

Models for testing regenerative therapies – focus on explants as models for osteoarthritis.

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Background. Osteoarthritis (OA) is a degenerative disease that progressively involves all joint compartments leading to destruction and loss of function. Regenerative medicine (RM) aims to introduce revolutionary therapies dedicated to drastically improve the way we treat degenerative diseases including OA. Explanted cartilage tissue has been proposed as a modality to study cartilage ontogeny and to understand cartilage repair as well as modality to screen new drug/therapies for the treatment of OA.

Objectives. To establish a working protocol for obtaining human osteochondral tissue explants in co

-culture with synovial tissue to be used as ex vivo models for OA and to demonstrate explant reactivity to conditioned media (CM) from adipose mesenchymal cells (ADSC) tested as a modality

for bone and cartilage rescue.

Methods. Human osteochondral samples were collected from patients undergoing total knee replacement were kept in incomplete chondrogenic media (ICM) or in serum free DMEM. Explant-released cytokines were quantified by ELISA for Human tumor necrosis factor alpha (TNF α) and interleukin -6 (IL-6) and qPCR-based immunoassay for Human IL-17A and Human IL-1 β (ProquantumTM immunoassay kit Invitrogen). Histology, Western blot and Immunohistochemistry studies to detect Collagen type II (Col II) matrix metalloprotease 1 and 13 (MMP I, MMP-13), Perlecan and beta galactosidase (BGAL) are going on.

Results. We found that culture media as well as synovial tissue presence influences the level of detectable IL-6, IL-17A CM increased IL-6 presence up to 29 days in culture; TNF α and IL1 β levels decrease after 7 days in culture; CM treatment significantly decrease TNF α in both synovium containing DMEM and ICM cultured explants Histology revealed presence of active chondrocyte with enlarged hyperchromatic nuclei in CM treated explants.

Conclusion. Time in culture, type of culture media and synovial presence influence explant reactivity. Presence of synovial tissue increase explant reactivity especially for situation when an anti-

inflammatory effect is expected. Histology and immunohistochemistry can detect markers of tissue regeneration. Explant culture can serve as a reliable ex vivo model for testing both anti-inflammatory

as well as tissue remodeling intervention for articular joint repair.

Keywords: osteoarthritis, regenerative medicine, explant culture, adipose derived mesenchymal cells