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. Herefore, 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 laboratories.

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-
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 (281) 483-1003. Refer to MSC-24314-I.