Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Towards tissue engineering of meniscus substitutes: selection of cell source and culture environment

A Marsano, G Vunjak-Novakovic, I Martin

Abstract— **With the ultimate goal to engineer a meniscus substitute based on autologous cells, we aimed this work at identifying (i) a human cell source capable of generating fibrocartilaginous tissues and (ii) a culture environment promoting the development of bi-zonal constructs, resembling the complex structure and function of a meniscus. The post-expansion differentiation capacity of different chondrogenic cells readily available by knee arthroscopy, namely inner meniscus, fat pad, synovial membrane cells and articular chondrocytes (AC), was assessed within hyaluronan based non-woven meshes. Under our experimental conditions, only expanded AC generated tissues containing relevant amounts of glycosaminoglycans (GAG) and with cell phenotypes compatible with those of the inner and outer meniscus regions. Physical conditioning of constructs generated by expanded AC was applied using mixed flasks. The hydrodynamic environment of mixed flasks was instrumental to promote the formation of bi-zonal tissues, with an inner region rich in GAG and stiffer in compression and an outer rim rich in collagen and stiffer in tension. Therefore, the use of AC cultured within porous scaffolds in mixed flasks allowed engineering of constructs resembling some aspects of the phenotype and function of meniscus tissue.**

I. INTRODUCTION

In the complex fibrocartilaginous tissue of meniscus, two main regions can be distinguished: an outer vascular zone, mainly exposed to circumferential tensile loads, and an inner avascular zone, predominantly exposed to compressive forces. The meniscus outer region is characterized predominantly by large bundles of type I collagen fibers, circumferentially organized, whereas the inner zone contains the highest content of GAG and type II collagen (1), which is specific of this region and is organized in a unique fiber network (2). Injuries to the meniscus are often treated by partial or total meniscectomy, which is known to be associated with detrimental changes in joint function, ultimately increasing the risk of early degenerative joint diseases (3, 4, 5). Surgical approaches currently in use to substitute the damaged meniscus (e.g., the use of allografts or of a collagen-based material) can initially restore a stable and pain-free joint, but long-term clinical results are still uncertain (6, 7). Promising tissue engineering strategies have been proposed for the generation of meniscus substitutes (8, 9, 10). The ultimate aim of these strategies is to generate a living functional meniscus replacement, which immediately after implantation could substitute meniscus in its main functions within the knee joint (i.e., shock absorption, protection of articular cartilage, maintenance of knee stability).

Towards this final goal, in this work we aimed at identifying a human cell source capable of differentiating into meniscus cell phenotypes and a hydrodynamic condition capable to modulate the spatial distribution of specific matrix molecules (11); in particular, we aimed to generate bi-zonal tissues resembling some features of the native meniscus structure and function.

II. METHODS

Cell source selection- Human inner meniscus cells (IMC), fat pad cells (FPC), synovial membrane cells (FPC) and articular chondrocytes (AC) were loaded into disk shaped hyaluronan-based scaffolds (Hyaff®-11) and induced in static culture to form fibrocartilaginous tissues for 6 weeks (as described in Marsano et al., in press, Osteoarthritis and Cartilage, 2006). Resulting tissues were assessed histolgically and immunohistochemically.

Hydrodynamic culture condition- Bovine articular chondrocytes were loaded into disks of Hyaff®-11 and cultured for 4 weeks in mixed flask and static condition (as described in Marsano et al., in press, Biorehology, 2006). Resulting tissues were assessed histologically, immunohistochemically, by scanning electron microscopy (SEM) and mechanically in different regions. Local

HYAFF®-11 scaffolds were kindly provided by Fidia Advanced Biopolymer, Abano Terme, Italy. This work was supported by the Swiss Federal Office for Education and Science (B.B.W.) under the Fifth European Framework Growth (MENISCUS REGENERATION, contract G5RD-CT-2002-00703).

I Martin is with the Departments of Surgery and of Research at the University Hospital Basel, Switzerland (corresponding author to provide phone: 0041-61-265-2384; fax: 0041-61-265-3990; e-mail: imartin@uhbs.ch).

A Marsano was with the Departments of Surgery and of Research at the University Hospital Basel, Switzerland. She is now with the Department of Biomedical Engineering, at the Columbia University, New York, USA.

G Vunjak-Novakovic is with the Department of Biomedical Engineering, at the Columbia University, New York, USA.

mechanical analysis in compression was performed by indentation-type scanning force microscopy (as described in Marsano at al. submitted in Biomaterials).

III. RESULTS

Tissues generated by AC were positively stained for GAG, collagen types I and II. Tissues formed by IMC, FPC and SMC were negatively stained for type II collagen, faintly stained for GAG and positively stained for collagen type I (see figure 1) (Marsano et al., in press in Osteoarthritis and Cartilage, 2006).

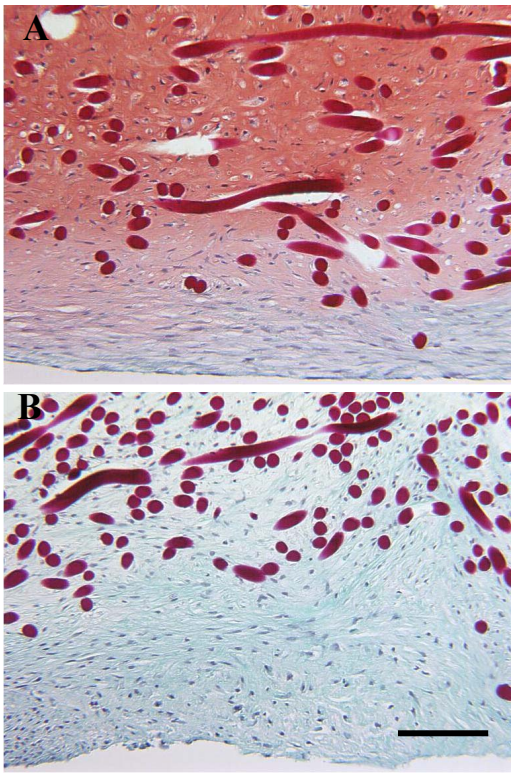

Figure 1. Representative histological stain for GAG with Safranin O of cartilaginous tissues generated by culture of AC (A) and SMC (B) into Hyaff®-11 for 6 weeks. Histological stains were similar in tissues generated by IMC, FPC and SMC. Scale bar = 300µm.

Tissues generated by AC in the hydrodynamic environment of mixed flask displayed a bi-zonal structure: an outer fibro-cartilaginous capsule and an inner hyaline-like region. The outer zone (outgrown from the original scaffold size for about 0.5 mm) was strongly stained for collagen type I, and the inner region (corresponding about to the space of the original scaffold) was intensively stained for GAG and type II collagen, and only faintly stained for type I collagen. Inner and outer regions of statically grown constructs were similarly positively stained for GAG, collagen types I and II (see figure 2) (Marsano et al., in press, Biorehology, 2006).

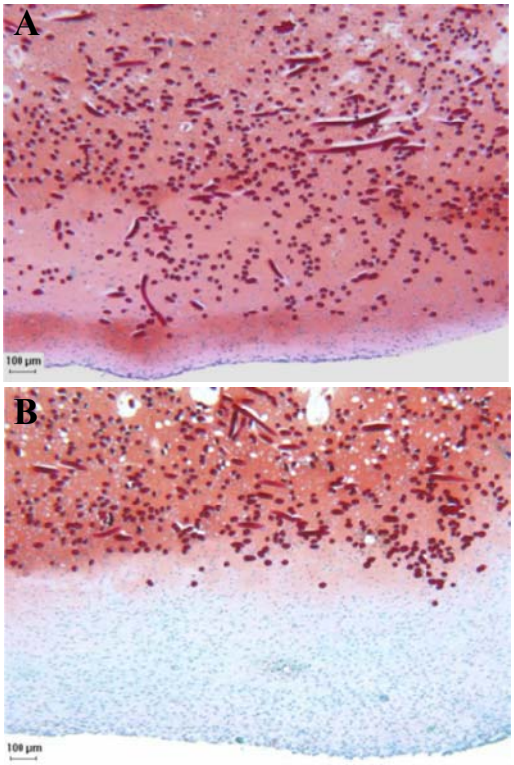

Figure 2. Histology of chondrocyte-based engineered tissues. Engineered tissue sections stained for GAG with Safranin O, generated after 4 weeks of culture statically (A) or in the mixed flask (B). Representative fields displaying the to the inner and outer region of bi-zonal tissue. Scale bar = 100µm.

SEM analysis indicated the presence of anisotropic collagen fibers in the inner region of all constructs, as well as in the outer region of statically grown tissues. Instead, the outer region of tissues cultured in mixed flask, selectively included bundles (up to 2µm diameter) of collagen fibers appeared oriented parallel to the construct periphery (Marsano et al., submitted in Biomaterials).

In tissues generated in the mixed flasks, elastic moduli in compression were significantly higher in the inner region as compared to the outer region. Elastic moduli in tension in the outer region of tissues generated within mixed flask were significantly higher than in the inner region. In contrast, stiffness in compression and tension was not statistically different between the regions of statically grown constructs (see figure 3) (Marsano et al., submitted in Biomaterials).

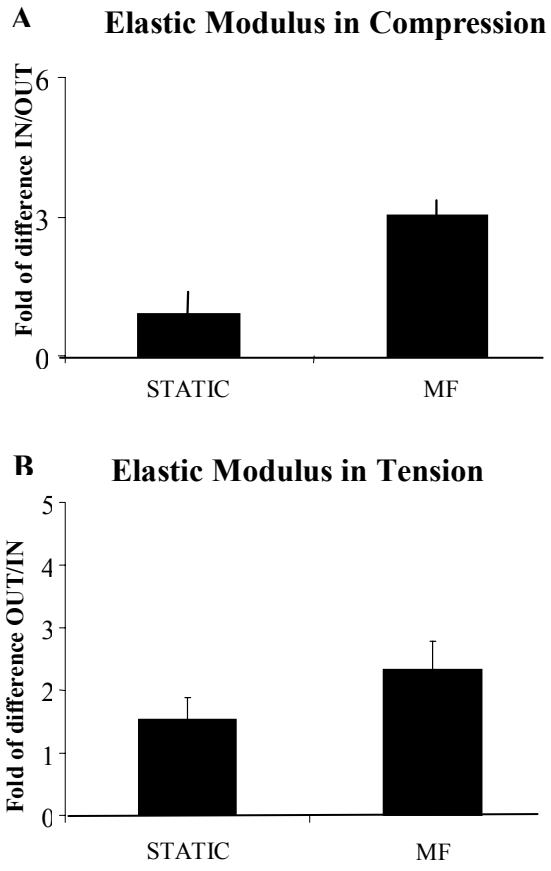

Figure 4. Ratios of Young's modulus in compression (A) for inner and outer region (IN/OUT) of constructs cultured statically (STATIC) or in the mixed flask (MF). Values of the elastic modulus in compression for the outer regions were 0.089MPa ±0.053 or 0.028MPa ±0.010, respectively for culture in static condition and in MF. Ratios of Young's modulus in tension (B) for outer and inner region (OUT/IN) of constructs cultured statically (STATIC) or in the mixed flask (MF). Values of elastic modulus in tension of the inner regions were 0.118MPa ±0.03, and 0.101MPa ±0.088, respectively for culture in static condition or in mixed flask (MF). All values are presented a mean ± standard deviation.

IV. CONCLUSIONS AND DISCUSSION

In our experimental conditions, we found that only AC are able to generate fibrocartilaginous tissues containing collagen types specific of the inner and the outer regions of the native meniscus, indicating therefore compatibility of their phenotype with meniscus cells. Our results are consistent with other studies which already showed the potential of AC in meniscus repair (12, 13).

We were then able to obtain a bi-zonal spatial organization of the collagen types and GAG by culturing AC-based constructs in hydrodynamic fluid flow. Bi-zonal generated tissues were characterized by locally different composition and mechanical properties, resembling some aspects of the complex structure and function of the outer and inner zones of the native meniscus. In particular, the higher stiffness in compression of the inner as compared to the outer region was directly related to the presence of GAG (14). Increased tensile stiffness in the outer region of the bi-zonal tissues was likely due not only to the higher collagen content and presence of specific collagen types, but also to the organization of collagen fibers in bundles parallel to the construct edges.

Although the engineered tissues only remotely mimic the highly complex structure and function of native meniscus, it is possible that the composition and mechanical properties of the graft will correctly develop by exposure to local mechanical forces upon implantation, as described during the process of embryonic joint development (15). In this context, studies are ongoing in a sheep model to assess the efficacy of autologous AC loaded into appropriately shaped scaffolds and culture in mixed flasks as engineered substitutes for total meniscectomies.

REFERENCES

[1] Cheung HS. - Distribution of type I, II, III and V in the pepsin solubilized collagens in bovine menisci. - Connect Tissue Res 1987;(4):-56.

[2] Kambic HE, McDevitt CA. - Spatial organization of types I and II collagen in the canine meniscus. - J Orthop Res 2005;(1):-9.

[3] Burr DB, Radin EL. Meniscal function and the importance of meniscal regeneration in preventing late medical compartment osteoarthrosis. Clin Orthop Relat Res 1982;171:121-6.

[4] Cox JS, Nye CE, Schaefer WW, Woodstein IJ. The degenerative effects of partial and total resection of the medial meniscus in dogs' knees. Clin Orthop Relat Res 1975;109:178-83.

[5] Higuchi H, Kimura M, Shirakura K, Terauchi M, Takagishi K. Factors affecting long-term results after arthroscopic partial meniscectomy. Clin Orthop Relat Res 2000;377:161 -8.

[6] Rijk PC. Meniscal allograft transplantation--part I: background, results, graft selection and preservation, and surgical considerations. Arthroscopy 2004;20:728-43.

[7] Rijk PC. Meniscal allograft transplantation--part II: alternative treatments, effects on articular cartilage, and future directions. Arthroscopy 2004;20:851-9.

[8] Ibarra C, Koski JA, Warren RF. Tissue engineering meniscus: cells and matrix. Orthop Clin North Am 2000;31:411-8.

[9] Adams SBJ, Randolph MA, Gill TJ. Tissue engineering for meniscus repair. J Knee Surg 2005;18:25-30

[10] Martinek V, Ueblacker P, Braun K, Nitschke S, Mannhardt R, Specht K, et al. Second generation of meniscus transplantation: in-vivo study with tissue engineered meniscus replacement. Arch Orthop Trauma Surg 2005; 8:1-7.

[11] G. Vunjak-Novakovic, "Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage" in J Orthop Res. 1999 Jan;17(1), pp 130-8.

[12] Peretti GM, Caruso EM, Randolph MA, Zaleske DJ. Meniscal repair using engineered tissue. J Orthop Res 2001;19:278-85.

[13] Peretti GM, Gill TJ, Xu JW, Randolph MA, Morse KR, Zaleske DJ. Cell-based therapy for meniscal repair: a large animal study. Am J Sports Med 2004;32:146-58.

[14] Aufderheide CA, Athanasiou AK. Comparison of scaffolds and culture conditions for tissue engineering of the knee meniscus. Tissue Engineering. 2005;11:1095-104.

[15] Mikic B, Johnson TL, Chhabra AB, Schalet BJ, Wong M, Hunziker EB. Differential effects of embryonic immobilization on the development of fibrocartilaginous skeletal elements. J Rehabil Res Dev 2000;37:127e33.