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Dietary Selenium Facilitates Skeletal Muscle Regeneration by Promoting Cell Proliferation

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ABSTRACT

Muscle stem cells (satellite cells) are the main contributor to muscle regeneration. Like most stem cells long-term expansion of muscle progenitors in vitro is troublesome. The in vivo muscle regeneration skills are quickly lost when culturing in vitro, that prevents the potential applications of muscle derived cells in cell-based therapies. Selenium is an integral a part of the enzyme antioxidant, that is an inhibitor enzyme that helps to regulate levels of hydrogen peroxide and lipid peroxides that are created throughout normal metabolic activity. Expanded muscle progenitors will replenish the endogenous stem cell pool and can repair multiple rounds of muscle injuries in vivo after a single transplantation. Proliferative ability was estimated by the count and proliferative activity of viable cells employing cell count and enzyme assay at completely different time points after and it has been identified that the proliferative effect of dietary source of selenium on Muscle progenitor cells is dose dependant.

Key words: *Muscle progenitor cells, Dietary Selenium – cells – in vitro- dose dependant*

INTRODUCTION

Stem cells have the potential to develop into a variety of different cell types in the body during early life and growth. In addition, they serve as a sort of internal repair system, dividing essentially without limit to replenish other cells if the person or animal is still alive. Stem cells are unique from other cell types by two important characteristics. First, they are unspecialized cells capable of renewing itself through cell division, sometimes after long periods of inactivity. Second, under certain physiologic or experimental conditions, they can be induced to become tissue- or organ-specific cells with special functions. Stem cells play a huge part in the body's healing process, and the introduction of stem cells has showed great promise in the treatment of many conditions. They undergo what is called asymmetric division, forming not one but two daughter cells: one cell often an exact replica of itself, a new stem cell with a relatively clean slate, and another stem cell that is ready to turn into a specific type of cell. This trait is known as self-renewal and allows stem cells to proliferate or reproduce rapidly[1].

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Stem cells are basically categorized into two types, embryonic stem cells and adult stem cells.

Embryonic stem cells are described as "pluripotent", meaning that they can generate almost all the different types of cells in the body. Embryonic stem cells are obtained from the blastocyst, a very early stage of development. At this stage, there are no organs nor specialised tissue, just an "inner cell mass" from which embryonic stem cells can be obtained. These cells can differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include every cell type in the adult body. While embryonic stem cells can generate all cell types in the body, adult stem cells are multipotent and can produce only a limited number of cell types[2]. Additionally, under defined conditions, embryonic stem cells can propagate themselves indefinitely. This allows embryonic stem cells to be employed as useful tools for both research and regenerative medicine, because they can produce limitless numbers of themselves for continued research or clinical use.

Adult stem cells are undifferentiated cells, found throughout the body after development, that multiply by cell division to replenish dying cells and regenerate damaged tissues[3]. Also known as somatic stem cells, they can be found in juvenile as well as adult animals and humans, unlike embryonic stem cells[3].Efforts are underway to stimulate these adult stem cells to regenerate missing cells within damaged tissues[4]. This approach will utilize the existing tissue organization and molecules to stimulate and guide the adult stem cells to correctly regenerate only the necessary cell types. Alternatively, the adult stem cells could be isolated from the tissue and grown outside of the body, in cultures[4]. This would allow the cells to be easily manipulated, although they are often relatively rare and difficult to grow in culture.

Mesenchymal stem cells (MSCs) are of stromal origin and may differentiate into a variety of tissues[3]. MSCs have been isolated from placenta, adipose tissue, lung, bone marrow and blood, Wharton's jelly from the umbilical cord, muscle and teeth. MSCs are attractive for clinical therapy due to their ability to differentiate, provide trophic support, and modulate innate immune response.

Skeletal muscle consists predominantly of syncytial fibres with peripheral, post mitotic myonuclei. Its intrinsic repair potential in adulthood relies on the persistence of a resident reserve population of undifferentiated mononuclear cells, termed satellite cells. In mature skeletal muscle, most satellite cells are quiescent and are activated in response to environmental cues such as injury to mediate postnatal muscle regeneration[5]. After division, satellite cell progeny, termed myoblasts, undergo terminal differentiation and become incorporated into muscle fibres[6].

Muscle satellite cells are located between the basement membrane and the sarcolemma of muscle fibres. Standardized protocols for the isolation and culture of satellite cells are key tools for understanding cell autonomous and extrinsic factors that regulate their performance. Knowledge gained from such studies can contribute important insights to developing strategies for the improvement of muscle repair following trauma and in muscle wasting disorders[7]. Muscle atrophy caused by disuse, malnutrition and diseases such as muscular dystrophies, diabetes, cancer and HIV infection, affects millions of patients. There is no effective cure for muscle atrophy caused by diseases thus far. Muscle progenitors are the major force that drives postnatal muscle injury repair[6].

Investigations of toxicity using existing models are always hindered by the physiochemical properties of dietary biomolecules in nano scales. Models for assessing toxicity of these dietary sources assililated in nano structures should ideally be designed such that the properties of the nanomaterial have a minimal influence on the testing system. The growing number of applications of nano scale dietary materials has increased the urgency of establishing a range of in vitro models that could allow researchers to assess the hazardous effects of

nanomaterials. Over the past several years, interest in the use of cell models for testing the safety of drugs and materials has led to improved outcomes. Systems that can be used as preclinical and clinical models during drug development have the ability to reduce the unknown toxic effects of nanomaterials. Currently, nanomaterial-induced responses are interpreted using cell-based (primary cell lines/immortalized cells) in vitro platforms. These platforms can be employed to assess benchmarking, IC50 values (50% inhibition concentration) and lethal concentrations for cytotoxicity and genotoxicity. Sublethal concentrations are thought to be appropriate for mechanistic endpoints but not for cytotoxicity and genotoxicity assays. A general approach is to assess the toxicity of nanoparticles by measuring cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay principally determines the number of viable cells by measuring the activity of enzymes released from the mitochondria.

Novel approaches are being implemented to understand the hazards and toxicity of nanomaterials, to safeguard human health and preserve the ecosystem. When coping with the rapid development of nanotechnology, both economic and practical outcomes should be sought. Ultimately, the advanced technology of stem cell research will be able to provide promising and sustainable modalities that can replace animal models in drug development and drug screens, in addition to guiding humane innovations in validation, regulatory acceptance and the implementation of non-animal testing methods.

Selenium is an important trace element, it was previously considered toxic but a decade ago researchers proved its importance to the human health[8].Importance of selenoproteins to human health has been identified by single nucleotide polymorphisms (SNPs) in genes encoding these proteins[5].Selenium is a trace element essential in animal nutrition and exerts multiple actions related to animal production, fertility, and disease prevention[9]. Selenium is an integral part of the enzyme glutathione peroxidase, which serves as an antioxidant enzyme that helps to control levels of hydrogen peroxide and lipid peroxides that are produced during normal metabolic activity. In addition, dietary selenium is essential for the activity of virtually all arms of the immune system[10].

MATERIAL AND METHODS

Isolation of muscle progenitor cells

The pectoral muscle of *Gallus domestics* was carefully excised with the help of sterile blade. The isolated muscle tissue was washed with 20% FBS medium and excised, trimmed of visible connective tissue, and minced with fine sharp scissors in dishes. Minced muscle was treated for 60 min with 0.2% collagenase in a 37^{o}C water bath with spin. This was followed by centrifugation at 4500rpm and 4^{o}C for 10 min. The pellet was washed with 20% FBS medium and then passed through a 70 mm filter. Subsequently, a 50-mm filter was utilized to separate mononuclear cells from the muscle fibres and myofibril fragments. The resulting supernatants were centrifuged at 4500 for 10 min. At this point, the supernatant fluid was discarded. The cells were pre-plated repeatedly to remove fibroblasts. The muscle SCs was plated in a growth medium (GM) containing 10% fetal bovine serum (FBS), penicillin-streptomycin and L-glutamine. The cells were incubated at 37 ^{o}C and 5% CO_2 in a standard cell culture incubator. The confluent monolayer cells were sub cultured and third passage cells werte used for proliferation analysis for a period of 26, 48 and 72 hrs.

Preparation of dietary organic selenium for *in vitro* culture

Organic selenium (Selenoprecise) commercially used as food supplement were used for the study. Different concentrations were prepared by dissolving 100µg, 50µg in 1ml of media to make concentrations of 100µg/ml and 50µg/ml. The cells were treated with these concentrations of selenium and cell proliferation was analysed.

Cell Proliferation Analysis

Cell count Assay

The cells were cultured in a 6-well plate with growth medium. After 24hrs of seeding equal volume of cells (5µL) and tryphan blue dye (5µL) were mixed well and used for Viable cell count. Counted cells using a haemocytometer under a light microscope after cultured at 24, 48, 72 hrs were plotted for proliferation analysis.

Dye based proliferation assay

The cells were cultured in a 96-well plate at a density of $2x10^6$ cells/mL. Twenty microliters of MTT (5 mg/ ml) solution were added to each well and incubated for 4 h. To each well, 200 µL of DMSO working solution was added, and the OD of the reaction product was evaluated in an ELISA reader at a 570-nm wavelength. At least 3 independent experiments from different samples were performed to examine proliferation.

RESULT AND DISCUSSION

The meat quality recent concerns are focussing with deep pectoral muscle disease and white striping that has impact on product appearance and impair the water holding capacity during meat processing and storage as well as poor toughness and cohesiveness related to immaturity of intramuscular connective tissue[11]. Hence addressing the factors to improve pectoral muscle growth and proliferation through diet is appropriate for enhancing meat quality. The muscle progenitor cells were isolated and cultured *in vitro* showed initial round and glistening morphology that were elongated in subsequent culture (Fig1). New muscle cells normally originate from quiescent satellite cells between basal lamina and scrcolemma in the muscle tissue fibre. The myoblast cells started to proliferate and fuse with other cells which were evidenced by the multinucleated syncytia formation 1(b).

Microphotographs of developing Chicken skeletal muscle progenitor cells.
Primary myogenic progenitors were grown to near confluence from (a) to (d) during different culture days from three to 10days (d) Proliferating confluent myogenic progenitors with Fluorescent di acetate . Scale bar represents (a) 44 µm (b) 39 µm (c) 200x (d) 25 µm

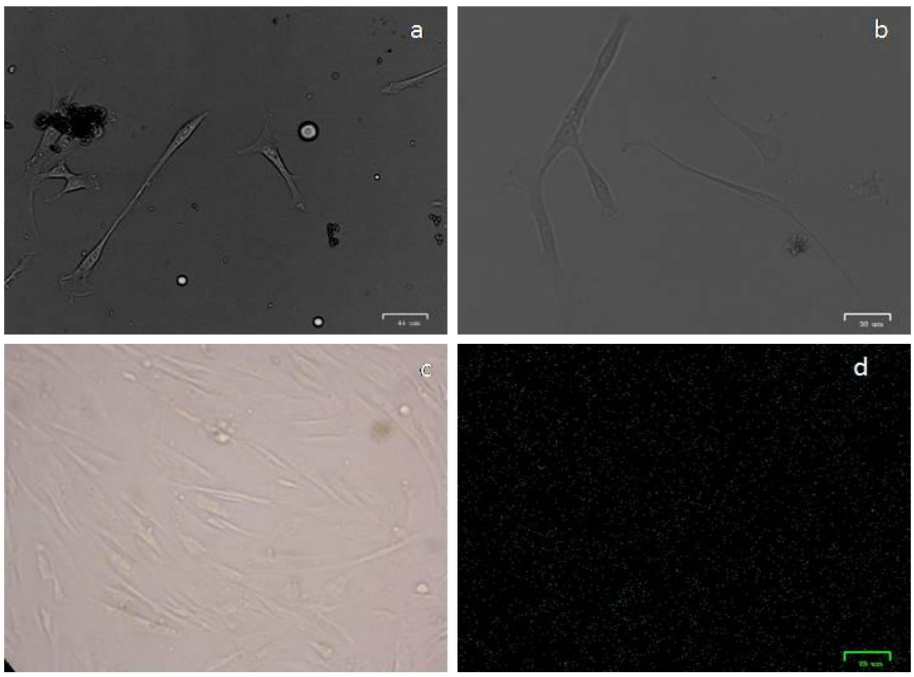

Fig. 1: Representative microphotograph of muscle progenitor cells in culture

Based on morphologic analysis of the cells observed under the inverted phase contrast microscope (Nikon), differences in the number of viable cells by cell count analysis (Fig2) and MTT assay proliferation (Fig3) of viable cells were done. Our results showed that when

the different time points were analysed separately, there is a significant increase in the muscle progenitor cell population over the course of 24, 48 and 72hr.

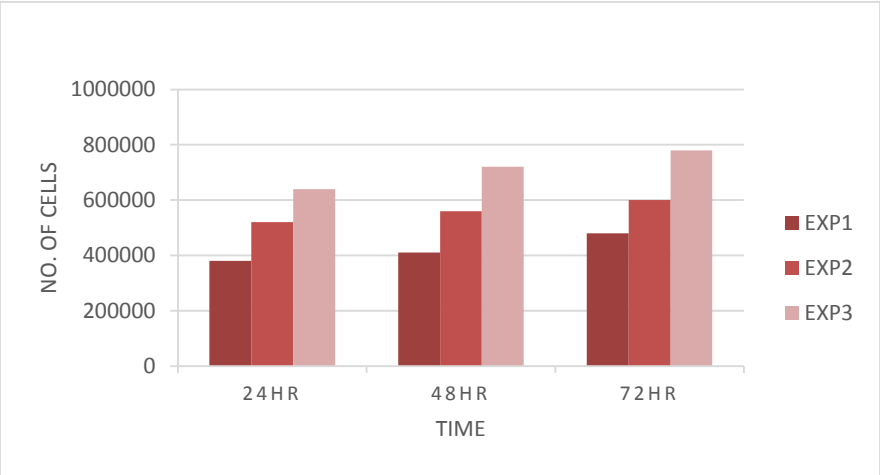

Fig. 2: Proliferative analysis of muscle progenitor cells during *invitro* culture,expt 1 indicates the control, expt 2 indicates 100 µg/ml concentration, expt3 indicates 50 µg/ml concentration

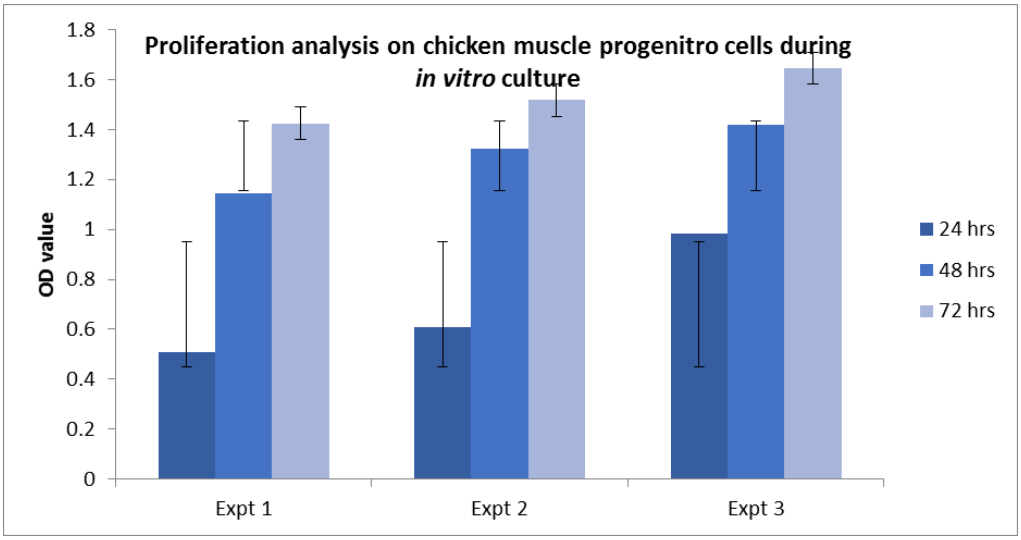

Fig. 3: Proliferative analysis of muscle progenitor cells different time points *invitro* expt 1 indicates the control, expt 2 indicates 100 µg/ml concentration, expt3 indicates 50 µg/ml concentration

To test the difference in muscle progenitor cell proliferation in the different concentrations of dietary organic selenium, *in vitro* primary muscle progenitor cell culture system was used. In this study, the progenitor cells were isolated from the muscle of *Gallus gallus domestics*. In this system, proliferative ability was measured as the number of viable cells over a short period of 3 days. Although not significant for all time points, a tendency towards an overall difference in proliferation 100µg/ml and 50µg/ml concentrations of organic selenium was found in reference to the control and specifically, the number of viable cells. There was a significant difference in the proliferative ability between the cells grown with 100µg/ml and 50µg/ml implying that the proliferation rate was slower in 100µg/ml concentration. The proliferative ability at 100µg/ml concentration is lower than that in the control indicating that at that concentration it is repressing the growth of the muscle progenitors. The proliferation of muscle progenitors were found to increase above the

control at 50µg/ml of organic dietary selenium which showed a decreasing trend at 100µg/ml concentration which supports the earlier reports on the use of dietary selenium that intake of selenium in excess could result in adverse health[15]. Authors reported that dietary modulation through selenium intake in excess was related to oxidant –antioxidant imbalance that may be induced by dietary mosulation.

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