Impact loading of articular cartilage during transplantation of osteochondral autograft

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Surgical reconstruction of articular surfaces by transplantation of osteochondral autografts has shown considerable promise in the treatment of focal articular lesions. During mosaicplasty, each cylindrical osteochondral graft is centred over the recipient hole and delivered by impacting the articular surface. Impact loading of articular cartilage has been associated with structural damage, loss of the viability of chondrocytes and subsequent degeneration of the articular cartilage. We have examined the relationship between single-impact loading and chondrocyte death for the specific confined-compression boundary conditions of mosaicplasty and the effect of repetitive impact loading which occurs during implantation of the graft on the resulting viability of the chondrocytes.

Fresh bovine and porcine femoral condyles were used in this experiment. The percentage of chondrocyte death was found to vary logarithmically with single-impact energy and was predicted more strongly by the mean force of the impact rather than by the number of impacts required during placement of the graft. The significance of these results in regard to the surgical technique and design features of instruments for osteochondral transplantation is discussed.

Articular cartilage has a limited capacity for repair. Several methods of treatment have been used for lesions of the articular surface, but none has shown consistent long-term success. Surgical reconstruction of the articular surface by transplantation of osteochondral autograft has often been performed with considerable success for the treatment of focal articular lesions of the knee and ankle. While concerns regarding the lateral integration of transplanted cartilage and the morbidity of the donor site are commonly cited, operations involving transplantation of osteochondral autografts are commonly performed.

Osteochondral mosaicplasty is designed to repair focal lesions of an articular surface using cylindrical osteochondral grafts harvested from regions of the knee which bear a low load. The use of several smaller grafts rather than a single large one is intended to minimise adverse effects at the donor site(s) and to improve the ability to achieve congruency of the articular surface at the site of repair. The initial fixation of the graft is generally provided by a press-fit obtained by inserting each graft into a predrilled hole of slightly smaller diameter. During insertion, each graft is centred over the recipient hole and tapped into place by impacting a tamp which is in contact with the articular surface.

Impact loading of articular cartilage has commonly been associated with structural damage, loss of viability and changes in the metabolism of chondrocytes with subsequent degeneration of the articular cartilage. The methods used to assess injury to the cartilage from impact loading have included histological assessment of the structural integrity, measurement of tissue swelling related to disruption of collagen fibrils, assessment of death or apoptosis of chondrocytes and release of cartilage macromolecular constituents during subsequent tissue culture. In general, the differences in the extent of injury associated with a given magnitude of impact load have been attributed to changes in the strain or loading rate, the presence or absence of subchondral bone and to boundary conditions of the impacted cartilage.

We have examined the relationship between impact loading and the death of chondrocytes which occurs within the articular surface of autologous osteochondral grafts because of the process of implantation during mosaicplasty. We investigated the association between impact loading and chondrocyte death for a single impact applied to the articular surface of an autologous osteochondral graft under the
same conditions as in mosaicplasty. We also measured the loads imparted to the articular surface of autologous osteochondral grafts during transplantation of the graft using three different commercially-available instruments and assessed their relationship with the resulting viability of the cells of the cartilage layer.

**Materials and Methods**

**Single impact.** Five fresh bovine knees from different animals (approximately two years old) were collected on the same day from a local abattoir. After disarticulation, 14 cylindrical osteochondral plugs were created in each knee under continuous irrigation with phosphate-buffered saline using a hollow reamer (of 4.5 mm inner diameter) and left in situ. A defined impact at one of seven different levels of energy (Table I) was applied to the articular surface of seven randomly-chosen test plugs from each knee by impacting the plunger with a custom-made instrument (Fig. 1). The impacts were administered to the surface of the cartilage in a confined compression configuration as occurs during the surgical procedure. These plugs were then removed from the condyles for analysis of cell viability and structural damage. A control plug which had not been impacted was harvested directly adjacent to the donor site of each impacted plug. All the osteochondral plugs were then placed in phosphate-buffered saline embedded in agar (3% aquades; Fluka, Buchs, Switzerland) and sectioned with a tissue slicer (Sorvall TC-Z, Newtown, Connecticut) for analysis of the cells. Slides of 200 µm were incubated for one hour in calcein AM/ethidium homodimer-1 (Molecular Probes, Eugene, Oregon) and analysed by confocal microscopy (Zeiss 400, Zeiss GmbH, Jena, Germany). Calcein AM is a fluorogenic esterase substrate which can be passively loaded into cells. Once inside the cell, non-fluorescent calcein AM was converted by non-specific intracellular esterases into fluorescent calcein. Thus, green fluorescence was an indicator of cells which had esterase activity as well as an intact membrane to retain esterase products. Ethidium homodimer-1 strongly binds to dsDNA, ssDNA, RNA, and oligonucleotides to produce a red fluorescent enhancement. Because ethidium homodimer-1 will not permeate an intact cell membrane it serves as a very potent marker for cells with a compromised membrane (e.g. dead cells). The depth of cell death was measured as the distance from the articular surface to the line of demarcation between live and dead cells expressed as a percentage of the full thickness of the cartilage and was obtained from the mean of three evenly-spaced measurements taken on a centrally-located slide from each plug.

The remaining tissue from each plug was fixed with 4% buffered formalin and evaluated histologically for structural damage, using Masson-trichrome, Safranin-O, and Alcian Blue stains. Control specimens were subjected to the

<table>
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<th>Impact energy (mJ)</th>
<th>Peak impact force (N)</th>
<th>Peak impact stress (MPa)</th>
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<td>15</td>
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<td>1309.09</td>
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same tissue preparation. For each control which exhibited more than 2% to 3% of dead cells we excluded the corresponding impact-loaded sample from the analysis, in order to avoid assessment of areas which might have been damaged before the experiment. Accordingly, of the 35 paired specimens, eight were excluded from analysis.

**Statistical analysis.** Observation of scatterplots suggested a logarithmic relationship between the percentage depth of cell death and the energy of the impact. A linear regression was therefore performed between the percentage depth of cell death and the logarithm of impact energy using NCSS 2.0 statistical software (NCSS, Kaysville, Utah). The significance of the hypothesis that the slope of the regression line was zero was tested for $p < 0.05$.

**Multiple impacts during delivery of osteochondral autograft.** A custom-made surgical hammer was fitted with a uniaxial accelerometer (B&K 4366; BK Precision, Yorba Linda, California) and a piezoelectric load cell (PCB 203B; PCB Piezotronics, New York, New York) in order to characterise impact loads during implantation of the graft (Fig. 2). Since the transition stiffness between the impact hammer and the stainless-steel plunger was assumed to be much larger than that between the plunger and the osteochondral plus ($C_{\text{HP}} \gg C_{\text{PP}}$), the acceleration of the plunger was approximately that of the impact hammer ($a_p \approx a_H$) while the two were in contact. Accordingly, the impact force delivered to the layer of cartilage of the osteochondral plug ($F_{\text{plug}}$) could be corrected for the inertial effects of different plunger masses associated with each instrument using the formula:

$$F_{\text{plug}} = F_H \cdot a_H \cdot M_p$$

where $F_H$ is the impacted force experienced by the force transducer of the impact hammer, $a_H$ is the tangential acceleration of the impact hammer during contact with the plunger and $M_p$ is the mass of the plunger.

Five fresh porcine (approximately six months old) and five fresh bovine (approximately 2 years old) knees were disarticulated and were constantly irrigated with phosphate-buffered saline during the procedure. For each knee, one autologous osteochondral graft was transplanted into a random location within the central region of the medial femoral condyle with each of three commercially-available instruments, giving a total of 15 transplantations in the bovine and 15 in the porcine knees. Recipient holes were drilled with each instrument set (outside diameter approximately 4.5 mm). The corresponding hollow reamer (inside diameter approximately 4.6 mm) was used to create cylindrical autologous osteochondral grafts. These were then harvested housed in the hollow cylinder associated with each instrument set, and implanted using the standard surgical procedure by centreing the cylinder over the recipient site and gently tapping a tamp with the instrumented hammer in order to insert the graft. To assess viability of the chondrocytes a vertical slide of thickness of approximately 1 mm was cut manually with a razor blade from the middle of the plug, perpendicular to the articular surface. Analysis of the viability of the cells was then carried out as described for the single-impact specimens. Force data were imported into Matlab 6.1 (The Mathworks Inc., Natick, Massachusetts) for examination of each impact trial, the calculation of mean and maximal impact forces within each trial, and provision of graphical output.
Statistical analysis. Multiple regression analyses were performed using NCSS 2.0 statistical software in order to assess the prediction of percentage cell death in the cartilage layer by measurement of the number of impacts and the mean impact force, as measured by the sum of the impact force for each hit divided by the number of hits required to insert the plug. The maximum impact force was not included in the regression analysis because of collinearity with the mean impact force. Three bovine specimens and two porcine specimens were excluded from the regression model because of 100% cell death.

Results

Single impact. Of the 27 specimens included in the single-impact analysis, all the associated control plugs appeared to have normal cell viability, with less than 2% to 3% of dead cells and a normal structure of the hyaline cartilage. A logarithmic relationship (Fig. 3) between single-impact energy and the depth of cell death as a percentage of the thickness of the cartilage was observed. The assumption of constant residual variance was verified by the modified Levene test and the normality of the residuals by the Shapiro-Wilk test. Although several data points were collected from the same animal, we have assumed independence of the criterion measures because of the variability of the mechanical properties of cartilage and subchondral bone within a joint. The value of R-squared, the proportion of the variation in the depth of cell death which can be accounted for by variation in the energy of impact, was 0.92 while the correlation between the depth of cell death and the energy of impact was 0.96. A significance test of the hypothesis that the resultant slope was zero was rejected ($t = 4.8420, p < 0.001$). The estimated slope of the regression was 0.18. The lower limit of the 95% confidence interval (CI) for the slope was 0.16 and the upper limit 0.19. The estimated intercept was -0.43. The lower limit of the 95% CI for the intercept was -0.08 and the upper limit 0.11.

Figure 4a – Oblique fissures demonstrate failure of the articular surface with impact loading (Massons Trichrome, x10). Figure 4b – Chondrocyte death (dark red) is concentrated around a fissure in the articular surface (live/dead stained specimen (Calcein AM/Ethidium homodimer-1, x10)). Figure 4c – Cell death was consistently observed to occur to a given depth and was measured as the distance from the articular surface to the line of demarcation (dashed line) between dead (dark red) and live (bright green) cells (Calcein AM/Ethidium homodimer-1, x10).
Fissures in the matrix which were orientated approximately 45° to the surface of the cartilage, were observed within the impacted region in several high-impact-energy specimens (Fig. 4). A greater concentration of cell death was observed consistently in regions where the structure of the matrix was damaged. At the sites of highest impact, some superficial fissures extended parallel to the surface such that pieces of cartilage were almost detached from the underlying tissue.

**Multiple impact.** Under the conditions of multiple impacts during implantation of the graft in mosaicplasty, disruption of the matrix and extensive cell death occurred throughout the full depth of the layer of cartilage whenever a graft made contact with the bottom of the recipient bed or ‘bottomed out’. The maximum value of the force of the impact associated with a graft ‘bottoming out’ was an order of magnitude larger than other forces within the same trial and was clearly distinguishable from impacts which were associated with translation of the graft during implantation (Fig. 5). In order to avoid violating the assumptions of equal variance and normal distribution of residuals in the regression models, specimens from these trials were excluded from further analysis because of a complete loss of sensitivity of the criterion measure to changes in the predictor variables. As a result, three trials were removed from the bovine regression model and two from the porcine regression model. Descriptive statistics for the number of impacts, maximum impact force, and mean impact force which occurred during implantation of the graft are shown in Table II.

Multiple linear regression was used to assess the importance of the mean impact force and the number of hits recorded to implant an osteochondral plug for the prediction of the percentage depth of cell death. Both the bovine and porcine regression models passed tests for normality (skewness, kurtosis, omnibus tests) at a threshold level of 5%, and displayed no obvious homoscedacity (degree of uncorrelated equal variance) in the scatterplots. The regression coefficients and basic descriptive statistics for each predictor are shown in Table III. For both regression models, the hypothesis that all regression coefficients were not different from 0 was rejected indicating that both the bovine (p = 0.002) and porcine (p < 0.001) models were significant predictors of the depth of cell death. An adjusted multiple correlation statistic \( R^2_{\text{adj}} \) of 0.625 for the bovine and 0.935 for the porcine model indicated that the linear regression model was able to account for 63% of the variance in the depth of cell death in the bovine model and 94% of the variance in the porcine model.

**Discussion**

Confocal microscopic examination allowed detection of cell death from a single impact in samples without histo-

![Figure 5a](image1.png)  
Figure 5a – Typical force profile of multiple impacts associated with implantation of a mosaicplasty graft. Figure 5b – Profile of implantation of a mosaicplasty graft indicating a sudden increase in impact force indicating contact between the graft and the bottom of the recipient hole.

![Figure 5b](image2.png)
logical evidence of matrix disruption. As a result, considerable damage was observable at a cellular level, even with low-energy trauma. Spatially, cell death was consistently observed to occur from the cartilage surface downwards and in regions surrounding structural failure, such as fissures in the surface of the cartilage or at the cut edges of the graft. The oblique orientation of the fissures and delamination of the superficial layer of cartilage observed after high impact is consistent with the observations of other authors. Our results demonstrate further that measures of cell viability are sensitive to changes in impact loading of articular cartilage in the confined compression configuration of autologous osteochondral transplantation. However, the absolute magnitude of these results should be interpreted with caution since samples of young bovine cartilage have different mechanical properties from those of adult human articular tissue.

During implantation of the graft the force produced between the graft and the impact hammer was limited by the press-fit strength of the graft-recipient interface. When the force reaches the threshold capable of being supported by the press-fit, the graft translates within the recipient hole. Contact between the bottom of a graft and the base of the recipient hole creates reaction forces much larger than those limited by the press-fit. The forces observed in these trials were of an order of magnitude larger than those in trials in which the plug did not contact the bottom of the recipient site. In all the trials involving both porcine and bovine specimens, extensive cell death and damage to the matrix were observed when the plug contacted the bottom of the recipient site. As a result, the criterion of cell death was no longer sensitive to changes in impact force, and such trials were therefore removed from the regression model. For trials in which the graft did not 'bottom out', significant regression models indicated a strong linear relationship allowing prediction of the depth of cell death. The mean impact force delivered to the graft during a trial proved to be the only significant predictor for the bovine and porcine regression models. The positive regression coefficient for mean impact force indicated that, by reducing the mean impact force, the amount of cell death could be reduced. The number of impacts was not a significant predictor.

Both bovine and porcine models were used in our study in order to investigate impact-related cell death in articular cartilage with different mechanical properties. Since porcine tissue has a higher proteoglycan content and a less organised arrangement of the collagen fibrils than bovine cartilage, it is not surprising that it demonstrates less cell death than bovine tissue for impacts delivered in a confined compression configuration. This relationship persisted regardless of the higher maximum and mean impact force delivered in implantation of the porcine grafts. The regression models demonstrated that mean impact force was a significant and positive predictor of the death of chondrocytes during mosaicplasty with cartilage from different species.

In order to minimise chondrocyte death these observations suggest important considerations for the technique of mosaicplasty and the design of instruments as follows:

1) A recipient bed which is slightly deeper than the length of the plug prevents bottoming out of the graft from disrupting the ability to achieve congruency with the surrounding articular surface and serves to limit the magnitude of the force applied to the layer of cartilage in a graft during implantation, resulting in a more viable construct.

2) When the plug did not contact the bottom of the recipient bed, we observed a direct relationship between the percentage of chondrocyte death and the mean impact force. Since the number of impacts was not a significant predictor of cell death, we recommend delivering a greater number of lighter blows to implant a graft and preserve the viability of the chondrocytes rather than using a smaller number of heavier impacts.

3) The instruments used should be designed to reduce the force applied to a mosaicplasty graft in order to increase the potential for preservation of viability. The use of a lighter hammer head may reduce the momentum of its use leading to a lower transference of forces to the graft during implantation.

Other factors which influence the force required to deliver the graft include the material and structural properties of subchondral bone, the implantation of adjacent grafts, since the procedure generally involves several grafts implanted close together, and the geometry of the press-fit. While a reduction in press-fit interference will reduce the forces required to implant a graft, tight contact at the level of the bone is important in order to avoid intrusion of synovial fluid into the bone-marrow space which may lead to the formation of synovial cysts and concomitant bone resorption. Also, the load-bearing capacity of the initial

<table>
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<th>Predictor</th>
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<th>95% CI</th>
<th>p value</th>
<th>Standardised coefficient</th>
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<td>Mean impact force</td>
<td>0.083</td>
<td>0.020</td>
<td>0.038 to 0.129</td>
<td>0.002*</td>
<td>1.005</td>
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<td>0.733</td>
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<td>0.213</td>
<td>0.325</td>
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<td><strong>Porcine model</strong></td>
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<td>0.029 to 0.042</td>
<td>&lt; 0.001*</td>
<td>1.018</td>
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<tr>
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<td>0.103</td>
<td>-0.083 to 0.374</td>
<td>0.187</td>
<td>0.116</td>
</tr>
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* significant, p < 0.05
graft and the maintenance of the congruency of the surgically-established articular surface are primarily dependent on the strength of fixation provided by the press-fit.

Cell death by necrosis and/or apoptosis has been implicated as a possible cause of osteoarthritis. Since normal articular cartilage is avascular and has no nerve supply, any repair response to damage to the internal matrix, for example denaturation of collagen or release of proteoglycans, is probably dependent on the metabolism of chondrocytes and, therefore, on their viability. Sufficient synthesis of cartilage proteins during the normal turnover of the matrix is necessarily dependent on cell viability. Indeed, changes in glycosaminoglycan content and cell death have been associated with repeated impact on articular surfaces. Intercellular signalling has also been implicated in the propagation of cell death from damaged to healthy tissue within the joint. 

The presence of cell death does not necessarily preclude osteochondral transplantation. The possibility of repopulation by chondrocytes, or migration of stem cells originating either from the synovial membrane or the underlying bone, have been suggested as mechanisms of repair. Different forms of chondroprotection may also play a role in preventing, or at least minimising, the spread of cell death to healthy areas of the joint. Transfection of chondrocytes to prevent apoptosis and tissue degradation has been demonstrated, however, an expensive and complicated procedure with ready access to specialised laboratory facilities would be required in clinical use. A more practical approach may be to target metabolic processes which lead to apoptosis or necrosis, such as caspase activity, by intra-articular injection.

We conclude that the act of impacting osteochondral grafts during mosaicplasty can produce significant cell death in the cartilage layer of the grafts. Although pharmaceutical interventions may assist in preventing the progression of cell death, the level of cell death in the transplanted grafts can be minimised by the adoption of a surgical technique which prevents ‘bottoming out’ of the graft and favours a higher number of lighter impacts rather than a few powerful blows during placement. Designs of surgical instruments which serve to reduce the force required to translate the graft into the recipient site for a given press-fit may also help to minimise chondrocyte death.

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References