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Contact pressures at grafted cartilage lesions in the knee

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Abstract The use of tissue-engineered cellular constructs is currently under clinical evaluation for the surgical treatment of articular cartilage lesions in the knee. The primary failure mode in such cartilage repair techniques is related to fixation. In addition, the repair tissue is believed to be very fragile in the post-operative period, and unable to support the intra-articular loads. We have developed a laboratory testing protocol in order to quantify the contact pressure distribution that develops on fibrin glue grafts applied to full-thickness cartilage lesions. The contact pressure distribution has been mapped on the contact surface of specimens subject to compression, in three configurations (intact, defect and grafted), at increasing load levels. All the maps show stress concentrations at the rim of the defect and a more uniform stress distribution around the rim after defect grafting. At a contact load of 180 N, the peak contact pressure measured on cartilage is 2.5 MPa. In presence of the graft, the peak pressures on the cartilage area surrounding the defect are reduced by 16%, on average. In contrast, both the mean contact pressure on the graft and the graft's contact area increase. The graft was found to carry around 80% of the total applied contact load, at all load levels tested. Fibrin glue was chosen as a grafting material in our study because it shows material properties very representative of currently-implanted cellular constructs. Thus, the results of this study have quantified aspects of recipient graft sites that may assist in optimising such grafting procedures from a biomechanical point of view.

Keywords Tissue engineering · Articular cartilage · Autograft · Contact pressure · Biomechanical testing

Introduction

Advanced treatments for symptomatic cartilage lesions aim to deliver cells to the defect site, which will contribute in developing a repair tissue [12]. In autologous chondrocyte transplantation therapies, currently in clinical use for the treatment of cartilage lesions in the knee, the cells are isolated from a cartilage biopsy and expanded in monolayer, the lesion site is covered with a periosteal flap and the cells are delivered to the site in a liquid suspension [6, 7]. To allow early weight-bearing and a more aggressive rehabilitation, which would accelerate patient recovery, the defect may be filled with a solid cell carrier, or scaffold. The scaffold could possibly withstand the forces within the knee and protect the graft. The development of new biodegradable materials has renewed the interest for this treatment approach [10, 11, 19]. Experimental investigations have also shown that graft placement can be

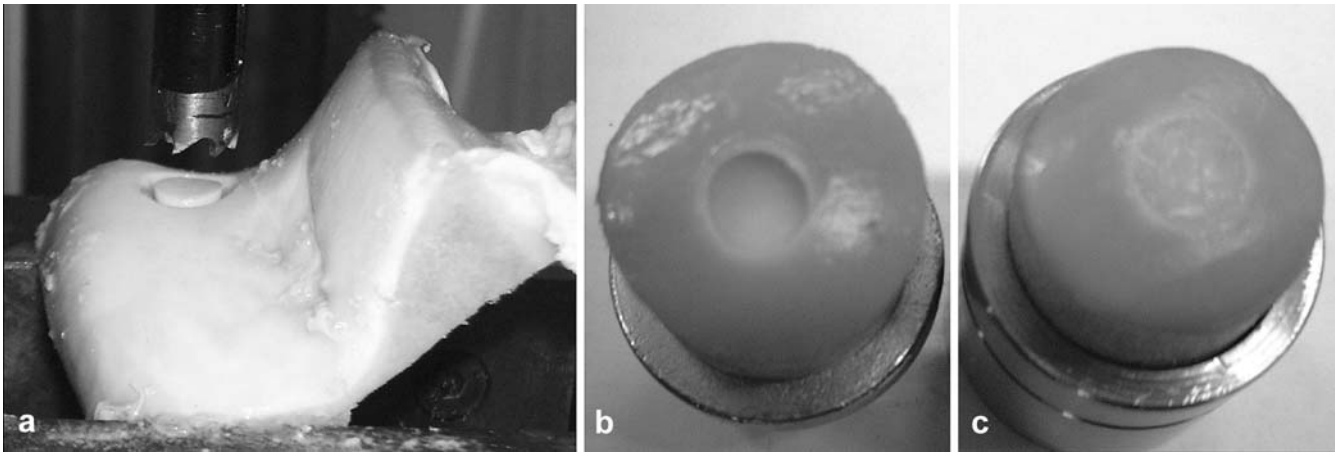

Fig. 1 Specimen preparation: **a** cylindrical specimens are obtained from the condyles of fresh bovine knees, **b** simulated 12-mm diameter full-thickness cartilage lesion, **c** the lesion filled with fibrin glue

performed with an arthroscopic approach. Various autologous cellular constructs, such as chondrocyte-seeded collagen sponges, PGA meshes and hyaluronic acid-derived fibre pads, are already commercially available and approved for implant in humans. However, the repair tissue is believed to be very fragile in the post-operative period, and unable to support the intra-articular loads. The primary failure mode using such cartilage repair techniques, as evaluated on animal models, is related to fixation [17].

Experimental methods have been extensively used to study the contact area and pressure distribution pattern in the intact knee [9, 15, 18]. A few studies have applied such methods to the optimisation of donor-site choice in autologous osteochondral grafting [2, 8, 23]. One animal study explored the biomechanical role of fibrocartilaginous repair tissue at various stages of spontaneous healing [21]. However, to date no clear analysis of the biomechanical effect of graft placement has been performed using a scaffold material.

The target of this study is the biomechanical evaluation of the effect of a full-thickness articular cartilage lesion and of treatment with a fibrin-glue graft replacement. To this purpose we developed a laboratory model simulating the contact mechanics occurring in a tibio-femoral joint, and we quantified the contact-pressure distribution at the articular surface in various configurations simulating the intact condition, the presence of a lesion and the presence of a grafted lesion. The results obtained and the limitations of the method are presented and discussed.

Materials and methods

Five fresh bovine knees were obtained at a local abattoir. For each knee, a set of two cylindrical specimens of articular cartilage with the underlying bone, of diameter 32 mm, was obtained using a cylindrical crown saw (Fig. 1a). Each set was composed of one specimen obtained from the medial condyle of the femur and one from the corresponding tibial emi-plate. To prevent tissue dehydration, specimens were kept in Dulbecco's modified Eagle Medium (Euroclone, Devon, UK) at 37° during any pause between testing campaigns. Each set of two specimens was mounted on a Mini Bionix 858 servo hydraulic axial testing machine (MTS Systems Corporation, Minneapolis, MN, USA) with the two cartilage surfaces facing (Fig. 2). A piece of Fuji Prescale pressure-sensitive film, pressure grade LLW (Fuji Photo Film Co. Ltd, New York, NY, USA), was placed between the cartilage surfaces. The anterior, posterior and lateral borders were marked on the grips of the testing machine, on the specimens and on the film. An axial load was progressively imposed, then kept constant for 30 s and progressively released, according to instructions given by the film manufacturer. The test was repeated at three levels of constant load: 100, 180 and

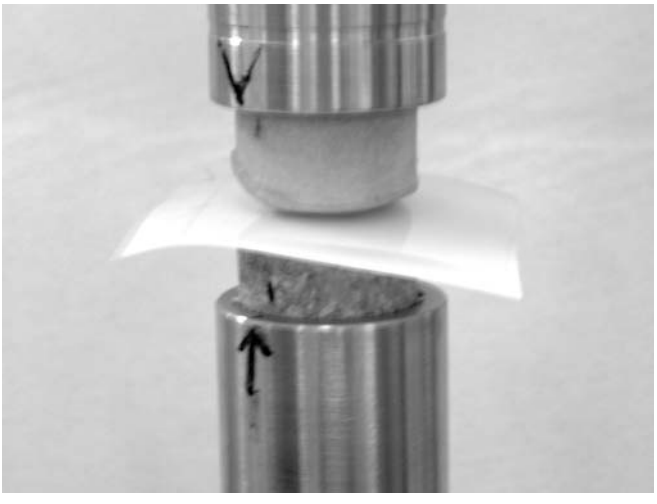

Fig. 2 The experimental set-up for contact pressure measurement

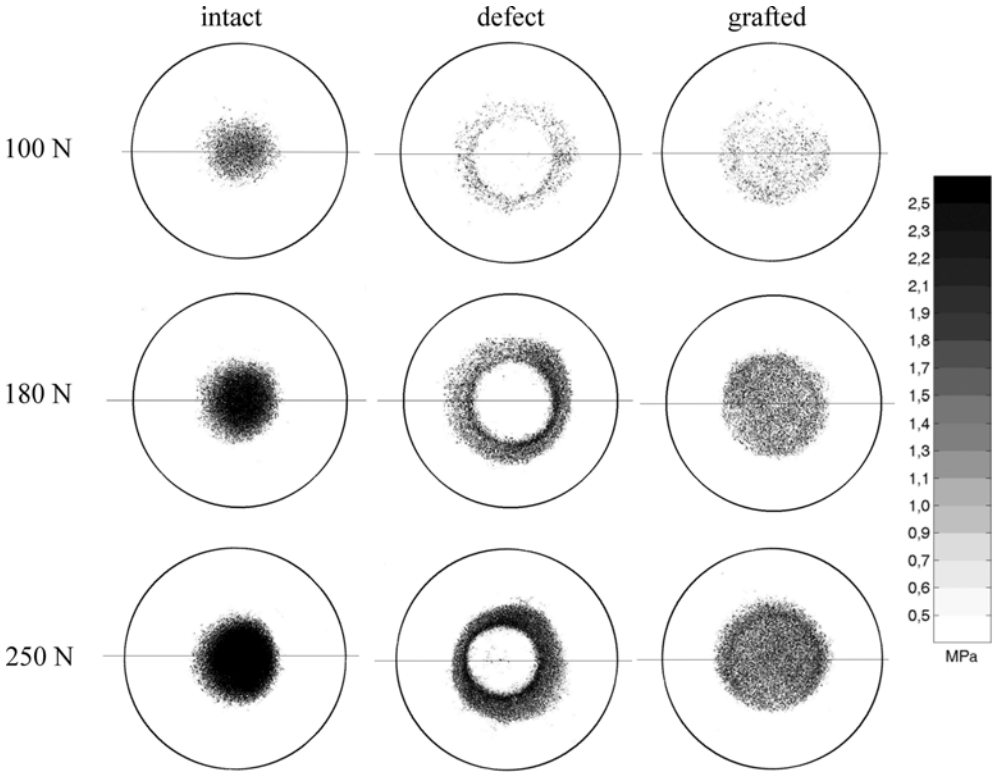

Fig. 3 Contact pressure distribution measured at increasing levels of contact load, on a set of specimens in three configurations: (*left*) intact, (*middle*) with a 12-mm diameter full-thickness cartilage defect and (*right*) with the defect grafted using fibrin glue

250 N. The grips carrying the specimens were disassembled from the testing machine, and a full-thickness chondral defect, 12 mm in diameter, was drilled on the specimen derived from the femoral condyle (Fig. 1b). Specimens were hydrated for 1 h then tested again as described above. The defect was then filled with fibrin glue (Fig. 1c) using the commercial surgical sealant Tissucol 2.0 (Baxter AG, Vienna, Austria). Specimens were again hydrated for 1 h and tested.

A digitised image of each coloured pattern developed on the film was obtained using a scanner. The images were processed using a custom-made software which permitted the transformation of the colour-intensity value of each pixel into a contact-pressure value, on the basis of a calibration curve. The calibration curves used were determined prior to each testing campaign, using flat metal specimens of known geometry which were mounted on the testing machine with an interposed piece of pressure-sensitive film. The film was axially compressed and replaced, and the test was repeated at increasing loads in order to develop colour patterns of increasing colour density.

Results

Figure 3 shows a representative contact-pressure distribution mapped on the contact surface at the three configurations tested (intact, defect and grafted) and at the three load levels tested. All the maps show stress concentrations at the rim of the defect and a more uniform stress distribution around the rim after defect grafting. A qualitative representation of the pressure variation along a diameter is plotted in Fig. 4. On cartilage, the normal intact condition generates a parabolic distribution of contact pressures. The defect generates two areas of high pressures, which, after insertion of the fibrin graft, maintain similar shapes with reduced peak values. The pressures on the fibrin graft are quasi-uniform.

Characteristic pressure values measured are given in Tables 1 and 2. At a contact load of 180 N, the peak contact pressure measured on cartilage is 2.5 MPa. On

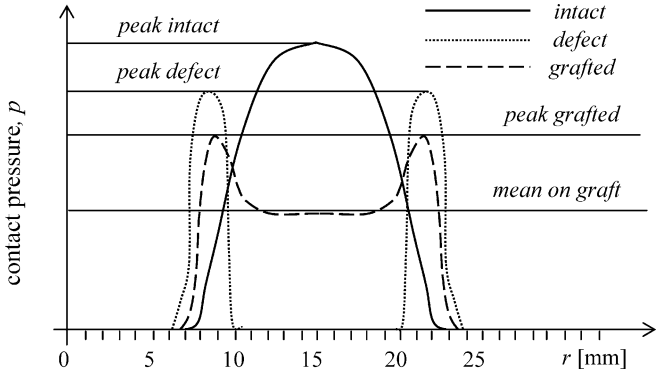

Fig. 4 Qualitative representation of the measured contact pressures along a specimen diameter

Table 1 Contact pressures measured at a contact load of 180 N

Test number	Peak p on cartilage (intact) (MPa)	Peak p on cartilage (defect) (MPa)	Peak p on cartilage (grafted) (MPa)	Mean p on fibrin graft (MPa)
1	2.40	1.75	1.30	1.05
2	2.50	2.50	1.75	1.05
3	2.50	2.25	2.15	0.50
4	2.50	2.40	2.15	0.50
5	2.50	2.30	2.10	0.55
Mean	2.48	2.24	1.89*	0.73
SD	0.04	0.29	0.37	0.29

*$p < 0.05$

Table 2 Results of the contact pressure measurements at increasing levels of contact load

Contact load F (N)	Peak p on cartilage (defect) (MPa)	Peak p on cartilage (grafted) (MPa)	Mean p on fibrin graft (MPa)	Graft contact area (mm^2)	Contact load on graft (N)	Load carried by the graft (percentage of F)
100	1.18 (0.15)[a]	0.80 (0.16)	0.60 (0.24)	131	78.5	78.5
180	2.24 (0.29)	1.89 (0.37)	0.73 (0.29)	135	141.7	78.7
250	2.33 (0.30)	1.88 (0.38)	1.45 (0.58)	141	204.4	81.8

[a]Values are given as mean (SD) of five measurements

average, the peak pressures on the cartilage area surrounding the defect are reduced by 16% in presence of the graft. Measurement results at increasing levels of contact load are shown in Table 2. In presence of the graft, the peak contact pressure on the cartilage rim is reduced at all load levels tested. In contrast, both the mean contact pressure on the graft and the graft's contact area increase. At all the load levels tested, the load carried by the graft is roughly 80% of the applied contact load.

Discussion

The target of this study was to give an estimate of the change in contact pressures occurring in the knee in three conditions: intact cartilage, in the presence of a full-thickness lesion and after lesion filling with a scaffold-type material. A 12-mm diameter defect at the inferocentral portion of the medial femoral condyle was chosen because this is a common size and location for a cartilage lesion and is weight-bearing during the stance phase of level walking [2, 16]. A 12 mm defect decreased peak contact stresses in the cartilage surrounding the defect, because the contact surface increased. Thus, in our experiments, the cartilage adjacent to the defect did not experience increased stresses, if compared to the intact condition, in accord with other studies [21]. All the maps showed stress concentrations at the rim of the defect, coherently with previous reports [8], but we could also demonstrate a more uniform stress distribution around the rim after defect grafting with fibrin glue. A repair in terms of local geometry decreased contact stresses on the cartilage because most of the peak stresses were borne by the graft.

The level of contact pressure was chosen in order to fall in the range of those obtained by other authors using pressure-sensitive film in the study of complete knee joints [9, 15, 18]. Only a general agreement may be expected with literature data in this respect, and only with data on meniscectomized knees subject to moderate loading conditions. An important limitation of our model is that the simulation results would be less reliable to study the contact mechanics at higher loading conditions, where the menisci play an important role. Furthermore, the stresses on cartilage in vivo are predominantly compressive, but the tissue must also withstand shear [4]. Compression was chosen in this study because the load sharing between graft and surrounding cartilage was the main issue. During articular sliding, compression may induce frictional shear on the graft, causing loss of the graft's lateral and basal bonding. Further work will elucidate other crucial aspects influencing the stability of the graft post-operatively , for instance the resistance to traction and shear of the grafts' lateral and basal interfaces.

Instead of considering the complete joint, only a small contact neighbourhood was studied under load. In our model, the applied loads imposed to obtain such pressure values are lower (100–250 N) than those applied in complete joint studies (500–1,000 N) [9, 15, 18], due to the fact that a small mono-condylar contact area, rather than the complete tibio-femoral interface, is considered. This simplification allowed realistic simulations of the condylar contact mechanics, while excluding many limitations associated with using pressure-sensitive

film on complete knee specimens [18], such as complex load-application systems simulating a functional range of knee motion, and also complex methods of inserting, exposing and removing the film.

The recorded pressure distribution depends on both the geometry and the material properties of the two surfaces between which the film is loaded. In the area where the specimens were sawed from the bovine condyles, the surfaces showed radii of curvature in the order of 30 mm, a value consistent with the curvature of the distal surface in human condyles [2]. The bovine knees used in this study were from animals 12 months old, which have been shown to have subchondral bone strength and elastic modulus comparable to middle-aged people [3]. In addition, adult oxen and humans have comparable cartilage thickness in the medial femoral condyle (of the order of 2 mm [2]), although the biomechanical properties of ox cartilage are superior to those of adult human cartilage, due to a higher content of sulphated glycosaminoglycan. Finite-lement models show that in a regimen of small deformations, variations in cartilage elastic modulus are similar to variations in cartilage thickness, in terms of the resulting contact pressures [5]. The average variability of cartilage thickness in human medial condyles can reach values of 30% [1]; thus a more precise mimicking of the biomechanical properties of human cartilage seemed unnecessary in relation to the comparative purpose of our study.

The standard deviations calculated for the measurements are reported in Table 1 in the last line for measurements at a contact load of 180 N and in Table 2 for measurements at all the load levels tested. The standard deviations are very low in all measurements carried out on intact cartilage (see first column in Table 1), because in this configuration the facing specimens were able to auto-align on the testing machine in the direction of the applied load. The calculated standard deviations are slightly higher for measurements carried out in presence of a defect and in the case of grafted cartilage, due to difficulties in perfectly centring the facing specimens on the testing machine in these configurations. Another source of inaccuracy of the measurements may be related to variations in the mechanical properties of different fibrin-glue grafts among different testing campaigns, probably related to slight variations in properties between different manufacturing batches. Despite all these limitations, the level of standard deviation is found to be acceptable and generally lower than those reported by others [9, 15, 21] in similar biomechanical studies, confirming that the choice of using five specimens was appropriate in relation to the biomechanical testing protocol adopted.

In our study, the levels of applied load were selected based both on the need to perform a realistic simulation of condylar contact mechanics and on the need to closely center the recorded values about the midpoint of the measurable range of the pressure transducer, i.e. 0.5–2.5 MPa. This was achieved in all measurements pertaining to the defect and the grafted configurations, whilst the pressure-sensitive film was found to be slightly saturated, i.e. the maximum threshold overcome, in the intact cartilage configuration, in some experiments, at the highest load level imposed, 250 N. In these cases the measurement was repeated using a low-grade film (LW), with range 2.5–10 MPa, which allowed us to verify that the actual pressure value in the saturation areas was 2.5 MPa on all the maps. Many limitations related to the use of pressure-sensitive film have been partially overcome in our study by processing the experimental colour patterns after a detailed calibration procedure, which was repeated prior to each testing campaign.

The main issue in our study was to obtain comparative information on contact pressures in intact cartilage, in presence of a lesion and in presence of a lesion grafted with a scaffold material for the cells to grow on. In natural cartilage, the pericellular region provides means for chondrocyte attachment to the extra-cellular matrix (ECM) and protection of cells during loading. This mechanical protection is not present in chondrocyte-seeded polymer constructs because the mechanical properties of current artificial matrices are far inferior to those of the natural one [22]. Although it would be tempting to allow early weight-bearing and a more aggressive rehabilitation in patients with a cartilage lesion treated using a solid cellular graft, compared to patients treated using autologous cells plus periosteal cover, the level of contact stresses generated by the articular load may jeopardize the viability of the graft. Cartilage damage and chondrocyte death has been reported at peak stresses as low as 4.5 MPa in bovine cartilage [20]. Loads used in our study are fairly benign, i.e. representative of the stance phase of level walking. In these conditions, the average stress measured on the fibrin graft was of the order of 1.45 MPa, a stress level well below the 4.5 MPa tolerable limit suggested by Loening et al. [20]. However, much higher loads are generated with stair ascent and descent, chair rising and more vigorous activities such as cycling and jogging. Our most significant result is that the fibrin grafts carried 80% of the contact load, at all load levels tested. This result implies that the graft contributed appreciably to weight-bearing. Assuming that the relationship between mean pressure on the graft and contact load is linear for higher loads than those used here, a contact load of 850 N would produce mean pressures above the tolerable limit.

Fibrin glue is a surgical sealant which was chosen as a grafting material in our study because it shows material properties very representative of currently-implanted cellular constructs. The elastic modulus of the commercial fibrin glue sealant used in our experiments, measured in dynamic shear, is in the order of 5 kPa [22],

a value comparable to levels reported in literature for various types of cellular constructs after few days of incubation, such as chondrocyte-seeded PGA meshes [24], collagen sponges [22] and hyaluronic acid-derived meshes [14]. The load sharing measured is therefore likely to be very representative of the load sharing occurring after graft insertion using current commercial cellular constructs. In addition, fibrin glue is often used in conjunction with the above-mentioned solid-cell carriers, as a fixing mean for the graft. In grafts obtained using traditional autologous cells plus periosteum techniques, cells are delivered to the lesion site in a liquid suspension. Contact mechanics in such a system are guided by different laws, because at the contact site the articular load would be distributed on a fluid domain, not on a solid one, and the resulting contact pressures are likely to be quite lower compared to those measured in our study. The main risk induced by high articular loads would be leakage of the cellular suspension from the periosteum suture, rather than an increase in stresses possibly harmful to the cells.

Transplantation of autologous cellular grafts obtained with tissue-engineering techniques is a procedure already clinically applied on humans and believed to be a potential alternative to conventional replacement strategies for the treatment of cartilage lesions in the knee. Our results suggest that early weight-bearing after graft placement could negatively affect cell survival and eventual repair for activities inducing higher loads on the joint.

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