Progress in Histochemistry and Cytochemistry 50 (2015) 19–36

Contents lists available at ScienceDirect

Progress in Histochemistry and Cytochemistry

journal homepage: www.elsevier.de/proghi

Review

Comprehensive analysis of translational osteochondral repair: Focus on the histological assessment

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ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 7 October 2015
Accepted 7 October 2015

Keywords:
Histology
Articular cartilage
Subchondral bone
Osteochondral unit
Animal model

ABSTRACT

Articular cartilage guarantees for an optimal functioning of diarthrodial joints by providing a gliding surface for smooth articulation, weight distribution, and shock absorbing while the subchondral bone plays a crucial role in its biomechanical and nutritive support. Both tissues together form the osteochondral unit. The structural assessment of the osteochondral unit is now considered the key standard procedure for evaluating articular cartilage repair in translational animal models. The aim of this review is to give a detailed overview of the different methods for a comprehensive evaluation of osteochondral repair. The main focus is on the histological assessment as the gold standard, together with immunohistochemistry, and polarized light microscopy. Additionally, standards of macroscopic, non-destructive imaging such as high resolution MRI and micro-CT, biochemical, and molecular biological evaluations are addressed. Potential pitfalls of analysis are outlined. A second focus is to suggest recommendations for osteochondral evaluation.

Contents

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http://dx.doi.org/10.1016/j.proghi.2015.10.001
0079-6336/

1. Introduction

Hyaline articular cartilage guarantees for an optimal functioning of the joint by providing a gliding surface for a smooth articulation, distributing weights evenly to the bone, and absorbing shocks (Buckwalter and Mankin, 1998; Hunziker et al., 1997; Hunziker et al., 2002; O'Driscoll, 1998). Once damaged, for example as a result of trauma, articular cartilage does not regenerate, i.e. having an identical quality than normal hyaline articular cartilage (Caplan and Goldberg, 1999; Hunziker, 2002). The unsolved problem of the inferior structural and functional quality of the repair tissue instigates further research.

In this regard, translational animal models of articular cartilage defects are main pillars in the field of experimental articular cartilage repair (Orth et al., 2012c, 2013a) as they allow for evaluation of the entire osteochondral unit. Here, not only the repair of articular cartilage is of importance, but also the problem of the underlying subchondral bone has been recognized (Orth et al., 2012b, 2013a; Orth and Madry, 2015a). The choice of the appropriate animal model mainly depends on the aim of the investigation and needs to take into account numerous factors such as accessible joints, cartilage thickness, defect location (Orth et al., 2013c), or feasibility of joint immobilization (Cook et al., 2014). The age of skeletal and cartilage maturity of the animal is crucial (Hurtig et al., 2011). In general, small animal models such as rodents or rabbits are recommended for analyses of mechanisms and proofs of principle while large animal models allow for true translational research aiming at clinical use in patients (Cook et al., 2014). Furthermore, the inclusion of relevant control groups and sufficient study duration are mandatory.

A broad range of different modalities can be applied to evaluate experimental osteochondral repair among which the histological scoring represents the gold standard. According to the American Society for Testing and Materials (ASTM) and the United States Food and Drug Administration (FDA), most relevant outcome measures include macroscopic, histological, biochemical, radiological, and biomechanical assessments (http://www.astm.org/Standards/F2451.htm; http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM288011.pdf).

The aim of this review is to give a detailed overview of the different methods for a comprehensive evaluation of osteochondral repair. The main focus is on the histological assessment as the gold standard, together with immunohistochemistry, and polarized light microscopy. Additionally, standards of macroscopic, non-destructive imaging such as high resolution MRI and micro-CT, biochemical, and molecular biological evaluations are addressed. Potential pitfalls of analysis are outlined. A second focus is to suggest recommendations for osteochondral evaluation.

2. Applied histology of the normal osteochondral unit

Articular cartilage consists of articular chondrocytes that are surrounded by an extracellular matrix (ECM), chiefly containing proteoglycans and type II collagen fibres (Buckwalter and Mankin, 1998). Microscopically, hyaline articular cartilage contains four separate zones with each having a specific composition of chondrocytes and extracellular matrix (Buckwalter, 1983): the superficial zone (10–20%) contains flat chondrocytes producing collagen, high water content, and collagens fibrils which are orientated parallel to the surface. The intermediate zone (40–60%) shows a chondrocyte morphology and collagen fibre orientation in transition between superficial and deep zone. The deep zone (30%) exhibits round chondrocytes and collagen fibres which are orientated perpendicular to the surface and crossing the tidemark to establish a strong connection with the calcified cartilage zone. Most basally, the zone of calcified cartilage (10%) consists of hypertrophic chondrocytes within a calcified matrix. Here, collagen fibres are orientated perpendicular to the surface, passing the tidemark (apical) and the cement line (basal), and anchoring the calcified zone to hyaline cartilage and subchondral bone plate, respectively.

Important boundaries between some of these zones are (1) the tidemark, a basophilic and histologically identifiable line separating the deep zone of the hyaline articular cartilage from the calcified cartilage zone (Broom and Poole, 1982),