Three Dimensional Screening of Tissues in vivo Decreases the Numbers of Experimental Animals Necessary in Cartilage Regeneration Studies

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Introduction

Despite promising *in vitro* models that could partially replace *in vivo* testing of conditions relevant to cartilage regeneration, current systems fail to recapitulate biological complexity making *in vivo* experiments, its associated costs and ethical burden still necessary. In this study, we have explored an alternative route that can abate the number of lives and expenses in animal testing for tissue regeneration applications through implantable and three-dimensional (3D) screening systems.

Materials and Methods

Free-form fabrication and biomaterials furnish the versatility necessary to design and produce wells organized in column × row arrays (Macroarrays).

To test the macroarray devices, a total of 36 extracellular matrix (ECM)-producing experimental conditions comprising various cell numbers of human mesenchymal stem cells (hMSCs), primary bovine chondrocytes and cocultures of these two cell types were dispensed in separate wells of macroarrays, impanted in one immunodeficient mouse (n=10 mice), examined through histological sections and screened for cartilage through glucosaminoglycans (GAG-alcian blue) .

Results and Discussion

Depending on the size of the animal, these platforms can be tailored to fit the required dimensions of an implantation site.Thus, the bigger the animal, the higher the number of conditions that can be investigated simultaneously (Figure 1) with various biomaterials and architectures for a macroarray device.

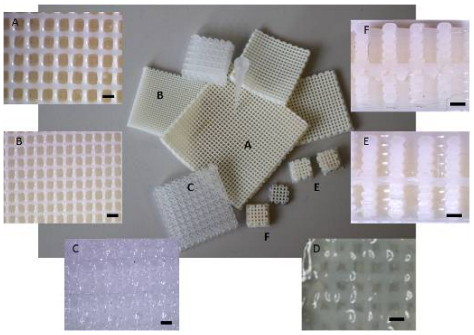

Figure 1. Macroarrays with different dimensions can be fabricated: 1024 wells (A) and 100 wells made of PEOT/PBT (B); 100 wells made of of poly lactic acid (C); 100 wells made of alginate (in PBS) (D). Different architectures could also be created: double matrix (top and bottom of a system) with a solid polymer layer in between (E); wells with a porous layer of material in the middle to enable co-culture (F). Scale bar: 1 mm.

ECM-producing conditions were implanted in mice and screened for cartilage through (GAG) (Figure 2). Out of the 36 conditions implanted, the results showed a significantly higher quality of ECM for the hMSCs:Chondrocytes at 80:20 ratio on week 4 compared to the control. Recently, an *in vitro* study (1) indicated the potential of these coculture ratios for cartilage research. Through the macroarray device, the translation of conditions from *in vitro* models of tissue are possible. By implanting multiple conditions in relatively few animals, resources are optimized while screening for ideal conditions in true 3D tissues.

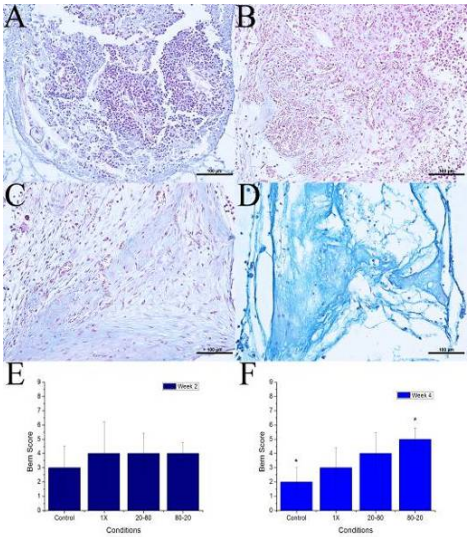

Figure 2. Cartilage screening in wells and evaluated through GAG (Blue) and cytoplasm (Red). Control (empty macroarray system) (A); 25,000 hMSCs (B); co-culture of hMSCs:Chondrocytes at 20:80 ratio (C); hMSCs:Chondrocytes at 80:20 ratio (D). Quantification of cartilage quality (mean Bern score, n= 10 mice) from conditions on weeks 2 (E) and 4 (F). Scale bars: 100 µm. One-way Anova was followed by Tukey's to evaluate significance (*p = 0.03).

Conclusion

With macroarray devices the use of vertebrate animal lives and costs could be drastically dwindled for tissue regeneration research, among others.

Reference

1.Wu L. et al., *Tissue Eng Part A* (2011). 17 (9-10):1425-36.

Acknowledgements

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