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# Decellularized tissue and cell-derived extracellular matrices as scaffolds for orthopedic tissue engineering

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ABSTRACT

The reconstruction of musculoskeletal defects is a constant challenge for orthopedic surgeons. Musculoskeletal injuries such as fractures, chondral lesions, infections and tumor debulking can often lead to large tissue voids requiring reconstruction with tissue grafts. Autografts are currently the gold standard in orthopedic tissue reconstruction; however, there is a limit to the amount of tissue that can be harvested before compromising the donor site. Tissue engineering strategies using allogeneic or xenogeneic decellularized bone, cartilage, skeletal muscle, tendon and ligament have emerged as promising potential alternative treatment. The extracellular matrix provides a natural scaffold for cell attachment, proliferation and differentiation. Decellularization of in vitro cell-derived matrices can also enable the generation of autologous constructs from tissue specific cells or progenitor cells. Although decellularized bone tissue is widely used clinically in orthopedic applications, the exciting potential of decellularized cartilage, skeletal muscle, tendon and ligament cell-derived matrices has only recently begun to be explored for ultimate translation to the orthopedic clinic.



**Contents**



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## 1. Introduction

Orthopedic injuries and degenerative diseases are common reasons for emergency room and office visits in the United States. There are more than 33 million orthopedic injuries each year (Mamaril et al., 2007). In the United States alone the estimated incidence of long bone fractures is about 1,500,000 per year (Friedlaender et al., 2001), anterior cruciate ligament (ACL) injuries affect more than 120,000 athletes every year (Hewett et al., 2013) and worldwide estimates for symptomatic osteoarthritis are 9.6% of men and 18% of women greater than 60 years old of age (Woolf and Pfleger, 2003). Loss of musculoskeletal tissue and function can occur as a result of athletic or traumatic injuries, degenerative changes, infections or tumor resection in bone, cartilage, skeletal muscle or tendon and ligament. Occasionally these injuries may result in large boney non-unions or large tissue voids requiring repair with either autologous grafts or allografts. However, autologous and allogeneic grafting techniques each have their own benefits and disadvantages. Autologous grafts have a low risk of transmitting diseases, good histocompatibility and are nonimmunogenic (Gazdag et al., 1995). Unfortunately, there is a limit to the quantity of autologous graft tissue that can be harvested before compromising the donor-site. Although allografts may eliminate donor-site morbidity and decrease operating time, they are associated with the risk of severe immune response, disease transmission and slower integration with native tissue compared to autologous grafts (Gazdag et al., 1995). For these reasons, there is a growing interest in engineering musculoskeletal tissues that can avoid donor site complications, are available in large quantities and have good histocompatibility.

During the past decade, there has been increased interest in creating biological scaffolds composed of extracellular matrix (ECM) derived from the decellularization of tissues or organs. The use of decellularized ECM from donor tissue has been utilized in the repair of skin (Livesey et al., 1995), bladder (Sutherland et al., 1996), heart valve (Dohmen et al., 2011) and small intestinal submucosa (Badylak et al., 2011). Furthermore, several commercialized decellularized scaffolds have received FDA approval for use in humans, including dermis tissue (Alloderm®; LifeCell), porcine heart valves (Synergraft®; Cryolife) and porcine urinary bladder (Urinary bladder matrix; ACell) (Gilbert et al., 2006; Yang et al., 2008). In preclinical trials, decellularized scaffolds made from porcine small intestinal submucosa (SIS) have been used in orthopedic surgical applications for repair of rotator cuff (Dejardin et al., 2001), anterior cruciate ligament (ACL) (Badylak et al., 1994, 1999) and Achilles tendon (Badylak et al., 1995). Although tissue-derived biologic scaffolds are commonly used to repair non-homologous anatomic sites, studies of skeletal muscle and liver tissue engineering have suggested that biologic scaffolds derived from site-specific homologous tissues may be better suited for constructive tissue remodeling than non-site specific tissue sources (Sellaro et al., 2007; Zhang et al., 2009). This has motivated the development of orthopedic tissue engineering strategies that utilize biologic scaffolds derived from specific homologous orthopedic tissues.

ECM components differ between bone, cartilage, skeletal muscle, ligament and tendon. The use of homologous orthopedic tissues as scaffolds for tissue engineering would provide tissue-specific ECM compositions, which can influence the behavior of resident and/or transplanted cells. ECM is a product of cells that functions to maintain tissue and organ structure, organization and function. It is a complex network of proteins and polysaccharides forming an intricate meshwork within tissue that interacts with the resident cells to regulate cell behavior, such as migration, proliferation and differentiation. The ECM exists in a state of dynamic equilibrium with its resident cells and is constantly being built, reshaped and degraded in response to changing environmental conditions and to cellular, tissue and organ demands (Bissell et al., 1986). Musculoskeletal tissues require proper organization of resident cells and ECM to withstand loads and produce adequate forces for everyday activities. Decellularized tissue explants may provide a naturally occurring three-dimensional scaffold with tissue-specific orientations of ECM molecules that are not easily created synthetically in the laboratory. This manuscript provides an overview of biological scaffolds created from decellularized ECM of musculoskeletal tissues and in vitro cell-derived matrices and their use in in vitro and in vivo applications of tissue engineering.

## 2. ECM immunogenicity

The decellularization process is crucial for eliminating cellular components and antigenicity from tissue explants in order to avoid disease transmission, reduce inflammatory and immune responses towards the scaffold and decrease the risk of rejection after implantation, particularly with xenogeneic or allogeneic donor tissues (Badylak et al., 2011). Unlike cellular material, ECM components are predominantly conserved among species and are therefore well tolerated when used as allografts or xenografts (Bernard et al., 1983; Constantinou and Jimenez, 1991; Exposito et al., 1992). The ideal decellularization technique would remove cellular remnants without the destruction of the original tissue architecture or the removal of ECM components, and thus maintaining the mechanical properties of the natural ECM.

DNA and the cell surface oligosaccharide molecule α-Gal (Galα1,3-Galβ1-4GlcNAc-R) also known as “Gal epitope” are two common antigens known to trigger an inflammatory response against biological scaffolds (Badylak and Gilbert, 2008). In most tissues, cells are embedded within a dense ECM making it difficult for complete removal of cellular material. In fact, most commercially available decellularized biological scaffold material, such as Restore™, GraftJacket™, and TissueMend™, contain trace amount of remnant DNA that are less than 300 bp in length (Derwin et al., 2006; Gilbert et al., 2009; Zheng et al., 2005). Although the majority of the commercially available biologic scaffolds contain DNA remnants, the clinical efficacy of these scaffolds has been largely positive (Badylak and Gilbert, 2008). Therefore, the small amount of DNA remaining may not be enough to elicit an immune response or adversely affect the remodeling process. There may be a threshold amount of cellular material that is required to trigger a severe immune response, and further studies are needed to determine this threshold.

Gal epitopes are cell surface molecules that are commonly found in most species except humans and Old World monkeys due to mutations in the α1,3-galactosyl-transferase gene (Badylak and Gilbert, 2008). As a result of the lack of Gal epitopes, humans produce a large amount of anti-Gal antibodies due to constant exposure to intestinal bacteria carrying Gal epitopes (Badylak and Gilbert, 2008). This is particularly important when creating decellularized biological scaffolds using xenografts for human implantation. Gal epitopes have been found in porcine ACL (Stone et al., 2007a), cartilage (Stone et al., 1998), SIS-ECM (McPherson et al., 2000) and bioprosthetic heart valves (Konakci et al., 2005). Konakci et al. demonstrated that patients receiving porcine bioprosthetic heart valves have a xenograft-specific immune response with elevated levels of cytotoxic IgM antibodies directed against α-Gal. The authors speculate that this may contribute to the failure



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