Characterization of Progenitor Cells Isolated from the Subchondral Bone of Rabbit Trochlea and Condyle

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Disclosures:

Introduction: Bone marrow stimulation initiates repair by fracturing or drilling into subchondral bone at the base of a debrided cartilage defect, typically leading to the formation of a fibrocartilaginous repair tissue. Incomplete regeneration, high inter-individual variability and poorer outcome in older animals are observed with this procedure. Cartilage repair outcomes are affected by defect location and age, suggesting that the structural and biological characteristics of underlying subchondral bone strongly influence the repair response. Specifically, better repair and increased chondrogenic potential were observed in rabbit trochlea compared to medial femoral condyle in previously published studies [1,2]. The aim of this study was to carry out a comprehensive analysis of the progenitor cells present in the subchondral bone of rabbit condyle and trochlea, taking into account location and age.

Methods: Progenitor cells were isolated from the distal femurs of female New Zealand White rabbits (n = 3 young animals aged 4 months and n = 2 retired breeders aged > 36 months). Bone chips were digested with collagenase enzyme to obtain a first cell population (Collagenase-derived cultures) and subsequent outgrowth from the digested bone explants led to a second cell population (Explant-derived cultures). Cells were isolated separately from trochlear and condylar regions of subchondral bone. It was also previously observed that drilling to 6 mm provides access to metaphyseal marrow and improves repair in rabbit trochlea [3], therefore, cells were isolated from two clearly distinct trochlear regions: 1) Above and 2) Below the growth plate in the young animals or epiphyseal line in the old animals. In order to compare the growth characteristics of the cells from different regions of subchondral bone, cell yield was determined at the end of P0. Stemness of cultures was verified by means of CFU-f assay. Flow cytometry analysis was carried out to determine the stem cell marker characteristics. Finally, the multilineage differentiation potential of the progenitor cells was determined. Cells were differentiated into a cartilaginous phenotype in 3D culture as pellets by addition of TGF-βIII. Distribution of GAGs and collagen type I and II in pellets was characterized. Confluent monolayer cultures were stimulated in the presence of dexamethasone for osteogenic differentiation and the presence of osteogenic matrix was determined by Alizarin Red S staining.

Results: Cells grew as colonies for both collagenase and explant-derived cultures and possessed same fibroblastic morphology. P0 cell yield was significantly higher for trochlear samples compared to condylar samples in both young and old animals (Fig. I). The cells isolated from rabbit trochlea also had higher stemness than condyles as observed by CFU-f assay (Fig. II). Growth rate, total cell yield at the end of P0 and clonogenic potential were found to decrease with increasing age. All cells expressed surface markers characteristic of bone marrow stem cells (CD44+ and CD34-). The cells differentiated into osteogenic phenotype as determined by Alizarin Red S staining (Fig. III). On stimulation in the presence of TGF-βIII, progenitor cells differentiated into cartilaginous phenotype. The collagenase-derived cultures exhibited a more complete differentiation into cartilaginous phenotype than explant-derived cultures, as evidenced by higher Safranin-O staining. A greater expression of cartilaginous phenotype was similarly observed for cultures originating from the trochlea versus condyles (Fig. IV). Finally, the cells from older animals were found to possess poor chondrogenic and osteogenic potential (Fig. IVb and IVv).

Discussion: The variability in cartilage repair outcomes has been found to be influenced by age, species as well as defect location. Structural and biological differences in the subchondral bone may have a profound effect on the properties of the progenitor cells and their subsequent ability to effect repair. Here, we isolated cells from three structurally distinct regions of the subchondral bone to study the effect of location on progenitor characteristics. We found clear biological differences between cells isolated from trochlea and from condyles, with trochlear cells exhibiting increased cell yield, more clonogenic potential and increased chondrogenic differentiation potential. Young and old animals were used in order to assess the influence of age on repair outcome. As expected, growth rate, cell yield and multilineage differentiation potential decreased with age. Since the origin of the cells involved in repair procedure during microfracture is still unclear, the role of marrow derived and bone-lining (collagenase isolation) cells versus those resident in bone matrix (explants-derived) was also explored. Although the clonogenic potential of explant-derived cells was found to be lower than collagenase-derived cells, no difference was detected in the osteogenic and chondrogenic differentiation potential. We expect to further establish a link between the location of the defect and the age of the individual and the efficiency of cartilage repair. We believe that this will be an important consideration when treating cartilage defects in clinical scenarios and might be used to improve cartilage repair outcome.

Significance: We believe that at the end of this study, clinicians will have a better understanding of the correlation between the structural and biological properties of the cells participating in the repair of cartilage defects. With the support of imaging

techniques like MRI, these observations will allow clinicians to improve clinical strategies based on the properties of the underlying subchondral bone which in turn will improve the efficiency of the cartilage repair.

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**References:**
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