

## Local Compressive and Tensile Stiffness Measured in Tissues with Regular Patterns of Hyaline-Fibrocartilage Regions

[A.Marsano](#)<sup>1</sup>, [D.Wendt](#)<sup>1</sup>, [R.Raiteri](#)<sup>2</sup>, [R.Gottardi](#)<sup>2</sup>, [S.Dickinson](#)<sup>3</sup>, [A.P.Hollander](#)<sup>3</sup>,  
[D.Wirz](#)<sup>4</sup>, [A.U.Daniels](#)<sup>4</sup>, [T.M.Quinn](#)<sup>5</sup>, [M.Stolz](#)<sup>6</sup>, [I.Martin](#)<sup>1</sup>

<sup>1</sup> *Institute for Surgical Research&Hospital Management, University Hospital Basel, Basel, CH*

<sup>2</sup> *University of Bristol, AMBI, Avon Orthopaedic Center, Southmead Hospital, Bristol, UK*

<sup>3</sup> *Department of Biophysical and Electronic Engineering, University of Genoa, I*

<sup>4</sup> *Laboratory for Orthopaedic Biomechanics, Felix Platter Hospital, University of Basel, CH*

<sup>5</sup> *Cartilage Biomechanics Group, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH*

<sup>6</sup> *Maurice E. Mueller Institute for Structural Biology, Biozentrum/University of Basel, CH*

**INTRODUCTION:** With the ultimate goal of engineering a meniscus substitute, we generated bi-zonal hyaline-fibrocartilaginous tissues using articular chondrocytes loaded on hyaluronan-based meshes and cultured in the presence of different hydrodynamic conditions [1, 2]. Specific aim of this study was to demonstrate that the different local biochemical composition of the bi-zonal tissues results in a different local biomechanical function, resembling that of native meniscus.

**METHODS:** Bovine articular chondrocytes were loaded into hyaluronan-based meshes (Hyalograft-C<sup>®</sup>, Fab, Italy) and cultured for 4 weeks in either (i) a static culture system, (ii) a mixed flask (MF), or (iii) a Rotary Cell Culture System (RCCS, Synthecon Inc., USA) [1]. Engineered tissues were assessed histologically, immunohistochemically, biochemically and by scanning electron microscopy. Local compressive stiffness was determined using atomic force microscopy-based,  $\mu\text{m}$ -scale indentation tests [3] at different regions of bisected cartilaginous constructs. Local tensile stiffness was determined by tensile tests performed on concentric rings punched out from the inner and outer regions of engineered tissues.

**RESULTS:** Hyaluronan meshes-based constructs generated in the bioreactor-based systems (MF or RCCS) were characterized by a fibrocartilage-like outer capsule, containing abundant collagen type I (CI), versican and negligible glycosaminoglycans (GAG) or collagen type II (CII), and a hyaline-like inner core, containing abundant CII and GAG, with little CI and versican. In contrast, constructs cultured in the static system did not display any regular pattern in the deposition of CI, CII, GAG or versican. Scattered areas with bundle of parallel collagen fibers were observed only in the outer capsule of MF- or RCCS-grown tissues. Compressive stiffness measured in the inner regions of constructs generated in the MF or the RCCS were significantly higher (respectively 3.8-

and 4.3-fold) than those measured in the corresponding peripheral regions. Conversely, tensile stiffness of the external rings of tissues engineered in the MF or the RCCS were higher than those of the corresponding internal rings (respectively 1.9- and 2.5-fold higher). Control constructs cultured statically had compressive and tensile stiffness that were similar in the inner and outer regions.

**DISCUSSION & CONCLUSIONS:** We found that differences in the local biochemical composition of cartilaginous tissues, introduced by using MF or RCCS, were reflected in different local biomechanical properties. In particular, the inner hyaline-like zones of bioreactor-based constructs had higher strength in compression than in tension, whereas the outer fibro-cartilaginous regions were stronger in tension than compression. Since these structural, biochemical and functional properties resemble the heterogeneous nature of the meniscus, we are currently investigating the use of MF or RCCS for the development of meniscus substitutes.

**REFERENCES:** <sup>1</sup> A. Marsano (In press 2006) Bi-zonal cartilaginous tissues engineered in a rotary cell culture system *Biorheology* <sup>2</sup> Vunjak-Novakovic G, et al (1999) Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage, *J Orthop Res.* *J Orthop Res* 17(1):130-8. <sup>3</sup> Stolz M, et al. (2004) Dynamic elastic modulus of porcine articular cartilage determined at two different levels of tissue organization by indentation-type atomic force microscopy *Biophys J*; 86(5):3269-83.

**ACKNOWLEDGEMENTS:** The study was funded by the Swiss Federal Office for Education and Science (B.B.W.), under the Fifth European Framework Growth Program (Meniscus Regeneration, contract G5RD-CT-2002-00703).