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In vitro evaluation of menstrual blood-derived endometrial stem cells for standard laboratory cell-based assays, an economic approach

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The standard *in vitro* laboratory cell-based assays are commonly performed using various cell lines of human or mammalian lineage that are often expensive for small scale research laboratories. Hence an alternative approach is needed. In this study we utilized the endometrial stem cells (MnSC) that were isolated from the menstrual waste blood samples. These MnSCs were further evaluated for *in vitro* cell-based assays. We focused on the toxicity profile evaluation of natural plant-based extracts and synthetic organic compounds such as drugs using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) dye in an MnSC-cell-bassed assay. Natural extracts from turmeric and grapes along with synthetic organic compounds (MA27) were evaluated in a 96-well plate for their cytotoxicity profiles. Our results indicate that the MnSCs behave similar to the other mammalian and/or human cell lines that are commonly used for the cell-based MTT assays. Thus, the MnSCs isolated from menstrual waste can be used for MTT assays with minimal cost. In future we plan to perform more assays focused on cell proliferation, apoptosis, etc.

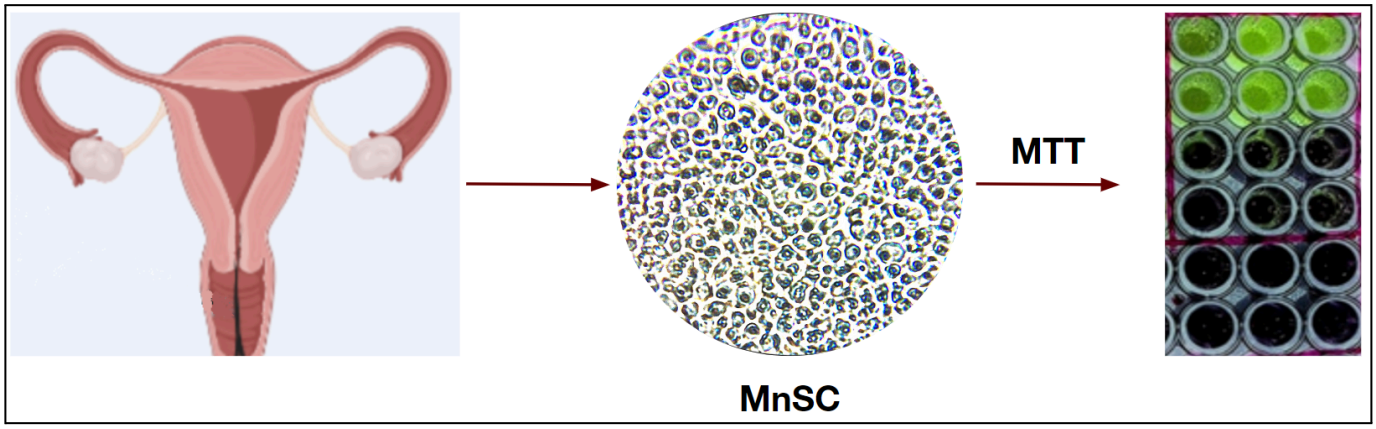

Figure 1. Endometrial stem cells were isolated from menstrual waste and purified cells were used for standard MTT assay.

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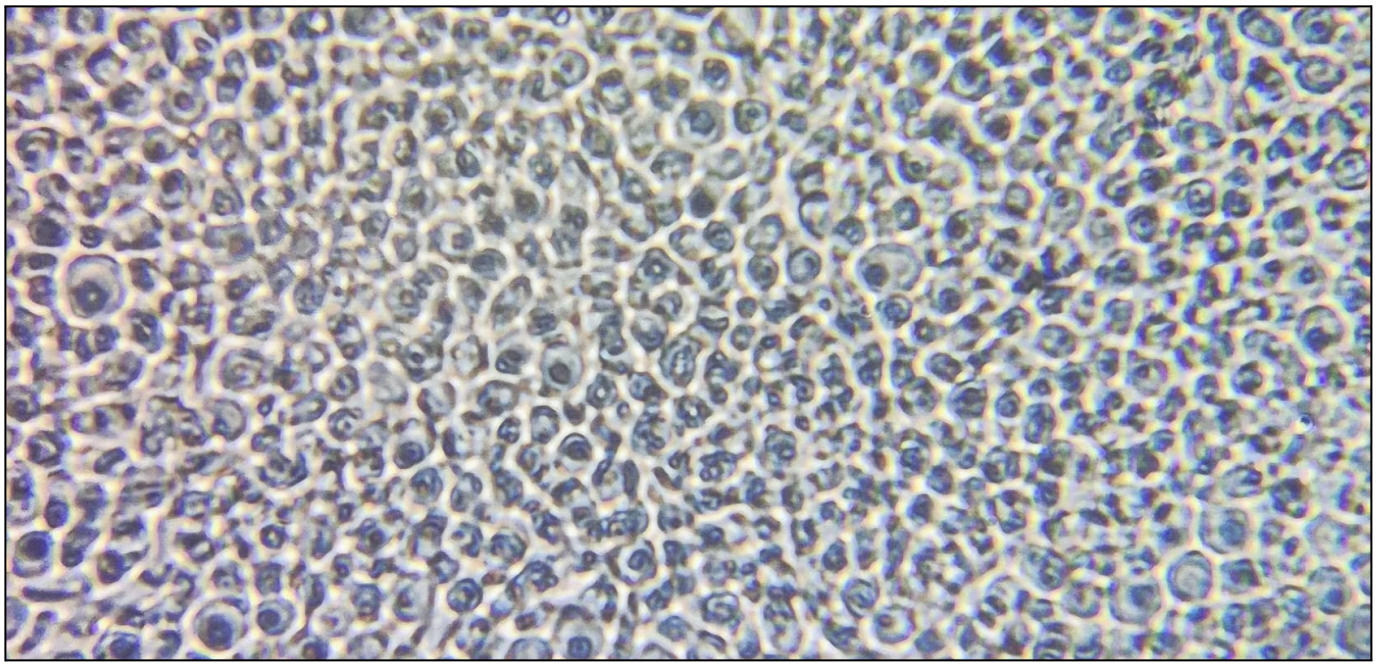

Figure 2. High resolution microscopic image (100X magnification) of menstrual endometrial stem cells purified from menstrual waste blood.

The most commonly used method for the determination of drug/natural product toxicity is the standard laboratory MTT (3-(4,5-di methyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay that utilizes the color change of the MTT dye in response to live and dead cells in the presence of the drug [1]. While the MTT dye can be readily purchased from multiple vendors for reasonable prices, one needs mammalian cells, preferably human cells that are very expensive [2]. Human cells can be purchased from certain vendors for cheaper prices but they often come with various contaminations such as the mycoplasmas, etc. [3]. Government and private funding can certainly help to some extent to acquire high quality human cells but their storage (liquid nitrogen tanks) is also a challenge for many research labs [4]. Many research labs often outsource the MTT assays to companies but the charge per assay is also not reasonably within the reach for many research labs as of today [5]. Hence the availability of high quality human cells for regular cell-based assays is still a much needed commodity for many research labs [6].

Women throughout the world throw away their menstrual blood considering it as a waste product [7]. Most of the women still use the sanitary pads in which the menstrual waste gets dried and separated from the liquid part that renders the cells useless [8]. However, recently women started using the menstrual cups that can easily collect the menstrual waste blood and keep it in the liquid form [9]. While the sanitary pads generate plastic waste that has to be properly disposed of, the menstrual cups are ecofriendly and reusable for a long time [10].

In this study we collected the menstrual waste blood from volunteers that are using menstrual cups along with appropriate consents. The waste blood was then used for extraction and purification of endometrial stem cells. Details of the process will be published elsewhere. The purified endometrial stem cells were characterized using the standard biomarkers (details will be published elsewhere). Briefly, modified Dulbecco's medium supplemented with 10% fetal bovine serum and antibiotic/antifungal solution was used for culture. The cells were counted and plated into a sterile 96-well plate @ 20,000 cells per well in a total volume of 200 µl per well. Cells were treated with drugs in triplicates and then MTT dye (HiMedia TC191). Dye was added to each of the wells containing cells according to the manufacturer's protocol.

As shown in Figure 3, the positive control (hydrogen peroxide) showed dead cells with bright yellow color of the MTT due to the lack of actively growing cells. Similarly, the negative control shows dark blue color MTT due to metabolically active and proliferating cells. In the case of the MA27 drug, higher concentrations were toxic and exhibit dull yellow color while the lower concentrations of MA27 were completely safe and comparable to the negative control. A panel of toxic substances will be evaluated in future to further validate these preliminary results.

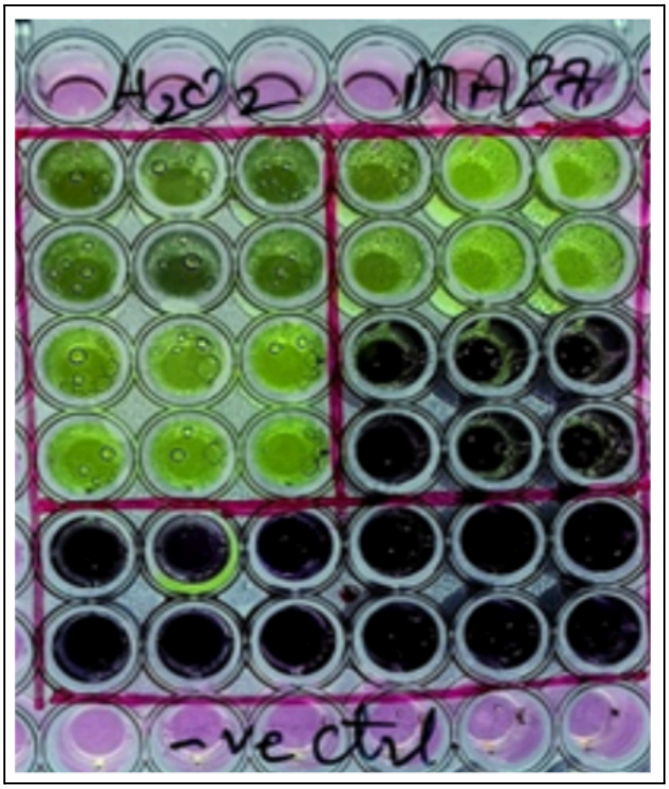

Figure 3. A 96-well plate showing MTT assay results.

In conclusion, the current methodology offers an excellent alternative to one of the most expensive cell-based assays that is routinely needed to be done in many research laboratories. Considering the highly economic source of the cells and their cooperativity in the MTT assay, the current method can be easily adopted into various research laboratories. Further, these cells can also be frozen easily and thawed whenever needed. However, freezing has its consequences on the recovery of the thawed cells like any other cell lines. The advantage is that a fresh batch of cells can be readily prepared as per the needs in the laboratory and hence repeated freeze-thaw cycles can be conveniently ignored. Along these lines, the cost involved in the cryopreservation and related consumables will be reduced and is highly advantageous for small scale research laboratories. One caveat is that a constant supply of the menstrual waste from women using menstrual cups is needed and additionally, they should be willing to consent and donate the menstrual waste. We believe that obtaining the menstrual waste from donors with their consent is ethical and one does not have to go through the complex ethical approvals. Support from the society is also highly valuable in this scenario without which, it may not be possible to obtain the menstrual waste. Awareness camps could help solve this problem.

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Conflict of interest: The authors declare no conflict of interest in this study.

Author contributions: C.G., C.V.M.G. and J.J. performed all the wet lab experiments. V.Y.P. and M.A. synthesized MA27. R.S.Y. is the principal investigator who designed the project, trained all co-authors, secured required material for the project, provided the laboratory space and facilities needed. R.S.Y. wrote, edited and finalized the manuscript. All authors approve the final manuscript.

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