

# Easily-injectable shear-thinning hydrogel provides long-lasting submucosal barrier for gastrointestinal endoscopic surgery

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## ABSTRACT

Submucosal injection material has shown protective effect against gastrointestinal injury during endoscopic surgery in clinic. However, the protective ability of existing submucosal injection material is strictly limited by their difficult injectability and short barrier time. Herein, we report a shear-thinning gellan gum hydrogel that simultaneously has easy injectability and long-lasting barrier function, together with good hemostatic property and biocompatibility. Shear-thinning property endows our gellan gum hydrogel with excellent endoscopic injection performance, and the injection pressure of our gellan gum hydrogel is much lower than that of the small molecule solution (50 wt% dextrose) when injected through the endoscopic needle. More importantly, our gellan gum hydrogel shows much stronger barrier retention ability than normal saline and sodium hyaluronate solution in the *ex vivo* and *in vivo* models. Furthermore, our epinephrine-containing gellan gum hydrogel has a satisfactory hemostatic effect in the mucosal lesion resection model of pig. These results indicate an appealing application prospect for gellan gum hydrogel utilizing as a submucosal injection material in endoscopic surgery.

## 1. Introduction

According to the global cancer statistics, an estimated 19.3 million new cancer cases occurred worldwide in 2020 [1]. The two most common types of gastrointestinal cancers are gastric cancer and colorectal cancer, ranking as the third and sixth commonly diagnosed cancers,

respectively [1]. Actually, the wall of gastrointestinal tract can be divided into four layers, including mucosa, submucosa, muscularis propria, and serosa [2]. Early gastrointestinal tumor is defined as the lesion that only infiltrates within the submucosa and can be resected by endoscopic surgery [3,4]. Benefiting from the screening strategy toward gastrointestinal lesion, more and more early-stage tumors can be found

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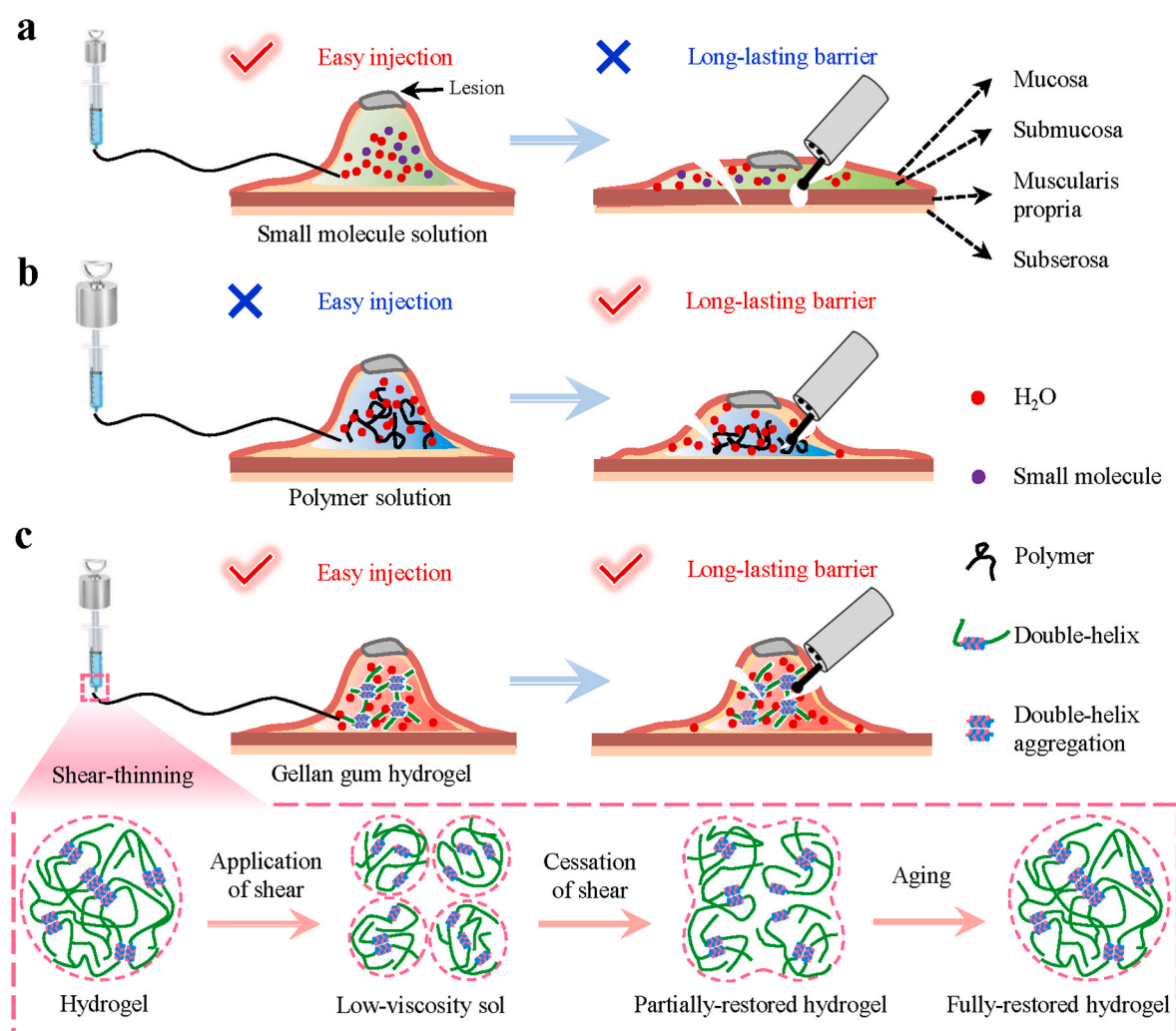
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and treated with endoscopic resection [5–10]. Endoscopic resection has been indicated to be a popular and effective method for the treatment of early-stage gastrointestinal tumors.

Due to the foldable and thin features of gastrointestinal tract, the resection of lesion during endoscopic surgery still faces huge challenges such as positive margin, perforation, and bleeding [11–13]. The application of submucosal injection material (SIM) to form submucosal barrier is a crucial method to satisfy the demand of endoscopic resection [5, 6]. SIM is able to elevate lesion by producing a thick liquid cushion, which creates adequate operation spaces between the mucosa and muscularis propria, and shields the electrical and thermal damage generated during the endoscopic resection procedure [14,15]. Normal saline (NS) has been used to elevate mucosal lesion after submucosal injection through the slender tract of endoscope with proper injection pressure [16]. However, the submucosal cushion height of NS will quickly reduce after injection because of the rapid diffusion of small molecules, and result in a fast weakening of the barrier function, which can increase the difficulty of operation and the risk of perforation and bleeding (Fig. 1a) [17–19]. Therefore, new SIM with long-lasting barrier

function to expose the edge of tumor and prevent muscularis propria damage is in urgent need.

Recently, progress has been made to achieve long-lasting barrier function through the injection of hypertonic liquids, polymer solutions, and hydrogels. However, most SIMs will encounter two bottleneck problems. On one hand, the effect of maintaining submucosal cushion height by injecting hypertonic liquids such as dextrose solution (DS) is unsatisfactory; the height will be quickly reduced to less than 50% within 30 min, and the hypertonic property can cause serious tissue damage (Fig. 1a) [20–22]. On the other hand, most polymer solutions with high viscosity such as sodium hyaluronate (SH) could create more lasting barrier, but they are difficult to inject due to their high viscosity (Fig. 1b) [23–30]. Similarly, some hydrogels are found to provide moderate submucosal cushion height because of their high-water content and stiffness, but the inconvenient operation still restricts their applications [31]. For example, the hydrogels formed by two or more components are inconvenient to be simultaneously injected [5,32,33], and photocurable hydrogels require ultraviolet light sources, making the operation difficult under the endoscope [34–37]. What's worse, the



**Fig. 1.** Schematic illustration of injectability and barrier function of submucosal injection materials (SIMs) for endoscopic injection. a) Conventional small molecule solution could be easily injected into the submucosa through the slender endoscopic needle, but could not maintain the submucosal cushion for a long time to provide long-lasting barrier function. b) Although conventional polymer solution is difficult to be injected into the submucosa with the endoscopic needle, it could offer a more lasting barrier function than the small molecule solution. c) Benefitting from shear-thinning characteristic, gellan gum hydrogel could be easily injected into the submucosa through the slender endoscopic needle and form long-lasting submucosal cushion simultaneously. Thus, it could elevate lesion satisfactorily and provide durable barrier effect to reduce the risk of perforation and bleeding. The shear-thinning characteristic could be explained as follows: when a shear force is applied, the hydrogel is broken into particles, leading to formation of low-viscosity sol; once the shear is ceased, the gel network is partially restored and then fully restored after a period of aging.

temperature-sensitive hydrogels have the risk of clogging [38–42]. Recently, few studies began to focus on the *ex vivo* barrier function of shear-thinning materials, but their biocompatibility and operability of endoscope are still unknown [6,43]. Therefore, how to balance the easy operability and long-lasting barrier property still remains a great challenge in SIMs.

In this work, we successfully develop a shear-thinning gellan gum hydrogel (GGH) with excellent biocompatibility, easy endoscopic injectability, long-lasting *in vivo* barrier function, and good hemostatic property (Fig. 1c). The GGH is formed by the double-helix aggregation as cross-linking point and thus presents a shear-thinning hydrogel characteristic. Once applying the shear, the hydrogel can be quickly transformed into low viscosity sol, providing easy endoscopic injectability; once the shear is ceased after completing submucosal injection, the hydrogel can entirely restore the robust hydrogel network, providing long-lasting barrier cushion. We find that the injection pressure of the shear-thinning GGH is significantly lower than that of the SH polymer solution and even lower to that of the small molecule solution (50 wt% dextrose). As such, GGH is easily injected into the stomach of a pig through the endoscope. The injected GGH with a robust restored network can create a high submucosal cushion, and perform very well as a submucosal barrier cushion for more than 30 min, a time enough for most endoscopic resection procedures. Our GGH contains a hemostatic agent (epinephrine) and then demonstrates satisfactory hemostatic activity in the mucosal lesion resection model of pig. Moreover, our GGH has good biocompatibility and does not inhibit cell growth, demonstrating a potential to heal wound after resection. We hope that our shear-thinning hydrogel could be an ideal SIM for clinical endoscopic surgery.

## 2. Results and discussion

Gellan gum is a natural polymer produced by the bacterium *Sphingomonas elodea*, and approved for food and medical usage by US FDA and European Union (E418) [44,45]. Our GGH was facilely obtained by heating a high acyl gellan gum solution at 170 °C, followed by cooling

down to room temperature. The high acyl gellan gum is a type of linear polysaccharides with a repeating unit of tetra-saccharide (Fig. S1, Supporting Information) [46]. It could exist in the form of random coils above 85 °C [47]; the coils turn into double-helices firstly during cooling, and then the helices aggregate to form a three-dimensional hydrogel network, which could come apart at low stress, leading to a great shear-thinning property [45,48,49].

The as-obtained GGH is a “weak gel”, which has the pourable property of viscoelastic fluid but maintains the network of the gel [47–49]. Considering that 0.4 wt% SH was widely used as a SIM in endoscopic treatments [19,21,28], we used it as the control sample. The rheological and viscosity properties of GGH were studied to investigate its gel characteristics and shear-thinning property [50,51]. In order to determine whether the sticky gellan gum liquid is a hydrogel, the frequency sweep measurement was done. It was found that in the low frequency range of 0.1–1.0 Hz, storage modulus ( $G'$ ) of GGH was always greater than loss modulus ( $G''$ ) (Fig. 2a), while  $G'$  of SH was always smaller than  $G''$  (Fig. 2b), indicating that GGH was indeed a hydrogel ( $G' > G''$ ) whereas SH was a solution ( $G' < G''$ ) [47]. Shear viscosity test was then used to indicate GGH presented a shear-thinning characteristic. As shown in Fig. 2c, when shear force increased from 2.0 to 7.0 Pa, shear viscosity of GGH dropped rapidly from 125000 to 10 mPa s, which was reduced by 12500 times. More importantly, under shear force of 7.0 Pa, the viscosity of the shear thinned GGH (10 mPa s) was 4.3 times as small as that of SH (43 mPa s). As a result, the injection pressure of GGH was as low as 127.8 kPa, which was significantly smaller than those of polymer solutions including sodium alginate (SA, 566.2 kPa), SH (547.2 kPa) and sodium carboxymethyl cellulose (SCC, 507.8 kPa), and even comparable to those of small molecule solutions including NS (37.9 kPa), glycerol fructose sodium chloride injection (GF, 55.7 kPa) and DS (353.3 kPa), when injected through a 22-gauge endoscopic injection needle (Fig. 2d). Obviously, such a low injection pressure could greatly decrease the operation difficulty of endoscopic injection when utilizing GGH as a SIM.

In addition to the easy injection performance, our injected GGH also demonstrated a strong shape retention function, because the hydrogel

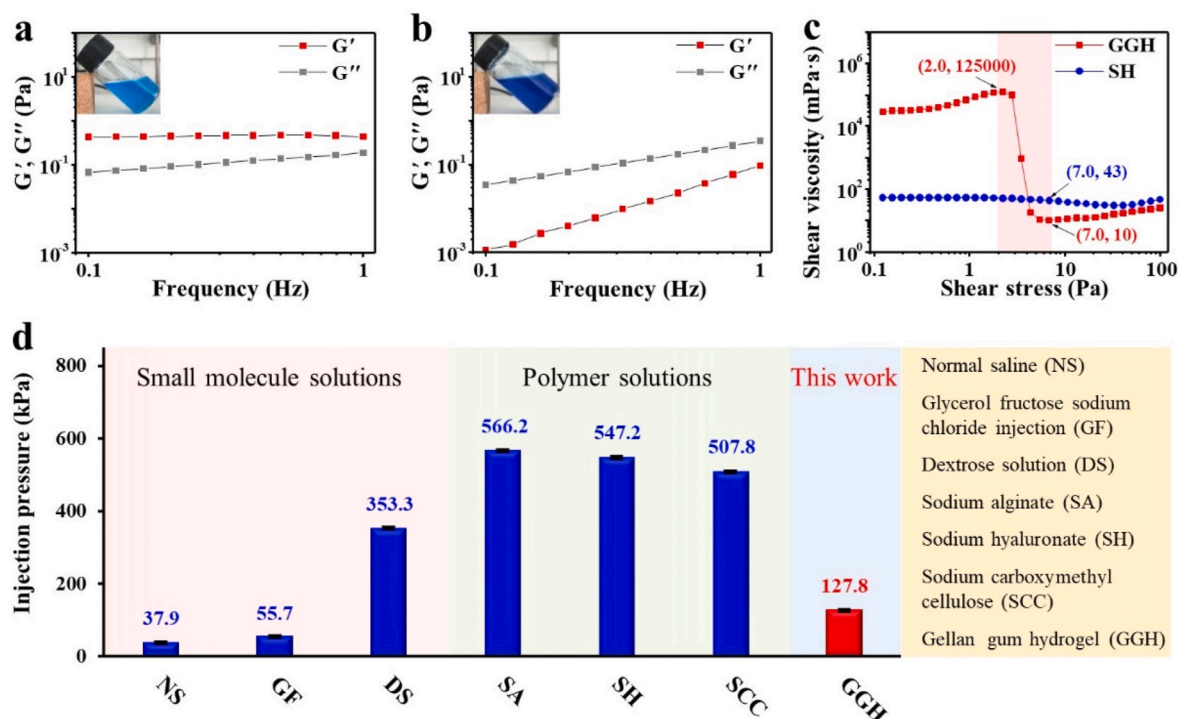
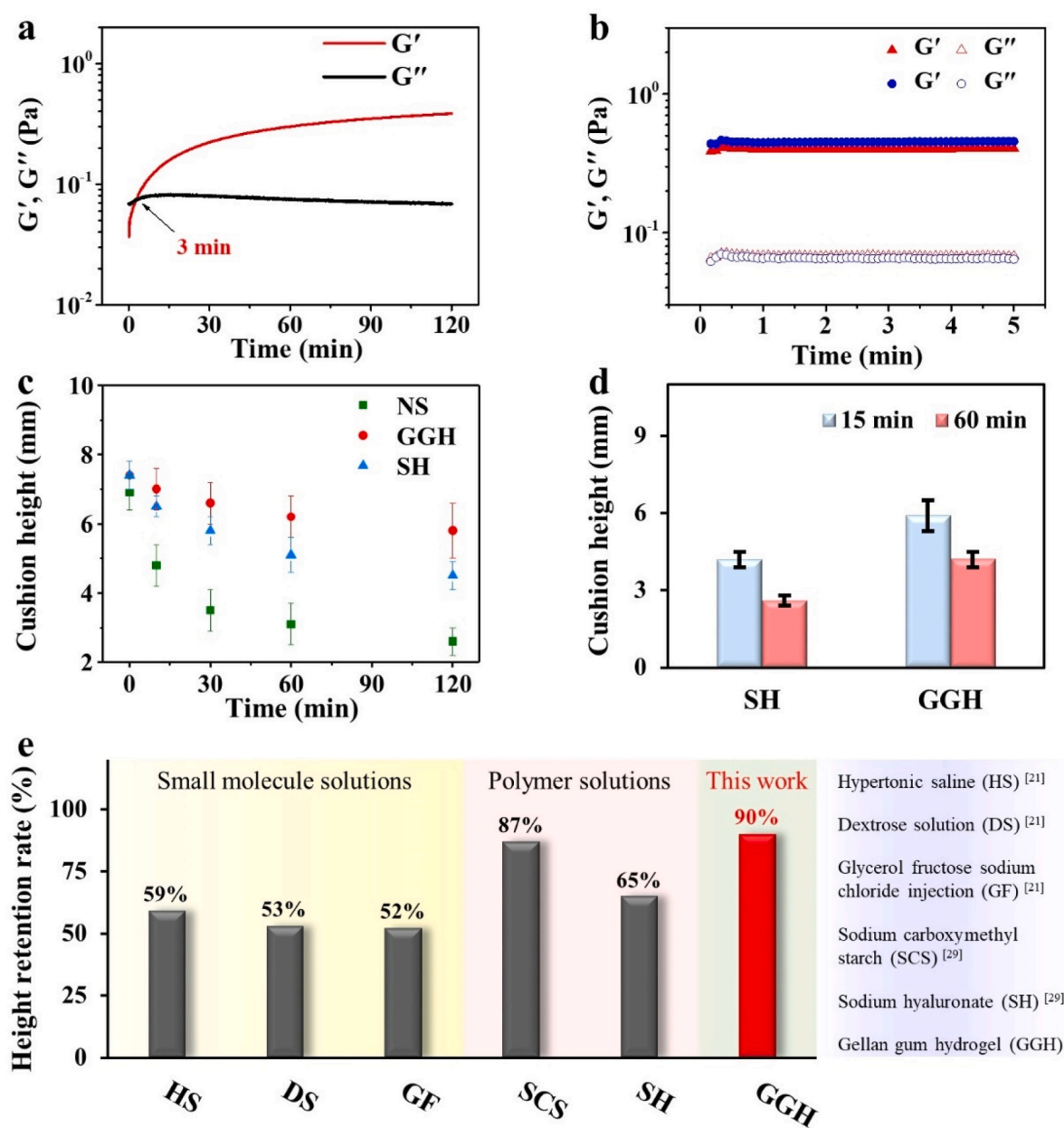


Fig. 2. Rheological properties, shear-thinning characteristic, and injection pressure of submucosal injection materials. Oscillatory frequency sweeps and digital photos (inset) of a) GGH and b) SH. c) Shear viscosity-stress curve of GGH and SH. d) Comparison of GGH with other SIMs in terms of injection pressure.

could entirely restore the robust hydrogel network once the injection shear was ceased. As shown in Fig. 3a–b, GGH rapidly underwent sol-gel transition and then  $G'$  went back to the original modulus before injection. The result indicated that GGH had a robust restored network and could create a high submucosal cushion after submucosal injection. As shown in Fig. 3c, when GGH and control samples (NS and SH) were injected into the *ex vivo* porcine stomach, their submucosal cushion height was very close at 0 min. However, the submucosal cushion height of NS and SH quickly decreased to 50% and 80% at 30 min, respectively, while our GGH still kept 80% of submucosal cushion height after 120 min (Fig. 3c). Furthermore, to the best of our knowledge, the height retention rate (90%) of GGH is higher than those of reported small molecule solutions and polymer solutions at 30 min after submucosal injection [21,29] (Fig. 3e). The superior cushion retention ability of GGH was also confirmed by *in vivo* rat model. After 1.0 mL of GGH was

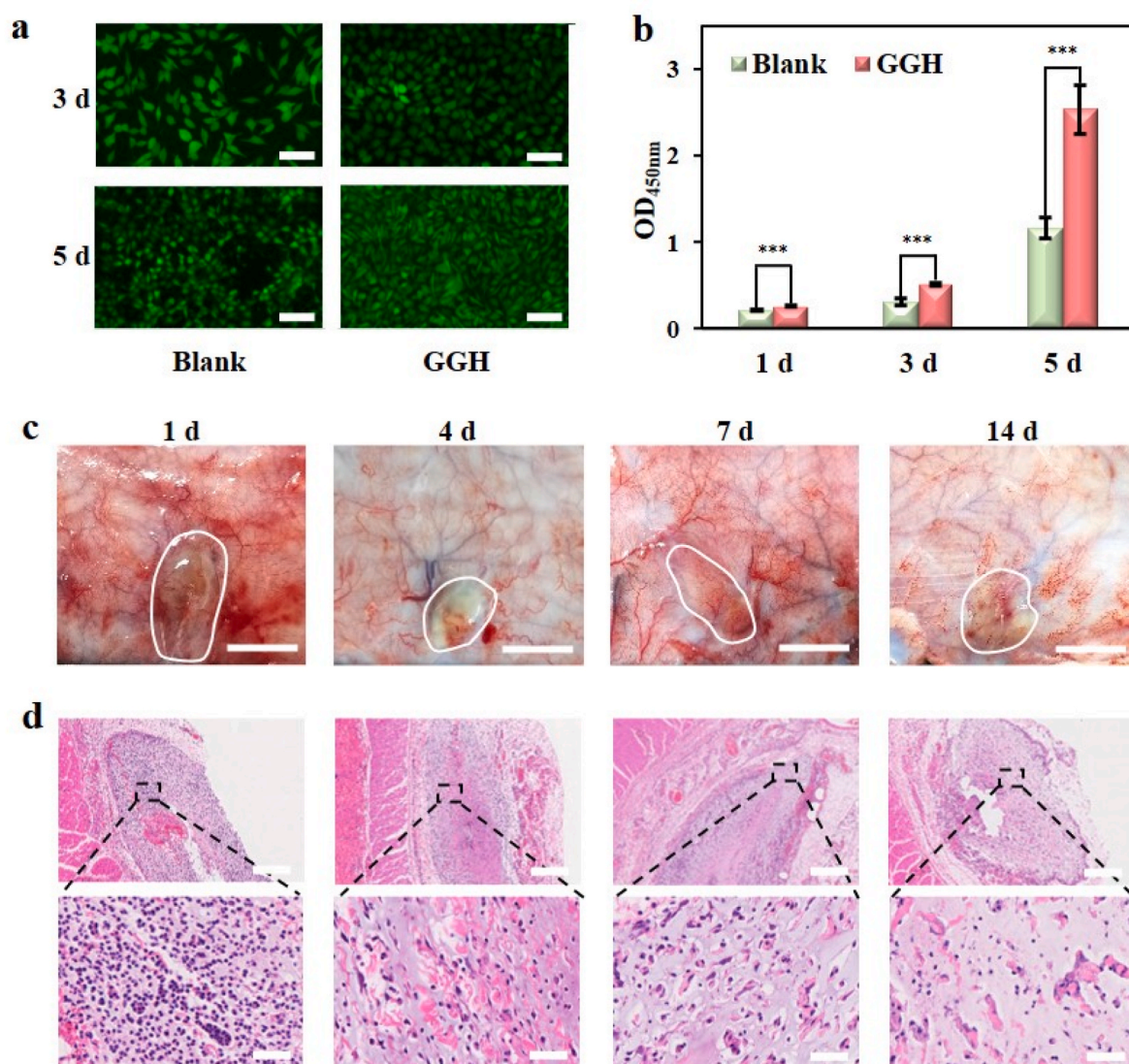
subcutaneously injected into the back of rat, the cushion height of GGH was still up to 5.9 and 4.2 mm at 15 and 60 min, respectively (Fig. 3d). In contrast, the height of SH quickly dropped to 4.2 and 2.6 mm at 15 and 60 min, respectively (Fig. 3d). The *ex vivo* and *in vivo* cushion retention comparisons clearly revealed our GGH had long-lasting barrier performance as a SIM.

The biocompatibility of SIM is an important concern for their biomedical application. The live/dead cell double staining kit experiment exhibited that after being cultured with our GGH, L929 fibroblast cells grew well and maintained their normal spindle shape (Fig. 4a). Cell Counting Kit-8 (CCK-8) assay indicated that L929 fibroblast cells in our GGH group grew better than the blank group (Fig. 4b), demonstrating the good biocompatibility and no inhibition of cell proliferation for GGH. To further illustrate the biocompatibility *in vivo*, GGH was injected subcutaneously on the back of the rat to form a cutaneous hillock about



**Fig. 3.** Rheological properties and shape retention ability of GGH. a) Oscillatory time sweep of GGH after injection through the endoscopic needle. b) Comparison of  $G'$  and  $G''$  for GGH before (blue) and after (red) injection for 2 h. c) Submucosal cushion height as a function of time for NS (green), SH (blue) and GGH (red) in the *ex vivo* model. d) Comparison of subcutaneous cushion height for SH and GGH at 15 and 60 min in the *in vivo* model. e) Comparison of GGH with the reported SIMs in terms of height retention rate.



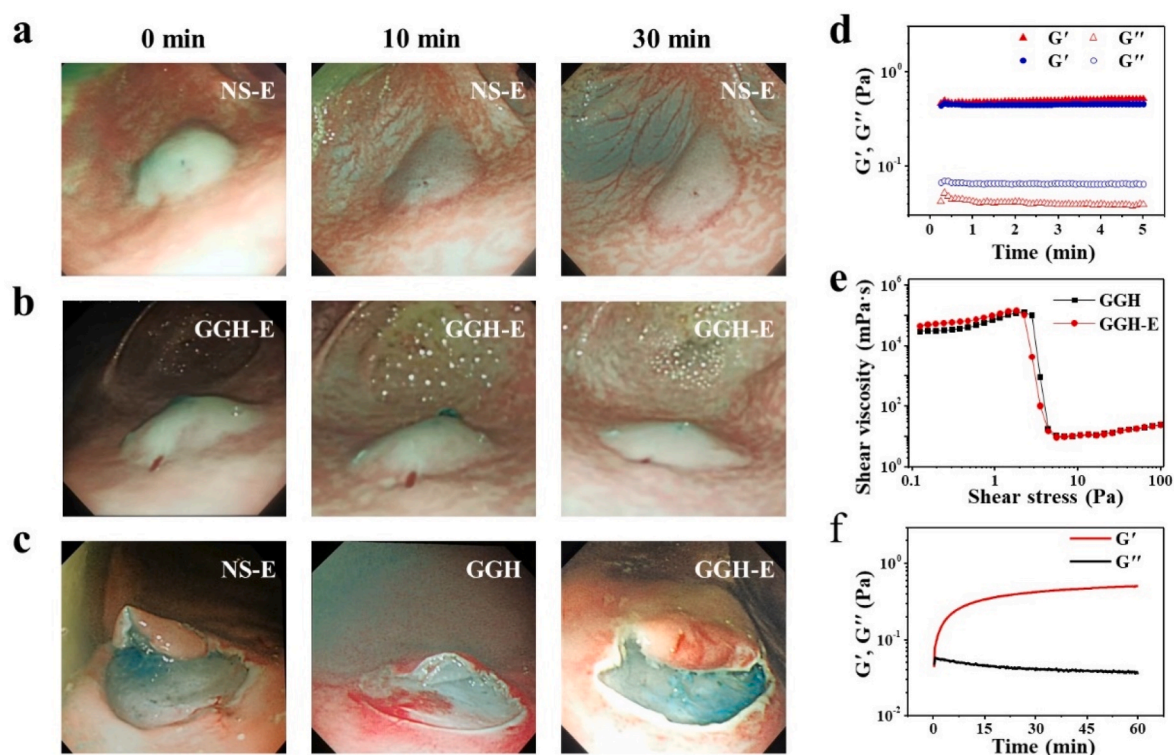


**Fig. 4.** Biocompatibility of GGH. a) Inverted fluorescence microscope images of L929 fibroblasts after incubation for 3 and 5 d (bar = 100  $\mu$ m). b) CCK-8 assay about the cytotoxicity of GGH to L929 fibroblasts after incubation for 1, 3 and 5 d. c) Images of subcutaneous tissues around the injection site of GGH at different times after subcutaneous injection (bar = 1 cm). d) H&E staining of GGH at different times (upper bar = 500  $\mu$ m, bottom bar = 50  $\mu$ m).

2.0 cm in diameter. It was found that there was no tissue edema, ulcer, purulent secretion, and other obvious foreign body reaction in the surrounding area of GGH within 14 d (Fig. 4c). The subcutaneous tissue containing GGH was cut into slices and assessed by haematoxylin and eosin (H&E) staining. Local infiltration of inflammatory cells was found inside and around the hydrogel on day 1; however, the inflammatory response attenuated to slight level on day 4, and a very few inflammatory cells could be found on day 7 and day 14 (Fig. 4d). The result was similar to that of previous studies [52–55], confirming the acceptable biocompatibility *in vivo*.

In order to assess *in vivo* submucosal barrier ability of our GGH, the submucosal injection model was performed by endoscopic procedure in the stomach of pig. 3.0 mL of GGH or NS containing 10 mg/L of epinephrine hemostatic agent (denoted as GGH-E or NS-E) was injected into gastric submucosa through the endoscopic equipment. It was found that a good submucosal cushion was formed after injection of NS-E or GGH-E; the cushion of NS-E flattened dramatically within 10 min and almost completely flattened at 30 min, while that of GGH-E remained nearly unchanged in 10 min and still retained an acceptable height at 30 min (Fig. 5a–b). In addition to the barrier function, hemostasis is another necessary index for SIMs during the endoscopic resection. To

explore the effect of GGH on the release rate of epinephrine, an *in vitro* drug release experiment was conducted. It was found that there was no significant difference in the release rate of epinephrine between NS-E and GGH-E (Fig. S4, Supporting Information). The result indicated that the epinephrine in GGH could be released smoothly to play a hemostatic effect in time. In order to verify the actual hemostatic effect of GGH-E, we performed a simulated endoscopic resection in a pig stomach model. 5.0 mL of GGH, GGH-E or NS-E was injected into the gastric submucosa and the mucosa was resected by endoscope electrosurgical system. According to Fig. 5c, as compared to GGH without epinephrine, both GGH-E and NS-E showed satisfy hemostatic effect during endoscopic resection of mucosa. It is worth mentioning that addition of epinephrine had no adverse effect on the gel properties and shear-thinning characteristic of GGH; there was no significant difference in comparison of  $G'$  and  $G''$  for GGH and GGH-E, their shear viscosity-stress curve almost coincide, and GGH-E also could quickly undergo sol-gel transition after injection with endoscopic needle (Fig. 5d–f). These results suggested that our epinephrine-containing GGH could generate long-lasting barrier in stomach of big animal to protect stomach from perforation and bleeding during endoscopic surgery, and thus might be an ideal material for gastrointestinal endoscope procedure in clinic.



**Fig. 5.** Submucosal barrier ability of GGH *in vivo*. Endoscopic images of submucosal cushions developed by a) NS-E and b) GGH-E at 0, 10 and 30 min after endoscopic injection. c) Comparison of hemostatic effect for submucosal cushions generated by NS-E, GGH, and GGH-E during resection by endoscope electro-surgical system. d) Comparison of  $G'$  and  $G''$  for GGH (blue) and GGH-E (red). e) Shear viscosity-stress curve for GGH and GGH-E. f) Oscillatory time sweep of GGH-E after injection through the endoscopic needle.

### 3. Conclusion

In summary, we report the development and application of GGH, which is a shear-thinning hydrogel, facilely prepared by heating high acyl gellan gum polysaccharide solution and then cooling down. Benefiting from the shear-thinning characteristic, the as-obtained easily-injectable GGH shows long-lasting submucosal barrier performance *in vivo* as a SIM, and simultaneously has good hemostatic property and biocompatibility. Moreover, GGH does not inhibit cell proliferation and growth, demonstrating a potential to heal wound. Based on these characteristics, our GGH could be a promising hydrogel material for broad application in endoscopic resection techniques, and potential development in luminal constriction, drug delivery, and tissue engineering.

### 4. Materials and methods

#### 4.1. Materials

Sodium chloride (>99.5%), sodium alginate (90%), sodium hyaluronate (>99.0%,  $M_w = 450$  kDa) and high acyl gellan gum (>99.0%) were purchased from Macklin, China. Sodium carboxymethyl cellulose was purchased from Guangzhou Qihua Medical Equipment Co., Ltd., China. Dextrose monohydrate (>99.7%) was purchased from Guangzhou Chemical Reagent Factory, China. Glycerol fructose sodium chloride injection was purchased from Jiangsu Chia Tai Fenghai Pharmaceutical Co., Ltd., China. Epinephrine hydrochloride injection (1 mg/mL) was purchased from Grandpharma Co., Ltd., China. Methylene blue injection (10 mg/mL) was purchased from Jiangsu Jichuan Pharmaceutical Co., Ltd., China. 10% chloral hydrate was purchased from Jin Clone Biotechnology Co., Ltd., China. Xylazine hydrochloride was purchased from Jilin Huamu Animal Health Product Co., Ltd., China. Propofol was purchased from Xi'an Libang Pharmaceutical Co., Ltd.,

China. MTT reagent was provided by Thermo Fisher Scientific, USA. Phosphate buffered saline (PBS) was purchased from Corning, USA. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin solution (PSS), and trypsin-ethylene diamine tetraacetic acid (EDTA) were obtained from Gibco, USA. Calcein-AM (2  $\mu$ M) and propidium iodide (8  $\mu$ M) were obtained from Sigma, USA.

#### 4.2. Preparation of SIMs

**Preparation of GGH:** Firstly, 1.0 mL of methylene blue injection (10 mg/mL) was dissolved in 1 L of deionized water to obtain a methylene blue stock solution with a concentration of 10 mg/L. Subsequently, 60 mg of high acyl gellan gum was added into 100 mL of methylene blue stock solution, and then the mixture was sealed and heated at 170 °C for 1 h with a heating stirrer (LC-DMS-H, Lichen, China). Thereafter, GGH with a concentration of 0.06 wt% was obtained by naturally cooling down the solution to room temperature.

SH (0.4 wt%) or NS (0.9 wt%) was prepared by dissolving 400 mg of sodium hyaluronate or 900 mg of sodium chloride in 100 mL of methylene blue stock solution, and then underwent the same sterilization process as GGH. DS with a concentration of 50 wt% was made by dissolving 11 g of dextrose monohydrate into 9 g of deionized water. SA with a concentration of 0.4 wt% was made by dissolving 400 mg of sodium alginate into 100 mL of deionized water. SCC with a concentration of 0.4 wt% was made by dissolving 400 mg of sodium carboxymethyl cellulose into 100 mL of deionized water.

For preparation of GGH-E, 60 mg of high acyl gellan gum was added into 100 mL of methylene blue stock solution, and then the mixture was purged with  $N_2$  for 30 min to remove  $O_2$ . Next, the mixture was sealed and heated at 170 °C for 1 h with the heating stirrer, and then 1.0 mL of epinephrine hydrochloride injection (1 mg/mL) was added into the mixture. Thereafter, the obtained solution was naturally cooled down to room temperature. On the other hand, NS-E was prepared by dissolving



900 mg of sodium chloride into 100 mL of methylene blue stock solution, and then underwent the same preparation process as GGH-E.

#### 4.3. Measurement of rheological and viscosity properties for SIMs

Shear viscosity, and oscillatory time, frequency, and strain sweeps were performed using a rotational rheometer (Kinexus pro+, Malvern, UK) with a set of DIN standard C25 coaxial cylinder measuring system. According to the guidelines of instrument, pre-prepared SIMs were put into cylinder. All rheological and viscosity tests were performed at 25 °C. Oscillation frequency sweep was conducted at the strain of 1.0% and frequency range of 0.1–1.0 Hz. Additionally, oscillation time sweep experiment was performed to record  $G'$  and  $G''$  of SIMs after injection through the endoscopic needle. Frequency and shear strain were set to 0.1 Hz and 1.0%, respectively. Shear viscosity-stress curve was obtained through a yield viscosity measurement. Shear stress and action time were set as 0.1–100.0 Pa and 10 min, respectively.

#### 4.4. Measurement of injection pressure for SIMs

Injection pressure of each SIM was evaluated with a system, in which an endoscopic injection needle (22-gauge, 1.8 m-long, NET2422-C4, Endo-Flex, Germany), a digital pressure gauge (HT-1895, Xinsite, China) and a syringe were connected to a three-way stopcock, followed by fixing the syringe with a syringe pump (LSP01-1A, Longer, China) to set injection speed (Fig. S2, Supporting Information) [17]. Injection pressure was measured by a 5 mL syringe at an injection speed of 0.1 mL/s, when the pressure value was stable for 3 s. Six independent injection pressure measurements were performed for each SIM, and the obtained results were expressed as the mean and standard deviation (Table S1, Supporting Information).

#### 4.5. Measurement of submucosal cushion height in resected porcine stomach

Submucosal cushion height was measured with a flat head spiral micrometer (S5210–25B, Wenzhou Weidu Electronics Co., Ltd., China) (Fig. S3, Supporting Information). Gastric specimen (5 cm × 5 cm) was collected from the upper third of the fresh porcine stomach, whose thickness was close to that of the human stomach [17]. 2.0 mL of GGH or control groups (SH and NS) was injected into submucosa from center of specimen by using a 5 mL syringe with a 23-gauge needle. Cushion height was measured with the spiral micrometer at 0, 10, 30, 60 and 120 min after injection. Seven independent measurements were performed, and the obtained results were expressed as the mean and standard deviation (Table S2, Supporting Information). Height retention rate of submucosal cushion at different times after injection was calculated, according to the following equation:

$$\text{Height retention rate (\%)} = \frac{\text{Mean cushion height at measurement time}}{\text{Mean cushion height at initial time}} \times 100$$

#### 4.6. Biocompatibility of GGH injected subcutaneously in rat model

The Sprague-Dawley (SD) rats (200–240 g) were purchased from the Laboratory Animal Center of Sun Yat-sen University, China. All the experimental operations involved in animals were approved by the guidelines of the Animal Ethics Committee for Guangzhou Huateng Biomedical Technology Co., Ltd., China. All the rats were treated following the Laboratory Animal Care and Use Guidelines strictly. Anesthesia was induced with intraperitoneal injection of 10% chloral hydrate. Firstly, back of rat was prepared for subcutaneous injection by clipping the hair and cleaning the operative area with medicinal alcohol solution. Subsequently, 1.0 mL of GGH or SH was injected into the back skin under sterile condition. Next, skin of whole injection area with

subcutaneous tissue was dissected at 15 min, 60 min, 1 d, 4 d, 7 d, and 14 d after injection. After placing a ruler next to cushion, it was photographed to record, and then cushion height was measured with the ruler. Four rats in each group were tested in parallel, and the obtained results were expressed as the mean and standard deviation (Table S3, Supporting Information).

#### 4.7. Epinephrine-release profile *in vitro*

The release of epinephrine from NS-E and GGH-E groups was determined by a dialysis method *in vitro* [56]. 20 mL of NS-E or GGH-E was placed in dialysis bag (3500 Da, Shanghai yuanye Bio-Technology Co., Ltd., China). Then the dialysis bag was incubated in 80 mL of NS (pH 5–6) at 37 °C with 120 rpm shaking. 5 mL of incubation medium was entirely replaced by the same volume of fresh NS at predetermined time points. Epinephrine was determined with high-performance liquid chromatography (HPLC) [57,58]. A HPLC system (Waters, USA) equipped with a Waters 1525 binary pump, a Waters 2489 UV-visible detector and a Waters 2707 autosampler was used. The separation was done on a C18 column (Diamonsil Plus 5 μm C18–B, DICKMA, China). The elution was performed on isocratic solvent system with formic acid solution (0.1% (v/v)) and acetonitrile as 80% and 20%, respectively, as a mobile phase at a flow rate of 0.5 mL/min. The UV-visible detector was set at the wavelength of 279 nm and injection volume was 20 μL for every sample.

#### 4.8. Performance of GGH in pig model

A Tibet miniature pig (16–22 kg) was provided by Dongguan Songshanhu Experimental Animal Technology Co., Ltd., China. All the experimental procedures in this study were approved by the guidelines of the Animal Ethics Committee for Yin She Guangzhou Medical Technology Co., Ltd., China. The pig was treated following the Laboratory Animal Care and Use Guidelines strictly. The pig was only allowed to drink water at 2 d before experiment. The pig was anesthetized by xylazine hydrochloride and propofol, endotracheal intubation was performed with continuous oxygen intake of 2 L/min. An endoscope was entered into the pig's stomach for observation. Subsequently, 3.0 mL of GGH-E or NS-E was injected into submucosa with an endoscopic needle (NET2422-C4, Endo-Flex, Germany) at front wall or back wall of the gastric antrum. Thereafter, shape change of submucosal cushion was observed by the endoscope at 0, 10 and 30 min after endoscopic injection, and the obtained images were recorded and analyzed. On the other hand, 5.0 mL of NS-E, GGH or GGH-E was injected into submucosa and then mucosa was resected by an electrosurgical knife (KD-655L, Olympus, Japan). The obtained images were recorded to observe the hemostatic effect.

#### 4.9. Biocompatibility assessments of GGH *in vitro*

Proliferation of L929 fibroblast cells (Chinese Academy of Sciences, China) was assessed by Counting Kit-8 (CCK-8) method. Briefly, experiment was divided into blank and GGH groups. In blank group, L929 fibroblast cells were seeded into 96-well plates (Corning, USA) with a density of 3000 cells/100 μL/well and incubated for 1, 3 and 5 d at 37 °C in a 5% CO<sub>2</sub> humidified incubator (Thermo, USA) to obtain a monolayer of cells. As for GGH group, 50 μL of GGH (without methylene blue) was additionally added into 96-well plate after seeding cells, and the cells were then incubated for 1, 3 and 5 d under the same conditions as blank group. After incubation, culture medium was removed, and 100 μL of fresh medium (10 μL of CCK-8 reagent) was added into each well and then incubated at 37 °C for 2 h. Thereafter, absorbance of sample was measured by a microplate reader (Thermo, USA) at 450 nm.

As for live/dead cell double staining kit experiment, two groups were divided as above. For blank group, L929 fibroblast cells were seeded into 12-well plates (Corning, USA) with a density of 20000 cells/1 mL/well

and incubated for 3 and 5 d at 37 °C in the 5% CO<sub>2</sub> humidified incubator. As for GGH group, 200 μL of GGH (without methylene blue) was additionally added into the plates after seeding cells, and then the cells were incubated for 3 and 5 d under the same conditions as blank group. During the incubation, all the culture mediums were replaced at 3 d after seeding cells. After incubation, culture medium was removed and every well was washed with PBS for three times, and then 500 μL of solution including calcein-AM (2 μM) and propidium iodide (8 μM) was added into each well and incubated for 30 min. Thereafter, each well was washed by PBS and observed by an inverted fluorescent microscope (IX73, Olympus, Japan).

#### 4.10. Statistical analysis

All statistical analyses were conducted using Excel software (Microsoft). Data were expressed as mean ± standard deviation. Statistical differences were determined using one-way analysis of variance (ANOVA). A p-value of less than 0.05 was considered statistically significant, and the levels of significance were labeled with \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

#### Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information file.

#### Credit authorship contribution statement

**Yinxiang Tang:** Preparation and characterization of materials, *Ex vivo* test, Writing - original draft. **Minhui Hu:** Writing - original draft, ESD pig model, *In vivo* tests, Writing. **Fuxin Tang:** Cell culture and toxicity analysis. **Rongkang Huang:** Conceptualization, Supervision, ESD pig model, Writing - original draft, Writing - review & editing. **Hui Wang:** Conceptualization, Supervision, Writing - review & editing. **Dingcai Wu:** Conceptualization, Supervision, Writing - review & editing. **Ping Lan:** Conceptualization, Supervision, Writing - review & editing.

#### 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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#### Appendix A. Supplementary data

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