Construction and evaluation of a phospholipid-based phase transition in situ gel system for brexpiprazole

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Abstract

The objective of this study was to develop phospholipid-based injectable phase transition *in situ* gels (PTIGs) for the sustained release of Brexpiprazole (Brex). Phospholipid (Lipoid S100, S100) and stearic acid (SA) were used as the gel matrix which was dissolved in biocompatible solvent medium-chain triglyceride (MCT), *N*-methyl pyrrolidone (NMP), and ethanol to obtain PTIGs solution. The Brex PTIG showed a solution condition of low viscosity in vitro and was gelatinized *in situ* in vivo after subcutaneous injection. Both *in vitro* release assay and *in vivo* pharmacokinetics study in SD rats displayed that Brex in PTIGs could achieve a sustained release, compared with brexpiprazole solution (Brex-Sol) or brexpiprazole suspension (Brex-Sus). The Brex-PTIGs had good degradability and biocompatibility *in vivo* with rare inflammation at the injection site. Among the three Brex-PTIG formulations, Brex-PTIG-3 with the SA in the formulation had the greatest gelation viscosity, the lowest initial release rate, and the most stable release profile with sustained release of up to 60 days. The above results indicated that, as a novel drug delivery system, the Brex-PTIGs offered a new option for the clinical treatment of patients with schizophrenia.

1. Introduction

As one of the main types of central nervous illness, schizophrenia has a prolonged course and a high relapse rate, making it necessary to take long-term medication [1, 2]. Aripiprazole is the mainstream drug for schizophrenia but still suffers from problems such as high doses and poor tolerability [3]. Brexpiprazole (Brex) as the first multi-target antipsychotic that exerts agonism at dopamine receptors, partial agonism of 5-HT_{1A} receptor, and antagonism of 5-HT_{2A} receptor [4], is a new option for the treatment of schizophrenia [5]. Research data had shown that Brex was approximately 7.5 to 10 times more potent than aripiprazole. It has a higher affinity towards 5-HT receptors, and less activity on D_2 receptors, which means better tolerability performance and lower incidence of sedentary inability side effects [3, 6]. Rexulti®, a brexpiprazole tablet, was approved by the FDA in 2015 [7]. Another orally disintegrating tablet of brexpiprazole was approved by PMDA in 2018 [8]. Both preparations of brexpiprazole should take once a day, which leads to poor compliance with long-term treatment [6, 9]. Therefore, it is necessary to develop a long-acting formulation of brexpiprazole with good adherence.

The drug depot is a long-acting, sustained-release drug delivery system that can last for days or even months with a single administration, it has drawn tremendous attention for its advantages [10, 11]. Injectable in situ gels are drug depots that can undergo non-covalent cross-linking phase transitions at the injection site and become local drug delivery depots following administration due to changes in external environmental conditions (e.g., light, temperature, pH, hydrophobicity, ionic strength, etc.) [12, 13]. In situ gel drug delivery systems offer more benefits, such as multiple routes of administration (subcutaneous, intramuscular, nasal, or ocular, etc.), simple manufacturing techniques, and low-cost manufacturing, than other drug depots such as suspensions, microspheres, or implants [14]. Currently, available gel injections included OncoGel®, Eligard®, AtridoxElyzol®, SomatulineDepot®, etc. All these gel injections provided a delayed release and prolonged the duration of action [15–17]. This strategy may also apply to enhancing treatment outcomes in schizophrenia.

Most of the long-acting gel injectable products used poly (lactic-co-glycolic acid) (PLGA) gel technology (Atrigel®) owning advantages such as ease of minimally invasive administration and reduced dosing frequency [18, 19]. However, some adverse effects hindered the practical use of PLGA systems, such as inflammation and potential toxicity [20, 21]. In contrast, phospholipid-based phase transition *in situ* gels showed excellent clinical potential to address the deficiencies of PLGA gel [21–23]. As is well known, phospholipids have excellent biocompatibility and safety [24]. Xiang et al. developed a phospholipid-based phase transition *in situ* gelation system to treat hyperlipidemia [25]. This system could release pitavastatin slowly with good biocompatibility and a mild inflammatory response. Moreover, solid lipids have been widely applied in pharmaceutics for sustained-release drug delivery [26, 27]. Zhang et al. developed an *in situ* hybrid implant

composed of stearic acid (SA) and PLGA. The unique advantage of incorporating SA was the improved drug release profiles and reduced burst release caused by morphology altering and phase inversion of the implant [28]. Medium-chain triglycerides (MCTs) lacked the hydrophilic domains, but they could act as dopants to replace some molecules in the liquid crystal structure of phospholipids and form a better drug depot. [14, 29].

This study aimed to develop phospholipid-based phase transition *in situ* gel systems of brexpiprazole (Brex-PTIGs) with good biocompatibility and improved medication adherence in patients with schizophrenia. Brex-PTIGs consist of phospholipid (S100), medium-chain triglyceride (MCT) or stearic acid (SA), and biocompatible solvents *N*-methyl pyrrolidone (NMP) and ethanol (Table 1). NMP is one of the FDA-approved solvents with a high safety profile and could dissolve brexpiprazole extremely well. The excipients in the Brex-PTIGs are biodegradable and have been approved by the FDA for approved marketing [30]. The Brex PTIG showed a solution condition of low viscosity in vitro and was gelatinized *in situ* in vivo after subcutaneous injection. The Brex-PTIGs were characterized by viscosity, *in vitro* release of brexpiprazole, *in vivo* biocompatibility, and biodegradability. In addition, the gelation conditions, rheological properties, and stability were also evaluated. The sustained-release preparations of brexpiprazole in this study have the potential for industrial production and clinical selection.

2. Materials And Methods

2.1. Materials

Brexpiparzole (Brex) and Aripiprazole were supplied by Yaopharma Co., Ltd (Chongqing, China). Phospholipid (S100) was purchased from Lipoid Co. (Germany). Ethanol was supplied by Chengdu Kelong Chemical Co., Ltd (Chengdu, China). Medium-chain triglycerides (MCT) were purchased from Liaoning Xinxing Pharmaceutical Co. (Liaoning, China). *N*-methyl pyrrolidone (NMP) was supplied by Honeywell Co. LTD (USA). Other chemicals and reagents were of an analytical or higher grade.

2.2. Animals

Healthy male SD rats, 170 ± 22 g in body mass, were provided by the Experimental Animal Centre of Chongqing Medical University. All rats were provided with standard housing conditions and maintained at 20℃ and 70% RH with a normal 12 h light/dark cycle starting 1 week before the experiment. The care of laboratory animal and the animal experimental operation had conforming to Chongqing Management Approach of Laboratory Animal (chongqing government order NO.195).

2.3. Preparation of the Brex-PTIG system

Brex was dissolved in NMP, and different amounts of S100, SA, MCT, and ethanol were added sequentially to obtain a gel solution by stirring at 37°C for 30 min. The composition of the formulations was presented in Table 1. To obtain samples in the gel state, different volumes of phosphate buffer (PBS, 0.01 M, pH 7.4) were added to the Brex-PTIGs solution and stirred for 10 min at room temperature [31].

2.4. Determination of Brex

The Brex in Brex-PTIGs or releasing medium were analyzed by high-performance liquid chromatography (HPLC) (Agilent 1260infinity, USA). The samples were diluted with methanol and separated on an Agilent Poroshell EC-C18 column (50 mm x 4.6mm, 2.7 μm). The mobile phase was potassium dihydrogen phosphate (pH 2.0)-methanol (35:65) at a flow rate of 1.0 ml/min. The detection wavelength was 213 nm. The concentration of Brex was calculated by the established standard curve (0.1–8.1 μg/ml).

The Brex in plasma was analyzed by LC-MS (Agilent 1290 + 6460). 100 µl of plasma sample was transferred into a 2 ml centrifuge tube, 300 µl of precipitant acetonitrile was added, and centrifuged at 13000 rpm for 5 min. 50 µl of the supernatant was taken and 25 µl of internal standard solution (Aripiprazole, 240 ng/ml) and 150 µl acetonitrile were added.

After vortexed for 1 min and then centrifuged at 13000 rpm for 3 min, the supernatant was collected for determination. A liquid chromatographic column (4.6 × 150 mm, 3 µm) (Luna PFP) was employed for the analytical separation. The mobile phases A and B were 0.5 vol% formic acid aqueous and formic acid - water – acetonitrile (0.5:15:85), respectively. The flow rate of the mobile phase was 0.5 ml/min, the injection volume was 20 µl, and the temperature of the chromatographic column was 40°C. For the mass spectroscopic measurements, an electrospray ionization source (ESI), positive ion mode detection, full ion scanning mode (100–500 m/z), and a capillary voltage of 7 V were used. The concentration of Brex was calculated by the established standard curve (10–180 ng/ml).

2.5. Characterization of the Brex-PTIG system

The viscosities of Brex-PTIGs before and after gelation were determined at room temperature using a rotational viscometer (Shanghai Fangrui Instruments Co., Ltd.). The morphological images after gelation were also taken by scanning electron microscopy (Thermo).

The rheological behavior of Brex-PTIGs was investigated using a rheometer (Anton Paar). The energy loss modulus G" and storage modulus G' were enumerated from vibration measurements at the constant strain in the angular frequency range of 0.01-10 Hz [14, 32].

2.6. In vitro release

The release of Brex from Brex-PTIGs was monitored according to the literature [33]. The release medium was PBS containing 30% ethanol (pH 6.5) [32, 34, 35]. Brex powder was dissolved in the release medium to a concentration of 30 mg/ml (Brex-Sol) which served as the control.

100 µl of Brex-PTIGs or Brex-Sol was added to dialysis cassettes (Thermo) with a cut-off molecular weight of 10,000, and each dialysis cassette was immersed in 300 ml of fresh release medium under magnetic stirring at a constant speed of 200 rpm at 37°C. At each time point, 50 ml of release medium was collected and the same amount of fresh release medium was replenished. The collected release medium was filtered through a 0.22 µm filter and then analyzed by HPLC according to the method mentioned in section 2.4. The cumulative release of Brex was calculated and the cumulative release-time profile was plotted.

2.7. In vivo pharmacokinetic studies in rats

Brexpiprazole solution (Brex-Sol) and Brexpiprazole suspension (Brex-Sus) were prepared as controls. Brex powder was dissolved in NMP to form Brex-Sol at a concentration of 30 mg/ml. Brex-Sus was prepared by dissolving the drug in a mixture of pure water containing 25% NMP at a concentration of 30 mg/ml. The rats were divided into 6 groups randomly and administrated subcutaneously with saline, or Brex-PTIG-1, Brex-PTIG-2, Brex-PTIG-3, Brex-Sol, and Brex-Sus at a Brex dosage of 100 mg/kg, respectively. Blood was taken from the retroorbital plexus of the rat at each time point and collected into heparinized tubes. The plasma was obtained after centrifuging (5500 rpm, 5 min) at 4°C. The concentration of Brex in plasma was analyzed by LC-MS according to the method mentioned in section 2.4. The pharmacokinetic parameters of different formulations were fitted using DAS 2.0 software.

2.8. Biocompatibility and biodegradability studies

Rats were shaved and randomly divided into 6 groups, injected subcutaneously with saline, Brex-PTIG-1, Brex-PTIG-2, Brex-PTIG-3, Brex-Sol, or Brex-Sus (100 mg/kg for Brex), respectively. The appearance of the rat’s skin in each group was

photographed at 1, 7, 14, and 21 d after injection. A 2×2 cm area of skin around the injection site was cut after executing the rats. The gels maintained at the injection site were separated and weighed.

2.9. Histological analysis

The skin removed was fixed in 4% paraformaldehyde for 48 hours. Paraffin sections were processed with hematoxylin-eosin (HE) staining and observed.

2.10. Data and statistical analysis

All experiments were repeated at least three times and data are presented as mean ± standard deviation (SD). Differences between experimental groups were assessed using a two-way analysis of variance by the analysis software GraphPad InStat 3.3 following a normal distribution test. $P < 0.05$ was considered statistically different. [*, $p < 0.05$, *, $p < 0.01$ and *, $p < 0.001$]

3. Results And Discussion

3.1. Preparation of the Brex-PTIG system

Brex is a novel multi-target antipsychotic drug for the treatment of schizophrenia, and its preparations on market are oral tablets [7]. To enhance medication adherence in patients with schizophrenia, there is an urgent need to develop psychotropic drug delivery systems that can deliver medication that lasts for months rather than days. Phospholipid-based organogels are of interest due to their non-toxicity, good biocompatibility, and injectability [36]. In this study, we designed *in situ* gel systems for Brex based on the phenomenon that high concentrations of phospholipids form *in situ* drug depots by precipitating, prolonging the duration of drug action. The Brex-PTIGs consist of S100, MCT or SA, NMP, and ethanol (Table 1).

Brex-PTIGs were obtained only by mixing and stirring of various components, which makes the preparation process simple, cost-effective, and suitable for mass production. As shown in Fig. 1, the obtained Brex-PTIGs solution were all clear yellow homogeneous solutions. After mixing with PBS, the Brex-PTIGs solution underwent a fast phase transition and convert to a semi-solid state (Fig. $1A_2$, $1B_2$, and $1C_2$).

Although extensive research was focusing on various in-situ implant delivery systems, only solvent diffusion-based in-situ polymer precipitation systems are commercially available, such as doxycycline (AtridoxElyzol®) for periodontal delivery and leuprolide (Eligard®) for the treatment of prostate cancer [33, 34]. In this study, we used S100 as the main gel matrix, which is a biodegradable and biocompatible excipient with low toxicity that has been approved by the FDA for marketing [27].

Brexpiprazole was a weakly basic compound with very poor solubility, but it is well dissolved in NMP (an FDA-approved solvent for injection) [32]. A marketed *in situ* phase transition gel product, a long-acting injection for buprenorphine (SUBLOCADE®), contained up to 50% NMP [37]. Therefore, the content of 45%-50% NMP in Brex-PTIGs was considered to be safe.

Ethanol is a good solvent in injections, but when injections contain high concentrations of ethanol, they often cause severe pain and even local necrosis at the injection site [14, 33]. To improve tolerance and reduce adverse effects in patients with schizophrenia, we strictly control the ethanol dosage to less than 10%. Moreover, studies had shown that the higher concentration of ethanol in sustained-release gels, the more severer the initial burst release of drugs [36]. Meanwhile, MCT and SA were also used to regulate viscosity, improve the release profile of the Brex and alleviate the irritation response caused by ethanol [32, 38].

Table 1
Percentage of materials for three brexpiprazole phospholipid-based phase transition in situ gels.

Samples	Brexpiprazole	S100	SA	MCT	ethanol	NMP
Brex-PTIG-1	5%(w/v)	35%(w/v)	\	5%(v/v)	10%(v/v)	45%(v/v)
Brex-PTIG-2	5%(w/v)	40%(w/v)	\	\	10%(v/v)	45%(v/v)
Brex-PTIG-3	5%(w/v)	40%(w/v)	2%(w/v)	\	8%(v/v)	45%(v/v)

3.2. Characterization of the Brex-PTIGs

Different volumes of PBS were added to investigate the effect of water on the gelation of the Brex-PTIGs. As shown in Fig. 2A, the viscosity of the Brex-PTIGs increased with the addition of PBS when the PBS content was less than 7% (w/w). The insolubility of phospholipids in an aqueous solution may be the main reason for the increase in viscosity of the Brex-PTIGs [39]. Ethanol, NMP, and water are miscible, providing the basis for the rapid precipitation of S100 and phase separation of Brex-PTIGs. It was demonstrated that water would enter and diffuse into the gel after injection, thus triggering an exchange of solvent and water [38]. However, when the PBS content was higher than 7% (w/w), the dilution effect of PBS caused the viscosity of the Brex-PTIGs to decrease (Fig. 2A). After gelation, the viscosity of the Brex-PTIGs increased significantly ($p < 0.001$, Fig. 2B).

Viscosity is an important factor in evaluating long-lasting injectable gels. The viscosity of injections needed to be less than 300 cP, while a higher viscosity is required for the gel to form a depot after injection for sustained release of the drug [32, 40]. Therefore, the gel must undergo a significant viscosity shift before and after injection. As shown in Fig. 2B, Brex-PTIGs were all suitable for injectable use, with viscosities below 300 cP in the solution state, and underwent effective phase transition after injection, with a significant increase of viscosity. Among the three Brex-PTIGs, BPPG-3 showed the greatest change in viscosity, increasing from a viscosity of 27.11 cP in a solution state to 1634.96 cP in a gelled state. The steep increase in viscosity suggested a shift in the state of the gel solution, which could help reduce the initial release of Brex and prolong its release time [41].

The surface morphologies of the gelation gels were photographed by scanning electron microscopy (Fig. 3A and 3B). Brex-PTIG-3 exhibited an angular-shaped surface, while the surface of Brex-PTIG-1 was sparse and porous, and Brex-PTIG-2 was smooth. This might be related to the viscosity of the gel after the phase transition [33]. The more gel matrix added to the PTIG of Brex, the more viscous the gel semi-solid was, and the less porous and more angular the gel surface was.

The complex modulus of Brex-PTIGs after gelation was also measured (Fig. 4). The magnitude of the coefficient of storage elasticity (G') and the coefficient of loss elasticity (G") was associated with the denaturing of the materials[42, 43]. Viscoelastic behavior (G' > G") was perceived for Brex-PTIGs following dynamic strain scan experiments, and the results indicated that all Brex-PTIGs were more elastic than viscous (solid-like).

3.3. Drug release studies in vitro

Brex is insoluble in water and its release could be facilitated in release media containing ethanol. In addition, ethanol in the release medium increases the concentration-dependent diffusion outside the gel matrix, thus accelerating the degradation of the gel matrix [38, 44]. We also adjusted the pH of the release medium to 6.5 [35]. The above conditions were set to mimic the biodegradation situation of *in situ* gels *in vivo* since there might be certain acids and enzymes at the injection site [14, 35].

The release profiles of the Brex-PTIGs were shown in Fig. 5. Brex in Brex-Sol was released rapidly, with a cumulative release percentage of over 90% within 1 h. In Brex-PTIG-1, Brex-PTIG-2, and Brex-PTIG-3, 29.26%, 15.59%, and 10.97% of Brex were released respectively, with significant differences compared to Brex-Sol (all $p < 0.001$). After 144 h, the cumulative release percentages of Brex in Brex-PTIG-1, Brex-PTIG-2, and Brex-PTIG-3 achieved 91.46%, 85.49%, and 80.62%, respectively, showing a significantly delayed release compared with Brex-sol. These results demonstrated the sustained release of Brex from Brex-PTIGs. The water-insoluble character of S100 might be the reason for the retarded release effect.

The release profiles of the three Brex- PTIGs were generally similar (Fig. 5). However, among the three Brex-PTIGs, Brex-PTIG-3 had the slowest release rate. The release rate of Brex in Brex-PTIG-3 was significantly lower than that of Brex-PTIG-1 at 1 h ($p < 0.05$). This indicated the SA was necessary for improving the initial sudden release of Brex from Brex-PTIG.

3.4. Pharmacokinetic studies

To investigate the pharmacokinetic properties of Brex-PTIGs *in vivo*, healthy SD rats were injected subcutaneously with Brex-Sol, Brex-Sus, Brex-PTIG-1, Brex-PTIG-2, or Brex-PTIG-3 with a single dose of Brex at 100 mg/kg. As shown in Fig. 6, the plasma concentration of Brex in the Brex-Sol group showed a significant increase within 1 h after injection, with a C_{max} of 875.52 ng/ml followed by rapid clearance and Brex could not undetectable after 24 h. Similarly, the concentration of Brex in the Brex-Sus group reached a peak quickly, with a C_{max} of 548.68 ng/ml (Table 2), and then cleared quickly (Fig. 6), and Brex was undetected in plasma after 7 d. In contrast, as shown in Table 2 and Fig. 6, the Brex-PTIGs groups had significantly delayed peaking, compared with Brex-sol and Brex-sus (all $p < 0.001$). The peak concentration of Brex was observed around 2 h post-injection for Brex-PTIG-1, Brex-PTIG-2, and Brex-PTIG-3, with the C_{max} of 139.30 ng/ml, 113.83 ng/ml, and 89.45 ng/ml respectively, which were significantly lower than that of Brex-sol (875.52 ng/ml) and Brex-sus (548.68 ng/ml) (all $p < 0.001$). A major challenge in the clinical implementation of *in situ* molded implants was the control of the initial burst release of drugs, particularly for *in situ* precipitation systems. Compared to solution and suspension, the Brex-PTIGs provided a significantly improved initial burst release of Brex and were able to release Brex smoothly for two months. Undoubtedly, the decreased initial burst release was attributed to the formation of Brex-PTIGs gel depots. There was no significance between the three Brex-PTIGs in the area under the concentration-time curves ($AUC_{(0-\infty)}$), indicating the same degree of absorption of the three Brex-PTIGs. All Brex-PTIGs released Brex stably for more than 60 d, which might facilitate medication adherence in patients with schizophrenia.

Among the three Brex-PTIGs (Table 2), the C_{max} of Brex-PTIG-3 was significantly lower than Brex-PTIG-1 ($p < 0.01$), and the $t_{1/2z}$ of Brex-PTIG-3 was significantly longer than that of Brex-PTIG-1 ($p < 0.05$). This was correlated with the release assay of Brex-PTIGs *in vitro*, which indicated that although a sustained-release profile of Brex-PTIGs was observed, the composition of the gel matrix might have influenced the initial burst release. The results of *in vitro* release and pharmacokinetics assay suggested that MCT in Brex-PTIG-1 might lessen the initial burst release of phospholipid-based phase transition *in situ* gel, but it was not as effective as that of SA in Brex-PTIG-3, suggesting SA was a more suitable component for Brex-PTIGs.

Table 2

Pharmacokinetic parameters after a single administration of three Brex-PTIGs (mean±SD, n=15).

Parameter value	Brex-Sol	Brex-Sus	Brex-PTIG-1	Brex-PTIG-2	Brex-PTIG-3
C_{max} (ng/mL)	875.52±79.45	548.68±55.86	139.30±50.49$^{***,###}$	113.83±20.59$^{***,###,\$}$	89.45±22.50$^{***,###.\$\$}$
T_{max} (h)	0.96±0.17	0.96±0.09	1.92±0.38$^{***,###}$	2.00±0.05$^{***,###}$	2.36±0.21$^{***,###}$
$t_{1/2z}$ (d)	0.45±0.06	1.21±0.08	12.48±2.69$^{***,###}$	20.75±3.66$^{***,###,\$}$	22.07±3.54$^{***,###,\$}$
$AUC_{(0-\infty)}$ (μg·d/L)	358.50±85.94	770.42±79.65	3303±118.95$^{***,###}$	3344.00±169.69$^{***,###}$	3339.37±186.69$^{***,###}$
$MRT_{(0-\infty)}$ (d)	0.28±0.13	1.23±0.20	26.64±3.61$^{***,###}$	31.92±2.95$^{***,###}$	42.47±1.98$^{***,###,\$}$

Abbreviations: ***p<0.001 compared to Brex-Sol; $^{###}$p<0.001 compared to Brex-Sus; $^{\$}$p<0.05 and $^{\$\$}$p<0.01 compared to Brex-PTIG-1.

3.5 Biodegradability and biocompatibility of the Brex-PTIG system in vivo

The state of the skin around the injection site was observed on days 0, 7, 14, and 21 after the injection. As shown in Fig. 7, on Day 0 an injection bulge could be seen in the Brex-PTIGs and Brex-sus groups, while the skin seemed smooth and flat with no discernable bulge in the Brex-sol group. On Day 7 after the injection, the volume of the injection bulge significantly decreased in all Brex-PTIGs groups. By Day 14, the skin at the injection site was almost flat in the Brex-PTIGs group.

To further evaluate the degradation of Brex-PTIGs *in vivo*, the depots formed after subcutaneous injection of Brex-PTIGs were dissected from the rat skin and weighed on different days after injection. The weight change of the residual gel was displayed in Fig. 8A. The weight of the residual Brex-PTIG-1 and Brex-PTIG-2 increased during the 0-5 d after injection and then decreased. This was consistent with the previous study. In a comparative study of *in situ* gels performed by Zhang et. al., the gel formulation with the best performance (better viscosity and best sustained-release characteristics) showed a slow increase and degradation of the gel weight *in vivo* [14]. The residual weight of Brex-PTIGs at Day 60 all decreased to 10% of the initial weight.

Organic solvents located in the *in situ* precipitation system may irritate the injection site. The inflammatory reaction is the main side effect after the local injection of *in situ* gel [45]. Thus, we examined the compatibility and inflammatory effect of Brex-PTIGs at the injection site after a single subcutaneous injection. At first, the skin around the injection site was dissected and observed on 7 d after injection. As shown in Fig. 8B, the yellowish gel-like depot of Brex-PTIGs was with smooth and flat edges and was covered by a transparent biofilm. However, in Brex-Sol and Brex-Sus groups, signs of bleeding and inflammations were observed. A partial white fatty accumulation at the injection site was also observed.

Then, the skin tissues at the injection site were fixed, sliced, and stained with hematoxylin and eosin (HE) for histological analysis. As shown in Fig. 9, none of the Brex-PTIGs showed a severe inflammatory response at the injection site over the 21 d following administration. However, for Brex-Sol and Brex-Sus groups, on the first day after injection, the structure of adipocytes was severely disrupted with massive cell necrosis, in addition, infiltration of lymphocytes, granulocytes, and other inflammatory cells into the tissues was also observed on Day 7 after injection, with the formation of large amounts of granulation and fibrous connective tissue. On Day 21, the skin conditions of all groups recovered to normal. These results indicated the Brex-PTIGs were more biocompatible than solution and suspensions *in vivo*.

The biocompatibility and mild inflammatory response of Brex-PTIGs might be owing to the biocompatible excipients and their reasonable adding amount. The matrix in Brex-PTIGs was all biocompatible, including S100 and SA. The solvents used in the Brex-PTIGs were MCT, ethanol, and NMP, which were seen as safe [45, 46]. As an FDA-approved solvent for injectable use, the safety of NMP could be effectively assured, and the low percentage of ethanol (less than 10%) might help reduce skin irritation, as demonstrated in previous studies [32]. The content of solvents in Brex-PTIGs was controlled strictly to ensure the safety of Brex-PTIGs.

4. Conclusions

In this study, an injectable *in situ* gel of Brexpiprazole (Brex) based on phospholipid-based phase transition consisting of S100, MCT/SA, NMP, and ethanol was constructed. Brex-PTIGs in solution state were suitable for injection, and when got in contact with water *in vivo*, they rapidly transformed to a semi-solid gel state. The Brex-PTIGs had a good sustained release of Brex *in vitro* and *in vivo*. The preliminary safety profile was also confirmed in this study. This study for the first time confirmed the feasibility of injectable *in situ* gel based on phospholipid-based phase transition for the sustained delivery of Brex, which holds high potential in developing a valid, safe, easy-producing, and low-cost preparation of Brex for the long-term treatment of schizophrenia.

Declarations

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Author contributions

Ran Tao: Conceptualization, Methodology, Investigation, Data curation, Writing an original draft, Visualization. Li Liu: Methodology, Investigation, Data curation, Visualization**,** Supervision, Writing - review & editing. Yingxin Xiong: Methodology, Investigation, Resources, Supervision. Qianyu Zhang: Methodology, Investigation**,** Writing - review & editing. Xiangyu Lv: Data curation, Formal analysis. Linbo He: Data curation, Formal analysis, Resources. Fang Ren: Validation, Methodology. Lu Zhou: Methodology, Software. BaoYan Chen: Formal analysis, Software. Kexin Wu: Validation, Visualization. Yan Zhang: Project administration, Resources, Supervision. Huali Chen: Funding acquisition, Conceptualization, Supervision, Writing - review & editing.

Ethics approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of Chongqing Management Approach of Laboratory Animal (chongqing government order NO.195).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available upon request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

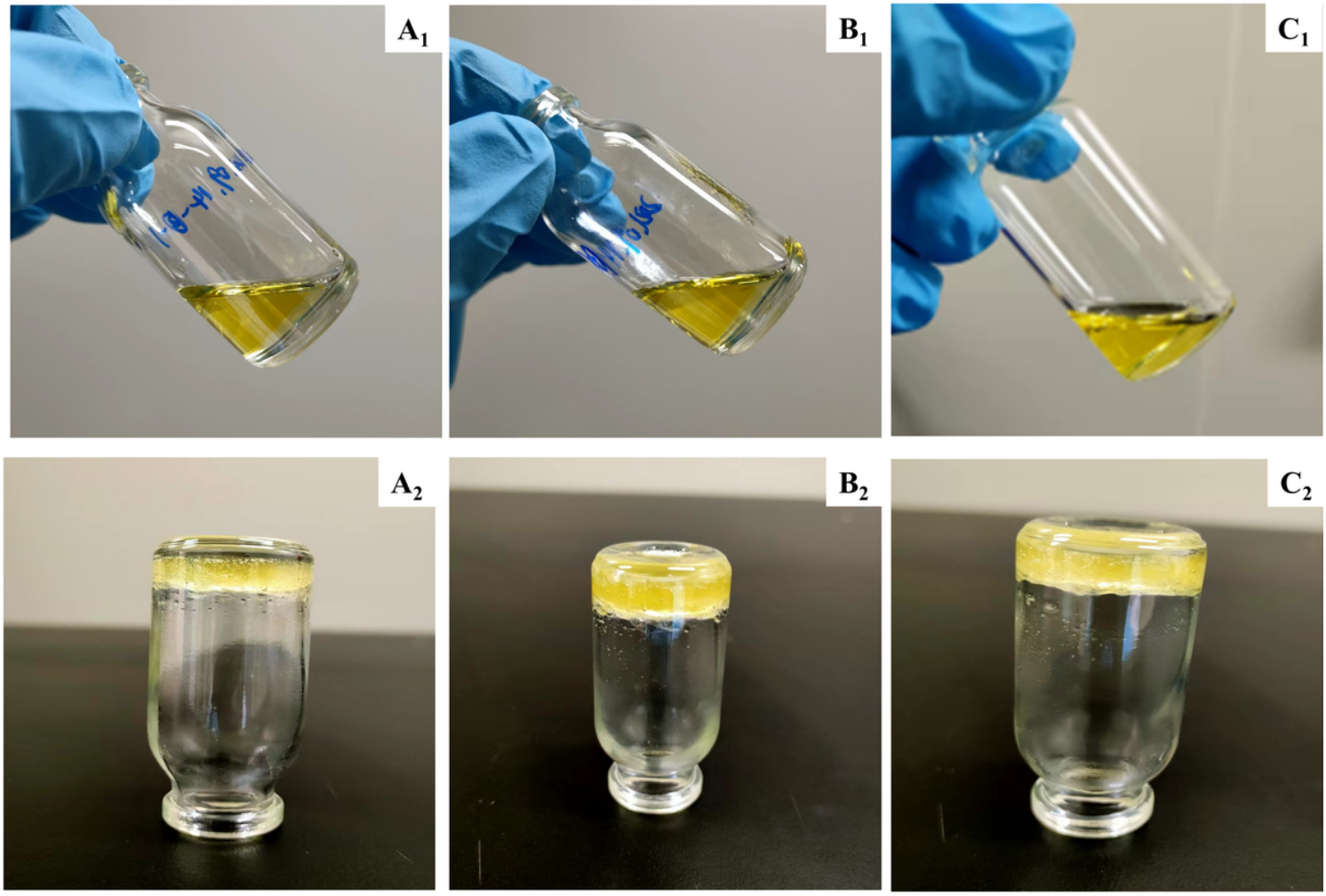

Figure 1

Morphology of Brex-PTIG-1, Brex-PTIG-2, Brex-PTIG-3 before (A1, B1, C1) and after (A2, B2, C2) phase transitions.

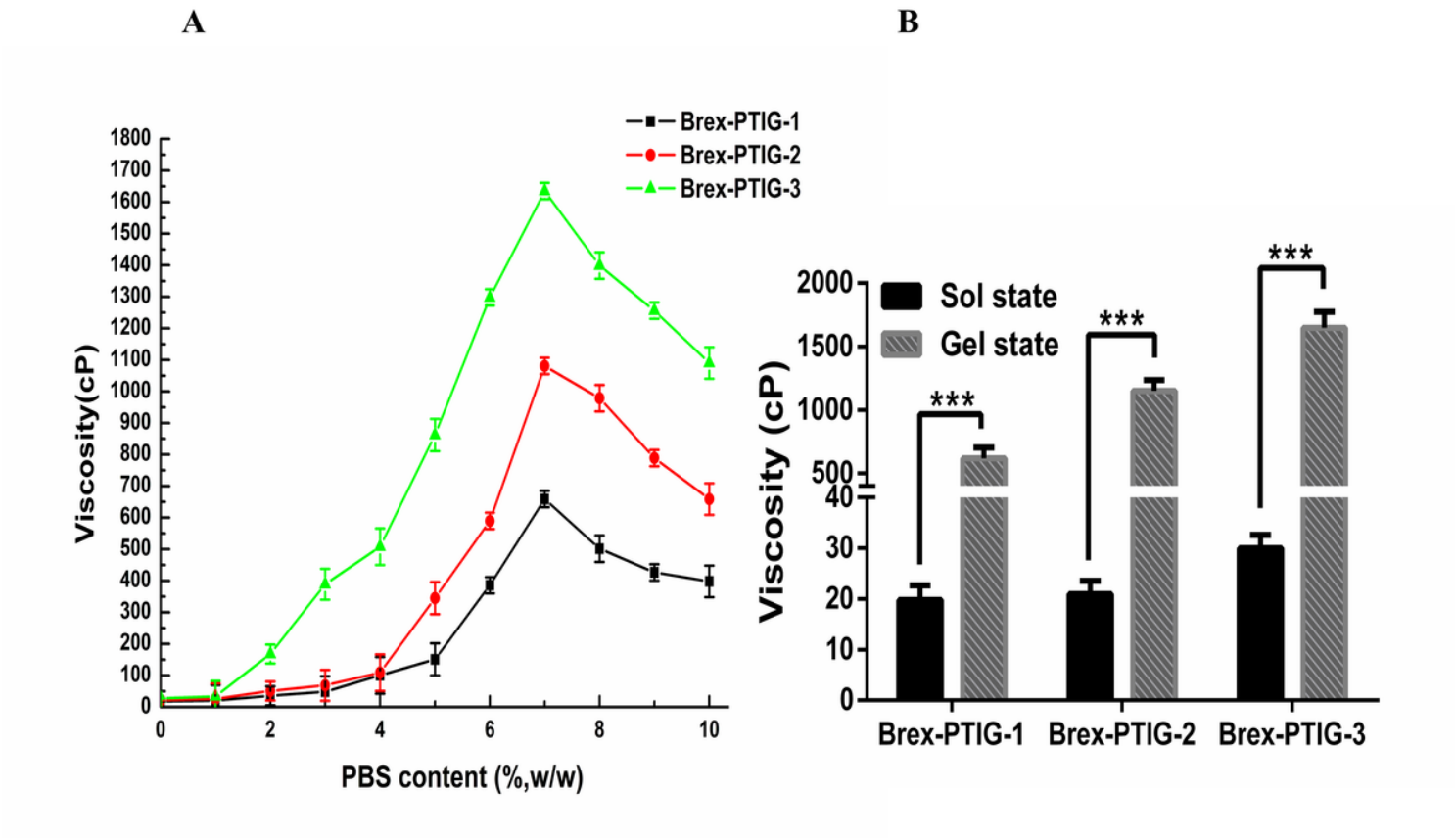

Figure 2

(A) The viscosity of Brex-PTIG-1, Brex-PTIG-2, and Brex-PTIG-3 with different PBS content (%, w/w). (B) Viscosity Changes of Gels at 25°C. Data represent mean ± SD (n = 3).

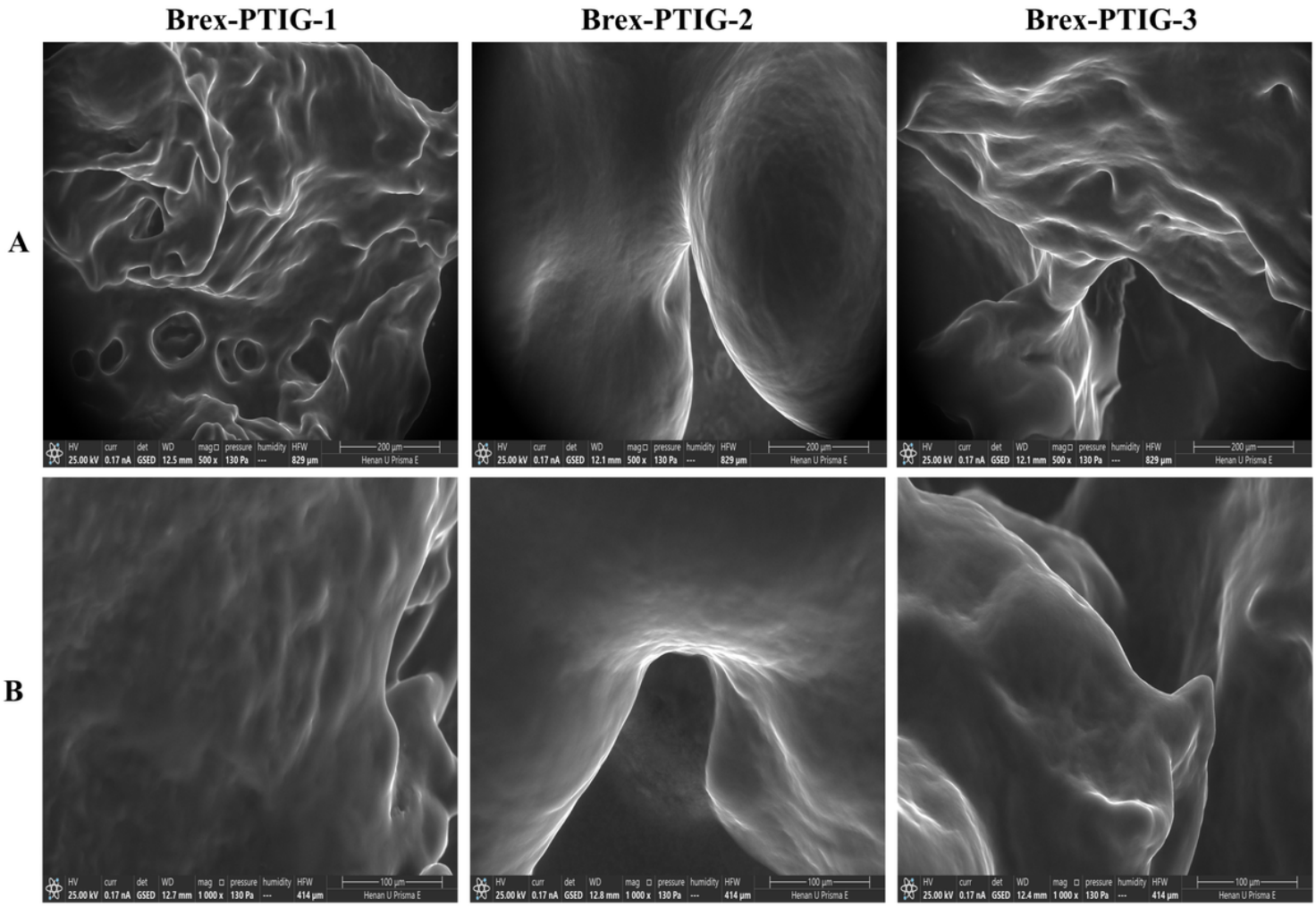

Figure 3

Scanning electron microscope (SEM) images of Brex-PTIGs magnified 500 × (A)(Scale bar: 200 μm) and 1000 × (B)(Scale bar: 100 μm).

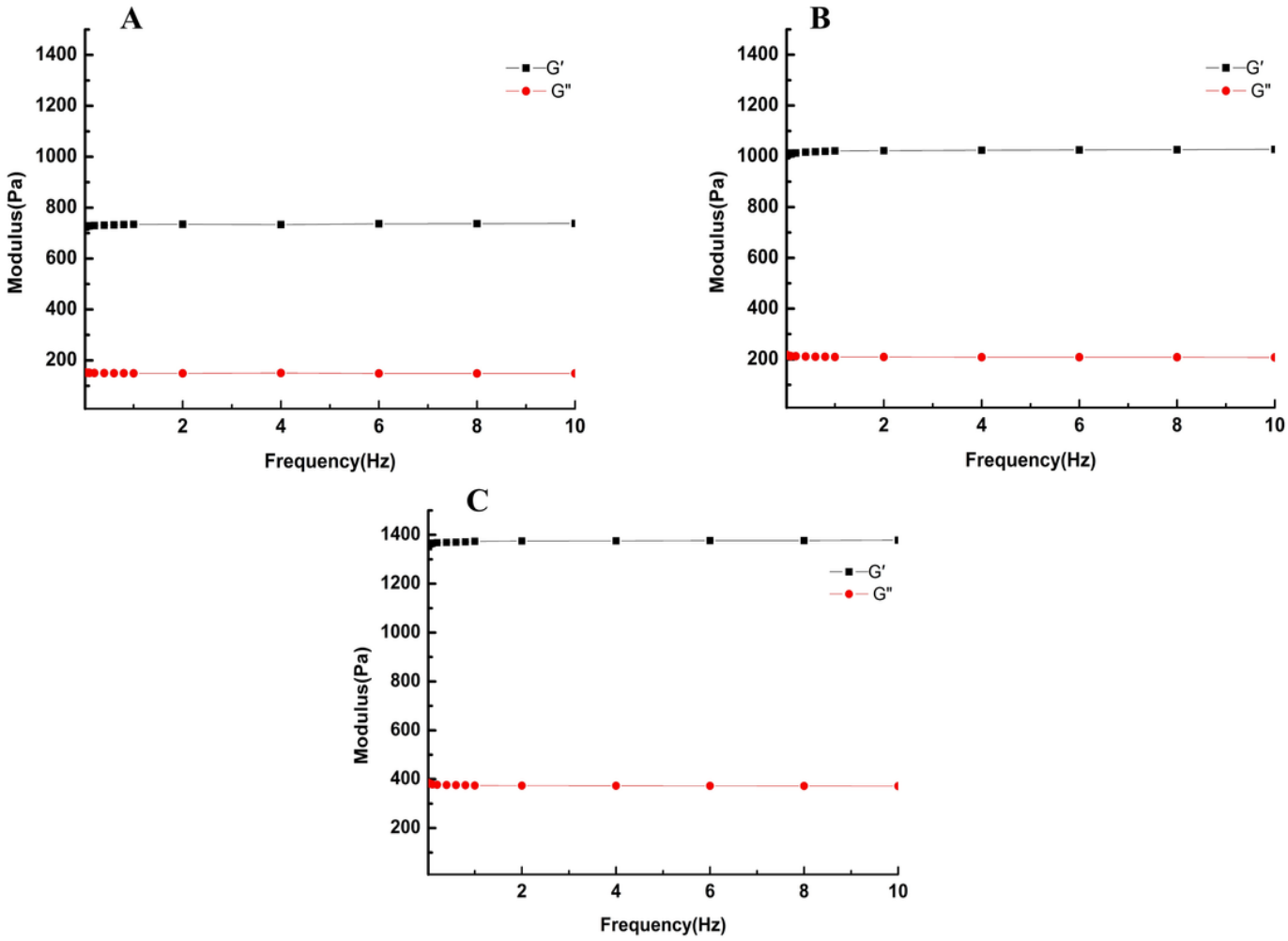

Figure 4

Complex modulus of Brex-PTIG-1 (A), Brex-PTIG-2 (B), and Brex-PTIG-3 (C) after gelation. Data represent mean ± SD (n = 3).

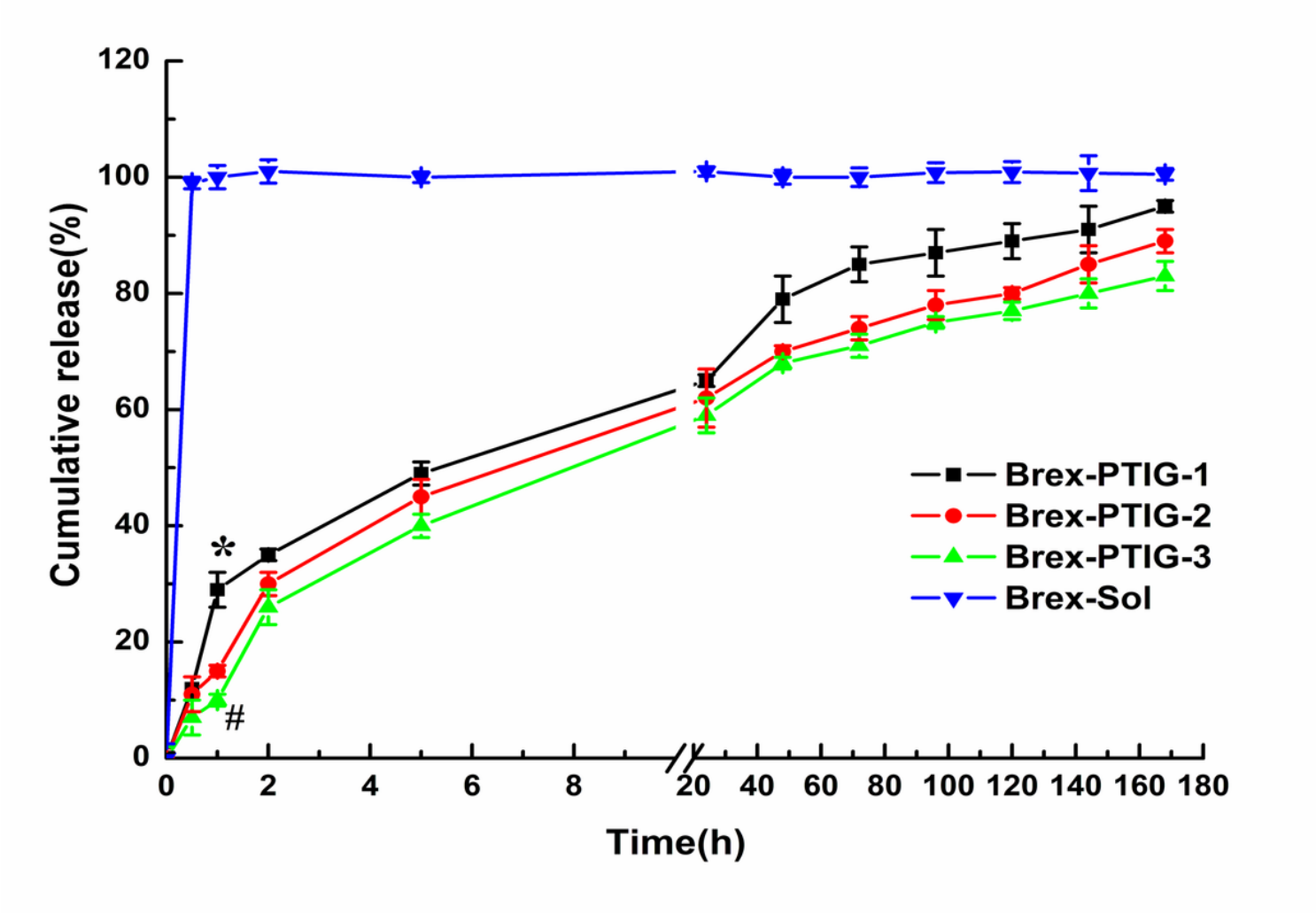

Figure 5

Release profiles of the formulations PBS containing 30% ethanol (pH 6.5) over 168 h, Data represent mean ± SD (n = 3). *p<0.05 compared to Brex-Sol; #p<0.05 compared to Brex-PTIG-1. Abbreviations: Brex-Sol, Brexpiparzole solution.

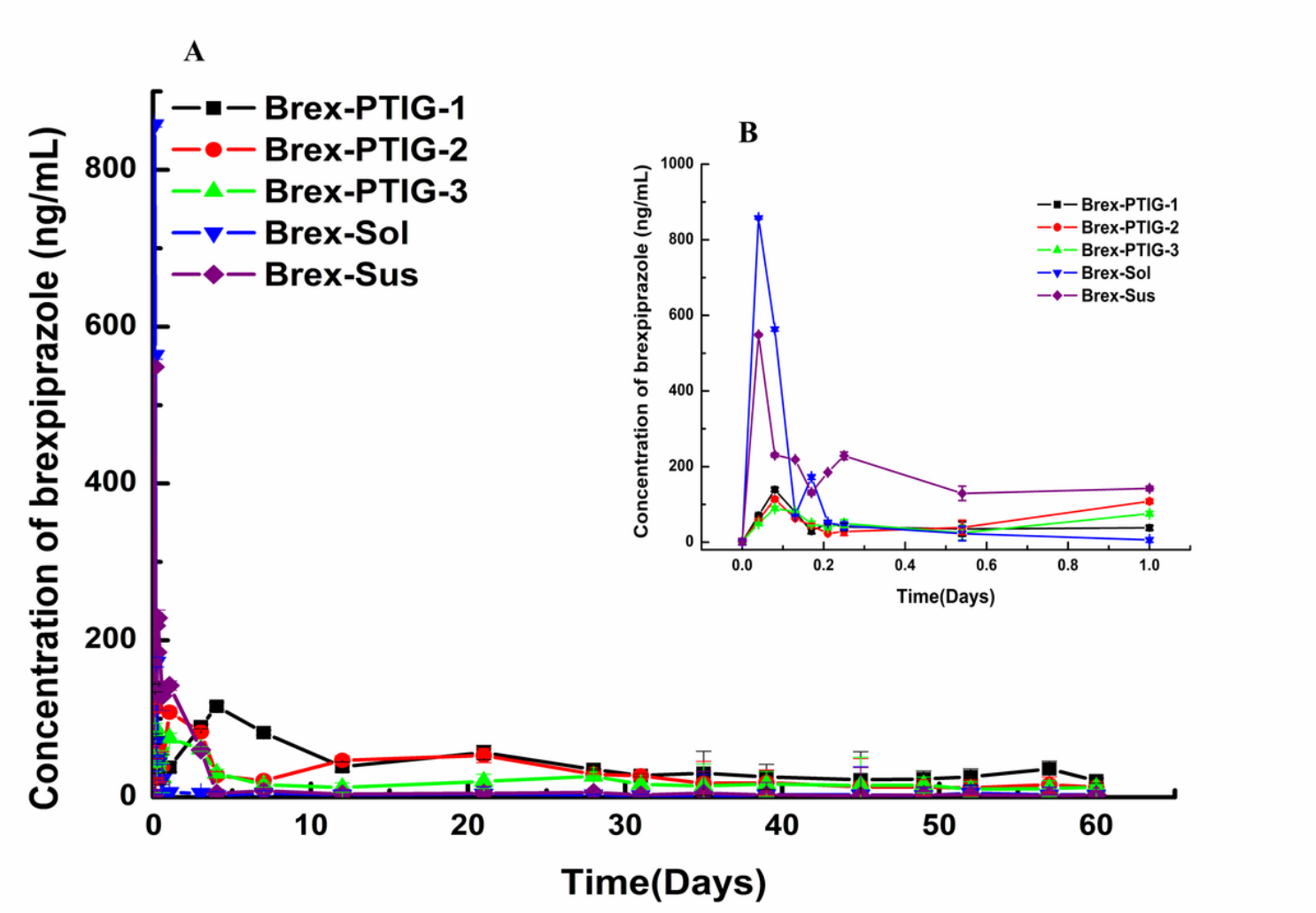

Figure 6

Plasma concentration-time curves of Brex samples: 1-60 d (A); The first day (B). Data were presented as mean ±SD (n = 15). Abbreviations: Brex-Sol, Brexpiparzole solution; Brex-Sus, Brexpiparzole suspension.

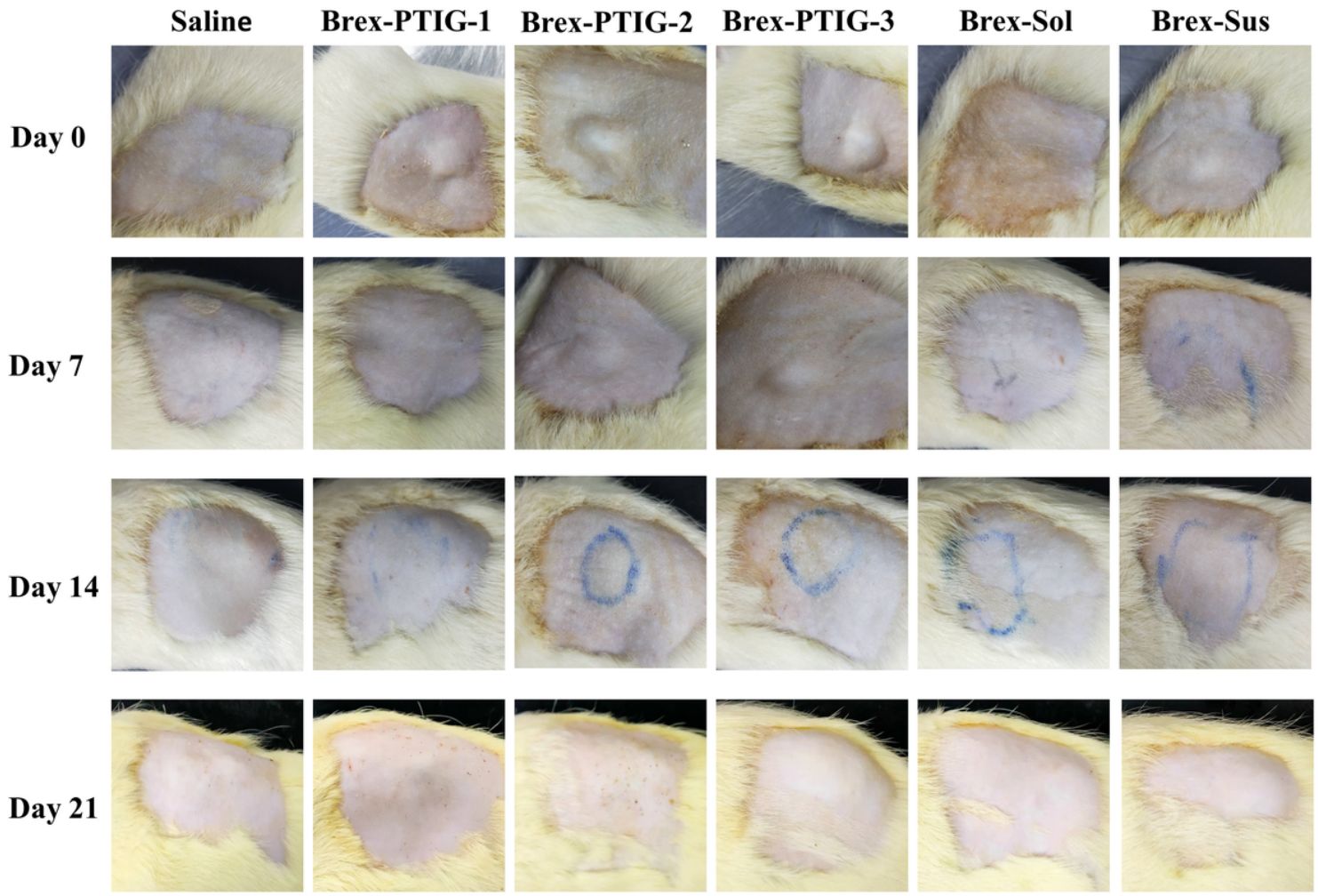

Figure 7

Representative photos of samples after a single subcutaneous injection in rats on days 0, 7, 14, and 21. Data were presented as mean ±SD(n = 4). Brex-Sol, Brexpiparzole solution; Brex-Sus, Brexpiparzole suspension.

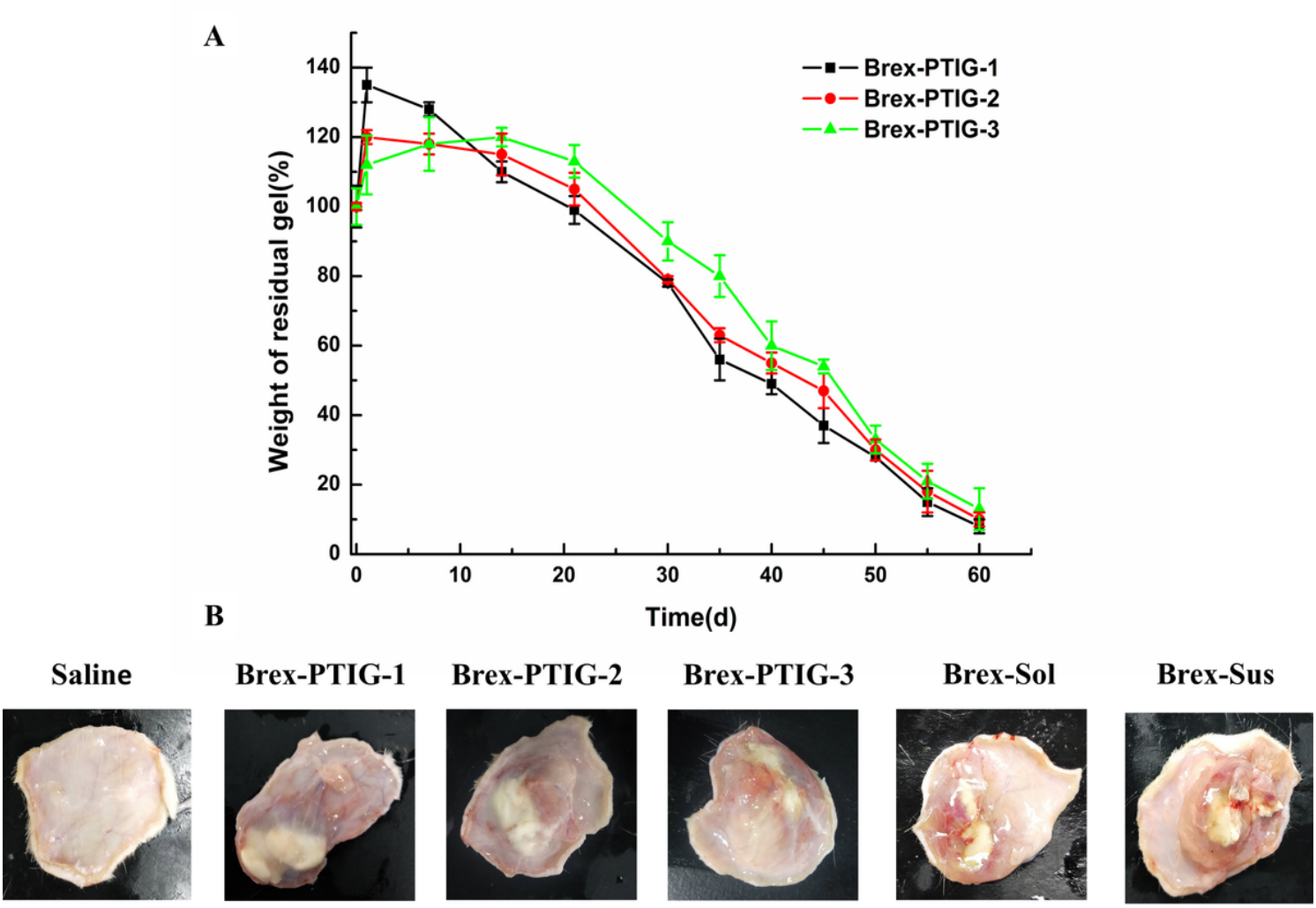

Figure 8

Weight of residual gel(%) *in vivo* (A); State of local injection site on the 7th day of administration (B). Data were presented as mean ±SD (n = 4).

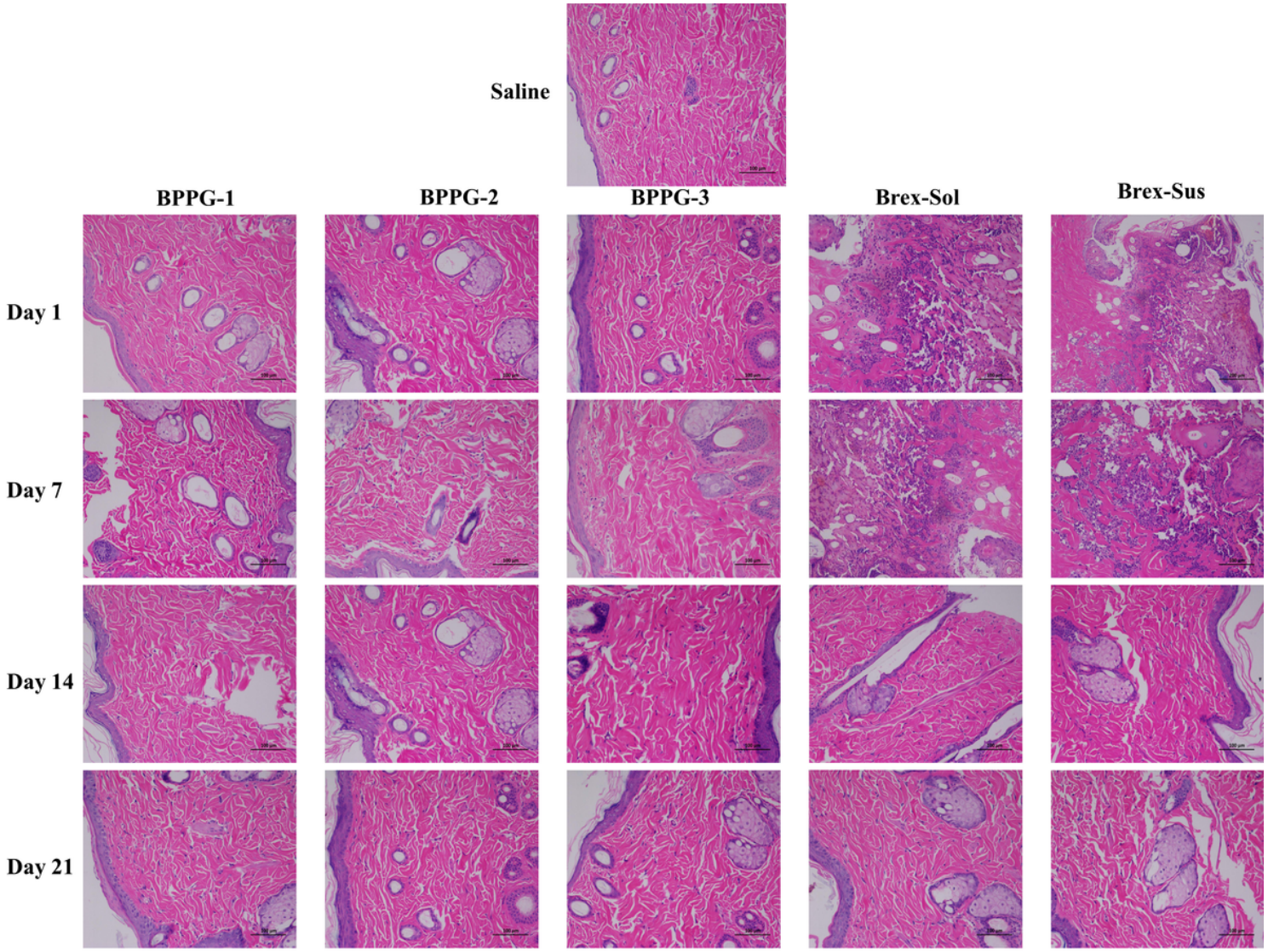

Figure 9

HE staining results of saline, Brex-BTIG-1, Brex-BTIG-2, Brex-BTIG-3, Brex-Sol, and Brex-Sus, Scale bar: 100 μm.

Supplementary Files

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