Acta Biomaterialia xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Review article

Functionality of decellularized matrix in cartilage regeneration: A comparison of tissue versus cell sources

Yu Sun [a,b], Lianqi Yan [b], Song Chen [c], Ming Pei [a,d,e,*]

[a] Stem Cell and Tissue Engineering Laboratory, Department of Orthopaedics, West Virginia University, Morgantown, WV 26506, USA
[b] Department of Orthopaedics, Orthopaedics Institute, Subei People's Hospital of Jiangsu Province, Yangzhou, Jiangsu 225001, China
[c] Department of Orthopaedics, Chengdu Military General Hospital, Chengdu, Sichuan 610083, China
[d] Exercise Physiology, West Virginia University, Morgantown, WV 26506, USA
[e] WVU Cancer Institute, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV 26506, USA

ARTICLE INFO

Article history:
Received 9 January 2018
Received in revised form 20 April 2018
Accepted 23 April 2018
Available online xxxx

Keywords:
Cartilage regeneration
Chondrocyte
Chondrogenic differentiation
Decellularized matrix
Extracellular matrix
Proliferation
Stem cell

ABSTRACT

Increasing evidence indicates that decellularized extracellular matrices (dECMs) derived from cartilage tissues (T-dECMs) or chondrocytes/stem cells (C-dECMs) can support proliferation and chondrogenic differentiation of cartilage-forming cells. However, few review papers compare the differences between these dECMs when they serve as substrates for cartilage regeneration. In this review, after an introduction of cartilage immunogenicity and decellularization methods to prepare T-dECMs and C-dECMs, a comprehensive comparison focuses on the effects of T-dECMs and C-dECMs on proliferation and chondrogenic differentiation of chondrocytes/stem cells *in vitro* and *in vivo*. Key factors within dECMs, consisting of microarchitecture characteristics and micromechanical properties as well as retained insoluble and soluble matrix components, are discussed in-depth for potential mechanisms underlying the functionality of these dECMs in regulating chondrogenesis. With this information, we hope to benefit dECM based cartilage engineering and tissue regeneration for future clinical application.

Statement of Significance

The use of decellularized extracellular matrix (dECM) is becoming a promising approach for tissue engineering and regeneration. Compared to dECM derived from cartilage tissue, recently reported dECM from cell sources exhibits a distinct role in cell based cartilage regeneration. In this review paper, for the first time, tissue and cell based dECMs are comprehensively compared for their functionality in cartilage regeneration. This information is expected to provide an update for dECM based cartilage regeneration.

Contents

* Corresponding author at: Stem Cell and Tissue Engineering Laboratory, Department of Orthopaedics, West Virginia University, PO Box 9196, One Medical Center Drive, Morgantown, WV 26506-9196, USA.
E-mail address: mpei@hsc.wvu.edu (M. Pei).

https://doi.org/10.1016/j.actbio.2018.04.048
1742-7061/

1. Introduction

Cartilage is an avascular load-bearing tissue consisting of chondrocytes distributed throughout a dense extracellular matrix (ECM), which is a structurally complex environment composed of many components including collagen, glycosaminoglycans (GAGs), proteoglycans and other elements such as fibronectin and laminin [1,2]. The low cellularity and avascular properties of cartilage result in the limited potential for self-repair following cartilage injury [3]. Current repair strategies, including microfracture, osteochondral autograft or mosaicplasty and autologous chondrocyte implantation, have achieved success in regenerating functional cartilage [4–7]. However, the limited availability of graft tissue, donor site morbidity, graft subsidence at the surface and fibrocartilage formation affect the quality of repair [4,8–10]. Recently, cartilage tissue engineering, combining cartilage-forming cells (chondrocytes and stem cells), growth factors and scaffolds, has provided promising approaches for cartilage regeneration [11,12].

As a basic element in cartilage tissue engineering, scaffolds play an important role in providing structural support and a micromechanical environment as well as biochemical cues for cell growth and chondrogenic differentiation. Numerous synthesized and natural materials, such as poly(l-lactic acid), poly(l-lactic-co-glycolic acid) (PLGA), collagen derivatives and fibrin glue [13], have been used as scaffolds for cartilage regeneration [12,14,15]. Increasing evidence has shown that ECM can provide not only physical support but also biological signals to cells that can facilitate cell attachment, proliferation and differentiation [16–19]. Decellularized ECMs (dECMs) from various tissues, such as heart, skin, bladder, nerves and tendons, have been used for tissue engineering applications with promising results [20,21]. Tissue-specific ECM derived from target tissues was reported to promote cell proliferation and lineage-specific differentiation through retaining biophysical and biochemical cues within native tissues [22,23]. For instance, dECMs derived from cartilage tissues (T-dECMs) have been extensively investigated as biological scaffolds for cartilage engineering due to their inherent components and unique structure and micromechanical properties, which provide a niche-like nanostructured microenvironment to aid in chondrogenesis [24–27].

Recent evidence showed that, different from T-dECMs that induce chondrogenic differentiation directly, dECMs derived from chondrocyte/stem cells (C-dECMs) benefited cartilage regeneration by promoting expanded cell proliferation and chondrogenic potential [25,27–32]. However, few review papers are available comparing the differences between these two dECMs when they serve as substrates for cartilage regeneration. In this review, cartilage immunogenicity and decellularization methods of T-dECMs and C-dECMs are introduced followed by a comprehensive comparison of the roles of T-dECMs and C-dECMs on proliferation and chondrogenic differentiation of cartilage-forming cells *in vitro* and *in vivo*. Also discussed are the potential influential factors within dECMs, including microarchitecture characteristics and micromechanical properties as well as retained insoluble and soluble matrix components such as collagen, GAGs and bioactive factors, which may contribute to differences between these two dECMs in regulating chondrogenesis (Fig. 1).

2. Decellularization approach

Cartilage tissue properties, such as avascularity and high density, are unique which render its decellularization more complicated than other connective tissues.

2.1. Cartilage immunogenicity and necessity of decellularization

The avascular and dense nature of articular cartilage has led to the prediction that articular cartilage is immunoprivileged, whereby the cartilage's immune system is limited because the cells are deeply encapsulated within the matrix and not easily reachable by immune cells [2,33–38]. Allogeneic cartilage transplantation from cadaveric origin is well tolerated and clinical results have validated a high success rate (60–95%) as construed by graft survival and good/excellent patient evaluations [38]. Animal studies also showed that chondrocytes of cartilage grafts maintained within their matrix are nearly nonimmunogenic [39]. Moreover, engineered cartilage using allogeneic chondrocytes with natural and synthetic scaffolds demonstrated successful repair of cartilage defects without significant signs of rejection and immune response [40-42].

However, the immunoprivileged nature of cartilage has been challenged by other findings showing that both chondrocytes and their embedded ECM contain antigens and elicit varying degrees of immune reactions [34,38,43–45]. Chondrocytes are liable to be attacked by natural killer cells [43,46] and also express major histocompatibility class (MHC) II antigens to trigger CD4 T lymphocytes and provoke cell or antibody-mediated immune responses [47–49]. Various degrees of immune response were reported after implantation of allogeneic chondrocytes grown on engineered scaffolds in osteochondral defects [50,51]. Interestingly, physically devitalized cartilage fragments supported chondrogenesis without significant inflammation *in vivo* [52]. Therefore, a threshold amount of cellular material remains in implanted scaffolds that can trigger a severe immune response. Eliminating donor cells through the decellularization processes is thought to be desirable to reduce the risk of immune response from recipients, particularly for xenogeneic or allogeneic donor tissues [53].

Furthermore, due to the intrinsic nature of cartilage tissue that consists of dense ECM with nanosized pores, chondrocytes/stem cells are unable to infiltrate and repopulate a cartilage scaffold in its native form. The matrix alone may not be adequate for tissue regeneration, while the low porosity limits cell infiltration which, in turn, limits new matrix deposition. Therefore, the decellularization process is necessary to remove cell components and immunogenic antigens as well as to improve reseeded cell infiltration for

Please cite this article in press as: Y. Sun et al., Functionality of decellularized matrix in cartilage regeneration: A comparison of tissue versus cell sources, Acta Biomater. (2018), https://doi.org/10.1016/j.actbio.2018.04.048