



A novel injectable tissue adhesive based on oxidized dextran and chitosan

Biji Balakrishnan, Dawlee Soman, Umashanker Payanam, Alexandre Laurent,
Denis Labarre, Athipettah Jayakrishnan

► To cite this version:

Biji Balakrishnan, Dawlee Soman, Umashanker Payanam, Alexandre Laurent, Denis Labarre, et al.. A novel injectable tissue adhesive based on oxidized dextran and chitosan. *Acta Biomaterialia*, Elsevier, 2017, 53, pp.343 - 354. 10.1016/j.actbio.2017.01.065 . hal-02626969

HAL Id: hal-02626969

<https://hal.inrae.fr/hal-02626969>

Submitted on 26 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives| 4.0
International License

Accepted Manuscript

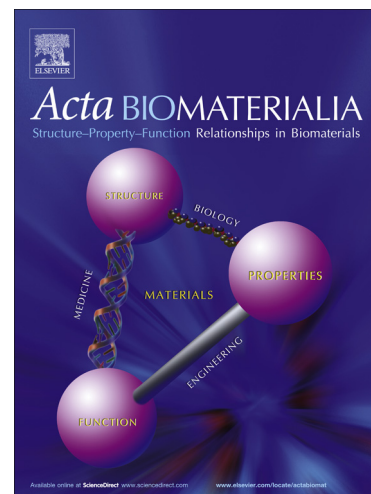
A Novel Injectable Tissue Adhesive Based on Oxidized Dextran and Chitosan

Biji Balakrishnan, Dawlee Soman, Umashanker Payanam, Alexandre Laurent, Denis Labarre, Athipettah Jayakrishnan

PII: S1742-7061(17)30074-0
DOI: <http://dx.doi.org/10.1016/j.actbio.2017.01.065>
Reference: ACTBIO 4700

To appear in: *Acta Biomaterialia*

Received Date: 17 August 2016
Revised Date: 6 January 2017
Accepted Date: 23 January 2017



Please cite this article as: Balakrishnan, B., Soman, D., Payanam, U., Laurent, A., Labarre, D., Jayakrishnan, A., A Novel Injectable Tissue Adhesive Based on Oxidized Dextran and Chitosan, *Acta Biomaterialia* (2017), doi: <http://dx.doi.org/10.1016/j.actbio.2017.01.065>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Comment citer ce document :

Balakrishnan, B., Soman, D., Payanam, U., Laurent, A., Labarre, D., Jayakrishnan, A. (Auteur de correspondance) (2017). A novel injectable tissue adhesive based on oxidized dextran and chitosan. *Acta Biomaterialia*, 53, 343 - 354. , DOI : 10.1016/j.actbio.2017.01.065

A Novel Injectable Tissue Adhesive Based on Oxidized Dextran and Chitosan*

Biji Balakrishnan^b, Dawlee Soman^{c†}, Umashanker Payanam^c, Alexandre Laurent^d, Denis Labarre^e, Athipettah Jayakrishnan^{a*}

^a Corresponding author; *Biomaterials Laboratory, Department of Biotechnology, Bhupat and Jyoti Mehta School of Biosciences, Indian Institute of Technology Madras, Chennai, Tamil Nadu 600 036, India. Email: ajk@iitm.ac.in. Tel : 91-44-2257-4129*

^b *Department of Biosciences & Bioengineering, Indian Institute of Technology Bombay, Mumbai, Maharashtra 400 076, India.*

^c *In Vivo Models and Testing Division, BMT Wing, Sree Chitra Tirunal Institute for Medical Sciences & Technology, Thiruvananthapuram, Kerala 695012, India.*

^d *Centre de Recherche en Imagerie Interventionnelle (Cr2i) APHP-INRA, Bâtiment 402, Domaine de Vilvert 78352 Jouyen Josas, Cedex, France.*

^e *Centred'Etudes Pharmaceutiques, UMR CNRS 8612, Université Paris-Sud, 5 rue J.B. Clément, 92296 Chatenay-Malabry Cedex, France.*

Abstract

A surgical adhesive that can be used in different surgical situations with or without sutures is a surgeons' dream and yet none has been able to fulfill many such demanding requirements. It was therefore a major challenge to develop an adhesive biomaterial that stops bleeding and bond tissues well, which at the same time is non-toxic, biocompatible and yet biodegradable, economically viable and appealing to the surgeon in terms of the simplicity of application in complex surgical situations. With this aim, we developed an *in situ* setting adhesive based on

biopolymers such as chitosan and dextran. Dextran was oxidized using periodate to generate aldehyde functions on the biopolymer and then reacted with chitosan hydrochloride. Gelation occurred instantaneously upon mixing these components and the resulting gel showed good tissue adhesive properties with negligible cytotoxicity and minimal swelling in phosphate buffered saline (PBS). Rheology analysis confirmed the gelation process by demonstrating storage modulus having value higher than loss modulus. Adhesive strength was in the range 200-400 gf/cm² which is about 4-5 times more than that of fibrin glue at comparable setting times. The adhesive showed burst strength in the range of 400-410 mm of Hg which should make the same suitable as a sealant for controlling bleeding in many surgical situations even at high blood pressure. Efficacy of the adhesive as a hemostat was demonstrated in a rabbit liver injury model. Histological features after two weeks were comparable to that of commercially available BioGlue[®]. The adhesive also demonstrated its efficacy as a drug delivery vehicle. The present adhesive could function without the many toxicity and biocompatibility issues associated with such products.

Keywords: Tissue adhesive, injectable, hemostat, dextran, chitosan

*The work reported in this publication is protected by one or more patents: *EP Patent, 2,231,134, 2010, W.O. Patent, WO/2009/072,146, 2009, US Patent Appl., 12/746,625, 2008.*

† deceased

1. Introduction

Recent advancements in wound management have led to the development of various tissue adhesives that could possibly address the problems associated with the traditional sutures, staples and wirings, in terms of lengthy application time, additional tissue damage, immunological response and insufficient prevention of fluid or gas leakage of challenging wounds. Having such multipurpose advantages, the tissue adhesives have been explored as hemostats, as drug delivery systems for the delivery of bioactive agents, sealants to prevent fluid or gas loss from the body, and as wound dressings [1,2]. Besides this, tissue adhesives have also been employed for fixation of surgical meshes for hernia repair [3], treatment of meniscus tears [4], abdominoplasty [5], plastic surgery [6] and as bone cements [7]. Though there are numerous tissue adhesives commercially available, none of them is completely devoid of shortcomings. Fibrin glue appeared to be the first successful surgical adhesive marketed as Tissucol[®], Beriplast[®], Bolheal[®] and Biocol[®], but its wide usage is restricted due to poor tissue adhesion, low strength and greater chances of contaminations with HIV and Prion [8,9]. Cyanoacrylate glues (eg: Histoacryl[®]) also attracted the attention of the medical community due to their bonding strength and ability to bond in wet environments, but problems with cell toxicity, foreign body reaction, burning sensation in the application area and low tensile strength limited their use [10-13]. The GRF glue based on gelatin, resorcinol and formaldehyde [14] and the FDA approved Bioglue[®] consisting of bovine serum albumin and glutaraldehyde [15] are associated with formaldehyde and glutaraldehyde toxicity [16]. Attempts have been made to crosslink gelatin using various other compounds such as carbodiimides, polyepoxy compounds and naturally occurring molecules such as genipin in order to overcome the cytotoxic nature of formaldehyde and glutaraldehyde [17-18], but ended up with long setting times [19]. The

preparation of mussel adhesive protein (MAP) based wet tissue adhesive is tedious, employs less efficient protein isolation and purification methods [20]. MAP mimicking systems prepared by inclusion of catechol groups to the polymers require oxidizing agents such as hydrogen peroxide, sodium periodate or enzymes such as horseradish peroxidase or mushroom tyrosinase for the polymerization of dopamine to give adhesive property [21-23]. While polyethylene glycols (PEG) based tissue sealants demonstrate fast setting, good adhesion and tissue compatibility, their preparation requires extensive chemical manipulations; functionalization with thiol and maleimide [24], nitrodopamine [25], or amine groups followed by cross-linking with dextran dialdehyde [26], tyramine followed by grafting with chitosan etc [27]. Moreover, when used as a sealant in closed cavities, these sealants create pressure build up on surrounding tissues due to their high swelling ratio [30]. PEG-based photopolymerizable sealants (FocalSeal™) require UV illumination and long curing times limit their use in many clinical applications such as in hemorrhages [31]. Low internal strength, tendency to crystallize making it brittle and non biodegradability are other limitations of PEG-based sealants [25, 30]. Various strategies employed for the preparation of tissue adhesives and its various applications have been described in detail elsewhere [1-7, 31-33].

There are numerous unmet challenges in the making of an ideal functional tissue sealant that works in a variety of surgical settings. An important requirement of tissue adhesive is the rapid formation of a strong bond on the tissue without retarding wound healing. The ideal tissue adhesive would be safe and biodegradable so that no risks would be incurred by its use or the presence of its metabolites. Ease of preparation, application, sterilizability, good tissue bonding characteristics and low cost are some of the features of a good adhesive. Based on all these criteria, we demonstrate here a rapidly setting biopolymers-based surgical adhesive without any

extraneous toxic cross-linking agents, photoinitiator or enzymes, and is rather simple, inexpensive, non-toxic, biocompatible, and biodegradable that can be used in a variety of clinical situations. Naturally occurring polymers such as dextran and chitosan were chosen based on their industrial availability, purity, non-toxicity, biodegradability, ease of chemical modification and general behavior in the biological milieu. Both biopolymers have a long history of use in humans. The strategy employed was to manipulate one biopolymer in such a way that it enters into a chemical reaction with the other resulting in the formation of the adhesive. In this regard, periodate oxidized dextran (DDA) was reacted with chitosan hydrochloride (ChitHCl) leading to *in situ* gelation via Schiff's base formation. The system was evaluated for its bonding and burst strengths *in vitro*. Its ability to function as a drug delivery vehicle was examined *in vitro* using a model drug such as 5-fluorouracil (5-FU) and a model protein such as bovine serum albumin. The hemostatic potential of the adhesive was evaluated in a rabbit liver parenchymal injury model.

2. Materials and Methods

2.1. Materials

Chitosan (Viscosity average molecular weight 311 kDa, degree of deacetylation 74%) was a gift from Central Institute of Fisheries and Technology, Cochin, India. Dextran (from *Leuconostoc mesenteroides*, M.W 500 kDa), sodium metaperiodate, FITC albumin, and 5-FU were purchased from Sigma Chemical Co., St. Louis, MO, USA. Dialysis tubing (Spectra/Por[®], MWCO 3500) was from Spectrum Laboratories Inc., CA, USA. Fibrin glue, Reliseal[®] was purchased from Reliance Life sciences, Mumbai, India. Phosphate buffered saline (PBS, pH 7.4, 0.1 M) was prepared by dissolving 17.97 g di-sodium hydrogen phosphate, 5.73 g monosodium

hydrogen phosphate and 9 g sodium chloride in 1 L distilled water. All other reagents were of analytical or equivalent grade.

2.2. Methods

2.2.1. Preparation of Chitosan Hydrochloride (ChitHCl)

ChitHCl was prepared by the method of Austin and Sennett [34]. Chitosan (10 g) dispersed in 100 mL of 60% ethanolic HCl was kept stirring magnetically for 3 h at 20 °C. The hydrochloride salt thus formed was then filtered off, washed extensively with acetone-water mixture (6:2) and dialysed against milli Q water until the dialysate reached neutral pH. The product was then freeze dried and stored at 4 °C until use. Approximate yield of ChitHCl was 14 g (0.88 mole acid per mole Chitosan).

2.2.2. Preparation of Dextran Dialdehyde (DDA)

Into 5 g dextran, dissolved in 100 mL distilled water, sodium metaperiodate of varying equivalents was added so as to obtain oxidized DDA of different degrees of oxidation. The reaction was allowed to proceed at 20 °C for 6 h in the dark with constant stirring. The degree of oxidation was found by determining the concentration of periodate left unconsumed by iodometry after 6 h [35]. Briefly, a 5 mL aliquot of the reaction mixture was neutralized with 10 mL of 10 wt% sodium bicarbonate solution, and iodine was liberated by the addition of 20 % potassium iodide solution (2 mL). After keeping in dark for 15 min, the liberated iodine was titrated with standardized sodium thiosulphate solution using starch as the indicator. After reaction, solutions were dialyzed against distilled water (2.5 L) for 48 h with several changes of water till the dialyzate was periodate-free. The absence of periodate was checked by adding a 0.5 mL aliquot of the dialyzate to 0.5 mL of a 1 wt % solution of silver nitrate and ensuring the

absence of any precipitate. The dialyzate was then freeze dried. Typical yield of the oxidized products ranged from 80 to 90 %.

2.2.3. Viscosity Measurements

Viscosities of ChitHCl and DDA solutions were measured using a Brookfield Viscometer Model DV-II (Brookfield, USA), Spindle No. S-31 in a small sample adaptor at 37 °C at 50 rpm.

2.2.4. Preparation of DDA-ChitHCl Gel and Gelling Time Measurements

DDA of different percentage oxidation was made to react with ChitHCl to form the cross-linked gel. Into 1 mL of DDA (5, 10 or 15 wt% solution in PBS) 1 mL of ChitHCl (5 or 10 wt% solution in milliQ water) was added in a 15 mL flat bottom vial (diameter 26 mm) and stirred using a Teflon magnetic stir bar (diameter 5 mm, length 10 mm at 50 rev/min). Gelling time was noted as the time required for the stir bar to stop using a stop watch. All the gelling experiments were carried out at 37°C. A 5 wt% solution of ChitHCl in MilliQ water and a 10 wt% solution of DDA in 0.1 M PBS were prepared and kept at 37 °C in water bath in sterile polypropylene centrifuge tubes. Solutions were aspirated into the syringes before application. The gelation time of the two-component glue was also tested in an Actalyke ACT tester in G-ACT tubes in every time before the solution was applied on animals in order to assess the gelling time.

2.2.5. Rheology analysis

Rheology analysis of hydrogel was done using parallel plate geometry in a Physica MCR 301 rheometer (AntonPaar, Graz, Austria) and analyzed using Rheoplus/32 software version V3.21 (Anton Paar, Graz, Austria). The hydrogels (10 mm diameter and 5 mm thickness) were incubated with PBS at 37 °C for 1 h before strain and frequency sweep analysis, and placed on the lower plate of the rheometer. Gap size was kept as 4.5 mm. The strain sweep was performed

at constant frequency 1 rad/s to see the strain range in which the gels showed linear visco-elastic behavior with the storage (G') and loss (G'') moduli of gels independent of strain. The frequency sweep was performed by varying the angular frequency from 1 to 100 rad s⁻¹ at 1% strain. For time sweep analysis, the mixing of 0.5 mL each of DDA (10 wt%) and ChitHCl (5 wt %) were done at the lower plate of the rheometer and moduli was noted as function of time at a frequency 1 rad/s and 1% strain. Time sweep analysis of gels prepared and incubated in PBS for 1 h at 37 °C was also performed at 1% strain and angular frequency of 1 rad/s.

2.2.6. Swelling analysis

One half mL 10 wt% DDA (5, 50, 90% oxidized) and one half mL ChitHCl solution (5 wt % solution in water) were mixed using a vortex mixer in a glass vial of 15 mL capacity and allowed to form gel of approximately 26 mm diameter and 20 mm thickness. It was then kept for 10 min at 37°C and 5 mL of PBS (0.1 M, pH 7.4) was added to the gel and incubated at 37 °C. At regular intervals of time, the weight of the gel was noted after removing PBS using Pasteur pipette. The percentage swelling was calculated using the equation:

$$\text{Swelling (\%)} = \frac{(W(t) - W(0))}{W(0)} \times 100$$

Where the $W(t)$ is weight of swollen gel at time (t) and $W(0)$ is the weight of lyophilized gel before swelling.

2.2.7. Degradation studies

The degradability of gel was evaluated by measuring the change in weight of gels at regular time intervals, after incubation with PBS at 37 °C. Concisely, 0.5 mL each of DDA (10 wt%) in PBS and ChitHCl (5 wt%) solution was mixed to obtain gels. After 10 min, 5 mL of PBS was added to the vial and incubated at 37 °C. Degradation was examined by removing the medium after regular intervals of time, followed by freeze-drying the gels and normalizing the values obtained to the initial weight. All the experiments were done in triplicate.

2.2.8. Surface and internal morphology analysis

Surface and internal morphology of DDA-ChitHCl gels were examined by scanning electron microscopy (SEM). Lyophilized gels were cut using a razor blade to expose the inner region, placed on double-sided tape, sputter coated with gold and examined in the microscope (Hitachi, Model S-2400, Japan) for internal structure.

2.2.9. Cytocompatibility evaluation

In vitro cytotoxicity testing was done using the direct contact method with the test sample based on ISO 10993-5 standards. Gels prepared using 50% oxidized DDA (10 wt% solution in PBS) and ChitHCl (5 wt% solution) were lyophilized and subjected to direct contact cytotoxicity evaluation on L929 mouse fibroblast cell lines. Gels which were washed with water after preparation and lyophilized also subjected to tests. Briefly, L929 cells were subcultured from stock culture (National Centre for Cell Sciences, Pune, India) by trypsinization and seeded onto multi-well tissue culture plates (Nunc, Denmark). Cells were fed with Dulbecco's minimum essential medium supplemented with bovine serum and incubated at 37 °C in 5% carbon dioxide atmosphere. When the cells attained a monolayer, the material was kept in contact with the cells in triplicate. After incubation of cells with test samples at 37 °C for 24 h, cell culture was examined microscopically (Leica, WILD MPS32, Germany) for cellular response around test samples.

Cytotoxicity of gels was quantitatively assessed by MTT staining assay. Toxicity was evaluated after preparing extract of the material by incubating the gel with media containing serum at an extraction ratio of 0.75 cm²/mL for 24 h at 37 ± 1°C. Cells were cultured in 96-well tissue culture plates and when monolayer was attained, culture medium was removed, rinsed with PBS and 100 µL each of extract of gel, 5 wt % ChitHCl and 10 wt% DDA50 in PBS were

added to different wells containing cells. Cells with medium alone served as control. Plates were incubated for 24 h at $37 \pm 1^\circ\text{C}$, in 5% carbon dioxide atmosphere. After 24 h, the extracts/media were removed and 200 μL of MTT (5 mg/mL) was then added into each well. Plates were wrapped with aluminum foil and incubated at 95% humidified atmosphere at 37°C for 4 h. After removing the reagent solution and rinsing with PBS, 200 μL of DMSO was added to each well and incubated for 15 min at 37°C in a shaker incubator (Labline Instruments, Melrose Park, USA). The absorbance of the resulting solution was recorded immediately at 570 nm using automated micro plate reader (Bio-Tek Instruments, Vermont, USA). Reported values are mean of three replicates.

$$\text{Metabolically active cells (\%)} = \frac{\text{Absorbance}_{(\text{control})} - \text{Absorbance}_{(\text{sample})}}{\text{Absorbance}_{(\text{control})}} \times 100$$

2.2.10. Blood compatibility evaluation

Blood compatibility evaluation of the hydrogels was carried out using blood from human volunteers which was collected into sodium citrate as the anticoagulant in the ratio 9:1. The hemolytic potential of the material was determined according to the procedure reported by O'Leary and Guess [36]. This was performed to see whether the gel exerts hemolysis when it comes in contact with blood. One mL of citrate anticoagulated human blood was added to 7.5 mL of PBS containing gel or ChitHCl or DDA50 (~ 0.15 g) in different test tubes. Equal volume (7.5 mL) of PBS and 0.1 wt% sodium carbonate solution without any material was served as negative and positive control, respectively. Each set of experiments was done in triplicate. All the test tubes containing samples and the control were incubated for 1 h at 37°C . After incubation, the tubes were centrifuged at 300 rpm for 5 min and percentage hemolysis was

calculated by measuring the optical density (OD) of the supernatant solution at 545 nm in a UV-vis spectrophotometer (Lamda 25, Perkin Elmer, USA)

$$\text{Hemolysis (\%)} = 100 \times \frac{\text{OD of the test sample} - \text{OD of negative control}}{\text{OD of positive control}}$$

For evaluating the effect of material on blood coagulation, partial thromboplastin time (PTT) was noted on DDA-ChitHCl gel exposed to fresh citrated human blood after 30 min of exposure. Hydrogels were equilibrated for 1 h in PBS, exposed to blood under agitation at 100 rpm using the environmental bath shaker at 37 °C. After 30 min exposure, blood was centrifuged to obtain platelet poor plasma. Plasma was then mixed with Cephalin reagent and incubated for 3 min before adding CaCl₂ solution to initiate clotting. Clotting time was noted using an automated coagulation analyzer (Diagnostica Stago, France). The PTT of blood on fibrin glue was also determined in a similar way in triplicates.

2.2.11. In Vitro Bonding Strength Measurements

Measurements were carried out on rat (Wistar male) skin. The fatty layer of rat skin was removed using a scalpel and the fatty layer free skin was cut into approximately 1 x 2 cm pieces. One hundred µL of ChitHCl solution (5 wt% solution) was spread over the dermal side of one of the skin slices and 100 µL of DDA solution (10 wt% in PBS) on the other slice. The two skin slices were then overlapped with a bonding area of 1 x 1 cm². After loading a weight of 50 gm on the slices for regular intervals of time, the skin sheets were then connected to a pulley through a non-absorbable surgical suture U.S.P (Ethibond, Code NW 636) on one side and to a small weighing pan on the other side. Distance from pulley to tissue piece, tissue to weighing pan and weighing pan to platform were maintained as 15, 16 and 13 cm respectively. A schematic of the system used is shown in Figure 1a. Bonding strength was measured as the load required for

peeling off the skin from the other piece by adding standard weights. Bonding strength was examined by varying the setting time and employing DDAs with different degrees of oxidation. Each experiment was performed at least six times. The negative control used was chitosan alone. The results were compared with values obtained for fibrin glue.

2.2.12. Burst test experiments

In order to examine the efficacy of the system as a tissue sealant, a burst test was performed using the custom designed apparatus similar to the one reported by Prior *et al* [37] after making slight modification. To establish a uniform surface for testing the strength of the gel and its adhesion to a test substrate, a circular sample plate with a central orifice of 2 mm diameter having inlet and outlet tubings was fabricated using commercial poly(methyl methacrylate). Inlet tubing was connected to peristaltic pump (Master Flex, USA) that maintained flow of PBS at rate of 5 mL min^{-1} and outlet was connected to a pressure gauge which records in line pressure (Figure 1b). The sample plate was then covered with excised rat skin and fastened to the plate by a gasket seal. An incision of 2 mm hole was pierced on rat skin. The skin incision was visible, and the pressure in line when the incision was not sealed was recorded as 0 mm Hg. Test formulation (5 wt% ChitHCl in water and 10 wt% DDA in PBS, 40 μL each) was dropped onto the tissue surface and allowed to gel for about 5 min. Pressure in the line was measured on the pressure gauge and the pressure at which water burst through the gel was recorded. The experiments were done in triplicate.

2.2.13. Release studies of 5-Fluorouracil (5-FU) and FITC-albumin from the gel

Gels prepared using DDAs of different degrees of oxidation were loaded with an anti-cancer drug, 5-FU (50 mg/g of gel) and cumulative release was followed. Briefly, gels were prepared by mixing 150 μL each of DDA (10 wt% solution in PBS, pH 7.4) and ChitHCl (5 wt%

solution in water) containing 5-FU in screw-capped test tubes kept at 37 °C. After 10 min, 10 mL of PBS was added and incubated at 37 °C. At regular intervals, 1 mL aliquots were withdrawn and replenished with 1 mL PBS and the absorbance of released 5-FU was read at 265 nm in a UV-Visible spectrophotometer. Cumulative release was then calculated.

To study the release profile of high molecular weight proteins, different types of gels were loaded with FITC-labeled bovine serum albumin and cumulative release was followed. Briefly, gels were prepared by mixing 150 µL each of DDA (10 wt% solution in PBS, pH 7.4) and ChitHCl (5 wt% solution in water) containing FITC-albumin (25 mg/gm of gel) in screw-capped test tubes kept at 37 °C. After 10 min, 10 mL PBS was added and incubated under dark at 37 °C. At regular intervals, 1 mL aliquots were withdrawn and replenished with 1 mL PBS and the absorbance of released FITC-albumin was read at 496 nm in a UV-Visible spectrophotometer. Cumulative release was then calculated. All the experiments were done in triplicate.

2.2.14. In vivo Evaluation of the Adhesive in Rabbit Liver Parenchymal Injury Model

The DDA-ChitHCl adhesive system was evaluated for its performance by examining its hemostatic effect on liver injury and safety by studying tissue response at 14 days. Experimental procedures were performed at the Center of Research in Interventional Radiology (Centre de Recherche en Imagerie Interventionnelle), Cr2i/APHP/National Institute of Agronomic Research INRA, Jouy-en-Josas, France). The experiment protocol was approved by the Institutional Animal Care and Use Committee (IACUC ref No: A78-322-1) of the Center and was conducted according to European Community Rules of Animal Care. The experimental design consisted of a rabbit liver injury model under normal physiological conditions. Bioglue® (CryoLife Inc., USA) was used as control. New Zealand white rabbits weighing 3-4 kg were employed in the

study. A total of 6 animals were used with 3 injuries in one animal. Animals were fed with standard rabbit pellets and water ad libitum. Test glue was applied in 3 animals and control glue was applied in the remaining three. DDA (50% oxidized) and ChitHCl were ETO-sterilized using standard protocols and solutions of appropriate concentrations were prepared in sterile media (PBS or water). Under general anaesthesia (Ketamine and thiopentone sodium, controlled on dorsal recumbence), the ventral abdomen of the animals was draped for aseptic surgery. Liver was assessed by a right paracostal incision of nearly 4 to 5 cm length. The following types of injuries were made on the liver.

1. Liver lobe: Liver lobe edge resection of approximately 1.5 cm length at two sites.
2. Liver lobe circular excision of approximately 1cm diameter at one site.

The site was cleaned free of blood. Test or control glue was applied and examined for hemostasis. The two component test glue was applied serially. First, 0.5 mL of DDA (10 wt %) was applied followed by 0.5 mL of ChitHCl (5 wt %) using separate syringes. Control BioGlue[®] was applied using the applicator provided by the manufacturer. The abdomen was closed as routine. Analgesics (Tidigesic) and antibiotic (Tetracycline) coverage was given. The animals were returned to their individual cages and observed daily for any adverse clinical symptoms. The animals were sacrificed at the end of 14 days and tissue response was studied on paraffin sections stained with hematoxylin and eosin after staining.

2.2.15. Statistical analysis

Statistical analysis of the data was performed by one-way analysis of variance, assuming a confidence level of 95% ($p < 0.05$) for statistical significance. All data are expressed as mean \pm standard deviation (SD).

3. Results and discussion

3.1. Preparation of ChitHCl-DDA hydrogel

Though chitosan has mucoadhesive property, hemostatic and anti-infective activity with low toxicity and significant biodegradability [38], its rigid crystalline structure, make it insoluble in aqueous medium which hinders its utility in various applications. Grafting with PEG [39], conjugation with isopropyl-s-acetylthioacetimidate [40] and acrylic acid [41] are few methods adopted to make chitosan soluble in aqueous medium. However, chitosan salts can be easily obtained by the direct action of inorganic acids on chitosan dispersed in an organic medium. These solid chitosan salts or complexes are soluble in water, offer advantages of convenience, ease of control and simplicity in handling. Dextran has been used clinically to prevent vascular occlusion, as a plasma expander, and for anticoagulation [42]. Dialdehyde derivatives of dextran were prepared by periodate oxidation, where the cleavage of the vicinal glycols occurred leading to the formation of dialdehydes. The extent of periodate oxidation was followed by iodometric titration of the periodate present in the reaction mixture (Table 1). Degree of oxidation was found to increase with increase in the periodate equivalents. DDAs of 5.16 ± 0.2 , 50.14 ± 0.6 and 90.4 ± 0.43 % degree of oxidation were prepared and are designated respectively, as DDA5, DDA50 and DDA90. The yield of the products obtained was almost the same irrespective of the degree of oxidation and ranged between 80 to 90%. The pH of a 10% solution of the ChitHCl in water was found to be 6. The viscosity of the DDAs at all concentrations irrespective of the degree of oxidation was in the range 3.6-4.2 cps in PBS at a speed of 50 rpm. Viscosity of chitosan solutions measured under the same conditions is shown in Table 2. The viscosities of solutions up to 50 cps are believed to be suitable for application using a syringe needle of 20- 22 gauge [43]. Therefore, concentration of ChitHCl up to 10% was used for all further evaluation.

Hydrogels were prepared by mixing equal volumes of aqueous solution of ChitHCl with DDA in PBS. Cross-linking was predominantly due to Schiff's base formation by the reaction between the amino groups of chitosan and the available aldehyde of DDA (Figure 2). Gelation was instantaneous and the gelling time obtained for all gels were within 4 secs irrespective of the degree of oxidation of DDA and concentration of ChitHCl and DDA (Table 3). Gels prepared by Schiff's linkages are known to undergo hydrolysis under acidic pH. However, gels formed from slightly acidic (pH=6) ChitHCl were found to be stable. This could be due to the buffering action of PBS used for preparing DDA solution. To further confirm this, we prepared gels out of chitosan dissolved in 1% HCl and DDA dissolved in aqueous medium. The gelation was instantaneous but the gel was not stable and slowly underwent dissolution. In a study by Mo *et al* [44], a glue based on chitosan solution in acetic acid (3%) and DDA (10%) having 70% dialdehyde content formed rapidly within 4 s, but demonstrated low adhesive strength. Gels prepared using DDA and ChitHCl upon keeping at room temperature demonstrated color change initially from colorless to yellowish at 24 h and brownish at 2 weeks (Figure 1d), which is a commonly observed phenomenon in case of gels formed via Schiff's base reaction due to the generation of C=N chromophore. Since the chitosan solution has been observed to undergo gelation at high concentrations, we assume that physical gelation also contributed to the fast gelation of the system. It has been reported that partially oxidized dextran and N-carboxyethyl chitosan undergo gelation at physiological temperature and pH within 30 s to 10 min [45]. However, in the case of DDA and ChitHCl system, gelation occurred instantaneously within 4 s for all polymer concentrations leading to the formation of a stable gel, indicative of more kinetically and thermodynamically favored conformation of polymers to undergo the Schiff's reaction and physical gelation.

3.2. Characterization of DDA-ChitHCl hydrogel

3.2.1. Rheological Studies

Since the gelling time for all the concentrations of DDA and ChitHCl was found to be within 4-6 s, all the further evaluations were done on gels prepared using 5 wt% ChitHCl and 10 wt% DDA50 solutions. The strain sweep rheology analysis of gels prepared by using 10 wt% solution of DDA50 and 5 wt% solution of ChitHCl were done in order to find the strain region at which they show linear viscoelastic behavior. It was seen that the modulus was independent of the strain up to 10% (Figure 3a). Therefore all the further analyses were performed at 1% strain. The time sweep rheology analysis was performed by mixing 0.5 mL each of DDA50 (10 wt%) and ChitHCl (5%) on the lower plate of the rheometer. Since the gelation was too rapid, it was difficult to record the gelling time at the crossing over point of the storage and loss modulus of the gel. However, it was seen that the storage modulus (G') of the gel was greater than the loss modulus (G'') at a given time (date not shown). The damping factor of gel was found to be less than 1 leading to a phase angle value $<45^\circ$ clearly demonstrating the elastic nature of the gels (Figure 3b). After 800 s, upon raising the upper plate of the rheometer, the gel was found to be stretched between two plates of the rheometer without undergoing breakage (Figure 3c) demonstrating the adhesive nature of lightly cross-linked polymer when comes into contact with the surface of rheometer plate. Similarly, cohesive nature was also observed between two instantaneously formed hydrogels in which one hydrogel was prepared in the presence of basic fuchsin dye for distinct identification (Figure 3c). For a polymeric adhesive to function properly, it should have appropriate liquid-like nature initially while applying to achieve good molecular contact and solid-like features so as to resist an applied stress once the bond is formed [46], which is well satisfied in the case of DDA-ChitHCl system. In order to see the frequency

dependence of storage modulus of DDA-ChitHCl gel after formation, change in moduli by varying the frequency from 1 to 100 rad/s was monitored. It was seen that storage modulus (G') was greater than loss modulus (G'') (Figure 3d) and both values reached plateau. This could be due to the rearrangement of the polymers in gel and sol phase in such a way that physical entanglements were created and broken quickly without making a change in moduli. (Figure 3d). Time sweep analysis of preformed gel was done at strain 1% and frequency 1 rad/s. It can be seen that the storage modulus remained unchanged with time, giving a plateau value of 865 ± 5 Pa (Figure 3e).

3.2.2. Swelling Studies

The rate of swelling of the gels prepared from a 5 wt% solution of ChitHCl in water and 10 wt% solution of DDA of different degrees of oxidation (5, 50 and 90%) in PBS is given in Figure 4a. After preparation, all the gels had about 90% water content which upon continued equilibration in PBS slightly decreased, but the decrease was statistically insignificant ($p > 0.05$). Slightly reduced fluid content can be due to the deprotonation of some of amino groups of chitosan at pH 7.4. This is interesting from the point of view of the intended application as continued swelling in the presence of body fluids such as blood is not desirable for the application of the material as a surgical adhesive. Statistical analysis (ANOVA) showed that there was no significant difference ($p > 0.05$) between swelling percentage of gels prepared with different degrees of oxidization of dextran. However, gels prepared using DDA 5 has comparatively less percentage swelling. DDA5 has only 5% di-aldehyde content and the gels formed out of it were weak and less stable compared to the gels prepared using DDA with higher oxidations. Weakly cross-linked gels swell more leading to an increase in swollen weight. However, in the case of gels prepared using DDA5, number of uncross linked polymer chains is

more, due to the less number of available aldehyde groups for Schiff's base formation with chitosan chains. Owing to the dissolution of uncross-linked polymer chains to the surrounding medium upon incubation in PBS, net effect is a decrease in swollen gel weight at time t . Since, $W(0)$ is almost same in all gels, only variable is $W(t)$. $W(t)$ of DDA5 gels was found to be lesser than other gels, resulting in high value of $W(0)/W(t)$ and lesser values of swelling (%). Cross-linking density (v_e , mol/cm³) of the hydrogels was subsequently calculated from the Flory-Rehner equation [47]. Cross-linking densities of gels prepared by using DDA50 (10 wt% solution in PBS) with 5 and 10 wt% solution of ChitHCl were estimated to be 68.0 ± 12.3 and 218.1 ± 9.3 $\mu\text{mol}/\text{cm}^3$ respectively. When the concentration of chitosan was increased, cross-linking density also increased since the number of amino functions that entered into Schiff's reaction with the aldehyde functions increased with increase in concentration of chitosan as expected.

3.2.3. *In vitro degradation*

The degradability of the gels was studied by noting the weight change upon incubation in PBS at body conditions. It has been observed that the gel started degrading with a 28 % weight loss observed within 7 days (Figure 4b). The weight of gel remaining after 6 weeks was only 43.10 ± 1.6 % demonstrating the biodegradability of the gel, which is one of the prerequisite of an ideal tissue adhesive. The time required for the gels to completely degrade can be predicted by extrapolating the graph, (weight remaining (%) vs time) to $y = 0$ and it is found to be 73 days (roughly 10 weeks and 3 days).

3.2.4. *Morphology and Cytotoxicity*

The surface morphology and internal structure of the representative lyophilized gel prepared from 5 wt% ChitHCl and 10 wt% DDA50 is shown in Figures 5 a & b. It was

interesting to note that the gel has a highly porous structure with an average pore size of $280 \pm 80 \mu\text{m}$. Qualitative cytotoxicity evaluation of the DDA-ChitHCl gel was carried out by direct contact assay using L₉₂₉ mouse fibroblast cell line as per ISO 10993 part 5. L929 mouse fibroblast NCTC clone 929 strain L is one of the ISO endorsed cell lines for the in vitro cytotoxicity evaluation. L₉₂₉ mouse fibroblasts cells maintained normal spindle shape indicating the cytocompatibility of gels (Figure 5c). Quantitative assessment of cytotoxicity was done by MTT assay of cells after contact with material extract. From the absorbance noted, it was found that cells after contact with the material extract showed $95.8 \pm 8.06 \%$ metabolically active cells compared to cells without the material extract for 24 h of contact. ChitHCl (5 wt%) and DDA (10 wt%) demonstrated 97.6 ± 7.12 and $102.3 \pm 5.9 \%$ metabolically active cells after 24 h (Figure 5d).

3.2.5. Blood compatibility

The hemolysis assay revealed that the DDA-ChitHCl gel and its components were non-hemolytic in nature. Table 4 shows the percentage hemolysis of blood in contact with different samples at 37 °C for 1 h. The extent of hemolysis was found to be lower than the permissible level of 5%. PTT is a standard test for analyzing the material's effect on intrinsic coagulation pathway. The PTT for the blood without any material has been found to be 26 ± 2.08 . It can be seen that PTT for fibrin glue (Reliseal®) and DDA-ChitHCl gel is 29.3 ± 2.3 and 35.6 ± 1.2 s, respectively. The normal reference laboratory value of PTT is in the range 30-40 s, showing that PTT value obtained for the DDA-ChitHCl is in acceptable range.

3.3. Evaluation of DDA-ChitHCl hydrogel as a tissue adhesive

3.3.1 Bonding strength measurements

The bonding strength of the two-component glue to tissue was determined by using excised rat skin. Experiments were done with 10 wt% solution of DDA in PBS and 5 wt% solution of ChitHCl in aqueous medium. Bonding strength was studied by varying the setting time and employing DDA having different degrees of oxidation. *In situ* gelling DDA-ChitHCl hydrogel system demonstrated superior adhesive property with bonding strength in the range of 200-400 gf/cm² for different setting times and concentrations employed (Figure 6a). The commercially available fibrin glue demonstrated negligible bonding strength on excised rat skin in similar experimental set up. However, it has been reported that the bonding strength of commercially available Fibrin glue (Bolheal[®]) after a setting time of 5 and 30 min was 45± 10 and 50 ± 5 gf/cm² on mouse skin and its bonding strength to rabbit skin was 93.4 ± 14.3gf/cm² after 10 min of setting time [48, 49]. It can thus be seen that the bonding strength of the present glue is at least 4-5 times greater than fibrin glue at comparable setting times. In fact, the glue sets in very rapidly as compared to fibrin glue which was very evident in the *in vivo* experiments (discussed below) and there was hardly any difference in the bonding strength at setting times of 5 and 10 min. We have recently reported that adhesive nature of *in situ* gelling hydrogel based on alginate dialdehyde and gelatin can be due to possible Schiff's reaction between the unreacted aldehyde groups of alginate dialdehyde and amino groups of collagen present in the cartilage tissue [50]. The superior adhesive property of DDA-ChitHCl could also be attributed to the Schiff's reaction between DDA and amino groups of collagen of the host tissue [50]. Chitosan being cationic in nature can interact with negatively charged collagen present in the skin tissue [51]. This could also contribute to the adhesive nature of these gels. Bonding strengths obtained on further increasing the setting time to 20 and 30 min (data not shown) were found to be not significantly different ($p>0.05$) for all compositions. Since the bonding strength obtained is

independent of the setting time ($t \geq 5$ min), it can be inferred that the interaction of the polymer chains with the adjacent tissues occurs within 5 min, sufficient for giving a high bonding strength.

3.3.2. Burst strength measurements

The strength and adhesive properties of the gels were also assessed by the burst test on excised rat skin (Figure 6b). Burst strengths on 2 mm diameter punch defects in rat skin for DDA-ChitHCl system showed no significant difference ($p > 0.05$) while using DDAs of different degrees of oxidization. Gels showed burst strength of 400-410 mm Hg. Burst test results on rat skin *in vitro* implied that this sealant formulation will be effective in preventing leakage of blood vessels. It was seen that the sealant was able to withstand significant pressure, higher than hypertension seen (140-159 mm of Hg) [52] in humans and therefore should be suitable as sealant for controlling the bleeding in many surgical situations. These results point to the possibility of realizing good tissue adhesion *in vivo*. The burst test also gave an indication of the cohesive and adhesive strength of the gel.

3.3.3. Evaluation as a drug delivery vehicle

For examining the potential of the system as an injectable drug delivery vehicle, 5-FU and FITC-albumin were incorporated in the system and their release profiles were examined. The cumulative release of 5-FU from gels prepared using DDAs of different degrees of oxidation are shown in Figure 7a. Release after 1 h was fast for gels prepared using DDA5 and DDA50 compared to that from DDA90. The cumulative drug release from DDA50 and DDA5 for first 1 h was $47.7 \pm 6 \%$ and $40.7 \pm 3 \%$, respectively and was found to be not statistically significant ($p > 0.05$). This could be due to weak structure of gel prepared with DDA having less number of aldehyde groups. However, about $92 \pm 2 \%$ drug release was observed from all the gels within 10

h (Figure 7a). We envisage that adhesive gels of the type described here can be effectively used with anti-cancer drugs for chemo-embolization of inoperable carcinomas. The release of FITC-albumin from gels prepared from DDA5, DDA50 and DDA90 is shown in Figure 7b. Release was slow from all the gels and lasted over many days. After 39 days, only about 25 % albumin was found to be released from gels prepared from DDA50 and DDA90, while about 40 % release was observed from gels prepared using DDA5 which is significantly different ($p < 0.05$). The release from DDA50 and DDA90 gels were slowed down due to the highly cross-linked nature of matrix as well as better protein conjugation due to the availability of more aldehyde functions. The release reached almost an asymptotic phase at the end of 40 days and possibly more will be released when the material undergoes further biodegradation. This was not pursued further. However, the preliminary results showed that the system will be suited for controlled release of therapeutic peptides and proteins.

3.3.4. Evaluation of DDA-ChitHCl as a hemostat

The DDA-ChitHCl adhesive system was evaluated for its performance by examining its hemostatic effect on rabbit liver injury and safety by studying tissue response at 14 days. Figure 8 (a) and (b) show the creation of circular excision on rabbit liver and the bleeding from the excised site. Upon application of the DDA-ChitHCl glue (Figure 8c) on the resected site, the wound was immediately covered by the gel formed. The hemostasis on liver edge resection injury could be observed with glistening appearance of glue on the injury site (Figure 8d). Hemostasis of the DDA-ChitHCl gel was comparable with that obtained after BioGlue[®] application on the injury site. Figure 8 (e, f) shows the test glue and BioGlue[®] respectively at 14 days. The adhesion of liver into abdominal wall in both control and test glue was seen in all animals.

Histologically, at the end of two weeks, in the case of control glue (BioGlue[®]), a thick layer of glue was seen as pink homogenous material (Figure 9a. arrow). Necrosis (exploding star) was noticed directly beneath the glue surrounded by inflammation (pentacle) consisting of macrophages, lymphocytes and eosinophils (Figure 9b). Giant cells were noticed near the glue. Subcapsular inflammation and fibrosis were also noticed. In the case of DDA-ChitHCl glue, a thin layer of glue was seen as pink homogenous material (Figure 9c, arrow). Adjacent to glue the area of necrosis (star), inflammation and fibrosis noticed were less compared to control. Some giant cells and macrophages were also noticed (Figure 9d). Capsular thickening and fibrosis were seen beneath the capsule affecting superficial parenchyma. Localized suppurative necrosis near glue was also seen.

In summary, the test and control glue could effectively control the hemostasis of liver injury. The tissue response elicited by test and control glue was comparable. It has been reported that glutaraldehyde used in BioGlue[®] can lead to various toxicities as well as vascular strictures at the site of application and embolization of adhesive material from the site of application to distant sites such as arm or leg [53-55]. Since DDA-ChitHCl gel system is solely based on biopolymers having hemostatic activity, without any toxic cross-linking agents, we envisage that this adhesive could be a potential substitute for commercially available tissue adhesives.

4. Conclusions

The development of a surgical adhesive that stops bleeding and bond the tissues well, which at the same time is simple, inexpensive, non-toxic, biocompatible, biodegradable, economically viable, and appealing to the surgeon in terms of the simplicity of application in complex surgical situations, has posed a major challenge. In this report, the DDA-ChitHCl system was shown to fulfil this objective to a significant extent. In addition to its tissue sealing

properties, and as a hemostat, the system can also act as vehicle for delivery of drugs and therapeutic peptides and proteins. The glue was able to seal bleeding in the rabbit liver injury model and the tissue response elicited at 14 days was comparable to that of commercially available glue such as BioGlue®. The adhesive developed could function without the many toxicity and biocompatibility issues associated with such products.

Acknowledgement:

Financial support from Indo-French Centre of Advanced Research (IFCPAR PROJECT No. 2603-2), New Delhi is gratefully acknowledged.

Disclosure

Authors declare no conflict of interest.

References

- [1].Quinn, J.V. editor. Tissue Adhesives in Clinical Medicine, BC Decker Inc; Hamilton: (2005) p. 1 4.
- [2].Wheat J. C., Wolf J. S. Jr. Advances in bioadhesives, tissue sealants, and hemostatic agents. Urol. Clin. North. Am. 36 (2009) 265.
- [3].Fernández-Gutiérrez M., Rodríguez-Mancheño M., Pérez-Köhler B., Pascual G., Bellón J.M. Román J. S. Structural Analysis and Application of n-Alkyl Cyanoacrylate Surgical Adhesives to the Fixation of Meshes for Hernia Repair. Macromol Biosci. 2016 (in press)
- [4]. Bochyńska A. I., Hannink G., Janssen D., Buma P., Grijpma D. W. Development of a fast curing tissue adhesive for meniscus tear repair. J Mater Sci Mater Med. 2017 (in press)
- [5]. Gilbert T.W., Badylak S. F., Beckman E. J., Clower D. M., Rubin J. P. Prevention of seroma formation with TissuGlu® surgical adhesive in a canine abdominoplasty model:

- Long term clinical and histologic studies. *J Plast Reconstr Aesthet Surg.* 66 (2013) 414–22.
- [6]. Toriumi D. M., Chung V. K., Cappelle Q. M. Surgical Adhesives in Facial Plastic Surgery. *Otolaryngol. Clin. North Am.* 49 (2016) 585-99.
- [7]. Farrar D. F. Bone adhesives for trauma surgery: A review of challenges and developments. *Int. J. Adhes. Adhes.* 33(2012)89-97.
- [8]. Sierra D. Fibrin sealant adhesive systems: A review of their chemistry, material properties and clinical applications. *J. Biomat. Appl.* 7 (1993) 309-352.
- [9]. Janmey P.A., Winer J.P., Weisel J.W. Fibrin gels and their clinical and bioengineering applications, *J. R. Soc. Interface.* 6 (2009) 1-10.
- [10]. Shivamurthy D. M., Singh S., Reddy S. Comparison of octyl-2-cyanoacrylate and conventional sutures in facial skin closure. *Natl. J. Maxillofac. Surg.* 1 (2010) 15–19.
- [11]. Matsumoto T. The chemistry of cyanoacrylate adhesives. In: Matsumoto T, ed. *Tissue adhesives in surgery*. Flushing, NY: Medical Examination Publishing Co., (1972)154-188.
- [12]. Sidentop. K. H. Tissue Adhesive Histoacryl® (2-cyanobutyl acrylate) in experimental middle ear surgery. *Am. J. Otol.* 2 (1980) 77-87.
- [13]. Mizrahi B., Stefanescu C. F., Yang C., Lawlor M. W., Ko D., Langer R., Kohane D. S. Elasticity and safety of alkoxyethyl cyanoacrylate tissue adhesives. *Acta Biomater.* 7 (2011) 3150-7.
- [14]. Nomori H., Horio H., Morinaga S., Suemasu K. Gelatin-resorcinol-formaldehyde-glutaraldehyde glue for sealing pulmonary air leaks during thoracoscopic operation. *Ann. Thorac. Surg.* 67 (1999) 212-6.

- [15]. Chao H. H., Torchiana D. F. BioGlue: Albumin/glutaraldehyde sealant in cardiac surgery. *J. Card. Surg.* 18 (2003) 500-3.
- [16]. Shaffer M.P., Belsito D.V. Allergic contact dermatitis from glutaraldehyde in health-care workers. *Contact Dermatit.* 43 (2000) 150–156.
- [17]. Otani Y., Tabata Y., Ikada Y. A new biological glue from gelatin and poly (L-glutamic acid). *J. Biomed. Mater. Res.* 31 (1996) 158-66.
- [18]. OldeDamink L. H., Dijkstra P. J., Van Luyn M. J., Van Wachem, P. B., Nieuwenhuis, P., Feijen, J. Crosslinking of dermal sheep collagen using a water soluble carbodiimide, *Biomaterials* 17 (1996) 765-773.
- [19]. Sung H. W., Huang, L. L. H., Tsai, C. C. In vitro evaluation of cytotoxicity of a naturally occurring crosslinking reagent for biological tissue fixation. *J. Biomater. Sci. Polymer Ed.* 10 (1999) 63-78.
- [20]. Messersmith, P. B., Lee, B. P., Dalsin, J. L. Synthesis and gelation of DOPA-modified poly(ethylene glycol) hydrogels. *Biomacromolecules* 3 (2002) 1038-47.
- [21]. Zhang H, Bré L. P., Zhao T., Zheng Y., Newland B., Wang W. Mussel-inspired hyperbranched poly(amino ester) polymer as strong wet tissue adhesive. *Biomaterials* 35 (2014) 711-9.
- [22]. Sung H. W., Huang D. M., Chang W. H., Huang R. N., Hsu, J. C. Evaluation of gelatin hydrogel crosslinked with various crosslinking agents as bioadhesives: In vitro study. *J. Biomed. Mater. Res.* 46 (1999) 520-530.
- [23]. Meng H., Liu Y., Lee B. P. Model polymer system for investigating the generation of hydrogen peroxide and its biological responses during the cross-linking of mussel adhesive moiety. *Acta Biomater.* 2016 Oct 12. (in press)

- [24]. Serban M. A., Panilaitis B., Kaplan D. L. Silk fibroin and polyethylene glycol-based biocompatible tissue adhesives. *J. Biomed. Mater. Res. A.* 98 (2011)567-75.
- [25]. Shafiq Z, Cui J., Pastor-Perez L., San Miguel V., Gropeanu' R. A., Serrano C., del Campo A. Bioinspired underwater bonding and debonding on demand. *Angew. Chem. Int. Ed.* 51 (2012)4332–4335.
- [26]. Artzi N., Shazly T., Crespo C., Ramos A. B., Chenault H. K., Edelman E. R. Characterization of Star Adhesive Sealants Based On PEG/Dextran Hydrogels. *Macromol. Biosci.* 9 (2009)754-65.
- [27]. Lih E., Lee J. S., Park K. M., Park K. D. Rapidly curable chitosan PEG hydrogels as tissue adhesives for hemostasis and wound healing. *Acta Biomater.* 8 (2012)3261-9.
- [28]. Mehdizadeh M., Yang J. Design strategies and applications of tissue bioadhesives. *Macromol. Biosci.* 13, (2013)271-88.
- [29]. Annabi N., Tamayol A., Shin S.R., Ghaemmaghami A. M., Peppas N. A., Khademhosseini A. Surgical Materials: Current Challenges and Nano-enabled Solutions. *Nano Today.* 9 (2014)574-589.
- [30]. Harris J. M. Poly(ethylene glycol) chemistry: biotechnical and biomedical applications. New York: Plenum Press; (1992). p. 385.
- [31]. Ryu J. H., Hong S, Lee H. Bio-inspired adhesive catechol-conjugated chitosan for biomedical applications: A mini review. *Acta Biomater.* 27(2015) 27:101-15.
- [32]. Annabi N., Yue K., Tamayol A., Khademhosseini A. Elastic sealants for surgical applications. *Eur J Pharm Biopharm.* 95 (2015) 27-39.

- [33]. Marchioni M., Ingrosso M., De Francesco P., Primiceri G., Manco R., Tenaglia R. L. The Use of Haemostatic Agents and Sealants for the Prevention of Lymphocele After Urological Surgery: A Review of the Literature. *Surg Technol Int.* 27 (2015) 45-50.
- [34]. Austin P. R, Sennett S. Dry chitosan salts and complexes of aliphatic carboxylic acids. In: Muzzarelli R., Jeuniaux C., Gooday G. W.(eds), *Chitin in Nature and Technology*, Plenum Press, New York, (1986); pp.279-286.
- [35]. Bassett J, Denny R. C., Jeffery G. H., Mendham J. *Vogel's Text book of quantitative Inorganic analysis*, 4th ed.; Longman: London, (1978) 370-375.
- [36]. O'Leary R. K., Guess W. L. Toxicological studies on certain medical grade plastics sterilized by ethylene oxide. *J. Pharm. Sci.* 58(1969)1007.
- [37]. Prior J. J., Wallace, D. G. Harner A., Powers N. A sprayable hemostat containing fibrillar collagen, bovine thrombin, and autologous plasma. *Ann. Thorac .Surg.* 68 (1999) 68:479.
- [38]. Ueno H., Mori T., Fujinaga T. Topical formulations and wound healing applications of chitosan. *Adv. Drug. Deliv. Rev.* 52 (2001) 105–15.
- [39]. Leithner, K., Bernkop-Schnürch, A. Chitosan and derivatives for biopharmaceutical use: Mucoadhesive properties. Chapter 10. In *Chitosan-based systems for biopharmaceuticals. Delivery, targeting and polymers therapeutics*. Sarmento, B. ; DasNeves, J. Eds. ; Wiley: Chichester. (2012) 159-180.
- [40]. Krauland A. H., Hoffer M. H., Bernkop-Schnürch A. Viscoelastic properties of a new in situ gelling thiolated chitosan conjugate. *Drug. Dev. Ind. Pharm.* 31 (2005) 885–893
- [41]. Weng L., Romanov A., Rooney J., Chen W. Non-cytotoxic, in situ gelable hydrogels composed of N-carboxyethyl chitosan and oxidized dextran. *Biomaterials* 29 (2008) 3905–3913.

- [42]. Mehvar R. Dextran for targeted and sustained delivery of therapeutic and imaging agents. *J. Control. Release* 69 (2000) 1–25.
- [43]. Miller M. A., Engstrom J.D., Ludher B. S., Johnston K. P. Low viscosity highly concentrated injectable non aqueous suspensions of lysozyme microparticles. *Langmuir* 26 (2010)1067–1074.
- [44]. Mo X., Iwata H., Ikada Y. A tissue adhesives evaluated in vitro and in vivo analysis. *J Biomed. Mater. Res. A* 94 (2010) 326-32.
- [45]. Weng L., Chen X., Chen W. Rheological characterization of in situ cross-linkable hydrogels formulated from oxidized dextran and N-carboxyethyl chitosan. *Biomacromolecules* 18 (2007) 1109-1115.
- [46]. Grillet A. M., Wyatt N. B., Gloe L.M. Polymer gel rheology and adhesion, Chapter 3, In *Rheology*. Vicente J. D. (Ed.), InTech, (2012) 59-80.
- [47]. Balakrishnan B., Jayakrishnan A. Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. *Biomaterials* 26 (2005) 3941-3951.
- [48]. Otani Y., Tabata Y., Ikada Y. A new biological glue from gelatin and poly(L-glutamic acid). *J. Biomed. Mater. Res.* 31 (1996) 157-166.
- [49]. Otani Y, Tabata Y, Ikada Y. Rapidly curable biological glue composed of gelatin and poly(L-glutamic acid). *Biomaterials*. 17 (1996) 1387-91.
- [50]. Balakrishnan B., Joshi N., Jayakrishnan A., Banerjee R. Self cross-linked oxidized alginate/gelatin hydrogel as injectable, adhesive bio-mimetic scaffolds for cartilage regeneration. *Acta Biomater* 10 (2014) 3650-3663.
- [51]. Sionkowska A., Wisniewski M., Skopinska J., Kennedy C. J., Wess T. J. Molecular interactions in collagen and chitosan blends. *Biomaterials* 25 (2004) 795-801.

[52]. URL:

http://www.heart.org/HEARTORG/Conditions/HighBloodPressure/AboutHighBloodPressure/Understanding-Blood-Pressure-Readings_UCM_301764_Article.jsp# (accessed on December 9, 2016)

[53]. Fürst W, Banerjee A. Release of glutaraldehyde from an albumin-glutaraldehyde tissue adhesive causes significant in vitro and in vivo toxicity. *Ann. Thorac. Surg.* 79 (2005) 1522–8.

[54]. LeMaire S. A., Schmittling Z. C., Coselli J. S., Undar A., Deady B. A., Clubb F. J., et al. BioGlue surgical adhesive impairs aortic growth and causes anastomotic strictures. *Ann. Thorac. Surg.* 73 (2002) 1500.

[55]. URL: http://www.fda.gov/ohrms/dockets/ac/01/briefing/3790b2_01_cryolife.pdf (accessed on August 2, 2016)

Figure Legends:

Figure 1: Experimental set up for bonding strength (a) and burst strength measurement (b); gelation of the two-component glue tested in an Actalyke ACT tester (c); photographs of gel demonstrating color change from colorless to yellowish at 24 h and brownish at 2 weeks (d).

Figure 2: Schematic representation of periodate oxidation of dextran (a) and Schiff's base reaction between DDA and ChitHCl to form hydrogel (b).

Figure 3: Rheology analysis of DDA-ChitHCl hydrogel; strain sweep analysis (a), variation of damping factor and phase angle with time (b), adhesive and cohesive nature of gel (c) frequency sweep (d) and time sweep analysis of gel (e).

Figure 4: Variation in percentage swelling of hydrogels with time for hydrogels prepared using DDAs having different degree of oxidation (a), degradation profile of the DDA-ChitHCl hydrogel prepared using 10 wt% solution of DDA50 and 5 wt% solution of ChitHCl (b).

Figure 5: SEM images showing surface morphology (a) and internal structure (b) of the representative lyophilized gel prepared using ChitHCl (5 wt%) and DDA50(10 wt%); Optical microscope images of L929 mouse fibroblast cells exposed to gels (c) and variation in percentage of metabolically active cells upon exposure to the gel extract and its components (d).

Figure 6: Variation in bonding strength (a) and burst strength (b) of hydrogels with change in degree of oxidation of dextran

Figure 7: Variation in cumulative release (%) of 5FU (50 mg/gm of gel) and FITC albumin (50 mg/gm of gel) with time from hydrogels

Figure 8: Photographs demonstrating the creation of circular excision on rabbit liver (a), bleeding from the excised site (b), application of DDA-ChitHCl gel on the resected site (c), glistening appearance of DDA-ChitHCl glue on the injury site (d) and gross appearance of DDA-ChitHCl gel (e) and Bioglue® (f) after 14 days.

Figure 9: Histology H & E stained liver tissue sections treated with Bioglue® and DDA-ChitHCl gel after 14 days. A thick layer of Bioglue® seen as pink homogenous material (a, arrow); Necrosis (exploding star) as noticed directly beneath the Bioglue® surrounded by inflammation (pentacle) consisting of macrophages, lymphocytes and eosinophils (b); a thin layer of glue seen as pink homogenous material in the case

of DDA-ChitHCl gel, (c, arrow); giant cells and macrophages were noticed in the DDA-ChitHCl gel (d).

Table legends

Table 1: Details of periodate oxidation of dextran

Table 2: Viscosity of ChitHCl solution with change in its concentration.

Table 3: Variation in gelling time of DDA-ChitHCl hydrogel with change in degree of oxidation.

Table 4: Hemolytic potential of DDA-ChitHCl gel and its components

Table 1. Details of periodate oxidation of dextran

Sample	Periodate equivalents (%)	Degree of oxidation (%)	Yield (%)
DDA5	5.24	5.16 ± 0.2	85.49 ± 2
DDA50	50.19	50.14 ± 0.6	86.73 ± 2
DDA90	90.74	90.4 ± 0.43	89.63 ± 5

Table 2. Viscosity of ChitHCl solutions at different concentrations.

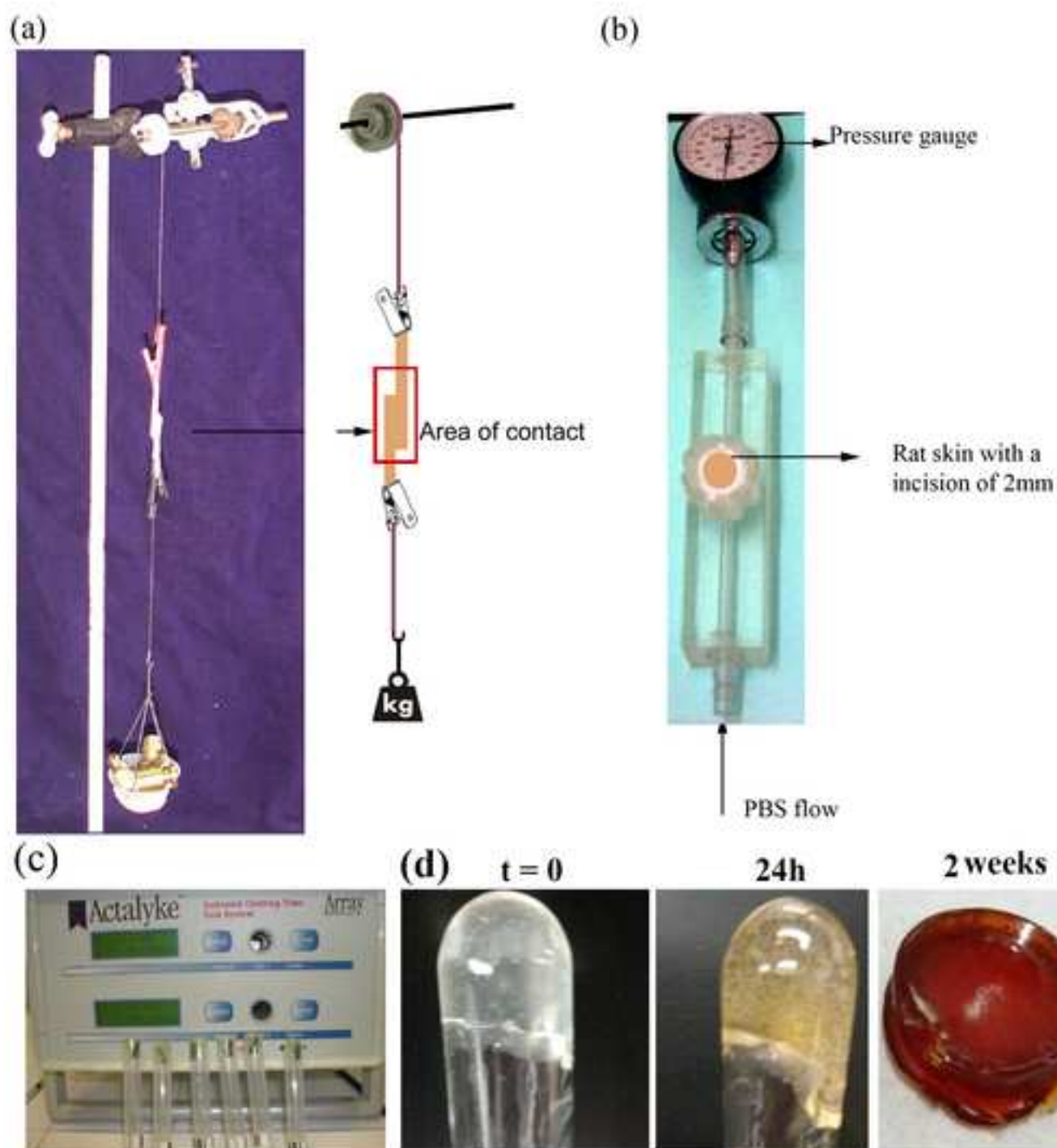
Concentration in water (%)	Viscosity (cP)
5	<1
10	45.2 ± 4
15	86.8 ± 2

Table 3. Variation in gelling time of DDA-ChitHCl hydrogel with change in degree of oxidation.

Sample	Concentration of DDA in PBS (wt%)	Concentration of ChitHCl in water (wt%)	Gelling Time at 37°C (sec)
DDA5	5	10	3 - 4
DDA50	10	10	3 - 4
DDA90	15	10	3 - 4
DDA5	5	5	3 - 6
DDA50	10	5	3 - 4
DDA90	15	5	3 - 6

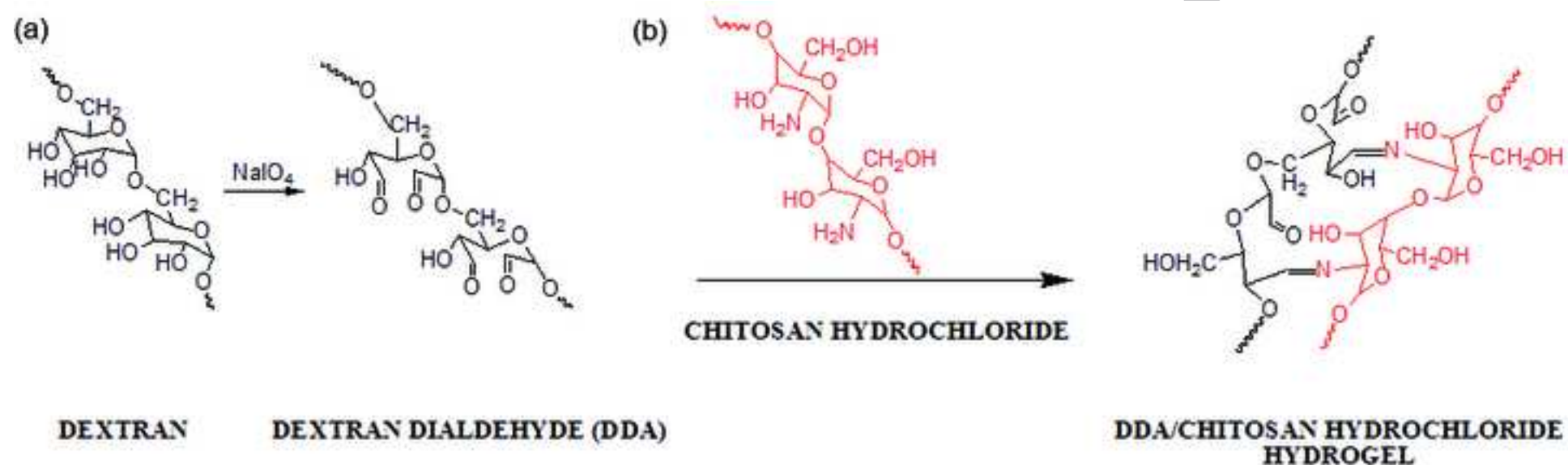
Table 4: Hemolytic potential of DDA-ChitHCl gel and its components

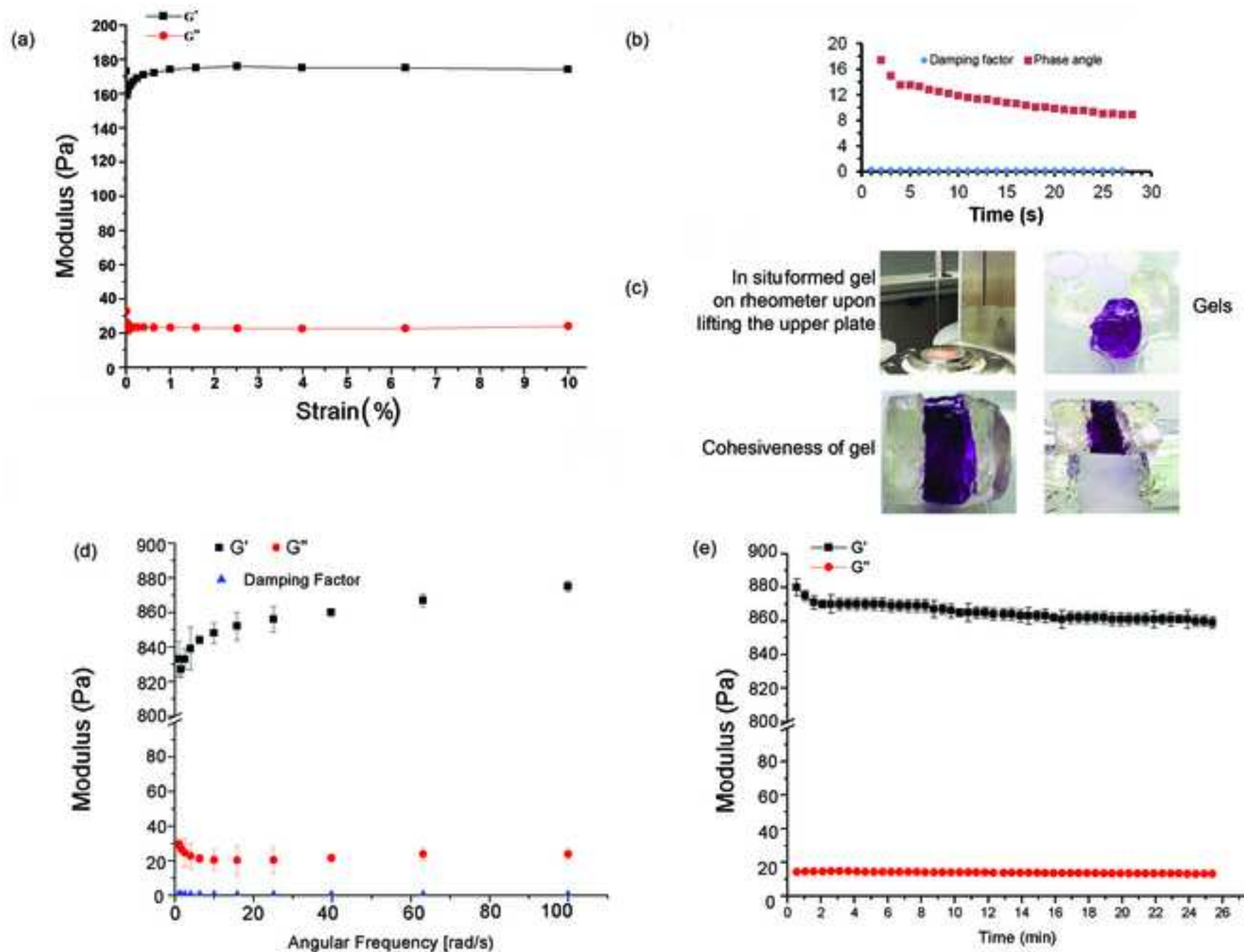
Samples	Hemolytic potential
PBS (negative control)	0
DDA-ChitHCl gel	1.55 ± 0.1
DDA	0.03 ± 0.04
ChitHCl	1.8 ± 0.4
0.1 wt % Sodium carbonate (Positive control)	100

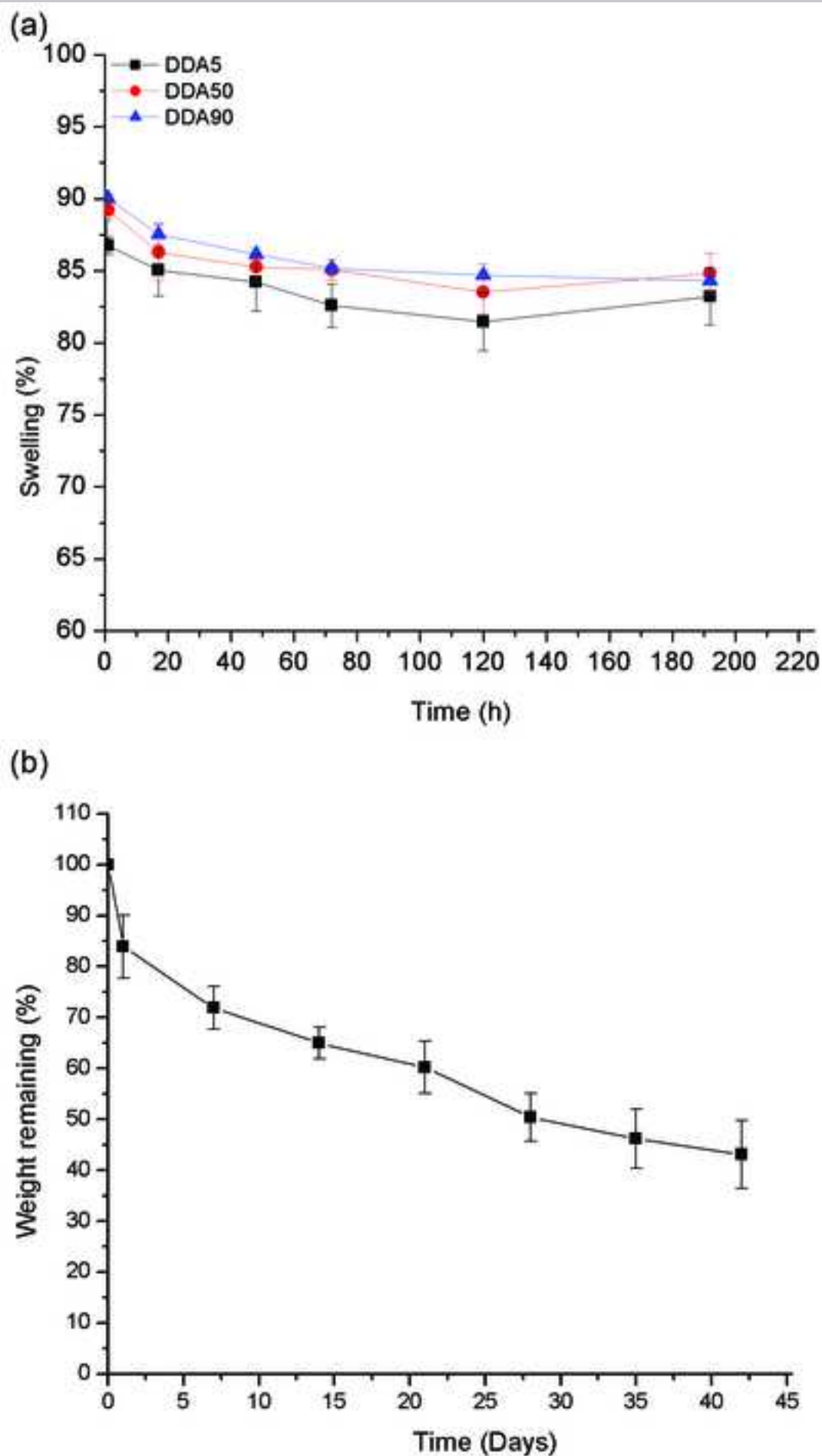


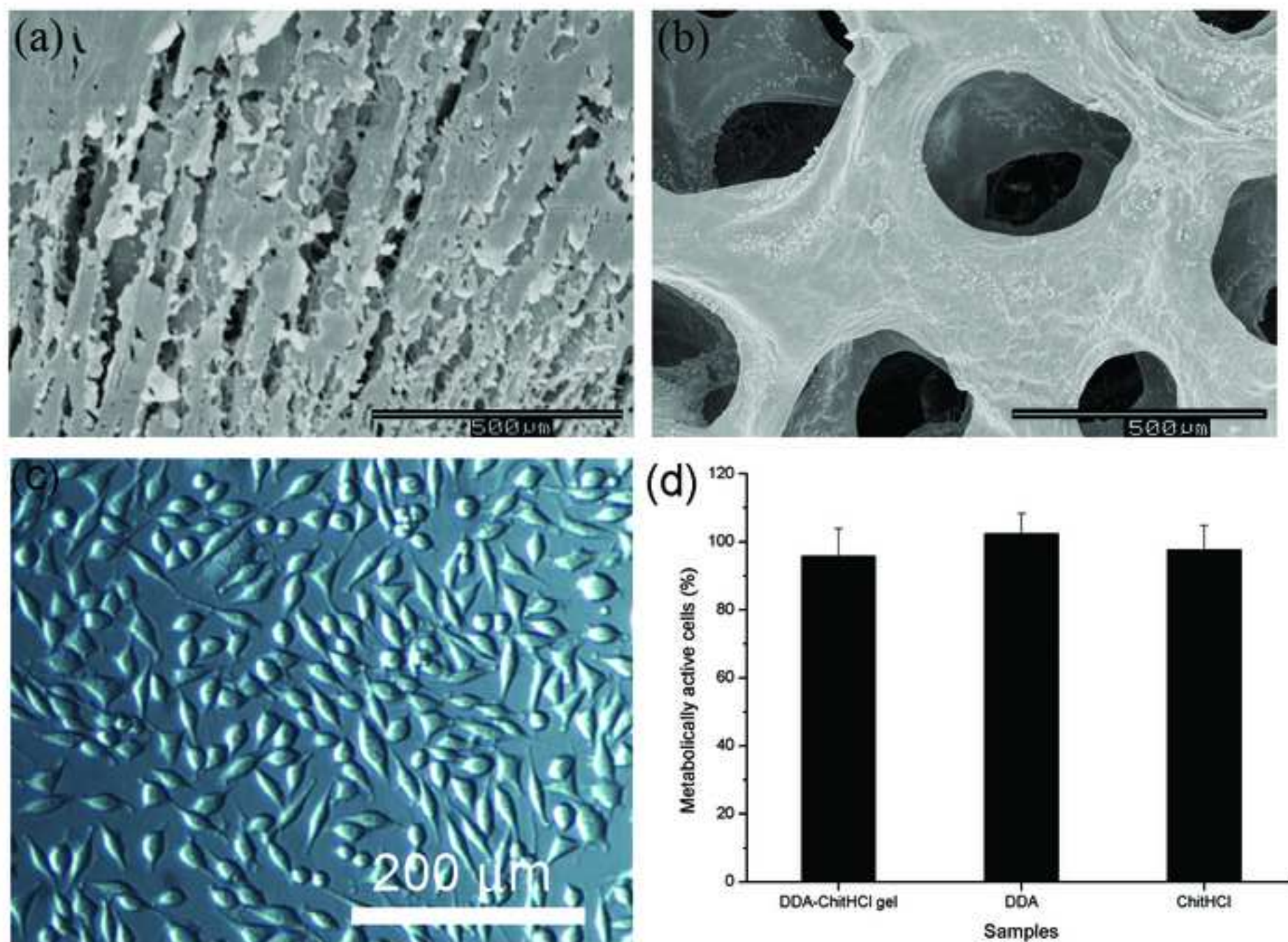
Comment citer ce document :

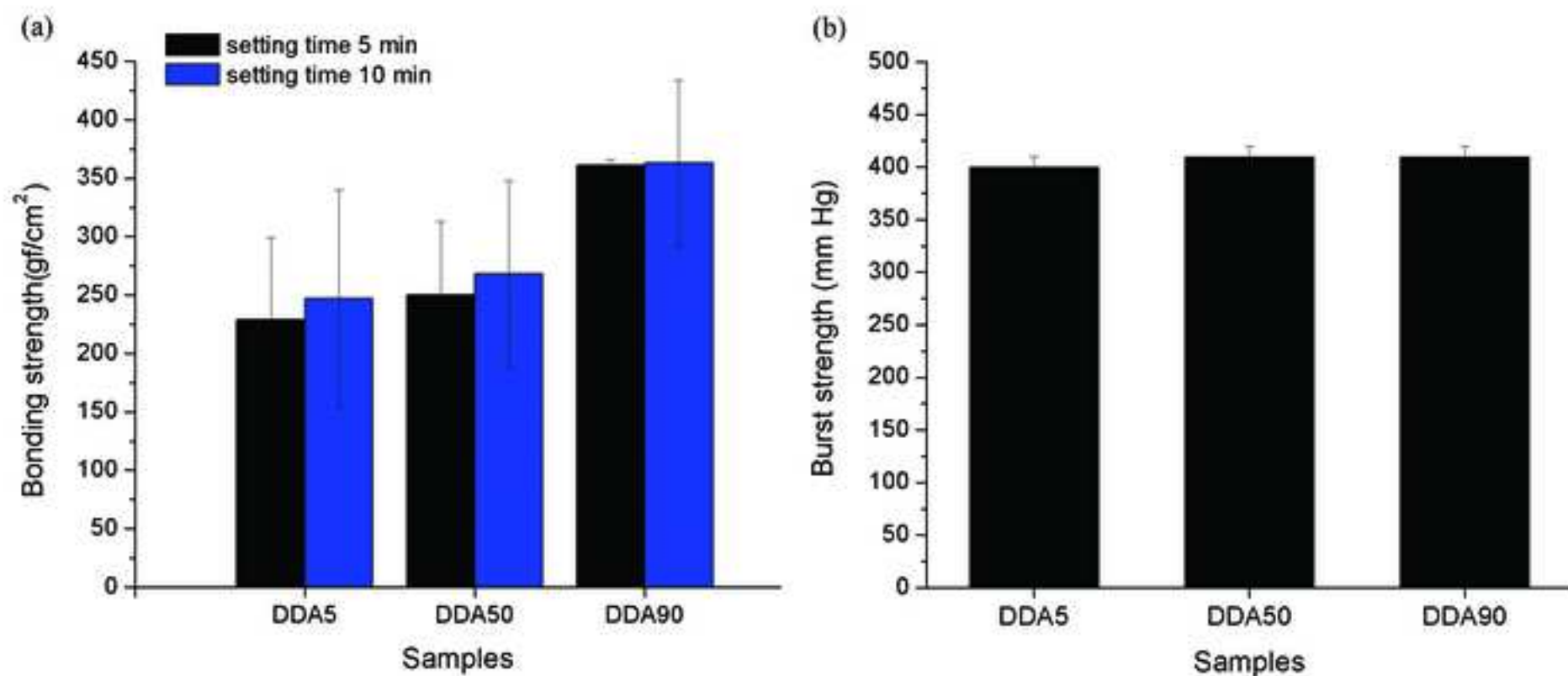
Balakrishnan, B., Soman, D., Payanam, U., Laurent, A., Labarre, D., Jayakrishnan, A. (Auteur de correspondance) (2017). A novel injectable tissue adhesive based on oxidized dextran and chitosan. *Acta Biomaterialia*, 53, 343 - 354. , DOI : 10.1016/j.actbio.2017.01.065

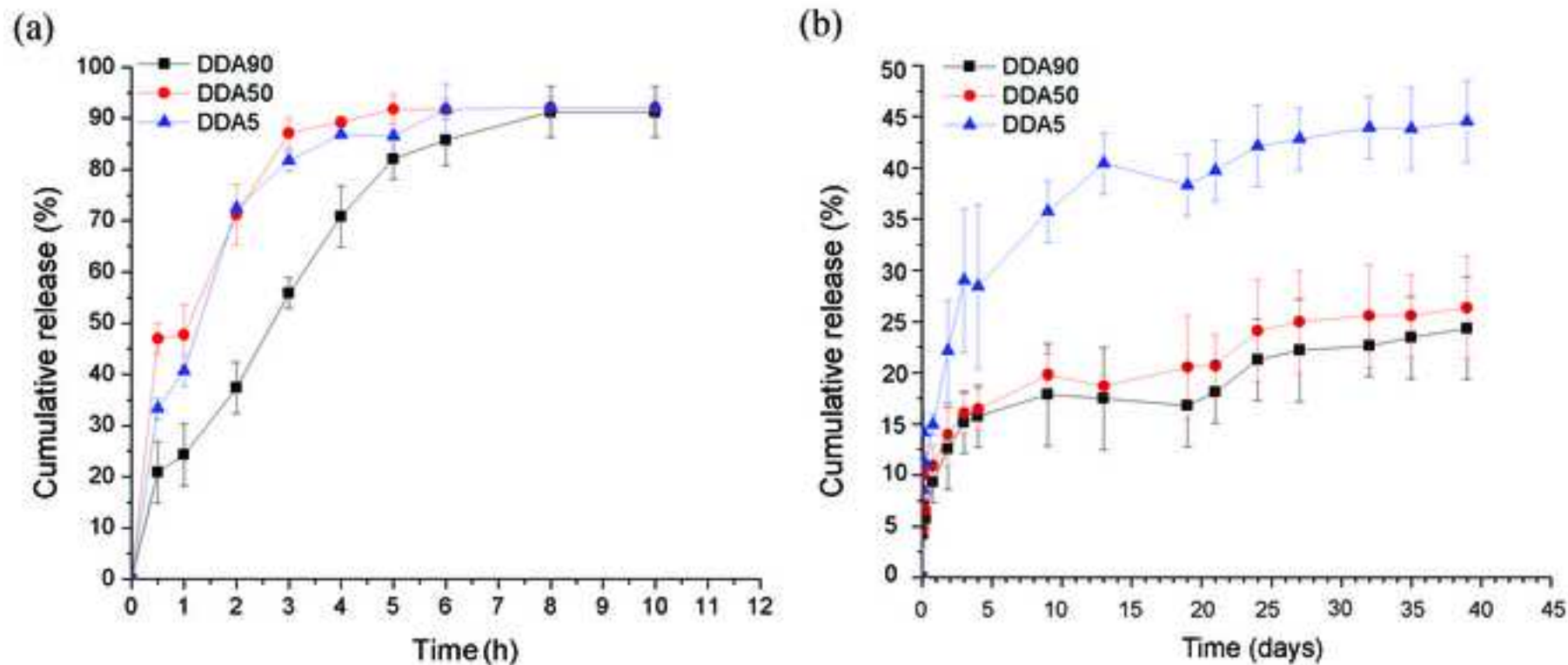


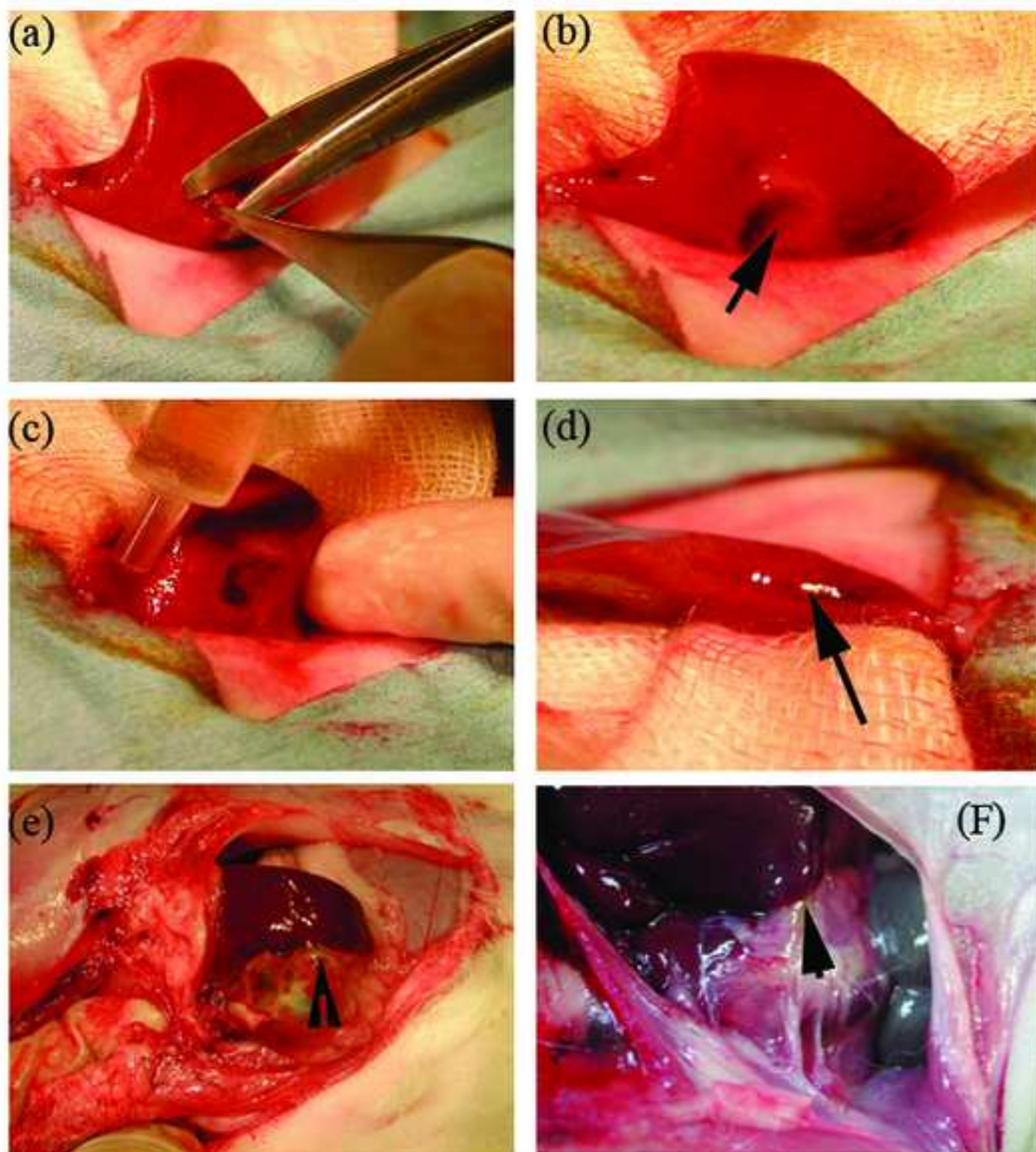






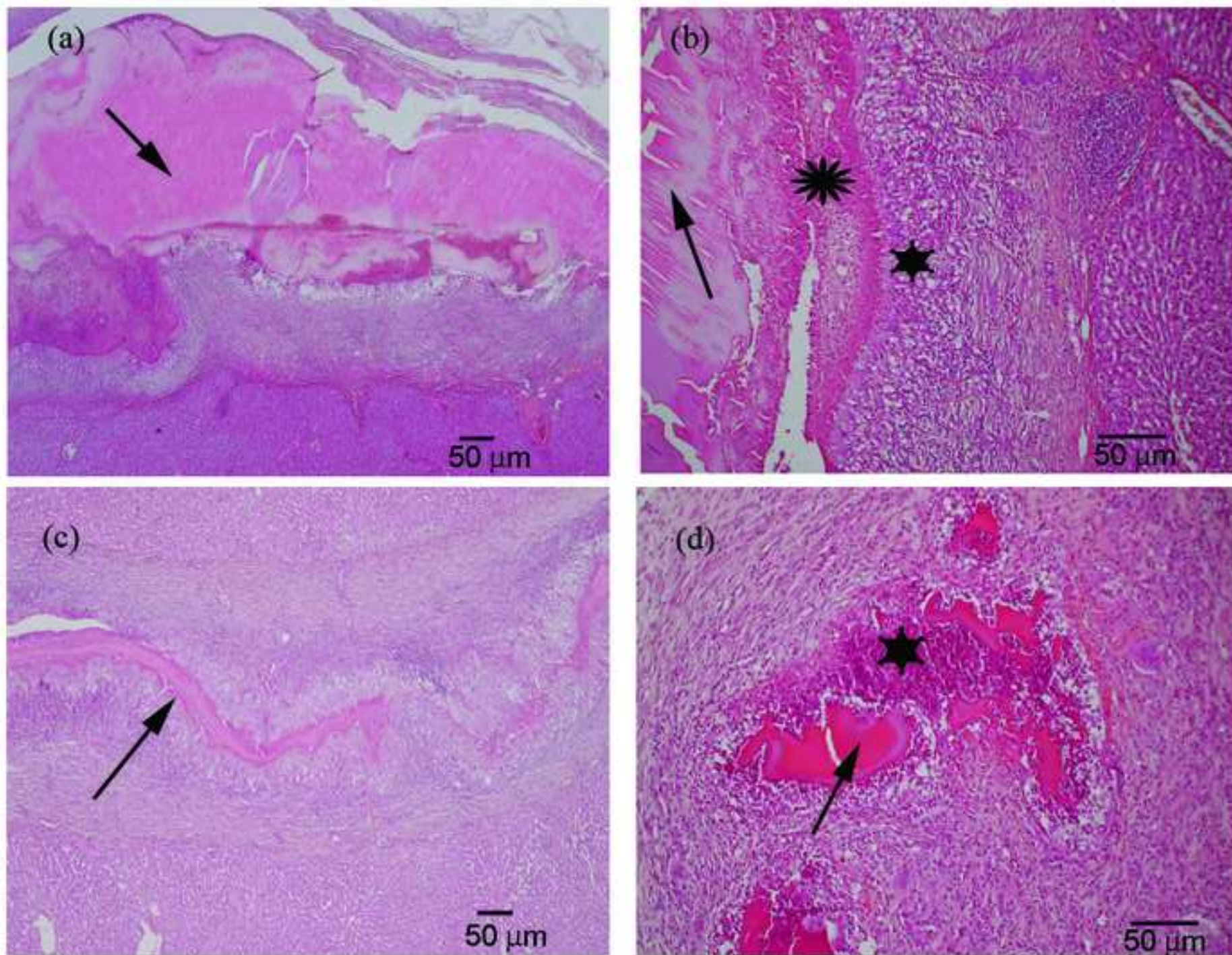


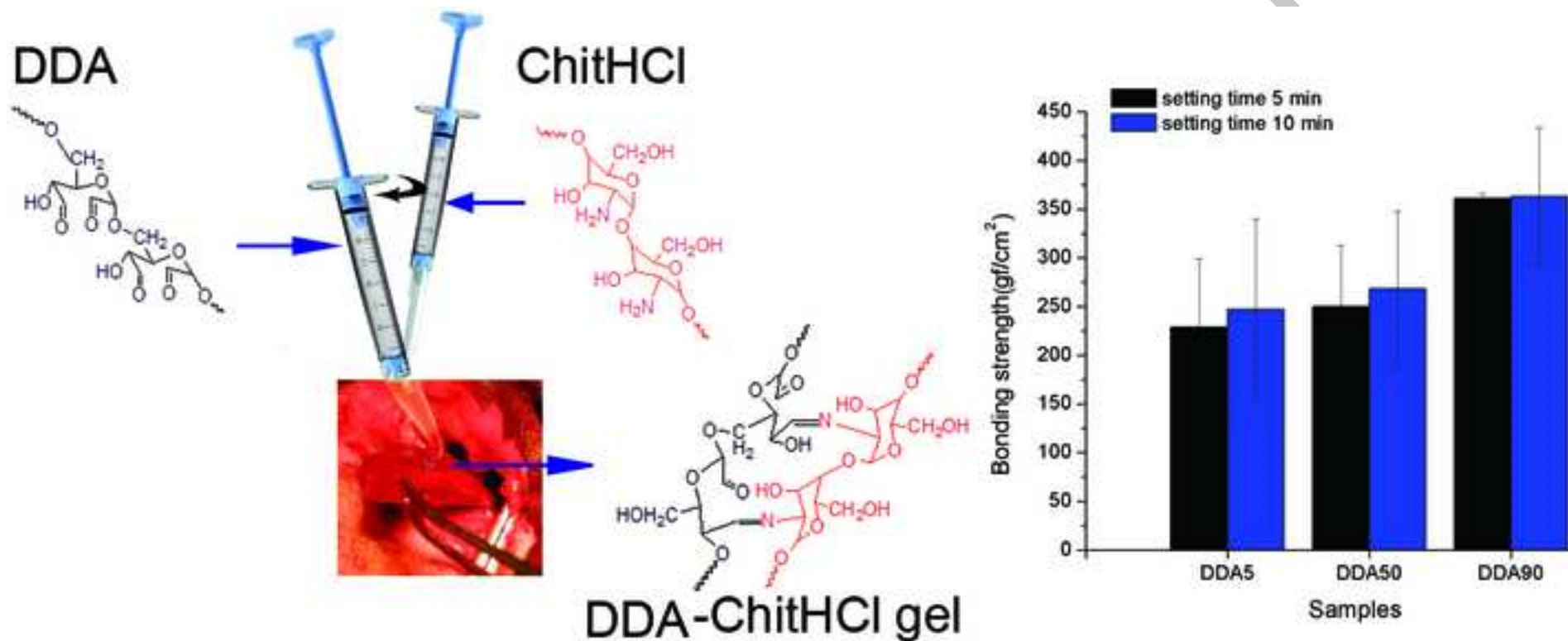




Comment citer ce document :

Balakrishnan, B., Soman, D., Payanam, U., Laurent, A., Labarre, D., Jayakrishnan, A. (Auteur de correspondance) (2017). A novel injectable tissue adhesive based on oxidized dextran and chitosan. *Acta Biomaterialia*, 53, 343 - 354. , DOI : 10.1016/j.actbio.2017.01.065





Statement of significance

Though there are many tissue adhesives available in market, none are free of shortcomings. The newly developed surgical adhesive is a 2-component adhesive system based on time-tested, naturally occurring polysaccharides such as chitosan and dextran which are both biocompatible and biodegradable. Simple polymer modification has been carried out on both polysaccharides so that when aqueous solutions of both are mixed, the solutions gel in less than 10 seconds and forms an adhesive that seals a variety of incisions. The strength of the adhesive is over 5-times the strength of commercially available Fibrin glue and is more tissue compliant than Bioglue®. This adhesive biomaterial showed excellent tissue bonding, was hemostatic, biocompatible and biodegradable. The significance of this work lies on the features of the developed tissue adhesive that it stops bleeding, bond the tissues well, can act as a drug delivery vehicle and would appeal to the surgeon in terms of the simplicity of application in complex surgical situations. There is no need for special delivery systems for application of this adhesive. The two-component adhesive can be applied one over the other using syringes. There is also no need for light curing with UV or visible light and the gelation between the two components spontaneously takes place on application leading to excellent tissue bonding.