

**Immobilization of the soluble domain of human complement receptor 1 (sCR1) on
agarose-encapsulated islets for the prevention of complement activation**

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ABSTRACT

The transplantation of islets of Langerhans has been successfully applied to the treatment of insulin-dependent diabetes. However, a shortage of human donors is the hardest obstacle to overcome. We aimed to develop a bioartificial pancreas that can realize xeno-islet transplantation. The islets were encapsulated in agarose microbeads carrying the soluble domain of human complement receptor 1 (sCR1), which is an effective inhibitor of the classical and alternative complement activation pathways. When naked rat islets were cultured in rabbit serum, large amounts of insulin leaked from the damaged islets over the course of a few days incubation, but no damaged cells were observed among islets in sCR1-agarose microbeads cultured in rabbit serum for 4 days. Although low levels of insulin were detected in the rabbit serum, the insulin did not leak from damaged β -cells, it was physiological insulin secreted by the β -cells.

Keywords: Islets of Langerhans, Bioartificial pancreas, Agarose, Soluble complement receptor 1 (sCR1), Microencapsulation, Immuno-isolation, Xenotransplantation

1. Introduction

The transplantation of islets of Langerhans has been successfully applied to the treatment of patients with insulin-dependent diabetes mellitus (type 1 diabetes). However, several obstacles still remain, such as a shortage of human donors, low efficacy of islet isolation, and side effects of immunosuppressive drugs, among which the shortage of human donors is the most difficult to overcome. Recently, Hering et al. [1] reported that normoglycemia can be maintained for more than 100 days in STZ-induced diabetic cynomolgus macaques after intraportal transplantation of adult porcine islets. The study suggested opening up the use of pig islet xenograft transplantation to human patients. However, rationalizing the use of a large dose of immuno-suppressive drugs, which are required to control graft rejection in xenotransplantation, is difficult. The side effects of immunosuppressive therapy are expected to exceed the merits of islet transplantation. The transplantation of islets enclosed in a semi-permeable membrane as a bioartificial pancreas has been studied as a method of islet transplantation free from immunosuppressive therapy. Islets can survive and control glucose metabolism for a long period of time in a host without immunosuppressive therapy because islets are isolated from the host immune system by a semi-permeable membrane. Various types of bioartificial pancreas have been proposed and developed, including islets microencapsulated within an alginate/poly(L-lysine) polyion complex membrane [2] or agarose hydrogel [3], or macroencapsulated by porous membranes [4-6] or living cells [7,8]. Our group previously developed a microcapsule-type bioartificial pancreas using agarose hydrogel and demonstrated its efficacy in allotransplantation models [3, 9-11]. However, the agarose hydrogel is not sufficient to protect xenogeneic islets from

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4 rejection. A combination of islet microencapsulation and mild immunosuppressive
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6 therapy could achieve xeno-islet transplantation [12].
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9 In the present study, agarose hydrogel carrying a complement regulatory protein, the
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11 soluble form of complement-receptor type 1 (sCR1-agarose), was prepared and applied to
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13 encapsulate rat islets. Complement receptor type 1 (CR1) has been reported to be an
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15 effective inhibitor of the classical and alternative complement activation pathways [13-
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17 20]. The protective effect of sCR1-agarose on xeno-islets against antibody-complement-
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19 dependent destruction was examined by incubating the microencapsulated islets in rabbit
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21 serum.
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28 **2. Material and methods**

29 *2.1. Materials*

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32 A Chinese hamster ovary (CHO35.6) cell line expressing sCR1 (CRL-10052TM) [16] was
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34 obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Alexa
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36 488-labeled goat anti-mouse antibody and mouse anti-human sCR1 monoclonal antibody
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38 (J3D3) were purchased from Invitrogen (Carlsbad, CA, USA) and Beckman Coulter (CA,
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40 USA), respectively. 2-Iminothiolane hydrochloride (Traut's reagent) was purchased from
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42 MP Biomedicals (Illkirch, France). The *N*-(*p*-maleimidophenyl) isocyanate (PMPI) was
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44 purchased from Thermo Fisher Scientific (Rockford, IL., USA). Agarose (Taiyo Agarose,
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46 AG LT-600) was obtained from Shimizu shokuhin KK (Shimizu, Japan), and paraffin oil
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48 (107162) was purchased from Merck (NJ, USA). Enzyme-linked immunosorbent assay
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50 (ELISA) kits for the insulin assay and CH50 autokit were purchased from Shibayagi
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52 (Gunma, Japan) and Wako Pure Chemical (Osaka, Japan), respectively. Collagenase was
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4 purchased from Nitta Gelatin (Osaka, Japan). The sources of other chemicals and culture
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6 media are listed in the supplemental information.
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10 11 2.2. sCR1 purification 12 13

14 Human sCR1 was purified from the culture medium of CHO cells expressing sCR1 as
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16 previously described [21]. Briefly, the cells were first cultured in α -MEM medium
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18 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μ g/ml
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20 streptomycin, and 500 nM methotrexate (MTX). After the cells reached confluence, they
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22 were washed three times with phosphate buffered saline (PBS) and cultured in serum-free
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24 ASF104 medium (Ajinomoto Co. Inc., Tokyo, Japan) supplemented with 500 nM MTX
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26 for an additional 2 days. The culture medium was collected and centrifuged at 1000 rpm
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28 for 5 min, the supernatant filtered through a membrane filter (ϕ :0.45 μ m), and stored at
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30 4°C before purification.
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35 sCR1 was collected from the CHO culture medium by affinity column chromatography
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37 using a heparin sepharose 6B column. A 500 ml sample of the medium was loaded onto
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39 the heparin column and contaminants washed out using a 10 mM NaH₂PO₄ (pH 7.0)
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41 buffer solution. Bound sCR1 was eluted with an elution buffer composed of 10 mM
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43 NaH₂PO₄ and 1 M NaCl (pH 7.0). The protein concentration of the eluent was
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45 determined using a micro BCA protein assay kit. The collected sCR1 was subjected to
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47 SDS-PAGE analysis with coomassie brilliant blue (CBB) staining. The sCR1 solution
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49 was diluted with PBS and divided into 100 μ l aliquots in 50 tubes and stored at -20°C
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51 until assayed. The sCR1 activity in the solution was evaluated with the CH50 assay using
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53 the CH50 autokit according to the manufacturer's instructions and Yamamoto et al. with
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4 slight modifications [22]. Briefly, 250 μ l hapten-liposome solution was added to a
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6 mixture of 9 μ l of normal human serum (NHS) and 1 μ l sCR1 solution (25 to 300 μ g/ml)
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8 in PBS. The solution was incubated at 37°C. After 5 min, 125 μ l of an antibody–substrate
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10 mixture was added to the solution. After incubating at 37°C for 4.6 min, absorbance was
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12 measured at 340 nm. The complement activation level (CH50 value) of the NHS-sCR1
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14 mixture was determined from the absorbance using a standard curve. In the case of rabbit
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16 serum, a serum-sCR1 mixture was prepared by mixing 27 μ l rabbit serum and 3 μ l sCR1
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18 solution in PBS (25 to 300 μ g/ml). The CH50 assay was conducted as described for NHS.
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26 *2.3. Chemical modification of sCR1 and agarose*

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28 sCR1 was immobilized on the agarose hydrogel as shown in Scheme 1. First, sCR1 was
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30 thiolated using Traut's reagent by mixing 1 ml of 2 mg/ml sCR1 solution in PBS with 10,
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32 20, or 50 μ l of 10 mg/ml Traut's reagent solution in PBS by agitating for 1 h at room
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34 temperature. Thiolated sCR1 (sCR1-SH) was purified using a sephadex G25 column (GE
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36 Healthcare, Buckinghamshire, UK). The number of thiol groups introduced to the sCR1
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38 molecule was determined using Ellman's assay. The complement inhibition function of
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40 sCR1 before and after thiolation was determined by the CH50 assay.
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46 The hydroxyl groups of agarose were modified to maleimide groups using PMPI as
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48 follows. PMPI (2 to 5 mg) was added to an agarose solution in dry DMSO (100 mg
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50 agarose in 5 ml DMSO) and stirred at room temperature under dark condition overnight.
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52 The modified agarose (Mal-agarose) was collected by pouring the reaction mixture into
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54 acetone. After decanting, the precipitated product was washed with acetone twice and
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56 dried under reduced pressure.
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2.4. Protective effect of sCR1 immobilized on agarose hydrogel

Hapten-liposomes encapsulated in 2.5% agarose microbeads (LAMs) were utilized to examine the protective effect of sCR1 immobilized on agarose. Mal-agarose (150 mg) was dissolved in 3 ml PBS in a glass centrifugal tube using a microwave oven to prepare a 5% agarose solution. The agarose solution was left at 40°C for 7 min. The hapten-liposome suspension, which was supplied as a component of the CH50 assay kit and kept at 40°C, was added to an equal volume (3 ml) of the 5% agarose solution and mixed well. Pre-warmed liquid paraffin (15 ml, 40°C) was added to the glass tube, and the tube was vigorously agitated on ice for 3 min to induce gelation of the agarose microdroplets. Cold PBS (15 ml) was added to the glass tube and the suspension was centrifuged at 2000 rpm for 5 min at 4°C. The paraffin oil and supernatant were removed and the microbeads washed twice with PBS. The volume of the microbead suspension was adjusted to 500 µl after removing the supernatant. For immobilization of sCR1 on the agarose microbeads, 800 µg of sCR1-SH in 1 ml PBS was added to 500 µl of the microencapsulated liposome suspension, and the reaction mixture was incubated at 37°C for 2 h with gentle shaking every 30 min. After 2 hours, the microbeads were suspended in PBS and centrifuged at 2000 rpm for 5 min at 4°C. This procedure was repeated 5 times to remove free sCR1-SH. As a control, a cysteine solution (1 mM), instead of the sCR1 solution, was added to agarose solution. The LAMs treated with or without sCR1 were stored in 10 ml PBS overnight to remove unreacted sCR1 and cysteine.

The CH50 assay using the CH50 autokit was modified to evaluate the inhibitory effect of sCR1 immobilized on agarose microbeads on complement activation. The sCR1-

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4 immobilized LAMs (200 μ l) were mixed with 1 ml of 5-times diluted NHS in veronal
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6 buffer and was incubated at 37°C for 5 min. Then, 125 μ l of the substrate solution was
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8 added to the mixture and kept at 37°C. Two hundred microliters of supernatant was
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10 collected at 25, 45, and 65 min during the incubation and the absorbance at 340 nm
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12 determined. The percentage of lysis in the LAMs was calculated from the absorbance
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14 value of completely lysed LAMs, which were incubated for 3 h in serum.
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18 The amount of sCR1 immobilized to the agarose microbeads was determined by the
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20 micro BCA assay using microbeads without encapsulation of the hapten-liposome.
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25 26 *2.5. Isolation and microencapsulation of islets*

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28 Islets were isolated from male, 8-week-old ACI/N rats by the ductal injection of a
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30 collagenase solution [23]. Briefly, a rat was anesthetized by intraperitoneal injection of
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32 Nembutal (pentobarbital, 75 mg/kg). Approximately 10 ml of collagenase solution (0.5
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34 mg/ml) was carefully injected through the common bile duct to the pancreas to distend
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36 the tissue. The pancreas was removed and kept at 37°C for 19 min to digest the
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38 pancreatic tissue. RPMI-1640 (5 ml) containing 10% FBS was added to the sample and
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40 the tissue was pipetted vigorously to release islets from the exocrine tissues. The
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42 disintegrated tissue was washed twice with HBSS and the supernatant removed after
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44 centrifugation. The tissue was suspended in 5 ml Ficoll/Conray solution (density: 1.1
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46 g/ml) and transferred to a test tube. Ficoll/Conray solutions with a density of 1.075 g/ml
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48 and 1.050 g/ml (3 ml and 2 ml, respectively) were sequentially layered on the suspension
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50 to make a discontinuous density gradient. The test tube was centrifuged at 2500 rpm for 8
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52 min. Islets were collected at the interface between the 1.075 g/ml and 1.050 g/ml layers.
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4 After washing the islets, they were transferred to culture medium (RPMI-1640)
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6 supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin and
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8 cultured for 2 days before encapsulation with agarose microbeads.
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10 Islets were encapsulated in sCR1-agarose microbeads using the same method as hapten-
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12 liposomes detailed above. Microencapsulated islets were cultured in RPMI1640
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14 supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C
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16 in 5% CO₂. The sCR1 immobilized in microbeads was visualized by immunostaining.
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18 After 2 days of culture, microbeads containing islets were kept in culture medium
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20 containing anti-human sCR1 monoclonal antibody (1 µg/ml, J3D3 mouse antibody) for 6
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22 h at 37°C. The islets were washed and incubated in antibody-free medium for 1 day to
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24 complete remove unbound antibodies. The sCR1 in the microbeads was visualized by
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26 incubation in culture medium containing Alexa 488-labeled anti-mouse antibody (1
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28 µg/ml, goat) for 6 h at 37°C, and then the Alexa 488-labeled antibody was extensively
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30 removed by culturing the microencapsulated islets in antibody-free medium for 24 h.
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32 The agarose microbeads were observed by confocal laser scanning microscopy
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34 (Fluoview, FV500, Olympus Optical Co. Ltd., Tokyo, Japan).
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37 The ability of the microencapsulated islets to release insulin was examined by the static
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39 glucose-stimulated test [24]. Briefly, 50 microencapsulated islets were sequentially
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41 incubated in Krebs-Ringer solutions with glucose concentrations of 0.1 g/dl, 0.3 g/dl, and
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43 0.1 g/dl for 1 h each at 37°C. At the end of each incubation, supernatants were collected
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45 and stored at -20°C until ELISA analysis of insulin concentrations.
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4 *2.6. Inhibition of the complement-dependent destruction of islets by sCR1 immobilized on*
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6 *agarose*
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9 Rabbit serum was used to examine the sCR1 protection of rat islets from complement-
10 dependent destruction. Twenty naked islets and 20 islets encapsulated in agarose
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12 microbeads with or without sCR1 were cultured in 100% normal rabbit serum at 37°C.
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14 Serum was collected every 24 h and replaced by fresh serum after three washes with
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16 HBSS. The insulin contents of the collected sera were determined by ELISA. Islet
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18 morphology was observed by phase contrast microscopy (IX71, Olympus Optical Co.
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20 Ltd., Tokyo, Japan).
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29 *2.7. Statistical analysis*
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31 Comparisons between two groups were performed using the Student's t-test. $P < 0.05$ was
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33 considered significant. All statistical calculations were performed using the software JMP
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35 ver.5.1.1.
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3. Results

3.1. Isolation and function of CHO sCR1

The SDS-PAGE of fresh ASF104 medium, conditioned CHO cell medium, and purified sCR1 is shown in Fig. 1A. A band appeared in the conditioned medium at roughly 250 kDa was assigned to sCR1 (Mw: 220 kDa). Bands at 80 kDa in the fresh and conditioned medium were assigned to transferrin. After sCR1 was purified using a heparin column, most contaminants were removed (Fig. 1A, line c). Approximately 10-20 µg of sCR1 was isolated from 1 ml of culture medium.

The inhibitory effect of sCR1 on complement activation was determined with the CH50 assay. The CH50 value decreased with increasing sCR1 concentrations (Fig. 1B), indicating that sCR1 isolated from the conditioned CHO cell medium can effectively inhibit complement activation.

In xenotransplantation, complement regulatory proteins, such as decay accelerating factors and CD46, are used to impair the assembly of the membrane attack complex. In some donor and recipient combinations, however, the inhibitory properties of these molecules are not effective due to species restriction [25]. We examined the inhibitory effects of human sCR1 on complement activation in rabbit serum. With the addition of sCR1 to the serum, the increased absorbance at 340 nm remained low (Fig. 1C). This finding indicates that sCR1 can also effectively inhibit complement activation in rabbit serum.

3.2. Immobilization of sCR1 on agarose hydrogel

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4 The number of thiol groups introduced on sCR1 increased with increasing Traut's reagent
5 concentration (S-Fig. 1). The CH50 value of NHS was 38.9 U/ml. When naïve sCR1 was
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9 added to NHS at 2 µg/ml, the CH50 value decreased to 24.6 U/ml. When sCR1-SH
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11 carrying 4.6, 7.6, or 10.8 thiol groups per molecule were added to NHS at 0.26 µg/ml, the
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14 CH50 value decreased to 26.46, 26.18, and 28.09 U/ml, respectively (Fig. 2). Although
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16 the inhibitory function of sCR1 tended to decrease with an increasing number of
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18 introduced thiol groups, it did not exert a deteriorative effect on sCR1 function. The
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21 CH50 value of sCR1 with 4.6 thiol groups per molecule was almost the same as that of
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23 naïve sCR1. sCR1 carrying 4.6 thiol groups was used in the following experiments.
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25 Maleimide groups were introduced to agarose by PMPI and sCR1-SH was covalently
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27 immobilized on agarose through the thiol-maleimide reaction. Microbeads made of
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29 agarose modified with PMPI were immersed in a sCR1-SH solution to immobilize sCR1
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31 to the agarose microbeads. The amount of sCR1 in the sCR1-agarose conjugates was
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34 determined using the micro BCA method (S-Fig. 2).
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41 *3.3. Inhibitory effect of immobilized sCR1 on agarose*

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43 Hapten-liposomes were enclosed in 2.5% sCR1-agarose microbeads and then incubated in
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45 NHS to examine the inhibitory function of immobilized sCR1. When the complement
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47 system is activated on the hapten-liposome, the enzyme enclosed in the liposome is
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49 released into the solution and subsequently releases chromophore from the substrate,
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51 increasing the absorbance of the supernatant at 340 nm. The percentage of damaged
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53 liposome in NHS was evaluated from the changes in absorbance at 340 nm (Fig. 3).
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57 Hapten-liposomes enclosed in agarose microbeads without sCR1 were also incubated in
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4 NHS and NHS supplemented with 50 µg/ml sCR1. Although the absorbance of the
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6 supernatants increased with time in all three conditions, the absorbance for sCR1-agarose
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8 microbeads increased much more slowly than those without immobilized sCR1.
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10 Immobilized sCR1 effectively inhibited the complement activation, comparable to sCR1
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12 in NHS.
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19 *3.4. Islet morphology*

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21 Islets were encapsulated in microbeads made of agarose modified with PMPI. The
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23 microencapsulated islets were immersed in a solution of sCR1-SH immobilized to aCR1
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25 in the agarose microbeads. To examine the immobilization of sCR1 in agarose
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27 microbeads, the microbeads were immuno-stained using anti-sCR1 antibody. Clear green
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29 fluorescence was observed on the agarose microbeads (Fig. 4B). However, no
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31 fluorescence was observed for agarose not activated with PMPI. These results indicate
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33 that sCR1 was covalently immobilized on the agarose hydrogel through the
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35 thiol/maleimide reaction.
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40 Rat-rabbit is a discordant combination [26], as antibodies that react with rat tissue exist in
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42 rabbit serum. When naked rat islets are exposed to fresh rabbit serum, the antibodies form
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44 antigen-antibody complexes on islets and the immune complexes activate the
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46 complement system through the classic pathway, resulting in the destruction of islet cells.
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48 The protective effect of sCR1 immobilized on agarose in regards to the antibody-
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50 complement dependent destruction of islets was examined by incubating
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52 microencapsulated islets in rabbit serum. Naked rat islets were rapidly destroyed over a
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54 few days in rabbit serum, whereas no morphological change was observed in islets
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1 encapsulated in sCR1-agarose microbeads (Fig. 4A). The sCR1-agarose microbeads
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6 effectively protected the islets. Figure 5 also includes images of islets encapsulated in 5%
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9 agarose microbeads without sCR1 immobilization. Although some cells located at the
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11 periphery were swollen, the islets kept their intact round shape even after 4 days in rabbit
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13 serum. Agarose microbeads alone have some protective effect against the antibody-
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15 complement-dependent destruction of islets.
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21 *3.5. Insulin release from islets*

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23 The glucose stimulation test was carried out to determine the effects of
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25 microencapsulation and sCR1 immobilization on islet function (Fig. 5). The release
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27 insulin from islets in microbeads with or without sCR1 at high glucose concentrations
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29 was about 20% less than that of naked islets, with no difference observed between the
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31 two groups of islets in microbeads. Although the microencapsulation procedure exerted
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33 some deteriorative effects on islet function, islets in microbeads carrying sCR1 still
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35 maintained the ability to release insulin and control the amount released in response to
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37 the glucose concentration.
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43 β -cells in islets contain insulin as granules. When islets are damaged in rabbit serum,
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45 insulin leaks from the damaged β -cells. The amount of insulin in rabbit serum was
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47 determined to quantitatively evaluate islet cell death (Fig. 6). From 20 naked islets
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49 cultured in rabbit serum, 551.1 ng insulin leaked during the first 24 h period and 239.4 ng
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51 insulin leaked during the second 24 h period. After two days, the amount of insulin
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53 released drastically decreased because most of the β -cells were destroyed during the
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55 initial two days. The amount of insulin released from the islets in microbeads was much
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4 less than that of naked islets over the course of two days in culture. The amount of insulin
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6 leaked from islets in microbeads carrying sCR1 was half that leaked from islets in
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8 microbeads without sCR1 during. These observations indicate that the agarose hydrogel
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10 effectively protects islets from antibody-complement-dependent cell damage and sCR1
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12 immobilized on agarose exerted an additional protective effect.
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19 **4. Discussion**

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21 Our group has been examining agarose as a material to microencapsulate islets and
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23 demonstrated that agarose microbeads effectively protect allogeneic islets from the
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25 immune attack of recipient mice; thus, islet graft survival was realized without
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27 immunosuppressive therapy for a long period of time [3]. Though hamster islets enclosed
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29 in agarose microbeads cannot survive so long in recipient mice, we demonstrated that a
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31 combination of islet microencapsulation and B cell suppression by the administration of
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33 15-deoxyspergualin can realize long normoglycemia in islet xenotransplantation between
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35 hamster and mouse [12]. However, the administration of 15-deoxyspergualin is not a
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37 good choice for diabetic patients when its side effects are taken into consideration.
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39 In order to improve xenograft survival, some groups have examined the immobilization
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41 of bioactive substances to hydrogel macrocapsules [27-30]. An anti-inflammatory peptide
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43 was immobilized to functionalized hydrogel crosslinked by the thioester/thiol reaction
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45 [31]. In another study, anti-Fas mAb was conjugated to PEG hydrogel using
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47 photopolymerization [32]. These approaches have shown promising results for the down-
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49 regulation of inflammatory reactions and are expected to improve graft survival.
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51 However, the chemical reaction employed and necessary UV exposure are expected to
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4 exert deteriorative effects on cell viability and protein function. In addition, the activation
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6 of the complement system should be controlled for the success of xenogeneic islet
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8 transplantation because antibody-complement-dependent cytotoxicity plays a major role
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10 in xenograft rejection.
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14 In this study, islets were enclosed in sCR1-agarose microbeads because sCR1 has the
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16 ability to bind complement C3b/C4b, controls the dissociation of C3 and C5 convertase,
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18 and also serves as a co-factor for the proteolytic cleavage of C3b/C4b by factor I. sCR1 is
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20 also able to inhibit complement activation in both the classical and alternative pathways,
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22 and it has been reported that sCR1 can prolong the survival of xenografts in a dose-
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24 dependent manner in several animal models and pre-clinical trials [13-20]. However,
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26 controlling the sCR1 level in the local microenvironment of the graft is difficult with
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28 systemic administration of sCR1 [33]. In this study, sCR1 was immobilized on agarose by
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30 the thiol/maleimide reaction and sCR1-agarose was used to microencapsulate islets. The
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32 local concentration of sCR1 surrounding the islets increased for the effective regulation
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34 of antibody-complement-dependent cytotoxicity. The advantage of the thiol/maleimide
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36 reaction is that agarose is modified with maleimide groups before microencapsulation of
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38 islets, and proteins carrying thiol groups can be immobilized onto the agarose microbeads
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40 containing living islets under physiological conditions.
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48 When naked rat islets were cultured in rabbit serum, large amounts of insulin leaked from
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50 the damaged islets over the course of a few days incubation (Fig. 6). However, no
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52 damaged cells were seen on the islets enclosed by sCR1-agarose microbeads (Fig. 4).
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55 Low levels of insulin were detected in the rabbit serum, but this was not due to leakage of
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57 insulin from damaged β -cells, it was physiological insulin secretion by β -cells.
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5. Conclusion

Although the protective efficacy of sCR1-agarose in islet xenotransplantation should be carefully examined using *in vivo* models, the results obtained in this study suggest that it is a promising material to prepare a bioartificial pancreas that can be applied to islet xenotransplantation.

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SCHEME AND FIGURE CAPTIONS

Scheme 1. Schematic illustration of the immobilization of sCR1 on agarose. (a) Thiolation of sCR1 by Traut's reagent. (b) Activation of agarose by PMPI. (c) Conjugation of sCR1-SH with the activated agarose.

Fig. 1. Analysis of sCR1 isolated from CHO35.6 cell culture medium. (A) SDS-PAGE of sCR1 before and after purification using a heparin column. a: ASF104 medium, b: CHO culture supernatant, c: fraction from the culture medium collected through the heparin column. (B) CH50 values of 90% normal human serum supplemented with certain amounts of sCR1. (C) Inhibitory effect of sCR1 in 90% rabbit serum.

Fig. 2. Effect of thiolation on sCR1 activity. CH50 values are shown for 90% normal human serum and 9 μ l serum supplemented with 1 μ l naïve sCR1 or thiolated sCR1 (0.26 μ g/ml) as indicated.

Fig. 3. Inhibitory effect of sCR1 on the complement-dependent lysis of hapten-liposome. Dark circles indicate agarose microbeads, white circles indicate sCR1-agarose microbeads, and squares indicate agarose microbeads suspended in a medium supplemented with sCR1 (50 μ g/ml). The average diameter of the agarose microbeads was 200 μ m.

Fig. 4. Phase contrast microscope images of microencapsulated islets (A) and Immunofluorescence staining of sCR1 using anti-sCR1 antibody (J3D3 primary mAb) and Alexa 488 labeled anti-mouse antibody (B). (A) Protection effect of sCR1-agarose microbeads. Islets were incubated in 100% normal rabbit serum for the indicated time a: Islet sCR1-agarose microbeads, b: islets in agarose microbeads without sCR1, c: naked islets. Scale bars = 200 μ m. (B) a: sCR1-agarose microbead, b: agarose microbead without sCR1. Scale bars = 100 μ m.

Fig. 5. Glucose stimulation tests of naked islets and microencapsulated islets (MIs) with or without sCR1.

Fig. 6. The amount of insulin release was determined to measure β -cell lysis during islet incubation in rabbit serum for 4 days. ■: Naked islets, □: Islets in agarose microbeads without sCR1, ▨: Islets in sCR1-agarose microbeads.

Scheme 1

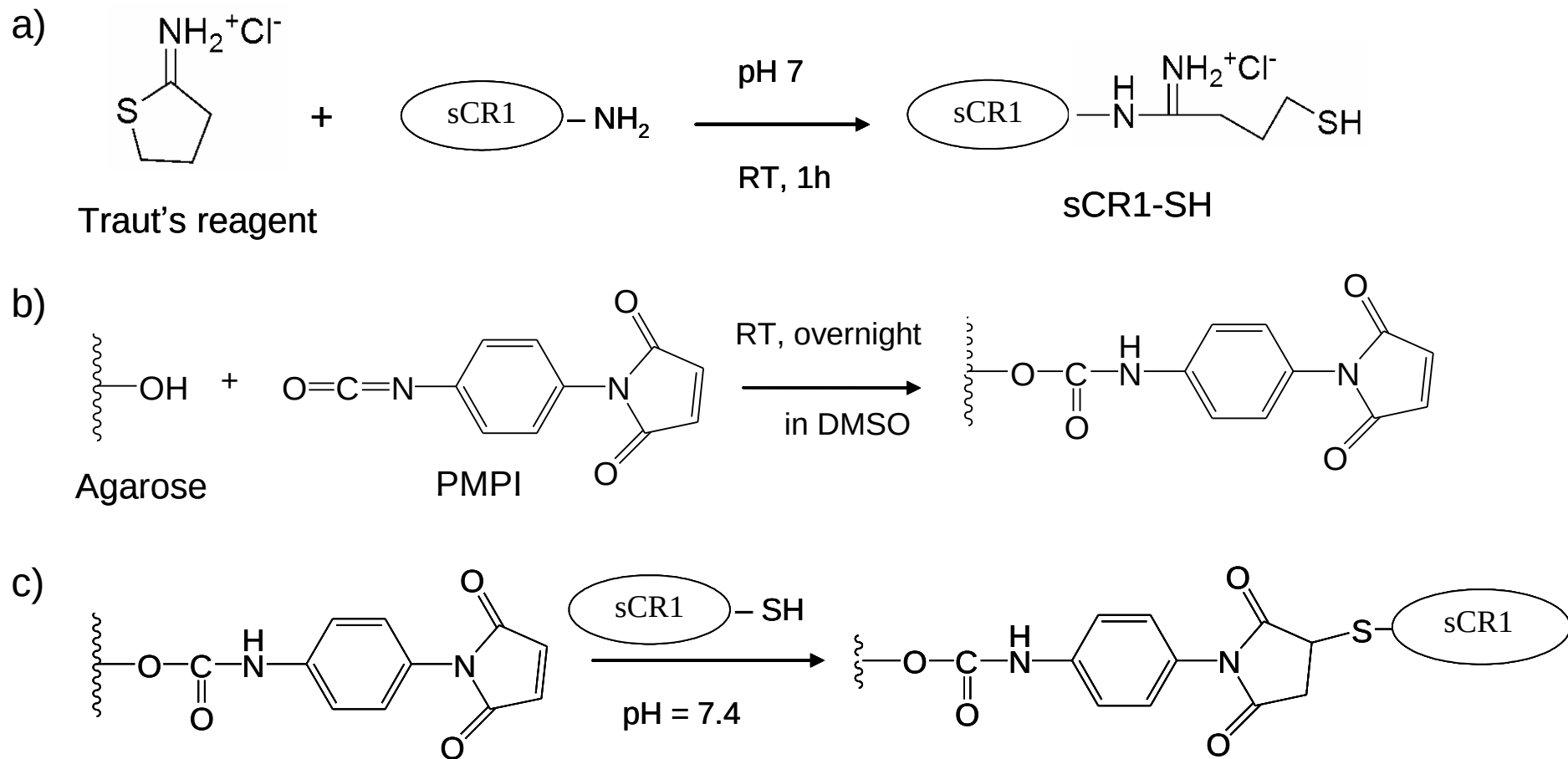


Figure 1
Figure 1 (A)

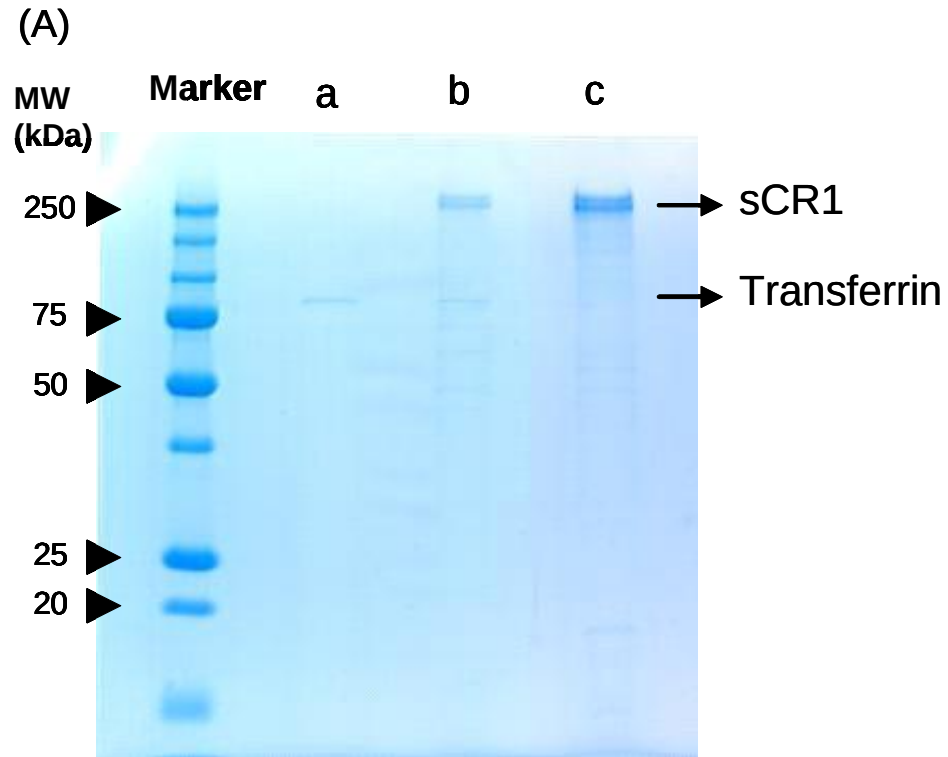


Figure 1 (B)

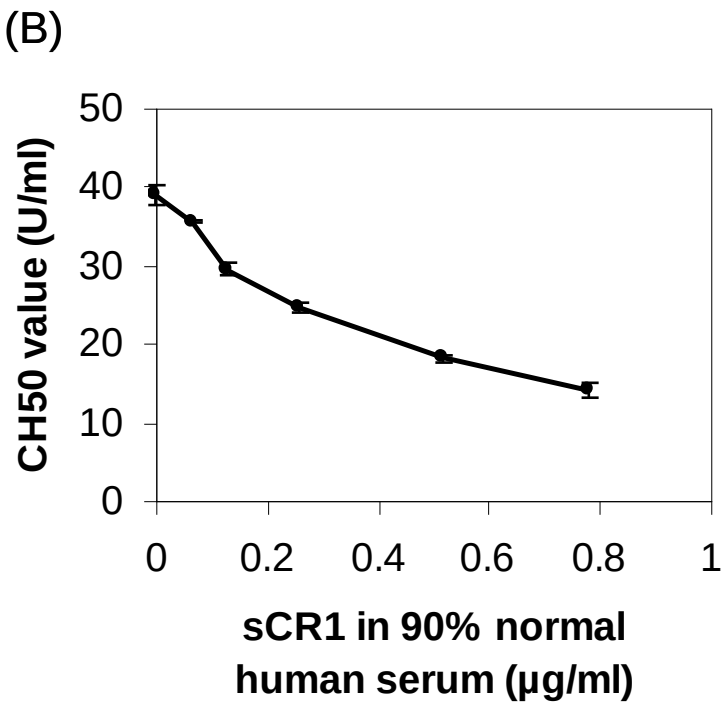


Figure 1 (C)

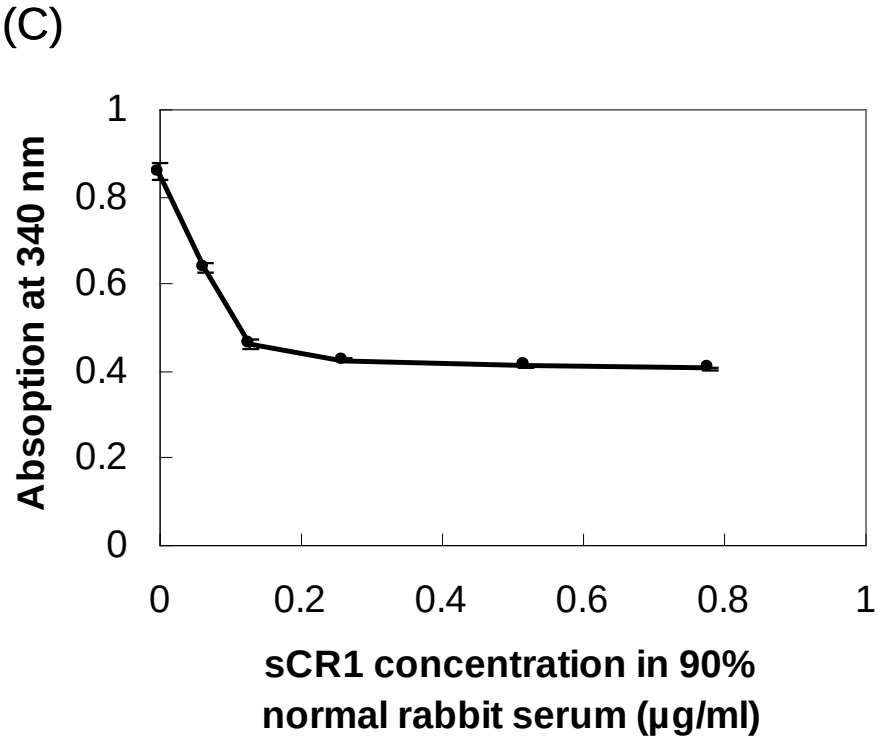


Figure 2
Figure 2.

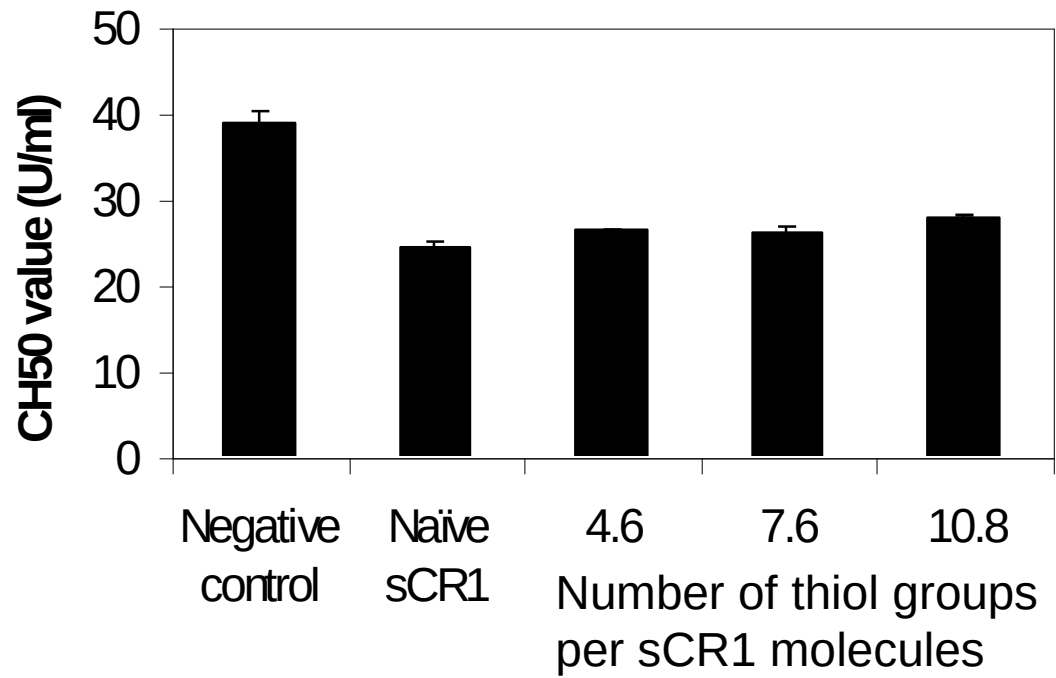


Figure 3

Figure 3

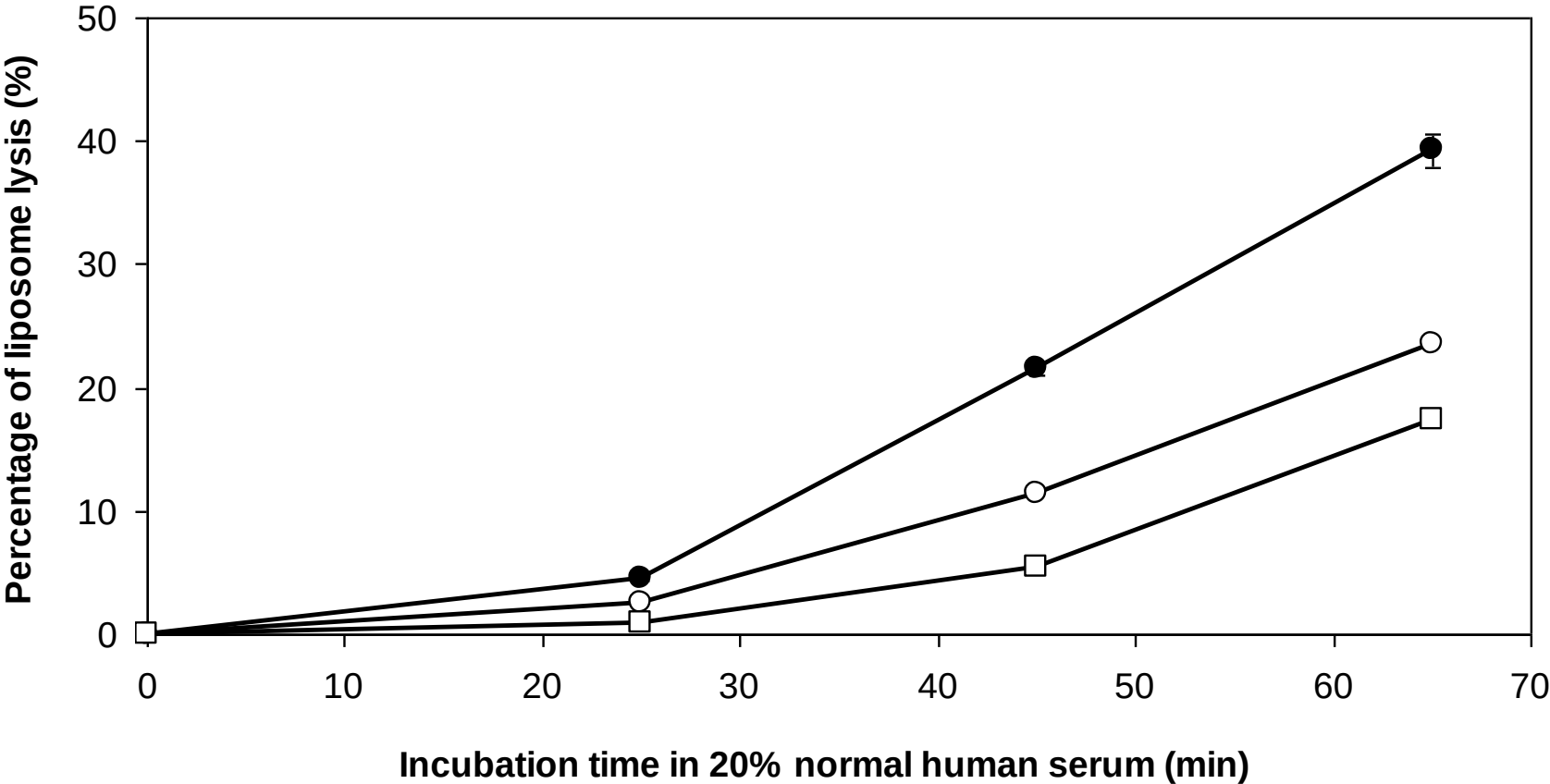
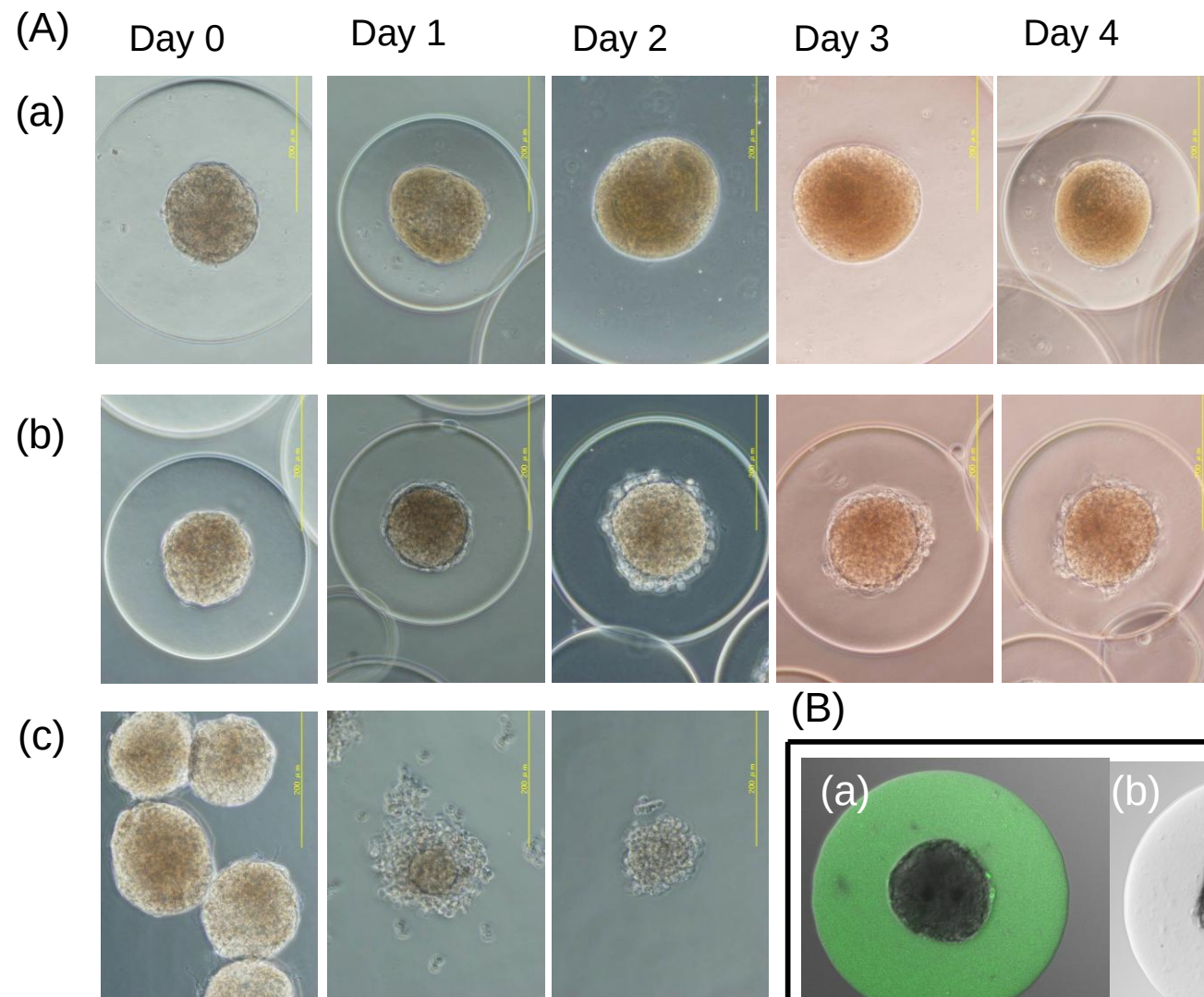


Figure 4

Figure 4



(B)

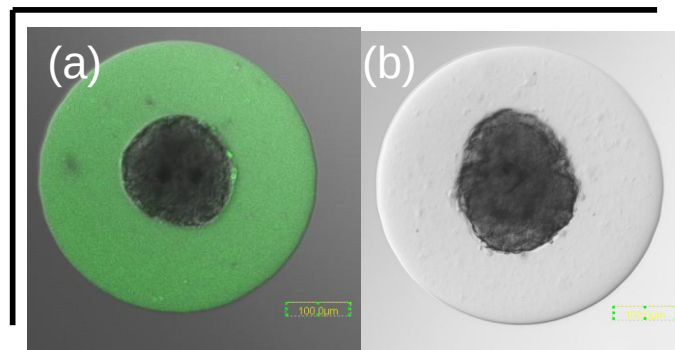


Figure 5

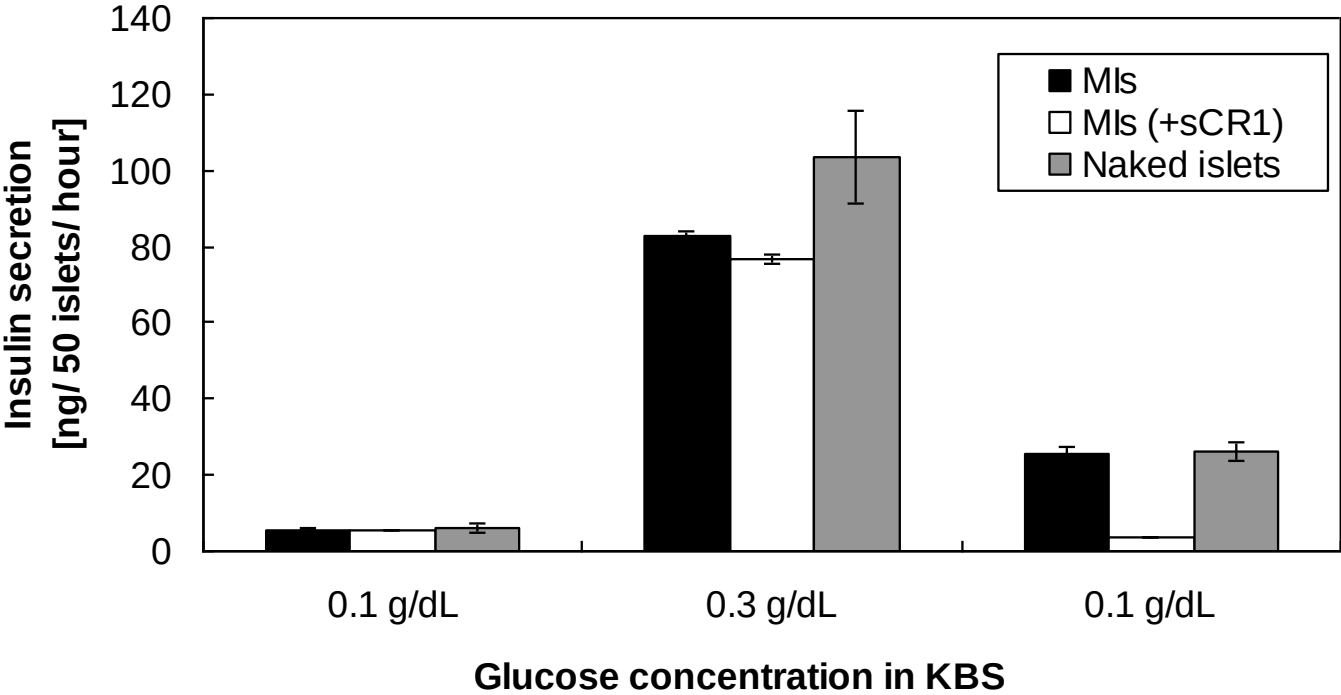
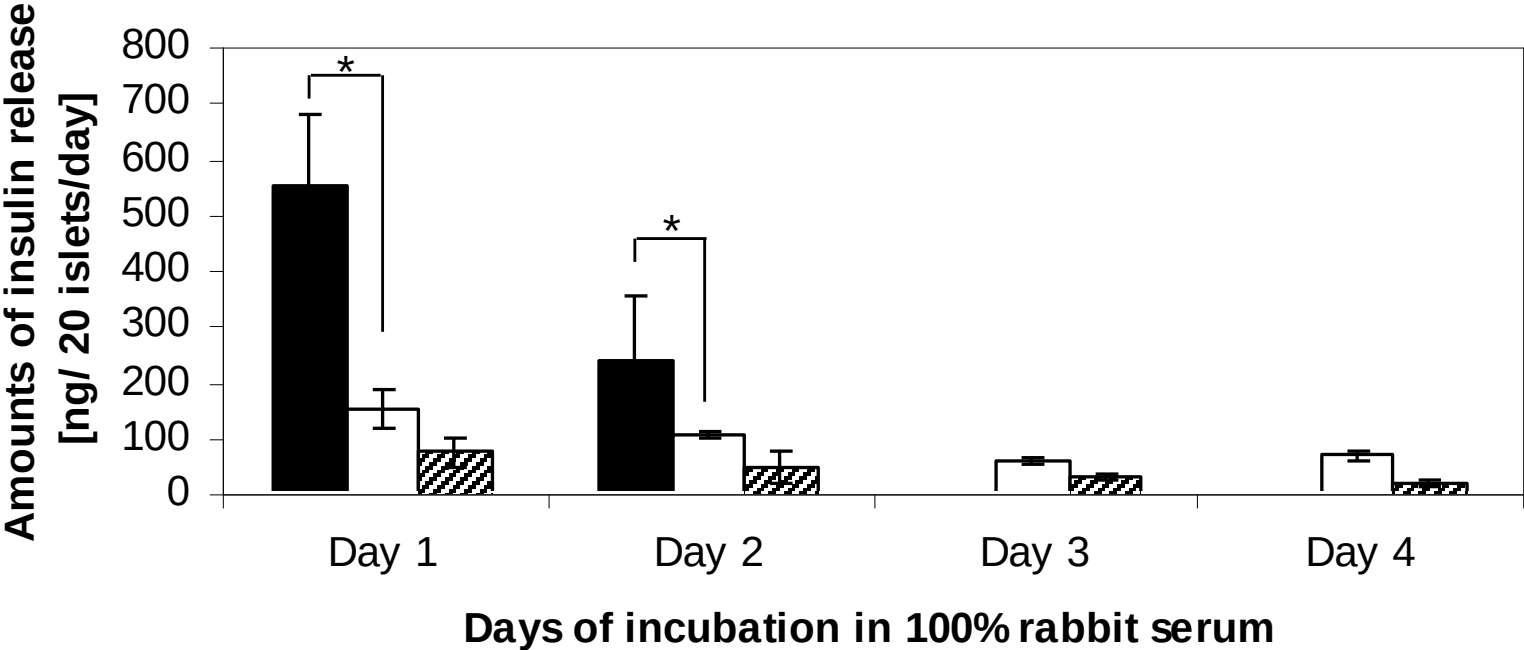


Figure 6

Figure 6



Immobilization of sCR1 on agarose-encapsulated islets for the prevention of complement activation

Nguyen Minh Luan^a, Yuji Teramura^b and Hiroo Iwata^{a*}

Supplemental data

Supplemental information

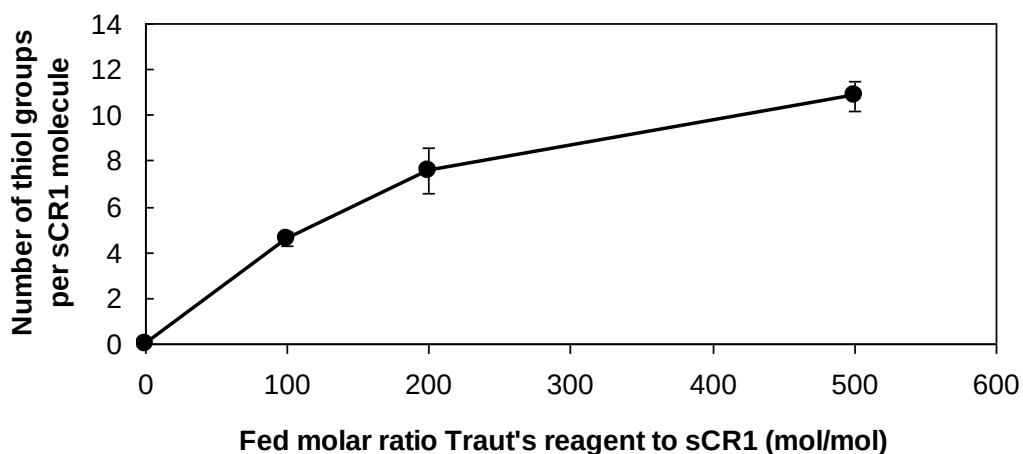
Materials

MEM Alpha (α -MEM) medium, RPMI-1640 medium, Hanks' balanced salt solution (HBSS), penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS) was purchased from Equitech-Bio (Kerrville, TX, USA), ASF 104 medium from Ajinomoto (Tokyo, Japan), phosphate-buffered saline (PBS) from Nissui Pharmaceutical (Tokyo, Japan), methotrexate from Sigma-Aldrich (St. Louis, MO, USA), and the micro BCA protein concentration kit from Thermo Fisher Scientific (Rockford, IL., USA). 5, 5'-Dithiobis (2-nitrobenzoic acid, Ellman's reagent), DMSO, and acetone were purchased from Nacalai Tesque (Kyoto, Japan). Ficoll was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden), Conray from Daichi Sankyo (Tokyo, Japan), EDTA from Dojindo (Kumamoto, Japan), Nembutal (pentobarbital) from Dainippon Sumitomo (Osaka, Japan), and cysteine from ICN Biomedical (Ohio, USA). Heparin sepharose 6B and sephadex G25 columns were purchased from GE Healthcare (Buckinghamshire, UK). SDS-PAGE materials were purchased from NAG Research Laboratories.

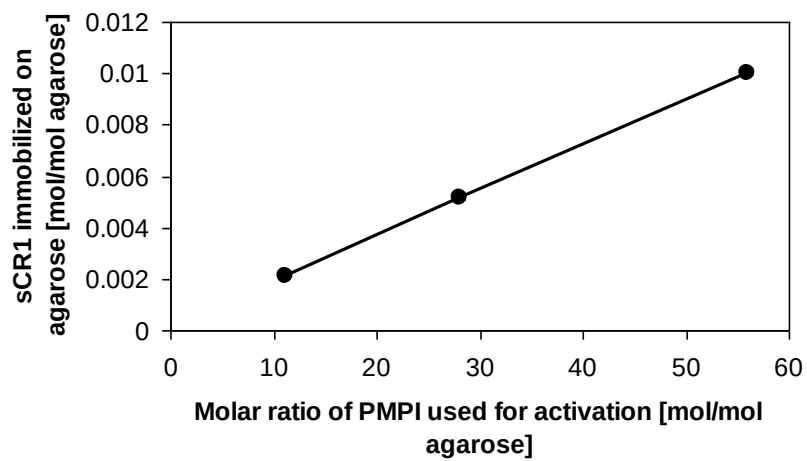
S1. Immobilization of sCR1 on agarose hydrogel

Thiol groups were introduced to sCR1 by converting amino groups with Traut's reagent. Because sCR1 has 74 lysine residues, a number of amino groups are converted to thiol groups. The number of introduced thiol groups was plotted against the molar ratio of Traut's reagent to sCR1 (S-Fig. 1). Although the number of thiol groups introduced on sCR1 increased with increasing Traut's reagent concentration, a few thiol groups were introduced even at higher molar ratios of Traut's reagent to sCR1.

Maleimide groups were introduced to agarose by PMPI. The sCR1-SH carrying 4.6 thiol groups was covalently immobilized on agarose through the thiol-maleimide reaction. The amount of immobilized sCR1 on agarose was plotted against the molar ratio of PMPI to agarose (S-Fig. 2). The amount of immobilized sCR1 increased with an increasing amount of PMPI used to modify the agarose, suggesting that sCR1 was immobilized through thiol/maleimide bonding.



S-Fig. 1. Number of thiol groups introduced per sCR1 molecule compared to the molar ratio of Traut's reagent to sCR1.



S-Fig. 2. Amount of immobilized sCR1 on agarose activated with varying amounts of PMPI. sCR1-SH carrying 4.6 thiol groups/sCR1 was used.