428q Isolated, Perfused Organ Model for Studying Stem Cell Survival and Differentiation

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Regeneration of tissue is, for many diseases, the preferred method for restoration of health. Many current approaches lead to a lesser and impermanent result and are used only because adequate methods of tissue regeneration remain undiscovered. The task is difficult because most tissues are comprised of different cell types. Two contemporary approaches are aimed directly at tissue regeneration: alteration of the genome of deficient cells by gene transfection and introduction of stem cells capable of growth and differentiation of function within diseased tissue. These cells may come from the unhealthy organism or from another organism, with much attention having been focused on the use of cells from fetal donors. Both of these approaches depend on the proliferation and functional integration of a new cell population within existing tissue. Rational approaches to tissue regeneration are nearly impossible without an understanding of proliferation and integration of new and transformed cells. The work described here is directed toward the placement and maintenance of skeletal muscle in an in vitro perfusion circuit over times long enough to permit study of how introduced stem cells spread, multiply, and integrate with host cells. Skeletal muscle was chosen because its resting metabolic requirements are very small, its natural function (chemomechanical transduction) is easily measured, and because the pathways by which natural processes lead to satellite cell activation and cell replacement in adult tissue are relatively well understood. Initial studies have been conducted on the biceps muscles of the rabbit and rat. These studies are seen as a necessary preamble to the use of cadaveric human skeletal muscle, obtained from organ donors. In all cases, it is necessary to remove the muscle with its blood supply intact, in such a manner that the warm ischemic time is minimized. The biceps was chosen because of its accessibility and relatively simple blood supply. In both species, the pectoral muscle in the foreleg of an anesthetized animal is transected to expose the biceps and its blood vessels. Using microsurgical techniques, the artery is separated from the adjacent nerve and vein, the branches of the artery proximal and distal to those branches that supply the long head of the biceps are ligated and severed, and the main branch of the radial artery is cannulated with a polyurethane tube and perfusion is begun. The long head of the biceps is freed from the short head and surrounding tissue and removed with as much tendon as possible at each end. The cannulated, perfused muscle is moved to the perfusion chamber, where both tendons are vertically attached to hooks: the top hook is connected to a low-compliance force transducer and the bottom one to a micro positioning device. The constrained muscle is placed under light pretension in the chamber maintained near 37 C and 100% relative humidity. Two electrodes fashioned from silver foil are placed semi-circumferentially near the ends of the muscle and are used to apply various patterns of electrical stimulation for two purposes: to exercise the muscle in order to prevent atrophy and death, and to obtain feedback of the liveliness of the muscle. Perfusion and stimulation is continued until the muscle no longer responds to stimulation. It is then removed from the chamber and weighed, and compared to the weight of the contra lateral muscle. To date, the rat biceps has shown a stable response to stimulation for periods of time up to 12 hours. The reasons for muscle death are still under investigation; however edema appears to be a common problem among all muscle perfused so far, with weight gains rarely less than 20%. The short term goal of this research is to extend the lifetime of the isolated muscle to days, perhaps weeks. To achieve this goal, studies are undertaken to find the optimal perfusion conditions and exercise regime for the muscle for sustained metabolic and mechanical function, and to identify the causes of edema and vasodilatation. The basic perfusate used thus far is Waymouth medium adjusted to a final composition of 5% albumin. The level of antioxidants, antibiotics, proteins and buffer in the perfusate will be adjusted. By monitoring the rate of glucose and oxygen consumption and lactate, creatine phosphatase, and carbon dioxide production, under both quiescent and stimulated conditions, the adequacy of the perfusate can be measured. The response of weight gain and effective delivery of the perfusate to the tissue will be monitored while changing the concentration of albumin in the perfusate as well as changing the flow rate and flow resistance of the

perfusate. To establish the ideal stimulation pattern for this fast twitch muscle, intermittent, brief, high frequency patterns will be applied, while changing the frequency, number of pulses per burst, and pulse duration of the signal and changing the pretension of the muscle. Thus far, tetanic tension has been measured under supra-maximal stimulation to prevent atrophy and death of the muscle, and chronaxie and rheobase measurements have been used to monitor the livelihood of the muscle. Other, perhaps better, stimulations will be developed, and their effects on the muscle will be monitored with the chemical analyses described above. In skeletal muscle, so-called satellite cells are believed to be the normal precursors of new myocytes in adult tissue, but only unusual conditions are believed to cause their proliferation and development. The long term goals of our research call for isolation of satellite cells from other muscles of the donor animal, vital staining of these cells with a fluorescent dye, and their placement by injection into perfused muscle. After different periods of perfusion, the tissue will be recovered. Tissue surrounding some injection points will be disintegrated and the number of stained cells determined by fluorescence-activated cell sorting. Tissue surrounding other injection points will be examined to determine the spatial distribution of cells around the point of introduction. When perfusion over several days is possible, it is planned to study differentiation in recovered, stained cells in terms of both cell-surface markers and RNA profiles. The use of less differentiated stem cells and human tissue will be undertaken when useful experimental data can be obtained routinely from the tissues of laboratory animals. Data from human tissue will be much more useful, not only because of their greater relevance but also because far more and better antibodies are available for cell-surface markers of human tissue and because more precise and complete genomic information is available for human muscle tissue.