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CHONDROCYTE DEATH AFTER DRILLING AND ARTICULAR SCREW INSERTION IN A BOVINE MODEL.

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ABSTRACT

Objective

Intra-articular screws are used for internal fixation of osteochondral fragments after fracture or osteochondritis dissecans. This causes cartilage injury potentially leading to chondrocyte death. We have visualised/quantified the hole and zone of cell death (ZCD) in cartilage after drilling/insertion of various articular screws.

Method

Using an *ex vivo* bovine model with transmitted light and confocal laser scanning microscopy (CLSM), the holes and ZCD following drilling/insertion of articular screws (cortical screw, headless variable pitch metallic screw, headless variable pitch bioabsorbable screw) were evaluated. *In situ* chondrocyte death was determined by live/dead cell viability assay. An imaging/quantification protocol was developed to compare hole diameter and ZCD from drilling/insertion of screws into cartilage. The effect of saline irrigation during drilling on the ZCD was also quantified.

Results

Screw insertion created holes in cartilage that were significantly ($p \leq 0.001$) less than the diameters of the equipment used. With a 1.5mm drill, a ZCD of $580.2 \pm 124 \mu\text{m}$ was produced which increased to $637.0 \pm 44 \mu\text{m}$ following insertion of a 2mm cortical screw although this was not significant ($p > 0.05$). The ZCD from insertion of the variable pitch headless screws (diam. 3.5mm) was lower for the metallic compared to the bioabsorbable design (800.9 ± 159 vs. $1236.4 \pm 212 \mu\text{m}$, respectively; $p < 0.01$). The ZCD from drilling was reduced ~50% ($p < 0.001$) by saline irrigation.

Conclusions

Cartilage injury during intra-articular screw fixation caused a ZCD around the hole irrespective of screw design. Saline irrigation significantly reduced the ZCD from drilling into cartilage.

INTRODUCTION

Intra-articular screws are commonly used for internal fixation of osteochondral fragments in fractures of different bones (e.g. scaphoid, radial head, capitellum, distal femur, talus¹). They are also used for fixation of osteochondral fragments in chronic joint disorders e.g. osteochondritis dissecans². Cortical and variable pitch headless screws have been used extensively for fragment fixation¹⁻⁵. Recently, bioabsorbable systems (screws, nails, tacks, pins) have been developed which do not require a second surgical intervention for removal⁶⁻⁸. The surgical goals are to achieve stable fixation between the fragment/lesion bed, restore articular congruity and preserve articular cartilage viability. The clinical goals are to alleviate pain, return patients to normal function and ultimately, prevent cartilage degeneration. However, mixed results have been obtained for internal fixation e.g. unsatisfactory results have been reported in approximately 30% of patients after internal fixation for osteochondritis dissecans^{2-4,6,7}.

There are several challenges to achieving a balance between the surgical goals of stable fixation, restoration of the articular surface and preservation of articular cartilage viability. Firstly, there is considerable heterogeneity in the presentation and complexity of articular pathology amenable to internal fixation. Poor results may occur even with stable fixation and restoration of the articular surface because the severity of the initial cartilage injury prevents subsequent repair as the tissue has poor intrinsic reparative potential⁹⁻¹⁴. Secondly, even if the articular cartilage on the fragment is viable, lateral integration of the articular cartilage needs to occur at the interface between the fragment and native tissue^{9,12,13}. Chondrocyte viability at this injured interface is poor and lateral integration often occurs by fibrocartilage formation¹²⁻¹⁵. Any residual incongruity at the articular surface may contribute to progressive cartilage degeneration¹⁶. Finally, loss of fixation from hardware failure results in a poorer prognosis as any fragment loosening or detachment is unlikely to heal anatomically due to loss of vascularity and interposed fibrous tissue².

The use of variable pitch screws and interfragmentary lag screw techniques designed to achieve compression between the fragment and host bone improves fixation stability and decreases the risk of fragment detachment¹⁻³. However, screw insertion into the articular fragment invariably results in articular cartilage injury. Although cartilage has an extraordinary capacity to withstand physiological mechanical loads, its ability to bear mechanical injury from iatrogenic surgical interventions is poor. For example, chondrocyte death and changes to matrix properties occur at wound margins following injury caused by a trephine⁹, scalpel cut¹⁰, surgical suturing¹¹, and partial thickness defects¹². The nature and extent of chondrocyte death in articular cartilage following insertion of an intra-articular screw has not been characterised. Although the natural history of cartilage defects created from insertion of the screw is unknown, focal tissue defects have the potential to progress and contribute to cartilage degeneration¹⁴. If an intra-articular screw is used for stable fixation of an osteochondral fragment, an appreciation of the extent of the associated articular cartilage injury sustained during the procedure would help surgeons develop techniques that minimise chondrocyte death and optimise the survival of the restored articular surface.

The aim of this study was to visualise and quantify the hole and zone of cell death (ZCD) produced in articular cartilage after drilling and insertion of various articular screws. We have utilised transmitted light images and confocal scanning laser microscopy (CLSM) to compare the holes and ZCD caused by a standard orthopaedic drill, with that following insertion of a cortical screw, a metallic headless screw and a bioabsorbable headless screw.

MATERIALS AND METHODS

Biochemicals and solutions. Biochemicals, including fluorescent indicators 5-chloromethylfluorescein-diacetate (CMFDA) and propidium iodide (PI), were obtained from Invitrogen Ltd. (Paisley, U.K.) unless otherwise stated. Dulbecco's Modified Eagle's Medium (DMEM; 340mOsm/KgH₂O; pH 7.4) was used for osteochondral explant culture. Saline (0.9% w/v) was from Baxter's Healthcare (Thetford, U.K.) and para-formaldehyde (4% v/v in saline; pH 7.3) from Fisher Scientific (Loughborough, U.K.).

Orthopaedic equipment. Details of the drills and screws are summarised in Table 1. Standard 1.5mm drills (Ortho Solutions Ltd., Essex, U.K.) were used either alone, or to prepare holes for the self-tapping cortical screws (2.0mm; Synthes, Hertfordshire, U.K.; Table 1, Fig. 1). Holes for the variable-pitch metallic (Acutrak) screws were prepared using the Acutrak long drill, whereas for the bioabsorbable (Biotrak) screws, the Biotrak tapered long drill was used with the necessary equipment for insertion by Acumed (Table 1, Fig. 1). Drill bits were coupled to a precision drill (18,000rpm; Radio Spares, Northamptonshire, U.K.). For some experiments, saline irrigation was used for cooling while drilling.

Bovine model and osteochondral explants. Metacarpophalangeal joints of three-year-old cows from a local abattoir, were washed, skinned and de-hoofed within 24hrs of slaughter, and joints opened and secured in a vice. Typically, the 1.5mm drill was placed firmly on the first condyle, drilled to a depth of 25mm and removed (Fig. 1). This was completed within 1-2secs, and was associated with a rise in temperature (see below). For the second condyle, the 1.5mm guide hole was prepared identically, and a self-tapping cortical screw (2mm diam; 20mm length) inserted. The screw was driven firmly onto the cartilage surface using a flat-headed screwdriver. Due to the screw head, the screw could not be inserted below the cartilage surface and therefore it had to be removed

before osteochondral strips could be harvested. For the third condyle, a metallic headless screw (Acutrak, head diam. 3.5mm; tip diam. 3.0mm; length 20mm) was inserted using an abbreviated version of the manufacturer's guidelines. The Acutrak long drill bit (3.5mm diam.) was placed firmly on the cartilage surface and drilled to approximately 25mm and removed. This was completed within 3-5secs, and was also associated with a rise in temperature (see below). The Acutrak handle was coupled with the hexagonal driver and the screw driven to the full depth so the head was recessed by 5mm below the cartilage surface. For the fourth condyle, a headless bioabsorbable screw (Biotrak, head diam. 3.5mm; tip diam. 3.0mm; length 20mm) was inserted into a guide hole prepared with the 3.5mm Biotrak mini drill bit, and subsequently threaded with the Biotrak mini tap to the full depth. The bioabsorbable screw was then inserted using the mini driver to the full depth so the head was recessed by 5mm below the cartilage surface, and the mini ejector used to disengage the driver from the screw upon completion.

Drilling was carried out perpendicular to the cartilage surface with DMEM used to keep cartilage moist and reduce deleterious effects of cartilage drying on chondrocyte viability¹⁷. Despite these measures, the drills and cartilage/bone were hot after drilling pilot holes. Preliminary measurements using a thermal imaging camera suggested that the surface temperature of the cartilage/bone increased to approx. $220\pm 20^{\circ}\text{C}$ immediately after drilling with the 1.5mm drill bit to a depth of 25mm, but decayed rapidly after removal from the hole to room temperature after approx. 5secs. Copious irrigation during drilling using saline (0.9%; 18°C) markedly reduced the peak temperature to $130\pm 40^{\circ}\text{C}$, with the temperature returning to initial values after approx. 2 secs. (Data are means \pm s.d.; for 5 separate joints). A greater rise in temperature, with a slower decay was noted when drilling with the 3.5mm drills. After drilling and screw insertion/removal, osteochondral strips containing the holes (with the full thickness of articular cartilage attached to 1-2mm of subchondral bone) were harvested from the joint using a chisel¹⁰. The strips were placed in DMEM (37°C ; 5% CO_2 ; pH7.4; 10mins) and the edges trimmed with a scalpel to produce osteochondral explants measuring approximately 6mm x 6mm.

Cell viability assay and fixation. CMFDA and PI were used to assess the extent of *in situ* chondrocyte death around the drill/screw holes in articular cartilage¹⁰. CMFDA is membrane-permeant, and once inside intact cells, reacts with intracellular components to produce an intense green fluorescence indicating a living cell. PI is impermeant, only binds to DNA if the plasma membrane is disrupted and is therefore indicates dead cells. Osteochondral explants were then incubated in DMEM containing CMFDA and PI (10 μ M each; 60mins; 37°C). Explants were rinsed in fresh medium and immersed in para-formaldehyde (4% v/v; 30mins; 21°C) for tissue fixation. Samples were then rinsed and finally immersed in phosphate-buffered saline (PBS), and visualised by CLSM.

Confocal laser scanning microscopy (CLSM). CLSM rejects out-of-focus light and can image thin 'optical sections' within the depth (i.e. z-axis) of cartilage¹³. An upright Zeiss Axioskop LSM 510 (Carl Zeiss Ltd, Welwyn Garden City, U.K.) with a x10 Plan Neofluar objective lens was used to image *in situ* chondrocytes around the drill/screw holes on explants. Excitation of CMFDA and PI, was achieved utilising Argon (EX λ =488nm) and Helium-Neon (EX λ =543nm) lasers respectively. Upon excitation, CMFDA emits light at EM λ =520nm (green) and PI emits light at EM λ =600nm (red) which were captured using 500-550nm band pass and >560nm long-pass filters respectively¹⁰. After optimising image quality, 20 sequential axial optical sections were taken starting from the cartilage surface at intervals of 10 μ m in the z-axis. The images therefore primarily represent chondrocytes within the superficial zone of cartilage.

Quantification of hole diameter from transmitted light images. Images were acquired using a transmission detector on the CLSM which was placed in the transmitted light path of the microscope. This allowed detection of light (from a halogen bulb) which was transmitted through an osteochondral explant. The resulting images (x5 objective) consisted of a white region (the hole,

i.e. where light was transmitted fully) and a dark border (the cartilage, which blocked light). Due to the shape of the hole and mechanism of trauma, initial CLSM images showed a poorly-defined edge. Using the Zeiss LSM image browser software (Carl Zeiss Ltd, Welwyn Garden City, U.K.) the contrast of the images was increased to 100%. This created a clear black/white image with a definite hole edge (Fig. 2). Lines were overlaid which bisected the image horizontally and vertically. Where these crossed, two 45° lines were overlaid. A mark was placed where these four lines touched the hole edge and the diameters measured (Fig. 2). An average of the four lines and yielded a value for the hole diameter.

Quantification of the zone of cell death (ZCD). The consecutive series of optical sections (200µm z-stack comprising 20x10 µm thick optical sections) acquired during CLSM were overlaid using the Zeiss LSM image browser to create 'CLSM reconstructions' representing the imaged cartilage volume. A standardised method was devised for the quantification of the ZCD from the CLSM reconstructions (Fig. 2). Reference lines, originating at a defined central point which was 200µm from the first dead (red) cell, identified on the 90° line, were overlaid at 45°, 65°, 90°, 115° and 135° (Fig. 2). A 50µm² region of interest (ROI) was overlaid at the live/dead cell border at each of these positions. Manual live/dead cell counts were performed within this ROI, which was then gradually moved outwards until the dead cell fraction comprised <50% of the total cell count. A line parallel to the reference line was then overlaid measuring the distance from the last dead cell within the ROI to the first cell along the reference line at the drilled edge. An average of the five measurements was taken. At x10 magnification, the maximum CLSM image area was 1300µmx1300µm, and because of this and the extent of the ZCD, the measurements from two images i.e. the left and right sides of the hole, were taken and averaged.

Statistical analysis. Data are presented as means with 95% confidence interval (CI) shown, with *n* as the number of different animals used. Statistical tests were performed using SigmaStat (Systat

Software Inc., San Jose, U.S.A.). Paired, two-tailed, Student's *t*-tests were used to compare observations between paired data sets, ANOVA for one-way comparisons between groups, and the post-hoc Holm-Sidak method for all pair-wise multiple comparisons. A significant difference was indicated when $p \leq 0.05$.

RESULTS

Qualitative assessment of drill and screw holes. Transmitted light images of the drill/screw holes gave an overview of the cartilage injury (Fig. 2). The 1.5mm drill bit consistently produced relatively smooth hole edges, with occasional minor irregularities. The appearance of cartilage following insertion/removal of the cortical screw into the 1.5mm pilot hole was, however, different (Fig. 2). The hole edges were markedly irregular both in terms of shape and degree of loose cartilage material, which was probably produced by the screw threads. Additionally, occasional cartilage splitting at the hole edge was observed both by transmitted light and CLSM (Fig. 2). Although the metallic headless screw (Acutrak) produced reasonably uniform holes, there was screw thread damage in the ZCD, but less marked compared to the cortical screw (Fig. 2). The hole produced by the bioabsorbable screw (Biotrak) was similar to the metallic headless screw but the hole edges demonstrated larger irregularities related to injury from either the tap or screw.

Hole diameter. This was determined using the 100% contrast transmitted light images to compare with the diameter of the equipment used to create the holes (Fig. 2). Holes produced by the 1.5mm drill were smaller than the drill diameter although this was not significant (Fig. 3). However, the holes produced by the cortical screw, metallic headless screw and bioabsorbable screw were significantly ($p \leq 0.001$) smaller than the diameter of the screw inserted (Fig. 3) suggesting a 'swelling' of the damaged extracellular matrix back into the hole.

ZCD. Quantitative CLSM measurements from fluorescently-labelled *in situ* living/dead chondrocytes demonstrated a ZCD with a 1.5mm drill measuring $580.2 \pm 124 \mu\text{m}$ ($n=6$, Fig. 4). This increased by approximately 10% to $637.0 \pm 44 \mu\text{m}$ ($n=4$) following insertion/removal of a 2mm cortical screw but this was not significant ($p > 0.05$). Although there was a 26% increase in the ZCD between the 2mm cortical screw and the 3.5mm Acutrak screw ($800.9 \pm 159 \mu\text{m}$; $n=6$), this was also

not significant ($p>0.05$). The ZCD from insertion of the headless bioabsorbable screw (Biotrak) of identical dimensions to its metallic design, was $1236.4\pm 212\mu\text{m}$ ($n=6$) and this was significantly greater than the ZCD of the Acutrak and the Biotrak screws ($p<0.001, p<0.01$ respectively; post-hoc Holm-Sidak tests; Fig. 4). There was a significant increase (one-way ANOVA; $p<0.001$) in the extent of the ZCD in the order: 1.5mm drill, cortical screw, Acutrak screw, Biotrak screw.

Comparison of the ZCD with or without saline irrigation. The same techniques and quantification methods were used to determine whether or not saline irrigation whilst drilling with the 1.5mm drill reduced the ZCD. CLSM reconstructions showed a clear decrease in the extent of cell death and quantification of the images demonstrated a significant reduction (approximately 50%) with saline irrigation compared to drilling without irrigation ($204.6\pm 132\mu\text{m}$; $455.0\pm 124\mu\text{m}$ respectively; $p<0.001, n=7$; Fig. 5).

DISCUSSION

This study described a novel method using CLSM for the quantification of *in situ* chondrocyte death associated with the insertion of drills/screws into articular cartilage. A qualitative description was also possible based on the transmitted light images. The two main findings were firstly, a marked ZCD and surface damage was observed around holes arising from using a drill and articular screws of different designs (cortical screw, headless metallic screw, headless bioabsorbable screw). Secondly, chondrocyte death arising from drilling could be significantly reduced using saline irrigation for cooling. The results suggest current methods of internal fixation may compromise the viability of chondrocytes within osteochondral fragments.

We are not aware of any literature on the extent of *in situ* chondrocyte death from the application of drills/screws to cartilage. Previous work has described a ZCD in cartilage around injuries from trephines⁹, circular osteotomes for mosaicplasty¹³ and along a scalpel cut¹⁰. The resulting chondrocyte death appears to be less than that reported here. Our observation that saline irrigation markedly reduced, but did not abolish chondrocyte death (Fig. 5) suggests that thermal injury during drilling could be a major cause of cell death. For bone, a variety of improved drill bits and finely-tuned drilling parameters, including external irrigation have been developed to reduce ‘thermal necrosis’¹⁸. Thermal damage delays bone healing, increases resorption, and causes implant loosening/failure¹⁹. It is also recognised that using an irrigation medium may decrease cell death in bone²⁰. However there are few studies on the response of chondrocytes/cartilage to heat²⁰, and while bone can remodel/repair following mechanical injury/cell death²¹, cartilage has very poor repair potential²². In cartilage, regions of tissue devoid of living chondrocytes, particularly in the superficial zone as studied here following the insertion of screws, result in focal cartilage defects that may progress to more extensive cartilage degeneration¹⁶. For example, in the knee joint, incidental findings of focal cartilage defects on MRI have been shown to be predictive of cartilage loss within 2 years²³. In the ankle joint, focal chondral defects following fractures were closely

associated with the development of post-traumatic arthritis of the ankle at approximately 12 years following injury²⁴. These clinical findings indicate that minimising the extent of focal articular injury during insertion of intra-articular screws may help to decrease the risk of chondral defects progressing to secondary osteoarthritis.

There was no significant increase ($P > 0.05$) in the ZCD between that produced by the 1.5mm orthopaedic drill alone and the cortical screw (Fig. 4). Although there appeared to be an increase in the ZCD (of approx. 160µm) between the 2mm cortical screw and the headless metallic screw, this also was not significant ($P > 0.05$; Fig. 4). However for the headless screw, there was a marked significant increase (54%; $P < 0.001$) in the ZCD between the Biotrak screw compared to the Acutrak screw. Overall, there was a significant increase (ANOVA $P < 0.001$) in the ZCD in the order; drill, cortical screw, Acutrak screw and Biotrak screw (Fig. 4) however it is important to note the different methodologies that were used. Thus, the drill used for the Acutrak and Biotrak screws had a larger diameter for the cortical screw (3.5mm vs 1.5mm) and the Biotrak screw also required a tap (see Table 1) and it is possible that these procedures accounted for the additional chondrocyte death. In addition, the cortical screw had to be removed before osteochondral explant harvest, as the screw head could not be buried below the subchondral bone. By contrast, the headless screws could be buried and explants harvested without the need for screw removal. Hence the ZCD for the cortical screw represented cartilage injury sustained as a result of cartilage drilling and following both insertion and subsequent removal of the screw, and may therefore overestimate chondrocyte death. The results are therefore the cumulative effect of the procedures on chondrocyte death and it would clearly be of interest to assess the contribution of the individual steps (including drilling and the use of the tap) on the ZCD which are required for the insertion (and removal) of orthopaedic screws. This may assist in developing improved orthopaedic equipment with the ultimate aim of minimising chondrocyte injury and death.

The finding that there was no significant difference between the extent of chondrocyte death following drilling alone and that after insertion/removal of the cortical screw (Fig. 4 drill vs cortical

screw) is of particular interest. This might suggest that the mechanical trauma resulting from manipulating the cortical screw was irrelevant compared to the trauma from drilling which would comprise both thermal and mechanical injury. Although there is the strong likelihood that the majority of chondrocyte death arises from cartilage heating during drilling, we cannot at present rule out the possibility that some of the cell death is due to mechanical trauma independent of a rise in temperature. For example, following cartilage drilling with copious irrigation, we still observed a marked ZCD (approx. 200µm; Fig. 5) although it was not possible to prevent a transient rise in temperature (see Materials and Methods). It has previously been reported that chondrocytes are sensitive to a rise in temperature above the physiological level, and in particular there is a marked decrease in chondrocyte viability when temperatures between 50-55°C²⁰ are applied for 5 mins²⁰. We have previously shown using a single scalpel cut¹⁰ and others have reported using a trephine⁹ or surgical suturing¹¹ that there is significant chondrocyte death to cartilage where there will be negligible thermal injury. Thus we feel that it is not unreasonable to suggest that the underlying mechanical trauma *per se*, could potentially contribute to the ZCD, although we are not able to assess its contribution with the present data. In order to address this point unequivocally, it would be necessary to undertake a further study to precisely determine the contributions made by thermal injury and the various mechanical insults to the ZCD in cartilage which occur during the application of orthopaedic drills and screws.

We emphasise that our data do not favour a particular screw design as there was a marked ZCD with all three screws studied. Nevertheless, the bioabsorbable headless screw demonstrated the greatest cartilage injury during insertion. While it may not require subsequent removal, further research is necessary before adopting it for widespread use in articular surgery. It may also be possible to reduce the ZCD resulting from mechanical injury *per se* by increasing the osmolarity of the irrigating solution¹⁰ and we are currently investigating chondroprotective strategies as they may maintain chondrocyte viability in osteochondral fragments and prevent cartilage degeneration.

Although care was taken, the ZCD in replicate samples was variable. The ability of the surgeon to maintain perpendicular insertion and steady pressure could have an impact on the final injury produced. It was also likely that the complex interaction between the drill/screw and the heterogeneous nature of the matrix surrounding chondrocytes, also caused variability. It was therefore important to have a standardised method for comparing the extent of cartilage injury statistically and the methodology was aimed at providing a relatively unbiased representation of the ZCD as detailed in Fig. 2. We also noted that cartilage hole diameters were significantly smaller than those created by the equipment inserted (Fig. 3). It is likely that mechanical damage to the collagen network allowed proteoglycan swelling²⁵ causing the injured matrix to spread into the hole leading to an apparent reduction in diameter. This could potentially improve the ‘sealing’ between the inserted screws and cartilage however it is probable that the vast majority of the mechanical holding strength of the screws will be by its interaction with bone.

There are certain limitations to our study. We compared the effects of three different methods of internal fixation and inserted the equipment as close to the clinical setting as possible which meant both drill diameter and design were not identical. Thus for example, a 1.5mm guide hole was required for the cortical screw, whereas a 3.5mm hole was necessary for the metallic headless and bioabsorbable screws (Table 1). It is likely that the larger diameter drills caused more heating trauma resulting in a greater degree of chondrocyte death²⁶. Additionally, these experiments were performed on an animal joint, and human cartilage might respond differently because, for example, it is thicker than bovine cartilage¹⁴ and rises in temperature during drilling might be dissipated more effectively. Nevertheless, all three techniques tested were clinically relevant and indicated for osteochondral fragment fixation. The extent of chondrocyte death associated with the insertion of articular screws suggests that further consideration of the design of orthopaedic drills/screws could be beneficial to minimise iatrogenic damage²⁶. The imaging methods described here could therefore be of value in providing quantitative and statistically comparable data. This study also demonstrated the protective effects of saline irrigation during

cartilage drilling through its cooling effect, which may improve the long-term viability of internally-fixed osteochondral fragments.

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AUTHOR CONTRIBUTIONS

Conception and design: DAH, AKA, TOW, ACH

Collection and assembly of data: DAH, AKA, IDMS, ACH

Analysis and interpretation of data: DAH, AKA, IDMS, ACH

Drafting of the manuscript: DAH, AKA, TOW, ACH

Critical revision: DAH, AKA, TOW, IDMS, ACH.

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CONFLICT OF INTEREST

All authors declared no competing financial interest regarding this manuscript.

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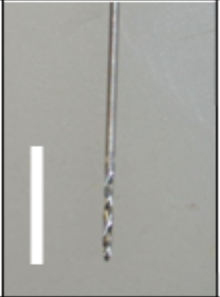



ID	Short Name	Drill type / dimensions	Screw type / dimensions	Example
GI	Drill	Ortho Solutions, AO QC Drill bit diameter – 1.5mm length – 85mm (Ortho Solutions Ltd, Essex, U.K.)	No screw used	
GII	Cortical Screw	Ortho Solutions, AO QC Drill bit diameter – 1.5mm length – 85mm (Ortho Solutions Ltd, Essex, U.K.)	Synthes, self-tapping cortical screw. diameter – 2.0mm length – 20mm (Synthes, Hertfordshire, U.K.)	
GIII	Acutrak	Acutrak, long drill diameter – 3.5mm length – 85mm (Acumed, Oregon, U.S.A.)	Acutrak, headless, variable pitch, metallic screw. diameter – tip: 3.0mm head: 3.5mm length – 20mm (Acumed, Oregon, U.S.A.)	
GIV	Biotrak	Biotrak, tapered long drill diameter – 3.5mm length – 85mm (Biotrak tap also used) (Acumed, Oregon, U.S.A.)	Biotrak, headless, variable pitch, bioabsorbable screw. diameter – tip: 3.0mm head: 3.5mm length – 20mm (Acumed, Oregon, U.S.A.)	

Table 1. Orthopaedic drills and screws used in this study. The procedures performed are identified (GI – GIV) with the drills and screws utilised as indicated. Also shown are the dimensions of the drills/screws, and supplier with an illustration of their appearance. Scale bar = 1cm.

Fig. 1. Insertion of the drill/screws on the bovine metacarpophalangeal joint. From left to right: hole produced by the 1.5mm drill, Acutrak screw, Biotrak screw and a self-tapping cortical screw. Bar = 1cm.

Fig. 2. Quantification of the hole diameter and zone of cell death (ZCD). Left panel: Transmitted light images with improvement in the demarcation of the edges with adjustment of contrast (top and middle images). The reference lines and measurements of hole diameter are superimposed on the bottom image. Right panel: CLSM reconstructions of the articular surface (top image) – note the dead (red) and live (green) *in situ* chondrocytes are clearly distinguished with a circumferential ZCD extending from the edge of the hole into normal tissue. The reference lines and $50\mu\text{m}^2$ region of interest (ROI) used to determine the live/dead border are shown in the middle image. The distance from the last dead cell in the ROI, to the first dead cell at the hole edge was measured, and shown as the ZCD (bottom image).

Fig. 3. Comparison between cartilage appearance, equipment diameters and holes produced.

Upper panels show transmitted light images used to determine hole diameters (see Results section for a qualitative description of the holes). Bar = $500\mu\text{m}$. The graph shows that there was no significant difference between the actual diameter of the drill and the diameter of the hole in articular cartilage caused by the drill ($1471\pm 181\mu\text{m}$; $n=6$). In contrast, the holes in articular cartilage after insertion of the cortical screw ($1381\pm 250\mu\text{m}$; $n=6$), Acutrak screw ($2569\pm 409\mu\text{m}$, $n=6$) and Biotrak screw ($2702\pm 272\mu\text{m}$; $n=6$) were significantly ($p\leq 0.001$) smaller than the actual screw diameters.

Fig. 4. CLSM images and the ZCD after drilling and articular screw insertion. Upper images show CLSM reconstructions of drill and screw holes in articular cartilage. Individual CLSM reconstructions have been combined to provide a composite view of the holes. The ZCD is the band of red stained cells surrounding the black hole created by the insertion of a drill or screw. Top left - 1.5mm drill; upper right - cortical screw; lower left – Acutrak screw; lower right - Biotrak screw. (Bars = $1000\mu\text{m}$). The graph compares the width of the ZCD after drilling alone and the three

different articular screws. Note the ZCD was least for the cortical screw and greatest for the Biotrak screw (Data are means with the 95% confidence interval shown, with $n=6$ for 1.5mm drill, Acutrak screw, Biotrak screw, $n=4$ for cortical screw).

Fig. 5. Reduction in the ZCD using saline irrigation during drilling. The CLSM reconstructions show the ZCD following drilling using a 1.5mm drill without (left) and with (right) saline irrigation (0.9%; 18°C). The graph summarises the pooled data demonstrating an approximately 50% reduction in the ZCD with saline irrigation. (Data are means with 95% CI, for $n=7$, white bar = 250 μ m).

Figure 1.



Figure 2.

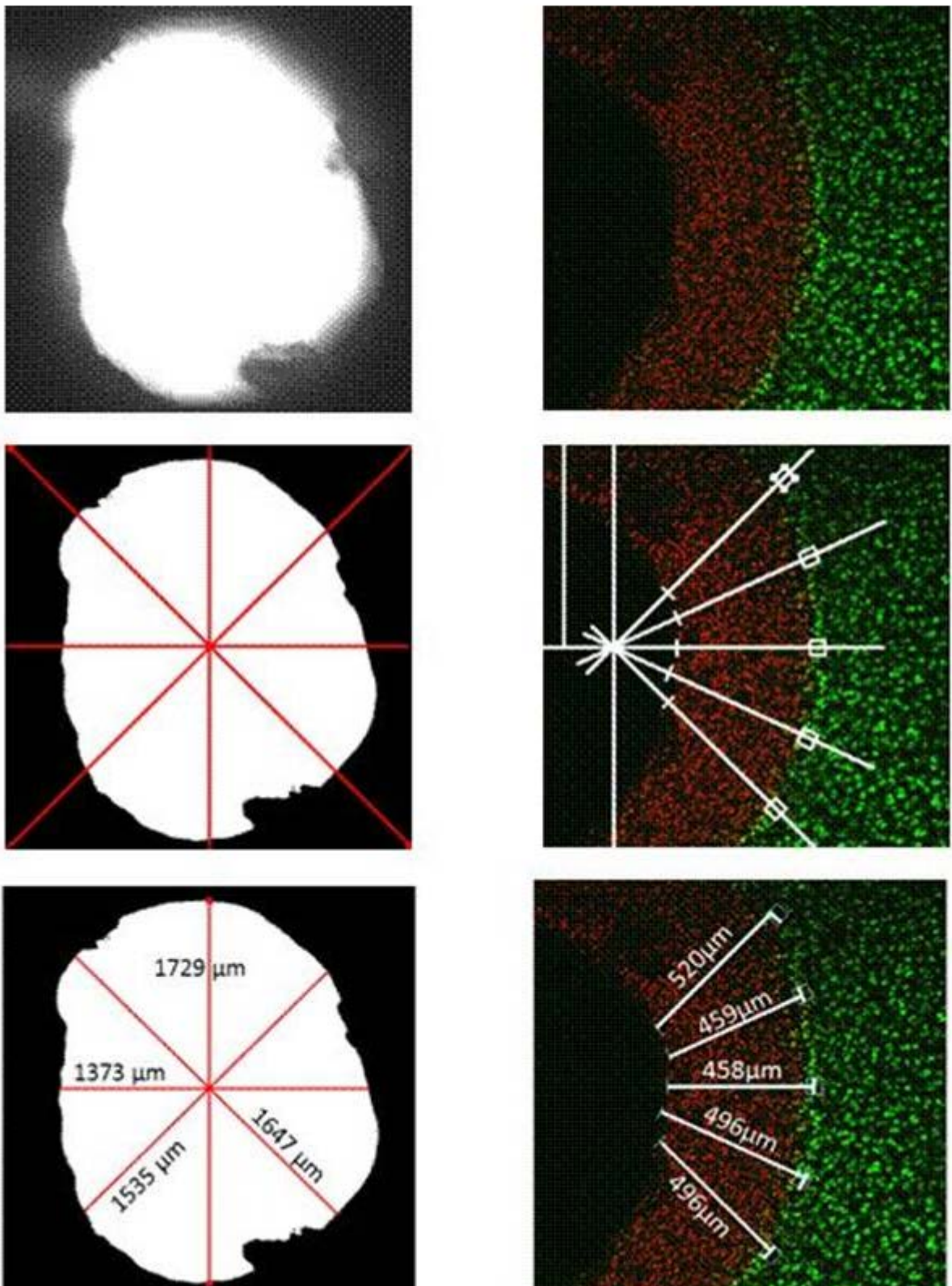


Figure 3.

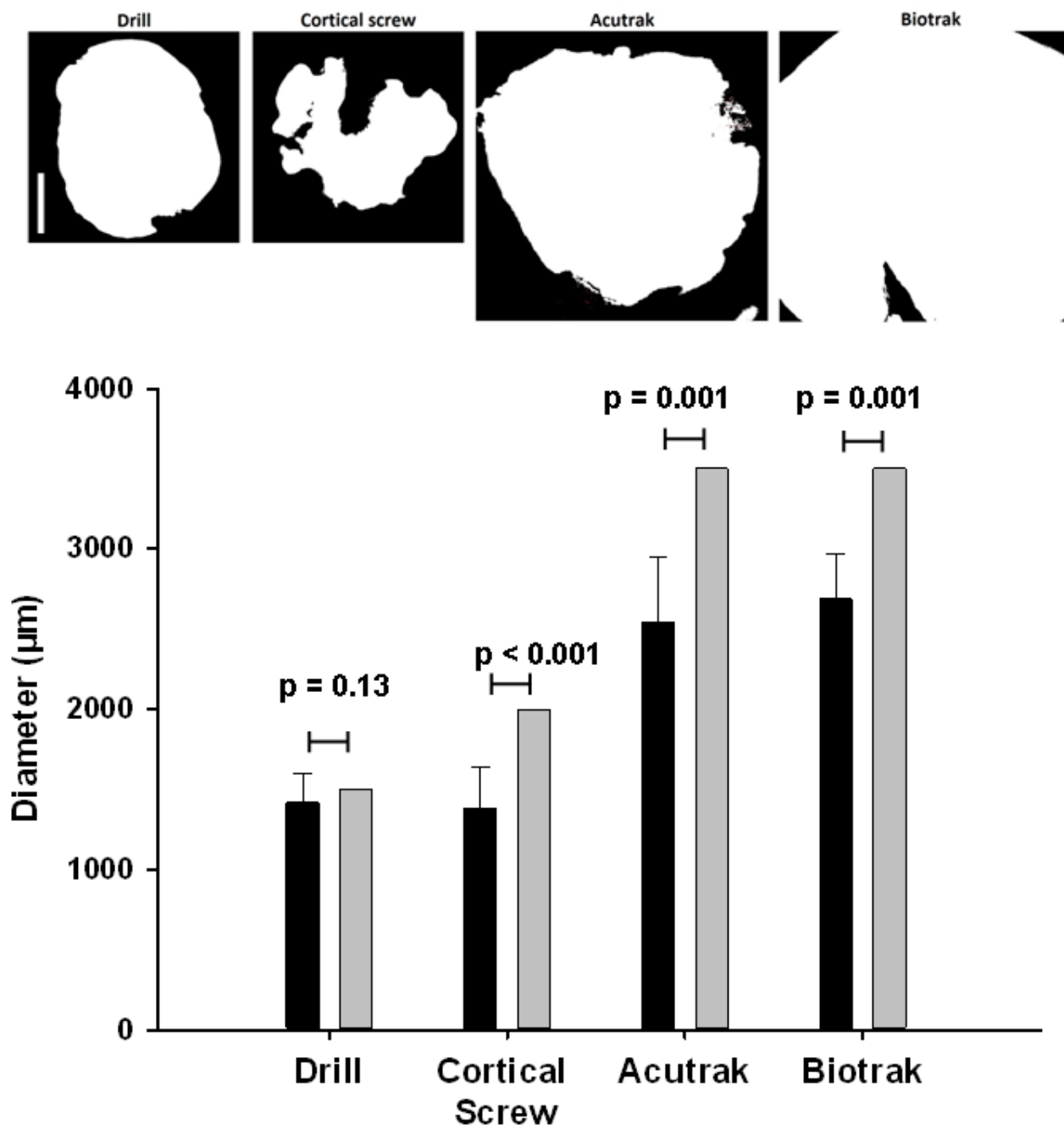


Figure 4.

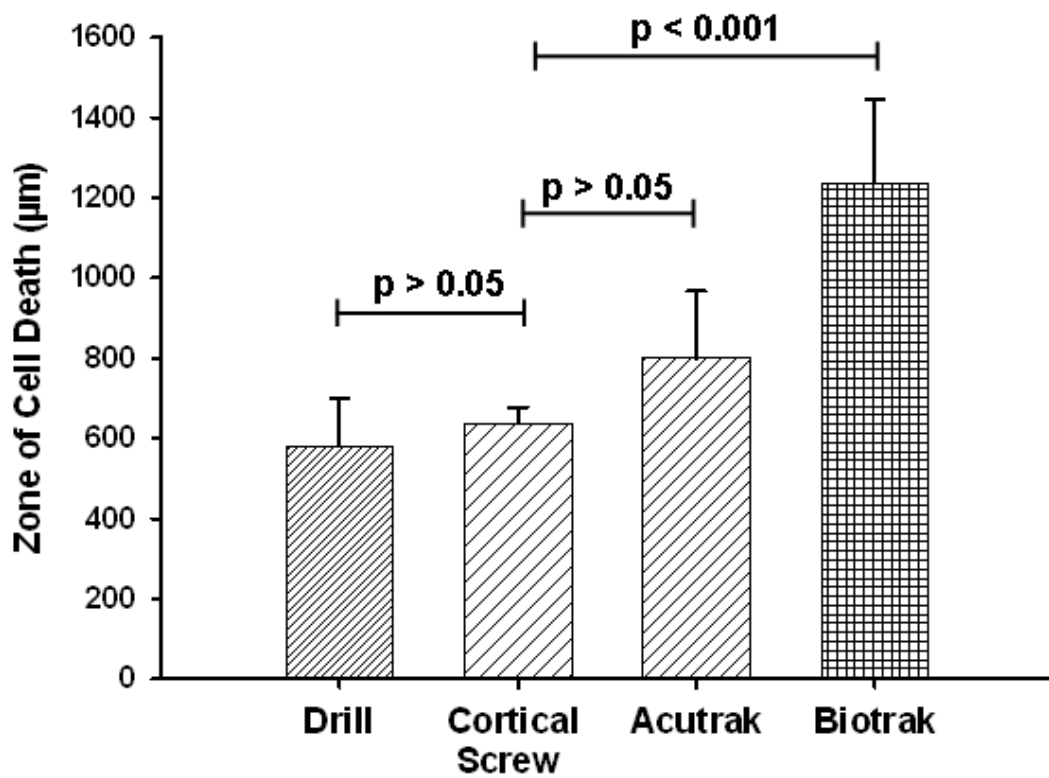
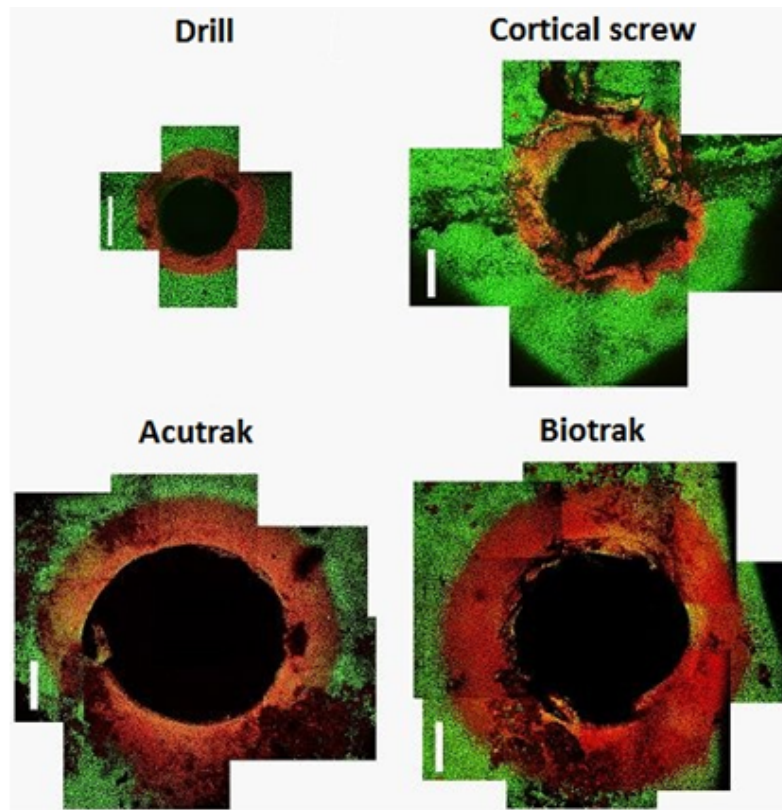


Figure 5.

