Osteoarthritis In Vitro Models

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Osteoarthritis (OA) is a complex multi-target disease with an unmet medical need for the development of therapies that slow and potentially revert disease progression. Intra-articular (IA) delivery has seen a surge in osteoarthritis research in recent years. As local administration of molecules, this represents a way to circumvent systemic drug delivery struggles. When developing intra-articular formulations, the main goals are a sustained and controlled release of therapeutic drug doses, taking into account carrier choice, drug molecule, and articular joint tissue target. Therefore, the selection of models is critical when developing local administration formulation in terms of accurate outcome assessment, target and off-target effects and relevant translation to in vivo.

Keywords: Osteoarthritis ; in vitro models ; synoviocytes ; chondrocytes

1. Introduction

Osteoarthritis (OA) is a chronic disease with worldwide incidence in the population aged 65 years and higher, representing a significant economic burden in terms of global health [1][2]. As the most common form of arthritis and one of the leading causes of disability in the elderly population, OA is characterized by chronic inflammation, articular cartilage degeneration and structural changes of whole joints. There is currently an unmet need for disease-modifying drugs (DMOADs) that slow or even revert disease progression [3][4][5]. Pharmacological treatment options focus on symptom management. Oral analgesic and nonsteroidal anti-inflammatory drugs (NSAIDs) are first-line treatments for pain and inflammation. However, since OA mainly affects the joint as a whole closed structure, systemic drugs result in less than optimal efficacy rates [6][7]. A known alternative that circumvents most of the drawbacks associated with systemic drug administration is the delivery of drugs locally, by intra-articular (IA) injection. IA allows for higher drug doses and prolonged delivery of drug molecules directly into affected joints. By this approach, more effective relief of symptoms may be attained, while systemic adverse effects are generally avoided. Different drug delivery systems (DDSs) have grown in the field to improve the delivery of small molecules locally to joints. These include different formulations such as polymeric nano and microparticles, hydrogels, liposomes and micelles, which have been extensively reviewed [8][9][10][11]. Due to its local administration, maintaining the selectivity of drug molecules and the carrier system towards biological tissue targets in the joint while avoiding off-target effects is critical when developing IA formulations. In this regard, the design of predictive in vitro OA models is crucial in characterizing and understanding the studied drug delivery systems for OA treatment. Different cellular models represent different tissues of the joint: synoviocytes, the synovium, and chondrocytes are used to model articular cartilage [12]. The different types of in vitro cellular models (i.e., monolayer, three-dimensional or explant) have various applications according to the final goals of IA formulation. Thus, a deep understanding of their intricacies is very important in this field.

2. 3D Cellular Models

2.1. 3D Cellular Models without Scaffold

Three-dimensional cellular pellets circumvent some of the disadvantages of monolayer cultures, especially as they allow a structure, maintaining cellular growth in all dimensions and synthesis of articular cartilage ECM. In this approach, chondrocytes can be centrifuged together in conical bottom wells or tubes or cultured under stirring using bioreactors. Inducing cell clustering forms cartilage tissue-like pellets, after a specific incubation time, with sizes up to 5 mm [13][14]. These pellets can mimic articular tissue as a whole, providing insights into cell-to-cell and cell-to-ECM relationships. Like in a monolayer culture, HBMSCs pellets can replace 3D chondrocyte pellets. As an in vitro model for IA DDSs development, 3D pellets have been applied in the evaluation of chondrogenesis and chondroprotective effects after IL-1β stimulation by GAG content quantification and gene expression of collagen II, aggrecan, and MMPs [15]. A primary reason as to why pellets are not a standard in vitro OA model is linked to difficulties in maintaining 3D cellular cultures in terms of cost and quantity. 3D articular dedifferentiated chondrocytes are not associated with high proliferation rates and derive

from low monolayer passages restricting cellular amounts. Culture media is supplemented with a high amount of growth factors and chondrogenic stabilizers, representing higher costs compared to monolayer culture [16]. Additionally, pellets have short viability spans, where nutrients have difficulties in penetrating the pellet, inducing cell death at its core. As a model for IA DDSs, interaction of formulations with the tissue as a whole is essential in characterizing target specificity. The inability to fully penetrate the pellets poses a limitation to the use of this model in the IA setting [17]. Bypassing these shortcomings is, however, made possible by establishing this type of 3D cellular growth in external structures—scaffolds.

2.2. 3D Cellular Models with Scaffold

Cells can be cultured directly into external scaffolds, gaining three-dimensional features. As an in vitro model for IA DDSs development, this alternative has great potential for targeted delivery. Not only does it provide structural support for 3D cellular growth by mimicking features of joint structure, making it a good model of loading and weight-bearing in OA, as the nature of the scaffold (biologic or synthetic) can play a role in cellular growth and maintenance. The most commonly used scaffolds are hydrogels due to their high water content and the extensive ability to tailor their mechanical and physicochemical properties. Biopolymers like agarose, chitosan, alginate and hyaluronic acid have been applied to grow chondrocytes, mimicking articular cartilage, and osteoblasts, aiming to model the osteochondral plate. Combining the growth of both these types of cells has also been explored, forming bilayer scaffolds, in an attempt to represent the whole articular joint [18]. As such, and after cytokine stimulation and exposure to therapeutic molecules, different cartilage markers can be assessed by different assays: GAG content (alcian blue assay), collagen II, aggrecan, MMPs (gene expression analysis) and even pro-inflammatory cytokines (enzyme-linked immunosorbent assay ELISA) [19][20]. Rheological measurements (elastic Young's and G moduli) help investigate the mechanical properties of chondrocytes in hydrogels (agarose) [21]. Synthetic hydrogels and polymers can be applied as scaffolds, with advantages like mechanical features and support. 3D printing has been applied in this field with promising results in cartilage regeneration [22][23]. Compared to 2D models, scaffold-based 3D culture is expensive, difficult to maintain and hard to standardize, given the many options for scaffolds. Depending on their nature, problems may arise with how these influence results. For example, biopolymer-based hydrogels may themselves have a chondroprotective effect on cultured chondrocytes, skewing effects of tested drugs. The nature of the scaffold may also translate into differences between in vitro and in vivo models. For instance, hydrogels are rich in water, unlike subchondral bones of joints; thus, the growth of osteoblasts in such scaffolds is not an accurate representation of in vivo conditions [24].

3. Conclusions and Future Perspectives

Improved design and development of efficacious IA DDSs relies on the use of accurate, predictive in vitro and in vivo models. However, to date, there is no OA gold standard in vitro model and few guidelines or models adapted specifically to IA DDSs formulations. Presently, monolayer models, despite being easy to establish and ideal for rapid screening of molecules, fail in representing accurate OA conditions, such as cross-talk between different tissues. This could be bypassed by co-culture of two types of cell lines, like synoviocytes and chondrocytes, but aspects like cell de-differentiation and ECM growth are not negligible. Three-dimensional models are considered better representations of in vivo OA, as in these models, three-dimensional structures of tissues and cellular phenotype and growth are preserved. However, with or without scaffold, 3D models are difficult to establish and maintain, and outcomes vary greatly according to the source and nature of scaffold. For studies in articular tissues, explants are considered best in correlation to in vivo OA conditions. However, viable replicates and maintenance of tissues in in vitro environments are important limitations. Recently, a bioengineering approach combining 3D cell culture and microfluidics—organ-on-chip (OoC)—has been in the field of OA. Cartilage-on-chip and osteochondral-tissue-on-chip have been developed to perfectly mimic joint microenvironments, allowing for better reproductions of in vivo conditions. Promising results have been described testing the drug alone, making this a promising approach for the better development of IA DDSs in the future [25][26][27]. In this context, considerations have to be taken into account when designing and developing IA DDSs, especially when deciding outcome readouts. To this extent, the type of formulation and mode of action of drug molecules play a critical role. Monolayer models are better suited for testing anti-inflammatory activity, whereas 3D chondrocyte models are preferred to evaluate chondroprotection activities. When testing hydrogels, it is important to assess the nature of the scaffold in 3D models and even occlusion in explants. In the future, research advancements should focus on improving the design and development of OA in vitro models for better prediction of in vivo and, eventually, clinical results. This should be done while always considering the tailoring of in vitro models to specific IA DDSs formulations, like maintaining cellular viability conditions for testing of sustained prolonged drug release delivery systems.

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