Hyaline Cartilage Production Independent from Osteoarthritic Status and Age

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Background

Cell therapy of cartilage lesions is an important frontier in biomedical science. 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) has hitherto not been produced from older patients > 55 years of age, nor from osteoarthritic (OA) joints harvest.

Objectives

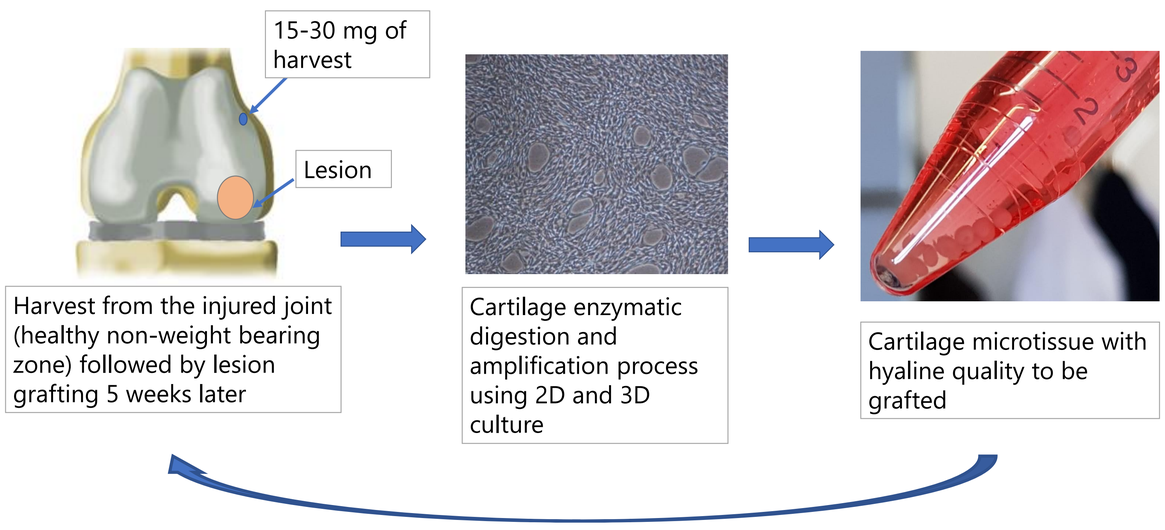
Develop a technique to engineer high quality cartilage for articular grafting, which :

1) allows to produce cartilage grafting material from elderly patients as well as patients with osteoarthritis.
2) shows preclinical safety and efficacy in animal models (mouse, minipig).

Methodology

A dedifferentiation/redifferentiation approach was used to generate cartilage for articular grafting from biopsies.

Harvested chondrocytes were amplified and processed through 2D/3Dculture to generate cartilage microtissues.



In vitro human chondrocyte study

Cartilage samples : 19 samples from donors aging between 18-80 y-o. Arthritic and non-arthritic harvests from knee and ankle (15-30 mg).

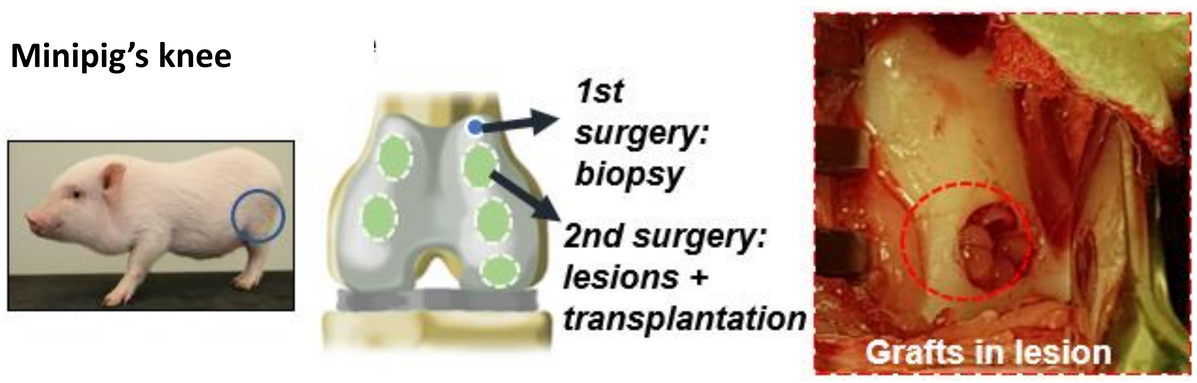
Cartilage produced from these samples and tested by Safranin-O staining (GAG), immuno-staining (collagen II vs collagen I), and quantified by GAG/DNA ratio.

In vivo preclinical trial

Safety in rodents (SCID mice): human cartilage microtissue was implanted in 56 mice compared to 14 positive controls (human cancer cells).

Efficacy in large animals : pig cartilage microtissue implanted in 6 minipigs followed overt 3 to 6 months.

Minipig's knee



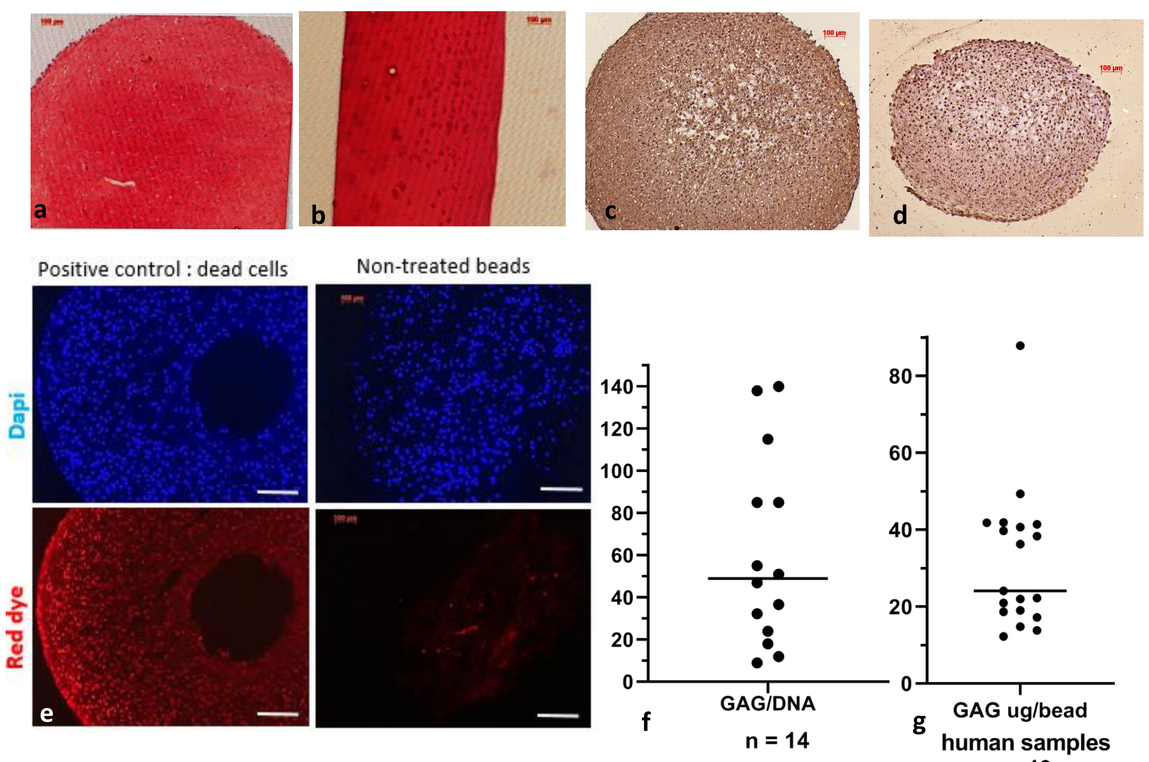
Results

❖ Tissue engineered cartilage results

Hyaline cartilage can be produced independently of the patient's age, arthritis status at the time of harvest with only 30mg of cartilage from knee or ankle.

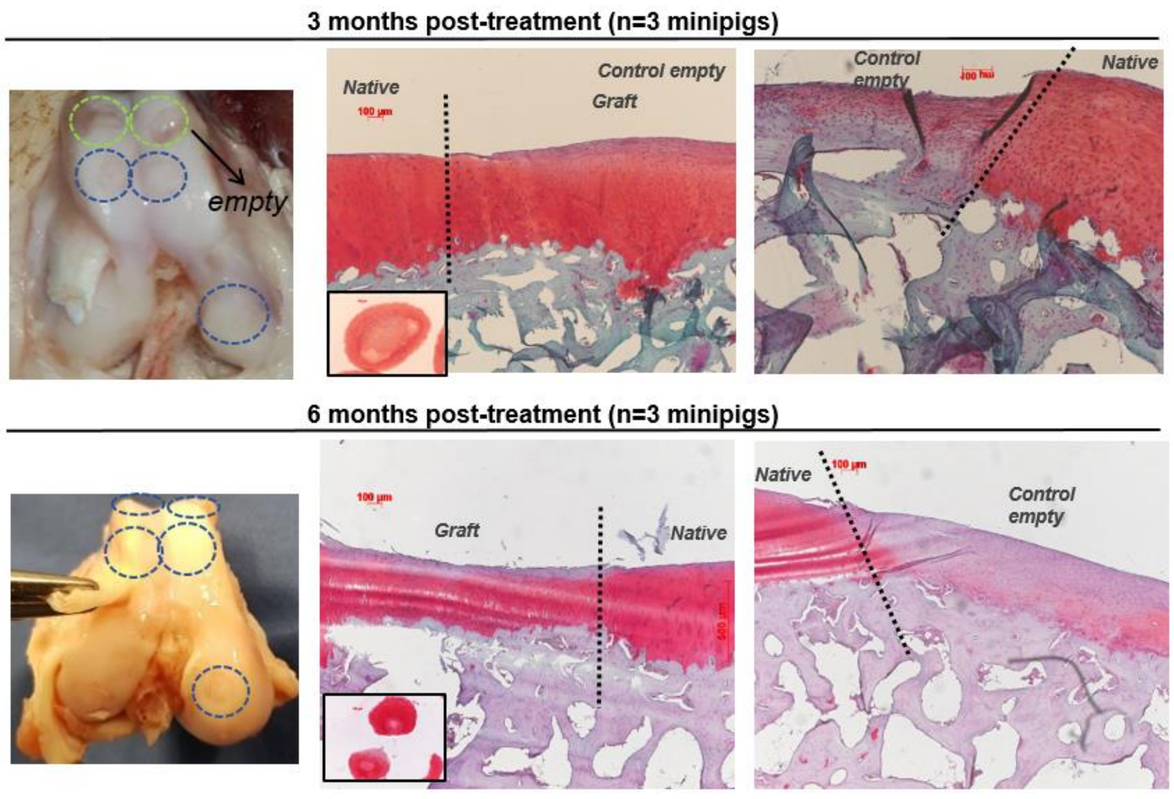
Quality control showed:

1) presence of GAG (Safranin-O) when compared to non-OA cartilage (a,b)
2) collagen II strongly present (immuno-staining) (c,d)
3) cell viability detection inside the beads (IF) (e)
4) average GAG/DNA is 50 [10;140], and GAG per bead is 24 [10;88] (f,g)



❖ Pre-clinical safety and efficacy results

- Safety proven on SCID mice with absence of tumor at 6 months post grafting.
- Efficacy studies on minipigs up to 3 and 6 months post graft, without limb immobilization. Histological analysis showed hyaline quality preservation and integration with native cartilage and subchondral bone.



Conclusion

- ✓ Cartilage for articular grafting can be produced from patients up to 80 years of age even from arthritic joint, from both knee and ankle.
- ✓ Our protocol yielded stem cell-like, "rejuvenated" chondrocytes after dedifferentiation and high quality cartilage microtissues after redifferentiation
- ✓ The produced cartilage proved to be safe in rodents and minipigs (fusion and integration with native cartilage and subchondral bone).

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.