Opinion Statement of the Effect of Mechanical Stress on Cartilage Tissue Engineering

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Abstract: Articular cartilage is characterized by its poor capacity for self-repair. Once articular cartilage is injured and defected, it cannot be spontaneously repaired and finally develops osteoarthritis (OA). OA is a major leading cause of severe activity limitations and disability, resulting in worldwide socio-economical burden. At present, there is no established therapy for adequate repair of damaged articular cartilage. Researchers have therefore attempted to establish the cartilage tissue engineering as an effective alternative treatment of cartilage repair. However, the articular cartilage repair still remains a clinical and scientific challenge.

In cartilage tissue engineering, it is believed that cell source, scaffold and growth factors are three key factors for the desired result of cell therapy for the damaged cartilage repair. However, increasing evidence is showing that these key factors are not enough and other factors may be required to achieve the optimal outcome. Since normal articular cartilage is always subjected to mechanical stress in daily activities, mechanical stress has attracted much attention as fourth key factor in cartilage tissue engineering. However, the real impact of mechanical stress on cartilage tissue engineering is far from complete understanding.

In this review, we summarize the accumulating knowledge of the effect of mechanical stress on cartilage tissue engineering and discuss about the challenges for the future.

Keywords: Articular cartilage, tissue engineering, mechanical stress, and chondrocytes.

INTRODUCTION

Articular cartilage, consisting of chondrocytes and hydrated extracellular matrix (ECM) such as type II collagen, glycosaminoglycan (GAG) and water is characterized by its poor capacity of self-repair due to the lack of blood supply and nerves [1]. Unfortunately, once articular cartilage is injured and defected, it cannot be spontaneously repaired [2]. Indeed, the damage of articular cartilage caused by trauma often accelerates its degenerative process and finally develops osteoarthritis (OA) [3].

OA, one of the most common joint diseases, seriously interferes with activity of daily living (ADL) and quality of life (QOL). Indeed, approximately 20 million of people in the United States are affected by OA [4]. The World Health Organization (WHO) estimates that approximately one in every ten people over 60 years in the world suffers OA [5]. OA is therefore one of the major leading causes of severe activity limitations and disability, thus resulting in worldwide socio-economical burden.

Once severe OA is established, currently available exclusive treatment is prosthetic joint replacement. However, prosthetic joint replacement has just "replaced", not "repaired" the involved joint, and it has several potential problems such as loosening and infection [6-9]. In turn, to establish the optimal treatment for OA, repair of the damaged cartilage is essential. A number of therapeutic techniques for damaged cartilage have been developed, *e.g.*, drilling [10], microfracture [11], osteochondral graft [12], periosteal graft [13], or autologous chondrocyte implantation (ACI) [14]. However, a successful articular cartilage repair still remains a clinical and scientific challenge.

In turn, the progress in the field of tissue engineering has shown the possibilities for the treatment of cartilage defects [14]. We have summarized representative animal studies of tissue engineering for articular cartilage defect using chondrocytes (Table **1**) [15-26]. Since the pioneering work of the ACI, a diversity of cell therapies has been invented [27-29].

In 1960s, it was found that chondrocytes cultured in monolayer condition rapidly dedifferentiated and lost their characters [30, 31]. After the dedifferentiation of chondrocytes, the cells lose the ability of maintaining the cartilage-specific ECM such as GAG and type II collagen, whereas they acquire fibroblastic morphology and mainly synthesize type I collagen [32-34]. Subsequent studies [34, 35] have revealed that three-dimensional (3D) culture with scaffold (*i.e.*, collagen gel) reduces this dedifferentiation process (Fig. **1**). However, 3D culture could not completely eliminate cell-dedifferentiation. Additional modifications which ameliorate the quality of tissue-engineered cartilage were required. Several factors were tested to increase the quality of tissue-engineered cartilage [32-44]. In turn, it is believed that cell source (*i.e.*, mesenchymal cell) [35-39], scaffold (*i.e.*, collagen gel, agarose gel) [32-34] and growth factors (*i.e.*, basic fibroblast growth factor (bFGF), bone morphogenetic protein-2, insulin-like growth factor-I, transforming

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Table 1. Representative Animal Studies of Tissue Engineering for Articular Cartilage Defect Using Chondrocytes

Author	Cells	Graft	Animal	Joint	Scaffold	Main findings	Reference
Chesterman PJ	chondrocytes	allo	rabbit	shoulder	free	fibrous tissue	[15]
Wakitani S	chondrocytes	allo	rabbit	knee	collagen gel	hyaline-like tissue	[16]
Hendrickson DA	chondrocytes	allo	horse	knee	fibrin glue	hyaline-like tissue	[17]
Kawamura S	chondrocytes	allo	rabbit	knee	collagen gel	hyaline-like tissue	[18]
Wakitani S	chondrocytes	allo	rabbit	knee	collagen gel	hyaline-like tissue	[19]
Katsube K	chondrocytes	allo	rabbit	knee	collagen gel	hyaline-like tissue	[20]
Grigolo B	chondrocytes	auto	rabbit	knee	hyaluronic acid	hyaline-like tissue	[21]
Mierisch CM	chondrocytes	allo	rabbit	knee	alginate beads	mix of hyaline and fibrous	[22]
Lee CR	chondrocytes	auto	canine	knee	collagen gel	mix of hyaline and fibrous	[23]
Willers C	chondrocytes	auto	rabbit	knee	collagen gel	hyaline-like tissue	[24]
De Franceshi L	chondrocytes	auto	rabbit	knee	collagen gel	fibrocartilagenous tissue	[25]
Dorotka R	chondrocytes	auto	ovine	knee	collagen gel	hyaline-like tissue	[26]

growth factor-β1) [40-44] are three key factors for the better quality of tissue-engineered cartilage, which govern the result of cell therapy for the damaged cartilage repair [32-44]. However, accumulating results have shown that these key factors are not enough and additional factors may be required to achieve the optimal outcome.

Normal articular cartilage is always subjected to mechanical stress in daily activities. In turn, mechanical stress has attracted much attention as fourth key factor in cartilage tissue engineering. However, the effect of mechanical stress on cartilage tissue engineering is far from complete understanding (Fig. **2**). In this review, we summarize the accumulating knowledge of the effect of mechanical stress on cartilage tissue engineering and discuss the challenges for the future.

MECHANICAL STRESS ON ARTICULAR CARTILAGE IN PHYSIOLOGICAL CONDITION

Articular cartilage is always exposed to various types of mechanical stimuli. During routine activities under normal physiological conditions, mechanical stimuli on articular cartilage can exert peak dynamic mechanical stresses of up to 18 megapascals (MPa) [45]. Furthermore, static physio-

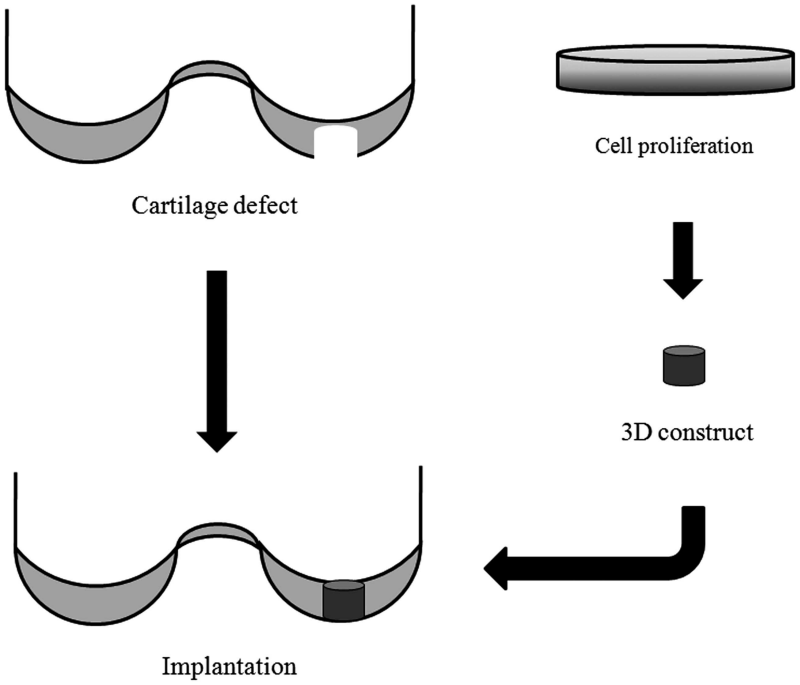

Fig. (1). Schematic presentation of cartilage repair using tissue-engineered 3D constructs.

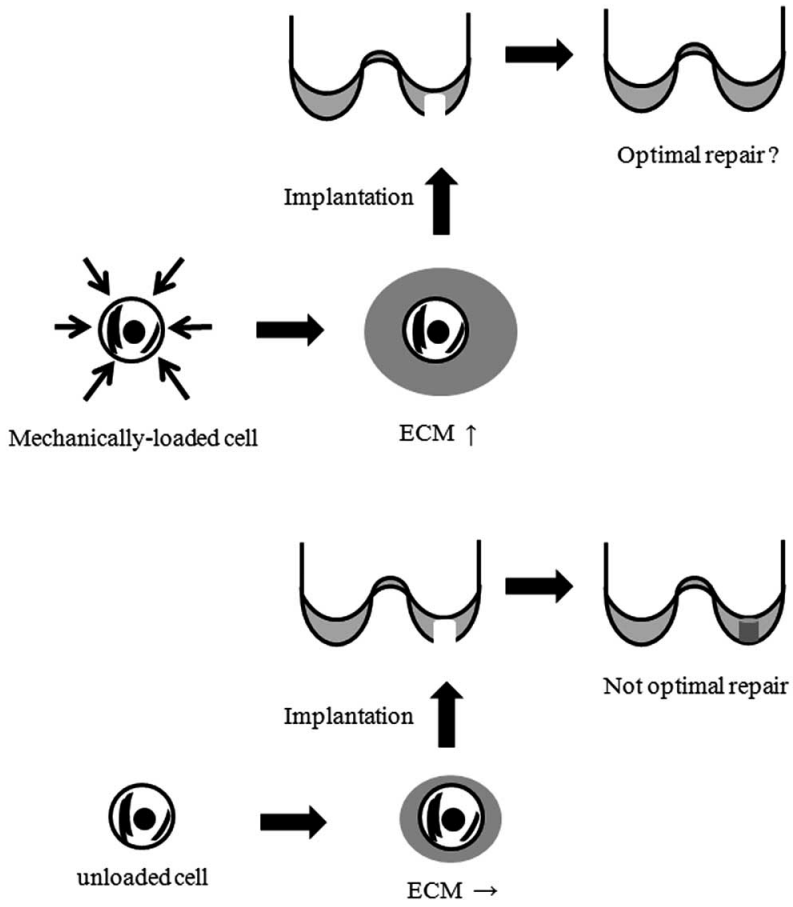

Fig. (2). Schematic presentation of cartilage repair using tissue-engineered chondrocytes. The quality of tissue-engineered chondrocytes before implantation governs the result of cell therapy for the damaged cartilage repair. Better quality of tissue-engineered chondrocytes [extracellular matrix (ECM) rich] under mechanical stress compared to those without mechanical stress might contribute to the optimal repair.

logical stresses applied to knee joints for 5-30 min can result in approximately compressive strains of 40% in certain knee cartilages [46]. On the other hand, *in vivo* joint immobilization and reduction of joint loading resulted in a rapid loss and degradation of ECM content, whereas moderate exercise stimulated ECM synthesis [47-49]. Accordingly, it is considered that mechanical stress plays an important role in cartilage homeostasis [50-52] and the lack of appropriate mechanical stress in previous culture systems in cartilage tissue engineering might be one of the causes of failure.

Mechanical stress can be divided into roughly two types; static and dynamic loading. The former is stimulus represented by standing; the latter is stimulus represented by walking or running. In recent studies [60-80], many researchers have attempted to determine the influence of these mechanical stresses on cartilage tissue engineering.

STATIC COMPRESSIVE LOAD

Previous studies have revealed that the ECM synthesis by chondrocytes under static compression load varied depending on the time length of stimulation [53, 54, 60, 61].

In particular, Ragan *et al.* [53] showed that both aggrecan and type II collagen mRNA expressions were up-regulated during the first 30 min of static compression, whereas they were significantly down-regulated 4 h to 24 h after the initial static compression. Valhmu *et al.* [54] reported that aggrecan mRNA expression temporarily increased 1 h after the initial static compression, however after 24 h long-term static compression had no significant change on it. Similarly, Fitzgerald *et al.* [61] demonstrated that ECM proteins were increased 2-3 fold during the first 8 h of 50% static compression. However, after 24 h of the static compression, ECM proteins were down-regulated, whereas ECM proteinases were highly up-regulated.

It remains unknown why chondrocytes showed differential response depending on the stimulating time.

DYNAMIC COMPRESSIVE LOAD

A number of studies [51, 52, 55-60, 63-73, 75-78, 80] have demonstrated that dynamic compressive loads enhanced the cartilage-specific ECM synthesis by chondrocytes in 3D scaffolds (*i.e.,* collagen gel, agarose gel) or cartilage explants. Most researchers in these studies have reported that amplitude (*i.e.,* 5-15%) and/or frequency (*i.e.,* 0.01-1 Hz) of dynamic compressive road govern ECM synthesis by chondrocytes.

Specifically, Buschmann *et al*. [57] demonstrated that 6% maximum strain of cyclic compression elevated GAG synthesis by chondrocytes embedded in agarose gel at 0.01-1 Hz. Elder *et al*. [63] demonstrated that low amplitude cyclic compression at 0.33 Hz promoted GAG synthesis of mesenchymal cells from chick limb bud embedded in agarose gel. Furthermore, we also found that cyclic compressive loading of 5% amplitude in cycles of 3s stimulated cartilage-specific ECM synthesis by chondrocytes embedded in type I collagen gel [76].

In contrast, only a few researchers reported that dynamic compressive loads were ineffective [58-60, 67]. Lee *et al*. [58] found that 15% dynamic compressive loading inhibited PG synthesis by chondrocytes embedded in agarose gel at 0.3 Hz and had no effect on it at 3 Hz, whereas stimulated it at 1 Hz. Hunter *et al*. [60] also demonstrated that 25% dynamic compressive loading at 1 Hz had no effect for cartilage-specific gene expressions in 3D collagen gel.

The cause of this discrepancy remains to be determined. However, the differences of animal species, experimental conditions or lack of strict control have been suggested as likely explanations [67]. Thus, the optimal conditions, (*i e.*, frequency, amplitude or timing etc.) for up-regulation of cartilage-specific ECM synthesis by chondrocytes to achieve "optimal tissue-engineered cartilage" remains to be determined. For the moment, most researchers seem that dynamic compressive loads with moderate frequency (0.01-1 Hz) and low amplitude (up to 15% peak to peak compression) achieve the best results.

Furthermore, recent studies [62, 81-86] have also revealed how mechanical stimulation can act on chondrocytes. Mechanical stimulation is converted to biochemical signal *via* mechanotransduction, which results in the activation of intracellular signaling pathways such as mechanoreceptors (*i e.*, integrins) [81], ion channels (slow conductance Ca^{2+} sensitive K^+ and stretch-activated ion channels) [82], soluble mediators [bFGF, interleukin-4 (IL-4)] [83, 84], and intracellular protein kinases (mitogen-activated protein kinase (MAPK) family) [62, 85]. Consequently, these intracellular signaling pathways modulate various biochemical activities in chondrocyte behavior. Salter *et al*. have demonstrated that integrin-associated signaling pathways, activation of stretch-activated ion channels and autocrine/paracrine activity of IL-4 are involved in the cellular response of human articular chondrocytes cultured in monolayer condition to dynamic load [86]. Further investigation to clarify the precise mechanisms of signaling pathways activated by mechanical stress might contribute to achieve a "better tissue-engineered cartilage".

SUMMARY AND FUTURE VISION

Many cells in our body are exposed to mechanical stress during physiological activities and respond to them in different ways. Especially, tissues which function as a supportive tissue, *i e.*, bone or cartilage use this stimulation during tissue formation and maintenance. As mentioned above, a number of studies have shown the effects and mechanisms of mechanotransduction in cartilage or three dimensional engineered tissues. Furthermore, these biological responses to mechanical stimulation are thought to enhance cartilage formation or regeneration. In turn, mechanical stimulation of a cultured tissue is thought to be a feasible strategy to develop a new and most effective cartilage therapy. Further studies are needed to elucidate precise mechanism as well as optimal conditions of mechanical stress for "best tissue-engineered cartilage".

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