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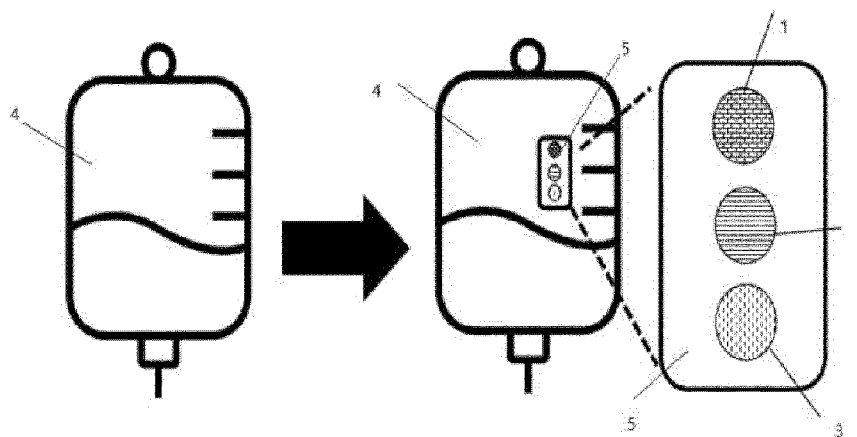


Figure 1

(57) Abstract: The present invention relates to the use of a biosensor to detect a postoperative leak with a patient's drain fluid, relating to an operation selected from the group consisting of pancreatic anastomosis, pancreatic resection, small colon anastomosis (also called small intestine anastomosis), large intestine anastomosis, colorectal surgery, colon bypass surgery, gastric bypass surgery, gastric resection surgery, cholecystectomy, bileduct surgery and oesophageal surgery, and wherein said biosensor comprises at least one gel which is placed on a support layer, and wherein the at least one gel is sensitive to a digestive enzyme and comprises a colorant selected from the group consisting of an organic dye, an inorganic dye, an organic pigment and an inorganic pigment, and wherein said at least one gel degrades upon contact with a drain fluid comprising one or more digestive enzymes, allowing a visual detection of discoloration of the gel or disappearance at the place where the gel was before, and/or a coloration of the drain fluid.

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Biosensor

The present invention relates to the use of a biosensor that allows the detection of a postoperative leak with a patient's drain fluid, relating to an operation selected from the group
5 consisting of pancreatic anastomosis, pancreatic resection, small colon anastomosis (also called small intestine anastomosis), large intestine anastomosis, colorectal surgery, colon bypass surgery, gastric bypass surgery, gastric resection surgery, cholecystectomy, bileduct surgery and
10 oesophageal surgery, and in particular pancreatic anastomosis, small colon anastomosis (small intestine anastomosis), colon bypass surgery and gastric bypass surgery.

An anastomotic leak occurs when there is a breakdown or separation of the connection (anastomosis) between two
15 segments of the gastrointestinal tract that were surgically joined together. Drain fluid refers to the fluid that accumulates at the surgical site and drains out through a surgical drain or a wound. An anastomotic leak is a serious complication of surgery, in particular after gastrointestinal
20 surgery, and the danger lies in the fact that it can cause severe infection, which could disseminate rapidly and adversely impact other organs. Manifestations of anastomotic leakage include fever, abdominal pain, nausea, vomiting, and tachycardia.

25 Prompt diagnosis and treatment are crucial to prevent severe complications like sepsis that may culminate in fatalities. The standard therapy involves administering antibiotics to tackle the infectious agents, followed by surgical

intervention aimed at rectifying the damaged anastomotic site.

Presently available diagnostic techniques for the identification of anastomotic leaks are characterized by inadequate sensitivity and specificity. They are only able to
5 identify leaks at the advanced stage of disease progression when the leakage is already fully developed, leading to severe clinical consequences like septic shock. State-of-the-art techniques involve analyzing several inflammatory biomarkers such as C-reactive protein, comparing them with the reference
10 range of healthy individuals. The elevated marker levels are not primarily due to the leak but due to the ensuing repercussions like sepsis or infection and the surgery itself, leading to delayed detection. Furthermore, conventional biomarker quantification necessitates access to clinical
15 chemistry laboratories and requires considerable resources, including materials, skilled personnel, and capacities.

These clinical chemistry-based measurements are frequently augmented by abdominal computer tomography (CT) imaging, particularly in situations where an anastomotic leak is
20 suspected (Gray, M. et al., Predictive and Diagnostic Biomarkers of Anastomotic Leakage: A Precision Medicine Approach for Colorectal Cancer Patients. J. Pers. Med. 2021, 11 (6), 471)).

Roser, M. V. et al (Roser et al. Evaluation of an Integrated
25 Smart Sensor System for Real-Time Characterization and Digitalization of Postoperative Abdominal Drain Output: A Pilot Study, Surg. Innov. 2022, 29 (3), 438-445) disclose a study which utilized spectroscopic techniques to detect biomarkers in drain fluid via in-line measurements. Absorption

measurements were employed to assess a variety of biomarkers, and these results were subsequently correlated with laboratory measurements.

- Ben-David et al (Ben-David et al: Implantation of an impedance
5 Sensor for Early Detection of Gastrointestinal Anastomotic
Leaks. J. Surg. Res. 2022, 278, 49-56) describes a method which
involves the implantation of biodegradable electrodes near the
suture site within the peritoneal cavity to monitor the
impedance changes caused by contact with gastric fluid.
10 However, this technique has limitations in terms of
invasiveness and spatial coverage since it can only detect
leaks occurring in the vicinity of the electrodes and is
susceptible to interference from pH changes that naturally
occur in the body.
- 15 Anthis et al. Nature Comm 2022. have reported an implantable
pH and enzyme responsive hydrogel sensor for the early
detection and containment of gastrointestinal anastomotic
leaks. However, this approach has limitations as it requires
the surgical implantation of the hydrogel sensor into the
20 abdomen of the patient.

- Linden, P. A. et al (Linden et al: A Simple and Versatile
Method of Detecting Esophageal Anastomotic Leaks. Ann. Thorac.
Surg. 2022, 113 (6), 1794-1800) have reported that amylase
present in the drain fluid is a promising biomarker for
25 predicting anastomotic leak. Studies have shown that drain
amylase levels are a reliable indicator of anastomotic leak
between the 3rd to 7th postoperative day, regardless of the
surgical site or patient comorbidities. However, like other
biomarkers, the assessment of drain amylase levels requires

specialized laboratory testing.

Pasquardini, L. et al (Pasquardini, L. et al : A Surface Plasmon Resonance Plastic Optical Fiber Biosensor for the Detection of Pancreatic Amylase in Surgically-Placed Drain
5 Effluent. Sensors 2021, 21 (10), 3443) have outlined a method for detecting amylase by employing surface plasmon resonance in conjunction with amylase-specific antigens. This technique requires a specialized setup comprising a light source and an optical spectrophotometer.

10 CN101473217 discloses optical sensors designed for assessing the age and/or quality of natural products, including both food items and cosmetic products. In one aspect, a device is provided for analyzing the age or quality of natural products, consisting of a reflection layer, a nanoparticle layer, and a
15 biodegradable polymer layer situated between the reflection layer and the nanoparticle layer.

US8278064 describes a protease-responsive biosensor that operates through the degradation of a hydrogel polymer coating on its surface. The methodology involves detecting a protease
20 by introducing a biological sample to a detector that includes a substrate at least partially coated with a hydrogel-based synthetic polymer matrix. An electronic device is necessary for the readout.

GB 2 350 677 outlines a technique for the detection of
25 enzymatic presence which involves exposing the sample under test to a substrate, a portion of which is coated with a biodegradable polymer film. An electronic device is necessary for the readout.

WO 2022/031558 introduces a sensor designed to quantify enzyme activity. This sensor includes a degradable conductive pathway featuring a first end, a second end, and an intermediate segment situated between these two ends. The intermediate
5 segment contains conductive particles embedded within it. An electronic device is necessary for the readout.

US2016061830 reveals a biosensor aimed at detecting microorganisms within a sample, which encompasses a polymer matrix adorned with a fixed bacteriophage layer atop the
10 matrix's surface. An electronic device is necessary for the readout.

The problem of the present invention is to provide a real time monitoring of leak occurrence, progression and early detection of infection.

15 The problem is solved by the use of a biosensor according to claim 1. Further preferred embodiments are subject of the dependent claims.

It was found that the use of a biosensor according to the present invention allows to detect a postoperative leak with
20 a patient's drain fluid, relating to an operation selected from the group consisting of pancreatic anastomosis, pancreatic resection, small colon anastomosis (also called small intestine anastomosis), large intestine anastomosis, colorectal surgery , colon bypass surgery, gastric bypass
25 surgery, gastric resection surgery, cholecystectomy, bileduct surgery and oesophageal surgery, and wherein the biosensor comprises at least one gel which is placed on a support layer, and wherein the at least one gel is sensitive to a digestive enzyme and comprises a colorant selected from the group

consisting of an organic dye, an inorganic dye, an organic pigment and an inorganic pigment, and wherein said gel degrades upon contact with a drain fluid comprising one or more digestive enzymes allowing a visual detection of discoloration of the gel and/or disappearance of the gel at the place where the gel was before, and/or a coloration of the drain fluid. Thus, said gel degrades upon contact with a body fluid comprising one or more digestive enzymes resulting in a change of structure of the gel. The support layer immobilizes the gel and allows therefore a better readout by naked eye.

This invention is based on the fact, that in a healthy individual there are no digestive enzymes found inside the peritoneal cavity. However, in the case of an anastomotic leak digestive enzymes are typically present. The drain fluid, also called body fluid, is typically collected in a drainage bag after gastrointestinal surgery, to remove excess fluid from the surgical site. The detection with the biosensor according to the present invention takes always place *ex vivo*, i.e. outside the body. The biosensor according to the present invention allows a real time monitoring of leak progression, by directly detecting the leak by the naked eye based on structural changes of the at least one gel by leak-indicating enzymes present in the drain fluid. Possible changes that may be detected are for example the disappearance of the gel, a color change of the gel or an opacity change of the gel. Due to the catalytic activity of enzymes and the fact that a digestive enzyme can react with multiple substrates the response can be amplified. The detection is rapid and requires no expensive supplementary instrumentation or calibration. In particular, the readout is easy to learn, reducing the need

for specialized training or expertise. Besides, since it does not rely on expensive electronic equipment, it is not dependent on a stable power supply, internet connectivity, or complex maintenance procedures. Furthermore, a measurement of inflammatory biomarkers is not necessary. In addition, the biosensor is noninvasive, as it is only employed with pre-existing drains and does not come into contact with other parts inside the body and will therefore have fewer requirements that need to be met before regulatory agency approval. Finally, the biosensor according to the present invention is highly specific, as there is no response if no leak is present.

Within the context of the present invention the term "gel" stands for a three-dimensional network of molecules or particles that trap a liquid phase such as water or drain fluid. Undigested, this network has a solid-like consistency, allowing it to maintain its shape and resist flow. Beside classical gels comprising a three-dimensional network and water as liquid phase, the term "gel" also includes lyophilized gels or electrospun fibers and beads that form together with the drain fluid a gel.

Biosensors according to the present invention comprising at least one lyophilized gel have an enhanced stability, an extended shelf life, and a facilitated storage. By removing the water, the gel becomes more resistant to degradation, microbial growth, and chemical reactions. The resulting lyophilized gel can be rehydrated and returned to its original gel state after contact with the drain fluid.

Within the context of the present invention the term "support layer" stands for a solid material that immobilizes the at

least one gel. It may be out of any material as long as its stability is not negatively impacted during the use of the biosensor. This means that the support is stable to enzyme and bacteria degradation for at least 24h. The support layer has preferably a structure selected from the group consisting of holes, nets and sub-layers or a combination thereof. Holes for example hold the at least one gel in place and also allow a better diffusion of the fluid which enhances the digestion. A net allows to cover one or both sides with the at least one gel which has also a positive influence on the diffusion. The support layer can also comprise a set of different sublayers, for example a first sublayer can be used to hold the gel in place, a second sublayer can be used to improve readout and a third sublayer can serve as attachment means.

Particular good results could be obtained for operations selected from the group consisting of pancreatic anastomosis, small colon anastomosis (also called small intestine anastomosis), colon bypass surgery and gastric bypass surgery.

Possible examples of such a support layer are synthetic gauze (woven and non-woven) made from synthetic polymer fibers, such as polyester, nylon, or viscose. Preferably, the support layer has a mesh-like structure, which allows for fluidflow and facilitates the fluid transport of the drain liquid. The composition of drain fluid can vary depending on the type of surgery, the location of the surgical site, and the individual patient. Generally, drain fluid consists of a mixture of blood, lymphatic fluid, and serous fluid. Typically, it is free of digestive enzymes and bacteria. However, in case of a leak the drain fluid usually contains a variety of enzymes that are involved in the digestion of food. These enzymes comprise

proteases, lipases, and amylases, which are secreted by the pancreas and released into the small intestine to break down proteins, fats, and carbohydrates.

In case of a leakage after gastrointestinal surgery, common proteases that can be found in the drain fluid are preferably selected from the group consisting of trypsin, chymotrypsin, carboxypeptidases, enterokinase, aminopeptidases and dipeptidyl peptidases, most preferably trypsin. Examples are trypsin-1, trypsin-2 and trypsin-3.

In case of a leakage after gastrointestinal surgery, common lipases that can be found in the drain fluid are preferably selected from the group consisting of pancreatic lipase, gastric lipase, bile salt-dependent lipase, phospholipase A2, lysosomal lipase, hepatic lipase, lipoprotein lipase, hormone-sensitive lipase, endothelial lipase and lingual lipase. Examples are pancreatic lipase related protein 2 and pancreatic lipase related protein 1.

In case of a leakage after gastrointestinal surgery, common amylases that can be found in the drain fluid after gastrointestinal surgery are preferably selected from the group consisting of salivary amylase and pancreatic amylase. Examples are alpha-amylases such as AMY1A, AMY1B, AMY1C, AMY2A and AMY2B.

Preferably, the support layer has at least partly a colored, opaque, structurally colored, transparent and/or patterned surface. Due to this surface, the readout can be significantly improved as the contrast is enhanced. The surface may, for example, have a pattern with colored dots. The gel is arranged on the surface in such a way that dots are covered. When the

gel dissolves, the dots become visible, which improves visibility.

The at least one gel comprises a dye and/or a pigment. Thus, said at least one gel, which is responsive to one of the human digestive enzymes is modified in such a way that it displays colors, which provide an optical readout through a color change upon digestion of the gel by the corresponding enzyme. Preferably, the color change can be read out by the naked eye or a smartphone camera, not relying any expensive electronic equipment.

By tuning the chemical characteristics of the gel, the sensitivity as well as the response time of the material can be influenced. Examples are but not limited to the addition of low amounts of chemical crosslinker such as PEG-diacrylate during gel formation in order to enhance network stability. Or a higher number of functional groups given to the polymer during the synthesis of the hydrogel starting material (e.g. more methacrylates on the starch).

Preferably, the biosensor comprises at least two, more preferably at least three different gels. Within the context of the present invention the expression "different gel" means that the gels are sensitive to different enzymes or different concentrations of enzymes. The biosensor according to the present invention can comprise for example an amylase sensitive gel and a lipase sensitive gel or an amylase sensitive gel and a trypsin sensitive gel. It is also possible to comprise two different amylase sensitive gels, wherein one amylase sensitive gel is very easily digestible in the presence of amylase and the second one is less digestible. Through this a

semiquantitative enzyme concentration estimate can be obtained.

Due to the presence of said different gels, the specificity and sensitivity of the biosensor can be significantly increased. This allows to detect an anastomotic leak at an early stage. Early detection of an anastomotic leak can lead to faster initiation of appropriate treatment, such as antibiotics and drainage of the affected area, which can prevent the spread of infection and minimize tissue damage. In addition, early intervention can prevent the need for more invasive procedures, such as reoperation or colostomy, which can be associated with increased morbidity and mortality. Furthermore, early detection and management of an anastomotic leak can facilitate earlier hospital discharge and reduce the length of hospital stay, leading to lower healthcare costs.

In one embodiment, the biosensor according to the present invention comprises two or more gels that are sensitive to different enzymes and two or more gels that can detect different concentrations of enzymes.

In a preferred embodiment the biosensor according to the present invention comprises at least one amylase sensitive gel, at least one lipase sensitive gel and at least one protease sensitive gel. The protease sensitive gel is preferably a trypsin sensitive gel. Thus, for each of the three major digestive enzymes amylase, protease, and lipase at least one separate gel is provided. Amylase breaks down carbohydrates into smaller sugars such as glucose. It does this by cleaving the α -1,4-glycosidic bonds in the carbohydrate molecules. Protease breaks down proteins into amino acids by cleaving the

peptide bonds, while lipase breaks down lipids into fatty acids and glycerol by cleaving the ester bonds. Since their concentration can vary on the type and amount of the food consummated, the presence of said three different gels make
5 the biosensor according to the present invention independent of the consummated food. It is important to note that a gel that is for example amylase sensitive, is stable in the presence of a lipase or a protease, thus it is highly selective.

10 The gels comprise a conventional organic or inorganic dye or an organic or inorganic pigment. Due to the degradation of the gel a leaking takes place which either results in a disappearance of the gel, and/or in a discoloration in the place where the gel was before and/or a coloration of the drain
15 liquid. This either results in discoloration at the site where the gel was previously situated, coloration of the liquid within the drain, or both. In other words, the breakdown of the gel can cause the colorant to both bleach the area where the gel was originally and stain the draining liquid
20 simultaneously. Non-limiting examples of suitable dyes and pigments include FD&C colors, such as blue no. 1, blue no. 2, green no. 3, red no. 3, red no. 40, yellow no. 5, yellow no. 6, and the like; natural colors, such as caramel coloring, annatto, chlorophyllin, cochineal, betanin, turmeric, saffron,
25 paprika, lycopene, elderberry juice, pandan, butterfly pea and the like; titanium dioxide; and any suitable food colorant known to the skilled person. Non-limiting examples of suitable pigments are iron oxide pigments, titanium dioxide, zinc oxide and carbon black.

30 Preferably, the biosensor according to the present invention

comprises a gel which changes its color upon contact with a protease, i.e., a protease sensitive gel. Within the context of the present invention the term "change of color" refers to any alteration in the appearance or hue of the gel, including
5 a shift to a different color, a darkening or lightening of the original color, the appearance of new colors or color patterns, and the reduction or disappearance of color intensity (i.e. color loss which also includes complete disappearance of the color). Such a gel can be selected from the group consisting
10 of gelatin, a gelatin-based gel, chemically crosslinked gelatin, chemically modified gelatin (such as methacrylated gelatin) gels comprising amino acid sequence crosslinkers, elastin, collagen, laminin, fibrin, silk fibroin, and globular proteins. Said gels have peptide bonds that are hydrolyzed by
15 a protease resulting in the structural change that allows the optical readout. Said protease is preferably selected from the group consisting of a serine protease, a metalloprotease, a cysteine protease, an aspartic acid protease, and a glutamic acid protease, most preferably a serin protease and ideally
20 trypsin. Within the context of the present invention a gelatin-based gel is a type of gel that is formed by using gelatin as one gelling agent, i.e., at least one gelling agent present in the gel is gelatin. A chemically crosslinked gelatin is a type of gelatin that has been modified by crosslinking. The
25 crosslinking can be obtained for example by radical polymerization, chemical crosslinking agents, radiation crosslinking, thermal crosslinking and photochemical crosslinking. Possible crosslinking agents are for example formaldehyde or glutaraldehyde. These crosslinking agents
30 react with the amino groups in the gelatin molecules, creating crosslinks between them and forming a three-dimensional

network structure. All gels mentioned above are compatible with photonic colorants, dyes or pigments.

Preferably, the biosensor according to the present invention comprises a gel which changes its color upon contact with amylase. Such an amylase sensitive gel is preferably selected from the group consisting of starch, a starch-based gel, glycogen, a glycogen-based gel, dextrin, a dextrin-based gel, cyclodextrin, and a cyclodextrin-based gel or mixtures thereof. Said gels have α -1,4-glycosidic bonds that are hydrolyzed by amylase resulting in the structural change that allows the optical readout. All gels mentioned above are compatible with photonic colorants, dyes or pigments. Within the context of the present invention a starch-based gel is a type of gel that is formed by using starch-based gel as one gelling agent, i.e., at least one gelling agent present in the gel is starch. An example of a starch-based gel is a gel comprising 5% methacrylated starch and 15% acrylamide. The same applies for glycogen-based gels, dextrin-based gels and cyclodextrin-based gels.

Preferably, the biosensor according to the present invention comprises a lipase sensitive gel. Such a lipase sensitive gel comprises preferably one or more compounds selected from the group consisting of plant oils, animal fats, phospholipids, monoacylglycerols and diacylglycerols or mixtures thereof. All gels mentioned above are compatible with photonic colorants, dyes or pigments. Within the context of the present invention the term "plant oils" includes derivatives coming from plant oils such as esters as well as lipids derived from plant sources. Examples of plant oils include crop oils, oilseeds and vegetable oils. Plant oils include, but are not

limited to, flax, soybean, safflower, sunflower, sesame, canola, rapeseed, jatropha, primrose, poppy, camelina, crambe, olive, coconut, palm, cotton, corn, and nut oils.

Within the context of the present invention the term "animal
5 fat" relates to lipids derived from animals. Examples of animal fats include poultry fats, pork fat, horse fat, yellow grease, butter and tallow.

Examples of phospholipids include soy lecithins or egg, phospholipids from bovine or swine brain or dermis,
10 phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine in which the acyl groups may be the same or different and are mostly derived from palmitic, stearic, oleic, linoleic and linolenic acids.

Monoacylglycerols are glycerol esters that contain a single
15 long-chain fatty acid esterified to one of the three hydroxyl groups of the glycerol molecule. In contrast, diacylglycerols are glycerol esters that contain two long-chain fatty acids esterified to two of the three hydroxyl groups of the glycerol molecule.

20 Preferably, such a lipase sensitive gel is an oleogel. An oleogel is a type of gel that is formed by dispersing a liquid oil or fat within a solid matrix. The solid matrix is typically made up of an ethylcellulose or a combination of natural waxes, such as beeswax, and/or synthetic waxes, such as hydrogenated
25 vegetable oil or microcrystalline wax. They are typically produced by melting the solid matrix ingredients and the liquid oil while stirring continuously. The mixture is then cooled to allow the solid matrix to form a gel. The texture and firmness of the oleogel can be adjusted by varying the type and amount

of solid matrix ingredients used.

In another embodiment of the present invention the at least one gel is printed on a support layer in a specific pattern which allows an automate readout. For example, a QR like code
5 arises or disappears upon degradation of the gel. Such a pattern can be obtained for example by 3D print of the gel.

The biosensor according to the present invention may be placed inside the drain system depending on the needs. The sensor may be placed in the drainage bag itself. It is compatible with
10 all drainage bags commonly employed for postsurgical care after surgery.

Alternatively, one sublayer of the biosensor according to the present invention may be attached to the outside of the drainage bag, said drainage bag comprising at least one opening
15 through which the at least one gel on a further sublayer is inserted to the inside.

Alternatively, the support layer of the biosensor according to the present invention may be equipped with drain bag perforating inlets, through which the at least one gel on a
20 support layer is connected to the inside.

A further embodiment relates to a drainage bag comprising a biosensor according to the present invention. Preferably such a drainage bag is made of a flexible material, preferably of
25 plastic. In such a way multiple bags can be stockpiled without any problems. The biosensor can be placed into drainage bags routinely employed in post-surgical care. Optionally, said drainage bag contains a stirring system (such as a continuously

stirred tank reactor like system), to maximize contact between drain liquid and sensor.

In one embodiment the biosensor comprises attachment means that allow a firm attachment to the drainage bag, which allows
5 a better visibility, since the drain fluid might be slightly colored. Possible attachment means are selected from the group consisting of adhesive strips, magnetic strips, hook-and-loop tapes, suction cups, and textured surfaces that grip into the bag (such as microneedles). Alternatively, the biosensor can
10 be integrated in the surface of the bag wall, for example by gluing, vacuum suction, suturing, plasma fusion, heat fusion or gel adhesion.

Alternatively, the biosensor can be attached to the drainage bag directly before use for example by a commercial adhesive,
15 a clip, a clamp, a needle or by thread/wire.

In a further embodiment, the biosensor floats freely in the drain bag.

The biosensor according to the present invention can be produced in a very easy and an inexpensive manner. Typically,
20 it includes a classical gel preparation.

A further aspect of the present invention relates to the use of a biosensor as described above for the postoperative detection of a leak, in particular an anastomotic leak. Such leaks can occur in the gastrointestinal tract. Preferably, the
25 biosensor according to the present invention is used after pancreatic anastomosis, pancreatic resection, small colon anastomosis (also called small intestine anastomosis), large intestine anastomosis, colorectal surgery , colon bypass

surgery, gastric bypass surgery, gastric resection surgery, cholecystectomy, bileduct surgery and oesophageal surgery, and in particular pancreatic anastomosis, small colon anastomosis (small intestine anastomosis), colon bypass
5 surgery and gastric bypass surgery.

Alternatively, the biosensor according to the present invention comprises at least one gel and a support layer on which the at least one gel is placed, whereby said gel degrades
10 bacteria, resulting in a change of structure of the gel. The support layer immobilizes the gel and secures that it does not freely float in the drainage liquids and allows therefore a better readout by naked eye. Alternatively the support layer helps the sensor to freely float.

15 In a healthy individual there are no gut bacteria found inside the peritoneal cavity. However, in the case of an anastomotic leak gut bacteria are typically present. The drain fluid, also called body fluid, is typically collected in a drainage bag after gastrointestinal surgery, to remove excess fluid from
20 the surgical site. The biosensor allows a real time monitoring of leak progression, by directly detecting the leak by the naked eye based on structural changes of the at least one gel by leak-indicating bacteria present in the drain fluid. Possible changes that may be detected are for example the
25 disappearance of the gel, a color change of the gel or an opacity change of the gel.

In this embodiment the least one gel comprises a dye and/or a pigment. Thus, said at least one gel, which is responsive to at least one gut bacteria is modified in such a way that it

displays structural colors, which provide an optical readout through a color change upon digestion of the gel by the corresponding bacteria. Preferably, the color change can be read out by the naked eye or a smartphone camera, not relying
5 any expensive electronic equipment.

Common bacteria that can be found in the drain fluid after gastrointestinal surgery are preferably selected from the group consisting of bacteroides fragilis, bacteroides melaninogenicus, bacteroides oralis, enterococcus faecalis,
10 escherichia coli, enterobacter sp., klebsiella sp., bifidobacterium bifidum, staphylococcus aureus, lactobacillus, clostridium perfringens, proteus mirabilis, clostridium tetani, clostridium septicum, pseudomonas aeruginosa, salmonella enterica, faecalibacterium prausnitzii,
15 peptostreptococcus sp., peptococcus sp., streptococcus, staphylococcus, lactobacillus, peptostreptococcus. staphylococcus epidermidis, staphylococcus aureus, staphylococcus warneri, streptococcus pyogenes, streptococcus mitis, cutibacterium acnes, corynebacterium spp.,
20 acinetobacter johnsonii and pseudomonas aeruginosa. Some of these bacteria are part of the normal gut flora, but they can cause infections if they enter the bloodstream or other parts of the body, while others are known pathogenic bacteria.

The biosensor can comprise a gut bacterium sensitive gel
25 comprising collagen and collagen derivatives, indigestible starches, cellulose, cellulose derivatives, lignin, lignin derivatives, chitin, chitin derivatives, xylan, xylan derivatives, carragenans, carragenans derivatives, alginate, alginate derivatives, fucoidan, fucoidan derivatives,
30 chitosan, chitosan derivatives, ulvan, ulvan derivatives and

mixtures thereof. Within the context of the present invention indigestible starches are a type of carbohydrate that resists enzymatic digestion in the small intestine and passes through to the large intestine or colon. Instead, they undergo
5 fermentation by gut bacteria. Further, the expression derivative means compounds such as collagen, cellulose, lignin, chitin xylan, carragenans, alginate, fucoidan, chitosan or ulvan are chemically modified through reactions such as esterification, etherification, or hydroxyalkylation.
10 Such reactions are known to the skilled person.

Figures

- Fig. 1: Schematic drawing of a drainage bag comprising the biosensor according to the present invention
- 15 Fig. 2A and 2B: Prototype of the biosensor according to the present invention
- Fig. 3: Biosensor according to the present invention, wherein the support layer has a surface with a pattern.
- 20 Fig. 4: Biosensor according to the present invention, wherein the support layer comprises several sub-layers.
- Fig. 5 to 7: Gel digestion by different enzymes.
- Figure 8 LOD of the different enzymes.
- 25 Figure 9 Response of the different gels on the different enzymes

Figures 10A to 10C Schematic drawing of a biosensor
attacheable to a drainage bag;

Figures 11A to E: Different prototype designs and their
features;

5 Figure 12 Simulated anastomotic leak;

Figures 13A-13B: Data from a small clinical pilot trial with
human samples;

Figure 14: Detailed images of gel-patches native and
partially digested;

10 Figure 15: Manufacturing of patches for a biosensor
according to the present invention;

Figure 16: IR-spectra of gelatin, Gel-MA and its
corresponding hydrogel;

15 Figure 17: IR-spectra of acrylamide, Starch and its
corresponding starch-MA hydrogel;

Figure 18: IR-spectra of coconut oil, ethylcellulose
and its corresponding oleogel;

Figure 19: A further simulated anastomotic leak;

20 Figure 20A: Top view of one embodiment of the present
invention where the sensing elements are
encased in a 3D printed circular element
printed on an also 3D printed support mesh.
The white color corresponds to an amylase
responsive hydrogel while the black color
25 corresponds to hydrogels responsive to

trypsin. Each circular sensing hydrogel has a different chemical modification making each circular element have a different enzymatic degradation time and resistance. The number of individually responsive elements is interchangeable and can be any number including one or higher;

Figure 20B: Angular view of the sensor according to present invention;

Figure 20C: Side view of the sensor according to present invention. The circular elements protrude on both sides allowing more hydrogel attachment and safeguards the hydrogels from detaching from the backing due to movement of the sensor;

Figure 21: Placement of a sensor according to the present invention into a commercially available drain bag. In this case the sensor is not attached to the bag but free floating;

Figure 22: Placement of a sensor according to the present invention by means of heat fusing the sensor to the bag. Image shows a sensor with three trypsin responsive elements, each having different sensitivity towards trypsin through chemical modification;

Figures 23A-D: Additional temperature and pH dependent kinetic data of the gels responsive to

trypsin and amylase according to the present invention.

Figure 24: Kinetic data of 7% wt. starch functionalized with glycidyl methacrylate (50% degree of functionalization) at 37°C and room temperature;

Figure 25: Kinetic data of 7% wt. and 10% wt. starch functionalized with glycidyl methacrylate (25% degree of functionalization) at 37°C and room temperature.

Figure 1 shows a schematic drawing of a drainage bag comprising the biosensor according to the present invention. The support layer of the biosensor comprises three different gels, a trypsin sensitive gel 1, an amylase sensitive gel 2 and a lipase sensitive gel 3.

Figure 2 shows a prototype of the biosensor according to the present invention. The support layer of the biosensor comprises three different gels, a trypsin sensitive gel 1, an amylase sensitive gel 2 and a lipase sensitive gel 3. Figure 2A shows a biosensor according to the present invention before contact with a drain fluid. Figure 2B shows the biosensor after contact with a trypsin containing drain fluid. In figure 2B the red color of the trypsin sensitive gel is no longer visible, and the gel almost disappeared.

Figure 3 shows a schematic drawing of a biosensor according to the present invention wherein the support layer has a surface with a pattern with 24 colored dots.

Figure 4 shows a schematic drawing of a support layer of

the biosensor according to the present invention consisting of three sub-layers. A first sub-layer 11 is a gel attachment layer that holds the gel in place. A second sub-layer 12 is a detection layer or a visualization aid that improves the quality of the readout. A third sublayer 13 serves as attachment means to allow a firm attachment to the drainage bag.

Figure 5 shows a gelatin-MA with and without PEG digestion.

Figure 6 shows the amylase digestion of a starch MA.

Figure 7 shows the lipase digestion of an oleogel.

Figure 8 shows the LOD of the different enzymes. Within the context of the present invention the term "LOD" refers to "limit of detection." It represents the lowest concentration or amount of a substance that can be reliably distinguished or detected by the biosensor of the present invention within 24h.

Figure 9 shows the selectivity of the different gels on the different enzymes.

Figures 10 A and 10B depict the profile and frontal perspectives, respectively, of one embodiment of the biosensor according to the present invention comprising three different gels, a trypsin sensitive gel 1, an amylase sensitive gel 2 and a lipase sensitive gel 3 which are hold in place by a support layer 10. The support layer 10 is equipped with drain bag perforating inlets 14A, 14B and 14C, through which the gels 1, 2, 3 on the support layer 10 are connected to the inside of the drainage bag 15.

Figures 11A to 11E show different prototype designs and their

features. Figure 11A shows a seamless integration of a biosensor according to the present invention into common drain bags. As illustrated in Figure 11B) a trypsin spike inside drain fluid can be detected by naked eye (dotted circle - Gel-MA digested). Figure 11C) illustrates that a semiquantitative readout of different trypsin concentrations in drain fluid (after overnight incubation) through Gel-MA patches containing different amounts of PEG-DA (PEG diacrylate) is possible with the biosensor according to the present invention. The lower the PEG-DA content the less enzyme is necessary to digest the patches. Circles mark the patches remaining intact. Figure 11D illustrates the visibility of the biosensor according to the present invention in drainage bag. (I): No fluid in drain. In Figures 11D (II) to 11D (IV), the drain is filled with liquids of various colors. (II): yellow from bile and digestive enzymes. (III) and (IV) pictures different shades of red. In this case Gel-MA sensor is preferably colored with TiO_2 (or carbon black/charcoal) to make it better visible in red liquid in case of blood in drain. Figure 11E) displays a biosensor according to the present invention with one missing element in the middle to show the support layer (also called backing) and its properties. Figure 11E(I) shows a support layer with a reflecting support layer improving visibility after patch digestion. Figure 11E (II) shows a support layer with a fluorescent support layer improving visibility after patch digestion. Figure 11E (III) shows a support layer with a QR-Code support layer improving visibility after patch digestion. Figure 11E (IV) shows a support layer with a colored support layer improving visibility after patch digestion.

Figure 12 presents a simulated anastomotic leak. Line (I):

Simulation of leak occurring after 12h. Start with a clear fluid containing the biosensor according to the present invention (image (1)). For the first 12h no leak is present (amylase concentration (from pancreatin, contains also trypsin and lipase, additional bile (5.3 mg/ml) is added) is 0.5 U/ml with incubation in RT) and all patches remain intact (image (2)). After 12h a leak is simulated, and the concentration is elevated to 5 U/ml of amylase. After an additional 15h (overall 27 h, (image (3))) the trypsin and amylase responsive patches are digested and only the lipase responsive element remains. Line (II): Simulation of a leak right after surgery. Amylase levels are 5 U/ml and trypsin, and amylase responsive patches disappear after 15h incubation at room temperature (image (4)) Only the oleogel patch remains. Line (III): Simulation of a severe leak right after surgery. Amylase levels are 50 U/ml. After 5h incubation at RT trypsin and amylase responsive patches are digested, leaving only the lipase responsive element (image (5)). After incubating for 24h at RT the lipase responsive patch is also digested (image (6)).

Figures 13A and 13B illustrate data from a small clinical trial: Figure 13A shows an overview and the principle of sample handling. The two graphs show the amylase and lipase activity values of 32 human samples collected from University Hospital Zurich and analysed by the Institute of Clinical Chemistry (IKC) of the University Hospital of Zurich. Samples were furthermore sorted into samples that contained a leak and samples that did not (after performing the measurements and unblinding). Figure 13B shows ROC (receiver operating curve) of trypsin responsive elements (Data from experiments where Gel-MA patches with 1% PEG-DA were subjected to the human

samples for 24h at 37°C. In case of a leak the patches were digested while in the case of no leak the patches remained intact) as well as of amylase and lipase responsive elements. For amylase and lipase, the ROC was generated with the above
5 shown data, as determined by IKC.

Figure 14(I) shows the native and partially digested Gel-MA patch, Figure 14(II) the native and partially digested starch-MA patch and Figure 14(III) the native and partially digested oleogel patch. Thus, partially digested means that parts of
10 the patch are still visible, whereas when fully digested, the patch has completely disappeared.

Figure 15 illustrates the manufacturing of biosensor active moieties in the form of black and white Gel-MA and oleogel patches according to the present invention. Figure 15A shows
15 the manufacturing of thin film Starch-MA patches. Figure 15B shows the manufacturing of thin film oleogel patches. Figures 15C and D show the manufacturing of thin film Gel-MA patches. Figure 15E shows the manufacturing of thin film Gel-MA patches colored with active charcoal (left) and TiO_2 (right) to give a
20 black and white colored patch respectively to make it better visible in red or brown liquid in case of blood in the drain . Figure 15F depicts thin film oleogel patches colored with active charcoal (left) and TiO_2 (right) to give a black and white colored patch respectively to make it better visible in
25 red or brown liquid in case of blood in drain.

Figure 16 shows an IR-spectra of gelatin, Gel-MA and its corresponding hydrogel.

Figure 17 shows an IR-spectra of acrylamide, starch and its corresponding starch-MA hydrogel.

Figure 18 shows an IR-spectra of coconut oil, ethylcellulose and its corresponding oleogel.

Figure 19 shows the same experiment as illustrated in Figure 12 (same sequence and concentration) but with closeup of
5 biosensor outside drain.

Figure 20 shows the top view of one embodiment of the present invention where the sensing elements are encased in a 3D printed circular element printed on an also 3D printed support mesh. The white color corresponds to an amylase responsive
10 hydrogel while the black color corresponds to hydrogels responsive to trypsin. Each circular sensing hydrogel has a different chemical modification making each circular element have a different enzymatic degradation time and resistance. The number of individually responsive elements is
15 interchangeable and can be any number including one or higher.

Figure 20B shows the angular view of the sensor according to present invention.

Figure 20C shows the side view of the sensor according to present invention. The circular elements protrude on both sides
20 allowing more hydrogel attachment and safeguards the hydrogels from detaching from the backing due to movement of the sensor.

Figure 21 shows the placement of a sensor according to the present invention into a commercially available drain bag. In this case the sensor is not attached to the bag but free
25 floating.

Figure 22 shows the placement of a sensor according to the present invention by means of heat fusing the sensor to the bag. Image shows a sensor with three trypsin responsive

elements, each having different sensitivity towards trypsin through chemical modification.

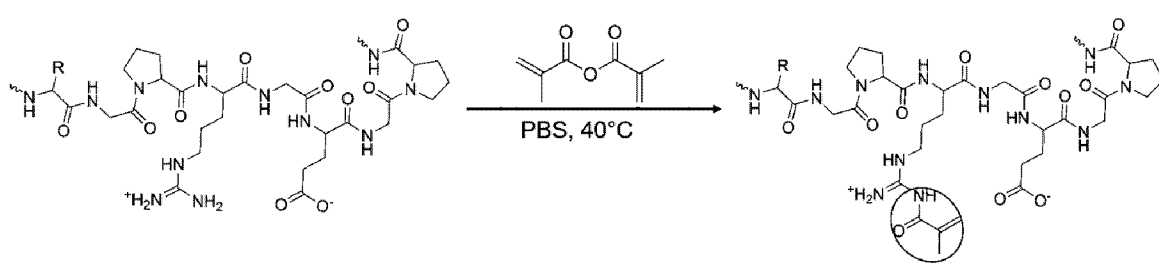
Figures 23A-D shows additional temperature and pH dependent kinetic data of the gels responsive to trypsin and amylase according to the present invention.

Figure 24 shows the kinetic data of 7% wt. starch functionalized with glycidyl methacrylate (50% degree of functionalization) at 37°C and room temperature.

Figure 25 shows the kinetic data of 7% wt. and 10% wt. starch functionalized with glycidyl methacrylate (25% degree of functionalization) at 37°C and room temperature.

Experimental part

Gelatin-MA synthesis and hydrogel formation



For the synthesis of Gelatin-MA a slightly modified version of the procedure from Dubruel et. al. was used. In brief, to a 100 ml two-necked round bottom flask equipped with a reflux condenser, stir bar and a stopcock 50 ml of PBS (Dulbecco modified without CaCl₂ and MgCl₂) were added. While stirring vigorously, 5g of gelatin (type A from porcine skin) was added and the mixture was heated to 40°C and stirred until the gelatin was fully dissolved. Afterwards 580 µl of Methacrylic Anhydride was added via syringe and stirring was continued for

3h. During this period the color changed to a light brown. Next 50 ml Milli-Q water (Metrohm) was added and stirring was continued for an additional 20 min. The whole mixture was then pipetted into dialysis tubing (3.5 kDa cutoff) and dialyzed
5 against deionized water for 2 days at RT with frequent water changes. Afterwards the solution was frozen, freeze dried and a white crystalline foam was obtained.

For the preparation of various Gel-MA hydrogel used in digestion experiments, 5 wt.% gelatin was dissolved in either
10 water or gold nanoparticle suspension (prepared as reported by Puntès et. al. with one growth step and used without further purification) by stirring and heating on a hotplate set to 50°C until everything was dissolved. Afterwards 1 wt.% PEG-DA (PEG diacrylate, 700 Da) as well as 10 vol.% LAP (Lithium
15 phenyl-2,4,6-trimethylbenzoylphosphinate, 6.33mg/ml) was added and briefly stirred. For gels not containing PEG-DA The mixture was then brought into the corresponding shapes and put into the fridge for 20 min to solidify. To achieve double network formation, the gelatin hydrogels were then exposed to
20 UV light for 5 min.

Starch methacrylate synthesis and Hydrogel formation

For this synthesis, a DMAP (4-(Dimethylamino)pyridine) catalyzed synthesis was performed, similar to the method used by Kessler et. al. to functionalize alcohols. To a 250ml three-
25 necked round bottom flask, equipped with a stir bar, reflux condenser and a thermometer, 4g of starch (from potatoes, SIGMA) as well as 150 ml of Milli-Q was added. After stirring briefly, 27 mg of 4-(Dimethylamino)pyridine (DMAP) was added and the mixture was heated to 50°C (inside the flask) for 15

minutes to achieve partial swelling of the starch granules. Next, the mixture was cooled to 40°C and 1.25 ml Methacrylic Anhydride was added dropwise. During the whole reaction, the pH of the solution was carefully monitored. After two hours, the pH of the reaction has dropped to 4-5. By adding 5M NaOH, the pH was adjusted to 6. One hour later the pH was readjusted by addition of more 5M NaOH upon which the reaction pH was around 9. The reaction mixture was then allowed to stir overnight in the dark. The next day, the solution was pipetted into dialysis tubing (3.5kDa cutoff) and dialyzed against 10L of deionized water. The water was changed once after 6h and then left for 72h. After freezing the solution, it was freeze dried until a white powder was obtained.

Starch glycidyl methacrylate synthesis and Hydrogel formation

For this synthesis, the process was similar to the starch methacrylate synthesis and hydrogel formation. Here a roundbottom flask equipped with a magnetic stirbar was filled with 30ml DMSO, in addition to DMSO, DMAP and 850 µL of GMA was also added, bringing the ratio of reagents to be GMA : Starch : DMAP = 1:2:1. Everything was added, in a 50 mL three-necked round-bottomflask, equipped with a stir bar and thermometer. The next day, dialysis was performed exactly as for the starch methacrylate. The water was changed daily for 3 days. After freezing the solution, it was lyophilised and, in this case, a white solid was obtained. To reduce the hydrogels with the new starch, it was sufficient to mix 5% starch with water without using acrylamide. After mixing and heating, the mixture was ready to use. Next, LAP was added to the mixture and briefly stirred. The mixture was then transferred to a glass plate and a thin film was created,

followed by immediate polymerisation under UV light (UVASPOT400/T mercury lamp, Höhnle) for 5 minutes. Circular elements were then cut out with a 9mm biopsy punch and stored in a refrigerator for several days until further use.

5 Plant-oil oleogel synthesis

Coconut oil was placed in a 25ml round bottom flask in an oil-bath set to 175°C together with different weight percentages ethylcellulose (300cp) and active charcoal. The mixture was heated and stirred for 2h. Next, the oleogel mix was taken up
10 by a glass pipette and spread thinly on a glass surface (a blade knife was used to create a thin uniform layer). The oleogel was then cooled in the fridge for 2h before further use.

Digestion of Gels, timeseries and concentration

15 Gelatin-MA: Gels were mixed as mentioned above (without PEG-DA) using gold nanoparticles (2x concentrated from synthesis). The mix was poured onto a glass plate and a thin film applicator was used to create a thin uniform layer. After cooling in the fridge, the sheets were irradiated by UV light (see above) and
20 a 9mm biopsy punch was used to cut out thin round patches. For the digestion experiments, only gold nanoparticle suspension was used to make the gels. Patches were then put into 12 well cell culture plates (TPP) filled with PBS (Dulbecco modified with CaCl_2 and MgCl_2). Trypsin (from porcine pancreas) was then
25 added in the right concentration by adding the corresponding volume of stock (stock 1mg/ml). The overall volume in the wells at the start of the experiment was 3ml for all samples. Tubes were then put either into an incubator set at 37°C, where they were gently mixed with an orbital shaker or shaken at RT with

the same speed. The digestion process was recorded automatically using a tablet and a timelapse app. Pictures were taken every 10 min and for each concentration triplicates were examined (see Figure 5). A patch was counted as digested when it either fully disappeared or showed significant alterations (loss of color and or structure) compared to the control patches (PBS only). The control patches did not significantly change over the observation period, while the digested patches gradually lost structure. First, they became softer and afterwards they lost the round shape, until they finally disappeared.

Starch-MA: For this Gel gold nanoparticles (2x concentrated from synthesis) were mixed with 5%wt. Starch-MA and 15%wt. Aam (Acrylamide) in a glass vial and put into an oven heated to 80°C. The vial was regularly vortex and heated for 30 min. Next 10%vol. LAP (6.33mg/ml) is added and heating is continued for another 5 min. Then the mix was poured onto a preheated glass plate and a thin film applicator was used to create a thin uniform layer and the sheets were irradiated by UV light (see above) and a 9mm biopsy punch was used to cut out thin round patches. Digestion experiments were conducted similar to Gelatin-MA with the difference being that the amylase (porcine pancreas 11U/mg) was added as a powder directly to the PBS (Dulbecco modified with CaCl_2 and MgCl_2) for higher concentration (>10U/ml) while for the lower concentration a 1mg/ml stock was diluted accordingly. The overall volume in the wells at the start of the experiment was 3ml for all samples. Plates were then put either into an incubator set at 37°C, where they were mixed with an orbital shaker or shaken at RT with the same speed. The digestion process was recorded

automatically using a tablet and a timelapse app. Pictures were taken every 10 min and for each concentration triplicates were examined. A patch was counted as digested when it either fully disappeared or showed significant alterations (loss of color and or structure) compared to the control patches (PBS only). The control patches did not significantly change over the observation period, while the digested patches gradually lost structure. First, they became softer and afterwards they lost the round shape, until they finally disappeared.

Plant oleogels: Oleogels were prepared as mentioned above. Patches were placed in 6 well plates filled with 6 ml PBS (Dulbecco modified with CaCl_2 and MgCl_2), Lipase (from porcine pancreas) in the corresponding concentration (from 1mg/ml stock) and 32mg bile (from porcine sources). Plates were then put either into an incubator set at 37°C, where they were mixed with an orbital shaker or shaken at RT with the same speed. The digestion process was recorded automatically using a tablet and a timelapse app. Pictures were taken every 10 min and for each concentration triplicates were examined. A patch was counted as digested when it either fully disappeared or showed significant alterations (loss of color and or structure) compared to the control patches (PBS only). The control patches did not significantly change over the observation period, while the digested patches gradually lost structure. First, they became softer and afterwards they lost the round shape, until they finally disappeared.

Clinical Samples

Samples were collected from USZ Zurich with a valid ethics approval in place. Volumes, colors as well as consistencies of

the samples varied. The only patient data gathered was the type of operation/complication, date collected and which postoperative day the sample was collected. From each sample 3-10ml were decanted into heparin coated tubes, that were then frozen until usually the next day when they were brought to the Institute for Clinical Chemistry (IKC) of USZ, where amylase and lipase activity was determined. Table 1 shows the determined amylase and lipase activity levels across samples where no patch response was measured.

Table 1:

Amylase activity	Lipase activity	Amylase activity	Lipase activity
3.264	12.604	0.021	0.052
*	0.128	0.01	0.005
0.027	0.015	0.057	0.027
0.022	0.012	*	*
0.011	0.013	0.01	0.021
0.015	0.014	0.105	0.893
0.032	0.052	0.03	0.013
0.029	0.011	0.028	0.015
0.082	0.383	0.01	0.021

Table 2 shows the amylase and lipase activity levels of samples where the patch did digest and disappear. All values are given as units per milliliter (U/ml).

Amylase activity [U/ml]	Lipase activity [U/ml]
*	15.819
*	6.462
115.64	455.3

*	15.694
8.125	1.436
82.201	20.58

Furthermore, bilirubin content was measured. The rest of the sample (generally 15-20ml) was then put into a falcon tube into which a 500µl Gel-MA patch (1% PEG-DA, only gold nanoparticle suspension) was thrown. The samples were then incubated overnight at 37°C while shaking gently on an orbital shaker. Digestion was determined the next day and again after 24h in the case of undigested patches.

Prototype proof of principle experiments

Prototype gel holders were first 3D printed and then used as negative molds for hot pressing plastic sheets over them. The molds were open on one side and had a hole over each of the patches on the other side. See example in Figures 2A and 2B (1 stands for a trypsin sensitive gel, 2 for an amylase sensitive gel and 3 for a lipase sensitive gel). Patches were then put into the plastics and a net was attached to the backside in order to avoid the patches to slip out of the mold.

For the digestion experiment a Jackson Drain (provided by USZ) was used. The cap was removed in order to place the patch strip inside. The drain bag was then filled with 100ml PBS (Dulbecco modified with CaCl_2 and MgCl_2) and enzymes in the corresponding concentration were added. A septum was hotglued on top of the drain to seal it and the bag was gently mixed with an orbital shaker at 37°C for 12h.

Figure 2A shows a biosensor according to the present invention before contact with a drain fluid. Figure 2B shows the biosensor after contact with a trypsin containing drain fluid. In figure 2B the red color of the trypsin sensitive gel is no longer visible, and the gel almost disappeared. As neither amylase nor lipase were present in the drain fluid, said gels remain unchanged.

Clauses:

1. Use of a biosensor to detect postoperative leak with a patient's drain fluid, relating to an operation selected from the group consisting of pancreatic anastomosis, pancreatic resection, small colon anastomosis (also called small intestine anastomosis), large intestine anastomosis, colorectal surgery, colon bypass surgery, gastric bypass surgery, gastric resection surgery, cholecystectomy, bileduct surgery and oesophageal surgery, wherein the biosensor comprises at least one gel which is placed on a support layer, and wherein the at least one gel is sensitive to a human gut bacteria and comprises a colorant selected from the group consisting of an organic dye, an inorganic dye, an organic pigment and an inorganic pigment, and wherein said gel degrades upon contact with a drain fluid comprising one or more human bacteria allowing a visual detection of discoloration at the place where the gel was before and/or disappearance of the gel, and/or a coloration of the drain fluid.

2. Use of the biosensor according to clause 1, wherein the operation is selected from the group consisting of pancreatic anastomosis, small colon anastomosis (small intestine anastomosis), colon bypass surgery and gastric bypass surgery.

3. Use of a biosensor according to any of the preceding clauses, wherein the support layer has at least partly a colored, opaque, structurally colored, transparent and/or patterned surface.

5 4. Use of a biosensor according to any of the preceding clauses, wherein said gel is a gut bacterium sensitive gel comprises one or more compounds selected from the group consisting of collagen, collagen derivatives, indigestible starches, cellulose, cellulose derivatives, lignin, lignin
10 derivatives, chitin, chitin derivatives, xylan, xylan derivatives, carragenans, carragenans derivatives, alginate, alginate derivatives, fucoidan, fucoidan derivatives, chitosan, chitosan derivatives, ulvan, and ulvan derivatives or mixtures thereof.

15 5. Use of a drainage bag comprising a biosensor according to any of clause 1 to 4.

6. Use of a drainage bag according to clause 5, wherein the biosensor is integrated in the surface of the bag wall.

Claims

1. Use of a biosensor to detect a postoperative leak with a patient's drain fluid, relating to an operation selected from the group consisting of pancreatic anastomosis, pancreatic resection, small colon anastomosis (small intestine anastomosis), large intestine anastomosis, colorectal surgery, colon bypass surgery, gastric bypass surgery, gastric resection surgery, cholecystectomy, bileduct surgery and oesophageal surgery, and wherein said biosensor comprises at least one gel which is placed on a support layer, and wherein the at least one gel is sensitive to a digestive enzyme and comprises a colorant selected from the group consisting of an organic dye, an inorganic dye, an organic pigment and an inorganic pigment, and wherein said at least one gel degrades upon contact with a drain fluid comprising one or more digestive enzymes, allowing a visual detection of discoloration of the gel or disappearance at the place where the gel was before, and/or a coloration of the drain fluid.
2. Use of the biosensor according to claim 1, wherein the operation is selected from the group consisting of pancreatic anastomosis, small colon anastomosis (small intestine anastomosis), colon bypass surgery and gastric bypass surgery.
3. Use of the biosensor according to any of the preceding claims, wherein the digestive enzyme is selected from the group consisting of amylase, lipase and protease.
4. Use of a biosensor according to any of the preceding claims, comprising at least two, preferably at least three

different gels which are sensitive to different enzymes or different concentrations of enzymes.

5. Use of a biosensor according to any of the preceding claims, comprising an amylase sensitive gel comprising
5 several α -1,4-glycosidic bonds, a lipase sensitive gel comprising several ester bonds and a protease sensitive gel comprising several peptide bonds.

6. Use of a biosensor according to any of the preceding claims, wherein the support layer has at least partly a
10 colored, opaque, structurally colored, transparent and/or patterned surface.

7. Use of a biosensor according to any of the preceding claims, comprising a protease sensitive, preferably trypsin sensitive gel comprising a gel selected from the
15 group consisting of gelatin, a gelatin-based gel, a modified gelatin based gel, chemically crosslinked gelatin, gels comprising amino acid sequence crosslinkers, elastin, collagen, laminin, fibrin, silk fibroin, and globular proteins.

20 8. Use of a biosensor according to any of the preceding claims, comprising an amylase sensitive gel selected from the group consisting of starch, a starch-based gel, a modified starch-based gel, chemically crosslinked starch glycogen, a glycogen-based gel, dextrin, a dextrin-based
25 gel, cyclodextrin, and a cyclodextrin-based gel or a mixture thereof.

9. Use of a biosensor according to any of the preceding claims, wherein said lipase sensitive gel comprises one or more compounds selected from the group consisting of
30 plant oils, animal fats, phospholipids, monoacylglycerols and diacylglycerols or mixtures

thereof.

10. Use of a biosensor according to any of the preceding claims, wherein the lipase sensitive gel is an oleogel.
11. Drainage bag comprising a biosensor according to any of
5 claims 1 to 10.
12. Drainage bag according to claim 11, wherein the biosensor is integrated in the surface of the bag wall or floats freely in the bag.

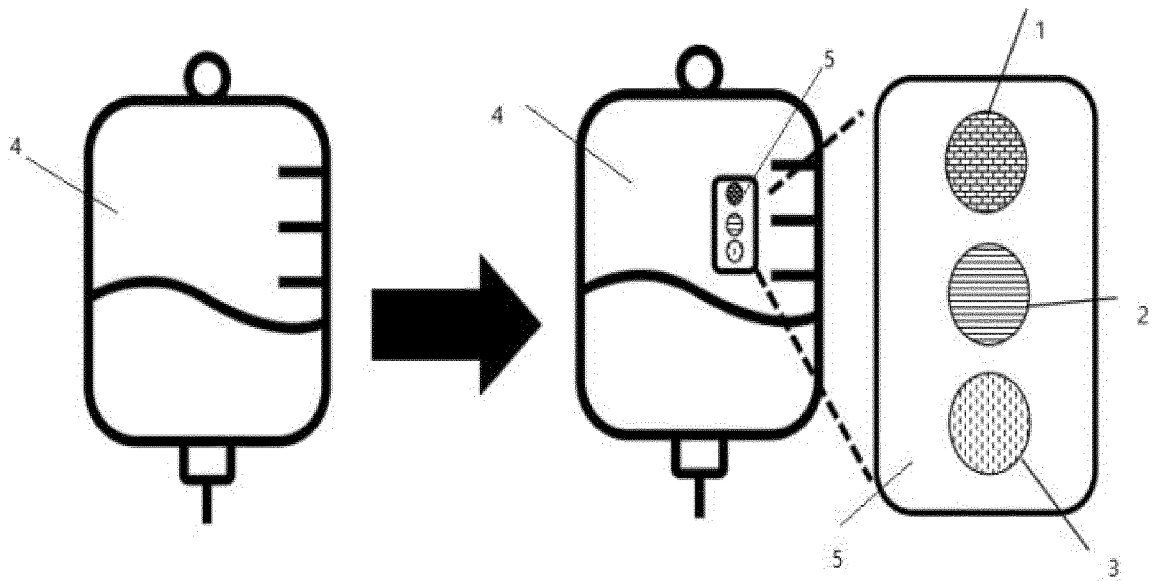


Figure 1

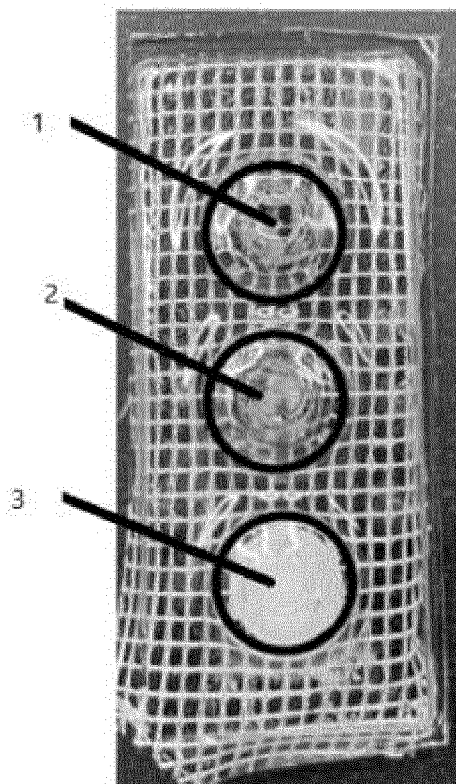


Figure 2A

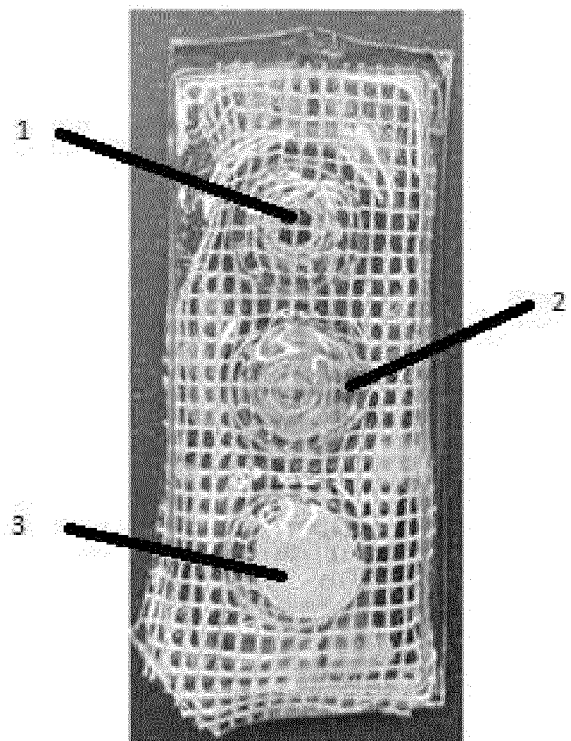


Figure 2B

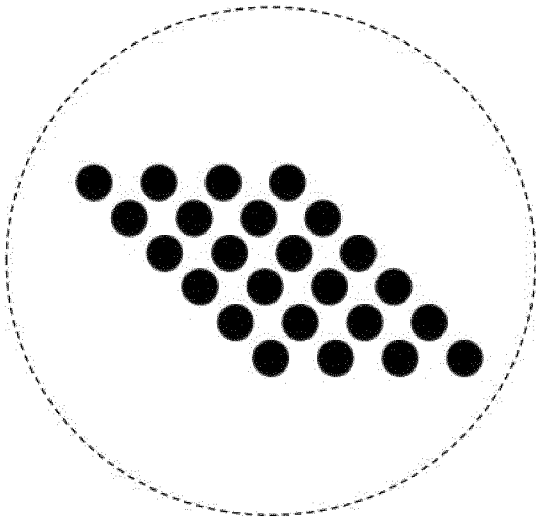


Figure 3

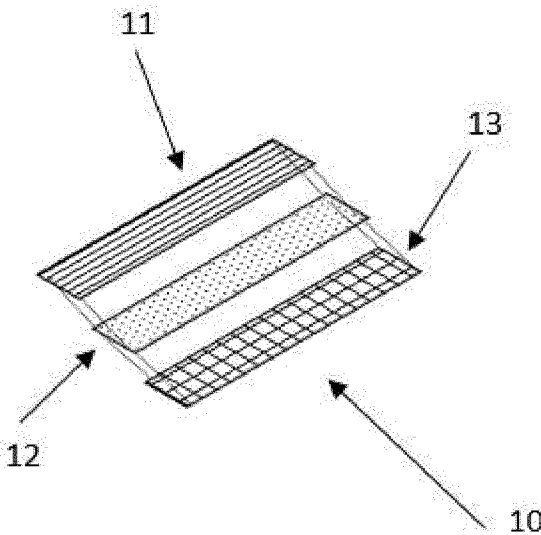


Figure 4

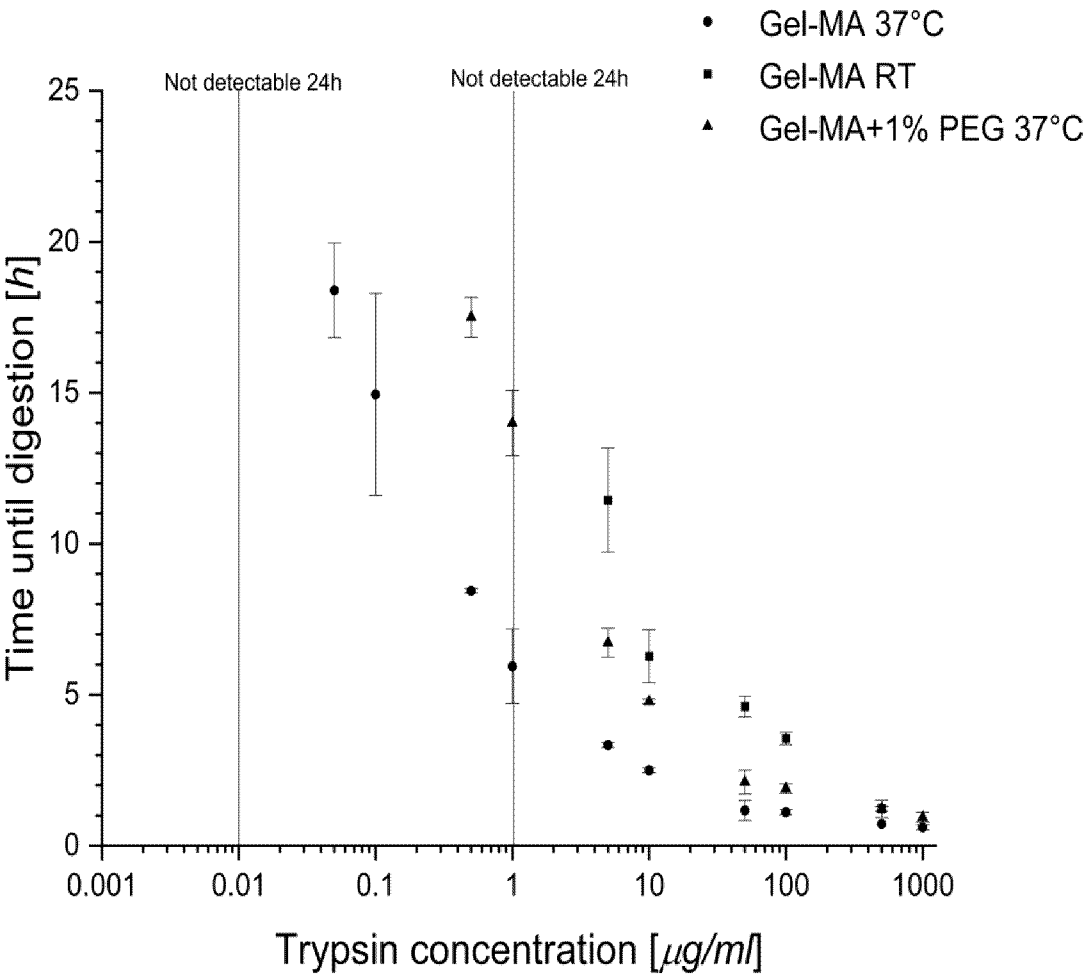


Figure 5

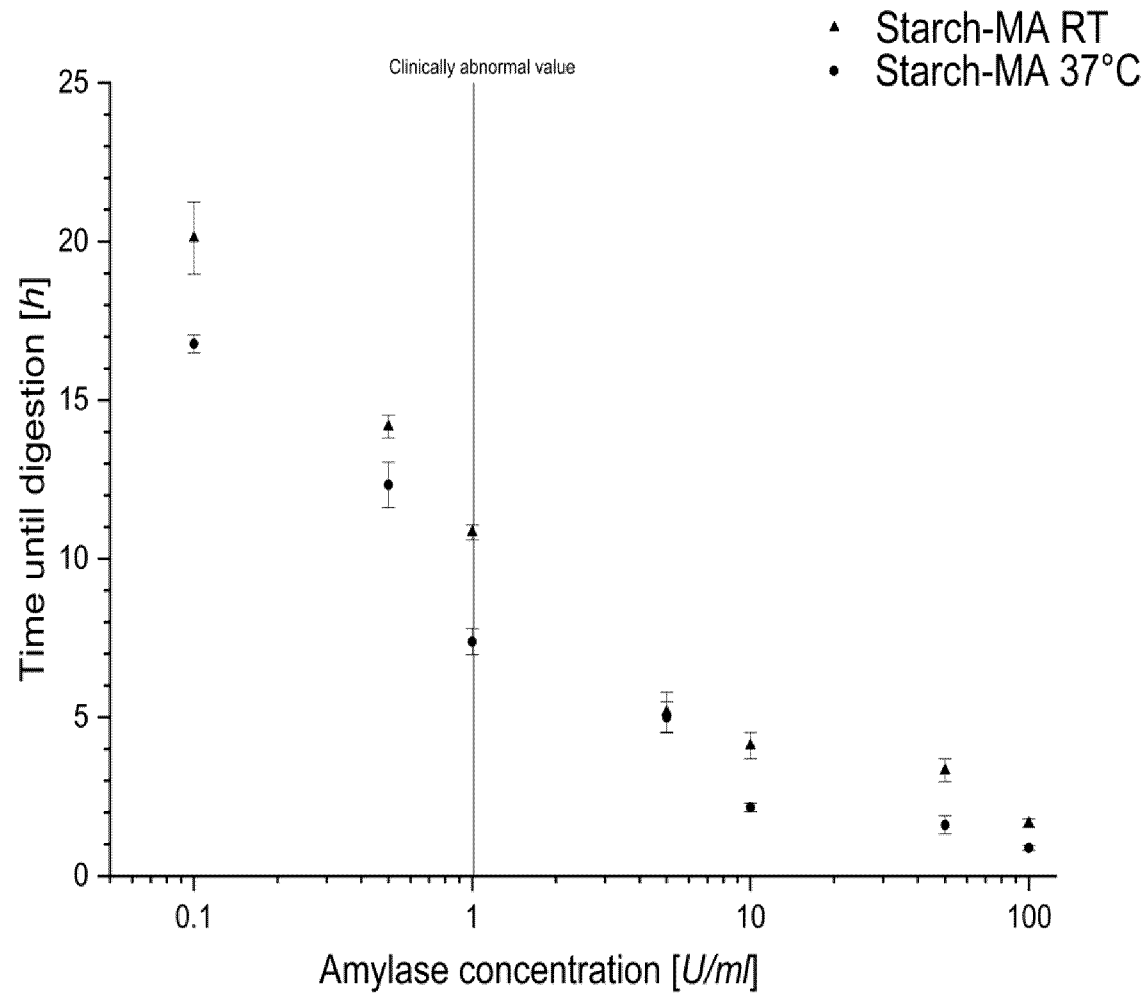


Figure 6

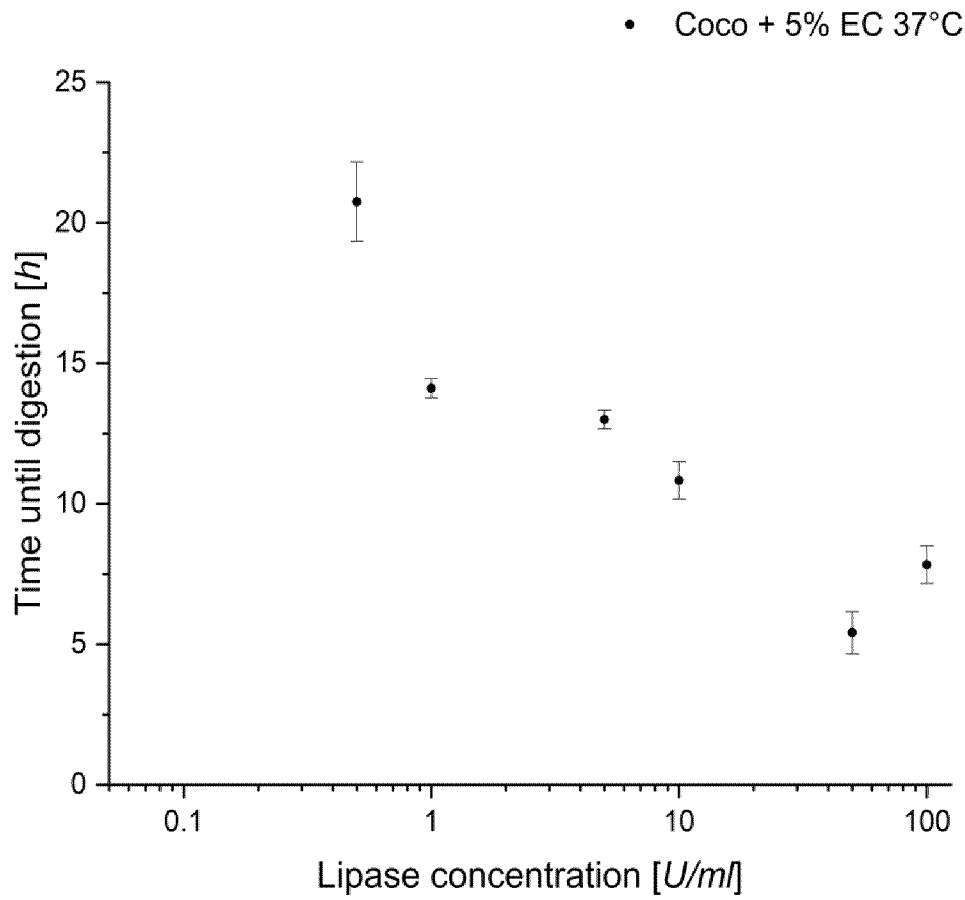


Figure 7

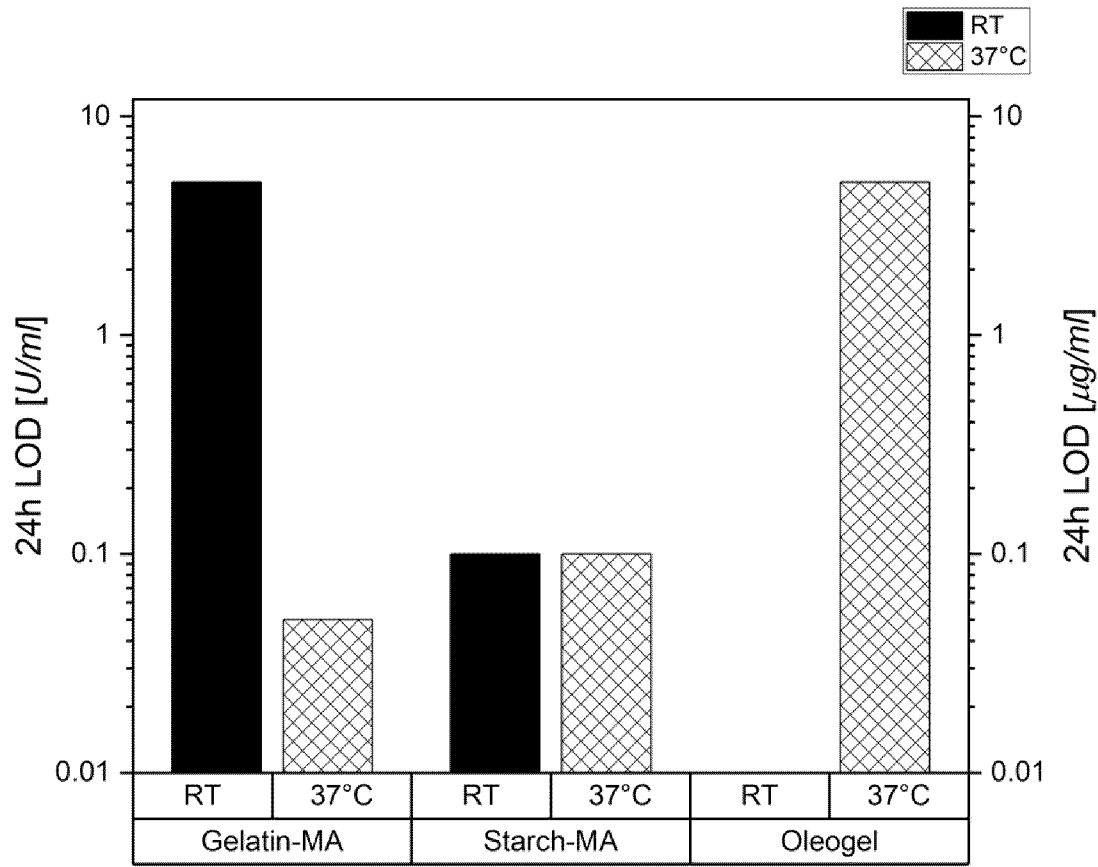


Figure 8

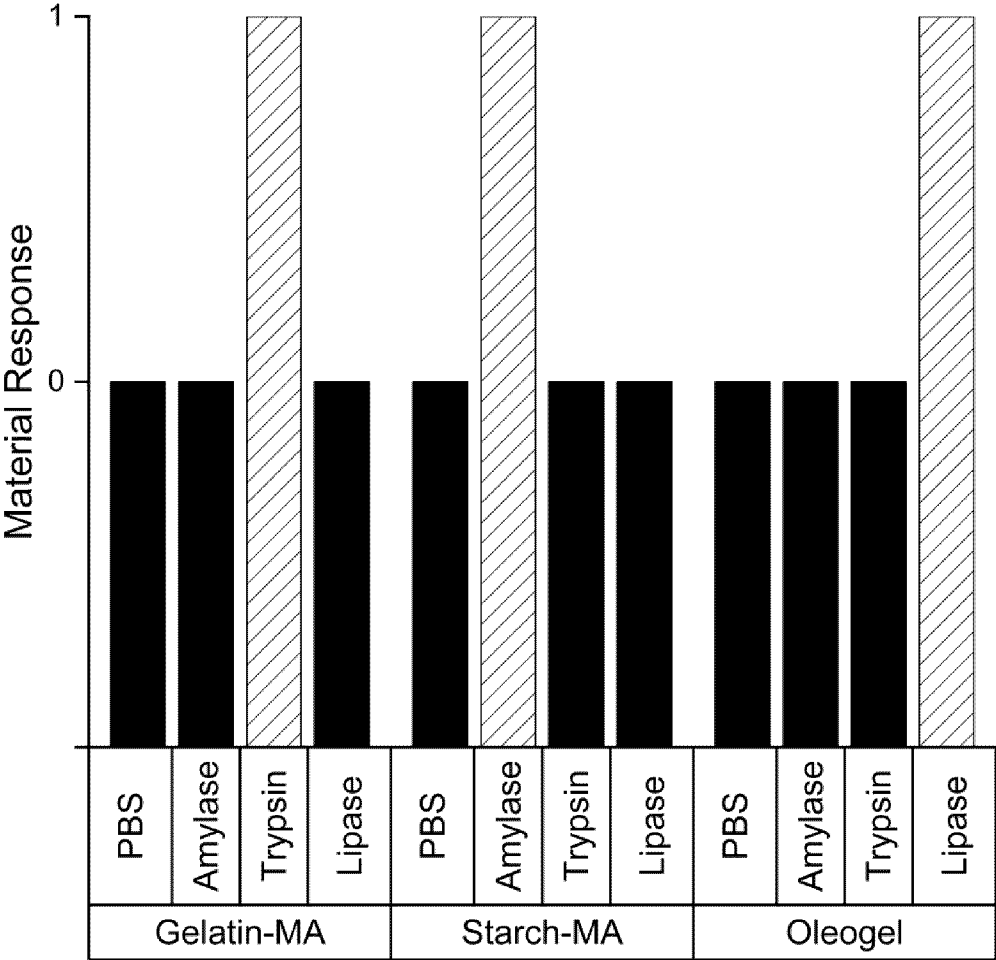


Figure 9

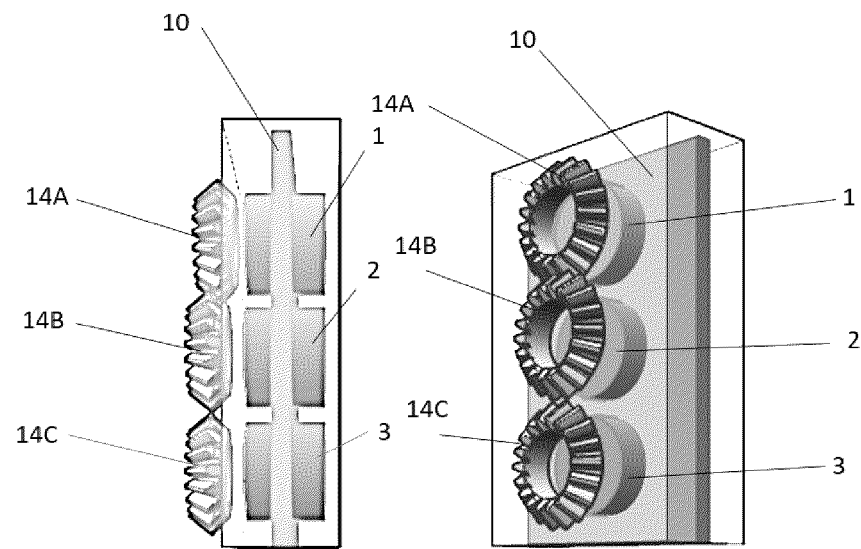


Figure 10 A

Figure 10B

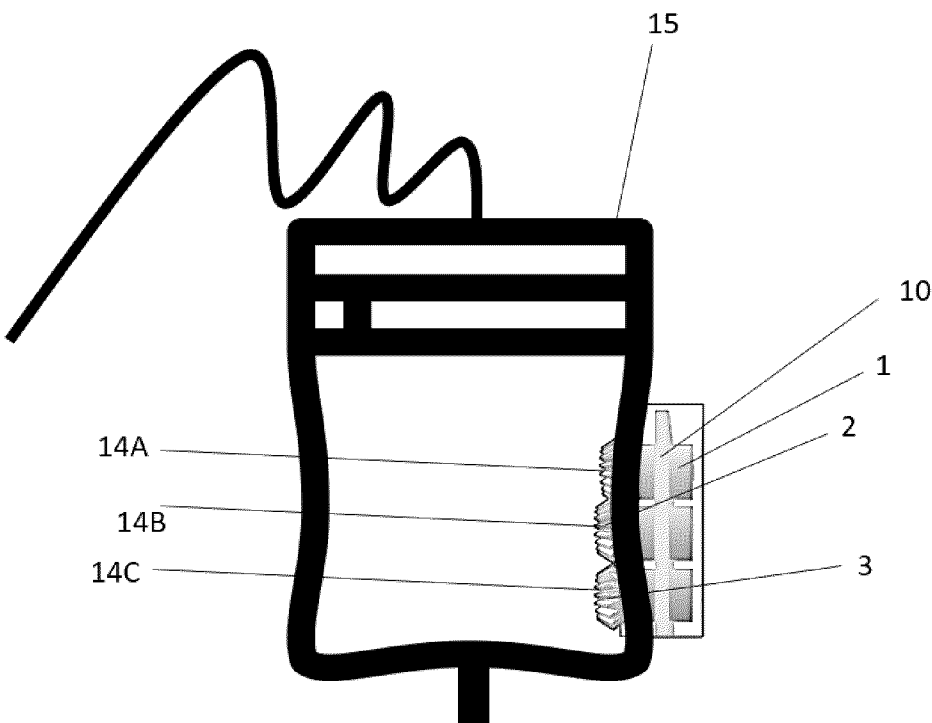


Figure 10C

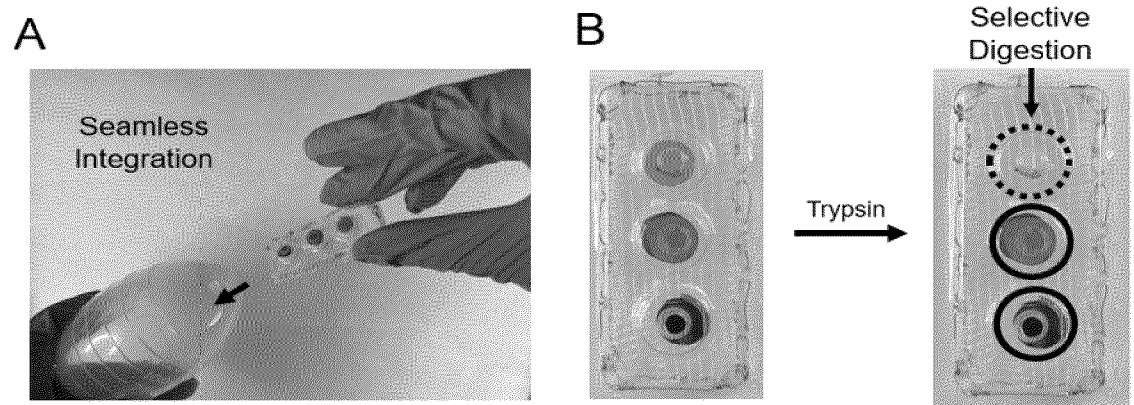


Figure 11A

Figure 11B

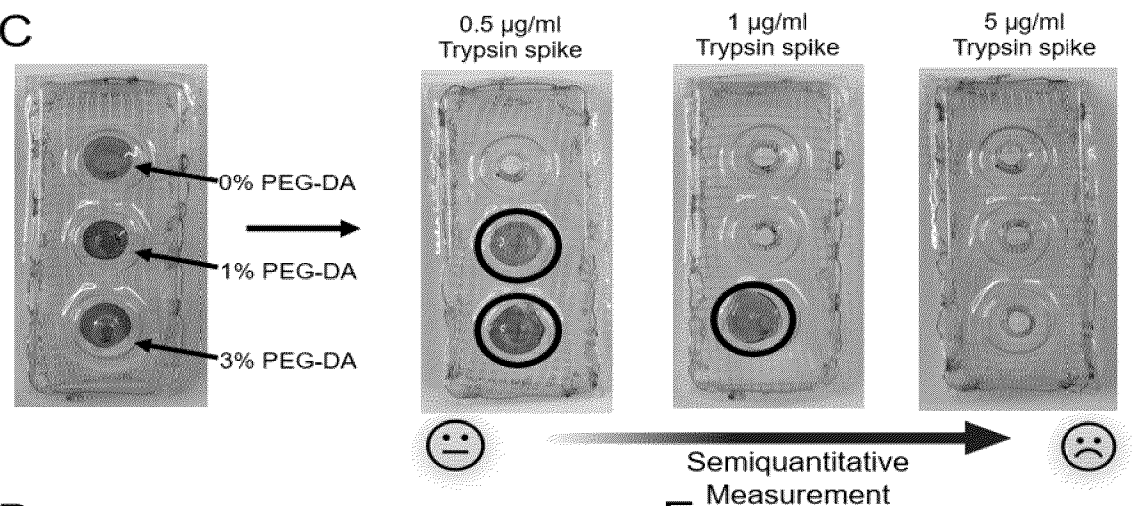


Figure 11C

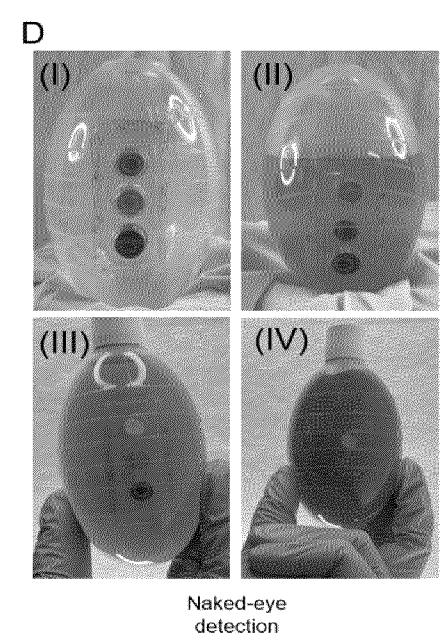


Figure 11D

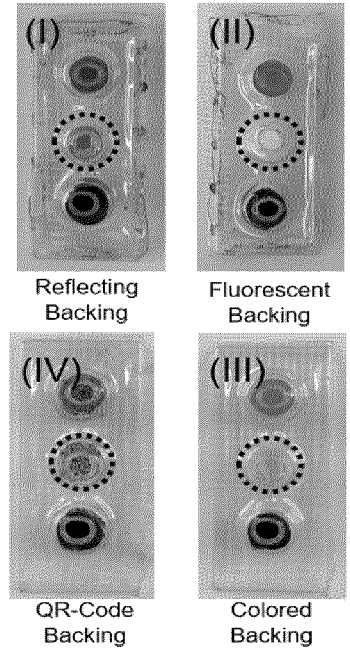


Figure 11E

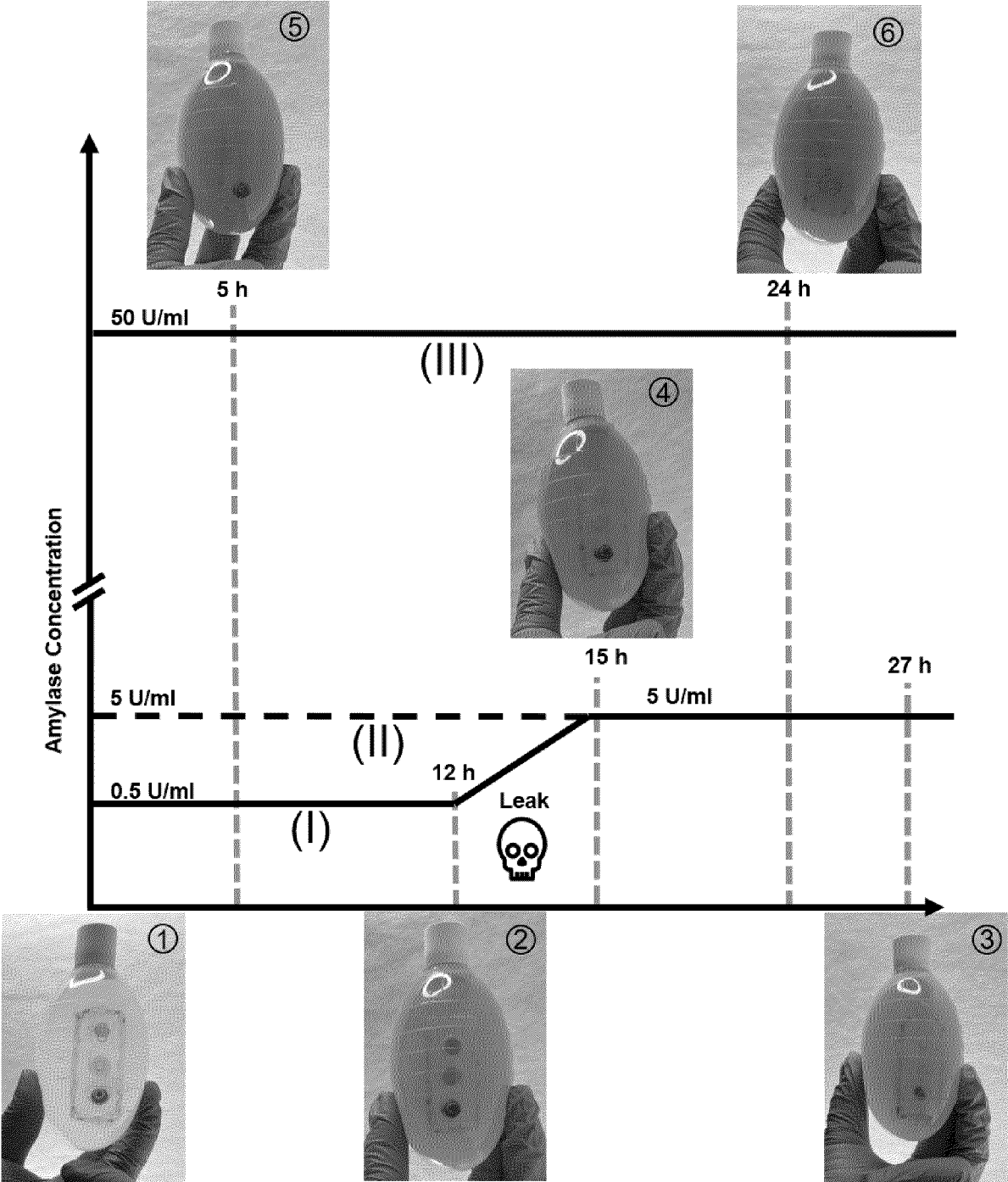


Figure 12

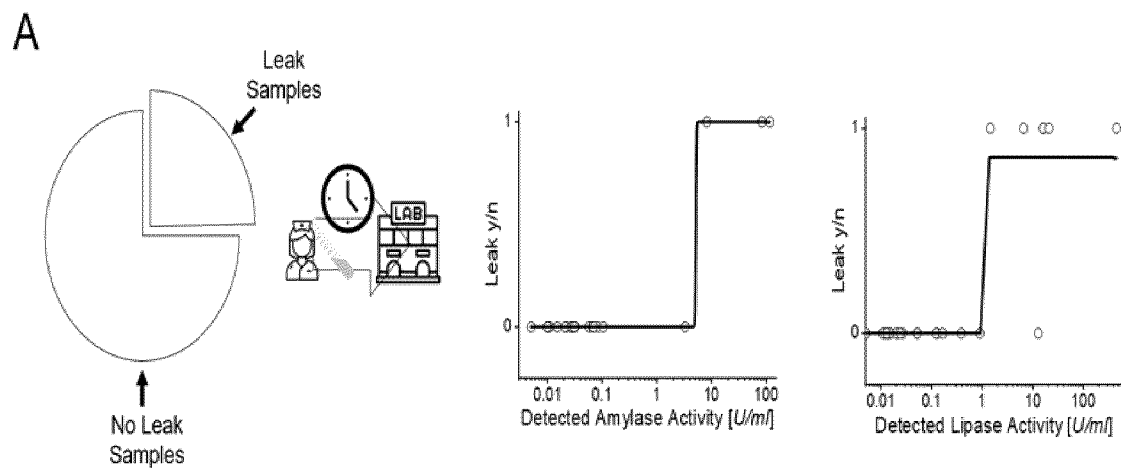


Figure 13A

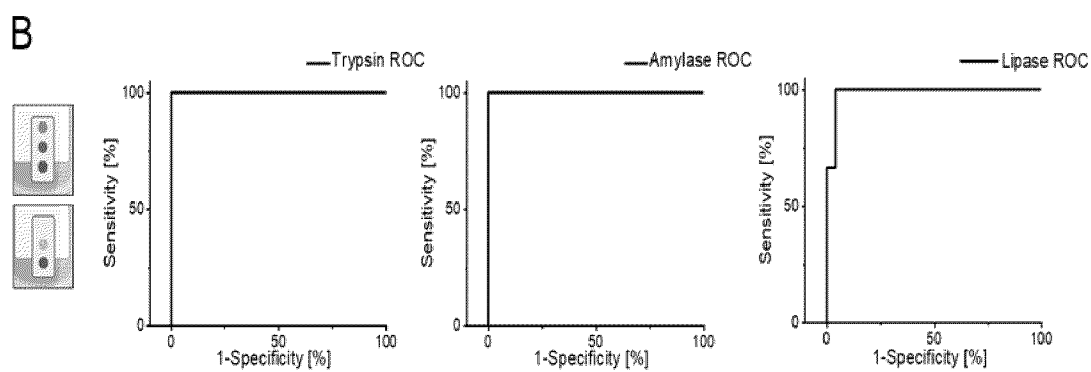


Figure 13B

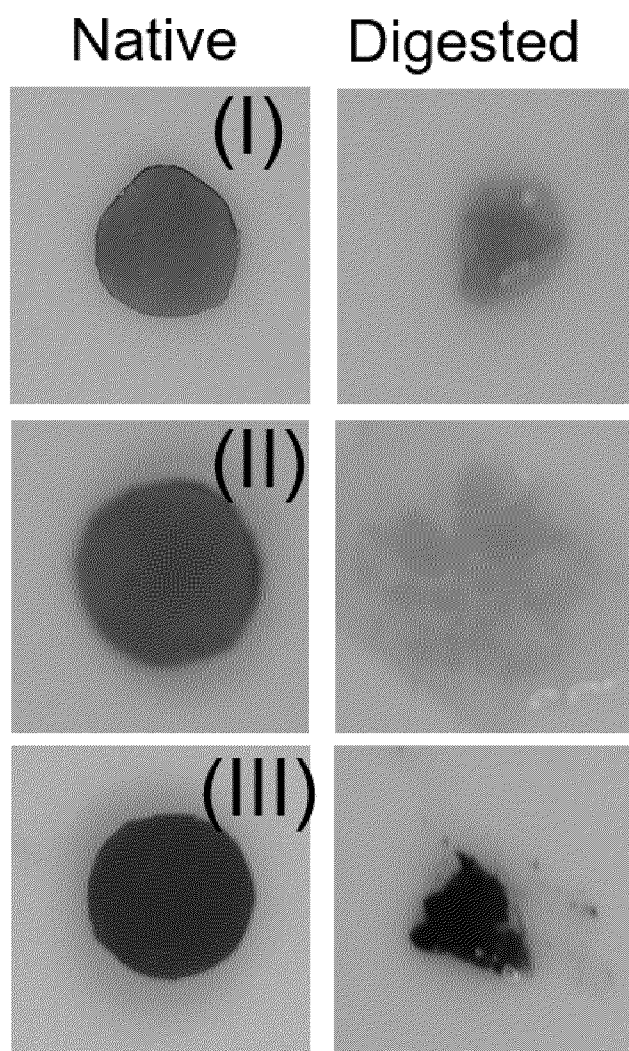


Figure 14

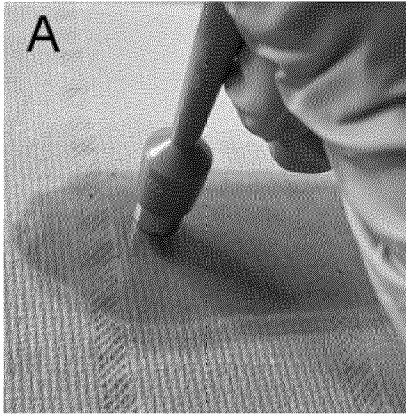


Figure 15A

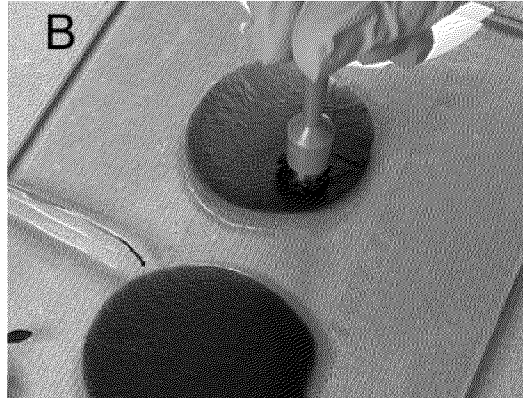


Figure 15B

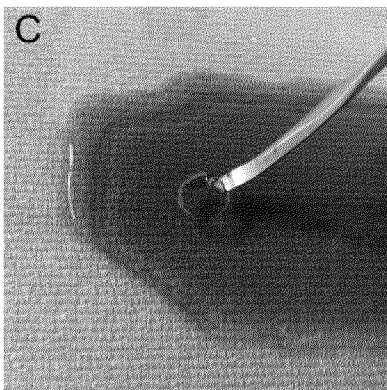


Figure 15C

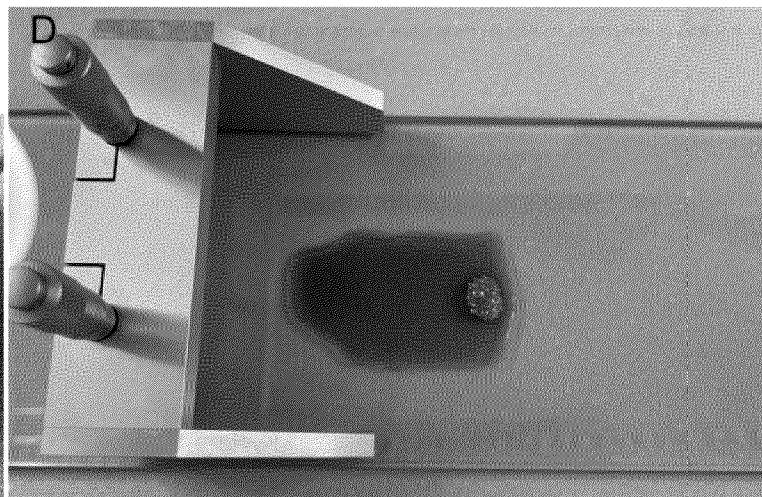


Figure 15D

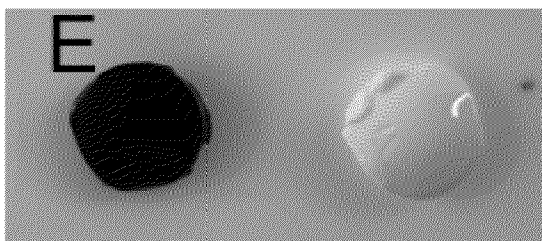


Figure 15E

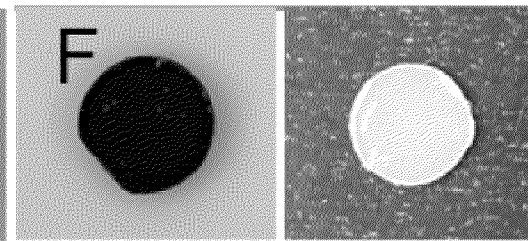


Figure 15F

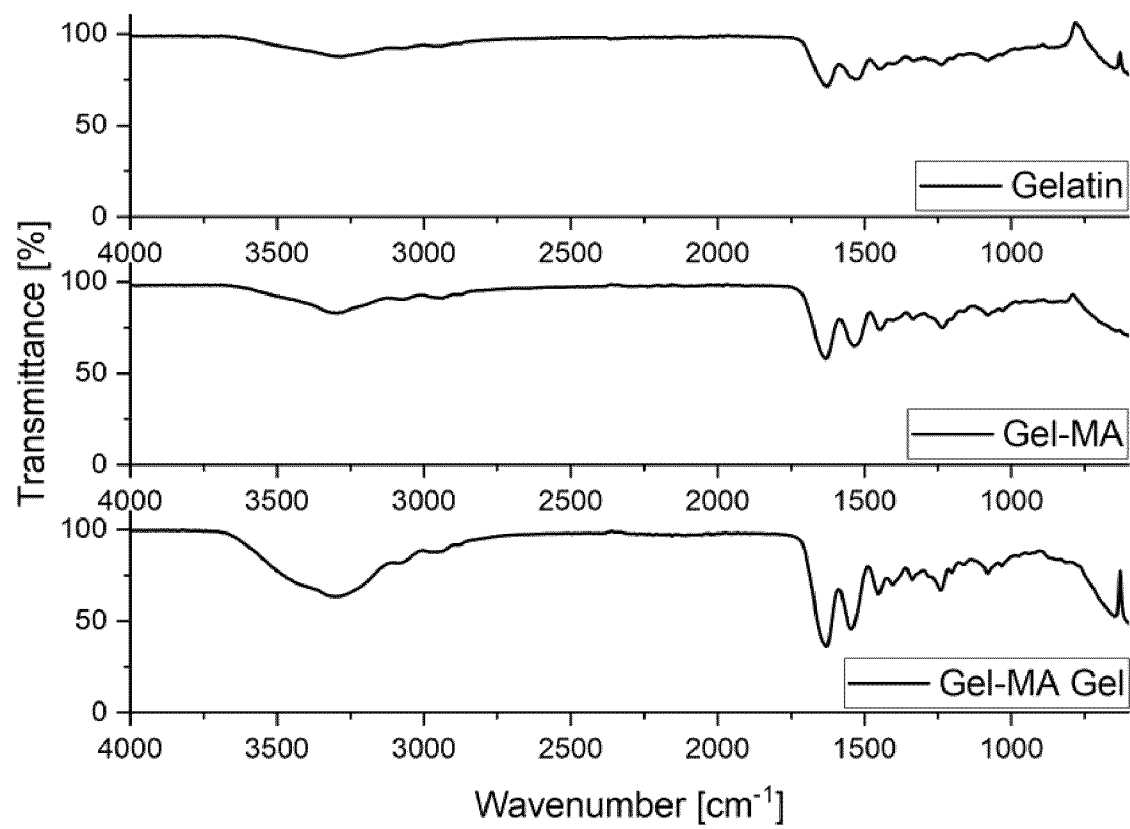


Figure 16

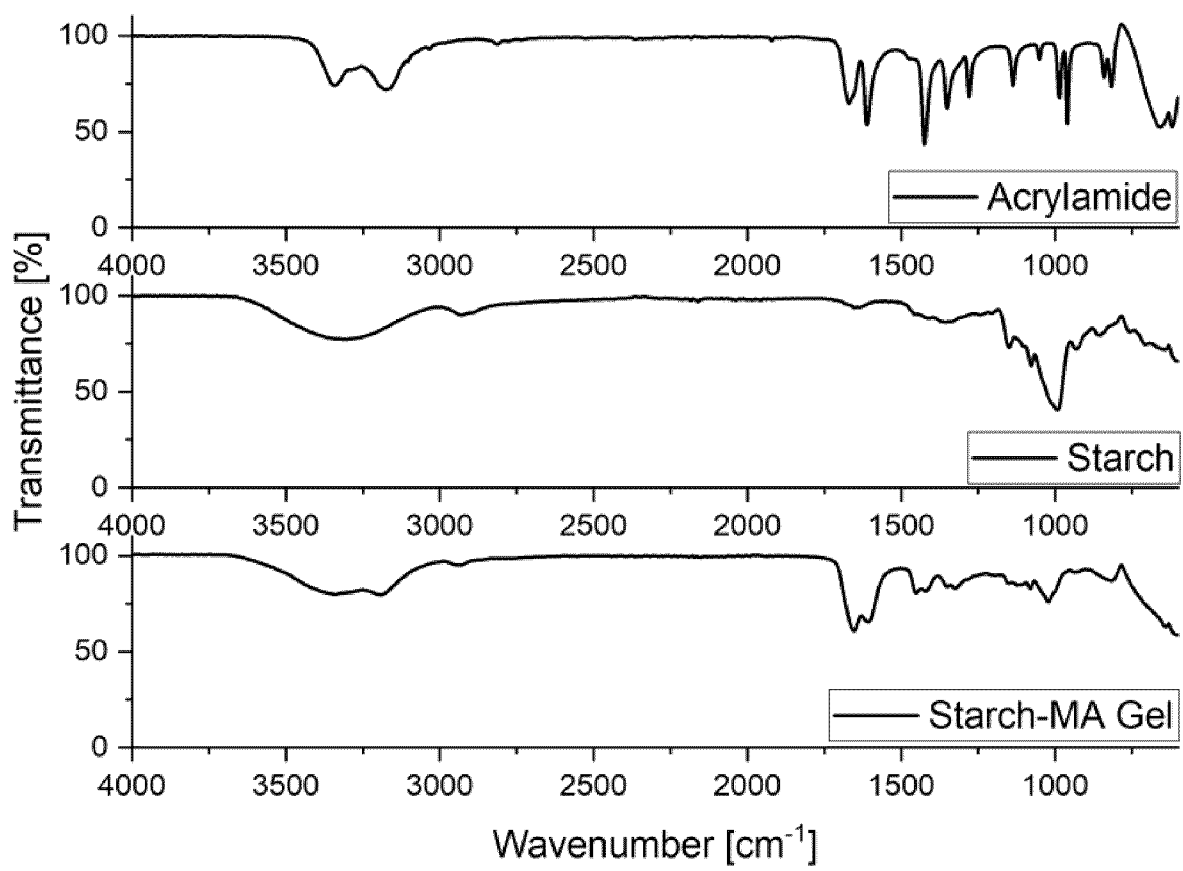


Figure 17

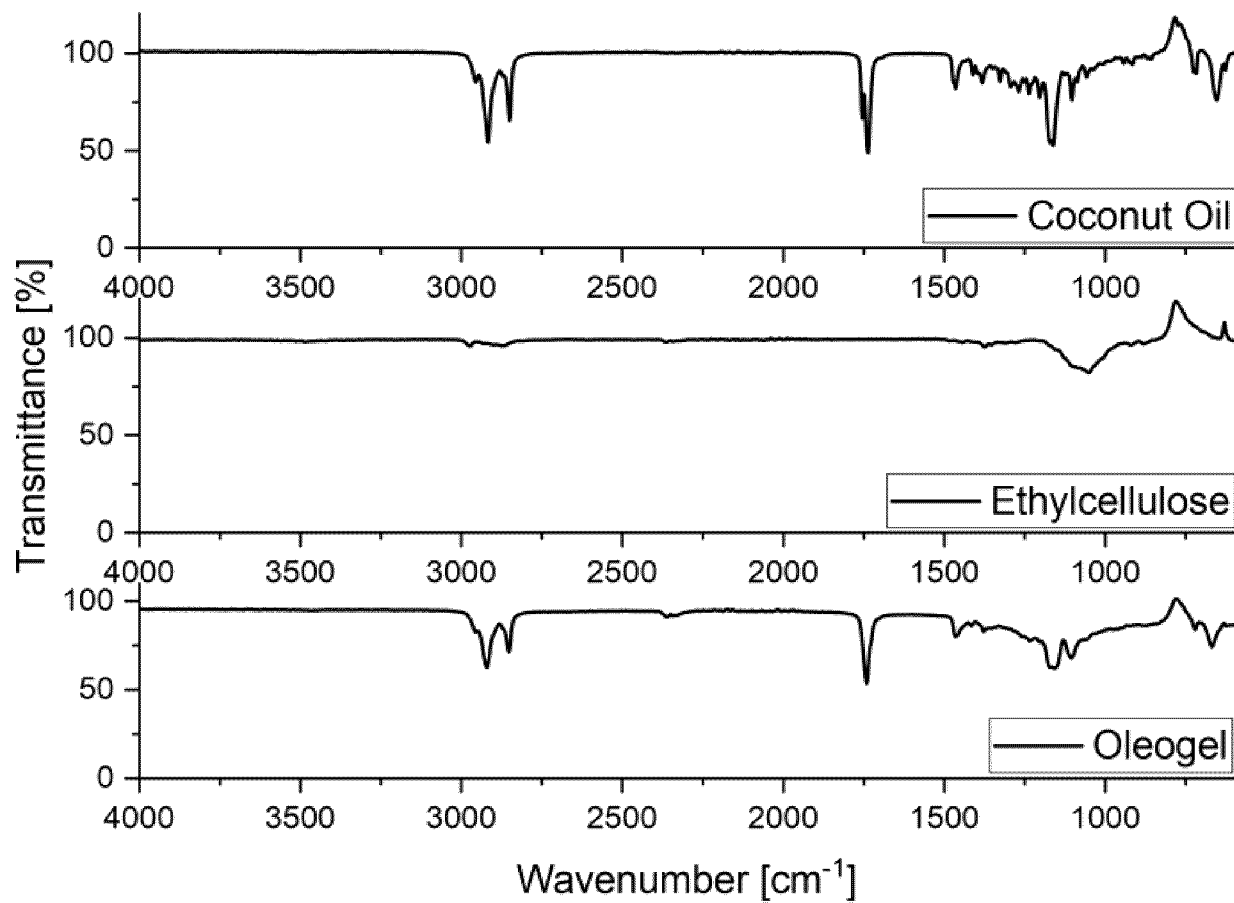


Figure 18

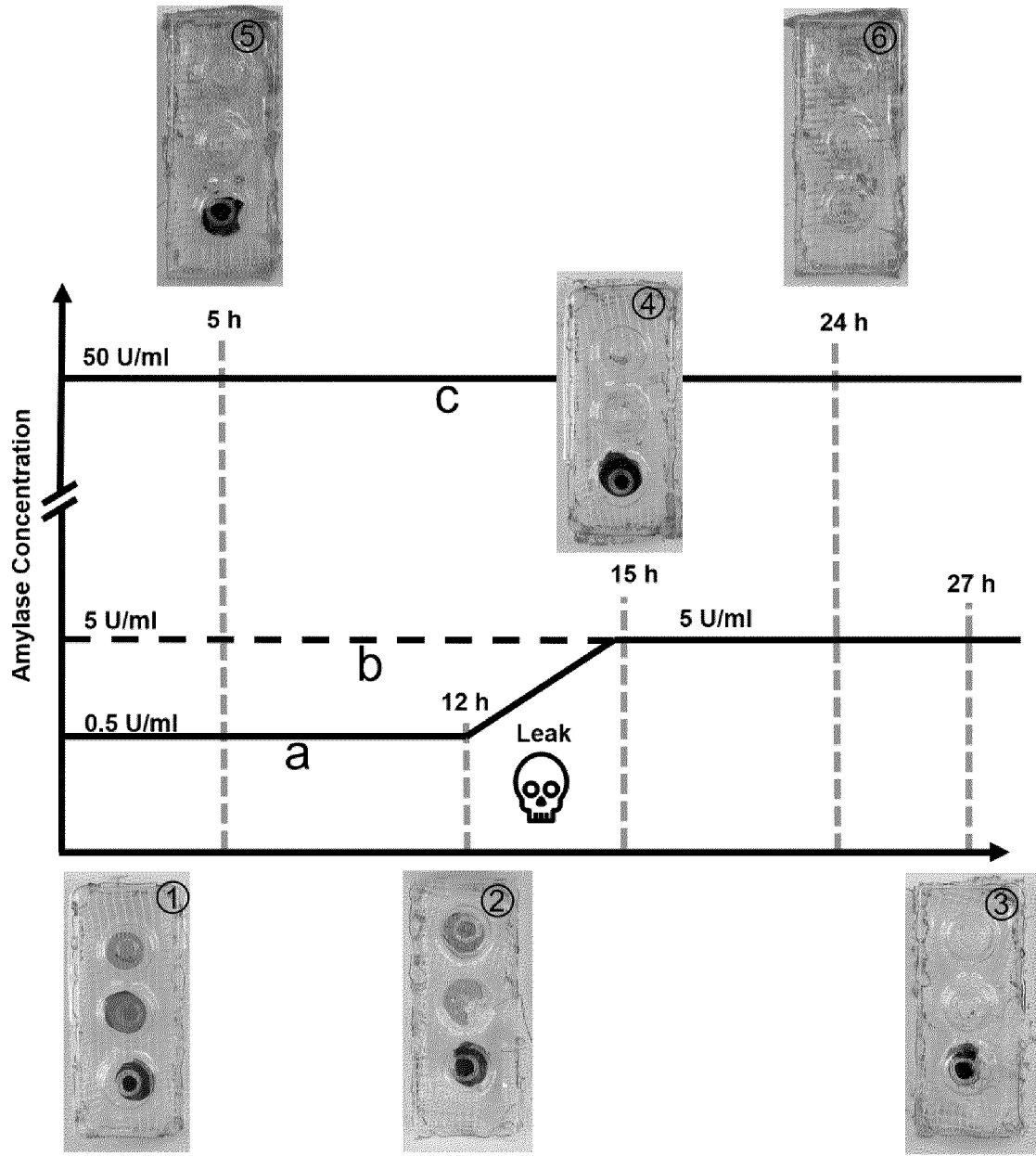


Figure 19

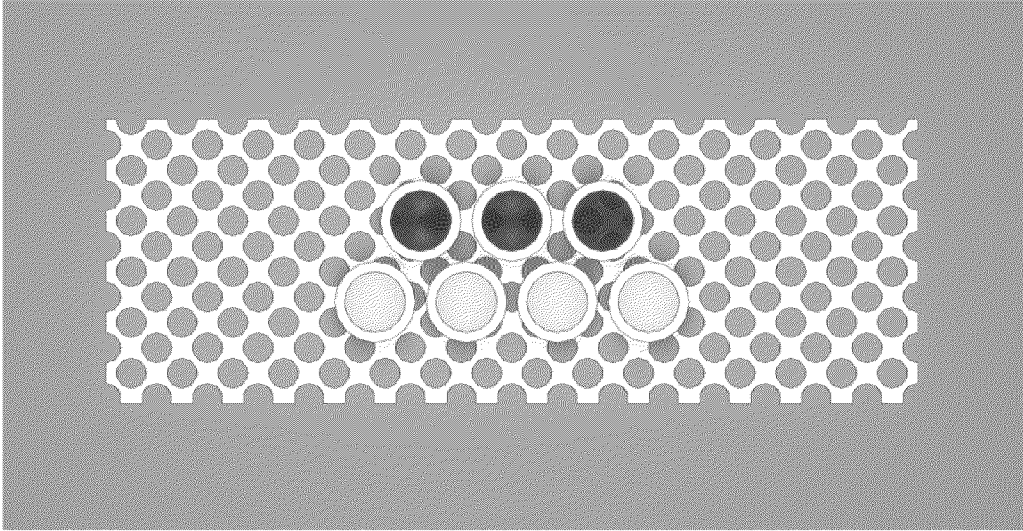


Figure 20A

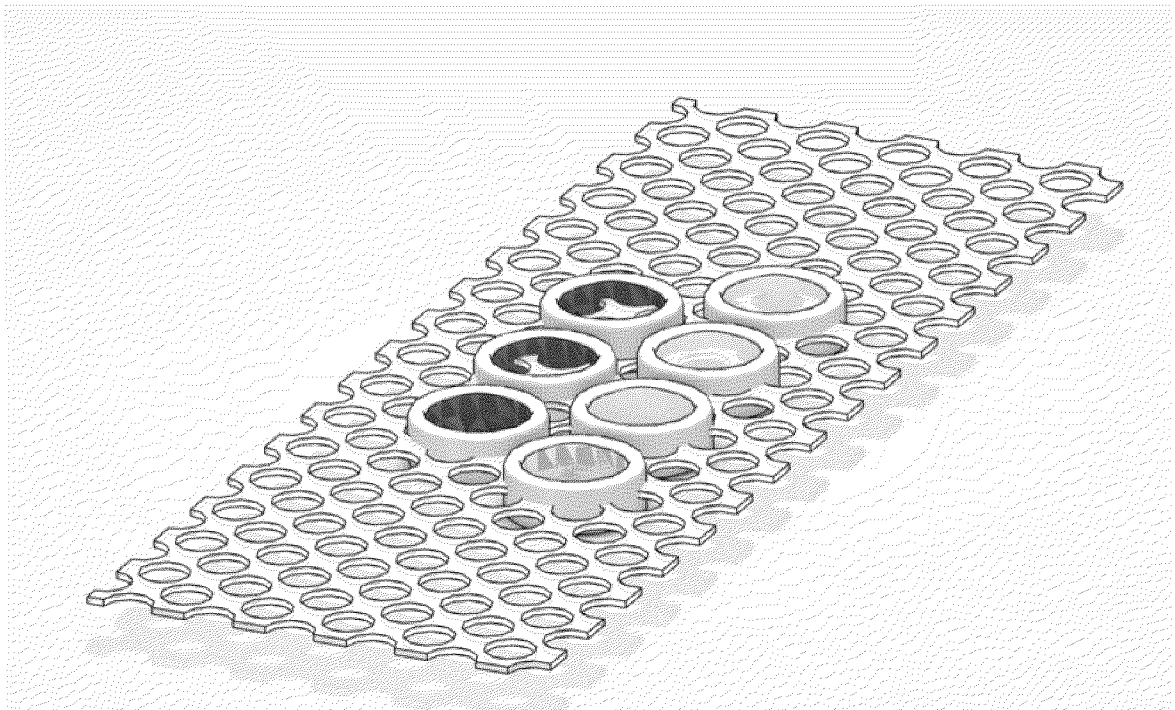


Figure 20B

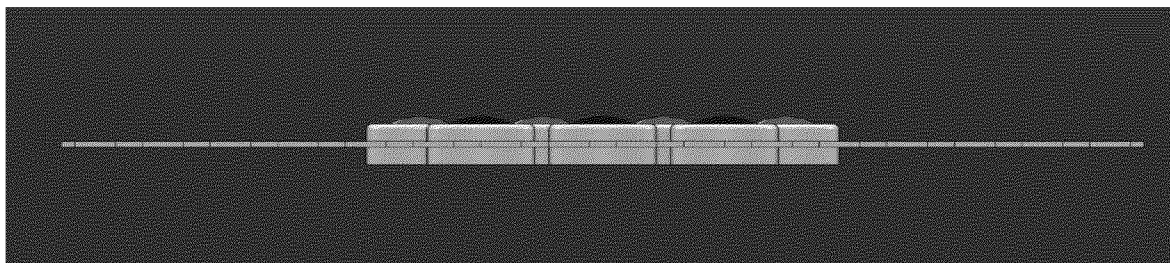


Figure 20C

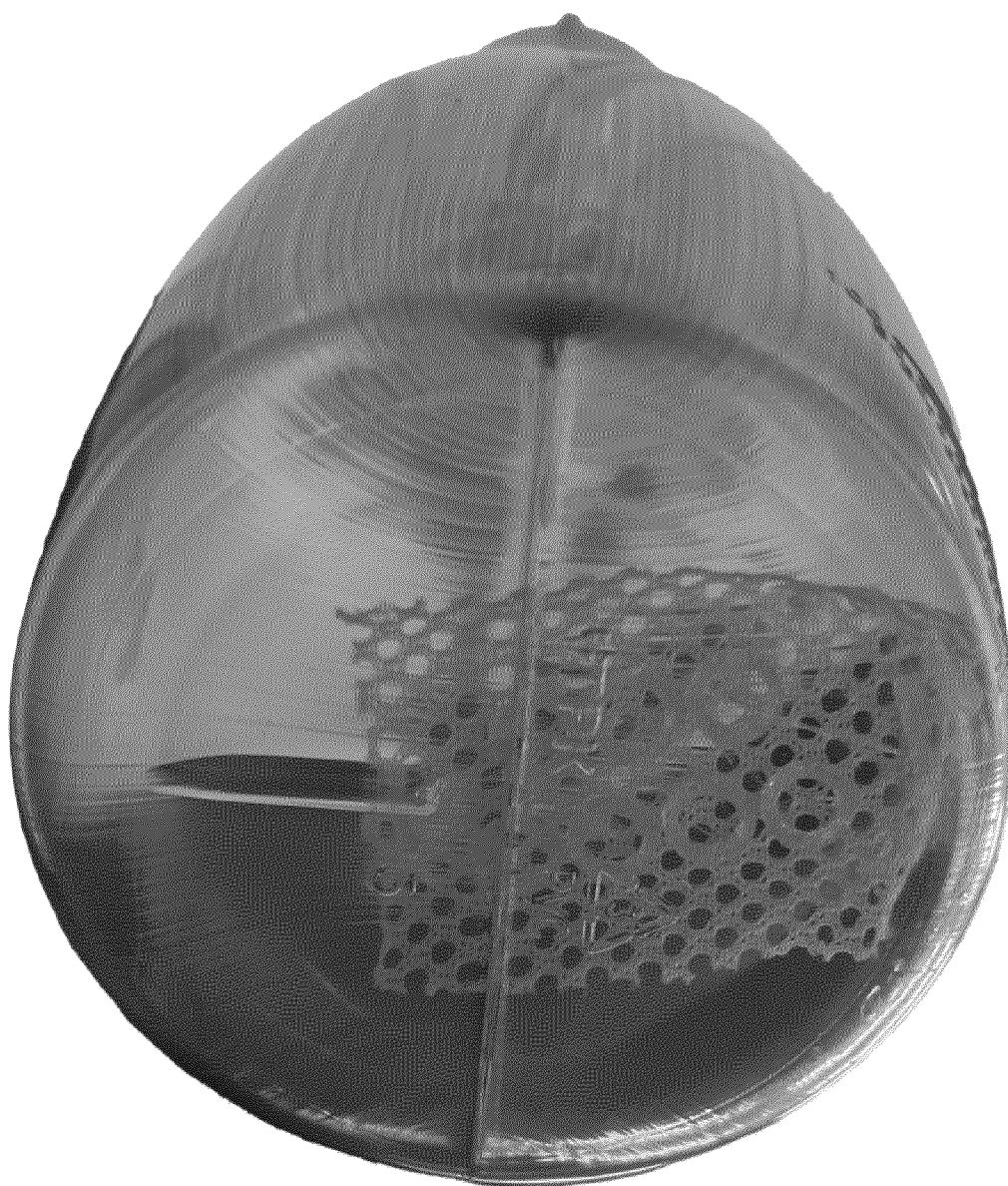


Figure 21

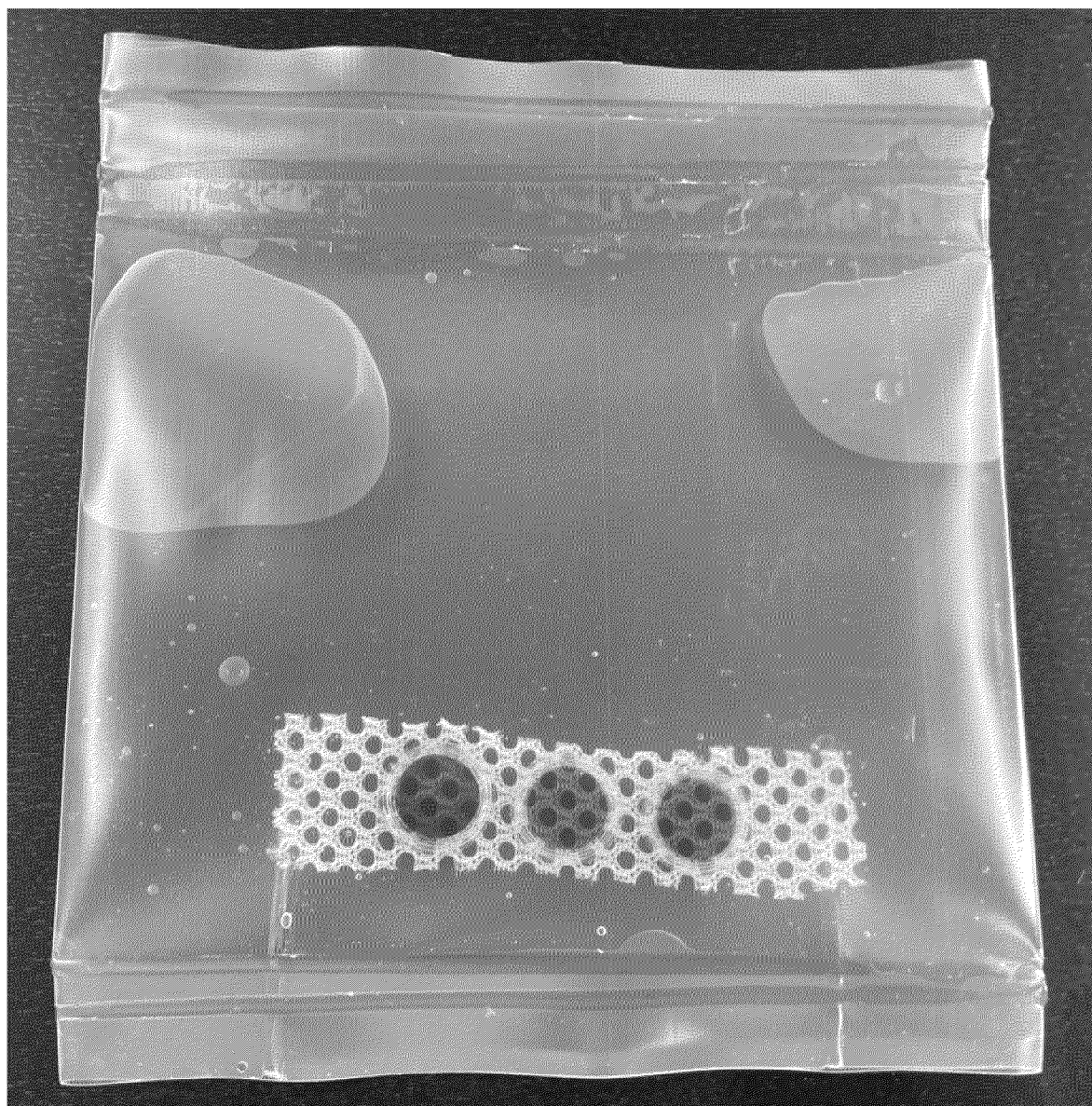


Figure 22

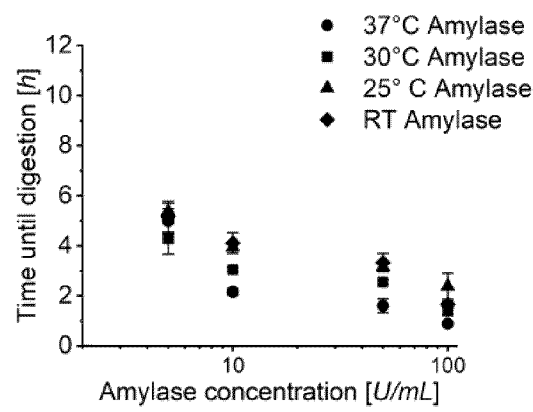


Figure 23A

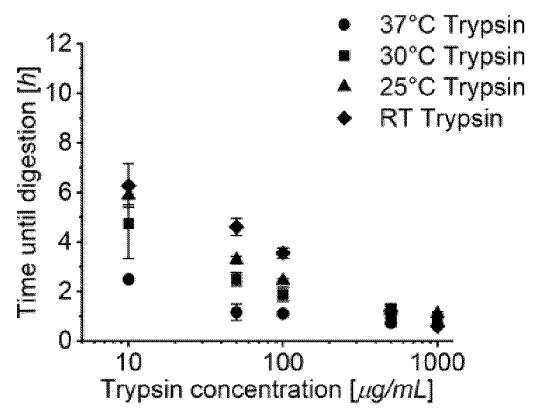


Figure 23B

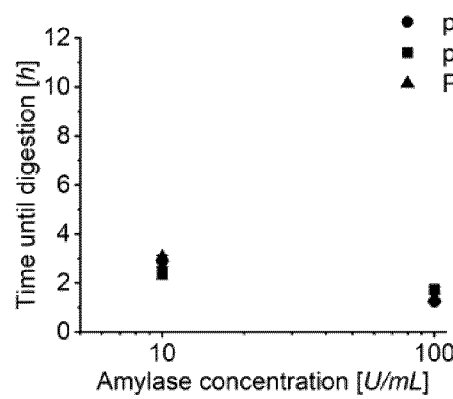


Figure 23C

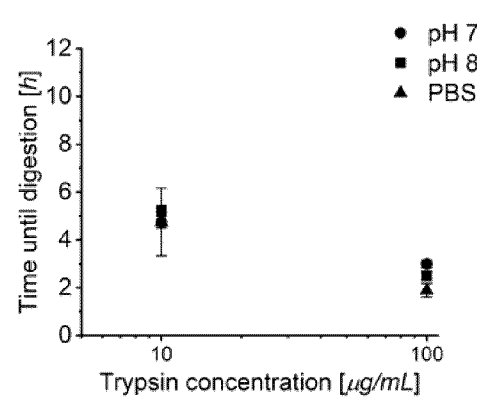


Figure 23D

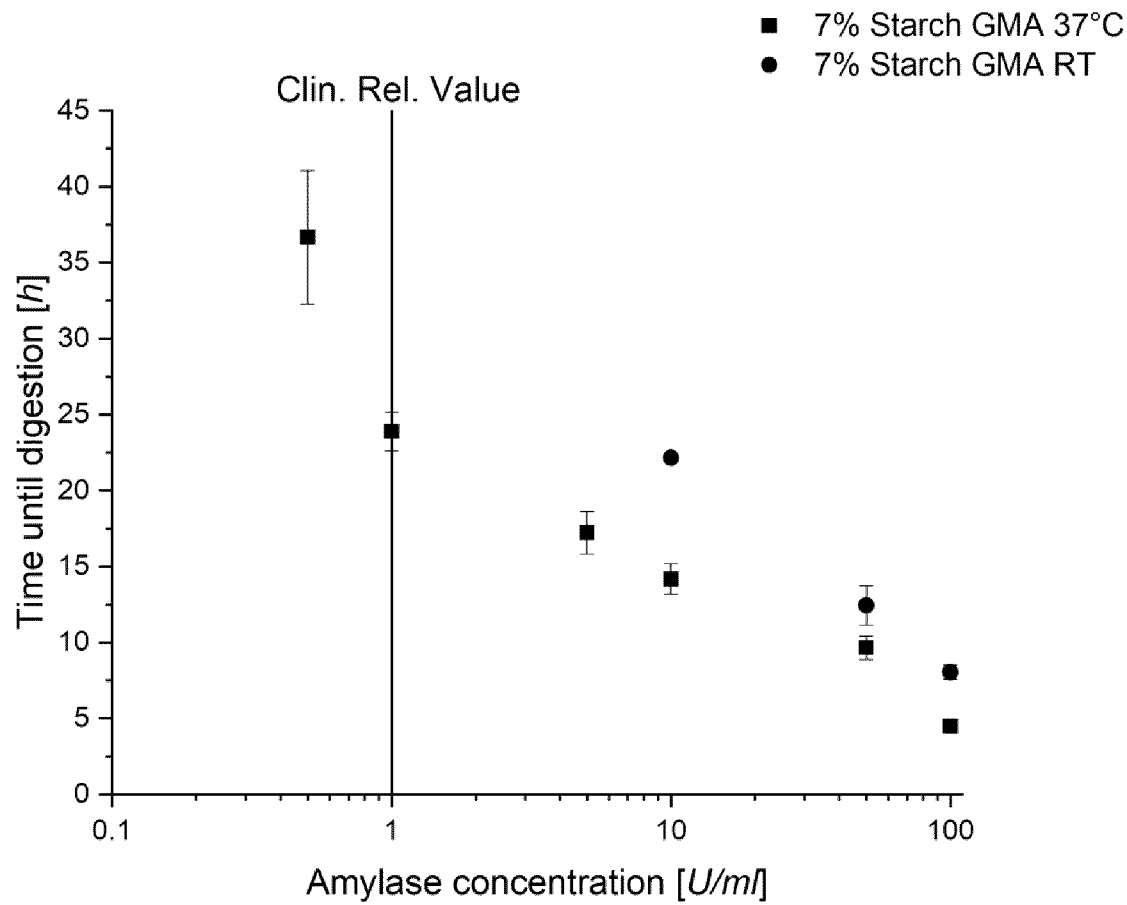


Figure 24

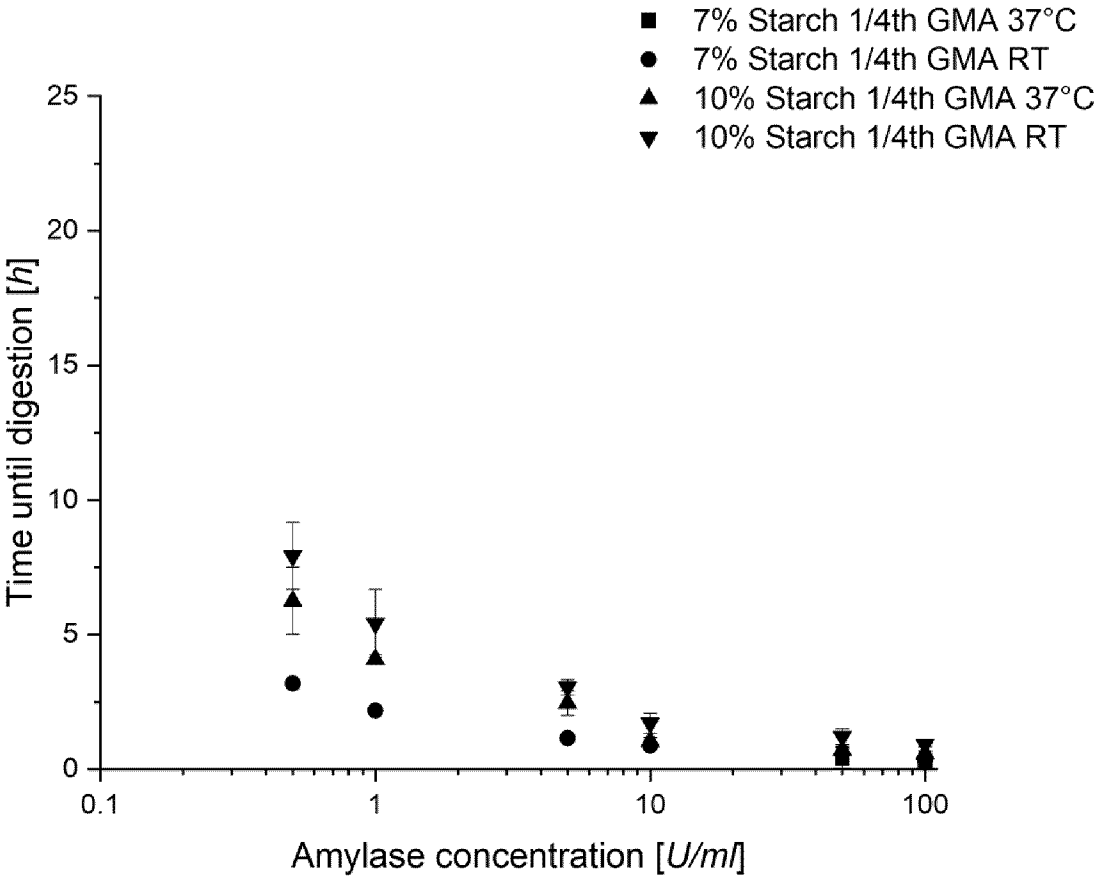


Figure 25

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065742

A. CLASSIFICATION OF SUBJECT MATTER INV. C12Q1/00 A61B5/145 A61B5/00 G01N33/52 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12Q A61B G01N Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 111 228 627 A (3RD AFFILIATED HOSPITAL SUN YAT SEN UNIV) 5 June 2020 (2020-06-05) Figs. 1-2; claims 1-4, 9 -----	1-12
A	US 2022/265175 A1 (HELWA YOUSSEF [CA] ET AL) 25 August 2022 (2022-08-25) par. 175-193; Fig. 5C -----	1-12
X	CN 101 473 217 B (UNIV WIEN) 5 October 2011 (2011-10-05) Fig. 1; Embodiment 1&2 -----	11,12
A		1-10
A	GB 2 350 677 A (CAMBRIDGE LIFE SCIENCES [GB]) 6 December 2000 (2000-12-06) claims 11, 14, 17 -----	1-12
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 27 August 2024		Date of mailing of the international search report 05/09/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Hohwy, Morten

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/065742

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 111228627 A	05-06-2020	NONE	

US 2022265175 A1	25-08-2022	CA 3134925 A1	01-10-2020
		CN 114007502 A	01-02-2022
		EP 3946055 A1	09-02-2022
		JP 2022528558 A	14-06-2022
		US 2022265175 A1	25-08-2022
		WO 2020191494 A1	01-10-2020

CN 101473217 B	05-10-2011	CN 101473217 A	01-07-2009
		DK 2035813 T3	17-09-2012
		EP 2035813 A1	18-03-2009
		US 2010068749 A1	18-03-2010
		US 2013177939 A1	11-07-2013
		WO 2007144367 A1	21-12-2007

GB 2350677 A	06-12-2000	EP 1185688 A2	13-03-2002
		GB 2350677 A	06-12-2000
		WO 0075360 A2	14-12-2000
