Laboratory study

The biocompatibility of BioGlue with the cerebral cortex: a pilot study

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Summary An *in vivo* pilot study investigating the biocompatibility of BioGlue as a dural sealant is described. Craniotomies were performed in adult Wistar rats. After excising the dura, BioGlue was applied directly onto the cerebral cortex and sterile Surgicel (Ethicon Inc., Somerville, NJ) placed over the brain in control rats. All rats were culled 14 days post-surgery. An inflammatory cell infiltrate was found overlying the pia-arachnoid, but this did not extend into the brain parenchyma except in instances of mechanical disruption. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) showed an increase in gliosis in the BioGlue treated rats but the degree of positive staining as well as the observed surface inflammation was not significant and possibly would not be clinically significant.
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INTRODUCTION

A watertight closure of the dura to prevent cerebrospinal fluid (CSF) leakage is a critical component of most intracranial and intradural spinal procedures. It is frequently difficult to obtain a direct primary closure of the dura, and often a dural graft is necessary in order to obtain satisfactory closure. Tissue glues, such as BioGlue® Surgical Adhesive (BioGlue, CryoLife, Inc., Kennesaw, GA), have been reported as aiding in dural closure, either by reinforcing a primary closure of the dura or sealing the edges of the graft.[1,2]

In using a dural sealant, there is a concern as to its toxicity, if it inadvertently leaks onto the underlying cerebral cortex. Even though biologically based sealants comprised of fibrin or gelatin have had some success in certain surgical disciplines[3–5] including application to the cranium, soft tissue of the cranial base, para-nasal sinuses, subdural and epidural spaces,[6–8] side effects such as neurotoxicity, induction of oedema or seizures, intense inflammation of vessels and possible arterial occlusions have been reported.[9–11]

In this study, we have investigated the effect of BioGlue, a surgical adhesive that is comprised of bovine serum albumin (BSA) and glutaraldehyde (GA) on rat cerebral cortex.

MATERIALS AND METHODS

Twenty-three adult wistar rats (200–250 g) were sedated using penthrane inhalation and then anaesthetized using an intraperitoneal administration of xylene (8 mg/kg)/ketamine (44 mg/kg). The scalp was incised, reflected and a dental drill used to perform a craniotomy measuring approximately 6 mm × 6 mm anterior to the coronal suture over the left cerebral hemisphere.

A fine nerve hook was used to lift the dura at the posterior end of the craniotomy while a microsurgical knife and scissors were used to excise the dura. The dura was then reflected back over the edge of the craniotomy to reveal the underlying cortex. A volume of 0.05–0.1 ml (1–2 drops) BioGlue was applied to the exposed cortex after the applicator tip was primed and the initial volume of BioGlue was discarded. The BioGlue was allowed to polymerize for a period of 2–3 min. The reflected skin and subcutaneous tissue was then sealed with 9 mm wound clips. Control or 'sham' rats underwent the identical surgical procedure except that moistened sterile Surgicel gauze was placed over the craniotomy prior to scalp closure. All the rats were observed daily until the endpoint of this pilot study at 2 weeks.

At the end of the 2-week period, the rats were euthanised using CO_2 inhalation and the skull removed to expose the brain. The brains were cut in a coronal plane at the site of the BioGlue application with a scalpel and both pieces placed in a solution of 10% neutral buffered formalin (NBF) prior to histological processing as paraffin embedded tissue. A second group of BioGlue and 'sham' rats were treated in the same manner to allow the skull and brains to be decalcified prior to histological processing which allows the BioGlue plug to remain attached at the surface of the brain. The BioGlue detached when the surrounding skull was removed in the first group of animals.

The decalcification procedure softens the skull and ultimately allows the whole skull and brain to be processed and sectioned without detaching the BioGlue. This was done with the use of a 12% formic acid/6.25% formalin solution. The softened tissue was sectioned at a thickness of 6 μm and stained with haematoxylin/eosin (H&E) for histological analysis or left unstained for immunohistochemical analysis for the antigen, glial fibrillary acidic protein (GFAP).

The sections were deparaffinized in xylene prior to washing in phosphate-buffered saline (PBS), blocking with a rabbit serum, and incubation with the GFAP antibody at a 1:100 dilution at 37 °C for 1 h. The presence of the antigen was detected via a sensitive streptavidin biotinylated method using a rabbit anti-cow GFAP primary antibody (DAKO – Z033401). The sections were incubated at 37 °C with a 1:100 dilution of this antibody after which they were washed and a 1:500 dilution of a biotinylated

swine anti-rabbit (DAKO – E0353) was added for 1 h at room temperature. Further washing and a 30-min incubation with a peroxidase conjugated streptavidin reagent (DAKO – P0397) at room temperature were carried out prior to chromogenic detection via a DAB kit from DAKO (K3468). The sections were then mounted with a DePex mounting medium under a coverslip. Twelve images were taken at a magnification of 400× on an Olympus IX50 microscope connected to an Olympus DP-10 digital camera. The area of positive staining was determined using the Image Pro V4.5 analysis software on a Windows-based IBM compatible computer. The figures were then averaged out from the 12 images for each rat.

RESULTS

No adverse reactions, deaths or seizure activity were noted in any of the animals.

Observations of the operative site

During examination of the BioGlue applied craniotomy on day 14 at the time of culling, the plug appeared smooth and firmly adhered to the skull.

Histological analysis

The results from the histological analysis can be summarized in the following four groups: Group A – BioGlue (non-decalcified – 7 rats), Group B – Sham (non-decalcified – 7 rats), Group C – BioGlue (decalcified – 5 rats), Group D – Sham (decalcified – 4 rats). All rats in each group were culled at a 2 week time point post-BioGlue application or sham operation. Groups C and D were smaller than the A and B due to the availability of rat stocks at the time.

In Group A, there was a moderately intense chronic inflammatory cell infiltrate covering the surface of the pia-arachnoid. This was confined to the pia-arachnoid. Dystrophic calcification was noted within the inflammatory cell infiltrate in one rat. In two of the rats, the underlying brain parenchyma was unremarkable with no involvement by the inflammatory cell infiltrate. There appeared to be mechanical disruption of brain parenchyma in two rats with the inflammatory cell infiltrate extending into the defect as far as the immediate sub-cortical white matter. In sections where a small fragment of BioGlue was attached onto cortex, the cortex immediately in contact with the BioGlue was intact, but there was an inflammatory cell infiltrate deep into the intact cortex.

In Group B, the coronal sections of brain showed focal mild chronic inflammation of the pia-arachnoid. In areas where the pia-arachnoid is intact, there was no involvement of underlying brain parenchyma. In one rat, there was mechanical disruption of the pia-arachnoid and the underlying brain parenchyma with the inflammatory cell infiltrate extending into this defect.

The decalcified coronal sections of Group C showed the brain surrounded by decalcified skull bone with attached soft tissue. In all rats, there was a defect in the vertex of the skull overlying the sagittal midline which was plugged with BioGlue. In general, there was an intense acute inflammatory cell infiltrate on the deep surface of the BioGlue plug extending to intact pia-arachnoid and into immediately adjacent skull bone. Scattered foreign body type multinucleated giant cells were noted within the acute inflammatory cell infiltrate overlying the pia-arachnoid. There was intense congestion of a large calibre thin walled vascular channel situated in the midline immediately deep to the BioGlue plug which was surrounded by the inflammatory cell infiltrate but no

evidence of vessel wall necrosis was seen. No extension of the inflammation into underlying brain parenchyma was seen nor was there any inflammation of pia-arachnoid from the site of the BioGlue plug.

Group D coronal sections also showed the brain surrounded by decalcified skull bone with attached soft tissue. All sections showed a small defect in the skull bone on one side of the sagittal midline. In some sections, this was filled by immature scar tissue consisting of plump fibroblasts and collagen with scattered chronic inflammatory cells. There was no inflammation noted of the pia-arachnoid away from the bone defect except in one animal where there had been minor mechanical disruption of the brain.

Fig. 1 shows macro images taken from BioGlue and sham treated rats. The top panel is taken from a Group C rat and it shows three distinguishable layers, the BioGlue layer at the top of the image, the area of acute inflammatory response on the surface of the brain and the underlying normal brain parenchyma. The middle panel is an image taken from another decalcified rat brain (Group C). Even though the tissue processing has resulted in the brain detaching from the BioGlue and skull bone, there is still evidence of the inflammatory reaction on the surface of the brain and on the BioGlue attachment to the bone. The bottom panel is an image taken from a decalcified brain of a sham rat. There is a
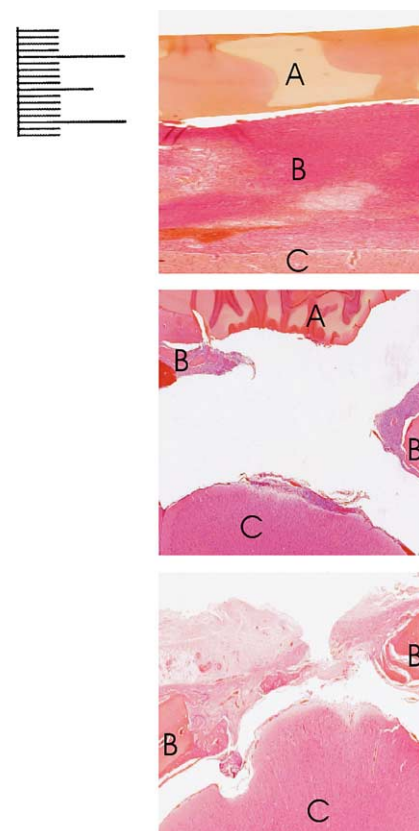

Fig. 1 Two weeks post-BioGlue application to the cortex. (Top) Macro image taken of a H&E stained rat brain section from a BioGlue rat (A: BioGlue layer; B: acute inflammatory response on surface of brain; C: normal brain parenchyma. (Middle) Macro image taken of a H&E stained section from a BioGlue rat (note that BioGlue has detached from brain surface during processing) – (A: BioGlue layer; B: skull bone; C: normal brain parenchyma) – both bone and the surface of the brain show evidence of inflammatory response. (Bottom) Macro image taken from a sham operated rat – note that the degree of inflammation that has occurred from the mechanical disruption from the removal of the dura is not as severe as the response from the BioGlue (B: skull bone; C: normal brain parenchyma). The scale on the left indicates a 1 mm division between the two major gridlines.
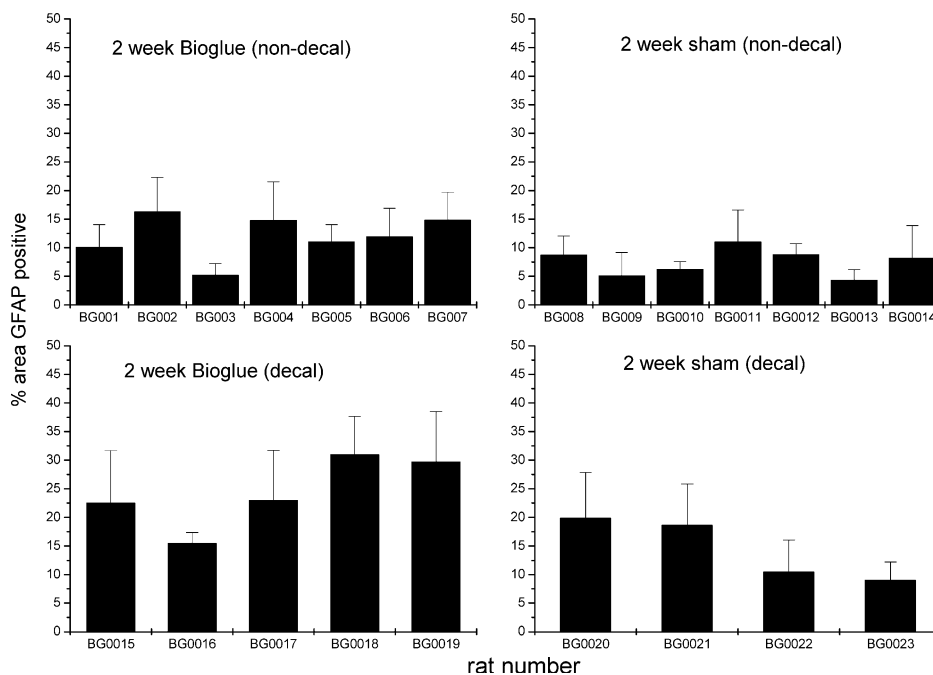
Fig. 2 GFAP immunohistochemistry staining results of non-decalcified brains (non-decal) and decalcified brains (decal). Each bar represents the average percentage area of GFAP positive staining taken from 12 images for a single rat which was allocated a study number as designated on the *x*-axes.

small degree of inflammation present that was caused by the mechanical disturbance during the removal of the dura.

GFAP immunohistochemical analysis

The results for the GFAP immunohistochemical analysis in all groups (A–D) are shown in Fig. 2. The results are shown as the average percentage area of positive staining for the GFAP antigen in the 12 images taken per rat. The results in Fig. 3 show that there was an overall higher positive stain for GFAP in the BioGlue groups of rats (non-decal and decal groups). Increased positive staining in the decal groups may be explained by the possibility of greater mechanical disturbance during the craniotomy/dura removal in the decalcified group or rats. An increase in GFAP staining was observed between the two 'sham' groups as well as the BioGlue groups and would therefore not attribute the increase in the BioGlue groups to be due to any effect from the BioGlue. It is possible that a greater degree of mechanical disruption occurred on the surface of the brain during the removal of the dura by the different researcher in the decalcified groups. Even allowing for the difference between the non-decalcified and decalcified groups, there was an average increase in GFAP staining (or gliosis) of 65.70% between the sham and BioGlue rats (60% increase – non-decalcified group; 71.40% increase – decalcified group) as shown in Fig. 3. Independent one-way ANOVA tests show a significant difference between the sham and BioGlue rats in each group. Fig. 4 shows images taken from the immunohistochemically stained sections for GFAP in a BioGlue treated rat (top panel) and a 'sham' rat (bottom panel). The staining of reactive astrocytes in the BioGlue rat is more intense and prominent throughout the coronal section.

DISCUSSION

BioGlue is an adhesive which is a combination of BSA and GA in the ratio of 45% and 10%, respectively. BSA possesses lysine groups which contain amine groups that can be linked by the aldehyde groups in glutaraldehyde. The GA acts as a connector
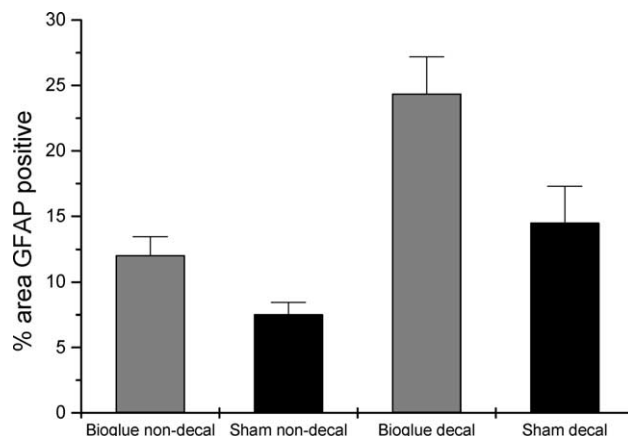

Fig. 3 GFAP immunohistochemistry staining results. Each column corresponds to the average of all the animals within a group that were represented as single entities within the four groups in Fig. 2. Independent one-way ANOVA analyses at a level of significance of $p = 0.05$ show a significant difference between BioGlue and sham rats using both methods of brain extraction.

molecule by binding the lysine groups of the BSA as well as those present in the extracellular matrix and cell surface allowing the BSA to bind to tissue. The high concentration of BSA present in BioGlue coupled with the rapid reaction between aldehydes and amines allows for the glue to set within 20–30 s and maximum binding strength to be obtained after 2–3 min.

The BSA was isolated from bovine spongiform encephalopathy free cattle used in this pilot study to December 2003. The process of purification is a three-step procedure involving heat precipitation, chromatography and γ-radiation exposure that reduces and inactivates viruses. A controlled delivery device is first primed and then used to dispense the two component mixture in a predefined ratio at the site of application to form a mechanical seal. BioGlue can be stored at room temperature and has a relatively long shelf life of 3 years. It was first approved and launched
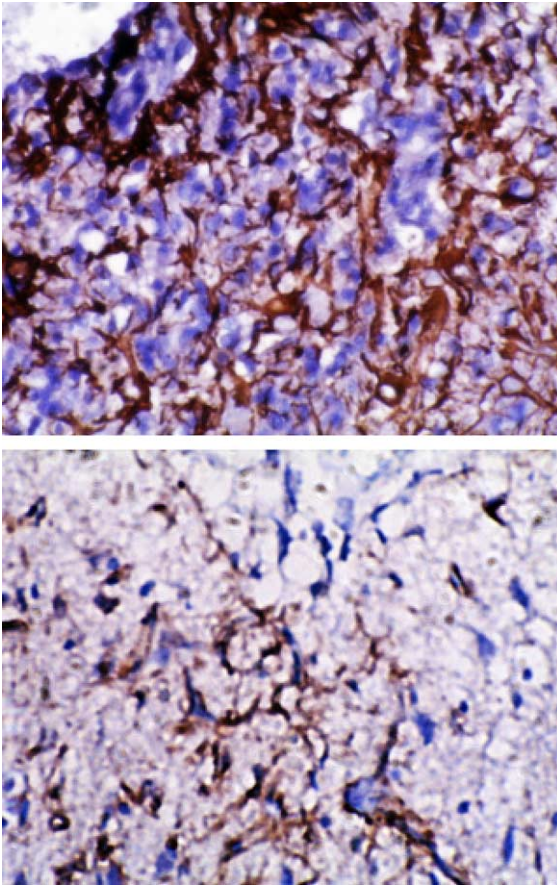
Fig. 4 Images taken from the coronal sections of a BioGlue rat (top) and a sham rat (bottom) that have undergone immunohistochemical analysis for the antigen, GFAP. Increased GFAP staining is seen by the areas of brown staining in both panels (magnification: 400×).

for vascular repair in Europe in 1998. Since then it has received approval for mainly vascular, pulmonary and soft tissue repair in countries such as Canada, USA, Australia as well as throughout Europe. We have previously reported its use in neurosurgery.[1,2]

The BioGlue was well tolerated and there were no visible physiological or neurological effects observed over the 2-week period. Histologically, a local inflammatory cell infiltrate was present in the rats that were subjected to BioGlue application. The inflammation was only minimal when compared to the sham rats which generally did not show signs of inflammation unless there was mechanical disruption of the brain during removal of the dura. We believe that the inflammatory response in the BioGlue rats is related to the time from application of the BioGlue as in general terms, acute inflammation would be seen for up to 10 days post-application. After this time, the inflammatory cell infiltrate would be composed or more chronic than acute inflammatory cells. Inflammation was also confined to the pia-arachnoid layer and did not extend into the brain parenchyma unless there was evidence of mechanical disruption. Minimal inflammation through the use of BioGlue was also noted by Hewitt et al.[12] in the repair of thoracic aorta in a sheep model. On histological examination of the tissue explanted after 3 months, the BioGlue was relatively inert and demonstrated a minimal inflammatory response. This is in contrast to Agarwal et al.[13] who used synthetic cyanoacrylate and gelatine. They both initiated an intense inflammatory reaction on canine dura and the intense inflammatory reaction persisted until the 11th week. Calcification was also seen following the use of these synthetic substances until the last observation at 11 weeks.

We did observe dystrophic calcification in one rat from the A group (BioGlue) but this was accepted as a normal by-product of wound healing by the senior neuropathologist.

GFAP is a member of the class III intermediate filament protein family. It is heavily and specifically expressed in astrocytes and certain other astroglia in the central nervous system, in satellite cells in peripheral ganglia, and in non-myelinating Schwann cells in peripheral nerves. Cellular damage to the brain may result in reactive gliosis as a result of the activation of the damaged glial cells. GFAP staining was present in both BioGlue and sham rats with a significant increase when comparing the positively stained areas of both groups. Overall, the staining was minimal and did not progress considerably beyond the BioGlue–brain interface.

CONCLUSIONS

The 2-week pilot study on the biocompatibility of BioGlue with rat cerebral cortex tissue can be summarized by the following points: all rats tolerated the surgical procedures well, remained in excellent general health throughout their post-operative course, were neurologically intact throughout the study and no seizures were noted; BioGlue applied directly to the exposed surface of the normal brain parenchyma may result in a minimal inflammatory response that is seen only on the surface of the brain, unless there is mechanical disturbance of the cortex below; activated glial cells expressing GFAP resulting in gliosis were present in higher numbers in the BioGlue rats adjacent to the area of the brain that was in contact with the BioGlue but the overall induction was minor; no necrosis of brain tissue was observed in any BioGlue rat.

BioGlue appears to cause only a minimal inflammatory response in cerebral cortex but further time points need to be investigated to monitor the inflammatory response.

REFERENCES

1. Kumar A, Maartens NF, Kaye AH. Reconstruction of the sellar floor using BioGlue following transphenoidal procedures. J Clin Neurosci 2003; 10: 92–95.
2. Kumar A, Maartens NF, Kaye AH. Evaluation of the use of BioGlue® in neurosurgical procedures. J Clin Neurosci 2003; 10: 661–664.
3. Bachet J, Gigou F, Laurian C, Bical O, Goudot B, Dubois C, Brodaty D, Guilmet D. Four year clinical experience with gelating-resorcinal formol glue in acute dissections of the ascending aorta. Arch Mal Couer Vaiss 1983; 76: 87–94.
4. Sierra DH, Nissen AJ, Welch J. The use of fibrin glue in intracranial procedures: preliminary results. Laryngoscope 1990; 100: 360–363.
5. Suzuki Y, Kurada Y, Morita A, Fujino Y, Tanioka Y, Kawamura T, Saitoh Y. Fibrin glue sealing for the prevention of pancreatic fistulas following distal pancreatectomy. Arch Surg 1995; 130: 952–955.
6. Shaffrey CI, Spotnitz WD, Shaffrey ME, Jane JA. Neurosurgical applications of fibrin glue: augmentation of dural closure in 134 patients. Neurosurgery 1990; 26: 207–210.
7. Nistor RF, Chiari FM, Maiser H, Hehl K. The fixed combination of collagen with components of fibrin adhesive: a new hemostyptic agent in skull base procedures. Skull Base Surg 1997; 7: 23–30.
8. Sawamura Y, Terasaka S, Ishii N, Tada, Abe H. Osteoregenerative lateral suboccipital craniectomy using fibrin glueir. Acta Neurochir (Wien) 1997; 139: 446–451.
9. Schlag MG, Hopf R, Redl H. Convulsive seizures following subdural application of fibrin sealant containing tranexamic acid in a rat model. Neurosurgery 2000; 47: 1463–1467.
10. Lee KR, Drury I, Vitarbo E, Hoff JT. Seizures induced by intracerebral injection of thrombin: a model of intracranial hemorrhage. J Neurosurg 1997; 87: 73–78.

11. Herrera O, Kawamura S, Yasui N, Yoshida Y. Histological changes in the rat common carotid artery induced by aneurysmal wrapping and coating materials. Neurol Med Chir (Tokyo) 1999; 39: 134–140.

12. Hewitt CW, Marra SW, Kann BR, Tran HS, Puc MM, Chrzanowski Jr FA, Tran JL, Lenz SD, Cilley Jr JH, Simonetti VA, DelRossi AJ. BioGlue surgical adhesive for thoracic aortic repair during coagulopathy: efficacy and histopathology. Ann Thorac Surg 2001; 71: 1609–1612.

13. Agarwal A, Varma A, Sarkar C. Histopathological changes following the use of biological and synthetic glue for dural grafts: an experimental study. Brit J Neurosurg 1998; 12: 213–216.