

CLAIMS

1. A method for *in vitro* production of 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 induction/maturation culture medium that maintain the inactivation of Wnt signalling pathway.
2. The method of claim 1 wherein said dedifferentiation culture medium comprises FGF-2 and redifferentiation culture medium and induction/maturation culture medium are FGF-2 free medium.
3. The method of 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 FGF2.
4. The method according to any one of claims 1 to 3 wherein said redifferentiation and maturation culture medium comprises TGF- β , preferably TGF- β 3, more preferably TGF- β 3 and FGF7.
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, redifferentiation and/or maturation culture medium comprise serum.

7. The method according to any one of claims 1 to 6 wherein said maturation 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 day, 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.
- 15 11. An engineered cartilage tissue in a form of spheroid presenting a GAG/ double strand DNA ratio of at least 15.
12. An engineered cartilage tissue of claim 11 for use in the treatment of cartilage defects and cartilage degenerative disease in a subject in need thereof, preferably for use in autologous transplantation.
- 20 13. A method of screening molecules inhibiting the cartilage degenerative process comprising :
- 25 i) Contacting cartilage tissue of claim 11 with one or more candidate molecules, and
ii) Selecting molecules inhibiting cartilage degenerative process.