Biomaterials and Medical Applications

An Elusive *In vitro* Native-like Microenvironment-Is a Decellularized Extracellular Matrix the Answer?

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Native microenvironment (NME) provides the mechanophysiological condition to the cells or tissues in their local microenvironment, where cells do grow, proliferate, differentiate, and migrate as well as undergo the natural apoptosis process. All the processes happen in cellular level to maintain a tissue or an organ homeostasis. In addition, NME controls a cell or a tissue function, for example secretion of bioactive molecules, deposition of extracellular matrix proteins (ECMs), organization of different kinds of cells, and hierarchical mechanisms on cell cross-talk networks [1-3]. NME is normal as in a normal tissue or abnormal as in a cancerous tissue. As *in vivo*, cells, their spatial arrangements, cell-cell interactions and cell-matrix interactions are indispensable to create and maintain the NME [4,5]. In other words, cells determine their NME and the NME controls the function of cells or vice versa. Generally, mesenchymal stem cells (MSCs), tissue specific stem cells, fibroblasts, immune cells, endothelial cells, lymphatic cells, adipocytes and migrating cells participate in the formation of NME, depending on the organ specificities. In tumor microenvironment (TME), presence of tumor cells and their control on other cells is vital for tumor growth and vasculogenesis [2,6].

Mimicking native-like microenvironment in *in vitro* system is a prime concern in tissue engineering and other studies. Many approaches have been attempted to provide a 3D spatial condition to cells, growing in *in vitro* system using various kinds of biomaterials that are biodegradable and biocompatible. Physical properties (e.g. topography, stiffness, and elasticity), chemical properties (e.g. hydrophilicity, and hydrophobicity), and presence of cell binding ligands of each biomaterial directly play the significant role in the cell attachment, proliferation, protein secretions and cell growth as well as differentiation. Synthetic biomaterials like polycaprolactone (PCL), poly(lactic co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), poly(vinyl chloride) (PVC) and poly(dimethyl silane) (PDMS), and natural biomaterials like chitosan, silk fibroin, starch, cellulose, alginate, gelatin, collagens, ECM and decellularized extracellular matrix (dECM) are popular in tissue engineering and biomedical applications [7-11]. Various blended forms from both synthetic and natural biomaterials have been used extensively in considering that composites or hybrid scaffolds can provide better native-like microenvironment to the cells compared to the individual biomaterial

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[12-15].

Biochemical and biophysical cues of synthetic biomaterial hydrogels can be temporally tuned to mimic the native-like extracellular matrix for the normal cellular process by modifying certain reactive groups such as thiols, NHS esters and azides under cytocompatible conditions through numerous reactions like azide alkyne cycloaddition and chain polymerization. Adhesiveness of synthetic biomaterials is increased by introduction of adhesive peptide sequences in the biomaterials such as binding of RGD peptide sequences in PEG by N-hydroxy succinamide ester on one end and acrylate functional group on other side [16,17]. Furthermore, some peptide units and different ECM components are incorporated in synthetic biomaterials to maintain the self-renewal of stem cells and to facilitate the interaction with cell surface glycans as a hybrid or composite hydrogels or 3D scaffolds, for instance incorporation of vitronectin-derived peptide units (GKKQRFRRHRNRKG) in polyacrylamide hydrogel [18,19]. Addition of functional groups, for example silk fibroin and curcumin in PCL demonstrated the better cell attachment. PCL scaffolds have been coated with cell-laid ECM, alginate or dECM to make hydrophilic and native mimicking NME to the cells [12,13]. Importantly, synthetic biomaterials or hybrid or composite hydrogels or scaffolds provide favorable condition and mechanical support to the cells. Though, they are excellent in providing the bioprinting hydrogels for precise scaffoldings with a control on size, shape and porosity through the 3D-bioprinting and conventional 3D scaffold systems, they are usually in limit providing a bioink, where cells are admixed with the hydrogels. The limitation in bioink formation is mainly because of their insolubility in water and solubility in organic solvents like chloroform or acid or alkalies, which harm the cells.

Selection of biomaterials turns towards the natural materials, which are inert, biocompatible and biodegradable as the better alternatives for the synthetic biomaterials. Studies have shown that natural derived biomaterials like silk fibroin, ECM and dECM provide the better favorable condition to the cells compared to synthetic biomaterials. Every native biomaterial has its pros and cons as of synthetic biomaterial. The choice of biomaterials depends on the purpose of tissue engineering studies, for example chitosan is usually selected for hard tissue engineering whereas, alginate is for soft tissue regeneration [20,21]. Explanation of each biomaterial is beyond the scope of this editorial article. Introduction of collagen in tissue engineering has changed a lot of paradigms in research as the better ECM for tissue engineering since it is a product of native tissue [22]. The hope in getting the native-like microenvironment in *in vitro* system using only collagen as the scaffold has not been fulfilled. Collagen as a single type ECM protein, loss of native configuration, and loss of cell binding ligands during extraction and purification process may be some of the factors that hinder the collagen as an ideal scaffold. Because of collagen limitation in tissue engineering, dECM has been emerged as the ideal hydrogel for 3D scaffolding system. dECM contains almost all the ECM proteins that play the vital role in the formation of native like microenvironment [23,24]. Like other natural biomaterials, dECM has been used as the bioinks for different biomedical research. It has been using in versatile applications both *in vitro* and *in vivo*. Based on ECM constituents, dECM provides the excellent microenvironment to the cells compared to any other

biomaterial. Since studies have shown that cells behave differently in different dECM, specific dECM has been selected for specific tissue engineering for example skin dECM for skin regeneration [25].

dECM is generally prepared from native tissue by decellularization process, where different types of detergents, acids, alkalies, or enzymes are used. The purpose of using those chemicals is to remove the nuclear contents and DNA completely from the tissue [23,26]. But, during decellularization process, ECM proteins have lost their physical configurations and chemical constituents, resulting in the loss of binding ligands, hide or loss of active sites, and loss of native topographical properties [27,28]. Therefore, there is the variation in result outcomes from a single type of cells even if there is a use of same dECM materials that prepared from different decellularization approaches. Considering this limit, ECM tissue should be decellularized to make dECM that should exactly mimic the native ECM, keeping all the proteins as they are in native tissue. In spite of a clear understanding that dECM is only the best alternative scaffolding system till date, we are far behind for the fabrication of the 3D functional system using dECM through fabrication process like bioprinting. There is still a challenge to fabricate the porous scaffold using only dECM as a bioink because of weak mechanical strength of dECM and contractile nature of certain proteins like collagens present in dECM that cause not only the collapse of precise porosities required for the cell migration and nutrition diffusion, but hinder proper cell adhesion and migration [29,30]. Further, the constant change in ECM constituents and interconversion of natured and denatured state of proteins in live tissue for various regulatory functions will be the great concern for making native tissue environment for growing cells in *in vitro* system [31-33].

Successful in mimicking tissue like microenvironment in *in vitro* system not only depends on the scaffolding biomaterials, its nature and properties, but also relies on other most important factors for example, physiological conditions (e.g. pH, temperature, oxygen and carbon dioxide content, and metabolites), growth factors (e.g. cytokines, hormones, and minerals), and energy supply (e.g. ATP, and nutrients). Optimization of cultural condition depending on the tissue type is very important that should let multi-type cells grow in a way as they do in native tissue. The author hopes that, after fundamental improvement on native ECM isolations and preparation of fully functional dECM, there is a great prospect of making fully native 3D spatial *in vitro* environment dECM systems for the cells that support for any type of tissue engineering and cancer studies for better improvement of public health.

Acknowledgments

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