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A histological comparison of the repair tissue formed when using either Chondrogide® or periosteum during autologous chondrocyte implantation

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SUMMARY

Objective: In this study, we compare the clinical and histological outcome between periosteum and Chondrogide® during autologous chondrocyte implantation (ACI).
Method: This study consisted of 88 patients having received ACI in the knee; 33 treated with Chondrogide® (ACI-C) and 55 with periosteum (ACI-P). Post-operative biopsies were taken at a mean of 16.6 ± 8 months (range 7–37 months) and 19 ± 18.4 months (range 4–114) for ACI-C and ACI-P respectively. Histological assessment was performed using the ICRS II and OsScore scoring systems. The immunolocalisation of elastin and collagen types I and II was analysed using specific antibodies. Lysholm scores, a measure of knee function, were obtained pre- and post-operatively at the time of biopsy and annually thereafter.
Results: Compared with ACI-P, the repair tissue formed from patients treated with ACI-C demonstrated a significantly higher score for cellular morphology (ICRS II score), significantly better surface morphology from medial femoral condyle treated defects (ICRS II score) and a significantly higher proportion of hyaline cartilage formation (OsScore). Elastin fibres were present in both ACI-C and ACI-P samples, although their presence was very variable in quantity, distribution, orientation, thickness and length. Patients treated with ACI-C demonstrated significantly more collagen type II immunolocalisation compared with ACI-P. Both groups exhibited a significant increase in Lysholm score post-ACI.
Conclusions: This study demonstrates a significantly better quality of repair tissue formed with ACI-C compared with ACI-P. Hence Chondrogide® is perhaps a better alternative to periosteum during ACI.
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Introduction

Hyaline cartilage is a highly organised tissue composed primarily of proteoglycans and type II collagen with a small amount of elastin[1], which lacks the capability for spontaneous self-regeneration. Symptomatic full-thickness chondral defects in articular joints such as the knee have been treated for many years with autologous chondrocyte implantation (ACI), a two-stage process whereby the patient's chondrocytes are culture-expanded *in vitro* and then implanted into the defect beneath a membrane.

Traditionally, the membrane was autologous periosteum[2], harvested from the medial proximal tibia. Periosteum has an outer "fibrous layer" consisting of fibroblasts and an inner "cambium layer" consisting of osteogenic progenitor cells[3]. It therefore has osteogenic capacity, but in a chondrotrophic environment it can also be used to promote cartilage formation[4,5]. Whilst ACI is generally considered to provide clinical relief in most patients[6,7], continued follow-up of patients treated with a periosteal patch during ACI has noted an increased rate of hypertrophy of the graft[8,9], thus often requiring shaving of the repair tissue overgrowth.

More recently, in an attempt to limit complications observed with periosteal based ACI and removing the need for any incision over the tibia, Chondrogide®, a commercially available membrane (Geistlich Pharma AG, Switzerland) consisting of porcine collagen types I and III, has been used[10]. This is described by the manufacturers as a bilayer membrane, naturally resorbed within 24 weeks[11], with a compact smooth surface preventing cells from diffusing through the membrane and a porous layer of collagen fibres which promotes cell invasion and attachment.

A prospective randomised clinical trial by Gooding *et al.*,[8] comparing the use of periosteum and Chondrogide® for the repair of full-thickness chondral defects in the knee concluded no

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significant difference between the two patches, based on clinical outcome and arthroscopic assessment at 2 years. However, the use of a collagen membrane was advocated as it is attributed with reducing graft failure, complications and re-operation rates associated with the use of periosteum[10,12].

Whilst the patient's clinical outcome following treatment such as ACI is paramount, follow-up can often be lengthy and time consuming. Therefore, the ability to use histological parameters to assess the quality of the repair tissue at an earlier time point may prove beneficial for predicting outcome. Histology or repair tissue biopsies has been performed in several other studies, but usually limited to overall matrix morphology[8,13,14,15] with few scoring individual parameters[16,17]. While studies have reported on hypertrophy of the graft, they fail to comment on the surface architecture.

The purpose of this study therefore was to compare the histological outcome of repair tissue when using the two different patch types, periosteum and Chondrogide® and determine if this impacted upon clinical outcome. In addition, the presence of elastin fibres in the repair tissue was investigated to see if the elastic network was replenished. Immunolocalisation of collagen types I and II was also investigated.

Methods

Patients and ACI procedure

This ethically approved retrospective study (09/H1203/90) consisted of 88 fully informed and consented patients who underwent ACI treatment of cartilage defects in the knee. ACI was performed as described by Brittberg *et al.*[2], using a two-stage procedure, where the first stage required an arthroscopic removal of a small piece of macroscopically healthy cartilage, usually from either the intercondylar notch or trochlea. Chondrocytes were isolated from the harvested cartilage in the GMP-approved John Charnley Laboratory and cultured in autologous serum[18]. After an average of 21 days in culture, the autologous chondrocytes were implanted beneath a periosteal or collagen (Chondrogide®) membrane covering the defect.

Of these 88 patients (66 men and 22 women), 33 patients received Chondrogide® (ACI-C) and 55 patients received periosteum (ACI-P). In total there were 110 defects treated, 48 treated with ACI-C and 62 treated with ACI-P with varying locations (see details in Table I). To maximise the sample number, all sequential patients with a biopsy of repair tissue were included, even though this resulted in different numbers of samples for ACI-C and ACI-P.

Post-operative tissue biopsies and clinical outcome

A juvenile bone marrow biopsy needle was used during arthroscopy to obtain a core biopsy of the repair tissue as close as possible to the centre of the treated defect, with the aim of acquiring both the cartilage repair tissue and the underlying subchondral bone. A total of 107 repair tissue core biopsies (45 ACI-C, 62 ACI-P) of <2 mm diameter, were taken at a mean of 16.6 ± 8 months (range 7–37 months) and 19 ± 18.4 months (range 4–114 months) post-operatively for ACI-C and ACI-P respectively. Biopsies were snap frozen in liquid nitrogen-cooled hexane, embedded in OCT compound (Tissue-Tek®, Zoeterwoude, The Netherlands) then cryosectioned to produce 7 μm thick sections which were collected onto poly-L-lysine coated slides. The remaining sample was stored in liquid nitrogen.

Lysholm scores, a measure of knee function[19], were obtained pre- and post-operatively at the time of biopsy and annually thereafter. The score ranges from 0 (poor outcome) to 100 (best outcome).

Table I
Patient, defect and biopsy details for both Chondrogide® (ACI-C) and periosteum (ACI-P) treated patients

	ACI-C	ACI-P
Number of patients	33	55
Age at ACI (years)	38.7 ± 8.1	34.2 ± 10.1*
Size of treated defect (cm^2)	6.7 ± 5.6 (range 1.1–33.75)	4.5 ± 3* (range 0.96–13.5)
Number of defects treated	48	62
Number of cells implanted	$5.9 \pm 1.9 \times 10^6$ (range $3–10 \times 10^6$)	$5.7 \pm 2.4 \times 10^6$ (range $1.1–12 \times 10^6$)
Number of patients: single defects	22	47
Number of patients: multiple defects	11	8
Medial femoral condyle	19	39
Lateral femoral condyle	11	12
Trochlea	8	5
Patella	4	3
Tibial plateau	5	3
Number of biopsies	45	62
Time of biopsy (months post-ACI)	16.6 ± 8 (range 7–37)	19 ± 18.4 (range 4–114)
Latest follow-up (months post-ACI)	56.5 ± 20.1 (range 15–98)	85.2 ± 32.6* (range 13–157)

* Significant differences between patch types.

Histology

Frozen sections were defrosted at room temperature and stained with either haematoxylin and eosin (H&E) for histological assessment of the general morphology of the repair tissue, or safranin O/fast green (Saf-O/FG) and/or toluidine blue (TB), to indicate glycosaminoglycan content as per standard protocols[20]. Sections were then analysed under polarised light microscopy to examine collagen fibre orientation and distinguish between hyaline cartilage and fibrocartilage[21]. The histological quality of the repair tissue was assessed and scored using (1) the ICRS II visual analogue scale[22] (VAS, each of 14 parameters scored 0–10) and (2) the OsScore[17] (total score 0–10), assessing the parameters listed in Table II. A higher score in each scoring system represents tissue resembling healthy articular cartilage.

Immunohistochemistry

Immunolocalisation of elastin was performed on eight biopsies (five ACI-C and three ACI-P) and for collagen types I and II on 74 biopsies (28 ACI-C and 46 ACI-P). Cryosections were incubated with

Table II
Parameters assessed in the ICRS II and OsScore histology scoring systems. For the ICRS II scoring system, each of the 14 parameters is marked out of 10 on a VAS[22]. For the OsScore system, there is a total score of 10 with different parameters contributing a different weighting to the overall score[17]

Scoring parameter	ICRS II	OsScore
Tissue morphology	●	●
Matrix metachromasia	●	●
Cell morphology	●	
Cell clusters	●	●
Surface architecture	●	●
Basal integration	●	●
Calcification front/tidemark	●	
Subchondral bone abnormalities	●	
Inflammation	●	
Calcification	●	●
Vascularisation	●	●
Surface/superficial assessment	●	
Mid/deep zone assessment	●	
Overall assessment	●	

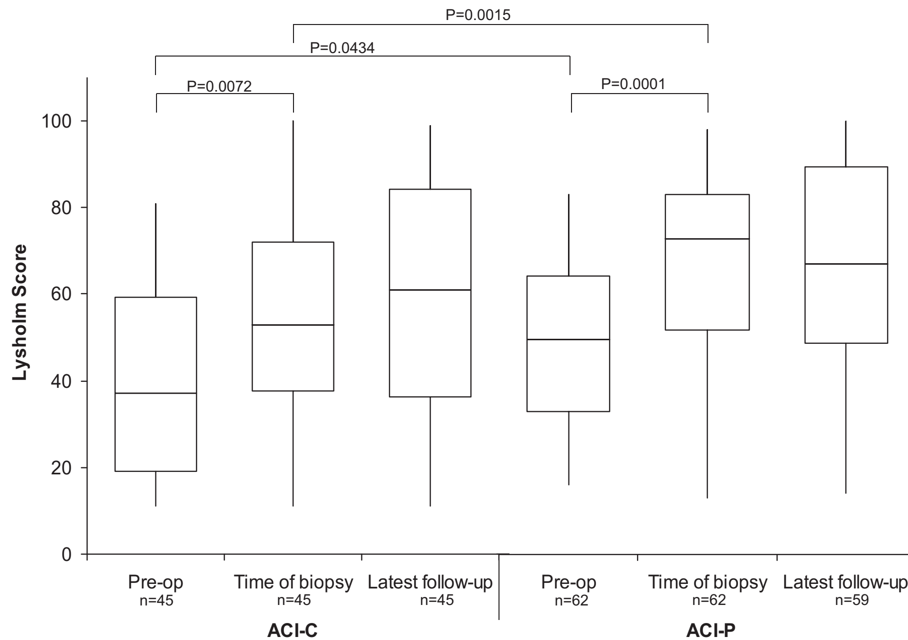

Fig. 1. Lysholm scores taken at pre-op, at the time of the biopsy and the latest follow-up for both ACI-C and ACI-P treated patients.

hyaluronidase prior to fixing in 4% formaldehyde. A rabbit polyclonal anti-alpha human-elastin antibody (1:50, Biogenesis, Oxford) was applied to the sections for 90 min followed by the secondary goat anti-rabbit biotinylated antibody for 60 min (Vectastain Elite ABC kit, Vector Laboratories, Peterborough)[1]. Antibodies against collagen type I (1:500, clone I-8H5, MP Biomedicals, Cambridge) or type II (1:10, clone CIIC1, Developmental Studies Hybridoma Bank, Iowa) were incubated for 60 min prior to the secondary goat anti-mouse biotinylated antibody for 60 min (Vectastain Elite ABC kit, Vector Laboratories, Peterborough)[23]. Blocking steps to minimise non-specific binding and endogenous peroxidase activity were incorporated as appropriate with normal goat and normal human serum in 3% bovine serum albumin and 0.3% hydrogen peroxide in PBS or methanol.

All incubations were performed at room temperature and sections washed with PBS three times between steps. Labelling was enhanced with streptavidin-peroxidase (Vectastain Elite ABC kit, Vector Laboratories, Peterborough) and visualised with diaminobenzadine (DAB). Adjacent sections were stained with an isotype-matched murine IgG1 (Dako, Cambridge) for collagen staining and rabbit IgG1 (Dako, Cambridge) for elastin staining in place of the primary antibody as a normal control. Cryosections of Chondrogide® and periosteum were also prepared and stained for elastin for comparison to the repair tissue.

To analyse the distribution of collagens types I and II, positive extracellular matrix staining was recorded as a percentage of the total area of the cartilage[23].

Statistical analysis

Statistical differences for the comparison of various parameters between the two patch types were tested for via a Mann–Whitney *U* test and correlations were tested for via a Spearman's Rank correlation. Statistical differences between grouped frequency data of the OsScore parameters were tested for using a Chi-squared test of independence. All statistical analyses were performed using the software programme Analyse-it® Software Ltd, Leeds, UK. Statistical significance was considered when $P < 0.05$.

Results

Patient characteristics and clinical outcome

Patients having received ACI-C were significantly older (mean age 38.7 ± 8.1 years) than those having received ACI-P (mean age 34.2 ± 10.1 years) at the time of treatment ($P = 0.0395$). The mean size of defect treated with ACI-C was also significantly larger than that treated with ACI-P ($P = 0.0092$). There was no significant difference in the number of cells implanted between each of the two patch types. The mean time at which post-operative biopsies were taken was shorter for ACI-C than for ACI-P, although not significantly different. The total follow-up time was significantly longer for ACI-P than ACI-C patients ($P = 0.001$). Patient baseline demographics and medical data are represented in Table I.

ACI-C treated patients had a significantly lower Lysholm score both pre-operatively ($P = 0.0434$) and at the time of biopsy than ACI-P patients ($P = 0.0015$), but no significant difference between the scores at the latest follow-up (Fig. 1). Within each patch type group, there was a significant improvement in Lysholm score from pre-operatively to that taken at the time of biopsy ($P = 0.0072$ and $P = 0.0001$ for ACI-C and ACI-P respectively), but the level of improvement at the time of biopsy and at latest follow-up was not significantly different between the two groups.

Histology of the repair tissue

Of the 107 post-operative biopsies analysed, there was no significant difference between the overall histology scores (either the ICRS II or OsScore) of the repair tissue between the two groups. Additionally, there were few significant differences between the individual parameters of either scoring mechanism for the two patch types. Whilst the ICRS II scoring mechanism is performed on a VAS, the OsScore is a nominal scoring mechanism, thus allowing the frequencies of each individual scoring parameter between the two patch types to be analysed.

Whilst the morphology of the matrix produced in the repair biopsies was found to not significantly differ between ACI-C and

ACI-P by ICRS II, the OsScore demonstrated a larger proportion of ACI-C biopsies to be of either hyaline or mixed hyaline and fibrocartilage morphology compared to ACI-P [Fig. 2(A)]. Thus, the presence of any hyaline cartilage (either alone or a mixture of hyaline and fibrocartilage) was observed in significantly more biopsies from patients treated with ACI-C than those treated with ACI-P ($P = 0.0103$). Patients treated with ACI-P demonstrated a significantly larger proportion of solely fibrocartilage repair tissue than those treated with ACI-C ($P = 0.0011$). However, fibrous tissue (the worst quality of repair tissue) was observed in 9% of ACI-C biopsies and only 2% of ACI-P biopsies. Neither of the scoring methods used detected any significant differences in the proteoglycan content between the two patch types with over 80% of biopsies from both ACI-C and ACI-P groups exhibiting normal or near normal matrix metachromasia.

Repair tissue biopsies from patients treated with ACI-C had significantly higher score for cellular morphology than those treated with ACI-P ($P = 0.0057$, [Fig. 2(B)]), with no significant difference in the presence of chondrocyte clusters between the two patch types. Small clusters were relatively common whereas large clusters were rare.

Collectively, the surface architecture of the repair tissue formed post-ACI did not differ significantly between the two patch types. However, when analysing biopsies from individual locations, patients with a single defect on the MFC, had significantly better surface architecture when treated with ACI-C than with ACI-P ($P = 0.0223$ (OsScore), [Fig. 2(C)]; $P = 0.0451$, (ICRS II) [Fig. 2(D)]). No other location yielded any significant difference in surface architecture between the two patch types.

No significant differences were found between ACI-C and ACI-P repair tissue with regards to tidemark formation or subchondral bone abnormalities. Collectively, over 90% of all biopsies, regardless of defect location, from both ACI-C and ACI-P treated patients exhibited either good or average basal integration of the repair cartilage with the underlying subchondral bone (OsScore, [Fig. 2(E)]). However, with respect to the different defect locations, patients having received ACI for a single defect on the LFC had significantly better basal integration of the repair tissue with the underlying subchondral bone when treated with ACI-P than ACI-C when scored with the ICRS II scoring system ($P = 0.024$, [Fig. 2(F)]). No other location demonstrated a significant difference in basal integration. The majority of biopsies in both groups were free of ectopic calcification and vascularisation and the occurrence of extensive calcification or vascularisation were rare. No evidence of inflammation was observed in any of the 107 biopsies.

For ACI-C treated patients, only the ICRS II parameter for tidemark formation correlated significantly with the Lysholm score reported at the time of the biopsy ($P = 0.0179$, $R^2 = 0.1914$, [Fig. 3(A)]). For ACI-P treated patients, only the ICRS II overall parameter was found to correlate with the Lysholm score at the time of biopsy ($P = 0.05$, $R^2 = 0.0311$, [Fig. 3(B)]).

Nine patients (three ACI-C and six ACI-P) had multiple biopsies taken at two different time points with an average of 34.8 ± 31.1 months (range 12–106 months) between each biopsy. While some

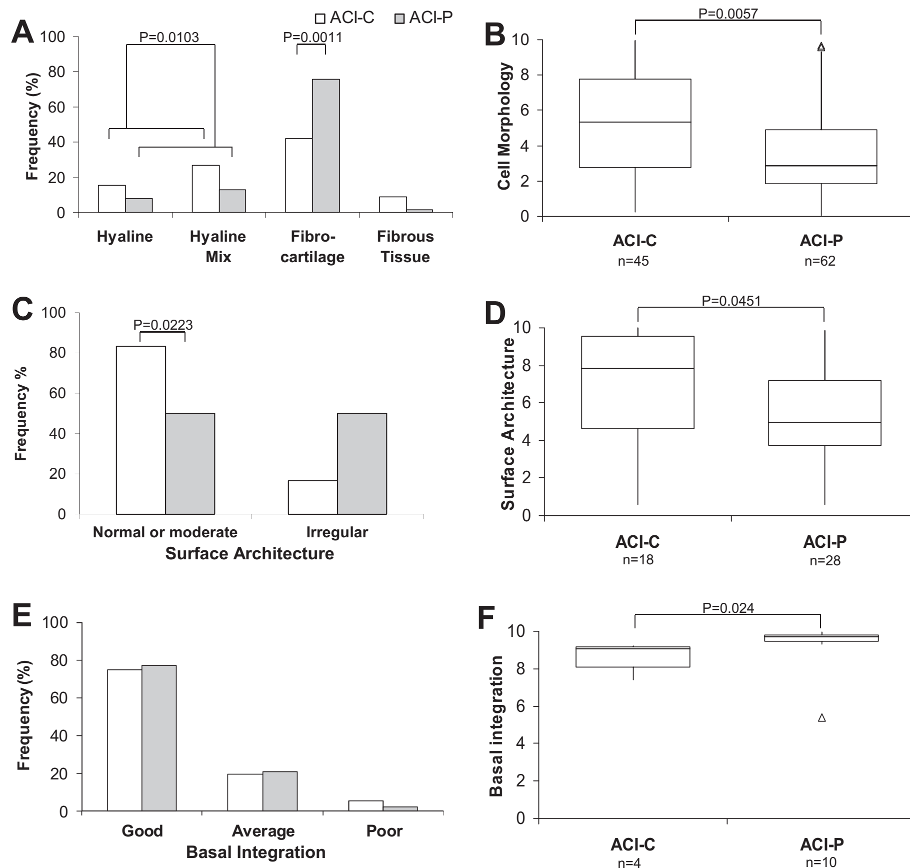

Fig. 2. Histological and morphological differences of the repair tissue biopsies from ACI-C and ACI-P treated patients. ACI-C treated patients demonstrated a higher proportion of hyaline cartilage formation in the repair tissue than ACI-P treated patients (A), with significantly better cellular morphology (B), significantly better surface architecture on MFC defects (C and D) and significantly better basal integration on LFC defects (E). Collectively, over 90% of all biopsies, regardless of patch type or defect location, demonstrated good or average basal integration (F). Graphs are presented as the percentage frequency of occurrence as determined by the OsScore scoring system (A, C and E) or the mean ICRS score (B, D and F).

patient's histology improved with time, others did not and some remained the same, likewise with the Lysholm scores obtained at the time of each biopsy. There was no correlation between either the OsScore or ICRS II overall parameter, with the Lysholm score.

Immunohistochemistry for elastin, collagen type I and collagen type II

Elastin fibres were visible in approximately 31% of H&E-stained sections (nine ACI-C and seven ACI-P). These were confirmed as elastin by performing immunohistochemistry on five ACI-C and three ACI-P. The size of the elastin fibres varied from wide fibres, visible on H&E-stained sections ([Fig. 4(A) and (B)]) and stained light blue with Saf-O/FG [Fig. 4(C)], to apparently long and very fine fibres, only visible with immunostaining [Fig. 4(D)]. Under polarised light, some elastin fibres could be seen to run parallel with collagen fibres [Fig. 4(E)]. The presence of elastin fibres in the repair tissue biopsies was very variable in quantity, distribution, orientation, thickness and length. Elastin could be observed throughout the depth of the cartilage or confined to one, but no specific area in both tissues with hyaline cartilage morphology and fibrocartilage [Fig. 4(F) and (G)]. Elastin fibres were abundant throughout the section of both Chondrogide® [Fig. 4(H)] and periosteum [Fig. 4(I)].

Immunolocalisation of collagen types I and II was variable throughout the repair biopsies (see Fig. 5). The area of positive staining for both patch types ranged from 0 to 100% of the extracellular matrix. There was a significantly greater amount of positive extracellular matrix staining for collagen type I compared to collagen type II for both ACI-C ($P = 0.0252$, Table III) and ACI-P ($P < 0.0001$). Whilst there was no significant difference in positive extracellular matrix staining for collagen type I between the two patch types, there was significantly more staining for collagen type II in ACI-C biopsies than ACI-P biopsies ($P = 0.0008$). The majority of biopsies, even those with hyaline cartilage morphology, stained positive for both collagen type I and II, but to varying degrees. Ninety-three per cent of ACI-C biopsies and 85% of ACI-P biopsies stained for collagen type I exhibited over 90% positive extracellular matrix staining throughout the depth of the tissue. However, for those biopsies which exhibited less than 90% positive extracellular matrix staining in either patch type group, staining was restricted to the superficial zone. Two biopsies, one from each patch type, failed to show any positive immunolocalisation for collagen type I.

Immunolocalisation of collagen type II however was more varied. Sixty-one per cent of ACI-C biopsies and 25% of ACI-P biopsies stained for collagen type II exhibited 100% positive extracellular matrix staining throughout the depth of the tissue. For the remaining biopsies with less than 100% positive extracellular staining, no staining was observed in the superficial zone except for two biopsies (ACI-P only). Only three biopsies, all from the ACI-P group, failed to show any positive immunolocalisation for collagen type II.

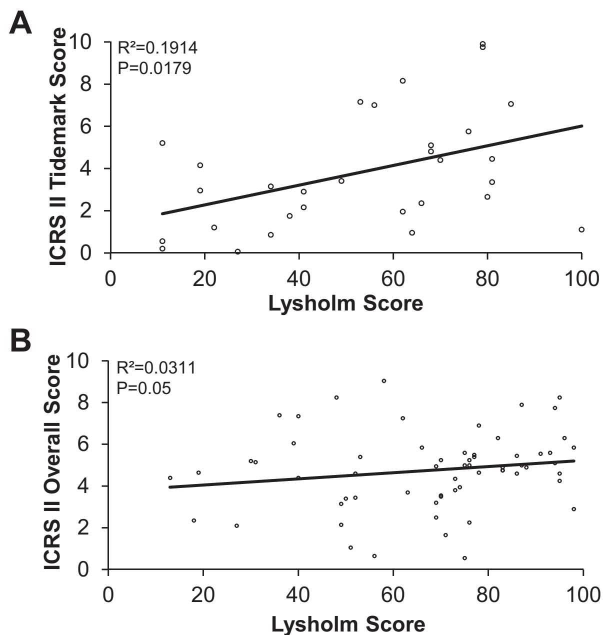

Fig. 3. Positive, but weak correlations were observed between the ICRS II scoring parameters for tidemark formation for ACI-C patients (A) and overall score for ACI-P patients (B) vs the Lysholm score obtained at the time of the biopsy.

Discussion

Periosteum was the traditional membrane used when the technique of ACI was first developed[2], but in more recent times, a commercially available collagen membrane (Chondrogide®) is often used in its place. The extent to which the patch used in ACI procedures contributes to the repair tissue is unknown and few studies have compared these two patches in terms of the quality of the repair tissue formed. It is known that periosteum contains mesenchymal stem cells[24] and has both osteogenic[25,26] and chondrogenic potential[27]. How much these or other cells present within the periosteum might contribute to the repair tissue, either directly or indirectly, has been debated[5,28] but recent *in vivo* studies have demonstrated the ability of periosteal cells to infiltrate scaffolds and produce a cartilaginous matrix[29].

Cost-effectiveness analysis in the USA comparing the use of a periosteal patch with a commercially available collagen membrane revealed that whilst both treatments were cost-effective in their own right, ACI-C was marginally better value due to fewer patch-related complications such as graft hypertrophy[30]. Although no similar analyses have been performed in the UK, it is highly likely the same results apply. While autologous periosteum is biologically compatible with the patient, is cost-free and may be a source of cells and growth factors, harvesting periosteum does require the patient to endure a further incision, thus prolonging both theatre time and potentially, patient recovery time as well as increasing the risk of infection. Periosteum can also vary greatly between patients, partly due to its autologous nature and partly due to the surgeon-to-surgeon variability during the harvesting procedure. Chondrogide® on the other hand eliminates the patch type variability between patients, removes the need for an extra incision and saves theatre time, but obviously incurs a cost for purchasing. Chondrogide® is reported to be easier to handle in surgery than periosteum and is less likely to tear during stitching and handling (personal communication with Prof JB Richardson).

Clinical and macroscopic results with Chondrogide® suggest that less hypertrophy occurs than seen when using a periosteal patch[10,12,31,32]. *In vitro* studies have proposed that dysregulated proteinase expression within the repair tissue may result in degradation, hypertrophy or rejection of a periosteal graft[33]. Although further procedures for graft hypertrophy or rejection were not analysed in the present study, we have demonstrated for the first time, significantly better surface architecture of the repair tissue (for defects on the MFC) when using Chondrogide® compared to periosteum by two different histological scoring methods (although the sample sizes of the two groups were not identical). This is despite ACI-C treated defects being both larger on average and biopsied earlier than ACI-P treated defects. It is

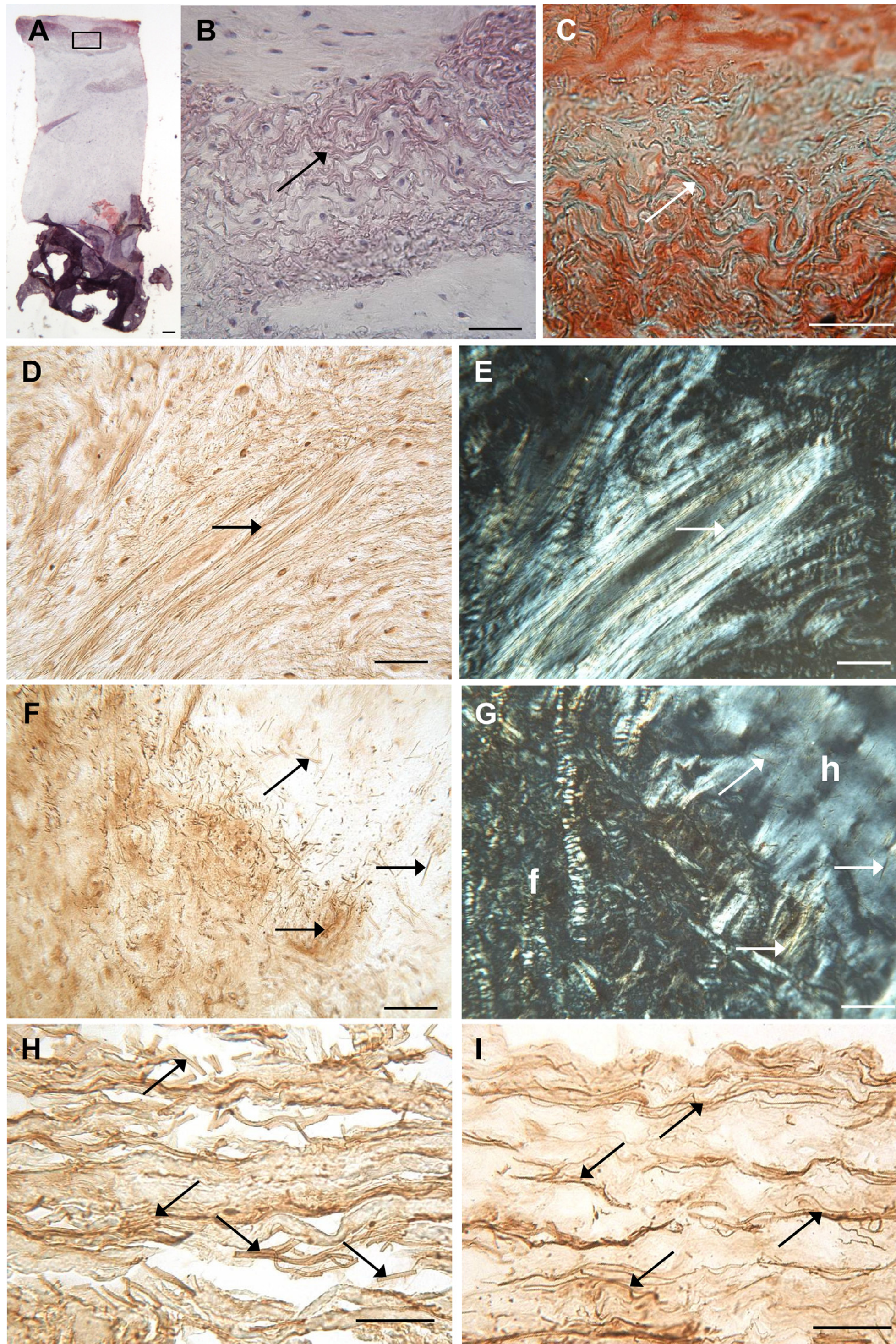

Fig. 4. Thick elastin fibres could be observed on H&E-stained (A, B) and Saf-O/FG stained sections where they stained light blue (C). The presence of elastin fibres was also determined by immunohistochemistry (D–I). Fibres could sometimes be seen to run in close proximity to each other in "bundles" (D) and when viewed under polarised light (E, which is a polarised image of D), they appeared to run parallel with collagen fibres. In the repair tissue biopsies, elastin fibres sometimes appeared short and very fine (F) and could be observed in both hyaline cartilage "h" and fibrocartilage "f" (G, polarised image of F). Images A-C and F-G are of repair tissue from patients treated with ACI-C, whilst D and E are from patients treated with ACI-P. The presence of elastin fibres was also noted in both types of patch materials *per se*, as seen in similarly prepared immunostained sections of Chondrogide® (H) and periosteum (I). Scale bars represent 500 μm (A) and 50 μm (B–I). Boxed section in A denotes area of higher powered image in B. Arrows point to elastin fibres.

believed that maturation of the tissue improves with time and this may impact on joint function[23].

The use of periosteum in treating cartilage defects by ACI has previously been reported to stimulate the remodelling process in the underlying subchondral bone in a sheep model[34]. Russlies *et al.*[34], demonstrated a significant increase in the bone density of the subchondral bone in those defects treated with periosteum compared to those treated using a collagen membrane, with no significant difference in the histology of the repair tissue formed between the two patch types. Although the biopsies analysed in

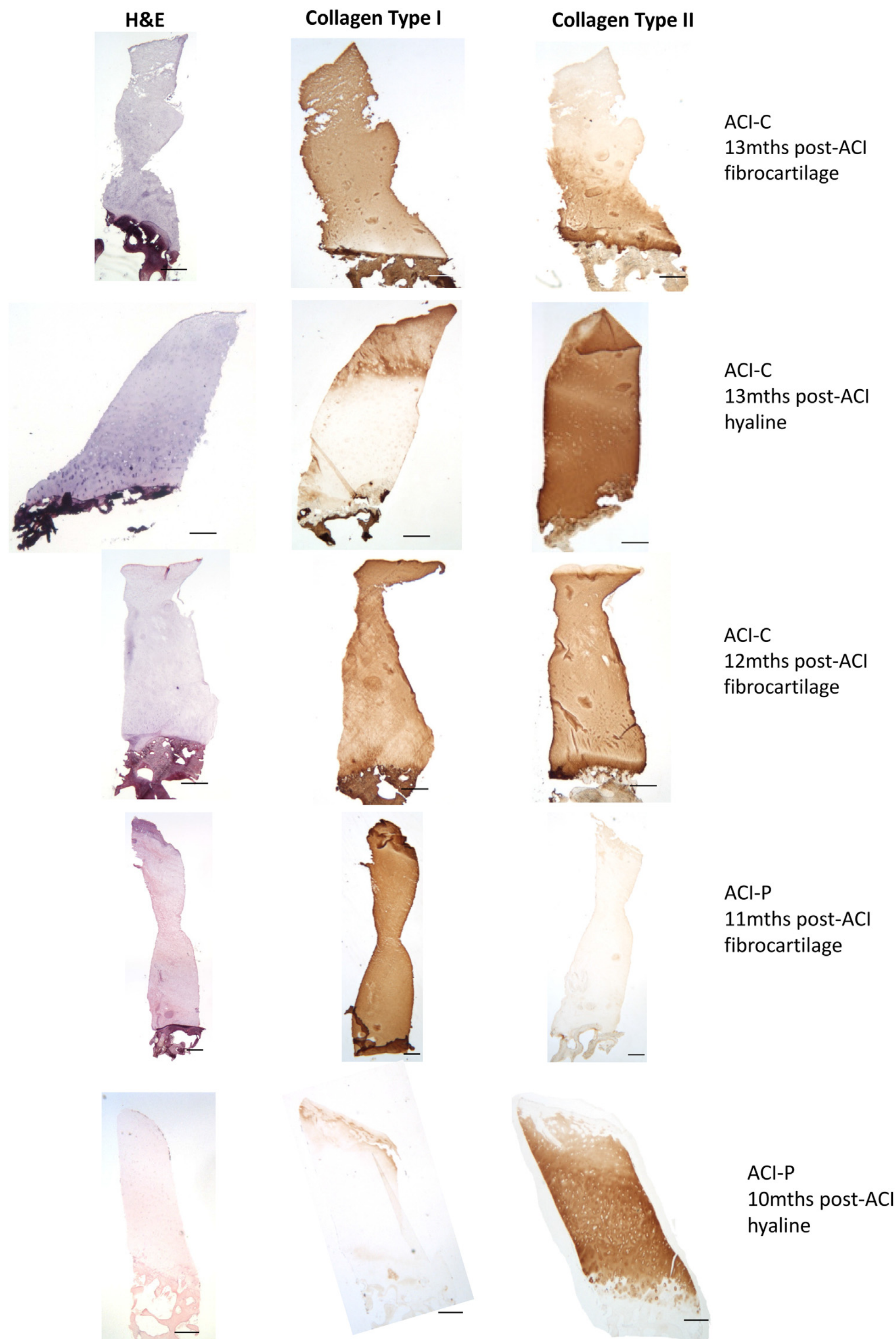

Fig. 5. Representative images of full depth core biopsies immunostained for collagen type I and type II. Scale bars represent 500 μm.

this present study were not assessed solely for the density of the subchondral bone, one of the ICRS II scoring parameters assesses subchondral bone abnormalities (including bone marrow fibrosis, see Table II). When scoring this parameter, any thickening of the subchondral bone in these biopsies was taken into account. There was not, however, any significant difference between the two patch types and over 90% of all biopsies analysed had good or average integration between the repair tissue and the underlying subchondral bone, regardless of defect location.

The periosteum has also been associated with ossification of the repair tissue due to its osteogenic capacity[35] which is not surprising given it contains both osteogenic progenitor cells and osteoblasts[36]. The periosteum is also highly vascularised and contains an abundance of endothelial pericytes which also have the capacity to differentiate into osteoblasts[37]. Periosteal-derived cells have been demonstrated to secrete several isoforms of vascular endothelial growth factor[38] (VEGF). VEGF has been suggested to act as an autocrine growth factor for the osteoblastic

Table III
Mean percentage area of positive extracellular matrix staining for collagen types I and II in both ACI-C and ACI-P biopsies

	ACI-C	ACI-P
Collagen type I	94.2 ± 22.0 (range 0–100)	88.0 ± 29.3 (range 0–100)
Collagen type II	91.1 ± 14.9* (range 50–100)	67.4 ± 32.2*,† (range 0–100)

* Significantly more staining for collagen type II than collagen type I.
† Significantly less staining for collagen type II in ACI-P than ACI-C.

differentiation of human periosteal-derived cells *in vitro*[38]. Ectopic calcification and vascularisation was observed, although infrequently, in repair tissue biopsies from both ACI-C and ACI-P treated patients. This is in contrast to a small study by Briggs *et al.*[13], who failed to observe any cases of ectopic calcification following ACI-C. While the use of periosteum could have contributed to the formation of such features during the repair process following ACI-P, it does not explain the presence of either ectopic calcification or vascularisation following ACI-C. Laboratory studies however have demonstrated that osteoblasts from sclerotic OA subchondral bone can induce OA chondrocyte hypertrophy and subsequent matrix mineralisation via the downregulation of SOX9, COL2, PTHrP and PTH-R and the upregulation of OSF-1[39]. In spite of this, due to the method of ACI stage 1 where a macroscopically healthy piece of cartilage is harvested from the knee for chondrocyte extraction, it is anticipated that the culture-expanded implanted chondrocytes are not of an osteoarthritic phenotype and so may be more resistant to the influences of the underlying subchondral bone, even if sclerotic.

The purpose of elastin in articular cartilage remains an area of interest and although its actual function is unknown, it is thought to have both a mechanical and biological role, such as acting as a repository for growth factors like TGFβ[40]. Previously, elastin fibres have been observed in both the superficial zone parallel to the articular surface and in the pericellular matrix of cells within the superficial zone in human, bovine, and equine articular cartilage[1,40,41]. It has long been established that collagen fibres cross-link with elastin fibres[42] and so the light blue elastin fibres observed in Saf-O/FG sections is perhaps to be expected. The variability in the thickness and alignment of elastin fibres observed within the repair tissue biopsies could be representative of the level of maturity of the repair tissue. Consistent with results presented here, elastin fibres within the periosteum have previously been described[43]. As far as we are aware, this is the first published study to reveal the presence of elastin in Chondrogide®. It is possible therefore, that the elastin observed within the repair tissue, particularly those samples where the elastin was confined to one particular region, could be remnants of the patch used during ACI.

Elastin is very resistant to degradation and turnover is typically very slow in other related connective tissues[44]. Synthesis of new elastin, for example in the intervertebral disc, is exceptionally slow[44], thus further supporting the hypothesis that the elastin observed in these repair tissue biopsies has been derived from the original patch. Whilst Chondrogide® has been reported to be resorbed during repair[11], we do not know what happens to the periosteal patch. It is possible that periosteum is remodelled to become part of the regenerated cartilage. If this is the case, then our proposal of the elastin in the repair tissue (especially in the mid and deep zones) having been derived from the patch, is plausible. If however, the periosteum remains on the surface of the repair tissue, then we would expect to see elastin fibres in the superficial layer only (as observed in normal human articular cartilage[1]); hence we suggest that the elastin we observed could be attributed to being produced as part of the repair process. If indeed the observed elastin is found to be derived from the patch, its presence in biopsies as much as 15 months post treatment, implies that, particularly in ACI-C patients, Chondrogide® is not completely resorbed within the 24 weeks as previously reported[11].

Collagen type I is present in large amounts in fibrocartilage and is generally either absent from healthy articular hyaline cartilage or present in very small amounts, whereas native hyaline cartilage contains in excess of 90% collagen type II[45]. Despite this, its presence alone is not recommended as a marker for hyaline cartilage[23] as it can also be seen in fibrocartilage, such as the intervertebral disc[46]. Collagen type I and type II have previously been shown in repair tissue biopsies following ACI with both fibrocartilaginous and hyaline cartilage morphologies[15], consistent with the results presented here. In addition, we show a significantly greater amount of collagen II immunolocalisation in biopsies from the ACI-C group compared with ACI-P, suggesting that perhaps Chondrogide® is superior to periosteum for producing a repair tissue with native-like properties. However, it has been hypothesised that the repair tissue formed following cell implantation resembles young, immature cartilage with chondrogenic potential[23] and may take some considerable time to mature. Therefore, as mentioned previously, obtaining further biopsies at a later stage in recovery could be beneficial for investigating the maturity of the repair tissue over time and its impact on clinical function.

Conclusion

In this study we have analysed and compared the repair tissue formed after ACI treatment for defects in the knee when using either a Chondrogide® or periosteal patch. Ideally, the number of patients and site of defect per patch type group would be identical; whilst this is possible in an animal study, the current study was performed in human patients. We believe this to be more relevant to the clinic, but it has the disadvantage of being less able to control for the presentation of patients such as the site of the lesion or the change in clinical practice from using a periosteal flap to the more common use of Chondrogide®. Whilst the uneven sample numbers could have affected the statistical analyses, the histological quality of repair tissue formed with ACI-C differed little from that seen with ACI-P. This is despite ACI-C patients being significantly older at treatment, having significantly larger defects treated and having significantly lower pre-operative Lysholm scores than ACI-P patients. Lysholm scores obtained at the time of biopsy were maintained in both groups up to 13 years later and so can be predictive of long-term ACI success, regardless of the patch type used.

These results have demonstrated for the first time, significantly better surface architecture and cellular morphology within the repair tissue when treated with ACI-C compared with ACI-P. Additionally, a significantly greater proportion of biopsies were found to be of hyaline or hyaline mix morphology with a significantly greater amount of positive extracellular matrix staining for collagen type II with ACI-C compared to ACI-P. Chondrogide® therefore provides a suitable and perhaps more appropriate alternative to periosteum for use as a patch in the procedure of ACI.

Author contributions

Helen S McCarthy: Conception and design, analysis and interpretation of data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, statistical expertise, collection and assembly of data.

Sally Roberts: Conception and design, analysis and interpretation of data, critical revision of the article for important intellectual content, final approval of the article, statistical expertise and obtaining of funding.

Role of the funding source

The funding body, Arthritis Research UK, had no involvement in the study design, collection, analysis and interpretation of data, in the writing of the manuscript or the decision to submit the manuscript for publication.

Conflicts of interest

The authors declare no conflicts of interest.

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