An osteochondral bio-engineered model to *in vitro* mimicking osteoarthritis

Giorgia Cerqueni,[1] Annachiara Scalzone,[2] Xiao Nong Wang,[3] Caterina Licini,[1] Kenny Dalgarno,[2] Ana Marina Ferreira-Duarte,[2] Piergiorgio Gentile,[2] Monica Mattioli Belmonte[1]

[1]Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy; [2]School of Engineering, Newcastle University, Newcastle upon Tyne; [3]Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom

Abstract

To date, treatments of Osteoarthritis are not able to provide the pathology regression. *In vitro* models are therefore necessary to: i) investigate the mechanisms involved in the disease evolution, ii) identify pharmacological targets, and iii) perform predictive tests for new drug delivery strategies.

Introduction

Osteoarthritis (OA) is a joint degenerative pathology characterised by an intricate interaction of inflammatory processes affecting synovium, Articular Cartilage (AC) and Subchondral Bone (SB).[1,2] With the increase of life expectancy, OA ranks the fourth among the cause of worldwide disability and needs significant national spending.[3] The OA pathophysiology is difficult to be understood due to a not recognised early phase of the disease, and the lack of reliable and consistent *in vivo* and *in vitro* models able to reproduce the entire pathology.

This work aimed to develop an engineered *in vitro* model of osteochondral human tissues mimicking the main features of OA as a tool for investigating the disease physiology, biology, and progression.

Materials and Methods

A Polylactic Acid (PLA) porous trabecular-like construct was 3D printed via Fused Deposition Modelling (Rokit INVIVO Bioprinter) and functionalised with gelatin (Gel) and hydroxyapatite (nHA) to obtain the SB layer. To mimic the AC deep zone, a photocurable methacrylated Gellan Gum (GG; 3%w/v) multi-channels hydrogel was obtained via soft-lithography, adherent to the PLA scaffold. Chondrocytes (differentiated from Y201 bone marrow mesenchymal stromal cells) (4) were encapsulated in each channel of the GG layer within a chondroitin sulfate-based hydrogel, while Y201 cells were seeded on the PLA scaffold. The OA environment was simulated by adding IL-6, IL-1β, and TNF-α. Cell behavior was assessed, for both phases of the construct, via Live/Dead, and analysis of metabolic activity and cytoskeleton. qRT-PCR, Alcian blue, and Alizarin Red were used to assess cell chondrogenic potential and the effect of cytokines.

Results

GG hydrogel displayed high water uptake capability (initial rapid uptake of 1433±57% at 3 h then achieving plateau), high porosity (30% of pores diameter is between 100-150 µm, only 5% of pores showed less than 100 µm while the percentage remained constant at approximately 15% in 150-200, 200-250, 250-300 and > of 300 µm), and appropriate mechanical properties for AC application (48±5kPa as compressive Young's modulus). All the materials were capable to sustain cell viability and metabolic activity for up to 7 days. The presence of cytokines decreased the mechanical properties at day 14, altered the expression of chondrogenic markers (COL2A1, ACAN, SOX9) and the mucopolysaccharide production (0.36±0.01µg in the Healthy model compared to 0.22±0.02µg in the OA samples at day 21). OA model showed an increase of OA-related molecule expression (COLL1, MMP9-13, ADAMTS5) and Alizarin Red values (680±20µM for the OA-model compared to 550±10µM of the Healthy model).

Discussion and Conclusions

We developed a high reproducible 3D *in vitro* OA model endowed with quickly manufacturing, easy manipulation, and large availability. The model was design respecting the architecture of the interest area, carrying out a careful analysis of the histological and structural traits of the two tissues of interest. Biomaterials that were capable of interfacing both with the characteristics of the designed model and with the biological and structural parameters of the tissues, were identificated. Then biomaterials practical applicability for the designed model was tested and the biocomapibilty was assessed. To investigate the biomimetic properties of the 3D bio-engeneered structure, the physiological tissue-related cell behaviour was analysed.

The obtained data confirmed that the proposed model was able to re-create an ideal environment for physiological cell behaviour, avoiding cell dedifferentiation and encouraging the expression of appropriate tissue-related markers. The proposed model was also an optimal tool for studying cell variation after an inflamed stimulus mimicking OA.

Further in-depth analysis of specific tissue-related markers in pathological conditions as well as of the possibility to reverse OA cell features and restore physiological behaviour, are forecasted. This construct could be applied to test new therapeutics and to study cell crosstalk interactions.

Correspondence: Giorgia Cerqueni, Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy.
E-mail: g.cerqueni@univpm.it

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