progression and hyaline-like tissue repair compared to a modified ACI technique. Human clinical studies evaluating the safety of CAIS are currently ongoing.

Exogenous growth factors, such as members of the TGF-β superfamily, could be co-delivered with the minced cartilage fragments to further stimulate or accelerate the production of cartilagenous matrix by the outgrown chondrocytes. We have explored the prochondrogenic potential of recombinant human Growth and Differentiation Factor 5 (rhGDF-5) to enhance cartilage matrix production of both isolated chondrocytes in vitro and minced cartilage fragments in a heterotopic model. In both systems, a robust, dose-dependent enhancement of neo-cartilaginous tissue formation was observed following rhGDF-5 treatment, suggesting that this combined cell-biologic therapeutic strategy may further improve chondral defect repair.

CAIS, a one-stage cartilage regeneration therapy, offers many practical advantages compared to other cell-based techniques. Delivery of minced cartilage provides a direct source of primary, autologous chondrocytes to the site of repair as opposed to marrow-derived cells arising from microfracture procedures. These minced cartilage-derived chondrocytes subsequently outgrow and proliferate in situ, obviating the need for ex vivo cell expansion and thus eliminating the associated costs and safety concerns. Further, as minced cartilage-derived chondrocytes are not subjected to ex vivo culture, it is possible they may retain a more natural or even more potent chondrogenic phenotype than culture-expanded cells. Most importantly, CAIS is a first-line cartilage regeneration therapy to provide intra-operative treatment immediately upon diagnosis during the arthroscopy, thereby limiting the patient’s surgical regimen to a single procedure.

In vitro results showed that micromass pellets comprising 20% primary chondrocytes and 80% bone marrow cells, contain significantly more GAG compared to the expanded chondrocyte and bone marrow controls, and a comparable (not significantly different) amount of GAG compared to the 100% primary chondrocyte control. These findings were in agreement with safranin O histologies; we detected positive staining for the cell combination groups, and negative staining for the expanded chondrocyte and bone marrow controls. GAG content per added primary chondrocytes increased on average 3 fold for the 10%/90% chondrocyte/bone marrow combination group, compared to the 100% primary chondrocyte control. In vivo results were consistent with in vitro results. Moreover, collagen type II staining was positive, and GAG content per added primary chondrocytes increased 7 fold (for a 10%/90% ratio), compared to the 100% primary chondrocyte group. Interestingly, GAG per added primary chondrocytes increased with decreasing added chondrocyte content, for both in vitro and in vivo studies. A sube isolated within 10-30 min from cartilage of donors of an average age of 69 years (range 47-83).

We have demonstrated a combination of primary chondrocytes and bone marrow cells to synergize in enhancing cartilage formation, compared to expanded chondrocytes, in vitro and in vivo. We further demonstrated an increased GAG content per added primary chondrocytes for the cellular synergy system compared to primary chondrocytes alone. Finally, we have established chondrocyte isolation within less then an hour. Provided these findings can be verified for a combination of chondrocytes with non expanded bone marrow cells, cellular synergy provides the basis for a one surgery cartilage defect treatment without the need for cell culture expansion, comprising: cartilage and bone marrow harvest, chondrocyte isolation, cell combination, seeding of the cell combination into a biomaterial scaffold, and, implantation. Further studies will be required to understand the mechanism of cellular synergy. Enhancement of chondrogenesis may depend on, either, i) chondrocytes instructing bone marrow cells to undergo chondrogenesis, ii) bone marrow cells enhancing chondrogenesis of chondrocytes, or iii) a combination of the two. Regardless of the exact mechanism, the potency of the cellular synergy system forms a highly suitable basis to advance the effectiveness of current cartilage repair.

References:
J Hendriks et al., Trans Orthop Res Soc 2005, 1792

29.2 Cellular synergy for 1 step cartilage repair
J. Hendriks, E. de Bruijn, R. Schotel, C.A. van Blitterswijk, J. Riesle, Netherlands

One of the current treatments for cartilage defects is based on expanded chondrocytes (ACI). Expansion of chondrocytes is, however, expensive, requires a complicated procedure including 2 surgeries, and hampers cellular quality as it results in cell dedifferentiation. This has prevented widespread acceptance of this treatment. We previously established a synergetic effect of a combination of primary chondrocytes with expanded chondrocytes to substantially enhance cartilage formation compared to expanded chondrocytes (Hendriks et al, 2005). To address the drawbacks of ACI, we hypothesized that primary chondrocytes and bone marrow cells synergize in cartilage formation. We further hypothesized that the required number of primary chondrocytes can be isolated from a small biopsy within surgery time. Together with an intra-operative source for bone marrow cells, a simple one surgery treatment for cartilage defects may be developed. The aim of this study was to investigate i) if a combination of primary chondrocytes and bone marrow cells synergize in enhancing cartilage formation, compared to expanded chondrocytes, in vitro and in vivo, and, ii) to establish chondrocyte isolation within less then an hour.

For the first in vitro study, we combined human primary chondrocytes and human expanded bone marrow cells at various ratios into micromass pellets, cultivated in standard medium without growth factors for 2 weeks, and analyzed for glycosaminoglycans (GAG), histologically and biochemically. Average chondrocyte and bone marrow donor age was 69 years (range 47-78) and 42 years (range 24-55), respectively. For the in vivo study, we combined bovine or human primary chondrocytes and human expanded bone marrow cells, seeded them into PEGT/PBT polymer scaffolds, implanted into nude mice, and analyzed for GAG and collagen type II eight weeks post-implantation. For the second study, we dissected human cartilage, enzymatically digested for various timeperiods, and analyzed for cell number and viability.