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Gelatin/gentamicin sulfate-modified PMMA bone cement with proper mechanical properties and high antibacterial ability

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Abstract

With the aging of the population, the risk of osteoporotic vertebral compression fractures (OVCF) caused by osteoporosis increases rapidly. Surgeons often fill the bone defect with injectable polymethylmethacrylate (PMMA) bone cement through vertebroplasty. However, compared with cancellous bone, the higher mechanical properties of PMMA bone cement can easily lead to the fracture of the adjacent cone. Besides, the wound infection caused by surgery is also a serious problem. In order to solve these problems, we designed a new type of PMMA bone cement, by adding gelatin as a pore former, 5% (w/w) gentamicin sulfate (GS) for antibacterial purpose, and 30% (w/w) barium sulfate (BaSO4) to provide excellent radiopacity. Compared with the traditional PMMA bone cement, with the dissolution of gelatin after being immersed in phosphate buffered saline (PBS) for 14 d, the mechanical properties of modified PMMA bone cement decreased by approximately 67%, which is close to the human cancellous bone. Besides, the release of GS increased 3.8 times, and the GS concentration remained above the minimum inhibitory concentration (MIC) for 12 d. In addition, the setting properties, contact angle, antibacterial ability, and cell compatibility of PMMA bone cement also maintained well. The integration and dissolution of gelatin were observed by a scanning electron microscope (SEM). All results indicate that the new type of gelatin-modified PMMA bone cement is a potential candidate material for vertebroplasty.

1. Introduction

The world has entered an aging society, and it is estimated that the number of patients with osteoporosis may reach 1.55 billion by 2050 [1]. Therefore, there is a great demand for vertebroplasty to treat osteoporotic vertebral compression fractures (OVCF) caused by osteoporosis [2, 3]. Polymethylmethacrylate (PMMA) bone cement is the most common bone cement in vertebroplasty. In the later 1930s, PMMA was firstly used in clinic to fill cranial defects of monkeys [4, 5]. Since then, PMMA bone cement soon gained great attention as orthopedic biomaterials due to ease of handling, cheap, appropriate curing time, and good biocompatibility [6–8]. PMMA bone cement is usually composed of two components. One is PMMA powder, which is mainly PMMA beads, and it also contains a small amount of benzoyl peroxide (BP, used as initiator) and barium sulfate/zirconium oxide (used to obtain radiopacity). The other one is liquid phase, mainly composed of MMA monomer, but also includes a small amount of N, N-dimethyl-p-toluidine (DMT, used as reaction accelerator) and hydroquinone (used to prevent premature polymerization) [7]. After mixing and stirring these two components, the MMA monomer will quickly polymerize to form a self-curing bone cement.

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However, it also has some urgent problems that need to be solved. For example, the high mechanical properties of bone cement compared with human bone will cause stress shielding effects [9, 10]. Moreover, the maximum temperature (up to 120 °C) generated during the polymerization process will damage the surrounding tissues [11]. Currently, the risk of infection caused by the orthopedic surgery related to the application of bone cement can reach up to approximately 1%–2% [12, 13]. In the beginning, the prevention of surgical infection was achieved through oral antibiotics or intravenous antibiotics, but the antibiotic concentration of the infected region is often lower than the minimum inhibitory concentration (MIC) and could lead to antibiotic abuse by using these methods [14]. Later, researchers added antibiotics into bone cement and this method performs local and effective antibacterial ability and achieves a good effect in the early stage [15]. However, this method did not significantly reduce the infection risk during the one-year postoperative examination [16, 17]. The main reason is that during the rapid polymerization of bone cement, antibiotics are evenly distributed on the surface and inside of bone cement, and the antibiotics on the surface are released rapidly in the first few hours, resulting in a good antibacterial effect, but the internal antibiotics are almost impossible to release [18, 19]. The final release of antibiotics does not exceed 20% of the total [15]. Therefore, it is necessary to find a way to release the antibiotics inside the bone cement. H van de Belt et al pointed out that the release of antibiotics is largely affected by porosity and surface roughness, and the release of antibiotics inside the bone cement can be accomplished by adding pore formers [20]. M R Virto et al successfully added lactose to PMMA bone cement to increase the porosity and the release of antibiotics from the bone cement [18]. Julia Schnieders et al added antibiotics to microspheres made of poly (lactic-co-glycolic acid), and the addition of microspheres to calcium phosphate cement also increases porosity to enhance the release of antibiotics [21].

Gelatin, as a natural polymer hydrolyzed from collagen, is chemically composed of 18 amino acids including glycine, alanine, and arginine. Its unique amino acid structure has many medical benefits, such as excellent biocompatibility, biodegradability, and hydrophilicity, so it can be used for drug release [22–24]. Gentamicin sulfate (GS) is one of the most common antibiotics in bone cement implants. It has a wide range of antibacterial ability, excellent water solubility, and high temperature stability [18, 25].

Owing to the advantages of gelatin and GS, we employed them both into a new type of PMMA bone cement for vertebroplasty in this study. Since the antibiotic concentration of commercial antibiotic bone cement does not exceed 5% [19], 5% (w/w) GS was added to PMMA powder to obtain antibacterial PMMA bone cement. Different proportions of ball-milled gelatin (particle size: 150–500 μm) was added as a pore former to improve the release behavior of GS. Besides, the pores caused by the gelatin involvement reduce the mechanical strength and modulus of the new type of PMMA bone cement, which prevents stress shielding effects caused by the excessive strength of traditional PMMA bone cement. Additionally, bone cement for vertebroplasty should have better radiopacity than that for joint replacement, usually the bone cement for vertebroplasty contain about 30 wt% barium sulfate [26, 27]. The basic physicochemical properties, mechanical properties, GS release behavior, antibacterial ability, and cell compatibility of the new type of PMMA bone cement were systematically studied. In addition, the integration and dissolution of gelatin during the long-term immersion were observed by a scanning electron microscope (SEM).

2. Materials and methods

2.1. Materials
PMMA bone cement powder and liquid, GS, and BaSO₄ were donated by Shandong Mingde Biological Co., Ltd. O-phthalaldehyde (AR, 98%) and isopropanol (AR, 99.5%) were purchased from Meryer (Shanghai, China). Sodium borate (AR), 2-mercaptoethanol (GC, ≥98%), and methanol (LC-MS, ≥99.9%) were purchased from Macklin (Shanghai, China). Phosphate buffered saline (PBS) (AR, ≥98%) was purchased from Solarbio (Beijing, China). Gelatin was purchased from Sigma (China). Gelatin needs to be ball milled to 150–500 μm to match human bones before using [24].

2.2. Preparation of PMMA antibacterial bone cement with gelatin
30% (w/w) BaSO₄, 5% (w/w) GS, and 10% /20% /30% (w/w) gelatin were added to the PMMA powder, then the powder was stirred in the polypropylene (PP) cup for 3 min. The liquid phase was poured into the powder with the powder-to-liquid ratio of 2 g:1 ml, followed by quickly stirring by stirring rod. After mixing the powder and liquid, and before the formation of dough, a syringe was used to inject the mixture into the polytetrafluoroethylene mold which was stored at room temperature for 24 h. The preparation process is carried out at 23 ± 1 °C.
2.3. Setting properties and contact angle
According to ISO-5833-2002, dough time refers to the time from the mixing of the powder and liquid to the clear separation between the mixture and the finger gloved with an unpowdered non-water-rinsed latex surgical glove. After mixing the powder and the liquid for approximately 3 min, the surface of the cement was gently touched with a gloved finger every 10 s till the gloved finger first separates cleanly from the cement and the time was recorded. After each contact, the cement was gently mixed to expose a new fresh surface for the next contact. Each sample was measured in duplicate. If the time difference is not more than 30 s, the average time was reported (ISO-5833-2002).

According to ISO-5833-2002, setting time refers to the time from mixing of the powder and liquid to the time when the temperature first reaches the average temperature of the maximum temperature and room temperature. A thermal imager (FLIR, E50, USA) was used to record the temperature every 5 s till the temperature reaches the highest point. Each sample is measured in duplicate. If the maximum temperature difference is not more than 10 °C and the setting time difference is not more than 30 s, the average temperature and time was reported (ISO-5833-2002). The above tests are all carried out at room temperature of 23 ± 1 °C and air relative humidity not lower than 40%.

The wettability of PMMA bone cement is characterized by the static water contact angle (JC2000D2, China). 1 μl of distilled water was dropped on the surface of PMMA bone cement by falling method, and the result was obtained after the angle measurement. Each sample was measured in quintuplicate.

2.4. Mechanical properties
According to ISO-5833-2002, compressive strength and modulus were tested by placing a cylindrical sample with a diameter of 6 mm and a height of 12 mm on a universal electronic testing machine (WDW-20, China) till it reached the upper yield point or the sample failed. The indenter speed was 20 mm min⁻¹. The compressive strength was obtained by dividing the corresponding pressure at the upper yield point by the original cross-sectional area, and the compressive modulus was obtained from the slope of the elastic phase in the stress-strain curve. Each sample was measured in quintuplicate.

Bending strength and modulus were tested by placing a strip sample (length/width/height: 75/10/3.3 mm) on a universal electronic testing machine (WDW-20, China) through four-point bending test until the sample failed. The indenter speed was set as 5 mm min⁻¹. The detailed calculation formulas were:

\[
B = \frac{3Fa}{bh^2}
\]

\[
E = \frac{\Delta Fa}{4fbb^3} \cdot (3l^2 - 4a^2)
\]

Where \(B\) is the bending strength, \(F\) is the force when sample fails, \(a\) is the distance between inner and outer load points, \(b\) is the sample width, \(h\) is the sample height. \(E\) is the bending modulus, \(\Delta F\) is the load range, \(I\) is the distance between external loads, \(f\) is the difference of the corresponding deflection when the load is 15 N and 50 N.

2.5. GS release behavior
The PMMA bone cement was made into a cylindrical sample with a diameter of 6 mm and a height of 12 mm. The sample was immersed in a 10 ml Phosphate buffered saline (PBS) solution and placed in a constant temperature shaker (37 °C; 200 rpm). 1 ml leaching solution was taken every predetermined time (4 h, 12 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 192 h, 240 h, 288 h, and 336 h), and then 1 ml fresh PBS solution was added to keep PBS volume unchanged. The release cycle time was 14 d and three independent PMMA bone cement samples were test for each component.

In this study, Zhang’s modified method [28] based on the o-phthaldialdehyde solution proposed by Sampath [29] was used to test GS. Specifically, 2.5 g o-phthaldialdehyde was dissolved in 62.5 ml methanol, then 3 ml 2-mercaptoethanol and 560 ml (0.04 M) sodium borate solution was added successively. The solution was stored in the dark for at least 24 h before using and the reagents can no longer be used after 3 d. Before the test, the test solution, o-phthaldialdehyde solution, and the isopropanol solution (to avoid precipitation) were mixed at a volume ratio of 1:1:1. 1 h later, the ultraviolet spectrophotometer (UV-2700, Japan) was used to test the absorbance at 332 nm. A standard curve was drawn and the GS concentration was obtained according to the Lambert Beer’s law, and the influence of gelatin on absorbance was excluded before testing. Each sample was measured in triplicate.

2.6. Surface morphology and porosity of PMMA bone cement
The surface morphology of PMMA bone cement was analyzed by a scanning electron microscope (SEM, S-4800, Japan). The sample surface was sprayed with gold for 100 s to increase its conductivity. The surface porosity of PMMA bone cement was obtained from SEM images.
2.7. Antibacterial ability test
PMMA bone cement with a diameter of 6 mm and a height of 12 mm was immersed in 10 ml PBS, and the leaching solution (from PBS) was used to test the antibacterial ability. Specifically, Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) were inoculated in Luria-Bertani (LB) liquid medium, and agitated overnight in a constant temperature shaker (37 °C, 200 rpm). The concentration of the bacterial cells was measured by optical spectrometry at 600 nm. When in use, E. coli and S. aureus were diluted in LB liquid medium to $1 \times 10^{10}$ CFU ml$^{-1}$ and $1 \times 10^8$ CFU ml$^{-1}$, respectively. Then, 50 μl of bacterial solution was inoculated evenly on agar plates. PMMA bone cement was immersed in PBS for 3, 7, and 14 d. The PBS solution was changed at day 3 and day 7. Subsequently, 10 μl of leaching solution was dripped on a filter paper (diameter = 6 mm) which was placed on the agar plates in advanced. The agar plates were incubated at 37 °C for 24 h. Then the inhibition zone was observed and photographed. The picture was processed to obtain the inhibition zone’s diameters (ZID). Each sample was measured in triplicate.

2.8. Cell compatibility test
This experiment was performed following the Chinese standard GB/T16886. 5-2017. For extract preparation, a PMMA bone cement sample with a diameter of 6 mm and a height of 3 mm was placed in a 48-well plate, and 500 μl of cell culture medium (89% (v/v) α-minimum essential medium (α-MEM), 10% (v/v) fetal bovine serum (FBS), and 1% (v/v) double antibody (penicillin and streptomycin)) was poured into each well. After 3 d of soaking, the culture solution was collected then filtered with a 0.2 μl filter. The extract (from cell culture solution) was used for subsequent cell experiments. Osteoblastic cell line of mouse (MC3T3-E1; ATCC CRL-2593), which was provided by NanKai University (Tianjin, China), was selected for evaluating the cell compatibility of the bone cements. Specifically, 5 $\times$ 10$^4$ cells/well were seeded in a 48-well plate. After culturing for 24 h, the culture medium was removed and replaced by the extract. After 1 d and 3 d of culture, the culture medium was changed by 0.5 mg ml$^{-1}$ thiazolyl blue tetrazolium bromide (MTT), and the cells were incubated in a cell incubator for 4 h. Next, the MTT solution was discarded and an equal volume of dimethyl sulfoxide was added. After being fully dissolved, the absorption at 490 nm was recorded by a microplate reader (Synergy H1, USA). Each sample was measured in quintuplicate, and the cell survival rate was calculated by the following formula.

\[
Cell\ viability\% = \frac{OD_{extract}}{OD_{control}} \times 100\%
\]  

2.9. Statistical analysis
The experimental data was represented by the mean ± standard deviation, and the data of different groups were analyzed by one-way variance (ANOVA). When the $P < 0.05$, the data of different groups were significantly different. The best-fitting formula of GS release used in this experiment was obtained by the Gauss-Newton method.

3. Results and discussion
3.1. Setting properties and contact angle
Figure 1 (A) shows the relationship between the dough time and the proportions of gelatin added. It took PMMA bone cement without gelatin 8 min to reach the dough time, which is slightly longer compared to other results other researchers made before (approximately 4 min) [30, 31]. The main reason is that the proportions of BaSO$_4$ in the cement powder reached 30% (w/w), which is higher than 10% (w/w) in other experiments. BaSO$_4$ did not participate in the polymerization reaction in PMMA bone cement, but hindered the mixing of the powder and liquid. With the increase of gelatin proportion, the dough time prolonged significantly (up to approximately 10 min). The reason is that gelatin also did not participate in the reaction and acted as a retarder.

Figure 1 (B) shows the relationship between reaction temperature and time. With the addition of gelatin, the maximum temperature of the reaction dropped from approximately 76.2 °C to 64.5 °C. One reason is that gelatin and inorganic BaSO$_4$ can absorb part of the heat. Besides, the addition of gelatin led to a reduction in the total amount of reactants and ultimately caused the reduction in the exotherm of the reaction.

Figure 1 (C) shows the relationship between the setting time and the proportions of gelatin added. The setting time of PMMA bone cement without gelatin was approximately 18 min, and the maximum setting time increased up to approximately 21 min with the addition of gelatin. The reason is that more BaSO$_4$ and gelatin can hinder the mixing of the powder and liquid and subsequently act as two retarders. The extension of the dough time and setting time can give the surgeon enough time for surgery, and a small increase in the mixing time can make the mixing more evenly. Finally, the lower reaction heat can reduce the harm to the human healthy tissue around the application region of the PMMA bone cement.
Figure 1 shows the relationship between the contact angle and the proportions of gelatin added. A high contact angle cannot lead to a proper cell or protein adhesion [32], while a low contact angle is more likely to promote cell-substrate rather than cell-cell interaction [33]. Some studies highlight that the contact angle between 40° and 60° is the preferred contact angle range to promote cell adhesion [34, 35]. The contact angle of PMMA bone cement without gelatin reached 75.7 ± 2.39° due to the hydrophobicity of PMMA. Since gelatin is a hydrophilic material, the contact angle became smaller and smaller with the increase of gelatin proportion. The contact angle of PMMA bone cement with 30% (w/w) gelatin was reduced to 59.3 ± 1.12°, which is more suitable for cell and protein attachment.

3.2. Mechanical properties

The compressive strength and modulus of PMMA bone cement are in the range of 85–114 MPa and 1.7–3.7 GPa, respectively, which are much higher than the strength and modulus required for vertebroplasty [9, 24, 26, 36]. Excessive mechanical properties will lead to stress shielding effects and increase the risk of adjacent cone fracture, so lower compressive strength and modulus are required.

Figure 2 shows the mechanical properties of PMMA bone cement with different proportions of gelatin after being immersed in PBS for 0, 3, 7, and 14 d. On day 0, the compressive strength of PMMA bone cement without gelatin reached 75.7 ± 2.39° due to the hydrophobicity of PMMA. Since gelatin is a hydrophilic material, the contact angle became smaller and smaller with the increase of gelatin proportion. The contact angle of PMMA bone cement with 30% (w/w) gelatin was reduced to 59.3 ± 1.12°, which is more suitable for cell and protein attachment.
The insigniﬁcant reduction in compressive strength was that PMMA bone cement did not degrade. After the addition of gelatin, the gelatin on the surface of PMMA bone cement gradually degraded in PBS and precipitated out to produce pores. Then the internal gelatin of PMMA bone cement will also degrade to form a porous structure. For mechanical properties, a large number of pores can easily lead to stress concentration and a signiﬁcant decrease in mechanical properties. The more gelatin is added, the more the strength was reduced. After 14 d of degradation in PBS, the strength of PMMA bone cement with 30% (w/w) gelatin was 31.77 ± 1.90 MPa, showing a decrease of 66.9%.

From Figure 2(B) we can see that the compressive modulus of PMMA bone cement was 1.47 ± 0.09 GPa before being immersed in PBS, and its changing trend was the same as the compressive strength shown in Figure 2(A). After 14 d of degradation in PBS, the compressive modulus of PMMA bone cement with 30% (w/w) gelatin was 0.46 ± 0.03 GPa, demonstrating a decrease of 68.7%. Besides, bending strength and modulus had also decreased signiﬁcantly as shown in Figures 2(C) and (D). Both the compressive and the bending properties decreased signiﬁcantly after being immersed in PBS for 14 d, which effectively reduced the risk of adjacent cone fracture caused by PMMA bone cement.

3.3. GS release behavior

The total release of GS in PMMA bone cement is less than 20%. And the release process is mainly divided into two stages. The ﬁrst stage is a burst release, during which the PMMA bone cement quickly released the antibiotics on the surface of PMMA bone cement. However, in the second stage, because PMMA did not degrade, the internal GS of PMMA bone cement will hardly be released, so the traditional PMMA bone cement will only have an antibacterial effect in the ﬁrst few hours.

From Figure 3(A), we can see that PMMA bone cement without gelatin released 15.04% GS after being immersed in PBS for 14 d. The release on the ﬁrst day accounted for 53.84% of the total release (figure 3(B)), and the release during the next 13 d accounted for 46.16% of the total release. The minimum inhibitory concentration (MIC) of GS was 1 µg ml⁻¹ [21]. As can be seen from Figure 3(D), PMMA bone cement without gelatin only worked for the ﬁrst 12 h. After the addition of gelatin, in addition to the burst release of GS on the surface of PMMA bone cement, the internal GS will also be released after the gelatin was degraded and precipitated out. For PMMA bone cement with 30% (w/w) gelatin, the release of GS reached 57.22% after being immersed in PBS for 14 d, which is 3.8 times higher than that of the PMMA bone cement without gelatin. The release on the ﬁrst day accounted for 30.90% of the total release, and the release rate slowed down during the next 13 d. It can still maintain a higher GS concentration. PMMA bone cement with 10%, 20%, and 30% (w/w) gelatin can maintain a good antibacterial effect for the ﬁrst 4 d, 6 d, and 12 d relatively (Figure 3(D)). For the
prevention of surgical infection, 3–7 d is often the golden period of prevention, so the new type of PMMA bone cement with the gelatin and GS can meet the needs of surgery.

The best-fitting formula between the relative amount of GS released and time (t) was found to be [39]:

\[
M_t = a + b \cdot (1 - \exp(-k \cdot t)) + c \cdot \sqrt{t}
\]  

(4)

Where \( M_t \) is the relative amount of GS released at time \( t \), \( k \) is the apparent kinetic rate constant of a first-order process, and \( a, b \), and \( c \) are parameters that have physical meanings.

The fitting results are shown in Table 1. \( R^2 \geq 0.95 \) in the table indicates that the GS release can be expressed by the above formula. The \( k \) and \( c \) in the table increased significantly after adding gelatin, indicating that the dissolution of gelatin significantly increased the long-term release of antibiotics.

### 3.4. Surface morphology and porosity of PMMA bone cement

Figure S1 (available online at stacks.iop.org/MRX/9/035405/mmedia) showed the FTIR spectra of gelatin, PMMA, and PMMA bone cement with gelatin. The stretching vibration of the C=O bond located at approximately at 1650 cm\(^{-1}\), the coupling of the bending of the N–H bond and the stretching of the C–N bond located at approximately at 1540 cm\(^{-1}\). These typical bands of gelatin can be found in PMMA bone cement with gelatin, which confirm the presence of unaltered gelatin in PMMA bone cement with gelatin [40]. The gelatin particles after ball milling are shown in figure 4(A). The gelatin has different shapes with size ranging from 150 to 500 μm. Figure 5 shows the surface morphology of the PMMA bone cement before degradation. There were some clear particles (marked by yellow dotted lines). Firstly, the particles can be found in figure 5(B), (C), and (D), and its number is growing. We cannot find the particles in figure 5(A). The main difference between them is
the existence of gelatin. Secondly, the particles showed similar shape and size compared with gelatin particles (figure 4(A)). Thirdly, we cannot find aggregation of barium in figure 4(D). So, the particles were not the aggregation of barium sulfate but the gelatin particles. In figures 5(B)–(D), the gelatin particles were completely embedded between the cement matrix, and with the addition of gelatin, the embedded gelatin particles increased significantly, indicating that the new type of PMMA bone cement was successfully prepared.
The surface morphologies of PMMA bone cement after being immersed in PBS for 14 d are shown in figure 6. The surface morphologies of PMMA bone cement without gelatin (figure 6(A)) showed a little defect (porosity: 0.98%) compared with that before immersion (figure 5(A)). The defects may be caused by the generation of bubbles or wear during the PMMA bone cement preparation process. There were obvious traces of particle dissolution on the surface of PMMA bone cement with gelatin (in figures 5(B)–(D)). Comparing figure 6 with figure 5, it can be confirmed that the pores were formed by the dissolution of the original gelatin particle (the pores are pointed out by the yellow arrows). The porosity became larger and larger with the addition of gelatin. For the PMMA bone cement with 30% (w/w) gelatin, the porosity reached 27.42% which is the highest in this study.

3.5. Antibacterial ability test

Figure 7 shows the ZID of PMMA bone cement after being immersed in PBS for different times (0–3 d, 3–7 d, and 7–14 d). The images (figures 7(A) and (C)) were enlarged for distinguishing, and original images were shown in figure S2. Firstly, whether gelatin was added or not, the ZID of E. coli/S. aureus were very obvious in 0–3 d. With the addition of gelatin, the ZID of E. coli/S. aureus increased from 13.45 mm/12.43 mm to 17.78 mm/16.25 mm, respectively. The good antibacterial ability was attributed to the burst release of GS in the first few hours. For 3–7 d and 7–14 d, PMMA bone cement without gelatin can release little GS inside PMMA bone cement, so the ZID of E. coli/S. aureus were 0 mm. While for the new type of PMMA bone cement, the ZID of E. coli/S. aureus maintained large. For example, PMMA bone cement with 30% (w/w) gelatin, its ZID of E. coli/S. aureus reached 12.80 mm/17.65 mm in 3–7 d and 14.97 mm/18.67 mm in 7–14 d. The good antibacterial ability was attributed to GS released from the inside of PMMA bone cement. In addition, the antibacterial ability of PMMA bone cement showed better effect on gram-negative bacteria than gram-positive bacteria. It’s because the thicker peptidoglycan cell membrane of S. aureus is more difficult to be destroyed [41, 42]. The changing trend of ZID was consistent with the trend of the GS.

Figure 6. SEM images of (A) 0% Gelatin; (B) 10% Gelatin; (C) 20% Gelatin; and (D) 30% Gelatin after degradation. (E) The porosity of PMMA bone cement with different proportions of gelatin. The yellow arrow points to the pores. The error bar indicates means ± standard deviations: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
release. It proved that the addition of gelatin in the PMMA bone cement can make GS release continuously in a large quantity.

3.6. Cell compatibility test

Figure 8 shows the cell viability of PMMA bone cement under different culture time. The cell viability of control group cultured for 1 d was regarded as 100%. For the first day, the cell viability of PMMA bone cement with 20% (w/w) gelatin reached highest and PMMA bone cement with 30% (w/w) gelatin slightly decreased. The reason may be that the gelatin lysate was beneficial to cell proliferation, but the release of GS hindered cell proliferation. When the proportion of gelatin was small, it mainly promoted the cell proliferation. But for 30% gelatin, cell proliferation was hindered due to excessive release of GS. For the third day, both the control group and the experimental group had a small decrease in varying degrees. The main reason was that MC3T3-E1 increased so rapidly that it reached its peak before day 3 and then dropped. But the overall cell survival rate was still above 80%, so all PMMA bone cements have a good cell compatibility.

4. Conclusion

In this study, 5% (w/w) GS, 30% (w/w) BaSO₄, and gelatin (150–500 μm, 10%, 20%, 30% (w/w)) were added to PMMA powder and mixed with PMMA liquid to make an antibacterial PMMA bone cement for vertebroplasty. For the setting properties of PMMA bone cement, the addition of gelatin effectively delayed the reaction time of the powder-liquid mixing. Both the dough time and the setting time had been significantly increased. And it provided sufficient time for clinical operations to mix and stir the powder and liquid. Besides, the maximum temperature of PMMA bone cement decreased with the addition of gelatin, which caused less harm to surrounding healthy tissues. Proper water contact angle allowed better cell or protein adhesion. After being immersed in PBS, the compressive and bending strength/modulus of PMMA bone cement quickly dropped to the lowest of 31.77 MPa/0.46 GPa and 21.30 MPa/2.24 GPa (PMMA bone cement with 30% (w/w) gelatin), which effectively reduced the probability of adjacent cone fracture. The release of GS in the PMMA bone cement with gelatin was increased by up to 3.8 times compared with the traditional PMMA bone cement without gelatin, and the release curve conformed to the dissolution-diffusion equation. The first-order kinetic constant k increased with the addition of gelatin and GS concentration could be kept higher than MIC for 12 d (PMMA...
bone cement with 30% (w/w) gelatin). SEM images show that the gelatin particles were embedded in the cement matrix and the pores left after the dissolution of gelatin particles. The remaining pores explain the significant reduction in mechanical properties and improvement in the release of GS. Finally, antibacterial experiments show that PMMA bone cement with 30% (w/w) gelatin can maintain a good antibacterial effect for at least 14 d, which is much better than the traditional PMMA bone cement without gelatin. PMMA bone cement possesses a good cell compatibility no matter whether the gelatin was added or not. These results show that the PMMA bone cement made by adding BaSO₄, GS, and gelatin to PMMA powder has a good application prospect in vertebroplasty.

Data availability statement

The data generated and/or analysed during the current study are not publicly available for legal/ethical reasons but are available from the corresponding author on reasonable request.

Declaration of competing 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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