

METHODS AND COMPOSITIONS FOR GENERATING CHONDROCYTE LINEAGE CELLS AND/OR CARTILAGE LIKE TISSUE

Abstract

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A method for generating chondrocytes and/or cartilage, optionally articular like non-hypertrophic chondrocyte cells and/or cartilage like tissue and/or hypertrophic chondrocyte like cells and/or cartilage like tissue, the method comprising: a. culturing a primitive streak-like mesoderm population, optionally a CD56+, PDGFRalpha+ KDR- primitive streak-like mesoderm population, with a paraxial mesoderm specifying cocktail comprising: i. a FGF agonist; ii. a BMP inhibitor; optionally Noggin, LDN-193189, Dorsomorphin; and iii. optionally one or more of a TGFbeta inhibitor, optionally SB431524; and a Wnt inhibitor, optionally DKK1, IWP2, or XAV939; to specify a paraxial mesoderm population expressing cell surface CD73, CD105 and/or PDGFR-beta; b. generating a chondrocyte precursor population comprising: i. culturing the paraxial mesoderm population expressing CD73, CD105 and/or PDGFR-beta at a high cell density optionally in serum free or serum containing media; ii. culturing the high cell density CD73+, CD105+ and/or PDGFRbeta+ paraxial mesoderm population with a TGFbeta3 agonist in serum free media to produce a high cell density Sox9+, collagen 2+ chondrocyte precursor population; and c. either i. culturing the high cell density Sox9+, collagen 2+ chondrocyte precursor population with the TGFbeta3 agonist for an extended period of time to produce an articular like non-hypertrophic chondrocyte cells and/or cartilage like tissue; or ii. culturing the high cell density Sox9+ collagen2+ chondrocyte precursor population with a BMP4 agonist for an extended period of time to produce a hypertrophic chondrocyte like cells and/or cartilage like tissue.

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Fig 1A.