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A novel biomimetic composite substitute of PLLA/gelatin nanofiber membrane for dura repairing

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ABSTRACT

Objective: Biomimetic design will significantly improve growth and regeneration of dural cells and tissue for better repairing effects and fewer complications in repairing the native dura. This study designed a novel composite, biomimetic substitute based on the characteristics of native dura extracellular matrix.

Methods and results: This substitute is expected to rapidly induce cell adhesion, migration, and fast regeneration of neotissue. The material characteristics (contact angle, surface charge, and zeta potential were evaluated), in vitro biological characteristics (cell stretch, connections between cells, cell proliferation) and in vivo tissue regeneration capability of this substitute were evaluated, compared to those of collagen dura substitute, the mostly used dura substitute. The results showed that the surface properties of this composite substitute were more biomimetic to native extracellular matrix than collagen substitute did, together with better cytocompatibility, tissue ingrowth, and neoangiogenesis. This composite substitute further demonstrated in clinical case study its ideal repair effect with no CSF leakage or other adverse reactions.

Conclusion: In conclusion, the new biomimetic composite substitute provides alternative substitute for dura repairing.

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KEYWORDS
Dural substitute; composite substitute; collagen substitute; tissue regeneration

1. Introduction

Dura mater, the outermost of three layers of meninges surrounding the brain and spinal cord, can be damaged by trauma, surgery or tumor intrusion [1,2]. Both synthetic polymer and naturally derived collagen matrix have been used as biomaterials for dural repair. Dural closure is one of the most critical steps in neurosurgical procedures to prevent CSF leakage, infection, and herniation of neural content [3–5]. Although primary suture closure of native dura is the preferred method, tissue injury, loss or shrinkage sometimes makes this approach impossible [6,7]. A variety of biomaterials have been tested to optimize dural closure and the biomaterials tested include autograft, allograft, xenograft, and synthetic materials [8–11]. Autografts are nontoxic and integrate rapidly into native tissues [12–14]. However, limited availability and additional incision at the graft harvesting site hinders the use of autografts [9]. Allografts, although more readily available, became less favorable after several reported cases of Creutzfeldt–Jacob disease (CJD) transmission [15].Thus, dural substitutes based on xenografts or synthetic materials are considered to be alternatives for neurosurgeons.

Collagen substitute, one of the most commonly used xenogenic dural substitutes derived from bovine pericardium, bovine Achilles tendon, bovine skin, or porcine small intestinal submucosa, is typically composed of animal extracellular matrix (ECM) rich in collagen processed to remove cells and other immunogenic components [16,17]. Collagen is a chemoattractant for fibroblasts [18], this feature, coupled with the porous structure of the ECM, promotes fibroblast ingrowth and neovascularization [19]. However, collagen is mechanically too weak to maintain the desired shape until newly formed tissue matures and it also degrades rapidly [16,20,21]. Additionally, animal origin complications such as bovine transmissible spongiform encephalopathy added risks to the use of xenografts [22–24].

Bioabsorbable synthetic polymers such as poly (lactic acid) (PLA), poly-3-hydroxybutyrate (PHB), poly (glyclic acid) (PGA), and poly (ε-caprolactone) (PCL), are nontoxic and have tunable degradation properties, thus are frequently used for medical applications [25–27]. Poly(L-lactic acid) (PLLA) has been attracting much attention from the academic viewpoint of structural interest as well as for practical applications [28].

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Synthetic polymers have relatively good mechanical strength with easily modifiable shape and degradation rate. However, they usually have hydrophobic surfaces and lack signals that can be recognized by cells [26].

A synthetic substitute with biomimetic design has been developed recently [29]. Furthermore, this substitute is suggested to be hydrated before use, after hydration, it can be used as both an onlay or suturable substitute [30]. But both the hydration and suturing steps can increase the operation time. Furthermore, due to the large difference between synthetic material and native tissue, a new substitute which has more similar characteristics to native tissue is need. This new substitute can rapidly induce cell adhesion, migration, and induce fast regeneration of neotissue. Since dura repair is associated with high risk, the longer the operation time and the slower new tissue regeneration is, the more risky the operation becomes, and the longer patient recovery time is needed.

To overcome these limitations , a novel composite, biomimetic substitute based on the characteristics of native dura extracellular matrix has been designed. This substitute is expected to rapidly induce cell adhesion, migration, and induce fast regeneration of neotissue. In this study, material characteristics such as contact angle, surface charge, and zeta potential of this composite substitute were compared to and those of a commercially available collagen-based substitute, which were not done previously. Using confocal laser microscopy and other methods, the manuscript focuses on evaluating cell stretch, connections between cells, cell proliferation, and the mechanical properties of the regenerated tissue in the *in vivo* large animal experiment to analyze the difference in tissue repair and to further evaluate the mechanism of the modified surface of the new composite substitute on guided tissue repair. Here we present a prospective study for the discovery of an ideal dural substitute.

2. Materials and methods

2.1. Materials

The composite substitute utilized in this study was Neodura™ (Medprin Biotech GmbH, Germany), which is fabricated with absorbable materials PLLA and gelatin. PLLA and gelatin fibers are deposited layer by layer in a fleece-like structure. The fiber microstructure resembles the extracellular matrix (ECM) of human dura mater. The control group is DuraGen™ Dural Graft Matrix (Integra Life Sciences, Plainsboro, NJ, U.S.A.), a commercially available dural substitute based on collagen. Fluorescein diacetate (FDA), propidium iodide (PI), and thiazolylblue tetrazolium bromide (MTT) were purchased from Sigma. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were acquired from Gibco BRL (Gaithersburg, MD).

2.2. Characterization of physical and mechanical properties

2.2.1. Tensile strength

Tensile strength and elongation were evaluated according to ISO 527-3 standard using electronic tensile testing machine (HY3080, Shanghai Heng Wing Precision Instrument Co. Ltd). 1 cm × 6 cm samples were soaked in distilled water before installed on the tensile testing machine. Five samples per group were tested with an elongation rate of 200 mm/min, the tensile strength and elongation at break were recorded by the testing machine.

2.2.2. Suture retention strength

Suture retention strength was examined following ISO 7198 standard using electronic tensile testing machine (HY3080, Shanghai Heng Wing Precision Instrument Co. Ltd). After infiltrating the samples (1 cm × 3 cm) in distilled water, a No. 4-0 suture was used to thread through the patch, 2–3 mm from the edge to a semi-ring. Samples were tested with a testing speed of 200 mm/min, the suture retention strength was recorded by the testing machine when the suture was pulled out.

2.2.3. Contact angle

The wettability of the experimental and control materials was determined by static contact angle measurement with sessile drop method and digital image analysis using optical contact angle goniometer OCAH200 (Dataphysics, Germany). 1 μl deionized water was dropped onto the two types of materials with surface area of 1×2 cm^2. The average contact angle of five test points for each sample was taken as the measurement result.

2.2.4. Zeta potential

Zeta potential was analyzed by SurPASS Electrokinetic Analyzer (Anton Paar GmbH, Austria). Diluted simulated body fluid (SBF) with 18 mS/m conductivity and an adjustable gap cell was used for the measurement. Samples (10 cm × 20 cm) installed on the blue sample module were loaded in the sample cells and connected with SurPASS analyzer. Gap height was adjusted to 100 ± 5 μm in the rinsing process. Each experiment was repeated three times. Means and corresponding standard deviations (mean ± SD) were shown as results.

2.3. Cytotoxicity of the substitute

2.3.1. Cell culture

L929 mouse fibroblast cells and rabbit mesenchymal stem cells were cultured in DMEM supplemented with 10% FBS, 1% antibiotic and antimycotic solutions at 37 °C in humidified atmosphere with 5% CO_2. Each of the sterilized dural substitutes was soaked in culture medium overnight followed by placing in a 24-well plate before cell seeding. Cells were seeded at 1×10^5/ml in 0.5 ml medium followed by culturing in incubator for different times.

2.3.2. Cytotoxicity test

Following the ISO10993.5-2009 standard, cytotoxicity was evaluated with L929 mouse fibroblast cell line using the MTT assay. Nine samples of each type of substitute, three for each time point, were prepared and extracted, respectively, in the DMEM culture medium for 24 h. The resulting extract (1 ml per one well) was separated into 24-well plates seeded by L929 cells. In each well, 2×10^3 cells were seeded. The same experiment was repeated with DMEM and phenol (5%) as the culturing media, serving as negative and positive controls, respectively. The plates were then placed in the incubator for 1, 3, and 7days, respectively, followed by MTT assay. The plates were read by microplate reader (BIO-RAD Model 550, BIO-RAD, U.S.A.) at a wavelength of 490 nm and the absorbance for each well was recorded. The cytotoxicity was expressed by optical density (OD) value corrected by blank measurements of wells without cells.

2.3.3. Cell viability

The novel composite and collagen substitutes were placed in the 24-well microplate and seeded with passage 3 rabbit mesenchymal stem cells (MSC) at a density of 2×10^3 cells/well. After culturing for 3 or 7days, samples were rinsed with PBS twice followed by double staining with FDA and PI for 5 min at 37 °C. After final washes in PBS, samples were mounted for confocal laser scanning microscope(CLSM) observation (Olympus Fluoview 300, Japan).

2.3.4. Cell morphology

Morphology of the *in vitro* cultured MSC on dural substitutes of different materials was observed. After 3 or 7 days of culturing, the cell-cultured materials were processed for scanning electron microscope (SEM) observation. The materials were rinsed three times with PBS and then fixed with 4% glutaraldehyde for 2 h at 4 °C, followed by three rinses with distilled water. The samples were dehydrated through a series of graded ethanol solutions (30, 50, 70, 90, and 100%, twice in each solution for 15 min each), and then left in a fume hood for air drying. Dried samples were sputter coated with gold and observed by SEM (ESEM XL30 HY-3080, FEI, U.S.A.).

2.4. Animal experiments

2.4.1. Animal model

The biologic responses, remodeling, and performance of materials were evaluated in the canine bilateral duraplasty model. Adult Chinese domestic dogs, provided by Suibei Medical Animal Testing Center (Guangzhou, China) were used in this study. The study was designed in full compliance with the policies and procedures set by Institutional Animal Care and Use Committee of the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). A total of 15 healthy adult dogs, weighted 15–20 kg and aged 6 months were randomly divided into three groups of five dogs, for 2, 4, and 12 weeks observation periods. The dogs included in this study were physically and neurologically normal. Dural substitutes were used to repair standard dura mater defects on the left and right sides of the brain for a total of 30 implanted patches (Left: composite patch, Right: collagen patch). At each time point, the implanted patches were retrieved, evaluated, and processed for histological analyses. Qualitative and quantitative comparisons were made between the composite and collagen patch repaired groups.

2.4.2. Surgical procedure

General anesthesia was induced by slow intravenous injection of 3% pentobarbital sodium (1 ml/kg). Bilateral frontotemporoparietal craniotomies were performed. Briefly, each dog was held to prone position on the operating table with hair shaved off. After top midst scalp was cut vertically, monopolar electrotome was used to cut laterally for 0.5 cm along sagittal suture. Muscle was detached to expose the bilateral temporoparietal calvarium (4×3 cm^2). Two bone flaps (~3.5×2.5 cm^2) were removed by high-speed drill and dura defects (2.5×1.5 cm^2) were created by microscopic scissor. Duraplasties using the composite and collagen devices were performed according to the manufacturers' instructions. Repair patches were cut into proper sizes to completely cover the dural defects, the bone flaps were placed back, followed by closing of the muscle layer. Epidural shunts were placed on both sides and the scalp was closed.

2.4.3. Post-operation observation and tissue biomechanics

At different time points after surgery, the animals were sacrificed and the implanted samples were retrieved. Briefly, the skulls of the dogs were reopened surgically and the implants and surrounding tissues were dissected and removed. The retrieved samples were inspected carefully and two samples, each measured to be 10×10 mm^2 were obtained. One was used for tensile strength test and the other was fixed in formalin solution for histological analyses, including Hematoxylin-Eosin (H&E) staining and Masson Trichrome staining.

2.5. Clinical case study

To further confirm the safety and efficacy of this new device, five clinical cases with a six-month follow-up were studied, those five patients had undergone repair of their dural defects with composite substitute in Zhujiang Hospital (Guangzhou, China). The China Food and Drug Administration (CFDA) and the medical ethics committee of Zhujiang Hospital of South Medical University approved the neurosurgical procedure of the clinical case using the composite substitute.

2.6. Statistical analysis

All results were expressed as mean ± S.D. Statistical analysis was performed using SPSS statistical program version 13 (SPSS Inc., Chicago, IL). Difference between two groups was analyzed by Student's t-test and the difference between three or more groups was analyzed by one-way ANOVA multiple comparisons followed by Tukey–Kramer *post hoc* analysis. Difference with $P <$ 0.05 (*) was considered statistically significant.

3. Results

3.1. Mechanical properties

Basic mechanical properties of the composite substitute and collagen substitute are shown in Figure 1. The average tensile strength of composite substitute was 3.8 ± 0.34 MPa (N = 5), and the average stretching elongation was 84.87 ± 8.45% (N = 5). Average suture strength reached 3.03 ± 0.12 N (N = 5) showing excellent mechanical properties. Attempts to quantify the mechanical properties of the collagen substitute were unsuccessful as the material was too fragile to be tested using the same setup.

3.2. Surface property

Interactions between medical implants and their surrounding tissues are affected by the physicochemical surface properties such as surface chemistry, charge, and topography of the implants [31–33]. Contact angle reflects the wettability of substitute surface. As shown in Figure 2(A), the shape of the water droplets on the two types of patches was similar. The mean contact angles of the composite substitute and collagen substitute was 102° and 98°, respectively, showing no significant difference. This result indicated that both types of substitutes exhibited hydrophobicity, which may be beneficial for the prevention of CSF leakage in the repair of dural defect.

Surface Zeta potential of material is a property that reflects surface charge. The Zeta potential of composite construct was −9.51 mV, and that of collagen composite was 0.34 mV, approaching neutral potential.

3.3. Cytocompatibility

To compare the cytocompatibility of the composite and collagen substitutes, cell proliferation was examined by MTT assay. Generally, optical values of different groups increased during the culturing period and the proliferation trend of the substitute groups was similar to that of the negative groups (Figure 3). OD value of the positive group was extremely low. On day 7, the OD values of composite patch and collagen patch were 1.17 and 1.25, respectively, which were about three times of the values at day 1, indicating that cell proliferated rapidly during the culture process. Composite and collagen samples both had good cytocompatibility, providing suitable growth environment. There was no significant difference between these two materials on promoting L929 cell proliferation.

As one kind of vital stem cells in soft tissue reconstruction, MSCs play important role in the dura repairing process. We characterized the behavior of MSCs after seeding on composite and collagen materials. The normal growth of MSCs was observed at day 3 and 7 by CLSM (Figure 4). Both materials provided proper environment for MSCs adhesion and proliferation. On day 3, most of MSCs appeared to adhere well on both patches. The adhered cells on composite substitute showed round or fusiform morphology under high magnification accompanied with few death cells. Cells on collagen patch mainly displayed round and polygonal shape. On day 7, cell number rose significantly and cell death reduced. MSCs with fusiform or round shape were seen grew densely on collagen scaffold. In contrast, on the composite patch, more cells presented a larger and extended shape with projected affluent pseudopodia which are mainly filopodia, implying that the structure

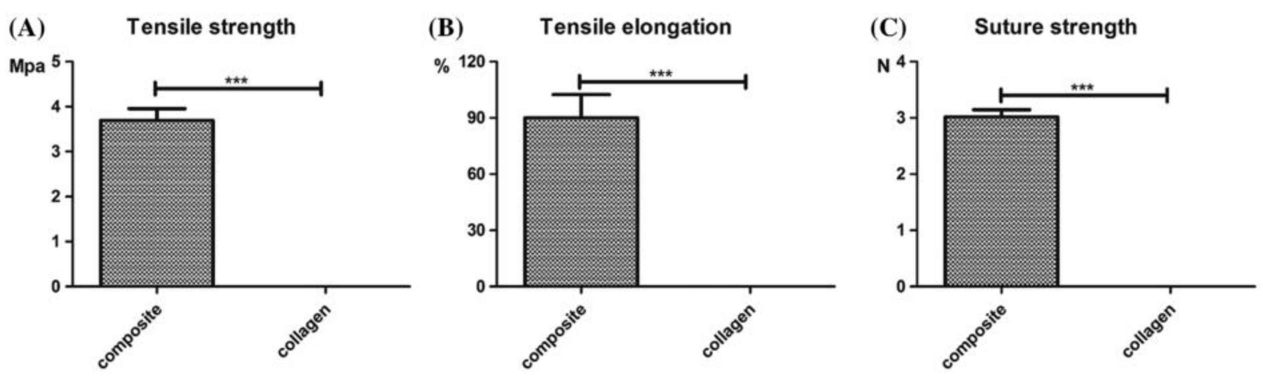

Figure 1. Basic mechanical properties of the composite substitute and collagen substitute. Source: ***: collagen substitute was too weak to be tested.

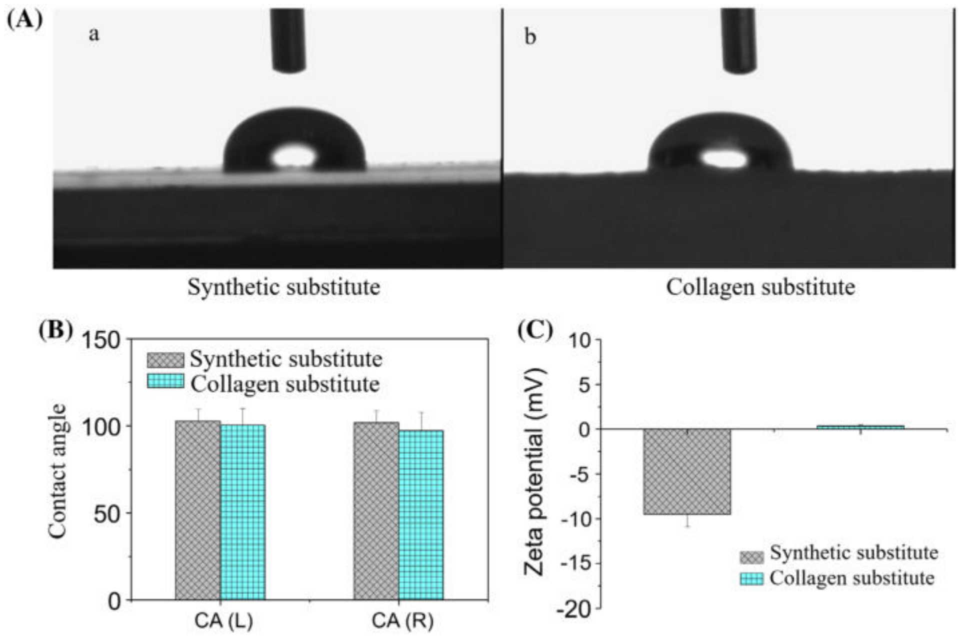

Figure 2. (A): Water droplet images on composite (a) and collagen (b) substitutes. (B): Contact angle of the composite and collagen substitutes. (C): Zeta potential of composite and collagen substitutes surface.

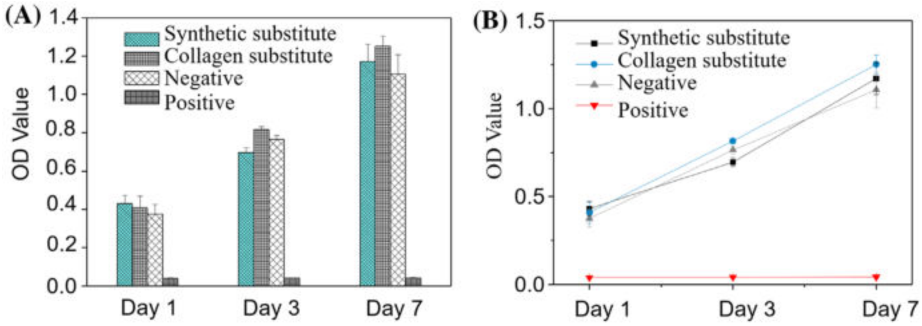

Figure 3. L929 cell proliferation of composite and collagen dural substitutes.

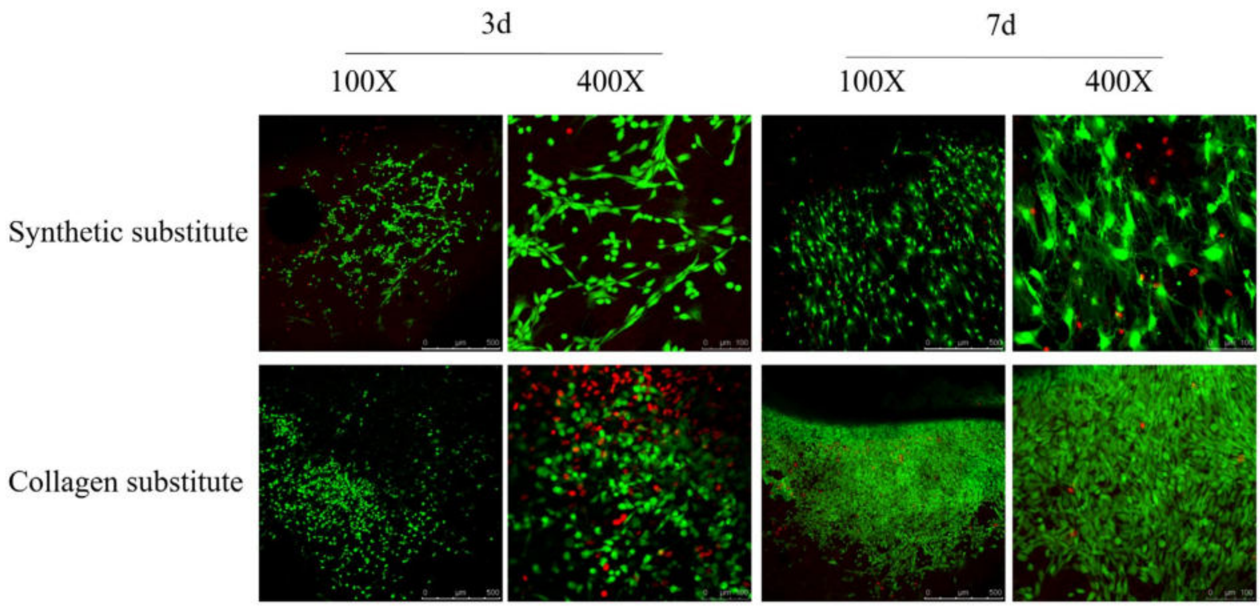

Figure 4. CLSM images of MSC growth after seeded on two dural substitutes for 3 and 7 days.

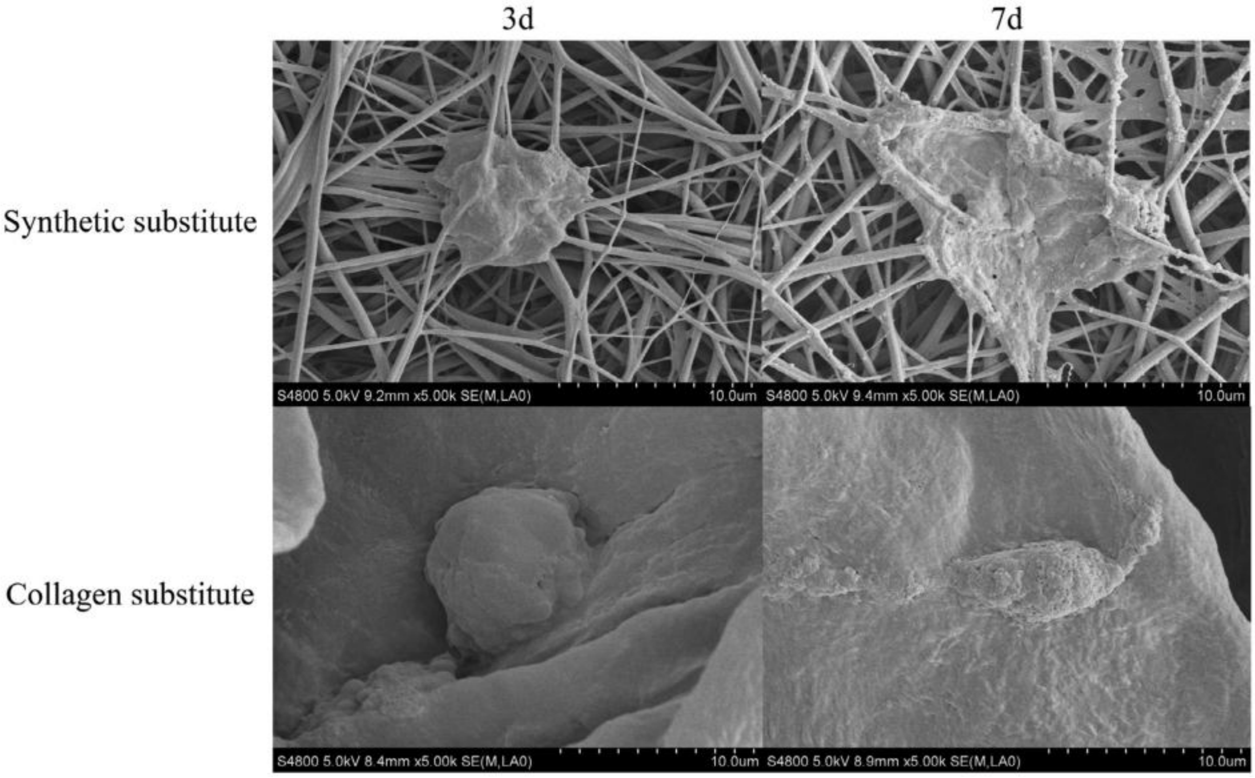

Figure 5. SEM images of MSC growth behavior after seeded on two kinds of dural substitutes for 3 and 7 days.

of the composite scaffold is beneficial for MSCs adhesion and migration.

Consistent with CLSM results, SEM images demonstrated that cells on composite fibrous scaffold displayed a polygonal shape and cells on smooth collagen surface were all nearly spherical on day 3 (Figure 5). It seemed that MSCs adhered more firmly to composite scaffold than to collagen material, exhibiting a better initial adhesion. On day 7, cells spread well with pseudopodia tightly adhering to nanofibers of the composite sample, which was a sign for cell migration. Nevertheless, cell on the flat surface of collagen material showed a fusiform morphology unfavorable for cell adhesion and migration.

3.4. Post-operation tissue regeneration

All of the experimental animals recovered from anesthesia and completed the study with appropriate weight gain and wound healing without abnormal neurologic behavior, epilepsy or CSF leakage. After the surgery, the implants were retrieved at different time points to observe the repair condition of dura mater (Figure 6(A)).

Two weeks post-operation: both constructs integrated to native dura with a clear patch–dura interface observed. The dura defects were closed completely without CSF leakage or adhesion to brain tissue. No new subdural or epidural masses were observed.

Four weeks post-operation: both substitutes exhibited favorable integration efficacy and complete closure. The edges of the patches were further merged with the native dura. The anatomical features were similar to those observed at two weeks with soft sense of touch.

Twelve weeks post-operation: the composite dural substitute integrated perfectly with the surrounding tissues and promoted remodeling of native dura. The flexible regenerated dura mater showed no adhesion to the cerebral cortex. Collagen substitute also closed the dura defect completely, whereas the patch–tissue interface was still observable. No CSF leakage or new subdural or epidural masses were observed.

Tensile strength of the repaired dura mater was tested at 2, 4, and 12 weeks (Figure 6(B–C)). As shown in Figure 6(B–C), at week 2, tensile strength of dura mater repaired with the composite material was 6.05 N, much greater that of the collagen substitute at the same time point. It indicated that composite patch provided higher initial strength for the injured dura. At weeks 4 and 12, tensile strength of dura mater repaired by the composite substitute increased significantly to 12.85 and 14.08 N, respectively, which eventually became comparable with native dura and attained functional replacement. The dura mater repaired by collagen substitute resulted in a relatively low repair level, with 9.5 and 8.7 N tensile strength observed on week 4 and 12.

H&E staining and Masson Trichrome staining were used for histopathological analysis of the retrieved dura implant with surrounding tissue. Twelve weeks after implantation, abundant fibroblasts and microvessels populated at the exterior of collagen layer accompanied with minimal inflammatory cell infiltration (Figure 7(A)). Collagen layer was observed at the composite

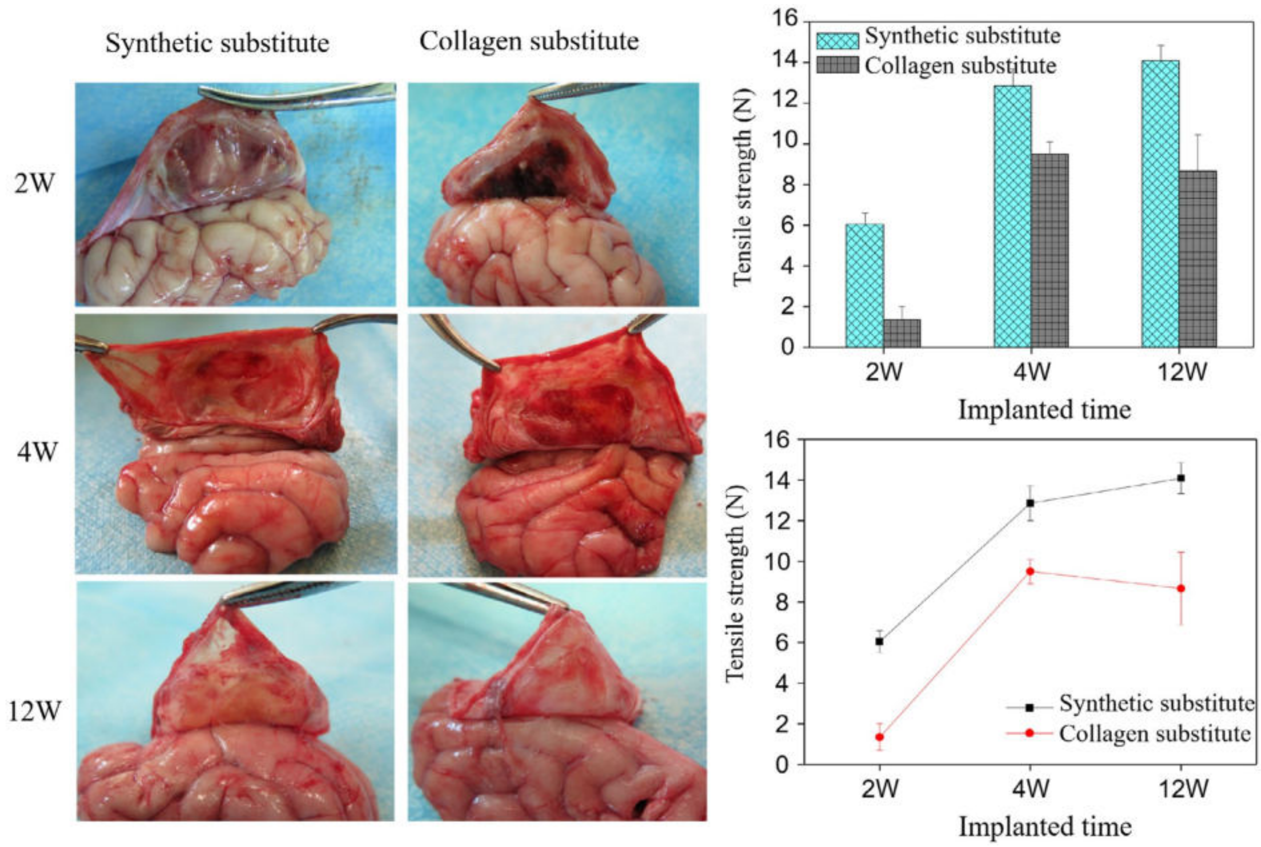

Figure 6. A: Gross observation of repaired dura tissues by different materials on 2, 4, and 12 weeks. B-C: Mechanical strength of repaired tissues at indicated time point.

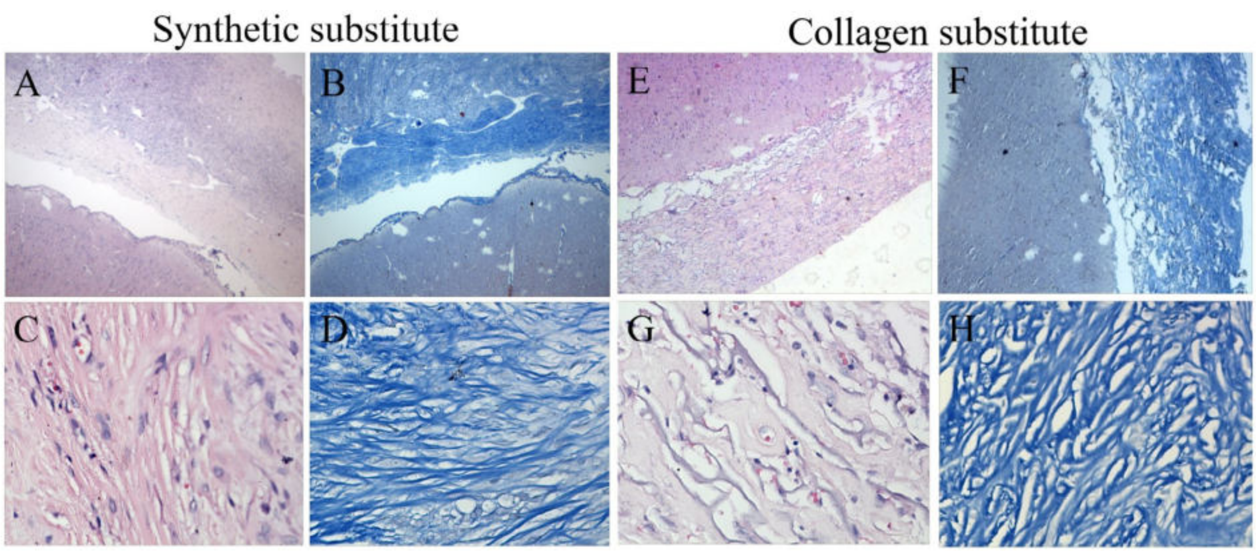

Figure 7. A–D: Histopathological analysis of composite substitute repaired tissues at week 12 (A–B: 40 × 10 magnification; C–D: 400 × 10 magnification). E–F: Histopathological analysis of collagen substitute repaired tissues at week 12 (E–F: 40 × 10 magnification; G–H: 400 × 10 magnification).

material repaired side without adhesion with pia mater or arachnoid (Figure 7(B)). At high magnification, fibers of composite patch were seen to provide scaffold for the adhesion and migration of fibroblasts. Fibroblasts grew and migrated following the orientation of substitute fibers (Figure 7(C)). In Masson Trichrome staining, collagen fibers secreted by fibroblasts presented orderly arrangement to remodel dura maters together with fibroblasts and microvessels (Figure 7(D)). In the collagen group, an area of thin and loose collagen with less fibroblast infiltration could be identified at the explants side (Figure 7(E–F)). Penetration of blood vessels and immature fibroblasts into the substitute was observed (Figure 7(G)). Collagen fibers of the substitute

Table 1. Clinical post-operation observation.

Post-operation (Days)	Major treatment efficacy indicator	Minor assessment indicators			
	CSF & subcutaneous fluids(major indication)	Headache	Fever	Nausea & vomiting	Wound heal
1 (5 patients)	No	Mild	No	Felt	–
11 (5 patients)	No	No	No	No	Good
90 (5 patients)	No	No	No	No	Good
180 (5 patients)	No	No	No	No	Good

*From lumbar puncture examination.

dispersed disorderly with regenerated collagen scattered in the materials (Figure 7(H)). Although all implants were well tolerated by the animals and were effective in repairing the dura mater defect, the composite material implants displayed much better and stronger repairing performance.

3.5. Clinical research

Five clinical case with a six-month follow-up were studied, those five patients had undergone repair of their dural defects with composite substitute in Zhujiang Hospital. The results of major/minor treatment efficacy indicator were shown in Table 1. The body temperature of the patient was normal and there was no obvious infection or CSF leakage after surgery. The other features remained the same as pre-operation.

At 90- and 180-day clinical follow-ups, the patient showed no fever, headache, nausea, vomiting, meningeal irritation, epilepsy, and CSF leakage, and good healing was observed.

4. Discussion

According to previous reports, an ideal dura mater substitute used in clinics must have proper mechanical properties such that the strength and flexibility of the substitute are similar to those of the native human dura mater, and it needs to provide a watertight closure to prevent CSF leakage [34]. Besides, the criteria also includes biodegradable for tissue remodeling and highly biocompatible to prevent inflammation and infection [35]. Numerous autogenic, allogenic, xenogenic, absorbable, and nonabsorbable composite materials have been used with varying degrees of success in the search of the perfect dural substitute [8,36–38]. In the present study, the unique type of biodegradable composite materials derived from PLLA and gelatin was compared to a commercially available collagen dura substitute to verify its repair performance.

Excellent mechanical strength of dural substitute is essential for positioning or repositioning of the patch in operative handling. Moreover, craniotomy associated complications such as increased intracranial pressure and encephalocele will exert forces on the implant materials. Ingrowth of new tissues is relatively slow after initial implantation, thus mechanical property of the patch is assumed all the functions for defect closure to prevent CSF leakage. Hence, mechanical strength of materials is crucial at the early stage of implantation. Mechanically, the composite substitute exhibited tensile strength of 3.6 ± 0.2 MPa and suture strength of 3.0 ± 0.1 N with 74.2% ± 1.3 stretching elongation (Figure 1). Whereas, attempts at quantifying the mechanical properties of the collagen substitute were unsuccessful as the material was too fragile to be tested using the testing system. In clinical application, this substitute is easy for surgeons to handle and it can solve the problem that collagen substitute cannot be sutured; in addition, this substitute can reduce the risk of cerebrospinal fluid leakage of collagen substitute due to weak mechanical properties (it is common that the intracranial pressure increases post-surgery, the integrity of collage substitute can be easily compromised by this high pressure due to weak mechanical properties). Prevention of CSF leakage is crucial in dural repair and physical and chemical properties of dural substitute are essential for leakage proof.

Besides, studies have indicated that the physical and chemical properties of material surface such as surface topography, functional groups, energy state, hydrophilicity, and hydrophobicity, and surface charge influence the biological behaviors such as adhesion, migration, proliferation, and differentiation [31,33,39] of cells. Surface wettability and surface charge are important characteristics responsible for specific tissue–material interaction mechanisms. We examined these two properties by measuring the contact angle and surface Zeta potential of the substitutes. The composite and collagen substitutes were hydrophobic with contact angles of 102° and 98°, respectively, which may be advantageous for the prevention of CSF leakage in the repair of dura defect (Figure 2(A–B)). The electric charge of a material surface is considered to be one of the main physical factors involved in the biological evolution of the tissue around an implant [40,41]. Surface Zeta potential of material is a property that reflects surface charge. Zeta potential of composite sample was −9.51 mV, and the collagen surface presented 0.34 mV potential (Figure 2(C)). It is reported that negative Zeta potential on biomaterials enhanced human osteoblastic cell growth and negatively charged surfaces were favorable toward vessel ingrowth [42,43]. Thus, rapid regeneration may be achieved with fast cell growth and vessel remodeling on the composite substitute.

One of the criteria for an ideal dural mater substitute is highly biocompatible. In the present study, cytocompatibility was investigated by MTT, CLSM, and SEM assay to confirm cell growth and adhesion on composite and collagen materials. The results revealed that cell proliferated rapidly on both, indicating good cytocompatibility and suitable growth environment provided by the two patches (Figure 3–4). Nevertheless, composite materials with porous 3D microfibrous biomimetic structure benefited rapid migration and function of the cells (Figure 5). Cells on composite patch were flat and tightly attached with affluent pseudopodia supportive for migration, whereas cells on smooth collagen surface were polygonal or fusiform of inferior migration ability (Figure 4). It verified that the composite substitute was more suitable for cell regeneration than the collagen construct.

We further compared the biocompatibility and regeneration capability of composite and collagen substitutes in the canine dura model. The composite dura patch integrated perfectly with the native dura mater and promoted the remodeling of native dura at the experimental end point (Figure 6(A). The flexible regenerated dura mater showed no adhesion to the cerebral cortex. Collagen substitute also completely closed the dura defect, the integration was incomplete and the graft–tissue boundary was still visible. Moreover, composite substitute showed much better regeneration of new tissue led to a trend of increasing in mechanical strength than collagen substitute (Figure 6(B–C)). Cells seeded on the composite substitute attached tightly to the nanofibers and spread with the formation of filopodia, which was not observed in the cells seeded on the collagen substitute. It is believed cell spreading is beneficial for cell proliferation and matrix formation. During the repair process, the tensile strength of composite patch repaired tissue was higher than that of the collagen construct at all time points. This higher strength indicates better resistance to increased intracranial pressure.

Histology showed that collagen layer regenerated at the repaired side of the composite patch group and abundant fibroblasts growth and microvessels ingrowth were visible at the external of collagen at the end point (Figure 7(A–B)). Porous, fibrous structure of composite patch provided scaffold for the adhesion and migration of fibroblasts. Fibroblasts grew and migrated along the direction of substitute fibers, secreting orderly arranged collagen fibers (Figure 7(C–D)). In the collagen substitute group, collagen fibers of the patch dispersedly arranged with regenerated collagen scattered in the materials (Figure 7(E–F)).The new collagen layer was weak and thin with less plump immature fibroblast infiltration (Figure 7(G–H)). It can be concluded that the composite substitute promoted efficient repair and regeneration of new dura mater.

Taken together, the fibrous composite substitute was mechanically and biologically more suitable for repairing dura mater than the collagen patch does. Higher mechanical property of the composite substitute is significant for positioning or repositioning of the substitute during operation. Hydrophobic surface of the newly designed substitute may contribute to the prevention of CSF leakage and the slightly negative surface charge is reported to be beneficial for cell growth and vessel ingrowth. Take together, the porous fibrous structure supported cell adhesion and migration, rapid and perfect regeneration of new dura tissue and has a great potential for application in dura tissue repair.

The safety and effectiveness were further confirmed by clinical application in five patients who underwent repair of the dural defect. All major clinical observations, including absence of CSF leakage and local infection, as well as the observation of wound healing were indicative of its positive effects. The surgery was completed more than six months ago and patients remained healthy and with normal neurological functions.

5. Conclusion

In this study, a novel composite substitute derived from poly-L-lactide (PLLA) and gelatin has been discussed. The material characteristics (contact angle, surface charge, and zeta potential were evaluated), biological characteristics *in vitro* (cell stretch, connections between cells, cell proliferation) and tissue regeneration *in vivo* were evaluated, comparing to collagen substitute, the most popular dura substitute. The results verified the composite substitute has better mechanical strength, better surface properties which can be beneficial for cell adhesion and migration, than collagen substitute, and could decrease the risk in clinical application. The safety and effectiveness were further confirmed by animal and clinical applications , all major clinical observations, including absence of CSF leakage and local infection, as well as the observation of wound healing were indicative of its positive effects. In conclusion, the novel composite substitute with biomimetic designed could be an ideal dural substitute and provide a potential more safety choice for dura closure.

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Disclosure statement

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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