

# The Use of Fibrin Glue in Skin Grafts and Tissue-Engineered Skin Replacements: A Review

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Fibrin glue has been widely used as an adhesive in plastic and reconstructive surgery. This article reviews the advantages and disadvantages of its use with skin grafts and tissue-engineered skin substitutes. Fibrin glue has been shown to improve the percentage of skin graft take, especially when associated with difficult grafting sites or sites associated with unavoidable movement. Evidence also suggests improved hemostasis and a protective effect resulting in reduced bacterial infection. Fibrin, associated with fibronectin, has been shown to support keratinocyte and fibroblast growth both in vitro and in vivo, and may enhance cellular motility in the wound. When used as a delivery system for cultured keratinocytes and fibroblasts, fibrin glue may provide similar advantages to those proven with conventional skin grafts. Fibrin glue has also been shown to be a suitable delivery vehicle for exogenous growth factors that may in the future be used to accelerate wound healing. (*Plast. Reconstr. Surg.* 108: 1713, 2001.)

### THE HISTORY OF FIBRIN GLUE USE IN WOUNDS

Fibrin was first noted to have a hemostatic effect on wounds by Bergel<sup>1</sup> in 1909 and was subsequently used by Gray,<sup>2</sup> who applied fibrinogen to cerebral hemorrhage. In 1940, Young and Medawar<sup>3</sup> used fibrin as a glue to repair peripheral nerves, and 4 years later the first use of fibrin glue to secure skin grafts was reported by Tidrick and Warner.<sup>4</sup> Initially, the glue was made by soaking the graft in citrated plasma; this was then placed on the wound, which had been coated with a thrombin solution, producing a relatively weak bond.

Fibrin glue was used very little for the next 30 years. This was partly because of the difficulty in obtaining autologous plasma intraoperatively and also because the plasma contained a low fibrin concentration, such that only a weak bond could be achieved. In 1972,

Mahas et al.<sup>5</sup> introduced commercially available fibrin glue with a higher fibrin content. Since then, refinements have added to the strength, efficacy, and safety of fibrin glues.

Fibrin glue is now a popular tool in many aspects of modern day surgery.<sup>6-10</sup> This review is focused on the potential for the use of fibrin glue to improve and complement reconstructive plastic surgery of the skin.

### FIBRIN GLUE PATHOPHYSIOLOGY

Fibrin and fibronectin are initially deposited into wounds from the circulation shortly after injury. A few days after injury, fibronectin deposition is continued by wound fibroblasts, macrophages, or migrating keratinocytes.<sup>11</sup> In response to injury, resident fibroblasts in the surrounding tissue proliferate for the first 3 days and then at day 4 migrate into the wounded site.<sup>12</sup> Once within the wound, fibroblasts produce type I procollagen as well as other matrix molecules and deposit these extracellular matrix molecules in the local milieu. Fibroblasts can use a fibrin and fibronectin matrix to move through the wound.<sup>12</sup> When exposed to a chemotactic gradient, they will migrate along, rather than across, the fibronectin fibrils. In this way it can be seen how a greater degree of cross-linking may increase the rate of fibroblast migration through a matrix.

In contrast to normal epidermal cells, wound keratinocytes express functionally active integrin receptors for fibronectin.<sup>13</sup> Thus, wound keratinocytes can pave the wound sur-

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face with a provisional matrix and express cell surface receptors that facilitate their migration across this matrix.<sup>14</sup> Fibronectin has been shown to increase the rate of keratinocyte spreading and replication,<sup>15</sup> and an increase in the degree of cross-linking may be important, as cross-linking by factor XIII promotes a similar effect. In fact, fibronectin binds specifically to fibrin and is covalently cross-linked to the fibrin alpha chain by activated factor XIIIa,<sup>16</sup> which is produced by specific dermal dendrocytes.<sup>17</sup> It has also been shown that fibrin will inhibit keratinocyte spreading and replication unless fibronectin is present *in vitro*.<sup>15</sup> This may explain the findings of Shakespeare and Shakespeare<sup>18</sup> who showed that fibrin blocked the attachment of keratinocytes to collagen *in vitro*.

Fibrin glue works as an adhesive by emulat-

ing the exudative phase of wound healing. Early products were made with human fibrin concentrate and thrombin. When the two substances are mixed the thrombin, in the presence of calcium, converts fibrinogen to fibrin (Fig. 1). A fibrin polymer is formed that has a stable structure that facilitates the growth of collagen-producing fibroblasts.<sup>19</sup> Further development has led to the addition of factor XIII, a fibrin-stabilizing factor present in blood, and aprotinin, an antiplasmin that will protect the fibrin polymer clot from premature fibrinolysis. Fibrin deposition depends on the relative rates of formation and destruction. Plasmin and other proteolytic enzymes degrade both fibrin and fibrinogen to progressively smaller polypeptide fragments called fibrin degradation products. Fibrin degradation products in turn inhibit fibrin formation by

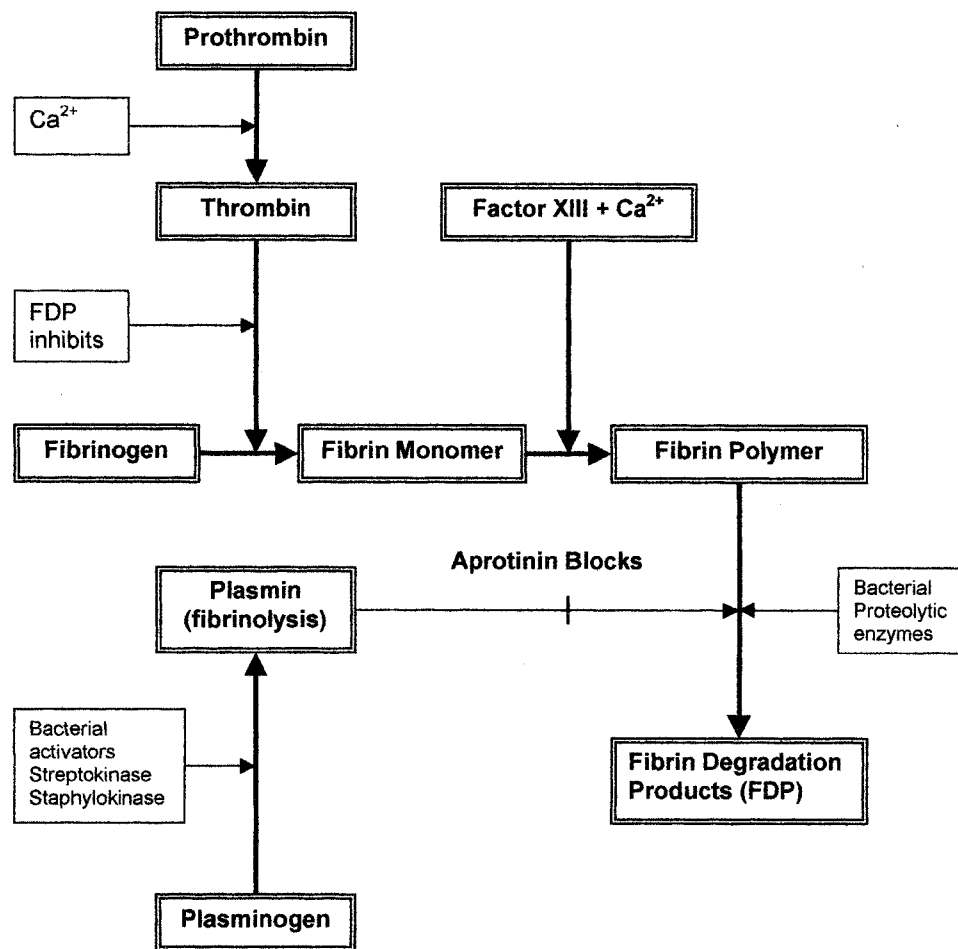


FIG. 1. Factors influencing fibrin deposition and breakdown. Fibrinogen is broken down to fibrin monomer by the action of thrombin. The fibrin monomer then polymerizes in the presence of factor XIII and calcium to form a fibrin polymer, which is precipitated as fibrin fibrils in the tissue. The fibrin polymer is broken down to fibrin degradation products by plasmin (which is inhibited by aprotinin) and bacterial proteolytic enzymes.

competitive inhibition of the action of thrombin as well as inhibiting polymerization of the fibrin monomers directly. It has been shown that bacteria produce proteolytic enzymes, which can increase the rate of fibrin polymer breakdown directly. These proteolytic enzymes also convert plasminogen to plasmin and thus increase the rate of fibrin polymer breakdown indirectly. Aprotinin blocks this indirect pathway by inhibiting plasmin breakdown of fibrin polymer.<sup>20</sup>

#### TYPES OF FIBRIN GLUE

In its simplest form, fibrin glue can be made by spinning down 50 ml of a patient's blood in

a citrated tube and removing the plasma supernatant, which contains fibrinogen. Plasma can be added to a solution of thrombin and calcium to form fibrin on a wound bed.<sup>21</sup> Plasma prepared in this way has a relatively low fibrinogen content. It has been shown that increasing the fibrinogen concentration of the glue increases the shear adhesive strength.<sup>22,23</sup> Fibrinogen concentration can be increased in two ways, either by concentrating the fibrinogen taken from a single donor or by pooling the fibrinogen taken from many donors.

The majority of commercially produced fibrin glues (e.g., Tisseel, Baxter Hyland Immuno, Glendale, Calif.; Beriplast, Centeon,

TABLE I  
Types of Commercially Available Fibrin Glue\*

| Trade Name                               | Manufacturer                           | Constituents†  | Country of License  | Licensed Intended Use   |
|--|--|--|---|---|
| Beriplast                                | Centeon, Marburg, Germany              | Fibrinogen, factor XIII, aprotinin (bovine), thrombin, calcium chloride  | Austria, Germany  | Hemorrhage, wounds<br>Surgical bleeding   |
| Biocol                                   | Bio-transfusion, Lille, France         | Fibrinogen, fibronectin, factor XIII, aprotinin bovine), calcium chloride  | France  | Hemorrhage  |
| Haemocomplettan                          | Centeon                                | Fibrinogen   | Austria, Switzerland, Germany   | Hemorrhagic disorders   |
| TachoComb                                | Nycomed Pharma, Roskilde, Denmark      | Collagen, fibrinogen, thrombin, aprotinin (bovine)   | Austria, Germany  | Hemorrhage  |
| Tisseel<br>Tisseel VH<br>(United States) | Baxter Hyland Immuno, Glendale, Calif. | Fibrinogen, fibronectin, factor XIII, plasminogen, aprotinin (bovine), thrombin, calcium chloride                          | United Kingdom<br>Canada, Ireland<br>United States  | Cardiac hemorrhage only<br>Wound gluing, sealing, and closure<br>Hemostasis in cardiac and splenic surgery, tissue sealing in colostomy closure |
| Tisseel Duo Quick                        | Baxter Hyland Immuno, Glendale, Calif. | Fibrinogen, fibronectin, factor XIII, plasminogen, aprotinin (bovine), thrombin, calcium chloride                          | Sweden, Germany   | Hemorrhage  |
| Tissucol                                 | Baxter Hyland Immuno, Glendale, Calif. | Fibrinogen, fibronectin, factor XIII, plasminogen, aprotinin (bovine), thrombin, calcium chloride                          | Austria, Belgium, Italy, Spain, Switzerland, South Africa, South America<br>France<br>Germany | Hemostasis, support of wound healing, tissue gluing<br>Hemostasis   |
| Tissucol Duo S                           | Baxter Hyland Immuno, Glendale, Calif. | Plasma protein fraction, fibrinogen, fibronectin, factor XIII, plasminogen, aprotinin (bovine), thrombin, calcium chloride | France<br>Germany   | Wounds  |
| Fibrin Sealant FS (human)                | Baxter Healthcare, Glendale, Calif.    | Fibrinogen, fibronectin, factor XIII, human thrombin, calcium chloride   | United States   | In clinical trials only at time of writing  |
| Hemaseel APR                             | Haemacure, Inc., Quebec, Canada        | Fibrinogen, fibronectin, factor XIII, thrombin (bovine), calcium chloride  | Canada, United States   | Hemorrhage  |
| Hemaseel HMN                             | Haemacure, Inc., Quebec, Canada        | Fibrinogen, fibronectin, factor XIII, human thrombin, calcium chloride   | Canada, United States   | In clinical trials only at time of writing  |

\*This table is correct to the best of the authors' knowledge at the time of writing, but clinicians should check details with the individual manufacturers.

†All proteins are of human origin unless otherwise stated.

Marburg, Germany) (Table I) consist of pooled cryoprecipitated fibrinogen and fibronectin combined with factor XIII and aprotinin, which are added to thrombin and calcium. Thrombin and factor XIII promote fibrin and fibronectin cross-linking and promote its adherence to wound collagen, whereas aprotinin reduces plasmin breakdown of the fibrin polymers formed. These products have been used extensively in Europe and have recently been given Food and Drug Administration approval in the United States. Pooled blood products obviously carry a risk of disease transmission, particularly hepatitis B and more recently human immunodeficiency virus. Donor screening, heat-treating, and the use of a solvent/detergent suspension for inactivation of lipid-enveloped blood-borne viruses in plasma derivatives<sup>24</sup> have made these products safer. Alternatively, single-donor products improve the safety margin. A multicenter trial to evaluate the safety of pooled human fibrin sealant for the treatment of burn wounds showed no rise in viral titers for human immunodeficiency virus; hepatitis A, B, and C; Epstein-Barr virus; or cytomegalovirus.<sup>25</sup> Wilson et al. recently reviewed all published data relating to the spread of variant Creutzfeldt-Jakob disease by blood products.<sup>26</sup> It was concluded that there was no evidence to suggest any risk of Creutzfeldt-Jakob disease transmission via blood transfusion. However, some commercial products contain bovine aprotinin and thrombin and there is a theoretical risk of variant Creutzfeldt-Jakob disease from these sources. Autologous fibrinogen preparations completely avoid the risk of viral transmission and would be inherently safer, provided that no exogenous thrombin or aprotinin are used.

Several methods have been described to isolate autologous fibrinogen. To be of clinical use, the technique must be relatively simple, quick, and preferably inexpensive. Saltz et al. used a cryoprecipitation technique, which took about 2 days to complete, and required around 300 ml of plasma.<sup>22</sup> Two cycles of freezing to  $-18^{\circ}\text{C}$  produced a fibrinogen concentration of approximately 45 mg/ml. In a previous article, these authors achieved a concentration of 6 mg/ml with a single freeze cycle. A much faster method has been described using ammonium sulfate<sup>27</sup> or ethanol<sup>28</sup> to precipitate out the fibrinogen. These methods produced a fibrinogen level of around 20 mg/ml and required only around 50 ml of blood to produce

up to 5 ml of glue. These methods of fibrinogen isolation did not achieve the bonding strength of commercial pooled fibrinogen products, which contain between 70 and 100 mg/ml fibrinogen.

An automated system for producing 5 ml of autologous fibrin sealant from 120 ml of blood in 30 minutes has been developed by Convatec (Skillman, N.J.). The Vivostat system<sup>29,30</sup> relies on biotin-batroxobin, which catalyzes the release of fibrinopeptide A from fibrinogen and does not activate factor XIII. This results in the formation of a fibrin I polymer that is acid soluble. Fibrin I polymer is then isolated after removing the biotin-batroxobin with avidin-agarose. The acid solution containing fibrin I polymer is neutralized with alkaline bicarbonate by co-spraying the two solutions onto the wound. On the wound surface, in the presence of calcium ions, endogenous thrombin cleaves fibrinopeptide B from fibrin I to form fibrin II. Thrombin also activates factor XIII, which acts on the fibrin II polymer to form a stable fibrin II polymer, which acts as a glue.

Most commercial pooled fibrin glue products and products derived from autologous fibrinogen contain thrombin for the activation of the fibrinogen. Initially, bovine thrombin was used, but several cases of anaphylaxis to the bovine thrombin have been reported.<sup>31</sup> Antibody formation to bovine factor V (contained in bovine thrombin preparations) causing depletion of human factor V in several patients has also been described resulting in morbidity and mortality.<sup>32</sup> Human thrombin preparations are now widely used as an alternative, though some commercial products still contain bovine aprotinin (see Table I).

#### THE USE OF FIBRIN GLUE WITH SKIN GRAFTS

The use of fibrin glue for fixing skin grafts has been investigated by a number of authors. The potential benefits can be grouped into three areas: hemostasis, graft adherence and take, and antibacterial action.

#### *Hemostasis*

Work by Ihara et al. in 1984 demonstrated an advantage of fibrin glue for reducing the hemorrhage and improving graft adhesion during burn excision.<sup>33</sup> Fibrin glue was used in 10 patients with up to 75 percent burns who underwent a total of 27 operations. The average transfusion volume per operation was shown to be 1226 ml in comparison with a control group

of 2038 ml per operation. However, no comparison of age or burn size was made between the two groups. The glue was reported to be most effective when used on the limbs under tourniquet. When the tourniquet was released, the glue was found to have had an effective hemostatic action in comparison with its application on actively bleeding tissue. Several authors have since investigated the effects of fibrin glue on wound hemostasis. Two studies used fibrin glue on graft donor sites as a model.<sup>25,34</sup> Auchaur et al. sprayed half the donor site with fibrin glue and thrombin and the other half with thrombin and placebo. No significant difference in the bleeding was found between groups. Likewise, Greenhalgh et al. failed to show any improvement in hemostasis of the fibrin-treated donor sites by measuring blood-soaked dressings. Both groups did notice a subjective reduction in bleeding. A larger study in the United States involving 95 burn victims also investigated the hemostatic effect of fibrin glue.<sup>35</sup> Fibrin glue used as a topical hemostatic agent to secure skin grafts resulted in an estimated blood loss of 0.5 ml/cm<sup>2</sup> of skin graft, compared with 0.98 ml/cm<sup>2</sup> in the control group (fibrin sealant FS, Baxter Healthcare Corp., Glendale, Calif.).

#### *Graft Adherence and Take*

Autologous skin graft take is inversely proportional to graft thickness, and cosmetic and functional success is directly proportional.<sup>36</sup> Anything that improves graft take, function, and cosmesis with no detrimental effect would be a useful adjunct to surgery. Spangler popularized the use of fibrin glue in Germany in the early 1970s,<sup>37</sup> and claimed that hemostasis was also obtained by sealing off bleeding surfaces with a fibrin layer. A rat model was used to demonstrate an improved rate of graft take when using fibrin glue.<sup>38</sup> Fibrin represents a biologic system that is completely absorbable, contrary to the commonly used synthetic adhesives. In 1983, Vibe and Pless<sup>39</sup> demonstrated an improvement in split skin graft area take from 83 percent to 92 percent with the use of fibrin glue in 20 patients. More significantly, they noted an improvement in graft take from 44 percent to 88 percent for grafts placed at difficult areas, such as over mobile muscle or close to skin folds. This is a relatively constant finding throughout the literature and shows a slight improvement in simple graft take, but

significant improvement in areas that are difficult to graft.<sup>40-42</sup> No authors have demonstrated a detrimental effect of fibrin glue on split skin graft survival, though excessive glue could conceivably reduce nutrient diffusion to the graft.

Several authors have looked at the use of fibrin glue for skin grafting hand burns. The improvement in graft fixation should allow earlier mobilization, which would result in better functional results with less hand stiffness. Stuart et al. used a single donor adhesive in 16 patients and achieved a 99 percent graft take.<sup>43</sup> Boeckx et al. showed an improvement in two-point discrimination, touch recognition, and mobility in a group of 15 patients with dorsal hand burns grafted with fibrin glue compared with a similar control group.<sup>44</sup>

Chemotherapeutic agents such as cyclophosphamide and adriamycin have been shown to alter the permeability of small blood vessels in wounds, allowing less fibrin deposition and as a result a reduced incidence of split skin graft take.<sup>45</sup> Matos and Cruz showed a reversal of this poor graft take using fibrin glue in a rat model.<sup>46</sup> Similarly, grafts treated with postoperative irradiation in rats have been shown by immunohistochemistry and electron microscopy to have a reduction in fibrinogen, fibrin, and fibronectin deposition in the wound.<sup>47</sup>

Burn patients may have an additional benefit from the use of fibrin glue in grafting compared with nonburn patients. It has been shown that partial thickness burns often progress to deeper burns within the first 72 hours of wounding. This is thought to be because of the suppression of fibrinolysis that occurs in burn patients causing progressive clot formation in damaged endothelium around the wound.<sup>48</sup> Fibrinolytic parameters have been monitored in plasma and burn wound exudate showing that fibrinolysis is activated 2 hours after burn but inactivated at 24 hours until day 10 after injury. Therefore, in this time period one might expect fibrin glue to have a longer residency and thus a greater efficacy, although this is yet to be proven in the clinical situation.

Is there any scientific evidence for an improvement in scar formation with the use of fibrin glue in skin grafts? Fibrin glue has been shown to be undetectable on histologic and immunohistochemical section by about 14 days.<sup>22,49</sup> Reduced inflammation in wounds covered by fibrin has been demonstrated in an

experimental dog model,<sup>50</sup> which may correspond to reduced scarring. Brown et al. have shown a reduction in skin graft contraction when fibrin glue was used in a rat model.<sup>51</sup>

#### *Antibacterial Action*

Fibrin glue has been shown to improve skin graft adhesion and take in the presence of bacteria in a rat model.<sup>52</sup> It was demonstrated that fibrin glue would restore graft adherence to normal levels in graft sites infected with greater than  $10^5$  bacteria per gram of tissue. Similar results were found in a clinical series looking at fibrin glue use for grafts in infected areas of 23 patients (axilla, perineum, and gluteal fold) with favorable outcomes.<sup>41</sup> It was postulated that the beneficial effect might be because of an improvement in phagocyte motility in the fibrin or because of a saturation of the bacterial proteolytic enzymes by the exogenous fibrin. Bacterial growth has been shown to be slower in a clot of fibrin glue than in a physiologic clot.<sup>53</sup>

Early skin graft survival depends on stabilization of the graft by the fibrin network between the graft and the recipient bed. Fibrin glue provides an immediate, highly cross-linked fibrin network that will stabilize the graft and facilitate graft nutrition by serum imbibition (plasmatic circulation) with subsequent ingrowth of vascular buds (neovascularization). A successful autograft leads to sterilization of a wound, and the ability of fibrin to stimulate phagocytosis may be an important factor in achieving this.<sup>54</sup> Skin graft failure may be because of bacteria causing dissolution of fibrin via proteolytic enzymes acting on plasminogen to increase the plasmin level as well as acting directly on fibrin polymers.<sup>55</sup> *Staphylococcus aureus*, beta-hemolytic streptococci, and *Pseudomonas aeruginosa* have all been found to be associated with high levels of fibrin degradation products when detected in wounds. Their presence is followed by a delayed rise in wound plasmin activity and a reduction in fibrinogen levels with subsequent graft loss. Although the bacterial load is an important factor in graft survival, the ability of certain bacteria to produce proteolytic enzymes may have more significance.

Teh found that attempts to increase the fibrin deposition artificially (by addition of thrombin or fibrinogen, or measures to prevent the digestion of fibrin by inhibiting the action of plasmin and proteolytic enzymes)

had no effect on graft survival.<sup>55</sup> Conversely, Perry et al. found that fibrin clot dissolution was enhanced by addition of plasminogen to bacterial culture with a rise in the fibrin degradation product levels of the supernatant.<sup>20</sup> Both aprotinin (a plasmin inhibitor) and epsilon-aminocaproic acid (an inhibitor of plasminogen activators) were capable of reducing clot destruction by bacteria.

#### FIBRIN GLUE AS A TEMPLATE FOR CELLULAR MIGRATION

Wound healing can be modified by changes in cell motility, angiogenesis, and modification of matrix production.<sup>56</sup> Tissue-engineered skin implants are designed to optimize these parameters. Changing the fiber size and the fiber spacing can alter the scaffolding properties of cross-linked polymer skin implants. Collagen dermal replacements were shown to have an optimal pore diameter of between 20 and 125  $\mu\text{m}$ .<sup>57</sup> This allows optimal cellular ingrowth while maintaining a dermal scaffold sufficient to retard wound contracture and scarring until cellular colonization has occurred.

A study involving fibrin glue as an implant to promote cellular migration compared pooled donor fibrin glue (60 mg/ml fibrinogen) with modified fibrin glue. The fibrin (60 mg/ml fibrinogen) was modified by addition of water-soluble polymer beads of 100 to 150  $\mu\text{m}$  to obtain a porous and rough structure of similar pore size. The modified fibrin scaffold increased the volume fraction of fibroblasts and the number of blood vessels compared with a nonmodified fibrin scaffold.<sup>58</sup> The functional mechanisms allowing fibroblasts to leave the collagenous matrix of normal connective tissue and invade the provisional matrix of the fibrin clot have not been fully defined. It has been demonstrated in vitro that recombinant platelet-derived growth factor (PDGF) at physiologic concentrations will stimulate migration of fibroblasts from a collagen gel onto a surface coated with fibrin fibrils.<sup>59</sup> Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor- $\beta$  (TGF- $\beta$ ) are thought to be important growth factors involved in endothelial cell migration and proliferation in the fibrin matrix.<sup>60,61</sup> However, the three-dimensional architecture of the fibrin matrix may be more important for capillary formation.<sup>62</sup>

The rate of implant degradation is an important factor when considering its ability to act as

a template for cellular migration and proliferation. Fibrin glue is present in wounds for at least 4 days but is difficult to detect after 10 days.<sup>49,63</sup> The fibrin glue degrades through enzymatic and phagocytic pathways and is therefore an ideal delivery system for cultured cells,<sup>64</sup> growth factors,<sup>65</sup> and even antibiotics.<sup>66</sup>

#### FIBRIN GLUE AS A DELIVERY SYSTEM FOR CULTURED KERATINOCYTES AND FIBROBLASTS

Since 1975 it has been possible to cultivate human keratinocytes *in vitro* using lethally irradiated 3T3 mouse fibroblasts in a specific culture medium.<sup>67</sup> It is now possible to culture enough keratinocytes over a 5-week period to cover the entire body surface of an adult with sheets of autologous keratinocytes several cells thick. This was a significant advance in the treatment of patients with major burns, with one study showing a reduction in mortality from 48 percent to 14 percent associated with its use.<sup>68</sup> However, sheets of autologous keratinocytes have several drawbacks in clinical use. A relatively unstable epidermis is produced that is often prone to blister on minor trauma.<sup>69</sup> Keratinocytes take a significant time to cultivate, during which the burn patient's condition may deteriorate. Surgical "take" of the sheets is relatively poor, ranging from 15 to 70 percent,<sup>70-73</sup> and it is now commonly accepted that the quality of epidermis can be improved by providing a dermal layer for skin replacements.<sup>74-77</sup>

Culturing cells to a nonconfluent state *in vitro* and then delivering them to the wound in suspension can reduce the time needed to cultivate epithelial sheets. This has the advantage of providing cells that have not undergone phenotypic changes associated with contact inhibition and should therefore have undiminished adhesive and proliferative potential. It also avoids the use of Dispase (Life Technologies, Grand Island, N.Y.), which is used to release epidermal sheets in conventional keratinocyte technology. Dispase may reduce the surface antigen expression of the keratinocytes and reduce their adhesive potential.<sup>78</sup>

Hunyadi reported the first use of fibrin glue with cultured keratinocytes in 1988. It was demonstrated that fibrin glue (Beriplast) could be used to effectively deliver autologous keratinocytes mixed with the fibrinogen component to the wound. A marked increase was found in the rate of leg ulcer healing compared with a control group.<sup>79</sup>

Ronfard et al. developed a technique in 1991 for culturing sheets of autologous keratinocytes on fibrin glue and reported its use in two burn patients. The only difference from conventional techniques was that the last subculture before grafting was set up on a Petri dish coated with fibrin glue (Biocol, Bio-transfusion, Lille, France) and seeded with irradiated 3T3 mouse fibroblasts that are a standard component of keratinocyte culture.<sup>80</sup> It was found that *in vitro* there was no destruction of the fibrin matrix for up to 15 days, probably because of the aprotinin in the product. The autologous keratinocytes could then be transferred to the patient on the fibrin sheets, alleviating the need to use Dispase. Inverted fibrin sheets were used on all but one area, with the keratinocytes closest to the wound. The worst graft take was on the noninverted area, and in areas the graft had been inverted, no apparent alteration of anchorage or growth of the keratinocytes was found on histologic analysis. The technique was claimed to accelerate the standard process of handling fragile cultured keratinocyte sheets.

The benefit of using fibrin glue to secure traditionally produced sheets of cultured keratinocytes was described in an athymic mouse model.<sup>63</sup> Fibrin glue (Hemaseel, Haemacure, Inc., Quebec, Canada) was sprayed onto the wound bed before deposition of the epidermal sheets and compared with a control group in which no fibrin glue was used. Seven days after transplantation, compared with controls, the percentage of graft take over the total surface area grafted was greater in animals that had received the tissue sealant application. No difference was found 14 and 21 days after grafting. In contrast, the percentage of graft take over the bony area (spinal) was significantly increased in animals grafted with previous application of sealant compared with controls at 7, 14, and 21 days after grafting. Immunohistologic and ultrastructural analysis showed that the evolution of the cultured human epidermis after transplantation was similar in both groups. The basement membrane was well-structured 21 days after transplantation. The sealant was present at 4 days but not at 21 days after grafting. Therefore, it was concluded that the application of fibrin sealant before cultured epidermal sheet deposition on a nude mouse graft bed is innocuous and enhances their mechanical stability. Auger et al. showed a 20 percent improvement in cultured epithe-

lial graft take in a similar model using Tisseel fibrin glue.<sup>49</sup>

Fibrin glue has also been used to deliver cultured human keratinocytes to a wound in suspension. In vitro studies showed keratinocytes to remain viable in suspension in fibrin for at least 5 days.<sup>81</sup> When compared with standard cultured epidermal sheet grafts in a nude mouse model, reepithelialization was similar but reconstitution of the dermoepidermal junction zone, as shown by electron microscopy and immunohistochemistry, was significantly enhanced by the fibrin-glue suspension technique.<sup>82</sup> It was concluded that the fibrin glue not only delivers highly proliferative keratinocytes but also provides an optimal milieu for their migration, proliferation, and differentiation.

The same authors have used cultured autologous keratinocytes suspended in fibrin glue (KFGS) with allogeneic skin overgraft in several burn patients.<sup>83-85</sup> Cultured cells that are 70 percent confluent before trypsinization were mixed with the fibrin component of the fibrin glue (Tissucol, Baxter Hyland Immuno) and the cell-containing suspension was used to secure the allograft skin to the debrided burn wound. The allogenic epidermis underwent immunologic rejection and cultured autologous keratinocytes replaced them. The fate of the allogenic dermis was less clear. Histologic evidence indicated integration of allodermis into the wound, although it was stated that further immunohistochemical studies were needed to verify this finding. The Vivostat system has been used in a similar way in an animal model to deliver subconfluent cultured autologous keratinocytes to a wound in an autologous fibrin spray.<sup>86</sup>

Fibrin gels have been used as a "dermal matrix" containing fibroblasts, keratinocytes, or a combination of the two. Meana et al. created a fibrin gel by adding 3 ml of fibrinogen cryoprecipitate to 12 ml of Dulbecco modified Eagle medium 10% fetal calf serum containing  $0.5 \times 10^6$  human fibroblasts and bovine aprotinin.<sup>87</sup> This was combined with thrombin and calcium to form a gel and then seeded with cultured human keratinocytes. Keratinocytes would not proliferate efficiently without the human fibroblasts in the matrix. These gels were successfully grafted onto athymic mice. Stains for the two basement membrane proteins, type IV collagen and laminin, were only

positive when both fibroblasts and keratinocytes were used.

Pellegrini et al. showed that keratinocytes cultured on fibrin glue maintained the relative percentage of holoclones, meroclones, and paraclones, proving this fibrin technique does not induce clonal conversion and consequent loss of epidermal stem cells.<sup>88</sup> Fibrin glue cultured keratinocyte autografts bearing stem cells applied "cells-up" to massive full-thickness burns (initially treated with allodermis) displayed a high keratinocyte take rate, which was reproducible and permanent, and maintained long-term proliferative potential.

A recent article reported a system for the cultivation of keratinocytes on acellular human dermis using fibrin glue and 3T3 feeder cells, hence avoiding the need for a two-stage procedure to create a dermal and epidermal component.<sup>89</sup> Deepithelialized dermis was used in which the fibroblasts were inactivated by repeated freeze-thaw cycles. Keratinocytes were delivered to the dermis and then fixed in position with a fibrin glue spray. This complex was cultured at the air-liquid interface in a medium that contained 3T3 fibroblasts. It has yet to be proven whether this in vitro product works in a clinical situation.

#### FIBRIN GLUE AS A DELIVERY SYSTEM FOR GROWTH FACTORS

Fibroblasts and endothelial cells will readily migrate into fibrin clot.<sup>58</sup> This process of migration and subsequent proliferation is controlled by a variety of growth factors. Exogenous growth factors have been used experimentally to modify wound healing. Fibrin glue provides a useful carrier, which both delivers the growth factors to the wound and releases them at a steady rate. It also acts as a scaffold for subsequent tissue regeneration as outlined earlier. Individual growth factor modifications of the wound-fibrin-cellular interactions are outlined below.

##### *Platelet Releasate*

Human platelets contain PDGF, TGF- $\beta$ , FGF, epidermal growth factor (EGF), platelet factor-4, platelet-derived angiogenesis factor, and  $\beta$ -thromboglobulin in their alpha granules.<sup>90</sup> Platelets from stored blood maintain their growth factors, and a releasate can be relatively easily made from platelet concentrate with the addition of thrombin. Commercially available pooled donor fibrin glue contains no active

platelets or growth factors because of the viral inactivation processes necessary during manufacture (see product data sheets). However, many methods of autologous glue production maintain viable platelets that will produce growth factors on application to the wound bed.<sup>91-94</sup> Clinical trials combining platelet releasate and fibrin glue have been performed to deliver growth factors to bone graft sites,<sup>95</sup> although no such studies exist for skin grafts. However, the releasate has been shown to increase fibroblast proliferation in culture.<sup>96</sup> Interestingly, a study examining the effect of fibrin glue *or* platelet releasate on the strength of meshed hernia repairs in a rat model concluded that they both improved the mechanical strength of the repair.<sup>97</sup> Fibrin glue and platelet releasate also independently increased the collagen content of the wounds. The authors did not examine the effects of combining the two.

#### *Vascular Endothelial Growth Factor*

VEGF is a potent mitogen for endothelial cells, but is not mitogenic for fibroblasts or vascular smooth muscle cells.<sup>98</sup> A dynamic interaction occurs between endothelial cells, angiogenic growth factors (VEGF, FGF, TGF- $\beta$ ), and the extracellular matrix.<sup>99,100</sup> VEGF, also known as vascular permeability factor, stimulates the endothelial cells of the microcirculation to proliferate and migrate. These endothelial cells are also rendered hyperpermeable so that they spill plasma proteins into the extravascular space, with subsequent fibrin deposition.<sup>100</sup> The integrin  $\alpha(v)\beta3$  is expressed on the tip of angiogenic capillary sprouts,<sup>101</sup> and growth appears to be better supported *in vitro* on fibrin gel than on collagen gels. Although the growth factors basic FGF and VEGF primarily stimulate the proliferation of endothelial cells, the configuration (three-dimensional architecture) and rigidity of the fibrin matrix is also essential for capillary morphogenesis.<sup>62</sup>

Fibrin glue has been used with exogenous VEGF in several experimental models. *In vitro* studies have clearly shown VEGF to stimulate angiogenesis in a fibrin clot.<sup>99,102</sup> Heparin (5 U/ml) and VEGF (1000 ng/ml) in a fibrin glue have been shown to promote human endothelial cell proliferation while inhibiting smooth muscle cell proliferation.<sup>103</sup> Recombinant  $\alpha$ -endothelial growth factor in fibrin glue has been shown to create newly grown

vascular structures when implanted between the aorta and myocardium of the left ventricle in an animal model.<sup>104</sup> VEGF in fibrin glue has also been used as an intramuscular injection in arteriopathies to stimulate angiogenesis, with angiographic evidence of new vessel formation.<sup>105,106</sup>

A novel approach to deliver VEGF has been adopted by Rio et al. They used porcine keratinocytes transfected with cDNA for VEGF driven by a keratin promoter to overexpress the growth factor.<sup>107</sup> The genetically modified pig keratinocytes were expanded on fibroblast-containing fibrin gels and transplanted to nude mice model as a composite material. A strong angiogenic response was demonstrated with immunostaining.

#### *Fibroblast Growth Factor*

FGF exists in two forms, acidic FGF (aFGF) and basic FGF (bFGF), both are commonly bound to heparin. Fibroblasts, endothelial cells, smooth muscle cells, and chondrocytes produce FGF. FGF is a potent mitogen for endothelial cells, fibroblasts, keratinocytes, chondrocytes, and myoblasts.<sup>90</sup> The angiogenic effect of bFGF *in vitro* is greatest when combined with VEGF and tumor necrosis factor- $\alpha$ .<sup>102</sup> Fibroblast replication has been shown to increase in the presence of FGF-heparin incorporated into fibrin matrices.<sup>108</sup> These composite fibrin matrices were subsequently implanted into collagen sponges, creating a fibroblast-infiltrated tissue resembling a normal dense connective tissue.

A fibrin-based FGF delivery system has been tested on a nerve regeneration model.<sup>109</sup> This was designed so that release of FGF occurs primarily in response to cell-associated enzymatic activity during healing, providing a localized release in a biomimetic manner. The drug delivery system involved a bidomain peptide with an N-terminal transglutaminase substrate and a C-terminal heparin-binding domain derived from antithrombin III. The bidomain peptide was covalently cross-linked to fibrin matrices during coagulation by the transglutaminase activity of factor XIIIa, which served to immobilize heparin electrostatically to the matrix. This in turn immobilized the heparin-binding growth factor and slowed its passive release from the matrix.

Pandit et al. have determined the effective angiogenic stimulatory dose of FGF-1 using a pooled modified fibrin glue delivery system.<sup>110</sup>

They used a rabbit ear ulcer model to demonstrate a dose-dependant healing response by measuring angiogenesis, fibroblastic responses, and epithelialization rate histologically. A dose of 8  $\mu\text{g}/\text{ml}$  was suggested as optimal. The same authors have previously demonstrated that aFGF increased angiogenesis, enhanced epithelialization, and reduced contraction rate when delivered through a collagen scaffold<sup>111</sup>; however, a higher inflammatory response was indicated in the collagen scaffold-treated group. Fibrin scaffold was found to be a more suitable vehicle for the delivery of aFGF, as demonstrated by the lack of inflammation and improved mechanical properties of the healed tissue.<sup>65</sup>

#### *Epidermal Growth Factor*

EGF is produced by platelets and is found in high quantities in the early stages of wound healing.<sup>90</sup> EGF stimulates the production of proteins, such as fibronectin, and stimulates the migration of epithelial cells. EGF does not stimulate collagen production, but it does increase the number of fibroblasts in the wound, and these produce collagen and increase wound strength.<sup>112</sup> EGF in silver sulfadiazine has been used in two clinical trials, one on skin graft donor sites<sup>113</sup> and the other on chronic wounds,<sup>114</sup> with some evidence of improved epithelialization rates in both trials.

A novel fibrin glue delivery system for EGF has been described using genetically modified cells to secrete the growth factor. Clonally selected human fibroblasts were transfected with a novel chimeric expression plasmid in which the biologically active portion of the human EGF gene is fused in-frame to the human granulocyte colony-stimulating factor signal sequence. This construct secretes biologically active EGF.<sup>115</sup> The gene-transfected fibroblasts were suspended in fibrin glue and then given a nonlethal irradiation dose to prevent replication and transplanted onto murine full-thickness wounds. EGF was demonstrated for at least 7 days in the wounds, slowly decreasing from 470  $\text{pg}/\text{ml}$  to 140  $\text{pg}/\text{ml}$  on day 7. EGF was not found in the wound at day 14.

#### CONCLUSIONS

Fibrin glue is potentially an extremely useful tool for the plastic surgeon. It has a hemostatic effect, increases the percentage of graft take, and may have a protective effect against infection. It also has great potential as a delivery

system for cultured cells and growth factors. So why is it not universally used on all skin grafts?

There is a potential risk of infection or hypersensitivity when using any blood product. This is minimized by use of donor selection, screening, and viral inactivation. The potential benefit has to be weighed against that risk. There is little point in exposing a patient to risk if undertaking a simple procedure with a high chance of success, such as a simple skin graft. However, if there is a low chance of success for the procedure, or there is a risk of a catastrophic loss should the procedure fail, then it seems reasonable to consider the use of fibrin glue if it will improve the chance of success. Therefore, we need to define which grafts are likely to fail. As previously mentioned, these may be grafts in areas of mobility or "dirty" areas, but it is beyond the scope of this review to set such criteria. Similarly, we need to define what grafts constitute a catastrophic loss should they fail. Financial considerations also have to be taken into account. Commercial glues cost approximately £300 for 5 ml in the United Kingdom. Autologous glues are more difficult to cost, as they are often incorporated into a preexisting blood bank, though one author quotes a setup cost of US\$2500.<sup>116</sup>

The decision to use fibrin glue for difficult split skin grafts is currently one taken by a few individual clinicians. Cost, storage, preparation time, and transfusion-transmitted disease all play a role in this choice, but the purpose of this review has been to suggest that there is evidence to support the use of fibrin glues to improve results in selected clinical situations. In the future, it is possible that fibrin glue will become an important component of tissue-engineered skin replacements, allowing early closure of burn wounds with an acceptable cosmetic result. In the future, the use of exogenous growth factors may have a significant role in accelerating wound healing. Fibrin glue has been shown to be a suitable delivery system for both growth factors and genetically modified cells expressing them.

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