INTRODUCTION

Early post-traumatic arthritis of the knee and ankle is a debilitating condition posing a reconstructive challenge for the young and active patient. Inevitable loosening of joint arthroplasty in a young active population has lead to the search for a biologic method of repair.

Bipolar fresh osteochondral allograft (BFOA) represents a fascinating alternative to arthrodesis and prosthetic replacement and, to date, the use of fresh osteochondral allografting to replace damaged articular cartilage has been well documented.

BFOA provide viable cartilage supported by an intact subchondral bony structure which is progressively replaced by host bone over time. Nevertheless, little is known regarding cartilage behavior following transplantation and the survivability of the transplanted cartilage has not been fully investigated. Few reports exist claiming the long term survivorship of the donor chondrocytes and the inability of the host cells to colonize the cartilage layer.

Aim of this study was to investigate the survivability of donor chondrocytes, the repopulation of the transplanted cartilage by host cells and the nature of these cells by genetic typing and mRNA expression analysis at 18 months follow up after transplantation.

METHODS

Eighteen patients, aged 42±12 years, received BFOA, 3 of total knee and 15 of total ankle. Patients evaluation was carried out clinically and by X-Rays, CT scans and MRI. Cartilage biopsies were obtained during revision (18 ± 5 months) or second look surgeries (18 ± 8 months) under patient informed consent. Biological samples were evaluated by histological and immunohistochemical analyses or used for genetic typing and mRNA expression analyses.

Histochemistry. Biopsies of the grafted areas were fixed in 10% buffered formalin and then embedded in paraffin. Cartilage sections were stained with Safranin-O, Fast Green and haematoxylin/eosin.

Immunohistochemistry. Anti-human type I and II collagens, metalloproteinase-1 (MMP-1), caspase-3, anti-Von Willebrand factor, anti-TRAP were used. The samples had different unmasking treatments, depending by the analyzed antigen.

Genetic typing. Total DNA from allograft biopsies, donor cartilage and recipient peripheral blood were compared. Genetic typing was performed at five short tandem repeat (STRs) markers as described.

Gene expression. Messenger RNA expression of specific cartilage markers was evaluated on selected samples by Real-Time RT-PCR and results were reported as means ± standard deviation (SD) of mRNA relative expression, calculated as the ratio between the signal of the RNA of interest and the corresponding GAPDH signal.

RESULTS

The 3 knee allografts were analyzed at revision for failure, while 12 out of 15 ankle allografts were analyzed at second look surgeries and 3 at revision for failure.

Histology and immunohistochemistry. All the three knee biopsies showed the presence of cartilage tissue with many degenerative features as confirmed by immunohistochemical analyses of the presence of some catabolic markers. For what is concerning ankle allografts, cartilage specimens showed fibrocartilaginous tissues with double tidemark crossed by bundles of cells from subchondral bone; immunohistochemical analyses revealed a strong positivity for type II collagen at the extracellular level in the majority of the samples.

Genetic typing and mRNA expression. All the cartilage tissues from knee allografts showed a mixed DNA profile confirming the presence of both donor and recipient cells. On the contrary, almost all ankle allografts typed (12 out of 15, including the three cases of failure) showed the presence of recipient DNA into the allograft; 1 allograft showed a mixed DNA profile and 2 allografts matched with donor DNA profile. Preliminary data on mRNA expression showed that allograft cartilage tissues express cartilage specific markers such as collagen II and SOX9.

CONCLUSIONS

The colonization of human viable knee and ankle allografts by recipient cells was investigated by genetic typing and mRNA expression. There is an evidence of persistence of donor cells particularly in knee allografts. This event is rare in ankle allografts, where the prevailing presence of host DNA suggests the ingrowth of recipient cells into the allograft, presumably migrating from the subchondral bone, in accordance with histological findings. The observed synthesis of cartilage specific RNAs in some of the analysed samples argues for the acquisition of a chondrocyte-like phenotype by some of these cells.

SIGNIFICANCE

BFOA transplanted cartilage is colonized by recipient cells and these cells synthesize chondrocyte-specific molecules.

AKNOWLEDGEMENTS

This work was supported by grants from Progetto Regione Emilia-Romagna Università “Regenerative medicine in osteoarticular diseases” PRRI 2007; from Progetto Monte dei Paschi “Prevenzione e cura dell’osteartite: patogenesi e terapie innovative” foundation and from MIUR and Centro Nazionale Traipanti.

REFERENCES