Kent Academic Repository

Brako, Francis, Raimi-Abraham, Bahijja Tolulope, Mahalingam, Suntharavathanan, Craig, Duncan Q.M. and Edirisinghe, Mohan (2018) *The development of progesterone-loaded nanofibers using pressurized gyration: A novel approach to vaginal delivery for the prevention of pre-term birth.* International Journal of Pharmaceutics, 540 (1-2). pp. 31-39. ISSN 0378-5173.

Downloaded from

https://kar.kent.ac.uk/78032/ The University of Kent's Academic Repository KAR

The version of record is available from

https://doi.org/10.1016/j.ijpharm.2018.01.043

This document version

Publisher pdf

DOI for this version

Licence for this version

CC BY (Attribution)

Additional information

Versions of research works

Versions of Record

If this version is the version of record, it is the same as the published version available on the publisher's web site. Cite as the published version.

Author Accepted Manuscripts

If this document is identified as the Author Accepted Manuscript it is the version after peer review but before type setting, copy editing or publisher branding. Cite as Surname, Initial. (Year) 'Title of article'. To be published in *Title of Journal* , Volume and issue numbers [peer-reviewed accepted version]. Available at: DOI or URL (Accessed: date).

Enquiries

If you have questions about this document contact ResearchSupport@kent.ac.uk. Please include the URL of the record in KAR. If you believe that your, or a third party's rights have been compromised through this document please see our Take Down policy (available from https://www.kent.ac.uk/guides/kar-the-kent-academic-repository#policies).

The development of progesterone-loaded nanofibers using pressurized gyration: A novel approach to vaginal delivery for the prevention of pre-term birth

Francis Brako[a], Bahijja Tolulope Raimi-Abraham[b,1], Suntharavathanan Mahalingam[a], Duncan Q.M. Craig[b,*], Mohan Edirisinghe[a]

[a] Department of Mechanical Engineering, University College London, Torrington Place, London WC1E 7JE, UK
[b] University College London School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, UK

ARTICLE INFO

ABSTRACT

Recent evidence has continued to support the applicability of progesterone in preventing preterm birth, hence the development of an appropriate vaginal delivery system for this drug would be of considerable interest. Here, we describe the development of progesterone-loaded bioadhesive nanofibers using pressurized gyration for potential incorporation into a vaginal insert, with a particular view to addressing the challenges of incorporating a poorly water-soluble drug into a hydrophilic nanofiber carrier. Polyethylene oxide and carboxymethyl cellulose were chosen as polymers to develop the carrier systems, based on previous evidence of their yielding mucoadhesive nanofibers using the pressurized gyration technique. The fabrication parameters such as solvent system, initial drug loading and polymer composition were varied to facilitate optimisation of fiber structure and efficiency of drug incorporation. Such studies resulted in the formation of nanofibers with satisfactory surface appearance, diameters in the region of 400 nm and loading of up to 25% progesterone. Thermal and spectroscopic analyses indicated that the drug was incorporated in a nanocrystalline state. Release from the drug-loaded fibers indicated comparable rates of progesterone dissolution to that of Cyclogest, a commercially available progesterone pessary, allowing release over a period of hours. Overall, the study has shown that pressurized gyration may produce bioadhesive progesterone-loaded nanofibers which have satisfactory loading of a poorly water-soluble drug as well as having suitable structural and release properties. The technique is also capable of producing fibers at a yield commensurate with practical applicability, hence we believe that the approach shows considerable promise for the development of progesterone dosage forms for vaginal application.

1. Introduction

Preterm delivery, as defined by the WHO, refers to babies born alive before 37 weeks of pregnancy are completed; the associated sub-categories, according to gestational age, are extremely preterm (< 28 weeks), very preterm (28 – 32 weeks) and moderate to late preterm (32 – 37 weeks) (WHO, 2004, 2015). Common predetermining factors for this condition include multiple pregnancies, infections and chronic conditions such as hypertension and diabetes, although for a significant number of cases the cause is not known (Vogel et al., 2016). Preterm births occur among 5–18% of all live births around the world, with about 60% of all incidence recorded in low income countries, particularly in Asia and Africa, and is a major contributing factor to neonatal mortality and morbidity as well as long-term adverse outcomes on health and quality of life. For example, a meta-analysis conducted for the Global Burden of Disease Study 2010 data attributed 77 million (3.1%) disability-adjusted life years to preterm birth, an estimate comparable to burden of HIV or malaria (3.3% each) (Murray et al., 2012; Lee et al., 2013; Romero et al., 2014). This condition is therefore a global health challenge and requires the development of effective interventions to mitigate its impact, especially in low income countries where the most damaging effects are seen (Beck et al., 2010).

Progesterone, an endogenous steroidal hormone crucial for human reproduction can be administered orally as a solid dosage form, parentally (subcutaneous and intramuscular as oil-based injections) or topically (as a pessary, cream or vaginal gel) for a variety of indications

(Brayfield, 2017). In the 1980s and 90s, progesterone was widely used in the prevention of preterm labour until safety concerns halted its use (Fuchs et al., 2014). Promising results from recent randomised controlled clinical trials have however, revived interest in the use of progesterone for the prevention of preterm labour. Indeed, a Cochrane Database of Systematic Review study concluded that progesterone significantly reduced the incidence of preterm labour, especially for women with history of spontaneous preterm birth (Dodd et al., 2008).

Given the necessity of finding new and effective means of preventing preterm labour, there is a clear need to develop a wider range of progesterone based formulations, particularly in order to overcome some of the limitations of the currently available products. In terms of vaginal delivery, existing approaches have been hindered by poor retention and intermittent leakages, often leading to reduced residence time and ultimately poor bioavailability (Neves et al., 2014). A number of approaches have been used to deliver drugs topically to the vagina, including inserts such as vaginal rings which may overcome the issue of retention while allowing controlled release. Bioadhesive monolith systems may also be used whereby the drug is incorporated into an insert which allows prolonged contact with the vaginal mucosa (Neves et al., 2014). Medicated tampon devices have also been explored as microbicide delivery systems for prevention of HIV transmission (Ball et al., 2012) The approach described in this study is to develop a bioadhesive nanofibrous system which could be processed into suitable dosage forms (particularly, we suggest, as a medicated tampon) for vaginal drug delivery. We believe that such an approach holds several potential benefits including ease and convenience of use, prolonged retention and reduced leakage due to the bioadhesive nature of the carrier polymer and prolonged release due to gel formation of the polymer which provides a diffusion barrier to release. Such a system would potentially allow release over a period of hours which may potentially optimise the treatment regimen and allow daily use for women deemed to be at high risk of early labour.

Nanofibrous approaches are attracting increasing interest within the pharmaceutical arena, particularly as the issue of poor yield is now becoming more effectively addressed, thereby rendering large scale production feasible. Pressurised gyration (PG) is a recently developed processing method which involves simultaneous centrifugal spinning and solution blowing to produce polymeric nano- and microfibers (Mahalingam and Edirisinghe, 2013). The laboratory-scale PG equipment consists of a cylindrical aluminium vessel of approximately 60 mm in cross-sectional diameter and 35 mm in height, capable of rotational speed of up to 36,000 rpm. The fibers are rapidly extruded as a polymeric solution under conditions of high rotation and enhanced pressure via a gas input through a series of holes with diameter of 0.5 mm, resulting in rapid evaporation of the solvent and collection of the resulting nano- or microfibers on a surround plate (for more details see Mahalingam and Edirisinghe, 2013). PG has successfully been used to manufacture nanofibers for a variety of pharmaceutical applications including the generation of amorphous drug dispersions (Raimi-Abraham et al., 2014) and microbubbles (Mahalingam et al., 2014).

PG represents a promising development for large scale production; while electrospinning remains the most widespread method of producing fibers (Eatemadi et al., 2016), the limitations of scale-up have restricted the use of this approach within the pharmaceutical arena. PG has advantages of simplicity, low cost, high yield and versatility, having already been used for the production of a range of materials from ceramics (Mahalingam et al., 2015) to shape memory 3D mats (Wu et al., 2017). Here we further develop the technique for pharmaceutical applications, with a particularly view to addressing the persistent question of how hydrophobic drugs may be incorporated into hydrophilic polymers. In our previous work, we demonstrated the possibility of generating nanofibers with suitable mucoadhesive properties from a range of polymers using PG (Brako et al., 2015). These included bioadhesive polysaccharides (sodium alginate) and synthetic or semi-synthetic polymers (polyacrylic acid and carboxymethyl cellulose

(CMC)), using a spinning agent polyethylene oxide (PEO) to elicit sufficient chain entanglement in the liquid state so as to ultimately allow for fiber formation. The study discussed here takes the work described above into the arena of drug-loaded systems in that it explores the possibility of incorporating progesterone into nanofibers as a potential precursor for a bioadhesive vaginal drug delivery system. More specifically, the aim of the study was to explore the manufacturing conditions (solvent choice, material composition and drug loading) for the production of drug-loaded PEO fibers so as to develop an optimised prototype for bioadhesive delivery, with associated inherent advantages of high surface area for both adhesion and release. PEO and CMC blend yielded fibers with optimal prospects for mucoadhesion and was therefore chosen for drug loading and compared with PEO in this study. Given the hydrophobicity of progesterone, a particular challenge and objective was to examine the incorporation of the drug into the water-miscible polymeric carrier systems. However, if it were possible to successfully incorporate the drug into such polymers the possibility of then using the product as the basis for a delivery system then becomes feasible. Our aim was therefore to examine the manufacture, drug incorporation, characterization and release characteristics of progesterone-loaded nanofibers using a blend of bioadhesive polymers.

2. Materials and methods

2.1. Materials

Sodium carboxymethylcellulose (CMC) (Mw ~ 250,000 g mol^{-1}), poly (ethylene oxide) (PEO) (Mw ~ 200,000 g mol-1), progesterone (Mw ~ 314 g mol^{-1}, aqueous solubility: 8.81 mg/L (at 25 °C), log P: 3.87) and ethanol were obtained from Sigma-Aldrich UK. All were used without further purification. Purified distilled water and ethanol mixtures (as specified) were used as solvents throughout the study. Simulated vaginal fluid (pH = 4.7), prepared according to a formula developed by Owen and Katz (1999); contained sodium chloride (3.51 g/L), potassium hydroxide (1.40 g/L), calcium hydroxide (0.222 g/L) and bovine serum albumin (BSA) (0.018 g/L). Other components were acetic acid (1.00 g/L), lactic acid (2.00 g/L), glucose (5.0 g/L), urea (0.4 g/L), glycerol (0.16 g/L) and porcine mucin type II (1.5% w/v). All of these were also obtained from Sigma-Aldrich. Cellulose acetate membranes of pore size 0.2 μm were used for permeation studies; these were obtained from Sartorius, Gottingen, Germany. Progesterone released from fibers were compared to Cyclogest® 200 mg, a vaginal pessary formulation (weighing 1.825 g) obtained from Actavis, Barnstaple, UK.

2.2. Methods

2.2.1. Preparation and characterisation of polymer solutions

Uniform mixtures of PEO, blends with CMC and progesterone were obtained by continuous magnetic stirring followed by sonication for 10 min using a Branson Sonifier 250 (Danbury, Connecticut, USA). Specifically, water and ethanol (equal part mixture) was added to premixed powders of progesterone and polymers in quantities shown in Table 2. The viscosities of the resulting polymer-drug solutions were measured using Brookfield DV-111 rotational viscometer (Harlow, UK) with spindle size 18 at a shear stress of 3.5 Pa. Surface tension was measured using Kruss K9 tensiometer (Hamburg, Germany). All experiments were conducted at 25 °C and five separate measurements were taken and their means calculated. Equipment were calibrated prior to measurements. The viscometer was calibrated automatically by running a complete cycle without the spindle in place while the tensiometer was calibrated by taking the surface tension of distilled water at room temperature, comparing with literature values and adjusting accordingly.

Table 1
Percentage drug loading in fibers produced using water and ethanol mixtures in different ratios, 15% w/w PEO and 1% w/w progesterone. Drug content refers to loading in fibers following production.

Sample	Solvent system (% w/w) Ethanol	Water	Drug content (% w/w)
1	10	90	0.5
2	20	80	2.0
3	30	70	3.0
4	40	60	4.5
5	50	50	7.7

2.2.2. Fiber preparation and characterisation

Fibers were produced by pressurised gyration at ambient temperature (25 °C) using a method described previously (Mahalingam and Edirisinghe, 2013). Briefly, 3 ml of drug-polymer mixture prepared using 1:1 mixture of water and ethanol (selected based on data shown in Table 1) was placed in the aluminium vessel and spun at a rotational speed of 24,000 rpm and a working pressure of 0.15 MPa to produce progesterone-loaded fibers. The morphology and size of fibers were studied using scanning electron microscopy, while their material compositions were analysed using thermal and spectroscopic techniques. Details of various techniques used in further characterisation studies are described below.

2.2.3. Drug loading

The progesterone content within the fibers generated was determined following the standard assay procedure of dissolution in ethanol for detection by UV at 241 nm (NCBI, 2004). UV analysis was chosen as PEO has no active UV chromophores and hence minimal interference with assay of progesterone was anticipated. 1 mg of fibers were dissolved in 10 ml of absolute ethanol in a volumetric flask. The flask was swirled gently over a period of 24 h to ensure complete removal of progesterone from fibers into the ethanol. 3 ml of resultant solution was drawn and analysed spectrophotometrically at 241 nm using a Jenway 6305 UV/Visible spectrophotometer (Bibby Scientific, Staffordshire, UK). The% loading was calculated by expressing the drug content in a given weight of fiber as a percentage.

2.2.4. Hot stage microscopy

Hot-Stage Microscopy (HSM) studies were conducted on a Leica DM 2700 M microscope (Leica Microsystems, Wetzlar, Germany) fitted with Infinity 2 digital camera (Lumenera Corporation, Ottawa, Canada). The unit was used to visually observe the melting of fibers on a FP5/FP52 heating stage unit controlled by FP90 central processor unit, both from Mettler-Toledo Ltd, Leicester, UK. Fibers containing progesterone were heated from 50 to 130 °C at a rate of 2 °C/min while unloaded PEO fibers were heated from 50 to 70 °C at the same rate.

2.2.5. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

The chemical composition of the fibers was investigated using Attenuated Total Reflection Fourier Transform Infrared spectroscopy

(ATR–FTIR) measurements (Bruker Vertex 90 spectrometer), and spectrographs were interpreted using OPUS Viewer version 6.5 software. Each sample had 32 scans at a resolution of 4 cm^{-1}. The same parameters were used for background scans before each sample was analysed. The CMC spectra partially overlapped with the PEO and progesterone and is present as a minority component in the fibers, thereby considerably complicating analysis; consequently, the results presented here are for PEO and the active only.

2.2.6. X-ray powder diffraction

Structure and crystalline forms of the content of fibers were studied using D/Max-BR diffractometer (RigaKu, Tokyo, Japan) with Cu Kα radiation. Analyses were conducted at 40 mV and 30 mA over 2θ range of 5–60° at a rate of 2°/min. Data obtained were converted to diffractograms and evaluated using OriginPro 7.0 software (OriginLab Corporation, MA, USA).

2.2.7. Release studies

In vitro drug release studies were carried out using Franz diffusion cells with cellulose acetate membranes of pore size 0.2 μm previously immersed in simulated vaginal fluid (SVF) for 30 min. The basis for selecting this approach to the study of release is the high correlation that exists between drug permeation through a 0.2 μm acetate membrane and the mucosal membrane, demonstrated in a study investigating the use of artificial membranes as a rapid screening alternative to natural mucosa (Khdair et al., 2013). The membrane was mounted onto the diffusion cell with the help of a pair of tweezers after 3 ml of phosphate-buffered saline (PBS, pH 7.4) and a magnetic stirrer was placed in the receptor chamber to ensure adequate mixing of progesterone transported through the membrane into the buffer solution in the accepting chamber. Systems containing 25 mg of progesterone in 1 ml of SVF were placed in the donor chamber. The apparatus was kept at a constant temperature of 37 °C. The amount of drug released through the membrane at various predetermined time points were determined after drawing 1 ml aliquots from the receptor chamber and quantified using UV spectroscopy. Sample volumes were replaced with fresh PBS solution. The possibility of membrane deterioration and pore plugging after an extended period of use (Gekas and Hallström, 1990) was anticipated and hence measurements were performed over a period under 4 h to minimise the chances of this eventuality occurring. Results were compared to those obtained by replacing fibers with a sample of the progesterone pessary (Cyclogest) containing an equivalent amount of drug (25 mg); the temperature of the analysis allowed spreading of the material on the membrane.

3. Results and discussion

3.1. Processing and characterisation of progesterone-loaded nanofibers

Fiber formation by the PG process is generally influenced by a number of factors including solution properties (e.g. viscosity and surface), processing parameters (i.e. working pressure and rotational speed) and environmental parameters (i.e. temperature and humidity)

Table 2
Viscosity and surface tension of drug-polymer mixtures used in generating fibers.

Fiber composition (wt.%)			Viscosity (mPa s) ± SD	Surface tension (mNm^{-1}) ± SD
Polymer		Drug		
PEO	CMC	Progesterone		
15	0	1	4065 ± 3	47.8 ± 0.2
13.75	1.25	1	5284 ± 9	53.5 ± 0.5
15	0	5	7593 ± 13	54.0 ± 0.2
13.75	1.25	5	8220 ± 30	55.7 ± 0.4

(Mahalingam and Edirisinghe, 2013). In this study, production conditions such as solvent choice, polymeric materials used (single or blend of polymers) and drug loading were investigated to determine their influence on fiber outcome and drug encapsulation.

3.1.1. Choice of solvent system

As PG is a solvent-based method for manufacturing nanofibers, solvent choice is a key parameter for consideration. Progesterone is soluble in ethanol (up to 100 mg/ml) and virtually insoluble in water (< 1 mg/ml at 19 °C) while PEO and CMC are poorly soluble in ethanol but soluble in water. Solvent systems with varying proportions of ethanol and water were therefore investigated to select the type with highest possible drug loading while also producing satisfactory fibers. Specifically, solvent systems of ethanol and water containing 10, 20, 30, 40, and 50% of ethanol were used to prepare solutions containing a standard PEO and drug composition (15% w/w PEO and 1% w/w progesterone) from which nanofibers were generated. Drug loading in the dry fibers (as opposed to the initial solutions) was assessed for each composition and is shown in Table 1, noting that the theoretical maximum is 6.25% w/w.

As seen in Table 1, the amount of drug encapsulated in the fibers increased with ethanol content, reflecting the higher solubility of drug in such solvents; notably, the solvent system containing 50% ethanol resulted in fibers with considerably higher drug loading (the excess over maximum probably reflecting some polymer not forming fibers). Systems with more than 50% ethanol resulted in mixtures with undissolved polymer and were hence unsuitable for fiber formation. On the basis of these observations, a 1:1 mixture of water and ethanol was chosen for further investigations.

3.1.2. Solution properties

Various drug-polymer solutions (using 1:1 ethanol water as solvent) using either PEO only or PEO/CMC blends, with 1 or 5 wt% progesterone, were prepared and characterised prior to spinning in order to examine the influence of solution properties (viscosity and surface tension) as well as initial drug loading on the drug-loaded fiber formation. While a direct correlation between viscosity and fiber outcome has been established (high viscosity typically yielding fibers of greater diameter), surface tension typically determines the upper and lower boundaries of solution spinnability. Outside this range, fibers may be heavily beaded at high surface tension or may not form at all at the lower values (Mahalingam and Edirisinghe, 2013; Padron et al., 2013). In this work, all solutions were within a suitable surface tension range. A summary of the drug-polymer solutions from which fibers were generated and their properties is given in Table 2.

Both drug and polymer constituents influenced the solution properties, with changes in viscosity considerably more marked than those in surface tension. The inclusion of CMC, primarily to improve the bioadhesive properties of fibers (Brako et al., 2015), increased the viscosity of the systems; CMC is well known as a viscosity modifier, the extent being dependent on the degree of methyl substitution (DS) on the cellulosic backbone (Barba et al., 2002). More surprisingly, the increase from 1 to 5 wt% progesterone loading also had a significant effect on viscosity, with an increase > 40% noted for this parameter. Fiber images and size (diameter) distribution generated from drug-polymer solutions with 5 wt% progesterone initial loading are shown in Fig. 1.

In our previous study (Brako et al., 2015), whereby fibers were produced from similar polymer composition without progesterone, the average diameters for PEO and PEO/CMC blends were 172 and 194 nm respectively. Here, the same polymer constituents yielded fibers averaging 394 and 404 nm respectively, demonstrating the influence of progesterone inclusion on fiber production by PG. This observation is also in keeping with the increase in viscosity caused by the inclusion of the drug as noted in Table 2.

3.1.3. Drug entrapment in PEO and PEO/CMC systems

The effects of initial drug concentration on entrapment efficiency in progesterone-loaded fibers was examined. The solvent system chosen for this study allowed initial drug loading up to 5 wt%, beyond which the system did not produce satisfactory fibers. Progesterone content in fibers produced from either 1% or 5% wt. initial drug loading was determined by UV/Vis spectroscopy as described in Section 2.2.3. The results are shown in Fig. 2; in all cases the loading was close to the theoretical values of 6.25% and 25% w/w for the 1% and 5% w/w loading respectively. A further observation was that polymer composition did not appear to affect drug loading.

3.2. ATR-FTIR analysis

To further characterise the fibers and confirm drug entrapment, ATR-FTIR analyses were conducted on systems with an initial (solvent) loading of 5% progesterone and 15% PEO in order to confirm the composition of the product via characteristic functional groups. In addition, the approach is useful for identifying the possibility of drug-polymer interactions.

The ATR-FTIR spectra of PEO and progesterone is shown in Fig. 3. For the progesterone, peaks indicating carbonyl stretching bands at C_3 and C_{20} (characteristic of two ketone groups at C_3 and C_{20} which typically distinguishes progesterone from other steroids) within the molecule can be seen at 1661 and 1693 cm^{-1} respectively (Benoit et al., 1986) The characteristic peak at 843 cm^{-1} resulting from –CH$_2$–CO rocking/stretching in PEO is also shown (Frech and Huang, 1995; Li and Hsu, 1984). All characteristic peaks for both compounds can be seen in ATR-FTIR spectra of progesterone loaded PEO fibers (Fig. 3). Identifying characteristic bands confirms the residual material seen beyond the melting point of PEO from the HSM as progesterone. No evidence was seen of polymer-drug interactions via shifts in characteristic peaks, nor was evidence found for amorphous drug via broadening of peaks.

3.3. Microscopic assessment of drug incorporation

When observed using optical microscopy, the fibers could be visualised as bundles rather than individual fibers. The crystalline properties of the drug within the polymeric carrier was investigated by examination of such fiber bundles using hot stage microscopy. PEO is a semi-crystalline polymer which has a melting temperature of ~60 °C whilst progesterone has a melting temperature of ~120 °C. The difference in melting points makes possible the visualisation of how various components of drug-loaded fibers will react to temperature elevation in terms of differential temperatures of melting, thereby potentially allowing visualisation of the drug crystals released from the fibers above the melting point of the latter.

Hot stage microscopy (HSM) studies were conducted on PEO and PEO-progesterone fibers to visualise any changes that occurred on heating. PEO (Fig. 4a, stages 1–4) and progesterone loaded PEO (Fig. 4b, stages 1–4) fibers were heated from 50 to 95 °C. HSM studies confirmed that PEO fibers melted at ~ 65 °C and completely without a trace of any other component at 95 °C (stage 4). In contrast, Fig. 4b stage 4 (i.e. 95 °C), shows the presence of particles (or more specifically particle aggregates, as confirmed by SEM (data not shown)) which we ascribe to progesterone crystals as they are visible above the melting point of PEO. This in turn indicates that the progesterone is incorporated as crystals rather than as a molecular dispersion within the fibers. Further analysis by X-ray diffraction (Section 3.4 below) was performed to confirm the crystalline structure and composition of the fibers.

3.4. X-ray diffraction (XRD) studies

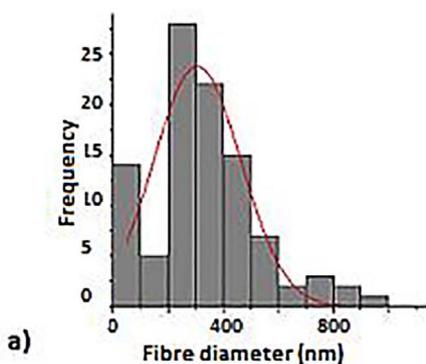XRD studies were conducted on the unloaded 15% PEO and 5% w/v

Fig. 1. SEM images and size distribution curve for a) PEO progesterone-loaded fibers (produced from solution containing 5% wt. progesterone and 15% wt. PEO), average size 349 nm with 22% dispersity and b) PEO/CMC progesterone-loaded (produced from solution containing 5% wt. progesterone, 13.75% wt. PEO and 1.25% wt. CMC), average size 404 nm with 15% dispersity.
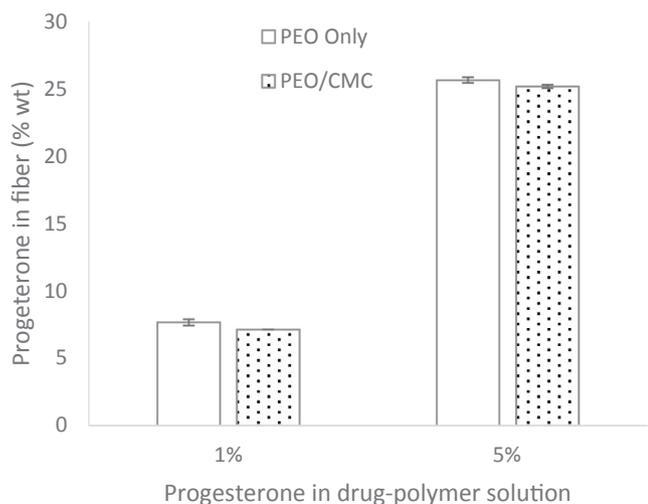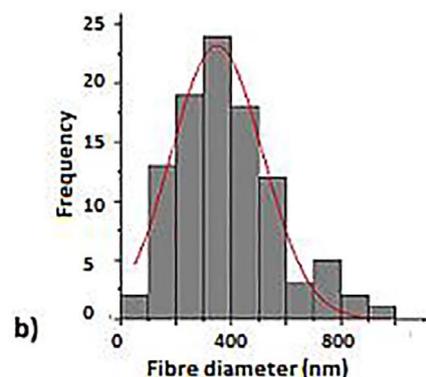

Fig. 2. Comparison of progesterone content in fibers produced from 15% wt. PEO (PEO only) or 13.75% wt. PEO and 1.25% wt. CMC (PEO/CMC) and with an initial drug loading of either 1% or 5% wt. progesterone.

Fig. 3. TR-FTIR spectra of PEO and progesterone in the 600–2600 cm^{-1} region showing characteristic peaks and that of drug-loaded PEO (with initial solvent loading of 5% progesterone) fibers confirming the presence of encapsulated progesterone.

progesterone loaded systems (% w/w referring to initial solvent loading) to confirm the solid state of encapsulated progesterone in the PEO fibers.

Several diffraction peaks are seen in both the PEO and progesterone XRPD patterns (Fig. 5). Peaks A and B at 22.4 and 18.7 2Θ degrees indicates crystallinity for PEO while peaks C and D at 12.7 and 16.9 confirms crystallinity in the progesterone powders used in this study (Oliveira et al., 2013; Sangawar and Bhagat, 2013).

The progesterone loaded PEO fiber (Fig. 5) shows all the characteristic peaks (A, B, C, and D) seen in each of the pure samples indicating that the progesterone was indeed encapsulated in the PEO in crystalline form. The halo diffraction pattern seen between 10 and 40
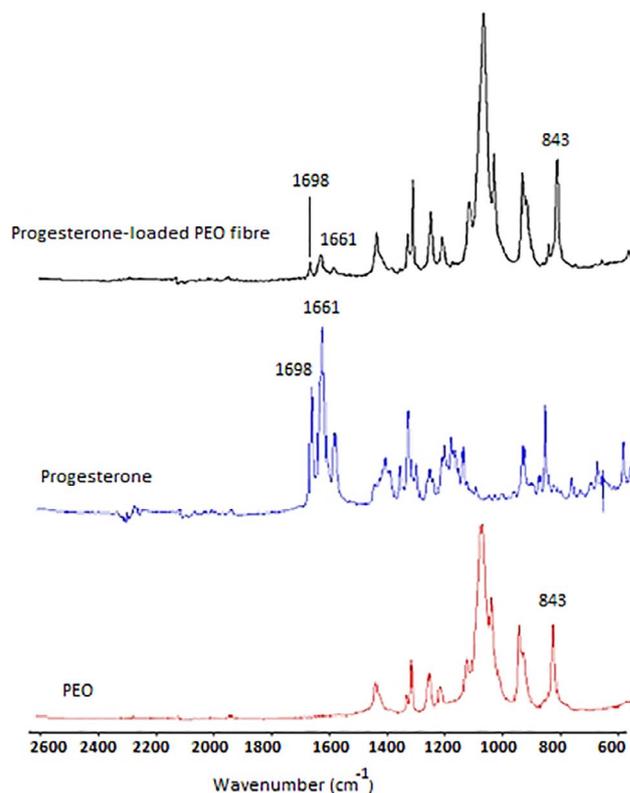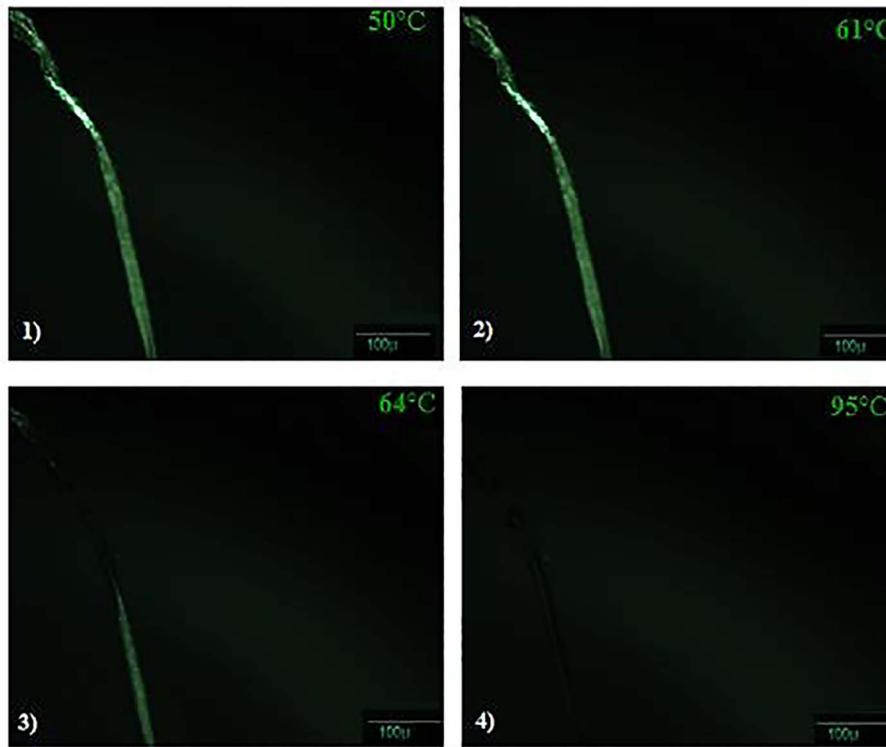
Fig. 4a. Melting of PEO fiber bundle without progesterone (stages 1–4 representing temperatures 50 °C, 61 °C, 64 °C and 95 °C) visualised by hot-stage microscopy. At stage 4, the PEO fiber had melted completely.
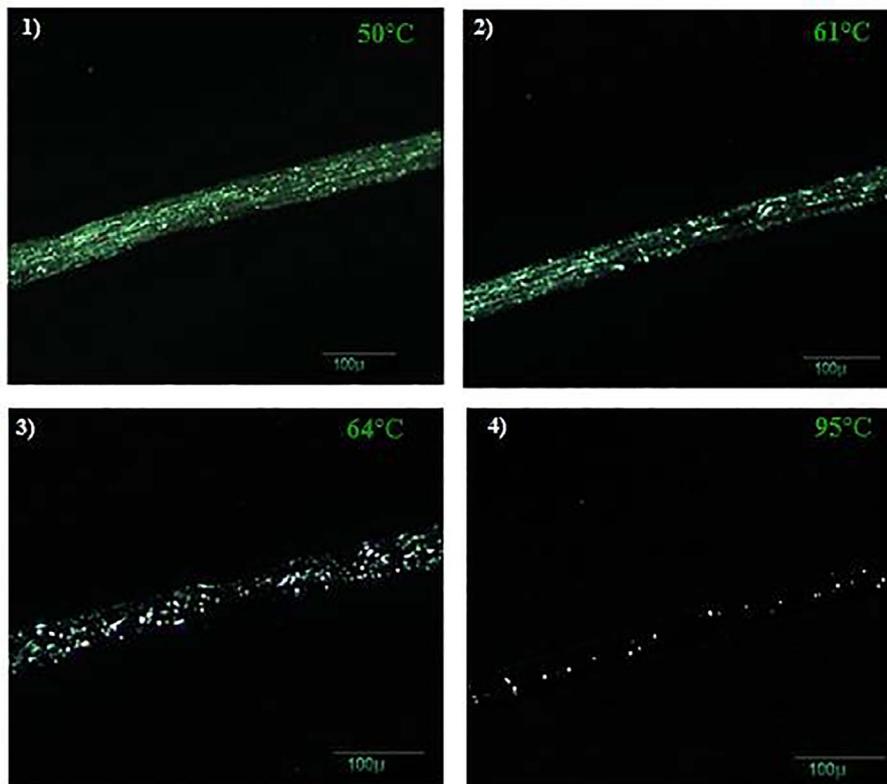

Fig. 4b. Melting of PEO fiber (with initial solvent loading of 5% Progesterone) (stages 1–4 representing temperatures 50 °C, 61 °C, 64 °C and 95 °C) visualised by hot-stage microscopy.
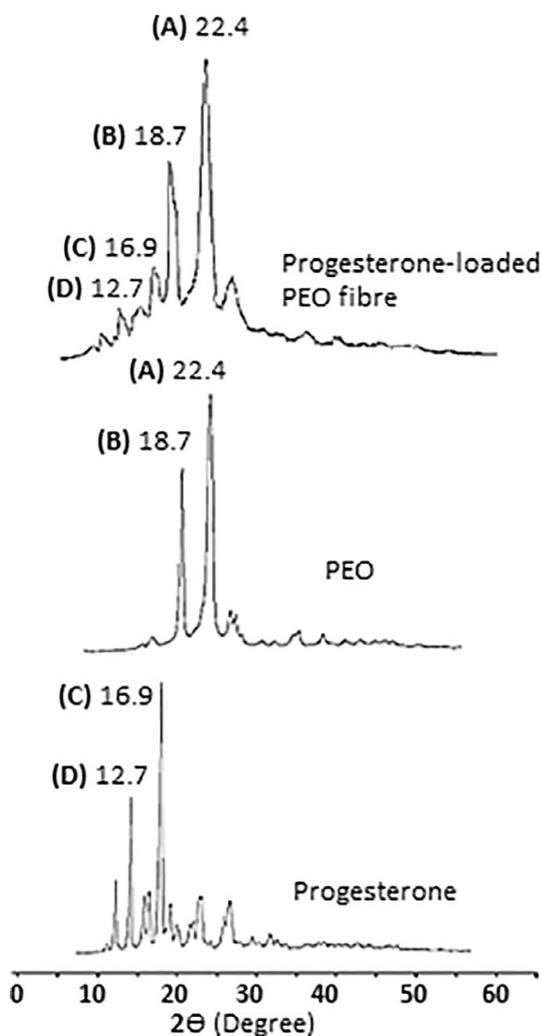
Fig. 5. X-ray diffractograms comparing the characteristic reflection peaks of their crystalline nature of PEO and progesterone powders to those in progesterone-loaded PEO fiber indicating the presence of crystalline drug.

2Θ in the drug loaded fibers is characteristic of amorphous material (Jain et al., 2008; Young, 2012) confirming a degree of amorphous characteristics in the drug-loaded fiber, most likely associated with the polymeric carrier.

3.5 In-vitro drug release studies

In vitro drug release studies were conducted on progesterone loaded PEO and PEO/CMC fibers (solvent loading 5% w/v active) and compared (under the same experimental conditions) with that of Cyclogest, a commercial pessary containing progesterone. The experimental approach was designed to observe progesterone release from nanofibers across an artificial membrane mimicking the mucosal environment of the vagina, particularly to ascertain whether the fibers showed release over a period of time commensurate with that required clinically (which would be a period of hours for daily administration).

As seen in the release profiles (Fig. 6), the release of the drug from the fibers and the commercial pessary were comparable although the release from the fibers was somewhat more rapid. Given the high surface area and hydrophilicity of the polymer this is to be expected, although the presence of the crystalline form of the drug and the propensity for gel formation in the microenvironment surrounding the hydrated nanofibers augers well for a release rate that is sufficiently slow as to provide day-long protection for the patient. The correlation coefficients from release profiles of nanofiber systems and Cyclogest when fitted in a zero-order model were 0.955, 0.979 and 0.893 for PEO, PEO/CMC and Cyclogest formulations respectively, indicating that over the time period under study the release profiles are effectively linear. Furthermore, it was observed that the inclusion of CMC had a limited effect on drug release in that some slowing of release was observed compared to the PEO alone.

3.6. Overarching comments

Taken together, the data show that the fibers may both incorporate progesterone and release the drug at a rate commensurate with a commercial product. Furthermore, the ability to now manufacture nanofibers at scale presents this approach to delivery as a viable addition to the current available approaches. While fiber-based vaginal delivery systems are being explored for interventions in other therapeutic areas such as infection management (Sharma et al., 2016) and the prevention of HIV transmission (Ball and Woodrow, 2014; Blakney et al., 2013; Krogstad and Woodrow, 2014), previous work on developing progesterone delivery systems for vaginal application have not thus far utilised drug-loaded fibers. Indeed, the development of new delivery systems for progesterone has been more focused on hormone replacement therapy and contraception (Friend, 2016). Considering the promise of fiber-based vaginal delivery systems in other therapeutic areas, there is a compelling argument for utilising the formulation approach for this particular drug and application. Indeed, our approach does present some tangible potential advantages, notably in the ability to combine mucoadhesion with a flexible delivery system which can reshape itself
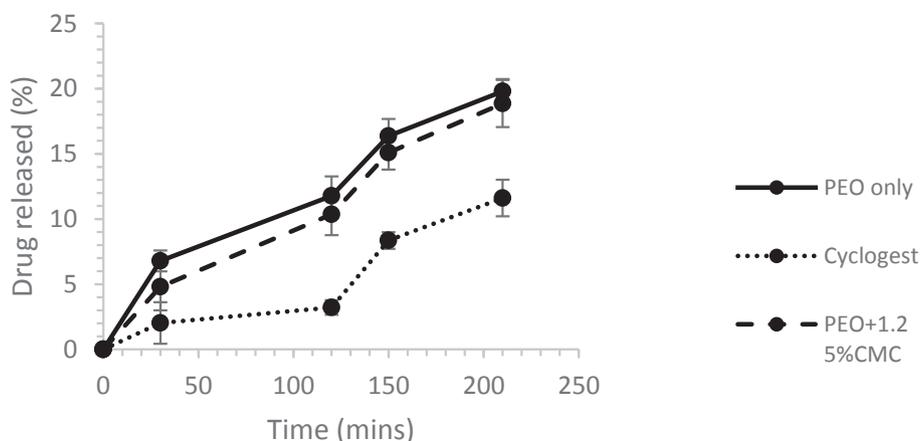

Fig. 6. Release profiles of progesterone loaded PEO and PEO/CMC fibers and Cyclogest in stimulated vaginal fluid.

to fit on to the vaginal wall while also allowing control of release via gel formation. In this particular study we have focused on the incorporation of the poorly water-soluble drug progesterone rather than the bioadhesion and swelling aspects; our previous work (Brako et al., 2015) has studied the adhesive properties of the fibers and we will shortly be reporting on a novel AFM-based technique to examine both swelling and adhesion. Here, we have demonstrated the incorporation of a relevant yet poorly water-soluble drug into hydrophilic nanofibers, the possibility of manufacture using the novel technique of pressurised gyration which indicates commercial production is feasible and finally the establishment of a release profile which is in line with a commercial formulation. Taken together, the study has shown that the nanofiber approach to vaginal delivery is a feasible methodology from a manufacturing viewpoint. Issues such as irritation and cytotoxicity would obviously need to be considered, although materials used for the manufacture of the fibers are widely used as food and pharmaceutical excipients as they are confirmed to be non-toxic and non-irritant (Rowe et al., 2006). Indeed, PEO and CMC are currently in use as excipients for vaginal formulations or on their own, for instance in managing vaginal dryness (das Neves and Bahia, 2006). We therefore suggest that fiber–based approaches to vaginal delivery have considerable potential, both therapeutically and commercially and, while the focus in the present case is pre-term birth, the possibilities for a range of conditions such as infection and inflammation is clear.

4. Conclusion

The poorly water-soluble drug progesterone has been successfully loaded in nanofibers generated by pressurised gyration. A solvent system composed of ethanol and water in equal parts enabled the formation of a drug-polymer solutions from which fibers were formed. Fibers produced had diameters from 40 to 1000 nm with average diameter dependent on amounts of polymer and progesterone therein. By varying production condition such as solvent system and initial drug loading, up to 25% wt. drug loading was achieved. Drug entrapment efficiency across batches with different polymer compositions were similar, though decreased in batches with higher amount of initial drug loading. Thermal and spectroscopic analyses confirmed that some of the entrapped remained in the crystalline state. The drug-loaded fibers showed more uniform release of higher amount of progesterone when compared to release Cyclogest, but nevertheless indicated release over a period of several hours. These drug-loaded fibers have demonstrated potential for efficient drug loading and prospects of optimal release kinetics. In addition, their surface properties and mucoadhesive prospects could be explored further for both prolonged localisation against the mucosal membrane and, potentially, enhanced trans-mucosal delivery aided by adhesion. The progesterone-loaded fibers produced and analysed have therefore proved to be promising materials for the delivery of progesterone to the vaginal mucosal membrane.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2018.01.043.

References

Ball, C., Krogstad, E., Chaowanachan, T., Woodrow, K.A., 2012. Drug-eluting fibers for hiv-1 inhibition and contraception. PLoS One 7, e49792.

Ball, C., Woodrow, K.A., 2014. Electrospun solid dispersions of maraviroc for rapid intravaginal preexposure prophylaxis of hiv. Antimicrob. Agents Chemother. 58, 4855–4865.

Barba, C., Montané, D., Farriol, X., Desbrières, J., Rinaudo, M., 2002. Synthesis and characterization of carboxymethylcelluloses from non-wood pulps II. Rheological behavior of cmc in aqueous solution. Cellulose 9, 327–335.

Beck, S., Wojdyla, D., Say, L., Betran, A.P., Merialdi, M., Requejo, J.H., Rubens, C., Menon, R., Van Look, P.F., 2010. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull. World Health Organ. 88, 31–38.

Benoit, J., Courteille, F., Thies, C., 1986. A physicochemical study of the morphology of progesterone-loaded poly (d, l-lactide) microspheres. Int. J. Pharm. 29, 95–102.

Blakney, A.K., Ball, C., Krogstad, E.A., Woodrow, K.A., 2013. Electrospun fibers for vaginal anti-hiv drug delivery. Antiviral Res. 100, S9–S16.

Brako, F., Raimi-Abraham, B., Mahalingam, S., Craig, D.Q., Edirisinghe, M., 2015. Making nanofibers of mucoadhesive polymer blends for vaginal therapies. Eur. Polym. J. 70, 186–196.

Brayfield, A. (ed.) 2017, Martindale: The complete drug reference. [Online] London: Pharmaceutical Press < https://www.medicinescomplete.com/mc/martindale/current/9098-x.htm > (accessed on 11/01/2018).

Das Neves, J., Bahia, M.F., 2006. Gels as vaginal drug delivery systems. Int. J. Pharm. 318, 1–14.

Dodd, J.M., Flenady, V.J., Cincotta, R., Crowther, C.A., 2008. Progesterone for the prevention of preterm birth: a systematic review. Obstet. Gynecol. 112, 127–134.

Eatemadi, A., Daraee, H., Zarghami, N., Melat Yar, H., Akbarzadeh, A., 2016. Nanofiber: synthesis and biomedical applications. Artif. Cells Nanomed. Biotechnol. 44, 111–121.

Frech, R., Huang, W., 1995. Conformational changes in diethylene glycol dimethyl ether and poly (ethylene oxide) induced by lithium ion complexation. Macromolecules 28, 1246–1251.

Friend, D.R., 2016. Development of controlled release systems over the past 50years in the area of contraception. J. Control. Release 240, 235–241.

Fuchs, F., Audibert, F., Senat, M.-V., 2014. Progesterone and preterm delivery: back to the future? Gynecol. Obstet. Fertil. 42, 112–122.

Gekas, V., Hallström, B., 1990. Proceedings of the symposium on membrane technologymicrofiltration membranes, cross-flow transport mechanisms and fouling studies. Desalination 77, 195–218.

Jain, S., Jain, A., Gupta, Y., Kharya, A., 2008. Design and development of a mucoadhesive buccal film bearing progesterone. Pharmazie 63, 129–135.

Khdair, A., Hamad, I., Al-Hussaini, M., Albayati, D., Alkhatib, H., Alkhalidi, B., 2013. In vitro artificial membrane-natural mucosa correlation of carvedilol buccal delivery. J. Drug Deliv. Sci. Technol. 23, 603–609.

Krogstad, E.A., Woodrow, K.A., 2014. Manufacturing scale-up of electrospun poly (vinyl alcohol) fibers containing tenofovir for vaginal drug delivery. Int. J. Pharm. 475, 282–291.

Lee, A.C.C., et al., 2013. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. Lancet Glob Health 1, e26–e36.

Li, X., Hsu, S., 1984. An analysis of the crystallization behavior of poly (ethylene oxide)/poly (methyl methacrylate) blends by spectroscopic and calorimetric techniques. J. Polym. Sci. Part B: Polym. Phys. 22, 1331–1342.

Mahalingam, S., Edirisinghe, M., 2013. Forming of polymer nanofibers by a pressurised gyration process. Macromol. Rapid Commun. 34, 1134–1139.

Mahalingam, S., Pierin, G., Colombo, P., Edirisinghe, M., 2015. Facile one-pot formation of ceramic fibers from preceramic polymers by pressurised gyration. Ceram. Int. 41, 6067–6073.

Mahalingam, S., Raimi-Abraham, B.T., Craig, D.Q., Edirisinghe, M., 2014. Formation of protein and protein–gold nanoparticle stabilized microbubbles by pressurized gyration. Langmuir 31, 659–666.

Murray, C.J.L., Vos, T., Lozano, R., Naghavi, M., Flaxman, A.D., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J.A., Abdalla, S., Aboyans, V., Abraham, J., Ackerman, I., Aggarwal, R., Ahn, S.Y., Ali, M.K., AlMazroa, M.A., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Bahalim, A.N., Barker-Collo, S., Barrero, L.H., Bartels, D.H., Basáñez, M.-G., Baxter, A., Bell, M.L., Benjamin, E.J., Bennett, D., Bernabé, E., Bhalla, K., Bhandari, B., Bikbov, B., Abdulhak, A.B., Birbeck, G., Black, J.A., Blencowe, H., Blore, J.D., Blyth, F., Bolliger, I., Bonaventure, A., Boufous, S., Bourne, R., Boussinesq, M., Braithwaite, T., Brayne, C., Bridgett, L., Brooker, S., Brooks, P., Brugha, T.S., Bryan-Hancock, C., Bucello, C., Buchbinder, R., Buckle, G., Budke, C.M., Burch, M., Burney, P., Burstein, R., Calabria, B., Campbell, B., Canter, C.E., Carabin, H., Carapetis, J., Carmona, L., Cella, C., Charlson, F., Chen, H., Cheng, A.T.-A., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahiya, M., Dahodwala, N., Damsere-Derry, J., Danaei, G., Davis, A., Leo, D.D., Degenhardt, L., Dellavalle, R., Delossantos, A., Denenberg, J., Derrett, S., Des Jarlais, D.C., Dharmaratne, S.D., Dherani, M., Diaz-Torne, C., Dolk, H., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Edmond, K., Elbaz, A., Ali, S.E., Erskine, H., Erwin, P.J., Espindola, P., Ewoigbokhan, S.E., Farzadfar, F., Feigin, V., Felson, D.T., Ferrari, A., Ferri, C.P., Fèvre, E.M., Finucane, M.M., Flaxman, S., Flood, L., Foreman, K., Forouzanfar, M.H., Fowkes, F.G.R., Fransen, M., Freeman, M.K., Gabbe, B.J., Gabriel, S.E., Gakidou, E., Ganatra, H.A., Garcia, B., Gaspari, F., Gillum, R.F., Gmel, G.,

Gonzalez-Medina, D., Gosselin, R., Grainger, R., Grant, B., Groeger, J., Guillemin, F., Gunnell, D., Gupta, R., Haagsma, J., Hagan, H., Halasa, Y.A., Hall, W., Haring, D., Haro, J.M., Harrison, J.E., Havmoeller, R., Hay, R.J., Higashi, H., Hill, C., Hoen, B., Hoffman, H., Hotez, P.J., Hoy, D., Huang, J.J., Ibeanusi, S.E., Jacobsen, K.H., James, S.L., Jarvis, D., Jasrasaria, R., Jayaraman, S., Johns, N., Jonas, J.B., Karthikeyan, G., Kassebaum, N., Kawakami, N., Keren, A., Khoo, J.-P., King, C.H., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Laden, F., Lalloo, R., Laslett, L.L., Lathlean, T., Leasher, J.L., Lee, Y.Y., Leigh, J., Levinson, D., Lim, S.S., Limb, E., Lin, J.K., Lipnick, M., Lipshultz, S.E., Liu, W., Loane, M., Ohno, S.L., Lyons, R., Mabweijano, J., MacIntyre, M.F., Malekzadeh, R., Mallinger, L., Manivannan, S., Marcenes, W., March, L., Margolis, D.J., Marks, G.B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B.M., McAnulty, J.H., McDermott, M.M., McGill, N., McGrath, J., Medina-Mora, M.E., Meltzer, M., Memish, Z.A., Mensah, G.A., Merriman, T.R., Meyer, A.-C., Miglioli, V., Miller, M., Miller, T.R., Mitchell, P.B., Mock, C., Mocumbi, A.O., Moffitt, T.E., Mokdad, A.A., Monasta, L., Montico, M., Moradi-Lakeh, M., Moran, A., Morawska, L., Mori, R., Murdoch, M.E., Mwaniki, M.K., Naidoo, K., Nair, M.N., Naldi, L., Narayan, K.M.V., Nelson, P.K., Nelson, R.G., Nevitt, M.C., Newton, C.R., Nolte, S., Norman, P., Norman, R., O'Donnell, M., O'Hanlon, S., Olives, C., Omer, S.B., Ortblad, K., Osborne, R., Ozgediz, D., Page, A., Pahari, B., Pandian, J.D., Rivero, A.P., Patten, S.B., Pearce, N., Padilla, R.P., Perez-Ruiz, F., Perico, N., Pesudovs, K., Phillips, D., Phillips, M.R., Pierce, K., Pion, S., Polanczyk, G.V., Polinder, S., Pope, C.A., Popova, S., Porrini, E., Pourmalek, F., Prince, M., Pullan, R.L., Ramaiah, K.D., Ranganathan, D., Razavi, H., Regan, M., Rehm, J.T., Rein, D.B., Remuzzi, G., Richardson, K., Rivara, F.P., Roberts, T., Robinson, C., De Leòn, F.R., Ronfani, L., Room, R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Saha, S., Sampson, U., Sanchez-Riera, L., Sanman, E., Schwebel, D.C., Scott, J.G., Segui-Gomez, M., Shahraz, S., Shepard, D.S., Shin, H., Shivakoti, R., Silberberg, D., Singh, D., Singh, G.M., Singh, J.A., Singleton, J., Sleet, D.A., Sliwa, K., Smith, E., Smith, J.L., Stapelberg, N.J.C., Steer, A., Steiner, T., Stolk, W.A., Stovner, L.J., Sudfeld, C., Syed, S., Tamburlini, G., Tavakkoli, M., Taylor, H.R., Taylor, J.A., Taylor, W.J., Thomas, B., Thomson, W.M., Thurston, G.D., Tleyjeh, I.M., Tonelli, M., Towbin, J.A., Truelsen, T., Tsilimbaris, M.K., Ubeda, C., Undurraga, E.A., van der Werf, M.J., van Os, J., Vavilala, M.S., Venketasubramanian, N., Wang, M., Wang, W., Watt, K., Weatherall, D.J., Weinstock, M.A., Weintraub, R., Weisskopf, M.G., Weissman, M.M., White, R.A., Whiteford, H., Wiebe, N., Wiersma, S.T., Wilkinson, J.D., Williams, H.C., Williams, S.R.M., Witt, E., Wolfe, F., Woolf, A.D., Wulf, S., Yeh, P.-H., Zaidi, A.K.M., Zheng, Z.-J., Zonies, D., Lopez, A.D., 2012. Disability-adjusted life years (days) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study 2010. Lancet 380, 2197–2223.

NCBI, 2004. Compound summary for progesterone. National Center for Biotechnology Information, Bethseda, Maryland.

Neves, J.D., Palmeira-de-Oliveira, R., Palmeira-de-Oliveira, A., Rodrigues, F., Sarmento, B., 2014. Vaginal Mucosa and Drug Delivery. John Wiley & Sons Ltd, New Jersey.

Oliveira, J.E., Medeiros, E.S., Cardozo, L., Voll, F., Madureira, E.H., Mattoso, L.H.C., Assis, O.B.G., 2013. Development of poly (lactic acid) nanostructured membranes for the controlled delivery of progesterone to livestock animals. Mater. Sci. Eng. C 33, 844–849.

Owen, D.H., Katz, D.F., 1999. A vaginal fluid simulant. Contraception 59, 91–95.

Padron, S., Fuentes, A., Caruntu, D., Lozano, K., 2013. Experimental study of nanofiber production through forcespinning. J. Appl. Phys. 113, 1–9 024318.

Raimi-Abraham, B.T., Mahalingam, S., Edirisinghe, M., Craig, D.Q.M., 2014. Generation of poly (n-vinylpyrrolidone) nanofibers using pressurised gyration. Mater. Sci. Eng. C 39, 168–176.

Romero, R., Dey, S.K., Fisher, S.J., 2014. Preterm labor: One syndrome, many causes. Science 345, 760–765.

Rowe, R.C., Sheskey, P.J., Weller, P.J., 2006. Handbook of Pharmaceutical Excipients. Pharmaceutical Press, London.

Sangawar, V.S., Bhagat, R.N., 2013. Synthesis and structural properties of polyethylene oxide complexed with cadmium sulfide. Int. J. Innov. Res. Sci. Eng. Technol. 2, 8.

Sharma, R., Garg, T., Goyal, A.K., Rath, G., 2016. Development, optimization and evaluation of polymeric electrospun nanofiber: a tool for local delivery of fluconazole for management of vaginal candidiasis. Artif. Cells Nanomed. Biotechnol. 44, 524–531.

Vogel, J.P., et al., 2016. Global, regional and national levels and trends of preterm birth rates for 1990 to 2014: Protocol for development of world health organization estimates. Reprod. Health 13, 76.

WHO, 2004. International Statistical Classification of Diseases and Related Health Problems. World Health Organization, Geneva.

WHO, 2015. Preterm Birth Fact Sheet. World Health Organisation, Geneva. [Online]. http://www.who.int/mediacentre/factsheets/fs363/en/.

Wu, X., Mahalingam, S., VanOosten, S.K., Wisdom, C., Tamerler, C., Edirisinghe, M., 2017. New generation of tunable bioactive shape memory mats integrated with genetically engineered proteins. Macromol. Biosci. 17.

Young, A.L., 2012. Xrd-powder x-ray diffraction and its application to biotherapeutic formulation development. Am. Pharmaceut. Rev. 15, 74.