Optimization of skeletal myocyte expansion for potential use in cell therapy for stress urinary incontinence

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Introduction: Skeletal progenitor cells are regarded as an optimal candidate for cell therapy in stress urinary incontinence due to urethral sphincter injury. However, it is a big challenge to expand a large amount of functional skeletal muscle cells in vitro from a small piece of tissue sample via tissue biopsy because the myocytes decrease the normal expression of their phenotypes in the conservative culture conditions. Our previous studies demonstrated that tissue specific extracellular matrix (ECM) provides stem cell niche (microenvironment) to promote cell growth and retain phenotypes of the functional cells (such as skeletal myocytes, hepocytes and skin epidermal cells) in 2D culture condition. The goal of this study is to determine whether skeletal muscle tissue ECM induce skeletal progenitor cells to differentiate into skeletal myocytes and enhance the cell expansion of functional myocytes in 3D culture.

Materials and Methods: Cellular components were removed from porcine skeletal muscle, kidney and liver tissues, respectively. The resulting acellular matrices were homogenized and dissolved. The ECM solutions were mixed with heparin-decorate, hyaluronic acid-based hydrogel. Human skeletal progenitor cells were grown on each 3D ECM gel to assess cell proliferation assessed by MTT, and evaluate their phenotypes and myotubular formation by Western bolt and immunofluoresce staining at several time points.

Results: Decellularized ECM contained less 15% DNA. Each tissue specific ECM coating solution and ECM gel are cell friendly and no cytotoxicity. Skeletal muscle ECM 3D gel significantly enhanced human skeletal progenitor cell proliferation, compared to liver, kidney or non ECM gels. More myofibers and myotubues were formed in muscle ECM gel than those in other gels. Numbers of cells express specific muscle cell markers (i.e. myosin, desmin, myoD and myf4) significantly increased when human skeletal progenitor cells cultured on muscle-ECM on 3D gel.

Conclusions: Our data demonstrated that skeletal muscle ECM provides an optimal culture microenvironment that is similar to the in vivo environment when used as culture substrates, which offer the potential use for skeletal muscle tissue regeneration in cell-based therapy of stress urinary incontinence.