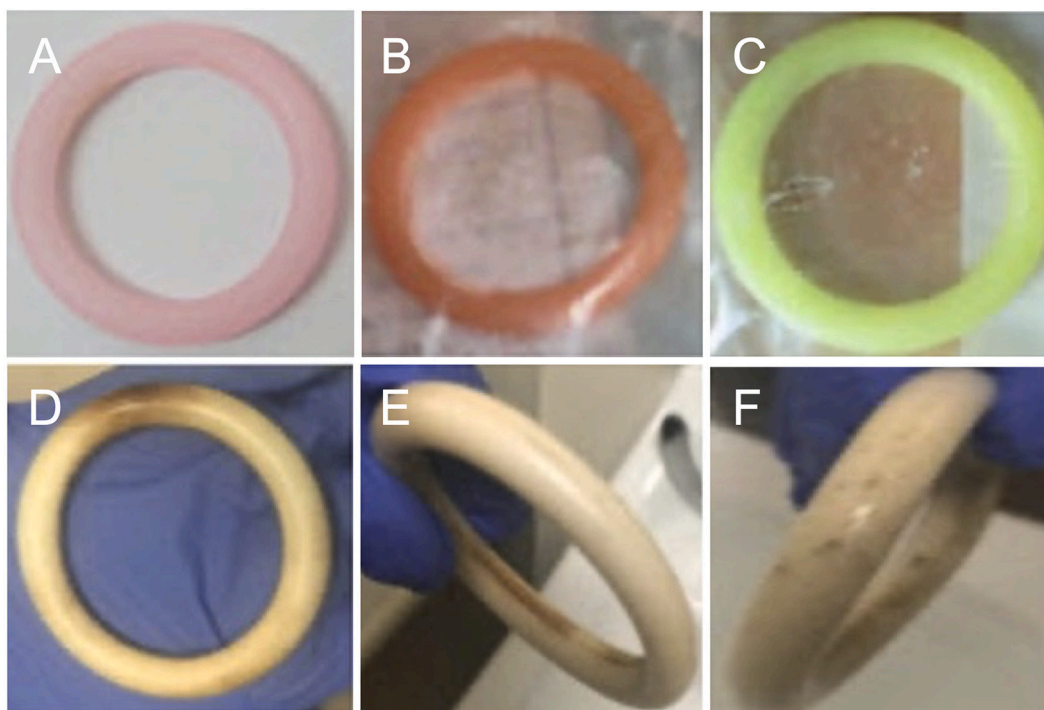


Fig. 1. Examples of post-use discolouration of silicone vaginal rings. These photographs were taken at the clinical site using a low-resolution camera. However, even at the current resolution, the photographs clearly illustrate the bright uniform discolourations (Rings A–C) and the dark, non-uniform staining (Rings D–F). All rings have the same dimensions: 56 mm outer diameter and 7.7 mm cross-sectional diameter.

2016). In 2020, the dapivirine vaginal ring received a positive scientific opinion from the European Medicines Agency (EMA) through the Article 58 procedure for the product's use by cisgender women ages 18 and older in developing countries. Also, in January 2021, the World Health Organization recommended the dapivirine vaginal ring be offered as an additional prevention choice for women at substantial risk of HIV infection as part of combination prevention approaches.

More recently, the field has focused its attention on the development of new drug-releasing multipurpose prevention technology (MPT) vaginal ring products intended to simultaneously prevent HIV infection, unintended pregnancy, and/or other sexually transmitted infections (Boyd et al., 2016; Baum et al., 2012; Woodsong and Holt, 2015; Celum and Baeten, 2020; Friend et al., 2013; Fetherston et al., 2013a; Thurman et al., 2018; Young Holt et al., 2018). A matrix-type silicone vaginal ring developed by IPM and containing 200 mg DPV and 320 mg levonorgestrel (LNG; a synthetic progestogen commonly used in contraceptive products) is currently in clinical development and provides continuous release of DPV and LNG for up to 90 days (Fetherston et al., 2014; Boyd et al., 2016; Dallal Bashi et al., 2019; Murphy et al., 2016). In a 14-day first-in-human Phase I safety and pharmacokinetic trial (MTN-030/IPM 041) of this 200/320 mg DPV + LNG ring, levonorgestrel plasma concentrations were maintained at levels considered suitable for contraception (Achilles et al., 2018). A second 90-day Phase I study (MTN-044/IPM 053/CCN019) of the same DPV + LNG ring was completed in October 2019 (PK Study of 90-Day Use of Vaginal Rings Containing Dapivirine and Levonorgestrel, 2018).

Acceptability studies have demonstrated that vaginal rings releasing steroid hormones or antiretrovirals are generally well-accepted, particularly after women have gained experience using the products (Montgomery et al., 2017; RamaRao et al., 2018; van der Straten et al., 2012; Novák et al., 2003; Kestelyn et al., 2018; Nel et al., 2016b). However, some women have voiced concerns regarding vaginal ring hygiene, particularly during menses (van der Straten et al., 2016; Duby et al., 2020; Watnick et al., 2018). Surface discolouration of silicone elastomer vaginal rings during clinical use has been reported in the scientific

literature (Kiser et al., 2012; van der Straten et al., 2016; Kestelyn et al., 2018; Ballagh, 2001; Murphy et al., 2019a; Das et al., 2015; Murphy et al., 2019b). In the absence of confirmatory studies, it has been widely assumed that this discolouration is due to cervicovaginal fluid and/or menstrual blood; in truth, little is known about the causes of discolouration. Although there is no evidence that discolouration affects the clinical performance of rings (including drug release and mechanical performance), women have expressed concerns about rings absorbing blood, rings getting “dirty” or stained, and ring malodour (van der Straten et al., 2016; Duby et al., 2020; Watnick et al., 2018; Stifani et al., 2018). As a consequence, some women report removing rings for cleaning, particularly during menses, which can lead to non-compliance (van der Straten et al., 2016; Montgomery et al., 2017; Nel et al., 2016b; Duby et al., 2020; Montgomery et al., 2012; Guthrie et al., 2015). However, clinical education and guidance and peer support are often enough to allay women's fears and positively influence adherence behaviours (Montgomery et al., 2017; van der Straten et al., 2012; Kestelyn et al., 2018; Griffin et al., 2019).

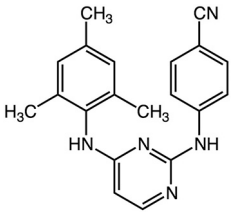
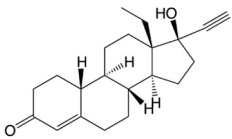
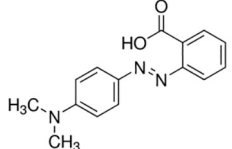
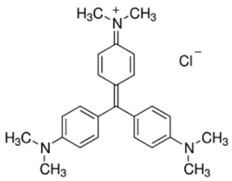
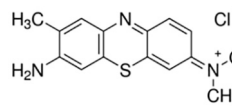
In this study the potential causes and factors leading to discolouration of rings during clinical use were investigated by exposing four different matrix-type silicone elastomer ring formulations – a placebo ring, a 25 mg DPV ring, a 200 mg DPV ring, and a DPV + LNG ring – to various simulated vaginal fluid and simulated menstrual fluid media at 37 °C for either 30 or 60 days *in vitro*. Digital photography and digital microscopy were used to evaluate the type and extent of discolouration.

2. Materials and methods

2.1. Materials

Placebo DDU-4320 rings ($n = 33$), 25 mg DPV DDU-4870 rings ($n = 36$), 200 mg DPV DDU-4320 rings ($n = 33$), and DPV + LNG DDU-4320 rings ($n = 36$) – all having 56 mm outer diameter and 7.7 mm cross-sectional diameter – were supplied by the IPM (Silver Spring, USA). DDU-4320 and DDU-4870 are addition-cure drug delivery grade silicone

Table 1
Physicochemical properties of ring APIs and synthetic dyes used in *in vitro* discolouration studies.

API / Dye Name(s) (CAS #)	Chemical structure	Molecular weight (g/mol)	Log P/Log K _{o/w}	Solubility in water
Dapivirine, TMC120 (244767-67-7)		329.40	2.27 at 20 °C ^a 5.3 (Jespers et al., 2007; Swedrowska et al., 2017)	0.02 µg/mL (Fetherston et al., 2013b) (<1 µg/mL) ^a
Levonorgestrel, norgestrel, D-norgestrel (797-63-7)		312.45	3.5 (Fick et al., 2010)	Practically Insoluble ^b 1.4 µg/mL (Schulz et al., 2011)
Methyl Red, Acid Red 2 (493-52-7)		269.30	3.9 ^c	Slightly soluble ^c
Crystal Violet, Basic Violet 3, Gentian Violet (548-62-9)		407.98	1.17 at 25 °C ^c	16 mg/mL at 25 °C ^d 50 mg/mL at 27 °C ^c
Toluidine Blue, Toluidine Blue O, Basic Blue 17, Tolonium chloride (92-31-9)		305.83	2.27 ^e	30 mg/mL at 25 °C ^f

Log P / Log K_{o/w} – n-octanol/water partition coefficient; ^a Ajinomoto Omnicem NV material safety data sheet (MSDS); ^b Industriale Chimica s.r.l. MSDS; ^c Sigma-Aldrich MSDS; ^d ThermoFisher Scientific MSDS; ^e ChemSpider; ^f Merck MSDS.

elastomer materials supplied by NuSil™ Technology Inc. (Carpinteria, CA, USA). SVF components (sodium chloride, calcium hydroxide, bovine serum albumin, lactic acid, acetic acid, glucose), xanthan gum, hydrogen peroxide (H₂O₂), methyl red (MR), toluidine blue (TB) and crystal violet (CV) were purchased from Sigma-Aldrich (Gillingham, UK). SVF was prepared according to Owen & Katz (Owen and Katz, 1999). SMF comprised a 1:1 mixture of defibrinated horse blood (Lab Supplies & Instruments Ltd., Antrim, UK) and SVF containing 0.5% w/v xanthan gum. A Millipore Direct-Q 3 UV Ultrapure Water System (Watford, UK) was used to obtain ultrapure water. Copper T380A intrauterine devices (IUD) (SMB Corporation of India, Mumbai, India) were purchased from a local pharmacy store.

2.2. Ring appearance

Initially, rings were weighed and both sides of each ring photographed. At scheduled timepoints during the study, rings were removed from the media in which they were immersed and photographed (both sides) alongside ultrapure water and untreated control rings. Consistent lighting conditions were used for all photographs to enable direct comparisons.

2.3. Ring immersion in simulated vaginal fluid and simulated menstrual fluid media

Individual rings were placed into 250 mL sterile glass flasks (three rings per formulation per media type). Various control and SVF media

were prepared – ultrapure water, SVF-only, SVF + 20 µM H₂O₂, SVF + 20 µM H₂O₂ + copper IUD, SVF + MR, SVF + TB, SVF + CV – and 100 mL added to the sterile flasks. Dye solutions (0.1 mg/mL) were prepared in deionised water and filtered (0.4 µm) immediately prior to addition to flasks. For flasks containing the copper IUD, the medium was first added to the flask followed by placement of the IUD at the centre of the ring; this ensured that the IUD was fully immersed. Flasks were incubated in a SciQuip Incu-Shake FL16–2 orbital shaking incubator at 37 °C/60 rpm for either 30 or 60 days. Media were replenished with 100 mL of a freshly prepared solution on a weekly basis. At scheduled timepoints (30 or 60 days), rings were removed from the media, rinsed with deionised water, and blotted dry using lint-free tissue paper. Discolouration and changes to the ring surface were noted. All rings were photographed using the procedure described in Section 2.2. Rings exposed to ultrapure water and untreated rings (freshly removed from the packaging) were used as study controls. Rings were weighed and any unusual odour noted. Similar protocols were adopted for the SMF media (SMF-only, SMF + 20 µM H₂O₂, SMF + 20 µM H₂O₂ + copper IUD), except for a reduction in the medium volume to 25 mL after two weeks of testing. SMF media was prepared immediately prior to addition to the flasks.

2.4. Microscopy analysis

Segments (~1.25 cm thickness; *n* ≥ 3) were cut from each treated and control ring using a CRD135 benchtop tubing cutter (Clean Room Devices, Inc. USA). Segments having surface discolouration or spotting were specifically selected and examined for surface discolouration and

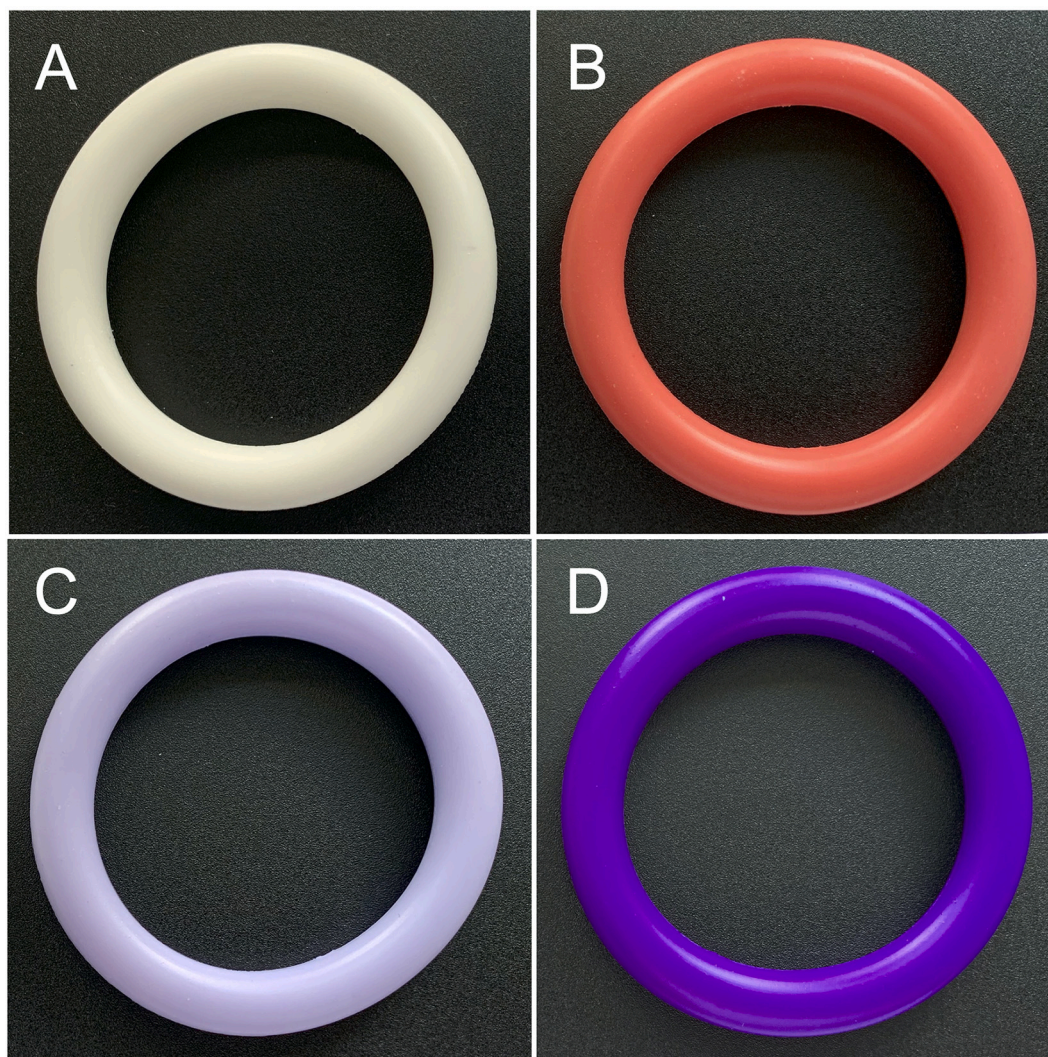


Fig. 2. Representative images of 200/320 mg DPV + LNG rings: A – untreated control ring; B – exposed to SVF + MR; C – exposed to SVF + TB; D – exposed to SVF + CV media for 30 days. Ring dimensions: 56 mm outer diameter, 7.7 mm cross-sectional diameter.

dye uptake using a Keyence VHX-700F series Digital Microscope (Keyence Limited, UK) fitted with a top-lit Z20 (20–200 \times) zoom lens. From each ring segment, a further thin cross-section (\sim 3 mm thick) was cut and analysed by digital microscopy. Different fields of view were recorded with both microscopy methods.

3. Results and discussion

This study was intended to help shed light on ring discolourations observed during clinical testing. In our experience, ring staining generally falls into one of two categories: yellow-tan-brown staining (associated with body fluids) and other non-natural colour staining (e.g., pink, purple, orange, etc.). The latter has been linked to use of personal care products (i.e.e.g., bath salts) or other products with intense colour characteristics, as described in our previous work (Murphy et al., 2019b).

3.1. Ring appearance

Rings were used as supplied. DDU-4320 placebo ($n = 33$), DDU-4320 200 mg DPV ($n = 33$; contains only DPV) and DDU-4320 DPV + LNG ($n = 36$) rings were uniformly white in appearance; a representative image of a DPV + LNG ring is presented in Fig. 2A. DDU-4870 25 mg DPV ($n = 36$) rings were uniformly off-white in appearance. No major surface

defects (e.g., holes, pittings, raised mold parting lines, weld lines, etc.) were visibly discernible on the supplied rings, although, based on previous work, high magnification microscopy is capable of revealing surface features such as mold parting lines and injection points. The surface roughness (R_a ; arithmetic average roughness) of the mold tools used to manufacture the DPV + LNG ring and 25 mg DPV ring were 0.54 μm and 1.97 μm , respectively.

3.2. Ring experiments in simulated vaginal fluids

A huge range of household and personal care products are available to women in Africa, and number of which may have been used to wash rings during/after clinical studies. In a previous study to evaluate the impact of common household and personal care products used by trial participants in Africa on drug release and stability of the 25 mg dapivirine ring, we observed uniform pink staining of some rings that corresponded with exposure to a highly coloured Sols bath salt product (Murphy et al., 2019b). Similar uniform colour staining – including pink, orange and green staining – was observed in this study (Fig. 1A–C).

Silicone elastomers are chemically inert and thus any discolouration observed with rings is solely due to the diffusion/ingression of solutions of coloured molecular species onto the ring surface or into the silicone elastomer matrix. The extent of dye ingress into the silicone elastomer is dependent on the physicochemical properties of the dye/

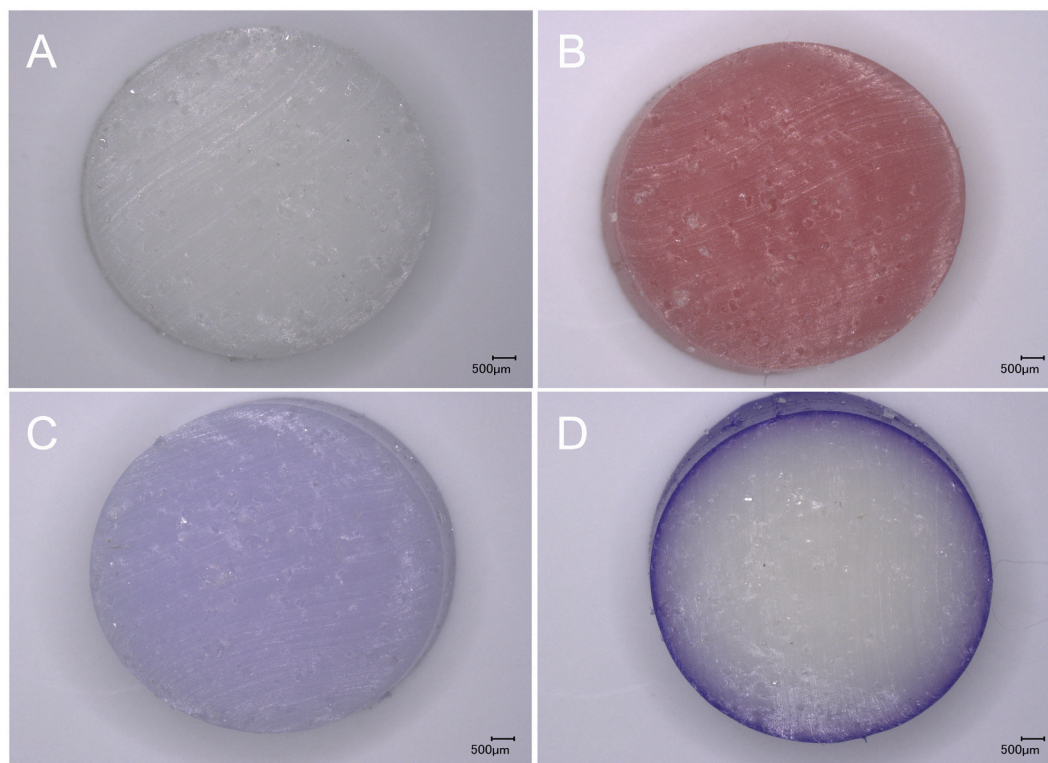


Fig. 3. Representative images of 200/320 mg DPV + LNG ring cross-sections (diameter 7.7 mm): A – untreated control; B – exposed to SVF + MR; C – exposed to SVF + TB; D – exposed to SVF + CV media for 30 days. Images were recorded using $\times 30$ magnification.

coloured substance, most notably its molecular weight (which influences diffusion rate) and the relative hydrophobicity of the molecule. Thus, we tested three model dye molecules – methyl red (MR), toluidine blue (TB) and crystal violet (CV) – operating under the assumption that if discrete dye substances can colour silicone elastomer vaginal rings, then so too can coloured household/personal care products. In an effort to represent the broad range of coloured dyes found in household/personal care products, these dyes were selected to have different hydrophobicities (log P values) and different degrees of aqueous solubility (Table 1).

In healthy women of reproductive age, the vaginal environment is normally dominated by lactic acid producing *Lactobacillus* species, bacteriocins and H_2O_2 (Eschenbach et al., 1989; Greenbaum et al., 2019; Mijač et al., 2006; Ceccarani et al., 2019). For this reason, H_2O_2 was included in the study to assess its role in ring discoloration. H_2O_2 concentrations vary between women with healthy flora and women experiencing bacterial vaginosis. We sought to explore whether the presence or absence of H_2O_2 would impact discoloration of the ring. The concentrations used were based on those reported by O'Hanlon et al. who identified $\sim 23 \mu M$ as the mean H_2O_2 concentration produced by lactobacilli in cervicovaginal fluid (O'Hanlon et al., 2010). Therefore, $20 \mu M H_2O_2$ was included or not included to simulate the peroxide presence under either normal flora or dysbiosis, respectively.

The Copper T 380A IUD – a popular contraceptive device among women in developing countries (Buhling et al., 2014) – was included in the study to assess the contribution of copper ions to vaginal ring discoloration. Rings (placebo, 25 mg DPV, 200 mg DPV and DPV + LNG) placed in the following media – SVF-only, SVF + $20 \mu M H_2O_2$, and SVF + $20 \mu M H_2O_2$ + copper IUD – showed no surface discoloration after immersion for 30 or 60 days (images not shown). By comparison, the DPV + LNG ring formulation showed significant surface coloration after 30-day exposure to the SVF + MR, SVF + TB, and SVF + CV media (Fig. 2). Similar colour staining of the ring surface was observed for placebo, 200 mg DPV and 25 mg DPV ring formulations (images not

shown). Uniformity and intensity of surface staining was not different after 60-day exposure to the SVF + dye solutions.

Ring cross-sections were examined to assess the extent of dye penetration into the ring body. Fig. 3 shows representative images for the untreated control sample and the DPV + LNG ring samples exposed to the various SVF + dye media for 30 days. For rings immersed in the SVF + MR and SVF + TB media, dye ingress occurred throughout the entire ring interior (Figs. 3B–C), while rings exposed to SVF + CV showed limited dye ingress (Fig. 3D). These results demonstrate the ability of highly coloured compounds/products to cause surface discoloration of silicone elastomer rings, and to permeate into the entire ring body to an extent (MR > TB > CV) that follows the trend in their log partition coefficient values (Table 1). The composition of the rings – specifically, the grade of silicone elastomer and the type and loading of active agents – appears to have little impact, at least based on the materials tested in this study.

3.3. Ring experiments in simulated menstrual fluids

A simulated menstrual fluid comprising a 1:1 mixture of defibrinated horse blood with SVF containing 0.5% w/v xanthan gum was developed for use in this study to mimic the composition and viscosity of human menstrual fluid (Hood et al., 2014; Fraser et al., 2001). Use of human blood for preparation of the simulated menstrual fluid was considered. However, the quantities required and the very considerable obstacles in securing a regular supply throughout the study period proved insurmountable. However, the components present in horse blood are entirely similar to those in humans, comprising erythrocytes, leukocytes, and thrombocytes. Thrombocytes and leukocytes do not differ significantly between humans and other vertebrates; the only reported deviation is that horse blood shows increased level of aggregation kinetics and adhesive forces between erythrocytes compared with human blood (Weng et al., 1996). The red colour of oxygenated blood in both humans and horses is due to the presence of hemoglobin molecules in

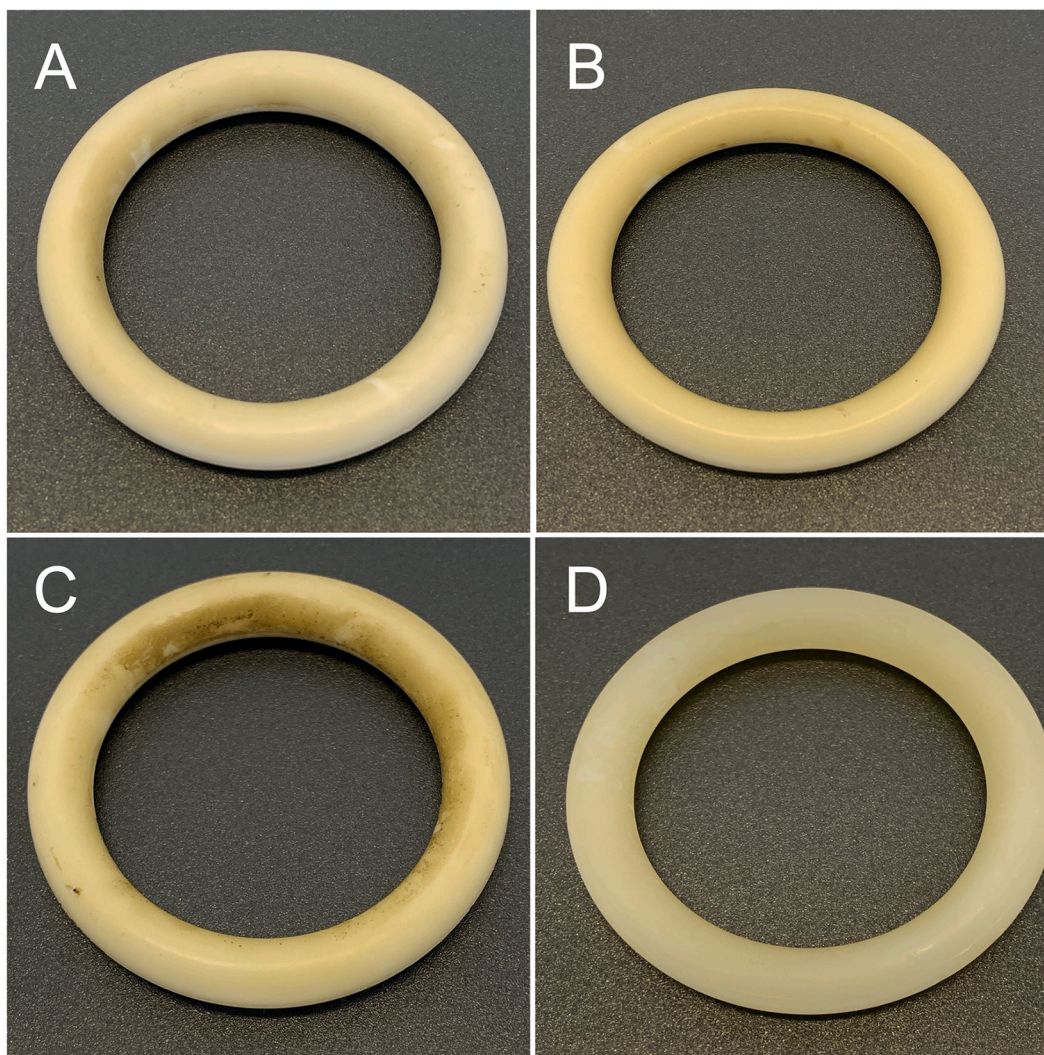


Fig. 4. Representative images of vaginal rings: A – placebo; B – 200 mg DPV; C – 200/320 mg DPV + LNG; D – 25 mg DPV exposed to SMF + 20 μM H_2O_2 treatment media for 60 days. Ring dimensions: 56 mm outer diameter, 7.7 mm cross-sectional diameter.

the erythrocytes. Thus, we concluded that horse blood would be a suitable replacement for human blood for the purposes of this study.

All rings (placebo, 25 mg DPV, 200 mg DPV and DPV + LNG) exposed to the SMF-only and SMF + 20 μM H_2O_2 media for 30 or 60 days showed a yellowed surface appearance compared to untreated control rings, with the intensity of the yellow surface colour increasing with duration of exposure; representative images are presented in Fig. 4. Also, non-uniform red-brown staining and spotting were observed to varying degrees on the surface of all rings exposed to the SMF-only and SMF + 20 μM H_2O_2 media. Darker staining was evident around the inner circumference of some rings (Fig. 4C), consistent with staining patterns observed on some of the DPV + LNG clinical rings (Fig. 1E). Similar staining patterns were observed across all four ring formulations (images not shown). It is likely the hemoglobin component of blood that is causing the yellow-tan-brown discoloration observed on the surface of the clinical rings. These red-brown marks were identified by digital microscopy as SMF blood debris adhered to the ring surface (Figs. 5B & 5C). Microscopic evaluation of these darker areas of staining and spots showed no surface feature (surface pitting or patterning) that could be the causative reason for this staining. No significant staining of the ring interior was observed for rings exposed to any of the SMF media; representative images are presented in Fig. 6. Since the staining pattern varied between rings of the same type as well as across the four different

ring formulations, it was not possible to correlate the staining with any particular surface feature. The extent and variability of staining observed for the ring samples in this *in vitro* study were entirely similar to those observed for rings returned to the clinic by women after use.

IUDs were included in the *in vitro* study as the first reports of ring discoloration were made only by trial participants who used an IUD (unpublished data). For this reason, copper ion deposition was initially considered as a potential source of discoloration and included in both the SVF and SMF study arms. Subsequently, discoloration reports were also made by several patients who did not use IUDs (unpublished data). Flasks containing rings exposed to IUDs and H_2O_2 solution were pulled from the study after Day 14 due to excessive microbial growth (blood is an excellent culture medium for bacterial growth), aggregation/clotting of material around the IUD, and unpleasant smell. After 14 days, all ring formulations showed a slightly yellowed surface appearance. Additional brown-black marks presenting in discrete pairs were observed on some ring surfaces (Fig. 7). These dark marks corresponded to the distance between the copper coils on the arms of the IUD (Fig. 7B) and were consistent with points of direct physical contact between the IUD and the ring surface. Since similar dark marks were not observed with any of the rings exposed to the SVF + H_2O_2 + IUD treatment solutions, we attributed them to an interaction between the copper coil of the IUD and some component of the SMF media. Since it would be physically

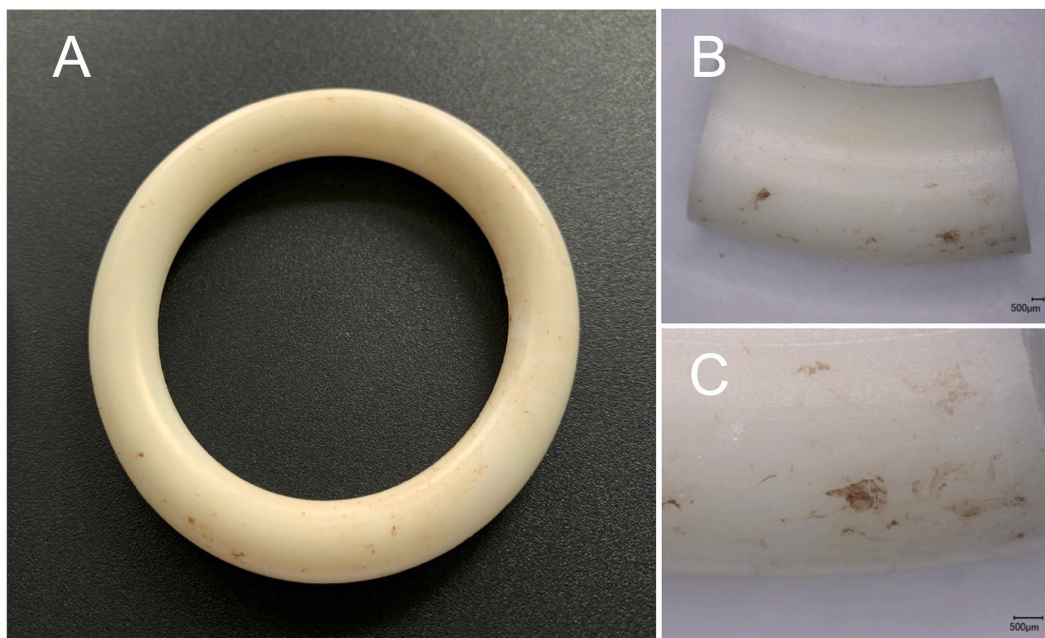


Fig. 5. Comparison of photographic and Keyence microscopy images recorded for 200/320 mg DPV + LNG rings exposed to an SMF + 20 µM H₂O₂ treatment media for 30 days: A – full ring photograph; B – Keyence image of segment (×20 magnification); C – Keyence image of segment (×50 magnification). Ring dimensions: 56 mm outer diameter, 7.7 mm cross-sectional diameter.



Fig. 6. Representative images of 200/320 mg DPV + LNG ring cross-sections (7.7. mm diameter): A – untreated control; B – exposed to SMF-only; C – exposed to SMF + 20 µM H₂O₂ media for 30 days. Images were recorded using ×30 magnification.

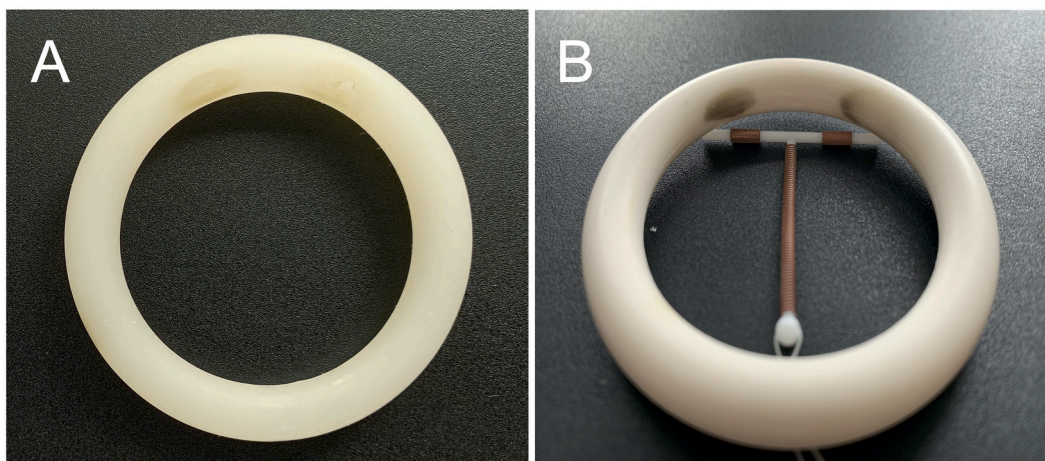


Fig. 7. A – 25 mg DPV ring and B – placebo ring overlaid with a copper IUD. Both images display dark oval marks that correspond with the contact points between the ring surface and the copper component of the IUD. Ring dimensions: 56 mm outer diameter, 7.7 mm cross-sectional diameter.

impossible for an IUD located in the uterus to be in direct contact with a vaginal ring device during clinical use, the marks due to the IUD are unlikely to contribute to the discolouration observed in clinical ring samples, unless copper ions transferring from the uterus to the vagina are implicated.

3.4. General discussion

It is well known that other silicone elastomer devices for vaginal administration – including drug-releasing vaginal rings, vaginal pessaries (for treatment of pelvic organ prolapse) and cervical diaphragms – are prone to discolouration as a result of prolonged use (Kiser et al., 2012; van der Straten et al., 2016; Kestelyn et al., 2018; Ballagh, 2001; Murphy et al., 2019a; Das et al., 2015; Murphy et al., 2019b; Atnip, 2009). However, the extent of vaginal ring staining is highly variable among women. Since women can easily remove and inspect rings, any observed discolouration may be a concern for some users (van der Straten et al., 2016; Duby et al., 2020; Watnick et al., 2018; Stifani et al., 2018). Patient education may help to reduce the levels of concern or non-adherence associated with discolouration.

Bleeding is widely reported and consistent with use of progestin-only contraceptives (Abdel-Aleem et al., 2013; d'Arcangues, 2000; Zigler and McNicholas, 2017). Spotting and irregular or heavy bleeding are common side effects associated with the use of progestin-only IUDs (such as Skyla® and Mirena®) and subdermal implants (such as Jadelle®), often occurring up to 6 months following initial administration of the products (Abdel-Aleem et al., 2013; d'Arcangues, 2000; Zigler and McNicholas, 2017; FSRH, 2015; Mirena (levonorgestrel-releasing intrauterine system), 2008; SKYLA (levonorgestrel-releasing intrauterine system), 2017; Jadelle (levonorgestrel implants) for subdermal use, 2016). The discolourations noted in this study when rings are immersed in SMF are consistent with those reported for rings after clinical use (van der Straten et al., 2016; Kestelyn et al., 2018; Murphy et al., 2019a; Das et al., 2015) and suggest that menstrual blood exposure is the primary cause.

4. Conclusions

In vitro exposure of silicone elastomer rings to aqueous solutions containing dyes (representing common household cleansing and personal care products) caused surface and interior staining of silicone elastomer vaginal rings similar to that observed in the clinic. Also, *in vitro* exposure of silicone elastomer rings to SMF solutions at 37 °C for up to 60 days produced yellow surface discolourations, dark markings, and non-uniform red/brown staining similar to those observed in the following clinical use of DPV + LNG rings. Exposure to menstrual blood was the most probable cause of the surface staining observed during the DPV + LNG ring Phase I trials. While these discolourations do not affect product performance nor are known to be related to adverse events, they may have an impact on acceptability and user behaviour. Strategies to either mask or reduce the extent of menstrual blood discolouration with silicone elastomer vaginal rings are warranted, including the use of coloured pigments/masterbatches that are specially designed for use with silicone elastomer medical devices.

Author contribution to manuscript

All authors contributed to the design of the study and drafting of the manuscript for submission. CFM, DJM, and YDB performed the experimental work. All authors approved submission of the manuscript.

Author statement

All authors contributed to conceptualisation, methodology, writing, reviewing and editing of the manuscript. Clare McCoy, Yahya Dallal Bashi, Diarmaid Murphy and Peter Boyd were responsible for resources and performing the experiments. Patrick Spence, Kyle Kleinbeck, Brid

Devlin, Tiffany Derrick, Peter Boyd and Karl Malcolm supervised various aspects of the work. Bindi Dangi was responsible for project management and administration.

Declaration of Competing Interest

All authors declare no actual or potential conflicts of interest.

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