The decellularized extracellular matrix in regenerative medicine

“The biocompatibility, biodegradability and bioinductivity of decellularized extracellular matrix are important factors to be considered in surgical practice and research.”

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The extracellular matrix (ECM) is a complex nonliving biomaterial, secreted by cells, which acts as a specific, functional and protective bed for cells. It is composed of many types of protein, which form a complex together with water-soluble and -insoluble molecules. The physical nature of ECM may be semifluid or solid depending on the protein types and their complexes.

The ECM's components, its stiffness and the presence of ECM-bound inducers are key factors, which are necessary for the attachment, proliferation, migration and function of cells. The major components, such as collagen, fibronectin, laminin, elastin and various complexes (e.g., glycosaminoglycans) play vital roles in maintaining the native-like environment of the cells growing in *in vitro* or *in vivo* systems. The ECM has the viscoelasticity and mechanoconduction abilities to regulate dynamic cellular behaviors, such as proliferation and migration, and to influence rapid growth of the cells [1]. Besides the fundamental role of the ECM, the cells growing on the ECM bed generate forces spatiotemporally on the ECM that trigger signaling pathways to regulate the biological processes of proliferation and expansion [2].

Removal of cellular components from the ECM leaves the water-insoluble matrix, generally known as decellularized ECM (DECM). Usually mild detergents like

sodium dodecyl sulfate and Triton X-100 are used to decellularize the tissue, along with physical and enzymatic treatment. The DECM has physiological cues to mimic the native microenvironment and is, therefore, more appropriate for refunctionalization and better recovery of a tissue as a replaceable model compared with commonly used biomaterials. DECM is therefore in high demand in both basic research and clinics. The biocompatibility, biodegradability and bioinductivity of DECM are important factors to be considered in surgical practice and research. The DECM tissue/organ, DECM hydrogel and DECM microparticles are all used in regenerative medicine.

DECM can be prepared from any organ or tissue according to the purpose of the research or a clinical application [3]. DECM from bone, breast, skin and bladder is most commonly used in clinical practice. Whole organ decellularization and its use as a scaffold to regenerate functional organs after loading of scaffold-specific cells have been practiced significantly in recent years. Whole tissues or organs can now be recellularized with the desired stem cells for functional tissue/organ development [4].

The use of DECM as a hydrogel or scaffold has significantly increased in the fields of regenerative medicine, cancer research, and drug and cell delivery [5]. Once a DECM

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hydrogel is prepared, it can be coated onto coverslips/culture plates or onto a solid scaffold (usually porous) prepared through fabrication techniques such as 3D prototype printing, conventional mould method and stereolithographic methods. Coating the porous scaffold prepared from the synthetic biomaterials (e.g., polycaprolactone [PCL]) provides a hydrophilic surface for the cells, with the environment required for the initial cell-matrix interactions, which are of foremost importance for successful cell attachment and growth [6]. The DECM hydrogel coating in 3D porous PCL scaffold has been successfully studied. The preparation of a bioink, a mixture of hydrogel with the cells, is nowadays a hot topic in the field of bioengineering and regenerative medicine. DECM is considered as the ideal biomaterial to use as a bioink. DECM has also been used comprehensively to study stem cell differentiation in *in vitro* models, for example differentiation to osteogenic, neural and renal progenitor cells [7,8].

Despite *in vitro* results from these scaffolds showing promise for better tissue growth and drug release, their actual contributions in the hosts for the better recovery, replacement and normalization of cellular/tissue functions are minimal [9]. The utilization and naturalization of delivered factors, and natural growth of delivered cells without any host immune response are the main concerns for successful tissue engineering [10].

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Injectable DECM gel from full native ECM to regenerate the tissue as demonstrated in the *in vivo* study has been implemented using pericardial matrix gel [11] and tendon hydrogel [12] to address a few of the concerns mentioned above. Recently, a functional porous scaffold prepared by coating a PCL scaffold with a mixture of DECM and sodium alginate, containing the active catalase, has been tested for successful *in vivo* tissue regeneration [13]. The catalase within the scaffold helps to detoxify the hydrogen peroxide released by cells during the metabolic process and protects the cells and tissues to better support tissue growth and angiogenesis [13]. This indicates progress toward successful regenerative medicine.

The concept of using a region-specific ECM is a focus for regenerating specific tissue types. For example, inner meniscus ECM has shown enhancement of differentiation of human bone marrow mesenchymal stem cells to fibrocartilaginous cells, whereas an outer meniscus ECM differentiated human bone marrow mesenchymal stem cells to fibroblast cell types [14].

Meanwhile, decellularized brain ECM improved behavior through the increased expression of neurotrophic proteins. Liver DECM for liver tissue engineering and cardiac DECM for cardiac repair are some additional examples of using specific DECM in bioengineering. Nonspecific, but relevant, types of DECM also show promise in differentiating stem cells and augmenting tissue, similar to those already used DECM [15,16]. Nonspecific ECM has been used to repair organs, but postgraft consequences may ultimately lead to graft rejection [17,18]. Overall, stem cells have the tendency to differentiate into cell types suited for DECM used in bioengineering. The matrix and its compliance are, therefore, very important in signaling pathways, via cell-matrix interactions, in the development of particular tissue types.

The use of naturally derived cross-linkers, for example heparin and photosensitive vitamin B2, to cross-link the ECM and trap a growth factor is a great advancement in tissue engineering models. It helps to reduce the use of non-native cross-linkers and synthetic polymers, which have more cons than pros in tissue regeneration and a high possibility for graft rejections. Heparin also improves endothelialization and minimizes thrombogenesis, allowing for a successful supply of oxygen and nutrients to a growing tissue [19]. However, the ECM microparticles have more binding sites compared with hydrogel and cross-linked ECM and are useful for cell and drug delivery, yet are not considered as a good graft model because of the technical difficulties in adhering them on a deeply localized defect tissue, as well as their dispersion after grafting. The antioxidant properties of DECM itself and the use of antibiotic drugs in the preparation of DECM grafts are some of the indicators that support successful *in vivo* tissue engineering.

Currently, DECM is used in various formats and studied extensively to reveal its potential role in regenerating functional tissue. A whole organ/tissue DECM with mechanical supports, recellularization with stem cells, along with supplements of growth and inducing factors indicate future success for functional organ formation *in vitro*. The implantation of an *in vitro* functional organ to a particular system and the restoration of physiological function by a grafted organ may be achievable thereafter. However, the protocol for preparation of a porous, flexible and spongy scaffold that could mimic the native tissue from pure DECM is still lacking for translation into regenerative medicine. Such a scaffold should have the capacity to maintain its own biomechanical integrity and provide a native-like environment for the cells, while growing in an *in vitro* system to regenerate tissue types according to needs. A soft functional tissue

regenerated on DECM scaffold could be subsequently grafted to restore tissue functions. In addition, for a regenerated hard functional tissue like bone, DECM scaffold should be hardened in a way that mimics native hard tissue that could provide minimum mechanical support. There is much hope that such an ideal 3D DECM scaffold will be invented for functional tissue regeneration and for the restoration of a functional organ after grafting in the near future.

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