An emerging tissue engineering approach is based on the use of spheroids as building blocks for damaged tissue repair. We aim to produce spheres with cellular, molecular and extracellular matrix (ECM) characteristics of cartilage tissue, with regular shape and in a consistent amount to treat articular cartilage defects. Samples of human nasal septum cartilage were collected, approved by local research ethics committee. Cartilage progenitor cells were isolated after collagenase digestion and expanded in vitro. Cell suspension was seeded in agarose hydrogel with micromolded resections and maintained in vitro for up to 3 weeks without inductive factors. After 24 hours, 21 spheroids were fabricated per cm2, with one spheroid formed per resection and showed a slightly increase in its diameter during culture time (from 381.26 ± 31.62µm after one week to 423.47 ± 102.61µm after 3 weeks). Viability was assessed qualitatively, using a Live & Dead Kit (Invitrogen) followed by confocal laser microscopy analysis, and quantitatively by 7-Acidine D (7AAD) exclusion evaluated by flow cytometry (BD™ Accuri C6), showing most viable cells (96.2%) with a few dead cells aside the spheroid. Histological analysis revealed cells with a preferably rounded morphology inside the spheroid while peripheral cells were flat. Cells in spheroids presented Sox genes mRNA (Sox-9, -5 and -6), known to able to activate genes of cartilage ECM components. Chondrogenic factors TGF-β1 and -β2 were secreted by spheroids after 3 weeks in culture. Besides, spheroids expressed ECM components typical of cartilaginous tissue, like collagen types I, II and VI, besides glicosaminoglicans aggrecan and chondroitin sulfate, all detected by immunohistochemistry. The association of the method described with cartilage progenitor cells cultivated without inducing factors enabled chondrospheres fabrication with high yield, viability and low cost. In vivo studies are in course to evaluate its regenerative potential.