Introduction Adult stem cells gradually lose their stemness once they are removed from their in vivo niche for plating in plastic flasks. Synovium-derived stem cells (SDSCs) are a tissue-specific stem cell for chondrogenesis. Our previous study indicated that SDSC-derived extracellular matrix (ECM) can serve as an in vitro three-dimensional (3D) niche, greatly enhancing SDSC propagation and allowing restoration of stem cell stemness toward chondrogenesis [34]. Considering articular chondrocytes reside in a hypoxic niche, we hypothesized that some important microenvironmental factors such as oxygen tension and mitogens are assumed to contribute to the regulation of these seeded cells. In this study, we hypothesized that in vitro microenvironment can be optimized by modulating oxygen tension and mitotic signal in ECM deposited by SDSCs to rejuvenate expanded SDSCs’ proliferation and chondrogenic differentiation capacity.

Methods Porcine synovial membrane was used to isolate SDSCs as described in our previous study [4]. SDSCs were used to prepare ECM [1]. Passage 3 SDSCs were plated at 3,000 cells per cm² in with (“P”) or without (“E”) ECM-coated flasks and treated with low oxygen (5% O₂, or “5”), normoxic oxygen (21% O₂, or “21”), and/or 10 ng/mL FGF-2 (or “F”) for one passage. The basal medium consisted of αMEM, 10% fetal bovine serum, and 1x penicillin and streptomycin. There were a total of eight groups: “21P”, “5P”, “21PF”, “5PF”, “21E”, “5E”, “21EF”, and “5EF”. Cell numbers were counted and cell morphology was photographed. The pretreated SDSCs (0.3 × 10⁶ cells) were centrifuged to form a pellet followed by incubation in a serum-free chondrogenic medium with 10 ng/mL of TGF-β3 for 14 days. At days 0, 7, and 14, pellets were collected for evaluation of the pretreatments on expanded SDSC chondrogenesis, Safranin O and Alcian blue staining for sulfated GAGs and immunostaining for types I and II collagen. Biochemical analysis was used to assess cell proliferation (DNA amounts) and chondrogenic differentiation (GAG amounts). TaqMan® PCR was used to evaluate chondrogenic markers (types I, II, and X collagen, aggrecan, and Sox9) and hypertrophic markers (type X collagen and MMP13) at an mRNA level (data not shown).

Results In vitro microenvironment to rejuvenate SDSC proliferation After one passage of the eight treatments, the expanded SDSCs exhibited flattened and irregular shapes (characteristic of aged cells) in “21P” and “5P” groups, shorter and glistening shapes in “21PF” and “5PF”, and longer and glistening fibroblast-like shapes in all ECM groups (“21EF”, “21EF”, “5E”, and “5EF”). Compared to the random arrangement of cells plated on plastic flasks, ECM-expanded cells were more organized (Fig.1). After 6-day-expansion, cell number increased 2.39-fold in “5P”, 2.61-fold in “21P”, 6.90-fold in “21PF”, 12.50-fold in “5PF”, 34.52-fold in “21E”, 54.52-fold in “21EF”, 77.38-fold in “5E”, and 93.45-fold in “5EF” (Fig.2). Histology for SDSC pellets is shown in Fig.3.

Pretreated effect with ECM on expanded SDSC chondrogenesis ECM-treated SDSCs yielded pellets with much higher chondrogenic index (GAG/DNA) than those from plastic-expanded SDSCs when SDSCs were incubated in a normoxic incubator (7.49 ± 1.22 versus 2.07 ± 0.39 at day 7 and 28.31 ± 2.88 versus 5.52 ± 0.88 at day 14) or in a hypoxic incubator (33.07 ± 0.89 versus 5.60 ± 0.53 at day 14). Surprisingly, with supplementation of FGF-2, chondrogenic index in ECM-treated SDSCs was not statistically different from that of plastic-expanded SDSCs, in either the normoxic or hypoxic condition.

Conclusion Our study, for the first time, evaluated three important microenvironmental parameters (ECM, low oxygen, and FGF-2) and their interaction on SDSC proliferation and chondrogenic potential. We found that pretreatment with SDSC-derived ECM could dramatically increase SDSC cell number while retaining their typical stem cell shape, which is much stronger than FGF-2 alone. The combination of ECM, low oxygen and FGF-2 yielded the highest cell number than the other groups. Pretreatment with low oxygen did not show any significant difference in cell number compared to the normoxic condition. When incubated in a serum-free chondrogenic medium, ECM-treated SDSCs exhibited much higher chondrogenic index than those from plastic flasks, no matter at normoxia or hypoxia. Although pretreatment with low oxygen did not show any significant difference in chondrogenic index from normoxic treatment when SDSCs were plated on plastic flasks, we found that pretreatment with combined ECM and low oxygen could increase subsequent SDSC chondrogenic differentiation capacity. Despite that pretreatment with FGF-2 alone could dramatically increase SDSC chondrogenic potential, the supplementation of FGF-2 in the combination of ECM and low oxygen did not benefit further SDSC chondrogenic capacity.

Reference

Disclosures Authors have nothing to disclose.