Australian Government

Department of Health and Ageing

Therapeutic Goods Administration

Australian Public Assessment Report for Fibrin Sealant

Proprietary Product Name: Tisseel VH/SD

Submission No: PM-2009-00290-3-4

Sponsor: Baxter Healthcare Pty Ltd

April 2010

Contents

I. Introduction to Product Submission

Submission Details

Type of Submission	Extension of Indications
Decision:	Approved
Date of Decision:	15 March 2010
Active ingredient(s):	Fibrin sealant
Product Name(s):	Tisseel VH/SD
Sponsor's Name and Address:	Baxter Healthcare Pty Ltd 1 Baxter Drive Old Toongabbie NSW 2146
Dose form(s):	Two deep frozen solutions
Strength(s):	1 mL, 2 mL and 5 mL of each solution.
Container(s):	Both Sealer Protein Solution and Thrombin Solution are contained in two separate chambers of a single use double chamber syringe made of polypropylene.
Pack size(s):	2 mL, 4 mL and 10 mL
Approved Therapeutic use:	as a sealant and/or adhesive for use in autologous chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI) procedures.
Route(s) of administration:	Topical
Dosage:	The approximate surface area covered by each package size of Tisseel are up to 8 cm^2 (2 mL), up to 16cm^2 (4 mL) and up to 40 cm^2 (10 mL).

Product Background

This application was submitted by Biotech Regulatory Solutions as an agent to the sponsor (Baxter Healthcare Pty Ltd) for Tisseel Duo 500 but has been carried over to Tisseel VH/SD, a later version of the product registered in Australia on 9 February 2009. Tisseel VH/SD (Vapour Heated, Solvent Detergent Treated), so named to distinguish it from earlier formulations, is manufactured with an additional solvent/detergent viral inactivation step and has less factor XIII than Tisseel Duo 500. Approval of Tisseel VH/SD in 2009 was based on comparable efficacy to Tisseel Duo 500 in haemostasis in humans and in closure of colostomies in animals. At that time, there were no comparative data in Autologous Chondrocyte Implantation (ACI)/ Matrix-induced Autologous Chondrocyte Implantation (MACI).

Tisseel VH/SD is a fibrin glue/sealant product which is already registered. It is a mixture of human plasma-derived coagulation factors, which when mixed together result in the formation of a solid fibrin clot. The product as presented as two separate solutions which are mixed at the site of application. The active ingredients in the currently registered formulation are as follows:

1. "Sealer Protein Solution"

· Fibrinogen (human)	72 – 110 mg per mL	Coagulation factor
· Factor XIII (human)	1.2 – 10 IU per mL	Coagulation factor
· Aprotinin (bovine)	3000 KIU per mL	Fibrinolysis inhibitor

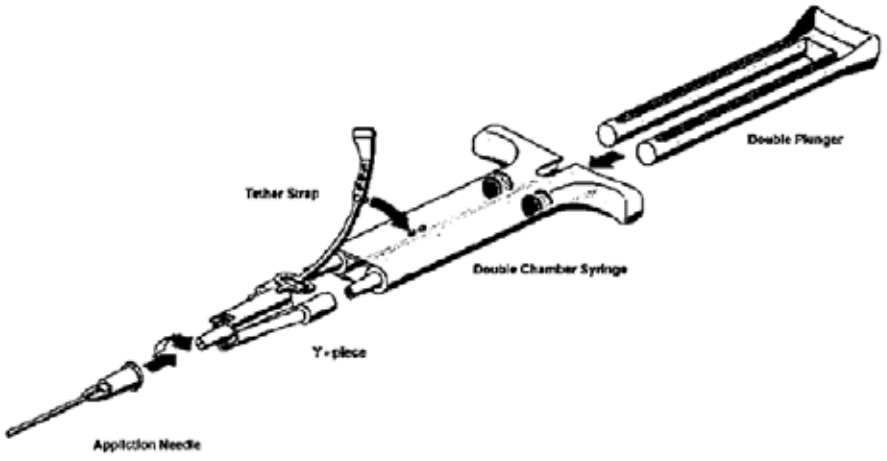
2. "Thrombin Solution"

· Thrombin (human)	500 IU per mL	Coagulation factor
· Calcium chloride	40 mmol per mL	Clotting activator

The human coagulation factors are derived from human plasma. Each solution is presented in a separate pre-loaded chamber of one double-chamber syringe (see Figure 1). Three pack sizes are registered in Australia:

· 2 mL – containing 1 mL of each solution;
· 4 mL – containing 2 mL of each solution; and
· 10 mL – containing 5 mL of each solution.

Figure 1 – Tisseel VH/SD Double Chamber Syringe



No changes to the formulation are proposed in the current application.

There have been numerous formulations and presentations of Tisseel marketed around the world since it was first marketed in Europe in the 1990's. These have varied in the source of the thrombin (bovine versus human), the number and type of viral inactivation steps included in the manufacturing process, the content of Factor XIII and the presentation (deep frozen vs freeze dried).

The original formulation approved in Australia in 2002 was named "Tisseel Duo 500'. The currently approved formulation, named 'Tisseel VH/SD" and which incorporates a reduced Factor XIII content and a solvent-detergent viral inactivation step was approved in February 2009.

None of the clinical studies in the current submission identified the particular formulation or presentation of Tisseel product used.

Tisseel VH/SD is currently registered for the following indications:

- As an adjunct to haemostasis during surgical procedures, when control of bleeding by conventional surgical techniques is ineffective or impractical; and
- As a sealant as an adjunct for the closure of colostomies.

The current application seeks approval of the following new indication:

- As a sealant as an adjunct for the adhesion of chondrocyte seeded scaffold to the subchondral bed of articular cartilage defects, for the initiation of articular cartilage repair following ACI / MACI.

The application seeks approval for use of Tisseel VH/SD for use in the surgical procedures of ACI and MACI. These are orthopaedic procedures used to treat full thickness defects of the articular cartilage, usually in the knee joint. According to the literature submitted with the application, ACI was first described in 1987 and the procedures have since become common practice. The sponsor estimates that over 6,000 patients received the MACI procedure in Australia between 1998 and 2008.

According to the sponsor, ACI / MACI procedures, which included the use of Tisseel, were funded in Australia under the Medicare Benefits Schedule (MBS) between 2002 and 2007. In September 2007, the Medical Services Advisory Committee (MSAC) decided to place a "hold" on funding for the procedures because this use of Tisseel was considered to be outside the TGA-approved indications for the product.

The current application therefore seeks TGA approval for the use of Tisseel in these procedures, presumably to enable resumption of Medicare funding. The application is a literature-based submission.

ACI and MACI

As indicated above, ACI and MACI are surgical procedures developed to treat defects in the articular cartilage of joints. Typically these defects arise as a result of trauma, but may also result from avascular necrosis, osteochondritis dissecans and various cartilage disorders. The procedures have been most commonly described in the treatment of such lesions in the knee. Articular cartilage has a poor capacity for repair, and these defects persist and cause ongoing joint pain, swelling and catching in the joint. The submission included review articles describing the surgical techniques.[1,2,3] The following description of the procedures is based on these papers.

ACI

ACI involves two stages of surgery. Patients initially undergo an arthroscopy, during which full thickness biopsies of healthy articular cartilage tissue (down to subchondral bone) are taken. In the knee joint these biopsies are taken from non-weight-bearing surfaces such as the outer edge of the superior medial or lateral femoral condyle, or the inner edge of the lateral femoral condyle at the intercondylar notch. Two or three biopsies with a total weight of 200 – 300 mg are required.

The biopsies then undergo an *in-vitro* culture process aimed at expanding the number of viable chondrocytes 10- to 12-fold. This process usually takes about three to four weeks and the final product is delivered as a vial of 0.3 to 0.4 mL of fluid medium containing 12 million autologous chondrocytes.

[1] Alford JW,, Cole BJ. Cartilage restoration, part 2: techniques, outcomes, and future directions. Am J Sports Med 2005; 33: 443-460.
[2] Brittberg M. Autologous Chondrocyte implantation – technique and long-term follow-up. Injury 2008; 39: S40-S49.
[3] Gillogly SD, Voight M, Blackburn T. Treatment of articular cartilage defects of the knee with Autologous chodrocyte implantation. J Orthop Sports Phy Ther 1998; 28: 241-251.

When the autologous chondrocytes are ready for implantation, the patient undergoes an arthrotomy. The cartilage defect to be treated is debrided circumferentially back to a healthy rim of surrounding normal cartilage, and any remaining cartilage or fibrous tissue in the base of the lesion is removed. A patch of periosteum is then obtained from the anteromedial surface of the tibia, via a separate incision distal to the knee joint. The patch is sized to match the size of the cartilage defect. The patch is then placed over the cartilage defect and secured to the surrounding normal cartilage with multiple interrupted 5-0 or 6-0 absorbable sutures. The suture line is then further sealed with the fibrin sealant to ensure a "watertight" closure. A small opening is retained to allow injection of the autologous cells into the space between the healthy cartilage, the subchondral bone and the patch.

The patient's autologous chondrocytes are then aspirated from the vial and injected into the cartilage defect, beneath the periosteal patch, ensuring complete fill of the defect with the cells. The small opening is then closed with one or two final sutures and sealed with the fibrin sealant. The arthrotomy incision is then closed. A diagram of the procedure is at Figure 2.[3]

The role of fibrin sealant in this procedure is therefore to:

1) provide additional adhesive support, and reduce the number of sutures required, in fixing the periosteal graft to the joint cartilage; and

2) obtain a watertight seal overlying the cartilage defect. This effect is analogous to the currently approved indication for Tisseel as a sealant as an adjunct to the closure of colostomies.

MACI

The MACI procedure (also referred to in the literature as 'second generation ACI') also involves an initial arthroscopy, cartilage biopsy and *in vitro* culture of autologous chondrocytes. Following culture, the cells are loaded / seeded onto a collagen membrane or scaffold. Chondrocytes adhere to collagen and differentiate. Following the loading / seeding, the scaffold with cells is cultured for another 2 to 5 days. At least one such collagen scaffold product has been registered in Australia as a therapeutic device (MatricelACI-MAIX Collagen Membrane; Verigen Australia Pty Ltd; ARTG 121056).

At the time of the second surgery, the cartilage defect is debrided as per the ACI procedure. The seeded collagen scaffold is cut with scissors to match the size and shape of the debrided cartilage defect. The seeded scaffold is then fixed into the cartilage defect using the fibrin glue as an adhesive. No suturing is required. The collagen scaffold acts as a carrier for the chondrocytes and also serves to fill the defect. Several layers of the scaffold may need to be applied in order to fill up the defect. Unlike ACI, the second stage of the MACI procedure can be performed via an arthroscope.

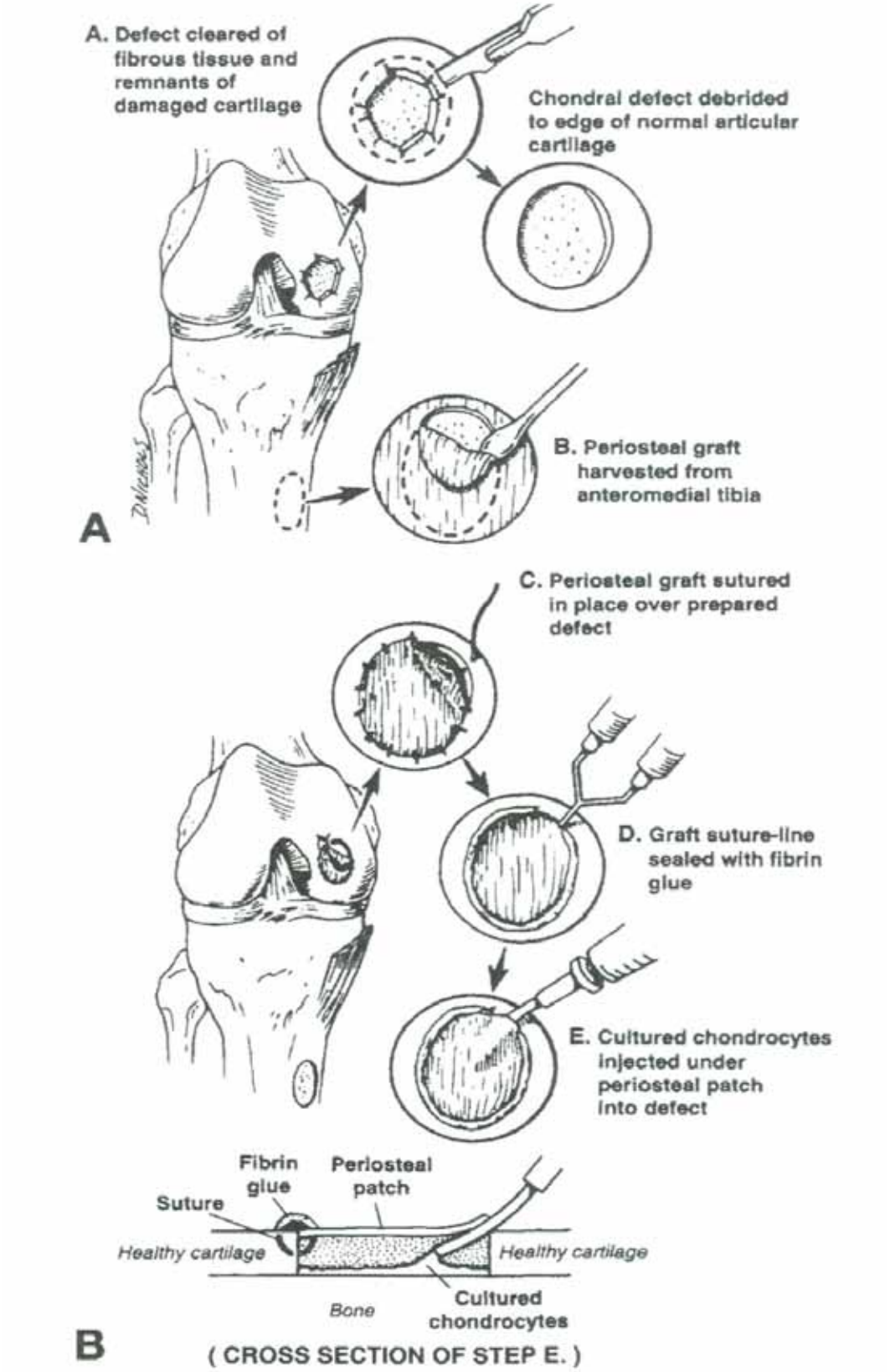
In this procedure the role of the fibrin is to act as an adhesive.

The dose of fibrin glue/sealant used in the procedures was not stated in any of the submitted publications.

Regulatory Status

This application is unique to Australia. However, in some foreign markets the product is approved for fairly broad surgical indications which would encompass the proposed new indication in Australia. For example, in the UK the product is approved for: *"Supportive treatment where surgical techniques are insufficient …..… as a tissue glue to promote adhesion / sealing or as a suture support."* Similarly, in Canada the product is approved for: *" … in addition to standard measures to achieve haemostasis, to seal or glue tissue, and to support wound healing. Indications include: ….. orthopaedic surgery …."*.

Figure 2 – Autologous Chondrocyte Implantation



(ACI)

Product Information

The approved product information current at the time this AusPAR was prepared is at Attachment 1.

II. Quality Findings

Quality Summary and Conclusions

There was no requirement for a quality evaluation in an application of this type.

III. Nonclinical Findings

Introduction

This submission is for an additional indication to the existing indications of Tisseel to be used as an orthopaedic glue of chondrocyte seeded scaffold to the subchondral bed. As for the previous indications, the product is expected to be used locally and mainly on a single occasion. There are no changes in dose for the newly proposed indication. However, the product is to be applied to areas of the body (articular cartilage) in which the product has no direct contact with blood vessels, compared to the currently approved indications (haemostasis and closure of colostomies). Therefore, the main concerns for this application were the pharmacodynamics and local tolerance of the product as well as its potential effects on chondrocytes.

This application is a literature-based submission. For the nonclinical data, 68 published papers were provided. Among these, directly relevant nonclinical data to the proposed indication are only discussed in this report. The data mainly focused on the characteristics and/or efficacy of commercial and non-commercial fibrin sealants alone, with articular chondrocytes or with chondrocytes in combination with matrix scaffold to be used for articular cartilage generation. For commercial fibrin sealants, a number of different Tisseel products were studied with/without modifications of ingredient contents. However, all these products are manufactured by Baxter and have the same active ingredients including their contents. Only a few papers contained information on nonclinical safety (acute toxicity and local tolerance) of the fibrin glue constructs.

Pharmacology

Primary pharmacodynamics

Fibrin sealant formulation for fibrin glue constructs

As a fibrin sealant, the bonding power of the fibrin glue was stronger with commercial fibrin sealants including Tisseel than fibrin adhesives prepared from autologous or single donor plasma (Siedentop *et al*., 2001), although the contents of active ingredients contained in their fibrin sealants were not compared.

Masses of the fibrin glue were greater as fibrinogen (porcine) concentration was increased (20- 80 mg/mL tested) and when aprotinin (3000 KIU) was contained in the fibrin sealant, following implantation of the fibrin gels in the subcutaneous tissue of mice for 4 weeks (Silverman *et al*., 1999).

As the time required for the onset of clot depends on the amount of thrombin used for a fibrin glue, Schlag and Redl (1986) have explained in their review article that low concentrations (that is, 4 IU/mL) of thrombin may be beneficial for slow sealing (that is, bone or tissue) and high concentrations (that is, 500 IU/mL) for instant clotting (that is, haemostasis). This suggests that the thrombin concentration (350-500 IU/mL) contained in Tisseel approved for haemostasis may require an adjustment to be used in articular cartilage defects for the proposed indication. However, an unmodified Tisseel series of products containing 500 IU/mL thrombin were used, with chondrocytes or with chondrocytes and collagen membrane for sealing knee defects in rabbits and dogs (Brittberg *et al*., 1997; Hashimotto *et al*, 1992; Ishimura *et al*., 1997; Willers *et al*., 2005).

Furthermore, implantation of a construct prepared with Tisseel, autologous chondrocytes and collagen membrane enhanced treatment of osteochondral defects in the rabbit knee, compared to no treatment (Willers *et al*., 2005). In addition, Tisseel containing 500 IU/mL thrombin in 40 mM calcium chloride is also used in tissue gluing/sealing in humans. From these observations, Tisseel containing a thrombin concentration of 350-500 IU/mL in 40 mM calcium chloride is considered to be suitable for use in the articular cartilage for the proposed indication.

The function of aprotinin contained in fibrin sealants for fibrin glue constructs is explained in the *Pharmacokinetics* section below.

Fibrin sealant in fibrin glue constructs

Effect of fibrin sealant on articular chondrocytes

In fibrin constructs prepared with fibrin sealants including an unmodified Tisseel series of products and articular chondrocytes with/without matrix scaffolds, the fibrin sealants were not cytotoxic to cells.

Many studies have shown that articular chondrocytes at cell numbers ranging from $1 \times 10^4 - 5 \times 10^6$ cells in fibrin/cell or fibrin/cell/scaffold constructs were viable and homogenously distributed in the constructs. They multiplied while retaining their morphology and produced extracellular matrix (ECM) components (that is, glycosaminoglycan [GAG] and collagen type I/II) in the construct *in vitro* and *in vivo* (Eyrich *et al*., 2007:13 and 2007:28; Homminga *et al*., 1993). In addition, migration (from collagen membrane towards fibrin glue) and proliferation of cells were observed in the Tisseel/ type I/III collagen membrane construct containing human articular chondrocytes of 5×10^6 cells (Kirilak *et al*., 2006). In this study, the authors suggested that these effects in the construct might be mediated, at least in part, via thrombin-induced protease-activated receptor (PAR)-1 signalling in human chondrocytes. It has also been reported that there was no ingrowth of cells when low numbers (1000 cells) of chondrocytes were contained in the fibrin/chondrocyte construct in an *in vitro* culture for 17 days (Brittberg *et al*. (1997).

Effect of fibrin sealant on the fibrin/chondrocyte/scaffold construct

To observe this effect, fibrin/cell or fibrin cell/scaffold constructs prepared with pre-cultured bovine articular chondrocytes were implanted in the flanks of mice (xenotransplantation). In this study, there were less homology of cells and ECM distribution in the construct prepared without fibrin sealant than that in the fibrin/cell/scaffold construct for 6 months implantation (Eyrich *et al*., 2007:13), suggesting that homogenous distribution of cells and ECM in the fibrin construct was contributed by the fibrin sealant, in addition to the gluing effect. In addition, stiffness and resistance of the implant over mechanical compression was greater when the fibrin construct consisted of fibrin sealant, lamb articular chondrocytes and lamb cartilage chips than the construct without fibrin sealant following implantation in mice for 9 weeks (Peretti *et al*., 2000).

Articular chondrocytes in fibrin glue constructs

As discussed in the *Fibrin sealant in fibrin glue constructs* section above, articular chondrocytes in the fibrin/cell or fibrin/cell/scaffold construct are directly involved in expression of ECM components and neocartilage formation in the construct. Hendrickson *et al.* (1994) have reported that cell differentiation ratio was greater at high cell numbers than low cell numbers (that is, 1×10^5 vs 5×10^6 equine neonatal articular chondrocytes) in an *in vitro* culture of fibrin/cell constructs. The homogenous distribution of cartilaginous ECM was observed in the fibrin/cell construct prepared with bovine chondrocytes at an initial seeding of $\geq 3 \times 10^6$ cells in the 5 weeks culture (Eyrich *et al*., 2007:28). In this study, cell differentiation was greater when the cells were pre-cultured than non-cultured prior to preparation of the construct.

The above observations together suggest that cell propagation and production of cartilaginous ECM in the fibrin/cell or fibrin/cell/scaffold construct are related to chondrocyte numbers seeded on the construct. However, it was difficult to estimate the number of chondrocytes required per size of construct, due to different formulations of fibrin sealants at various sizes of constructs with/without scaffold and different types of scaffolds tested, when used. Furthermore, autologous, allogeneic and xenogenic cell transplantation would result in different outcomes. According to the proposed indication, a fibrin/cell/scaffold construct prepared from autologous chondrocytes is transplanted and, as explained by Schlag and Redl (1986) in their review article, outcomes from this autologous graft would be expected to be better than those from heterogeneous grafts used in most studies above. The outcomes from transplantation of a fibrin/chondrocyte/scaffold construct containing autologous chondrocytes are explained further in the *Fibrin/chondrocyte/scaffold construct for generation of articular cartilage* section below.

Matrix scaffold in fibrin glue constructs

For effects of the matrix scaffold on a fibrin/chondrocyte/scaffold construct, there were no great differences in the cell growth or production of ECM components (GAG and collagen) in the fibrin/bovine chondrocyte constructs with/without polymer scaffolds in an *in vitro* culture for 4 weeks (Eyrich *et al*., 2007:13). However, in an *in vivo* study, the size and mass of fibrin glue construct were greatest when the construct consisted of a fibrin sealant (ingredient concentrations not indicated), chondrocytes (lamb articular cartilage) and cartilage chips (same cartilage), compared to the construct without cartilage chips, after implantation of the construct in the subcutaneous pouch of mice for 9-12 weeks (Peretti *et al*., 2000). There were also some reports on shrinkage of fibrin constructs (fibrin/chondrocytes) prepared without scaffold in *in vitro* culture (Eyrich *et al*., 2008) and *in vivo* after implantation in mice (Xu *et al*., 2004), which might be prevented by addition of scaffold to the constructs. Similarly, Peretti *et al.* (2006) have explained in their review article that an addition of devitalised cartilage matrix chips to the fibrin/chondrocyte construct provided stability to the volume of the implant and conferred biomechanical integrity (that is, size, stiffness and resistance) to the implant.

Fibrin/chondrocyte/scaffold construct for generation of articular cartilage

When Tisseel was added to pre-cultured (2-3 days) human articular chondrocytes (5×10^6 cells for seeding) seeded on polymer scaffold, cartilage was visible in the construct (fibrin/cell/scaffold) within 5 days culture *in vitro* (Doolin *et al*., 2002).

Although there were no direct *in vivo* studies conducted on Tisseel for the proposed indication, implantation of autologous rabbit articular chondrocytes (1×10^4-10^6 cells/cm_2) mixed with Tisseel and seeded on porcine type I/III collagen membrane was effective for treatment of osteochondral defects in rabbit knees during a 12 weeks implantation period (Willers *et al.*, 2005). The assessment was based on defect filling, articular surface continuity, restoration of osteochondral architecture, repair tissue integration, cellular morphology of new tissue and production of type II collagen and proteoglycan in the implant in the treated animals, compared to the non-treated animals. The difference in cell numbers (10^4 or 10^6 cells) in the construct did not have any effects on treatment outcomes at 8 weeks post-implantation.

In another study, Eyrich *et al*. (2007) have found that a construct prepared with a non-commercial fibrin sealant (fibrinogen 100 mg/mL, aprotinin 10,000 KIU/mL and thrombin 5 U/mL), bovine articular chondrocytes and a polymer scaffold enhanced early production (1 month compared with 6 months) of newly developed cartilaginous tissue with expression of ECM components (GAG and collagen type I/II), compared to the construct without fibrin sealant or cells, when implanted in the flanks of mice (xenotransplantation). This effect was greater when the construct was pre-cultured *in vitro*, compared to non-cultured prior to implantation. However, the duration (1 or 4 weeks) of incubation did not affect outcomes. In the construct without fibrin sealant, there was inhomogeneous distribution of cells and ECM observed.

In addition, when constructs prepared with fibrin sealants (other than Tisseel formulation), lamb or swine articular chondrocytes, and lamb or swine articular chips, respectively, were implanted in the subcutaneous tissues of mice (xenotransplantation), the formation of neocartilage was seen along the surface of chips (Peretti *et al*., 2000; Silverman *et al*., 2000). The neocartilage matrix was directly adjacent to the normal cartilage matrix without gaps and collagen fibres in the neocartilage were similar to normal collagen matrix (Silverman *et al*., 2000).

From the above studies, evidence indicated that implantation of a fibrin construct prepared with a fibrin sealant containing Tisseel formulation, articular chondrocytes (autologous or xenogenic) and scaffold produced neocartilage in the implant in mice and rabbits. The functions of individual components contained in the construct can be referred to in the relevant sections above.

Secondary pharmacodynamics

In the original report for Tisseel, concerns for anaphylactic reactions, due to foreign proteins contained in the product, were discussed as secondary pharmacodynamics. In the papers provided to support the current application, tissue reactions (inflammation) were also observed at fibrin glue implanted sites in animals. This is explained in detail in the *Local tolerance* section below.

There was an explanation in the sponsor's Non-clinical Overview that the bovine aprotinin contained in the product might be replaced with synthetic aprotinin in the future.

Pharmacokinetics

The solidified fibrin gel transplanted into the body is degraded by plasmin, which is produced by the activation of plasminogen by tissue plasminogen activator, and the degraded products are absorbed into the body (Pipan *et al*., 1992; Schlag and Redl, 1986). To delay the degradation process, the protease inhibitor aprotinin inhibits enzymes such as plasmin. Homminga *et al*. (1993) have reported that the degradation of a fibrin construct prepared with Tisseel containing aprotinin 3000 KIU/mL, thrombin 4 IU/mL instead of 350-500 IU/mL and rabbit articular chondrocytes ($1 \times 10^4 - 2 \times 10^6$ cells) started at 3 days post-preparation in an *in vitro* culture, and accelerated degradations were seen at high cell numbers in the construct. In an *in vivo* study, degradation rates of the fibrin glue alone (porcine fibrinogen 80 mg/mL, aprotinin 3000 KIU/mL, thrombin 50 IU/mL and $CaCl_2$ 40 mM) transplanted in the mice subcutaneous tissue were fast at an early stage (that is, Weeks 0-2) of implantation, then gradually slowed at later stages (Silverman *et al*., 1999).

For the current indication, there was no information on tissue distribution of degraded products in the body following articular application of fibrin glue. Given the nature of this product, no extensive tissue distribution would be expected for the new indication.

In relation to haemostasis, one of the original indications, tissue distribution of fibrinogen contained in a fibrin sealant (fibrinogen 80 mg/mL, Factor XIII 60 U/mL, aprotinin 1000 KIU/mL, thrombin 300 IU/mL and calcium chloride 40 mM) following pericardial application (0.2 mL in total) in rats was reported (Hattori *et al*., 2000). The distribution of ^{125}I-labelled fibrinogen in the heart was rapidly decreased from 48% on Day 1 to 0.01% on Day 14. The radioactivity was negligible ($\leq 1\%$) in the blood, liver, spleen, and kidney throughout the observation period, except for the thyroid in which the radioactivity increased to 7.9% and 4.3% on Days 7 and 14, respectively. Dense and thick fibrin network, observed on Day 1, had dissipated and was thinner with collagen formation by Day 7 post-operation.

Relative exposure

In the submitted research/review papers, there were various combinations of active ingredient concentrations for fibrin sealants with/without scaffold and different types of scaffold tested. Furthermore, many studies did not include clear information on doses of fibrin sealants used (that is, "2 drops" or "covered surface"). Therefore, relative exposures of the Tisseel formulation between animals and humans for the treatment of articular cartilage defects could not be calculated,

although fibrin sealants including this formulation were, in broad, efficacious (production of neocartilage) in animal studies.

Toxicology

General toxicity

Tisseel is expected to be used mainly on a single occasion, with repeated application in exceptional cases only. No information was provided for the repeated dose toxicity. In the single dose toxicity studied following application of a fibrin sealant (concentrations of individual components not indicated) to the colon of rats, all animals survived during a 10 day observation period (Sanal *et al*., 1992). For the Tisseel series product formulation, acute toxicity of Tisseel (unmodified) was studied in one paper following a single dose administration (Mikami *et al*., 1984). In this study the product was administered systemically (orally [PO], subcutaneously [SC], intraperitoneally [IP], or intramuscularly [IM]) to mice, rats and rabbits. All animals administered Tisseel by PO, SC, IP, or IM at doses of 20-50 mL/kg survived by the 14 days observation period.

Tisseel is largely composed of human proteins and is for local application for the current and proposed indications. The differences between these two indications include no direct contact of the product with vascular vessels for the proposed indication (used in the articular cartilage), whilst direct contact with vascular vessels for the existing indications (haemostasis). This suggests less acute toxicities may be expected for the proposed indication than those for the existing indications at the same dose. For the proposed indication, dose was not changed. In addition, Tisseel series fibrin sealants have a relatively long history of clinical use (7 years on the Australian Register of Therapeutic Goods [ARTG] and 10 years in the USA) for haemostasis, and some products have been used as a tissue adhesive in the European Union (EU). In consideration of available information, the product is unlikely to have severe toxicity and is generally expected to have similar or less toxicities for the proposed indication than those for the current indications.

Genotoxicity, carcinogenicity and reproductive toxicity

No new nonclinical data were included in the papers submitted.

Use in children

No data generated from implantation of fibrin/chondrocyte/scaffold constructs prepared with Tisseel series of products in juvenile animals were included in the papers submitted. However, a caution stating that *Safety and efficacy in children have not been established* is included in the *Use in children* section of the product information (PI). This is acceptable.

Local tolerance

Tissue inflammation was seen at the fibrin sealant sites including Tisseel (contained bovine and human originated active ingredients) implanted sites in rats at 2-30 days post-implantation (Erkan *et al*., 2007; Pinholt *et al*., 1992; Sanal *et al*., 1992; Schwarz *et al*., 1993). The testing sites included abdominal muscle and colon of rats, and nasal septum of rabbits. However, as the implantation period increased (that is, 6 weeks), the inflammation observed at early stages (that is, 3 weeks in the nasal septum of rabbits) of implantation disappeared (Erkan *et al*., 2007). Interestingly, in one study, there was no evidence of tissue reaction even at early periods of implantation (3-21 day), following exposure to a fibrin sealant in the rat colon (Siedentop *et al*., 2001).

Nonclinical Summary and Conclusions

This application is a literature-based submission and the published papers submitted were mainly focused on characteristics and efficacy of fibrin sealants alone, with articular chondrocytes or with chondrocytes in combination with matrix scaffold for cartilage generation. However, the available information was sufficient to evaluate the current application.

For physical properties, bonding power was greater in the fibrin glue prepared from Tisseel (same active ingredients but a different product) than that prepared from autologous or single donor plasma. The size and mass of a fibrin glue construct were greater when the construct consisted of a fibrin sealant, chondrocytes and cartilage chips, compared to the constructs without cells or chips, after implantation in mice.

Fibrin sealants including a formulation similar to Tisseel were not toxic to articular chondrocytes. In the fibrin/chondrocyte/scaffold construct, the cells propagated and produced extracellular matrix (ECM) components (glycosaminoglycans (GAG) and collagen type I/II) *in vitro* and *in vivo*. Implantation of this construct in mice enhanced early production (that is, 1 month compared with 6 months) of newly developed cartilaginous tissue, compared to the construct without fibrin sealant or cells. For major functions of individual components in the fibrin/chondrocyte/scaffold construct, the fibrin sealant contributed homogenous distribution of cells and ECM to the implant in addition to the gluing effect, and chondrocytes were directly involved in neocartilage formation. The scaffold would provide stability to volume and biomechanical integrity of the construct.

The fibrin sealant implanted is degraded and the degraded products are absorbed into the body. The degradation was fast at an early stage (0-2 weeks) of implantation, and then was gradually slow at later stages, when the fibrin glue was implanted in the subcutaneous tissue of mice. Accelerated degradations of the fibrin glue were seen at high cell numbers in the fibrin/articular chondrocyte construct in an *in vitro* culture.

Tissue inflammation was seen at the fibrin sealant including Tisseel implanted sites in rats and rabbits at early stages (2-30 days) of post-implantation. However, as the implantation period was prolonged (to 6 weeks in rabbits), the inflammation disappeared in the rabbit study. This tissue reaction was considered to be due to foreign proteins (bovine and human) contained in the fibrin sealant to the host animals. Therefore, Tisseel may have the potential for anaphylactic reactions, when used in humans.

There are no nonclinical objections to include the proposed indication for Tisseel in the registration.

IV. Clinical Findings

Introduction

The submission was a literature based submission. It included 49 references (48 published and 1 unpublished). The literature search strategy used by the sponsor was reviewed by the TGA Library and found to be acceptable.

The submission included:

- Five “pivotal” randomised controlled studies of efficacy and safety in which the ACI or MACI procedure was compared with other surgical techniques for the treatment cartilage defects;
- One supportive unpublished single-arm study of efficacy and safety;
- Another 13 published papers provided as supportive evidence of safety.

Pharmacokinetics

No new data on the pharmacokinetics of the active ingredients of Tisseel were included in the current application.

Pharmacodynamics

No new clinical data on the pharmacodynamics of Tisseel were included in the submission.

The submission included a published paper which described an *in vitro* test to investigate the use of a fibrin sealant for attaching periosteal patches to articular cartilage. The adhesive strength obtained in joining periosteum to cartilage was comparable to that obtained in joining dermis to dermis.

However, the fibrin sealant product used in this experiment was not Tisseel, and the periosteum and cartilage used were of bovine origin. The study is therefore not considered relevant to the application.

Efficacy

The efficacy studies included in the submission were not designed to specifically examine the contribution of fibrin glue/sealant to the efficacy of the ACI / MACI procedures. There were no studies which compared ACI/MACI with fibrin glue/sealant to ACI/MACI without fibrin glue/sealant. Rather, the studies sought to examine the efficacy of the ACI/MACI procedure (which included the use of fibrin glue/sealant) with other modalities for the treatment of cartilage defects.

The submission included five randomised controlled trials. These have been evaluated as 'pivotal' studies in support of the application. The sponsor also provided one single-arm non-comparative trial as supportive evidence of efficacy.

The published papers describing the trials did not always identify the fibrin glue/sealant used as being the Tisseel product which is the subject of this application. However, according to the sponsor's Clinical Overview, the authors of each study were contacted by the sponsor to identify the specific product used. The submission only included studies in which a Tisseel product was used.

Pivotal studies

Bentley et al

The first study was a randomised trial of ACI versus mosaicplasty in patients with cartilage defects in the knee.[4] The study was conducted in the United Kingdom and was published in 2003.

Mosaicplasty is an auto graft procedure in which several cylindrical osteochondral plugs are harvested from non-weight-bearing areas of the patellofemoral area, and then inserted into drilled tunnels in the cartilage defect. The plugs are each approximately 4.5 mm wide and 15 to 20 mm deep. The plugs are packed into the defect such that there is minimal spacing between them. During healing, the spaces become filled with fibrocartilage.

The study was a randomised parallel group design. All patients underwent an initial arthroscopy, where the suitability of the lesion was assessed. Only patients with a chondral or osteochondral lesion of greater than 1 cm in diameter, and an otherwise normal joint, were included. Patients were randomised in theatre using random sample numbers in sealed envelopes. If the patient was randomised to mosaicplasty, the procedure was performed under the same anaesthetic. If randomised to ACI, the biopsy was taken and the patient returned for the formal ACI procedure three to five weeks later. The article specifically stated that fibrin glue was used to render the ACI site watertight. Rehabilitation procedures in the two groups were identical.

The study was not blinded, as the ACI procedure involved two stages of surgery and mosaicplasty only one.

Endpoints

The following endpoints were used. None was identified as being the primary endpoint.

- The modified Cincinnati Rating System. Although not described in any detail, this appeared to be an examiner-determined assessment of knee function. Subjects could receive a score between 0 and 100 based on eight parameters, as shown in Table 1. The criteria for assigning a particular score within the eight parameters were not described.

Table 1: Modified Cincinnati Rating System (Bentley)

[4] Bentley G, Biant LC, Carrington RW, et al. A prospective, randomised comparison of autologous chondrocyte implantation versus mosaicplasty for oeteochondral defect in the knee. J Bone Joint Surg Br 2003; 85: 223-230.

Score	Maximum value
Pain	20
Giving way	20
Swelling	10
Walking ability	10
Stair-walking	10
Running	5
Jumping/twisting	5
Overall activity	20

> 80 = Excellent
55 – 79 = Good*
30 – 45 = Fair*
< 30 = Poor

**The paper did not describe how patients with a score between 46 and 54 were rated*

- Arthroscopy – ICRS grade. Patients underwent arthroscopy after one year. The appearance of the defect site was assessed using the International Cartilage Research Society (ICRS) grading system. This is a classification system for grading the macroscopic appearance of cartilage defects as summarised in Figure 4. The four grades were assigned descriptive terms: - grade 1 = "excellent", grade 2 = "good", grade 3 = "fair" and grade 4 = "poor".
- Biopsies. For patients in the ACI group, biopsies of the treated area were also taken at the 1-year arthroscopy.

Statistical methods

The article stated the following: *"Statistical comparison of outcome scores between the groups was by the Mann-Whitney U test for non-parametric data. A p value of < 0.05 was taken to be statistically significant."*

Patient enrolment, characteristics and disposition

A total of 100 consecutive patients were enrolled. Of these, 58 patients were randomised to receive ACI and 42 to mosaicplasty. No comment was made as to the discrepancy in patient numbers between the two groups.

Mean age was 31.6 (range 20 to 48) years in the mosaicplasty group and 30.9 (16 to 49) years in the ACI group. There were 57 men and 43 women.

The mean size of the osteochondral defect was 4.66 cm^2 (range 1 to 12.2). Subjects had had a long history of symptoms (mean 7.2 years), and most (94/100) had undergone previous surgical procedures, with a mean of 1.5 prior operations (range 0 to 4).

Efficacy results

- The modified Cincinnati Rating System. Results for this endpoint are shown in Table 2. When patients with ratings of "excellent" or "good" were grouped, the ACI procedure produced a numerically superior result (88% vs 69%), but the difference was not statistically significant. A subgroup analysis of results according to anatomical location of lesion suggested that ACI may be superior to mosaicplasty for lesions involving the medial femoral condyle.

Table 2: Results for modified Cincinnati Rating System – number of patients (%)

	Total	Excellent	Good	Fair	Poor

ACI	58	23 (40)	28 (48)	7 (12)	0
Mosaicplasty	42	9 (21)	20 (48)	6 (14)	7 (17)

	Excellent or Good – n (%)	**p-value**
ACI	51 (88)	p = 0.277
Mosaicplasty	29 (69)	

- Arthroscopy – ICRS grade. Results at one year are shown in Table 3. Data were available for a total of 60 of the 100 subjects who were enrolled. Grouping of patients with "excellent" or "good" grades suggested that ACI provided a superior outcome compared to mosaicplasty.
- Biopsies A total of 19 ACI subjects underwent biopsy at 1 year. Seven patients had hyaline cartilage of normal appearance, seven had a mixture of hyaline cartilage and fibrocartilage, and five had only fibrocartilage.

Table 3: Results for ICRS grade - number of patients (%)

	Total	**Excellent**	**Good**	**Fair**	**Poor**
ACI	37 (100)	6 (16)	24 (66)	6 (16)	1 (2)
Mosaicplasty	23 (100)	0 (0)	8 (34)	10 (44)	5 (22)

	Excellent or Good – n (%)	p-value
ACI	30 (81)	p < 0.01
Mosaicplasty	8 (34)	

Comment

The efficacy endpoints used in this study were subjective in nature, and the assessor appears not to have been blinded to treatment allocation. It is therefore difficult to draw any reliable conclusions regarding the comparative efficacy of the two procedures. Most subjects in the ACI arm achieved an "excellent" or "good" rating on the rating scale use, suggesting an improvement compared to baseline. However, the rating scale appears to have been based on the investigator's assessment of outcome, rather than the patient's assessment of symptoms.

Dozin et al

This study was also a randomised trial of ACI versus mosaicplasty in patients with cartilage defects in the knee.[5] The study was conducted in Italy and was published in 2005.

It had a randomised, parallel group design. The inclusion criteria specified that patients should have a Grade III or IV lesion according to the Outerbridge classification. This is a classification for assessing the severity of joint cartilage breakdown according to macroscopic appearance, and has ratings from 0 (normal) to IV (exposed subchondral bone).

Subjects underwent an initial arthroscopy at which suitability of the lesion to be treated was established. Subjects were randomised during this procedure, following telephone communication

[5] Dozin B, Malpeli M, Cancedda R et al. Comparative evaluation of autologous chondrocyte implantation and mosaicplasty: a multicentered randomized clinical trial. Clin J Sport Med 2005; 15: 220-226.

with the study co-ordinating centre. Treatment assignment was decided through the use of randomisation lists, stratified by surgeon. After randomisation all patients were treated with simple debridement of the lesion. Subjects allocated to ACI also had the biopsy of healthy cartilage. Patients then returned for definitive treatment (ACI or mosaicplasty) after 6 months. For patients randomised to ACI, cultured chondrocytes were stored in liquid nitrogen for the 6-month rest period.

The article specifically stated that fibrin glue was used to seal the periosteal flap during the ACI procedure. The same rehabilitation procedures were used in the two groups.

Endpoints

The planned primary endpoint for the study was based on the Lysholm Knee Scoring Scale (LKSS), as assessed by the operating surgeons. The LKSS is a scale ranging from 0 to 100, which is based on the assessment of 8 parameters as shown in Figure 3. The planned endpoint was a comparison of mean LKSS scores assessed at 12 months after the randomised surgery.

Statistical methods

The original statistical plan assumed that the minimum clinically significant benefit would be a difference in mean LKSS between groups of one standard deviation. A required sample size of 40 patients was calculated. This was increased to 60 patients to allow for an estimated one third of patients who would recover over the 6-month rest period, and who would therefore not be randomised.

Due to poor recruitment and loss to follow-up of a significant proportion of subjects, patient accrual was terminated early (after 47 of the planned 60 subjects). The method of analysis was changed, with outcome analysed as a categorical variable, with 5 classes of LKSS:

- LKSS < 60
- LKSS = 60 – 90
- LKSS > 90
- Subjective improvement (without an LKSS assessment);
- Lost to follow-up.

The distribution of patients in these five classes was compared in the two treatment groups using the c^2 test for heterogeneity.

Patient enrolment, characteristics and disposition

A total of 47 subjects were enrolled prior to the early termination of recruitment. Of these, 22 were randomised to ACI and 25 to mosaicplasty. Three subjects in the mosaicplasty arm were excluded from the analysis – two due to lack of baseline data to determine eligibility, and one who withdrew consent prior to randomised treatment. A total of 44 subjects were therefore considered evaluable, 22 in each arm.

The randomised surgical procedure was administered to only 23 of the 44 evaluable subjects. A large proportion of patients had clinical improvement over the 6-month rest period and no longer required surgery.

Figure 3: Lysholm Knee Scoring Scale

Limp (5 points)	
None	5
Slight or periodical	3
Severe and constant	0
Support (5 points)	

None	5
Stick or crutch	2
Weight-bearing impossible	0
Locking (15 points)	
No locking and no catching sensations	15
Catching sensation but no locking	10
Locking	
Occasionally	6
Frequently	2
Locked joint on examination	0
Instability (25 points)	
Never giving way	25
Rarely during athletics or other severe exertion	20
Frequently during athletics or other severe exertion (or	15
Occasionally in daily activities	10
Often in daily activities	5
Every step	0
Pain (25 points)	
None	25
Inconstant and slight during severe exertion	20
Marked during severe exertion	15
Marked on or walking more than 2 km	10
Marked on or walking less than 2 km	5
Constant	0
Swelling (10 points)	
None	10
On severe exertion	6
On ordinary exertion	2
Constant	0
Stair-climbing (10 points)	
No problems	10
Slightly impaired	6
One step at a time	2
Impossible	0
Squatting (5 points)	
No problems	5
Slightly impaired	4
Not beyond 90 degrees	2
Impossible	0

Efficacy results

For the 23 patients who underwent the procedure to which they had been randomised, the proportion of patients who achieved an LKSS score of 90 – 100 (designated a "complete success") was 91% (10/11) in the mosaicplasty arm and 58% (7/12) in the ACI arm. No statistical analysis of this finding was presented. No other analysis of this group of 23 patients was presented.

The authors presented efficacy results for the evaluable population in tabular format, which showed there was no significant difference between the two groups. This analysis is fairly meaningless in terms of comparing the efficacy of the two procedures, as only 23 of the 44 evaluable subjects actually received their randomised therapy.

Comment

This study is of very limited value in determining the efficacy of the ACI procedure compared to mosaicplasty, due to the numerous methodological problems. In the small proportion of patients who actually completed the study, ACI appeared to be less effective than mosaicplasty.

Knutsen et al

This study was a comparison of ACI against another surgical procedure known as microfracture, in patients with cartilage defects in the knee. It was conducted in Norway. The two-year results were published in 2004 and the five-year results in 2007.[6,7]

Microfracture is a procedure which aims to provide a blood supply and recruit bone marrow cells into the cartilage defect by creating a communication between the defect and the underlying bone marrow by penetration of the subchondral bone plate. This allows the formation of a clot in the cartilage defect, which is subsequently replaced by repair tissue. The marrow also provides access to pluripotent mesenchymal stem cells which can differentiate into cartilage or fibrous tissue. The procedure used in this study involved debridement of all damaged cartilage from the defect, down to the subchondral bone plate. This included the removal of all loose cartilage from the rim of the defect to form a stable perpendicular edge of healthy cartilage. Multiple holes were then created in the subchondral bone plate 3 to 4 mm apart. This results in the propagation of small microfractures around the holes, thereby increasing access to the marrow blood supply.

Microfracture is a procedure which can be performed through an arthroscope.

Design

The study had a randomised, parallel group design.

Subjects underwent an initial arthroscopy at which suitability of the lesion to be treated was established. Subjects were randomised during the arthroscopy, through the use of sealed envelopes. Patients randomised to microfracture had the procedure performed under the same anaesthetic, while those randomised to ACI returned for their implantation procedure after approximately 4 weeks. The article specifically stated that fibrin glue was used to form a watertight chamber during the ACI procedure. The same rehabilitation procedures were used in the two groups.

Endpoints

Several efficacy endpoints were used in this trial. None was specified as the primary endpoint.

[6] Knutsen G, Engebretsen L, Ludvigsen TC et al. Autologous chondrocyte implantation compared with microfracture in the knee. A randomised trial. J Bone Joint Surg Am 2004; 86: 455-464.

[7] Knutsen G, Drogset JO, Engebretsen L et al. A randomized trial comparing autologou chondrocyte implantation with microfracture. Findings at five years. J Bone Joint Surg Am 2007; 89: 2105-2112..

- Overall treatment failure. Surgery was considered to be a "failure" if the patient needed a re-operation because of symptoms due to a lack of healing of the operated defect. The need for shaving or trimming of a lesion was not considered a failure.
- Lysholm Knee Scoring Scale (LKSS), as described above and shown in Figure 3. The mean value was compared at baseline and at 1, 2 and 5 years after surgery.
- A Visual Analogue Score (VAS) for pain, with pain rated by the patient on a scale of 0 to 100. Results were presented for baseline and at 1, 2 and 5 years after surgery.
- The Short Form-36 (SF-36) questionnaire. This is a widely used quality of life instrument which assesses eight separate domains – vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning and mental health. The publication focussed on results of the physical component scores. Results were presented for baseline and at 1, 2 and 5 years after surgery.
- Tegner score. This is a score of between 0 and 10, assigned on the patient's level of activity. The instrument scores a person's activity level between 0 and 10 where 0 is 'on sick leave/disability' and 10 is 'participation in competitive sports such as soccer at a national or international elite level'. It is the most widely used activity scoring system for patients with knee disorders. However, it does not take into account that individuals may be able to participate at a higher level of activity but consciously choose not to or that some people will participate at a higher level of activity but with limitations.
- Arthroscopy. Subjects underwent a second look arthroscopy at 2 years and macroscopic appearance was scored according to an International Cartilage Repair Society (ICRS) scoring system. Scores could range between 0 and 12 with 1-3 rated as Grade IV (severely abnormal) and 12 rated as Grade 0 (normal).
- Histology. Biopsies (from the central part of the treated defects) were taken at the 2-year arthroscopy and results were classified into one of four treatment groups – Group 1: predominantly normal hyaline cartilage, Group 2: a mixture of hyaline and fibrocartilage, Group 3: fibrocartilage, Group 4: inadequate biopsy or no repair tissue (predominantly bone).

The LKSS, VAS and Tegner scores were assessed by an independent (but not blinded) assessor at the one and two year assessments. All assessments at the 5-year time point patients were conducted by the principal investigator (again in an unblinded manner). The histology at 2 years was assessed by two blinded assessors.

Statistical methods

The statistical methods used were summarised as follows:

"A sample-size estimation showed that forty patients in each group would be required to demonstrate a difference between the Lysholm and SF-36 scores of the two groups of at least 0.75 standard deviation of the mean, with an alpha level of 0.05 and a beta level of 90%.

The data were analysed with the SPPS statistical package (SPSS, Chicago, Illinois). T tests, the Pearson chi-square and Mann-Whitney U tests, analysis of covariance, and multiple analyses of variance were used. The level of significance was $p < 0.05$."

Patient enrolment, characteristics and disposition

The study enrolled 80 subjects, 40 in each treatment arm. All 80 subjects received their randomised treatment and were followed up for 5 years.

Efficacy results

- Overall treatment failure. After 5 years, nine patients (23%) in each treatment group had failed treatment.
- Lysholm Knee Scoring Scale (LKSS). There was no significant difference between the two treatment arms. Both treatment groups experienced significant improvement compared to baseline. For the total population, 80% had some improvement in the score.
- A Visual Analogue Score (VAS) for pain. Again there was no significant difference between groups at five years, but both groups improved significantly from baseline. For the total population, 72 % had some improvement.
- The Short Form-36 (SF-36) questionnaire. Results for the physical component scores of the SF-36 showed no significant differences between the two groups. The microfracture group had a significant improvement from baseline ($p < 0.001$), whereas the ACI group did not ($p = 0.309$).
- Tegner score. Both groups had a significantly improved Tegner score compared to baseline. Mean score improved from 3.28 to 4.05 in the ACI group ($p = 0.007$), and from 3.16 to 4.36 in the microfracture group ($p=0.002$). There was no significant difference between the two groups ($p = 0.323$).
- Arthroscopy. There was no significant difference between groups for the score assessing macroscopic appearance at 2 years.
- Histology. Biopsy was performed on 67 of the 80 patients at 2 years. There were no significant differences between groups in the histology grading, although there was a trend for the ACI procedure to be more likely to be associated with the production of normal hyaline cartilage.

Comment

This study was a better quality trial than the two studies reviewed above. It provided long term efficacy data on the two procedures under study. Histological assessment at 2 years was blinded and showed that ACI results had at least comparable efficacy to microfracture. Assessment of the other efficacy parameters was not blinded, but provided some supportive evidence of efficacy in that patients treated with ACI demonstrated statistically significant improvement from baseline on most endpoints. Overall, a reasonable conclusion would be that the ACI procedure produces at least comparable efficacy results to microfracture. The study authors recommend that microfracture should be preferred as first-line treatment on the grounds of lower cost and the fact that it involves only a single surgical procedure.

Visna et al

This trial compared a modified MACI technique with another procedure known as abrasion arthroplasty, in patients with cartilage defects in the knee. The study was conducted in the Czech Republic and published in 2004.[8]

Abrasion arthroplasty is similar to the microfracture procedure in that it creates a communication between the cartilage defect and the underlying bone marrow by penetration of the subchondral bone plate. The penetration is achieved by abrading the surface of the exposed subchondral bone with a burr via arthroscope to create a bleeding bony surface. In this trial the abrasion was done to a depth of 1 mm.

[8] Visna P, Pasa L, Cizmar I, Hart R, Hoch J. Treatment of deep cartilage defects of the knee using autologous chondrograft transplantation and by abrasive techniques – a randomized controlled study. Acta Chir Belg 2004; 104: 709-714.

Design

The study had a randomised, parallel group design.

Randomisation was performed using an envelope method (not further described). The time at which randomisation occurred was not stated but this presumably occurred at initial arthroscopy. For patients randomised to MACI, the time interval between initial arthroscopy and the implantation procedure was 21 to 28 days.

The method used in this study for implantation of the autologous chondrocytes was different to that described for the other studies. The chondrocytes were mixed directly with fibrin glue to form a matrix. The cartilage defect was debrided and the debridement procedure included penetration of the subchondral bone. The solidified chondrocyte/fibrin glue matrix was moulded and placed directly into the cartilage defect, and fixed with further application of the fibrin glue. The article identified the product used as "Tissucol" which is the tradename used for Tisseel in some foreign markets. No comment was made as to whether subjects in the two groups received the same rehabilitation procedures.

Endpoints

The endpoints studied were the following. None was identified as the primary endpoint.

- Lysholm Knee Scoring Scale (LKSS), as described above and shown in Figure 3. The mean value was compared at baseline and at 5 and 12 months after surgery.
- The International Knee Documentation Committee (IKDC) subjective knee score. This is a questionnaire completed by patients which grades knee symptoms and patient abilities to be active. The answers are converted to a numerical scale from 1 to 100. The mean value was compared at baseline and at 5 and 12 months after surgery.
- Tegner score as described above. The mean value was compared at baseline and at 12 months after surgery.

Statistical methods

The statistical methods used were summarised as follows:

"The results were subjected to the c^2 –test and its modifications (MANTEL-HAENSZEL, YATES, FISCHER), KRUSKAL-WALLIS analysis as a non-parametric test for distribution-free data and ANOVA analysis of normally distributed data were also done."

Patient enrolment, characteristics and disposition

A total of 50 subjects were enrolled and 25 randomised to each treatment group. The disposition of patients over the course of the trial was not reported.

Efficacy results

- Lysholm Knee Scoring Scale (LKSS). Results are summarised in Table 4. Possible scores on the LKSS range between 0 and 100. There was no significant difference between the groups at baseline. At both 5 and 12 months the results for MACI were superior to those for abrasion. The benefits were modest with differences in mean scores being 8 and 12 points respectively.

Table 4: Efficacy results – Lysholm Knee Scoring Scale

Lysholm knee score (points)	Preoperative value	5 months after surgery	12 months after surgery
Group I (MACI)	47.60	77.20 ($p<0.05$)	86.48 ($p<0.001$)
Group II (Abrasion arthroscopy)	52.60	69.20 ($p<0.05$)	74.48 ($p<0.001$)

- The International Knee Documentation Committee (IKDC) subjective knee score. Results are summarised in Table 5. Possible scores on the IKDC score range between 0 and 100. There were no significant differences between the groups at baseline or at 5 months. At 12 months the results for MACI were significantly superior to those for abrasion, although the difference again appeared modest.
- Tegner score. Results are summarised in Table 6. Possible results on the Tegner score range between 0 and 10. There were no significant differences between the groups at baseline. At 12 months the results for MACI were significantly superior to those for abrasion.

Table 5: Efficacy results – IKDC Subjective Knee Score

IKDC subjective score (points)	Preoperative value	5 months after surgery	12 months after surgery
Group I (MACI)	41.28	67.00 (NS)	76.48 ($p<0.05$)
Group II (Abrasion arthroscopy)	45.00	62.28 (NS)	68.08 ($p<0.05$)

NS: not significant

Table 6: Efficacy results – Tegner score

Tegner score (points)	Value before injury	Value before surgery	Value 12 months after surgery
Group I (MACI)	7.85	3.23 ($p<0.01$)	5.92 ($p<0.01$)
Group II (Abrasion arthroscopy)	7.10	2.30 ($p<0.01$)	4.20 ($p<0.01$)

Comment

The published report of this study lacked some detail (for example, no description of the inclusion/exclusion criteria, no comment on any differences in the rehabilitation procedures post surgery etc). The method of use of the fibrin glue/sealant (mixture with the autologous chondrocytes) was also very different from the other published studies included in the submission. However, the efficacy results suggested superiority of this MACI technique over abrasion arthroplasty.

Basad et al

This trial compared a MACI technique with microfracture, in patients with cartilage defects in the knee. The study was conducted in Germany and published in 2004.[9]

Design

The trial appeared to have a randomised, parallel group design.

The article specifically stated that Tissucol (a European tradename for Tisseel) fibrin glue was used to fix the chondrocyte-loaded collagen scaffold to the edges and surface of the cartilage defect. Differing rehabilitation procedures were used for the two groups. For example, in the MACI group, the joint was immobilised for one week followed by partial weight-bearing for 8 to 12 weeks. In the microfracture group it appears that the only restriction was partial weight bearing for 6 weeks.

Endpoints

The following endpoints were used. None were described as being primary.

- Meyers score. This endpoint was only described briefly and a reference was not cited by the authors. The scoring system apparently has a maximum of 18 points, with 18 classified as "excellent", 15 – 17 points as "good", 12 – 15 points as "satisfactory" and less than 12 points as "unsatisfactory". The criteria used to arrive at these scores were not described. Values were compared at 12 months after surgery.
- Lysholm Knee Scoring Scale (LKSS), as described above and shown in Figure 3. Values were compared at 12 months after surgery.
- Tegner score as described above. Values were compared at 12 months after surgery.
- Extended IKDC score. This score was only briefly described. It appeared to be a different scoring system to the IKDC subjective knee score described above. It was composed of both patient and investigator assessments. On the basis of these patients were categorised into one of four levels (Levels I – IV), in ascending order of the patient's symptoms

Statistical Methods

No statistical analysis was performed. The presentation of the results was descriptive.

Patient enrolment, characteristics and disposition

A total of 46 subjects were enrolled and treated. The paper did not state how many of these were randomised to each group. The publication provided results on the first 19 patients, of whom 10 had received MACI and 9 had received the microfracture procedure.

Efficacy results

- Meyers score. In the MACI group, this score improved by 6.5 points after 12 months, whereas in the microfracture group, the score rose by only 1.9. Although not stated, these figures presumably were changes in the mean scores.
- Lysholm Knee Scoring Scale (LKSS). In the MACI group there was an improvement of 48 points at 12 months after surgery, whereas the microfracture group the improvement was 15 points. Although not stated, these figures presumably were changes in the mean scores.
- Tegner score In the MACI group, this score improved by 1.6 levels after 12 months, whereas in the microfracture group there was no change. Again, although not stated, these figures presumably were changes in the mean scores.

[9] Basad E, Stürz H, Steinmeyer J. Treatment of chondral defects with MACI or microfracture – first results of a controlled clinical trial. Orthopädische Praxis 2004; 40: 6-10.

- Extended IKDC score. Results are summarised in Table 7. The MACI procedure was associated with a higher percentage of patients experiencing the lowest level of symptoms (that is, Level I – 60% vs 20%).

Table 7: Efficacy results – extended IKDC score – before and one year after treatment with MACI (n=10) and microfracture (n = 9).

IKDC score	Level I	Level II	Level III	Level IV	n.d.
Preoperative:					
MACI	3.1%	12.5%	54.9%[3]	37.5%	0.0%
MFX	0.0%	7.7%	46.2%	46.2%	0.0%
1 year postop.:					
MACI	60.0%[4]	30.0%	10.0%	0.0%	0.0%
MFX	20.0%	20.0%	40.0%	10.0%	10.0%

n.d. = not determined, MFX = microfracture,
MACI = matrix-supported autologous chondrocyte implantation

Comment

This was a poorly described study. It was an interim report only on a small number of patients. The different rehabilitation procedures may have biased the results. The limited data presented suggest some benefit for MACI over microfracture.

Supportive study

Marlovits et al

The sponsor also submitted as evidence of efficacy a report of an open, single-arm, non-comparative study of patients undergoing the MACI procedure for cartilage defects of the knee. It was performed in Austria between 2000 and 2006. The study has not been published, but a study report of 81 pages was provided.[10]

Design

The study was an open, non-comparative single arm trial. Subjects were followed for two years post surgery.

The report specifically stated that Tissucol (a European tradename for Tisseel) fibrin glue was used to fix the chondrocyte-loaded collagen scaffold.

Endpoints

The endpoints used in the study were the assessment of the clinical outcomes as follows:

1. Objective knee examination
   - IKDC knee examination form
   - Lysholm score
2. Intensity of knee pain
   - VAS scale

[10] Marlovits S. Clinical investigation on MACI for the treatment of articular cartilage defects of the knee. 2006 Clinical Study Report.

3. Subjective evaluation of the knee symptoms
    - KOOS (Knee injury and Osteoarthritis Outcome Score)
    - IKDC subjective knee evaluation form
4. Function and patient activity level
    - Britberg score
    - Activity score (Tegner and Lysholm)
    - Sport activity (Noyes)
5. Radiological screening (MRI)

Statistical Methods

All analysis was done descriptively.

Patient enrolment, characteristics and disposition

A total of 21 subjects were treated, including 18 males and 3 females. Mean age was 35.18 years. In 18 of the patients the cause of the cartilage defect was trauma, and in the other 3 the patient did not relate the symptoms to a specific traumatic event. A total of 16 patients had undergone some form of prior knee surgery. Of these, 5 had had previous surgery aimed at repair of the cartilage defect.

Efficacy results

Patients generally showed improvement compared to baseline on all efficacy endpoints at both 12 and 24 months. For example:

- Lysholm Knee Scoring Scale (LKSS). Mean score improved from 54.7 at baseline to 83.8 after 24 months (Table 8).
- VAS pain scale (0 to 10). Mean score improved from 7.14 at baseline to 1.95 after 24 months (Table 9).
- IKDC subjective knee score . Mean score improved from 31.5 at baseline to 68.6 after 24 months (Table 10).
- MRI scan. The study included assessment of the volume of the cartilage defect over time by MRI scan. Of 17 defects assessed, 11 (65%) had 76 – 100% filling by two years.

Table 8 : Lysholm Score at Baseline, 6 and 12 Months

	Status (months)		
	0	**6**	**12**
mean	54.7	77.5	83.8
median	52	75	80
minimum	41	56	64
maximum	76	100	100
changes to Status 0	better	19	21
	no change	1	0
	worse	1	0
n	21	21	21

Table 9 : VAS Pain Score at Baseline, 6 and 12 Months

	Status (months)		
	0	**6**	**12**
mean	7.14	2.57	1.95
median	7	2	2
minimum	5	0	0
maximum	9	8	6
changes to Status 0	better	20	20
	no change	1	1
	worse	0	0
n	21	21	21

Table 10 : IKDC Subjective Knee Score at Baseline, 6 and 12 Months

	Status (months)		
	0	**6**	**12**
mean	31.5	61.7	68.6
median	29.9	60.9	72.4
minimum	12.6	23.0	27.6
maximum	60.9	92.0	93.1
changes to Status 0	better	20	19
	no change	0	2
	worse	1	0
n	21	21	21

Comment

The lack of a comparator arm makes interpretation of efficacy in this study difficult, as improvement over time may have been to some extent the result of spontaneous healing processes. It provides some supportive evidence.

Conclusions regarding efficacy

None of the submitted studies directly examined the efficacy of Tisseel fibrin glue/sealant in the ACI / MACI procedures. The use of fibrin glue/sealant appears to have been an integral component of the ACI and MACI procedures since the original development of these techniques. Theoretically a trial could have been conducted comparing suturing plus fibrin sealant versus suturing alone, in the ACI procedure. However, an increased number of sutures would be required to obtain a 'watertight' seal of the periosteal membrane used to close the cartilage defect. This would result in increased trauma to the healthy joint cartilage surrounding the defect, with potential adverse outcomes.

For the MACI procedure, a comparative trial of fibrin glue versus another method of fixation (for example, suturing) may have been possible. The submission included a study in human cadavers of

four different techniques for fixation of a collagen scaffold into cartilage defects.[11] Cartilage defects (full thickness, 2.5 cm^2) were created on the medial femoral condyle of human cadaveric knee joints, and a collagen scaffold was fixed into the defects using four different techniques:

- simple implantation without fixation;
- use of fibrin sealant (the product used was Beriplast P, not Tisseel);
- absorbable sutures anchored to bone;
- use of a periosteal cover (similar to that used in the ACI procedure).

The knee joints were then subjected to 150 repeated cycles of motion (1 cycle was from full extension to full flexion back to full extension) by means of a continuous passive motion device. With the simple implantation procedure, the scaffold detached after 60 cycles, whereas with the other fixation techniques, the scaffolds remained in situ for the entire 150 cycles. Fixation strength was tested separately, and was found to be higher for the bone suture and periosteal cover techniques compared to the fibrin sealant technique. The authors concluded that all three techniques provided *sufficient* fixation, but expressed a preference for the fibrin sealant technique because of ease of use. The other two techniques were also inherently associated with damage to surrounding tissues.

A comparative trial against another fibrin glue/sealant could also have been conducted. However, there do not appear to be any other fibrin products that are accepted as standard treatment for these procedures, and a comparative trial would therefore be fairly meaningless.

In the submitted studies a total of 166 subjects received Tisseel as part of an ACI or MACI procedure (110 for ACI and 56 for MACI – Table 11). In the sponsor's Clinical Overview, the sponsor points out that amongst these patients there were no cases of apparent failure of the fibrin *glue* component. This would have manifested as movement of the periosteal membrane or collagen scaffold. This finding provides supportive evidence of the efficacy of Tisseel as an adhesive.

Table 11: Patient numbers enrolled in efficacy studies

Reference	N	ACI	MACI	Mosaicplasty	Microfracture	Abrasion
Bentley[4]	100	58		42		
Dozin[5]	23	12		11		
Knutsen[6,7]	80	40			40	
Visna[8]	50		25			25
Basad[9]	19		10		9	
Marlovits[10]	21		21			
Total	**293**	**110**	**56**	**53**	**49**	**25**

Overall the evaluator considered that the absence of a randomised controlled trial specifically examining the efficacy of the Tisseel component of the ACI / MACI procedures should not form an obstacle to approval of the application.

[11] Drobnič M, Radosavljevič D, Ravnik D, Pavlovčič V, Hribernik M. Comparison of four techniques for the fixation of a collagen scaffold in the human cadaveric knee. Osteoarthritis cartilage 2006; 14: 337-344.

In the absence of a randomised controlled trial specifically examining the efficacy of Tisseel, the sponsor has submitted five randomised controlled studies which compared ACI / MACI with other standard surgical procedures for the treatment of cartilage defects. These trials suffer from a number of limitations:

- Assessment of efficacy was generally not blinded. Blinding of the patient and surgeon to treatment allocation was not possible because the ACI / MACI surgical procedures involved two stages of surgery, whereas the comparator treatments only involved one surgical intervention. However, it may have been possible to obtain a blinded third party assessment of efficacy.
- There was no consistency across studies in the efficacy endpoints that were used;
- The studies were variable in the level of detail provided;
- Only relatively small numbers were included in the trials;
- All studies evaluated efficacy of ACI / MACI only in the knee joint.

These limitations make interpretation of the entire dataset problematic. Overall, if the studies are considered in total, a reasonable assessment would be that ACI and MACI procedures (which involve the use of fibrin glue/sealant) are comparable in efficacy to other techniques used in the treatment of cartilage defects of the knee.

Safety

Patient exposure

As indicated above, a total of 166 subjects were treated with Tisseel in the submitted efficacy studies. The sponsor also submitted the following studies, identified from the literature search, as evidence of safety of the product:

- A further 6 studies of ACI / MACI. These studies were case series or open single-arm trials, with one randomised controlled trial comparing ACI with MACI. These studies enrolled a total of approximately 500 patients.[12,13,14,15,16,17]
- A further 7 studies were selected because the literature search identified that the publication specifically reported on the safety of Tisseel. These were trials in which Tisseel was applied internally as a sealant, adhesive or haemostatic agent, in types of surgery other than orthopaedic surgery.[18,19,20,21,22,23,24]

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[12] Alfredson H, Thorsen K, Lorentzon R. treatment of tear of the anterior cruciate ligament combined with localised deep cartilage defects in the knee with ligament reconstruction and autologous periosteum transplantation. Knee Surg Sports Traumatol Arthrosc 1999; 7: 69-74.

[13] Bartlett W, Skinner JA, Gooding CR et al. Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee: a prospective, randomised study. J Bone Joint Surg Br 2005; 87: 640-645.

[14] Browne JE, Anderson AF, Arcerio R et al. Clinical outcome of autologous chondrocyte implantation at 5 years in US subjects. Clin Orthop Relat Res 2005; 436: 237-245.

[15] D'Anchise R, manta N, Prospero E, Bevilacqua C, Gigante A. Autologous implantation of chondrocytes on a solid collagen scaffold: clinical and histological outcomes after two years of follow-up. J Orthopaed Traumatol 2005; 6: 36-43.

[16] Minas T. Autologous chondrocyte implantation for focal chondral defects of the knee. Clin Orthop Relat Res 1999; 391: S49-S61.

[17] Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res 2000; 374: 212-234.

[18] Beierlein W, Scheule AM, Antoniadis G, Braun C, Schosser R. An immediate, allergic skin reaction to aprotinin after reexposure to fibrin sealant. Transfusion 2000; 40: 302-305.

[19] Oswald AM, Joly LM, Gury C, Disdet M, Leduc V, Kenny G. fatal intraoperative anaphylaxis related to aprotinin after local application of fibrin glue. Anesthesiology 2003; 99: 762-763.

[20] Scheule AM, Beierlein W, Lorenz H, Ziemer G. Repeated anaphylactic reactions to aprotinin in fibrin sealant.

Adverse events

The reporting of safety data in the submitted published studies was generally very limited. Due to the design of the studies it would not be possible to assign causality of any adverse event to Tisseel, as opposed to the surgical procedure itself.

Pivotal studies

Bentley et al

This study compared ACI (n = 58) with mosaicplasty (n = 42). Minimal safety data were reported. Three patients were reported as having delayed mobilisation. One patient developed a deep venous thrombosis, and one developed a superficial infection (site not stated) which resolved with antibiotics. The treatment arm to which these five adverse events belonged was not stated.

Dozin et al

No safety data were reported in this study.

Knutsen et al

No safety data were reported in this study.

Visna et al

The authors reported that no serious clinical complications were observed in either group (MACI or abrasion arthroplasty) during the post-operative period. Of note, five patients (20%) in the MACI group developed a "reactive synovitis with exudation". This settled in all patients within 4 weeks, after NSAID administration and elimination of weight-bearing for 3 weeks. No further details were provided.

Synovitis was not reported in any of the other studies in which fibrin sealant / glue was used. The technique of MACI used in this study was atypical in that the cultured chondrocytes were mixed directly with the fibrin glue to form a matrix which was moulded to fill the cartilage defect. The dose of fibrin glue / sealant used may therefore have been higher in this study than in the others.

Basad et al

No safety data were reported in this study.

Supportive study

Marlovits et al

The study report for this single-arm trial stated that there were no product specific adverse events recorded among the 21 patients enrolled.

Other studies

The adverse events in the 6 other trials described what would appear to be related to the surgical procedure and it would be difficult to assign causality to Tisseel. One patient (Browne et al)

Gastrointest Endosc 1998; 48: 83-85.

[21] Scheule AM, Beierlein W, Wendel WP, Eckstein FS, Heinemann MK, Ziemer G. Fibrin sealant, aprotinin, and immune response in children undergoing operations for congenital heart disease. J Thorac cardiovasc Surg 1998; 115: 883-889.

[22] Scheule AM, Beierlein W, Wendel WP, Jurmann MJ, Eckstein FS, Ziemer G. Aprotinin in fibrin tissue adhesives induces specific antibody response and increases antibody response of high-dose intravenous application. J Thorac Cardiovasc Surg 1999; 118: 348-353.

[23] Schievink WI, Georganos SA, Maya MM, Moser FG, Bladyka M. Anaphylactic reactions to fibrin sealant injection for spontaneous spinal CSF leaks. Neurology 2008; 70: 885-887.

[24] Shah HN, Hegde S, Shah JN, Mohile PD, Yuvaraja TB, Bansal MB. A prospective, randomized trial evaluating the safety and efficacy of fibrin sealant in tubeless percutaneous nephrolithotomy. J Urol 2006; 176: 2488-2492.

developed a deep venous thrombosis (with pulmonary embolus), however this occurred in the non-operated limb.[14]

The adverse events described in the 7 trials of other types of surgical procedures reported allergic reactions to Tisseel. The reports included one fatality. Such reactions have previously been documented with the product and the currently approved product information (PI) includes appropriate precautionary statements.

Other safety parameters

The submitted studies did not report on other safety parameters such as withdrawals, serious adverse events or laboratory parameters. There were no deaths reported in any of the ACI / MACI studies.

Post-marketing experience

The submission included a Periodic Safety Update Report (PSUR) for Tisseel. The date of the report was 23 January 2008 and it covered the 12-month period from 1 December 2006 to 30 November 2007. It reviewed all adverse reaction reports received by the sponsor during this period. During this time the sponsor estimated that 1,042,000 treatments with Tisseel (in its various forms) had been administered.

During the 12-month period covered by the PSUR, a total of 60 adverse event reports were received by the sponsor. Brief narratives of the reports which are not currently listed in the PI were provided. None of the reports related to use of Tisseel in ACI or MACI procedures. There was no pattern amongst the reported events that would warrant revision to the currently approved PI.

The PSUR also reviewed 14 studies published during the period covered. None of the studies related to use of Tisseel in ACI or MACI procedures. No new safety issues were raised.

Conclusions regarding safety

The submitted published trials document experience with Tisseel in over 600 patients receiving ACI or MACI procedures. The reporting of adverse events in the published trials was limited.

The only specific adverse event reported in the trials in ACI and MACI that might plausibly be related to Tisseel was reactive synovitis. This was only reported in one study and may have been a function of a higher dose of the product being used in this trial.[8]

ACI and MACI procedures have been described in the literature since the late 1980's. There do not appear to have been any specific safety concerns raised in relation to the use of fibrin glue/sealant products such as Tisseel in these procedures.

Overall the safety profile of Tisseel in ACI / MACI appears acceptable.

Clinical Summary and Conclusions

The sponsor has submitted this application to extend the approved indications of Tisseel to include use as a sealant and/or adhesive in ACI and MACI. The stimulus for the application was suspension of the funding of Tisseel use in these procedures by the Medical Services Advisory Committee (MSAC), on the grounds that such use did not fall within the TGA-approved indications for the product.

The submission was primarily based on published literature, although a report was also provided for one supportive efficacy study.[10]

Evidence of efficacy was based on five randomised controlled trials which compared the ACI or MACI procedures with other surgical techniques used in the treatment cartilage defects of the knee (mosaicplasty, microfracture, abrasion arthroplasty). Tisseel was used as part of the ACI /MACI procedure in these five studies. Although the studies had a number of limitations, a reasonable

conclusion drawn from them would be that the ACI and MACI procedures have comparable efficacy to these other techniques.

The major limitation of the submission is that there have been no studies conducted comparing the surgery with and without Tisseel.

In terms of safety, studies of ACI/MACI using Tisseel involving over 600 patients did not suggest any significant safety concerns. The ACI/MACI procedures have been described in the published literature for approximately 20 years without significant safety issues related to fibrin use having arisen.

Overall the evaluator considered that the evidence is sufficient to conclude that the benefits of Tisseel use in these procedures outweigh the associated risks. Approval of the application was therefore recommended.

V. Pharmacovigilance Findings

There was no Risk Management Plan submitted with this application as it was not a requirement at the time of submission.

VI. Overall Conclusion and Risk/Benefit Assessment

The submission was summarised in the following Delegate's overview and recommendations:

Quality

There was no requirement for a quality evaluation in an application of this type.

Nonclinical

Implantation of the fibrin/chondrocyte/scaffold construct in mice enhanced development of cartilage compared to the construct without fibrin sealant or cells.

There was transient inflammation at fibrin sealant (including Tisseel) implantation sites in rats and rabbits. This inflammation was likely related to the foreign proteins (bovine and human) in the sealant. There is potential for anaphylactic reactions.

Most of the studies reviewed did not use Tisseel VH/SD (or the most recent superseded formulation Tisseel Duo 500). The products used varied in the amounts of fibrinogen, fibronectin, factor XIII, aprotinin and thrombin compared with Tisseel VH/SD. Such differences are not expected to significantly change local tissue responses.

The evaluator recommended approval.

Clinical

Five published studies and an unpublished case series of the use of Tisseel (*formulation not specified*) in ACI/MACI were submitted in support of efficacy.[4-10] All studies involved cartilage defects in the knee. ACI was used in the Bentley, Dozin and Knutsen studies (n=120)[4-7] and MACI in the Visna, Basad and Marlovits studies (n=56).[8-10] The efficacy of ACI/MACI was compared with other procedures for repair of articular cartilage: mosaicplasty,[4,5] microfracture[6,7,9] and abrasion arthroplasty.[8] There is no gold standard. In one study, follow-up assessments were done by an independent assessor blinded to treatment allocation.[6,7] Patients were aged 16 to 50 years.

In the six trials, 166 patients were treated with Tisseel as an adjunct to ACI or MACI. The controlled trials except Dozin supported the comparability of ACI/MACI to the control treatment (Table 12) and indirectly supported the role of Tisseel. The uncontrolled Marlovits trial added further support. In the first 6 months after treatment, there were no reports of locking or catching of the knee joint indicative of graft instability. Knutsen provided the best evidence with long-term data to 5 years.

Table 12. Efficacy of ACI/MACI with Tisseel as Adjunct

Trial	Endpoint	ACI/MACI	Control	Difference
Bentley 2003[4] ACI vs Mosaicplasty	MCS excel/good	88% (n=58)	69% (n=42)	p=0.3 not significant
Dozin 2005[5] ACI vs Mosaicplasty	LKSS 90-100	58% (n=12)	90% (n=11)	not assessed
Knutsen 2007[6,7] ACI vs Microfracture	Fail at 5 yrs LKSS	23% *CE Part B,* (n=40)	23% *Fig 8* (n=40)	not significant not significant
Visna 2004[8] MACI vs Abras Arthroplasty	Mean LKSS at 1 yr	86 (n=25)	74 (n=25)	p<0.001
Basad 2004[9] MACI vs Microfracture	Mean LKSS gain at 1 yr	48 (n=10)	15 (n=9)	not assessed
Marlovits 2006[10] MACI	Mean LKSS gain at 2 yrs	29 (n=21)	-	-

MCS: Modified Cincinnati Score (0-100). LKSS: Lysholm Knee Scoring Scale (0-100). CE: Clinical

In the Marlovits case series, the volume of the cartilage defect was assessed by MRI over 2 years. Of 17 defects assessed, 11 (65%) were > 76% filled at 2 years.

With respect to safety, in addition to the 6 studies above, a further 13 published papers were submitted, 6 of which involved the use of Tisseel in ACI/MACI (approx 500 subjects). Reporting of adverse events was limited. Reactive synovitis in the Visna study was possibly related to Tisseel. Specific safety concerns regarding the use of Tisseel in ACI/MACI do not appear to have been raised over the 20 years since the procedure was first described.

The evaluator recommended approval with more concise wording for the indication.

Risk-Benefit Analysis

The literature submitted has several limitations which make assessment of efficacy difficult. However, four of the five controlled trials provide indirect support of the role of Tisseel as an adjunct to ACI or MACI in the repair of articular cartilage defects of the knee. In these trials, ACI or MACI (with Tisseel) was at least comparable to the control procedure. It would be reasonable to extrapolate efficacy in these trials to repair of cartilage in other joints.

The benefit of Tisseel itself over the normal healing process was not assessed since there were no controlled trials of ACI or MACI with and without Tisseel. The European Guideline is not specific in relation to ACI/MACI, but in general requires a controlled trial of standard treatment with and without Tisseel. The requirement for a controlled trial is specifically mentioned for the higher risk procedures vascular and gastrointestinal anastomoses and neurosurgery, where a high degree of adhesion and haemostasis is required. The Delegate agreed with the clinical evaluator that a control is not an absolute requirement for the present application. Tisseel is an established tissue glue and, as well as the clinical studies, there is support from the non-clinical studies. Therefore, the Delegate concluded that Tisseel was likely to be contributing to the efficacy of ACI/MACI. The extent to which Tisseel contributes to the efficacy of ACI/MACI has not been determined; however, the degree of adhesion is not as critical in ACI/MACI as in the higher risk procedures.

The safety data from the published literature was limited. Reactive synovitis in one of the trials is a possible adverse reaction.

The formulation of Tisseel used in the trials was not specified. From the dates of publication, it is likely to have been an earlier formulation than Tisseel VH/SD. However, based on similar composition and action, Tisseel VH/SD is likely to have similar efficacy to and greater safety (following refinements to the manufacturing process) than earlier formulations. This is supported by the comparability of Tisseel VH/SD to the recently superseded Tisseel Duo 500 in the registered indications.

Overall, the evidence submitted is borderline. However, there is an established extensive use of the product in ACI/MACI in Australia and overseas with no apparent efficacy or safety issues. On the basis of the literature and extensive use and the approval of broad indications encompassing ACI/MACI in the UK and Canada, the Delegate concluded that the benefit-risk profile of Tisseel (and specifically Tisseel VH/SD) in ACI/MACI is in favour of approval. The Delegate supported the clinical evaluator's concise wording for the indication and recommended approval of the extension subject to finalisation of product information.

The Advisory Committee on Prescription Medicines (ACPM) (which has succeeded ADEC), having considered the evaluations and the Delegate's overview, as well as the sponsor's response to these documents, agreed with the Delegate's proposal.

ACPM recommended approval of the submission with the indication:

Tisseel is indicated as a sealant and/or adhesive for use in autologous chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI) procedures

In making this recommendation, the ACPM noted that Tisseel Duo/ Tisseel VH/SD is an established tissue glue and, as well as the clinical studies, there is support from the non-clinical studies. Overall, although the evidence submitted is minimal, there is an established extensive use of the product in ACI/MACI in Australia and overseas with no apparent efficacy or safety issues.

Outcome

Based on a review of quality, safety and efficacy, TGA approved the registration of Tisseel VH/SD fibrin sealant syringe for the new indication

as a sealant and/or adhesive for use in autologous chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI) procedures.

Attachment 1. Product Information

Product Information.

T I S S E E L [Fibrin Sealant]

**Two-Component Fibrin Sealant,
Deep-Frozen, Vapour Heated (VH) and Solvent Detergent (S/D) Treated**

NAME OF THE DRUG

TISSEEL [Fibrin Sealant]
Two-Component Fibrin Sealant, Deep-Frozen, Vapour Heated (VH) and Solvent Detergent (S/D) treated, TISSEEL VH S/D[1]

DESCRIPTION

The active ingredients of TISSEEL are formulated as two sterile, deep-frozen solutions, the Sealer Protein Solution and Thrombin Solution (see Table 1 and PRESENTATION section). Each solution is presented in a separate preloaded chamber of one double-chamber syringe. The active ingredients are fractionated from pooled human plasma.

Table 1: Composition of the Active Ingredients of TISSEEL:

(1) Sealer Protein Solution 1 mL of the solution contains

Active Ingredients:		
As total protein	96 - 125	mg
thereof Fibrinogen (Clottable Protein)	72 - 110	mg
Factor XIII (human)	1.2 - 10	IU
Aprotinin, (bovine)	3000	KIU[2]
Excipients: (see Table 2)		

[1] The term 'Vapour Heated (VH) and Solvent Detergent (S/D) treated' is abbreviated as VH S/D
[2] KIU = Kallidinogenase Inactivator Unit

(2) Thrombin Solution: 1 mL of the solution contains

Active Ingredients:		
Thrombin (human)	500	IU[3]
Calcium chloride (2 H_2O)	40	µmol
Excipients: (see Table 2)		

Table 2: Composition of the Excipients of TISSEEL:

(1) Sealer Protein Solution: 1 mL of the solution contains, Human Albumin (10-20 mg), Histidine (10-25 mg), Sodium Citrate (4.8-9.7 mg), Polysorbate 80 (0.6–1.9 mg), Nicotinamide (3–9 mg), Water for injection q.s. to 1 mL.

(2) Thrombin Solution: 1 mL of the solution contains, Human Albumin (45–55 mg), Sodium Chloride (3.5–5.5 mg) and Water for injection q.s to 1 mL

The two deep frozen solutions comprising TISSEEL must be defrosted prior to use. After thawing and warming up to 37 ºC, the two solutions are mixed during application (see DOSAGE AND ADMINISTRATION section, heading Method of Application).

Chemical structures

The major component of the clottable protein (human origin) is fibrinogen. The fibrinogen molecule is a dimer composed of two symmetrical subunits linked by -S-S- bonds. It could be written in a simple formula as $(A\alpha, B\beta, \gamma)_2$ and has a molecular weight (MW) of about 340 000. The $A\alpha$-chain contains 610 amino acids (MW about 68 000), the $B\beta$-chain 461 amino acids (MW about 57 000), and the γ-chain 411 amino acids (MW about 47 000). Thus, the entire human fibrinogen contains 2964 amino acids.

Thrombin (human origin) is a glycosylated protein, consisting of two polypeptide subunits A and B, covalently linked by one -S-S- bond. The molecular weight is about 33 800. The human thrombin subunit A chain is made of 36 amino acids, whilst the B chain contains 259 amino acids.

Factor XIII (human origin), also called blood-coagulation factor XIII, is a tetramer composed of two a-chains and two b-chains (each of a molecular weight of about 80 000) which are non-covalently associated.

Aprotinin (bovine origin) is a protease inhibitor, a polypeptide consisting of one chain of 58 amino acids with a molecular weight of 6 500, also stabilized by -S-S- bonds.

[3] One International Unit (IU) of Thrombin is defined as the activity contained in 0.0853 mg of the First International Standard of Human Thrombin

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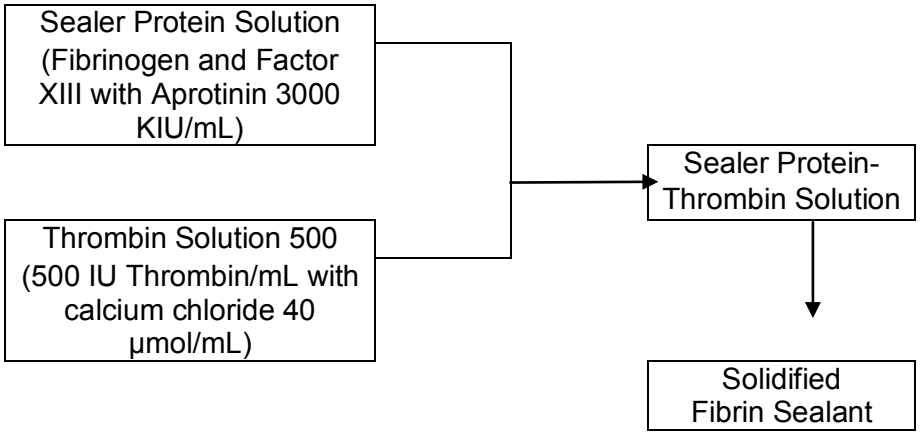
PHARMACOLOGY

Pharmacodynamics

TISSEEL contains two components, Sealer Protein Solution and Thrombin Solution. The Sealer Protein Solution contains fibrinogen as the main active ingredient, the active ingredient of the Thrombin Solution is human Thrombin. Thrombin is a highly specific protease that transforms the fibrinogen contained into fibrin monomers. These fibrin monomers are then polymerized in a linear fashion and stabilised by cross-linking (catalysed by factor XIII) to form an insoluble fibrin clot. Aprotinin (bovine), is a protease inhibitor which prevents the premature degradation of fibrin.

These reactions simulate the key features of the physiological coagulation process. The resulting fibrin clot appears as a white, elastic mass which firmly adheres to tissue and which can be used to achieve haemostasis or seal tissues.

When the two component solutions come into contact, conversion of fibrinogen to fibrin, and polymerization and cross-linking of fibrin monomers commences immediately and results in the clotting of the fibrin within seconds. The following diagram illustrates the process.



Pharmacokinetics

Solidified TISSEEL is intended for local application only, therefore systemic exposure or distribution to other organs or tissues is not expected and Pharmacokinetic Studies were not conducted.

Clinical Trials

TISSEEL VH S/D was evaluated in a prospective, parallel design, randomised (1:1), double-blind, multicenter clinical study against an earlier formulation of

the product, TISSEEL VH[4], in 317 subjects undergoing cardiac surgery requiring cardiopulmonary bypass (CPB) and median sternotomy. Patients were treated with TISSEEL VH S/D or the control product TISSEEL VH only when haemostasis was not achieved by conventional surgical methods. For the end point, haemostasis achieved at the primary treatment site within 5 minutes of treatment and maintained until closure of the surgical wound, TISSEEL VH S/D was non-inferior to the earlier formulation of the product using a one-sided 97.5% confidence interval on the difference in the proportion of subjects successfully treated.

Haemostasis within 5 minutes and maintained until surgical closure		
	TISSEEL VH S/D	TISSEEL VH
Intent to Treat Analysis	127/144 (88.2%)	129/144 (89.6%)
Per Protocol Analysis	108/123 (87.8%)	122/135 (90.4 %)

Virus Safety

To confirm virus safety of TISSEEL VH S/D, subjects were followed up for seroconversion due to virus infections. There were zero confirmed seroconversions for both TISSEEL VH S/D-treated subjects and TISSEEL VH-treated subjects: analysis of B19V seroconversion 1 month after surgery revealed a 0% (0/140) incidence of seroconversion in TISSEEL VH S/D-treated subjects and a 0% (0/138) incidence of seroconversion in TISSEEL VH-treated subjects. Analysis of HAV, HBV, HCV, and HIV-1/-2 six months after surgery revealed a 0% (0/128) incidence of seroconversion in TISSEEL VH S/D-treated subjects and a 0% (0/134) incidence of seroconversion in TISSEEL VH-treated subjects.

An earlier formulation of TISSEEL VH S/D, TISSEEL HT [5] (Fibrin Sealant heat-treated) was evaluated in an open-label crossover study against control topical haemostatic agents in 489 patients undergoing cardiovascular re-operation or re-sternotomy at 11 institutions. Patients were randomised to TISSEEL HT or control haemostatic agents when a topical haemostatic was needed at the conclusion of surgery and after all attempts of surgical haemostasis. Patients were crossed to the alternative therapy if bleeding continued after the 5 minute endpoint. At 10 centres, TISSEEL was used after administration of protamine sulfate. At one site, TISSEEL could be used before administration of protamine sulfate. 365 of the 489 patients had an eligible bleeding event, for the primary endpoint, successful haemostasis at 5 minutes, TISSEEL was statistically significantly superior to control topical haemostatic agents:

[4] Baxter commercialized several single virus inactivated, predecessor fibrin sealant products, utilizing heat treatment (HT) or vapor heat treatment (VH) for virus inactivation. Predecessor products were manufactured both in frozen or lyophilized presentation.

[5] Baxter commercialized several single virus inactivated, predecessor fibrin sealant products, utilizing heat treatment (HT) or vapor heat treatment (VH) for virus inactivation. Predecessor products were manufactured both in frozen or lyophilized presentation.

Haemostasis within 5 minutes	
TISSEEL HT[5]	Control Topical Hemostatic Agent
159/193 (82.4%)	76/172 (44.2%)
Pearson x^2, two sided; p <0.0001; intent-to-treat analysis	

Similarly, absolute time to cessation of bleeding was statistically significantly shorter for TISSEEL than for control topical haemostatic agents (p<0.0001, Wilcoxon-Gehan test, two sided).

In a single centre, prospective open label study of 120 patients randomised to standard of care (59 patients) or standard of care plus Fibrin Sealant (61 patients) for elective colostomy closure after temporary colostomy placement for treatment of traumatic injury to the colon, the earlier version of TISSEEL[6] plus standard of care was shown to be statistically significantly superior to standard of care alone (p = 0.0406, Jonckheere-Terpstra test for ordinal data, two sided) with regard to anastomotic complications (leakage, intra-abdominal abscess formation, re-operation, septic shock, and death).

A review of published literature was conducted studying the repair of defects of the articular cartilage in the knee; (n= 293 patients; 166 patients were treated with either Autologous Chondrocyte Implantation (ACI) or Matrix-Induced Autologous Chondrocyte Implantation (MACI); 127 patients were treated with either mosaicplasty or microfracture or abrasive arthroplasty). In all ACI/MACI procedures, TISSEEL Fibrin Sealant was applied topically. The efficacy of TISSEEL has been assessed indirectly by the efficacy outcome measures used to assess joint function following repair of cartilage defects. Outcome measures within the first six months of treatment are considered to be of particular importance because treatment failure attributed to graft movement (e.g., periosteal delamination or detachment of the collagen matrix) typically occurs within the first three to six months following implant. In addition, in the first 6 months post-implant, there were no reports by patients of symptoms which may be indicative of graft instability such as “locking” or “catching” of the knee joint. In one study MRI assessments, made at one and two months, showed that there was a high level of graft integration with the surrounding cartilage, and that grafts were present and in their original position in the majority of patients (15/17). These findings suggest that TISSEEL is an effective adhesive in this indication. Long term results (≥ 6 months) indicated that treatment with either ACI or MACI was at least as successful as the comparative treatment.

In a single centre, open label trial, an earlier formulation of TISSEEL[6] was compared to historical controls in patients undergoing laparotomy for blunt or penetrating traumatic injury to the spleen and/or liver. Use of TISSEEL

[6] Baxter commercialized several single virus inactivated, predecessor fibrin sealant products, utilizing heat treatment (HT) or vapor heat treatment (VH) for virus inactivation. Predecessor products were manufactured both in frozen or lyophilized presentation.

resulted in the need for statistically significantly fewer splenectomies than control haemostatic manoeuvres:

Splenectomy Rate		
Injury to:	TISSEEL[6]	Historic Controls
Spleen p <0.001	0/19	14/22
Spleen and liver p<0.001	1/26	19/34

TISSEEL did not result in statistically significantly reduced mortality in patients with blunt or penetrating trauma to the liver alone or to the liver and spleen (p = 0.067, χ^2 , one sided).

INDICATIONS

TISSEEL is indicated:

- as adjunct to haemostasis during surgical procedures, when control of bleeding by conventional surgical techniques is ineffective or impractical; and
- as a sealant as an adjunct for closure of colostomies.
- As a sealant and/or adhesive for use in autologous chondrocyte implantation (ACI) or matrix-induced autologous chondrocyte implantation (MACI) procedures.

CONTRAINDICATIONS

Known hypersensitivity to aprotinin (or other bovine proteins) or known hypersensitivity to any other component of TISSEEL.

Injection of TISSEEL into tissues is contraindicated. Such use has been associated with inadvertent intravascular injection, with thromboembolic complications.TISSEEL should be applied with caution to minimise any risk of intravascular application, for example in coronary bypass surgery. TISSEEL should only be applied topically.

PRECAUTIONS

Viral and Prion Risk

Sealer Protein Concentrate and Thrombin are made from human plasma. Products made from human plasma may contain infectious agents which can cause disease, such as viruses and theoretically Creutzfeld-Jacob Disease (CJD) agents. Standard measures to prevent infections resulting from the use of medicinal products prepared from human blood or plasma include selection of donors, screening of individual donations and plasma pools for specific

markers of infection and the inclusion of effective manufacturing steps for the inactivation/removal of viruses. Despite this, when medicinal products prepared from human blood or plasma are administered, the possibility of transmitting infective agents cannot be totally excluded. This also applies to unknown or emerging viruses or other pathogens.

The measures taken (including double virus inactivation by vapour heat treatment and solvent detergent treatment) are considered effective for enveloped viruses such as HIV, HBV, and HCV, and for the non-enveloped virus HAV.

The measures taken may be of limited value against small non-enveloped viruses such as parvovirus B19. Parvovirus B19 infection may be serious for pregnant women (foetal infection) and for individuals with immunodeficiency or increased red blood cell turnover (e.g., hemolytic anaemia).
It is strongly recommended that every time a patient receives a dose of TISSEEL, the name and batch number of the product are recorded in order to maintain a record of the batches used.

General

Administration of TISSEEL may result in allergic reactions in some patients. For patients with a known allergic diathesis, a history of hypersensitivity to medical products or a history of having previously received aprotinin-containing products (including previous use of TISSEEL) a careful risk-benefit assessment should be carried out prior to administration. The risk of immunisation against bovine-derived proteins such as aprotinin is increased if repeated exposure occurs within six months. If it is decided to proceed with treatment in such patients, prior administration of antihistamines should be considered.

Manifestations of hypersensitivity reactions to TISSEEL observed include: bradycardia, tachycardia, hypotension, flushing, bronchospasm, wheezing, dyspnea, nausea, urticaria, angioedema, pruritus, erythema, paresthesia. Fatal anaphylactic reactions, including anaphylactic shock, have also been reported with TISSEEL. Refer ADVERSE EFFECTS. Intravascular application might increase the likelihood and severity of acute hypersensitivity reactions in susceptible patients.

As Sealer Protein and Thrombin Solutions can be denatured following contact with solutions containing alcohol, iodine or heavy metals (e.g. in disinfectants), any such substances should be removed before application. **Refer Incompatibilities**.

TISSEEL alone is not indicated for the treatment of massive and brisk arterial or venous bleeding.

If possible, cover all tissue adjacent to the site of sealing before applying TISSEEL

TISSEEL should not be used for the sealing of neuroanastomoses, as the high aprotinin content of the TISSEEL solution delays absorption of the fibrin seal and it cannot be ruled out that this may cause fibrosis.

Injection into the nasal mucosa must be avoided, as severe allergic/anaphylactoid reactions have been observed and thromboembolic complications may occur in the area of the ophthalmic artery.

If fibrin sealants are applied in confined bodily spaces, the risk of compressive complications should be taken into account.

Genotoxicity

Studies of genotoxic potential of TISSEEL have not been performed.

Carcinogenicity

Animal studies to evaluate the carcinogenic potential of TISSEEL have not been performed.

Effects on Fertility

Studies of the effect of TISSEEL on fertility have not been performed.

Use in Pregnancy (Category B2)

Animal reproduction studies have not been conducted with TISSEEL. There are no adequate and well-controlled studies in pregnant women. TISSEEL should be used during pregnancy only if clearly needed and potential benefit justifies the potential risk to the foetus.

Use in Lactation

Studies on TISSEEL in lactating animals or women have not been conducted. TISSEEL should be used during lactation only when strictly indicated.

Paediatric Use

Safety and effectiveness of TISSEEL in paediatric patients have not been established. There has been a single report of disseminated intravascular coagulation occurring in a premature infant who received TISSEEL 3 mL during a laparotomy for peritoneal adhesions.

Use in the Elderly

Of the total number of subjects in a clinical study of TISSEEL, 71 out of 144 subjects were 65 and over. No overall differences in safety or effectiveness were observed between these subjects and younger subjects, and other reported clinical experiences have not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

Interactions with Other Drugs

There are no known interactions between TISSEEL and other drugs. Efficacy has been demonstrated in fully heparinised patients undergoing cardiopulmonary bypass. **Refer to Incompatibilities for more detailed information on interactions with substances other than drugs.**

ADVERSE EFFECTS

Anaphylactic and anaphylactoid reactions may occur in patients who have previously received a fibrin-based sealant, in those with a known hypersensitivity to aprotinin and those who have previously received aprotinin systemically. Even if the second treatment with TISSEEL was well tolerated, a subsequent administration of TISSEEL or systemic administration of aprotinin may result in severe anaphylactic reactions.

Symptoms associated with allergic/anaphylactic reactions include flush, urticaria, pruritus, nausea, drop in blood pressure, tachycardia or bradycardia, dyspnoea, severe hypotension, and anaphylactic shock. In the event of hypersensitivity reactions, administration of TISSEEL should be discontinued, the topical clot removed, and appropriate treatment instituted.

In rare cases, these reactions may also occur in patients receiving aprotinin or TISSEEL for the very first time.

Injection of TISSEEL into tissues has been associated with inadvertent intravascular administration and thromboembolic complications. Such use is therefore not recommended (see CONTRAINDICATIONS section).

The adverse reactions presented in this section were reported from clinical trials investigating the safety and efficacy of TISSEEL. In these trials, TISSEEL was administered for adjunct hemostasis in cardiac, vascular, and total hip replacement surgeries; and for the sealing of lymphatic vessels in patients undergoing axillary lymph node dissection. In these studies, a total of 499 patients were administered TISSEEL. The frequencies are based on the number of cases considered possibly/probably related by investigators.

Clinical Trial Adverse Reactions			
System Organ Class (SOC)	Preferred MedDRA Term	Frequency	Number of Cases (Frequency Percentage)
VASCULAR DISORDERS	Hypotension	Uncommon	1 (0.2%)
GASTROINTESTINAL DISORDERS	Nausea	Uncommon	2 (0.4%)
INVESTIGATIONS	Fibrin degradation products increased	Common	7 (1.4%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	Postprocedural pain	Common	7 (1.4%)

Legend: ADR frequency is based upon the following scale: Very Common (≥1/10); Common (≥1/100 - <1/10), Uncommon (≥1/1,000 - <1/100), Rare (≥1/10,000 - <1/1,000), Very Rare (<1/10,000)

The undesirable effects reported in the listing hereafter are based on post-market experience for this type of product. Their frequency has been evaluated by using the following criteria: very common (>1/10), common (>1/100, <1/10), uncommon (>1/1,000, <1/100), rare (>1/10,000, <1/1,000), and very rare (<1/10,000).

The undesirable effects listed below reflect the type of undesirable effects that have been reported with TISSEEL.

Their incidence rate is <1/10,000, i.e. very rare.

Cardiac disorders

- ·Bradycardia, tachycardia

Gastrointestinal disorders

- ·Nausea

General disorders and administration site disorders

- Hypersensitivity reactions

Immune system disorders

- Hypersensitivity reactions (including anaphylactic reactions, anaphylactic shock, and the following manifestations: angioedema, paresthesia, bradycardia, tachycardia, flushing, bronchospasm, dyspnea, wheezing, urticaria, pruritus, and erythema). Anaphylactic reactions and anaphylactic shock have included fatal outcomes.

Injury, poisoning and procedural complications

- Anaphylactoid reactions

Investigations

- Drop in blood pressure

Respiratory, thoracic and mediastinal disorders

- Dyspnoea

Skin and subcutaneous tissue disorders

- Pruritus, Impaired wound healing

Vascular disorders

- Flush, (severe) hypotension, ,thromboembolism (including cerebral artery embolism and venous thrombotic cerebral infarction) as a result of intravascular application.

Class Effects

Other adverse reactions associated with fibrin sealant/hemostatic products include, as manifestations of hypersensitivity or allergic reactions, application site irritation,chest discomfort, chills, headache, lethargy, restlessness, and vomiting.

DOSAGE AND ADMINISTRATION

Dosage

TISSEEL should only be administered topically. **Do not inject** .The required dose depends upon the size of the surface to be covered. To avoid the formation of excess granulation tissue, and to ensure gradual absorption of the solidified fibrin sealant, only a thin layer of TISSEEL should be applied.. Excessive thickness of the fibrin layer may negatively interfere with the product's efficacy and the wound healing process.

The application can be repeated, if necessary. However, avoid re-application of TISSEEL to a pre-existing polymerized TISSEEL layer as TISSEEL will not adhere to a polymerised layer. If used for tissue adherence, it is recommended that the initial application cover the entire intended application area.

The approximate surface areas covered by each package size of TISSEEL are listed in the following table.

Maximum size of the area to be sealed		Required package size of TISSEEL
8 cm^2 16 cm^2 40 cm^2		2 mL 4 mL 10 mL

Method of Preparation of TISSEEL Preloaded Syringe (Frozen)

Thaw preloaded syringe in one of the three following options:

Option 1 – Thawing on the sterile field
33°C to 37°C sterile water bath - transfer devices set and the inner pouch to the sterile field, remove devices set with preloaded syringes from inner pouch and place directly into sterile water bath. Ensure the contents of the syringe are completely immersed under the water.

Approximate thawing and warming times when using this method are:

Pack Size	Thawing/Warming Times 33°C to 37°C Sterile Water Bath (Pouches Removed)
2 mL	5 minutes
4 mL	5 minutes
10 mL	12 minutes

Option 2 – Thawing off the sterile field
33°C to 37°C non-sterile water bath in two pouches - maintain the devices set in both pouches and place into a water bath off the sterile field for appropriate time. Ensure the pouches remain submerged throughout thawing. Remove from the water bath after thawing, dry external pouch and transfer inner pouch and preloaded syringe onto the sterile field.

Approximate thawing and warming times when using this method are:

Pack Size	Thawing/Warming Times 33°C to 37°C Non-Sterile Water Bath (In Pouches)
2 mL	30 minutes
4 mL	40 minutes
10 mL	80 minutes

Option 3 – Thawing off the sterile field
incubator (33°C to 37°C) in pouches – maintain the devices set in both pouches and place into an incubator for appropriate time. Remove from incubator after thawing and transfer inner pouch and preloaded syringe onto the sterile field.

Approximate thawing and warming times when using this method are:

Pack Size	Thawing/Warming Times 33°C to 37°C Incubator (In Pouches)
2 mL	40 minutes
4 mL	85 minutes
10 mL	105 minutes

Do not microwave TISSEEL

TISSEEL should only be used when, after thawing, the Sealer Protein Solution has a viscous consistency similar to honey (air bubbles in the syringe chamber holding the Sealer Protein Solution slowly rise to the top when the double chamber syringe is tilted or turned upside down). If the Sealer Protein Solution has the consistency of a gel, it must be assumed to have become denatured due to an interruption of the cold storage chain. In this case, the fibrin sealant must not be used.

The protective syringe cap should not be removed until thawing is complete and application tip is ready to be attached. Do not use TISSEEL unless it is completely thawed and warmed (liquid consistency).

The solutions must be used within 72 hours after thawing and stored at or below 25 ^{o}C.

Any unused product and/or devices should be disposed of in accordance with local requirements.

Method of Application

Application of TISSEEL must be completed within 4 hours after opening the preloaded frozen double chamber syringe. Discard any unused product. Separate, sequential application of the two components of TISSEEL must be avoided.

Prior to application, TISSEEL must be warmed to 33-37°C. TISSEEL must not be exposed to temperatures above 37°C.

Before application, the surface of the wound should be as dry as possible. If application is interrupted, clogging occurs immediately in the cannula. Replace the application cannula with a new one only immediately before application is resumed. If the aperture of the joining piece (Y connector) facing the cannula is clogged, use the spare joining piece provided in the package.

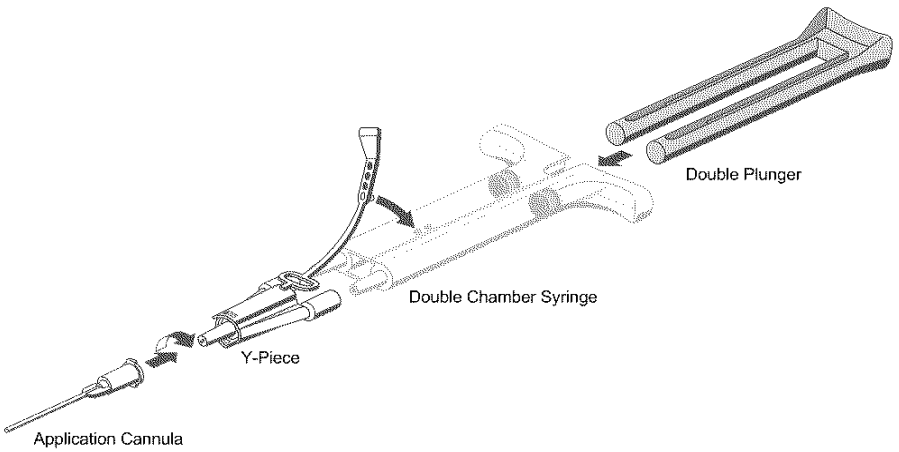
In cases where very small volumes (1 to 2 drops) of TISSEEL are administered, expel and discard the first several drops from the application cannula immediately before application, to ensure adequate mixing of the sealer protein and thrombin solutions.

Application beyond the intended area of application should be avoided.

After the two components have been applied, fix or hold the sealed parts in the desired position for at least three to five minutes to ensure the setting TISSEEL adheres firmly to the surrounding tissue.

Operating Instructions

For application, connect the double chamber syringe with the Sealer Protein Solution and the Thrombin Solution to a Y-piece and an application cannula (see diagram overleaf) as provided in the accompanying set of devices. The double plunger of the double chamber syringe ensures that the equal volumes are fed through the Y-piece before being mixed in the application cannula and ejected.



Device Set Instructions: firmly connect the double chamber syringe nozzles to the Y–piece and secure it by fastening the tether strap to the syringe. Fit an application cannula onto the Y-piece. To avoid clogging, do not expel the air remaining inside the Y-piece or application cannula until application.

Incompatibilities

Sealer Protein and Thrombin Solutions can be denatured following contact with solutions containing alcohol, iodine or heavy metals. If any of these substances have been used to clean the wound area, the area must be thoroughly rinsed before application of TISSEEL.

Oxycellulose-containing preparations may reduce the efficacy of TISSEEL and should not be used as carrier materials.

TISSEEL must not be mixed with other medicinal products.

OVERDOSAGE

TISSEEL should only be applied as a thin layer. Excessive clot thickness may negatively interfere with the product's efficacy and the wound healing process. In the event of overdosage, please contact the Poison Information Centre at Phone Number: 131126.

PRESENTATION

Nature and Contents of Container

Nature of containers:
Both Sealer Protein Solution and Thrombin Solution are contained in two separate chambers of a single use double chamber syringe made of polypropylene.
Contents:
Each pack TISSEEL contains

- One single use double chamber syringe, each chamber containing:
    - Chamber number [1]:Sealer Protein Solution (with aprotinin) deep frozen
    - Chamber number [2]: Thrombin Solution (with calcium chloride) deep frozen
- One set of devices (see below)

TISSEEL is available in the following pack sizes:

- TISSEEL, 2.0 mL (containing 1.0 mL of Sealer Protein Solution and 1.0 mL of Thrombin Solution)
- TISSEEL, 4.0 mL (containing 2.0 mL of Sealer Protein Solution and 2.0 mL of Thrombin Solution)
- TISSEEL, 10.0 mL (containing 5.0 mL of Sealer Protein Solution and 5.0 mL of Thrombin Solution)
- (See Table 3 below for formulation details)

Table 3: TISSEEL is supplied in three different package sizes of 2.0, 4.0 and 10.0 mL, containing the following components

	Package sizes	2 mL	4 mL	10 mL
Sealer Protein Solution	**Active Ingredients**:	**1 mL**	**2 mL**	**5 mL**
	as total Protein (mg) thereof:	96 – 125	192 – 250	480 – 625
	Fibrinogen (Clottable Protein)(mg)	72 – 110	144 – 220	360 – 550
	Factor XIII, human (Unit)	1.2-10 IU	< 20 IU	< 50 IU
	Aprotinin, bovine (KIU)	3000	6000	15000
	Excipients:			
	Human albumin (mg)	10 – 20	20 – 40	50 – 100
	Histidine (mg)	10 – 25	20 – 50	50 – 125
	Sodium Citrate (mg)	4.8 – 9.7	9.6 – 19.4	24.0 – 48.5
	Polysorbate 80 (mg) *	0.6 – 0.9	1.2 – 3.8	3.0 – 9.5
	Nicotinamide (mg)	3.0 – 9.0	6.0 – 18.0	15.0 – 45.0
	Volume Water for Injections to (mL)	**1.0**	**2.0**	**5.0**
Thrombin Solution	**Active Ingredients**:	**1 mL**	**2 mL**	**5 mL**
	Thrombin, human (IU)	500	1000	2500
	Calcium Chloride (µmol)	40	80	200
	Excipients:			
	Sodium Chloride (mg)	3.5 – 5.5	7.0 – 11.0	17.5 – 27.5
	Protein (mg) (by addition of Human Albumin			

	≥ 35 mg/ mL)	45 – 55	90 – 110	225 – 275
	Volume: Water for Injections to (mL)	**1.0**	**2.0**	**5.0**
	Combined Volume	**2.0**	**4.0**	**10.0**

* tested on the Drug Substance level

Shelf Life

Deep frozen TISSEEL has a shelf life of two years at temperatures < -20°C. The expiry date is stated on the final container and the package.
The thawed solutions may be used within 72 hours when stored at or below 25 °C in the unopened, undamaged sterile pack. After thawing, the solutions must not be refrozen! The TISSEEL solutions contain no antimicrobial agent. TISSEEL is intended for single use in one patient only and unused solution in the syringe should be discarded.

Special Precautions for Storage

Store in a freezer (**at -18°C or colder**). The cold storage chain must not be interrupted until use.

Keep container in the outer carton to protect from light.

Keep out of reach and sight of children.

For single use only. Do not re-sterilise!

Set of Devices

Each pack TISSEEL contains a double-sterile set of devices (DUO SET) consisting of one syringe double-plunger, two Y-pieces and four application cannulas. These devices are used for the simultaneous application of the fibrin sealant components. For details on application and complications associated therewith see DOSAGE AND ADMINISTRATION section, heading Operating Instructions using double-chamber syringe, double-plunger, Y-Piece and application cannulas.

The set of devices is sterile and non-pyrogenic in unopened and undamaged package. Sterilised by exposure to ethylene oxide.

POISON SCHEDULE

Unscheduled.

NAME AND ADDRESS OF THE SPONSORS

TISSEEL, Two component Fibrin Sealant, deep frozen, Vapour Heated (VH) and Solvent Detergent (S/D) treated, is manufactured by Baxter AG, Vienna, Austria, and supplied in Australia by:

Baxter Healthcare Pty Ltd
1 Baxter Drive
Old Toongabbie, NSW 2146. Ph: 9848 1111, Fax: 9848 1123

Date of TGA approval: 15 March 2010

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