Osteophytes - an alternative source of chondrocytes for transplantation?

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Summary

Objective: **The aim of this research was to evaluate the possibility of using osteophyte-derived chondrocytes for autologous chondrocyte implantation by comparing the behaviour of these chondrocytes with that of normal articular cartilage-derived chondrocytes in monolayer culture.**

Materials and Methods: **The scrapings from the cartilage mantle of osteophytes harvested during routine total knee replacement for osteoarthritis were enzymatically digested and grown in monolayer culture. Articular cartilage scrapings obtained from visually normal area of the femoral condyle (usually from the posterior cuts) were also enzymatically digested as for the cartilage from the osteophytes. The behaviours of these two sets of chondrocytes were evaluated in monolayer culture, by their gross appearance, matrix protein elaborated and collagen types with the articular cartilage-derived chondrocytes acting as controls.**

Results: **Osteophyte-derived chondrocytes confluenced earlier than normal articular cartilage-derived chondrocytes. The osteophyte-derived chondrocytes elaborated cartilaginous matrix as evidenced by positive staining for Toluidine blue and the cells were immunoreactive positive for collagen types I, II and III.**

Conclusion: **Osteophyte-derived chondrocytes are similar to normal articular cartilage-derived chondrocytes in monolayer culture. Since osteophytes are expendable tissues and often found in association with full thickness articular cartilage defects, they may provide an alternative source of chondrocytes for transplantation in cases where autologous chondrocyte transplantation (implantation) is embarked upon.**

Key words: ***Osteophyte, Chondrocyte, Osteoathritis, Autologus, Transplantation.***

Résumé

Objectif:- Le but de ce travail est d'évaluer la possibilité de l'utilisation des chondrocyte à travers l'ostéophite pour l'implant de chondrocyte autologouse en faisant la comparaison du comportement des chondrocytes avec celui des chondrocytes des articulation normale du cartilage dans la culture mono couche.

Matériels et méthodes:- Les mince couches de manteau du cartilage d'ostéophytes recoltés au cours du remplacement total du genou de façon systématique pour l'ostéoarthrite étaient digérées enzymeticalement et grandie dans la culture mono couche. Des mince couches du cartilage articulaire obtenues de la région normale du condyle femoral qu' on pourrait voir (d'habitude à partir du postérieur coupé étaient également digérées enzymeticalement comme pour le cartilage d'ostéophyte. Les comportements des deux séries de chondrocytes ont été évalués dans la culture monocouche, par leur grand apparition, protéine matrix élaborée et types de collagène avec des chondrocytes d'origine du cartilage articulaire suppléant agissant comme contrôles.

Résultats:- L'ostéophyte d'origine chondrocyte confluencée plus tôt que le cartilage d'origine chondrocytes normale articulaire. L'ostéophyte d'origine chondrocyte cartilagineux matrix élaboré comme en témoigne la teinture positive pour Toluidine bleu et les cellules étaient immunoreactives positives pour les type I, II, et III collagènes.

Conclusion:- Les ostéophytes d'origine chondrocytes sont semblables au cartilage d'origine chondrocytes normale articulaire dans la culture de monocouche. Puisque les ostéophytes sont des tisus élastiques et le plus souvent trouvé en association avec les défauts du cartilage articulaire en pleine épaisseur, ils peuvent fournir une souce alternative de chondrocytes pour un implant dans les cas ou on commence à opérer un implant chondrocyte autologeux.

Introduction

Circumscribed articular surface defects in the knee joints have been successfully treated with cultured chondrocytes using the autologous chondrocyte implantation technique popularised by Brittberg et al[1]. The chondrocytes used in this technique are obtained from cartilage that is harvested from normal areas of the knee joint (usually from the edge of the trochlear of the femur). The long-term changes at these cartilage donor sites are not known but there are fears that these sites may turn out to be areas of cartilage defects over time. Osteophytes on the other hand are expendable osteochondral tissues most commonly observed at the margins of osteoarthritic joints (including the edge of the trochlear of the femur) and also in association with full thickness articular cartilage defects[2].

Osteophytes are repair tissues which appear to be wrongly sited in the joints bedevilled by full thickness articular cartilage defects[2,3]. The aim of this study was to explore the possibility of obtaining chondrocytes from osteophytes and evaluating their suitability as an alternative source of chondrocytes for autologous chondrocyte transplantation.

Material and methods

The cartilage mantle of osteophytes obtained from the knee of 6 patients undergoing total knee replacement for osteoarthritis were scrapped off under aseptic conditions and washed in sterile α-MEM tissue culture medium

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supplemented with 10% foetal bovine serum, L-glutamine, penicillin and streptomycin (Gibco, Life Technologies, Paisley, UK). Visually normal articular cartilage scrapings were obtained from the parts of the joints least affected by the degenerative disease and washed as for the osteophytes cartilage scrapings. The specimens were then transferred into sterile universal containers and enzymatically dissociated in 2% w/v collagenase (Type 1a, Sigma, Poole, UK) at 37°C for 3-4 hours. Each universal container was vigorously agitated every 30 minutes using a Whirlmixer. The cells obtained at the end of the dissociation period were cultured in α-MEM tissue culture medium and plated out into 35mm tissue culture dishes (Nunc, Life Technologies, Paisley, UK) at two cell densities – Medium (3×10^5) and High (8×10^5) (Figure 1). Cultures were fed every third day with α-MEM tissue culture medium and examined daily using the Olympus phase contrast inverted microscope. Sample cultures were passaged at three-, one-, and two-weekly intervals (Figure 1). At the end of a total culture period of 10 weeks, the cultures were fixed in 10% formalin for 48 hours and processed for histology (using Toluidine blue) and immunocytochemical studies which localised types I, II and III collagens. Goat anti-type I, mouse anti-type II and goat anti-type III primary antibodies (Southern Biotechnology Associates, Alabama, USA) were used and rabbit anti-goat horse radish peroxidase (HRP) (DAKO Ltd. High Wycombe, UK) conjugated secondary antibodies were used for the evaluation of types I and III collagens and anti-mouse HRP conjugated secondary antibody was used for type II collagen.

Results

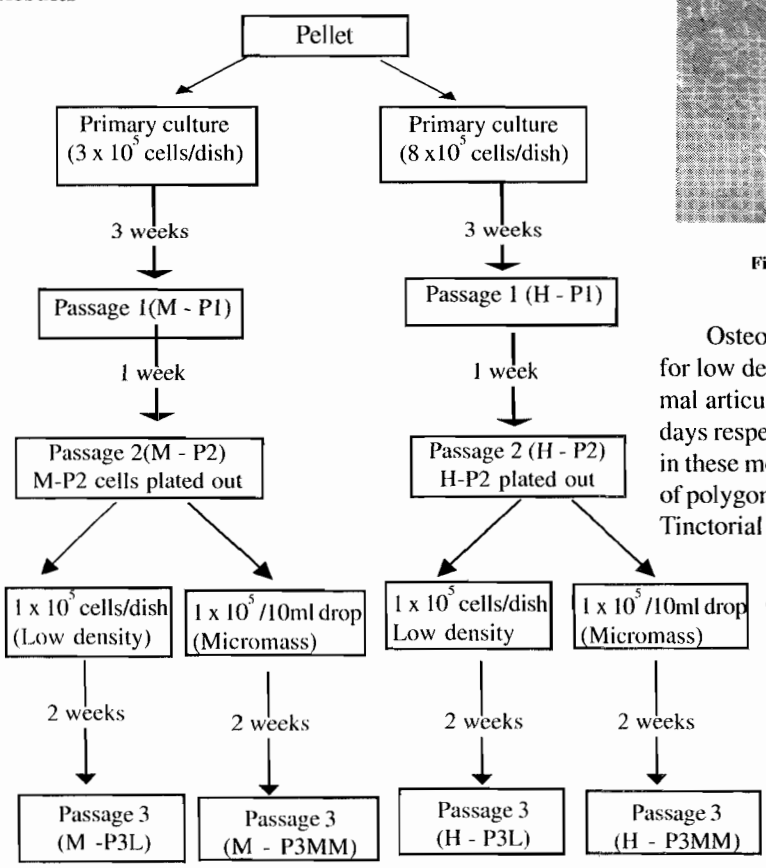

Fig. 1 *Scheme of cell cultures*

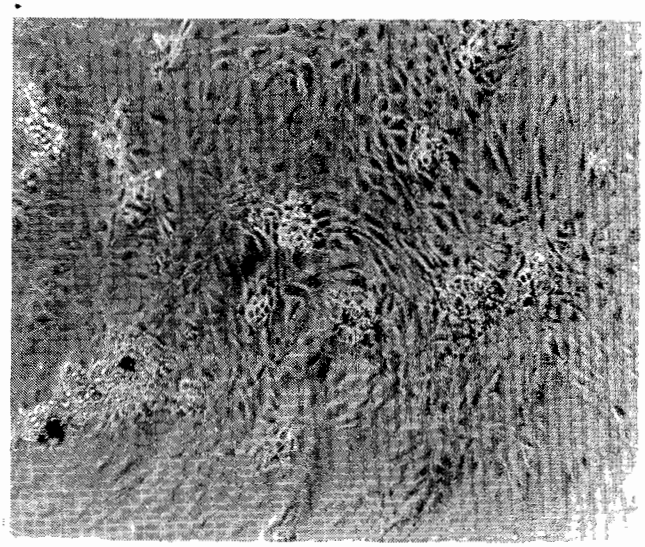

Fig. 2 *Photograph showing mainly fibroblastic cells in monolayer culture and some polygonal cell colonies embedded in a refractile matrix. (Magnification x 100).*

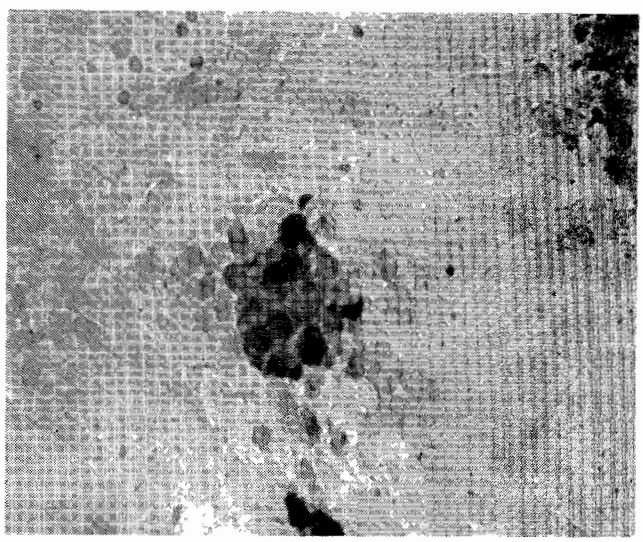

Fig. 3 *Photograph showing Toluidine blue staining of the matrix around the cultured chondrocytes. (Magnification x 100).*

Osteophyte-derived cells reached confluence at 7 days for low density and at 3 days for high density cultures; normal articular cartilage cells reached confluence at 10 and 5 days respectively. The predominant cell type was fibroblast in these monolayer cultures with cell colonies that consisted of polygonal cells embedded in a refractile matrix (Figure 2). Tinctorial staining with Toluidine blue (Figure 3) revealed that osteophyte-derived cells elaborated cartilaginous matrix. All the osteophytic cultures were immunoreactive positive for collagen type I (Figure 4a), collagen type II (Figure 4b) and collagen type III (Figure 4c). The intensity of the staining appeared to be more pronounced for collagen types I and III.

Discussion

This study shows that chondrocytes derived from the cartilage mantle of osteophytes can be grown

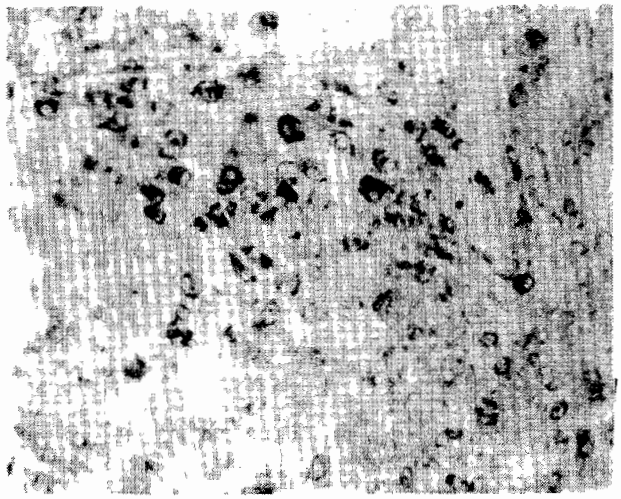

Fig. 4a *Photograph showing type I collagen staining of the matrix in monolayer culture. (Magnification x 100).*

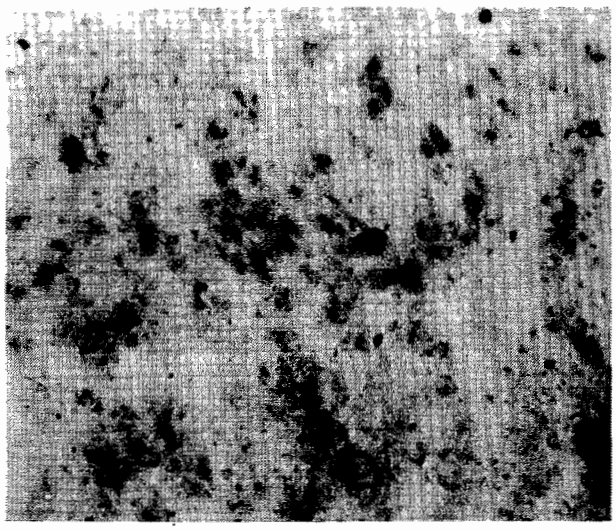

Fig. 4b *Photograph showing type II collagen staining of the matrix in monolayer culture. (Magnification x 100).*

in ordinary monolayer cultures and that the cells in these cultures appear to have similar morphological characteristics as those from normal articular cartilage. The early rate of confluence of osteophyte-derived chondrocyte may suggest that these cells have a higher proliferative ability compared to visually normal articular cartilage-derived cells. It is well known that the rate at which cultured cells confluence is related to the rate at which they proliferate[4]. Therefore, it is to be expected that if transplanted, osteophyte-derived chondrocytes may repair full thickness defects in the articular cartilage more quickly than normal articular cartilage-derived chondrocytes. Cell proliferation rate is also inversely proportional to the differentiation rate[5-8] so that a slower rate of cellular proliferation would be accompanied by early differentiation. It is therefore apparent that as a consequence of early differentiation, transplanted chondrocytes may be prevented from expressing their whole gamut of

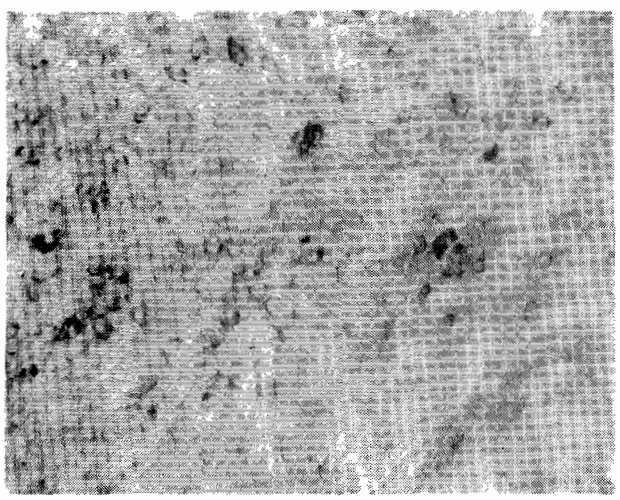

Fig. 4c *Photograph showing type III collagen staining of the matrix in monolayer culture. (Magnification x 100)*

differentiation. From this study, it would appear that osteophyte-derived chondrocytes might have an advantage over normal cartilage-derived chondrocytes on account of it's higher rate of proliferation and therefore the delay in the full differentiation of the chondrocytes. The ability of these cells to elaborate cartilage matrix as well as collagen type II suggests that they are able to maintain a chondrocytic phenotype. Thus, if osteophyte-derived chondrocytes are transplanted into full thickness articular cartilage defect, they can be expected to re-differentiate into cartilage and be anchored into the subchondral bone as has been shown by normal articular cartilage-derived chondrocytes. Whether or not the architecture of the normal articular cartilage will be reproduced under these conditions is not entirely clear and may be the subject of future research.

Conclusions

Osteophyte-derived chondrocytes are similar to normal articular cartilage-derived chondrocytes in monolayer culture. Since osteophytes are expendable tissues and often found in association with full thickness articular cartilage defects, they may provide an alternative source of chondrocytes for transplantation in cases where autologous chondrocyte transplantation (implantation) is embarked upon.

Acknowledgement

This research was funded by the British Association for the Surgery of the Knee (BASK)/Johnson and Johnson Knee Fellowship. The authors will like to thank Prof. J Harper, Head of the University Department of Orthopaedics, The Glenfield Hospital, Leicester, UK, and Mike Robert of the same department for their assistance with the provision of all the equipments required to carry out this research. The authors will also like to thank Johnson and Johnson for sponsoring this research.

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