HIGH-THROUGHPUT IN 3D
CELL AND TISSUE SCREENING *IN VITRO* AND *IN VIVO*

Gustavo A. Higuera, Joost van Dalum, Ling Wu, Liliana Moreira-Teixeira, Jeroen Leijten, Marcel Karperien, Clemens A. van Blitterswijk, Lorenzo Moroni

Tissue Regeneration Department, Institute for Biomedical Technology and Technical Medicine, University of Twente

Every year, millions of animals are used to evaluate the impact of substances, materials and treatments on the environment and human health. Despite the ethical concerns and financial costs, vertebrate animals will continue to be used in the chemical, biotechnological, pharmaceutical, and biomedical industries, among others. Here, we explored an alternative to reduce the number of lives and costs in animal testing. This approach involves the development of implantable high-throughput screening systems. The HTS system consists of μm^3 to mm^3 size wells organized in a matrix array consisting of wells (macroarray). Thousands of wells can be made with a wide range of materials, where the HTS size can be adapted to the implantation site of the tested animal. This invention opens the possibility to evaluate biology in three dimensions *in vitro* and *in vivo*.

Figure 2. HTS systems can be adapted to the size of the animal. This means that as the size of an animal increases, more conditions can be tested and fewer animals are required, providing the minimal amount of animals for statistical analysis are maintained. Here, for example, mice, rats, and rabbits are depicted.

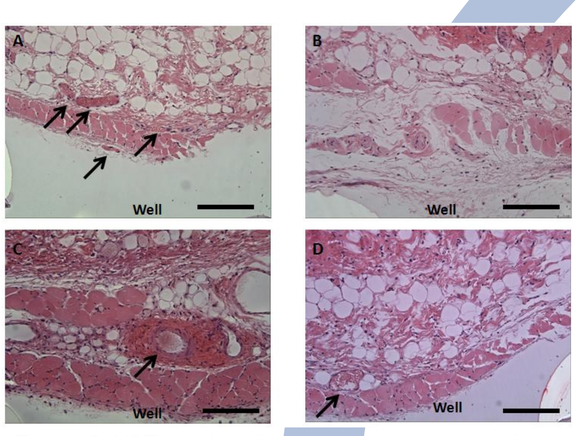

Figure 6. H&E staining of representative sections of host tissue above well macroarrays after 1 month implantation. Arrows point to vessel lumens, which were observed at the top of the well. The number of vessels formed depends on the condition tested. **A:** hMSCs. **B:** Differentiated hMSCs **C**: Primary Bovine Chondrocytes (PBCs). **D**: Co-culture hMSCs:Primary Chondrocytes. Scale bar: 100 µm.

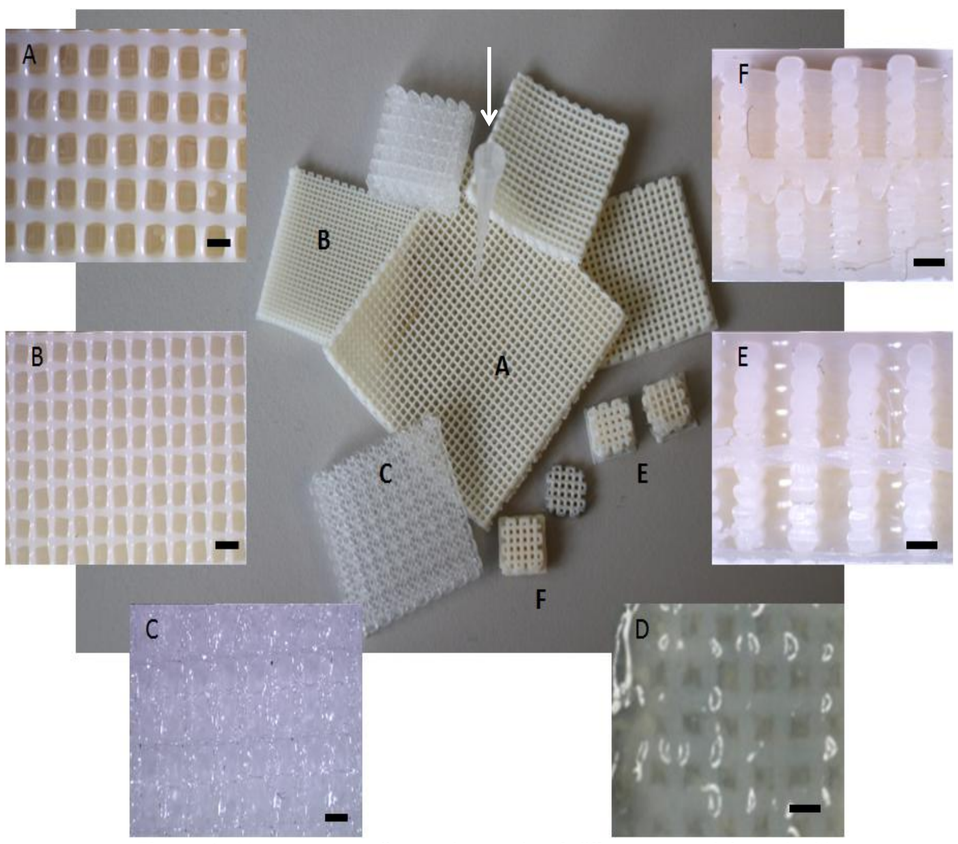

Figure 1. Macroarray wells can be made of different materials and with different architectures. Since these HTS systems can be custom-made, there are screening possibilities for research and industry. White arrow points to pipette tip size that fits into a well, thus wells can be seeded without the need of special equipment or operator. **A**: Top view of 1024 wells in a 32 × 32 array made of PEOT/PBT of 300/55/45 composition. **B**: Top view of 100 wells in a 10 × 10 array made of PEOT/PBT of 1000/70/30 composition. **C**: Top view of 100 wells in a 10 × 10 array made of poly lactic acid. **D**: Top view of 100 wells in a 10 × 10 array made of alginate (in PBS). **E**: Cross section of a double array with a closed polymer layer in between and opened bottom and top sides. **F**: Cross section of wells with a porous layer in the middle, closed bottom and opened top layers. Scale bar: 1 mm.

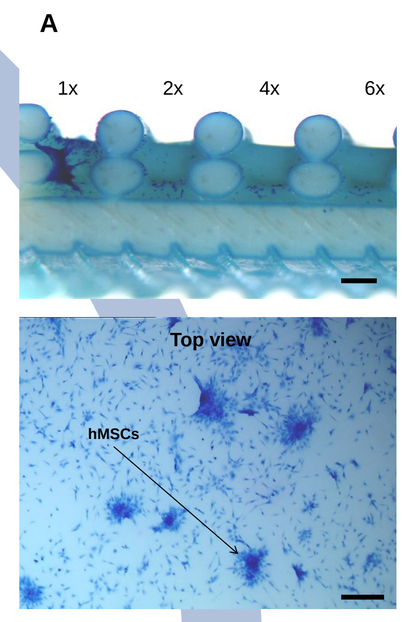

Figure 3. 3D aggregates of cells can be cultured in a few days in the macroarray system depending on the initial cell number, whereas on 2D surfaces 3D aggregates are not formed. **A**: Cross section of hMSCs dilutions seeded in a row. 1X was equivalent to 25,000 hMSCs, where a 3D construct can be observed. Scale bar: 0.5 mm. **B**: hMSCs on 2D PEOT/PBT disc. 300,000 hMSCs seeded. Culture time 5 days. hMSCs do not form a 3D construct. Scale bar: 0.1 mm.

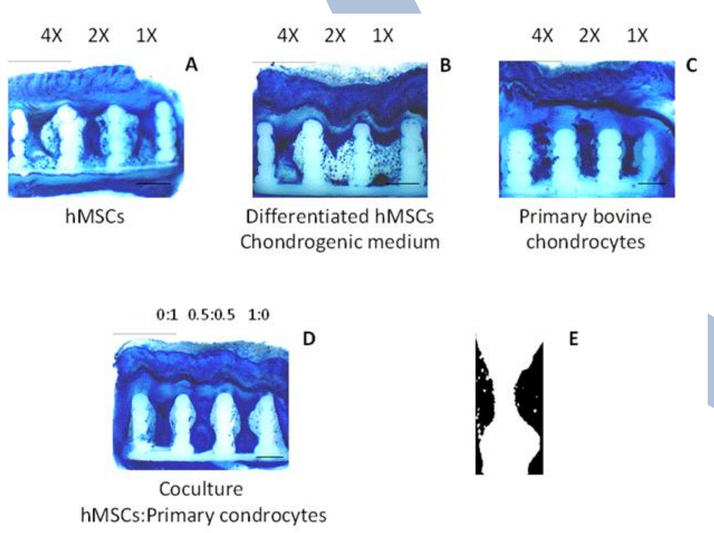

Figure 5. Subcutaneous implantation of macroarray systems. Four conditions were seeded in 3 × 3 macroarray and implanted in one mouse (n=10). **A**: hMSCs. **B**: Differentiated hMSCs **C**: PBCs **D**: co-culture hMSCs:PBCs in three ratios 0:1, 0.5:0.5, 1:0. We are determining parameters such as the tissue area inside of the well to assess the physical effects of implanting HTS systems in animals. **E**: Example of image used for tissue area quantification within each well. The image fits into the area contained in one well using the macroarray material as reference. Scale bar: 1 mm. 1X was equivalent to 25,000 cells seeded per well. 25,000 cells were the highest number of cells seeded in a well.

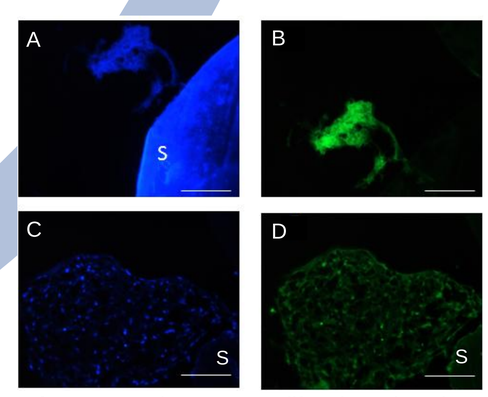

Figure 4. hMSCs proliferation in time produces aggregates of different sizes inside of the wells. 25,000 hMSCs seeded per well. S: Scaffold. **A**: 2 days of culture. Cell nuclei (Dapi) in aggregate. **B**: 2 days of culture. Proliferation marker (EDU) in aggregate. **C**: 5 days of culture. Cell nuclei (Dapi) in aggregate. **D**: 5 days of culture. Proliferation marker (EDU) in aggregate. Scale bar: 100 µm. Magnification 200 X.

MIRA

INSTITUTE FOR BIOMEDICAL TECHNOLOGY AND TECHNICAL MEDICINE

UNIVERSITEIT TWENTE.