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Controlling stem cell behavior with decellularized extracellular matrix scaffolds

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ABSTRACT

Decellularized tissues have become a common regenerative medicine platform with multiple materials being researched in academic laboratories, tested in animal studies, and used clinically. Ideally, when a tissue is decellularized the native cell niche is maintained with many of the structural and biochemical cues that naturally interact with the cells of that particular tissue. This makes decellularized tissue materials an excellent platform for providing cells with the signals needed to initiate and maintain differentiation into tissue-specific lineages. The extracellular matrix (ECM) that remains after the decellularization process contains the components of a tissue specific microenvironment that is not possible to create synthetically. The ECM of each tissue has a different composition and structure and therefore has unique properties and potential for affecting cell behavior. This review describes the common methods for preparing decellularized tissue materials and the effects that decellularized materials from different tissues have on cell phenotype.

1. Introduction

Decellularized tissues are widely used clinically for tissue repair and regeneration. A few tissues such as dermis, small intestinal submuscosa, urinary bladder, and pericardium from humans, pigs, and cows have been decellularized to create commercially available scaffolds such as AlloDerm, CuffPatch™, MatriStem, Pelvicol, and Dura-Guard® [1]. However, in basic research and preclinical studies, numerous tissues and organs have now been decellularized and used in various regenerative medicine applications [2]. The extracellular matrix (ECM), which remains after decellularization, plays a crucial role as a structural support for tissue as well as a source of biochemical and biophysical cues for the cells that reside within it. Through these two roles the ECM directs cell proliferation, migration, differentiation, and behavior [3]. The ECM of each tissue provides a unique tissue specific microenvironment for resident cells. This cell niche has been adapted by nature to provide the cells with the structure and biochemical cues that are required for their function [4]. It has therefore been hypothesized that decellularized tissue materials should have distinct effects on cell differentiation based on which tissue the material was sourced. Using nature's tailored cell niches to direct cells to differentiate toward specific cell lineages has become a popular avenue for tissue engineering studies. By understanding decellularized tissues' effects on cell differentiation more thoroughly, this technology could provide a useful platform for controlling cell fate and generating regenerative therapies.

This manuscript will review the different methods for creating decellularized tissue materials and the studied effects of these materials on cell behavior, with a focus on differentiation. These materials can be in the form of entire organ scaffolds, slices or blocks of ECM, hydrogels, and coatings, and have been derived from the following tissues: lung, liver, kidney, heart, central nervous system, adipose, tendon, skeletal muscle, cartilage, bone, nucleus pulposus, uterus, corneal stroma, musculofascia, trachea, and dermis (Fig. 1).

2. Preparation of decellularized materials

2.1. Decellularization methods

The goal of decellularizing tissues is to maintain all of the structural and biochemical cues in the ECM, but remove the cellular

Abbreviations: ASC, adipose derived stem cell; ECM, extracellular matrix; EDC, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide; ESC, embryonic stem cell; hASC, human adipose derived stem cell; hESC, human embryonic stem cell; hMSC, human mesenchymal stem cell; iPSC, induced pluripotent stem cell; mESC, murine embryonic stem cell; MSC, mesenchymal stem cell; NHS, N-hydroxysulfosuccinimide; NSC, neural stem cell; PDMS, polydimethylsiloxane.

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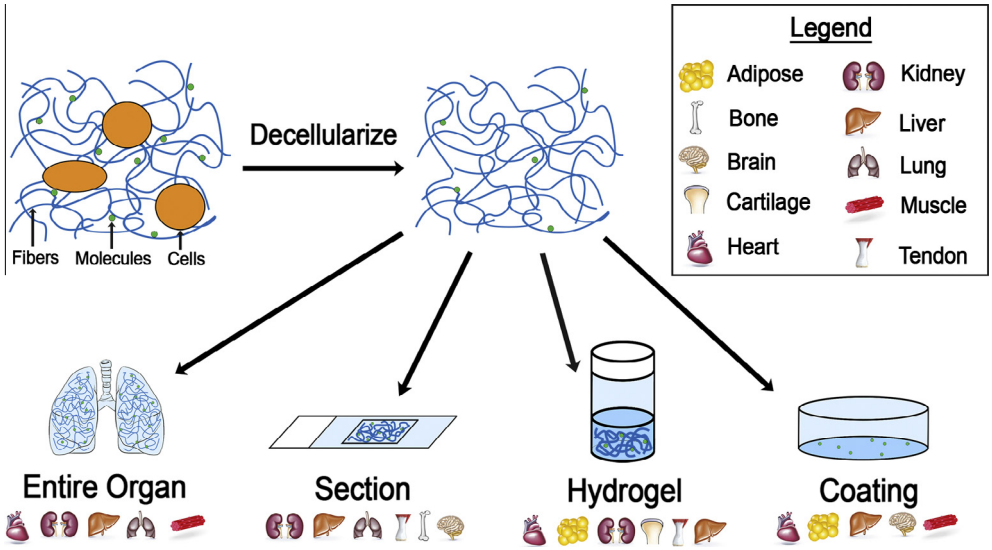

Fig. 1. Decellularized tissue materials made from various tissues. After removing the cellular contents of the native tissue, the extracellular matrix remains and can be left unprocessed as an entire organ, or be processed into a section, hydrogel, or coating. The tissues that have been processed for the purpose of testing cell differentiation effects are shown below each material category.

components that cause an adverse inflammatory response in the host and hinder regeneration [2]. The different methods used for decellularization utilize mechanical, chemical, enzymatic, or detergent approaches [2,3]. The protocols for decellularizing different tissues have been reviewed thoroughly, but there is no consensus for the best protocols to use with each tissue [2,5,6]. When choosing decellularization protocols it is important to realize that one method does not work for every tissue, since tissues vary in ECM density, cell density, and basic morphology [2]. Other reviews have more thoroughly examined different decellularization protocols and the effects of the commonly used methods. The reader is referred to several excellent reviews in this area [1,2,7]. Briefly, freeze–thawing can affect the ultrastructure of the ECM, pressure techniques may affect mechanical properties of the ECM fibers, alkaline and acidic solutions can degrade components of the ECM, ionic detergents (like sodium dodecyl sulfate) disrupt non-covalent bonds between proteins and can be problematic to remove from the matrix, and alcohol has been shown to crosslink collagen in the ECM increasing its stiffness [1,2,6–8]. All of these factors can also affect how cells interact with the materials because they change the environment with which the cells are in direct contact. Therefore, care must be taken to choose the ideal decellularization protocol for each application because it can affect the success of directing cells toward a specific lineage [9]. In short, considerations must be made in designing and selecting the optimal decellularization process before the materials are manipulated into their final form.

2.2. Material preparation

While decellularized tissue materials can be left as an intact whole organ, they can also be further processed by cutting into small sections or blocks, or digesting into a liquid to form a hydrogel or coat a substrate. Each of these categories of materials has been used with multiple tissues and may provide different benefits. Keeping the whole organ intact during the decellularization process is especially useful when the goal is to engineer a transplantable organ. Decellularizing cadaveric organs maintains the macro and micro architectures of the organ along with the natural ECM composition, thereby providing a platform for recellularization and regeneration of the organ [1]. Perfusion decellularization has been used on lungs, livers, kidneys, and hearts from rats, mice, pigs, and humans to create acellular organ scaffolds [10–19]. The process of perfusion decellularization requires flowing the decellularizing reagents through native arteries at physiological pressures in order to enter the innate blood vessel system and reach every cell [20]. Thin tissues, like mouse tibialis anterior, and ones that do not have easily accessible blood vessel systems, like cartilage, may use submersion and agitation techniques [21,22].

If an entire organ is not required for a study, then thin sheets of the decellularized organ can be used as a more convenient method for testing the ECM's effect on cells while still maintaining the tissue's architecture and biochemical composition. The two main approaches for creating these slices are either to first decellularize the entire organ then cut it into slices or do the opposite by sectioning the unprocessed organ and then decellularizing the slices. To section the organ after decellularization, the organ must first be stiffened either by embedding the organ in agarose, OCT freezing medium, or paraffin [9–12,23,24]. However, the most common approach to create decellularized sections is to first cut the tissue into the desired thickness and then decellularize the slices of tissue. This approach has been used for tendon, brain, kidney, lung, liver, corneal stroma, and dermis [25–30]. Decellularized materials can also be created with sizes between whole organ decellularization and thin slices, for example $4 \times 4 \times 3$ mm cubes of bone [31] or $4 \times 2 \times 0.5$ cm pieces of musculofascial tissue [32].

Decellularized tissue can also be processed further into hydrogels. Hydrogels are commonly used with the goal of injecting into sites of injury to promote regeneration, but are also a favorable platform for cell culture because they can be made in larger quantities and designed to retain cells more easily than ECM sections. The methods of preparation vary greatly depending on the application and the characteristics that need to be modified for an experiment. The strategies include using decellularized matrix as the only component of the hydrogel, using cross-linking agents, or creating composites with additional materials. A common method for making hydrogels with ECM as the only element in the material requires lyophilizing, milling, and digesting the ECM with pepsin. At this point the ECM can be brought to body temperature to form a hydrogel [30,33–37]. These materials have been used alone *in vivo* [37–40] or to create a 2D and 3D culture environments *in vitro* [36,40–42]. Another approach is to continue manipulating