Nanotechnology-based Cell Sheet Engineering for Regenerative Medicine

Masayuki Yamato
Tokyo Women's Medical University

In the 21st century, novel therapeutics is going to be established. Controlled drug delivery has been already commercialized in the limited area. Gene therapy has experimentally challenged to human patients. Furthermore, robotic surgery systems as well as computer-aided surgery navigation systems are also commercialized. Tissue engineering was proposed in 1980's by the collaboration of a chemist, R. Langer and medical doctors, J.P. Vacanti and C.A. Vacanti. As demonstrated in reconstruction of cartilage tissues that is world-famous human ears on mice, the key technology is the use of biodegradable polymer scaffolds for cell seeding preformed in the target tissue shapes. By the combination of preformed biodegradable polymer scaffolds and specific cell types, various tissues including cartilage, bone, blood vessel were reconstructed, although the therapeutic use has been very limited up to now. Here, we present another tissue engineering without biodegradable polymer scaffolds, named cell sheet engineering. Conventionally, proteolytic enzymes such as trypsin and dispase are used in cell harvest. These enzymes degrade cell adhesion molecules and deposited ECM to detach cultured cells. But at the same time, cell-cell junctional proteins as well as membrane proteins such as ion channels and growth factor receptors are often degraded. Therefore, cultured cell sheet harvest is achieved with the only exceptional cell types whose cell-cell junctions are less susceptible to such enzymes. In order to solve this problem, we first developed temperature-responsive culture dishes. A temperature-responsive polymer, poly(N-isopropylacrylamide), is covalently grafted on the surfaces. The grafted polymer thickness is controlled around 20 nm to achieve temperature-responsive cell adhesion/detachment control. To achieve such surface modification in a well-controlled manner, we utilize electron beam irradiation-initiated radical polymerization. The surfaces of temperature-responsive culture dishes are relatively hydrophobic at 37°C similarly to commercially available tissue culture dishes, but changes to hydrophilic below 32°C. Various cell types adhere, spread, and proliferate on the surfaces at 37°C. Only by reducing temperature, cells are spontaneously lifted up from the surfaces without the need for trypsin. Highly trypsin-susceptible cells such as hepatocytes and glial cells retained the differentiated native cell functions after the noninvasive cell harvest. Confluently cultured cells are recovered as a single contiguous cell sheet with intact cell-cell junctions and deposited extracellular matrix. Harvested viable cell sheets can be transferred to other surfaces of culture dishes in vitro or tissue surfaces in vivo since the ECM associated with the basal side of cell sheets shows adhesion. The harvested cell sheets can be stratified to reconstruct thicker or complex tissue architectures such as cardiac muscle, liver lobule, kidney glomeruli. We will demonstrate how to utilize these cell sheets for regenerative medicine including ocular surface reconstruction and cardiac tissue repair in clinical setttings.