Age Independent Cartilage Generation for Synovium Based Autologous Chondrocyte Implantation

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Introduction: In clinical practice, attempts to induce the repair of lesions within articular cartilage now commonly involve the implantation of mature autologous chondrocytes. However, the most popular approaches, namely, autologous-chondrocyte implantation (ACI) [1] and matrix-assisted autologous-chondrocyte implantation (MACI), are efficacious only in young adults. In elderly patients, they are less successful, owing to an age-related decline in the prolifervative and the differentiative capacities of the autologous chondrocytes that are used as the donor cells. Moreover, in removing the donor tissue from healthy sites of the targeted articular-cartilage layer, surgical damage is induced which not only fails to heal but which may also lead to progressive osteoarthritic lesioning. Furthermore, given the advisability of removing only a small mass of healthy tissue and the intrinsically very low cellularity of adult human articular cartilage, only limited numbers of cells can be generated by the in-vitro-expansion step that precedes ACI or MACI. Another disadvantage of the ACI- and the MACI- approaches is that two surgical or arthroscopic interventions are necessary, which not only raises the cost of the undertaking but also heightens the risk of infection. Against this background, the availability of an alternative approach whereby the aforementioned problems and restrictions could be circumvented would be welcome. In a number of studies, cells that have been derived from the synovium or the synovial membrane of adult human patients, with or without osteoarthritic joint pathologies, have been shown to sustain their very high prolifervative and differentiative capacities with increasing age [2]. On the basis of these data, we hypothesize that it should be possible to induce the formation of cartilaginous tissue within the subsynovial space of a joint by the local and temporary subcutaneous deposition (to avoid an opening of the joint cavity) of a spatially-confinable carrier bearing the signalling agent BMP-2 in a delayed-release system. The mass of synovium-derived cartilaginous tissue thereby generated could then be used for ACI or MACI, for which purpose only one intra-articular intervention would be necessary [(to implant the construct (MACI) or the isolated chondrocytes (ACI)]. By this means, a sufficient number of autologous chondrocytes could be generated in all patients, irrespective of their age.

Methods: The principles of this hypothesis were tested in Chinchilla rabbits. BMP-2 was trapped within small, fibrin-impregnated collagenous patches in a liposome-encapsulated form for slow release. The tissue mass that was generated within the subsynovial space was excised 5, 8, 11 and 14 days later for an analysis of its volume and composition. All but one of the thirty-four rabbits survived the postoperative course in a disease-free state. The specimens were examined in a Nikon Eclipse microscope. The areas containing neoformed tissue were serially sectioned. The first to the last sections of the series were collected for further microscopic and histomorphometric analyses.

Results: Our analyses revealed the neogenerated subsynovial-tissue masses to contain significant amounts of cartilaginous tissue (Figure 1), which could be readily implemented for synovium-based (sb) ACI (sb-ACI) or MACI (sb-MACI). The results of the histomorphometric analysis of the neoformed tissue
are summarized in Figure 2. The mean absolute volumes of connective tissue (Figure 2A), of adipose tissue (Figure 2B), of cartilaginous tissue (Figure 2C) and of osseous tissue (Figure 2D) are represented as a function of time for the experimental group (BMP-2-bearing patch) and the negative control (saline). Figure 2A reveals that in the negative-control and experimental groups, the mean absolute volumes of connective tissue were similar and peaked on day 8, values of approximately 12.6 mm³ and 14.6 mm³, respectively, having been attained by this juncture. The higher mean value on day 14 in the latter group did not differ significantly from that in the negative control (high SEM). Figure 2B depicts temporal changes in the absolute volumes of adipose tissue in the two groups. In the negative-control group, the mean absolute volume of adipose tissue changed erratically with time, although the differences were not statistically significant. At each time-point, the mean value for this parameter was 2- to 3-fold higher than in the experimental group. An inspection of Figures 2C and 2D reveals that in the negative-control group, neither cartilaginous nor osseous tissue was formed during the 14-day monitoring period. In the experimental group, the mean absolute volume of cartilaginous tissue increased erratically with time, from a value of 0.5 mm³ on day 5 to one of 11.9 mm³ on day 14. Osseous tissue first appeared on day 11, viz., one week after the initial deposition of cartilage.

**Discussion:** Sufficient quantities of cells for ACI or MACI could be generated subsynovially within a week of the chondrogenic-induction process, thereby permitting a curtailment of the waiting time between autodonation and the instigation of the therapy, as well as a reduction in the risk of joint infection and in the health-care costs. One of the greatest advantages of sb-ACI would be that it could be instigated in patients of all ages, which would considerably broaden the currently narrow scope of clinical indications to embrace also other fields of pathology. In humans, the biopsy of articular cartilage that is removed for ACI weighs about 280 mg, which corresponds to a volume of about 280 mm³. This volume of tissue is approximately 110-fold greater than that generated 2 weeks after the synovial implantation of one small BMP-2-bearing patch. However, the same pool-size of proliferating chondrocytes could be obtained within a week from a 150-fold lesser volume (1.8 mm³) of the cartilaginous tissue that is generated by a single BMP-2-bearing synovial patch (and in patients of all ages). On the basis of these approximate quantitative estimations, it is evident that sb-ACI or sb-MACI would offer many advantages over the classical approaches.

**Significance:** Our data indicate that a synovium-based ACI-approach could be implemented in patients of all ages, not just in young individuals, thereby broadening the scope of the clinical indications for cell-based cartilage repair.
Figure 1

BMP-2-Patch
Adipose Tissue
Cartilage
Synovium

Figure 2

A

Volume of Connective Tissue (mm³)

B

Volume of Adipose Tissue

C

Volume of Cartilaginous Tissue (mm³)

D

Volume of Bone (mm³)

ORS 2015 Annual Meeting
Poster No: 0386