



## Research

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# Autologous adipose stem cells and polylactide discs in the replacement of the rabbit temporomandibular joint disc

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The temporomandibular joint (TMJ) disc lacks functional replacement after discectomy. We investigated tissue-engineered bilayer polylactide (PLA) discs and autologous adipose stem cells (ASCs) as a potential replacement for the TMJ disc. These ASC discs were pre-cultured either in control or in differentiation medium, including transforming growth factor (TGF)- $\beta$ 1 for one week. Prior to implantation, expression of fibrocartilaginous genes was measured by qRT-PCR. The control and differentiated ASC discs were implanted, respectively, in the right and left TMJs of rabbits for six ( $n = 5$ ) and 12 months ( $n = 5$ ). Thereafter, the excised TMJ areas were examined with cone beam computed tomography (CBCT) and histology. No signs of infection, inflammation or foreign body reactions were detected at histology, whereas chronic arthrosis and considerable condylar hypertrophy were observed in all operated joints at CBCT. The left condyle treated with the differentiated ASC discs appeared consistently smoother and more sclerotic than the right condyle. The ASC disc replacement resulted in dislocation and morphological changes in the rabbit TMJ. The ASC discs pre-treated with TGF- $\beta$ 1 enhanced the condylar integrity. While adverse tissue reactions were not shown, the authors suggest that with improved attachment and design, the PLA disc and biomaterial itself would hold potential for TMJ disc replacement.

## 1. Introduction

### 1.1. Background

Internal derangement of the temporomandibular joint (TMJ) frequently involves displacement of the TMJ disc, the most common TMJ arthropathy [1,2]. The displaced or otherwise damaged TMJ disc is exposed to morphological changes and degeneration [2–4]. Similar to articular cartilage, the TMJ disc lacks an intrinsic regenerative capacity. Surgery is considered for patients suffering from significant joint dysfunction and persistent pain [1,5,6]. Discectomy is required when other treatment options are unsatisfactory or the TMJ disc has become morphologically too altered to be repositioned or repaired by other therapies [1,5–7]. After discectomy, the joint surfaces are exposed to direct mechanical loading and abrasion. This predisposes the joint to arthritic deformation, which may ultimately necessitate total joint replacement [1,8]. The development of a functional replacement for the TMJ disc is warranted.

The TMJ disc is composed of specialized fibrocartilaginous tissue differing from both hyaline and meniscal cartilage [9]. The cell population in the TMJ disc is referred to as fibrochondrocytes, because cells display both fibroblast and chondrocyte-like morphology [10,11]. The structural framework of the TMJ disc consists of collagens, predominantly type I collagen [4,10,12,13] and proteoglycans rich in chondroitin and dermatan sulphates [13–16]. The number and distribution of extracellular matrix (ECM) components in the TMJ disc are anisotropic, and dependent on the species and age-dependent variations [12–14].

Different materials, including autologous tissue grafts, natural and synthetic biomaterials, have been proposed for replacement the excised TMJ disc. Autografts from various sources such as auricular cartilage [17–19], dermis [20–22], fat [23], fascia and temporal muscle [24–26] have been used. In addition to morbidity caused by harvest, autografts have not been successful in treatment of the TMJ disorders [17–20,27,28]. Xenogenic ECM prepared from porcine urinary bladder has recently reported to support the formation of functional tissue resembling that of the native TMJ disc without signs of pathologic changes in the articulating surfaces [29].

The failure and detrimental effects of synthetic implants based on silicone and polytetrafluoroethylene [30–32] have directed interest towards biodegradable materials. The first attempt to engineer the TMJ disc was performed using type I collagen mesh [33]. Subsequently, synthetic polyglycolide (PGA) has been the most extensively studied biodegradable polymer used for tissue engineering of the TMJ disc [34–36]. PGA has good cytocompatibility with TMJ disc-derived cells, but exhibits too rapid resorption in order to sustain the mechanical integrity and open porosity as well supporting of the construct [34,36–38]. Polylactide (PLA) has a longer degradation time; and is therefore promising as a synthetic replacement for the TMJ disc [39,40].

Various cell sources have been examined for TMJ disc engineering. TMJ disc-derived fibrochondrocytes appear a natural choice [33,37,41–43]. These cells may be harvested during arthroscopic examination. Nevertheless, the use of the patient's own TMJ disc-derived cells is compromised by the degenerative changes in the TMJ disc [42]. Moreover, TMJ disc-derived fibrochondrocytes tend to change their phenotype during expansion in the laboratory [10,37,40,44]. Alternatively, chondrocytes from other cartilage types [36,37,45,46] or dermal fibroblasts [43] have been examined for TMJ disc engineering. The disadvantage of autologous cartilage is its limited availability owing to donor site scarcity and morbidity. These factors have increased interest towards the use of stem cells for the TMJ disc replacement [44,45,47,48]. Adipose tissue is an expendable, and abundant source of autologous adult stem cells capable of undergoing differentiation towards cells of mesenchymal origin, including the fibroblasts and chondrocytes found in the TMJ disc [40,49,50].

Tissue engineering aims to provide sustainable regeneration of damaged tissues by using live cells to produce new tissue possessing similar properties to the original tissue. This is achieved in an appropriate stimulatory environment in a three-dimensional culture system created with biodegradable scaffolds [42,51] or by cell self-assembly in a scaffold-free approach [43,45,52]. Ideally, the tissue-engineered TMJ disc would provide a structurally and functionally analogous equivalent to the native TMJ disc. Only few studies on TMJ disc engineering have been performed *in vivo* [27,36].

However, cell-based solutions have not so far been used for replacement of the TMJ disc in an animal model.

According to anatomical features, pig is suggested as the most suitable model for TMJ disc studies [2,37,53,54], but is far too expensive and laborious to handle in the primary experiments. With respect to the TMJ disc, the rabbit model also has many similarities to the human TMJ disc, including biochemical composition [53], morphology with biconcave parasagittal profile, internal collagen fibre organization [55] and attachments of the TMJ disc [56]. In addition, the rabbit TMJ exhibits significant lateral and anteroposterior movements in comparison with other mammals [57].

Our group has previously reported the potential of ASC-seeded non-woven PLA discs for TMJ disc engineering *in vitro* [40]. The aim of this study was to evaluate novel ASC-seeded PLA discs as a tissue-engineered replacement of the TMJ disc *in vivo*. Our hypothesis was that the PLA disc would provide a suitable basis for the formation of fibrous-like replacement tissue, and ASCs would further improve the fibrocartilaginous TMJ disc-like tissue formation. The study was performed with adult rabbits for extended follow-ups in order to better reflect the long-term effect of the implant in the adult patient population. Furthermore, because the dimensions of the implanted PLA discs were equal, the possible unbalance owing to bilateral effects remained negligible at the starting point. The treated sides differed only in the pre-culture conditions for the ASC discs which were compared with respect to TMJ disc regeneration.

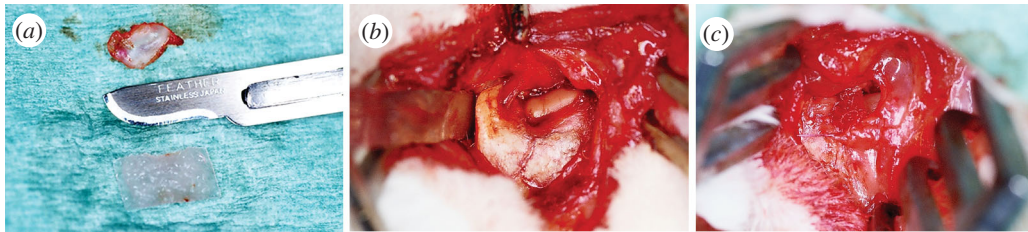
## 2. Material and methods

### 2.1. Preparation of the polylactide disc

The biomaterial disc designed to replace the rabbit TMJ disc was composed of two layers: an upper layer of non-woven mat of poly(L/D)lactide (P(L/D)LA) 96/4 was sealed from bottom side with a P(L/DL)LA 70/30 smooth foil to hold seeded cells inside the disc. Medical grade P(L/D)LA 96/4 with an intrinsic viscosity of  $3.5 \text{ dl g}^{-1}$  (Purac Biochem b.v., Gorinchem, The Netherlands) and medical grade P(L/DL)LA 70/30 with an intrinsic viscosity of  $6.1 \text{ dl g}^{-1}$  (LR 708, Boehringer, Ingelheim, Germany) were used to prepare the non-woven mat and the foil, respectively. The PLA disc was prepared according to a previously described protocol [40] to the dimensions of  $7 \times 5 \times 1.2 \text{ cm}$ .

### 2.2. Harvest and culture of cells

Subcutaneous adipose tissue was harvested from the neck area of 10 adult (2.5- to 3-year-old) female New Zealand White rabbits under anaesthesia. The mean weight of rabbits was 3.7 kg. ASC isolation was performed according to the previously described protocol [40]. ASCs were expanded in control medium containing Dulbecco's modified Eagle's medium (Sigma-Aldrich Chemie GmbH, Steinheim, Germany); 10 per cent foetal bovine serum (FBS; Gibco, Invitrogen Life Technologies, Paisley, UK); 1 per cent antibiotic/antimycotic ( $100 \text{ U ml}^{-1}$  penicillin,  $100 \mu\text{g ml}^{-1}$  streptomycin and  $250 \text{ ng ml}^{-1}$  amphotericin B; Gibco, Invitrogen); and 1 per cent L-glutamine (Gibco, Invitrogen) for two weeks. After expansion, ASCs were seeded as a suspension at a concentration of  $10^5 \text{ cells ml}^{-1}$  in a volume of  $40 \mu\text{l}$  into the PLA discs. ASCs were allowed to adhere to the fibres of the PLA disc for 3 h in a cell incubator in humidified atmosphere at  $37^\circ\text{C}$  in 5 per cent  $\text{CO}_2$  prior to media addition. One half of the ASC discs were maintained in control medium and the other half in chondrogenic medium comprising of DMEM/F12 supplemented with 1 per cent FBS, 1 per cent antibiotic/antimycotic, 1 per cent L-glutamine,  $6.25 \mu\text{g ml}^{-1}$  insulin



**Figure 1.** (a) The excised TMJ disc next to the ASC disc implant. (b) Zygomatic arch and condyle exposed after TMJ disc removal and the ASC disc in its position between the condyle and zygomatic arch after implantation. (Online version in colour.)

(Sigma, St Louis, MO), 50 nM ascorbic acid (Sigma) and 10 ng ml<sup>-1</sup> transforming growth factor (TGF)- $\beta$ 1 (Sigma). Chondrogenic medium was supplemented with TGF- $\beta$ 1 in the first media exchange after 24 h of culture. The medium was changed twice during one week culture prior to discectomy operation. ASCs at passage 5 were used for implantation. There were two parallel samples of the ASC discs representing each of the rabbits in both culture media. One of the samples was taken for implantation, and the other was used for qRT-PCR to measure gene expression at the time of implantation.

### 2.3. Implantation and excision of the adipose stem cell discs

All of the 10 test animals underwent bilateral discectomy of the TMJ disc without pre-operative fasting. Subcutaneous injection of 60 000 IU kg<sup>-1</sup> benzylpenicillin procaine/benzathine-penicillin (Duplocillin LA, Intervet International, the Netherlands) was given prior to the surgery as a prophylaxis for infection. The operation was performed under general anaesthesia using 0.5 mg kg<sup>-1</sup> medetomidate (Domitor 1.0 mg ml<sup>-1</sup>, Orion Inc., Espoo, Finland) and 25 mg kg<sup>-1</sup> ketamine hydrochloride (Ketalar 50 mg ml<sup>-1</sup>, Pfizer Inc., Espoo, Finland) which were administered subcutaneously (s.c.). In addition, 50 ml of saline was injected s.c. to prevent dehydration during surgery. The operation site was shaved and then scrubbed thoroughly with chlorhexidine gluconate solution (Klorhexol 5 mg ml<sup>-1</sup>, Leiras Inc., Finland). A vertical incision of 2 cm was made through the skin about 1 cm distally of the lateral canthus of the eye. Soft tissues were dissected to the bony surface, and the upper and lateral parts of the zygomatic arch were exposed. The lateral part of zygomatic arch was saved to maintain the anatomic structure as original as possible and to provide some lateral support for the implant in order to reduce the chance for the implant to dislocate. The TMJ capsule was then opened with a scalpel in order to expose the condyle head. The fibrous part of the TMJ disc was removed in both joints for subtotal discectomy (90%). The ASC discs were inserted into the joint space in front of the condyle. Photographs of the surgical protocol are shown in figure 1. The right and left TMJ joints were operated on the control and the differentiated ASC disc, respectively. The surgical procedure was exactly the same in order to eliminate the effect of different operation techniques to the results. Each animal was treated with autologous ASCs. The ASC disc was secured with a mattress suture (5/0 Monocryl, Johnson & Johnson Int., Belgium) around the zygomatic arch, and free movement of the lower jaw was verified. As the size of TMJ is very limited, it was not possible to stabilize the implant by suturing it to the periphery of the joint capsule or to use fixation in the medial area of the joint space. The joint capsule and the access incision were then closed in layers with multiple sutures (5/0 Vicryl rapid, Johnson & Johnson Int.). The 10 test animals were divided into two test groups of five animals each for follow-up periods of six and 12 months.

For post-operative analgesia buprenorphin, 0.03 mg kg<sup>-1</sup> (Temgesic 0.3 mg ml<sup>-1</sup>, Schering-Plough Europe, Brussels, Belgium)

s.c. was used. The analgesic medication was administered every 12 h for 2 days post-operatively. After the operation, all test animals had free access to their ordinary food: dry pellets, fresh vegetables, dry hay and water. All animals were housed individually in single cages. After the follow-up periods of six and 12 months, the animals were sacrificed by an intravenous overdose of pentobarbital sodium (Mebunat vet 60 mg ml<sup>-1</sup>, Orion Inc., Finland). The heads of the rabbits were removed and placed in 70 per cent ethanol.

### 2.4. Limited cone beam computed tomography

Radiological examination was performed using limited CBCT (dental micro CT/CBCT) with 3DX Accu-I-Tomo device (Accu-tomo, J. Morita MFG, Kyoto Japan) after the follow-up of six and 12 months. CBCT examination was performed with 1 mm slice thickness in 80 kV and 1.5 mA using voxel size of 80  $\mu$ m. A radiation field of 80  $\times$  80 mm was used to obtain both condyles into the same view.

### 2.5. Histology

After imaging, the TMJ area was examined histologically. For histological examination, the specimens were dehydrated by increasing series of ethanol and embedded into methylmethacrylate. The specimen blocks were cut in half sagittally through the whole condyle. The slices of 5  $\mu$ m in thickness were prepared and stained with haematoxylin-eosin and Masson's trichrome.

### 2.6. qRT-PCR

After the pre-culture, one of the parallel ASC discs including both control and differentiation treatment was analysed for messenger RNA expressions in order to measure the gene expression of the fibrocartilage ECM components at the time of implantation. Total RNA was isolated from the ASC discs using Trizol-reagent (Invitrogen, Paisley, UK) according to the manufacturer's instructions. First-strand cDNA was reverse transcribed from total RNA using the high-capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA). The mRNA level of aggrecan, types I and II collagen were analysed by qRT-PCR method. The sequences and AC numbers of the primers (Oligomer Oy, Helsinki, Finland) are presented in table 1. The qRT-PCR protocol followed the manufacturer's instruction. The parameters and calculations used are described elsewhere [40].

### 2.7. Statistical analyses

All data are presented as median and range. Data were analysed using GRAPHPAD PRISM v. 5.01 software. The Kruskal-Wallis test was used to assess differences between control and treated ASC discs at six and 12 months ( $n = 5$ ), and Dunn's test was performed for pairwise multiple comparison. Two-tailed  $p$ -values of less than 0.05 were considered significant.



**Table 1.** Primer sequences for qRT-PCR.

gene	accession number	primer sequence	product size (bp)
GAPDH	L23961	forward: 5'-GGG TGG TGG ACC TCA TGG T-3' reverse: 5'-CGG TGG TTT GAG GGC TCT TA-3'	57
aggrecan	L38480	forward: 5'-GGG ACG TGT GCG CAT CA-3' reverse: 5'-GTA GTT GGG CAG CGA GAC CTT-3'	54
type I collagen	D49399	forward: 5'-GGG ACA CAA CGG ATT GCA A-3' reverse: 5'-GCA CCT TGA TCA CCA TGT TGA C-3'	59
type II collagen	D83229	forward: 5'-CCC CGT CTG CCC TAC TGA-3' reverse: 5'-GTT CTC CTT TCT GCC CCT TTG-3'	67

### 3. Results

#### 3.1. Results of qRT-PCR

Relative changes in the expression of aggrecan and types I and II collagen genes were measured from the ASC discs after one week of pre-culture at the time of implantation (figure 2). The differentiation of ASC discs seemed to increase the relative expression of aggrecan to seven- and 14-fold in six- and 12-months' group, respectively. Minor decrease and increase were observed in type I collagen expression. In the 12-month group, the differentiation of the ASC discs seemed to induce the relative expression of all the measured genes but only the fivefold increase in type II collagen expression was statistically significant ( $p < 0.05$ ) compared with the control ASC discs. Donor-dependent differences were observed in the gene expressions of the rabbits.

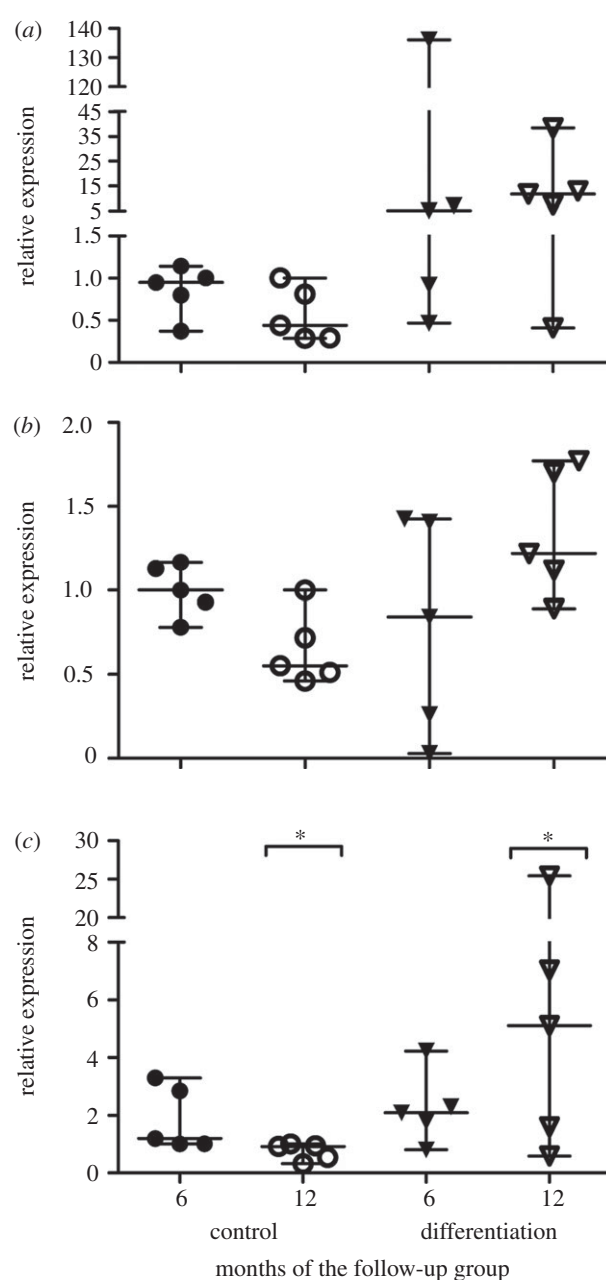
#### 3.2. Animal tests

All test animals healed uneventfully, and no adverse effects were noticed during follow-up period of six and 12 months. All animals maintained their weight as well. In both the six- and 12-month groups, the implanted ASC discs were found to be dislocated either anteriorly or posteriorly of the condylar head in the joint space.

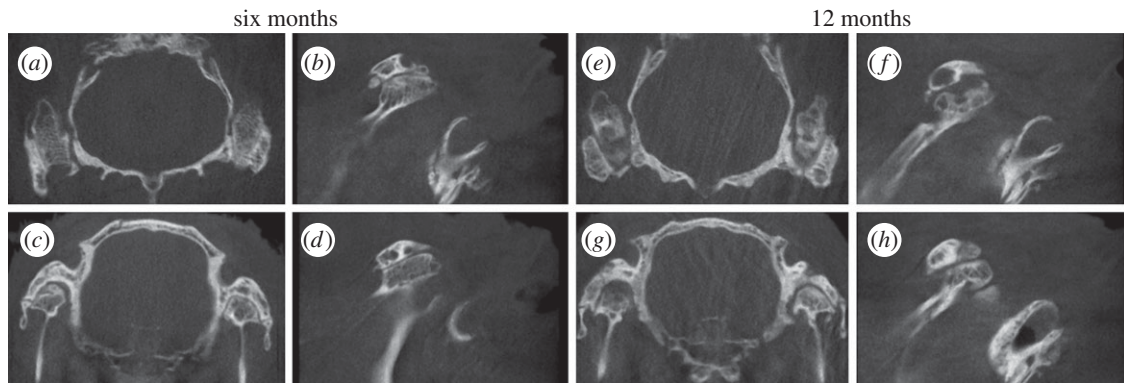
#### 3.3. Findings on cone beam computed tomography

Chronic arthrosis was visible in all the operated joints (figure 3); however, the morphological findings differed between the joint sides. The common feature was considerable hypertrophy of the condyles. In the six-month group, the shape of the right condyle treated with control ASC disc seemed consistently more irregular than that of the left condyle. The left condyle treated differentiated ASC discs appeared smooth and more calcified near the condylar surface. More microcysts were detected in the right than left joint. The bone in the temporal articulation surface was more radio-opaque in the TMJs treated with differentiated ASC disc than with the control ACS disc. The findings were similar in the 12-month study group, where the hypertrophy of the condyles was further increased. The temporal bone medially to the condyle showed thinning as well.

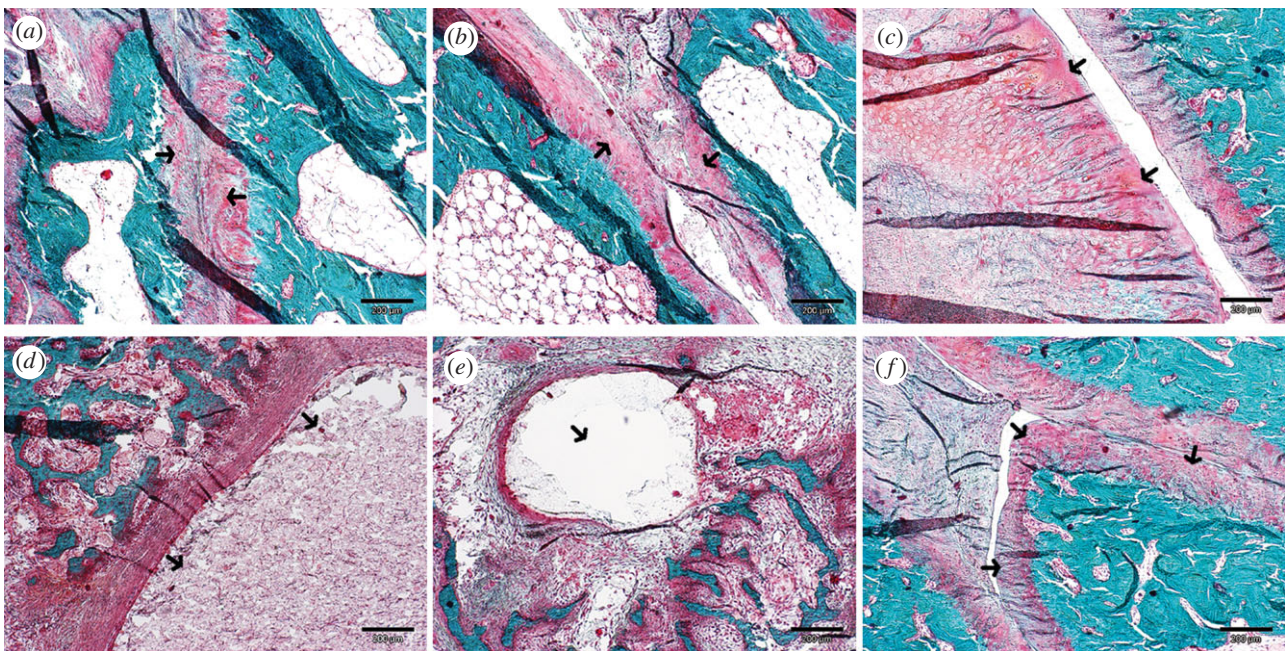
Calcified loose bodies were detected in the joint space in all the treatment groups. The size and shape of the loose bodies varied from 0.3 to 2 mm depending on the direction measurements were taken. The number of particles in a joint also varied ranging from 0 to 10. Particles were usually



**Figure 2.** The relative expression levels of (a) aggrecan, (b) type I and (c) II collagen of the ASC discs after one week pre-culture prior to implantation measured by qRT-PCR. The six and 12 months denote the division of the rabbits in the subsequent follow-up groups. The difference in type II collagen expression between the control and differentiated ASC discs in the 12-month group was found statistically significant and is denoted by an asterisk ( $p < 0.05$ ).



**Figure 3.** CBCT images of the operated TMJ joints in the six- and 12-month groups represented as (a,e) axial and (c,g) coronal views as well as sagittal views of the (b,f) right and (d,h) left TMJs treated with control and differentiated ASC discs, respectively.



**Figure 4.** The sagittal histological sections of the TMJ area stained with Masson's trichrome. (a) The TMJ joint space of the right treated with control ASC disc and (b) the left side treated with differentiated ASC disc in the six-month group. Hyaline cartilage covering the joint surfaces are pointed by arrows. (c) The substantial hypertrophy of condylar cartilage developed in the control ASC disc treated side of the TMJ at 12 months of the follow-up. (d) Microcysts developed in the condylar and (e) temporal bone sides and (f) calcified loose body covered with hyaline-like cartilage are shown in the control ASC disc treated joints after 12 months. The described details are indicated by arrows. Scale bar, 200  $\mu\text{m}$ . (Online version in colour.)

located superiorly of the condyle behind the zygomatic arc and secondly anteriorly of the zygomatic arch. The fragments seemed more numerous on the medial and lateral sides of the right condyle than on the left side. No signs of infection, inflammation or foreign body reaction were observed in any of the treated joints in either of the follow-up groups on the basis of CBCT findings.

### 3.4. Histological findings

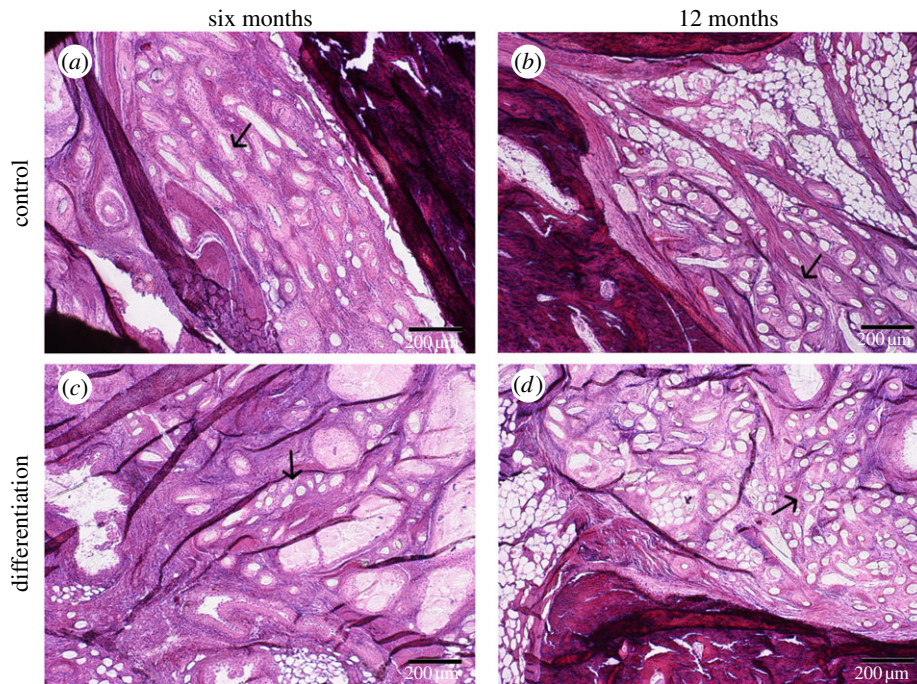
The signs of chronic arthrosis were also observable in the histological sections. The hyaline cartilage on the joint surfaces seemed smooth but the cartilage–bone interface appeared irregular, especially in the 12-month group (figure 4). The superficial cartilage of the left joint treated with differentiated ASC discs appeared more regular than in the right joint. In some areas, hypertrophy of the cartilage seemed considerable (figure 4c). The formation of microcysts was confirmed (figure 4d,e). Calcified loose bodies, also detected in the CBCT,

were covered with cartilaginous tissue (figure 4f). The remnants of the dislocated PLA disc were visible six and still after 12 months after implantation (figure 5). They were detected more frequently at the anterior part of the joint capsule than the posterior part of the joint capsule. PLA fibres of the dislocated ASC discs were surrounded by the fibrous tissue, but no adverse tissue reactions, such as foreign body reactions, were observed. As a biomaterial, PLA did not cause irritation and allowed the normal regeneration of adjacent tissue in the joint.

## 4. Discussion

There is ample evidence that discectomy will in many instances improve the condition for the patient on a short follow-up. The mouth opening will increase and thereby the function will improve and a number of patients have less pain and discomfort [6,58–61]. Yet, the underlying problem has not been solved by discectomy alone and the





**Figure 5.** (*a,b*) The remnants of the ASC discs stained with haematoxylin–eosin of the control and (*c,d*) differentiated ASCs discs after (*a,c*) six and (*b,d*) 12 months of implantation. Some of the PLA fibres are indicated by arrows. The scale bar is 200  $\mu\text{m}$ . (Online version in colour.)

patient will be left with increasing condylar head degeneration, crepitus and in time, an increasing restriction of function. Therefore, there is room for improvement, and tissue engineering has the potential for long-term cure, unlike the situation with discectomy only. This cure might include a tissue-engineered condyle as well [1,2]. In this study, we evaluated a novel construct composed of ASCs and the bilayer biodegradable PLA discs as a potential replacement of the TMJ disc after discectomy in rabbit animal model. To the best of our knowledge, this is the first *in vivo* study of a tissue-engineered TMJ construct consisting of both cells and biomaterial scaffolds.

The test group of aged rabbits was chosen to better reflect the effects of the ASC discs replacement on an actual patient population. Laboratory animals are often juvenile and tissue regeneration after surgery accordingly outperforms that in older animals. Moreover, the follow-up points of six and 12 months were considered long enough to better distinguish the reactions actually related to ASC discs tissue from those occurring normally in the healing tissue after surgery. All test animals recovered well from surgery and maintained good health for the six- and 12-month follow-up periods.

The PLA disc and ASCs used here demonstrated the potential of the TMJ disc engineering *in vitro* [40]. The non-woven fibrous mat was designed to roughly resemble the ECM framework in the native TMJ disc. The fibrous format of a scaffold was demonstrably beneficial with respect to cell number maintenance and ECM formation in fibrochondrogenic cultures [34]. In addition, one week pre-culture, as used here, has previously shown to be a suitable time for the adhesion and retention of rabbit perichondrial cells on the porous PLA scaffolds *in vitro* [62].

The stable positioning of ASC discs on the implantation site proved challenging. All the implanted ASC discs appeared to be displaced from the intended location in both six- and 12-month study groups. Accordingly, the condition in the operated TMJ partly resembled the situation seen after discectomy that lead to changes in the condylar

morphology [1,8,63]. Similar difficulties in anchoring any graft or biomaterial replacement to remaining tissues (retrodiscal tissue remnants, lateral pterygoid muscle attachments or the lateral pole of the condylar head) are known to be a technical challenge in TMJ surgery [20,23,24,64]. In this study, we aimed to secure fixation of the implant to the unmoving zygomatic part of the TMJ and we also placed the smooth foil inferiorly against condyle in order to reduce the effects of mastication movements which may also predispose the implant to dislocation. In this system, the ASCs in the non-woven mat would also have a direct contact with zygomatic hyalinous cartilage that could facilitate cell ingrowth and stimuli for ASCs to differentiate. Brown *et al.* [65] succeeded in securing the TMJ disc implant in its location suturing the implant via its marginal zones against the temporal fossa and joint capsule in canine animal model. In the rabbit model, used here, small holes could have been drilled through the zygomatic bone for additional stabilizing sutures. The stability of the PLA disc could also be improved by moulding it into a rounded shape with either integrated sutures or reinforced periphery area for these sutures. With such suturing the implant would be stabilized better against lateromedial and anterodistal displacement. Nonetheless, according to the histological findings, the PLA fibres of the displaced ASC discs did not cause adverse tissue reactions in the TMJ. Instead, it appeared that the PLA fibres were able to directly bond to bone located in the TMJ space. In this respect, as a biomaterial, PLA might offer good biocompatibility for TMJ surgery.

Given our results, progressive chronic arthrosis was evident in all operated joints. Histological findings similar to those of our study, including deformation and decrease in the morphological integrity of condylar cartilage, have previously been described in osteoarthritic TMJs [66]. Emergence of loose calcified bodies and erosion of the condyle has also been observed with TMJ disc transplants [19]. The considerable hypertrophy of the condyles was visible on both treated sides. Similar findings of condylar hypertrophy have been reported in

conjunction with deformation occurring after disc displacement [67,68]. Surgically induced disc displacement in rabbits has resulted in progressive enlargement of the condyle. Moreover, similar to our study, extensive hyperplasia of the condylar cartilage has been reported [67].

The presence of ASCs may have further facilitated the condylar hypertrophy. The potential of ASCs for bone regeneration has previously been clinically demonstrated in the reconstruction of a hemimaxillectomy patient [69], in large reconstruction of the mandible [70] and in the treatment of the large cranial defects [71] as well as cell culture *in vitro* [72–74]. *In vivo* findings of ASC induced osteogenesis have been contradictory [74–77].

Although condylar hypertrophy was apparent in both joints, the morphological findings differed between the right and left sides. The right TMJ, treated with the control ASC disc, was more irregular and microcystic than the left TMJ, which was visibly smoother and more sclerotic. The differences between the right and left sides increased from the six to 12-month follow-up groups. Because the dimensions of the ASC disc and surgical procedure were the same for all the samples, it can be assumed that possible bilateral effects between the TMJ sides remained negligible at the starting point. Accordingly, the detected side differences were mainly caused by different pre-culture conditions. An increase in both bone and cartilage formation *in vivo* has likewise been reported after local articular administration of TGF- $\beta$ 1 [78,79]. TGF- $\beta$ 1 is known either to promote or inhibit joint destruction *in vivo* depending on type, differentiation stage and health status of target cells, and on the environment [80].

TGF- $\beta$ 1 is a traditional differentiation factor used in chondrogenic cultures [81] and is also advantageous in fibrochondrogenic cultures of mature TMJ disc-derived fibrochondrocytes [39,45] as well as ASCs aiming at TMJ disc reconstruction [40]. There were some donor-dependent differences between autologous ASC disc implants at the time of implantation. The observed differences between the treatment groups after pre-culture remained mainly insignificant. The differentiation medium supplemented with TGF- $\beta$ 1 showed a tendency to increase the gene expression in most of the cases but only type II collagen expression showed a significant difference to control one in the 12-month group. Although type II collagen is the major type in hyaline cartilage, in the TMJ disc, it is found in lesser amounts [10,13,82]. The pre-culture parameters can further be optimized with respect to

cell seeding density, time of culture and supplements in the medium [34]. Here, the volume and cell concentration for scaffold loading was chosen on the basis of the most homogeneous and dense cell distribution attained in the preliminary pilot cultures. High cell density is essential for chondrogenic differentiation to occur in cell culture [83,84]. This is also demonstrated in fibrochondrogenic ECM formation in TMJ disc engineering [35]. Recently, different co-cultures of stem cells and mature fibrochondrogenic cells have been suggested to have potential route for the production of fibrocartilaginous tissue *in vitro* [45,48].

## 5. Conclusion

Chronic arthrosis was discernible in all operated joints, which may have been caused by the implanted ASC disc or its displacement or both. The presence of ASCs probably enhanced the significant hypertrophy of the condyles on both sides. The use of ASC in the TMJ disc engineering may be useful but an improved system for cell differentiation or utilization of additional cell source needs to be considered. The cell labelling would also ease estimation of the actual cell effects on the TMJ regeneration. Apparently, differentiated ASC discs treated with TGF- $\beta$ 1 lead to more regular morphology and increased calcification of the condyle in the TMJ. Nonetheless, PLA fibres were well tolerated in the TMJ space, no signs of inflammatory reaction appeared. Accordingly as a biomaterial, PLA has potential in TMJ surgery. In further studies the design of the PLA disc needs to be improved in order to ensure a better fit and stability of the PLA disc in the correct anatomical position. The mechanical properties of the ASC need to be measured as well as the performance evaluated against unseeded PLA disc and empty discectomy control.

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