High efficacy of tetra-PEG hydrogel sealants for sutureless dural closure

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ABSTRACT

Advances in meticulous dural closure technique remain a great challenge for watertight dural closure in the aged society, because the cerebrospinal fluid (CSF) leakage after spinal surgery is often accompanied with the disgusting wound infection, meningitis and pseudomeningocele. Here, a tetra-poly (ethylene glycol) (PEG)-based hydrogel sealant is developed with collective advantages of facile operation, high safety, quick set time, easy injectability, favorable mechanical strength and powerful tissue adhesion for effective sutureless dural closure during the surgery procedure. Impressively, this tetra-PEG sealant can instantaneously adhere to the irregular tissue surfaces even in a liquid environment, and effectively prevent or block off the intraoperative CSF leakage for sutureless dural closure and dura regeneration. Together, this sutureless tetra-PEG adhesive can be utilized as a very promising alternative for high-efficient watertight dural closure of the clinical patients who incidentally or deliberately undergo the durotomy during the spinal surgery.

1. Introduction

Urgent and effective watertight dural closure of preventing cerebrospinal fluid (CSF) leakage during spinal surgeries is significant important, because the dura has no chance to heal itself once cerebrospinal fluid leakage occurs, and CSF leakage can flow into extradural space to lead to the annoying neurological death [1]. In addition, continuous CSF leakage can also bring about many troublesome complications like wound breakdown, incision infection, arachnoiditis and epidural abscess, thus increasing the risk of morbidity and mortality [2–4]. The incidence of postoperative CSF leakage ranged from 1% to 21% depending on surgical technique, operative location, prior spinal surgery, etc [5–7]. Thus, when there is a dural incision intraoperatively, either intentional or incidental, watertight closure is essential to minimize the risk and potential sequelae of cerebrospinal fluid leakage.

To date, dural repairs were accomplished with many strategies including sutures, adhesives, hydrogels and dural substitutes. Direct suture was a most classical common dural closure technique during spinal surgery, but it could be theoretically elusive to achieve watertight closure through simply direct suture since the microscopic pinholes by the suture needle was inevitable, and it is very difficult to sew the dura after cerebrospinal fluid leakage in anterior cervical surgery [8–10]. In addition, autologous, allogeneic or xenogeneic collagenous connective tissue transplantation and synthetic connective tissue transplantation were frequently applied for the clinical setting, but they always possessed many potential disadvantages, such as nerve compression, insufficient closure and toxicity [11–14]. Although some off-label biological hydrogel products (fibrin glues, gelatin, collagen sponges, etc.) had also been applied in the dural sealing, they possessed insufficient tissue adhesion, difficulty of gel formation in liquid environment, fast degradation, excessive swelling ratio and troublesome preparation [15–17]. Additionally, as a typical representative of biological adhesives, DuraSeal Dural Sealant System is a commercialized synthetic polyethylene glycol (PEG) hydrogel sealant for watertight dural sealing in spinal surgery, but it was only worked after the primary repair with suturing. The primary closure was enhanced via covering the small
pores around the sutures with the absorbable gels to provide a more definite epidural seal [18]. In other words, it can’t be used alone as a sutureless dural sealant in clinical practice. In fact, its dural closure effect was still highly relied on the primary suture so far [19–21].

All the above methods were based on primary suture of dura mater, but intraoperative sutures required highly surgical techniques with risk of injuring spinal cord. Moreover, when a dural tear or defect occurred on ventral side of the dura or at branch of the nerve root, it may not be achievable for suture [22]. Based on the universal prevalence of dural defects and high requirement of immediate watertight closure during the spine surgery, sutureless dural repair appeared promising because it could significantly shorten operation time, reduce surgery risks and overcome difficulties related to the sutural pinholes without tediously labor-intensive suturing procedures [23]. Consequently, an ideal sutureless dural sealant should be able to be quick set time, easy to use, minimal expansion, competitively intrinsic strength, strong and persistent adhesion to the dura, suitable biodegradation and low toxicity or foreign body reaction, which was keenly desirable to prevent cerebrospinal fluid leakage and decrease the incidence of postoperative positional headache and pseudomeningocele.

Herein, we developed a kind of biocompatible tetra-PEG hydrogel as a sutureless sealant by an ammonolysis reaction between the four-armed poly (ethylene glycol) amine (tetra-PEG-NH₂) and four-armed poly (ethylene glycol) succinimideactivated ester (tetra-PEG-SS). These two components approved by the Food and Drug Administration (FDA) were entirely synthetic without other transferring disease. Once they were synchronously sprayed onto the dura mater tissues, amine groups of tetra-PEG-NH₂ polymer were quickly reacted with N-hydroxysuccinimide (NHS)-ester of tetra-PEG-SS polymer to generate the crosslinking amide bonds within seconds. Importantly, tetra-PEG-SS aqueous solution could also fill into the cavities of irregular surface of dura mater tissues that was enriched with numerous collagen fibers, elastic fibers and fibroblasts arranged in a parallel form [24], and therefore the amine groups of proteins in the dura mater tissue simultaneously reacted with tetra-PEG-SS polymer to offer the covalent adhesion onto dura mater and withstand the CSF pressure without additional suture (Fig. 1). Notably, this novel sutureless tetra-PEG sealant was able to maintain strong adhesion even under water environment, demonstrating the tissue adherence and compliance to wet tissue, which resolved the exasperating situation that fluctuations in CSF pressure inevitably caused seepage around defect area intraoperatively.

2. Materials and methods

2.1. Materials

Tetra-arm poly (ethylene glycol) (tetra-PEG-OH, \(M_w = 10\ \text{kDa}, \frac{M_w}{M_n} = 1.03\)) and tetra-arm PEG-amine (tetra-PEG-NH₂, \(M_w = 10\ \text{kDa}, \frac{M_w}{M_n} = 1.03\)) were purchased from Xiamen SINOPEG BIOTECH CO., LTD. Succinic anhydride, dimethylamino-pyridine (DMAP), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDCI) and N-hydroxyl succinimide (NHS) were purchased from Energy Chemical. All other reagents were purchased from Beijing Chemical Works without further purification.

2.2. Measurements

NMR spectrum was obtained on a Bruker DRX-400 spectrometer using tetramethylsilane (TMS) as internal reference. Scanning electron microscopy (SEM) image was obtained at acceleration voltage of 5 kV on a JSM-6700 F microscope (JEOL, Japan). The samples were sputter-coated with a thin layer of Pt for 120 s to make the samples conductive before testing.

2.3. Synthesis of tetra-PEG-SS polymer

Tetra-PEG-SS polymer was synthesized according to our previous literature [25]. First, tetra-PEG-OH (0.5 g), DMAP (56 mg) and succinic anhydride (50 mg) were dissolved in 25 mL of dry CH₂Cl₂. The system was stirred for 12 h and then directly washed with 2 M HCl aqueous solution, saturated NaCl aqueous solution and DI water for several times, and dried over anhydrous Na₂SO₄. The final product was further precipitated into diethyl ether for three times to afford white powder of tetra-PEG-COOH under vacuum.

Fig. 1. Schematic illustration of the sutureless tetra-PEG sealant for dural repair.
Then tetra-PEG-COOH (0.5 g), NHS (110 mg) and EDCI (184 mg) were dissolved in 25 mL of dry CH₂Cl₂. The system was stirred for 24 h and then directly washed with 2 M HCl aqueous solution, saturated NaCl aqueous solution and DI water for several times, and dried over anhydrous Na₂SO₄ to afford white powder of tetra-PEG-SS under vacuum.

2.4. Preparation of tetra-PEG hydrogel

The tetra-PEG-NH₂ and tetra-PEG-SS polymers were respectively dissolved in PBS (pH 7.4) in two sample bottles with the concentrations of 15 wt% according to our previous works [25]. Then, the injectable tetra-PEG hydrogel was prepared by mixing two components using a dual syringe. When the solutions were not flowed backward, the time was designated as the gelation time.

2.5. Adhesive strength

The adhesive strength was measured by the porcine skin. Firstly, strips of porcine were prepared with the size of 30 mm × 10 mm. Then, 20 μL of tetra-PEG-NH₂ solutions (150 mg/mL) were applied at the end of porcine strip on an area of 10 mm × 10 mm 20 μL of tetra-PEG-SS solutions (150 mg/mL) were applied at another porcine strip on the same area. Then the solution-coated places of the two porcine strips were attached together, after which the shear strength was performed by the universal tensile machine. The commercialized fibrin glue was used as the control.

2.6. Compression test

For the compression tests, the tetra-PEG hydrogel was prepared in a cylindroid mold with the diameter of 15 mm and height of 7.5 mm. Compression test was measured by the universal tensile machine (3365 Instron, USA). A digital caliper was used to measure the dimensions of the hydrogels before testing.

2.7. Rheological tests

Rheological measurements were carried out on a Thermo Haake Rheometer equipped with cone-parallel plate geometry (35 mm of diameter) at a gap of 0.5 mm. The sample was measured at 25 °C in a frequency range of 100–0.1 rad s⁻¹.

2.8. Adhesion in water environment

To evaluate the adhesive strength of tetra-PEG hydrogels, a dural defect in an ovine model in vitro was constructed. In briefly, a complete ovine dura of about 4 cm × 4 cm was obtained and attached to a 15-mm-diameter of glass tube with a rubber band. A 2–3 mm of hole was made with a Scalpel. Then, the imitated ovine dural defect was repaired using tetra-PEG hydrogel under the water environment. After repair, the glass tube with the dura mater was placed facing down and the water was added with red ink to detect whether the dural defect was watertight closure. Water was then gradually added until the dural defect cannot be sutured watertight, and the height of the water column when the water leaks from the hole was defined as the burst pressure.

2.9. Swelling behavior in vitro

The tetra-PEG hydrogels (5 mm in diameter and 6 mm in height) were immersed in 50 mL of PBS solution and incubated at 37 °C to evaluate their swelling behavior. To calculate the swelling ratio, the wet weights (Wₛ) of the swollen hydrogel were measured at each specific time point (1, 2, 3, 5 and 7 days) referred to as the initial weight of the hydrogel (Wₒ) after gelation, and the formula of swelling ratio using the following equation:

\[ \text{Swelling ratio (\%)} = \left( \frac{W_S - W_O}{W_O} \right) \times 100\% \]

2.10. In vitro cytotoxicity

The CCK-8 assay of fibroblasts was used to study the cytotoxicity of tetra-PEG hydrogels. Tetra-PEG extract was obtained after immersing tetra-PEG hydrogel (1.5 g) in cell culture medium (10 mL) for 24 h. The 3T3 mouse fibroblasts (Chinese Cell Line Resource Infrastructure) in the logarithmic growth phase were suspended in the cell culture medium. The density of cell suspension was adjusted to 1 × 10⁵ cells/100 μL, and 100 μL of cell suspension per well was seeded in a 96-well cell culture plate. The plates were cultured at 37 °C for 24 h in a 5% CO₂ humidified incubator. Then, the cells cultured with the tetra-PEG extract instead of the fresh cell culture medium were used as the tetra-PEG group, and the cells cultured in fresh medium were used as the control group. After the cells were cultured for the preset time (1 and 2 days), 10 μL of acetone containing CCK8 (1 mg/mL) was added per well, and the cell were incubated for an additional 2 h. Finally, the absorbance of sample solution was detected at 450 nm, with 6 replicate holes in each group, and the experiment was repeated 4 times. The ratio between the mean absorbance values of the tetra-PEG group and the control group was the relative cell viability.

2.11. Immunofluorescence staining of extracellular collagen

Fibroblast cells were seeded into the hydrogels in glass bottom culture plate at a density of 4.0 × 10⁵ cells/1 mL. The samples were placed at 37 °C in a 5% CO₂ humidified incubator. The culture medium was replaced every second day. After cultured for 1, 2 and 3 days, cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. The samples were then exposed to primary rabbit monoclonal antibody against COL-I in PBS (1:250 dilution, ab260043, abcam) overnight at 4 °C. Goat anti rabbit IgG (1:1000 dilution, ab150077, abcam) was used as the secondary antibody. The cell nucleus was stained with DAPI and scanned using fluorescence microscope.

2.12. Surgical procedure

All the animal experimental procedures were approved by the Animal Research Committee of Peking University (Permit number: LA2021190) and we confirmed that all methods were performed in accordance with the relevant guidelines and regulations. In total there were 17 adolescent male New Zealand white rabbits weighing 3.5–4.0 kg that were anesthetized using a ketamine hydrochloride (50 mg/kg, IM) and fentanyl (0.17 mg/kg, IM). A rabbit duraplasty model was conducted as previously described [26]. In brief, a middle skin incision was made to expose L4-6 laminae. The total laminectomy of L4-5 was performed to expose the dura mater. A 5 mm-length defect of dura mater was created, and further repaired using commercial biological membrane (BM) or tetra-PEG hydrogel. Those without any dural sealant implantation was used as control group. The wounds were sutured well and the prophylactic antibiotic together with the analgesics was given for three days.

2.13. Biodegradation and swelling rate in vivo

T2-weighted MR images of surgical site were applied at specific time interval postoperatively (0, 1, 2, 3, 5, 7, 9, 14 and 21 days) to evaluate the biodegradation time and swelling behavior in vivo. Irregularly shaped high intensity solids on T2-weighted images were considered as tetra-PEG hydrogels. Therefore, we measured the longest diameter of the PEG hydrogel in the mid-sagittal position of the spine. The Ld was defined as the longest diameter of the PEG hydrogel at d days.
postoperatively, and the formula of swelling ratio using the following equation:

\[ \text{Swelling ratio} (\%) = \frac{(L_f-L_0)}{L_0} \times 100\% \]

2.14. Postoperative CSF leakage

T2-weighted MR images of surgical site were applied for determining the presence of CSF leakage at 4 weeks and 6 weeks postoperatively. Epidural effusions areas showing high intensity on T2-weighted images were considered as CSF leakage.

2.15. Macroscopic assessment of epidural scar adhesions

Macroscopic assessment was performed at 6 weeks postoperatively. Three rabbits from each group were sacrificed by euthanasia. The surgical site was reopened and epidural scar adhesions were assessed in a double-blind manner based on the Rydell classification (Table S1, Supporting Information).

2.16. Histological analysis

Histological analysis was assessed at 6 weeks postoperatively as previously described [27]. In brief, the harvested specimens were washed by the saline, fixed in 10% neutral buffered formalin for 72 h and then decalcified for 1 week at room temperature. After complete decalcification and dehydration, the tissues were embedded with paraffin and horizontally sectioned in 5 μm of thick slices, and H&E and Masson’s trichrome staining were processed. These slides were made with a digital camera (NanoZoomer-SQ; Hamamatsu Photonics K.K., Hamamatsu, Japan).

2.17. Statistical analysis

Data are presented as the mean ± standard deviation (SD). Differences between two groups or multiple groups were statistically compared with unpaired Student t-test and analysis of variance (ANOVA), respectively. Significance levels were set at *p < 0.05, **p < 0.01, and ***p < 0.001. All data were analyzed using SPSS software (v.19.0).

3. Result and discussion

Tetra-PEG-SS polymer was feasibly prepared in two steps according to the previous work [25]. All the peaks in the FTIR spectrum (Fig. S1, Supporting Information) were attributed to the polymeric structure and the integration ratio \( \frac{I_a}{I_b} \) in the \(^1\)H NMR spectrum (Fig. 2A) was extremely close to 2:1:1:1, verifying the intact structure of tetra-PEG-SS polymer. In views of intrinsic properties of ammonolysis reaction between the amine and active ester groups, an injectable tetra-PEG hydrogel was quickly formed within 12 s through mixing the tetra-PEG-NH₂ and tetra-PEG-SS solutions using a dual syringe (Fig. S2, Supporting Information). Besides for the high biocompatibility and beneficial porous structures for nutrient exchange and cell growth (Fig. 2B), they possessed powerful adhesive force onto the tissue via the formation of chemical linkage among the amine groups of proteins within the tissue with NHS-activated tetra-PEG-SS polymer. Since the surface shape of hole in the dura was irregular, tetra-PEG hydrogels were advantageous to cover the whole wound rapidly and evenly by simultaneously spraying two components onto the tissue that was suitable for the intraoperative operation, which was confirmed by the rheology tests (Fig. 2C). The larger elastic modulus (‘G’) than viscous modulus (‘G”) proved the formation of hydrogel after mixing the two components. Although the average adhesive strength of tetra-PEG hydrogel (19.8 ± 4.5 kPa) was close to the commercialized fibrin glue (16.3 ± 4.3 kPa), they had a higher compression stress of 0.82 ± 0.22 MPa than fibrin glue (0.12 ± 0.06 MPa) (p < 0.05) in Fig. 2D and E, demonstrating the good stability and strong adherence for potential usage.

Another noteworthy advantage of tetra-PEG hydrogel was its capacity to maintain outstanding adhesion even in underwater environment, which was significantly important for the sutureless sealant to overcome the fluctuations in CSF pressure that inevitably caused the seepage around the defect area intraoperatively. Effective closure of the dural holes was achieved using tetra-PEG sealant without any compression in underwater environment by an ovine model (Fig. 2F and G, Fig. S3 and Videos S1 and S2 Supporting Information). The results showed that the burst pressure of tetra-PEG sealant was more than 500 mm of water (500 mmH₂O), significantly larger than normal range of CSF pressure (80–180 mmH₂O). It was mentioned that if the dural hole was adhered in the air, its burst pressure could reach 900 mmH₂O (Fig. 2H). Although there were currently some dural substitute claimed
to be used sutureless [23,28], almost all clinicians preferred to use them as adjuvant to suture in actual conditions. The main concern was that even though temporary watertight closure was obtained intraoperatively, delayed CSF leak may occur when the CSF pressure suddenly fluctuated postoperatively, especially the cerebrospinal fluid pressure can be up to 250 mmH\textsubscript{2}O when sneezing, coughing and emesis [29]. The powerful tissue adhesion of tetra-PEG hydrogel surpassed the upper limit of normal CSF pressure (250 mmH\textsubscript{2}O) when sneezing, which enabled itself to withstand the pressure of normal cerebrospinal fluid for the application of sutureless dural closure (Fig. S4, Supporting Information).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bioactmat.2021.06.022

The main problem with the application of a dural sealant is spinal cord compression due to the swelling of the residual hydrogel [30,31]. Therefore, we have performed the water absorption both in vitro and in vivo. As shown in Figs. S5–S7, the swelling rate of tetra-PEG hydrogel in vitro was 200–540% while that of less than 60% in the dura defect model in vivo, which was attributed to the great influence of aquatic environment around tissues and organs on the swelling behavior and biodegradation time of tetra-PEG hydrogels [25]. Actually, the maximum swelling rate appeared on the first day postoperatively, and then gradually decreased over time because of the initiate of hydrolysis. The size was almost the same as the original at 9 days postoperatively. At 21 days postoperatively, the tetra-PEG hydrogel was completely degraded in vivo. In all postoperative MR images, without occurrence of spinal cord compression by swelling of tetra-PEG hydrogel, and all of animals did not show up any symptoms of the lower limbs (Fig. S6, Supporting Information).

To the best of our knowledge, PEG hydrogel is approved by the FDA, non-toxic, and used extensively in engineering scaffold applications. In order to investigate its biocompatibility of tetra-PEG hydrogel, cytotoxicity assay was carried out through the 3T3 mouse fibroblasts. As it demonstrated in Fig. S8, fibroblasts showed high viability. Quantitatively, tetra-PEG not only maintained but also improved cell viability compared to the control group at the early stage of culture (1 day), revealing the low cytotoxicity on fibroblasts. During the dural healing process, fibroblasts play a vital role in it and it mainly secretes the type I collagen (COL-I), which is the major component of extracellular matrix (ECM) in the dura mater. Immunofluorescence studies were conducted to investigate the total COL-1 in fibroblasts on day 1, 2 and 3 after fibroblasts seeding on the tetra-PEG hydrogel. These results showed that fibroblasts could indeed grow into the tetra-PEG hydrogel benefiting from its porous structure (Fig. S9, Supporting Information).

To evaluate the efficacy of adhesives in the treatment of dural closure, tetra-PEG hydrogel was used to adhere the durotomy site using a rabbit duraplasty model in Fig. 3A. It was intuitively observed that tetra-PEG hydrogel was rapidly gelled and tightly blocked off to dural defect intraoperatively (Video S3, Supporting Information). As a control, the commercial BM exhibited the failure plugging effect because of the

Fig. 3. (A) Pictures showing the surgical procedure of sutureless dural closure using a rabbit duraplasty model. a: spinal cord at L4-5 level was clearly exposed during the operation. b: a 5-mm length defect of dura mater was performed, and those without additional treatment were used as control group. c: those with commercial BM applied at the durotomy site were used as BM group. d: those with tetra-PEG hydrogel applied at the durotomy site were used as tetra-PEG group. MRI images of CSF leakage in the laminectomy sites at (B) 4 weeks and (C) 6 weeks. The laminectomy sites were labelled by yellow dashed frame on sagittal images. The red arrow in sagittal and transverse images indicated the CSF leakage, and the blue arrow indicated the edema. Bar = 1 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
impaired physical interaction and weak adhesion strength with the tissues in the presence of leaky cerebrospinal fluid. Compared to many traditional approaches that should require the cumbersome tissue transplantation from a nearby site or longer preparation times [32], this tetra-PEG sealant could reduce the rate of cerebrospinal fluid leakage, the risk of wound infection and the overall incidence of surgical complications, demonstrating its unique advantages on faster preparation, easy usage and highly efficient for sutureless dural closure. Therefore, this covalently linked dural sealant was mechanically tough enough to not only meet the requirements of sutureless sealant during the surgery but also maintain stability during the dynamic movement of paraspinal muscles postoperatively.

Whether postoperative cerebrospinal fluid leakage occurs is the key indicator to evaluate the success of dural repair. To observe the CSF leakage, magnetic resonance imaging (MRI) was performed at 4 weeks and 6 weeks postoperatively. After surgery for 4 weeks, both the control group and the BM group formed a large posterior effusion like the cerebrospinal fluid, presenting high intensity signals in T2. The homogeneous (low complexity) fluid collection had direct communication with the thecal sac, which was specific MRI characteristics of CSF leakage [33]. However, tetra-PEG hydrogel group showed less high-intensity signals in T2 and the heterogeneous signals had no communication with the thecal sac (Fig. 3B), which was consistent with the signs of paraspinal muscle edema postoperatively in clinical [34]. Similar results were observed at 6-week postsurgery (Fig. 3C). In the control and BM group, obvious CSF leakage was formed in the MRI images, which was identified as homogenous high-intensity signals in T2 without delineation to thecal sac. For the tetra-PEG hydrogel group, only mild paraspinal muscle edema and obvious interstitial space between thecal sac and surrounding muscles were observed, convincingly demonstrated the sutureless tetra-PEG sealant could effectively prevent the occurrence of postoperative CSF leakage in vivo.

The desired outcome of dural prosthetics using a dural sealant is to facilitate dural regeneration and prevent epidural scar adhesion and spinal cord compression caused by residual materials [35]. After MRI evaluations, these rabbits were sacrificed at 6-week postoperatively and the degree of epidural scar adhesion was assessed using the gross evaluation. There was a decrease in epidural adhesion scores in tetra-PEG hydrogel group compared to the control and BM groups (Fig. S10, Supporting Information). The regeneration of dura and inhibition of extradural scar adhesion were evaluated using the hematoxylin eosin (H&E) staining and Masson’s trichrome staining (Figs. 4 and 5 and Fig. S11, Supporting Information). At 6 weeks after implantation, dural incision in tetra-PEG hydrogel group was healed to some extent. Although there was a little epidural fibrous tissue in the defect region, an intact dura-like fiber circle was obviously regenerated with an adequate subarachnoid space. The spinal cord’s morphology was observed to be almost natural without any residual tetra-PEG hydrogels. In comparison, both the control and BM groups had obvious dural defects by formation of extradural fibrous tissue, and the morphology of spinal cord was significantly altered by the adhesion of epidural scar. Macroscopic and histological evaluation confirmed that this sutureless tetra-PEG sealant was effective in terms of dural healing promotion and complication prevention.

4. Conclusion

In summary, we reported a sutureless tetra-PEG sealant for effective

![Fig. 4. H&E staining of laminectomy site at 6 weeks. Wherein, SC: spine cord, EF: epidural fibrosis and NB: new bone. The black arrow indicated the neodura tissue. The yellow arrow indicated fibrous hyperplasia with almost no subarachnoid spaces and the red arrow indicated adequate subarachnoid spaces. The images (D, E and F) represented the magnifications of yellow dashed frame in the uplink images (A, B and C) while the images (G, H and I) represented magnifications of yellow dashed frame in the uplink images (D, E and F). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image-url)
watertight closure, which combined high mechanical performance (compressive strength and bursting pressure) and outstanding tissue adherence with quick set time, feasible injectability, good biocompatibility and biodegradability. In vivo experiments demonstrated the effectively sealing the defect of dural mater even in underwater environment. In terms of safety, effectiveness, availability, cost and acceptability, we believe this tetra-PEG hydrogel sealant was an optimized choice for universal prevalence of dural defects and high requirement of immediate watertight closure during the spine surgery. It may provide more advantages to other clinically surgical techniques because it can take less time to prepare and use for the effective watertight dural closure in patients undergoing an accidental or intentional durotomy during the spinal surgery. Additionally, on account of its excellent injection performance, high mechanics, favorable biocompatibility and suitable degradability, this sutureless sealant can also be widely exploited to other bioadhesive applications of skin tissue, cartilage, meniscus, achilles tendon and transected peripheral nerve repair during the surgical operation.

CRediT authorship contribution statement

Tengjiao Zhu: Conceptualization, Investigation, Methodology, Validation, Funding acquisition, Writing – original draft. Hufei Wang: Conceptualization, Investigation, Methodology, Validation. Zehao Jing: Investigation, Methodology, Validation. Daoyang Fan: Investigation, Methodology, Validation. Zhongjun Liu: Conceptualization, Supervision. Xing Wang: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. Yun Tian: Validation, Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2021.06.022.

References

[1] N. Kögl, L. Schenk, C. Presas Hernandez, C. Thomé, O. Petr, Bed rest after incidental durotomy in lumbar surgery. A redundant measure in case of watertight dural repair, J Neurosurg Sci (2020 Nov 27), https://doi.org/10.23736/S0390-5616.20.05143-7, Epub ahead of print. PMID: 33245223.
[2] L. Ter Hout, M.C. Brussev, A. van der Ende, D. van de Beek, Community-acquired bacterial meningitis in adults with cerebrospinal fluid leakage, Clin. Infect. Dis. 70 (11) (2020) 2256–2261.
[3] S.M. Barber, J.S. Fridley, S. Konakondla, J. Nakhl, A.A. Oyelese, A.E. Telfeian, Z.L. Gokaslan, Cerebrospinal fluid leaks after spine tumor resection: avoidance, recognition and management, Ann. Transl. Med. 7 (10) (2019) 217.
[4] K.D. Kim, D. Ramanathan, J. Highsmith, W. Lavelle, P. Gerszten, F. Vale, N. Wright, DuraSeal exact is a safe adjunctive treatment for durotomy in spine: postapproval study, Global Spine J. 9 (3) (2019) 272–278.

Fig. 5. Masson’s trichrome of laminectomy site at 6 weeks. The black asterisk indicated residual dura mater. The black arrow indicated neodura tissue. The yellow arrow indicated the fibrous hyperplasia with almost no subarachnoid spaces and the red arrow indicated the adequate subarachnoid spaces. The images (D, E and F) represented the magnifications of yellow dashed frame in the uplink images (A, B and C) while the images (G, H and I) represented the magnifications of yellow dashed frame in the uplink images (D, E and F). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
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[5] S.K. Menon, C.U. Ozynia, A short review on a complication of lumbar spine surgery: CSF leak, Clin. Neurol. Neurosurg. 139 (2015) 248–251.

[6] D. Hannallah, J. Lee, M. Khan, W.F. Donaldson, J.D. Kang, Cerebrospinal fluid leaks following cervical spine surgery, J Bone Joint Surg Am 90 (5) (2008) 1101–1105.

[7] A.P. Wong, P. Shih, T.R. Smith, N.P. Slimack, S.G. Aoun, T.R. Smith, N.P. Slimack, N.S. Dahdaleh, P.J.M. Bouten, M. Zonjee, J. Bender, S.T.K. Yauw, R. Hoogenboom, The effectiveness of dural sealants for the management of symptomatic cranial dural leakage with subdural hematoma, World Neurosurg 81 (3–4) (2014) 634–640.

[8] F. Xu, F. Wu, R. Zhou, L. Guo, J. Zhang, D. Tao, Current developments in dural repair: a focused review on new methods and materials, Front. Biosci. 18 (2013) 1335–1343.

[9] J. Zhai, B.B. Panchal, Y. Tian, S. Wang, L. Zhao, The management of cerebrospinal fluid leak after anterior cervical decompression surgery, Orthopedics 41 (2) (2018) e283–e288.

[10] G.R. Cosgrove, J.B. Delashaw, J.A. Grotenhuis, J.M. Tew, H. Van Loveren, R. Byrne, A. Naborah, Safety and efficacy of a novel polyethylene glycol hydrogel sealant for watertight dural repair, J. Neurosurg. 106 (1) (2007) 52–58.

[11] J. Parizek, V. Mericka, Z. Husek, P. Soba, J. Spacek, S. Nemecek, J. Nemeckova, J. Zhai, R.R. Panchal, Y. Tian, S. Wang, L. Zhao, The management of cerebrospinal fluid leakage after intradural surgery: current status and future perspectives, Expert Rev. Med. Dev. 16 (7) (2019) 549–553.

[12] J.H. Guo, J.J. He, J.J. Li, Z.L. Sun, Y.H. Zhang, Y.H. Tu, S.Z. Wu, F.G. Huang, H.Q. Xie, Efficacy and safety of small intestinal submucosa in dural defect repair: a prospective, multicenter, randomized controlled study, Spine 40 (13) (2015) 3075–3080.

[13] K.E. Radcliff, G.D. Sidhu, C.K. Kepler, J. Gruskay, D.G. Anderson, A. Hilibrand, T. Payner, G. Rosseau, M.E. Shaffrey, L.N. Hopkins, R. Byrne, A. Ayyad, N. Simmons, Spinal sealant system provides better intraoperative watertight closure than standard of care during dural closure, J. Neurosurg. Spine 12 (4) (2010) 381–390.

[14] S.K. He, J.H. Guo, Z.L. Wang, Y. Zhang, Y.H. Tu, S.Z. Wu, F.G. Huang, H.Q. Xie, Detailed evaluation of 2959 allogeneic and xenogeneic dense connective tissue grafts (fascia lata, pericardium, and dura mater) used in the course of 20 years for duralplasty in neurosurgery, Acta Neurochir. 139 (9) (1997) 927–936.

[15] K.M. Preul, P.K. Campbell, D.S. Garlick, R.F. Spetzler, Application of a new model, Mater Sci Eng C Mater Biol Appl 73 (2017) 267–273.

[16] S.K. Menon, C.U. Ozynia, A short review on a complication of lumbar spine surgery: a prospective, multicenter, randomized controlled study, Spine 40 (5) (2015) 575–582.

[17] S.K. He, J.H. Guo, Z.L. Wang, Y. Zhang, Y.H. Tu, S.Z. Wu, F.G. Huang, H.Q. Xie, Efficacy and safety of small intestinal submucosa in dural defect repair: a prospective, multicenter, randomized controlled study, Spine 40 (13) (2015) 3075–3080.

[18] J. Albert, A.R. Vaccaro, Complications of flat bed rest after incidental durotomy, World Neurosurg 127 (2019) 567–574.

[19] J. Parizek, V. Mericka, Z. Husek, P. Soba, J. Spacek, S. Nemecek, J. Nemeckova, J. Zhai, R.R. Panchal, Y. Tian, S. Wang, L. Zhao, The management of cerebrospinal fluid leakage after intradural surgery: current status and future perspectives, Expert Rev. Med. Dev. 16 (7) (2019) 549–553.

[20] Z.A.F. Alshameeri, A. El-Mubarak, E. Kim, V. Jasani, A systematic review and meta-analysis on the management of accidental dural tears in spinal surgery: drowning in information but thirsty for a clear message, Eur. Spine J. 29 (7) (2020) 1671–1685.

[21] E.H. Choi, A.Y. Chan, N.J. Brown, B.V. Lien, R. Sabyouni, A.K. Chan, J. Roufail, M. Y. Oh, Effectiveness of repair techniques for spinal dural tears: a systematic review, World Neurosurg 149 (2021) 140–147.

[22] H. Kawai, I. Nakagawa, F. Nishimura, Y. Motoyama, Y.S. Park, M. Nakamura, H. Nakase, S. Suzuki, Y. Ikada, Effectiveness of a new gelatin sealant system for dural closure, Neurosurgery. Res. 36 (10) (2014) 866–872.

[23] K. Deng, X. Ye, Y. Yang, M. Liu, A. Ayyad, Y. Zhao, Y. Yuan, J. Zhao, T. Xu, Evaluation of efficacy and biocompatibility of a new absorbable synthetic substitute as a dural onlay graft in a large animal model, Neurol. Res. 38 (9) (2016) 799–806.

[24] M. Protasoni, S. Sangiorgi, A. Cividini, G.T. Culurivia, G. Torei, C. Dell Orbo, M. Raspanti, S. Balbi, M. Reguzzoni, The collagenic architecture of human dura mater, J. Neurosurg. 114 (6) (2011) 1723–1730.

[25] Y. Bu, L. Zhang, G. Sun, F. Sun, J. Liu, J. Yang, P. Tang, D. Wu, Tetra-PEG based hydrogel sealants for in vivo visceral hemostasis, Adv. Mat. 31 (28) (2019), e1901580.

[26] Y. Yu, W. Cui, Y. Zhang, P. Zhou, Y. Gu, X. Shen, B. Li, L. Chen, Hierarchical micro/nanofibrous bioscaffolds for structural tissue regeneration, Adv Healthc Mater 6 (13) (2017).

[27] Y. Cui, T. Zhu, A. Li, B. Liu, Z. Cui, Y. Qiao, Y. Tian, D. Qiu, Porous particle-reinforced bioactive gelatin scaffold for large segmental bone defect repairing, ACS Appl. Mater. Interfaces 10 (8) (2018) 6956–6964.

[28] M.T. Espiritu, A. Rhyne, B.V. Darden, 2nd, Dural tears in spine surgery, J. Am. Acad. Orthop. Surg. 18 (9) (2010) 537–545.

[29] J. Jito, N. Nitta, K. Nozaki, Delayed cerebrospinal fluid leak after watertight dural closure with a polyethylene glycol hydrogel dural sealant in posterior fossa surgery: case report, Neurol. Med.-Chir. 54 (8) (2014) 633–639.

[30] H.J. Han, J.H. Jeong, J.W. Kim, W.B. Seung, Postoperative thecal sac compression induced by hydrogel dural sealant after spinal schwannoma removal, Korean J. Nutr. 16 (1) (2020) 99–104.

[31] S.H. Lee, C.W. Park, S.G. Lee, W.K. Kim, Postoperative cervical cord compression induced by hydrogel dural sealant (DuraSeal®), Korean J Spine 10 (1) (2013) 44–46.

[32] S. Patel, E.C. Rodriguez-Merchan, F.S. Haddad, The use of fibrin glue in surgery of the knee, J Bone Joint Surg Br 92 (10) (2010) 1325–1331.

[33] K.E. Radcliff, G.D. Sidhu, C.K. Kepler, J. Gruskay, D.G. Anderson, A. Hildbrand, T. J. Albert, A.R. Vaccaro, Complications of flat bed rest after incidental durotomy, Clin Spine Surg 29 (7) (2016) 281–284.

[34] S. Sedaghat, F. Schmitz, J. Meschede, M. Sedaghat, Systematic analysis of post-treatment soft-tissue edema and seroma on MRI in 177 sarcoma patients, Surg Oncol 35 (2020) 218–223.

[35] M.C. Preul, P.K. Campbell, D.S. Garlick, R.F. Spetzler, Application of a new hydrogel dural sealant that reduces epidural adhesion formation: evaluation in a large animal laminectomy model, J. Neurosurg. Spine 12 (4) (2010) 381–390.