Chitosan/Gelatin Hydrogel Prolonged the Function of Insulinoma/Agarose Microspheres In Vivo During Xenogenic Transplantation

K.-C. Yang, C.-C. Wu, Y.-H. Cheng, T.-F. Kuo, and F.-H. Lin

ABSTRACT

Purpose. A chitosan/gelatin solution with glycerol 2-phosphate disodium salt hydrate in liquid phase at room temperature becomes a hydrogel at 37°C. The material can be used as an injectable cell carrier into the human body for gelation in situ. We hoped that the chitosan/gelatin hydrogel provided extra protection for insulinoma/agarose microspheres during xenogenic transplantation.

Materials and Methods. Mouse insulinoma was microencapsulated in agarose as microspheres, which were macroencapsulated in chitosan/gelatin hydrogel. Insulin secreting profiles were first demonstrated in vitro. Diabetic rats were injected subcutaneously with insulinoma/agarose microspheres or insulinoma/agarose microspheres suspended in chitosan/gelatin solution. The nonfasting blood glucose concentrations (NFBG) of diabetic rats were measured perioperatively. Rats were humanely killed 1 month postoperatively and the hydrogel was retrieved for histological examination.

Results. The insulinoma/agarose microspheres continually secreted insulin for 1 month when macroencapsulated in chitosan/gelatin hydrogel in vitro. The NFBG of diabetic rats injected with insulinoma/agarose microspheres decreased to euglycemic status albeit hyperglycemia was restored within 10 days. The NFBG of diabetic rats injected with chitosan/gelatin hydrogel, which contained insulinoma/agarose microspheres, was maintained at less than 200 mg/dL for 25 days. The histological section revealed immune cell infiltration and accumulation within the hydrogel and around the iusulinoma/agarose microspheres that may have contributed to the slowly increasing NFBG after day 25.

Conclusion. This study showed that chitosan/gelatin hydrogel can be used as a cell carrier for an injectable bioartificial pancreas; the hydrogel prolonged the function of cells encapsulated in agarose microspheres during xenogenic transplantation.

ALLOGENIC islet transplantation to compensate for pancreas dysfunction is an efficient therapy for type 1 diabetes, but the donor shortage restricts islet transplantation as a major treatment.[1] Xenogenic species may be an alternative due to the sufficient donor supply. However, the obstacle of islet transplantation is immune rejection. The recipient must receive lifelong immunosuppressive therapy to overcome immune rejection.[2]

Immunoisolation can facilitate the use of xenogenic cell sources to solve the problem of an insufficient donor supply, minimizing or eliminating the need for systemic immunosuppression.[3] Microencapsulation is one type of immunoisolation technique that has shown some encouraging outcomes in animal experiments. Microencapsulation can

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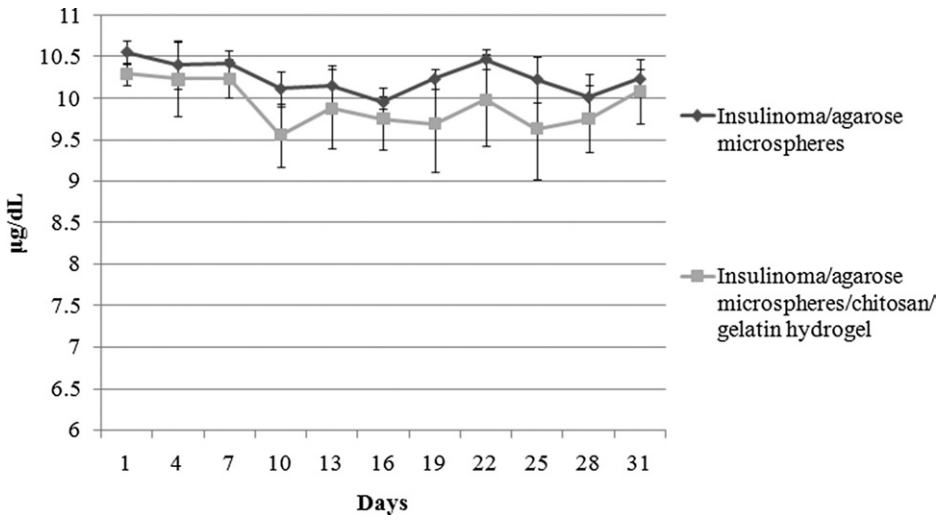

Fig 1. Insulin-releasing profiles of insulinoma in hydrogel. The insulinoma/agarose microspheres can secrete insulin continuously over 1 month when macroencapsulated inside the chitosan/gelatin hydrogel.

prevent attack by the recipient's immune system.[4] Hyperacute rejection is the major barrier to xenotransplantation even when microencapsulation is applied.[5] Extra protection is necessary for microencapsulation in xenogenic transplantation.

The chitosan/gelatin solution added with glycerol 2-phosphate disodium salt hydrate is a thermal sensitive material that is liquid at room temperature, becoming a hydrogel at 37°C.[6] In this study, we used chitosan/gelatin solution as a carrier to deliver insulinoma/agarose microspheres into diabetic rats. The purpose of this study was to evaluate the feasibility of using chitosan/gelatin hydrogel to provide extra protection for the insulinoma/agarose microspheres during xenogenic transplantation.

MATERIALS AND METHODS

In this study 2.5% chitosan (Kiotek, Hsinchu, Taiwan) and 2% gelatin (G1890, Sigma, St. Louis, Mo, United States) were dissolved in 0.1 mmol/L acetic acid (242853, Sigma). Glycerol 2-phosphate disodium salt hydrate (β-GP; G6251, Sigma) was dissolved in deionized water (0.8 weight/volume). The β-GP solution was added to the chitosan/gelatin solution during stirring until the pH value of the mixed solution was 7.4. The chitosan/gelatin solution with β-GP was used as a cell carrier.

Mouse insulinoma, NIT-1, was encapsulated as microspheres as described in a previous study.[7] One milliliter insulinoma/agarose microspheres was mixed with 1 mL chitosan/gelatin solution. The insulin release profiles of insulinoma/agarose microspheres macroencapsulated in chitosan/gelatin hydrogel were first evaluated in vitro by an insulin ELISA kit (Mouse insulin ELISA kit, Mercodia, Uppsala, Sweden).

Wistar rats were induced to be diabetic by a single intraperitoneal injection of 60 mg/kg Streptozotocin (S0130, Sigma). Under general anesthesia and after adequate skin preparation and sterilization, the chitosan/gelatin solution containing insulinoma/agarose microspheres was injected into the subcutaneous tissue of rats (n = 9) using a syringe with a 14-gauge needle. Each rat was injected with 1 mL chitosan/gelatin solution, which contained insulinoma/agarose microspheres (about 5×10^6 cells). Other rats (n = 9) were injected with insulinoma/agarose microspheres only (also 5×10^6 cells). The nonfasting blood glucose concentration (NFBG) of rats was measured perioperatively. The rats were humanely killed at 1 month postoperatively for histological examination of the hydrogel containing insulinoma/agarose microspheres. The host reaction to hydrogel was examined using optical microscopy.

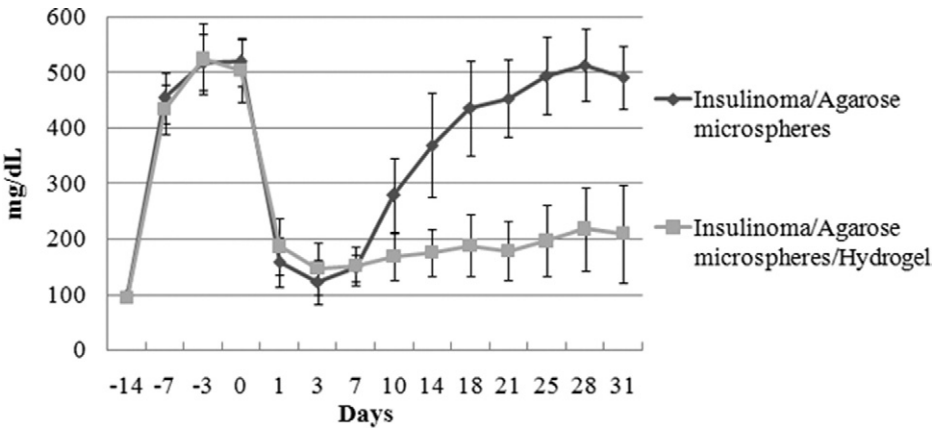

Fig 2. The rats were induced to diabetic status on day -14 and injected with chitosan/gelatin solution, which contained insulinoma/agarose microspheres, on day 0. The NFBG of diabetic rats were decreased from 503 ± 56 to 146 ± 48 mg/dL and maintained at 140–200 mg/dL for 25 days. The NFBG of rats injected with insulinoma/agarose microspheres only were also decreased to euglycemia status but were restored to hyperglycemia after day 10.

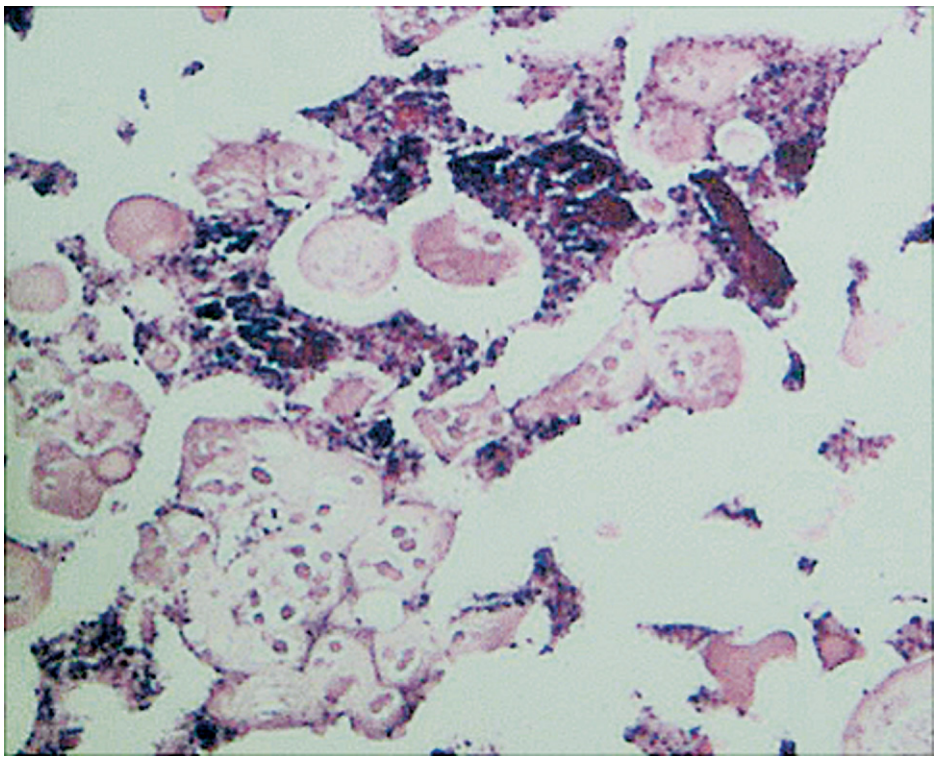

Fig 3. The histological section of retrieved hydrogel showed immune cell infiltration and accumulation; hydrogel was covered with monocytes and macrophages. This phenomenon was in foreign body reaction (H&E; 100×).

RESULTS

The insulin-secreting profiles are shown in Figure 1 indicating that the insulinoma/agarose microspheres continuously secreted insulin for 1 month in vitro when macroencapsulated inside chitosan/gelatin hydrogel. The amount of insulin secreted by insulinoma/agarose microspheres macroencapsulated in chitosan/gelatin hydrogel was similar to that released by insulinoma/agarose microspheres, indicating that the chitosan/gelatin hydrogel did not influence the insulin secretion function of the insulinoma.

The NFBG of rats injected with insulinoma/agarose microspheres was decreased from 519 ± 43 to 123 ± 40 mg/dL, but restored to hyperglycemic levels (360 ± 94 mg/dL) after day 10 (Fig 2) . The NFBG of diabetic rats decreased from 503 ± 56 to 146 ± 48 mg/dL after injection with the chitosan/gelatin hydrogel, which contained insulinoma/agarose microspheres. The NFBG were maintained at 140–200 mg/dL for 25 days. The NFBG increased to 200–230 mg/dL after day 28.

Histological sections (Fig 3: H&E; 100×) revealed immune cell infiltration and accumulation within the hydrogel and around the insulinoma/agarose microspheres that may have contributed to the slowly increasing NFBG after day 25.

DISCUSSION

Hydrogel can provide extra protection for xenotransplanted cells/agarose microspheres. Compared with the free insulinoma/agarose microspheres, which only functioned for 10 days in vivo, the insulinoma/agarose microspheres macroencapsulated in chitosan/gelatin hydrogel functioned for 25 days. The NFBG of rats injected with chitosan/gelatin hydrogel, which contained insulinoma/agarose microspheres, slowly increased to 215 ± 64 mg/dL at day 28 and 228 ± 88 mg/dL at day 31. The histological section of retrieved implants showed immune cell infiltration and accumulation; the hydrogel was covered with monocytes and macrophages. This phenomenon was a foreign body reaction that may have contributed to the slowly increased NFBG.[8] We believe that the implant will fail when the insulinoma/agarose microspheres are covered with immune cells.

Some additional advantages may be conferred by chitosan/gelatin hydrogel as a cell carrier. Although the insulinoma/agarose microspheres can be injected into an animal's body when suspended in isotonic liquid, the chitosan/gelatin hydrogel has the character of viscosity and is easier to inject. In addition, when the hydrogel is applied, microspheres are entrapped inside it and the gel is easy to remove if necessary. In contrast, if the microspheres are suspended in isotonic liquid and injected into an animal's body, the microspheres disperse and are difficult to retrieve.

This study showed that chitosan/gelatin hydrogel is a feasible cell carrier for an injectable bioartificial pancreas. The insulinoma/agarose microspheres secreted insulin normally when macroencapsulated in chitosan/gelatin hydrogel. The hydrogel also provided extra protection for cells encapsulated in agarose microspheres to prolong xenogenic transplant function. Some surface modifications of the hydrogel may be considered to avoid or retard the foreign body reaction and provide a future treatment for type 1 diabetes.

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