



Improved Cell Culture Method for Growing Contracting Skeletal Muscle Models

This method has great potential for biomedical research and medical treatment.

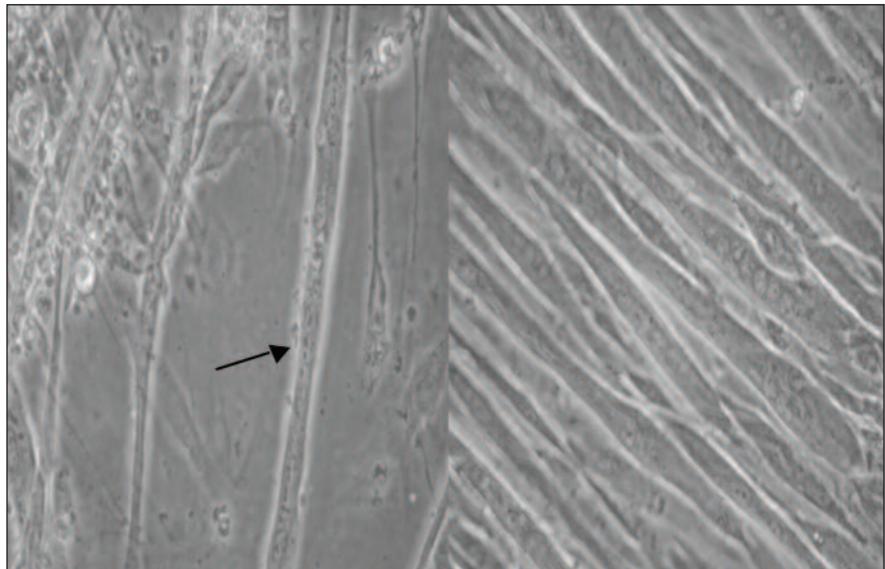
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An improved method for culturing immature muscle cells (myoblasts) into a mature skeletal muscle overcomes some of the notable limitations of prior culture methods. The development of the method is a major advance in tissue engineering in that, for the first time, a cell-based model spontaneously fuses and differentiates into masses of highly aligned, contracting myotubes. This method enables (1) the construction of improved two-dimensional (monolayer) skeletal muscle test beds; (2) development of contracting three-dimensional tissue models; and (3) improved transplantable tissues for biomedical and regenerative medicine applications. With adaptation, this method also offers potential application for production of other tissue types (i.e., bone and cardiac) from corresponding precursor cells.

In order to form or repair skeletal muscle tissue, myoblasts must proliferate, align, fuse to form myotubes (maturing, elongated, multinucleated cells), and differentiate to produce new proteins necessary for contraction. Heretofore, none of the typical culture methods has enabled the growth of skeletal muscle tissue possessing all of these characteristics. Notable limitations of prior methodologies include:

- Differentiation is usually induced by the use of undefined serum switches or deprivation, either of which can result in decreased cell viability, occasional unpredictable variations, and experimental bias.
- Alignment of cells is affected by means of time-consuming procedures that involve the use of expensive, complex, micropatterned substrates.
- Spontaneous contraction is a random, rare event.

In contrast, the present method enables the generation of large numbers of aligned, spontaneously contracting myotubes without the use of serum switches or special substrates. The resources necessary for practicing this method are readily available in most cell-culture lab-



Comparison of Skeletal Muscle Culture Methods: Typical culture using serum switches results in random distribution of myotubes (left). The arrow indicates one of many myotubes in the image. The improved cell culture methodology produces aligned myotubes (right) without the use of (micropatterned) substrate modification.

oratories.

The method is embodied in a cost-effective, multistep protocol of cell culture that results in the formation of the desired skeletal muscle tissue model and that, with additional time in culture, yields sheets of spontaneously contracting aligned muscle cells. Omitting most details for the sake of brevity, the protocol is summarized as follows:

1. From typical cell culture of the desired cell type, the cells are removed and concentrated. The cells are resuspended in culture medium to achieve a specific cell density.
2. Cells are seeded onto the bottoms of tissue culture Petri dishes.
3. New culture medium is added to the dish and cultured at 37 °C. After 3 days of incubation, myotubes are evident. At 5 to 6 days, myotubes are aligned and are in greater abundance.
4. From this point onward, Petri dishes should be monitored on a daily basis and medium should be replaced with culture medium on an as-needed basis.

5. By the end of a two-week culture period, spontaneously contracting myotubes are evident although contractions may have been noted as early as day 5.

6. Contracting myotubes can be maintained for an additional two weeks with daily culture medium replacement. During this additional culture time, sheets of aligned contracting myotubes appear to gain synchronicity.

The theory underlying this methodology includes the following elements:

- Low-density cell culture favors cell flattening. Flattened cell morphology, in the presence of growth factors found in the culture medium, encourages cell proliferation. In this new methodology, the lower-density cell population will also proliferate. The newly formed cells, crowded by adjacent newly formed cells, are forced to compete for attachment space and are forced to align.
- Simultaneously, the cell crowding caused by the high-density cell population prevents total cell flattening. In-

stead, the attached, rounded morphology, coupled with the high degree and duration of cell-to-cell contact, is thought to induce expression of genes that causes the cells to switch from proliferating to differentiating phenotype. This differentiating phenotype gains the ability to fuse and express contractile proteins.

Currently, this model can serve as an improved, yet easily generated, in vitro skeletal muscle test bed for evaluating signal transduction pathways, stretch activated ion channels, protein synthesis regulation, and interactions with the ex-

tracellular matrix and membrane proteins with the cytoskeleton. The ease and low cost of generation makes this model a valuable option to many laboratories that have the desire, but lack the sophisticated resources previously required to explore the mechanisms of muscle atrophy. Future development of the model into a three-dimensional construct provides a potential powerful tool for defining the mechanisms of microgravity-induced muscle atrophy, identification of molecular targets for novel countermeasure development, and testing of some countermeasures.

This work was done by Michele L. Marquette of the University of Texas Medical Branch, and Marguerite A. Sognier of the Universities Space Research Association for Johnson Space Center. For further information, contact the JSC Innovation Partnerships Office at (281) 483-3809.

This invention is owned by NASA, and a patent application has been filed. Inquiries concerning nonexclusive or exclusive license for its commercial development should be addressed to the Patent Counsel, Johnson Space Center, (281) 483-1003. Refer to MSC-24314-1

Hand-Based Biometric Analysis

Goddard Space Flight Center, Greenbelt, Maryland

Hand-based biometric analysis systems and techniques provide robust hand-based identification and verification. An image of a hand is obtained, which is then segmented into a palm region and separate finger regions. Acquisition of the image is performed without requiring particular orientation or placement restrictions. Segmentation is performed without the use of reference points on

the images. Each segment is analyzed by calculating a set of Zernike moment descriptors for the segment.

The feature parameters thus obtained are then fused and compared to stored sets of descriptors in enrollment templates to arrive at an identity decision. By using Zernike moments, and through additional manipulation, the biometric analysis is invariant to rotation, scale, or translation or an input

image. Additionally, the analysis uses re-use of commonly seen terms in Zernike calculations to achieve additional efficiencies over traditional Zernike moment calculation.

This work was done by George Bebis of University of Nevada, Reno for Goddard Space Flight Center. For further information, contact the Goddard Innovative Partnerships Office at (301) 286-5810. GSC-16141-1

The Next Generation of Cold Immersion Dry Suit Design Evolution for Hypothermia Prevention

The system design recovers warm exhaled air and re-circulates it inside the suit.

John H. Glenn Research Center, Cleveland, Ohio

A body at sea is vulnerable to hypothermia, which often leads to loss of life. Hypothermia is caused by the differences between the core body temperature and the surrounding air and seawater temperatures. The greater the differences between the body core temperature and the sea temperature, the more rapidly the core body temperature will drop, and hypothermia can quickly set in. Heat loss is primarily caused by conduction of heat away from the body. Most cold immersion suits on the market are passive designs that only insulate the body against the cold, although some cold immersion suits use special materials such as paraffin to absorb heat and to radiate the heat back to the body. This new utility patent is an active design that relies on the lung's role as an organic heat ex-

changer for providing deep body core heating of air. It is based on the fact that the greatest heat loss mechanism for an insulated human body immersed in a cold water environment is due to heat loss through respiration.

This innovation successfully merges two existing technologies (cold immersion suit and existing valve technologies) to produce a new product that helps prevent against the onset of hypothermia at sea. During normal operations, a human maintains an approximate body temperature of [98.6 °F (37 °C)]. A mechanism was developed to recover the warm temperature from the body and reticulate it in a survival suit. The primary intention is to develop an encompassing systems design that can both easily and cost effectively be integrated in all existing currently manufac-

ture cold water survival suits, and as such, it should be noted that the cold water immersion suit is only used as a framework or tool for laying out the required design elements.

At the heart of the suit is the Warm Air Recovery (WAR) system, which relies on a single, large Main Purge Valve (MPV) and secondary Purge Valves (PV) to operate. The main purge valve has a thin membrane, which is normally closed, and acts as a one-way check valve. When warm air is expelled from the lungs, it causes the main purge valve to open. Air forced from the MPV is dumped directly into the suit, thereby providing warmth to the torso, legs, and arms. A slight positive over-pressure in the suit causes warm waste air (or water if the suit is punctured) to be safely vented into the sea through large PVs located at the bot-