Amniotic membrane: Chondrogenic differentiation in toto

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Abstract

Human amniotic membrane has already been applied in a lot of clinical trials due to its beneficial properties and is still of great interest for a multitude of areas of research. Tissue engineering strategies usually require cell isolation and combination with a suitable carrier substrate. Alternatively, the cell sheet technology enables transplantation of expanded cells without the use of carrier materials. In contrast, our approach is to differentiate stem cells within intact amniotic membrane (AM), which constitutes a pre-formed sheet of stem cells, without prior cell isolation. We have previously demonstrated osteogenic differentiation of stem cells within intact human AM in vitro. Beside bone, regeneration of cartilage is an important scope in orthopedic trauma. For this reason, also the chondrogenic differentiation potential of human AM was investigated. In vitro chondrogenic differentiation of human AM biopsies was induced by culture in Mesenchymal Stem Cell Chondrocyte Differentiation Medium (C, Lonza) supplemented with transforming growth factor beta 3 (Lonza), optionally supplemented with fibroblast growth factor 2 (FGF-2, R&D Systems; CF), and the medium described by Tallheden (T) for 16 weeks. Membrane viability was quantified by EZ4U-Nonradioactive Cell Proliferation and Cytotoxicity Assay (Biomedica). To determine chondrogenesis, cartilage-specific collagen II and glycosaminoglycans (GAG) were demonstrated by alcian blue staining of paraffin embedded histological sections. Furthermore, the amount of GAG was quantified using BlyscanTM Sulfated Glycosaminoglycan Assay (Biocolor). The highest hAM-viability was sustained in the medium described by Tallheden [d56: control medium (Co): 15.12%±1.47%; C: 24.56%±4.26%; CF: 21.71%±13.31%; T: 56.18%±19.14% compared to d0]. Regardless of the medium applied, Alcian blue stainings of all AM-samples revealed accumulation of GAGs in the membranes. Nevertheless, stainings of samples cultured in control medium were less intense, whereas CF appeared to show the most intense staining. Quantitative evaluation showed that in the control medium (Co) the amount of GAG was decreasing during culture (52.0%±14.5% on d56 compared to d0). When culturing in C, the GAG-amount was increased compared to Co at any timepoint and remained stable throughout the culture period. Noticeable, 3-fold increased GAG production compared to Co was obtained when adding FGF-2 to the medium. Chondrogenic differentiation of intact human amniotic membrane could be induced by chondrogenic medium (C) and further increased with supplemented FGF-2. Thus, these results are another promising step towards using intact human amnion with its residing stem cells for tissue engineering.