COMPARISON OF THREE DIFFERENT FIBRIN TISSUE ADHESIVES AND THEIR EFFECT ON HUMAN ARTICULAR CHONDROCYTES

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Introduction
The influence of fibrin sealant to cartilage repair after ACT has been the focus of many discussions in the last few years (2,3). All of them focused on either the commercial Tissucol®, TissucolKit® (Immuno, Vienna, Austria) or autologous fibrin glue. Apparently there has never been a comparison between these sealants.

In our in vitro study we compared three fibrin glues in two types of experiments. Experiment A was set up to check the toxicity of the glues, Experiment B to see whether the sealant affects chondrocyte migration. The aim was to determine an exact statement what type of glue is suitable for ACT and MACI® (matrix induced autologous chondrocyte implantation).

Methods
For both experiments cartilage was harvested from patients (informed consent) underwent total hip replacement or MACI®, in addition 100 ml blood was collected in a tube without additives. The autologous fibrin glue was made using cryoprecipitation. The chondrocytes were isolated from the articular cartilage by sequential enzymatic digestion and cultured in Hams F-12 and 10% FCS to confluency in about 4-6 weeks.

Experiment A: A suspension of 1 million chondrocytes per ml was used. Each cell suspension was placed on top and into 2ml of autologous fibrin glue, Tissucol® or TissucolKit® w/o Aprotinin. The sealant-cell suspension was incubated at 37°C and 5% CO₂ for 24 hours and 3 days and fixed in Bouin solution.

Experiment B: One million chondrocytes were seeded on a 1cm² either collagen type I/III (Chondro-Gide®, Geistlich Biomaterials, Wolhusen, Switzerland) or collagen type II matrix for 3 days. The seeded membrane was given into a modified Boyden chamber with chondrocyte conditioned media as a chemotactic attractant and Thermax® plastic cover slides on the bottom of the chamber. The fibrin glue was placed between the media and the seeded side of the membrane on a 9 µm polycarbonate filter (“sandwich-like”). After 3, 6 and 9 days incubation the filter-glue-membrane composite was fixed.

In both experiments we used Mayer’s hematoxylin-eosine and Masson Goldner staining for light microscopy. For further histological analysis we used transmission and scanning electron microscopy to determine chondrocytes apoptosis a TUNEL test (Apoptag®, Oncor Appligene, Heidelberg), a procedure that detects endonuclease activity indicative of apoptosis was set up.

Results
Experiment A: There was a significant difference in the morphology of the chondrocytes placed in either one of the three fibrin glues. The cells seeded in Tissucol® showed no toxic reaction, but they were apoptotic in TUNEL staining. In light microscopy the cytoplasm was vacuolized and the nucleus was smaller than usual. In scanning electron microscopy the cells had a fibroblast like morphology (Fig 1). Also the cells in Tissucol® w/o aprotinin showed signs of apoptosis. In TUNEL staining about 2/3 of the chondrocytes had a brownish color as marker for apoptosis in both Tissucol® adhesives.

In autologous fibrin glue the cells did not undergo apoptosis, TUNEL staining was negative. The cells appeared in scanning and transmission electron microscopy to be more roundish and chondrocyte like, showing also vesicle-like protrusion (Fig 2). To a great extent the cells had an irregular nucleus and a granular endoplasmatic reticulum. In light microscopy we could not notice any swallowing or destruction of the nucleus. These cells appeared to be undistorted.

Furthermore the time difference of one to 3 days did not make any difference to the chondrocytes.

Experiment B: In our assay we could not see any migration of the membrane (collagen type I/III or collagen type II membrane) seeded cells through the Tissucol® adhesives. In contract after removing the membrane the Tissucol® as well as the Tissucol® w/o aprotinin seemed to functioned as a barrier for chondrocytes migration. No chondrocytes were found on HE stained Thermax® cover slides underneath the polycarbonate filter or even in the deeper layers of the Tissucol®.

In autologous fibrin glue there has been a migration of the chondrocytes into the sealant. We also found chondrocytes on the bottom of the polycarbonate filter. About 1/3 of the cells had migrated through the glue in the direction of the chondrocyte conditioned media. The TUNEL staining showed the same results as in Experiment A. Chondrocytes were apoptotic in Tissucol® sealant but not in autologous glue.

Discussion
The results clearly demonstrate that fibrin tissue adhesives has an remarkable influence on cartilage repair and chondrocyte migration, and hence on the success of autologous chondrocyte transplantation.

Even aprotinin is discussed as an important inhibitor of cartilage repair and so as a cause of toxic reactions (1), Tissucol® without aprotinin is also not suitable for ACT or MACI®. The study indicates that Tissucol® has an influence on the apoptosis of the cells, and on the morphology, by inducing a fibroblast like phenotype.

The results also show a positive effect of autologous fibrin glue as judged from migration and the histology of the cells. It is open to debate whether or not cytokines present in the fibrin glue function as chemotactic stimuli.

In conclusion, the future of autologous chondrocyte transplantation should focus on using autologous fibrin glue in order to benefit from the stimulated chondrocyte migration and to avoid apoptosis of the transplanted cells.

References
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