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Tissue Engineering of Skeletal Muscle

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Striated muscle structural anomalies, whether they evolve from a congenital condition, as the result of an accident, or from removal of a tumor, can affect physiological performance as well as the psychological health of a patient. A recent review indicates that there has been very little work in skeletal muscle tissue engineering for reconstruction of the head and neck [1]. The long-term goal of our laboratory is to develop a striated muscle implant, derived from a patient's own skeletal muscle satellite cells, that mimics the architectural organization and physiological function of intact muscle.

The prototypic skeletal muscle is composed of striated myotubes that are arrayed in parallel with one another along a common axis. Each multinucleated myotube functions as a single cell and spans the entire length of the muscle. Adjacent myotubes are tethered together by a connective tissue covering [2]. We believe that it is essential to develop an implant that displays an *in vivo*-like pattern of organization. To this end, we have used a modification of the aligned collagen gel system [3] to engineer a three-dimensional prosthesis of skeletal muscle cells. In the aligned collagen gel system, cells are plated onto a thin, polarized matrix of Type I collagen fibrils prepared on a solid substrate. Applying a solution of ice cold, neutral type I collagen to a culture dish or extruded collagen surface, at a concentration of 0.62μg/mm^2, produces this matrix. The substrate is created by "painting" the solution across the culture dish using a soft sterile nylon brush or when applying a second layer, the solution is allowed to drain across the surface. Once this application process is completed, the collagen is allowed to polymerize at 37 ºC for 1 hour. This procedure results in a thin layer of collagen fibrils that are arrayed in parallel with one another along the direction that the solution of collagen was originally painted (Figure 1).

Satellite cells were isolated from neonatal (3-4day old) rat hind limbs by 1.25% protease digestion on a reciprocating rocker at 37C for 60 min. The released cells were harvested by centrifugation (100x g) for 10 min and plated on the aligned collagen gels. Two to three days after plating on the aligned collagen gels the "fibroblast-like" satellite cells are aligned in the direction of the painted collagen and begin to fuse into myotubes. Over the next four to five days the myoblasts continue to fuse and a series of uniformly arrayed, densely packed myotubes with morphology reflective of skeletal muscle develop (Figure 2). If the cells are plated on plain tissue culture plastic or on non-aligned collagen gels, the satellite cells differentiate into skeletal myotubes (Fig 3), but the arrangement is haphazard and does not reflect what is seen in differentiated skeletal muscle. The majority of the cells placed on the aligned collagen are myoD positive myoblasts that fuse into multinucleate myotubes. Confocal microscopy of a phalloidin stained culture (figure 4) demonstrates parallel-aligned skeletal muscle cells with uniformly spaced sarcomeres. Located between the skeletal muscle cells are a population of fibroblast-like cells that are a mixture of

myoblasts and fibroblasts that appear in some cases to be producing what appears to be collagen (not shown). To increase the thickness of the aligned skeletal muscle cultures to form a tissue like construct, we apply an additional layer of collagen gel to the aligned layer of cells after a week, as described above and then add more satellite cells to the preparation. To determine the thickness of the skeletal cultures, we fixed them with paraformaldehyde and and processed the tissue engineered muscle for paraffin histology. Cross sections cut perpendicular to the long axis of the surface cells indicate that multiple layers of skeletal muscle cells were achieved (figure 5). In figure 5, the lower arrow marks the interface between the first plating of cells on the aligned collagen (below) and the second (above and arrow). Although the two platings are not perfectly aligned, it is apparent that multiple layers of myotubes exist in both platings. The majority of the elongated cells with multiple prominent nuclei in both layers are skeletal cells. The other cells cut in cross section are undifferentiated cells present in the primary culture.

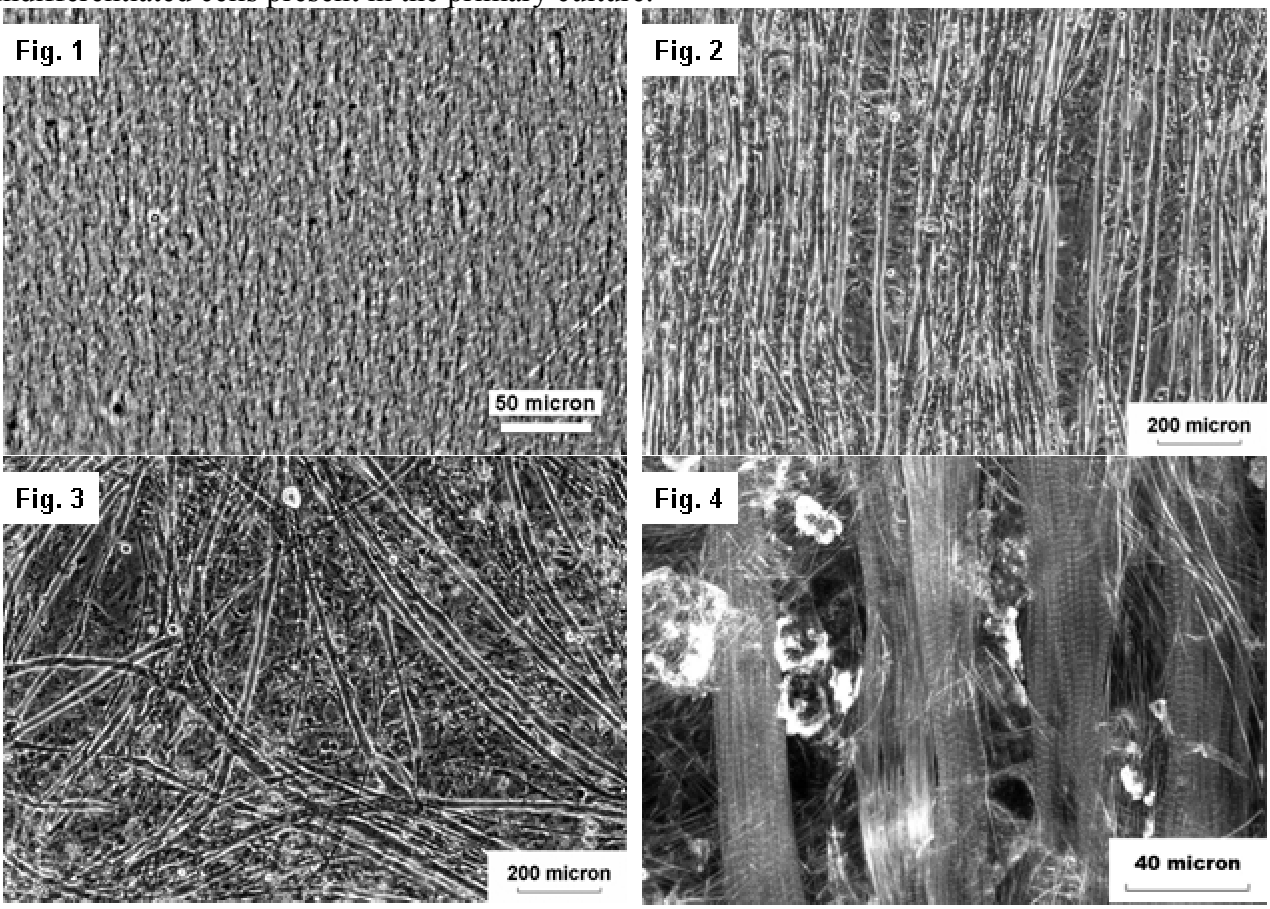

Figure 1: Phase contrast micrograph of collagen fibrils aligned in a parallel layer on a culture dish. Figure 2: Skeletal muscle myotubes in parallel array on the aligned on the collagen. Figure 3: Skeletal myotubes on a standard culture dish, exhibiting a random distribution. Figure 4: Confocal microscopy of phalloidin stained myotubes.

References:

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