Hyaline Cartilage Production Independent from Osteoarthritic Status and Age

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Background

Autologous Cartilage Implantation (ACI) showed better results at mid to long-term follow-up according to meta-analysis by Riboh et al. 2017[1] when compared to other treatment modalities. However, hyaline cartilage (shown through the presence of collagen type II, glycosaminoglycan (GAG), and GAG/DNA ratio) is not produced from older patients > 55 years of age, nor from osteoarthritic (OA) joints harvest.

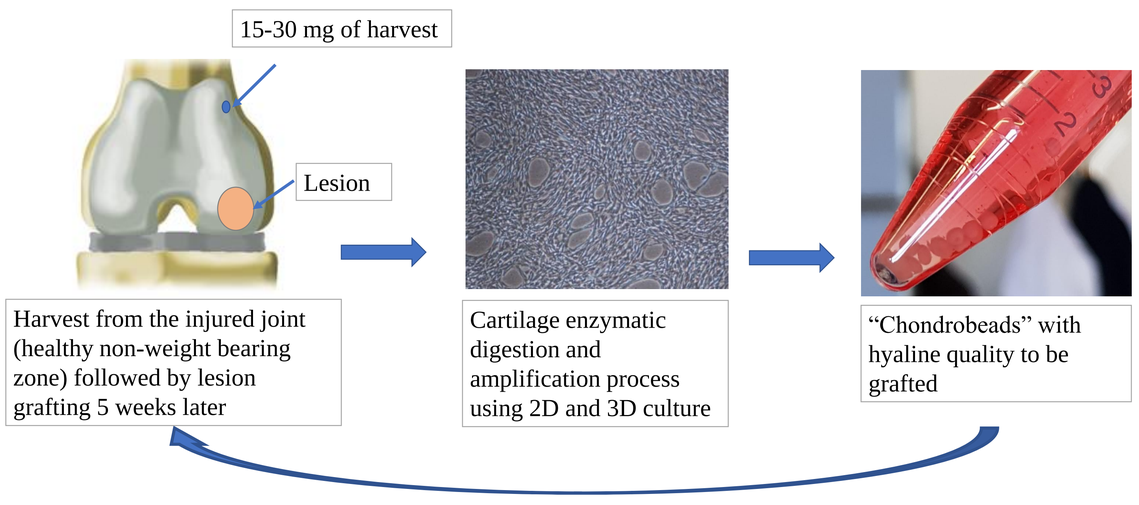
Objectives

To present a novel cartilage engineering procedure:

1) Showing that hyaline cartilage can be produced in vitro using cartilage from patients **between 18-80 y-o** and from **osteoarthritic joints**
2) proving its safety in a mice
3) proving its efficacy in a pig

Methodology

A Standardized protocol is set up to produce hyaline cartilage from chondrocytes extraction. Stem cell-based approach is applied to regenerate cartilage from samples harvested using patients postoperative material regardless of the arthritic status or age. The harvested chondrocytes are then rejuvenated and processed through 2D/3D culture creating a biomaterial called "chondrobeads" .



In vitro human chondrocyte study

Cartilage samplers were harvested from 24 patients aging between 18-80 y-o.

These samples are arthritic and non-arthritic harvests coming from knee and ankle post operative material (15-30 mg).

Cartilage is then produced from these samples and its hyaline quality is tested by Safranin-O coloration (GAG), immuno-staining (collagen II vs collagen I) and quantified by its GAG/DNA ratio.

In vivo preclinical trial

1) Safety in rodents (SCID mice): human chondrobeads are implanted subcutaneously on the back of 60 mice and compared to 14 positive controls implanted with human cancer cells in the same manner (Tumor formation)

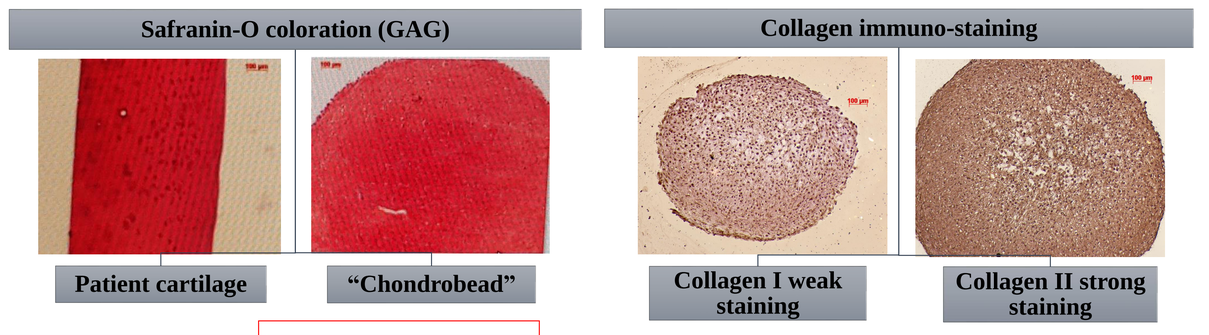
2) Efficacy in large animals (pigs): cartilage samples are harvested (pigs=2). The autologous cartilage beads are re-implanted in 6mm lesions (4-5 lesions) where a lesion per joint is left empty as a control. The 2 pigs are then analyzed at 2 weeks post grafting to verify the beads hold in the lesions (macro) and the quality of the graft (micro, Safranin-O staining).

Results

❖ Tissue engineered hyaline cartilage results

- Hyaline cartilage "Chondrobeads" can be produced independently of the patient's age, arthritis status at the time of harvesting with only 30mg of cartilage of either the knee or the ankle.
- "Chondrobeads" quality testing showed:

1) presence of GAG (Safranin-O) when compared to non OA cartilage sample
2) collagen II strongly present (immuno-staining)
3) GAG/DNA ratio mean was 62.9 (50.3-87.05) compared to patients cartilage (OA and non OA) mean 478 (270-1178)



Patient /age	GAG / dsDNA ratio
Pt 1 / 70 y-o	**87.05**
Pt 2 / 64 y-o	50.03
Pt 3 / 47 y-o	**52.40**
Pt 4 / 38 y-o	62.40
Pt 3 cartilage / 47 y-o	**296.2**

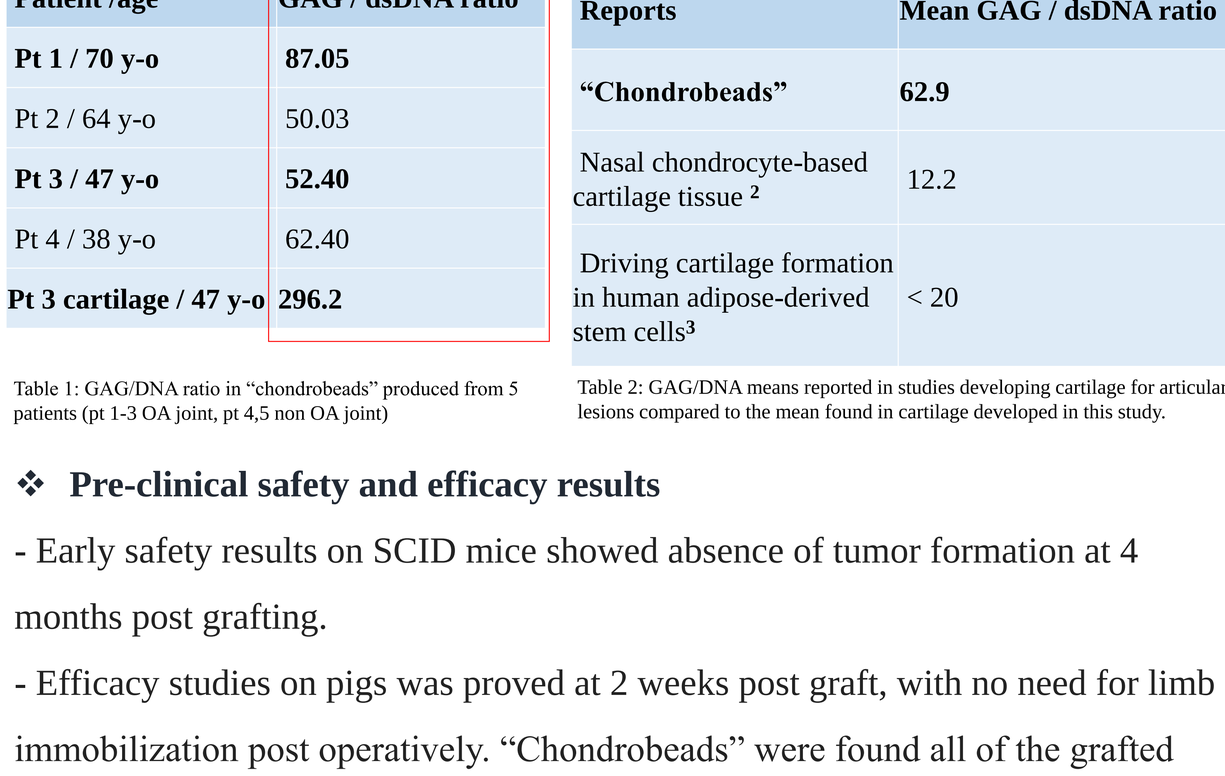
Table 1: GAG/DNA ratio in "chondrobeads" produced from 5 patients (pt 1-3 OA joint, pt 4,5 non OA joint)

Reports	Mean GAG / dsDNA ratio
"Chondrobeads"	**62.9**
Nasal chondrocyte-based cartilage tissue [2]	12.2
Driving cartilage formation in human adipose-derived stem cells[3]	< 20

Table 2: GAG/DNA means reported in studies developing cartilage for articular lesions compared to the mean found in cartilage developed in this study.

❖ Pre-clinical safety and efficacy results

- Early safety results on SCID mice showed absence of tumor formation at 4 months post grafting.
- Efficacy studies on pigs was proved at 2 weeks post graft, with no need for limb immobilization post operatively. "Chondrobeads" were found all of the grafted lesions. Histological analysis (Safranin-O) showed hyaline quality preservation, beads fusion among themselves and integration with surrounding native cartilage.



Conclusion

- ✓ Hyaline cartilage can be produced from patients up to 80 years of age and from arthritic joint, from the knee and ankle.
- ✓ The produced cartilage proved to be safe in rodents
- ✓ Proved efficacy in pigs in terms of graft hold in the lesions, beads fusion among themselves and attachment to the neighboring native cartilage while preserving their hyaline quality

References:

1 Riboh, J.C., et al., *Comparative efficacy of cartilage repair procedures in the knee: a network meta-analysis.* Knee Surg Sports Traumatol Arthrosc, 2017. **25**(12): p. 3786-3799. 2 Mumme, M., et al., *Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial.* The Lancet, 2016. **388**(10055): p. 1985-1994. 63 Dang, P.N., L.D. Solorio, and E. Alsberg, *Driving Cartilage Formation in High-Density Human Adipose-Derived Stem Cell Aggregate and Sheet Constructs Without Exogenous Growth Factor Delivery.* Tissue Engineering. Part A, 2014. **20**(23-24): p. 3163-3175.