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A human osteoarthritis osteochondral organ culture model for cartilage tissue engineering

P. Yeung [a], W. Zhang [a], X.N. Wang [a], C.H. Yan [b], B.P. Chan [a, *]

[a] Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong
[b] Department of Orthopaedics & Traumatology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

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ABSTRACT

Rationale: In vitro human osteoarthritis (OA)-mimicking models enabling pathophysiological studies and evaluation of emerging therapies such as cartilage tissue engineering are of great importance.
Objective: We describe the development and characterization of a human OA osteochondral organ culture. We also apply this model for evaluation of the phenotype maintenance of a human MSC derived engineered cartilage, as an example of emerging therapeutics, under long term exposure to the OA-mimicking environment. We also test the sensitivity of the model to a series of external factors and a potential disease-modifying agent, in terms of chondrogenic phenotype maintenance of the engineered cartilage, under OA-mimicking environment.
Method: Excised joint tissues from total knee replacement surgeries were carved into numerous miniaturized and standardized osteochondral plugs for subsequent OA organ culture. The organ cultures were characterized in detail before being co-cultured with a tissue engineered cartilage. The chondrogenic phenotype of the tissue engineered cartilage co-cultured in long term up to 8 weeks under this OA-mimicking microenvironment was evaluated. Using the same co-culture model, we also screened for a number of biomimetic environmental factors, including oxygen tension, the presence of serum and the application of compression loading. Finally, we studied the effect of a matrix metalloprotease inhibitor, as an example of potential disease-modifying agents, on the co-cultured engineered cartilage.
Results: We demonstrate that cells in the OA organ culture were viable while both the typical chondrogenic phenotype and the characteristic OA phenotype were maintained for long period of time. We then demonstrate that upon co-culture with the OA-mimicking organ culture, the engineered cartilage initially exhibited a more fibrocartilage phenotype but progressively reverted back to the chondrogenic phenotype upon long term co-culture up to 8 weeks. The engineered cartilage was also found to be sensitive to all biomimetic environmental factors screened (oxygen tension, serum and compression). Moreover, under the effect of a MMP inhibitor, the chondrogenic phenotype of engineered cartilage was better maintained.
Conclusion: We demonstrated the development of a human OA osteochondral organ culture and tested the feasibility and potential of using this model as an in vitro evaluation tool for emerging cartilage therapies.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease, which affects a large number of people across the globe. It affects multiple joints including the knees, hips, ankles, elbows and fingers [1,2]. The socioeconomic burden of OA is becoming more and more significant, especially as this disease is becoming increasingly prevalent with the aging population [3].

There is a strong correlation between aging and OA as it is speculated that the normally well-balanced metabolism in cartilage is altered in elderly joints [4]. Moreover, a history of trauma, obesity and malalignment also play a role in the etiology of OA [5–7]. The degradation of cartilage in the joints, with a loss of both proteoglycans and the collagen meshwork, are hallmarks of the disease. Moreover, OA joints are exposed to a harsh catabolic

* Corresponding author.
E-mail address: bpchan@hku.hk (B.P. Chan).

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environment, made up of various pro-inflammatory factors, such as TNF-α, IL-1 [8,9] and IL-6, as well as a vast range of matrix proteases, such as MMP13 and ADAMTS-4,5 [10]. Existing treatments for OA are mainly symptom-relieving and include both non-pharmacological modalities (such as exercise and physical therapy), and pharmacological modalities (such as analgesics and nonsteroidal anti-inflammatory drugs; NSAIDs) [5,11]. Surgical intervention for developing OA is currently very limited, and joint replacement is still so far the most cost-effective surgical intervention for patients with severe OA symptoms. However, the life span of an implant is only 10–15 years [12], and the risk of complications in the elderly is substantial [13]. There is therefore an urgent need for the development of more effective disease-modifying therapies for treating OA.

Autologous chondrocyte implantation (ACI) has shown a satisfactory outcome in the short term [14–17], but the formation of fibrocartilage repair tissue of inferior quality remains an unresolved issue. Other disease-modifying strategies that attempt to delay the progression of the disease have also been developed. These include chondroprotective small molecules [18,19], collagenase inhibitors [20,21], stem cell therapies [22,23], and tissue engineered cartilage [24,25]. Among these emerging strategies, tissue engineering is a promising option for the surgical replacement of defective tissues. However, these emerging OA treatments are heavily influenced by interactions with the native cells and with the extracellular matrix (ECM) via diverse mechanisms [26].

In order to evaluate the performance of emerging therapies, to screen for factors that will optimize their efficacy, and to predict the fate of these therapies in an OA-relevant environment, a valid OA-mimicking model is necessary. Animal models of OA, including both spontaneously arising and induced models, have been used for many years [27]. Spontaneously arising models better simulate the natural progression of human OA but they can take a long time to develop. Induced models can be produced either surgically or chemically. In surgically-induced OA models, the disease develops over a short period of time and progresses rapidly, with OA changes that are much more severe than they are in humans [27]. In addition, chemical induction methods (such as intra-articular chemical injections), lead to OA with a unique pathophysiology that is distinct from human OA [28]. Testing emerging therapies in relevant animal models is a necessary step prior to clinical trials. However, concerns about the dissimilarities between animal and human OA, and the costs and ethical issues related to the use of large numbers of animals for initial screening and optimization purposes, indicates the absolute requirement for the development and use of an effective in vitro OA model prior to conducting formal well-planned animal studies.

Using an in vitro models that can accurately mimic human OA as multiple physiological and environmental factors can be controlled [29], and so a simulated OA disease environment can be constructed to evaluate and predict the performance of emerging therapies. Early in vitro models developed to study OA involve human OA chondrocytes being cultured in a monolayer [29,30]; however these cells dedifferentiated and so their chondrogenic phenotype could not be maintained [29]. A cartilage explant culture system has also been used to study the mechanical and pharmaceutical dynamics of OA [31–36], but the subchondral bone (which is known to play an important role in the pathophysiology of human OA), is not usually included in these cartilage explant cultures. Thus, ideal OA-mimicking models should simulate human OA as closely as possible, and be easy to apply and quantify, as well as be high-throughput and low cost. The model should also be able to facilitate the optimization of the formulation design under a disease-relevant environment. Organ cultures usually retain the cells and their secreted factors, as well as the ECM and the native tissue structure, organization and configuration. Therefore, joint tissues harvested during joint replacement surgeries in human OA patients might intrinsically offer a highly biomimetic OA microenvironment. However, there are few reports on developing a human OA organ culture.

Ramakrishnan et al. made use of bovine osteochondral graft to establish an in vitro injury model [37]and have successfully use it to find a potential chemicals that could reduce chondrocyte death after injury [38]. In terms of cell therapy in cartilage repair, De Vries-van Melle et al. did a systematic study and evaluation of a cartilage repair method using bovine osteochondral model [39]. But bovine joint is fundamentally different from human joint in terms of joint mechanics, cartilage thickness and physiology. However, experiments using osteochondral grafts of human origin is even scarcer. Williams et al. have characterized the over time effect of culture on human osteochondral graft in terms of cartilage matrix content and chondrocyte viability [40] and they found that osteochondral graft could maintain its in vivo properties within a short period of time after harvest, showing the feasibility and potential of osteochondral graft in in vitro testing. But a systematic study on repair treatments using human osteochondral graft is lacking.

Here, we describe the development of a human osteochondral tissue-based OA organ culture model and its application as a platform to screen for multiple factors during the development of human MSC derived engineered cartilage. Specifically, we report the chondrogenic phenotype (typical cartilage ECM), the characteristic OA phenotype (specific catabolic enzymes) and its long term maintenance in cultures, and in this way we were able to provide a better understanding of the human OA phenotype and pathological environment. Moreover, we demonstrated the application of this organ culture model in evaluating emerging treatments such as tissue engineered cartilage, in an OA-mimicking microenvironment. We studied the phenotype maintenance of a mesenchymal stem cell derived and collagen-based engineered cartilage when implanted into and hence co-cultured with the human OA organ model for an extended period of time. We demonstrated that the engineered cartilage temporarily lost its chondrogenic phenotype particularly through reduced glycosaminoglycan content but remodeled over time and regained its chondrogenic phenotype. We also applied this in vitro human OA model in screening various external factors including a few key OA relevant microenvironmental factors (such as oxygen tension, serum supplementation and mechanical loading), and a potential disease modifying agent, a broad spectrum matrix protease inhibitor and demonstrated the responsiveness of the engineered cartilage in phenotype alterations. We anticipate that this robust in vitro model will contribute to the study of OA pathophysiology and the development of emerging therapies for OA.

2. Materials and methods

2.1. Overall research design

Fig. 1 shows the overall research design. The harvested OA specimens were cleaned and graded before they were carved into osteochondral plugs (OC plugs) using a 3-mm biopsy punch. The height of OC plugs were around 5–6 mm with the comparable cartilage and bone proportion. The OC plugs were either cultured directly as an organ culture model for subsequent detailed characterization, or else they were processed for organ co-culture model, using a tissue engineered cartilage for validation. To perform the organ co-culture, a focal defect of 1-mm diameter was first created at the center of the OC plug using a 1-mm biopsy punch. The engineered cartilage constructed was then inserted into the space created in the OC plug for long-term co-culture of up to