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Rheological Analysis of Cultured Cell/Matrix Sheet for Regenerative Medicine Using Novel Magnetic Indenter

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Extended Abstract

Regeneration of elastic fibers in extracellular matrix is highly demanded since their degradation causes various aging-related diseases. Recent progress in the technique for culturing and retrieving a cell sheet with a dimension exceeding square centimeters is expected to provide a breakthrough. It is quite essential to understand the relation between the pharmacological conditions in the culturing process and the mechanical properties of the regenerated sheet. These sheets typically have a single-cell-level thickness and are extraordinary compliant and fragile. A new technique to analyze their mechanical properties, not only a static one but also dynamical one, under physiological condition is required.

In the present research a novel magnetic indenter is proposed. The sheet sample is suspended over a circular aperture with a diameter of 4 mm and a steel sphere with a diameter of 0.8 mm is placed at its center. A magnetic force is exerted onto the sphere via an electromagnet with 2400 turns placed beneath the sample and the resultant displacement of the sphere is detected via an optical displacement sensor with a resolution of 1 μm that corresponds to a force resolution of ca. 0.1 μN for a typical sample. The electromagnet is driven with closed-loop regulation in order to suppress the effect by the inductance. The system possesses a bandwidth exceeding 100 Hz that provides potential for various rheological analysis.

In the present work creep response of the sample to a step loading/unloading of magnetic force was measured for two types of samples: a cultured sheet of skin fibroblasts including extracellular matrix (ECM) (a "cell sheet") and a similar one with cells removed to leave ECM only (a "matrix sheet"). Measurement was carried out at room temperature in phosphate buffered saline (PBS) solution. Loading and unloading cycle was repeated while gradually increasing the force setpoint from about 13 μN to about 110 μN.

Whether at loading or unloading, every measured response curve proved to be a synthesis of three modes, i.e., a quasi-instantaneous displacement and two delayed elastic processes having a time constant of roughly 6-30 sec. and 60-250 sec. These time constants exhibited a roughly increasing trend with increasing load. It implies that the sample responds in more viscous manner as it is deformed in large amount. It was found that the cells contribute rather the viscosity than the elasticity. In the case of the cell sheet the proportion of the quasi-instantaneous deformation was higher at unloading than at loading. This irreversibility markedly decreased as the load increased. On the other hand, the irreversibility of the matrix sheet was less pronounced and showed only moderate dependence on the load magnitude. It is speculated that the cells have undergone some change during the loading time exceeding 150 sec. and caused the sample to deform back in a less viscous manner upon unloading. Thus, the three deformation modes mentioned above are considered not to be of completely different nature but to be switchable flexibly to one another depending on the load magnitude and loading history.