Effect of dimethyl sulfoxide on *in vitro* proliferation of skin fibroblast cells

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Dimethyl sulfoxide is a preferred solvent for a wide range of compounds. It is used as a solvent of drugs for in-vitro toxicity testing using cell lines. Determination of safe solvent concentration for cell type to be used is a pre-requirement. Here we show that 0.1% DMSO in growth media is the safest concentration for skin fibroblast cell line used in this study. The low concentrations (0.01-0.001%) enhance proliferation of cells when cultured in-vitro on plastic surfaces for a 4-day period, whereas higher concentrations (0.5-3%) lead to reduction in cell-viability on a dose dependent manner. No cell survived beyond 3% DMSO.

Keywords: *in vitro* culture; DMSO; cell proliferation; skin fibroblasts; goat; cytotoxicity.

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Abbreviations: DMSO: Dimethyl sulfoxide; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal bovine serum.

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Introduction

Acetone, ethanol, dimethyl formamide, and dimethyl sulfoxide (DMSO) are organic solvents and are used to dissolve poor water soluble compounds. However, DMSO has become a preferred solvent due to its high boiling-point (189°C), relatively high freezing-point (18.5°C), and its miscible nature with water and wide range of other organic solvents. It is a colorless, polar solvent that dissolves both polar and non-polar compounds. It is considered as a super solvent for drug delivery, since it has no effect on drug binding. It is used as an effective paint stripper, cryoprotectant, differentiating agent, in polymerase chain reaction to prevent secondary structure formation, and in medicine as a topical analgesic for transdermal drug delivery [1]. Due to its ability to dissolve a wide range of compounds, DMSO is commonly used in sample management and high throughput drug screening using mammalian cell lines [2].

DMSO concentrations more than 1% have been shown to be toxic for most mammalian cell types when used for *in vitro* culture assays. Although, the exact toxic concentration is dependent on the cell type and its source, for example, 10% DMSO is non-toxic for lymphocytes whereas only 0.5% concentration is toxic for some cell types [3]. DMSO concentrations more than 0.1% have been shown to decrease cell proliferation which could be associated with cell cycle arrest, cell differentiation, and cell death [4].

Earlier studies show that lower concentrations of DMSO (0.05-0.2%) may stimulate proliferation in some cell types, for example, ovarian cancer cells [5]. Whether or not the lower concentrations affect proliferation of cell types, other than

cancer cells, is not adequately studied. For DMSO to be successful as an effective solvent, it should be compatible with growth media to obtain optimal benefit of an *in vitro* screening assay. Thus the inhibition or enhancement of growth, cytotoxicity testing, and to determine a safe concentration, study on each in-vitro cell model is essential including the skin fibroblast cells.

Fibroblast cells are present primarily in dermis layer of skin. They are responsible for generating connective tissue and allowing skin to recover during injuries. They are derived from mesenchymal stem cells within the body. In-vitro culture of these cells has multiple applications including bioengineering of skin to treat burn patients, to understand basic skin biology, to serve as feeder layers to help culturing stem cells, and for prescreening of drugs for toxicity testing using predominantly DMSO as a solvent. Here we report effect of various DMSO concentrations in growth media on in-vitro cell-viability and proliferation of goat skin fibroblast cells and discuss importance of optimal concentration to avoid any bias in drug toxicity screening programs in this cell model.

Material and methods

Cell culture

GSF3.2, a goat (*Capra hircus*) skin fibroblast cell line, previously established in the lab [6], was used to detect the effect of addition of DMSO to the growth media on *in vitro* cell-viability and culture proliferation. GSF3.2 cells stored in liquid nitrogen for 6 years at sixteenth passage (p16) level were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 50 units/ml of penicillin, and 50 µg/ml of streptomycin in 60 mm dishes for two passages before being used for experiments.

An aliquot of these actively growing cells was first cultured in chambered polystyrene slides (BD Falcon, Bedford, MA) overnight. Next day the cells were labeled with mouse monoclonal anti-vimentin-FITC antibodies (Abcam Inc., Cambridge, MA) to test for their true fibroblastic nature using immunofluorescence technique as per the manufacturer's protocol. Vimentin protein is expressed on fibroblast cells, and thus is a fibroblast specific marker. Every cell in our experiment was stained positive (figure 1, panel VMT) and therefore, suggested that these cells were pure fibroblastic cells.

DMSO treatment experiments

To test for the effect of DMSO, 50,000 cells from actively growing cultures were treated with 0, 0.001, 0.01, 0.1, 1, 3, 5, 7, and 9% of the DMSO (v/v) in 0.5 ml of DMEM media supplemented with 10% FBS. The treated cells were cultured in triplicate in a 24-well microtiter plate at 37°C, in a humidified 5% CO_2 incubator.

Cell viability assay

The growth morphology of the cells in respective treatments was captured in a TS100 inverted phase microscope after 4 days of incubation (figure 1). Subsequently the adhered cells were trypsinized and the number of live cells was counted by using the trypan blue dye exclusion assay [7] in a Countess II automatic cell counter (Thermo Fisher Scientific, Rochester, NY) following standard protocol described by manufacturer.

Statistical analysis

The mean and standard deviation of values in a category was calculated by using Microsoft Excel program.

Results and discussion

Mammalian skin is a tissue constantly exposed to environmental insults. Some of these insults cause diseases including skin cancers. Therefore, skin fibroblast cells were chosen in this study so that their *in vitro* culture can help screening treatments. There are also drugs that are applied topically on skin as a cream containing DMSO as a solvent. These cells, therefore, are choice

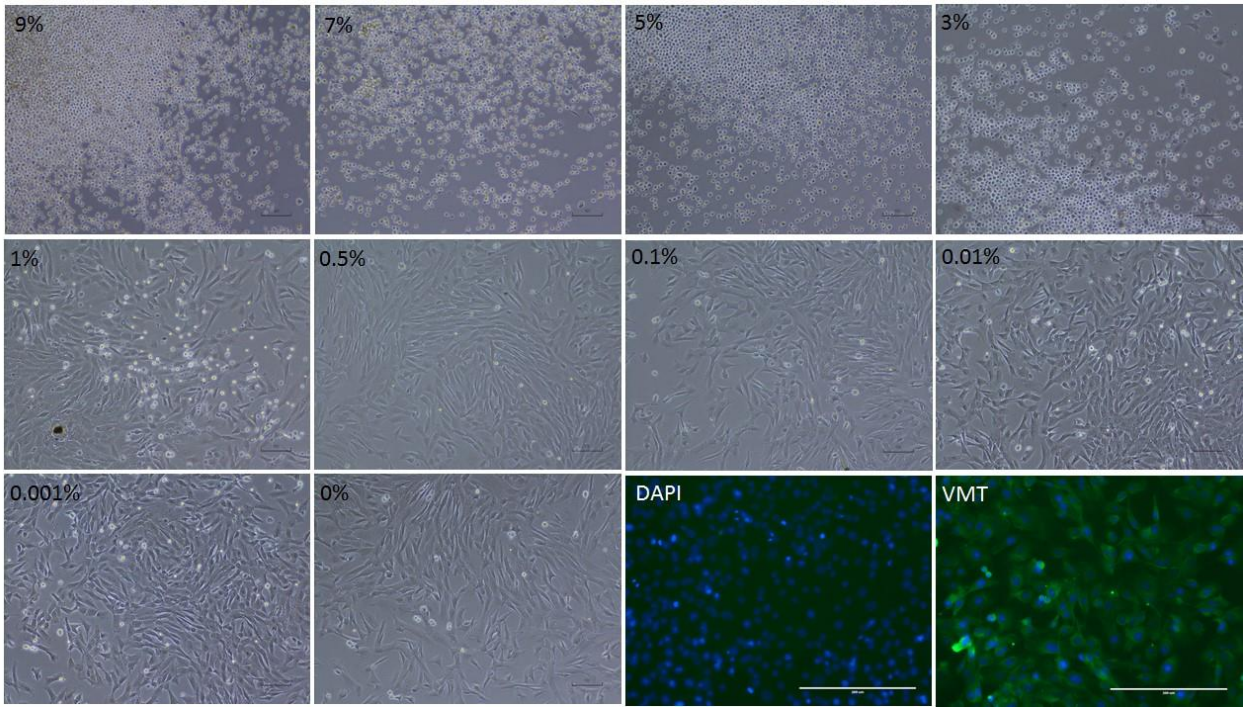

Figure 1. Morphology of cells after 4 days of *in vitro* culture in presence of different concentrations of DMSO. Images were captured in Nikon TS100 inverted phase microscope; magnification x100. Panel VMT shows staining for fibroblast specific marker antibodies (anti-vimentin-FITC) along with nuclear stain DAPI. Panel DAPI shows only nuclear staining and was without staining with anti-vimentin antibody which served as a negative antibody control.

targets for any drug screening protocol to treat human and veterinary subjects rather than other cell types. In addition, skin cells are easy to culture and the experimental tissue is easily accessible making them preferable. Skin fibroblast cells have been used earlier to study the protective effects of plant flavonoids on sun's ultraviolet-B light radiation damage in humans [8, 9]. Physiologically human and livestock skin cells are not much different and, therefore, any study undertaken in livestock fibroblast cells is translatable to human benefit. The aim of this study was to evaluate the effect of DMSO addition to growth media as a drug solvent on skin fibroblast cell's growth and proliferation when cultured *in vitro*. To study this phenomenon, we exposed cells to different concentrations of DMSO and evaluated their growth performance for a 4-day period. We observed that all cells died when cultured in the media containing DMSO concentrations beyond 3% (figure 1 and figure 2). Live cell count was found inversely correlated with increasing DMSO concentration from 0.5-3% (figure 2). It could be associated with cell cycle arrest, cell differentiation, or cell death as suggested previously [4]. Interestingly we observed an increase in number of live cells, as compared to untreated controls, when the cells were cultured in media containing DMSO concentrations below 0.1%. Maximum increase in the number of live cells was observed in 0.01% DMSO concentration (figure 2 and figure 3). Although, there was difference in the number of live cells in different passages, no difference was observed in overall trend of the effect on DMSO concentration on cell-viability and proliferation (figure 3) of cells from different passages. Low concentrations of DMSO have been shown to stimulate cell growth and *in vitro* transfection of human multiple myeloma cells [10]. It is possible that use of low concentration of DMSO in transdermal drug delivery creams may have positive effect on skin health. Low concentration

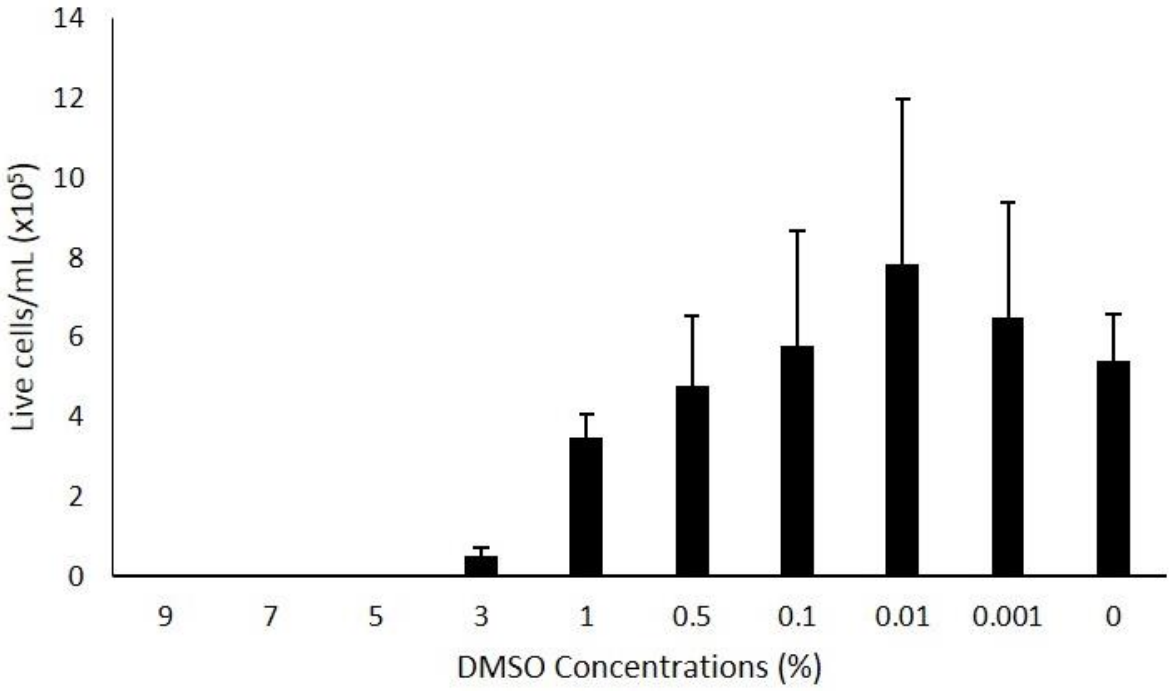

Figure 2. Effect of various DMSO concentrations on cell viability. Values represent mean ±SD of 6 independent experiments. Measurements were taken in the cells cultured on plastic dish surface for 4 days after treatment.

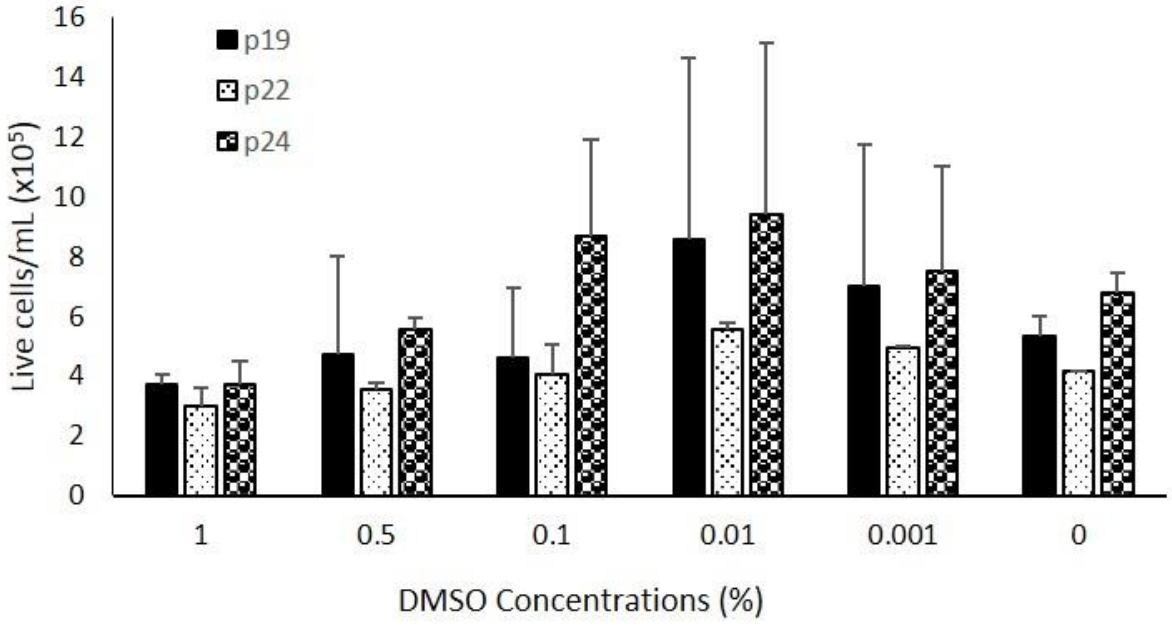

Figure 3. Effect of DMSO concentrations on viability of cell cultures from 3 different passages. Values are mean ±SD of 2 independent experiments for each passage.

of DMSO alone or in combination with UV radiation in sunlight may also lead to transformation of skin cells and/or skin cancer. However, it is not known at this time and could make an interesting study in future. Overall the use of *in vitro* cultured cell models is important to prevent use of live animals at the initial stage of drug screening, where thousands of compounds are screened, and thus reduce cost significantly in any drug developmental program.

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