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1 # Biodegradable smart materials with self-healing and
2 # shape memory function for wound healing

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10 Number of pages: 10

11 Number of figures: 4

12 Number of tables: 1

13 **Table of contents**

14 Table S1 Elastomers are synthesized by $mPEG_{113}$ to initiate MBC……………………………S3

15 Fig. S1 Mechanical properties of the $P_3$: (a) The Storage modulus (G′, solid line) and loss modulus
16 (G″, dotted line) of the $P_3$ vs. frequency at room temperature; (b) The G′ and G″ of the $P_3$ vs.
17 temperature at a fixed frequency of 1
18 Hz…………………………..……………………………………….………………………S8

19 Fig. S2 the self-healing behavior of the obtained SMPs with an almost fully healing within 130 min
20 at 37 °C or 10 min at 50 °C.…………………………………………..………………………S8

21 Fig. S3 The the mechanical strengths changed of the obtained $P_3$ after 8 days of
22 degradation……………………………………..……………………………………………S9

23 Fig. S4 H&E stained cross-sections of the self-healing of SMPs suture site skin of two groups on

24 the 5 th days after the surgery………………………………………..…………………………S9

25 **S1. Experimental Section**

26 **Materials**

27 2, 2-Bis(hydroxymethyl) propionic acid (99%) was purchased from Tianjin Bodi Chemical Co., Ltd.
28 (Tianjin, China). Benzyl chloride (99%) was purchased from Shanghai Chemical Reagent plant
29 (Shanghai, China). Methoxypolyethylene glycols ($mPEG_{113}$, 5.0 kDa) was purchased from Alpha
30 chemical co., Ltd. Triethylamine (99%) was purchased from Tianjin Damao Chemical Reagent
31 Factory. (Tianjin, China). Stannous octoate [$Sn(Oct)_2$] (99%) was purchased from SigmaAldrich.
32 Other solvents were obtained from commercial sources and used as received.
33 Ethics statement: Only volunteer blood donor was included. She was specifically informed that the
34 obtained materials would be tested for hemolysis test. Meanwhile, she offered signed written
35 informed consent.
36 **The synthesis of monomer and the self-healing elastomer**: The monomer was synthesized
37 according to our previous work. [1] It is noted that the monomer 2-methyl-2-benzyloxy carbonyl
38 propylene carbonate (MBC), the $mPEG_{113}$ and $Sn(Oct)_2$ are deemed as a hydrophobic monomer
39 (denoted as M), a macromolecular initiator (denoted as I), and a catalyst (denoted as C),
40 respectively. The detail was described as follows: $mPEG_{113}$, MBC and stannous octoate were put
41 into a dried silanized glass ampoule proportionally (Table S1). The ampoule was evacuated, purged
42 with nitrogen three times, and sealed. Then kept the ampoule in an oil bath at 85$^{o}$C for 24 h. After
43 polymerization, the as-prepared organogels were swelled in a large amount of methylene chloride
44 for 48 h to dissolve unreacted monomer. Then, products were shrunken in ethanol for 24 h and
45 shrank products were dried in a vacuum oven for four days to constant weight. Special note: before

46 the experiment, Teflon mold was put into the polymerization tube to ensure product molding, and

47 the resultant elastomers were named as $P_1$-$P_3$.

48 Table S1 Elastomers are synthesized by $mPEG_{113}$ to initiate MBC

| samples | M:I | M:C | reaction time /h | reaction temperature /°C | whether gel |
|---|---|---|---|---|---|
| $P_1$ | 2000:1 | 500:1 | 24 | 100 | √ |
| $P_2$ | 2000:1 | 600:1 | 24 | 100 | √ |
| $P_3$ | 2000:1 | 800:1 | 24 | 100 | √ |

49 Note: "×" will not be the formation of gelation, and "√" will be the formation of gelation

50 **The self-healing behavior of the elastomers:** The repeatability tests are carried out in order to

51 accurately quantify the self-healing efficiency, and the obtained elastomers square (5.000

52 mm×5.000 mm) were cut into three completely separate pieces. At least three parallel samples were

53 recorded for each sample, using a surgical blade. Then, the self-healing of every sample has been

54 completely cut and have applied a slight force to dock softly the two cut interfaces.

55 **Structure and thermal behavior characterization:** Unless otherwise noted, all tests are measured

56 at room temperature, and at least three parallel samples are recorded for each sample. FT-IR spectra

57 are determined on a PerkinElmer Spectrum One (B) spectrometer (PerkinElmer, Foster City, CA,

58 USA) over the wavenumber ranging from 4000 to 500 $cm^{-1}$. The differential scanning calorimetry

59 (DSC) is carried out with a NETZSCH DSC-204 thermal analyzer (Netzsch, Hanau, Germany), the

60 measurements are obtained over a temperature range from -50 to 200 °C at a rate of 10 °C/min under

61 flowing nitrogen. The thermal decomposition temperatures are performed under a nitrogen

62 atmosphere with a Netzsch 209C TGA (Netzsch, Hanau, Germany) at a heating rate of 20 °C/min.

63 **Mechanical properties characterization:** All tests were performed on a universal test machine

64 (AGS-X，Shimadzu, Japan) with a 2000 N load cells. For tensile strength studies, all tests were

65 performed at a constant speed of 10 mm/min at room temperature, at least five parallel samples were

66 recorded for each sample.

67 **Self-healing tests:** Optical microscope is used to observe the self-healing processes (Smartzoom 5,
68 Beijing, China) to record the changes of the scratch-width track. The procedure of self-healing at
69 room temperature, without requiring any external stimuli. The self-healing ability is monitored by
70 the detection of mechanical properties embracing modulus. Healing efficiency (HE) of elastomers
71 can be defined as the ratio of the stress or modulus of the healed ($M_h$) and pristine elastomer ($M_p$):
72 HE= ($M_h/M_p$) eqn. 1 [4]
73 **Shape memory performance.** To qualitatively evaluate the shape memory properties and
74 temperature responsiveness, $P_1$-$P_3$ were made into 3 cm rod-shaped materials. The plasticity was U-
75 shaped under external force, and at least three parallel samples were recorded for each sample. The
76 detailed preparation is shown as follows: the obtained elastomers were put into 7 $^oC$ ice water to fix
77 shape for 10 s, and a 10 g weight was loaded for 10 s without deformation. Then, the fixed shape
78 was immersed in 37 $^oC$ water for 10 s. To calculate the shape fixity ($R_f$): $R_f$=(180-α)/180 eqn. 2 and
79 shape recovery ($R_r$): $R_r$ =(180-α-β)/(180-α) eqn. 3, where α is the recovery angle, 180-α the fixed
80 temporary angle, β the residual angle. [5]
81 **Enzymatic degradation**
82 Enzymatic degradation of the obtained elastomer was carried out in lipase solutions
83 (Aspergillus oryzae,≥100,000 U/g) were purchased from Sigma-Aldrich) at 37 °C. The samples cut
84 into a cylindrical structure (ca. 3 mm (L)×2.000 mm (D)), and was put into a clean transparent
85 colorimetric tube. Then, 200 μL of enzyme solution were added into the tube, and the fresh enzyme
86 solution was exchanged every three days. The tube were incubated at 37 °C with constant shaking.
87 Furthermore, the extent of biodegradation was expressed in terms of weight loss percentage (eqn.
88 4). The loss weight ratio (%)=($m_d$-$m_i$)/$m_i$, where $m_d$ is the dried weight of after degradation, while

89 $m_i$ is the initial weight. Special note: before the fresh enzyme solution was put into the tube, the

90 sample was rinsed deionized water for three times.

91 **Biological recognition**

92 **In Vitro Cell Compatibility Test.** The experiments of cell counting kit-8 (CCK-8) and clone-

93 forming were used to evaluate the effect of the obtained materials on cell viability. The cell

94 compatibility of the obtained elastomer was performed in vitro.[6] The cell culture experiments were

95 determined by Human umbilical vein endothelial cells (HUVEC), which were purchased from the

96 Cell Bank of Chinese Academy of Sciences Type Culture Collection Committee (Shanghai, China).

97 Firstly, the HUVEC cells were digested, then centrifuged and counted. The HUVEC cells were

98 cultured in Dulbecco's modified Eagle medium (Gibco) contained 1% penicillin-streptomycin

99 solution (Gibco) and 10% fetal bovine serum (Gibco) in an incubator at 37 °C/5% $CO_2$. Special

100 note: all of the materials were cut into a cylindrical structure ( thickness of 1 mm, diameter of 6

101 mm) and sterilized by 75% ethanol for one night and washed with phosphate buffer saline (PBS).

102 Subsequently, the sterilized samples were put into a 96-well plate. 1 mL HUVEC with a density

103 of 8×105 cells/mL was seeded on them. The HUVEC cells were incubated in a thermostatic

104 incubator at 37 °C for 1 and 3 days, respectively. After the treatment, culture media was removed

105 and fresh media (500 μL) containing 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-

106 disulfonic acid benzene)-2H-tetrazole monosodium salt (CCK-8) solution (100 μL, 5 mg/mL) was

107 placed into each well to further incubate at 37 °C for 4 h. Finally, the absorbance of the solution

108 was measured at 450 nm by a microplate reader (ThermoFisher Scientific). Meanwhile, to

109 investigate the morphology of the cell proliferation on the specimens, the HUVECs cells after 3

110 days of incubation were stained with calcein AM (A017, USA) for 20 min. Then, the cells were

111 gently washed with PBS for third, and the spreading and attachments of cell on the specimens were

112 observed by the confocal laser scanning microscope (Eclipse Ts2-FL, Nikon, Japan).

113 **Clone-forming experiments.** The HUVEC cells in the logarithmic growth phase were used to

114 centrifuge and count the cells. Then, the cells were inoculated at 2000 cells/well in a 6-well plate,

115 and were drew cross to make the cells in the well plate evenly dispersed. The cells were placed into

116 an incubator for regular observation. After 3-5 days, when the appearance of obvious colonies were

117 found, the well plate was removed to terminate the culture. After carefully removing the culture

118 solution, the culture media was rinsed thoroughly with PBS for 3 times. Then, the Coomassie

119 Brilliant Blue staining fixative solution was added to stain at room temperature for 10 min. Discard

120 the staining solution, the deionized water was used to rinse for third and dry at room temperature.The

121 inverted phase contrast microscope (DMIL-PH1, Leica, Germany) was used to observe the clone-

122 forming condition of cells.

123 **Blood Compatibility.** The erythrocyte compatibility of the elastomers can be studied by the

124 hemolysis test. [7] First, 5 mL of the volunteer's blood was added into 10 mL of physiological saline

125 that does not contain calcium and magnesium ions. Then, the obtained saline containing the

126 volunteer's blood was centrifuged at 2000 rpm for 10 min to obtain red blood cells (RBCs). The

127 RBCs were diluted into physiological saline with a final volume of 100 mL for later use. Secondly,

128 1 mL of the RBC suspension was introduced into the sample immersed in deionized water and

129 physiological saline (sodium chloride solution), and incubated at 37°C for 3 h, and then the RBCs

130 were named as the positive control and negative control, respectively. Finally, the suspensions were

131 incubated in a shaking incubator at 37°C for 3 h, and then centrifuged at 8000 rpm for 5 min. The

132 absorbance of the released hemoglobin in the suspension is measured by the UV-Vis

133 spectrophotometer (UV-Eppendorf AG, Co. Ltd Germany) at a wavelength of 540 nm, and the

134 calculation formula for the hemolysis rate is eqn. 5: Hemolysis ratio (%)=($A_s$-$A_n$)/($A_p$-$A_n$)×100,

135 where $A_s$ is the absorbance of the tested sample suspension. $A_n$ and $A_p$ were regarded as the

136 absorbance of the negative control and the positive control, respectively.

137 **In vivo studies**

138 Wistar adult female rat (180 g, 12-14 weeks old) were purchased from Benxi Longevity Biology

139 (Benxi, China), and the animals were housed in cages at 25 °C, and fed with standard pellets and

140 water ad libitum. An incision of length 2 cm was made in the dermal skin of the rat's back near the

141 neck (wiped with 70% alcohol). Rats were anesthetized using thiopental sodium (dose of 25 mg per

142 kilogram of body weight) for all surgical procedures.[8] The animals were randomly divided into

143 two groups: the animals in group I (n=2) were sutured with traditional sutures, and the obtained

144 SMP sutures were used to suture the animals in group II (n=4). The obtained SMPs were made into

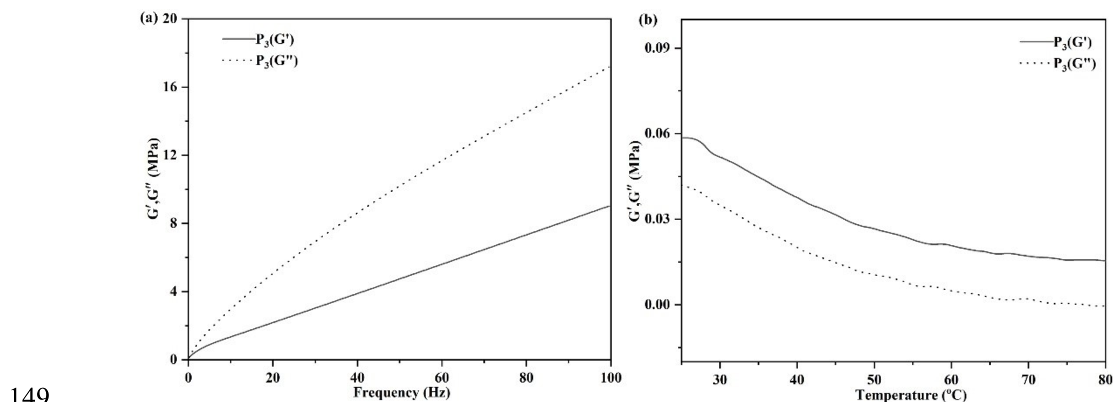
145 0.5 mm width and 0.2 mm thickness and autoclaved before the suturing experiment. Note: animal

146 experiments were performed according to the Guidelines of the Liaoning Administrative Committee

147 on Animal Research.

## 148 Supporting Figures

149

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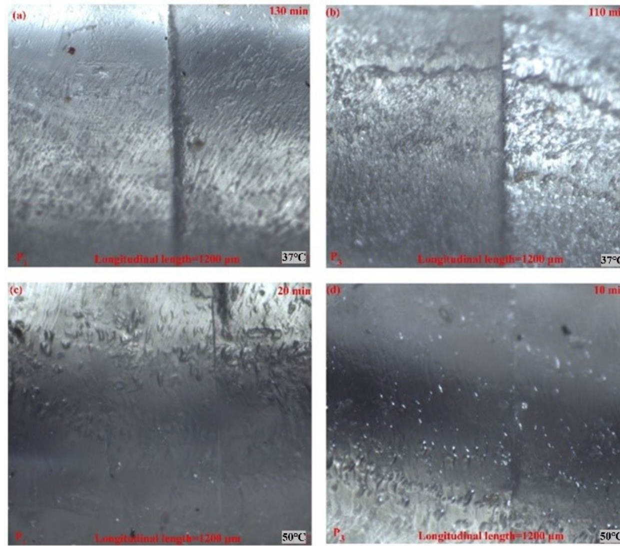
153 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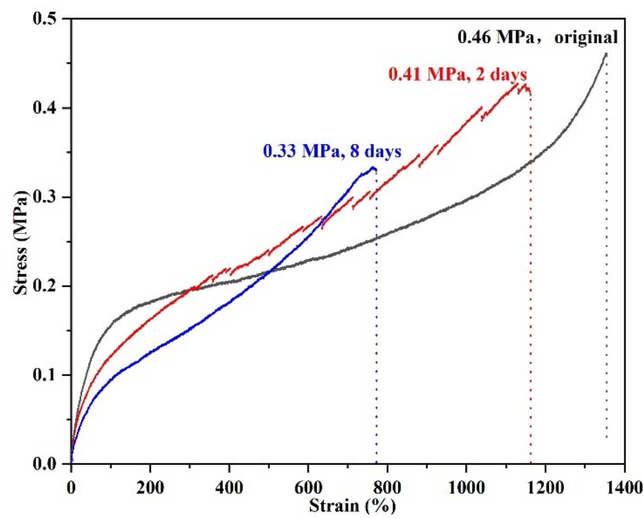
157 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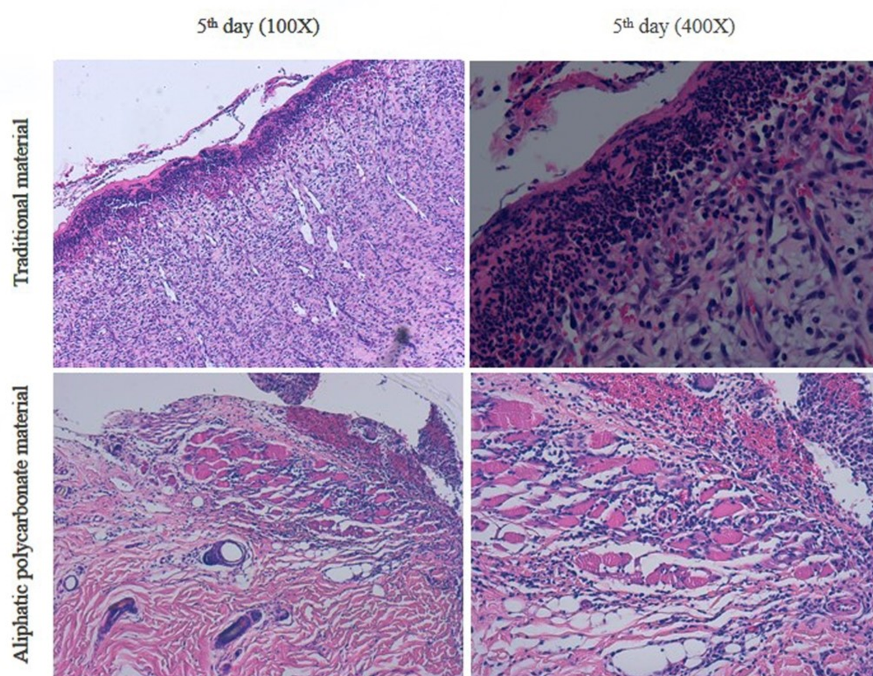


160 Fig. S4. H&E stained cross-sections of the self-healing of SMPs suture site skin of two groups on

161 the 5 th days after the surgery.

162 **Reference**


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