Materials Science & Engineering C 94 (2019) 94–107

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Impact of styrene maleic anhydride (SMA) based hydrogel on rat fallopian tube as contraceptive implant with selective antimicrobial property

Bhuvaneshwaran Subramanian[a,b], Arun Prabhu Rameshbabu[a], Kuntal Ghosh[e], Pradeep K. Jha[a], Rakhi Jha[a], Selvakumar Murugesan[c], Santanu Chattopadhyay[d], Santanu Dhara[a], Keshab C. Mondal[e], Piyali Basak[b], Sujoy K. Guha[a,*]

[a] Indian Institute of Technology, School of Medical Science and Technology, Kharagpur 721302, India
[b] Jadavpur University, School of Bio-Science and Engineering, Kolkata, West Bengal 700098, India
[c] Department of Chemical Engineering, Ecole Polytechnique, 2900 boul Edouard Montpetit, Montreal, Quebec H3C 3A7, Canada
[d] Indian Institute of Technology, Rubber Technology Centre, Kharagpur 721302, India
[e] Department of Microbiology, Vidyasagar University, Midnapore 721102, West Bengal, India

ARTICLE INFO

Keywords:
Hydrogel
Styrene maleic anhydride (SMA)
Primary uterine cell lines
Selective antimicrobial

ABSTRACT

Development of non-hormonal female contraception is a need to combat against increasing population growth. The presently available short term or long term female contraceptives and sterilization methods have their own restrictions and side effects. With this objective, herein, we describe an innovative insight about the use of hydrogel formulation consisting of Styrene Maleic Anhydride (SMA) dissolved in Dimethyl Sulfoxide (DMSO) as non-hormonal fallopian tube contraceptive implant. Firstly, *in vitro* behavior of SMA hydrogel was evaluated by *in vitro* swelling and rheological properties to comprehend the polymeric hydrogel property post implantation inside the fallopian tube. Simulated Uterine Fluid (SUF) was used to simulate female reproductive tract environment in this study. Mechanical strength of the hydrogel when subjected to dynamic environment post implantation in the fallopian tube was estimated by the G′ values demonstrated. SMA hydrogel expressed selective antimicrobial activity against opportunistic pathogens (*Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) while having limited consequence over the growth of *Lactobacillus* spp. After confirmation of cytocompatibility against primary rat endometrial cell lines, the polymeric hydrogel was implanted inside the uterine horns of Sprague-Dawley rats. *In vivo* biocompatibility of the hydrogel was confirmed by histological and immunohistochemical evaluation of uterine tissue sections. Hematology, blood biochemistry and organ toxicity (kidney, liver, spleen, lungs and heart) also revealed biocompatibility of SMA hydrogel. The results of the current study indicated that the SMA copolymer dissolved in DMSO to form hydrogel has excellent biocompatibility for application as female contraceptive gel which can be implanted in the fallopian tube.

1. Introduction

It was reported in world population prospects: the 2008 revision that the world's population is increasing 80 million yearly and by 2050, it will reach 9–10 billion. Population explosion is the principal reason of environmental dilapidation and human suffering from poverty and starvation. Unintended pregnancy plays major role in population increase. This increased incidence of unintended pregnancy is owing to inadequate access to the presently available contraceptive methods. The presently available short term or long term female contraceptives and sterilization methods have their own restrictions and side effects. Non-hormonal female contraception has become more attractive method as it avoids the adverse connotations associated with the use of hormones used in hormonal contraceptives. The process of non-hormonal female contraception is accomplished through the intra-uterine contraceptive device (IUCD) over the past decades. However, there is a need for potentially superior techniques as the known IUDs causes trauma to the lining of the internal cavity of the uterus. This mechanical trauma triggers nerves and pain sensors and bleeding, which causes uterus to become more susceptible to various endogenous or exogenous infections. In order to prevent IUCD associated bacterial infections FDA approved antibiotics are generally used. However these antibiotics also kill beneficial *Lactobacillus* population inhabiting in the female reproductive tract. These adverse side effects limit acceptance of known

* Corresponding author.
E-mail address: skguhasmst@gmail.com (S.K. Guha).

https://doi.org/10.1016/j.msec.2018.09.023
Received 25 February 2018; Received in revised form 24 August 2018; Accepted 7 September 2018
Available online 08 September 2018
0928-4931/

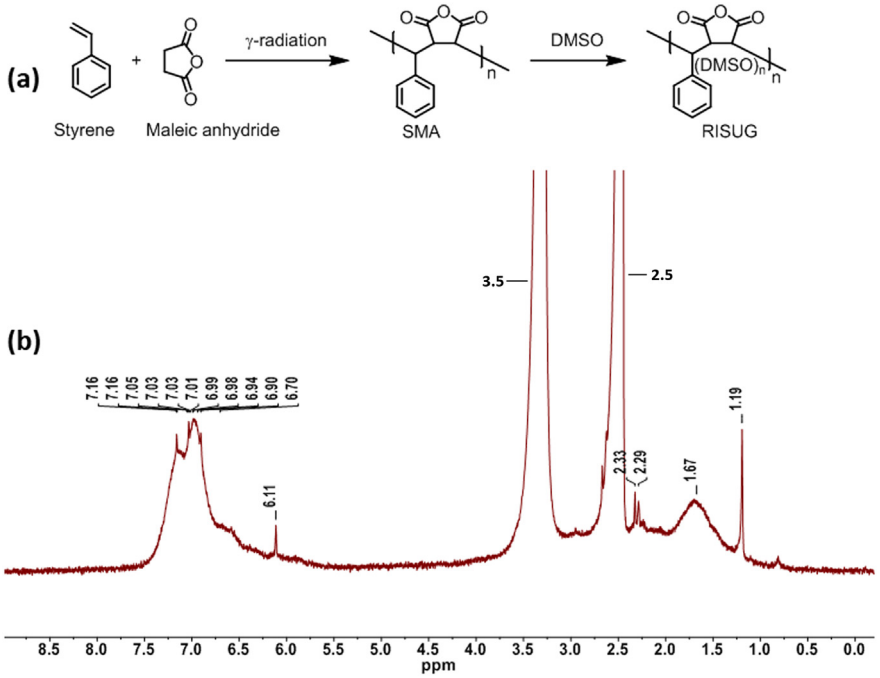

Fig. 1. (a) Schematic representation for the synthesis of SMA polymer.
(b) ^{1}H NMR spectra of SMA polymer in deuterated DMSO.

IUCDs by females in the reproductive age, and if accepted then often lead to the demand for early removal thereof.

RISUG® (Reversible Inhibition of Sperm Under Guidance) is a hydrogel made up with styrene maleic anhydride (SMA) and styrene maleic acid dissolved in DMSO is useful as vas deferens injected reversible male contraceptive [1]. This drug is presently under advanced phase III clinical trial in India [2]. The polymeric hydrogel RISUG® is not bio-inert material, but instead, the polymer has the property to reduce pH of the medium in its instantaneous vicinity. The polymeric hydrogel inactivates most of the sperm through damaging the acrosome by reducing the pH of the surrounding environment. The low pH not only damages the acrosome but also alter sperm morphology that results in poor motility due to which the fertilizing ability is lost in the sperm. The possibility of using RISUG® as a fallopian tube implant provides an opportunity to develop a novel and improved form of non-hormonal female contraceptive implant has not been explored yet. Besides providing contraception it may also act as an antibacterial agent which is considered as vital biological property for a synthetic bio-polymer [3–8].

The shape of the fallopian tube and the character of isthmic layers could restrict the use of RISUG® as human female contraceptive implant especially for the long term application. Interestingly RISUG® after deployment in the upper female reproductive tract will be converted to semisolid mass upon exposure with the reproductive tract fluid. The converted semisolid RISUG® should have sufficient structural strength to withstand under various shears which may vary from 0.1 s^{-1} to 100.0 s^{-1} due to gravity and capillary flow inside the female reproductive tract. These internal forces existing in the female reproductive tract and dilution of RISUG® due to the exposure with reproductive tract fluid will have significant direct effect on rheological properties of RISUG® [9–11].

Keeping these demands of rheological properties of RISUG® for application as female contraceptive gel, its rheological behavior has been evaluated. In order to mimic the fallopian tube environment, RISUG® was allowed to react with Simulated Uterine Fluid (SUF) in three different conditions based on proportion of RISUG® and SUF such as 1) RISUG® is higher than SUF (R_3U_1), 2) RISUG® and SUF are in equal proportion (R_1U_1), 3) RU is RISUG® only and 4) SUF is higher than RISUG® (R_1U_3) at 37 °C. All the rheological study was carried out in all four conditions. The descriptions of samples were given in Table S3. The present study also aims to identify the selective antibacterial profile of the test hydrogel RISUG® against opportunistic pathogens of the female reproductive tract for using as fallopian tube implantable hydrogel formulation which make RISUG® as a prime candidate for the development of the novel female contraceptive hydrogel. Furthermore, RISUG®, as an implantable hydrogel, should posses' significant biocompatibility without altering the biological properties of the female reproductive tract. Given the research hypothesis, *in vitro* toxicological profile of RISUG® was characterized using rat primary uterine cell lines. Investigation of *in vivo* toxicity of any biological implant is vital for its medical application. Despite the circumstance that fallopian tube of rat is extremely long-winded and significantly smaller when compared with that of human, the uterine horns of rat owns sole characteristics making it equivalent to the isthmic portion of human fallopian tube. The *in vivo* toxicity of RISUG® was investigated in uterine horns of female rat for 29 days post implanting the RISUG® hydrogel.

2. Materials and methods

2.1. Synthesis of RISUG®

The RISUG® used in this study was synthesized in our laboratory according to the method patented [12]. Styrene Maleic Anhydride (SMA) is prepared through gamma radiation polymerization. Styrene and maleic anhydride were taken in the ratio of 1:1 and dissolved in ethyl acetate ≥99% (monomer conc. 50% by wt.). The polymerization was carried after purging the nitrogen gas in to polymerization vessel in order to remove the dissolved oxygen. Samples were subjected to gamma irradiation (0.3 Gy/s at 37 °C with a total dosage of 2.4 Gy) in a Co-60 gamma radiation chamber. The co-polymers were precipitated and vacuum dried. The homopolymer of styrene was removed from precipitated SMA co-polymer by soxhlation using 1,2-Dichloroethane. Furthermore the homopolymers of polymaleic anhydride was removed from SMA co-polymer by washing in distilled water. Finally the SMA precipitate is dried, powdered and stored in stoppered sterile glass tubes inside vacuum desiccators. To obtain the final product RISUG®,