Effect of irrigation fluids, local anaesthetics, Glucosamine and Corticosteroids on human articular cartilage: an in vitro study

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Abstract

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Background: Animal studies have shown that the commonly used arthroscopic irrigation fluid, 0.9% normal saline, can be toxic to articular cartilage. There have been several reports of chondrolysis following arthroscopy especially with the use of local anaesthetic pain pumps post operatively. In vitro studies have shown severe toxicity of local anaesthetics to articular cartilage but there are currently no published studies looking at methods to prevent this toxicity.

Aims: To study the effect of different irrigation fluids and local anaesthetics on human articular cartilage and the ability of Glucosamine or Corticosteroids to protect against or recover from any potential toxicity.

Materials and Methods: Chondral explants obtained from human femoral heads were exposed to different irrigation fluids, local anaesthetics, Glucosamine, Methylprednisolone or culture medium (control) for one hour. After exposure, explants were incubated with radio-labelled $^{35}SO_4$and uptake was measured after 16 hours as an indicator of proteoglycan synthesis.

Results: The inhibition of $^{35}SO_4$uptake was 10% by Ringer's solution, 24% by 1.5% Glycine, 31% by 5% Mannitol ($p=0.03$)and 35% by Normal saline ($p=0.04$). Lidocaine 1 and 2%, Bupivacaine 0.25 and 0.5% and Levobupivacaine 0.5% were all toxic causing inhibition ranging from 61% to 85% ($p<0.001$). The addition of Glucosamine or Methylprednisolone at the same time as 0.5% Bupivacaine protected articular cartilage and reduced the inhibition by approximately 50% ($p<0.001$).

Conclusions: Ringer's solution was the least toxic arthroscopic irrigation fluid and should replace normal saline in clinical practice. Intra-articular local anaesthetic injections should only be used with careful consideration of risks and benefits. Further clinical studies are required to assess the potential damage to cartilage from local anaesthetics or normal saline and to investigate the protective effect of Glucosamine or Corticosteroids.

Publications

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Presentations

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East Midlands Orthopaedic Research meeting, Leicester, November 2009.
European Federation of National Associations of Orthopaedics and Traumatology (EFORT) annual conference, Madrid, May 2010.

Gulihar A, Hadi M, Taylor GJS. Can glucosamine protect against the toxicity of local anaesthetic solutions to human articular cartilage?
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East Midlands Orthopaedic Research meeting, Nottingham, November 2010– prize presentation (East midlands south trainees).
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Chapter 1 Background

Section 1.1. Articular cartilage

Articular cartilage is a highly specialized tissue that allows repetitive gliding motion in synovial joints. It provides a low friction surface and lubrication to allow range of motion. It also aids in shock absorption and transmission of load onto subchondral bone. Before we can discuss the effect of different substances on articular cartilage, it is important to understand its structure, composition and the mechanisms that allow it to bear the compressive stresses applied. This chapter also discusses the effect of ageing and osteoarthritis on articular cartilage because the cartilage specimens in this study were collected from elderly patients.

Section 1.1.1 Structure and Composition

The three main components of the articular cartilage are water, proteoglycans and collagen, which form the extra-cellular matrix (ECM). Specialised cells called chondrocytes, which are responsible for synthesis and metabolism of different cartilage components, are spread throughout the ECM. The articular cartilage is divided into four zones: the superficial zone, the middle or transitional zone, the deep zone, and the zone of calcified cartilage (Buckwalter et al., 2000). The concentration of water and proteoglycans and the structure and alignment of collagen, chondrocytes and proteoglycans vary in the four zones of articular cartilage.

The superficial zone forms the gliding surface of the cartilage. Water content is the highest in this zone and the proteoglycan content is the lowest. The collagen fibres and chondrocytes are elongated and arranged parallel to the articular surface (Figures 1.1 and 1.2). The middle zone, also called the transitional zone, contains rounded chondrocytes, which are randomly distributed within the ECM along with large diameter collagen fibers. The deep zone contains the lowest water content and the highest concentration of proteoglycans. The collagen fibers again have a large diameter but are arranged perpendicular to the articular surface. The chondrocytes are spherical in shape and are arranged parallel to the collagen fibres in a more perpendicular orientation to the articular surface. The zone of calcified cartilage is the deepest layer and is separated from the deep zone by a line called the tidemark. It contains small cells distributed within a cartilaginous matrix.

The extra cellular matrix is also classified into peri-cellular, territorial, or inter-territorial regions based on its proximity to the chondrocytes (Buckwalter et al., 2000). The chondrocytes are completely surrounded by the peri-cellular matrix, which contains mainly proteoglycans and no collagen fibres. Surrounding the peri-cellular matrix is the territorial matrix that contains thin collagen fibrils. The inter-territorial matrix contains the large collagen fibers and the majority of the proteoglycans and is therefore responsible for the mechanical properties of the articular cartilage.

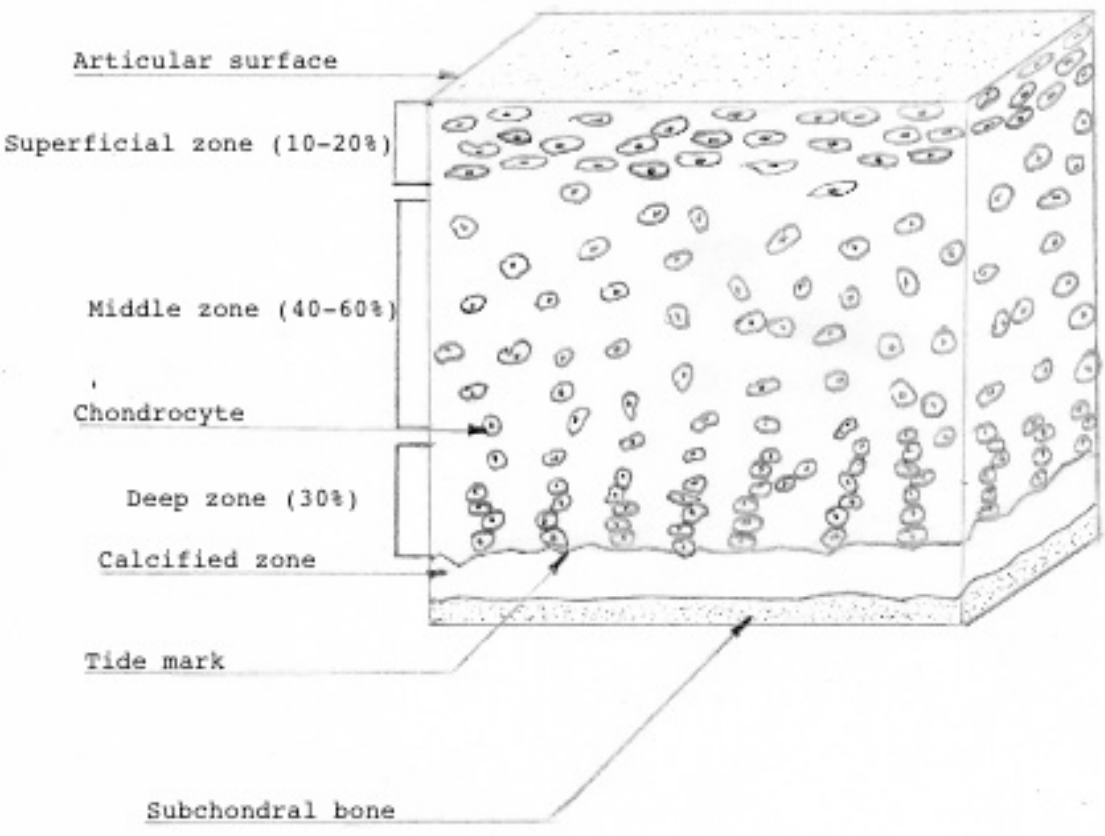

Figure 1.1 The distribution and orientation of chondrocytes in the different zones of articular cartilage

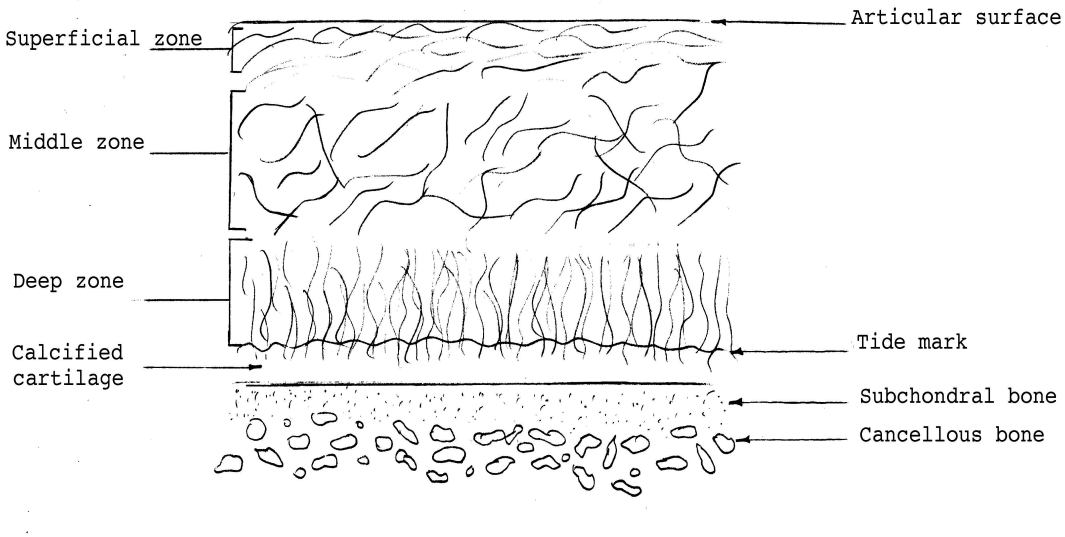

Figure 1.2 The arrangement of collagen fibres in the different zones of articular cartilage.

Chondrocytes

The chondrocytes account for less than 10% of the tissue volume of articular cartilage (Nordin and Frankel, 2001). They are specialised cells responsible for the formation and maintenance of various articular cartilage components. Their function is regulated by a variety of chemical and mechanical stimuli such as growth factors, interleukins, mechanical loads and hydrostatic pressure changes (Buckwalter et al., 2000). The factors that influence chondrocyte activity will be explained in more detail later on in this chapter.

Water

Water content varies throughout articular cartilage, decreasing in concentration from approximately 80% of its total wet weight at the surface, to 65% in the deep zone (Buckwalter et al., 2000). It helps in the movement of nutrients and waste products between chondrocytes and synovial fluid. It contains free mobile cations such as Na^+, K^+ and Ca^{2+} that are essential for the mechanical properties of cartilage.

Most of this water is extracellular, with a very small amount being present within the cells. About 70% is contained in the inter-fibrillar space where it is free to move through the ECM when a mechanical load is applied (Nordin and Frankel, 2001). While the water is free to move in the ECM, it encounters high resistance against this flow (Comper et al., 1983).This flow against resistance is chiefly provided by the proteoglycans (Comper and Zamparo, 1989) and is one of the mechanisms that allow the cartilage to sustain very high mechanical loads. The remaining 30% of water is contained within the intra-fibrillar space i.e. enclosed by the collagen fibrils.

Collagens

Collagens account for 15 to 22% of the wet weight and over 50% of the dry weight of articular cartilage. They are the major structural molecules of the ECM and therefore contribute greatly to its mechanical properties. Type II collagen is the most abundant type in articular cartilage and represents 90% to 95% of the total

collagen in the ECM. Other collagen types demonstrated in articular cartilage are types III, V, VI, IX, X, XI, XII and XIV (Eyre, 2002).

The collagen fibrils are formed by the polymerisation of much smaller tropocollagen molecules that coil around each other to form a triple helix (Nordin and Frankel, 2001). The tropocollagen fibrils are also bound together by covalent cross linkages, adding to the tensile strength of the collagen fibres (Eyre, 2002). The function of collagen is to provide the tissue's resistance to tensile and shear forces and also to immobilize the proteoglycans within the ECM.

The distribution of collagen in articular cartilage differs in the three zones (Figure 1.2). In the superficial zone, they are arranged as densely packed sheets parallel to the joint surface. In the middle zone, they are randomly oriented and dispersed within the ECM. In the deep zone, the fibres are again arranged as sheets or bundles that are perpendicular to the subchondral bone. These bundles cross the tidemark and enter the zone of calcified cartilage and therefore, anchor the cartilage to the underlying bone. This difference in arrangement of the collagen fibres may be due to the biomechanical properties of collagen fibres. Collagen fibres are strongest in tension and relatively weaker in compression (Li et al., 2005). This property may account for the compressibility of superficial layers of articular cartilage.

Proteoglycans

Proteoglycans (PG) are large complex macromolecules consisting of a protein core

to which Glycosaminoglycan (GAG) molecules are attached. GAGs consist of long-chain, unbranched, repeating disaccharide units. The main types of GAGs in articular cartilage are chondroitin sulphate (CS) and keratan sulphate (KS) while dermatan sulphate (DS) is less commonly found.

Aggrecans form the majority of PGs found in articular cartilage. They consist of a long, extended protein core with up to 100 CS and 50 KS GAG chains covalently bound to it (Nordin and Frankel, 2001). Several aggrecan molecules can bind non-covalently to a single Hyaluronan (HA) molecule via a specific HA- binding region (HABR) and this attachment is stabilised by a link protein (LP) (Figure 1.3) (Parkkinen et al., 1996). This aggregation of several aggrecan molecules promotes immobilization of the PGs within the collagenous matrix and this adds to the mechanical stability of articular cartilage.

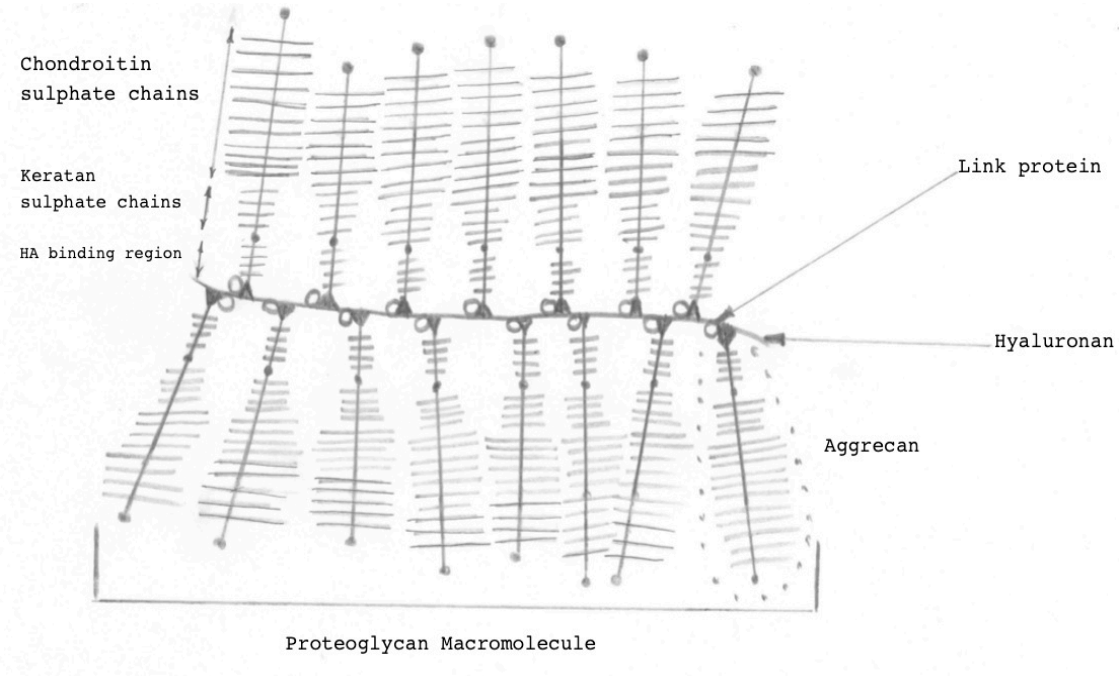

Figure 1.3 The structure of a large proteoglycan aggregate showing individual aggrecan units bound to a central hyaluronan unit

The other PGs found in articular cartilage are biglycan and decorin, which contain DS chains and Fibromodulin, that contains KS chains (Roughley and Lee, 1994). These smaller PGs are more concentrated in the superficial zone of articular cartilage (Buckwalter et al., 2000). They are thought to play a role in interacting with collagen fibres and therefore contributing to the general stability of the collagen-proteoglycan network in the ECM.

All the GAG chains found in cartilage have repeating carboxyl (COOH) and/or sulphate (SO4) groups. In solution, these groups become ionized (COOandSO3-) leading to a fixed negative charge along the PG molecule (Hall, 1999). This negative charge is countered by the cations such as Ca^{2+} and Na^{+} that are present in the

water leading to the development of a Donnan osmotic pressure effect (Donnan, 1924). The PGs are tightly packed within the ECM and this also leads to the development of a strong electrostatic repulsive force between the negative charges on the PG surfaces.

When a compressive force is applied to articular cartilage, an instant deformation is seen on the surface. This is due to a change in the PG concentration within the ECM. The external pressure leads to the development of internal stresses and fluid flows out of the cartilage. The PG concentration therefore rises and the resultant Donnan osmotic pressure also increases. This increase in the osmotic pressure continues until a state of equilibrium is reached with the external force applied on the cartilage. The ability of PGs trapped in collagen to resist compression and hold water derives from two main mechanisms: the development of Donnan osmotic forces and repulsive charge forces and secondly, the stiffness of the collagen-PG solid matrix which reduces the permeability of water (Nordin and Frankel, 2001).

Other matrix components

The ECM also consists of other non-collagenous proteins. These include Anchorin CII, which has a role in anchoring chondrocytes to type II collagen in the pericellular matrix (Mollenhauer et al., 1984) and Cartilage oligomeric protein (COMP), which has the capacity to bind to chondrocytes (Buckwalter et al., 2000). COMP is believed to be an important component of articular cartilage and increased degradation has been observed in osteoarthritis (Lohmander et al., 1994). Other proteins include Fibronectin and Tenascin, which are thought to have

a role in the response of articular tissues to inflammatory arthritis and osteoarthritis (Chevalier et al., 1994, Brown and Jones, 1990).

Lipids form less than 1% of the wet weight of adult articular cartilage (Buckwalter et al., 2000). Delipidised cartilage has been found to be stiffer than normal articular cartilage (Oloyede et al., 2004a, Oloyede et al., 2004b), indicating that lipids play a role in the deformability of cartilage and may influence the development of osteoarthritis.

Section 1.1.2 Cartilage Metabolism

The synthesis, assembly and distribution of the components of the ECM are regulated by the chondrocytes. The chondrocytes also continuously maintain the ECM by balancing the rate of synthesis, assembly and distribution of ECM components with the rate of their degradation and release from the cartilage. These processes are mediated by various chemical and mechanical factors. The chemical factors include growth factors such as Insulin-like growth factor (IGF-1) and platelet derived growth factor (PDGF) (Schmidt et al., 2006), inflammatory mediators such as interleukins and changes in composition of the ECM. In addition, mechanical loads and hydrostatic pressure changes can directly or indirectly influence the metabolic activities of the chondrocytes (Lee et al., 2002, Hall, 1999).

Cartilage nutrition

Articular cartilage is aneural and avascular i.e. it does not have a nerve or blood supply (T.G, 2006). The exact mechanism by which it derives nutrition is not clear. Part of it is through the underlying subchondral bone and the remainder is believed to be derived from the small amount of synovial fluid present in normal synovial joints (Buckwalter et al., 2000, Fam et al., 2007). Because of this lack of blood supply, chondrocytes are well adapted to survive in an environment of low oxygen tension (Rajpurohit et al., 1996).

Proteoglycan Synthesis

Chondrocytes are responsible for the synthesis, assembly, and sulphatation of proteoglycans. The synthesis of a proteoglycan molecule involves the synthesis of the protein core, GAG chains, the link protein and hyaluronate (Lohmander, 1988).

The first step in synthesis is expression of the PG gene and the transcription of the messenger RNA (mRNA) from the nuclear DNA. Translation of the mRNA occurs then in the endoplasmic reticulum and the protein core is synthesized at the ribosome. The protein core is then transported to the Golgi complex, where the GAG chains are added (Buckwalter et al., 2000).

After glycosylation and sulphatation, the proteoglycan and link protein are transported to the plasma membrane of the cell and from there into the ECM. Hyaluronate synthesis occurs separately at the plasma membrane, which is then

secreted into the ECM. The final step is the formation of aggregates by linking together of aggrecan, link protein and hyaluronate. Aggregate formation restricts the movement of proteoglycans within the ECM and therefore helps in retaining proteoglycans at a high concentration in the tissue (Hardingham et al., 1987).

Proteoglycan catabolism

Chondrocytes secrete various proteolytic enzymes that are needed for breakdown of the ECM. Proteoglycans and other components of the ECM undergo continuous degradation in articular cartilage as part of normal tissue maintenance. This degradation is increased in ageing, injury and in osteoarthritis. The important groups of proteinases involved in cartilage turnover are matrix metalloproteinases (MMP): (collagenase, gelatinase, and stromelysin), cathepsins (types B,D,L) and aggrecanases (Buckwalter et al., 2000).

Aggrecanases and cathepsins B and L are involved in the degradation of aggrecan in articular cartilage (Nguyen et al., 1990). Stromelysin is the principal enzyme involved in the breakdown of the protein core of aggrecan (Hughes et al., 1991).

Aggrecan consists of three globular domains, G1, G2 and G3 (Kiani et al., 2002). The major cleavage site on the protein core of aggrecan is between the G1 and G2 domains, separating the part of the proteoglycan involved in aggregation (binding to hyaluronate and link protein) from the part that contains the glycosaminoglycan chains (Kiani et al., 2002). The G1 domain and link protein also are susceptible to proteolytic degradation by MMPs. The degradation fragments created from

proteolytic cleavage of aggrecan are transported to the synovial fluid, from where they are taken up through the synovium to the lymphatic system. The glycosaminoglycan chains can even reach the bloodstream or urine (Buckwalter et al., 2000).

Collagen synthesis

Collagen synthesis starts with translation of mRNA for the constituent α chains to form a polypeptide chain. These are then transported to the rough endoplasmic reticulum where propeptide glycosylation, proline and lysine hydroxylation and lysine glycosylation occur. The propeptides are then secreted onto the plasma membrane where crosslinking and triple helix formation occur (Buckwalter et al., 2000). Propeptide cleavage occurs after transportation into the ECM and the resultant collagen molecules assemble to form a fibrillar network. The final step is the formation of covalent cross-links, which is catalyzed by the enzyme lysyl oxidase.

Collagen catabolism

The breakdown of collagen is regulated by chondrocytes via MMPs. While all three MMPs are involved in collagen breakdown, Collagenase is the only enzyme that can cleave the triple-helical part of collagen. Collagenase-3 (MMP 13) is the most active enzyme in breakdown of type II collagen (Murphy et al., 1999).

Section 1.1.3 Changes in Ageing and Osteoarthritis

Ageing

Several changes in cartilage structure and function have been observed with age. The majority of these changes are seen in the proteoglycan structure. With increasing age, the aggrecan aggregates are shorter because of a reduction in the number of aggregating monomers and are also much more variable in length. In young adults, chondroitin 4-sulphate is the predominant GAG molecule. With increasing age, the concentration of KS increases and chondroitin 6-sulphate is seen more than chondroitin 4-sulphate (Buckwalter et al., 1994).

It has been suggested that these changes in the structure of the articular cartilage may be related to an age-related alteration in the normal response to different growth factors such as PDGF, IGF-I, Transforming Growth Factor (TGFβ) and Fibroblast Growth Factor (bFGF) (Guerne et al., 1995). Martin et al (Martin, Ellerbroek et al. 1997) reported an age related decline in the ability of IGF-1 to stimulate chondrocytes to produce PGs and collagen. This is especially important because IGF-I is well known to stimulate cartilage matrix synthesis (Martin et al., 1997).

In addition to a reduced response to growth factors, increasing age is also associated with an increase in levels of MMPs and other degradative enzymes that accelerate cartilage catabolism (Wu et al., 2002). An increase in collagen turnover

has also been found in ageing cartilage. Aurich et al (Aurich et al., 2002) found increased degradation of type II collagen in macroscopically normal ankle cartilage.

Other age related changes in the molecular composition and structure of the articular cartilage matrix include increased collagen cross-linking and decreased water concentration. These structural, cellular and functional changes in articular cartilage with age lead to a reduction in its mechanical properties (Loeser, 2010, Loeser, 2009). Table 1.1 summarises the changes seen in articular cartilage with ageing.

Osteoarthritis

Osteoarthritis (OA) is the process of degeneration of articular cartilage and is characterised by alteration of the structure and composition of the ECM leading to a reduction in its mechanical properties. In the early phases of osteoarthritis, there is a loss of proteoglycans and an increase in water content to over 90% (Buckwalter et al., 2000). Even in the early stages of the joint degeneration, the stiffness of the articular cartilage declines and its permeability increases.

	Osteoarthritis	Ageing
Macroscopic	Fibrillation may extend to subchondral bone	Localized fibrillation
	Progressive loss of cartilage	
ECM	Initial increase in water content	Decreased water content
	Progressive degradation and loss of collagens	Increased collagen cross-linking and fibril diameter
	Progressive breakdown and loss of proteoglycans and hyaluronan	Reduction in Aggrecan aggregate size. Increased decorin concentration
	Increased fibronectin concentration	
	Increased permeability and loss of tensile and compressive stiffness and strength	Decreased tensile strength and stiffness in superficial layers
Cells	Loss of chondrocytes	Decreased chondrocyte density
Metabolism	Initial increase in synthesis and proliferation. Eventual decreased synthetic activity	Decreased synthetic activity
	Increased degradative enzyme activity	Decreased anabolic response to growth factors (IGF-I)

Table 1.1 Changes in articular cartilage seen in osteoarthritis and ageing

The tissue damage stimulates an increase in synthesis of proteoglycans and other ECM components as an attempt to maintain or restore the articular cartilage. This increase in anabolic response can continue for several years but can progress rapidly is some patients. Eventually the cartilage reaches a stage where anabolic activity declines and catabolic activity increases leading to progressive loss of articular cartilage (Sandell and Aigner, 2001).

There is evidence of increased catabolic activity mediated by degradative enzymes in osteoarthritis. Dahlberg et al (Dahlberg et al., 2000) obtained cartilage specimens from 11 patients with osteoarthritis and found that the digestion of type II collagen by different collagenases was increased. Forsyth et al (Forsyth et al., 2005) suggested that chondrocytes from older adults produced more MMP-13 (collagenase-3) after stimulation with either IL-1β or fibronectin fragments leading them to question whether an increased susceptibility to catabolism was responsible for the development of osteoarthritis in older patients.

Osteoarthritis is also characterised by an increase in inflammatory processes. Several pro-inflammatory cytokines are found in osteoarthritic cartilage such as IL-1, IL-6, IL-7, IL-8, and TNF-α (Loeser, 2010).

Buckwalter has classified osteoarthritis into three phases (Buckwalter et al., 2005). The first phase is characterised by changes at a molecular level that may be

initiated by a high impact mechanical insult or inflammatory/ metabolic disturbances. The second phase consists of the tissue reaction to these changes and consists of primarily anabolic activity but also some catabolic activity. The third phase is characterised primarily by catabolic activity. This stage is reached when the chondrocytes are no longer stabilised by an intact matrix. This may be associated with synthesis of substances that bind the anabolic cytokines and leads to a declining anabolic activity characterised clinically by the progressive loss of articular cartilage.

Changes seen in ageing do not necessarily lead to the development of osteoarthritis. However, the structural, molecular, cellular and mechanical changes that occur in articular cartilage with age make the cartilage vulnerable to developing osteoarthritis. Articular cartilage is thought to be less responsive to growth factors and anabolic cytokines with increasing age and hence, older articular cartilage is less able to repair and regenerate itself. Therefore, changes in articular cartilage in ageing increase the risk of joint degeneration, and decrease the ability of joint tissues to prevent progression once degeneration begins. The various changes in articular cartilage seen in osteoarthritis are summarised in table 1.1.

Section 1.2 Arthroscopy

Joint arthroscopy is one of the commonest orthopaedic procedures. In 2006, nearly a million arthroscopies of only the knee joint were performed in the United States (Kim et al., 2011b). Of these, 53% of the operations were performed for meniscal tears. Other common indications for knee arthroscopy included "chondromalacia" of patella, cruciate ligament injury and osteoarthritis. In the UK, this number was smaller but still substantial at 50000 in the year 2004 (Hawker et al., 2008). Meniscal tears can be present in young active patients secondary to sports injury and also in older patients where the tear is often degenerative in origin. In the above study by Kim et al., 53% of the procedures were performed in males and 75% in patients aged between the ages of 20 and 64 years.

Arthroscopic procedures are also commonly performed for other joints such as the shoulder, hip, ankle, wrist and elbow.

Section 1.3 Chondrolysis

Chondrolysis is a rare but devastating complication of arthroscopic surgery. It is characterised by progressive breakdown of cartilage evidenced by reduction of joint space on radiographs. Clinically this is manifested by progressive increase in pain and stiffness in the affected joint. There were only four documented cases of gleno-humeral chondrolysis prior to the advent of arthroscopy and these were related to the use of gentian violet leakage as a colour test for identifying rotator

cuff tears (Shibata et al., 2001, Tamai et al., 1997). Post arthroscopic chondrolysis has since been described most often in the gleno-humeral joint but has also been documented in the knee joint (Slabaugh et al., 2010, Fester and Noyes, 2009). While the exact cause of chondrolysis in these patients is unknown, infusion of local anaesthetic through intra-articular pain pumps has been implicated as the causative factor in many patients. Other possible factors include the use of thermal probes or bio-absorbable suture anchors.

Scheffel et al (Scheffel et al., 2010) published a literature review of 16 articles that had reported a total of 96 cases of gleno-humeral chondrolysis. They found that instability was the principal indication for surgery, 63 patients had suture anchors, radiofrequency probes were used in 34 and intra-articular pain pumps were used in 59 patients. In 50 out of these 59 patients, different concentrations of Bupivacaine had been used and Lidocaine was used in 2. The authors do not state whether there were any patients who did not have any of the above-implicated risk factors. Symptoms started between 42 and 730 days after surgery with an average of 254 days. The authors wondered whether irrigation with a non-isotonic solution under pressure contributed to articular cartilage damage.

Several laboratory studies have assessed the effect of different materials used in arthroscopic surgery, especially different irrigation fluids and local anaesthetic solutions, on articular cartilage.

Section 1.4 Effect of different Irrigation fluids

During arthroscopy, the joint is continuously irrigated with fluid to provide a clear bloodless field and to distend the joint. During this procedure, the physiological synovial fluid is replaced by the irrigation fluid and this may impair cartilage metabolism and proteoglycan synthesis. Different irrigation solutions have been used for this purpose. Various studies have been conducted using animal models to assess the safety and impact of these fluids on the articular cartilage

Arciero (Arciero et al., 1986) measured the uptake of radio-labelled sulphate ($^{35}SO_4$), as a measure of proteoglycan synthesis, by rabbit articular cartilage after irrigating the knee joints for two hours with normal saline, Ringer's solution, sterile water or an un-irrigated control group and found no difference between the three solutions.

Bert et al (Bert et al., 1990) compared the appearance of human knee articular cartilage on scanning electron microscopy after exposure to five different irrigation solutions. They found that specimens exposed to 1.25% Glycine had the most smooth appearance while those exposed to saline and Ringer's lactate showed fibrillation and ridges.

Yang et al (Yang et al., 1993) irrigated rat knee joints with saline, Ringer's lactate, 3% sorbitol or distilled water and observed the surface ultrastructure with a scanning electron microscope (SEM). No differences were seen, leading them to conclude that all four solutions were safe for use in arthroscopy.

Reagan et al (Reagan et al., 1983) compared $^{35}SO_4$ uptake by bovine articular cartilage specimens and found that the uptake was higher with Ringer's lactate compared to normal saline. They felt that the acidic pH (5.3) of the saline solution used in their study could be responsible for this difference in uptake.

Bulstra et al (Bulstra et al., 1994) compared inhibition of proteoglycan metabolism by measuring $^{35}SO_4$ uptake in rat patellar articular cartilage after exposure to five different irrigation solutions for one hour and a recovery period of 16 hours. They found that compared to the control solution (culture medium), all solutions caused some degree of inhibition of proteoglycan metabolism. This was least with Ringer's solution at 5%, 10% for Ringer's Glucose and 20% for saline and Ringer's lactate. They also felt that the acidic pH (5.5) of saline could be the reason for causing 20% inhibition. Based on their results, they recommended the use of Ringer's solution for irrigation during arthroscopy.

Jurvelin et al (Jurvelin et al., 1994) measured instant, total and creep deformation in bovine articular cartilage after applying an indenting force. Instant deformation was measured immediately after the indenting force was applied and the total deformation was measured after 95 seconds. Creep deformation was measured after allowing the cartilage to re-swell for another 95 seconds. They compared the deformation with four different fluids; a 6% dextran - 5% sorbitol solution, 5% fructose, 5% Mannitol and Ringer's solution. They observed that Ringer's solution produced increased instant and total deformation after immersing bovine cartilage for 2, 4 and 20 hours as compared to the other three solutions. They suggested that

non-ionic fluids such as Fructose and Mannitol might have potential for use as irrigation fluids in arthroscopy.

Gradinger et al (Gradinger et al., 1995) measured proteoglycan loss from bovine cartilage and found that it was higher with ionic solutions such as normal saline and Ringer's solution than non-ionic solutions such as 2% Mannitol and 20% Sorbitol. The surface of articular cartilage on SEM was also seen to be rougher with Ringer's solution than 5% Mannitol. They also found that proteoglycan loss was negligible with 0.1% NaCl but became significant when NaCl concentration reached 0.9% leading them to conclude that proteoglycan loss was dependent on the ionic concentration of the irrigating fluid and that non ionic fluids like Mannitol were better than ionic fluids like 0.9% saline and Ringer's solution.

Section 1.4.1 Effect of duration of exposure

The duration of exposure to an irrigation fluid may have an effect of the metabolism of articular cartilage. Duration of arthroscopy depends on the nature of the procedure and is usually shorter for diagnostic purposes than for therapeutic procedures. Jurvelin et al (Jurvelin et al., 1994) observed that creep deformation of articular cartilage increased as the immersion time in Ringer's solution was increased from 2 hours to 20 hours. Yang et al (Yang et al., 1993) observed similar morphology at 1 and 2 hours after exposure to four different fluids.

Section 1.4.2 Effect of temperature

Some authors have also studied the effect of temperature of the irrigation fluid. Cheng et al (Cheng et al., 2004) irrigated rat articular cartilage with normal saline and observed that irrigation at 37 °C caused less damage on SEM to articular cartilage than at 4 °C. Brand et al (Brand et al., 1991) incubated bovine articular cartilage explants at two different temperatures and found that the rate of proteoglycan synthesis and the release of newly synthesized proteoglycans were decreased in cultures incubated at 32 °C compared to 37 °C.

Section 1.4.3 Summary

There is no clinical evidence to suggest that any of the irrigation fluids are safer or produce better outcomes than other fluids. In vitro studies seem to suggest that non-ionic fluids are safer than ionic fluids. The effect of duration of exposure, pH and osmolarity of the fluid is not clear but using fluid at 37 °C seems to be safer than that at room temperature. A summary of the current literature is presented in table 1.2.

Variables tested	Study model	Findings
Normal saline, Ringer's solution, sterile water	$^{35}SO_4$ uptake- Rabbit knees, two hour exposure	No difference in uptake between the three solutions and unirrigated control (Arciero et al., 1986)
Normal saline, Ringer's lactate, sterile water, 1.25% Glycine, Synovisol	Scanning electron microscope – human knee cartilage biopsies	Fibrillation and ridges seen with all except 1.25% Glycine. (Bert et al., 1990)
Normal saline, Ringer's solution, distilled water, 3% Sorbitol Duration of exposure	Scanning electron microscope – rat knee cartilage	No difference in SEM appearances No difference in SEM appearances between 1 or 2 hour exposure (Yang et al., 1993)
Normal saline, Phosphate buffered saline, Ringer's lactate, Ringer's acetate	$^{35}SO_4$ uptake – Bovine articular cartilage	Ringer's acetate (pH 6.5) had higher uptake than PBS (pH 7.1) and normal Saline (pH 5.3) (Reagan et al., 1983)
Normal saline, Ringer's lactate, Ringer's Glucose, Ringer's solution	$^{35}SO_4$ uptake – Bovine articular cartilage	Inhibition of uptake - Ringer's solution at 5%, 10% for Ringer's Glucose and 20% for saline and Ringer's lactate (Bulstra et al., 1994)

6% dextran-5% sorbitol solution, 5% fructose, 5% mannitol and Ringer's solution. Duration of exposure	Creep deformation – bovine articular cartilage	Ringer's solution produced increased instant and total deformation compared to the other non ionic solutions Increased creep deformation as duration increased from 2 to 20 hours (Jurvelin et al., 1994)
Normal saline, Ringer's solution, 2% Mannitol, 20% Sorbitol Ionic concentration of NaCl (increased from 0.1 to 0.9%)	Proteoglycan loss and SEM appearance - bovine articular cartilage	More PG loss with ionic solutions: saline and Ringer's solution Rough cartilage surface with Ringer's solution NaCl only caused PG loss at concentration of 0.9% (Gradinger et al., 1995)

Table 1.2 Effect of irrigation fluids on articular cartilage – summary of current literature.

Section 1.5 Local anaesthetics

Local anaesthetics are used for inducing a reversible local loss of sensation, usually to provide local pain relief. A local anaesthetic molecule consists of an aromatic part, connected by an intermediate chain to an amine group (Rang, 2003). The intermediate chain is composed of either an ester or an amide linkage. The type of linkage can determine the duration of action of the anaesthetic and can therefore be used to classify different local anaesthetics. The aromatic portion is lipophilic and the amine portion has hydrophilic properties. The degree of lipid solubility of a local anaesthetic enables its diffusion through the nerve membrane and therefore, also determines its potency (Gmyrek, 2011).

An impulse is transmitted through a nerve fibre by the process of depolarisation, which involves the influx of sodium ions into the nerve cells through sodium channels. Once depolarisation is complete, there is an active transport of the sodium ions from the intracellular to the extracellular space along with an influx of potassium ions. Local anaesthetics block depolarisation and hence, the initiation and propagation of action potentials, by blocking the sodium channels as well as by inhibiting potassium ion movement across the nerve cell membrane.

Local anaesthetics are weak bases, with pK_a values mainly in the range 8-9 and are therefore, partially ionised at physiological pH. They require the addition of hydrochloride to be water soluble and therefore injectable. Their activity is increased at alkaline pH because of lower proportion of ionised molecules and decreased at acidic pH. This is because the compound needs to penetrate the nerve

sheath and the axon membrane to reach the inner end of the sodium channel. Penetration is very poor at acid pH as the ionized forms are not able to permeate the membrane but once the anaesthetic reaches inside the axon, it is the ionised form of the molecule that binds to the sodium channel. It is this pH-dependence that makes inflamed and infected tissues resistant to local anaesthetics because of the presence of an acidic environment.

Commonly used local anaesthetics include Lidocaine, Bupivacaine (Figure 1.4), Levo-Bupivacaine and Ropivacaine. Lidocaine has a rapid onset of action but has a short duration of action. The other three anaesthetics have a slower onset of action but this is sustained over a longer period of time. Levo-Bupivacaine has the advantage of reduced cardiac toxicity and CNS depression over Bupivacaine.

Intra-articular Bupivacaine has been shown to be more effective than placebo at achieving effective analgesia after arthroscopic surgery (Chirwa et al., 1989, Eroglu et al., 2010). However, several laboratory studies and clinical reports have cast doubts on their safety when used as an intra-articular injection.

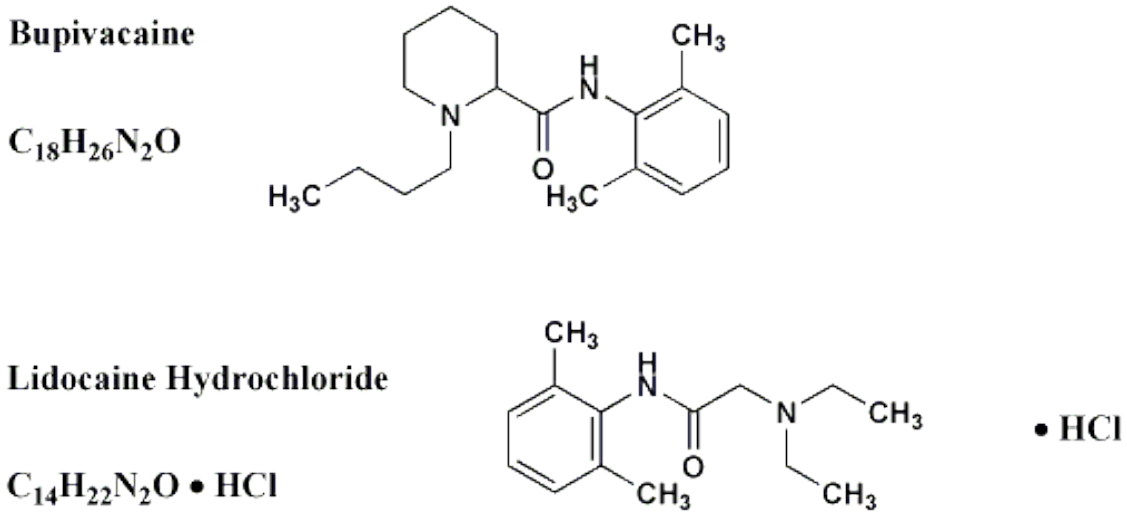

Figure 1.4 Molecular structures of Bupivacaine and Lidocaine

Section 1.6 Effect of local anaesthetics on articular cartilage: a systematic review

To further discuss the possible toxic effects of different local anaesthetics on articular cartilage, a systematic review of available clinical and laboratory studies was conducted.

Section 1.6.1 Introduction

Chondrolysis is a rare but devastating complication of arthroscopic surgery. It is characterised by progressive breakdown of cartilage evidenced by reduction of joint space on radiographs. Clinically this is manifested by progressive increase in pain and stiffness in the affected joint. There were only four documented cases of gleno-humeral chondrolysis prior to the widespread use of arthroscopy and these

were related to the use of gentian violet leakage as a colour test for identifying rotator cuff tears (Shibata et al., 2001, Tamai et al., 1997). Post-arthroscopic chondrolysis has since been described most often in the gleno-humeral joint (Wiater et al., 2011, Bailie and Ellenbecker, 2009, Anderson et al., 2010) but also in the knee joint (Slabaugh et al., 2010, Fester and Noyes, 2009). While the exact cause of chondrolysis in these patients is unknown, infusion of local anaesthetic through intra-articular pain pumps has been implicated as the causative factor in many patients. Other possible factors include the use of thermal probes or bio-absorbable suture anchors.

The aim of this review was to summarise all clinical and laboratory studies related to the safety of local anaesthetic use on articular cartilage. This was done in line with PRISMA guidelines (Moher et al., 2009).

Section 1.6.2 Methods

Criteria for considering studies for this review

This review included any laboratory studies that have investigated the effect of local anaesthetics on articular cartilage. These included human and animal studies. We also included all clinical studies describing chondrolysis or adverse effects associated with local anaesthetic use.

Letters to the editor, review articles and foreign language studies were excluded from this review. However, reference lists of all systematic and literature reviews were searched to identify relevant articles.

Search methods for identification of studies

A librarian assisted literature search was performed. A MEDLINE search including the years 1966 to February 2012 (inclusive) was used to identify all relevant studies. EMBASE was searched for the time period 1980 to February 2012 (inclusive). Reference lists of all identified papers and systematic reviews were manually searched. The controlled trials register was searched for any randomised controlled trials assessing the safety of different local anaesthetics.

The search strategy was as follows:

1. MEDLINE; (local AND anaesth* OR local AND anesth*).af; 53739 results.
2. MEDLINE; exp ANESTHETICS, LOCAL/; 83819 results.
3. MEDLINE; bupivacaine.ti,ab; 9112 results.
4. MEDLINE; bupivacaine.af; 11943 results.
5. MEDLINE; exp BUPIVACAINE/; 9295 results.
6. MEDLINE; (lidocaine OR lignocaine).af; 25708 results.
7. MEDLINE; exp LIDOCAINE/; 20195 results.
8. MEDLINE; (levobupivacaine OR chirocaine).af; 655 results.
9. MEDLINE; ropivacaine.ti,ab; 2287 results.
10. MEDLINE; ropivacaine.af; 2538 results.

11. MEDLINE; 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10; 114426 results.

12. MEDLINE; (articular AND cartilage).af; 26971 results.

13. MEDLINE; exp CARTILAGE, ARTICULAR/; 20737 results.

14. MEDLINE; chondrolysis.af; 404 results.

15. MEDLINE; 12 OR 13; 26971 results.

16. MEDLINE; 11 AND 15; 112 results.

17. MEDLINE; 11 AND 14; 24 results.

18. MEDLINE; 17 not 16; 6 results.

19. EMBASE; (local AND anaesth* OR local AND anesth*).af; 64662 results.

20. EMBASE; exp ANESTHETICS, LOCAL/; 155082 results.

21. EMBASE; bupivacaine.ti,ab; 9112 results.

22. EMBASE; bupivacaine.af; 25473 results.

23. EMBASE; exp BUPIVACAINE/; 24530 results.

24. EMBASE; (lidocaine OR lignocaine).af; 53170 results.

25. EMBASE; exp LIDOCAINE/; 50201 results.

26. EMBASE; (levobupivacaine OR chirocaine).af; 1708 results.

27. EMBASE; ropivacaine.ti,ab; 2943 results.

28. EMBASE; ropivacaine.af; 5625 results.

29. EMBASE; 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28;

187699 results.

30. EMBASE; (articular AND cartilage).af; 25233 results.

31. EMBASE; exp CARTILAGE, ARTICULAR/; 17407 results.

32. EMBASE; chondrolysis.af; 681 results.

33. EMBASE; 30 OR 31; 25233 results.

34. EMBASE; 29 AND 33; 141 results.

35. EMBASE; 29 AND 32; 53 results.

36. MEDLINE,EMBASE; Duplicate filtered: [11 AND 15], [29 AND 33]; 253 results.

37. MEDLINE,EMBASE; Duplicate filtered: [11 AND 15], [29 AND 33]; 253 results.

38. MEDLINE,EMBASE; Duplicate filtered: [11 AND 15], [29 AND 33]; 253 results.

39. MEDLINE, EMBASE; Duplicate filtered: [11 AND 15], [29 AND 33]; 253 results.

40. EMBASE; 35 not 34; 33 results.

Methods of the review

The list of articles was reviewed to exclude duplicate articles. Abstracts of all the remaining studies were reviewed for the pre-defined inclusion and exclusion criteria. Following this, full texts of the remaining articles were examined and final study selection was performed. Data including patient demographics, index procedure, risk factors for chondrolysis, treatment received and outcomes was extracted from all clinical studies. For the laboratory studies, data on study design, study population, main results and conclusions were recorded.

Section 1.6.3 Results

A total of 289 studies were identified using the two databases. Manual searching of reference lists revealed three more studies not picked up in the original search. The study selection process has been summarised in figure 1.5. There was only one foreign language study that satisfied the inclusion criteria but a translated copy was not available via the British library. After study selection, 41 studies including 18 case series and 23 laboratory studies were included. We did not find any

randomised controlled trials that had assessed the safety of different local anaesthetics.

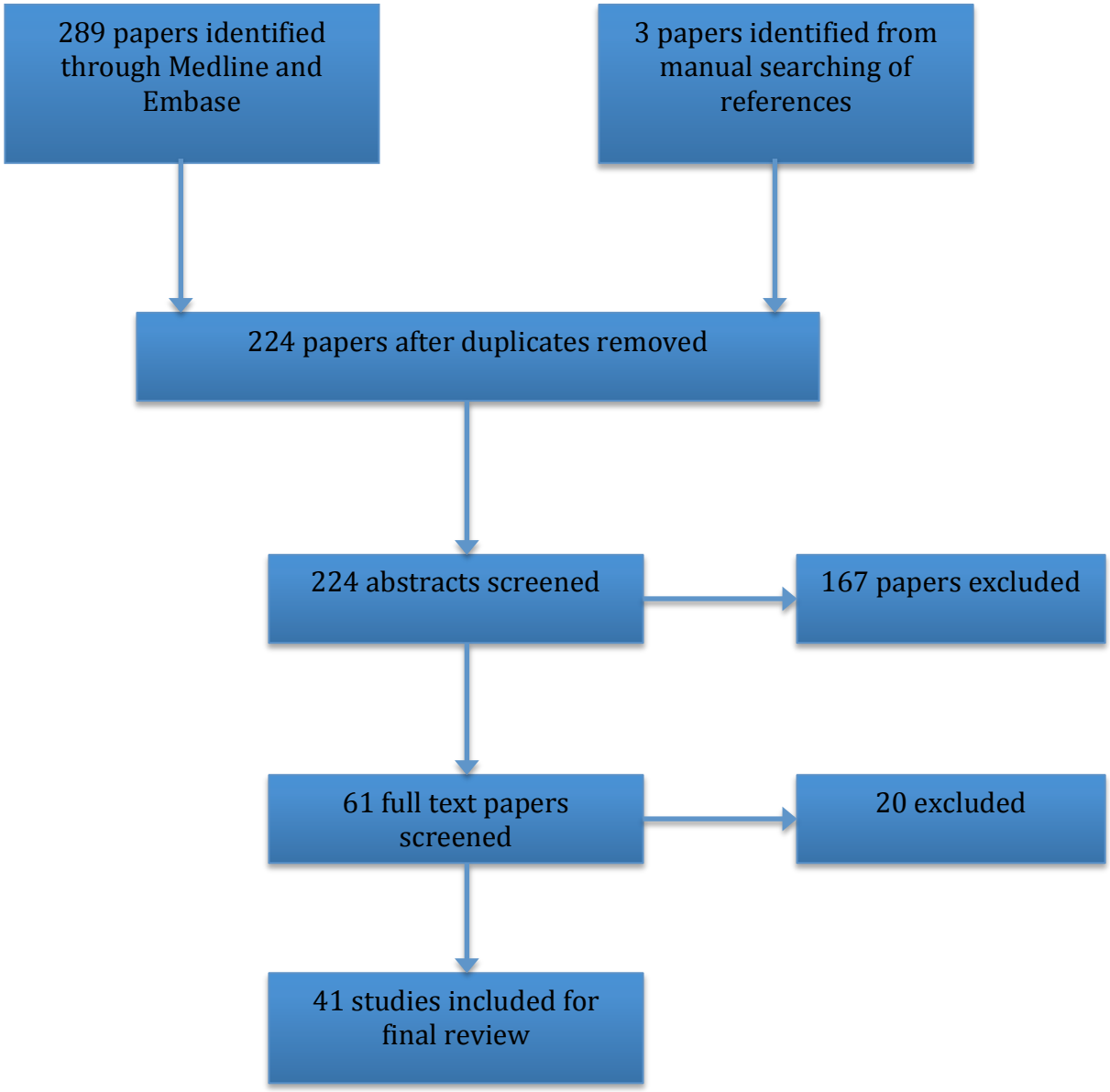

Figure 1.5 Flow chart demonstrating the study selection process used for the systematic review.

Clinical cases

We found 167 cases of chondrolysis in 163 patients associated with intra-articular local anaesthetic infusion or infiltration. Of these, 149 (89.3%) cases involved the Gleno-humeral joint. Other joints involved included the knee (16 cases) and one case each involving the ankle and elbow joints. 163 (97.7%) cases involved continuous intra-articular infusion via a pain pump.

There were only four cases of chondrolysis following a single intra-articular injection of Bupivacaine that not been delivered via a pain pump. Bailie et al (Bailie and Ellenbecker, 2009) described 23 cases of Glenohumeral chondrolysis who had undergone a variety of arthroscopic procedures. While 19 of these cases had documented use of intra-articular pain pumps, suture anchors or radiofrequency probes, four patients did not have any of the above treatments. However, all 23 patients had been given an intra-articular injection of 20 ml of 0.25% Bupivacaine with 1:200,000 Epinephrine.

The infusate was known in 105 cases. Bupivacaine was the agent infiltrated via the pain pump in 103 cases. The concentration of Bupivacaine varied from 0.25% to 0.5%. The rate of infiltration varied from 2ml/hr to 4.16ml/hr. Lidocaine 2% use was documented in one case. This was at a rate of 2ml/hr. Ropivacaine 0.2% was used in one case. Wiater et al (Wiater et al., 2011) documented the use of Lidocaine or Bupivacaine in 49 cases but did not confirm the breakdown of cases between the two anaesthetics. The local anaesthetic used was not specified in 9 cases. Epinephrine was used in the pump in 37 cases.

In some ways chondrolysis following exposure of articular cartilage to local anaesthetic appears to be an idiosyncratic response. However individual case series have reported alarmingly high rates. This incidence was 3 out of 29 (10%) (Rapley et al., 2009), 18 out of 45 (40%) (Anderson et al., 2010), 49 out of 109 (45%) (Wiater et al., 2011) and 12 out of 19 (63%) (Hansen et al., 2007) patients treated with pain catheters in the four studies where the total number of cases was known.

There may be a dose response-element. Anderson (Anderson et al., 2010) found that chondrolysis developed in 16 of 32 (50%) patients who received high-flow (5ml/hr) intra-articular Bupivacaine injection but only in 2 of 12 (17%) who received low flow (2ml/hr) infusion. Rapley et al (Rapley et al., 2009) found that chondrolysis did not develop in 13 cases where 100 ml of 0.5% Bupivacaine was infused at 2.08 ml/hour but developed in 3 out of 16 cases where 270 ml of 0.5% Bupivacaine was infused at 4.16 ml/hour.

The age was available in 91 cases. Mean age was 29.1 years (Range 14-60). Gender was discernible in 69 patients. There were 25 women and 44 men. The time period from the index procedure to the onset of symptoms was available in 85 cases. The mean time from exposure to re-presentation due to symptoms was 11 months (range 0-73 months).

The outcome of chondrolysis is catastrophic. Of the 112 patients (116 shoulders) for whom the outcome was determinable from the reports, 109 (113 shoulders)

underwent 148 procedures including 58 arthroplasty procedures (hemiarthroplasty, resurfacing, total or revision arthroplasty).

The phenomenon of this apparently inexplicable chondrolysis has previously been termed post-arthroscopic gleno-humeral chondrolysis (PAGCL) (Hansen et al., 2007). However, Serrato (Serrato et al., 2011) reported chondrolysis in 4 cases of women aged 52 to 60 years who underwent just manipulation under anaesthesia for frozen shoulder followed by infiltration of Bupivacaine. Arthroscopy no longer seems to be a criterion sine qua non for the evolution of the condition. The continuous intra-articular infiltration of local anaesthetic may alone be sufficient.

There is no direct causal link between local anaesthetic infusions and chondrolysis. However, some studies provide a compelling argument. Wiater et al (Wiater et al., 2011) observed chondrolysis only in those patients that had received a postoperative local anaesthetic pain pump out of a consecutive series of 365 patients. Hansen et al (Hansen et al., 2007) found that 12 out of 19 patients who had an arthroscopic shoulder stabilisation procedure developed PAGCL. The only new addition to the senior author's practice in all patients was the infusion of 0.25% Bupivacaine with Epinephrine for 48 hours. In addition, all patients had also received preoperative and postoperative intra-articular injections of 25 mL of 0.25% Bupivacaine with Epinephrine and 5 mg of morphine sulphate. PAGCL was not seen to develop in 13 patients who had arthroscopic stabilisation but no pain pump or in another 102 patients who had the same pre and postoperative protocol but the pain pump catheter was placed extra-articular in the sub-acromial bursa. Saltzman (Saltzman et al., 2009) gave an account of a patient who underwent

identical shoulder procedure bilaterally. The only difference in the post-operative regimen was that a Lidocaine pain pump was placed in left shoulder but not the right. Chondrolysis developed in the left but not the right.

In a number of cases there were potential confounders. There was documented use of suture anchors in 32 cases, radiofrequency probes in 8 cases, thermal devices in 7 cases and bioabsorbable devices in 17 cases. Wiater et al (Wiater et al., 2011) followed up a single surgeon case series of 365 patients. They found that chondrolysis only occurred in 49 patients out of 109 who all had local anaesthetic pain pumps. No chondrolysis was observed in the remaining 256 patients who did not have a pain pump but had other described risk factors such as suture anchors, thermal and radiofrequency devices. They concluded that pain pumps were the single most important risk factor for chondrolysis in their series of 365 patients.

Table 1.3 summarises all the cases series included in the review.

Author	Patients	Diagnosis	Surgery	Agent	Additional injections	Onset of symptoms	Further Surgery
Petty 2004	18 years, Female	Rotator cuff tear	Arthroscopic cuff repair	0.5% Bupivacaine + Epinephrine		3 months	
Bojescul 2005	21years, Male	Ankle instability	Ankle arthroscopy and lateral ligament reconstruction	Bupivacaine		11 months	
Hansen 2007	12 shoulders in 10 patients Mean age 28.9 years (range 16-47)	Instability	7 capsular shift, 3 posterior Bankart repairs, 2 anterior Bankart	0.25% Bupivacaine with Epinephrine 4.16ml/hr 48 hours	All had pre and post-operative 25 mL of 0.25% Bupivacaine with epinephrine and 5 mg of morphine sulfate without preservative.	Mean 4.3 months (Range 3 to 13 months)	8 patients needed 21 further procedures. 7 arthroplasty procedures.

Levy 2008	9 patients Mean age 41.1 years		2 SLAP repairs, 5 Bankart repair, 2 capsular release	Local anaesthetic pump		16 months	All 9 had total shoulder arthroplasty
Gries 2008	14 and18 years both bilateral, both Female	Instability	Arthroscopic plication	Bupivacaine infusion 0.5%, 4ml/hr for 48hrs	1 shoulder had 20ml 0.5% Bupivacaine with Epinephrine pre-injection	Mean 9 months (Range 4-24 months)	Both patients had 4 further procedures, 2 arthroplasty.
Bailie 2009	17 patients Mean 32 years (range 15-47) 12 Male	SLAP lesion, rotator cuff tear, frozen shoulder, Bankart lesion	Rotator cuff repair, Bankart/ labral repair, Arthrolysis.	250 to 300mL of 0.25%Bupivacaine – 48 hours		9 months	17 procedures - 8 arthroplasty, 9 debridement
	4 patients		Arthroscopy	20 to 30 mL of 0.25% Bupivacaine with 1:200,000 Epinephrine after the procedure			

Fester 2009	3 female patients, 17-21 years	ACL rupture	ACL repair, menisectomy	Bupivacaine pump for 48 hours		Mean 12.6 months (7-21)	One patient had visco-supplementation
McNickle 2009	16 cases Mean age 20.4 years (Range 13-37.4)		3 SLAP, 4 capsulorrhaphy, 4 Bankart repairs, 5 labral or capsular repairs	0.25% Bupivacaine	15 mL 0.5% Bupivacaine	Mean 26 months (Range 3-73)	16 procedures, 8 arthroplasty
Saltzman 2009	37 years, Female		SLAP repair, Bankart repair, capsulorrhaphy, acromioplasty	2% Lidocaine at a rate of 2 mL/hour		Pain never improved	hemiarthroplasty
Rapley 2009	3 patients	Instability	Posterior Bankart, Anterior Bankart, Capsular plication	270 mls of 0.5% Bupivacaine	30 mls of 0.25% Bupivacaine pre and post procedure	3.5 -12 months	2 procedures – 1 arthroplasty

Rey 2009	9 cases Mean age 37 years (Range 26-47)	Instability, cuff tear	Anterior stabilisation, SLAP repair, Cuff repair	Bupivacaine infusion 0.5%, 2 patients had Epinephrine			All 9 had arthroplasty
Anderson 2010	18 cases 15Male Mean age 28 years (Rage 18-30)	Instability`	18 Bankart repair	15 at 0.5% Bupivacaine + Epinephrine at 5 mL/hr and 3 at 2ml/hr	Most patients received 15 to 60ml Bupivacaine at 0.25%-0.5%.	Mean 9.5 months (Range 2-27.5)	19 procedures - 5 resurfacing
Anakwanze 2010	2 female patients, 19 and 26 years	Instability, Labral tear	Arthroscopic capsular plication, Labral repair	Bupivacaine infusion		Pain never improved	4 further procedures, both had arthroplasty.
Serrato 2010	4 female patients, 52-60 years.	Frozen shoulder	Manipulation Under Anaesthetic	0.5% Bupivacaine + Epinephrine at 2 mL/hr	3 patients received 2 mL of Betamethasone and 8 mL of 0.5% Bupivacaine	Mean 10.3 months (Range 3-17)	5 arthroplasty procedures

Slabaugh 2010	17 years, Female	ACL rupture	ACL reconstruction, meniscal repair	48 hours Bupivacaine pump		4 months	Valgus producing high tibial osteotomy with osteoarticular allograft
Wiater 2011	49 cases		Shoulder arthroscopy, cuff repair, labral repair	Bupivacaine or Lidocaine			
Kinkartz 2012	29 years, Male	Elbow arthro-fibrosis	Osteophyte debridement and capsular release	0.20% Ropivacaine for 48 hours		3.5 months	
Noyes 2012	21 patients, Mean age 23 years (Range 14-42), 18 Male		18 ACL Reconstruction, 1 Meniscal Repair 1 Arthroscopy 1 Tibial Tubercle osteotomy	High flow pump– 10, Low flow -10, 0.5% Bupivacaine-20, 0.25% Bupivacaine–1, Epinephrine -11		9 +/- 7 months	19 patients had 41 operations, one arthroplasty

Table 1.3 Summary of all clinical case series of chondrolysis associated with local anaesthetic use

Laboratory studies

The first of the laboratory studies was conducted by Nole et al (Nole et al., 1985) who recognised the increasing use of Bupivacaine following arthroscopy and therefore decided to assess whether it was safe to use on articular cartilage. They examined the effects of 0.5% Bupivacaine solution (diluted in isotonic saline) on articular cartilage of 6-week old pigs and adult dogs. They found that $^{35}SO_4$ incorporation was inhibited acutely compared to control in vitro but was not significantly different when tested 3 days after injection, in an in vivo model. They concluded that the acute inhibition caused by Bupivacaine is normalised by the third day after injection and hence, it could be used safely for intra-articular analgesia.

The potentially harmful effects of Bupivacaine on articular cartilage were first reported by Dogan et al (Dogan et al., 2004) who injected saline or 0.5% Bupivacaine into the knee joints of rabbits and examined cartilage specimens histo-pathologically after 24 hours, 48 hours or 10 days. They found that the specimens from animals that received Bupivacaine showed significantly more inflammatory changes in the form of inflammatory cell infiltration and synovial membrane cell hyperplasia and hypertrophy compared to saline control.

Anz et al (Anz et al., 2009) analysed cartilage and synovium in a canine in vitro model wherein the specimens were exposed to a saline control, 0.5% Bupivacaine or Morphine. They found that Bupivacaine reduced cell viability by 100% compared to saline but did not reduce the tissue concentration of water, collagen

or Glycosaminoglycans. Morphine did not cause any appreciable reduction in cell viability.

Piper and Kim (Piper and Kim, 2008) harvested cartilage explants as well as chondrocyte cultures from the femoral heads or tibial plateau of five patients and exposed them to 0.9% saline control, 0.5% Bupivacaine or 0.5% Ropivacaine for 30 minutes. The explants and chondrocytes were analysed using live/dead cell viability analysis after 24 hours. They found that Bupivacaine had significantly lower cell viability at 78% in the explants and 37% in the cell cultures compared to 94% and 64% for Ropivacaine. They found no significant difference between Ropivacaine and saline control in the explants. However, we believe that saline was a poor choice of control because it has been shown to inhibit proteoglycan metabolism in in-vitro studies (Bulstra et al., 1994, Gulihar et al., 2012). The use of a cartilage culture medium instead probably would have shown more toxicity with Ropivacaine.

Farkas et al (Farkas et al., 2010) found that even Ropivacaine can be harmful to articular cartilage when used at a higher concentration. They assessed the effect of 1% Lidocaine, 0.5% Bupivacaine or 0.75% Ropivacaine with or without Gluco-corticoids on human chondrocyte cultures or osteochondral explants obtained from four human femoral heads using cell viability and flow cytometry analysis. They found significantly increased number of dead/ necrotic cells with all anaesthetics and this toxicity worsened when Betamethasone was added to the solutions. Toxicity increased when duration of exposure was increased from 2 hours to 6 hours or 24 hours.

Miyazaki et al (Miyazaki et al., 2011) assessed the effect of increasing concentrations of Lidocaine from 0.125% to 1% on bovine articular cartilage after 1 hour, 12 hours and 24 hours exposure. They found that the number of viable cells using confocal microscopy reduced as the concentration and duration of exposure of Lidocaine was increased.

Two studies have assessed the effect of Mepivacaine and it appears to be less toxic to articular cartilage compared to other anaesthetics. Park et al (Park et al., 2011) compared the effects of 0.5% bupivacaine, 2% Lidocaine or 2% Mepivacaine on equine articular cartilage using cell viability and flow cytometry assays. They found that cell viability was 29% after 30 minutes of Bupivacaine exposure, 67% after Lidocaine exposure and 87% after Mepivacaine exposure. They suggested that Mepivacaine could be a safer alternative to Lidocaine or Bupivacaine. Bolt et al (Bolt et al., 2008) assessed the effect of Mepivacaine Hydrochloride on articular cartilage explants obtained from four patello-femoral joints of two adult horses. On histological assessment, they found increased number of pyknotic nuclei and empty lacunae but found no difference in Glycosaminoglycan content compared to control explants.

Chu et al (Chu et al., 2006) from the University of Pittsburgh, isolated bovine articular chondrocyte into alginate bead cultures and exposed them to 0.5% Bupivacaine or 0.9% saline for 15, 30 or 60 minutes. They used flow cytometry to analyse chondrocytes for apoptotic and dead cells 1 hour, 1 day, and 1 week after Bupivacaine exposure and found 99% cell death/apoptosis with Bupivacaine

specimens compared to 20% for saline. In a second study, Karpie et al (Karpie and Chu, 2007) exposed bovine chondrocytes in alginate bead cultures to 1% Lidocaine or 2% Lidocaine or 0.9% saline (at pH 5.0, 7.0 and 7.4) for 15, 30 or 60 minutes and assessed cell viability/dead/apoptotic cells using flow cytometry after 1 hour, 24 hours or 1 week. They also exposed 8 mm osteochondral cores to 1% Lidocaine or 2% Lidocaine or 0.9% saline at pH 7.4 for 30 minutes and assessed chondrocyte viability using fluorescent microscopy after 24 hours. In both assays, they found significant reduction in the number of live cells after 15-minute exposure to Lidocaine and this worsened after 30 and 60 min exposures. The cell viability was worse with 2% than 1% Lidocaine. Altering the pH of saline solution had no effect on the cell viability.

They then went on to evaluate, in a subsequent study, the effect of 0.125%, 0.25% and 0.5% Bupivacaine on human and bovine articular chondrocytes in both alginate bead cultures and chondral explants (Chu et al., 2008). Compared to a saline control, there was no difference in chondrocyte death or apoptosis after exposure to 0.125% Bupivacaine. Bupivacaine 0.5% had a drastic effect on chondrocytes with less than 5% viable cells remaining after exposure for 15, 30 or 60 minutes. Cell viability was found to be 52% one hour after a 30 minute exposure to 0.25% Bupivacaine. Hence, the effect observed was dose dependent i.e. the cell viability decreased as the concentration of Bupivacaine was increased and also as the duration of exposure was increased. Furthermore, no recovery was seen even a week after exposure where cell viability had decreased further with all three Bupivacaine concentrations.

In their final study, Chu et al (Chu et al., 2010) used an in-vivo technique and injected rat knee joints with 100 µl of 0.9% saline control or 0.5% Bupivacaine. They then analysed the articular cartilage at one week, 4 weeks, 12 weeks and 6 months using confocal live/ dead cell microscopy, histological analysis and quantitative cell density analysis. They found no significant differences between Bupivacaine and Saline in terms of cell viability and histological appearance but cell density reduced by up to 50% at six months after injection of Bupivacaine.

One more study has attempted to look at the medium term effects of local anaesthetic exposure. Gomoll et al (Gomoll et al., 2009) infused rabbit shoulder joints with saline, 0.25% Bupivacaine or 0.25% Bupivacaine with epinephrine for 48 hours. Three months after infusion, the rabbits were sacrificed and the articular cartilage was analysed for four parameters; radiological/macroscopic changes, proteoglycan synthesis using $^{35}SO_4$ uptake, proteoglycan content and cell viability using confocal microscopy. They found increased $^{35}SO_4$ uptake to 166% in cartilage exposed to Bupivacaine and to 210% in that exposed to Bupivacaine with Epinephrine. An increase in proteoglycan content was found in both Bupivacaine groups. They did not find any macroscopic/ radiological differences in any of the specimens or any differences in percentage of live/dead cells. Based on their results, they concluded that Bupivacaine did not cause any long-term harmful effects on articular cartilage.

Some of the laboratory studies have not found significant toxicity with local anaesthetics on their own and have tested the effect of epinephrine or preservatives used in local anaesthetic solutions. Dragoo et al (Dragoo et al., 2008)

cultured chondrocytes from the articular cartilage of two patients undergoing total knee replacement and exposed them to one of seven solutions: growth medium (control), 1% Lidocaine, 0.25% Bupivacaine or 0.5% Bupivacaine or the above anaesthetics with Epinephrine. These medications were infused using a standard pain pump but with a system designed to have continuous inflow and outflow of medication and culture medium to try and simulate normal synovial fluid turnover. They measured live: dead cell ratio after infusion for 24, 48 or 72 hours using a fluorescent microscope. They found that at all time periods, solutions containing Epinephrine were more toxic than control, of which, 1% Lidocaine with Epinephrine was the most toxic. None of the local anaesthetic solutions without Epinephrine were toxic at 24 hours, 1% Lidocaine produced reduced cell viability at 48 hours while 0.5% Bupivacaine produced reduced viability at 72 hours. The authors wondered whether the reduced pH (≤ 4.5) of solutions containing Epinephrine contributed to the toxicity or whether it could be due to the preservatives in the solutions containing Epinephrine. They suggested that single injections of any of the solutions without Epinephrine should be safe in clinical practice. In a follow up study, Dragoo et al (Dragoo et al., 2010) cultured human chondrocytes from three total knee replacement patients to assess the toxicity of low pH, epinephrine, and preservatives found in commonly used local anesthetics. They infused culture media at pH ranging from 4.5 to 6.5 or 1% Lidocaine or 0.25% Bupivacaine with/ without Epinephrine or two different preservatives for 24 hours after which they counted percentage of dead cells using fluorescence microscopy. They found that culture media at pH ≤ 5.0 (70% necrosis) and local anesthetics containing epinephrine at pH 4.0-5.5 (30-40% necrosis) had high cell death rates. The preservative 5mg/mL Sodium Metabisulphite caused 30% cell

death whilst the preservative Methylparaben had no significant effect. The authors felt that the toxicity was seen only with local anaesthetics containing Epinephrine and this was due to a combined effect of low pH and the preservative Sodium Metabisulphite. Hennig et al (Hennig et al., 2010) assessed the effect of 0.5% Bupivacaine with or without the preservative Methylparaben on osteochondral cores obtained from 20 Glenohumeral joints of 10 cadaveric canines. They observed more than 50% cell death with Bupivacaine and Bupivacaine with Methylparaben. Methylparaben did not significantly increase cell death.

Syed et al (Syed et al., 2011) suggested that the low pH of local anaesthetics was unlikely to be a contributor to toxicity. They tested the effect of 0.25% Bupivacaine on human femoral articular (cartilage explants and monolayer cultures) and the effects of buffering the pH to that of synovial fluid (pH 7.4). Bupivacaine 0.25% reduced cell viability to 72% in the monolayer cultures but buffering the pH resulted in worsening of cell viability to 22%.

Jacobs et al (Jacobs et al., 2011) suggested that Epinephrine might even have a protective effect on articular cartilage. They harvested articular cartilage from the knees of four human donors and three patients undergoing Total Knee Arthroplasty (TKA) and cultured the chondrocytes in alginate bead cultures. They tested the effects of 1 and 2% Lidocaine with or without Epinephrine on chondrocytes using Lactate Dehydrogenase (LDH) activity, Interleukin 6 (IL-6) production and live/ dead cell assay after 24 hours, 48 hours and 7 days. They found that LDH activity increased for all solutions at 24 hours but normalised for all except 2% Lidocaine at 7 days. IL-6 concentration was reduced for all solutions

at all durations except for 1% Lidocaine with Epinephrine. This was also seen in the live/ dead cell assay where more than 90% dead cells were seen for all solutions except 1% Lidocaine with Epinephrine. The toxicity of Lidocaine was dose and time dependent as found in previous studies.

Not all studies blame low pH or Epinephrine for this toxicity and have suggested alternative hypotheses. Bogatch et al (Bogatch et al., 2010) cultured bovine articular chondrocytes and exposed them to several different solutions: (i) phosphate buffered saline (PBS) (pH 7.1) or (ii) 1% Lidocaine, 0.25% or 0.5% Bupivacaine with or without Epinephrine or (iii) PBS at pH 4.5, 3.8, 3.4 and 2.4 or (iv) mixture of different local anaesthetics and culture medium or human synovial fluid. They analysed these cultured chondrocytes using flow cytometry to determine dead/live cell ratio. They found that Lidocaine and Bupivacaine with or without Epinephrine produced about 5% reduced cell viability compared to PBS (pH 7.1). Reducing the pH of PBS caused cell death only at pH≤ 3.4. Mixing 0.5% Bupivacaine or 1% Lidocaine with culture medium or human synovial fluid caused the formation of crystals and led to more than 90% cell death. Surprisingly, this precipitation and resultant cell death was not seen with 2% Lidocaine. The authors felt that local anaesthetics on their own or low pH or the presence of Epinephrine do not cause significant toxicity. The chemical incompatibility between the anaesthetic solution and human synovial fluid was suggested as a possible mechanism of toxicity.

The mechanism of chondrocyte toxicity at a molecular level has been studied by Grishko et al (Grishko et al., 2010), who used chondrocyte cultures from patients

undergoing total knee replacement and exposed them to 0.9% saline solution or different concentrations of Lidocaine, Bupivacaine or Ropivacaine for one hour. They then assessed the number of dead and apoptotic cells by flow cytometry after 24, 72 and 120 hours. They also assessed the exposed cultures for mitochondrial DNA damage, changes in Adenosine Triphosphate (ATP) production and mitochondrial protein levels. They found that 2% Lidocaine caused almost 100% cell necrosis at 24 hours while 1% Lidocaine and 0.5% Bupivacaine caused some reduction in cell viability. At this stage, 0.25% Bupivacaine, 0.5% Ropivacaine and 0.2% Ropivacaine did not cause a reduction in cell viability. Five days after exposure, all concentrations of Lidocaine, Bupivacaine, and Ropivacaine except 0.2% Ropivacaine caused a significant reduction in cell viability with an increase in the number of apoptotic cells. Mitochondrial dysfunction in the form of mitochondrial DNA damage, decrease in ATP and mitochondrial protein levels was seen with all local anaesthetics. Blockade of sodium channels was not thought to be a mechanism responsible for this toxicity. The authors concluded that mitochondrial DNA damage induced by the local anaesthetic solutions led to the development of apoptosis in these cells. They further plan to test whether the addition of DNA repair enzymes can help reduce this toxicity in chondrocytes.

Magnesium Sulphate ($MgSO_4$) has previously been suggested as an intra-articular analgesic. Baker et al (Baker et al., 2011b) assessed cell viability in human chondrocytes using light spectroscopy after exposure to Bupivacaine (0.13, 0.25, 0.5%), LevoBupivacaine (0.13, 0.25, 0.5%), Ropivacaine (0.19, 0.38, 0.75%), 10% Magnesium Sulphate or normal saline. They found reduced cell viability with all local anaesthetics except 0.13% Bupivacaine. $MgSO_4$ and Normal saline did not

cause a significant reduction in cell viability. They suggested that $MgSO_4$ could be used as an alternative intra-articular analgesia. Another study (Baker et al., 2011a) conducted by the same authors looked at the effect of the addition of $MgSO_4$to different local anaesthetics. They found that 0.5% Bupivacaine, 0.5% LevoBupivacaine, 0.75% Ropivacaine and 2% Lidocaine all reduced cell viability. When $MgSO_4$ was added in varying concentrations (10, 20 and 50%), the cell viability of all anaesthetics was seen to improve.

All the laboratory studies assessing the effect of different local anaesthetics on articular cartilage are summarised in table 1.4.

Author	Local Anaesthetic/ additive	Study design	Salient Findings
Nole 1985	Bupivacaine 0.5%	$^{35}SO_4$ uptake-in vitro study in pigs	Reduced uptake but normalised by 3 days
Dogan 2004	Bupivacaine 0.5%	Histo-pathological examination-rabbits	Histo-pathological changes at 10 days
Chu 2006	Bupivacaine 0.5%	Flow cytometry – bovine alginate bead cultures and cell viability- osteochondral cores	99% cell death or apoptosis after 60 minute exposure in alginate bead cultures and 42% cell death in osteochondral cores
Karpie and Chu 2007	1 and 2% Lidocaine	Flow cytometry – bovine alginate bead cultures and cell viability- osteochondral cores	Both concentrations caused chondrocyte death, 2% >1%.
Bolt 2008	Mepivacaine	Chondral explansts from equine patello-femoral joints Histological assessment and GAG content	Increased number of pyknotic nuclei and empty lacunae with Mepivacaine exposure No difference in GAG content after Mepivacaine

Chu 2008	Bupivacaine 0.125%, 0.25% and 0.5%	Cell viability using confocal microscopy - human and bovine bead cultures and chondral explants	Bupivacaine 0.125% not toxic compared to saline. 5% viability with 0.5% Bupivacaine and 52% with 0.25%.
Dragoo 2008	Lidocaine/ Bupivacaine with Epinephrine	Cell viability using confocal microscopy - human chondrocytes – continuous in vitro infusion	No toxicity with 1% Lidocaine, 0.25% Bupivacaine, 0.5% Bupivacaine at 24 hours but all three solutions toxic with epinephrine
Piper and Kim 2008	0.5% Bupivacaine and 0.5% Ropivacaine	Cell viability using Fluorescence microscopy – human explants	78% viability with Bupivacaine and 94% with Ropivacaine. No difference between Ropivacaine and saline
Anz 2009	Bupivacaine 0.5%	Cell viability using confocal microscopy - canine model	100% reduction in cell viability
Gomoll 2009	0.25% Bupivacaine with/without epinephrine	In vivo – rabbits-radiology, $^{35}SO_4$ uptake, PG content, cell viability at 3 months	Increased PG content in the Bupivacaine groups. Metabolism 166% of saline in Bupivacaine only group, 210% in Epinephrine group

Bogatch 2010	Lidocaine/ Bupivacaine with Epinephrine Combination of synovial fluid and local anaesthetic	Cell viability – bovine chondrocytes	No toxicity with 1% Lidocaine, 0.25% Bupivacaine, 0.5% Bupivacaine with or without epinephrine. Crystallisation when synovial fluid mixed with 0.5% Bupivacaine or 1% Lidocaine.
Chu 2010	Bupivacaine 0.5%	In vivo- rat knees. Cell viability, density and histology at 1,4,12 weeks and 6 months.	Reduced cell density with Bupivacaine at six months. No other differences with saline
Dragoo 2010	Effect of pH, 1% Lidocaine/0.25% Bupivacaine with Epinephrine, Preservatives	Cell viability using confocal microscopy - human chondrocytes – continuous in vitro infusion	Culture medium pH <5.0 – 70% cell death. Local anaesthetic with epinephrine – 40% cell death. Sodium Metabisulphite – 30% cell death. Methylparaben – no toxicity.

Farkas 2010	1% Lidocaine, 0.5% Bupivacaine or 0.75% Ropivacaine with or without Gluco-corticoids 2,6, 24 hours exposure	Human chondrocyte cultures or osteochondral explants - human femoral heads using cell viability and flow cytometry analysis	Increased cell death with all anaesthetics Cell viability reduced due to addition of Betamethasone Cell death increased with increasing duration of exposure
Grishko 2010	Lidocaine, Bupivacaine, Ropivacaine. Mechanism of toxicity.	Human chondrocytes – cell viability, mitochondrial function using DNA damage, ATP and mitochondrial protein levels.	2% Lidocaine the only one toxic at 24 hours. At 5 days, 1 and 2% Lidocaine, 0.25 and 0.5% Bupivacaine and 0.5% Ropivacaine were toxic. Damage to Mitochondrial DNA seen with all concentrations.
Henning 2010	Bupivacaine 0.5% with/ without Methylparaben	Osteochondral explants from Gleno-humeral joints of canine cadavers	More than 50% cell with Bupivacaine with/ without preservative. No increase in toxicity due to preservative.

Baker 2011	0.5% Bupivacaine, 0.5% LevoBupivacaine, 0.75% Ropivacaine and 2% Lidocaine Addition of $MgSO_4$ to all solutions above	Human chondrocytes – cell viability using light spectroscopy	All anaesthetics reduced cell viability Addition of $MgSO_4$ to all solutions increased the number of live cells i.e. reduced toxicity
Baker 2011	Bupivacaine (0.13, 0.25, 0.5%), LevoBupivacaine (0.13, 0.25, 0.5%), Ropivacaine (0.19, 0.38, 0.75%), 10% Magnesium Sulphate	Human chondrocytes – cell viability using light spectroscopy	All solutions except 0.13% Bupivacaine significantly reduced cell viability. No difference between control and $MgSO_4$

Jacobs 2011	1 and 2% Lidocaine with or without Epinephrine	Chondrocytes in alginate bead cultures - from TKA patients LDH activity, IL-6 concentration and cell viability	LDH activity increased for all solutions. IL-6 concentration reduced and increased cell death for all except 1% Lidocaine with Epinephrine.
Miyazaki 2011	Lidocaine 0.125% to 1% 1, 12 and 24 hours	Bovine articular cartilage – cell viability using confocal microscopy	Cell viability worsened with increasing concentration and time of expoure of Lidocaine
Park 2011	0.5% bupivacaine, 2% Lidocaine or 2% Mepivacaine	Equine articular cartilage - cell viability and flow cytometry	Cell viability - 29% withBupivacaine, 67% with Lidocaine and 87% with Mepivacaine
Syed 2011	Bupivacaine 0.25%, effect of buffering pH to 7.5	Human femoral condyles – chondral explants and monolayer cultures	Bupivacaine reduced cell viability to 72% Buffering worsened viability to 22%

Table 1.4 Effect of local anaesthetics on articular cartilage – summary of current literature.

Section 1.6.4 Summary of systematic review

1. Chondrolysis is a devastating complication of arthroscopic surgery especially in young patients. Almost all patients require further surgery and a large proportion of them will eventually end up having arthroplasty.
2. Intra-articular local anaesthetic pain pumps have a high risk of Chondrolysis and should be avoided.
3. There is minimal clinical evidence of chondrolysis resulting from a single injection of local anaesthetic.
4. Laboratory studies have demonstrated that Bupivacaine, Lidocaine, Ropivacaine and LevoBupivacaine are all toxic to cartilage.
5. No toxicity has been shown with Mepivacaine but the effect of different concentrations has not been studied.
6. Increase in dose or exposure time makes toxicity worse.
7. Effect of pH combined with local anaesthetics is currently not clear.
8. Effect of adding Epinephrine to local anaesthetics is not clear.
9. Effect of preservatives added to anaesthetics on articular cartilage is also currently not clear.
10. There is very limited evidence on mechanism of toxicity but Mitochondrial DNA damage or chemical incompatibility has been suggested.
11. Combining other drugs may offer some protection. One study has suggested that $MgSO_4$ may have a protective effect.

The findings from this review have been further discussed in Chapter 5.

Section 1.7 Glucosamine

Glucosamine (Figure 1.6) is a naturally occurring 6-carbon amino sugar and is a normal constituent of Glycosaminoglycans and Proteoglycans in articular cartilage (Kirkham and Samarasinghe, 2009). Glucosamine sulphate is a pharmacological derivative and is one of the pharmacological methods used for treatment of osteoarthritis.

Several authors have attempted to describe the pharmacokinetics of Glucosamine in animal and human models. Setnikar et al (Setnikar et al., 1984) demonstrated that Glucosamine sulphate was taken up by articular cartilage in rats after oral or intravenous intake. A further study in dogs showed that Glucosamine rapidly appeared in plasma after oral or intravenous administration and then disappeared into other tissues such as liver, kidney, articular cartilage and bone (Setnikar et al., 1986). Most of the Glucosamine was excreted as CO_2 from breathing, 34% in urine and only 2% in faeces.

Further animal studies have helped in determining an estimated bioavailability of 25%. Setnikar et al also studied (Setnikar et al., 1993) pharmacokinetics after oral, intramuscular and intravenous dosing in six human volunteers. The oral bioavailability of Glucosamine was shown to be 44% in spite of 90% absorption due to a significant first pass metabolism in the liver.

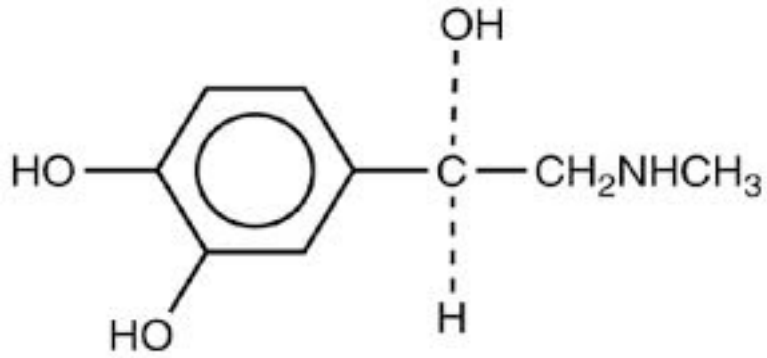

Figure 1.6 Molecular structure of Glucosamine

Persiani et al (Persiani et al., 2005) used liquid chromatography with mass spectrometry detection to determine plasma concentrations in 12 healthy volunteers and found that plasma Glucosamine levels increased as the oral once daily dose was increased from 750 mg to 1500 mg but not at 3000 mg. They estimated the half-life as 15 hours, which confirmed that once daily administration is ideal.

Several laboratory studies have shown that Glucosamine helps to protect and repair experimentally induced articular cartilage damage. Oegema et al (Oegema Jr et al., 2002) demonstrated that oral ingestion of Glucosamine for 8 weeks was able to recover GAG levels in rabbit knee articular cartilage following experimentally induced damage by an injection of the proteolytic enzyme chymopapain.

Dodge et al (Dodge and Jimenez, 2003) isolated chondrocytes from articular cartilage of patients with knee osteoarthritis and incubated them with increasing doses of Glucosamine Sulphate. They found that it increased aggrecan levels and

also decreased metallic metalloproteinase activity. However, chondrocytes from 40% of the patients with OA did not respond to this treatment.

Tiraloche et al (Tiraloche et al., 2005) fed Glucosamine HCl or placebo for 8 weeks to rabbits with or without OA induced by Anterior Cruciate Ligament (ACL) transection and found that PG levels were elevated in the lateral femoral and tibial condyles in the treatment group. They also observed that macroscopic changes in the form of fibrillation and erosions were less pronounced in the treatment group. Interestingly, they did not find a similar effect on the medial side of the knee.

Fenton et al (Fenton et al., 2000) found that equine cartilage degradation induced by lipopolysaccharide was inhibited by Glucosamine−3−sulfate and Glucosamine HCl, but not by N−acetyl− glucosamine. Bassleer et al (Bassleer et al., 1998) found that Glucosamine Sulphate stimulated Proteoglycan synthesis, but had no effect on DNA synthesis, in chondrocytes isolated from arthritic human femoral heads.

Panicker et al (Panicker et al., 2009) injected mouse knees with papain and measured cartilage PG levels and serum inflammatory cytokine levels after two weeks of oral Glucosamine or a normal diet. PG levels were higher in the Glucosamine group after 2 weeks. Levels of cytokines TNF-α, IL-1β and IL-6 peaked earlier and also decreased sooner to normal levels in the treatment group indicating that Glucosamine may also have an anti-inflammatory effect. This effect has been suggested in several other studies (Kim et al., 2011a, Kim et al., 2010, Largo et al., 2009).

Alternative mechanisms have been suggested to explain the protective effect of Glucosamine on chondrocytes. Valvason et al (Valvason et al., 2008) demonstrated that Glucosamine altered gene regulation in human chondrocytes and restored normal expression of haemoxygenase (HO−1) and inducible nitric oxide synthase (iNOS) genes after it was experimentally altered by the administration of IL-1β. The authors suggested that the reduction of oxidative stress might account for the protective effect of Glucosamine on chondrocytes.

Various clinical trails have been conducted to evaluate the effect of Glucosamine in pain and function in patients with arthritis. A randomised double blind trial (Reginster et al., 2001) showed that Western Ontario and McMaster Universities Arthritis (WOMAC) scores improved in patients who were given oral once daily Glucosamine for 3 years as opposed to deterioration in scores in the placebo group. This trial was however, sponsored by the Rotta research group who are one of the major manufacturers of this drug. A second study (Pavelka et al., 2002), also sponsored by Rottapharm, found that patients who had Glucosamine daily for three years had no joint space narrowing compared to 0.2 mm narrowing in the placebo group. These patients also had better scores on the Lequesne index and the WOMAC total index and pain, function, and stiffness subscales.

Trials have also tested parenteral routes of administration. Reichelt et al (Reichelt et al., 1994) demonstrated that symptomatic patients with knee OA who received intramuscular Glucosamine 400 mg for 6 weeks showed a better improvement in the Lequesne index compared to those who received placebo. D'Ambrosio (D'Ambrosio et al., 1981) recorded improvement in pain and range of knee

movement compared to placebo after intravenous or intramuscular Glucosamine for 7 days followed by oral Glucosamine for 2 weeks.

Two studies have tested intra-articular administration of Glucosamine. Crolle et al (Crolle and D'Este, 1980) administered intramuscular or intra-articular Glucosamine to 15 patients for one week followed by two weeks oral treatment. Compared to placebo treatment, both treatment groups had an improvement in pain scores and walking speed. Vajaradul (Vajaradul, 1981) injected 54 patients with once weekly intra-articular Glucosamine injections for five weeks and found that these patients showed a significant improvement in pain and range of flexion 4 weeks after treatment compared to placebo. However, the small number of patients, lack of objective scores and the small treatment period limit the results of these trials. Also, in clinical practice, patients are unlikely to tolerate weekly injections of Glucosamine.

In spite of several studies showing an improvement in symptoms of arthritis after Glucosamine use, it is still controversial as many trials have not been able to demonstrate a significant effect of Glucosamine on pain and function. Rozendaal (Rozendaal et al., 2008) observed that Glucosamine did not prevent deterioration of symptoms in 222 patients with hip osteoarthritis compared to placebo. A series of studies were published from the University of Utah as part of the Glucosamine/chondroitin Arthritis Intervention Trial (GAIT). Clegg et al (Clegg et al., 2006) could not demonstrate a significant effect of Glucosamine or Chondroitin Sulphate on knee pain scores but found that in patients with moderate to severe symptoms, a combination of the two drugs showed an improvement. Two years

later, Sawitzke et al (Sawitzke et al., 2008) did not find any difference in tibio-femoral joint space width between treatment groups and placebo. In their third report, Sawitzke et al (Sawitzke et al., 2010) found trends of improvement in WOMAC pain and function scores in the Glucosamine and Celecoxib groups but no significant differences between treatment groups and placebo.

Towheed et al conducted a Cochrane library review of published randomized controlled trials evaluating the effect of Glucosamine therapy for osteoarthritis in 2001 (Towheed et al., 2001) and published further updates in 2005 and 2008 (Towheed et al., 2005). They included 25 studies and 4963 patients, which showed an overall 22% improvement in pain and 11% in function on the Lequesne Index. They did not find a similar benefit in WOMAC pain and function outcomes but when the results were pooled separately for Rotta or non-Rotta preparations of Glucosamine, the Rotta preparation was found to be superior to placebo in the treatment of pain and functional impairment resulting from symptomatic OA. They found Glucosamine to be as safe as placebo.

The current literature on Glucosamine is summarised in Table 1.5

Variables tested	Study model	Findings
Glucosamine Sulphate	Rabbit knees – after chymopapain damage	Oral Glucosamine recovered GAG levels after 8 weeks ingestion (Oegema Jr et al., 2002)
Glucosamine Sulphate	Human osteoarthritic knee chondrocytes – incubation with Glucosamine	Increased aggrecan levels and reduced MMP activity (Dodge and Jimenez, 2003)
Glucosamine HCl	OA induced in rabbit knees by ACL transection	Increased PG levels and reduced macroscopic changes of OA (Tiraloche et al., 2005)
Glucosamine Sulphate and N-acetyl Glucosamine	Equine cartilage	Inhibition of lipopolysaccharide mediated cartilage damage (Fenton et al., 2000)
Glucosamine Sulphate	Chondrocytes from osteoarthritic femoral heads	Increased PG synthesis, no effect on DNA synthesis (Bassleer et al., 1998)
Glucosamine Sulphate	Mouse knees after feeding oral Glucosamine for 2 weeks	Increased PG levels, some inhibition of TNF-α, IL-1β and IL-6 (Panicker et al., 2009)

Glucosamine Sulphate	Human macrophages– measurement of pro-inflammatory cytokines and their regulation	Inhibition of synthesis of TNF-α, IL-1β and IL-6 and various other cytokines (Kim et al., 2011a)
Glucosamine Sulphate	Human chondrocytes – addition of IL-1β, effect of Glucosamine on gene regulation measured	Glucosamine restored normal expression of HO-1 and iNOS genes and hence reduced oxidative stress on chondrocytes (Valvason et al., 2008)

Table 1.5 Effect of Glucosamine – summary of literature

Section 1.8 Effect of intra-articular Corticosteroids

Corticosteroids such as Triamcinolone and Dexamethasone are often used as intra-articular injections in patients with osteoarthritis. While this is mainly due to their anti-inflammatory action, there also is evidence of Corticosteroids having a protective effect on chondrocytes and articular cartilage. Pelletier et al (Pelletier et al., 1987) found that chondral specimens from patients with knee osteoarthritis, who had received intra-articular steroids, had lower level of proteoglycan degrading MMPs compared to those who did not receive steroids. In a subsequent study, they induced osteoarthritis in dogs by transecting the ACL and manifested by the development of femoral cartilage erosions (Pelletier and Martel-Pelletier,

1989). Dogs that were given an intra-articular injection of Triamcinolone did not develop any lesions and did not show any evidence of cell death or degeneration. In a similar model, they further demonstrated that Methylprednisolone reduced osteophyte formation and histological changes of OA and also suppressed the synthesis of the metalloproteinase stromelysin (Pelletier et al., 1994). Butler et al (Butler et al., 1983) found that Triamcinolone delayed the onset of osteoarthritis in rabbits that had a partial lateral menisectomy and resection of the lateral collateral ligament. Triamcinolone also reduced fibrillation, osteophyte formation, histological abnormalities and chondrocyte loss in knees of guinea pigs injected with sodium iodoacetate (Williams and Brandt, 1985).

Corticosteroids also seem to have a protective effect on non-arthritic articular cartilage. Wang et al (Wang et al., 2004) investigated the effect of Hydrocortisone on chondrocytes obtained from femoral condyles of five donors. They found that levels of aggrecan, type II collagen and fibronectin were increased in the steroid treated cells and ILα and β were inhibited. This inhibitory effect of steroids on IL-1 has previously been well described (Lee et al., 1988, Knudsen et al., 1987).

Several authors have assessed the clinical effect of steroids on patients with osteoarthritis. Robinson et al (Robinson et al., 2007) found an improvement in WOMAC scores for pain, stiffness and disability in male and female patients with hip osteoarthritis 12 weeks after injection of Methylprednisolone. Their effects in patients with osteoarthritis of the knee were summarised by Bellamy et al in a Cochrane review (Bellamy et al., 2006). They found only a short-term improvement in pain that lasted for up to three weeks. They did not find sufficient

numbers to support evidence of their effect longer than 3 weeks. One of the studies included showed that the effect of Methylprednisolone on pain and Lequesne index score lasted for at least 8 weeks (Pyne et al., 2004). Raynauld et al (Raynauld et al., 2003) also demonstrated an improvement in WOMAC pain and stiffness scores with repetitive knee injections of Triamcinolone compared to saline injections every 3 months and this was sustained throughout their two-year study period.

The studies described above have been summarised in Table 1.6

Variables tested	Study model	Findings
Triamcinolone	Human chondrocytes, knee OA patients after intra-articular injection	Reduced MMP levels (Pelletier et al., 1987)
Triamcinolone	Dog knees after ACL transection	Inhibition of macroscopic changes of osteoarthritis (Pelletier and Martel-Pelletier, 1989)
Methylprednisolone	Dog knees after ACL transection	Reduced histological changes of OA and osteophyte formation, inhibition of stromelysin synthesis (Pelletier et al., 1994)
Triamcinolone	Rabbit knees – after lateral menisectomy	Delayed the development of osteoarthritis (Butler et al., 1983)
Triamcinolone	Guinea pig knees after sodium iodoacetate injection	Reduced fibrillation, osteophytes, histological abnormalities and chondrocyte loss (Williams and Brandt, 1985)
Hydrocortisone	Chondrocytes from femoral condyles of young patients	Increase in aggrecan, type II collagen and fibronectin levels, inhibition of ILα and β (Wang et al., 2004)

Table 1.6 Effect of corticosteroids on articular cartilage – summary of literature

Section 1.9 Deficiencies in literature

To our knowledge, there is currently no published literature on the effect of irrigation solutions on human articular cartilage; all previous studies have been in animals. At the time of study design, there were no published studies comparing the effects of different local anaesthetics on human articular cartilage. Soon after, Piper et al (Piper and Kim, 2008) and Chu et al (Chu et al., 2008) published their results followed by other authors. However, to date none of the studies have looked at the effect of Levo-bupivacaine, which is a commonly used local anaesthetic. Also, there are currently no published studies examining the effect of Glucosamine on the metabolism of chondrocytes damaged by the addition of local anaesthetics.

We hypothesised that the impaired chondrocyte metabolism caused by the local anaesthetic solution may be favourably influenced by the concurrent addition of Glucosamine or Corticosteroids.

Section 1.10 Aims

To study the effect of different irrigation fluids on human articular cartilage.
To study the toxic effect of different local anaesthetics on human articular cartilage and the ability of Glucosamine or Corticosteroids to protect against or recover from this toxicity.

Section 1.11 Clinical Significance

The use of toxic local anaesthetics and irrigation fluids during or after arthroscopic surgery may initiate articular cartilage damage in young patients and this may lead to early onset of osteoarthritis. It may also worsen or accelerate cartilage damage in older patients with osteoarthritis who have an arthroscopy for treatment of degenerate meniscal tears or loose bodies prior to a having a joint replacement.

There may be a role for injection of Glucosamine or Corticosteroid after arthroscopy to prevent or reduce articular cartilage damage.

Chapter 2 Materials and Methods

Section 2.1 Study approval

Ethical approval for this study was obtained from the Leicestershire, Northamptonshire and Rutland research ethics committee. Local approval was obtained from the University Hospitals of Leicester NHS Trust research and development department.

Section 2.2 Materials

Cartilage explants were obtained from two different sources:

(i.) The primary source of chondral explants was femoral heads of 24 patients, who had suffered an intra-capsular fracture neck of femur. These patients were scheduled to have a hemiarthroplasty and the femoral head would normally be disposed of as excess human tissue. This injury is generally seen in elderly patients and therefore arthritic changes were seen in some of the femoral heads, (Figure 2.1) while in others, macroscopically normal cartilage was seen (Figure 2.2). Patients with dementia were not included in this study as they were unable to consent for donating excess human tissue. In patients where there was a suspicion that the fracture could be pathological, the femoral head was sent for histology and hence could not be collected for analysis.

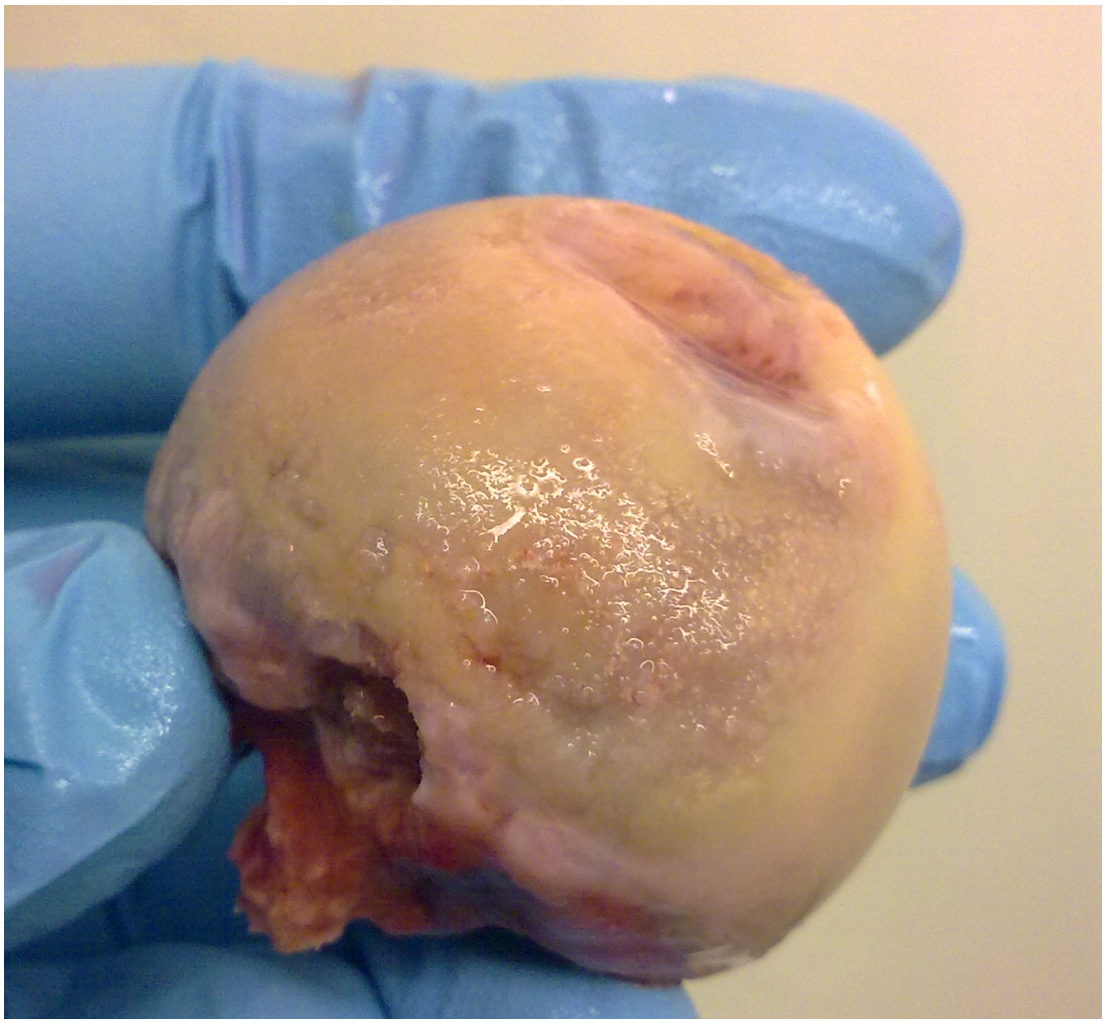

Figure 2.1 Femoral head with obvious macroscopic signs of osteoarthritis including thinning and focal loss of articular cartilage. Parts of the femoral head were spared allowing full thickness chondral explants to be collected.

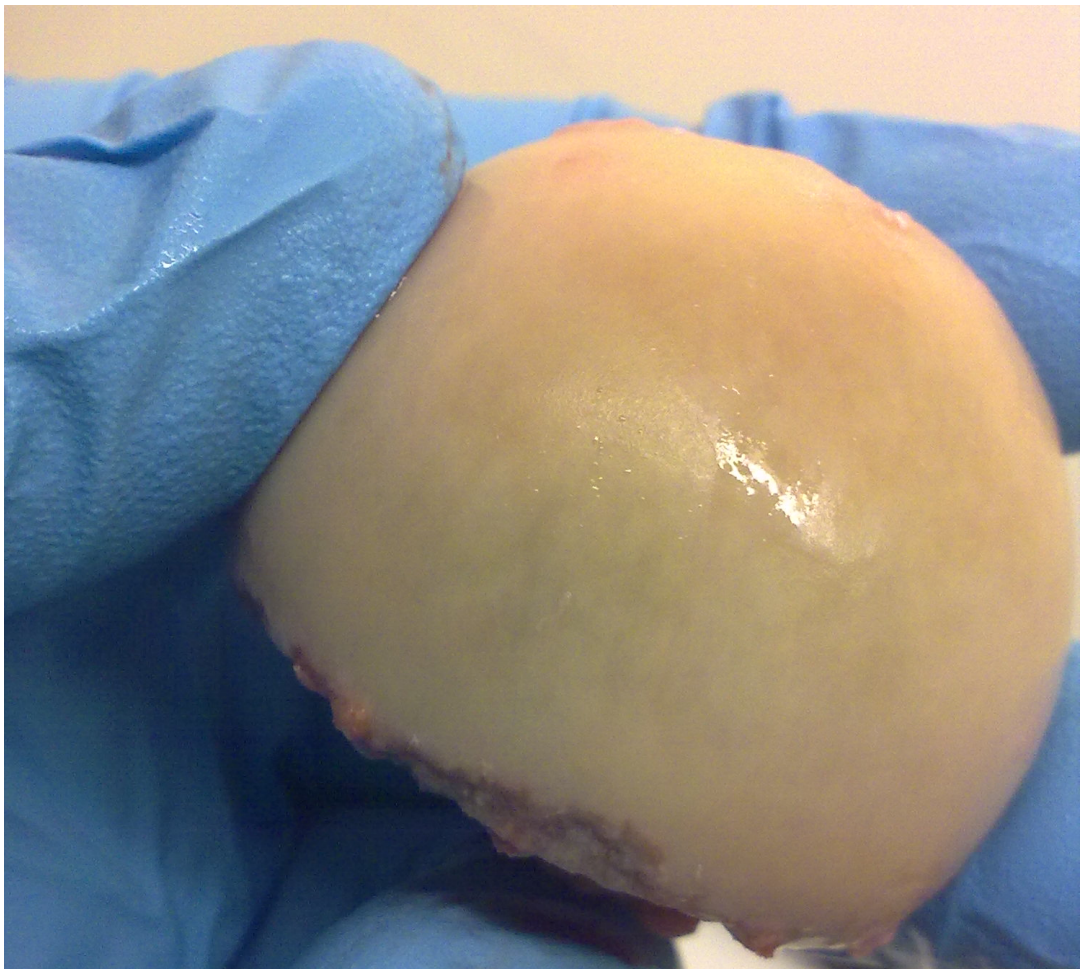

Figure 2.2 Femoral head with a smooth articular surface and no macroscopic signs of osteoarthritis.

(ii.) The second source of chondral explants was tibial plateau from three patients who had been admitted for a total knee replacement. The cartilage from the tibial articular surface in the knee replacement patients was found to be very variable in thickness and consistency. We felt that this could lead to variability in our results and therefore, it was decided to only include femoral heads for the rest of the study.

Trial runs were conducted with the first two femoral heads and the first tibial plateau to develop a study protocol and to be completely familiar with laboratory and radiation handling technique. This was an essential part of training prior to certification as a radiation worker.

Section 2.3 Study Sites

Femoral heads were collected from the trauma unit at the Leicester Royal Infirmary. Tibial plateaus were collected from total knee replacement patients at the Glenfield Hospital. All experiments were conducted in the University of Leicester laboratories based at Glenfield Hospital, Leicester. Initial steps including sterile tissue handling were conducted in a tissue culture laboratory and radiation work was conducted in a designated radioisotope laboratory.

Section 2.4 Preparation

The M199 culture medium was supplemented with 10% foetal calf serum, 250 μg/ml L-glutamine, 50 μg/ml ascorbic acid, 500 IU/ml penicillin and 500 μg/ml streptomycin in the tissue culture laboratory. This was stored at 4 °C once mixed. Prior to femoral head collection, culture medium was warmed to 37 °C and poured into a sterile bottle provided by the Leicester bone bank.

The choice of culture medium and supplements was guided by a previously published study in rat patellar cartilage (Bulstra et al., 1994) and also by two

previous studies conducted in our department. M199 is a widely used culture medium for human and animal tissues including cartilage (Moore et al., 1980, Zheng et al., 2012, Masri et al., 2007, Bulstra et al., 1994). It contains amino acids such as adenine, adenosine, hypoxanthine and thymine that give it a unique advantage over other culture media. It is frequently supplemented with foetal calf serum, which contains a variety of proteins and growth factors with a low level of antibodies. This allows the cells to survive and multiply in the medium. L-Glutamine provides an alternative source of energy for rapidly multiplying cells while Ascorbic acid is a commonly used antioxidant and cofactor. Since M199 is a well-accepted and widely used tissue culture medium, we did not feel that it was necessary to validate its use. However, we felt that the use of a negative control, 0.5% Bupivacaine, in the first part of the thesis would validate its use if it was demonstrated that M199 had the highest uptake while 0.5% Bupivacaine had the lowest uptake.

Section 2.5 Femoral head collection

Patients were identified with the help of the trauma operating theatre co-ordinator on the day of admission prior to surgery. The study was explained in detail to each patient and an information leaflet was provided. Written consent was obtained from each patient. One copy of the consent form was handed over to the patient, one copy was kept for the study records and another copy was filed in the patient's medical records.

Prior to surgery, the collection bottle containing the warmed culture medium was handed over to the operating theatre circulating nurse. After retrieval of the femoral head from the patient, the scrub nurse was instructed to place it straight into the collection bottle, which was held open by the circulating nurse, in order to prevent contamination. The bottle was then screwed tight to seal and transported to the tissue culture laboratory where it was stored in an incubator at 37 °C and 5% CO_2.

Section 2.6 Role of the candidate

For the first 14 patients (11 in experiment one and three trial runs), consenting, femoral head collection, transport and storage and all experiments were performed by the candidate. Dr V Codd supervised initial experiments and laboratory training. In the second part of the study, Mr Mohammad Hadi was recruited to help with femoral head collection to overcome some of the difficulties faced with collection over the weekends. Mr Hadi was able to consent all 13 patients but could only collect femoral heads from 7 patients due to his family commitments. The remaining 6 femoral heads were collected by me. Mr Hadi transported two of these femoral heads to the laboratory from Leicester Royal Infirmary to the Glenfield Hospital. All experiments including data entry were performed by the candidate. Statistical support for data analysis was provided by Dr N Taub and statisticians from the University of Sheffield.

Section 2.7 Technique

We used the established technique of measuring radio labelled sulphate uptake by chondrocytes to form proteoglycans (Meachim and Collins, 1962, Lane and Brighton, 1974, Mankin and Lippiello, 1969). The uptake of sulphur is proportional to the metabolic activity of the chondrocytes (Collins and Mc, 1960). One of the reasons for choosing this technique was to allow a direct comparison with the results of Bulstra et al (Bulstra et al., 1994) who had performed a similar study comparing different irrigation fluids, in rat patellar cartilage. We were also more familiar with this technique as previous studies utilising this method have been conducted in our unit under the supervision of the senior author (Best et al., 2007, Reading, 2000). We would have liked to use an assessment of cell viability to complement our study but the cost of confocal or fluorescent microscopy was well outside our study grant.

Femoral heads or tibial plateaus were retrieved from 27 patients who had suffered a fractured neck of femur or had been admitted for a knee replacement. These were stored immediately at 37 °C in M199 culture medium. The initial steps of the experiment were conducted in a clean air enclosure in a tissue culture laboratory (Figure 2.3).

Figure 2.3 The microbiological safety cabinet where the steps of chondral explant harvesting and exposure to different irrigation fluids and local anaesthetics were performed.

Articular cartilage explants, 4 mm in diameter, were harvested from the underlying subchondral bone using an osteochondral harvester (COR osteochondral repair system, Johnson and Johnson). Although the harvester was designed to collect osteochondral specimens (Figure 2.4), we used it to make a full thickness circular imprint down to bone (Figure 2.5) and then peeled off the cartilage explant using a sharp knife. The explants were then placed in 100 μl of culture medium and weighed. The specimens were then transferred onto a 24 well

plate (Figure 2.6) and exposed to one of several different experimental variables or control M199 solution for one hour.

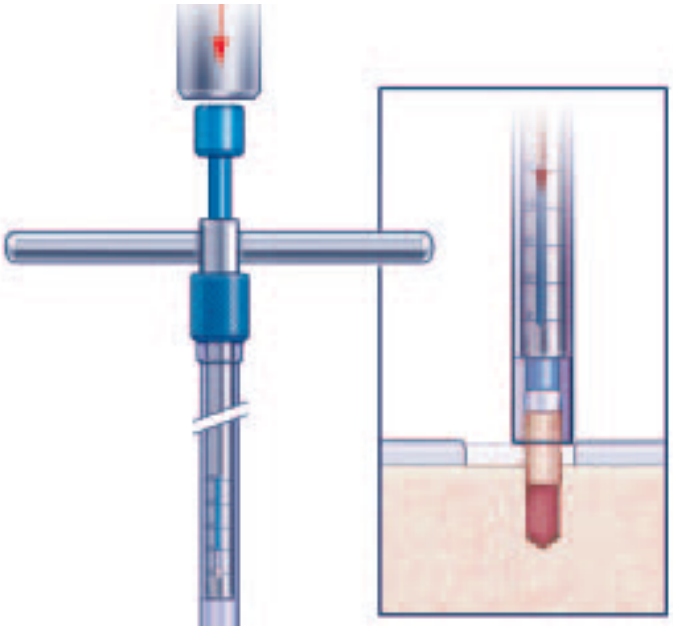

Figure 2.4 Osteochondral harvester used to harvest 4 mm chondral explants. The harvester was designed to take osteochondral specimens.

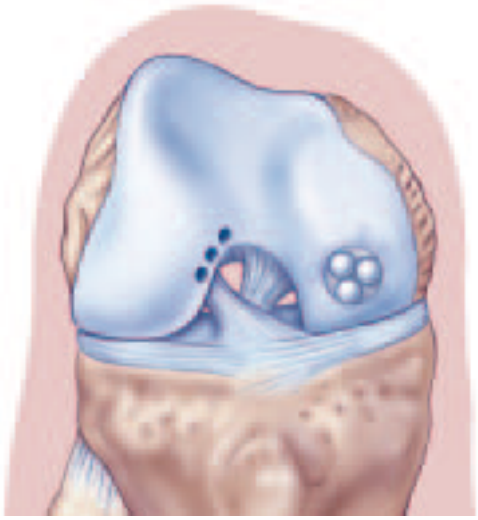

Figure 2.5 The harvester was used to make 4mm full thickness imprints in the cartilage and these explants were then peeled off the bone.

Figure 2.6 A 24-well plate used to expose chondral explants to different irrigation fluids and local anaesthetics.

It has been shown that chondrocytes in explants are viable for up to 60 days in culture media (Brighton et al., 1979). In the first part of this study, explant harvesting and experiments were conducted within 24 hours for all 11 femoral heads. In the second part, explants were harvested from six (Patients 15, 16, 21, 23, 24, 27) out of 13 femoral heads within 24 hours. For the remaining seven femoral heads (Patients 17, 18, 19, 20, 22, 25, 26), this was done between 48 and 72 hours.

It is possible that different areas of the articular surface may have different metabolism and this may affect the $^{35}SO_4$ uptake of the explant. To take this potential variability in metabolism into account, explants were harvested from different areas of the articular cartilage. It was not possible to accurately define specific loaded and non-loaded areas especially in arthritic femoral heads. Therefore, any area from where full thickness explants could be harvested was used. From each area, a number of explants equal to the number of variables being tested were collected. For example, where six solutions including one control were being tested, six explants were obtained from that area and one each was exposed to the test variables or control.

After this, the explants were washed three times with culture medium for twenty minutes each to remove any residual local anaesthetic. Samples were then incubated in M199 containing 5 mCi radio labelled $^{35}SO_4$ (35-S)(Perkin Elmer, Cambridge, UK) for 16 hours at 37 °C and 5% CO2.

The explants were then transferred to a radioisotope laboratory (Figure 2.7) where they were washed three times in phosphate-buffered saline (Sigma-Aldrich Company Ltd., Dorset, UK) for 20 min each. De Vries et al (de Vries Bj Fau - van den Berg et al.) calculated that each cycle of washing removed 95% of unbound radioactive sulphate. To measure the amount of radio-labelled sulphur taken up by the chondrocytes, the cartilage was broken down by proteinase-K (2.5 IU per ml in 0.05 M TriseHCl, 1 mmol CaCl2, pH 7.9; Sigma-Aldrich Company Ltd., Dorset, UK)) for 24 h. McKenzie et al (McKenzie Ls Fau - Horsburgh et al.) showed that this method liberated 95% of incorporated radio nucleotide. The liquid was then drawn off and the specimen centrifuged at 10 000 rpm for 1 min to remove debris. Three 100 μl aliquots from each sample were removed and added to 1 ml Biofluor scintillation fluid (Perkin Elmer, Cambridge, UK). Measurements were made as counts per gram of cartilage per minute (CPG), in a liquid scintillation counter (Figure 2.8). Average of the three readings was taken and used for analysis. This was done on the advice of Dr V Codd, Departmental Radiation Protection Officer (DRPO), and is the recommended standard method for measurement of scintillation counts. Figure 2.9 explains the distribution and demographics of the patients in the two different experiments and Figure 2.10 summarises the protocol used for the experiments.

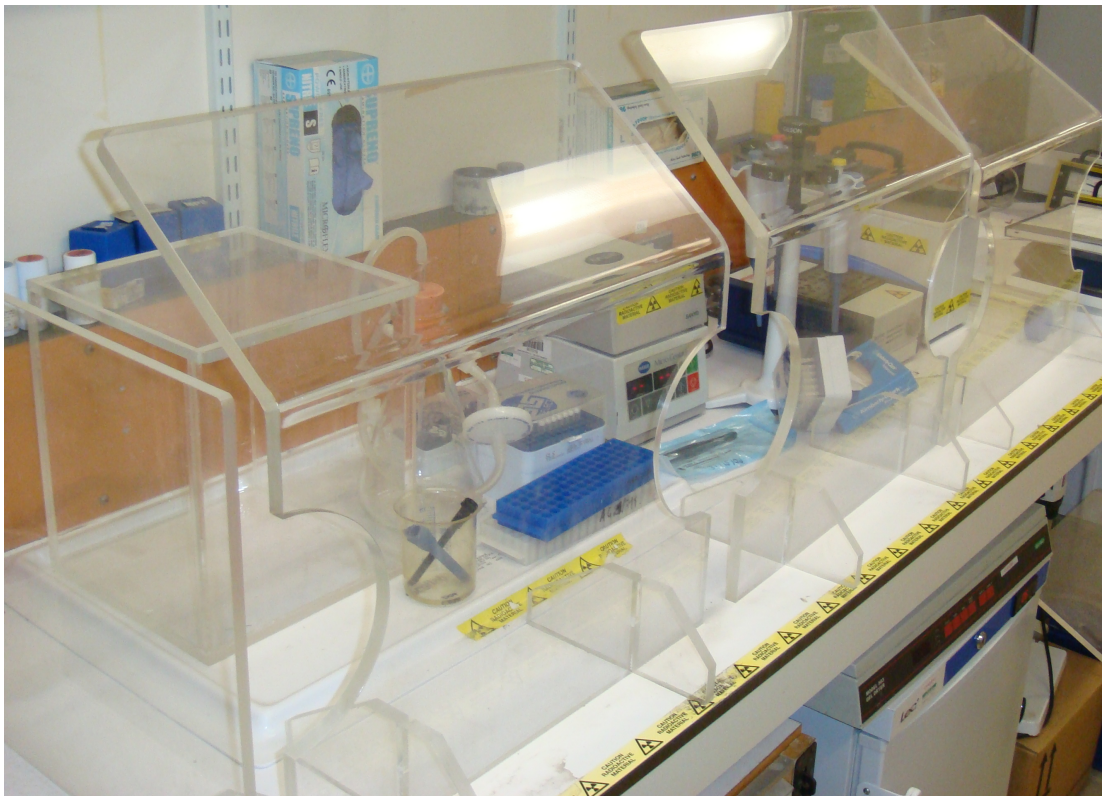

Figure 2.7 The radio-isotope laboratory with protective screens where explant digestion and addition of scintillation fluid was performed.

Figure 2.8 The Packard 1500 liquid scintillation analyser used to measure 35-S uptake by chondral explants.

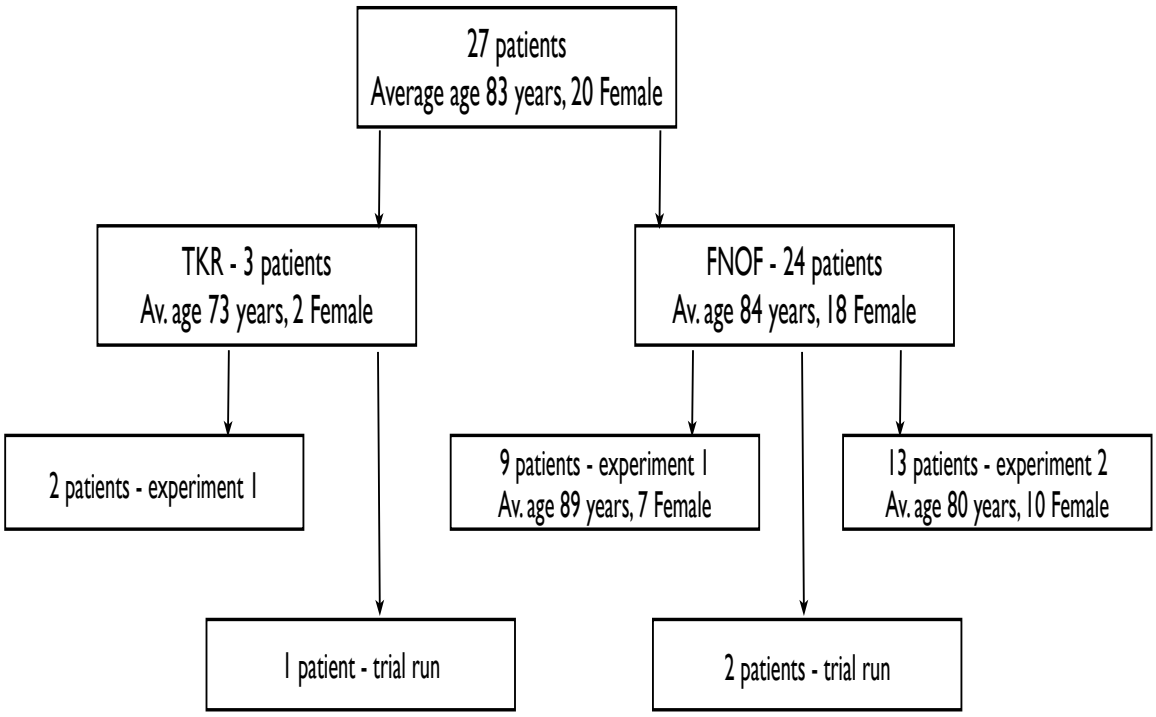

Figure 2.9 Distribution and demographics of patients in the two experiments (TKR – Total Knee Replacement, FNOF – Fracture Neck of Femur, Experiment 1 – comparison of irrigation fluids, Experiment 2 – comparison of local anaesthetics)

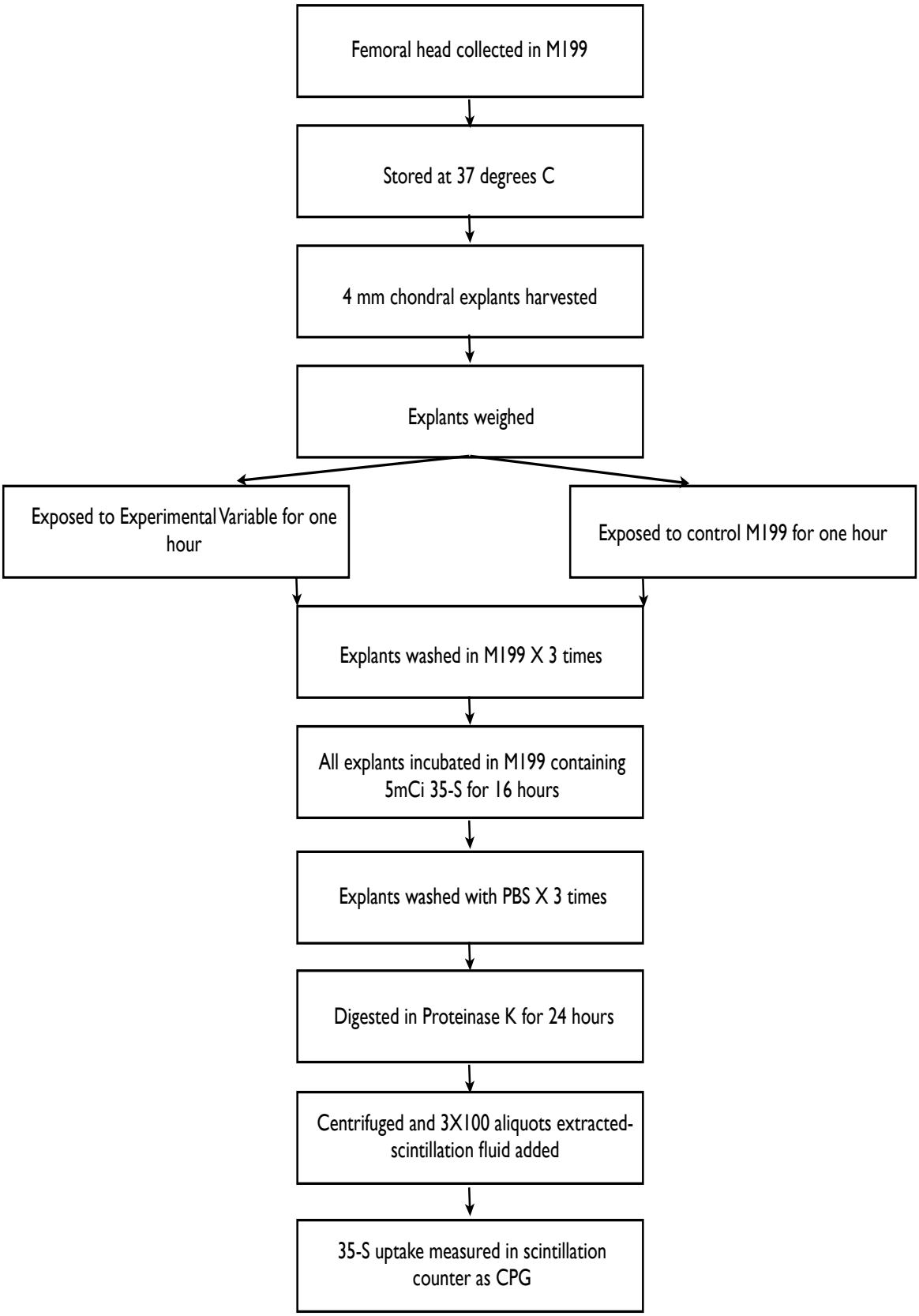

Figure 2.10 Protocol used for measuring 35-S uptake by chondral explants

Section 2.8 Statistical Analysis

Dr Nick Taub from the Trent Research and Development Support Unit (RDSU) had kindly performed the initial sample size estimation. For this purpose, I had obtained articular cartilage $^{35}SO_4$ uptake data (control specimens exposed to M199 medium) from a previous study on rat patellar articular cartilage performed in our department (Reading, 2000). However, this data only included a mean and standard deviation, the actual raw data was not available. Mr Reading, who was working as a Consultant Orthopaedic Surgeon in Birmingham at that time, was contacted for the data but he no longer had this available.

The formula used for sample size was $n = f(\alpha,\beta)(2s/\delta)^2$.

Where:

α is the significance level (0.05)

$1 - \beta$ = power of the test, chosen as 80% (0.8).

δ = the smallest difference regarded as being clinically relevant. We chose this as 10%.

s = standard deviation from previous study.

The sample size obtained was 1600, which was felt to be too high and not realistic since previous studies have demonstrated a difference with a sample size as low as 12 (Bulstra et al., 1994). Dr Taub felt that some pilot data from human articular cartilage was required to make an accurate estimation of sample size and

therefore, suggested that we should obtain pilot data of 15 specimens per variable from initial experiments and then approach him again for a sample size analysis.

Pilot data was collected from 11 patients but by then, the Trent research and development support unit (RDSU) had stopped providing statistical support. Therefore, I approached the university of Sheffield statisticians for further advice. They recommended that a logistic linear regression analysis was suitable for this kind of analysis. They further suggested that a minimum of 10 specimens per experimental variable was required for this analysis. Since I already had at least 36 specimens for each variable from 11 patients, they advised that no further data collection was required for this analysis.

We have analysed 532 chondral explants from 24 patients and this sample size is similar to or larger than most other published studies. Amongst animal studies, Bulstra et al (Bulstra et al., 1994) had 10 rat patellae for each variable, Jurvelin et al (Jurvelin et al., 1994) used 13 bull knees, Karpie and Chu (Karpie and Chu, 2007) had 9 osteochondral cores per variable from 2 bovine knees and Gomoll et al (Gomoll et al., 2009) used 10 rabbit shoulders per variable in their in vivo study. Amongst human studies, Dragoo et al used multiple specimens from two and five human knees in two consecutive studies (Dragoo et al., 2010, Dragoo et al., 2008) and Farkas et al harvested multiple chondral specimens from four femoral heads (Farkas et al., 2010).

There were other problems with femoral head collection such as not being able to consent patients with dementia or pre-operative confusion/ delirium who account

for a large proportion of patients who suffer a fracture neck of femur. Many patients had the femoral heads spent to histology if there was any suspicion of a pathological fracture. Since the processing time for one femoral head is 3 days, experiments could only be started on Monday, Tuesday or Wednesday. For the first part of the study, we only used femoral heads collected 24 hours before chondral explant harvesting. Therefore, patients admitted from Wednesday to Saturday could not be recruited for the study. For the second part of the study, we decided to allow up to 72 hours between femoral head collection and explant harvesting. This still meant patients admitted on Wednesdays and Thursday could not be recruited.

Descriptive statistics were calculated for the 35-S uptake (CPG) of each irrigation fluid and control. The data distribution was found to be skewed and was transformed to log base 10 to overcome skewness and produce a near normal distribution prior to statistical analysis. Geometric means were calculated for each variable and the percentage difference between the 35-S uptake of control and each variable was calculated as percentage inhibition of metabolism using the equation:

$$\frac{^{35}SO_4 \text{ uptake (M199)} - ^{35}SO_4 \text{ uptake (solution)}}{^{35}SO_4 \text{ uptake (M199)}} \times 100$$

Statistical comparisons were conducted between the 35-S uptake of cartilage specimens exposed to different irrigation fluids or local anaesthetics and that of the control group, separately for each fluid.

The independent samples t-test was used for initial comparisons using the log transformed data. However, this analysis does not take into account the potential variability between different patients and treats each cartilage specimen as similar. Therefore, a statistical mixed model taking into account the random effect of each patient was used for the final analysis. This analysis was performed by Dr Taub.

Section 2.8.1 Random and Fixed Effects

A fixed effects analysis assumes that the sample of the general population that are part of the study are identical and that differences between them are not of interest i.e. all the conditions that we are interested in are present in the experiment (Field). This model should ideally be applicable only to the population being studied. A random effects model is applied when the sample population is believed to be a random sample of the general population and that the variance between the subjects may be of interest and not fixed. This model allows for clustering of samples within a dataset (Petrie). In other words, a fixed effects model is more interested in the means of different variables while a random effects model is more interested in the variances. A mixed effects analysis takes into account both the random and fixed effects.

In this study, patients are a random factor because this is just a small sample that has been selected from the general population and there is a possibility of between-subjects variability that the investigator has no control over. Irrigation

fluids or local anaesthetics are the fixed factors because they can be altered by the investigator.

Another example of a mixed effects model would be a study measuring the effects of two different blood pressure medications on a sample population of 100 patients. In addition, several different physicians may prescribe the medications. In this study, the fixed factor would be the medications. The investigators may believe that different patients will respond differently to the same dose of the medications and that the different physicians may also account for some differences in thresholds for prescription. In such a model, both patients and physicians should be considered as random factors.

Section 2.8.2 Effect of osteoarthritis

The effect of osteoarthritis on the 35-S uptake of each fluid was calculated by measuring the interaction between (a) the comparison between each variable and control and (b) the presence or absence of osteoarthritis. Statistical software STATA (StataCorp LP, Texas, USA) was used for the analysis and significance was assumed at $p < 0.05$.

Section 2.8.3 Effect of different areas of femoral head

We wanted to investigate whether the different areas of the femoral heads had different metabolic rates and whether this had an effect on the 35-S uptake of the

chondral explants exposed to different irrigation fluids or local anaesthetics. We were unable to determine the orientation of the femoral heads after collection because they were spherical and we did not mark them for orientation at the time of retrieval. To determine whether the area made any difference to chondral metabolism, we looked at the goodness of fit of data using the mixed effects model for patient or for area. This analysis revealed that the goodness of fit of the data using the Bayesian and Akaike methods was slightly worse when the random effects model was applied by area than when it was applied by patient. This meant that there was no significant effect of the different areas of the femoral heads on 35-S uptake of different variables.

Explants from Experiment 2 (comparison of local anesthetics) were also subjected to another analysis. To confirm that the storage of femoral heads in culture medium for more than 24 hours did not have an adverse effect on 35-S uptake, comparison of explants exposed to M199 culture medium (control specimens) was done between the six femoral heads stored for less than 24 hours and the seven femoral heads stored for up to 72 hours. This was done using the independent samples t-test (log transformed data) because we were only interested in these 13 patients and not the rest of the population.

Chapter 3 Effect of irrigation fluids

Section 3.1 Introduction

This chapter was aimed at comparing the effects of different irrigation fluids on articular cartilage. Four irrigation fluids were tested, two ionic fluids: normal saline and Ringer's solution and two non-ionic fluids: Glycine and Mannitol (Table 3.1). Bupivacaine 0.5% was included as a negative control as we expected it to be toxic to articular cartilage and M199 was used as a positive control.

Section 3.2 Methods

Chondral explants were obtained from 11 patients. Two of these were total knee replacement patients and the remaining nine had suffered a fracture of neck of femur. Six out the nine femoral heads did not have any macroscopic signs of osteoarthritis such as cartilage fibrillation or defects. A total of 228 chondral explants were analysed from these 11 patients. Some of the explants had to be discarded because the osteochondral extractor was introduced too deep and into the bone and this resulted in a small quantity of bone being left attached to the deep surface of the explant. The explants were exposed to one of six solutions as explained in Figure 3.1. The number of specimens selected per irrigation fluid from each femoral head or tibial plateau is demonstrated in Appendix 1. The chemical composition, pH and osmolarity of the fluids are outlined in Table 3.2.

Fluid	Number of specimens
Normal Saline	40
1.5% Glycine	38
M199 (control)	39
Ringer's solution	38
5% Mannitol	36
0.5% Bupivacaine	37

Table 3.1 The different irrigation fluids tested including control solution (M199)

Statisticians from the University of Sheffield were approached with pilot data for sample size estimation. They advised that a logistic linear regression analysis would be suitable and for this kind of analysis, a minimum of ten specimens would be required per variable tested. We decided to collect as many explants as possible from the available patients so that variability due to patient factors could be reduced.

Fluid	Chemical composition	pH	Osmolarity (mosm/L)
0.9% Saline	Sodium Chloride: 9.0 g/L	5.0	308
Ringer's solution	Sodium chloride: 8.60 g/L Potassium chloride: 0.30 g/L Calcium chloride: 0.33 g/L	6.1	309
5% Mannitol	Mannitol 50 g/L	5.0	274
1.5% Glycine	Glycine 15 g/L	6.1	200
0.5% Bupivacaine	Bupivacaine 5.0 g/L Methylparaben (preservative) 1mg/ml	4.0-6.5	

Table 3.2 Chemical composition of the irrigation fluids tested

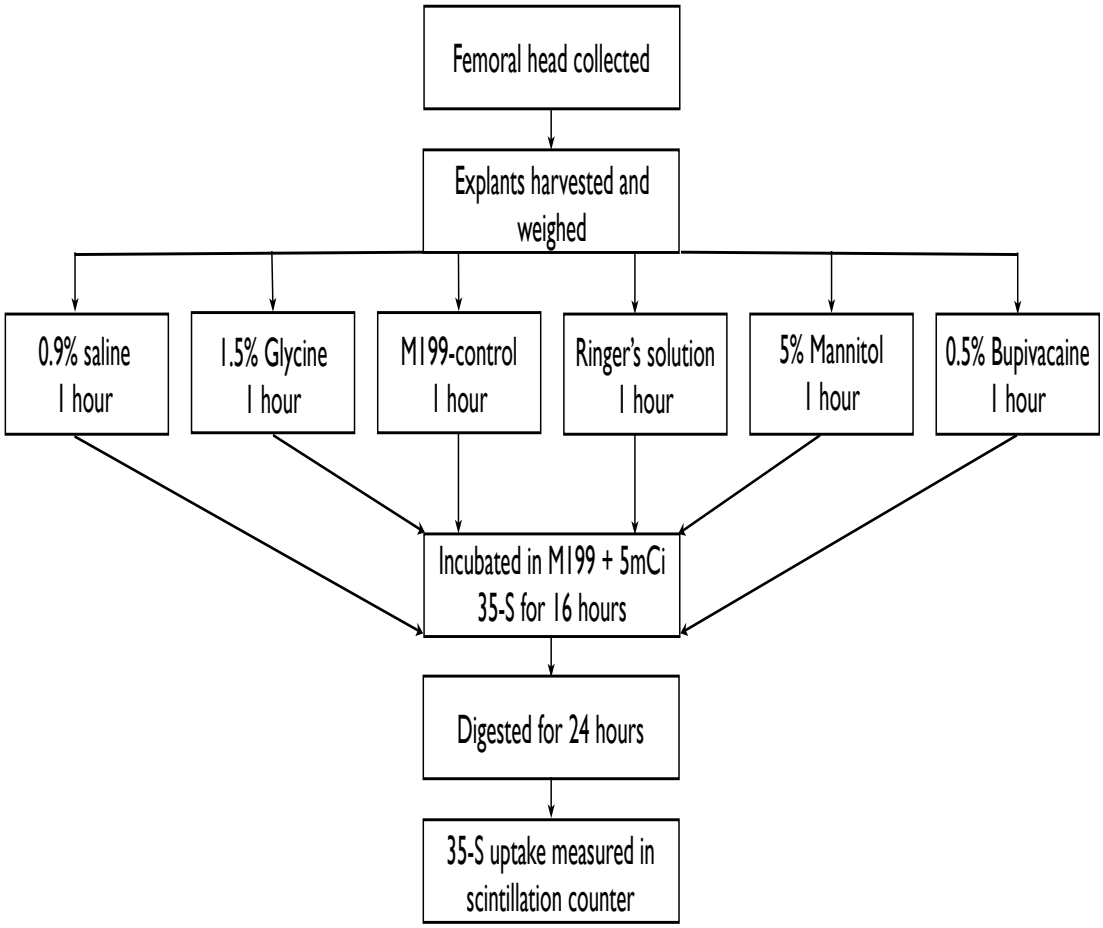

Figure 3.1 Protocol followed for investigating the effect of irrigation fluids on articular cartilage

Section 3.3 Results

The highest uptake of 35-S was seen with the control solution. All other solutions showed reduced uptake compared to the control solution (Table 3.3 and Figure 3.2). The raw data showing 35-S uptake for all specimens is presented in Appendix 2.

	Counts per gram per minute (CPG)			
Fluid	Mean	Std. deviation	95% confidence interval	
M199	65516	42449	55619	75414
Ringer's	57394	34299	47367	67421
5% Mannitol	47818	38783	37516	58120
1.5% Glycine	47649	25197	37622	57676
N saline	42952	26248	33178	52725
0.5%Bupivacaine	9283	9696	-878	19444

Table 3.3 Uptake of 35-S by chondral explants exposed to different irrigation fluids or a control solution

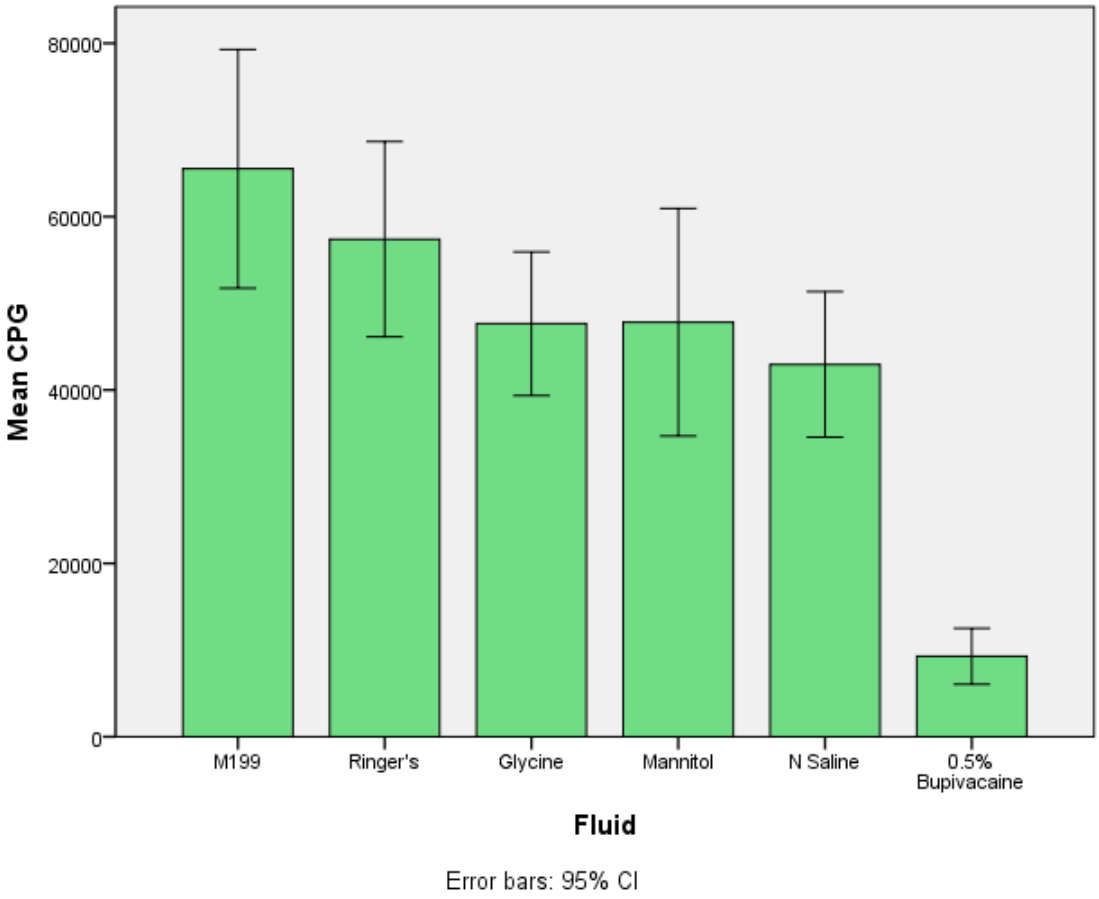

Figure 3.2 Bar chart displaying the uptake of 35-S by chondral explants exposed to different irrigation fluids.

The data was found to have a skewed distribution and log transformation was performed to achieve normal distribution prior to conducting statistical comparisons. Histograms were drawn for irrigation fluid data (Figure 3.3) and for all patients (Figure 3.4) to ensure that log transformation produced a normal distribution.

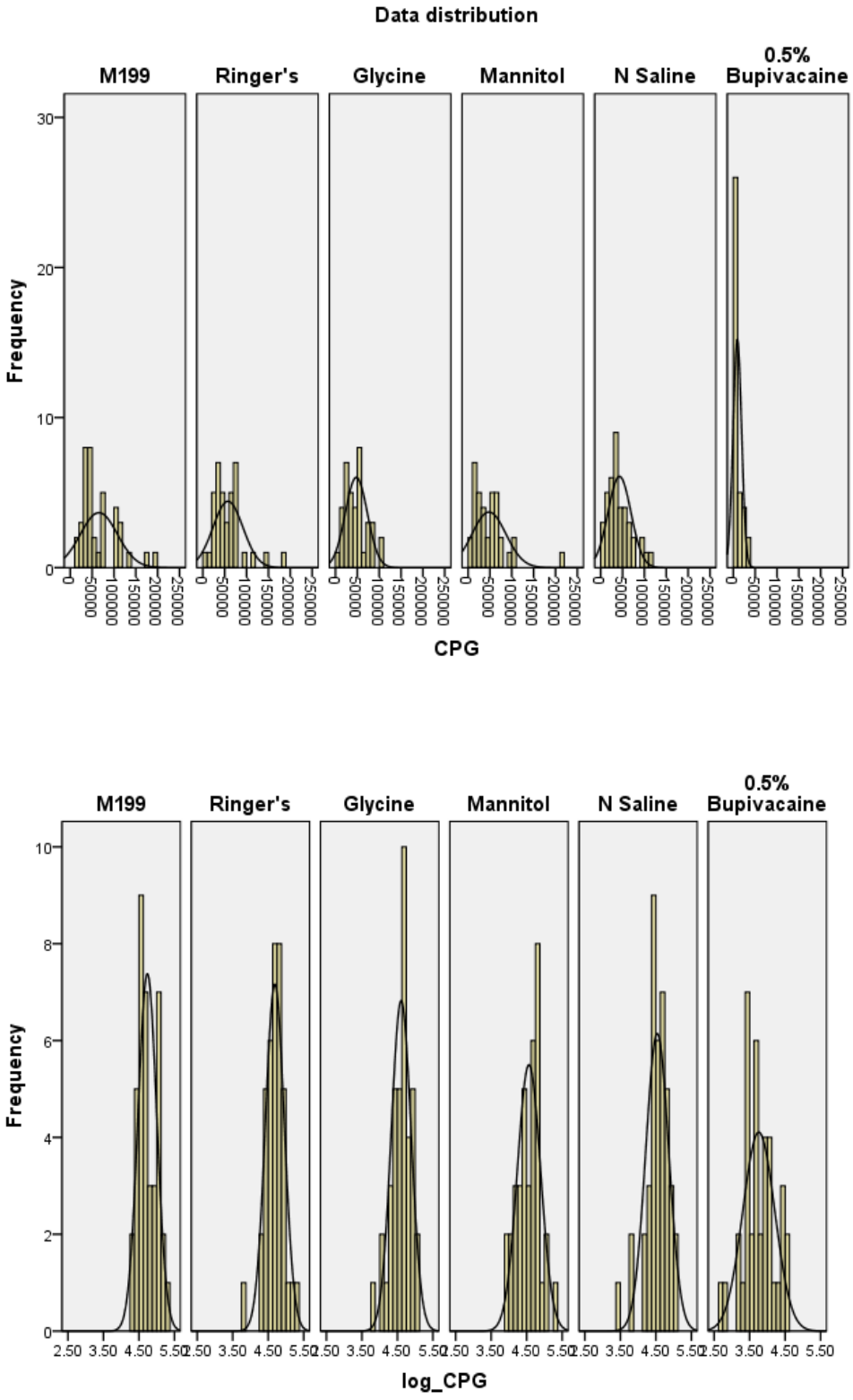

Figure 3.3 Histograms showing the data distribution of 35-S uptake of each irrigation fluid before and after log transformation; performed to convert a skewed distribution into normally distributed data.

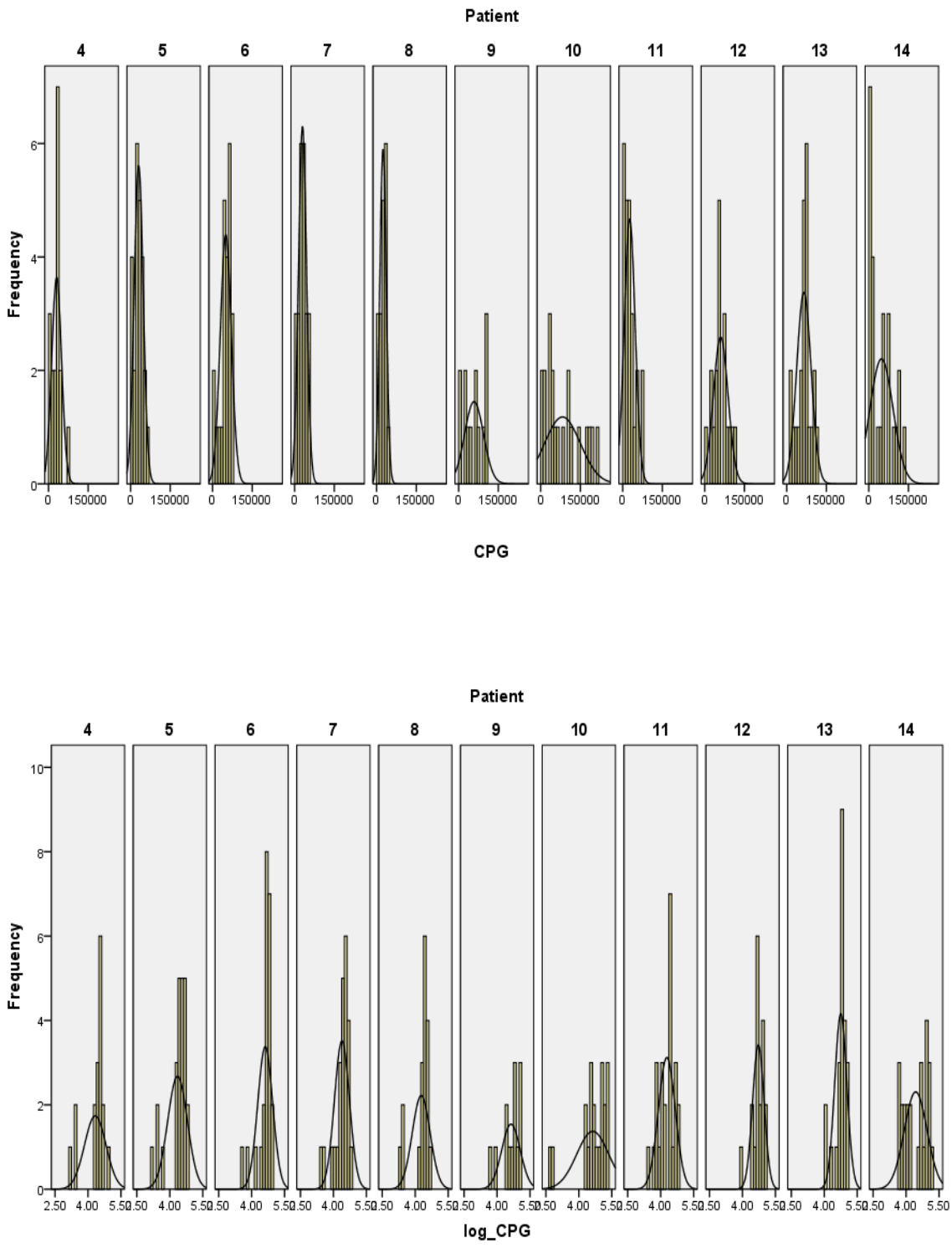

Figure 3.4 Histograms showing the data distribution of 35-S uptake of each patient before and after log transformation.

The reduction in 35-S uptake was expressed as percentage inhibition of metabolism. The percentage inhibition was calculated using the formula in Section 2.8. The inhibition was found to be the least with Ringer's solution at 10% and was the worst for saline at 35% (Table 3.4).

Initial analysis using the independent samples t-test showed that there was a significant difference between the 35-S uptake of normal saline and control ($p=0.004$), 1.5% Glycine and control ($p=0.04$) and 0.5% Bupivacaine and control ($p<0.001$). This difference was not statistically significant for Ringer's solution ($p=0.41$) and Mannitol ($p=0.14$).

Further analysis using the mixed effects model revealed that there was a significant difference between the 35-S uptake of normal saline and control, 5% Mannitol and control and 0.5% Bupivacaine and control. This difference was not statistically significant for Ringer's solution and Glycine (Table 3.4).

Irrigation fluid	%age inhibition*	95% confidence intervals		p-value**
Ringer's	10	-11	28	0.3
1.5%Glycine	24	-4	44	0.08
5%Mannitol	31	3	51	0.03
N Saline	35	1	58	0.04
0.5% Bupivacaine	90	81	95	<0.001

*Percentage based on geometric means.

**p-value compared to control, mixed effects analysis

Table 3.4 The percentage inhibition of metabolism in chondral explants exposed to different irrigation fluids

Chapter 4 Effect of Local Anaesthetics, Glucosamine and Corticosteroids

Section 4.1 Introduction

This part of the study was aimed at assessing the quantitative toxic effect of different local anaesthetics and the ability of Glucosamine or Corticosteroids to protect against or recover from this toxic effect.

Section 4.2 Methods

Chondral explants were harvested from 13 fracture neck of femur patients (patient numbers 15 to 27) as explained in Section 2.6. Seven femoral heads showed obvious signs of osteoarthritis while the remaining six had macroscopically normal cartilage. After weighing, the explants were exposed to one of 10 different experimental conditions for one hour each as outlined in table 4.1. The pH range for all local anaesthetics was 4.0 to 6.5. Explants exposed only to M199 culture medium were used as control. Due to a delay in the delivery of Glucosamine and Methylprednisolone, explants from the first two patients (number 15 and 16) were subjected only to the first six experimental conditions i.e. different local anaesthetics and control.

To assess its protective effect, 10mg Glucosamine-6-sulphate (100 mg/ml solution) was added along with 0.5% Bupivacaine to the specimens in

experimental condition 7. To assess its reparative effect, Glucosamine was added after the specimens had been exposed to 0.5% Bupivacaine for one hour (experiment 8). Similarly, to assess the protective effect of corticosteroid on cartilage, 4mg of Methylprednisolone (Depo-medrone, Pfizer limited, Surrey, UK) was added at the same time as 0.5% Bupivacaine (experiment 9) and to assess its reparative effect, Methylprednisolone was added after the specimens had been exposed to 0.5% Bupivacaine for one hour (experiment 10). Since corticosteroids are used clinically only for patients with osteoarthritis, conditions 9 and 10 were tested only in five femoral heads where the articular cartilage showed macroscopic signs of osteoarthritis.

The number of specimens selected per experimental condition from each femoral head is demonstrated in Appendix 3.

To assess the protective and reparative effect of Glucosamine, a comparison was made between the specimens exposed to 0.5% Bupivacaine only with those exposed to 0.5% Bupivacaine and Glucosamine using a random effects mixed model. Similarly, to assess the protective and reparative effect of Methylprednisolone, a comparison was made between the specimens exposed to 0.5% Bupivacaine only with those exposed to 0.5% Bupivacaine and Methylprednisolone.

Experiment Condition	Number of specimens	Test solution exposure (for one hour)	Recovery Incubation (for 16 hours)
1	38	1% Lidocaine (1ml)	M199 (1ml) + 35-S (5mCi)
2	38	2% Lidocaine (1ml)	M199 (1ml) + 35-S (5mCi)
3	38	0.25% Bupivacaine (1ml)	M199 (1ml) + 35-S (5mCi)
4	38	0.5% Bupivacaine (1ml)	M199 (1ml) + 35-S (5mCi)
5	38	0.5% Levo-bupivacaine (1ml)	M199 (1ml) + 35-S (5mCi)
6	38	Control - M199 (1ml)	M199 (1ml) + 35-S (5mCi)
7	26	0.5% Bupivacaine (1ml) + Glucosamine-6-sulphate (10mg/100μl)	M199 (1ml) + 35-S (5mCi)
8	26	0.5% Bupivacaine (1ml)	M199 (1ml) + Glucosamine-6-sulphate (10mg/100μl) + 35-S (5 mCi)
9	13	0.5% Bupivacaine (1ml) + Methylprednisolone (4mg/100μl)	M199 (1ml) + 35-S (5mCi)
10	13	0.5% Bupivacaine (1ml)	M199 (1ml) + Methylprednisolone (4mg/100μl) + 35-S (5 mCi)

Table 4.1 Experimental conditions used to test the effect of local anaesthetics, Glucosamine and Corticosteroids on articular cartilage. Radio-labelled sulphate (35-S) uptake was compared to the control solution (M199).

Section 4.3 Results

There was no difference between the 35-S uptake of M199 explants from femoral heads stored for less than 24 hours (Mean CPG 51058) and those stored for up to 72 hours (Mean CPG 47408) (p=0.22, independent samples t-test). This confirmed that there were no adverse effects of prolonged storage and all explants could be included for analysis.

Section 4.3.1 Effect of local anaesthetics

All local anaesthetic solutions caused a reduction in uptake of 35-S compared to the control solution. The means and standard deviation of the 35-S uptake (CPG) by the explants in the first six experimental conditions are outlined in table 4.2 and represented in decreasing order of uptake in Figure 4.1. The raw data showing 35-S uptake for all specimens is presented in Appendix 4.

	Counts per gram per minute (CPG)			
Fluid	Mean	Std. deviation	95% Confidence Intervals	
1% Lidocaine	22386	32054	11851	32922
2% Lidocaine	12215	13101	7909	16521
0.25% Bupivacaine	24433	26913	15587	33279
0.5% Bupivacaine	10741	13464	6315	15166
0.5% Levo-bupivacaine	13404	19683	6934	19874
M199	49041	44030	34569	63514

Table 4.2 The 35-S uptake of different local anaesthetics and control.

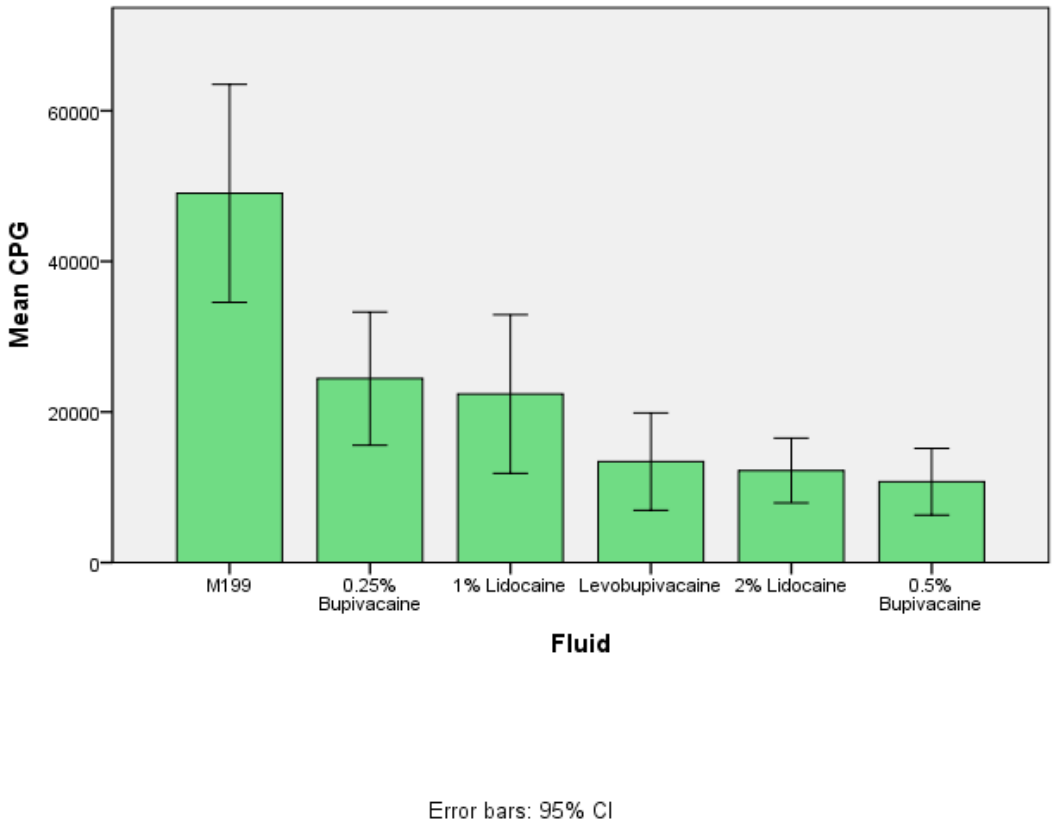

Figure 4.1 Bar chart representing the uptake of 35-S by chondral explants exposed to the different local anaesthetics in decreasing order of activity. Error bars represent 95% confidence intervals.

The data was found to have a skewed distribution and log transformation was performed to achieve normal distribution prior to conducting statistical comparisons. Histograms were drawn for each experimental condition (Figure 4.2) and for all patients (Figure 4.3) to ensure that log transformation produced a normal distribution.

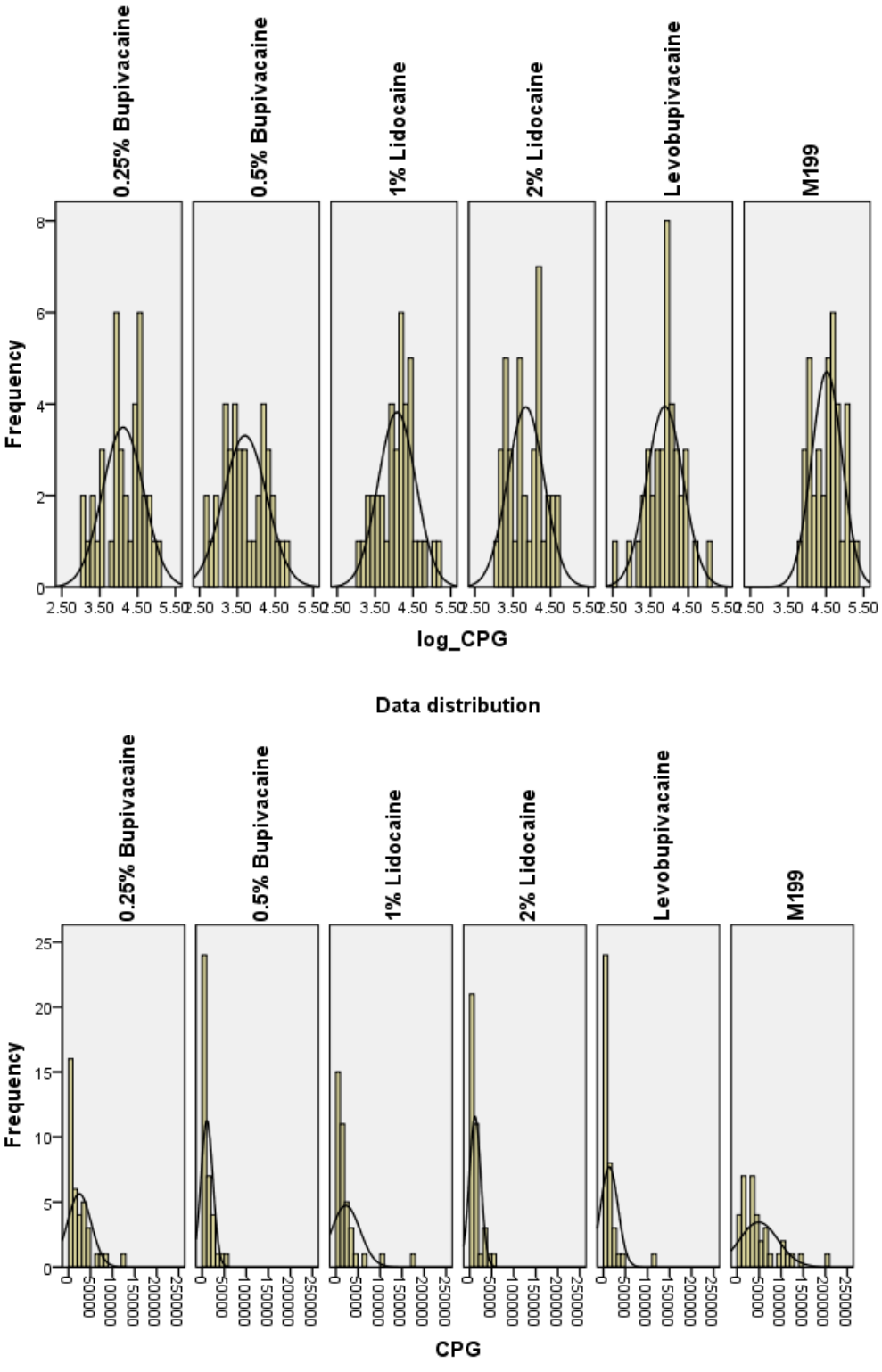

Figure 4.2 Histograms showing the data distribution of 35-S uptake of explants exposed to different local anaesthetics before and after log transformation, performed to reduce skewness and achieve a near normal distribution

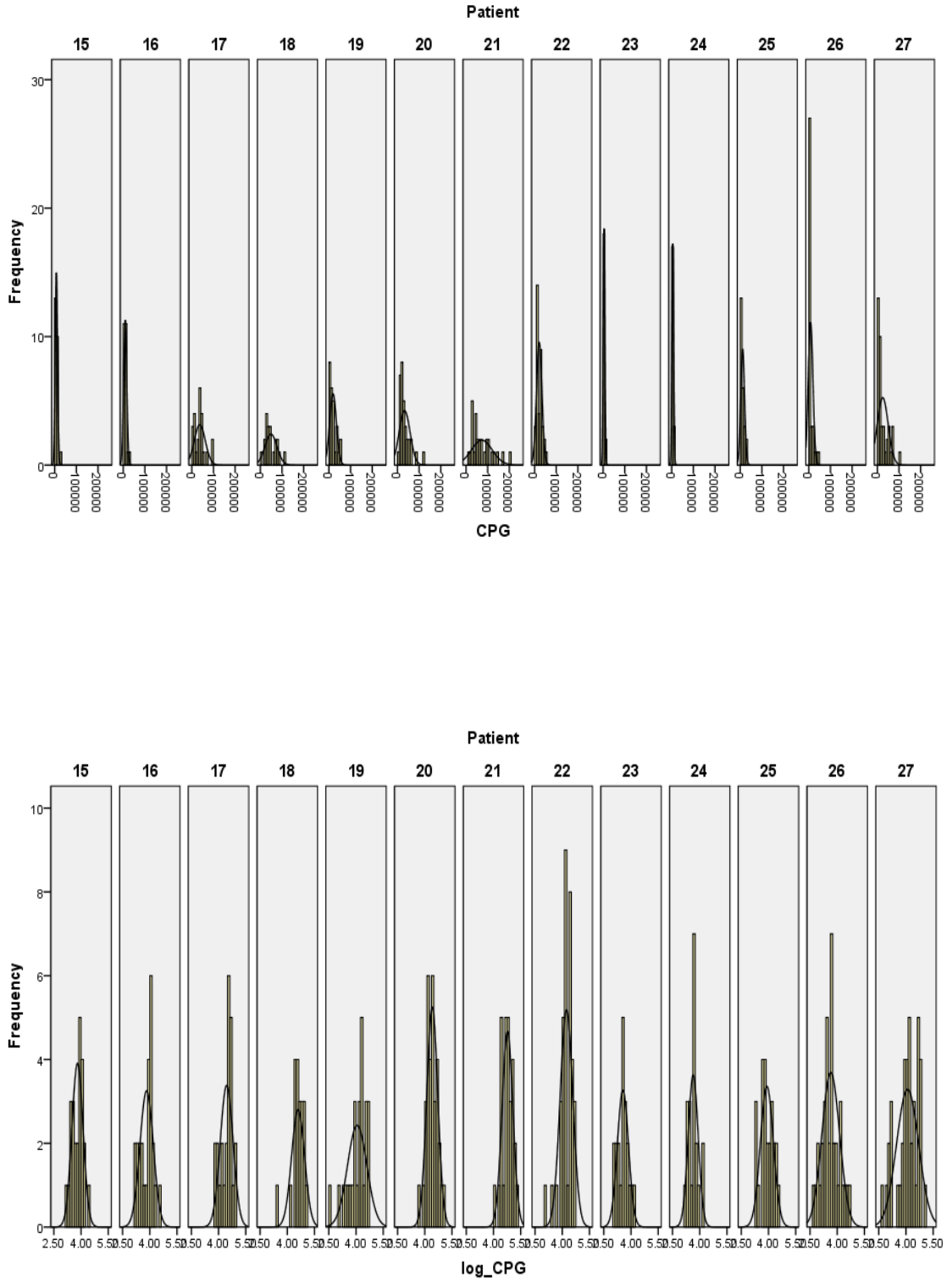

Figure 4.3 Histograms showing the data distribution of 35-S uptake of each patient before and after log transformation.

Initial analysis with the independent samples t-test showed that all local anaesthetics inhibited 35-S uptake significantly compared to the control M199 culture medium ($p<0.001$).

The mixed effects analysis further confirmed that all local anaesthetic solutions caused a significant inhibition of proteoglycan metabolism (Table 4.3). 2% Lidocaine was more toxic than 1% Lidocaine ($p<0.001$) and 0.5% Bupivacaine was more toxic than 0.25% Bupivacaine ($p<0.001$). There was no significant difference between the toxicity of 2% Lidocaine and 0.5% Bupivacaine. Similarly, there was no difference between 1% Lidocaine and 0.25% Bupivacaine.

Local Anaesthetic	%age inhibition*	95% confidence intervals		p-value**
1% Lidocaine	65	49	75	<0.001
2% Lidocaine	79	71	85	<0.001
0.25% Bupivacaine	61	40	75	<0.001
0.5% Bupivacaine	85	71	92	<0.001
0.5% Levo-bupivacaine	77	66	85	<0.001

*Percentage based on geometric means.

**p-value compared to control, mixed effects analysis

Table 4.3 Percentage inhibition of proteoglycan metabolism compared to a control solution after exposure to different local anaesthetic solutions.

Section 4.3.2 Effect of Glucosamine and Corticosteroids

Explants from patient numbers 15 and 16 were excluded from this analysis because of a delay in delivery of Glucosamine and Methylprednisolone. The uptake of 35-S by explants exposed to Glucosamine and Methylprednisolone is outlined in table 4.4 and represented in Figure 4.4. Addition of Glucosamine or Methylprednisolone at the same time as 0.5% Bupivacaine increased 35-S uptake of the chondral explants compared to 0.5% Bupivacaine alone. Adding these solutions after Bupivacaine exposure also increased 35-S uptake but this effect was not as marked.

	Counts per gram per minute (CPG)			
Experimental condition	Mean	Std. deviation	95% Confidence Interval	
0.5% Bupi	11680	14560	6243	17117
M199	58572	44848	41826	75319
Gluc-protect	33231	25637	22876	43586
Gluc-repair	17369	18954	9713	25025
Steroid-protect	34624	39599	10694	58553
Steroid-repair	18135	18212	7129	29140

Table 4.4 Uptake of 35-S by explants exposed to 0.5% Bupivacaine, M199, Glucosamine or Methylprednisolone.(0.5% Bupi - 0.5% Bupivacaine, Gluc-protect – adding Glucosamine and 0.5% Bupivacaine together, Gluc-repair –adding

Glucosamine after 0.5% Bupivacaine exposure, Steroid-protect - adding Methylprednisolone and 0.5% Bupivacaine together, Steroid – repair - adding Methylprednisolone after 0.5% Bupivacaine exposure)

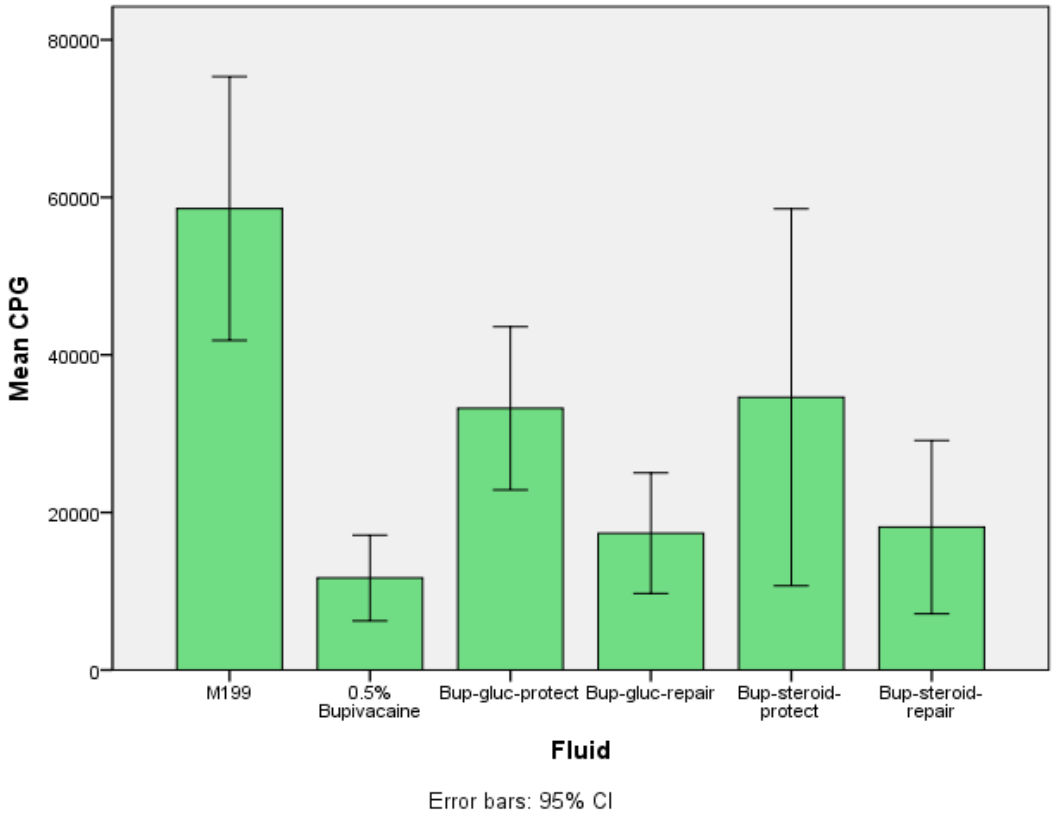

Figure 4.4 Bar chart representing the uptake of 35-S after exposure to 0.5% Bupivacaine, Glucosamine or Methylprednisolone

The data was transformed to log base 10 to achieve a near normal distribution prior to conducting statistical comparisons (Figure 4.5).

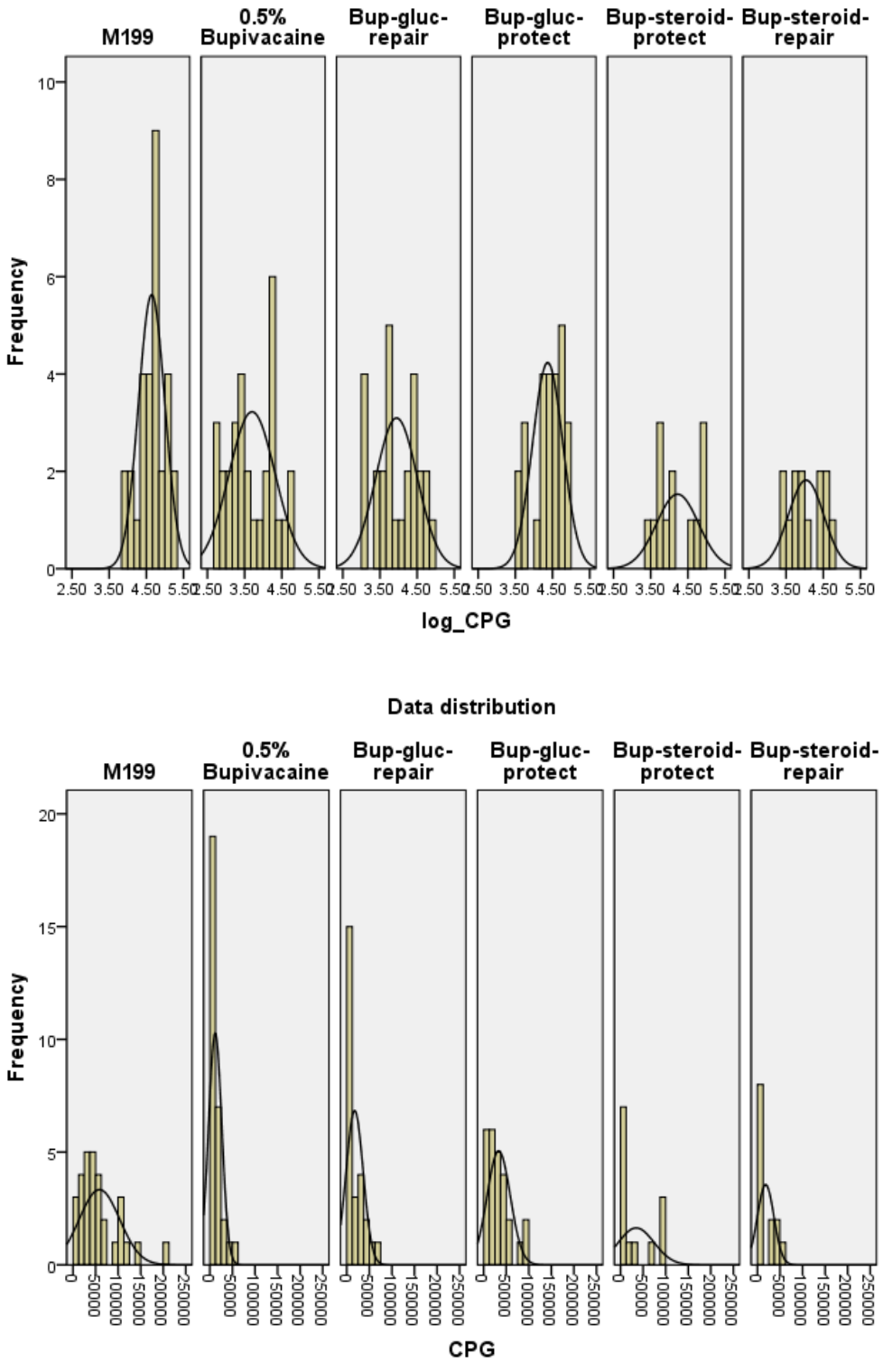

Figure 4.5 Histograms showing the data distribution of 35-S uptake of explants exposed to 0.5% Bupivacaine, Glucosamine or Methylprednisolone before and after log transformation.

This uptake was compared to that of explants exposed to 0.5% Bupivacaine only. The inhibition of metabolism due to 0.5% Bupivacaine was 80% with the exclusion of the first two patients ($p<0.001$).

Initial analysis using the t-test showed that both Glucosamine ($p<0.001$) and Methylprednisolone ($p=0.006$) offered significant protection against Bupivacaine toxicity. Adding Glucosamine or Methylprednisolone after Bupivacaine exposure appeared to reduce the toxicity but the differences were not significant ($p=0.06$ and 0.09 respectively).

Final analysis with the mixed effects model showed that the addition of Glucosamine at the same time as the local anaesthetic reduced the toxicity of 0.5% Bupivacaine from 80% to 43% ($p<0.001$). Adding Glucosamine to the culture medium after one-hour exposure to the 0.5% Bupivacaine helped reduce the toxicity from 80% to 70% ($p=0.004$).

The addition of Methylprednisolone at the same time as the local anaesthetic reduced the toxicity of 0.5% Bupivacaine from 80% to 41% ($p<0.001$). Adding Methylprednisolone to the culture medium after one-hour exposure to the 0.5% Bupivacaine helped reduce the toxicity from 80% to 69% ($p=0.004$).

Section 4.3.3 Effect of osteoarthritis

Overall, the 35-S uptake in M199 explants was higher in osteoarthritic cartilage (CPG mean 51029, 95% CI 42450 to 59607) than non-arthritic cartilage (CPG 60147, 95% CI 51444 to 68850) ($p<0.001$, random effects model).

We wanted to know whether the comparison of 35-S uptake by each irrigation fluid or local anaesthetic against control was different for those with or without osteoarthritis. The interaction of the effect of osteoarthritis on proteoglycan synthesis was measured for each irrigation fluid using the mixed model giving a random effect for each patient.

No difference was observed in the effect of irrigation fluids on arthritic and non-arthritic cartilage (Table 4.5). However, the 0.5% Bupivacaine data from experiment 1 revealed that its effect was worse on arthritic cartilage (Percentage inhibition 96%, 95% CI 88-99, $p<0.001$) than non-arthritic cartilage (Percentage inhibition 80%, 95% CI 72-86, $p<0.001$).

Irrigation fluid	p-value
0.9% saline	0.18
1.5% Glycine	0.22
Ringer's solution	0.46
5% Mannitol	0.22
0.5% Bupivacaine	<0.001

Table 4.5 Effect of presence of osteoarthritis on the comparison between 35-S uptake of explants exposed to different irrigation fluids and control.

Similarly, the effect of the presence of osteoarthritis on proteoglycan synthesis was measured for each local anaesthetic using the random effects mixed model. No difference was found in the effect of local anaesthetics on arthritic and non-arthritic cartilage (Table 4.6).

Local Anaesthetic	p-value
1% Lidocaine	0.67
2% Lidocaine	0.54
0.25% Bupivacaine	0.18
0.5% Bupivacaine	0.99
0.5% Levo-Bupivacaine	0.82

Table 4.6 Effect of presence of osteoarthritis on the comparison between35-S uptake of explants exposed to different local anaesthetics and control.

Chapter 5 Discussion

Several reports have now been published describing cases of chondrolysis following arthroscopic surgery. In the majority of these reports, one or more risk factors such as an intra-articular pain pump, radiofrequency thermal probe or bio absorbable suture anchors have been implicated. At the same time, there have also been cases where none of the above risk factors were found. However, the one common factor in all cases of post arthroscopic chondrolysis was the use of a solution for continuous joint irrigation. While concerns have been expressed from in vitro studies about the potential toxicity of irrigation fluids, they have not been thought of as a risk factor for chondrolysis in any of the case reports.

Arthroscopic surgery was initially described for the knee joint but its application has since spread to involve other joints of the body such as shoulder, hip, ankle, elbow and wrist. Even though knee arthroscopy is much more commonly performed than shoulder arthroscopy, more than a 100 cases of chondrolysis have been described in the shoulder with only a few reports in the knee joint. While there is no clear reason for this difference between the joints, this may be because intra-articular pain pumps and bio absorbable suture anchors are used more commonly in shoulders than in knees. It may also be due to the fact that pain pumps are predominantly used for the shoulder joint. Secondly as the shoulder joint is a non-weight bearing articulation, the local anaesthetic bathes the cartilage for protracted periods without being extruded during weight-bearing by the contact between the articular surfaces. Slabaugh (Slabaugh et al., 2010) and Fester

(Fester and Noyes, 2009) also suggested that the shoulder might be particularly vulnerable due to the fact that it is a small joint. This authors both described cases of knee chondrolysis following the administration of intra-articular Bupivacaine in young female sports women. It is suggested that the knee may more tolerant of local anaesthetic as it is large joint, within which there is relative ease of egress of fluid. In addition the postoperative haematoma would result in dilution of the local anaesthetic. Also, the shoulder joint has thinner articular cartilage than the knee joint (Fox 2008), which may further explain its susceptibility to chondrolysis.

Section 5.1 Irrigation fluids

We found that normal saline inhibited human cartilage activity by 35% and Ringer's solution by 10%. Our results are similar to those of Bulstra et al (Bulstra et al., 1994) who found that normal saline inhibited rat cartilage metabolism by 20% while Ringer's solution only caused 5% inhibition. Some studies have used normal saline as a control solution for comparison against local anaesthetic solutions. Chu et al (Chu et al., 2008) found 35% dead or apoptotic chondrocytes using flow cytometry in bovine and human alginate bead cultures after exposure to normal saline for 60 minutes. They then performed a live: dead cell analysis using confocal microscopy for osteochondral cores and found 11% cell death. Karpie et al (Karpie and Chu, 2007) also used normal saline as control for comparison against Lidocaine but observed 20% non viable cells after exposure to 0.9% saline for 60 minutes. We feel that if the above authors had compared different local anaesthetics to a cartilage culture medium, their results would have shown even

worse toxicity due to the local anaesthetic.

Previous studies have suggested that non-ionic fluids such as Glycine, Mannitol and Sorbitol may be less harmful than ionic fluids such as saline and Ringer's solution (Bert et al., 1990, Jurvelin et al., 1994, Gradinger et al., 1995). However, the methods used in these studies such as indentation creep testing, appearance on scanning electron microscope and measuring proteoglycan loss were less sensitive than measuring the metabolic activity of articular cartilage. We found that the non ionic fluids, 5% Mannitol and1.5% Glycine, inhibited metabolism by 31% and 24% respectively and therefore, caused more damage than the ionic Ringer's solution. Although the toxicity of Glycine was not statistically significant, a larger sample size may have converted the trend seen towards statistical significance. Based on our results, we cannot support that there is a difference between ionic and non-ionic fluids.

Bulstra et al (Bulstra et al., 1994) suggested that the inhibition of metabolism by normal saline might be due to its acidic pH. This was subsequently investigated by Karpie et al (Karpie and Chu, 2007), who tested chondrocyte viability with normal saline at pH 5.0, 7.0 and 7.4 and found no differences between the solutions at these three different pH levels. Bogatch et al (Bogatch et al., 2010) also found that phosphate buffered saline only caused cell death at pH <3.4. The pH of normal saline in our study was 5.5 and based on the results of the above two studies, is unlikely to be the cause of toxicity. Gradinger et al (Gradinger et al., 1995) increased the ionic concentration of NaCl from 0.1% to 0.9% and cell death in bovine cartilage was only seen at a concentration of 0.9%. This may suggest that

the osmolarity of the solution plays a role in its toxicity towards articular cartilage but this does not explain why Ringer's solution would be less toxic than normal saline as they both have a similar osmolarity. It is probably the concentration of specific ions in Ringer's solution especially the similarity to the cations in articular cartilage that makes this solution the least toxic to articular cartilage. It has been shown that the electrolyte activity in articular cartilage is approximately equal to Ringer's solution (Maroudas, 1970).

We discontinued the use of knee articular cartilage from patients with osteoarthritis because the cartilage was much more variable in thickness and consistency. The results of 35-S uptake, however, were similar between explants from arthritic knee joints and arthritic femoral heads. With hindsight, it may well have been acceptable to continue using arthritic knee explants.

Section 5.2 Local Anaesthetics

It is possible that local anaesthetic chondrotoxicity is an under-reported phenomenon, as clinicians may not intuitively make a link between the two. The onset of symptoms is frequently months or years after what apparently appears to be an uncomplicated procedure. The single case of ankle chondrolysis reported in 2005 is notable for the fact that the authors are at a loss to determine the cause or risk factors of chondrolysis in their young patient. However further analysis of the paper reveals that a pain pump was used, which may be significant in the light of recent literature. There may also be a reporting bias towards younger patients. It is

possible that the development of chondrolysis in older patients is presumed to be a natural progression of osteoarthritis secondary to the original condition for which the arthroscopy was performed.

An interest into the effect of local anaesthetics on articular cartilage was sparked by several reports of chondrolysis associated with the use of post-operative intra-articular pain pumps. While clinically this toxicity has only been seen with continuous infusions via pain pumps, even a single exposure to local anaesthetics has been found to be toxic in laboratory studies. Chu et al (Chu et al., 2006) found that exposure to 0.5% Bupivacaine for 30 minutes caused 42% chondrocyte death in bovine articular cartilage. Reduced chondrocyte viability has since been found with different concentrations of Lidocaine, Bupivacaine and Ropivacaine in a dose and duration dependent manner, with higher concentrations being more toxic (Karpie and Chu, 2007, Lo et al., 2009). Our results show that all local anaesthetic solutions tested were highly toxic to articular cartilage. We also found that 2% Lidocaine and 0.5% Bupivacaine were more toxic than 1% Lidocaine and 0.25% Bupivacaine respectively. However, at equivalent clinical concentration, both Lidocaine and Bupivacaine were equally toxic.

Chu et al (Chu et al., 2010) conducted an in vivo study on rat cartilage and found reduced chondrocyte density six months after a single exposure to 0.5% Bupivacaine, indicating that this toxic effect is maintained at least in the medium term. However, there are currently no clinical reports indicating damage to articular cartilage secondary to a single exposure to local anaesthetic. The effect may be subclinical. There may be damage leading to osteoarthritis many years

later but this is attributed to the pathology for which the original injection was initially given. Repetitive injections may cause cumulative damage but this has not been studied yet in either in vivo or in vitro studies.

Scheffel et al (Scheffel et al., 2010) summarized 100 cases of PAGCL and noted that symptoms started between 42 and 730 days after surgery whilst a radiographic diagnosis was made between 90-1095 days post operatively. In an in vivo model, this was supported by the results of Gomoll et al (Gomoll et al., 2009) who found that proteoglycan metabolism was increased in rabbit chondral explants even three months after a 48 hours infusion of Bupivacaine. While the authors of this paper believed that this was a sign that cartilage had fully recovered, the increased metabolism can be a sign of degenerative disease as it has been reported that proteoglycan metabolism is increased in osteoarthritic cartilage (Collins and Mc, 1960) and was also seen in this study. Our results show that proteoglycan metabolism was inhibited 16 hours after a single exposure to local anaesthetics. While a single injection of local anaesthetic may not lead to chondrolysis, it may stimulate a degenerative process. To date, this has not been investigated but will require an in vivo study similar to that of Gomoll et al with a longer recovery period of six or twelve months.

Whilst the use of Glucosamine is clinically controversial, very clear beneficial effects were observed in our laboratory-based study. The majority of clinical trials have examined the effects of oral Glucosamine but there is evidence that intra-articular injections are safe and can help improve symptoms of osteoarthritis (Vajaradul, 1981, Crolle and D'Este, 1980). We found that Glucosamine offered

protection against the toxicity of local anaesthetics to articular cartilage and reduced the inhibition of proteoglycan metabolism by 37%. It was also able to reverse some of this toxicity by 10%, sixteen hours after exposure to 0.5% Bupivacaine. While this reparative effect was not as dramatic as the protective effect, we only measured one recovery time period of 16 hours. More time may have provided more benefit. Even after the addition of Glucosamine, 43% toxicity was observed. Whilst this may still appear alarming, it is however, similar to the 35% toxicity seen with a simple solution such as normal saline, which is regularly used clinically to irrigate joints during arthroscopic surgery.

With so much evidence emerging that local anaesthetics may not be safe for intra-articular injection, questions have been asked whether they are at all necessary for postoperative analgesia. Townshend et al (Townshend et al., 2009) did not find any difference between visual analogue pain scores of patients who had Bupivacaine injection around the arthroscopic portals only and those who had an intra-articular injection. However, they assessed scores only at one time interval, one-hour after arthroscopy, and did not calculate the amount of oral opiate and non-opiate analgesia consumed in each group. Campo et al (Campo et al., 2011) injected patients' knee joints with 10mls of saline or 0.5% Bupivacaine or 0.75% Ropivacaine after arthroscopy and found only a small improvement in analgesia offered by the addition of local anaesthetics. They felt that systemic analgesia should be preferred to local anaesthetic intra-articular injection in view of the several published reports of chondrotoxicity.

Anecdotally, the use of levo-bupivacaine (Chirocaine) seems to be increasing because of less cardiotoxicity compared to Bupivacaine. We did not find any difference in its toxicity towards articular cartilage compared to Bupivacaine.

Some authors have attempted to investigate the mechanism of this toxicity. Dragoo et al (Dragoo et al., 2010) suggested that the toxicity could be due to the presence of epinephrine, the preservative Sodium Metabisulphite and the low pH of such solutions. Henning et al (Hennig et al., 2010) found that Bupivacaine with the preservative Methylparaben was no more toxic than Bupivacaine alone. Chu et al had previously demonstrated Bupivacaine toxicity in their earlier studies (Chu et al., 2008, Chu et al., 2006) but did not find any difference in vivo in superficial cell viability or histological scores between preservative free Bupivacaine and saline control at any time interval from one week to six months (Chu et al., 2010). They did, however, find reduced chondrocyte density six months after exposure indicating that the toxicity is not entirely due to preservatives. Bogatch et al (Bogatch et al., 2010) did not find any toxicity due to Bupivacaine with epinephrine or due to low pH of phosphate buffered saline (PBS) control. They found that there was a crystallisation reaction between the anaesthetic and the culture medium and with synovial fluid and wondered whether an incompatibility between the synovial fluid and the local anaesthetic was responsible for the toxicity. We wonder whether the damage was caused by the crystals or whether a third chondrotoxic chemical was formed as a result.

Grishko et al (Grishko et al., 2010) found that local anaesthetics caused mitochondrial DNA damage in chondrocytes leading to cell death. While this may

explain the mechanism of toxicity at a molecular level, the effect of addition of epinephrine or preservatives or low pH on local anaesthetic toxicity is yet unclear and needs to be investigated further.

The mechanism by which Glucosamine protected or repaired articular cartilage damaged by local anaesthetics is unknown. Such a protective effect could be due to a direct chemical interaction of Glucosamine with 0.5% Bupivacaine. It may have neutralised the anaesthetic effect of Bupivacaine. However this does not explain the marginal recovery of 35-S uptake when the reparative effect of Glucosamine was studied after the removal of 0.5% Bupivacaine. Therefore, it is probably more likely that this was due to a direct chondro-protective effect mediated via stimulation of proteoglycan synthesis.

Section 5.3 Corticosteroids

We found that the addition of Methylprednisolone at the same time as Bupivacaine reduced toxicity by 39% and addition after Bupivacaine exposure reduced toxicity by 11%. Our results are different from those of Syed et al (Syed et al., 2011) who tested cell viability after exposure and found that a combination of Triamcinolone and Bupivacaine was no less toxic than Bupivacaine alone. We used Methylprednisolone in our study, which is supplied as a sterile white aqueous suspension. This suspension was seen to coat the chondral explants when added to Bupivacaine or M199 and this may have physically protected the cartilage from the toxic effect of Bupivacaine.

Unlike Glucosamine, Corticosteroids are frequently injected, together with a local anaesthetic, into human synovial joints in clinical practice. There seems to be some clinical evidence (Pyne et al., 2004, Raynauld et al., 2003) that steroid and local anaesthetic injections provide a short-term improvement in symptoms of osteoarthritis. However, does this short-term benefit in pain lead to improvement or deterioration of arthritis?

Section 5.4 Effect of osteoarthritis

The cartilage metabolism seen in the M199 explants was higher in arthritic than non arthritic articular cartilage, which is similar to what has been shown in previous studies (Collins and Mc, 1960). Our results from the first experiment showed that the presence of osteoarthritis did not have any effect on the comparison between different irrigation fluids and control. The effect of 0.5% Bupivacaine in this data was found to be worse on arthritic than non-arthritic cartilage. However, when we analysed the effect of osteoarthritis on the comparison between local anaesthetics and control, no significant effect was found for any of the local anaesthetics. It is likely that the solitary significant effect seen for 0.5% Bupivacaine was a statistical error and our wider results do not support the hypothesis that osteo-arthritic cartilage is more susceptible to the toxic effect of different irrigation fluids or local anaesthetics.

Section 5.5 Strengths

This is the first study using an objective method to assess the effects of different irrigation fluids on human articular cartilage as previous studies have been conducted in animals, except for one study that used appearance on SEM as their outcome measure (Bert et al., 1990). We are the first to examine the effects of Levobupivacaine, which is a commonly used local anaesthetic, on articular cartilage.

In spite of several reports on the toxicity of local anaesthetics on articular cartilage, there have been no studies on methods to prevent this toxicity. Ours is the first study examining the ability of Glucosamine to prevent this toxicity.

We have used an established, well-described method to measure cartilage metabolism which is probably better than measuring the number of viable cells as this may not be an accurate reflection of cartilage metabolism. Meachim and Collins (Meachim and Collins, 1962) established that the 35-S uptake by chondrocytes was increased in osteoarthritis due to an increase in chondrocyte count and was therefore, proportional to the number of chondrocytes present in cartilage. However, this may not be true in the presence of apoptotic cells that may not be able to synthesise proteoglycans as effectively as normal cells.

Section 5.6 Limitations

We acknowledge the limitations of an in vitro model. Further clinical studies are required to confirm or refute our laboratory findings. We do not know if the addition of Glucosamine will have any neutralising effect on the analgesic properties of the local anaesthetic solution. This will need to be further established with a clinical study.

While it is not necessary to obtain in vitro evidence before conducting every clinical study, we believe that it is important step on the research ladder. The advantages of an in vitro study are that it allows comparison of a large number of samples and it allows more control over the variables being studied.

We have used cartilage from elderly patients wherein the cellular changes due to ageing would already have set in. We have based our results on the presence or absence of macroscopic signs of osteoarthritis. Histological examination may have allowed better categorisation.

We have estimated 35-S uptake as a measure of proteoglycan synthesis. Further analyses to measure proteoglycan catabolism as well as collagen synthesis and breakdown would have complemented our results. We did not have the resources to perform an assessment of cell viability such as confocal microscopy, which

would also have allowed us to investigate the effects of different solutions in the different zones of articular cartilage.

It can be argued that the explants represent only a very small proportion of a normal adult joint and 1 ml of solution might be too high a dose for exposure. Also, it is not known for how long the local anaesthetic remains in a joint post injection. We used a volume of 1 ml to ensure that the explants were completely submerged within the solution and all the surfaces would be exposed. This may mean that the extent of toxicity may be overestimated using this model. However, ours is a comparative study and having Bupivacaine as a negative control in the assessment of irrigation fluid toxicity strengthens the comparative assessment.

Section 5.7 Future research

Our results need to be tested further with a clinical study. An ideal study would be a randomised clinical trial comparing outcomes of arthroscopy in patients matched by age, gender and diagnosis who have an injection of local anaesthetic into the joint with or without an injection of Glucosamine or placebo. However, the differences between the three groups may not be evident at short-term follow up because a single injection of local anaesthetic is unlikely to cause severe articular cartilage damage or chondrolysis. Therefore, long-term follow up will be required with the development of osteoarthritis as an end-point to conclude whether patients who receive local anaesthetic will be clinically worse off compared to those who do not receive an injection or the ones who receive Glucosamine in

addition. Such a trial is unlikely to happen because of the ethical issue of deliberately injecting a potentially toxic agent. Even if it receives ethical approval, patients may not agree to being recruited once they have been provided the evidence about the potential toxicity of local anaesthetics. One practical way would be to conduct a retrospective review of patients who have received local anaesthetic into their joints for non-arthritic conditions such as post knee arthroscopy in young patients where no arthritis was identified. This can be compared to a similar group who have had arthroscopy but no local anaesthetic.

We do not know whether the addition of Glucosamine will influence the efficacy of a local anaesthetic. Postoperative pain relief can also be compared within the different groups described in the above trial.

Similarly, our results showing reduced toxicity with Ringer's solution compared to saline need to be tested further with a clinical study. This would again ideally be in the form of a randomised control trial comparing outcomes after arthroscopy using saline or ringer's solution as the irrigation fluid. If there is a toxic effect of saline, it is unlikely to manifest itself in the short term and therefore, a minimum of 5 years follow up will be required. A preliminary study comparing conversion to knee replacement and the time between arthroscopy and knee replacement between surgeons who use saline or Ringer's solution might provide some clinical evidence regarding the toxicity of saline.

We found that normal saline was the most toxic of the irrigation fluids tested and all local anaesthetics tested were toxic to articular cartilage. Since these two toxic

substances are often used in the same patient during or after arthroscopy, it is important to test the combined effect of saline and local anaesthetic and this can be done using the same model.

This method can be used to test the effect of other materials that are used as intra-articular injections. There have been reports of chondrolysis in glenohumeral joints following use of gentian violet dye (Shibata et al., 2001, Tamai et al., 1997). In our clinical practice, there has been one patient who developed chondrolysis following a distention arthrogram. Distention arthrography is one of the recognised treatments for frozen shoulder (Ibrahim et al., 2006) and involves injection of iodine based contrast (niopam), corticosteroid, local anaesthetic and air in the shoulder joint. The in vitro effect of niopam on articular cartilage could be studied further with our model.

Gadolinium contrast is also routinely used for Magnetic resonance arthrogram studies in different joints (Jazrawi et al., 2011). Effect of gadolinium contrast on articular cartilage has been studied in animals but not in human cartilage. Hajek et al (Hajek et al., 1990) found no difference between articular cartilage of rabbits that received gadolinium injections in their knees compared to those who did not have any injections. However, Kose et al (Kose et al., 2007) found that gadolinium caused more activation of chondrocytes than saline in rabbit knee cartilage explants and this activation slowly but incompletely improved over a two-week period. Greisberg (Greisberg et al., 2001) also found that gadolinium induced apoptosis in bovine chondrocytes. A further in vitro study to assess the effect of

gadolinium on human articular cartilage will provide further evidence on the safety of this contrast medium.

We have only looked at cartilage from knee and hip joints and not from shoulder joints. Since the majority of cases of chondrolysis have been reported in the glenohumeral joint, it would be interesting to test the effect of local anaesthetics on chondral explants from patients who have shoulder hemiarthroplasty for osteoarthritis and for complex fractures.

We have tested only one time interval of one-hour exposure to a toxic agent followed by 16 hours recovery. This method of testing 35-S uptake could be used to assess the effect of a single exposure at different time intervals such as three and six months in a live animal model and could also be used to test the effect of repetitive local anaesthetic injections.

We have only assessed the effect of corticosteroids on osteoarthritic cartilage in line with clinical practice. In retrospect, it would have been interesting to assess their effect on normal cartilage.

While we have tested the protective effect of Glucosamine on articular cartilage, the effect of other chondro-protective substances such as chondroitin sulphate and cod liver oil on articular cartilage exposed to local anaesthetics should also be tested.

Section 5.8 Summary of results

In an in vitro human model, we found that Ringer's solution was the best irrigation fluid as it caused the least inhibition of 35-S uptake and normal saline was the worst. All local anaesthetics tested were toxic to articular cartilage. Glucosamine and Methylprednisolone were shown to protect against this toxicity. Results are summarised in Figure 5.1.

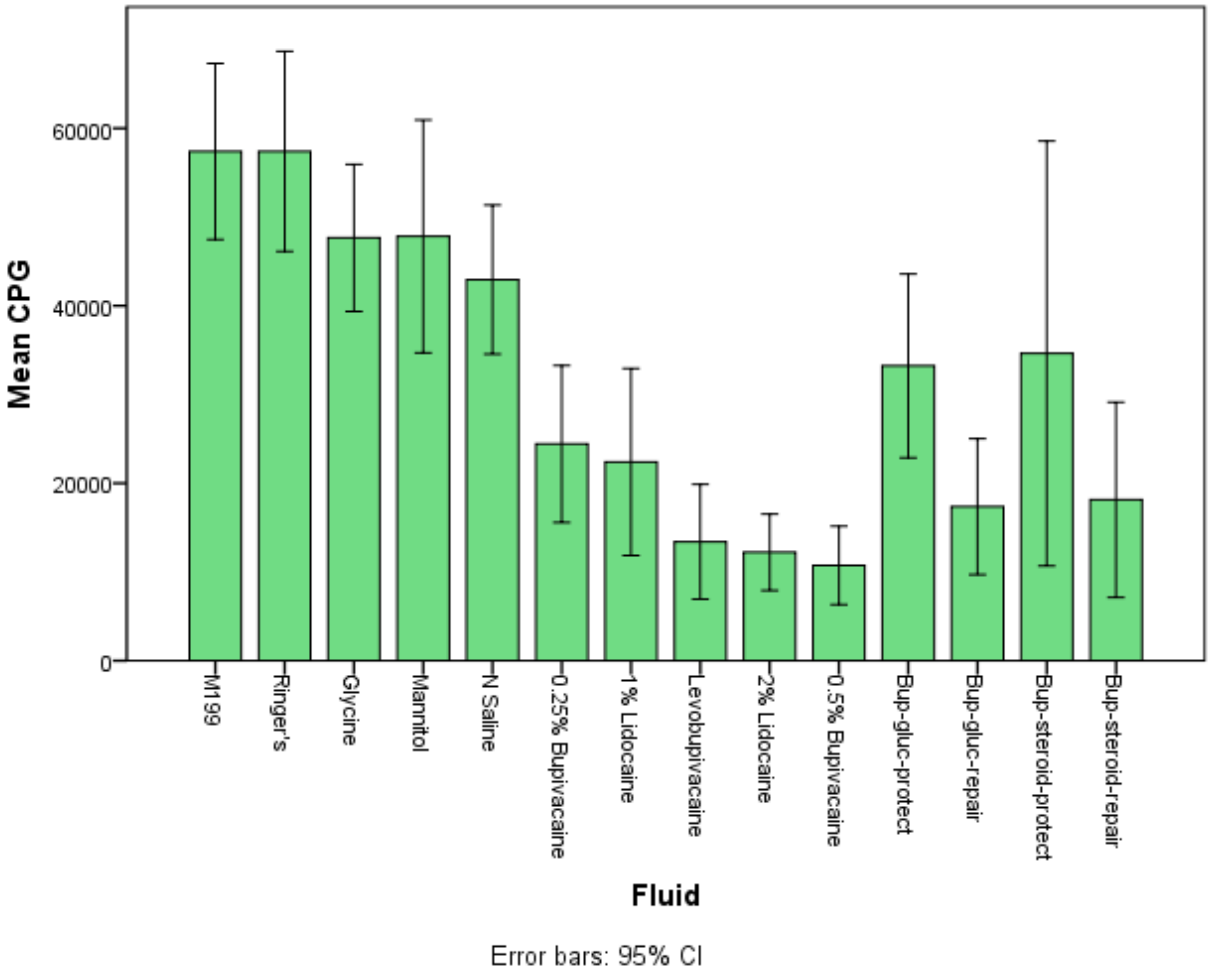

Figure 5.1 Summary of all results showing the 35-S uptake (CPG) of explants exposed to different irrigation fluids, local anaesthetics, Glucosamine or

Methlyprednisolone. (35-S uptake of M199 and 0.5% Bupivacaine have been combined from the two experiments)

Section 5.9 Conclusions and recommendations

Ringer's solution was the least toxic arthroscopic irrigation fluid and should replace normal saline in clinical practice. Injecting local anaesthetics into joints needs careful consideration of risks and benefits and should not be routine practice post arthroscopy. More clinical studies are required to assess whether any real damage occurs to a joint injected with local anaesthetic or irrigated with normal saline. If a clinical problem can be identified then the protective effect of Glucosamine or Corticosteroid could be assessed.

Appendix 1: Distribution of chondral explants per irrigation fluid from each patient

Patient	Number of specimens per fluid						Total from patient
	Normal Saline	1.5% Glycine	M199 (control)	Ringer's solution	5% Mannitol	0.5% Bupivacaine	
4	3	3	3	3	2	3	17
5	4	4	4	4	4	4	24
6	4	4	4	3	4	4	23
7	4	4	4	4	4	4	24
8	3	3	3	3	3	3	18
9	3	2	2	2	2	2	13
10	4	3	4	4	3	2	20
11	4	4	4	4	4	4	24
12	3	3	3	3	3	3	18
13	4	4	4	4	3	4	23
14	4	4	4	4	4	4	24
Total per fluid	40	38	39	38	36	37	228

Appendix 2: $^{35}SO_4$ uptake of chondral explants exposed to different irrigation fluids

Patient	Source	Experimental variable	Average CPG	CPG	Osteoarthritis
4	TKR	Normal Saline	34775	37789	OA
4				32574	OA
4				33960	OA
4	TKR	1.5% Glycine	36900	35424	OA
4				34280	OA
4				40996	OA
4	TKR	M199	31410	29192	OA
4				33154	OA
4				31885	OA
4	TKR	Ringer's solution	27063	28960	OA
4				27921	OA
4				24307	OA
4	TKR	5% Mannitol	19607	17888	OA
4				19006	OA
4				21925	OA
4	TKR	0.5% Bupivacaine	3113	3255	OA
4				3774	OA
4				2311	OA
4	TKR	Normal Saline	47348	45054	OA
4				43602	OA
4				53387	OA
4	TKR	1.5% Glycine	39939	35758	OA
4				43818	OA
4				40242	OA
4	TKR	M199	36810	32790	OA
4				38155	OA
4				39485	OA
4	TKR	Ringer's solution	48105	51105	OA
4				44632	OA
4				48579	OA
4	TKR	0.5% Bupivacaine	1457	1825	OA
4				1027	OA
4				1521	OA
4	TKR	Normal Saline	18507	18092	OA
4				18397	OA
4				19033	OA

4	TKR	1.5% Glycine	27467	24920	OA
4				26600	OA
4				30880	OA
4	TKR	M199	76160	73259	OA
4				74778	OA
4				80444	OA
4	TKR	Ringer's solution	33025	31667	OA
4				32148	OA
4				35259	OA
4	TKR	5% Mannitol	36435	39768	OA
4				35637	OA
4				33900	OA
4	TKR	0.5% Bupivacaine	2663	3476	OA
4				1280	OA
4				3232	OA
5	TKR	Normal Saline	43475	36293	OA
5				53282	OA
5				40849	OA
5	TKR	1.5% Glycine	23180	23486	OA
5				23649	OA
5				22405	OA
5	TKR	M199	43262	47554	OA
5				40971	OA
5				41259	OA
5	TKR	Ringer's solution	38819	35561	OA
5				38700	OA
5				42197	OA
5	TKR	5% Mannitol	24656	24772	OA
5				24531	OA
5				24665	OA
5	TKR	0.5% Bupivacaine	2497	2510	OA
5				2394	OA
5				2587	OA
5	TKR	Normal Saline	33829	25353	OA
5				34684	OA
5				41450	OA
5	TKR	1.5% Glycine	58348	47000	OA
5				59783	OA
5				68261	OA
5	TKR	M199	36176	39321	OA
5				24679	OA
5				44528	OA
5	TKR	Ringer's solution	49468	47474	OA

5				47680	OA
5				53247	OA
5	TKR	5% Mannitol	27176	29298	OA
5				25661	OA
5				26570	OA
5	TKR	0.5% Bupivacaine	4534	4939	OA
5				4211	OA
5				4453	OA
5	TKR	Normal Saline	27657	21815	OA
5				36700	OA
5				24455	OA
5	TKR	1.5% Glycine	28291	24467	OA
5				34467	OA
5				25939	OA
5	TKR	M199	24072	21274	OA
5				21415	OA
5				29528	OA
5	TKR	Ringer's solution	36308	25748	OA
5				42336	OA
5				40841	OA
5	TKR	5% Mannitol	18144	19281	OA
5				16587	OA
5				18563	OA
5	TKR	0.5% Bupivacaine	1667	1966	OA
5				1854	OA
5				1180	OA
5	TKR	Normal Saline	18761	19777	OA
5				17695	OA
5				18810	OA
5	TKR	1.5% Glycine	39445	35315	OA
5				39099	OA
5				43919	OA
5	TKR	M199	42573	44167	OA
5				45000	OA
5				38553	OA
5	TKR	Ringer's solution	52151	51802	OA
5				51221	OA
5				53430	OA
5	TKR	5% Mannitol	60256	70385	OA
5				51752	OA
5				58632	OA
5	TKR	0.5% Bupivacaine	2857	2449	OA
5				2755	OA

5				3367	OA
6	NOF	Normal Saline	49723	48133	No OA
6				48889	No OA
6				52133	No OA
6	NOF	1.5% Glycine	63181	66648	No OA
6				60989	No OA
6				61868	No OA
6	NOF	M199	75713	80531	No OA
6				70619	No OA
6				75929	No OA
6	NOF	Ringer's solution	62606	59188	No OA
6				57868	No OA
6				70711	No OA
6	NOF	5% Mannitol	55714	60000	No OA
6				56667	No OA
6				50476	No OA
6	NOF	0.5% Bupivacaine	13914	13440	No OA
6				14679	No OA
6				13578	No OA
6	NOF	Normal Saline	62016	57151	No OA
6				65116	No OA
6				63721	No OA
6	NOF	1.5% Glycine	56132	44009	No OA
6				52028	No OA
6				72311	No OA
6	NOF	M199	49758	47826	No OA
6				50932	No OA
6				50497	No OA
6	NOF	5% Mannitol	72739	68790	No OA
6				74713	No OA
6				74713	No OA
6	NOF	0.5% Bupivacaine	3704	3862	No OA
6				2804	No OA
6				4392	No OA
6	NOF	Normal Saline	64590	56803	No OA
6				68852	No OA
6				68033	No OA
6	NOF	1.5% Glycine	76972	66620	No OA
6				100704	No OA
6				63592	No OA
6	NOF	M199	49536	50309	No OA
6				49794	No OA
6				48454	No OA

6	NOF	Ringer's solution	68966	53448	No OA
6				63563	No OA
6				89828	No OA
6	NOF	5% Mannitol	51179	50714	No OA
6				50821	No OA
6				52000	No OA
6	NOF	0.5% Bupivacaine	24735	26254	No OA
6				23074	No OA
6				24841	No OA
6	NOF	Normal Saline	33237	25780	No OA
6				40636	No OA
6				33295	No OA
6	NOF	1.5% Glycine	49591	41500	No OA
6				49545	No OA
6				57727	No OA
6	NOF	M199	41846	36615	No OA
6				43077	No OA
6				45846	No OA
6	NOF	Ringer's solution	51196	39234	No OA
6				48612	No OA
6				65837	No OA
6	NOF	5% Mannitol	65758	66424	No OA
6				66242	No OA
6				64606	No OA
6	NOF	0.5% Bupivacaine	6425	6373	No OA
6				6010	No OA
6				6891	No OA
7	NOF	Normal Saline	37540	40714	No OA
7				33571	No OA
7				38333	No OA
7	NOF	1.5% Glycine	37405	34580	No OA
7				36870	No OA
7				40687	No OA
7	NOF	M199	37292	33958	No OA
7				31562	No OA
7				44375	No OA
7	NOF	Ringer's solution	38372	45426	No OA
7				37984	No OA
7				31783	No OA
7	NOF	5% Mannitol	16727	18970	No OA
7				15152	No OA
7				16121	No OA
7	NOF	0.5% Bupivacaine	2845	3707	No OA

7				1121	No OA
7				3707	No OA
7	NOF	Normal Saline	28077	21282	No OA
7				36282	No OA
7				26923	No OA
7	NOF	1.5% Glycine	18581	20387	No OA
7				20645	No OA
7				14839	No OA
7	NOF	M199	24110	22329	No OA
7				28493	No OA
7				21644	No OA
7	NOF	Ringer's solution	24027	22349	No OA
7				26376	No OA
7				23490	No OA
7	NOF	5% Mannitol	55185	57160	No OA
7				46049	No OA
7				62469	No OA
7	NOF	0.5% Bupivacaine	3415	4309	No OA
7				2439	No OA
7				3496	No OA
7	NOF	Normal Saline	28837	27364	No OA
7				29690	No OA
7				29457	No OA
7	NOF	1.5% Glycine	20884	20744	No OA
7				22605	No OA
7				19349	No OA
7	NOF	M199	40000	40992	No OA
7				39339	No OA
7				39669	No OA
7	NOF	Ringer's solution	31604	36415	No OA
7				32075	No OA
7				26415	No OA
7	NOF	5% Mannitol	23621	23534	No OA
7				21207	No OA
7				26121	No OA
7	NOF	0.5% Bupivacaine	9518	10422	No OA
7				8795	No OA
7				9398	No OA
7	NOF	Normal Saline	42466	43356	No OA
7				44041	No OA
7				40137	No OA
7	NOF	1.5% Glycine	51034	57011	No OA
7				43678	No OA

7				52414	No OA
7	NOF	M199	36111	38571	No OA
7				36190	No OA
7				33571	No OA
7	NOF	Ringer's solution	42770	46149	No OA
7				38243	No OA
7				43919	No OA
7	NOF	5% Mannitol	58412	54882	No OA
7				61176	No OA
7				59176	No OA
7	NOF	0.5% Bupivacaine	10760	11462	No OA
7				9532	No OA
7				11287	No OA
8	NOF	Normal Saline	37376	36170	No OA
8				38723	No OA
8				37305	No OA
8	NOF	1.5% Glycine	34688	32266	No OA
8				32266	No OA
8				39531	No OA
8	NOF	M199	18551	18841	No OA
8				19783	No OA
8				17101	No OA
8	NOF	Ringer's solution	32606	30282	No OA
8				36338	No OA
8				31197	No OA
8	NOF	5% Mannitol	15917	17750	No OA
8				13333	No OA
8				16667	No OA
8	NOF	0.5% Bupivacaine	2683	2439	No OA
8				2732	No OA
8				2927	No OA
8	NOF	Normal Saline	31395	30465	No OA
8				31473	No OA
8				32248	No OA
8	NOF	1.5% Glycine	25714	26894	No OA
8				31056	No OA
8				19255	No OA
8	NOF	M199	43497	42168	No OA
8				55455	No OA
8				32867	No OA
8	NOF	Ringer's solution	21218	26282	No OA
8				19423	No OA
8				17949	No OA

8	NOF	5% Mannitol	31308	25794	No OA
8				39813	No OA
8				28318	No OA
8	NOF	0.5% Bupivacaine	2358	1870	No OA
8				2683	No OA
8				2683	No OA
8	NOF	Normal Saline	27032	30065	No OA
8				30710	No OA
8				20387	No OA
8	NOF	1.5% Glycine	29076	39748	No OA
8				27395	No OA
8				20168	No OA
8	NOF	M199	18725	18523	No OA
8				20805	No OA
8				16980	No OA
8	NOF	Ringer's solution	35197	43543	No OA
8				30157	No OA
8				31969	No OA
8	NOF	5% Mannitol	26351	33311	No OA
8				27230	No OA
8				18649	No OA
8	NOF	0.5% Bupivacaine	3077	4396	No OA
8				2527	No OA
8				2363	No OA
9	NOF	Normal Saline	65335	63515	OA
9				67573	OA
9				64916	OA
9	NOF	1.5% Glycine	48664	48826	OA
9				46397	OA
9				50850	OA
9	NOF	M199	32651	37068	OA
9				31727	OA
9				29157	OA
9	NOF	Ringer's solution	73730	76475	OA
9				74836	OA
9				69918	OA
9	NOF	5% Mannitol	61732	65748	OA
9				60472	OA
9				59055	OA
9	NOF	0.5% Bupivacaine	9613	9021	OA
9				10387	OA
9				9433	OA
9	NOF	Normal Saline	91489	82939	OA

9				91985	OA
9				99618	OA
9	NOF	1.5% Glycine	103022	115822	OA
9				101022	OA
9				92267	OA
9	NOF	M199	106684	107487	OA
9				103743	OA
9				108877	OA
9	NOF	Ringer's solution	28013	32244	OA
9				29487	OA
9				22436	OA
9	NOF	5% Mannitol	101200	94857	OA
9				95600	OA
9				113143	OA
9	NOF	0.5% Bupivacaine	4536	4372	OA
9				3825	OA
9				4918	OA
9	NOF	Normal Saline	29110	28941	OA
9				27373	OA
9				31017	OA
10	NOF	Normal Saline	110380	137772	OA
10				93564	OA
10				99802	OA
10	NOF	1.5% Glycine	54317	53650	OA
10				60650	OA
10				48650	OA
10	NOF	M199	100331	109581	OA
10				103822	OA
10				87592	OA
10	NOF	Ringer's solution	61651	72752	OA
10				58257	OA
10				53945	OA
10	NOF	5% Mannitol	34205	34848	OA
10				34583	OA
10				33182	OA
10	NOF	Normal Saline	30855	29646	OA
10				33009	OA
10				29912	OA
10	NOF	1.5% Glycine	46813	43585	OA
10				55346	OA
10				41509	OA
10	NOF	M199	41203	48107	OA
10				36272	OA

10				39231	OA
10	NOF	Ringer's solution	17930	19263	OA
10				16105	OA
10				18421	OA
10	NOF	5% Mannitol	31895	32706	OA
10				33118	OA
10				29765	OA
10	NOF	0.5% Bupivacaine	487	232	OA
10				696	OA
10				534	OA
10	NOF	Normal Saline	19692	18103	OA
10				24410	OA
10				16564	OA
10	NOF	1.5% Glycine	82698	75318	OA
10				89364	OA
10				83410	OA
10	NOF	M199	193355	192303	OA
10				184408	OA
10				203487	OA
10	NOF	Ringer's solution	182677	181460	OA
10				186569	OA
10				180000	OA
10	NOF	5% Mannitol	217077	200231	OA
10				211769	OA
10				239231	OA
10	NOF	M199	172551	181173	OA
10				166369	OA
10				170112	OA
10	NOF	Normal Saline	108285	112573	OA
10				107801	OA
10				104481	OA
10	NOF	0.5% Bupivacaine	608	156	OA
10				833	OA
10				833	OA
10	NOF	Ringer's solution	140981	168824	OA
10				91765	OA
10				162353	OA
11	NOF	Normal Saline	30273	29808	No OA
11				23221	No OA
11				37788	No OA
11	NOF	1.5% Glycine	12976	12107	No OA
11				13793	No OA
11				13027	No OA

11	NOF	M199	30988	28663	No OA
11				34302	No OA
11				30000	No OA
11	NOF	Ringer's solution	24545	26938	No OA
11				18947	No OA
11				27751	No OA
11	NOF	5% Mannitol	14487	15085	No OA
11				14359	No OA
11				14103	No OA
11	NOF	0.5% Bupivacaine	6557	6274	No OA
11				6132	No OA
11				7358	No OA
11	NOF	Normal Saline	3077	2564	No OA
11				2756	No OA
11				4038	No OA
11	NOF	1.5% Glycine	6217	7116	No OA
11				5356	No OA
11				6217	No OA
11	NOF	M199	28681	35174	No OA
11				20938	No OA
11				29965	No OA
11	NOF	Ringer's solution	7113	7950	No OA
11				6276	No OA
11				7113	No OA
11	NOF	5% Mannitol	8690	8034	No OA
11				8379	No OA
11				9655	No OA
11	NOF	0.5% Bupivacaine	29055	28583	No OA
11				25984	No OA
11				32677	No OA
11	NOF	Normal Saline	27668	26324	No OA
11				28458	No OA
11				28300	No OA
11	NOF	1.5% Glycine	44785	50323	No OA
11				45161	No OA
11				38871	No OA
11	NOF	M199	54387	56323	No OA
11				51161	No OA
11				55677	No OA
11	NOF	Ringer's solution	52952	44762	No OA
11				57905	No OA
11				56190	No OA
11	NOF	5% Mannitol	12604	14201	No OA

11				9645	No OA
11				13964	No OA
11	NOF	0.5% Bupivacaine	10640	9040	No OA
11				12480	No OA
11				10400	No OA
11	NOF	Normal Saline	16778	16407	No OA
11				16778	No OA
11				17148	No OA
11	NOF	1.5% Glycine	28775	29069	No OA
11				32843	No OA
11				24510	No OA
11	NOF	M199	32677	31111	No OA
11				36667	No OA
11				30303	No OA
11	NOF	Ringer's solution	74222	104611	No OA
11				52556	No OA
11				65556	No OA
11	NOF	5% Mannitol	72573	70351	No OA
11				68772	No OA
11				78713	No OA
11	NOF	0.5% Bupivacaine	4834	5213	No OA
11				4076	No OA
11				5213	No OA
12	NOF	Normal Saline	51688	46926	No OA
12				50779	No OA
12				57359	No OA
12	NOF	1.5% Glycine	77014	79431	No OA
12				78246	No OA
12				73365	No OA
12	NOF	M199	110191	112823	No OA
12				102392	No OA
12				110191	No OA
12	NOF	Ringer's solution	73463	82878	No OA
12				78683	No OA
12				58829	No OA
12	NOF	5% Mannitol	26352	25409	No OA
12				24937	No OA
12				28711	No OA
12	NOF	0.5% Bupivacaine	27660	26117	No OA
12				27819	No OA
12				29043	No OA
12	NOF	Normal Saline	58207	57052	No OA
12				58008	No OA

12				59562	No OA
12	NOF	1.5% Glycine	82900	81082	No OA
12				88139	No OA
12				79481	No OA
12	NOF	M199	108034	104101	No OA
12				108427	No OA
12				111573	No OA
12	NOF	Ringer's solution	79845	75155	No OA
12				86443	No OA
12				79845	No OA
12	NOF	5% Mannitol	44363	48578	No OA
12				45049	No OA
12				39461	No OA
12	NOF	0.5% Bupivacaine	8912	6477	No OA
12				11088	No OA
12				9171	No OA
12	NOF	Normal Saline	55754	47989	No OA
12				58994	No OA
12				60279	No OA
12	NOF	1.5% Glycine	51891	49370	No OA
12				53613	No OA
12				51891	No OA
12	NOF	M199	53403	56639	No OA
12				55126	No OA
12				48445	No OA
12	NOF	Ringer's solution	46042	44917	No OA
12				44333	No OA
12				48875	No OA
12	NOF	5% Mannitol	91737	98743	No OA
12				103892	No OA
12				72575	No OA
12	NOF	0.5% Bupivacaine	38631	39544	No OA
12				35768	No OA
12				40581	No OA
13	NOF	Normal Saline	53877	45761	No OA
13				55870	No OA
13				60000	No OA
13	NOF	1.5% Glycine	53837	53023	No OA
13				51008	No OA
13				57481	No OA
13	NOF	M199	70000	69204	No OA
13				67389	No OA
13				70000	No OA

13	NOF	Ringer's solution	49776	49590	No OA
13				53433	No OA
13				46306	No OA
13	NOF	0.5% Bupivacaine	22778	25247	No OA
13				20216	No OA
13				22778	No OA
13	NOF	Normal Saline	70161	73817	No OA
13				72849	No OA
13				63817	No OA
13	NOF	1.5% Glycine	109550	118700	No OA
13				102800	No OA
13				107150	No OA
13	NOF	M199	78565	82261	No OA
13				76478	No OA
13				76957	No OA
13	NOF	Ringer's solution	112747	119485	No OA
13				108240	No OA
13				110515	No OA
13	NOF	5% Mannitol	64441	73882	No OA
13				58651	No OA
13				60789	No OA
13	NOF	0.5% Bupivacaine	10941	12892	No OA
13				11394	No OA
13				8537	No OA
13	NOF	Normal Saline	91224	88265	No OA
13				89286	No OA
13				96122	No OA
13	NOF	1.5% Glycine	70891	71977	No OA
13				66860	No OA
13				73837	No OA
13	NOF	M199	70751	75117	No OA
13				70329	No OA
13				66808	No OA
13	NOF	Ringer's solution	68249	67510	No OA
13				64825	No OA
13				72412	No OA
13	NOF	5% Mannitol	60374	56636	No OA
13				58178	No OA
13				66308	No OA
13	NOF	0.5% Bupivacaine	32365	27801	No OA
13				31411	No OA
13				37884	No OA
13	NOF	Normal Saline	75909	79682	No OA

13				72500	No OA
13				75545	No OA
13	NOF	1.5% Glycine	82157	87353	No OA
13				79069	No OA
13				80049	No OA
13	NOF	M199	64509	59191	No OA
13				61561	No OA
13				72775	No OA
13	NOF	Ringer's solution	65260	71823	No OA
13				62656	No OA
13				61302	No OA
13	NOF	5% Mannitol	101239	102051	No OA
13				98632	No OA
13				103034	No OA
13	NOF	0.5% Bupivacaine	10214	8248	No OA
13				11709	No OA
13				10684	No OA
14	NOF	Normal Saline	6391	6154	OA
14				7692	OA
14				5325	OA
14	NOF	1.5% Glycine	57566	61217	OA
14				55238	OA
14				56243	OA
14	NOF	M199	108833	112667	OA
14				104583	OA
14				109250	OA
14	NOF	Ringer's solution	79246	69497	OA
14				85930	OA
14				82312	OA
14	NOF	5% Mannitol	53869	58214	OA
14				50833	OA
14				52560	OA
14	NOF	0.5% Bupivacaine	4943	5682	OA
14				5227	OA
14				3920	OA
14	NOF	Normal Saline	15958	17422	OA
14				14669	OA
14				15784	OA
14	NOF	1.5% Glycine	13913	11594	OA
14				15870	OA
14				14275	OA
14	NOF	M199	137594	136031	OA
14				138000	OA

14				138750	OA
14	NOF	Ringer's solution	92702	91774	OA
14				94879	OA
14				91452	OA
14	NOF	5% Mannitol	10104	10415	OA
14				8549	OA
14				11347	OA
14	NOF	0.5% Bupivacaine	4889	4500	OA
14				6167	OA
14				4000	OA
14	NOF	Normal Saline	32403	36628	OA
14				29767	OA
14				30814	OA
14	NOF	1.5% Glycine	54000	49949	OA
14				51846	OA
14				60205	OA
14	NOF	M199	119384	122745	OA
14				121261	OA
14				114146	OA
14	NOF	Ringer's solution	78394	80843	OA
14				77912	OA
14				76426	OA
14	NOF	5% Mannitol	9641	11704	OA
14				9103	OA
14				8117	OA
14	NOF	0.5% Bupivacaine	5575	4598	OA
14				6954	OA
14				5172	OA
14	NOF	Normal Saline	6932	8580	OA
14				6648	OA
14				5568	OA
14	NOF	1.5% Glycine	11870	9350	OA
14				10528	OA
14				15732	OA
14	NOF	M199	114784	110275	OA
14				107961	OA
14				126118	OA
14	NOF	Ringer's solution	77950	82590	OA
14				74676	OA
14				76583	OA
14	NOF	5% Mannitol	45707	50000	OA
14				46859	OA
14				40262	OA

14	NOF	0.5% Bupivacaine	8021	6471	OA
14				7166	OA
14				10428	OA

Appendix 3: Distribution of chondral explants per experimental condition from each patient

Patient	Experimental condition										Total from patient
	1	2	3	4	5	6	7	8	9	10	
15	4	4	4	4	4	4					24
16	4	4	4	4	4	4					24
17	2	2	2	2	2	2	2	2	2	2	20
18	2	2	2	2	2	2	2	2			16
19	3	3	3	3	3	3	2	2			22
20	4	4	4	4	4	4	2	2			28
21	3	3	3	3	3	3	2	2	3	3	30
22	3	3	3	3	3	3	3	3	3	3	30
23	2	2	2	2	2	2	2	2			14
24	2	2	2	2	2	2	2	2			14
25	2	2	2	2	2	2	2	2	2	2	20
26	3	3	3	3	3	3	3	3	3	3	30
27	4	4	4	4	4	4	4	4			32
Total per solution	38	38	38	38	38	38	26	26	13	13	304

Appendix 4: $^{35}SO_4$ uptake of chondral explants exposed to different local anaesthetics, Glucosamine or Methylprednisolone.

Patient	Experimental variable	Average CPG	CPG	Osteoarthritis
15	1% Lidocaine	9302	10698	OA
15			8651	OA
15			8651	OA
15	2% Lidocaine	5359	4219	OA
15			6456	OA
15			5485	OA
15	0.25% Bupivacaine	10137	9622	OA
15			10069	OA
15			10653	OA
15	0.5% Bupivacaine	25041	27057	OA
15			25422	OA
15			22698	OA
15	LevoBupivacaine	3389	4086	OA
15			3754	OA
15			2326	OA
15	M199	9862	8858	OA
15			7474	OA
15			13253	OA
15	1% Lidocaine	11692	12000	OA
15			12308	OA
15			10769	OA
15	2% Lidocaine	2119	2203	OA
15			2203	OA
15			1949	OA
15	0.25% Bupivacaine	8301	9804	OA
15			7386	OA
15			7843	OA
15	0.5% Bupivacaine	1486	1886	OA
15			914	OA
15			1714	OA
15	LevoBupivacaine	7411	8036	OA
15			8304	OA
15			5893	OA
15	M199	11117	7606	OA
15			13457	OA
15			12394	OA

15	1% Lidocaine	7167	7167	OA
15			6667	OA
15			7750	OA
15	2% Lidocaine	2574	2941	OA
15			1912	OA
15			2941	OA
15	0.25% Bupivacaine	8654	5769	OA
15			11538	OA
15			8654	OA
15	0.5% Bupivacaine	3763	3548	OA
15			3871	OA
15			3871	OA
15	LevoBupivacaine	3000	1250	OA
15			6250	OA
15			1625	OA
15	M199	10732	9431	OA
15			11382	OA
15			11382	OA
15	1% Lidocaine	14097	13403	OA
15			14097	OA
15			14792	OA
15	2% Lidocaine	3626	2515	OA
15			3684	OA
15			4678	OA
15	0.25% Bupivacaine	8993	9799	OA
15			8255	OA
15			8926	OA
15	0.5% Bupivacaine	4510	2810	OA
15			4967	OA
15			5882	OA
15	LevoBupivacaine	2973	2703	OA
15			3784	OA
15			2432	OA
15	M199	14341	12636	OA
15			14419	OA
15			15969	OA
16	1% Lidocaine	9388	9038	OA
16			10000	OA
16			9125	OA
16	2% Lidocaine	14357	12357	OA
16			15571	OA
16			15214	OA
16	0.25% Bupivacaine	16658	17574	OA

16			16658	OA
16			15743	OA
16	0.5% Bupivacaine	2870	3625	OA
16			3202	OA
16			1813	OA
16	M199	10572	9611	OA
16			10206	OA
16			11899	OA
16	LevoBupivacaine	10669	11572	OA
16			11037	OA
16			9465	OA
16	1% Lidocaine	8977	9962	OA
16			8333	OA
16			8826	OA
16	2% Lidocaine	11861	11861	OA
16			11496	OA
16			12226	OA
16	0.25% Bupivacaine	19246	26754	OA
16			15738	OA
16			15279	OA
16	0.5% Bupivacaine	3411	4548	OA
16			3090	OA
16			2624	OA
16	M199	32346	31173	OA
16			31648	OA
16			34246	OA
16	LevoBupivacaine	11152	11198	OA
16			9516	OA
16			12742	OA
16	1% Lidocaine	4186	4488	OA
16			3628	OA
16			4488	OA
16	2% Lidocaine	1662	1662	OA
16			1939	OA
16			1385	OA
16	0.25% Bupivacaine	7808	7673	OA
16			8501	OA
16			7293	OA
16	0.5% Bupivacaine	12065	37552	OA
16			12773	OA
16			11386	OA
16	M199	5663	5233	OA
16			5125	OA

16			6667	OA
16	LevoBupivacaine	1808	1534	OA
16			1808	OA
16			2082	OA
16	1% Lidocaine	1506	1674	OA
16			1381	OA
16			1506	OA
16	2% Lidocaine	2609	16848	OA
16			2880	OA
16			2337	OA
16	0.25% Bupivacaine	2323	1806	OA
16			2452	OA
16			2774	OA
16	0.5% Bupivacaine	4605	5085	OA
16			4520	OA
16			4237	OA
16	M199	11763	12171	OA
16			12245	OA
16			10872	OA
16	LevoBupivacaine	9651	9884	OA
16			9302	OA
16			9884	OA
17	1% Lidocaine	11440	11320	OA
17			10920	OA
17			12120	OA
17	2% Lidocaine	5851	6224	OA
17			5104	OA
17			6349	OA
17	0.25% Bupivacaine	40481	40160	OA
17			41538	OA
17			39744	OA
17	0.5% Bupivacaine	13469	13531	OA
17			13313	OA
17			13625	OA
17	LevoBupivacaine	7562	7851	OA
17			7975	OA
17			6860	OA
17	M199	47714	42857	OA
17			48786	OA
17			51643	OA
17	Bup-Gluc-Protect	35154	32423	OA
17			39912	OA
17			33172	OA

17	Bup-Gluc-Repair	47746	50058	OA
17			44855	OA
17			48324	OA
17	Bup-steroid-protect	95233	97471	OA
17			94070	OA
17			94186	OA
17	Bup-steroid-repair	38029	36308	OA
17			34624	OA
17			43226	OA
17	1% Lidocaine	13496	13540	OA
17			13097	OA
17			13850	OA
17	2% Lidocaine	7175	5381	OA
17			7444	OA
17			8789	OA
17	0.25% Bupivacaine	47391	49275	OA
17			44203	OA
17			48768	OA
17	0.5% Bupivacaine	20043	19571	OA
17			19742	OA
17			20858	OA
17	LevoBupivacaine	9550	8800	OA
17			10300	OA
17			9650	OA
17	M199	37029	40171	OA
17			35029	OA
17			36000	OA
17	Bup-Gluc-Protect	31355	28805	OA
17			40757	OA
17			24542	OA
17	Bup-Gluc-Repair	25602	25994	OA
17			32319	OA
17			18554	OA
17	Bup-steroid-protect	97514	97229	OA
17			99314	OA
17			96000	OA
17	Bup-steroid-repair	38741	37889	OA
17			38630	OA
17			39741	OA
18	1% Lidocaine	24111	23741	No OA
18			25407	No OA
18			23185	No OA
18	2% Lidocaine	36100	35405	No OA

18			35714	No OA
18			37181	No OA
18	0.25% Bupivacaine	84634	86463	No OA
18			84024	No OA
18			83415	No OA
18	0.5% Bupivacaine	2520	2520	No OA
18			2920	No OA
18			2120	No OA
18	LevoBupivacaine	16732	17899	No OA
18			15837	No OA
18			16459	No OA
18	M199	62481	62636	No OA
18			62984	No OA
18			61822	No OA
18	Bup-Gluc-Protect	47910	47075	No OA
18			49075	No OA
18			47582	No OA
18	Bup-Gluc-Repair	73867	75600	No OA
18			70400	No OA
18			71600	No OA
18	1% Lidocaine	49738	51311	No OA
18			49625	No OA
18			48277	No OA
18	2% Lidocaine	34365	34985	No OA
18			34365	No OA
18			33746	No OA
18	0.25% Bupivacaine	32364	31603	No OA
18			34701	No OA
18			30788	No OA
18	0.5% Bupivacaine	29429	30204	No OA
18			29102	No OA
18			28980	No OA
18	LevoBupivacaine	25489	23484	No OA
18			25990	No OA
18			26992	No OA
18	M199	111301	109384	No OA
18			113014	No OA
18			111507	No OA
18	Bup-Gluc-Protect	80697	86307	No OA
18			84425	No OA
18			71359	No OA
18	Bup-Gluc-Repair	37716	38025	No OA
18			37407	No OA

18			37716	No OA
19	1% Lidocaine	20551	20441	No OA
19			20074	No OA
19			21176	No OA
19	2% Lidocaine	13986	14895	No OA
19			13392	No OA
19			13741	No OA
19	0.25% Bupivacaine	36607	38214	No OA
19			29286	No OA
19			42411	No OA
19	0.5% Bupivacaine	34625	29644	No OA
19			36601	No OA
19			37668	No OA
19	LevoBupivacaine	20395	18814	No OA
19			19368	No OA
19			23043	No OA
19	M199	34982	35055	No OA
19			33432	No OA
19			36531	No OA
19	Bup-Gluc-Protect	50868	80491	No OA
19			34340	No OA
19			37849	No OA
19	Bup-Gluc-Repair	18016	18492	No OA
19			18373	No OA
19			17183	No OA
19	1% Lidocaine	2925	3163	No OA
19			3503	No OA
19			2143	No OA
19	2% Lidocaine	1975	2160	No OA
19			2253	No OA
19			1543	No OA
19	0.25% Bupivacaine	1132	1226	No OA
19			755	No OA
19			1415	No OA
19	0.5% Bupivacaine	5830	7011	No OA
19			6273	No OA
19			4280	No OA
19	LevoBupivacaine	365	304	No OA
19			395	No OA
19			395	No OA
19	M199	22081	22013	No OA
19			23154	No OA
19			21141	No OA

19	Bup-Gluc-Protect	42857	44370	No OA
19			39496	No OA
19			44790	No OA
19	Bup-Gluc-Repair	16271	13531	No OA
19			17822	No OA
19			17492	No OA
19	1% Lidocaine	30381	29524	No OA
19			32667	No OA
19			29048	No OA
19	2% Lidocaine	4937	4906	No OA
19			5755	No OA
19			4182	No OA
19	0.25% Bupivacaine	7607	7493	No OA
19			7493	No OA
19			7863	No OA
19	0.5% Bupivacaine	4062	4370	No OA
19			4566	No OA
19			3249	No OA
19	LevoBupivacaine	19135	16955	No OA
19			19239	No OA
19			21211	No OA
19	M199	55572	57363	No OA
19			52239	No OA
19			57214	No OA
20	1% Lidocaine	35053	34000	No OA
20			30316	No OA
20			40842	No OA
20	2% Lidocaine	11392	9267	No OA
20			13040	No OA
20			11941	No OA
20	0.25% Bupivacaine	27040	26400	No OA
20			27200	No OA
20			27600	No OA
20	0.5% Bupivacaine	4536	4750	No OA
20			5000	No OA
20			3929	No OA
20	LevoBupivacaine	15906	17181	No OA
20			15436	No OA
20			15168	No OA
20	M199	121809	109894	No OA
20			110638	No OA
20			145000	No OA
20	Bup-Gluc-Protect	23667	22815	No OA

20			23556	No OA
20			24667	No OA
20	Bup-Gluc-Repair	50000	51563	No OA
20			48438	No OA
20			50000	No OA
20	1% Lidocaine	16696	16564	No OA
20			16123	No OA
20			17445	No OA
20	2% Lidocaine	17117	30061	No OA
20			10798	No OA
20			10613	No OA
20	0.25% Bupivacaine	35155	33292	No OA
20			30807	No OA
20			41366	No OA
20	0.5% Bupivacaine	10000	10573	No OA
20			9108	No OA
20			10382	No OA
20	LevoBupivacaine	10212	8783	No OA
20			10582	No OA
20			11270	No OA
20	M199	90656	93443	No OA
20			84672	No OA
20			93934	No OA
20	Bup-Gluc-Protect	49085	79577	No OA
20			53239	No OA
20			14507	No OA
20	Bup-Gluc-Repair	23969	24897	No OA
20			19897	No OA
20			27113	No OA
20	1% Lidocaine	19349	19642	No OA
20			18990	No OA
20			19414	No OA
20	2% Lidocaine	17746	17514	No OA
20			16358	No OA
20			19422	No OA
20	0.25% Bupivacaine	35045	30804	No OA
20			32143	No OA
20			42232	No OA
20	0.5% Bupivacaine	20154	12538	No OA
20			22308	No OA
20			25615	No OA
20	LevoBupivacaine	14949	16327	No OA
20			15816	No OA

20			12755	No OA
20	M199	49273	52485	No OA
20			50909	No OA
20			44424	No OA
20	1% Lidocaine	27150	29534	No OA
20			27098	No OA
20			24870	No OA
20	2% Lidocaine	30714	34921	No OA
20			23810	No OA
20			33571	No OA
20	0.25% Bupivacaine	61221	59008	No OA
20			56489	No OA
20			68168	No OA
20	0.5% Bupivacaine	15729	14583	No OA
20			14583	No OA
20			18021	No OA
20	LevoBupivacaine	13178	15194	No OA
20			13411	No OA
20			11085	No OA
20	M199	65818	59394	No OA
20			66242	No OA
20			71879	No OA
21	1% Lidocaine	69612	64509	OA
21			73031	OA
21			71413	OA
21	2% Lidocaine	27517	28859	OA
21			25168	OA
21			28523	OA
21	0.25% Bupivacaine	128510	131137	OA
21			131901	OA
21			122541	OA
21	0.5% Bupivacaine	48869	48216	OA
21			47808	OA
21			50663	OA
21	LevoBupivacaine	46022	44445	OA
21			49393	OA
21			44258	OA
21	M199	149040	118653	OA
21			133775	OA
21			194702	OA
21	Bup-steroid-protect	34428	35055	OA
21			35055	OA
21			33210	OA

21	Bup-steroid-repair	12228	13212	OA
21			9845	OA
21			13644	OA
21	Bup-Gluc-Protect	93494	86859	OA
21			96902	OA
21			96795	OA
21	Bup-Gluc-Repair	30968	29247	OA
21			31290	OA
21			32473	OA
21	1% Lidocaine	104773	102014	OA
21			111480	OA
21			100906	OA
21	2% Lidocaine	49078	47668	OA
21			49260	OA
21			50398	OA
21	0.25% Bupivacaine	45792	45560	OA
21			47490	OA
21			44402	OA
21	0.5% Bupivacaine	19763	19428	OA
21			21203	OA
21			18738	OA
21	LevoBupivacaine	28006	24881	OA
21			27350	OA
21			31814	OA
21	M199	106587	126735	OA
21			132992	OA
21			128328	OA
21	Bup-steroid-protect	65017	67684	OA
21			72768	OA
21			54689	OA
21	Bup-steroid-repair	26706	23333	OA
21			26667	OA
21			30196	OA
21	Bup-Gluc-Protect	90473	88166	OA
21			88462	OA
21			94872	OA
21	Bup-Gluc-Repair	30909	29221	OA
21			29113	OA
21			34416	OA
21	1% Lidocaine	172203	146799	OA
21			161488	OA
21			208381	OA
21	2% Lidocaine	50750	49908	OA

21			49352	OA
21			53056	OA
21	0.25% Bupivacaine	71825	73457	OA
21			73192	OA
21			68871	OA
21	0.5% Bupivacaine	56815	52757	OA
21			53992	OA
21			63786	OA
21	LevoBupivacaine	117037	111605	OA
21			121893	OA
21			117696	OA
21	M199	201667	206482	OA
21			189983	OA
21			208586	OA
21	Bup-steroid-protect	99501	97458	OA
21			99413	OA
21			101662	OA
21	Bup-steroid-repair	60048	59348	OA
21			56325	OA
21			64519	OA
22	1% Lidocaine	12509	12014	OA
22			13428	OA
22			12132	OA
22	2% Lidocaine	14043	13373	OA
22			13992	OA
22			14815	OA
22	0.25% Bupivacaine	27500	28041	OA
22			25563	OA
22			28942	OA
22	0.5% Bupivacaine	17743	16732	OA
22			18547	OA
22			18028	OA
22	LevoBupivacaine	8626	7899	OA
22			9795	OA
22			8373	OA
22	M199	30840	29692	OA
22			31513	OA
22			31373	OA
22	Bup-Gluc-Protect	16164	17352	OA
22			14916	OA
22			15068	OA
22	Bup-Gluc-Repair	10456	11579	OA
22			9825	OA

22			10059	OA
22	Bup-steroid-protect	9763	10057	OA
22			9265	OA
22			10057	OA
22	Bup-steroid-repair	2812	2130	OA
22			4047	OA
22			2343	OA
22	1% Lidocaine	16323	14798	OA
22			15546	OA
22			18685	OA
22	2% Lidocaine	19615	19743	OA
22			21795	OA
22			17436	OA
22	0.25% Bupivacaine	28047	30976	OA
22			27161	OA
22			26038	OA
22	0.5% Bupivacaine	17451	17124	OA
22			16471	OA
22			18824	OA
22	LevoBupivacaine	9507	9688	OA
22			10837	OA
22			8046	OA
22	M199	30283	29689	OA
22			31444	OA
22			29825	OA
22	Bup-Gluc-Protect	15060	11780	OA
22			17403	OA
22			16064	OA
22	Bup-Gluc-Repair	1019	1062	OA
22			637	OA
22			1486	OA
22	Bup-steroid-protect	10866	9318	OA
22			11024	OA
22			12336	OA
22	Bup-steroid-repair	4815	5247	OA
22			3858	OA
22			5401	OA
22	1% Lidocaine	29100	28258	OA
22			30335	OA
22			28835	OA
22	2% Lidocaine	16008	16601	OA
22			18313	OA
22			13175	OA

22	0.25% Bupivacaine	35824	34608	OA
22			34314	OA
22			38627	OA
22	0.5% Bupivacaine	10041	9504	OA
22			9367	OA
22			11295	OA
22	LevoBupivacaine	30401	27871	OA
22			29766	OA
22			33668	OA
22	M199	50069	48958	OA
22			46181	OA
22			55093	OA
22	Bup-Gluc-Protect	35338	31579	OA
22			37093	OA
22			37469	OA
22	Bup-Gluc-Repair	32564	32601	OA
22			32479	OA
22			32723	OA
22	Bup-steroid-protect	12979	11187	OA
22			12785	OA
22			15068	OA
22	Bup-steroid-repair	25250	22857	OA
22			28333	OA
22			24643	OA
23	1% Lidocaine	1277	1257	No OA
23			1118	No OA
23			1457	No OA
23	2% Lidocaine	1943	1741	No OA
23			3239	No OA
23			931	No OA
23	0.25% Bupivacaine	3173	2410	No OA
23			3735	No OA
23			3454	No OA
23	0.5% Bupivacaine	1660	2213	No OA
23			1028	No OA
23			1818	No OA
23	LevoBupivacaine	3701	4173	No OA
23			2598	No OA
23			4331	No OA
23	M199	7655	8325	No OA
23			7629	No OA
23			7036	No OA
23	Bup-Gluc-Protect	3363	4305	No OA

23			3408	No OA
23			2377	No OA
23	Bup-Gluc-Repair	3137	2745	No OA
23			2941	No OA
23			3725	No OA
23	1% Lidocaine	4286	4343	No OA
23			4914	No OA
23			3600	No OA
23	2% Lidocaine	1257	1639	No OA
23			1257	No OA
23			874	No OA
23	0.25% Bupivacaine	1356	1525	No OA
23			1102	No OA
23			1525	No OA
23	0.5% Bupivacaine	1980	1139	No OA
23			1782	No OA
23			3119	No OA
23	LevoBupivacaine	4350	4800	No OA
23			3650	No OA
23			4650	No OA
23	M199	11466	11675	No OA
23			10628	No OA
23			12199	No OA
23	Bup-Gluc-Protect	6101	6422	No OA
23			5046	No OA
23			6881	No OA
23	Bup-Gluc-Repair	3598	4167	No OA
23			3144	No OA
23			3523	No OA
24	1% Lidocaine	3733	4578	No OA
24			3822	No OA
24			2800	No OA
24	2% Lidocaine	1374	1099	No OA
24			2363	No OA
24			714	No OA
24	0.25% Bupivacaine	2965	3982	No OA
24			2655	No OA
24			2345	No OA
24	0.5% Bupivacaine	2487	2335	No OA
24			2843	No OA
24			2335	No OA
24	LevoBupivacaine	5048	4762	No OA
24			5857	No OA

24			4571	No OA
24	M199	14587	13761	No OA
24			14817	No OA
24			15275	No OA
24	Bup-Gluc-Protect	6047	5000	No OA
24			5000	No OA
24			8140	No OA
24	Bup-Gluc-Repair	2736	2972	No OA
24			2642	No OA
24			2642	No OA
24	1% Lidocaine	5185	5291	No OA
24			5450	No OA
24			4921	No OA
24	2% Lidocaine	2049	2131	No OA
24			1885	No OA
24			2131	No OA
24	0.25% Bupivacaine	2324	1831	No OA
24			1408	No OA
24			3732	No OA
24	0.5% Bupivacaine	1959	2216	No OA
24			2062	No OA
24			1701	No OA
24	LevoBupivacaine	5172	6092	No OA
24			4368	No OA
24			5172	No OA
24	M199	8357	8786	No OA
24			6893	No OA
24			9393	No OA
24	Bup-Gluc-Protect	4488	4594	No OA
24			3887	No OA
24			5053	No OA
24	Bup-Gluc-Repair	6358	5954	No OA
24			7283	No OA
24			5954	No OA
25	1% Lidocaine	7958	8042	OA
25			8583	OA
25			7333	OA
25	2% Lidocaine	4507	5035	OA
25			3873	OA
25			4683	OA
25	0.25% Bupivacaine	9623	8994	OA
25			10063	OA
25			9843	OA

25	0.5% Bupivacaine	2237	2711	OA
25			1842	OA
25			2184	OA
25	LevoBupivacaine	2180	1831	OA
25			2209	OA
25			2500	OA
25	M199	20570	21839	OA
25			18290	OA
25			21580	OA
25	Bup-Gluc-Protect	10060	9489	OA
25			10300	OA
25			10390	OA
25	Bup-Gluc-Repair	6655	7855	OA
25			6291	OA
25			5818	OA
25	Bup-steroid-protect	6278	7444	OA
25			6099	OA
25			5381	OA
25	Bup-steroid-repair	2257	2178	OA
25			1837	OA
25			2782	OA
25	1% Lidocaine	13493	13824	OA
25			13971	OA
25			12721	OA
25	2% Lidocaine	4353	4306	OA
25			4471	OA
25			4306	OA
25	0.25% Bupivacaine	13974	14359	OA
25			13231	OA
25			14359	OA
25	0.5% Bupivacaine	2766	2582	OA
25			3545	OA
25			2172	OA
25	LevoBupivacaine	6163	6089	OA
25			6510	OA
25			5941	OA
25	M199	32381	35000	OA
25			28386	OA
25			33757	OA
25	Bup-Gluc-Protect	24615	25481	OA
25			26274	OA
25			22115	OA
25	Bup-Gluc-Repair	5347	5099	OA

25			6188	OA
25			4777	OA
25	Bup-steroid-protect	4562	5199	OA
25			3873	OA
25			4668	OA
25	Bup-steroid-repair	7329	6832	OA
25			6832	OA
25			8344	OA
26	1% Lidocaine	2090	1716	OA
26			2463	OA
26			2239	OA
26	2% Lidocaine	3051	3814	OA
26			2246	OA
26			3093	OA
26	0.25% Bupivacaine	3206	3282	OA
26			1756	OA
26			4580	OA
26	0.5% Bupivacaine	903	903	OA
26			1111	OA
26			694	OA
26	LevoBupivacaine	2500	2031	OA
26			2344	OA
26			3125	OA
26	M199	48932	45437	OA
26			48058	OA
26			53398	OA
26	Bup-Gluc-Protect	16770	17764	OA
26			15714	OA
26			16957	OA
26	Bup-Gluc-Repair	7269	8148	OA
26			5833	OA
26			7870	OA
26	Bup-steroid-protect	5360	4640	OA
26			5440	OA
26			6000	OA
26	Bup-steroid-repair	8449	8182	OA
26			9626	OA
26			7647	OA
26	1% Lidocaine	2438	1413	OA
26			3993	OA
26			1979	OA
26	2% Lidocaine	1343	1060	OA
26			1873	OA

26			1166	OA
26	0.25% Bupivacaine	3540	2888	OA
26			3416	OA
26			4348	OA
26	0.5% Bupivacaine	542	813	OA
26			434	OA
26			434	OA
26	LevoBupivacaine	1623	1509	OA
26			2113	OA
26			1245	OA
26	M199	22090	18401	OA
26			28913	OA
26			18977	OA
26	Bup-Gluc-Protect	6609	6137	OA
26			6137	OA
26			7575	OA
26	Bup-Gluc-Repair	4805	4897	OA
26			4138	OA
26			5425	OA
26	Bup-steroid-protect	3039	2857	OA
26			2675	OA
26			3636	OA
26	Bup-steroid-repair	4308	3846	OA
26			4692	OA
26			4436	OA
26	1% Lidocaine	1960	2120	OA
26			2520	OA
26			1320	OA
26	2% Lidocaine	2020	2801	OA
26			1824	OA
26			1498	OA
26	0.25% Bupivacaine	1285	1493	OA
26			1389	OA
26			1042	OA
26	0.5% Bupivacaine	660	799	OA
26			556	OA
26			694	OA
26	LevoBupivacaine	824	691	OA
26			691	OA
26			1144	OA
26	M199	38759	38722	OA
26			35451	OA
26			42105	OA

26	Bup-Gluc-Protect	14974	14974	OA
26			15707	OA
26			14293	OA
26	Bup-Gluc-Repair	4888	4832	OA
26			5391	OA
26			4469	OA
26	Bup-steroid-protect	5573	5882	OA
26			5449	OA
26			5449	OA
26	Bup-steroid-repair	4788	5212	OA
26			3909	OA
26			5309	OA
27	1% Lidocaine	16172	11962	No OA
27			18341	No OA
27			18341	No OA
27	2% Lidocaine	10182	12727	No OA
27			7273	No OA
27			10707	No OA
27	0.25% Bupivacaine	6477	5699	No OA
27			5181	No OA
27			8636	No OA
27	0.5% Bupivacaine	1413	1576	No OA
27			1268	No OA
27			1449	No OA
27	LevoBupivacaine	7489	6926	No OA
27			7503	No OA
27			8081	No OA
27	M199	61077	58205	No OA
27			58590	No OA
27			66538	No OA
27	Bup-Gluc-Protect	25664	25408	No OA
27			25175	No OA
27			26573	No OA
27	Bup-Gluc-Repair	1389	1852	No OA
27			1852	No OA
27			694	No OA
27	1% Lidocaine	18989	20599	No OA
27			17041	No OA
27			19476	No OA
27	2% Lidocaine	4276	4276	No OA
27			5154	No OA
27			3509	No OA
27	0.25% Bupivacaine	26296	30865	No OA

27			21606	No OA
27			27161	No OA
27	0.5% Bupivacaine	515	368	No OA
27			735	No OA
27			490	No OA
27	LevoBupivacaine	9444	10031	No OA
27			8642	No OA
27			9722	No OA
27	M199	74545	76936	No OA
27			82155	No OA
27			64646	No OA
27	Bup-Gluc-Protect	35402	37946	No OA
27			33482	No OA
27			34821	No OA
27	Bup-Gluc-Repair	1314	762	No OA
27			1524	No OA
27			1714	No OA
27	1% Lidocaine	20813	20596	No OA
27			19512	No OA
27			22493	No OA
27	2% Lidocaine	7685	9568	No OA
27			5864	No OA
27			7716	No OA
27	0.25% Bupivacaine	13281	16927	No OA
27			8854	No OA
27			14323	No OA
27	0.5% Bupivacaine	1419	1351	No OA
27			676	No OA
27			2252	No OA
27	LevoBupivacaine	8210	6790	No OA
27			10082	No OA
27			7819	No OA
27	M199	49497	41341	No OA
27			68156	No OA
27			39106	No OA
27	Bup-Gluc-Protect	50443	55907	No OA
27			46203	No OA
27			49367	No OA
27	Bup-Gluc-Repair	3826	3478	No OA
27			4493	No OA
27			3623	No OA
27	1% Lidocaine	30583	29297	No OA
27			26906	No OA

27			35575	No OA
27	2% Lidocaine	17617	10071	No OA
27			23404	No OA
27			19433	No OA
27	0.25% Bupivacaine	12664	12355	No OA
27			13642	No OA
27			12098	No OA
27	0.5% Bupivacaine	797	1087	No OA
27			483	No OA
27			845	No OA
27	LevoBupivacaine	9529	9150	No OA
27			10588	No OA
27			8889	No OA
27	M199	100072	69892	No OA
27			121386	No OA
27			108961	No OA
27	Bup-Gluc-Protect	47351	42053	No OA
27			64901	No OA
27			35099	No OA
27	Bup-Gluc-Repair	1174	1252	No OA
27			1408	No OA
27			939	No OA

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