



## Intramedullary Cavity as Implantation Site for Bioartificial Pancreas: Preliminary In Vivo Study

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

---

### ABSTRACT

**Background.** The intramedullary cavity is a widely distributed well-vascularized micro-environment capable of sustaining grafts, and is a potential site for islet transplantation. The bone marrow offers sufficient space that may also be suitable for bioartificial pancreas (BAP) implantation.

**Objective.** To evaluate the feasibility of bone marrow as an implantation site for BAPs.

**Materials and Methods.** A calcium phosphate cement chamber satisfies the criteria for immunoisolation. Mouse insulinoma cells were suspended with agarose gel and enclosed in a calcium phosphate cement chamber to create a BAP, which was implanted in the intramuscular space in diabetic swine or the intramedullary cavity in diabetic dogs. Blood glucose and C-peptide concentrations were determined perioperatively.

**Results.** In the swine, the mean  $\pm$  SD blood glucose concentration decreased from  $413 \pm 24$  mg/dL to  $285 \pm 47$  mg/dL, and was maintained in the range of 285 to 336 mg/dL for 15 days. It increased to 368 to 450 mg/dL after the BAPs were implanted in the intramuscular space. In the dogs, the blood glucose concentration decreased from  $422 \pm 32$  mg/dL to  $247 \pm 52$  mg/dL, and was maintained in the range of 247 to 347 mg/dL after the BAPs were implanted in the intramedullary cavity. The C-peptide concentration increased from  $6.1 \pm 2.8$  pmol/L to  $104.7 \pm 16.4$  pmol/L when the BAPs were implanted in the intramedullary cavity.

**Conclusion.** This study indicates superior effectiveness of BAPs implanted in the intramedullary cavity compared with the intramuscular space. This observation may be attributed to the greater oxygen tension in the bone marrow. The BAPs in direct contact with the circulatory system receive sufficient blood flow for function and survival. This preliminary study demonstrates that the intramedullary cavity may be an implantation site for BAP transplantation.

---

**A** BIOARTIFICIAL PANCREAS (BAP) utilizes the strategy of immunoisolation to use xenogeneic islets as a donor supply in the absence of immunosuppression for type 1 diabetes.<sup>1</sup> However, most BAPs have been implanted in

subcutaneous sites, intramuscular spaces, or the peritoneal cavity. Implantation in these areas may result in failure because of hypoxia and fibrotic tissue envelopment.<sup>2-4</sup> A more promising site is necessary for successful BAP implantation.

---

From the Department of Organ Reconstruction (K.-C.Y.), Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; Institute of Biomedical Engineering (C.-C.W.), College of Medicine and College of Engineering, National Taiwan University, Taipei, Taiwan; Department of Orthopedic (C.-C.W.), En Chu Kong Hospital, Taipei, Taiwan; and the Institute of Veterinary (Z.-F.K.) Medicine, College of Veterinary Medicine, National Taiwan University, Taipei, Taiwan; Department of Traumatology and Department of Surgery (C.-Y.Y.), National Taiwan University

Hospital, Taipei, Taiwan; Institute of Biomedical Engineering (F.-H.L.), College of Medicine and College of Engineering, National Taiwan University, Taipei, Taiwan; Division of Medical Engineering (F.-H.L.), National Health Research Institute, Chunnan, Taiwan.

Address reprint requests to Feng-Huei Lin, Prof., Institute of Biomedical Engineering, College of Engineering and College of Medicine, National Taiwan University, No 1, Jen Ai Rd, Sec 1, Taipei, 10051, Taiwan. E-mail: [double@ntu.edu.tw](mailto:double@ntu.edu.tw)

0041-1345/10/\$-see front matter  
doi:10.1016/j.transproceed.2010.04.027

© 2010 by Elsevier Inc. All rights reserved.  
360 Park Avenue South, New York, NY 10010-1710

The intramedullary cavity has a widely distributed well-vascularized microenvironment that may be capable of sustaining transplanted islets.<sup>5</sup> The bone marrow offers sufficient space that also may be suitable for BAP implantation. Calcium phosphate cement (CPC) has an interconnected porous structure that satisfies the criteria for an immunoisolative device.<sup>6</sup> The final product of hydrated CPC is calcium-deficient hydroxyapatite, similar to the apatite of human bones, resulting in a suitable material for implantation in bone tissue.<sup>7</sup> In addition, islet cells enclosed inside the chamber exhibit normal viability and secrete insulin continually, which suggests that a CPC chamber might be used for a BAP.

In the present study, mouse insulinoma cells were enclosed in a CPC chamber to create a BAP. The BAPs were implanted in the intramedullary cavity in diabetic dogs, and in the intramuscular space in swine for comparison. The feasibility of bone marrow as an implantation site for BAPs was evaluated.

#### MATERIALS AND METHODS

The CPC chambers were prepared according to previously described methods.<sup>6</sup> Mouse insulinoma cells (NIT-1) were suspended in 5% agarose gel and transferred into the preformed CPC chamber as a BAP. Each BAP contained  $1.2 \times 10^7$  insulinoma cells.

In 3 swine (Lee-Sung pigs, aged 6–8 months, and weighing 8–10 kg), diabetes was induced via intravenous injection of 180 mg/kg of streptozotocin. After streptozotocin injection, the mean  $\pm$  SD nonfasting blood glucose concentration increased from  $104 \pm 13$  mg/dL to  $413 \pm 24$  mg/dL. The diabetic swine were implanted with 14 BAPs ( $1.68 \times 10^8$  cells total) in the intramuscular space between the biceps femoris and the vastus lateralis. In 3 dogs (English beagles, aged 1–2 years, and weighing 8–11 kg), diabetes was

induced via intravenous injection of 90 mg/kg of alloxan. The blood glucose concentration increased from  $121 \pm 12$  mg/dL to  $422 \pm 32$  mg/dL. The diabetic dogs were implanted with BAPs as follows. A longitudinal skin incision was made along the long axis of the knee, and a parapatella approach was used to expose the patellar groove of the distal femur. Seven BAPs were implanted through the canals created using a hand reamer into the intramedullary cavity of the distal femur. The same procedure was performed in another thigh, and 14 BAPs ( $1.68 \times 10^8$  cells total) were implanted. The blood glucose concentrations in the swine and dogs were measured, and venous blood samples were collected for determination of C-peptide.

#### RESULTS

In the swine, the mean  $\pm$  SD blood glucose concentration decreased from  $413 \pm 24$  mg/dL to  $285 \pm 47$  mg/dL at 1 day postimplantation, and was maintained in the range of 285 to 336 mg/dL for 15 days. Thereafter, it increased to 368 to 450 mg/dL, revealing implant failure (Fig 1). In the dogs, the blood glucose level decreased quickly from  $422 \pm 32$  to  $247 \pm 52$  mg/dL at 1 day postimplantation, and was maintained in the range of 247 to 347 mg/dL for 31 days, indicating that the implanted BAPs secreted insulin continuously.

The C-peptide serum concentration in diabetic animals was negligible when analyzed using a mouse C-peptide assay (3.3–9.6 pmol/L for swine, and 2.9–8.3 pmol/L for dogs). At 3 weeks postimplantation, the concentration was in the range of  $11.3 \pm 4.0$  pmol/L in the swine, and increased to  $104.7 \pm 16.4$  pmol/L in the dogs.

#### DISCUSSION

The effectiveness of BAPs implanted in the intramuscular space or intramedullary cavity was compared. Although the

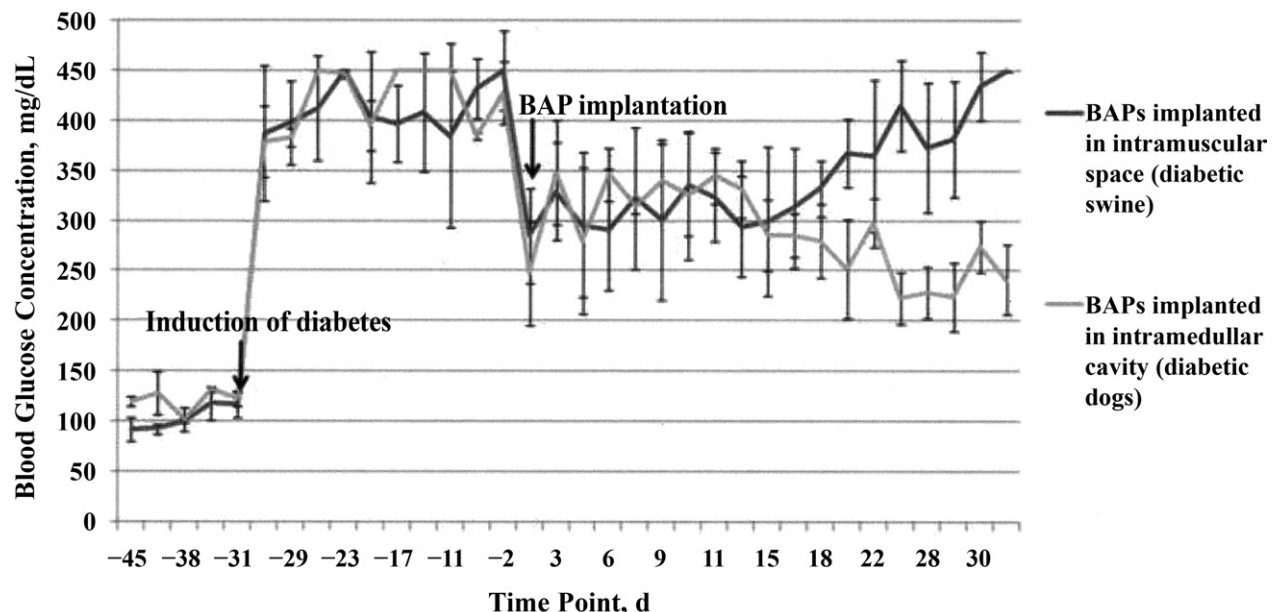


Fig 1. Blood glucose concentration in diabetic animals before and after bioartificial pancreas (BAP) implantation.

species of recipients were different, their body weight and initial blood glucose concentrations were similar. The mean  $\pm$  SD blood glucose concentration in diabetic animals decreased from  $413 \pm 24$  mg/dL to  $308 \pm 17$  mg/dL and was maintained for 15 days before increasing to  $398 \pm 35$  mg/dL. The BAPs exhibited only partial function for 2 weeks when implanted in the intramuscular space. It was assumed that insufficient oxygen tension at this site may have contributed to graft failure.<sup>8</sup> The blood glucose concentration decreased from  $422 \pm 32$  mg/dL to 247 to 347 mg/dL for 31 days when BAPs were implanted in the intramedullary cavity, which indicates that the implanted BAPs functioned continuously. This finding was consistent with the C-peptide concentration, which was low and negligible when the BAPs were implanted in the intramuscular space. The concentration in animals with BAPs implanted in the intramedullary cavity was one-third that in healthy animals.<sup>9</sup>

The BAPs implanted in the intramedullary cavity showed longer effectiveness than those implanted in the intramuscular space. This may be because the sufficient partial oxygen tension (40–60 mm Hg) in the bone marrow is significantly higher than that in the intramuscular space (<10 mm Hg).<sup>10,11</sup> BAPs require sufficient blood flow for function and survival. The blood glucose concentration in recipients with BAPs implanted in the intramedullary cavity was lower than in animals with BAPs implanted in the intramuscular space. This may be attributed to rapid insulin delivery by the circulatory system within the bone marrow. In contrast, insulin is only delivered by passive diffusion when the BAP is implanted in the intramuscular space. The blood concentration decreased over time, which it was assumed may be due to bone tissue growing progressively in the CPC chamber, accelerating mass diffusion between the graft and the recipient, subsequently enhancing the functions of the BAPs.

In conclusion, this preliminary study demonstrated that the intramedullary cavity as an implantation site is superior to the intramuscular space, and could represent an alternative site for implantation. An increased number of implanted BAPs should be considered to achieve insulin independence in recipients with diabetes.

## REFERENCES

1. Prokop A: Bioartificial pancreas: materials, devices, function, and limitations. *Diabetes Technol Ther* 3:431, 2001
2. Tashiro H, Iwata H, Warnock GL, et al: Functional assessment of microencapsulated porcine islets with agarose polystyrene sulfonic acid in vitro and in xenotransplantation. *Transplant Proc* 37:3512, 2005
3. Yang KC, Wu CC, Cheng YH, et al: Chitosan/gelatin hydrogel prolonged the function of insulinoma/agarose microspheres in vivo during xenogenic transplantation. *Transplant Proc* 40:3623, 2008
4. Anderson JM, Rodriguez A, Chang DT: Foreign body reaction to biomaterial. *Semin Immunol* 20:86, 2008
5. Salazar-Bañuelos A, Wright J, Sigale D, et al: The bone marrow as a potential receptor site for pancreatic islet grafts. *Arch Med Res* 39:139, 2008
6. Yang KC, Yang CY, Wu CC, et al: In vitro study of using calcium phosphate cement as immunoisolative device to enclose insulinoma/agarose microspheres as bioartificial pancreas. *Biotechnol Bioeng* 98:1288, 2007
7. Huipin Y, Li YB, de Bruijn JD, et al: Tissue response of calcium phosphate cement: a study in dogs. *Biomaterials* 21:1283, 2000
8. de Groot M, Schuurs TA, Keizer PP, et al: Response of encapsulated rat pancreatic islets to hypoxia. *Cell Transplant* 12:867, 2003
9. Montgomery TM, Nelson RW, Feldman EC, et al: Basal and glucagon-stimulated plasma C-peptide concentrations in healthy dogs, dogs with diabetes mellitus, and dogs with hyperadrenocorticism. *J Vet Intern Med* 10:116, 1997
10. Harrison JS, Rameshwar P, Chang V, et al: Oxygen saturation in the bone marrow of healthy volunteers. *Blood* 99:394, 2002
11. Carlsson P-O, Palm F, Andersson A, et al: Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 50:489, 2001