

CLAIMS

1. A method for *in vitro* production of hyaline cartilage tissue comprising:
 - i) culturing chondrocytes on an adherent culture system in a dedifferentiation culture medium that activates Wnt signaling pathway to obtain chondrocytes with a morphology of fibroblastic-like cells;
 - ii) culturing said fibroblastic-like chondrocytes on an adherent culture system in a redifferentiation culture medium that inactivates Wnt signaling pathway to obtain chondrocytes with full capacity to resynthesize hyaline matrix;
 - iii) culturing said chondrocytes obtained in step ii) in a three-dimensional culture system in an induction/maturation culture medium that maintains the inactivation of Wnt signalling pathway.
2. The method according to claim 1, wherein said dedifferentiation culture medium comprises FGF-2 and said redifferentiation culture medium and induction/maturation culture medium are FGF-2 free medium.
3. The method according to claim 1 or 2, wherein said dedifferentiation culture medium comprises at least one growth factor selected from the group consisting of: FGF-2, PDGF-BB, TGF- β and EGF, preferably said dedifferentiation culture medium comprises FGF-2.
4. The method according to any one of claims 1 to 3, wherein said redifferentiation culture medium and said induction/maturation culture medium comprises TGF- β , preferably TGF- β 3, more preferably TGF- β 3 and FGF-7.
5. The method according to any one of claims 1 to 4, wherein said redifferentiation culture medium comprises platelet lysate.
6. The method according to any one of claims 1 to 5, wherein said dedifferentiation culture medium, redifferentiation culture medium and/or induction/maturation culture medium comprise(s) serum.

7. The method according to any one of claims 1 to 6, wherein said induction/maturation culture medium comprises at least one component selected from the group consisting of: insulin, IGF-1, selenium, transferrin, and ethanolamine.
- 5 8. The method according to any one of claims 1 to 7, wherein said chondrocytes are cultured in step iii) in hypoxia atmosphere comprising less than 10% O₂ (v/v).
9. The method according to any one of claims 1 to 8, wherein said chondrocytes are cultured in step i) during 10 to 15 days, in step ii) during 4 to 8 days, and/or in step iii) during 10 to 15 days.
- 10 10. The method according to any one of claims 1 to 9, wherein said chondrocytes of step i) are isolated from a subject, preferably from cartilage tissue of a human or horse subject.
11. An engineered cartilage tissue in a form of spheroid presenting a glycosaminoglycan (GAG) content per spheroid between 10 and 100 µg/spheroid~~GAG/ double-strand DNA ratio of at least 15.~~
- 15 12. ~~Use of the~~An engineered cartilage tissue of claim 11 for the manufacture of a medicament for use in the treatment of cartilage defects and cartilage degenerative disease in a subject in need thereof, preferably for use in autologous transplantation.
13. The use according to claim 12, wherein said medicament is for autologous transplantation.
- 20 14. The use according to claim 12, wherein said medicament is for allogenic transplantation.
- 13.15. A method of screening molecules inhibiting the cartilage degenerative process comprising:-
 - 25 i) ~~c~~ontacting the engineered cartilage tissue of claim 11 with one or more candidate molecules, and
 - ii) ~~s~~electing molecules inhibiting cartilage degenerative process.