CELL-BASED THERAPY FOR THE TREATMENT OF FOCAL ARTICULAR CARTILAGE LESIONS: A REVIEW OF SIX YEARS OF STUDIES IN A MALAYSIAN UNIVERSITY MEDICAL CENTRE

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ABSTRACT

Autologous chondrocyte implantation (ACI) is a significant technique that has gained widespread use for the treatment of focal articular cartilage damage. Since its inception in 2004, the Tissue Engineering Group (TEG) of the Faculty of Medicine, University Malaya has been dedicated to carrying out extensive research on this cell-based therapy. The objective of this report, comprising one clinical case report, six animal studies and one laboratory study, is to summarise and discuss TEG's key findings. On the whole, we observed that the ACI technique was effective in regenerating hyaline-like cartilage in treated defects. Autologous chondrocytes and mesenchymal stem cells (MSC) were found to produce comparable tissue repair irrespective of the state of MSC differentiation, and the use of alginate-based scaffolding and oral pharmacotherapy (Glucosamine and Chondroitin Sulphate) was shown to enhance ACI-led tissue repair. ACI is suggested to be an efficient therapeutic option for the treatment of articular cartilage defects of the knee.

Keywords: articular cartilage, cell therapy, autologous chondrocyte implantation, animal studies, review

Introduction

The treatment of articular (hyaline) cartilage injuries is one of the most difficult challenges for orthopaedic surgeons. As aptly stated by William Hunter in 1743, 'an ulcerated cartilage is a troublesome problem and once destroyed, it never repairs'. This is because articular cartilage is avascular, with very limited healing capacity. Injury to articular cartilage begins an inexorable cascade of pathobiological and pathomechanical events that lead towards loss of tissue and formation of defect, in contrast with the process of inflammation and repair found in vascularized tissue. If left untreated, articular cartilage lesions invariably progress in size and lead to degenerative changes typical of osteoarthritis (1-2).

Articular cartilage lesions can result from sports injuries, trauma, osteoarthritis or osteochondritis. In Malaysia, symptomatic knee osteoarthritis is responsible for the largest burden of joint pain and is the single most important rheumatological cause of disability and handicap. A Community Oriented Program for Control of Rheumatic Diseases (COPCORD) survey conducted in 2007, involving a total of 2594 community members from Banting, revealed that 9.3% of adult Malaysians complain of knee pain, with

clinical evidence of osteoarthritis in more than half those examined for knee pain (3). The incidence was noted to be higher in the aging population, with a sharp increase in pain rate to 23% in those over 55 years of age, and 39% in those over 65%. For those who suffer from degenerative joint disease, the ability to regenerate cartilage would reduce the need for joint replacement, decrease pain, increase mobility and lead to a better quality of life.

As such, the restoration of articular cartilage defects is of paramount importance due to an urgent and expanding clinical need, especially with the expected 269% increase in the elderly (above 65 years) population in Malaysia between the years 2008 and 2040 (4). In recent years, the cell-based therapy termed autologous chondrocyte implantation (ACI) has emerged as a promising technique to repair full-thickness chondral defects. The early work done by Hui *et al* of the National University of Singapore (NUS) in 1999 involving 52 Singaporean patients reported a symptomatic improvement in 90.2% of patients, with no adverse reaction to surgery (5). However, there are minimal data on the efficiency of the ACI technique in Asian populations available at present. Since its inception in 2004, the Tissue Engineering Group (TEG) of the Faculty

of Medicine, University Malaya has been dedicated to carrying out extensive research on the ACI technique, with the aim of advancing knowledge and repair outcomes in the treatment of articular cartilage defects. In particular, we have directed our investigations towards focal articular cartilage lesion of the knee, defined as a circumscribed, full-thickness cartilage defect down to, but not through, the subchondral bone on a load-bearing surface of the femoral condyle or patellar facet.

The objective of this report is to present TEG's six years of experience in engineering cartilage cell therapy from the year 2007 to 2012. We have embarked on a collective appraisal of our centre-led trials (clinical, animal, laboratory studies) and scientific literature to discuss and compare our findings with previously published data. In particular, we aim to: 1) provide an overview of cartilage surgical repair techniques; 2) examine the clinical outcome and regenerative profile of conventional ACI; 3) discuss the chondrogenic potential of transplanted cells; 4) discuss the role of cell carriers and scaffolds; and 5) discuss the role of local and systemic pharmacological agents.

An overview of cartilage surgical repair techniques

A number of orthopaedic surgical procedures have been developed in an attempt to alter the otherwise dismal natural history of chondral defects. Arthroscopic debridement was the earliest technique utilised to treat articular cartilage defects of the knee. Although this procedure was shown to provide clinical improvements (6-9), the benefits were short-lived and the technique did not address the underlying pathology (10-12). Attempts were then made to improve the long-term results of cartilage repair by directing treatment towards exploiting the natural intrinsic repair response, observed upon penetration of the subchondral bone in full-thickness defects. Methods that accomplish this type of repair are termed marrow stimulation treatment (MST) techniques, and include subchondral drilling, abrasion arthroplasty and microfracture. In MST, subchondral bone is perforated to induce mesenchymal stem cell infiltration into a chondral defect, which leads to the formation of a clot that may differentiate into repair tissue. While MST procedures have demonstrated good to excellent results in 60-80% of patients (13-14), they typically promote the development of fibrocartilage that is biomechanically inferior to articular cartilage (10-12,15-16) and deteriorates over a short period of time postoperatively (16-18).

In 1965, Audrey Smith was able to culture chondrocytes in vitro after freezing, culminating in the advent of cryopreservation. This led to modern cartilage transplantation techniques, which include perichondral and osteochondral grafts. Perichondral grafts harvested from donor sites and implanted into chondral defects have been shown to lead to marked functional improvement (19, 20).

Osteochondral grafts, which harvest osteochondral plugs from low weight-bearing areas within the knee joint for implantation into chondral defects, have shown promising results with a success rate of up to 80% (21-25). Although both these techniques are able to retain the viability of hyaline tissue unlike previous surgical interventions (20, 26), perichondral grafts are susceptible to ossification and graft failure (10, 20) while osteochondral grafts are limited by donor site morbidity concerns (10) and the lack of lateral integration of mosaic plugs and recipients, which may lead to degeneration of the graft over time (17, 27).

In recent years, the numerous experimental attempts to reestablish structurally and functionally competent repair tissue which is of an enduring nature has led to the advent of autologous cell extraction for implantation into chondral defects. A Swedish group led by Lars Peterson and Mats Brittberg was the first to describe the technique of autologous chondrocyte implantation (ACI) in 1984 (28), which was the first application of a cell-based approach in orthopaedic surgery. As previous studies had shown the ability of autologous cells to regenerate cartilage, Peterson *et al* refined a three-step procedure where chondrocytes isolated from cartilage biopsy were harvested arthroscopically from a non weight-bearing area, isolated enzymatically and expanded in a monolayer culture, and then implanted into the cartilage defect using a periosteal patch over the defect as a method of cell containment. Encouraged by their early success in rabbit models, the Swedish group refined the technique to culture autologous chondrocytes from a small sample of patients' own normal articular cartilage, and began a clinical trial to treat full-thickness defects in patients' knees.

In a variation of ACI, Wakitani *et al* exploited the self-repair potential of mesenchymal stem cells (MSC) in treating chondral defects (29). MSC are pluripotent progenitor cells that can be ex vivo expanded and induced, either in vitro or in vivo, to terminally differentiate into chondrocytes. They have emerged as a potential cell source for cartilage cell therapy due to their multipotential capacity, easy isolation and culture, and high ex vivo expansive potential in governing the rapid and specific repair of skeletal tissues (30, 31). Aside from cells, recent advancements in cartilage cell therapy have focused on exploring the potential of chondro-conductive scaffolds and biofactors in further improving articular cartilage repair, giving rise to the terms 'second-generation' and 'third-generation' ACI.

The usage of ACI in the repair of cartilage has generated much interest within the orthopaedic community, and there have been numerous studies reporting on ACI. A systematic review published in 2011 by our centre suggested that there were at least 568 scientific papers reporting controlled and randomised clinical studies related to ACI, involving 1,644 patients in total (32). In this paper, comparison with other treatments suggested that ACI is associated with superior structural regeneration of cartilage tissues and better clinical outcomes when compared with microfracture, similar or superior outcomes

when compared with mosaicplasty, and comparable outcomes when compared with MSC transplantation and matrix-assisted autologous chondrocyte implantation (MACI), both of which are techniques adapted from ACI. Many authors comparing the ACI technique with another treatment modality reported more favourable results for ACI (33-38). However, there were also authors who reported comparable (39-41) or less favourable results (42) for ACI. Early systematic reviews examining the evidence for ACI concluded that there was insufficient evidence from the existing literature to determine whether conventional ACI was superior in the treatment of focal articular cartilage defects (43-49). However, the ACI technique has evolved considerably over the past few years. As mentioned earlier, the induction of biomaterial-based scaffolding, alternative cell sources and biofactors are anticipated to improve the outcome of ACI-led cartilage repair, which will be discussed in the following text.

An overview of the studies performed in TEG

The TEG studies investigating the ACI technique for the treatment of focal articular cartilage defects are illustrated in Table 1. There is a total of eight studies included in this paper, comprising a clinical case report, six animal studies and one laboratory study. There were no adverse effects observed in any of the studies.

The clinical outcomes and regenerative profile of the conventional ACI technique

Our first attempt to establish the clinical outcomes of conventional ACI (i.e. utilising autologous chondrocyte and periosteal patch) in our centre was a preliminary case report of two patients (50). In this study, we observed clinical improvements in both patients following ACI-led repair of articular cartilage defects as evidenced by the International Knee Documentation Committee score, Oxford Knee Score and American Knee Society Score at nine months postoperatively (Table 1). Complete filling of treated chondral defects with repair tissue was also demonstrated via magnetic resonance imaging at six months. No complications were observed in both patients during the study period. Our early clinical findings suggested that the ACI technique could be applied in clinical practice for the treatment of chondral defects.

As the compositional changes in repair tissues are known to have major influences on their physiological function, we sought to determine the quality of cartilage regenerated following conventional ACI repair. In order to perform a quantitative and qualitative assessment of regenerated tissue, we opted to examine the glycosaminoglycan (GAG) content and Brittberg score. GAG plays a significant role in regulating the chondrocyte phenotype and is a determinant of the biochemical and mechanical quality of regenerated cartilage (51), whilst the Brittberg score has been found to

be statistically reliable and repeatable for the macroscopic assessment of cartilage repair (52). In our later studies, we also used the O'Driscoll score, a validated measure for histological scoring (53), as an outcome measure.

Using nine rabbit models, identical full-thickness defects were created in the articular cartilage of both knees, and a month later the right knee was repaired via ACI while the left knee was left untreated (control group) (54). At three months post-surgery, enhanced macroscopic regeneration, significantly ($p=0.008$) higher cellular expression of GAG and significantly ($p=0.007$) higher mean Brittberg repair score were observed in the ACI-treated knees compared to the control group (Table 1). The study suggested that articular lesions treated by ACI repaired better than those which were not treated.

The work done by other centres has also reported a significant amount of cartilage reconstitution in ACI-treated defects in both preclinical (28,55-56) and clinical studies (57-61). The clinical trial conducted by the pioneering Swedish group led by Peterson and Brittberg, spanning a mean follow-up of 39 months, showed good or excellent clinical outcomes in 87% of cases (n=23) and 'hyaline-like' results in 11 out of 15 treated lesions (57). A more recent publication from this group has shown durable results up to 11 years, with 96% clinical durability (60), and a correlation of good and excellent clinical results with the generation of hyaline-like repair tissue (62). However, not all reports have been favourable. Horas *et al* (42) compared ACI with osteochondral cylinder transplantation, and reported slower recovery and fibrocartiliginous defect filling for ACI-treated patients. Moreover, despite its promising tissue repair potential, several complications arising from the use of conventional ACI have been reported. These include periosteal hypertrophy, disturbed fusion of regenerative cartilage and healthy surrounding cartilage, and dedifferentiation of cellular phenotypes cultured in a monolayer culture (63-67). Although ACI has been shown to produce satisfactory short and medium-term results, further technique improvements are needed to address the typical complications associated with ACI.

The chondrogenic potential of transplanted cells

Chondrocytes, responsible for the secretion of extracellular matrix, were initially the logical and preferred cell source for cell-based cartilage engineering. Although found to produce good outcomes, their use in various models was shown to be limited by issues of donor site morbidity and cellular dedifferentiation when cultured in vitro (65-67). In recent years, MSC has emerged as a promising alternative cell source for cartilage repair. MSC, responsible for bone and cartilage formation in the embryo, and repair and turnover in the adult, are multipotent stem cells with the potential of differentiating into adipocytes, chondrocytes or osteocytes. The multipotency of MSC enables them to be stable phenotypes in vitro as well as progenitors for

Table 1. TEG papers investigating the ACI technique for the treatment of focal articular cartilage defects, in chronological order

Authors	Year	Type	n	Objective	Assessment tools	Follow up	Findings	Adverse events	Conclusions
Abbas et al	2008	Clinical case report	2	To examine the clinical efficacy of ACI	IKDC score, OKS, AKSS, MRI	9 months	Improved clinical post-operative scores Patient 1: IKDC (34.5→79.3), OKS (22→12), AKSS (90→100) Patient 2: IKDC (29.9→87.4), OKS (22→12), AKSS (90→100) MRI: complete filling of treated knee defects with repair tissue	nil	Clinical and structural improvements seen after ACI at 9 months
Kamarul et al	2008	Animal study	9	To compare the efficacy of ACI vs. non-operative measure	GAG expression, Brittberg score	3 months	Significantly higher GAG expression in ACI-treated knees (1.12 vs. 0.81μg GAGs/mg protein, p=0.008) Significantly higher Brittberg score in ACI-treated knees (6.00 vs. 1.89, p=0.007)	nil	ACI superior to non-operative measure for repairing focal cartilage defects
Kamarul et al	2010	Animal study	18	To compare the efficacy of chondrogenic differentiated MSC (C-MSC) vs. undifferentiated MSC vs. autologous chondrocytes	Brittberg score, O'Driscoll score, GAG expression	3 and 6 months	Higher scores (all outcome measures) for defects treated with undifferentiated MSC. Treated knees produced significantly (p=0.0024) higher scores compared to untreated (control) group at 6 months. Hyaline cartilage regeneration at treated sites.	nil	Undifferentiated MSC shown to have better tissue repair outcomes compared to C-MSC and autologous chondrocyte
Tay et al	2011	Animal study	30	To compare the efficacy of allogenic undifferentiated MSC (allo-MSC) vs. autologous chondrocyte (auto-C)	Brittberg score, O'Driscoll score, GAG expression	6 months	Similar regenerative profile in both groups except for significantly higher Brittberg scores in the allo-MSC group (8.8 vs. 6.6, p=0.04) All outcome measures significantly higher (p<0.05) in both treatment groups compared to control group (untreated)	nil	Allo-MSC and auto-C have similar efficacy in repairing focal cartilage defects Both treatments superior to non-operative measure
Dashdtar et al	2011	Animal study	12	To compare the efficacy of allogeneic derived chondrogenic pre-differentiated MSC (C-MSC) vs. undifferentiated MSC	Brittberg score, O'Driscoll score, GAG expression	3 and 6 months	No significant difference in all outcome measures between C-MSC and undifferentiated MSC Mean Brittberg score is significantly higher (p<0.05) in treated knees compared to untreated knees (control) at 6 months, with hyaline-like cartilage regeneration	nil	C-MSC and MSC produced comparable treatment outcomes
Kamarul et al	2011	Animal study	18	To examine the effect of GS and CS on healing of ACI-treated articular cartilage defects	Brittberg score, O'Driscoll score, GAG expression	3 and 6 months	Significantly higher (p<0.05) Brittberg score and O'Driscoll score for GS and GS+CS groups compared to groups without pharmacotherapy at 3 and 6 months, and GAG values at 6 months. Histology revealed typical hyaline cartilage structure.	nil	Combination of ACI and pharmaco-therapy may improve healing of focal cartilage defects
Ab-Rahim et al	2013	Lab Study	9	To compare the extracellular matrix expression produced by chondrocyte-alginate constructs ± TGF-β1 vs. monolayer culture	Immuno-histochemical evaluation, histology exam, TEM, SEM	-	Significant increase in GAG/mg protein levels in chondrocyte cultures grown in alginate than in monolayer cultures (1.071μg/mg protein vs. 0.435μg/mg protein, p<0.05) and an abundance of ECM protein distribution was observed	nil	Use of alginate hydrogel beads in chondrocyte cultures (± TGF-β1 supplement) produced superior ECM expression than monolayer cultures
Dashdtar et al	2013	Animal study	24	To compare repair outcomes of MSC seeded in novel PVA-chitosan composite hydrogel vs. established alginate-transplanted models	Brittberg score, O'Driscoll score, GAG expression	6 months	Significant (p<0.05) results for groups treated with PVA-chitosan-MSC or alginate-MSC compared to PVA-chitosan-scaffold and untreated controls. No significant difference between PVA-chitosan-MSC and alginate-MSC group.	nil	Comparable outcomes with PVA-chitosan-MSC and alginate-MSC for cartilage repair.

TEG Tissue Engineering Group; *ACI* autologous chondrocyte implantation; *IKDC* International Knee Documentation Committee; *OKS* Oxford Knee Score; *AKSS* American Knee Society Score; *MRI* magnetic resonance imaging; *GAG* glycosaminoglycan; *MSC* mesenchymal stem cell; *GS* Glucosamine sulphate; *CS* Chondroitin sulphate; *TEM* transmission electron microscopy; *SEM* scanning electron microscopy; *ECM* extracellular matrix; *PVA* polyvinyl alcohol

other cell types despite being removed from their original environment (68-69). MSC can be isolated from various tissues, but bone marrow-derived MSC have been found to have better chondrogenic differentiation compared with other sources and are easily attainable (70-71).

To determine the cell source with the highest chondrogenic potential, we conducted a study to compare the treatment outcomes of alginate-embedded allogenic undifferentiated MSC against autologous chondrocytes in the repair of focal articular cartilage defects in 30 rabbit models (72). We found that, apart from significantly (p=0.04) higher Brittberg scores in the allogenic MSC treatment group, both treatments showed similar cartilage regenerative profiles (Table 1).

Similar to the findings of our study, many authors have reported that autologous chondrocytes and MSC produce comparable repair outcomes (29,73-74). Hui et al (73) from NUS compared the efficacy of chondrocytes, MSC, periosteal grafts and mosaicplasty for treating osteochondritis dissecans in rabbits, and reported that cultured chondrocytes and MSC had comparable enhancing effects on the repair of chondral defects, whereas mosaicplasty did well initially and periosteal grafts did less favourably. Yan et al (74) from Peking University evaluated the repaired tissue formed in 36 full-thickness rabbit cartilage defects implanted with chondrocytes, MSC, fibroblasts and human umbilical cord blood stem cells (hUSC-SC), and reported hyaline-like cartilage repair and significantly higher histologic scores in groups treated with chondrocytes and MSC compared with groups treated with fibroblasts and hUSC-SC. Similarly, Nejadnik et al compared the clinical outcomes of 72 patients treated with conventional ACI with patients treated with autologous MSC and found no discernable differences between the MSC and ACI group in terms of clinical outcomes (75).

Another issue to consider with regards to the choice of transplanted cells is the suggestion that lineage committed cells have superior chondrogenic expression markers compared with undifferentiated MSC (76-77). As in-vivo assessments of chondrogenic pre-differentiated MSC to repair cartilage defects had not yet been initiated, we decided to perform a preliminary study in which full-thickness focal cartilage defects were created in 18 rabbit models and treated with either chondrogenic differentiated MSC (CMSC), autologous chondrocytes or undifferentiated MSC (78). Quantification analyses demonstrated higher scores in terms of mean Brittberg score, GAG quantification and histological score for defects treated with undifferentiated MSC compared with ones treated with CMSC and autologous chondrocytes, although these findings were not significant (Table 1). On the other hand, in a subsequent study comparing cartilage repair potential between allogenic-derived CMSC and undifferentiated MSC for full-thickness cartilage defects of 12 rabbits, we observed no significant differences between the Brittberg, O'Driscoll scores, GAG and total protein content when comparing defect sites treated with MSC and CMSC (79) (Table 1). These findings suggest that

transplantation of MSC for the repair of articular cartilage defects produces a comparable repair to autologous chondrocytes, irrespective of their state of differentiation. This may have clinical implications forthe wider use of undifferentiated MSC in cell-based therapy, as this option is associated with more simplified and economical laboratory processing. However, as there are no available papers at present with which to compare our findings, these observations are preliminary.

The role of cell carriers and scaffolds

First-generation (conventional) ACI utilising periosteal grafts, as developed by Peterson et al, was observed to produce good repair outcomes, but this was complicated by periosteal hypertrophy, local morbidity for periosteal harvest and graft failures (63, 64, 80, 81). Second-generation ACI explored the use of collagen membranes as a replacement for periosteal grafts, and was found to eliminate the drawbacks associated with the use of periosteal flaps with no statistical reduction in clinical outcomes (61, 82). However, both first- and second-generation ACI techniques have been reported to be limited by the risk of cell leakage with inadequate sealing (83) and dedifferentiation of cellular phenotypes cultured in a monolayer culture (65-67).

Recent advances in tissue engineering have led to the development of third-generation ACI, which relies upon utilising scaffolds and cell carriers to mimic a matrix and provide the necessary information or signaling for cell attachment, proliferation and differentiation (84-85). Chondrocytes are embedded into three-dimensional (3D) constructed scaffolds for cell growth, resulting in an 'all-in-one' graft that does not need a periosteal cover or fixing stitches and can be trimmed to exactly fit the cartilage defect, effectively avoiding spillover or asymmetric distribution of chondrocytes following implantation. Additionally, it has been suggested that the use of certain scaffolds in chondrocytic cultures can eliminate the phenotypic drift associated with monolayer culture (65, 86). This is of particular importance considering that an alteration in the ability of chondrocytes to maintain extracellular matrix expression (ECM) may ultimately lead to inferior repair outcomes in patients.

We decided to study the use of alginate, one of the most studied and applied polymers in the tissue engineering and drug delivery field (85) which is known to improve chondrocyte differentiation in cultures (87-90). To this end, our centre conducted a controlled laboratory experiment where chondrocytes were isolated from rabbit knee articular cartilage and expanded in vitro using a monolayer culture system or alginate chondrocyte construct suspension (91) (Table 1). Results demonstrated a significant increase (p<0.05) in GAG/mg protein levels in chondrocyte cultures grown in alginate construct compared with monolayer cultures. In addition, an abundance of ECM protein distribution surrounding the chondrocytes cultured in alginate hydrogel was observed.

To support our findings, many other authors have reported encouraging alginate outcomes in in vitro and in vivo studies and concur that cultures of chondrocytes in alginate beads enable chondrocytes to maintain their spherical shape and typical chondrotypic appearance (87-88), resulting in significantly raised cartilage formation and particularly increased expression of type II collagen (89-90). Correspondingly, the application of alginate in ACI-treated patients has produced significant clinical improvements at two years, with predominantly hyaline-like repair tissue observed in the majority of patients (92). Other matrix and scaffold materials such as collagen (84, 93-95), hyaluronic acid (96-98), agarose (99), fibrin (100) and polyhydroxyacids (101) have shown equally favourable clinical results in cartilage regeneration. Four comparative studies comparing the use of periosteal-ACI with collagen (102-103), hyaluronic acid (104) and polyhydroxyacids (105) produced comparable short-term clinical results, suggesting that at present there is no obvious ranking among the currently available scaffolds for clinical use.

In recent years, hybrid scaffolds that combine synthetic and natural polymer have recently been developed in an effort to further enhance the clinical outcome of ACI-led cartilage repair. Naturally derived materials such as alginate often have desirable biological properties but limited mechanical strength or fat degradation profiles (for eventual integration into surrounding tissue). In contrast, synthetic polymers provide an appropriate 3D environment and have the desired mechanical strength but lack the bioactive properties of natural scaffolds. Composite hydrogels are thought to be able to retain the desirable characteristics of both materials, and have been observed to produce encouraging results in in vitro and in vivo experiments (106-109).

Poly-vinyl alcohol (PVA)-chitosan application has been previously described for wound healing (108, 109) but its role as a scaffold for MSC in cartilage tissue engineering has not been demonstrated. Our group investigated the efficacy of a novel PVA-chitosan composite hydrogel compared with previously established alginate-transplanted models (107). In this study, a medial femoral condyle defect was created in both knees of 24 rabbit models and transplanted, after three weeks, with PVA-chitosan-MSC, PVA-chitosan scaffold alone, alginate-MSC or alginate alone. We observed that the morphological and histological analysis showed significantly better (p<0.05) tissue repair when treated with PVA-chitosan-MSC or alginate MSC compared to the scaffold alone and the untreated control (Table 1). No significant difference was observed between the PVA-chitosan-MSC and alginate-MSC groups. These findings suggest that the use of scaffolds and cell carriers (e.g. alginate, PVA-chitosan) in the ACI technique may provide more favourable ECM expression compared to conventional ACI utilising monolayer cultures, by maintaining cell morphology within a conductive culture environment.

Although the induction of scaffolds and cell carriers into the ACI technique has been reported by many authors to be favourable, evidence suggests that while third-generation ACI, utilising scaffolds and cell carriers, seems to promote chondrocyte differentiation and formation of cartilage matrix, it has not resulted in superior clinical results when compared with conventional ACI. The five comparative controlled clinical trials available at present demonstrated similar results when comparing second- and third-generation ACI against first-generation ACI (82, 102-105). However, the authors did note that the use of scaffolds reduced the incidence of periosteal hypertrophy (82, 102).

The role of local and systemic pharmacological agents

Another approach in augmenting tissue differentiation and cell activity in cartilage tissues is via the introduction of biochemical modulators. Glucosamine sulphate (GS) and chondroitin sulphate (CS) are the two most frequently prescribed nutritional supplements for the treatment of osteoarthritis (110), and have been shown to have substantial clinical efficacy in several osteoarthritis studies (111-114). In vitro, GS and CS have been reported to stimulate the synthesis of proteoglycans by cultivated human chondrocytes (115-118). Although investigated extensively in osteoarthritis, the use of these pharmacological agents for the repair of focal chondral lesions has not been explored.

To elicit the role of GS and CS in enhancing ACI-led focal cartilage repair, our centre conducted a preliminary laboratory experiment to establish the effects of oral administration of GS and CS on the healing rate of ACI-repaired chondral lesions in 18 rabbits (119). We observed significantly higher (p<0.05) Brittberg scores and modified O'Driscoll scores in groups treated with a combination of ACI and pharmacotherapy (Group ACI+GS and Group ACI+GS+CS) compared with the ACI without pharmacotherapy group at three months, and significantly higher (p<0.05) GAG content at six months (Table 1). Based on the results of our study and those reported by others (120, 121), it is suggested that there may be a functional role for GS and CS in augmenting the restoration of ACI-repaired chondral lesions. However, as there are no available papers at present with which to compare our findings, these observations are preliminary.

Conclusion

Earlier systematic reviews examining the evidence for conventional ACI by other authors concluded that there was insufficient evidence in the existing literature to determine whether or not conventional ACI was superior in the treatment of focal articular cartilage defects (43-49). However, the ACI technique has evolved considerably over the past few years. Not only have biomaterials been introduced into the field, MSC has emerged as a promising potential alternative cell source to autologous chondrocytes. Our six years of experience in cell-based therapy, which consists of one clinical study, six animal

studies and one laboratory study, suggests that the ACI technique is a feasible technique for the treatment of focal articular cartilage defects. Our study has shown that the ECM expression produced by a chondro-conductive scaffold construct (e.g. chondrocyte-alginate construct) is superior to the ECM expression produced by chondrocytes grown in monolayer cultures. Moreover, by utilising a 3D chondro-conductive scaffold, the issues of periosteal hypertrophy, cell leakage and dedifferentation of cellular phenotypes cultured in a monolayer culture (63-67, 80-81, 83) associated with conventional ACI can be avoided. Our study has also demonstrated that autologous chondrocytes and MSC have comparable regenerative profiles, irrespective of the state of MSC differentiation. Owing to the ease of harvesting and their multi-potential ability, MSC represent a feasible alternative cell source that can be exploited in repairing articular cartilage defects. Finally, our study has also preliminarily shown the potential of oral pharmacotherapy (GS and CS) in enhancing the healing of ACI-led repair of focal cartilage defects. No adverse reactions were observed in any of our clinical and animal studies. On the whole, these findings collectively suggest that the ACI technique incorporating 1) autologous chondrocytes or MSC; 2) chondro-conductive scaffolds and cell carriers; and 3) oral pharmacotherapy, is a safe and efficient therapeutic option for the treatment of articular cartilage defects of the knee. We are excited to be embarking, in the near future, on research into further methods of refining the ACI technique, including mechanotransduction and gene manipulation. Based on our six years' experience in cell-based therapy, we are optimistic that we will be able to achieve an effective and reproducible solution to the repair of the notoriously difficult to treat chondral lesions.

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