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BIOMECHANICS OF HYBRID VASCULAR TISSUE IN BIOMOLECULAR AND CELLULAR LEVEL

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Vascular cells of arterial walls are continuously exposed to the mechanical stimulation induced by pulsatile blood flow. Understanding of mechanically induced cellular behavior *in vitro* may help us build a highly structured hybrid graft. In the present study, we investigated the effect of periodic stretch on orientation and morphology of 2-dimensionally (2-D) and 3-D cultured bovine aortic smooth muscle cells (SMCs).

i) SMCs 2-D seeded onto transparent elastomeric films were subjected to periodic stretch-relaxation under various amplitudes ranging from 5 to 20% and at frequencies ranging from 15 to 120 RPM for up to 24 hours. Time-lapse video-recorded images of stress-loaded cells under a phase contrast microscope were analyzed by a computer-aided morphometric system to quantitatively evaluate the cellular orientation responses and morphological changes. The stress-loaded cells tended to align perpendicularly to the direction of stretch with time. More pronounced orientation was attained under operating conditions with higher amplitude and frequency of stretching. Meanwhile, little morphological change was observed, irrespective of stress-loading or nonloading.

ii) Ring-shaped hybrid tissues were prepared by 3-D incorporating SMCs into type I collagen gels. Three different samples of gels, which were found spontaneously to shrink, were subjected to stress-loading in culture medium; floated (nonstressed), stretched isometrically (static stress-loaded) and periodically stretched and recoiled with 10% amplitude at 60 RPM frequency (dynamic stress-loaded). After 4-week stress-loading, the gels were morphologically investigated with light microscope and transmission electron microscope. Irrespective of static or dynamic stress-loading, SMCs in stress-loaded gels exhibited elongated bipolar spindle shape and oriented parallel to the direction of stretch, whereas those in nonstressed ones were polygonal-shaped and randomly oriented. Transmission electron microscopic observation showed that SMCs in non-stressed and static stress-loaded gels were intracellularly filled with organelles such as rough endoplasmic reticula, free ribosomes, Golgi complexes and mitochondria, which indicate that the cells were of the synthetic phenotype. On the other hand, SMCs in dynamic stress-loaded gels tended to have increased fractions of contractile organelles such as myofilaments, dense bodies and basement membranes. These findings suggest that periodic stretch plays an important part in phenotypic modulation of SMCs from the synthetic to the contractile state as well as cellular orientation.

[References]

1) Kanda K, Matsuda T, Oka T:Two-dimensional orientation response of smooth muscle cells to cyclic stretching. ASAIO J 38:M382-M385, 1992

2) Kanda K, Matsuda T, Oka T:Orientation Response of Vascular Cells to Periodic Stretch. Japanese J Artificial Organs 22(2):483-487, 1993.

3) Kanda K, Matsuda T: Behavior of Arterial Wall Cells Cultured on Periodically Stretched Substrates. Cell Transplantation, 1993 in press.

4) Kanda K, Matsuda T, Oka T: A highly structured "Hybrid artificial media: induced by mechaincal stimulation in vitro. Japanese J Artificial Organs 22(2):478-482, 1993

5) Kanda K, Matsuda T, Oka T: Mechanical Stress-induced Cellular Orientation and Phenotypic Modulation of 3D-cultured Smooth Muscle Cells. ASAIO J 1993 in press.