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A PILOT STUDY TO ASSESS THE POTENTIAL OF PLATELET LYSATE AS AN ALTERNATIVE TO FETAL BOVINE SERUM

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ABSTRACT
The culture of stem cells requires the addition of fetal bovine serum (FBS) in the culture media as a source of the required growth factors. However, the use of animal-derived supplement in clinical trials is unsafe as there is the possibility of immunoreactions and internalization of animal proteins by the human mesenchymal stem cells. Hence, there is a need to find better alternatives to FBS which are of human origin in order to avoid xenogenic contamination. One such alternative is the platelet lysate which has been proved to be a better alternative to FBS in the culture of human mesenchymal stem cells derived from bone marrow. Thus this study aims at proving the efficiency of platelet lysate in the culture of mesenchymal stem cells from the human epidermis. The use of platelet lysate in the isolation of mesenchymal stem cells from human epidermis was successful and the isolated cells showed the properties of plate-adherence and fibroblast-like appearance which are characteristic of mesenchymal stem cells.

KEYWORDS: Mesenchymal Stem cells, Fetal Bovine Serum, Platelet lysate, human epidermis.

1. INTRODUCTION
The use of *in vitro* human cell culture for tissue engineering, (adult) stem cell technology, and cell-based therapy has gained importance during the last decade.[1,2,3] Traditionally, *ex vivo* expansion of human mesenchymal stem cells (hMSCs) has been performed using basal culture media plus supplements to provide growth factors (GFs), proteins and enzymes to support cell growth [4]. Fetal bovine serum (FBS) [or fetal calf serum (FCS)] is most commonly used to supplement hMSC cultures, because the fetal milieu is enriched with GFs and poor in antibodies. However, for clinical use it is important to substitute animal-derived products, since hMSCs can internalize xenogeneic proteins, and thus carry the risk of infection (*via* viral or prion agents) and immunoreaction; it has been reported that a single injection of 100 million hMSCs expanded in 20% FBS-supplemented media is associated with approximately 7-30 mg of calf serum proteins.[5] Additionally, there are concerns regarding FBS sample-to sample inconsistency, and animal welfare in terms of the '3 Rs' principle (replacement, reduction, refinement).[6,7] Because of the undefined composition of FBS, the risk of contaminations, animal welfare concerns regarding its harvest and production, along with the problems of limited availability and the cost factor, the switch to serum-free alternatives is promoted by regulatory authorities, industry, and the research community in general.[8,9,10] The search for alternatives to replace fetal bovine serum (FBS) has become a major goal in the field of cell and tissue culture. Platelets are known to be a rich source of growth factors,[11,12,13] thus suggesting platelet lysate as a valuable animal serum substitute. Platelet lysate was tested on mesenchymal stem cells (MSCs), concluding that the lysate was more efficient in terms of costs and proliferation rate than using exogenous recombinant growth factors and retained their immunosuppressive[14] as well as their differentiation capability.[15]

2. MATERIALS AND METHODS
2.1 Skin sample procurement and processing
Skin sample was cordially donated by department of plastic surgery after informed consent from the patient undergoing skin graft for foot ulcer. Briefly, the skin sample was washed thoroughly 2 to 3 times with normal saline followed by PBS- antibiotic wash and the samples were stored at 4°C until use.

2.2 Preparation of platelet releasate and complete growth medium
Platelet concentrate was obtained from a registered blood bank for the preparation of platelet releasate. Around 50ml of platelet concentrate (from AB+ blood group) suspended in plasma from single whole blood

donation was procured and kept at 27°C with gentle agitation until use. Platelet concentrate was added to the basal media (HiPer DMEM, HiMedia laboratories, India) at final concentration of 10%. Antibiotic-antimycotic (HiMedia laboratories, India) was added at final concentration of 1%. The media was then kept at room temperature for about 4 hours to clot physiologically by the action of calcium present in the media. Then the media was kept at 4°C overnight for clot maturation and retraction. Finally the media was warmed to 37°C for 1-2 hours before removing the clot. The complete media was then filtered with 0.22µ sterile filter (Whatmann, USA). Filtered media was then aliquoted and stored at -20°C for further use. FBS containing media was kept as control throughout the study.

2.3 Isolation of mesenchymal stem cells from epidermis

Stored skin graft was washed with PBS (HiMedia laboratories, India) and treated with Trypsin-EDTA (0.25%, HiMedia laboratories, India) with epidermis side facing downwards in a petri dish for about 30 minutes at 37°C. The action of trypsin-EDTA was stopped by adding complete growth medium for about 5 minutes. The epidermis was then peeled from dermis using sterile forceps. Remaining epidermal tissue was gently scraped using sterile scalpel blades. The epidermis was then mixed thoroughly using Pasteur pipette to dislodge the epidermal cells from basement membrane. The single cell suspension was then filtered via cell strainer (70µ, HiMedia laboratories, India), centrifuged at 1500rpm for 5 minutes and the pellet was re-suspended with complete growth media and seeded into a 75cm 2 flask (CorningInc, USA).

2.4 Expansion and storage of epidermal stem cells

After 48 hours of seeding, complete media change was done with fresh pre-warmed media. Thereafter media replenishment was done every 2-3 days until 80% confluency. Once confluent, cells were harvested using Trypsin - EDTA for about 30-45 seconds. Suspended cells were then incubated with complete media to inhibit Trypsin-EDTA activity for about 1 minute. Cells were thoroughly mixed and split at 1:4 ratio with same size flasks. All flasks were maintained as earlier until confluence till passage 2 and cryopreserved for further use. Cryopreservation was done using the following media composition: 85% FBS (HiMedia laboratories, India) 5% Human Serum Albumin (20%Albumin, Reliance life sciences, India) and 10% DMSO (Sigma Aldrich, USA).

3. RESULTS AND DISCUSSION

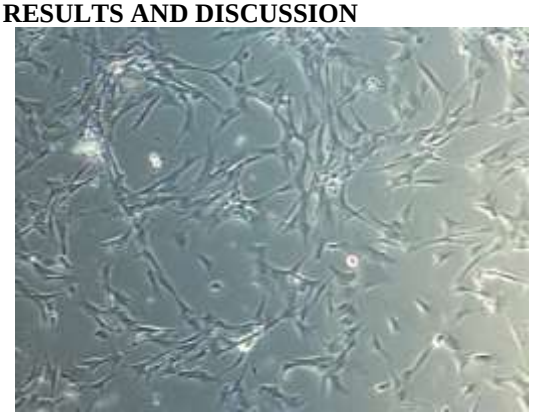

Figure 1: Control cells from human epidermis cultured in FBS-supplemented.

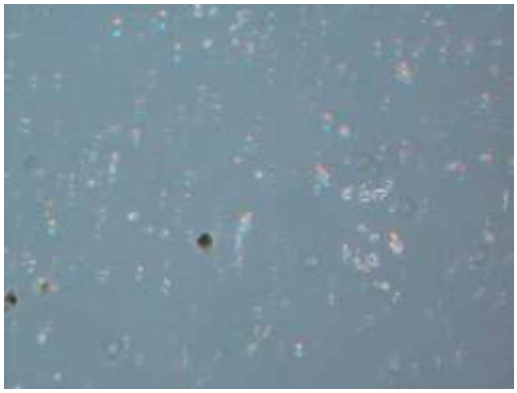

Figure 2: Primary culture cells from human epidermis.

Spherical, floating cells and plate-adherent, fibroblast-like cells was co-present from the first day of isolation and primary culture of cells. Most of the cells became plate-adherent after the third day of culture (Fig. 2). Homogenously plate-adherent, fibroblast-like, spindle shaped cells were obtained in the passage 1 & 2 around the end of the first and second week of the culture (Figs. 3,4). These cells were allowed for proliferation upto 3 weeks. The figures 3,4 show upto 80% confluent cells.

The isolated cells showed plate-adherence and fibroblast-like appearance which are characteristic of mesenchymal stem cells. The addition of 10% platelet lysate as supplement to the medium has promoted proliferation of the cells from the human epidermis. The cells had showed homogenous shape and reached a confluency of 80% at which point they were harvested. These cells were comparable to the control cells that were supplemented with 10% FBS and they showed excellent growth morphology. The results are in accordance with previous studies which support the use of 10% platelet lysate in the media over 5 & 20%.[16] The cultured cells showed good confluency in the presence of platelet lysate in the culture media and so supports studies previously done in the same aspect.

Figure 3: Passage 1 cells from human epidermis.

Figure 4: Passage 2 cells from human epidermis.

4. CONCLUSIONS

This study supports the Use of platelet lysate as a potential alternative to fetal bovine serum in the isolation of mesenchymal stem cells from human epidermis. Further study needs to be done to standardize the concentration of the platelet lysate for the isolation of mesenchymal stem cells from various other sources.

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