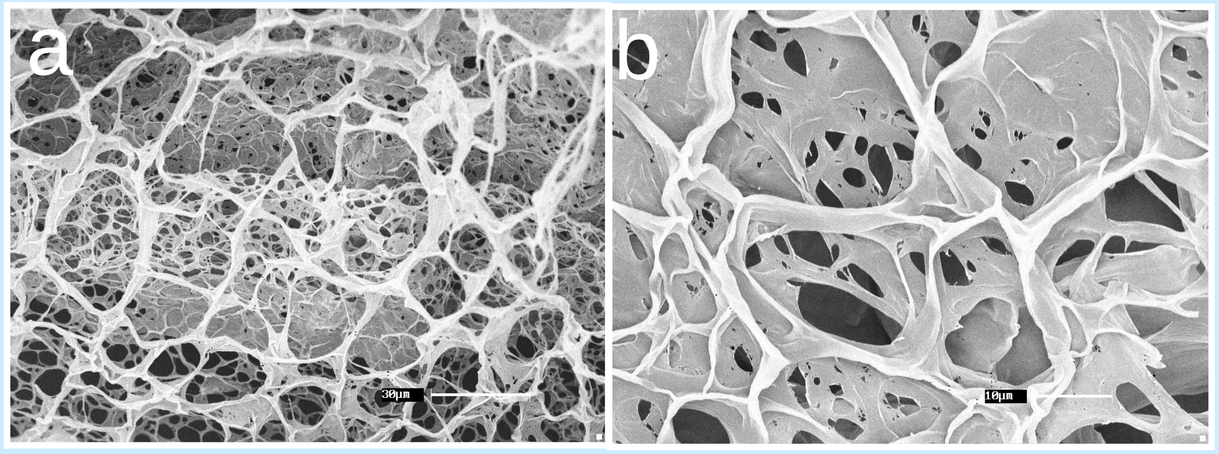
THREE DIMENSIONAL CO-COLTURE OF CHONDROCYTES AND ADIPOSE STROMAL VASCULAR FRACTION FROM KNEE HOFFA'S BODY FOR CARTILAGE REGENERATIVE THERAPY

F. Crovato[1], F. Mingotto[1], M. Bucco[2], T. Chlapanidas[2], I Ghidoni[2], P. Gaetani[3], M. Faustini[4], D. Vigo[4], M. Klinger[5], S. Faragò [6], M. Marazzi[1], V. Sansone[6], M.L. Torre[2]

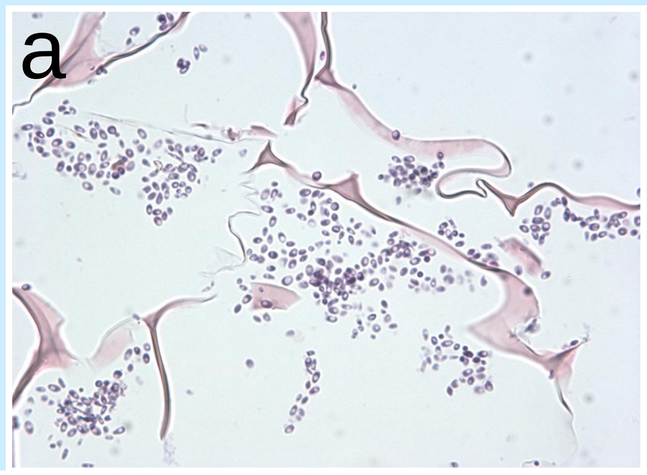
[1] Centro di Riferimento Regionale Colture Cellulari, Ospedale Niguarda Ca' Granda, 20133 Milan, Italy
[2] Dipartimento di Chimica Farmaceutica, University of Pavia, 27100 Pavia, Italy.
[3] IRCCS Istituto Clinico Humanitas, 20089 Rozzano, Italy.
[4] Dipartimento di Scienze e Tecnologie Veterinarie per la Sicurezza Alimentare, University of Milan, 20133 Milan, Italy.
[5] Istituto di Chirurgia Plastica, University of Milan, 20133 Milan, Italy
[6] Stazione Sperimentale per la Seta, 20133 Milan, Italy
[7]Dipartimento di Ortopedia, University of Milan, 20133 Milan, Italy

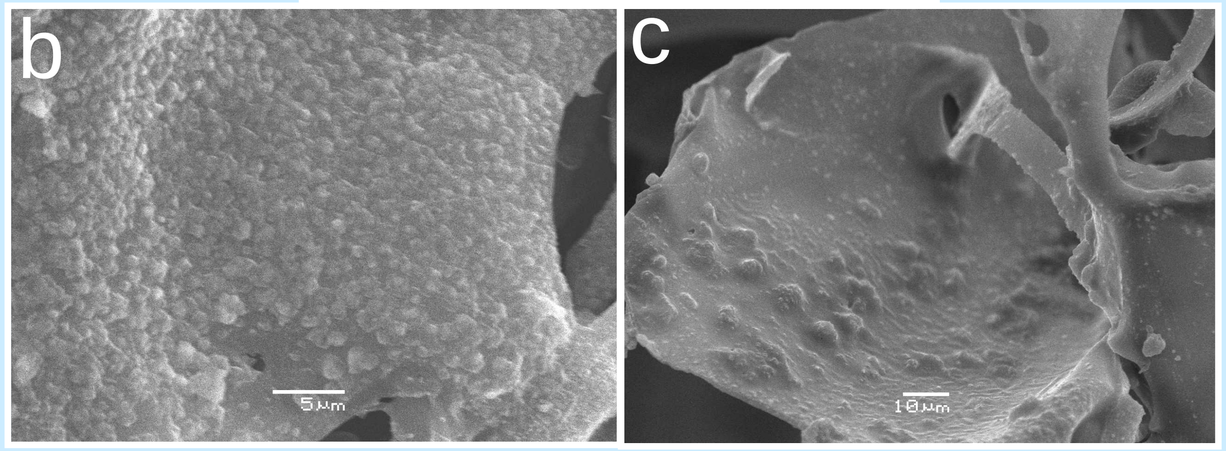
Adipose stromal vascular fraction (SVF) from human Hoffa's Body knee fat (HBF) was cultured on a silk fibroin fiber scaffold as a strategy for increasing cell adhesion for cartilage tissue engineering.

The HBF samples were obtained during surgery for knee articular diseases and knee articular cartilage harvested from cadaver donors. Samples were suspended in PBS and forwarded to a GMP cell factory at a temperature of 4°C. Tissues were digested in collagenase, and cell suspensions were filtered and centrifuged. Silk fibroin scaffold was seeded with SVF, incubated for 1 week, and then seeded with chondrocytes; co-culture were performed for 21 days. Culture of chondrocytes alone were performed in the same condition as a control..

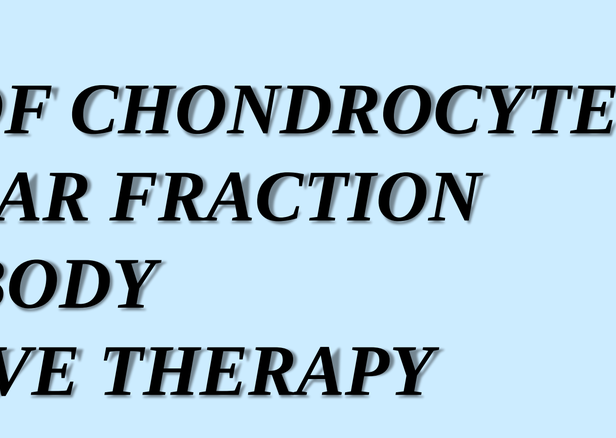


SEM images of fibroin scaffold: before (a) and after ethanol treatment (b). Silk fibroin ethanol conditioning provides a stable, uniform, smooth surface, which potentially promotes cell adhesion and proliferation.

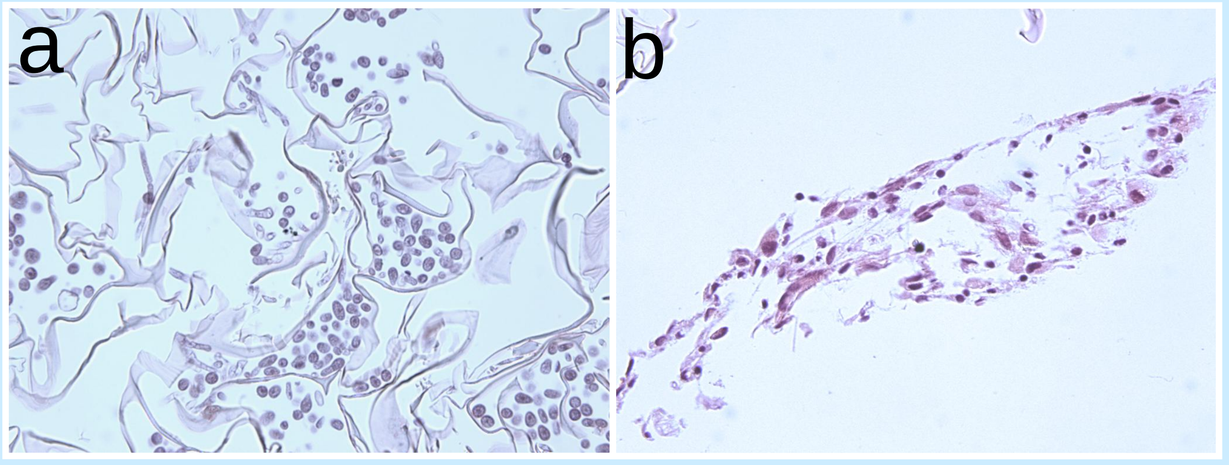




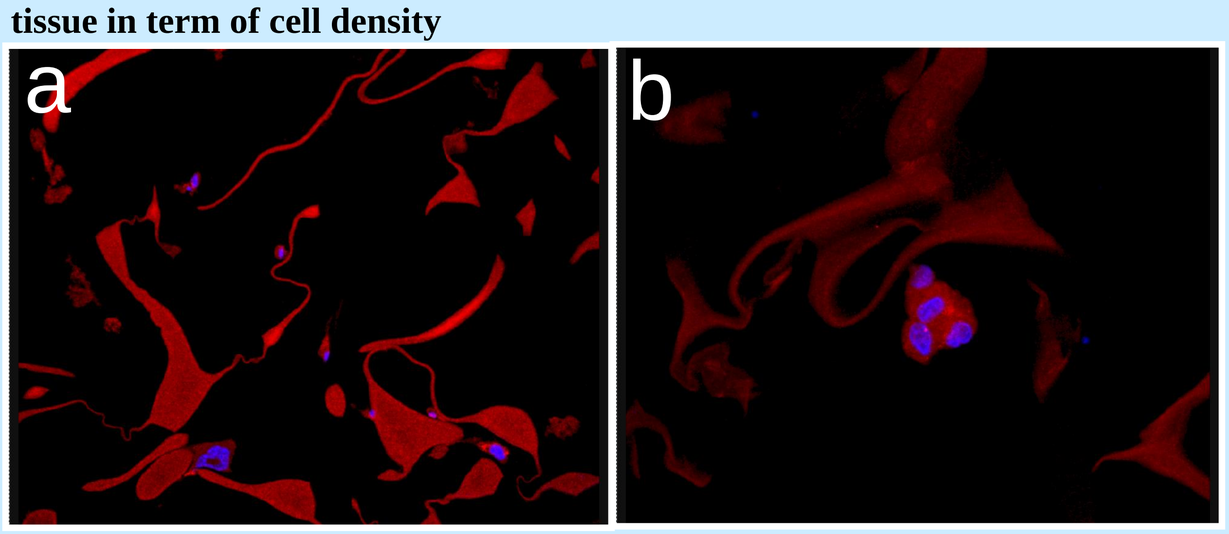
Hoffa's Body derived stromal vascular fraction cultured on fibroin scaffold for 7 days. (a) hematoxylin/eosin staining; original magnification: 40X. (b,c) SEM images. SVF cells adhere in wide clusters coating the scaffold surface after one week of culture.



Microphotograph of chondrocytes cultured on fibroin scaffold for 7 (a), 10 (b) and 21 (c) days, original magnification: 40X. While chondrocytes seem to adhere in a lesser amount without cluster formation after one week, they adhere in wide clusters after 10 and 21 days of colture.



Microphotograph of co-colture for 10 (a) and 21 days (b). Hematoxylin/eosin staining. Original magnification: 40X. Co-culture of SVF and chondrocytes improves the quality of the *in vitro* reconstructed tissue in term of cell density



Fluorescent microphotographs of co-colture for 10 (a) and 21 (b) days. Collagen II$^+$/TRITC (red) e DAPI (blue). Original magnification 40X. Co-culture of SVF and chondrocytes improves the quality of the *in vitro* reconstructed tissue in term of extracellular matrix production.

Co-culture of Hoffa's Body knee SVF and chondrocytes is a promising strategy to improve cartilage bioengineering. These results represent the premise for the development of an animal model.

REFERENCES 1. Zuk P.A. et al. Mol. Biol. Cell. 13, 4279, 2002.
ACKNOWLEDGEMENTS This study was supported by PRIN, Pavia and Milan Universities. and the Biotechnologies for Cell Therapy "Luigi Donati" Society.

Bone-Tec 2008 International Bone-Tissue-Engineering Congress. November 7–9, 2008. Hannover, Germany.