**In vitro** Digestion of Oil-containing Hydrogels Using Gastric Digestion Simulator: a Model Analysis for Oil Release Control inside Human Stomach

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Emulsion hydrogel that incorporates oil droplets are commonly used as food models due to easy manipulation of their mechanical characteristics and compositions. A novel *in vitro* gastric model called the "human gastric digestion simulator (GDS)" equipped with peristalsis enables the simulation and direct observation of the disintegration of food particles induced by peristalsis. The objective of this study was investigating the gastric digestion behavior of emulsion hydrogels with variable mechanical characteristics using the GDS. Four types of emulsion hydrogels containing soybean oil droplets were prepared, namely agar (AG), agar and native-type gellan gum (AG-NGG), deacetylated gellan gum (DGG), and deacetylated gellan gum and native-type gellan gum (DGG-NGG). During peristalsis in a GDS digestion experiment, DGG and DGG-NGG emulsion hydrogel shrank without releasing oil droplets, whereas AG and AG-NGG emulsion hydrogel disintegrated, releasing oil droplets. The disintegration and oil release rates for the AG-NGG emulsion hydrogel were lower than those for the AG hydrogel, where a linear relationship was observed between these two rates. The fracture stress and strain differed between these two hydrogels. The results indicate that lipid release from gels in the human stomach can be controlled by adjusting the gelling agent without changing the lipid amount.

**Keywords:** *in vitro* gastric digestion, peristalsis, gelling agents, O/W emulsion, release control of lipid

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**1. Introduction**

Ingested food undergoes digestion, adsorption, and excretion through the human digestive system. After each meal, we experience different digestion processes in the body. The human digestion process can be categorized into physical, chemical, and biological processes. The physical digestion process mainly involves the oral digestion process, which includes mastication and swallowing [1]. The chemical digestion process plays an important role in the small intestine including the duodenum [2]. In the small intestine, the physical digestion process is also required to mix chime (gastric digesta fasted from the pyloric sphincter) with digestive fluids and transport this mixture downstream [3]. A biological digestion process entailing a sort of microbial fermentation takes place in the microflora in the large intestine [4]. The digestion processes in the stomach differ from those in the other digestive organs, being strongly influenced by both the physical and chemical digestion processes [5].

Physical functions of the stomach include (i) the storage of a bolus (a masticated mixture of food particles and saliva) in the body (upper part of the stomach), (ii) the mixing, breaking down, and grinding of the gastric contents, and (iii) the emptying of chyme from the pyloric sphincter [5]. These functions are induced by gastric peristalsis, which is generated on the wall around the middle of the body [6]. This gastric peristalsis is called the antrum contraction wave (ACW) and slowly progresses toward the pyloric sphincter [6]. There are also chemical functions of the stomach such as chemical and enzymatic reactions of the gastric contents with gastric fluid secreted from the body and fundus (the uppermost part of the stomach). Through the gastric digestion process, food particles in the bolus undergo dramatic changes due to the combination of physical and chemical digestion.

To date, gastric digestion of foods has been intensively studied using *in vivo*, *in vitro*, and *in silico* approaches [7–10]. The *in vivo* and *in silico* approaches can analyze the physical gastric digestion processes under essential
restrictions; however, the disintegration of food particles cannot be analyzed in detail using these approaches [8,11–15]. It should be stated here that the in vivo approach using magnetic resonance imaging (MRI) provides the most reliable information about peristalsis and digestion phenomena in the stomach [11–13]. Numerous studies on gastric digestion of foods have been conducted by using the in vitro approach [10], as the in vivo approach has ethical constraints including restrictions on the number of subjects available for each investigation.

The simple and most commonly used in vitro approaches are shaking methods using test tubes or flasks [9]. However, the physical functions of the stomach cannot be reasonably considered using these methods. Research groups in Europe have developed automated in vitro gastric (and gastrointestinal) digestion models equipped with functions including wall peristalsis and/or segmentation, secretion of digestive fluid(s), and emptying of digesta [16,17]. Simpler in vitro gastric digestion models for solid and semi-solid foods have received much attention over the last decade. A human gastric simulator (HGS) developed by Kong et al. (2010) models the entire stomach and is equipped with quantitatively simulated ACWs and observation windows [18]. In recent years, several research groups in different countries have also proposed in vitro gastric digestion models that can quantitatively consider ACWs [19–21].

There has been growing interest in gaining insights into the design of novel foods whose digestibility is controllable based on life stages and health conditions. Potential food applications include health care, medical care, exercise, and sports. The release of nutrients and functional components incorporated in food particles is pronouncedly affected by their disintegration in the stomach due to physical and chemical effects [5]. In particular, the physical digestion would greatly affect foods having solid-like characteristics. There is the possibility that utilizing mechanical characteristics of foods enables to control the release of nutrients and functional components incorporated in food by varying the degree of physical digestion in the stomach. Emulsion hydrogels, each containing lipid droplets, are commonly used as food models due to the ease of manipulating their mechanical characteristics and compositions (e.g., nutrients and functional components) [22]. Emulsion hydrogel is a major food structure; representative food-emulsion hydrogels are cheese, daily desserts, and Tofu (soybean curd). The release of the major nutrient (lipid droplets) incorporated in an emulsion hydrogel can be shortened or prolonged by varying its mechanical characteristics. Protein-based emulsion hydrogels have been used for in vitro gastrointestinal digestion studies [23], while it should be stated that protein digestion in the stomach affects their disintegration. The use of gelling agents undigested by gastric pepsin allows one to simply investigate the relationship between the disintegration of food particles and the release of lipid droplets during in vitro gastric digestion.

Kozu et al. (2014) developed a novel in vitro gastric model called the “human Gastric Digestion Simulator (GDS)” that is equipped with quantitatively simulated ACWs and observation windows [19]. The GDS was designed based on the structure and functions of the antrum, where gastric peristalsis mainly acts. A unique advantage of the GDS is direct real-time observation of the disintegration of food particles in the presence of ACWs, enabling a detailed understanding of the gastric digestion behavior affected by both physical and chemical processes. Investigations using the GDS have been carried out with foods (e.g., cooked white or brown rice and Tofu) and a food model (agar hydrogel) [19,24,25]. To the best of our knowledge, in vitro gastric digestion models equipped with quantitatively simulated ACWs have not yet been used for directly observing the gastric digestion of emulsion hydrogels. Therefore, the objective of this study was to investigate the gastric digestion characteristics of emulsion hydrogels with variable mechanical characteristics using the GDS. We focused on the disintegration of emulsion hydrogel and on the release of oil (lipid) droplets incorporated in them during the GDS experiments.

2. Materials and methods

2.1 Materials

Agar powder (#010–15815), calcium DL-lactate pentahydrate, 1N hydrochloric acid (HCl), potassium chloride (KCl), sodium bicarbonate (NaHCO3), sodium chloride (NaCl), soybean oil, and sudan IV were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Native gellan gum and deacetylated gellan gum were kindly provided by San–Ei Gen FFI Inc. (Osaka, Japan). Decaglycerol monolaurate (ML750 (hydrophilic–lipophilic balance (HLB): 14.8)) was procured from Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan). a–Amylase (#02100447) and pepsin from porcine gastric mucosa (P7000) were purchased from MP Biomedicals, Inc. (Santa Ana, CA, USA) and Sigma–Aldrich Inc. (St.
Louis, MO, USA). Milli-Q water obtained through a Millipore filtration system (Merck Millipore Co., Darmstadt, Germany) was used for all of the experiments.

2.2 Preparation of emulsion hydrogels

An O/W emulsion was first prepared by rotor-stator homogenization. Soybean oil dyed with sudan IV (5 ppm) was used as the dispersed phase, and Milli-Q water containing 2.0 wt% decaglycerol monolaurate was used as the continuous phase. A liquid mixture consisting of the dispersed phase (40 g) and continuous phase (160 g) was homogenized in a 250–mL glass vessel using a Polytron homogenizer (PT 3100, Kinematica AG, Lucerne, Switzerland) at 5000 rpm for 5 min to prepare an O/W emulsion with a weight fraction of the dispersed phase of 20 wt%. The prepared O/W emulsion sample was warmed up to 60°C for preparing AG emulsion hydrogels and to 90°C for the other types of emulsion hydrogels.

One-hundred-fifty grams of hydrosol was prepared by dissolving gelling agent(s) into Milli-Q water at 60°C for preparing AG emulsion hydrogels and at 90°C for the other types of emulsion hydrogels. The composition and concentration of the gelling agent(s) were 3.0 wt% agar (AG), 1.5 wt% agar and 0.4 wt% native–type gellan gum (AG–NGG), 1.3 wt% deacetylated gellan gum (DGG), 0.25 wt% deacetylated gellan gum and 0.25 wt% native–type gellan gum (DGG–NGG). Calcium DL-lactate pentahydrate was added for preparing DGG and DGG–NGG hydrosols at a concentration of 0.2 wt%. The prepared hydrosol was kept around 60°C with gentle stirring for AG hydrosols and around 90°C for the other types of hydrosols.

An emulsion hydrogel was prepared using a slight modification of a previously described method [26]. One-hundred-fifty grams of the prepared O/W emulsion and hydrosol were mixed in a 300–mL beaker. The mixture was then cooled down in a water bath set at 8°C for 2 h to obtain an emulsion hydrogel (300 g). In this study, we obtained the following four different emulsion hydrogels: AG emulsion hydrogels, AG–NGG emulsion hydrogels, DGG emulsion hydrogels, and DGG–NGG emulsion hydrogels.

2.3 Measurements of mechanical characteristics of emulsion hydrogel

We selected fracture stress and fracture strain as the mechanical characteristics of the resulting emulsion hydrogels to be considered in this study. Prior to mechanical characteristics measurements, the emulsion hydrogel sample was cut into cylinders with a diameter of 16 mm and a height of 10 mm. Texture profile analysis was performed using a 16 mm flat-tipped probe attached to a texture profile unit (TPU, Yamaden Co., Ltd., Tokyo, Japan). The cylindrical sample was compressed up to 90% deformation at a probe speed of 2.5 mm/s.

2.4 In vitro digestion procedures

2.4.1 Preparation of simulated digestive fluids

Simulated saliva was prepared by dispersing 0.117 g/L NaCl, 0.14 g/L KCl, 2.1 g/L NaHCO3, and 2.0 g/L α–amylase in Milli-Q water at ambient temperature [24]. Simulated gastric fluid was prepared by dispersing 8.775 g/L NaCl and 1.0 g/L pepsin in Milli–Q water and subsequently adding 1N HCl to adjust its pH to 1.3 [24]. This simulated digestive fluid was warmed up to 37°C before being used for in vitro digestion experiments.

2.4.2 Preparation of emulsion hydrogel

The emulsion hydrogels prepared in Section 2.2 were cut into cubes of 5 mm on each side. The shape and size of the resulting emulsion hydrogel is the same as those of the food particles (e.g. Tofu and agar hydrogel) used for previous GDS studies [19,24]. According to Jalabert-Malbos et al. (2007), the larger portion of particles after mastication usually have a maximum size of 5 mm [27]. The use of the preceding emulsion hydrogel also permits easier direct observation of particle disintegration during experiments using GDS.

2.4.3 In vitro gastric digestion using GDS

The key components of the GDS used for this study are depicted in Fig. 1. A gastric vessel with a total volume of 550 mL models the dimensions of the human antrum, where gastric peristalsis mainly occurs. The motion of sponge rollers attached to metallic roller chains can generate ACWs on the rubber sidewalls of the gastric vessel at specific intervals. These rollers proceed toward the bottom of the gastric vessel that simulates the pylorus. The transparent parallel windows of the gastric vessel enable real-time monitoring of the disintegration of food particles during digestion. The temperature inside and around the gastric vessel can be kept at a core body temperature of ~37°C during the experiments using a heating unit. Details of the GDS are described in our previous publication [19].

Prior to in vitro gastric digestion, 120 g of emulsion hydrogel was mixed with 30 mL of simulated saliva (pH
7, 37°C) for 2 min to simply simulate oral digestion. Emulsion hydrogel mixed with the simulated saliva was then immediately introduced into the gastric vessel, initially containing 150 mL of simulated gastric fluid (pH 1.3, 37°C). Each in vitro gastric digestion experiment was performed at 37°C up to 180 min. Based on the literature data on ACWs for healthy human adults [13], the speed of simulated ACWs was set at 2.5 mm/s, and their generation frequency was set at 1.5 cycle/min. The gastric digestion of the emulsion hydrogel was monitored and recorded through the front transparent window using a video camera.

2.4.4 In vitro gastric digestion using flask-shaking method

Prior to each flask-shaking experiment, simplified in vitro oral digestion was performed by mixing 120 g of emulsion hydrogel with 30 mL of simulated saliva (pH 7, 37°C) for 2 min. In vitro gastric digestion using the flask-shaking method was performed based on the procedure described by Wang et al. (2013) with slight modifications [26]. One-hundred fifty mL of simulated gastric fluid (pH 1.3, 37°C) was introduced into a 300-mL Erlenmeyer flask. A mixture of emulsion hydrogel and simulate saliva was then added to the simulated gastric fluid in the flask, initiating in vitro gastric digestion at 37°C with a shaking frequency of 115 strokes/min up to 180 min.

2.5 Characterization of the digested samples

2.5.1 Classification and observation of emulsion hydrogel

After terminating the in vitro gastric digestion experiments, the upper creaming layer containing the oil droplets released from emulsion hydrogel was first collected for further quantification and measurement as described in Sections 2.5.2 and 2.5.3. The size distribution of the digested emulsion hydrogel was then measured using the wet sieving method [24]. The digesta without the top creaming layer was transferred on the top of a stack of metal sieves with mesh sizes of 0.6, 1.2, 2.4, and 3.4 mm. The emulsion hydrogel retained in the gastric vessel were carefully rinsed with Mill-Q water. The emulsion hydrogel on each sieve was also gently washed with Milli-Q water to prevent further particle breakdown during the operation. After sieving, the wet weight of four different size fractions was measured to understand the degree of particle disintegration during in vitro gastric digestion.

We collected a small portion of the digested emulsion hydrogel in each size fraction. The collected samples were then photographed to observe their particle shapes and to analyze the variation of emulsion hydrogel during in vitro gastric digestion.

2.5.2 Size distribution measurement of the released oil droplets

The size distribution of the oil droplets released from emulsion hydrogel was measured using a laser diffraction particle-size analyzer (LS 13 320, Beckman Coulter, Brea, CA, USA). The upper creaming layer containing the released oil droplets was collected as described in Section 2.5.1. Drops of this creaming layer were introduced into a universal liquid module of the particle-size analyzer. Each sample was measured in duplicate to obtain the average droplet diameter and droplet size distribution data. The size distribution of a freshly prepared O/W emulsion sample was also measured to understand the variation of droplet size distribution during in vitro gastric digestion.

2.5.3 Quantification of oil droplet release

The collected upper creaming layer containing the released oil droplets was de-emulsified by heating in a water bath at 90°C for 15 min and subsequent centrifugation (Avanti HP-25 centrifuge, Beckman Coulter) at 12,000 g for 10 min. After de-emulsification, the upper oil phase was carefully collected, and its volume was measured photographically in a 15-mL tube or a glass capillary.

2.6 Statistical analysis

A t-test was conducted to evaluate significant differences in the fracture stress and fracture strain of the emulsion hydrogel samples prepared using different gell-
Emulsion hydrogel disintegration by GDS

3. Results and discussion

3.1 Direct observation of digestion of emulsion hydrogel in GDS

Figure 2a depicts the variation of the gastric content containing AG emulsion hydrogel during in vitro gastric digestion using GDS. At 0 min, these particles are almost uniformly distributed in the simulated gastric fluid due to the incorporation of oil droplets of lower density into the hydrogel matrix. In contrast, we previously reported that AG hydrogel settled down on the bottom of the gastric

Table 1 Fracture stress and strain of prepared emulsion hydrogels.

| Sample    | Fracture stress (kPa) | Fracture strain (%) |
|-----------|-----------------------|---------------------|
| AG        | 28.1±2.8              | 23.6±1.4            |
| AG-NGG    | 14.6±1.3              | 25.9±1.7            |
| DGG       | 32.0±3.3              | 21.3±1.9            |
| DGG-NGG   | 5.6±0.9               | 29.7±3.3            |

Fig. 2 Snapshots of emulsion hydrogel digestion up to 180 min in GDS. a: Agar (AG) emulsion hydrogel. b: Agar-native gellan gum (AG-NGG) emulsion hydrogel. Blue arrows indicate the creaming layer of emulsion released from hydrogel. Soybean oil was stained red with Sudan IV.

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During 180 min of gastric digestion, the AG emulsion hydrogel broke down into smaller ones in the presence of simulated ACWs. At 90 and 180 min, the larger particles were mainly distributed in the upper region of the gastric content, while the smaller particles tended to distribute and pack in the lower region of the gastric content. As a result of visual observation, the smaller particles distributed in the lower region looked transparent, where the red color derived from oil droplets stained by Sudan IV became thin. The oil droplets inside smaller particles may be easy to be released compared to those of the larger particles because of the large surface area, which increases the density of smaller particles resulting in sedimentation. This observation result also indicated that oil droplets incorporated into the smaller particles could be easily squeezed out by the compression caused by simulated gastric peristalsis, which is due to the shorter distance between inner droplets and outer gastric fluid. The released oil droplets floated up in the gastric content, forming a creaming layer on the top. The thickness of this creaming layer gradually increased during the entire gastric digestion process. Oiling-off was not observed in the creaming layer containing oil droplets stabilized by a hydrophilic emulsifier (ML750). Using a test-tube shaking method, Wang et al. (2012) demonstrated that O/W emulsions stabilized by ML750 were stable during in vitro gastric digestion [28]. The oil droplets densely packed in the creaming layer had a Sauter mean diameter \(d_{3,2}\) of 17.6 µm, which is similar to the \(d_{3,2}\) of the fresh O/W emulsion (23.7 µm) to some extent. The larger oil droplets could be more easily remain in hydrogel compared to the smaller oil droplets, resulting in the preferential release of smaller oil droplets and the decreased \(d_{3,2}\) after digestion experiment. At least, the droplet size distribution did not shift toward larger sizes during gelation and gastric digestion as seen in Fig. 3. These results clearly indicate that the oil droplets released from AG emulsion hydrogel were stable against coalescence in the gastric content.

Figure 2b depicts the variation of the gastric content containing AG-NGG emulsion hydrogel during in vitro gastric digestion using GDS. At 0 min, we observed almost uniform distribution of these particles in the simulated gastric fluid, similar to the behavior of AG emulsion hydrogel (Fig. 2a). Although breakdown of AG-NGG emulsion hydrogel was observed during gastric digestion, the number of particles that broke down was less than that of AG emulsion hydrogel. At 180 min, the larger particles in Fig. 2b were still distributed over the entire gastric content, while a mixture of smaller and larger particles was packed in the lower region of the gastric content. Creaming of the released oil droplets without oiling-off was clearly observed at the end of gastric digestion. At a certain time, this creaming layer was thinner than that formed using AG emulsion hydrogel, indicating that a smaller amount of oil droplets was released from AG-NGG emulsion hydrogel.

An in vivo gastric digestion study using MRI demonstrated that ingested food emulsion forms a creaming layer on the top of the gastric content in the human stomach [29]. This in vivo result and our in vitro results (Fig. 2) indicate that mixing of the gastric content induced by ACWs is quite mild and that solid particles and liquid droplets heterogeneously distribute in the stomach.

We also investigated variations of the gastric content containing DGG or DGG-NGG emulsion hydrogel during in vitro gastric digestion using GDS (Fig. A1). These particles did not break down but shrank in the presence of simulated ACWs during gastric digestion. The cubic particles shrank slowly and floated up in the simulated gastric fluid, concentrating on the top of the gastric content. After 180 min, no creaming layer was observed. These results suggest that the ACWs squeezed water from the hydrogel matrix without releasing oil droplets, rendering the density of the shrunken particles lower than that of the initial particles. There is the possibility that this shrinking behavior was caused by the characteristics of DGG. The same shrinking behavior would also occur in the case of pure DGG and DGG-NGG hydrogels without oil droplets.

As demonstrated in this section, the type and composition of gelling agents pronouncedly affect the digestion
of emulsion hydrogel using GDS. The results obtained by direct observation are useful for better understanding the digestion of emulsion hydrogel in the presence of ACWs.

### 3.2 Disintegration profiles of emulsion hydrogel during in vitro gastric digestion

The disintegration profile of AG emulsion hydrogel during 180 min of in vitro gastric digestion using GDS is presented in Fig. 4a,i. During the first 30 min, the wet weight of the largest particles \((d>3.4 \text{ mm})\) decreased rapidly to 78.1 g, while that of large \((2.4<d\leq3.4 \text{ mm})\), medium \((1.2<d\leq2.4 \text{ mm})\), and small particles \((0.6<d\leq1.2 \text{ mm})\) increased to 5.6 g to 18.0 g. After 30 to 180 min, the wet weight of the largest particles decreased gradually from 78.1 g to 51.8 g, while that of small particles increased slowly from 18.0 g to 29.0 g. The internal diameter of the pylorus is about 2 mm [5], so particles larger than the pylorus size are assumed to remain in the stomach. The total wet weight of the digested particles smaller than medium fraction \((1.2<d\leq2.4 \text{ mm})\) was 25.8 g, indicating that 21.5% of the digested particles can pass through the pylorus.

The disintegration profile of AG-NGG emulsion hydrogel during 180 min of in vitro gastric digestion using GDS is presented in Fig. 4b,i. The wet weight of the largest particles decreased gradually to 73.6 g during 180 min of in vitro gastric digestion. In contrast, the wet weight of large, medium, and small particles increased slightly to 8.2 g to 12.1 g. The total wet weight of the digested particles smaller than medium fraction \((1.2<d\leq2.4 \text{ mm})\) was 25.0 g, indicating that 20.8% of the digested particles can pass through the pylorus.

Figures 4a,ii and 4b,ii depict the wet weight profiles of AG and AG-NGG emulsion hydrogel after in vitro gastric digestion using the flask-shaking method. The wet weight of the largest particles hardly decreased, to 119.2 g for AG emulsion hydrogel and to 115.6 g for AG-NGG emulsion hydrogel. These results demonstrate that AG and AG-NGG emulsion hydrogel is almost unaffected by flask shaking. As depicted in Figs. 5a,ii and 5b,ii, the digested particles almost kept their cubic shape and size, indicating that the shear stress generated by flask shaking scarcely affected particle disintegration.

The wet weight profiles of DGG and DGG-NGG emulsion hydrogels after in vitro gastric digestion using GDS are depicted in Fig. A2. The wet weight of the largest particles decreased to 94.0 g after 180min for DGG emulsion hydrogel and to 53.4 g for DGG-NGG emulsion hydrogel. For DGG emulsion hydrogel, slight increases
of large, medium, and small particles (<2 g) were observed. For DGG–NGG emulsion hydrogel, we observed a small increase of large particles (~6 g) and slight increases of medium and small particles (<2 g). The digested particles shrank while retaining their initial cubic shape (Fig. A2). The results obtained in Figs. A1 and A2 demonstrate that water retained in DGG and DGG–NGG emulsion hydrogels was squeezed by the ACWs.

### 3.3 Effect of mechanical characteristics on particle disintegration

Fracture stress and strain are important factors affecting physical gastric digestion. Several types of hydrogels with different fracture stress and/or strain have been used for *in vitro* gastric digestion experiments [17,23,24]. When a hydrogel sample is compressed in a one-dimensional direction, the fracture stress (strain) indicates the force necessary to fracture (the degree of deformation until fracture). The fracture strain of AG–NGG emulsion hydrogel was significantly higher ($p = 0.002$) than that of AG emulsion hydrogel (Table 1). When emulsion hydrogels were compressed by the ACWs simulated on the walls of the gastric vessel, the AG–NGG emulsion hydrogel may have deformed largely. As a result, the compression force that acts on the emulsion hydrogel can be distributed, suppressing the disintegration. In contrast, the fracture stress of the AG–NGG emulsion hydrogel was about half that of the AG emulsion hydrogel. As mentioned above, AG–NGG emulsion...
hydrogel tends to deform more easily than AG emulsion hydrogel. The compression force applied to the AG–NGG emulsion hydrogel could be distributed both vertically and horizontally within the hydrogel sample, leading to lowering the stress on AG–NGG emulsion hydrogel. Since this force distribution can also occur during in vitro digestion using the GDS, it may be difficult to generate the force necessary to disintegrate AG–NGG emulsion hydrogel.

The fracture strain of the DGG–NGG emulsion hydrogel was also significantly higher \( (p<0.001) \) than that of the DGG emulsion hydrogel (Table 1). The DGG–NGG emulsion hydrogel was deformed quite largely by the ACWs simulated on the walls of the gastric vessel. Water inside the DGG–NGG emulsion hydrogel may be squeezed out more easily than in the DGG emulsion hydrogel, resulting in large shrinkage (Fig. A2). The fracture stress of the DGG–NGG emulsion hydrogel is also about one fifth that of the DGG emulsion hydrogel. Water retention in a hydrogel may be weak at lower fracture stress, inducing the squeezing out of water from DGG–NGG emulsion hydrogel.

### 3.4 Oil release from emulsion hydrogel during in vitro gastric digestion

Figure 6 depicts the variation of the rate of the oil release from AG or AG–NGG emulsion hydrogel as a function of digestion time in the GDS. The rate of oil release after each digestion time \( (OR_t) \) was estimated using the following equation:

\[
OR_t = 100 \times \left( \frac{V_{t,r.o.}}{V_{i.o.}} \right) \quad (1)
\]

where \( V_{t,r.o.} \) is the volume of the oil released after each digestion time and \( V_{i.o.} \) is the initial oil volume incorporated in the emulsion hydrogel before in vitro gastric digestion. The \( OR_t \) for AG emulsion hydrogel increased with increasing digestion time, reaching 34% after 180 min. A slower increase in \( OR_t \) was observed for the AG–NGG emulsion hydrogel; their maximum \( OR_t \) was 16% at 180 min. The variation of \( OR_t \) for the AG and AG–NGG emulsion hydrogel gradually slowed down during 180 min of gastric digestion. The \( OR_t \) for the AG emulsion hydrogel was 1.9–2.4 times greater than that for the AG–NGG emulsion hydrogel after each digestion time. We consider that this difference is affected by the degree of particle disintegration during in vitro gastric digestion using the GDS (Fig. 4). Only a slight amount of oil droplets was released from the AG and AG–NGG emulsion hydrogel when using the flask-shaking method. Moreover, the DGG and DGG–NGG emulsion hydrogels released only a slight amount of the oil released during in vitro gastric digestion using the GDS. These results also indicate that particle disintegration (Fig. 5) affects the oil release during gastric digestion.

The results in Figs. 4 and 6 demonstrate that \( OR_t \) for AG emulsion hydrogel is higher than that for AG–NGG at each digestion time, as AG emulsion hydrogel was more easily disintegrated during in vitro gastric digestion using the GDS. Here, we investigated the effect of the disintegration rate of emulsion hydrogel on \( OR_t \) at each digestion time. The disintegration rate of emulsion hydrogel at a specific digestion time \( (DR_{t,e.h}) \) was defined as follows:

\[
DR_{t,e.h} = 100 \times \{1 - (W_{\text{classified,h.g}}/W_{\text{total,h.g}})\} \quad (2)
\]

where \( W_{\text{classified,h.g}} \) is the wet weight of the digested hydrogel larger than each mesh size and \( W_{\text{total,h.g}} \) is the total wet weight of the hydrogel before in vitro gastric digestion. Fig. 7 depicts the relationship between \( DR_{t,e.h} \) and \( OR_t \) for AG and AG–NGG emulsion hydrogel obtained from the flask-shaking and GDS experiments. \( OR_t \) increased linearly with increasing \( DR_{t,e.h} \), regardless of the mesh size and the types of emulsion hydrogels and in vitro gastric digestion. A small portion of oil droplets may be released from the hydrogels without disintegration: e.g. the release from a microcrack generated by the stress of peristalsis. However, if these phenomena domi-
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This result strongly indicates that the disintegration degree of AG and AG-NGG emulsion hydrogel during in vitro gastric digestion is a dominant factor affecting ORt.

Around 5% of the soybean oil droplets incorporated in emulsion hydrogel was also released from the particles during 180 min of the flask-shaking experiments (Fig. 7). It should be noted that no particle disintegration was observed in Figs. 5a,ii and b,ii. Based on the assumption that only the oil droplets in contact with the hydrogel surface are released into the gastric juice, the ORt after 180 min of the flask-shaking experiments was estimated by the following procedure. (1) Emulsion hydrogel density was estimated from water and soybean oil densities and the weight ratio of oil in the emulsion hydrogel. Since the final concentration of the gelling agents (agar and native-type gellan gum) in the emulsion hydrogels was low enough (approximately 1 wt%), emulsion hydrogel density was approximated as water and soybean density. (2) Total volume of 5 mm-cube emulsion hydrogel used in the digestion experiment was calculated from emulsion hydrogel density and total weight. (3) Total number of 5 mm-cube emulsion hydrogel used in the digestion experiment was estimated from the above-mentioned total volume and the volume per one 5 mm-cube. (4) Total surface area of 5 mm-cube emulsion hydrogel used in the digestion experiment was calculated from the total number of 5 mm-cube emulsion hydrogel and the surface area per one 5 mm-cube. (5) The volume of the layer where the droplets are in contact with the hydrogel surface was estimated by multiplying the total surface area of 5 mm-cube to the \(d_{32}\) of the fresh oil droplets (23.7 µm) as the layer thickness. (6) The weight of released oil droplets was estimated from the above-mentioned layer volume, emulsion hydrogel density, and the weight ratio of oil in the emulsion hydrogel. As a result, the ORt was calculated to be about 3%. Similar values between the measured and estimated ORt indicate that oil droplets were released only from the surfaces of emulsion hydrogel. New surfaces are formed when emulsion hydrogel is fractured in the presence of simulated ACWs, causing further release of oil droplets from the newly formed surfaces. We therefore consider that DRt,e,h increased linearly with increasing ORt. In contrast, the ORt for DGG and DGG-NGG emulsion hydrogels could not be obtained, as their particles did not break down but rather retained oil droplets within them during all GDS experiments.

The findings obtained in this section demonstrated that the release of oil droplets from the surfaces of emulsion hydrogel is considerably affected by their disintegration degree. We assume that the amount of oil released from emulsion hydrogel during gastric digestion can be varied by appropriately controlling their disintegration by varying the composition of the gelling agents.

4. Conclusions

The gastric digestion of emulsion hydrogel containing soybean oil was analyzed using GDS, an in vitro gastric digestion device simulating human gastric peristalsis. In the case of DGG and DGG-NGG emulsion hydrogels including deacetylated gellan gum as a gelling agent, the emulsion hydrogel shrank without releasing oil droplets in the presence of simulated gastric peristalsis during the GDS experiments. Gastric peristalsis is considered to compress the hydrogels and squeeze water from the hydrogel matrix. In contrast, in the case of AG and AG-NGG emulsion hydrogels including agar as the gelling agent, their particles were disintegrated by the simulated gastric peristalsis of GDS. Soybean oil droplets were observed to be released into the liquid phase of
gastric contents with the disintegration progress. Compared with the AG emulsion hydrogel, the disintegration degree of hydrogel and the oil release rate from the hydrogel both decreased in the case of the AG-NGG emulsion hydrogel during the GDS experiments. The measured mechanical characteristics (fracture stress and strain) differed between these two types of hydrogels, indicating that mechanical characteristics affect the disintegration of hydrogel during gastric digestion. Further analysis revealed that there was a linear relationship between the disintegration rate of hydrogel and the oil release rate. Oil droplets on the fracture surface were mainly released when hydrogel was fractured and disintegrated by gastric peristalsis. These results give us the possibility that lipid release amount and/or speed from hydrogels in the human stomach can be controlled by adjusting mechanical characteristics, without changing the amount of the lipid incorporated in the hydrogel. This study provides us with basic knowledge for controlling lipid release inside the stomach and subsequently controlling the absorption amount of lipid inside the small intestine by utilizing the characteristics of solid foods.

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Fig. A1  Snapshots of emulsion hydrogel digestion up to 180 min in GDS. 
a: Deacetylated gellan gum (DGG) emulsion hydrogel. b: Deacetylated gellan gum–native gellan gum (DGG–NGG) emulsion hydrogel. Soybean oil was stained red with Sudan IV.

Fig. A2  Time-course change of emulsion hydrogel in GDS digestion. Wet weights of each size fraction of emulsion hydrogel is indicated at each digestion time point. a: Deacetylated gellan gum (DGG) emulsion hydrogel. b: Deacetylated gellan gum–native gellan gum (DGG–NGG) emulsion hydrogel.
ヒト胃消化シミュレーターを用いた油含有ゲルの in vitro 消化試験：油の胃内放出制御のためのモデル解析

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微小油滴を包括させたエマルションハイドロゲルは、その力学特性と組成が容易に調整できることから、モデル食品として使われている。著者らが開発してきたヒト胃消化シミュレーター（Gastric Digestion Simulator; GDS）は、胃のぜん動運動を模擬可能であり、消化中の食品の崩壊過程の再現と観察をすることができる。本研究では、GDS を用い、様々な力学特性を有するエマルションハイドロゲルの胃消化挙動について検討することを目的とした。微小油滴 (大豆油) を包括させたエマルションハイドロゲルを、次の4種類のゲル化剤の組合せで調製した：寒天 (AG)，寒天＋ネイティブ型ジェランガム (AG-NGG)，脱アシル型ジェランガム (DGG)，脱アシル型ジェランガム＋ネイティブ型ジェランガム (DGG-NGG)。GDS を利用した in vitro 消化試験において、DGG と DGG-NGG のエマルションハイドロゲルは、ぜん動運動により発生微小油滴が放出されることなくゲル粒子が収縮した。一方、AG と AG-NGG のエマルションハイドロゲルでは、ゲル粒子が崩壊し、微小油滴が放出された。また、AG と比較して AG-NGG の方が、崩壊率と放出率が共に低くなることがわかり、崩壊率と放出率には線形の相関関係があることが明らかになった。これら2つのエマルションハイドロゲルでは、破断応力と破断歪率がそれぞれ異なりていた。本研究の結果により、ヒトの胃において、脂質の含有量を変えることなく、ゲル粒子からの脂質放出量を制御できる可能性が示唆された。

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