**Materials and Methods:** hPSCs cultured on IαI were transitioned to hNPSCs using modified RSeT and 2iGöY formulations in normoxic, feeder-free and coating-free conditions. Western blotting, qPCR and high-resolution confocal microscopy was performed to investigate the hNPSCs biomolecular profile. Seahorse X96 Mito-stress assay was performed to measure oxidative respiration.

**Results:** The hNPSCs obtained in IαI conditions show typical dome morphology, increased expression of naïve markers and relevant intracellular signalling. Integrin profile shows reduced total integrin protein content as well as reduced FAK phosphorylation. DNA hypomethylation was confirmed using 5-mC staining coupled with confocal imaging. Seahorse assay was performed to confirm switch from glycolysis to oxidative phosphorylation. Finally, hNPSCs were transferred to a chemically-defined hydrogel set-up (peptide-based, FEFEFKFK), with confocal microscopy showing naïve colonies growing into large 3D clusters expressing naïve markers KLF17, DPPA3 and SSEA-4.

**Discussion:** We present a defined and feeder-free method for naïve and primed pluripotent stem cells. The culture method is flexible to biomaterial formats and hydrogel technologies, as shown by the easy adaptation to a defined, naked hydrogel. This new culture method has the potential to widen the use of hNPSCs in basic research and in combination with tissue engineering for early development modelling and cell therapy strategies.

## TNF alpha-stimulated gene 6 (TSG-6) is weakly chondroprotective in murine OA but does not account for FGF2-mediated joint protection

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**Introduction:** Tumour necrosis factor alpha-stimulated gene 6 (TSG-6) is expressed in response to a range of pro-inflammatory mediators and has purported tissue-protective and anti-inflammatory effects. TSG-6 has been detected at high levels in the synovial fluid of patients with rheumatoid arthritis and osteoarthritis. We have previously shown that TSG-6 is regulated in the mouse joint following surgical destabilization of the medial meniscus (DMM), in a highly mechanosensitive and fibroblast growth factor 2 (FGF2)-dependent manner. As FGF2$^{-/-}$ mice develop accelerated OA, and injury-induced FGF2-dependent genes were downregulated in TSG-6$^{-/-}$ mouse joints, we speculated that the chondroprotective role of FGF2 may be mediated through TSG-6.

**Materials and Methods:** 42 genes were quantified by real-time PCR in whole joints post DMM (6h and 7 days). Joint pathology was assessed in male and female FGF2$^{-/-}$, TSG-6$^{-/-}$, TSG-6$^{tg}$ (overexpressing), FGF2$^{-/-}$;TSG-6$^{tg}$ (8 weeks only) mice with their respective controls at 8 and 12 weeks following DMM. Cartilage repair was tested 8 weeks following focal cartilage injury in TSG-6$^{tg}$ and control mice. FGF2 release after cartilage injury was measured by V-PLEX bFGF kit.

**Results:** 15 inflammatory genes were significantly upregulated in TSG-6$^{-/-}$ joints post DMM including *IL1α*, *Ccl2* and *Adamts5* compared with wild type. Six genes were significantly suppressed in TSG-6$^{-/-}$ joints including *Timp1*, *Inhibin βA* and *podoplanin*. Accelerated OA was seen at 12 weeks post DMM in male TSG-6$^{-/-}$ mice and a reciprocal improvement in disease was seen in TSG-6$^{tg}$ mice. There was no significant difference observed in cartilage repair between genotypes. FGF2 release in the conditioned medium after injury was not influenced by genotype. TSG-6 overexpression was unable to prevent accelerated OA in FGF2$^{-/-}$ mice.

**Discussion:** TSG-6 influences early gene regulation in the destabilized joint and is weakly chondroprotective in murine OA. Although strongly FGF2-dependent, TSG-6 does not account for FGF2-mediated joint protection.

## D-Net: An AI model for aligning volumes, developed for cartilage segmentation from CT scans contrasted with a type II collagen binding peptide containing di-iodotyrosine

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**Introduction:** Di-Iodotyrosinated Peptide Imaging of Cartilage (DIPIC) by computed tomography (CT) uses a type II collagen targeted radio-contrast agent to visualize cartilage by X-rays. Manual segmentation of cartilage from the contrast-enhanced images is time consuming, while semi-automated methods based on X-ray absorption only reliably segments out cartilage in the weight bearing region. We hypothesize subtraction of non-contrasted CT scan from DIPIC scans would yield better segmentation of cartilage, but this requires accurate alignment of both scans. We explore the use of Artificial Intelligence (AI) models for this alignment task.

**Materials and Methods:** The CT scans of mouse tibiae were obtained with and without contrast. The nature of the

animal scanner meant that no standardization of pose between scans was possible. Thus, in addition to being rotated within the field of view, the two scans may be to be rotated in any orientation compared to each other. We compared three previously published AI methods and two translational models and found that they were unable to cope with the full range of rotation. To address this, we developed D-Net. The networks were trained with 50 CT volumes, synthetically translated and rotated. The original 512x512x512 with resolution $10^3$ $\mu m^3$/vox scan was subsampled to 64x64x64 ($80^3$ $\mu m^3$/vox) before fed into the network due to memory limitations. Testing was on another 50 CT volumes unseen by the network and on paired volumes with and without contrast. We report the difference between the predicted and expected translation (TE), rotation (RE).

**Results:** D-Net significantly outperformed all other networks, achieving a TE of 70 μm (sub-voxel) and a RE 4°.

**Discussion:** While D-Net performed well on the downsampled volumes, it was impractical to train it on the original sized data. Further development is required to align the volumes at the original resolution. We postulate however, that trained on the appropriate dataset, D-Net would be able to perform alignment of any 2 volumes from any rotation. Additionally, the calculations for this to occur takes less than a second, enabling real-time applications.