Journal of Minimally Invasive Gynecology (2007) 14, 633–637

THE JOURNAL OF
MINIMALLY INVASIVE
GYNECOLOGY

# Phase I/II prospective trial of autologous platelet tissue graft in gynecologic surgery

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**KEYWORDS:**
Autologous platelet tissue graft;
Gynecologic surgery;
Pain

**Abstract**

**STUDY OBJECTIVE:** To perform the original phase I/II trial of autologous platelet tissue graft in gynecologic surgery to evaluate toxicity and efficacy on decreasing pain.

**DESIGN:** Prospective nonrandomized trial (Canadian Task Force classification II-A).

**SETTING:** Teaching hospital.

**PATIENTS:** Fifty-five consecutive patients undergoing major gynecologic surgery were entered into this prospective phase I/II trial and were matched with 55 patients from the previous 6 months.

**INTERVENTION:** After anesthesia was induced, peripheral venous blood (55 mL) was obtained from the patient producing, autologous platelet tissue graft (20 mL). At completion of surgery, autologous platelet tissue graft was directly applied to the surgical site.

**MEASUREMENTS AND MAIN RESULTS:** Median pain on the day of surgery was 2.7 (mild) in the autologous platelet tissue graft group vs 6.7 (severe) in the control group, $p < .001$. Likewise, pain on postoperative day 1 was 2.1 (mild) in the autologous platelet tissue graft group vs 5.5 (moderate) in the control group, $p \leq .001$. Median of morphine per hospital stay for the autologous platelet tissue graft group was 17 mg (range 1–98 mg) vs 26 mg (range 3–90 mg) in the control group, which was statistically significant at $p = .02$. There were no adverse effects associated with autologous platelet tissue graft.

**CONCLUSIONS:** In the original phase I/II prospective trial of autologous platelet tissue graft in gynecologic surgery, there were no apparent adverse effects, and pain was significantly reduced.
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More than 350 000 women undergo major gynecologic surgery in the United States each year. Major morbidity associated with gynecologic surgery includes pain, bleeding, infection, hernia, and wound dehiscence. Reduction in the incidence of these morbidities would improve patient care and decrease medical expense.

Recently, several autologous platelet-rich plasma preparations, including autologous platelet tissue graft (BioMet Biologics, Warsaw, IN), have been used clinically to enhance hemostasis and wound healing.[1,2] Application of autologous platelet tissue graft to the operative site has a 3-fold effect (Figure 1). First, the concentrated platelets form a fibrin clot that aids in hemostasis. Second, the platelets degranulate and release numerous chemotactic and mitogenic growth factors (including platelet-derived growth

Supported in part by a research grant funded by BioMet Biologics. There was no direct funding of this trial to Summa by BioMet Biologics. BioMet Biologics has provided funding to Summa for a swine postoperative adhesion study.

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Submitted March 27, 2007. Accepted for publication May 18, 2007.

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doi:10.1016/j.jmig.2007.05.014

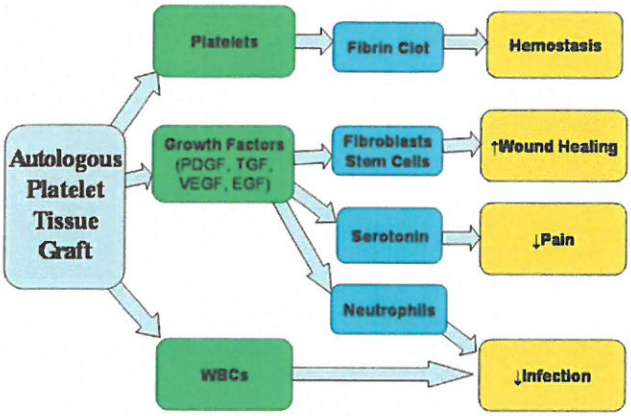


**Figure 1** Autologous platelet tissue graft: mechanism of action. EGF = epidermal growth factor; PDGF = platelet-derived growth factor; TGF = transforming growth factor; VEGF = vascular endothelial growth factor; WBC = white blood cells.

factor, transforming growth factor, vascular endothelial growth factor, and epidermal growth factor), which aid in wound healing and decrease infection and pain. Third, increased white blood cell (WBC) concentration also aids in decreasing infection. Recent studies have shown that autologous platelet tissue graft produced by the Gravitational Platelet Separation system (BioMet Biologics) contains an 8-fold increase in platelet count, 5-fold increase in white blood cell count, a 4- to 6-fold increase in growth factors, and a 7-fold increase in serotonin[3,4] compared with serum levels.

Because autologous platelet tissue graft is obtained in the operating room and immediately directly applied to the patient's own operative site, adverse effects should be minimal or nonexistent. Because of the small risk of adverse effects and encouraging initial empiric results, the use of autologous platelet tissue graft is increasing.[1,2] However, the clinical effectiveness and safety in gynecologic surgery have not been proven. In general, there are 3 phases of clinical testing of new therapeutic agents.[5] Phase I trials are designed to evaluate toxicity. Phase II trials determine efficacy. If phase I/II trials show safety and efficacy, a phase III trial is performed to determine whether the new treatment is more effective or safer than standard therapy. Therefore our purpose was to perform the original phase I/II trial of autologous platelet tissue graft in gynecologic surgery to evaluate toxicity and efficacy on pain.

## Materials and methods

### Eligibility

Patients undergoing major gynecologic surgery (laparoscopic-assisted vaginal hysterectomy, laparoscopic-assisted vaginal hysterectomy with laparoscopic lymphadenectomy, abdominal hysterectomy, advanced laparoscopic procedures, and advanced urogynecologic procedures requiring multiple repairs), aged 18 years or older, nonpregnant, and able to give informed consent comprised the study group. Institutional review board approval was granted, and written informed consent was obtained from all patients.

### Patient characteristics

From August 5, 2005, through February 6, 2006, 55 consecutive patients were entered into this phase I/II trial, and none were excluded. Two patients declined to enter the trial. Patients were recruited from 3 physician offices—1 private gynecology group, 1 academic urogynecologist, and 1 academic gynecologic oncologist. Patient characteristics are presented in Table 1. Median age was 55 years old (24–84 years old), median weight was 167 lbs (112–260 lbs), and 78% had medical comorbidities. Twenty-five percent of surgical procedures were laparoscopic-assisted vaginal hysterectomy, 20% were extended urogynecologic procedures, and 22% were laparoscopic gynecologic oncology procedures.

### Comparison group

Fifty-five patients from the previous 6 months were matched by surgeon and surgical procedure. As can be seen from patient characteristics in Table 1, there was no difference between the groups in age, weight, race, medical comorbidities, or types of surgery. We believed this comparison group was justified because both groups had the same prospective pain evaluation by the same nursing staff who were blinded to the study's objectives. Also, the same preprinted postoperative pain management protocol was used for both groups.

**Table 1** Patient characteristics

| | APTG | Control | p |
|---|---|---|---|
| Age (yrs) | 55 (24–84) | 49 (27–76) | .06 |
| Weight (lbs) | 167 (112–260) | 160 (67–250) | .13 |
| Race | | | .90 |
| White | 87% | 80% | |
| Black | 13% | 20% | |
| Comorbidity | 78% | 63% | .10 |
| Procedure (No., %) | | | .10 |
| LAVH | 14 (25) | 14 (25) | |
| LAVH nodes | 12 (22) | 10 (18) | |
| Scope | 4 (7) | 4 (7) | |
| TAH | 14 (25) | 12 (22) | |
| Urogyn | 11 (20) | 15 (27) | |

APTG = autologous platelet tissue graft; LAVH = laparoscopic-assisted vaginal hysterectomy; LAVH nodes = LAVH with lymphadenectomy; Scope = advanced laparoscopic procedure; TAH = abdominal hysterectomy; Urogyn = advanced urogynecologic procedure.

## Technique of autologous platelet tissue graft (BioMet Biologics)

After induction of general anesthesia, peripheral venous blood (55 mL) was obtained from the patient and mixed with acid-citrate-dextrose-A 5 mL, an anticoagulant, in a 60-mL syringe. The syringe contents were transferred to the Gravitational Platelet Separation system disposable separation tube (GPSII; BioMet Biologics) (Figure 2) and spun in a BioMet centrifuge for 15 minutes at 3200 rpm. After centrifugation, 2 aliquots of 7 mL of platelet-poor plasma and 3 mL of platelet concentrate (platelets and WBCs) were obtained and transferred to two 10-mL syringes. An activation solution was prepared by mixing 1000 units of topical bovine thrombin (Jones Pharma, St. Louis, MO) per milliliter of 10% $CaCl_2$ solution. The thrombin directly activates platelets, and the $CaCL_2$ deactivates the acid-citrate-dextrose-A anticoagulant. The activation solution was drawn into two 1-mL syringes. The 10-mL platelet-poor plasma/platelet concentrate syringe and the 1-mL activation syringe were connected, in tandem, to the BioMet Malleable Dual Cannula Tip, a dual-spray apparatus (Figure 3). This allows both syringe plungers to be advanced in unison, mixing the 2 sprays, which allows activation before reaching the wound bed. At completion of the surgical procedure and after ensuring adequate hemostasis, the autologous platelet tissue graft was directly applied to the surgical site (including the vaginal cuff, parametrium, and fascia). Depending on the surgical procedure, autologous platelet tissue graft was applied vaginally, laparoscopically, or transabdominally (Figure 4). Surgical application takes approximately 1 minute.

**Figure 2** Gravitational Platelet Separation (GPSII) tube.

**Figure 3** BioMet Malleable Duel Cannula Tip.

## Data collection

All hospital data were collected prospectively before discharge. Patients were followed up for 28 days with outpatient checkups scheduled for the seventh and twenty-eighth postoperative day. Pain was evaluated with the visual analog scoring system[6]: 0 = no pain, 2 = mild, 5 = moderate, 7 = severe, 10 = excruciating. At Summa, visual analog scoring is prospectively performed and recorded every 4 hours by the nursing staff. The highest visual analog score on postoperative day 0 and postoperative day 1 was used. Preprinted postoperative pain management protocol was the same for both groups: ketorolac 30 mg intravenously every 6 hours for 4 doses, morphine 2 to 5 mg intravenously every 2 hours as needed, and oxycodone/acetaminophen 5/325 mg 1 to 2 tablets orally every 6 hours as needed. For the few patients that required meperi-

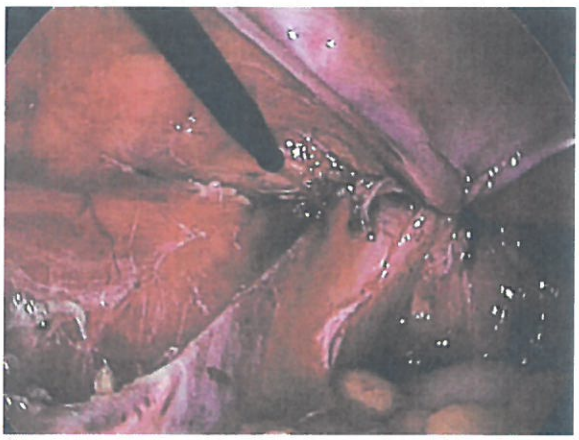

**Figure 4** Laparoscopic application of autologous platelet tissue graft.

dine, a conversion of meperidine 75 mg to morphine 10 mg was used. The oxycodone/acetaminophen to morphine conversion was 2 tablets to 4 mg of morphine. For the comparison group, hospital data were collected, as well as outpatient follow-up for 28 days. Because the control group was not prospectively evaluated after hospital discharge, accurate visual analog scores for pain on postoperative day 7 and 28 are not available.

## Statistics

Discreet variables were compared by use of $\chi^2$ or Fisher's exact test, and continuous variables were compared by $t$ testing. A power analysis was performed with a p value = .05, power of .80 and a 25% difference in pain as being medically significant. Sample size calculation estimated a required sample size of 35 patients per group.

## Results

Results are shown in Table 2. Median blood loss was 270 mL (10–500 mL), and median operative time was 163 minutes (75–330 minutes). Median change of postoperative hemoglobin was 1.8 g, and median length of stay was 1.3 days. Comparing these results with the 55 matched control subjects (Table 2); there was no statistically significant difference in blood loss, operative time, median change in hemoglobin, or length of stay.

Comparing patients treated with autologous platelet tissue graft to matched controls, postoperative pain was significantly reduced. Median pain on the day of surgery was 2.7 (mild) in the autologous platelet tissue graft group vs 6.7 (severe) in the control group, p <.001. Likewise, pain on postoperative day 1 was 2.1 (mild) in the autologous platelet tissue graft group versus 5.5 (moderate) in the control group, p ≤.001. Median pain in the autologous platelet tissue graft group was 0.3 on postoperative day 7 and 0 on postoperative day 28. The median dose of morphine per hospital stay for the autologous platelet tissue graft group was 17 mg (1–98 mg) vs 26 mg (3–90 mg) in the control group, which was statistically significant at p = .02.

**Table 2** Surgical characteristics

| | APTG | Control | p |
|---|---|---|---|
| Blood loss | 270 (100–1500) | 272 (50–1000) | .48 |
| OR Time (min) | 163 (75–330) | 143 (40–360) | .06 |
| Δ Hgb (g/dL) | 1.8 | 2.0 | .16 |
| LOS (days) | 1.3 (1–4) | 1.4 (1–5) | .17 |
| Pain day 0 | 2.7 (0–7) | 6.8 (1–10) | <.001 |
| Pain day 1 | 2.1 (0–6) | 5.6 (1–10) | <.001 |
| Pain day 7 | 0.3 (0–3) | | |
| Pain day 28 | 0 | | |
| Morphine (mg) | 17 (1–98) | 26 (3–90) | .02 |

APTG = Autologous platelet tissue graft; Δ Hgb = change in hemoglobin level; LOS = length of hospital stay.

No patient treated with autologous platelet tissue graft had a surgical site infection or postoperative dehiscence develop. Because the control group was not prospectively followed up, data on infection and dehiscence was not available. Autologous platelet tissue graft was not associated with any apparent adverse effects.

## Discussion

Autologous platelet tissue graft is being used clinically to enhance hemostasis and wound healing after surgery.[1,2] Concentrated platelets obtained from the patients own blood are applied directly to the operative site. The 8-fold increase in platelet count forms a fibrin clot, which aids in hemostasis[3,4] (Figure 1). When the concentrated platelets degranulate they release numerous chemotactic and mitogenic growth factors (including platelet-derived growth factor, transforming growth factor, vascular endothelial growth factor, and epidermal growth factor), which aid in wound healing, decrease infection, and decrease pain[3,4] (Figure 1). Growth factors have a chemotactic and mitogenic effect on fibroblasts and mesenchymal stem cells, which leads to reepithelialization, angiogenesis, and collagen formation, which accelerates wound healing. The 5-fold increase in white blood cell count along with the chemotactic and mitogenic effect of growth factors on neutrophils may help decrease infection. Also, the growth factor chemotactic effect increases the concentration of serotonin, which helps decrease pain.

Although autologous platelet tissue graft is clinically being used,[3,4] the clinical effectiveness and safety has not been proven, and thus we performed the original phase I/II trial of autologous platelet tissue graft (BioMet Biologics) in gynecologic surgery. None of the 55 patients treated developed a side effect from autologous platelet tissue graft. This was expected because autologous platelet tissue graft is obtained in the operating room from the patient and immediately directly applied to the patient's own operative site. In the phase II portion, we found a significant reduction in pain. Patients treated with autologous platelet tissue grafting had an approximate two-thirds reduction in pain resulting in mild postoperative pain versus moderate to severe postoperative pain in the control subjects (statistically significant, p <.001). Also, morphine use was reduced approximately 50% in the autologous platelet tissue graft group compared with the control subjects, which was statistically significant at p = .02. Because this was a phase I/II trial, blinding was not performed, and therefore placebo effect can not be excluded. Although our study did not have enough power to evaluate efficacy on surgical site infection or postoperative dehiscence, no patients treated with autologous platelet tissue graft developed these complications.

Multiple additional investigations of autologous platelet tissue graft are needed. We are presently evaluating postoperative adhesions in a swine model. In the future, we are planning to evaluate the effects of autologous platelet tissue grafting on

postoperative malignant growth. We are also planning to evaluate autologous platelet tissue grafting as a drug delivery system. Finally, because we performed a phase I/II trail, there was no control group. Therefore we are presently performing a phase III prospective randomized controlled trial on autologous platelet tissue graft in cesarean sections.

## Conclusion

In the original phase I/II prospective trial of autologous platelet tissue graft in gynecologic surgery, there were no adverse effects, and pain was significantly reduced. We are presently developing a phase III prospective placebo-controlled randomized trial to verify the results of our phase I/II trial, evaluate placebo effect and to evaluate the efficacy of autologous platelet tissue graft on surgical site infection and postoperative dehiscence.

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# Is the Use of Autologous Platelet-Rich Plasma Gels in Gynecologic, Cardiac, and General, Reconstructive Surgery Beneficial?

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**Abstract:** Tissue repair at wound sites begins with clot formation, and subsequently platelet degranulation with the release of platelet growth factors, which are necessary and well-regulated processes to achieve wound healing. Platelet-derived growth factors are biologically active substances that enhance tissue repair mechanisms, such as chemotaxis, cell proliferation, angiogenesis, extracellular matrix deposition, and remodeling. This review describes the biological background and results on the topical use of autologous platelet-rich plasma and platelet gel in gynecologic, cardiac, and general surgical procedures, including chronic wound management and soft-tissue injuries.

**Keywords:** Platelets, platelet-rich plasma (PRP), platelet gel (PG), growth factors, wound healing, cardiac surgery, gynecology, general surgery, adipose graft.

## 1.INTRODUCTION

The physiologic cascades of soft tissue wound healing and bone growth are only partially clarified, but it is clear that cellular and hormonal factors have fundamental roles in these processes [1]. In particular, platelet-derived growth factors (PDGFs), which are stored in various platelets vesicles, are pivotal healing factors [2, 3]. Currently, platelets can be retrieved and isolated from a volume of fresh, autologous whole blood with point-of-care devices which intraoperatively fractionate the blood into platelet-poor plasma (PPP), platelet-rich plasma (PRP), red blood cells, and other biological mediators [4]. In most cases, the PRP is a so-called buffy coat product and therefore consists of a small volume of plasma in most instances, in which the number of platelets and leucocytes in excess of baseline values can be measured. In general, PRP is a term used to describe a variety of techniques to produce blood components, which are enriched in platelets with the growth factors contained therein. The term PRP is a bit of a misnomer since the end product is not always a plasma fraction, but can also be a gel.

Most of the platelet growth factors are stored in the alpha granules of platelets and are inactive upon platelet activation. Platelet aggregation and activation can be accomplished with platelet agonists (e.g., thrombin, calcium, or other proteins) to create a viscous solution frequently termed platelet gel (PG). This platelet coagulum can be applied exogenously as a spray or as a solid gelatinous mass to soft tissues, chronic wounds, bone, or synthetic bone. The reason for applying PG to tissues is the delivery of platelet growth factors and other biological mediators (e.g., adhesive proteins, fibrinogen, fibronectin, vitronectin, and thrombospondin-1) to mimic and accelerate physiologic wound healing and regenerative tissue repair processes [5, 6]. This article reviews the use of PG in gynecologic, cardiac, and general and reconstructive surgery.

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## 2. PREPARATION OF PRP AND MECHANISMS OF ACTION

### 2.1. PRP Preparation and Growth Factor Release

In our institution, blood is drawn in the patient holding area or in the operating room prior to the induction of anesthesia, depending on the type of surgery. To draw blood, a venous infusion catheter is placed in the patient's antecubital vein. Blood is collected in syringes or blood bags containing an anticoagulant to prevent the blood from clotting. Thereafter, the pre-donated blood is sequestered with point-of care devices, including blood cell savers/separators or table top devices, to produce PRP. In our opinion, the preparation of PRP by blood banks through discontinuous plasmapheresis methods should be limited because of higher production costs and delayed availability of PRP compared to bedside devices. Furthermore, blood bank–prepared PRP is not accessible by the clinician, and demands a highly controlled logistic system to avoid product mismatch before administration to the patient.

With cell savers/separators, larger pre-donation blood volumes (150 to > 500 mL of whole blood) can be obtained, resulting in a PRP volume ranging from 15 to > 50 mL. Tabletop centrifuges have been used to manufacture smaller volumes of PRP from lesser amounts of whole blood (50–150 mL). The choice for system is mainly dependent on the type of surgical procedure, and thus the anticipated amount of PRP to be produced.

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PRP is stored in the patient's operating room at room temperature on a shaker until use. The PRP is placed in the surgical field in a sterile specimen cup when appropriate during surgery. To release platelet growth factors, the PRP needs to be activated. When platelet activators, such as thrombin, interact with PRP, a sticky platelet gel will be formed. At this stage, the semi-viscous PG can be applied to wounds or used during surgical wound closure.

### 2.2. Mechanisms of Action

Tissue repair and surgical wound healing are well-orchestrated, and a complex series of events involving cell–cell and cell–matrix interactions in which platelet growth factors serve as messengers to regulate various regenerative processes.

Initially, tissue repair begins with activation of the coagulation cascade, platelet clot formation, platelet aggregation, and degranulation. During this degranulation period, the platelets release a pool of biologically active proteins (PDGFs) and other substances into the extracellular milieu. In this environment, the biologically active proteins might bind to specific platelet growth factor receptors present in surgical tissues. Released growth factors interact and bind with the platelet tyrosine kinase receptor (TKR), which is present in the cell membranes of tissue cells (ligand-receptor interaction) [10]. Therefore, the actual binding site is on the outer surface of the cell membrane, and thus not directly on the cell nucleus. The TKR is a membrane spanning protein that extends into the cytoplasm of cells. After the platelet growth factor interacts with the external part of the TKR, activation of inactive messenger proteins occurs in the cytoplasm. Thereafter, the messenger proteins become activated and bind to the TKR cytoplasmic tail. Activated proteins are generated via an active signaling cascade in the cell nucleus where the genes responsible for control of cell division are triggered. Thus, transcription of messenger RNA is induced, producing a biological response that starts cascades, which in turn provoke tissue repair and tissue regeneration [11, 12].

### 2.3. Platelet Growth Factors in PRP

A variety of platelet growth factors are located in the alpha granules of platelets present in the PRP. Some of the most relevant platelet growth factors and their specific characteristics are summarized in Table **1**.

Platelet-derived growth factor was one of the first growth factors to be identified in platelets. Subsequently, additional platelet growth factors have been identified, including transforming growth factor (TGF)-α and -ß, fibroblast growth factor (FGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), vascular endothelial growth factor (VGEF), and connective tissue growth factor (CTGF) [13,14]. The platelet growth factors have their own specific function and biological activity. Cell studies in wound care have shown that multiple growth factors tend to be more effective than the use of a single growth factor [15]. The concept of harvesting and concentrating autologous platelets with subsequent transfer and fixation to the wound site within the medium of an autologous soft tissue graft allows access to higher concentrations of multiple growth factors placed directly into the wound site [16].

### 2.4. Infection Prevention

In addition to the PG delivery of growth factors, limited data are available that deal with the role of leucocytes present in PLG to act as an antimicrobial component. We and others have reported that buffy coat-prepared PRP not only comprises a high concentration of platelets containing platelet growth factors, but that it is also rich in concentrated leukocytes, in particular neutrophilic granulocytes, monocytes, and lymphocytes [17,18]. Neutrophilic granulocytes and monocytes contain numerous granules full of myeloperoxidase, which catalyzes the oxidation of chloride to generate hypochlorous acid and other reactive oxygen derivates that act as potent bactericidal oxidants toxic to micro-organisms and fungi [19, 20]. Furthermore, Yeaman *et al.* [21] and Tang *et al.* [22] have maintained the idea that platelets are also involved in microbicidal activity, suggesting that platelets take part in the platelet host defense mechanism by releasing a variety of platelet microbicidal proteins. The platelet microbicidal proteins have been shown to be released after platelet activation, demonstrating potent activities against pathogens that have a tendency to enter the bloodstream [23].

### 2.5. Wound Healing

During the initial days of wound healing, an inflammatory process is initiated by migration of neutrophils, and subsequently macrophages, to the wound site. In turn, activated macrophages release multiple growth factors, including platelet-derived growth factor, TGF-α and -β, interleukin-1, and FGF [24]. Angiogenesis and fibroplasia start shortly after day 3, followed by collagen synthesis on days 3-5. This process leads to an early increase in wound-breaking strength, which is the most important wound-healing parameter of surgical wounds, followed by epithelialization and the ultimate remodeling process leading to a tissue scar [25].

Based on the actions of the various platelet growth factors during the different stages in the wound healing cascade, the use of autologous PG to stimulate wound tissue repair and tissue regeneration is an interesting proposition. Clinicians have also used recombinant growth factor to stimulate wound healing [26]. However, as compared with recombinant single growth factor applications, PG has the advantage of being autologous. In addition, in PG the multiple platelet growth factors and other biological and adhesive proteins work together synergistically and promote mitogenesis of mesenchymal stem cells and growth factors at the surgical wound site, and therefore have the potential to accelerate and boost tissue healing [27].

## 3. PLATELET-RICH PLASMA GELS IN GYNECOLOGIC, CARDIAC, AND GENERAL AND RECONSTRUCTIVE SURGERY

### 3.1. Methods

Few articles are published on the use of autologous platelet growth factor applications to support wound healing, tissue regeneration, or tissue growth in gynecologic and cardiac surgery. Therefore, we performed a review of the literature, as recommended by the Cochrane Collaboration with studies

**Table 1. Sources of Growth Factors ad their Biological Actions on Wounds**

| Platelet Growth Factor Type | Growth Factor Source | Biological Actions |
|---|---|---|
| Platelet Derived Growth Factor, PDGF(a-b) | Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells | Mitogenetic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/ smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis |
| Transforming Growth Factor TGF (α-β) | Platelets, extracellular matrix of bone , cartilage matrix, activated $TH_1$ cells and natural killer cells, macrophages/ monocytes and neutrophils | Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte proliferation |
| Vascular endothelial growth factor, VEGF | Platelets, endothelial cells | Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells |
| Epidermal Growth Factor, EGF | Platelets, macrophages, monocytes | Stimulates endothelial chemotaxis/ angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis |
| Fibroblast Growth Factor, FGF | Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts | Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenetic for mesenchymal cells, chondrocytes and osteoblasts |
| Connective tissue growth factor CTGF | Platelets through endocytosis from extracellular environment in bone marrow. | Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion |
| Insulin-like growth factor-1 IGF-1 | Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells,osteoblasts, bone matrix | Chemotactic for fibroblasts and stimulates protein synthesis. Enhances bone formation by proliferation and differentiation of osteoblasts |

N.B. PRP also contains other proteins like fibrin, fibronectin, vitronectin, serotonin and thrombospondin, which are known to act as cell adhesion molecules, important for the migration of osteoblasts, fibroblasts, and epithelial cells [11].

published and indexed in the Cochrane, Embase, and PubMed databases until April 2010. The following criteria were established for the selection and inclusion of articles: clinical trials, complete articles, and articles published in international and national journals indexed in the databases mentioned above. Exclusion criteria were articles, editorials, and letters published in abstract form. Only sources available in English were used. The databases were searched for literature on PRP using the keywords platelet-rich plasma, PRP, platelet releasate, platelet gel, platelet concentrate, platelet-derived growth factor, gynecology, gynecologic surgery, and cardiac surgery.

The search for articles on PRP use in general surgery was more complex because this is a very diverse medical specialty, including many sub-specialties, like vascular surgery, laparoscopic surgery, oncologic surgery, and wound care management. For these reasons, we searched for appropriate studies using the same methodology as described above, with the exception of the use of PRP in wound care management. Data on diabetic foot ulcers, neuropathic foot ulcers, and chronic diabetic foot ulcers treated with PRP-like products are being presented as a summary and conclusion of existing systematic reviews from the last 5 years.

### 3.2. Gynecology

In the gynecology literature, we could only retrieve three relevant articles and one case report on the use of autologous prepared PRP, PG, or recombinant platelet growth factors on wound healing and tissue regeneration.

Fanning et al performed a prospective, non-randomized trial in 55 consecutive patients undergoing major gynecologic surgery [28]. The treated patients were compared with a control group consisting of 55 matched patients from the previous 6 months by surgeon and surgical procedure. They conducted a phase I/II trial of autologous platelet tissue graft in gynecologic surgery to evaluate toxicity and efficacy on decreasing pain. There were no adverse effects recorded in this study related to the application of the autologous platelet tissue graft. Median pain on the day of surgery and on postoperative day 1 was significantly less in the autologous platelet tissue graft group. Likewise, the median use of morphine per hospital stay was significant for the autologous platelet tissue graft-treated patients.

Shackelford *et al.* conducted a double-blind, randomized, placebo-controlled trial using topical recombinant human PDGF gel after abdominal wound separation [29]. They used the recombinant growth factor to treat the wound and studied the effects on wound healing. The patients in the placebo group closed 54 +/- 26 days post-operatively, whereas the wounds of patients in the treatment group closed in 35 +/- 15 days (P =.05). The preliminary study suggests that the topical application of 0.01% recombinant human PDGF gel accelerates healing of separated surgical wound significantly, as determined by Kaplan-Meier analysis.

In an *in-vitro* study, PRP was used to assess its ability to seal an iatrogenic fetal membrane defect [30]. If during pregnancy these membrane defects do not seal spontaneously, it is most likely that fluid leakage through the vagina may occur, resulting in infections and pregnancy loss.

The authors evaluated the sealing capability of PRP plugs in an *in vitro* model that mimics a fetoscopic membrane defect. Furthermore, the effect of PRP on membrane repair and cell proliferation in monolayer cell cultures and amnion-chorion tissue explants was determined. The fetal membranes were obtained from uncomplicated singleton pregnancies undergoing elective caesarean section, and PRP was obtained from healthy volunteers and produced by laboratory techniques, although we could not clearly elucidate a true definition of the PRP plugs used in the study. The results showed that PRP plugs persisted in amniotic fluid for a median of 7 weeks; they also demonstrated waterproof sealing of a fetoscopic membrane defect. In addition, PRP stimulated cell proliferation in a monolayer cell culture and resulted in a good matrix for cell proliferation and migration in amnion-chorion tissue explants. Sipurzynski-Budrass *et al.* published a case report in which a pregnant woman with ruptured membranes after genetic amniocentesis in the 16th gestational week was successfully treated with a platelet concentrate [31]. Complete closure of the rupture was realized 10 days after placement of the platelet plug.

Several endometrial tissue remodeling studies with the involvement of PDGFs have been performed [32]. In a study of Matsumoto *et al.* the effects of PDGF isoforms (PDGF-AA, PDGF-AB, and PDGF-BB) on the proliferation, motility, invasiveness, and gel contractility of cultured human endometrial stromal cells (ESC) were studied in well-established *in-vitro* models [33].

### 3.3. Cardiac Surgery

#### *3.3.1. Blood Component Platelet Plasmapheresis*

Peri-operative platelet-rich plasmapheresis with cell-saver devices has been used for decades in cardiac surgery in order to control bleeding. The rationale for employing these blood sequestration techniques has been that coagulopathy after cardiopulmonary bypass (CPB) and platelet dysfunction are the most common causes of non-surgical bleeding. Repeatedly, peri-operative transfusion of both allogeneic red blood cells and platelet concentrates are used to overcome these life-threatening situations. Pre-CPB whole blood sequestration, in order to produce autologous blood components (PRP, PPP, and red blood cell concentrate) is thought to be one of the potential solutions to the problem of bleeding and blood product transfusions in cardiac surgery. A meta-analysis of clinical outcomes and costs has been well-described by Mahoney in 1998 [34]. The concept of removing platelets from a patient immediately before CPB, thereby potentially sparing platelets due to the avoidance of these platelets with foreign surface materials of the extracorporeal circuit, followed by post-CPB platelet re-infusion, seems to be a reasonable approach to the problem of post-CPB platelet dysfunction and bleeding.

Carless *et al.*, in a Cochrane systematic review, suggested that PRP infusion therapy in general is effective in reducing allogeneic RBC transfusion in adult patients undergoing elective surgery [35]. However, there was considerable heterogenecity in treatment effects and the trials were of poor methodological quality. Their conclusion was therefore that the available studies provided inadequate data for firm conclusions with respect to the impact of PRP infusions on clinically important endpoints.

#### *3.3.2. Platelet Gel and Platelet-Poor Plasma Application*

The production of these fresh blood components at point of care was the starting point to apply activated PRP (i.e., PG) as an element of a blood management program in cardiac surgery on wound tissues to affect bleeding and contribute to improved wound healing and prevention of infection in select patients.

In cardiac surgery, PG is only used after CPB and antagonization of the systemic heparin effects in order to have it stick to the tissues. PG is applied on the cut sternum when both sternum sites are re-approximated and tightened, using either a single syringe technique or a dual spray tip catheter. Thereafter, PG can be subcutaneously applied prior to skin closure. Eventually, the vein harvesting donor site of the leg can also be injected with PG.

#### *3.3.3. Clinical Studies*

The efficacy and safety of PG and PPP use during sternal and saphenous vein harvest site closure was recently addressed in a retrospective analysis by Khalafi *et al.* [36]. Forty covariates were collected for each patient to determine the effect on infection and drainage of the sternal and leg wounds. Propensity scoring was used to adjust for baseline imbalance. The application of platelet-rich and platelet-poor plasma significantly reduced the rates of chest wound infection, chest drainage, and leg wound drainage. Additionally, no treatment-related adverse events were recorded. Englert *et al.* were the first to report on the use of autologous PG as a byproduct of platelet-rich plasma sequestration during cardiac surgery [37, 38]. The purpose of their prospective randomized pilot study was to examine the effects of PG on post-operative sternal and leg pain and tissue bruising, comparing the pre- and post-operative situation. A retrospective follow-up study by the same group revealed that the PG treatment group had significantly shorter intensive care unit and total hospital lengths of stay with less post-operative blood loss when compared with control patients. Furthermore, they reported less incisional wound infections in PG-treated patients. Another group also reported less wound infections in patients undergoing cardiac surgery in a large sample (n = 2259) retrospective study following PG applications [39]. The incidence of superficial infection was significantly lower in patients in whom PG was applied compared with non-PG-treated patients and a historical control group. A similar result occurred for deep sternal wound infections, and they concluded that the application of PG in patients undergoing cardiac surgery seems to present a level of protection against infection. An increased resistance to infection, and a significantly better hemostatic wound healing success rate was reported by Gunaydin *et al.* when PG was used in a prospective, randomized study in coronary artery bypass surgical patients [40]. Several studies also mention no effect of PG in cardiac surgical patients. A prospective, dou-

ble-blind study in 44 patients at risk for wound complications at thoracotomy, as well as at the site of saphenous vein harvesting, was conducted using a table top platelet separator [41]. The incidence of major and minor wound complications was not enunciated in either of the groups. Pain sense, blood loss, and intensive care were not significantly either. Intensive care unit stay and in-hospital mortality were also comparable for both groups. In similar studies, the incidence of post-operative wound healing disturbances was studied in PG and control patients. Buchwald and co-workers **also** determined PDGF AB and EGF levels originate from the PG [42]. In this study, wound healing was photographically documented after surgery, and the patients were monitored until the 50$^{th}$ post-operative day to obtain information on wound healing status. During the hospital stay, no statistically significant differences were recorded in the number of hematomas, post-operative leg swelling, or pain level, although large-area hematomas were less frequently observed in the PG group, whereas both PDGF AB and EGF concentrations were significantly higher when compared to whole blood levels. Vang *et al.* also reported no significant data on blood loss and wound bruising, although these parameters scored less in the PG-treated groups on the 2$^{nd}$ post-operative day [43].

The outcomes of multiple studies on the efficacy of PG treatment in cardiac surgery have been published. Proponents of PG application refer to improved wound healing, beneficial effects on pain, blood loss, and bruising. A reduction in the development of severe post-operative wound infections with the application of PG during incisional wound closure has also been reported. These observations indicate that this is a promising technique, with the result that the delivery of autologous platelet growth factors and vital neutrophilic leucocytes is now gaining more popularity. However, randomized controlled studies to support the use of PG in cardiac surgery are mandatory, but it is also difficult to execute such a study because these patients have a number of confounding factors which need to be compensated for, and therefore large patient groups are necessary order to achieve statistical significance [44].

### 3.4. General and Reconstructive Surgery

General surgery focuses on skills in a variety of medical areas, such as the abdomen and its contents, the vascular system, skin, breast, trauma, soft tissues, and reconstruction procedures. Despite large general surgical expertise with PRP-application, few well-documented studies are available in the literature. Therefore, we are limited in addressing the use of PRP gels in general surgery. We will describe PRP/PG applications in inguinal herniorraphies, to treat complications following endovascular surgery and diabetic wound care management. Furthermore, we would like to introduce the bioengineered adipose tissue (BEAT) graft as a recent development of PRP use. This graft is a combination of fat tissue, PRP, and a mixture of calcium chloride with autologous-prepared thrombin. The rationale to employ these grafts is to augment tissue regeneration in vascular-deprived areas (e.g., after radiotherapy following breast cancer), diabetic foot ulcers, and soft tissue deficits.

#### *3.4.1. General Surgery: Inguinal Herniorraphy*

Meshes are frequently used in inguinal herniorraphies and they have dramatically reduced the recurrence rate. However, chronic pain has become the main post-operative complication, probably due to the sutures or staples used to fix the mesh. A glue technique is an alternative for suturing to avoid these complications. De Hingh and co-workers were the first to publish a feasibility study in which they used autologous platelet-rich fibrin (PRF) Fig. (**1**), a prepared product similar to PRP without leucocytes, in order to study its ability to glue the mesh instead of using sutures and staples [45]. They assessed post-operative pain and impaired daily activities. The conclusion of the study was that it is technical feasible to use PRF to fix the mesh, and the visual analogue scale and disability pain scores were lower than they were pre-operatively for all patients with no chronic pain, sensory disorders, or discomfort at long-term follow-up. One patient underwent re-operation due to discomfort. If glue fixation becomes the standard to repair inguinal hernias with mesh, than autologous prepared materials should be further studied in randomized trials, with a focus on direct postoperative pain and costs.

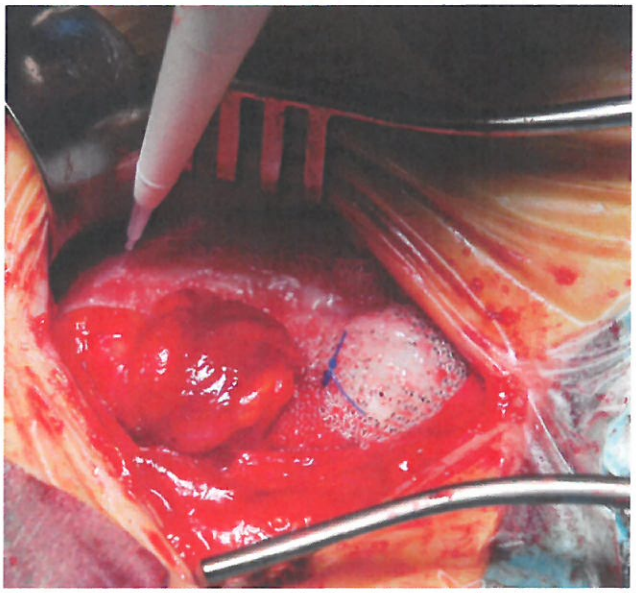

**Fig. (1).** Platelet-rich fibrin (white haze) is sprayed during inguinal hernia repair for mesh fixation. A total of 3 mL is applied.

#### *3.4.2. Vascular Surgery: Endovascular Repair*

Endovascular repair (EVAR) is an alternative technique for open surgical procedures in various vascular fields, including abdominal aortic aneurysms (AAAs) [46]. With EVAR it is necessary to use a percutaneous catheterization technique via the common femoral arteries.

Due to this technique, wound-related complications have been reported, including hematomas, seromas, infections, pseudoaneurysm formation, and arterial bleeding. Saratzis *et al.* conducted a patient- and assessor-blinded controlled trial involving 100 patients undergoing EVAR of AAAs [47]. A subcutaneous injection of autologous, non-activated PRP was injected bilaterally into the subcutaneous tissues during wound closure, and a final volume of PRP was injected per-

cutaneously while closing the skin. Safety and efficacy of PRP injections were evaluated in terms of duration of post-operative hospital stay and wound-related complications.

The post-operative hospitalization was significantly lower in the PRP-treated patient groups. The overall surgical wound–related complications rate was also significantly lower in the PRP group. In addition to this, the complications observed in the control group were of greater extent and severity than in the PRP group.

### *3.4.3. Vascular-Reconstructive Surgery: Chronic Wound Management*

Diabetic foot ulcers represent a major medical, social, and economic problem in many countries [48]. Approximately 15% of diabetics will develop at least one foot ulcer during their lifetime, and in 5%-8% of diabetics a major amputation is to be expected within 1 year [49]. The triad of vasculopathy, neuropathy, and immunopathy outlines the fundamental point on which the chronicity of diabetic wounds rests. Most of these wounds are typified by increased protease levels, particularly matrix metalloproteinase's (MMPs) and neutrophil elastases. Furthermore, tumor necrosis factor–alpha has been proven to increase the production of MMPs, while hindering the production of tissue inhibitory metalloproteinase [50]. The goal of diabetic foot ulcer treatment is to obtain wound closure as promptly as possible. Accepted standards of care for diabetic foot ulcers include pressure relief in the wound area, appropriate wound management, infection and ischemia management, management of co-morbidities, and wound debridement as needed. Aggressive sharp wound debridement is believed to convert chronic wounds to acute wounds and allows growth factors to function more effectively. This allows the wound to progress through the normal phases of wound healing (inflammatory, proliferative, and remodeling). These phases involve complex paracrine-mediated growth factors which influence mitogenic and cellular differentiation activity. In addition, Cooper *et al.* illustrated that a number of growth factors were strikingly reduced in wound fluids from chronic wounds as compared with acute wounds [51]. Moreover, FGF and TGF-β concentrations are significantly down-regulated in chronic wounds when compared with acute wounds.

Emerging cellular therapies, such as PRP, can have an adjunctive role in the standardized, patient treatment plan. The use of platelet growth factors for the topical treatment of chronic wounds is based on the fact that PRP growth factors aid the three phases of wound healing in the newly created "acute" wound Figs. (**2** and **3**).

Platelet releasates, PRP, and PGs, including multiple growth factors, have been used to treat chronic wounds since 1985 [52]. Since this period, a variety of studies have been published on the application of PRP gels in wound care management.

In 2001, Margolis published a retrospective study analyzing the treatment results of 26,599 patients with diabetic neuropathic foot ulcers who had been treated with an autologous platelet releasate [53]. One of the conclusions was that platelet releasate applications are more effective than standard therapy, and the effect is more pronounced in more severe wounds. Crovetti *et al.* performed a technique based on once-weekly application of PG [54]. They enrolled 24 patients with single or multiple cutaneous ulcers with a different ethio-pathogenesis. In each case, granulation tissue formation increased following the first PG applications, while complete re-epithelization was obtained later. An interesting observation was that pain was reduced in every patient treated with PG.

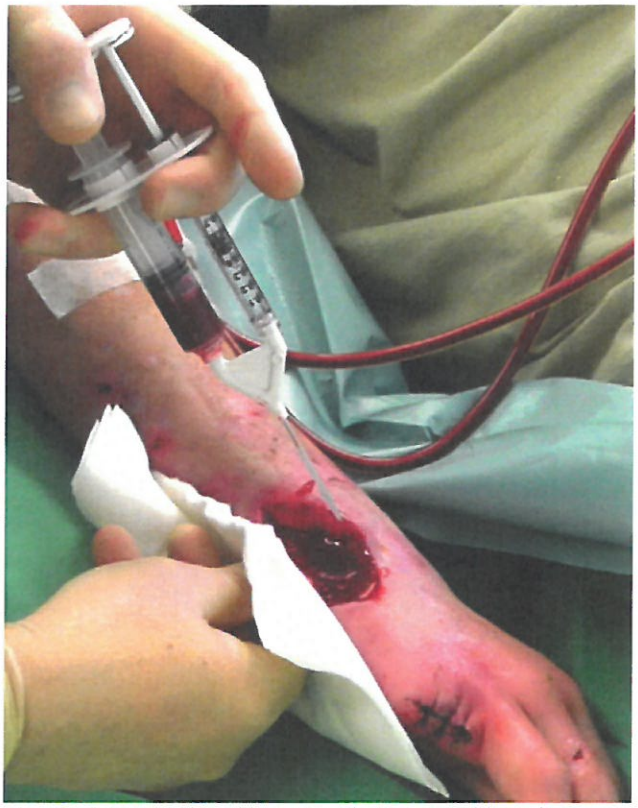

**Fig. (2).** PRP and thrombin are applied by a double syringe technique on a diabetic wound during while the patient is on renal dialysis.

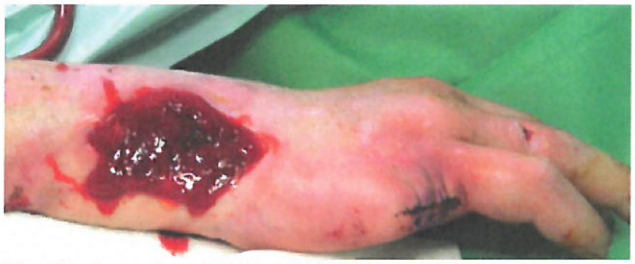

**Fig. (3).** An activated PRP gel cloth is covering the entire wound after application.

A prospective, randomized, controlled, blinded, multicenter clinical study was conducted by Driver and associates to evaluate the efficacy and safety of autologous PRP gel for the treatment of non-healing diabetic foot ulcers [55]. The primary study objective was the proportion of patients with a healed wound. Seventy-two patients were enrolled the study. The proportion of completely healed wounds was significantly higher in the PRP gel group when compared to the control group (81.3% and 42.1% in the PRP gel and control

treatment groups, respectively). Furthermore, no treatment–related adverse effects were noted, indicating safe PRP gel preparation and application. A variety of reviews have addressed the efficacy of autologous platelet growth factor applications in chronic and diabetic wounds [56, 57]. In general, all authors concluded that diabetic foot ulcers are a major health care problem, and that complications of foot ulcers are a leading cause of hospitalization and amputation in diabetic patients. In most of the reviewed papers, it was concluded that there is a rising body of evidence suggesting that wound healing in chronic diabetic foot ulcers is growth factor-dependent, and that the topical therapeutic delivery of these growth factors to wounds has the potential capability to speed up wound healing in combination with conventional wound care. In a systematic review by Villela and Santos, 18 studies were selected, from which 7 (39%) were randomized clinical trials, and 5 of which studied ulcers of diabetic etiology [58]. The results of a meta-analysis showed that PRP favors the healing process (95% CI: 2.94-20.31), demonstrating that there is scientific evidence regarding favorable outcomes of the use of PRP for the treatment of diabetic ulcers.

### *3.4.4. Reconstructive and Plastic Surgery: Adipose Tissue Grafting with PRP*

Despite modern advances in wound care treatment protocols, reconstructive surgical procedures, and cosmetic surgery, there is still a significant requirement for new methods to enhance healing processes, or to restore soft tissue contour defects. Clinically implemented tissue engineering protocols have emerged as a promising alternative to current clinical treatment plans. Many of these new therapies have included the use of human growth factors, with their known biological activities. One of the promising, yet clinically challenging areas of recent therapeutic development involves the injection of adipose tissue derived from a modified lipoculture technique [59]. However, successful fat graft techniques are frequently limited by the sometimes low, and often unpredictable, survival rates. As a consequence, clinicians tend to initially overcorrect fat grafts, and/or perform multiple operations to meet the recipient site volume and contour requirements. In an attempt to increase fat graft survivability, Cervelli and co-workers were the first to report on the enhancement of fat grafting with PRP during *in-vivo* tissue engineering applications [60, 61]. They applied the PRP-fat graft in plastic, reconstructive, and maxillofacial surgical procedures, and as a treatment option in patients with chronic lower extremity ulcers. The authors observed that in 16 of 20 patients with chronic ulcers, re-epithelialization occurred during an average period of 9.7 weeks when PRP was mixed with fat tissue with an improved functional fat graft. According to the authors, this underlying principle for applying PRP to fat tissue grafts is the delivery of autologous platelet growth factors to mimic and accelerate physiologic wound healing and reparative tissue processes. The platelet alpha granules release their growth factors into the extracellular milieu of the fatty tissue. In this environment, the growth factors bind to specific platelet growth factor receptors. Released platelet growth factors interact and bind with the platelet TKR on the cell membranes of fat cells (ligand-receptor interaction). The release of VEGF, TGF-ß, FGF, and IGF has been shown to stimulate human adipose-derived stem cells and human dermal fibroblast proliferation and differentiation [62]. Therefore, PRP might be suitable for clinical cell-based, soft-tissue engineering protocols in order to promote wound healing, when the appropriate ratio between activated PRP and fat tissue was used. Furthermore, Blanton *et al.* suggested that an important component of wound healing was induced by the combination of adipose stem cells and PRP, promoting enhanced vascularization of wound repair in an experimental wound model [63]. Increased VEGF levels were found when adipose stem cells were combined with PRP, contributing to a higher content of arterioles formed in healed wounds, resulting in neovascularization, an important process in the healing of wounds.

Despite the positive effects of PRP on fat survivability, Por and associates reported an animal study in which they did not observe statistically significant changes with regard to weight, volume, and histological parameters when PRP was mixed with fat tissue when compared to a mixture of fat tissue with a saline solution [64]. Closer analysis of the methodology revealed that the final platelet concentration in the fat graft was lower than the circulating whole blood platelet concentration. The therapeutic contribution of platelet growth factors might therefore be questionable.

### *3.4.5. Bio-Engineered Adipose Tissue (BEAT) Graft*

Based on the available results in the literature, and our long-term experience on PRP applications, we decided to develop a modified fat-PRP grafting, the bio-engineered adipose tissue (BEAT) graft [65]. Tumescent fluid infiltration of the donor sites is carried out with xylocaine and epinephrine. Microcannulas are used to harvest the fat tissue. During fat tissue harvesting, low-negative pressure is applied by limiting the plunger movement of a 10 mL syringe to one-half when the cannula is inserted. The syringe will be filled with fat and tumescent fluid, which will be placed in a holder for approximately 20 minutes to separate the fatty tissue by gravity in fluid and oil, which will be removed, leaving fat tissue behind.

Prior to the induction of anesthesia, a volume of anticoagulated whole blood is drawn from the antecubital vein for blood component sequestration. This process is performed manually in order to collect PRP with a high platelet count, approximately 5-6 times the baseline value.

Both, PRP and PPP are collected separately. When the fat harvesting is performed, these blood products are aseptically transferred to the sterile field. At the sterile OR table, PPP is mixed with 10% calcium chloride and placed in a glass Petri dish in order to create a viscous PPP cloth. After 25-40 min, a clot was formed inside the glass dish and compressed manually. The cellular clot debris was left behind on the dish and the liquid fraction containing, the thrombin is then aspirated with a syringe. The separated fat tissue will be transferred in a 60 mL syringe, to which PRP will be added in a ratio of 1:0.5. The mixture is gently, but frequently, agitated for good mixing Fig. (**4**). Prior to graft injection, 3 mL aliquots are drawn from the 60 mL syringe and mixed with 0.15 mL of autologous prepared thrombin in order to induce platelet degranulation shortly after the graft has been injected, as advocated by Kakudo. The activated BEAT graft is

injected in a fan-shaped manner Fig. (5). Rather than focusing on the mature adipocytes, it is our belief that the BEAT graft bioactivation aims to improve fat graft survival rates by stimulating the early ischemic phase with new mature adipocytes from the differentiation of cells under the influence of platelet growth factors by mechanisms, such as neoangiogenesis, neovasculogenesis, and adipogenesis.

**Fig. (4).** PRP is transferred in a syringe containing adipose tissue and then both components are gently but frequently mixed in order to achieve a homogenous graft mass.

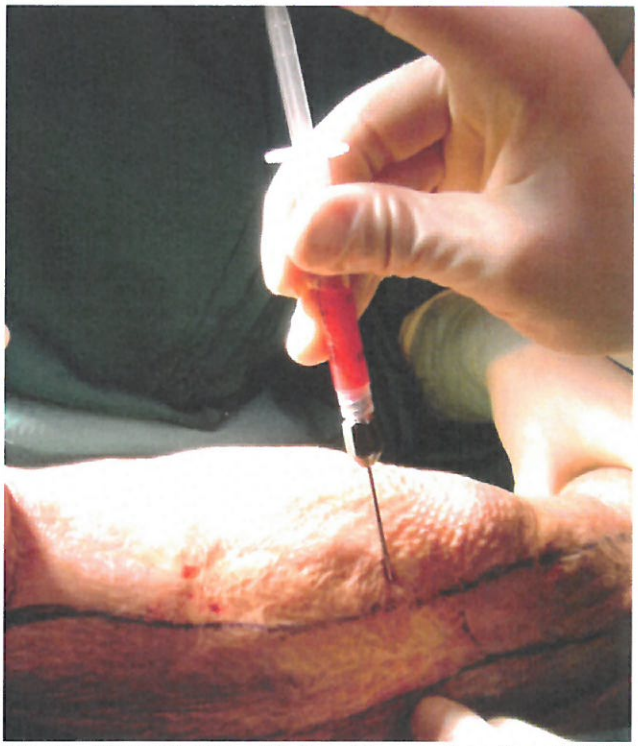

**Fig. (5).** After activation with thrombin a small portion of BEAT graft is injected with a large bore cannula during a reconstructive surgical procedure of a skin grafting procedure of the lower extremity following an accident.

## 4. CONCLUSION

From the literature it is clear that autologous PRP and PG have a wide and safe application within a variety of operative procedures as a tissue regenerative agent. Its application has extended to patients that are prone to higher surgical complications, and to patients suffering from chronic, often diabetic, wounds. The ability of PRP to deliver multiple growth factors with synergistic effects to wound sites is an attractive proposition. Activated PRP will result in a platelet plug, which acts as a barrier to microorganism invasion of wounds, achieved with the help of highly concentrated leukocytes present in the PG if prepared from the buffy coat volume. Platelet growth factors and other platelet cytokines promote mitogenesis of a variety of cells, such as macrophages, other circulating growth factors, and mesenchymal stem cells at wound sites. Ultimately, these mechanisms might boost primary wound healing during surgical wound closure, especially in patients who are at risk for wound healing disturbances, or they might contribute to wound healing in patients with chronic lesions.

However, the overall efficacy of PPR gels in treating wounds is likely to be a function of many variables, such as the platelet concentration of the PRP, PRP preparation device used, platelet activation, application volume to a wound, and the overall health status of the patient.

As a three-dimensional volumetric soft connective tissue replacement, the combination of thrombin-stimulated PRP with fat tissue provides a unique active tissue matrix for cell migration, proliferation, differentiation, and finally, tissue granulation formation.

Until now, few well-designed studies are available in a variety of procedures related to gynecology, cardiac and general surgery. Therefore, more randomized, controlled, blinded, studies on autologous growth factor applications are needed to demonstrate its effects in supportive healing during primary and delayed wound healing. Furthermore, cost-effectiveness studies of PRP therapy benefits are lacking in all medical disciplines.

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**Received:** ??????????? **Revised:** ???????????? **Accepted:** ????????????

INTERNATIONAL JOURNAL OF OBSTETRIC ANESTHESIA 17 (2008) 37–45

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Review Article

# Cell salvage in obstetrics

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*Keywords*:
Cell salvage
Blood salvage
Autologous transfusion
Autotransfusion
Caesarean section
Obstetric haemorrhage
Post-partum haemorrhage

*SUMMARY:* The safety of cell salvage in obstetrics has been questioned because of the presumed risk of precipitating amniotic fluid embolism and, to a lesser extent, maternal alloimmunisation. For these reasons, experience in this field is limited and has lagged far behind that in other surgical specialties. There has, however, been renewed interest in its use over recent years, mainly as a result of problems associated with allogeneic blood transfusion. Our aim was to review the medical literature to ascertain the principles of cell salvage, the ability of the process to remove contaminants, and its safety profile in the obstetric setting. The search engines PubMed and Google Scholar were used and relevant articles and websites hand searched for further references. Existing cell salvage systems differ in their ability to clear contaminants and all require the addition of a leucocyte depletion filter. Although large prospective trials of cell salvage with autotransfusion in obstetrics are lacking, to date, no single serious complication leading to poor maternal outcome has been directly attributed to its use. Cell salvage in obstetrics has been endorsed by several bodies based on current evidence. Current evidence supports the use of cell salvage in obstetrics, which is likely to become increasingly commonplace, but more data are required concerning its clinical use.

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doi:10.1016/j.ijoa.2007.08.001

## Introduction

The report on Confidential Enquiries into Maternal and Child Health (CEMACH) 2000-2002 has shown an increase in mortality due to haemorrhage from 3.3 to 8.5 per million maternities, such that it now exceeds hypertensive disease as the second most common cause of direct maternal death.[1] Furthermore, haemorrhage is often a contributing factor in deaths from other causes. It is estimated that severe haemorrhage occurs in 6.7 per 1000 deliveries[2] and that obstetric haemorrhage accounts for 3-4% of all red cell transfusions in the UK.[3] The ongoing demand for donor blood,[3,4] in the face of a threatened supply, has prompted government recommendations for conserving and using alternatives to donor blood wherever possible.[5] Every effort should therefore be made to reduce blood transfusion requirements. General measures include optimising haemoglobin levels pre-operatively with erythropoietin and iron, the use of antifibrinolytic agents and robust, local blood transfusion guidelines to ensure transfusion is appropriate. In pregnancy, oral and parenteral iron supplementation is probably effective in raising haemoglobin levels but its impact on reducing maternal anaemia[6] and the need for blood transfusion is uncertain. Additionally, dose requirements in both anaemic and non-anaemic mothers and effects of supplementation on fetal and maternal outcomes remain unclear.[7]

In addition to concerns over availability are the well-documented risks associated with allogeneic blood transfusion.[8–12] The greatest risk, as highlighted by the Serious Hazards of Transfusion (SHOT) reports, is that of incorrect blood transfusion due to clerical error in the laboratory or at the bedside. In the 2005 SHOT report,[13] there were 485 (79.6%) incidents of incorrect blood component transfusion, compared with only three confirmed reports of transfusion-transmitted infection. Although transmission of well known agents now represents a very small risk,[13,14] the threat from new or unscreened agents is ever present.[15,16] In particular, the recent probable transmission of variant Creutzfeldt-Jakob Disease (vCJD) in four patients,[17–20] three of whom have since died, highlights the need to develop mandatory donor screening for sub-clinical vCJD.[21] A positive finding on screening risks a significant and adverse impact on donor life insurance. This could deter donors in the future, diminishing the current donor pool and further compounding problems of blood availability and cost currently faced by the National Blood Service (NBS).

When blood transfusion is unavoidable, conserving and using autologous blood wherever possible is increasingly favoured.[22,23] This is mostly due to its improved safety profile over allogeneic blood and ready availability. Although immunological and infective complications may be reduced, the risk of process failures and errors in collection, delivery and administration notably still remain. A recent review by Catling[3] considered the effectiveness of pre-operative autologous donation (PAD), acute normovolaemic haemodilution (ANH) and intra-operative cell salvage (IOCS) as blood conservation tools in obstetrics. PAD, performed only at certain blood processing centres, has been successfully used in the past[24,25] and may be safely achieved without physiological compromise to both mother and baby. It is of limited value, though, as most cases of obstetric haemorrhage require blood transfusion far in excess of that which may be collected pre-operatively and the need can rarely be foreseen, while only a small proportion of patients are suitable. For these reasons, PAD is currently not recommended by CEMACH in obstetrics or by the NBS in most other forms of surgery. ANH can deplete iron stores and cause anaemia. It is only suitable for use in a minority of patients and good evidence regarding its safety and efficacy is lacking.

The use of IOCS however, may be a more effective[3] and life-saving tool in many cases of obstetric haemorrhage.[26] Indeed, the Obstetric Anaesthetist's Association/Association of Anaesthetists of Great Britain and Ireland (OAA/AAGBI) Guidelines for Obstetric Anaesthetic Services published in 2005,[27] state "*an increasing shortage of blood and blood products, and growing anxiety about the use of donor blood, is leading to an increasing interest in the use of cell salvage in obstetrics*". We reviewed the medical literature to ascertain the principles, efficacy and safety of cell salvage in the obstetric setting.

## Methods

The National Library of Medicine literature database accessed via PubMed (www.pubmed.gov), and the internet search engine Google Scholar (http://scholar.google.com/), were searched using the terms "obstetric*", "cesarean", "caesarean" or "pregnancy" AND "blood transfusion, autologous", "autotransfusion" or the text strings "cell saver" or "cell salvage". Relevant articles and websites were hand searched for further references.

## Principles of cell salvage

Autologous erythrocyte salvage, commonly termed "cell salvage," was developed in the 1970s and has become well established in cardiac, vascular and orthopaedic surgery. In this technique, blood shed at the time of surgery is collected and washed, and red cells are returned to the patient as an ongoing process.[28,29] In skilled hands, blood salvage can be quickly set up and the final product returned to the patient within minutes of collection.

In traditional devices, blood is aspirated from the operative site via a dual lumen anticoagulated suction tube, passed through a filter and collected in a reservoir before episodic or non-continuous haemoconcentration and washing in a differential centrifugation bowl. Less dense elements such as plasma, platelets, activated clotting factors and complement are all removed as effluent in the centrifugation and washing process. The quality of salvaged red cells depends on the size and speed of the processing bowls, the volume of saline wash used and the final concentration of red cells, all of which may vary between devices. Red cells from bloody swabs may also be salvaged after placement in sterile, anticoagulated saline. Washed red cells, with a haematocrit of 0.5-0.8, may be re-infused

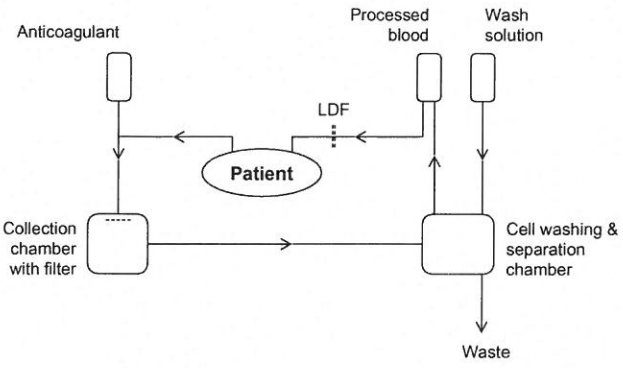


**Fig. 1 – Schematic representation of a blood salvage system. LDF = leucocyte depletion filter.**

immediately or within 6 h of collection. A newer cell salvage device (CATS: Continuous Autotransfusion System, Fresenius AG, Bad Homburg, Germany) washes and separates cells continuously,[30] using a spiral-shaped separation chamber. However, the general principle of collection, anticoagulation, washing/separation and re-infusion are the same (Fig. 1).

With the exception of small amounts of residual heparin, salvaged blood has been shown to be at least equal or physiologically superior to banked blood in terms of red cell osmotic resistance, morphology, pH and levels of 2,3-diphosphoglycerate.[31,32]

Two groups have assessed and compared the performance of these machines.[33,34] They have found differences in the ability to clear certain blood components such as leucocytes, and differences in the rate and mass of red cell recovery. However, these differences may not be clinically relevant.[34] Some currently available systems are listed in Table 1.

The salvage process itself is cost-effective, as the disposable items required cost less than a single unit of banked blood (currently £134 plus laboratory costs in the UK [NBS; personal communication]), regardless of the number of units salvaged, and there is a reduction in both the length of hospital stay[35] and the amount of allogeneic blood used.[36,37] An important practical limitation of its use however, is the need for adequately trained personnel who, ideally, should be available to provide 24-h emergency cover. Obtaining time for training may be difficult and costly and not viewed as a priority by some hospitals, and at present, few anaesthetists and operating department practitioners use cell salvage routinely. Although in experienced hands the device can be set up quickly and little intervention is required thereafter, its use should not unduly distract from care of the mother. Other considerations when using cell salvage are the initial high capital cost of the equipment and the risk of error during use.[38]

A particular advantage of the cell salvage apparatus is that it can be set up in continuity with the patient's own circulation, thus increasing its acceptability to Jehovah's Witnesses.[39,40] This has current relevance since two of the 17 maternal deaths due to haemorrhage listed in the CEMACH report occurred in patients who refused allogeneic blood transfusion.[1]

### *Complications*

Early problems of cell salvage in the general population included the development of coagulopathy, haemolysis and renal failure.[41,42] Following technological advances, more recent concerns have been the potential for precipitating embolic sequelae from the re-infusion product[43] and disseminating infection.[44] Autotransfusion has been associated with increased levels of inflammatory markers,[45,46] which may be linked to the occurrence of "salvaged blood syndrome,"[47] comprising disseminated intravascular coagulation (DIC) and acute respiratory distress syndrome (ARDS). It has been proposed that cell salvage may activate polymorphonuclear leucocytes, leading to endothelial damage, increased vascular permeability, coagulopathy and pulmonary dysfunction. However, Tawes and Duvall[48] proposed that factors such as shock, hypothermia and multiple transfusions are responsible for causing this syndrome, rather than the autotransfusor itself, because of the reperfusion injury that follows an ischaemic event. Encouragingly,

**Table 1 Key features and costs of currently available cell salvage systems**

| Manufacturer | Fresenuis AG, Bad Homburg, Germany | Haemonetics Corp., Braintree, Massachusetts, USA | *Sorin Group, Mirandola, Modena, Italy | Medtronic Inc., Minneapolis, Minnesota, USA |
|---|---|---|---|---|
| Cell saver system | C.A.T.S | Cell Saver 5+ | Electa Essential Concept | AutoLog Autotransfusion System |
| Approximate cost (£):‡ | | | | |
| Machine | 12,000 | 12,000 | 6850 | Available on request |
| Consumables | 85 | 102 | 62 | |
| Key features | Single spiral chamber, continuous cycling, low/high blood volumes, higher fat[27] removal | 3 bowl sizes, faster RBC recovery rate,[30] lower final haematocrit[31] | 4 bowl sizes for low/high blood volumes, in-built vacuum pump | 2 stage bowl-filling process, higher platelet + free haemoglobin removal,[30] faster processing time[31] |
| Published descriptions§ | 30, 49, 92 | 26, 65, 74, 75, 77, 83, 87, 88 | 61, 73 | 90 |

* Formerly Dideco SpA.

‡ Supplied by manufacturer. Consumables = per use.

§ Refer to previous models except C.A.T.S.

recent work has found that using the CATS, polymorphonuclear leucocytes are not activated to a priming threshold to induce endothelial damage.[49]

## Cell salvage in obstetrics

The delayed introduction of cell salvage in the obstetric arena, compared with the non-obstetric setting, stems from the historical and ongoing dispute concerning its safety in parturients, who are at theoretical risk of amniotic fluid embolism (AFE) and alloimmunisation.

### *Experimental evidence*

#### *Cell salvage and amniotic fluid*

The predominant concern of using cell salvage in obstetrics is that any amniotic fluid entrained into the circuit may be re-infused and so precipitate the syndrome of amniotic fluid embolism.[50,51] This phenomenon of hypoxia, cardiovascular collapse and coagulopathy continues to be associated with a high mortality, although recent improvements in outcome have been reported.[52] The pathophysiology of the condition remains controversial, as does the question of which, if any, of the components of amniotic fluid might be the primary trigger,[51,53–58] and even whether amniotic fluid has a role at all.[59] Various studies have examined the products of cell salvage for evidence of amniotic fluid contamination. In 1990, Zichella and Gramolini[60] salvaged blood at caesarean section with the Cell Saver 3 (Haemonetics Corporation, Braintree, Massachusetts, USA) and examined the re-infusion product for amniotic fluid phosphatidyl glycerol, both directly and indirectly (the latter by assessing clotting activity). Investigation revealed near disappearance on spectrophotometry and an "absolute lack of coagulant activity". Another laboratory-based study mixed amniotic fluid from healthy parturients with samples of blood before processing through an early Dideco model (formerly known as Dideco SpA, now Sorin Group, Mirandola, Modena, Italy).[61] In all post-wash samples, α fetoprotein was completely eliminated and trophoblasts, lanugo hair and vernix caseosa were absent. Fetal squames, however, were still present, although reduced in numbers after processing. Similarly, α fetoprotein was completely removed but some squamous cells remained following salvage of blood at caesarean section through a COBE BRAT-2 system (COBE Cardiovascular, Arvada, Colorado, USA).[62] Tissue factor is another constituent of amniotic fluid and is postulated to be an important trigger of DIC seen in this syndrome.[63] One study,[64] despite the investigators' attempts to minimise gross amniotic fluid contamination, was unable fully to eliminate tissue factor or α fetoprotein from post-wash samples. However, on analysing blood deliberately contaminated with amniotic fluid, the Cell Saver 4 (Haemonetics) has since successfully eliminated active tissue factor from all post-wash samples.[65]

The removal of lipid components such as lamellar bodies, which are phospholipids released from the maturing fetal lung, has also been studied. In the past, their removal from salvaged blood has been shown to be incomplete,[66] but the newer CATS device appears to be more efficient in this respect.[30]

#### *Cell salvage with leucocyte depletion filters*

The need to eliminate fully the particulate components of amniotic fluid has led to the introduction of leucocyte depletion filters to improve processing efficiency. Leucocyte depletion filters are used to remove white cells from donated blood, in order to improve the safety of allogeneic blood transfusion. Their mechanism of action relies on a combination of passive sieving through a microfibre web and active adhesion to a negative surface charge.[67] Since tumour cells have been demonstrated in salvaged blood, the potential contribution of these filters to the salvage process in patients with malignancy and infection has been examined. They have been shown to remove tumour cells from salvaged blood almost completely[68,69] and their use is well documented and mostly supported.[70]

In patients with infection, most studies have shown a reduction in bacterial contamination following the wash process[70,71] and even in cases of penetrating abdominal trauma,[72] morbidity does not appear to be significantly increased provided antibiotics are given. In obstetrics, bacterial contamination in post-wash, unfiltered samples[73,74] has been found to be minimal and clinically irrelevant. Waters et al.[75] compared unwashed blood collected from the surgical field during elective caesarean section with washed, filtered blood using the LeukoGuard RS filter (Pall Biomedical Products Company, East Hills, New York, USA). They demonstrated a significant reduction in bacterial contamination in the washed, filtered samples, to levels equivalent to those found in maternal central venous blood at the time of delivery.

With regard to lipid, a recent *in vitro* study using the PureCell leukocyte reduction filter (Pall Biomedical, Portsmouth, UK) has demonstrated complete removal of lipid from oil/blood mixtures following blood salvage.[76]

Two *in vivo* studies in the obstetric population, neither of which returned salvaged blood to patients, have examined the efficacy of these filters in removing the various components of amniotic fluid. The first, by Catling et al.[77] in 1999, involved cell salvage in 27 patients undergoing elective caesarean section using the Cell Saver 5 (Haemonetics) in conjunction with the Pall RC 100 leucocyte depletion filter (Pall Biomedical). Pre-wash, post-wash and post-wash plus filtration (post-filtration) samples were analysed. Levels of α fetoprotein were found to be significantly reduced in the post-wash compared with pre-wash samples, but remained unchanged following filtration. Leucocytes and trophoblastic tissue were completely eliminated from the post-filtration samples. Fetal squames, however, were present in all but two of the post-filtration samples. Similarly, amorphous debris was not cleared in any instance. The second study[75] again involved the Cell Saver 5 (Haemonetics), but this time in conjunction with the LeukoGuard RS filter, in 15 women undergoing elective caesarean section. As in the study by Catling et al., three sequential samples were taken, in addition to maternal central venous blood obtained from a femoral catheter at the time of feto-placental separation. Squamous cell

concentrations were significantly lower in the post-filtration samples compared with maternal blood. Lamellar-body count and potassium levels were also significantly reduced in the post-filtration samples and found to be significantly lower than those found in maternal blood. These two studies found a marked difference in the clearance of squamous cells from the post-filtration samples. This finding has been attributed to the use of different filters, which vary in design features such as fibre diameter and charge.[75,77]

*Cell salvage and alloimmunisation*

Despite the addition of leucocyte depletion filters to improve clearance of amniotic fluid, cell salvage systems remain unable to differentiate fetal from maternal red blood cells, and so the risk of alloimmunisation to the parturient must also be considered.

Fetal red cells may be entrained into the mother's circulation at any time during pregnancy or delivery,[78] so any incompatibility between maternal and fetal red cell antigens risks maternal alloimmunisation, with resultant erythroblastosis and fetal anaemia in future pregnancies.[79] Rhesus factor incompatibility is particularly common and Rhesus negative mothers are routinely given anti-D immunoglobulin to counteract this. Kleihauer-Betke testing[80] post-natally gives the degree of fetal red cell contamination of maternal blood so that adequate doses of anti-D may be given.

Fong et al.[62] demonstrated that all 10 post-wash samples derived from elective caesarean section in Rhesus negative mothers were positive on Kleihauer-Betke testing, indicating the universal presence of fetal blood cells in the re-infusion product. However, these post-wash samples, once mixed with pre-operative maternal blood samples, did not display any antigen-antibody reactions even 48 h after incubation, and crossmatching maternal serum with the samples was successful in all cases. None of these patients, however, were re-infused. On assessing the quality of processed blood salvaged during caesarean section, Rainaldi et al.[73] found that fetal haemoglobin was present at 1.8-2.0% in three samples (20%), but that these same mothers had comparable levels of fetal haemoglobin present in their circulation pre-operatively. The authors concluded that 3 mL, the maximum estimated contamination with fetal blood, was in keeping with that of spontaneous feto-maternal haemorrhage, thus the risk of alloimmunisation from salvaged blood is likely to be the same as during normal delivery. However, patients with materno-fetal blood group incompatibility were only re-infused after ensuring complete absence of fetal blood from the re-infusion product. There were no reported complications as a result of re-infusion.

The addition of leucocyte depletion filters to the salvage process has not improved contamination with fetal erythrocytes, which Catling et al.[77] described to be in the range of 2-19 mL (an average of 100 mL of blood was obtained after salvage) in 27 patients undergoing routine elective caesarean section. It was calculated that had a patient received maximally contaminated blood, she would require five times the standard dose of anti-D immunoglobulin. Interestingly, the more recent study by Waters et al.[75] showed post-filtration concentrations of fetal haemoglobin (1.9%) to be significantly higher than those in maternal central venous blood (0.5%). Again, none of the mothers in either study were re-infused with the salvaged product.

In summary, cell salvage in combination with a leucocyte depletion filter appears to provide a re-infusion product comparable to maternal blood in terms of particulate and bacterial contaminants, but is unable to clear fetal red cells to this extent.

***Clinical evidence***

Much evidence exists for the use of cell salvage in other forms of surgery. An earlier meta-analysis[36] of cell salvage in orthopaedic and cardiac surgery showed no difference in the frequency of adverse events compared to the control groups and a reduction in allogeneic blood transfusion in the orthopaedic group. A recent Cochrane review[37] has shown that, again in elective orthopaedic and cardiac surgery, cell salvage reduces overall allogeneic blood transfusion. No difference was found to exist between cell salvage and control groups in terms of postoperative complications, length of hospital stay or mortality. In vascular surgery, the effectiveness of cell salvage is less well defined.[81,82] However, because of practical difficulties in achieving blinding, in any cell salvage trial the strength of the evidence base may be questioned. The evidence relating to safety is favourable, with only coagulopathy reported as an adverse outcome. As the wash process is known to remove clotting factors from salvaged blood, a coagulopathy should be anticipated, particularly when coupled with massive haemorrhage. There is to date no proven case of any serious, adverse maternal outcome following the use of cell salvage in obstetrics.

Many cell salvage studies in the past have included small numbers of obstetric and gynaecology patients. An early retrospective review of 725 patients,[83] although predominantly involving cardiovascular cases, included in the miscellaneous group (5.9%) several women having surgery for ectopic pregnancy or caesarean section. Clotting profiles taken pre- and postoperatively were not significantly different, and none of the morbidity or mortality encountered were attributed to autotransfusion. No evidence of systemic sepsis, air or amniotic embolism or renal failure was found in this series. Tawes and Duvall[48] carried out a retrospective review of 36 000 cases receiving salvaged blood over an 18-year period. This included an undocumented number of obstetric and gynaecology patients. Overall, they found minor abnormalities in clotting to be "not uncommon" but the incidence of DIC and ARDS to be low at 0.05%. All 18 deaths occurred in non-obstetric, shocked, hypothermic patients receiving large amounts of blood, and were not attributed to cell salvage. An early case series,[84] involving 38 patients with ruptured ectopic pregnancy, concluded that cell salvage with autotransfusion was safe in this group of patients, despite clinically significant coagulopathy with re-infusion of large volumes of blood. No adverse reactions occurred in 18 patients undergoing laparoscopy for haemoperitoneum resulting from ectopic pregnancy or benign ovarian bleeding following intra-operative blood salvage and transfusion.[85]

**Table 2 Clinical outcomes of cell salvage and autotransfusion in obstetrics**

| Publication | Publication type | Number of subjects | Clinical setting | Clinical outcomes |
|---|---|---|---|---|
| Grimes,[93] 1988 | Case report | 2 | Abdominal pregnancy, PPH | Uneventful |
| Jackson,[87] 1993 | Retrospective series | 64 | CS | Uneventful |
| Rainaldi,[73] 1998 | Prospective, controlled | 68 (34 in salvage group) | CS | Salvage group: reduced length of stay + allogeneic blood transfusion, higher postoperative Hb |
| Rebarber,[88] 1998 | Historical cohort | 139 | CS | Heparin toxicity (n = 1) |
| Potter,[90] 1999 | Case report | 1 | Placenta praevia/CS | Pyrexia/endometritis |
| Catling,[26] 2002 | Case reports | 4 | Extrauterine placenta/CS | ARDS/Pneumonia |
| | | | PPH | Pyrexia/respiratory tract infection |
| | | | Jehovah's witness/CS | Uneventful |
| | | | Jehovah's witness/CS | Anaemia |
| Waters,[94] 2003 | Case report | 1 | Beta thalassaemia/CS | Uneventful |
| De Souza,[91] 2002 | Case report | 1 | Placenta praevia/CS | Uneventful |
| Oei,[92] 2000 | Case report | 1 | PET + HELLP/CS | Cardiac arrest/death |

CS = caesarean section; PPH = postpartum haemorrhage; HELLP = haemolysis, elevated liver enzymes and low platelets; ARDS = acute respiratory distress syndrome; Hb = haemoglobin.

Documented experience of cell salvage in a solely obstetric population is more limited and to date totals around 400 cases (Table 2).[86] The exact number studied is difficult to quantify, as not all published data specify numbers of obstetric cases included. The literature mostly comprises small series, retrospective studies, case reports and correspondence, with only one small reported controlled, prospective trial. The available information encompasses its use in elective and emergency caesarean section,[60,73,87,88] placenta praevia,[89,90] Jehovah's Witnesses,[26,91,92] and laparotomy for postpartum haemorrhage.[26,93] Both early and more recent reports of blood salvage and autotransfusion during caesarean section have been encouraging and deemed safe, although details are often incomplete.[60] Jackson and Lonser[87] reported 64 patients in 1993 who received a total of 136 units of salvaged blood at caesarean section, without any adverse sequalae. In 1998, Rebarber et al.,[88] in a triple-centre historical cohort study, compared 139 patients who received cell salvaged blood during caesarean section with a control group receiving allogeneic blood transfusion only. This was the first such study to assess the safety of cell salvage in the obstetric population. No differences existed between the two groups in terms of length of hospital stay, postoperative infection rates, need for ventilatory support and occurrence of ARDS, DIC or AFE. There was one suspected case of heparin toxicity in a patient re-infused with 45 units of salvaged blood; this coagulopathy was reportedly reversed with protamine sulphate. The one maternal death, which occurred in the study group, was attributed to massive, uncontrollable haemorrhage from a ruptured splenic artery aneurysm.

There has been only one prospective, controlled trial to date that has evaluated the use of cell salvage in elective and emergency caesarean section.[73] Thirty-four patients receiving autologous, salvaged blood were compared with a control group, who were to receive only allogeneic blood if transfusion was required. The mean volume of blood salvaged in the study group was 363 mL. The results revealed a significant reduction in the need for allogeneic blood transfusion, higher postoperative haemoglobin levels (despite lower initial baseline values) and shorter lengths of hospital stay in the study group. However, the postoperative haemoglobin threshold for allogeneic transfusion was not pre-defined, and as the study was not blinded, the findings may be subject to bias. This emphasises the need for clear and appropriate local transfusion guidelines both in practice and when conducting clinical trials. No serious complications were noted from the re-infusion of salvaged blood.

With regard to salvage in placenta praevia, a retrospective series[89] showed it to be a useful and safe tool. Another case report[90] described a successful outcome in a patient with placenta praevia who had experienced DIC and congestive cardiac failure after massive blood loss in a preceding pregnancy also complicated by placenta praevia. She developed a postoperative pyrexia that was attributed to endometritis, but remained otherwise well. More recently,[26] the use of the Cell Saver 5 in combination with the Pall RC 100 leucocyte depletion filter was reported in four obstetric patients. In one, the cell saver crucially allowed ongoing transfusion after depletion of the immediately available allogeneic blood supply because of torrential bleeding from an extra-uterine placenta discovered at caesarean section. The patient developed a coagulopathy (readily corrected by clotting factors), staphylococcal pneumonia and ARDS postoperatively, but made a full recovery. Another patient required emergency laparotomy for post-partum haemorrhage. As the patient was severely anaemic, the cell saver was used to facilitate immediate transfusion. The patient developed a postoperative pyrexia and clinical chest infection, but remained otherwise well. The other two cases involved Jehovah's Witnesses undergoing caesarean section. Apart from the associated clinical signs and symptoms of anaemia in one patient, both made an unremarkable recovery. Earlier cases of blood salvage during laparotomy in obstetric patients have also been reportedly free of postoperative complications.[93] Successful and safe blood salvage has also been described in unusual conditions such as β thalassaemia

intermedia[94] and in patients with rare red cell antibodies who present serious cross-matching difficulties.[95]

The only obstetric death in the literature to date, clinically attributed to amniotic fluid embolism by the authors, occurred in Holland in 2000.[92] The case involved cell salvage in a Jehovah's Witness requiring emergency caesarean section, who had declined allogeneic blood products. The patient was unwell with preeclampsia and HELLP syndrome, anaemia and coagulopathy. Shortly after starting to re-infuse the salvaged product, without the use of a leucocyte depletion filter, the patient "became very restless and dyspnoeic" and developed hypoxia and then cardiac arrest. This death has not generally been accepted in the literature as secondary to AFE, as the patient was at high risk in terms of obstetric co-morbidity and post-mortem examination was inconclusive of AFE. The risk of AFE in obstetrics therefore remains theoretical.

### *Current opinion*

Several bodies, such as CEMACH,[1] the American College of Obstetricians and Gynecologists (ACOG)[96] and the OAA/AAGBI,[27] have endorsed the use of cell salvage in obstetric haemorrhage, even in patients willing to accept allogeneic blood transfusion. The National Institute for Health and Clinical Excellence (NICE) issued a cautious endorsement in November 2005,[97] emphasising the importance of providing patients with information and gaining consent, and the need for experienced multidisciplinary teams who regularly practise cell salvage.

Some authors believe, pending further trials, that cell salvage should only be used in exceptional cases when there is no alternative for oxygen carriage,[98] that is in patients unable to receive allogeneic blood transfusion for personal, haematological or religious reasons. However, the risk-benefit ratio seems to have changed in favour of cell salvage.[3,86,99,100] In a recent debate, 58% of obstetric anaesthetists favoured the motion that facilities for cell salvage should be available in every obstetric theatre.[101] A survey of lead obstetric anaesthetists in the UK revealed that 11% had experience of cell salvage in obstetrics, and that 42% of units had access to a cell saver.[102] Our own experience reflects this changing attitude and, in our maternity unit, a protocol for the use of cell salvage has recently been approved and adopted.

There appears to be a consensus that certain precautions should be taken when using cell salvage in obstetrics. Most authors support the use of a separate suction device from the time of rupture of the amniotic membrane until complete delivery of the placenta and fetus in caesarean section,[73,77] in order to reduce amniotic fluid contamination of salvaged blood.[62] Experience of cell salvage is mostly confined to caesarean section, but it has been safely used in laparotomy for post-partum haemorrhage and ectopic pregnancy. Cell salvage is not advocated, however, in perineal or lower genital tract bleeding because of the risk of infection. Most authors consider the use of leucocyte depletion filters mandatory.[75,77] Furthermore, cell salvage produces a dilutional coagulopathy by removing clotting factors and platelets from the re-infusion product. They must therefore be replaced in cases of major bleeding. Kleihauer-Betke testing is essential as fetal blood cells and haemoglobin cannot be reliably removed during the wash process.[75,97] Following surgery, patients should be monitored in a high dependency unit[26] and any complications reported to the Medicines and Healthcare products Regulatory Agency (MHRA). A national survey into the use of cell salvage in obstetrics has recently been sanctioned by the OAA (www.oaa-anaes.ac.uk/surveys3.htm).

## Conclusion

Autotransfusion following cell salvage in obstetrics does not appear to increase the rate of AFE, infection or DIC.[73,88] At present its use is recommended in unexpected major haemorrhage,[86] or in cases at increased risk of major haemorrhage,[101] such as placenta praevia. Cell salvage may also decrease the incidence of infectious and non-infectious complications of allogeneic blood transfusion[97,99] and may even decrease mortality.[101] Its use is supported by national bodies and many clinicians and is likely to increase. However, well-designed, large prospective studies to evaluate further the balance of clinical effectiveness and safety of cell salvage are clearly needed before wider application in obstetrics. Clinical experience in the interim must continue to be carefully collected, to ensure that current practice is based on the best available evidence.

## Acknowledgement

We are grateful to Dr V. Clark and Dr S. Catling for helpful advice when drawing up our protocol.

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