Semmelweis Egyetem – Doktori Iskola

Semmelweis University – School of Ph.D. Studies

Immunoisolation of islets of Langerhans using a tissue engineered chondrocyte membrane

Summary of the Ph.D. Thesis

Dr. Jörg-Matthias Pollok

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Dr. Jörg-Matthias Pollok – University Hospital Hamburg-Eppendorf

Chief-Coordinator: Prof. Dr. Zsolt Tulassay – Semmelweis University

Budapest, 2001.

Diabetes mellitus is one of the most frequent metabolic disorders with increasing incidence. Current therapeutic options, including insulin supplementation improved the life expectancy, but short and long term complications like hyper- and hypoglycemic crisis, retinopathy, nephropathy and microvascular angiopathy are still devastating problems. Pancreas or islet transplantation offer a curative treatment, but clinical application remains restricted, because of insufficient methods to prevent rejection and autoimmune destruction of islet grafts. Immunoisolation is an experimental strategy to prevent immunorecognition or -destruction by separating transplanted cells or tissues from the host immune system, providing a barrier device. Applying the principles of tissue engineering we propose an immunoisolation method using recipient own chondrocytes as immunoisolation barrier. Cartilage is an avascular tissue and the chondrocyte matrix has immunoprivileged properties.

Islets of Langerhans were isolated from Lewis rats, seeded on biodegradable poly-glycolic acid (PGA) polymer and encapsulated with a membrane of bovine, porcine, or rat chondrocytes, previously grown in culture from primary isolated chondrocytes. The encapsulated constructs and controls were kept in culture for up to five weeks. One group was exposed to a glucose challenges. Preserved specific function and morphology of encapsulated islets were tested.

H&E, Toluidin blue and Heidenhain staining demonstrated viability and intact morphology of the encapsulated islets and the surrounding chondrocyte layers. Immunohistochemistry was positive for insulin within the β-cells of the islets. Both the encapsulated constructs and non-encapsulated controls showed increasing insulin levels after glucose challenge in vitro, as detected by RIA.

A chondrocyte encapsulation membrane which permits diffusion of glucose and insulin could be tissue engineered. Islets survive within the chondrocyte capsule and the glucose/insulin feedback mechanism remains intact. For possible clinical application autologous chondrocytes could be isolated and expanded from a cartilage biopsy to serve as an immunoisolation barrier for allogeneic or xenogeneic islets of Langerhans.

Összefoglaló

Langerhans szigetsejtek immunoizolálása tissue engineered chondrocyta membránnal

Dr. Pollok Jörg-Matthias – Egyetemi Klinika Hamburg-Eppendorf

Programvezető: Prof. Dr. Tulassay Zsolt – Semmelweis Egyetem

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A diabetes mellitus az egyik leggyakoribb anyagcserebetegség egyre emelkedő incidenciával. A jelenlegi terápiás lehetőségek, ideértve az inzulin alkalmazását, javítják ugyan az életkilátásokat, de a rövid- és hosszútávú szövődmények, mint például hyper- és hypoglycaemiás állapot, retinopathia, nephropathia és angiopathia változatlanul súlyos gondok maradnak. A pancreas vagy szigetsejt transzplantáció egy lehetséges gyógyítási mód, de klinikai alkalmazását korlátozza, hogy a beültetett sejtek elleni kilökődést és autoimmun reakciót nem lehet tökéletesen megakadályozni.

Az immunizoláció egy kísérletes módszer az immunrendszer által történő felismerés és elpusztítás megakadályozására azáltal, hogy a beültetett sejteket, vagy szövetet a befogadó (host) szervezet immunrendszerétől elválasztjuk. A tissue engineering elveinek alkalmazásával egy olyan immunizolációs módszert javaslunk, amely a befogadó szervezet saját porcsejtjeit használja fel mint immunizolációs membránt, lévén a porc egy avasculáris szövet, a porcsejt matrix pedig az immunrendszertől speciális védettséget élvez.

Langerhans szigetsejteket izoláltunk Lewis patkányokból és biodegradábilis poly-glycolsav (PGA) polymerre helyezve becsomagoltuk marha, sertés, illetve patkány porcsejt membránba, melyet előzőleg primer porcsejtek tenyésztésével állítottunk elő. A becsomagolt- és a kontroll szigetsejt-polymer készítményeket 5 hétig tenyésztettük. Egy csoportnál glucose provokációs tesztet végeztünk. A becsomagolt szigetsejtek functionális és morphológiai jellemzőit vizsgáltuk.

A H&E, Toluidinkék és Heidenhain festések élő és intact morphológiájú szigetsejteket és azokat körülvevő porcmembrán sejteket mutattak. Az immunhisztokémiai festés pozitív volt a β-sejtekben. Mind a becsomagolt, mind a kontroll készítmények fokozódó inzulinválaszt adtak glucose provokációra RIA-val meghatározva.

A tissue engineering módszereivel előállítható egy olyan izolációra alkalmas porcmembrán, melyen keresztül glucose és inzulin diffúziója lehetséges. A szigetsejtek életképesek maradnak a porcmembránon belül és a glucose/inzulin feed-back mechanizmus megtartott. Klinikai alkalmazás során autológ porcsejtek izolálása és tenyésztése porcbiopsziából nyert anyagból történne, majd ebbe az immunizolációs membránba allogen vagy xenogen Langerhans szigetsejtek kerülnének.

Introduction

Tissue engineering

Tissue engineering is the application of the principles and methods of engineering and the life sciences toward the fundamental understanding of structure/function relationships in normal and pathological tissues and the development of biological substitutes to restore, maintain, or improve tissue function.

Diabetes mellitus

Diabetes mellitus is the most frequent, medically relevant metabolic disorder. In Hungary with a population of 10 million inhabitants the estimated number of diabetic patients is 500,000 with a 3 fold increase in incidence of type-I diabetes in childhood during the last 2 decades (3.8/100,000 person/year in 1978 to 10.7/100,000 person/year in 1997).

Although intensified insulin supplementation offers a good symptomatic treatment, long term complications like polyneuropathy, microangiopathy, and retinopathy are still a major burden leading to blindness, renal failure, limb amputation and cardiovascular problems.

Pancreas and islet transplantation

Pancreas and islet transplantation has the potential as a curative therapy for patients with diabetes mellitus. Broad clinical application is restricted by the necessity of immunosuppression and its side effects, which so far is regarded by most experts not to be justifiable for pancreas and islet transplantation alone.

Immunoisolation

Immunoisolation is an experimental strategy to avoid immunosuppression, preventing immunorecognition and rejection by separating the transplanted cells from the host immune system (Figure 1).

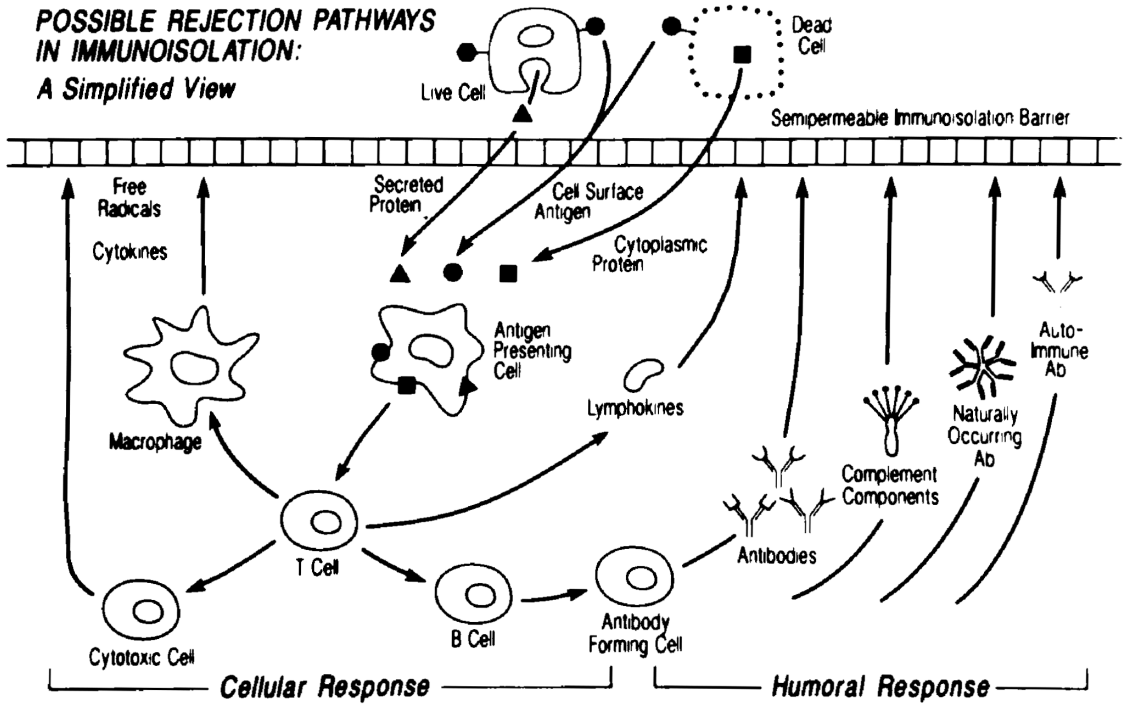

Figure 1: *Possible rejection pathways in cell transplantation. Displayed are the components of the cellular and the humoral response to which a transplanted cell will be exposed. The semipermeable immunoisolation barrier is meant to prevent the penetration of the different components of the immune system.*

Current methods of immunoisolation use artificial materials as barrier devices, like semipermeable membranes, hollow fiber devices, hydrogels, alginate capsules, agarose, or silicone diffusion chambers. These materials however are not completely inert and can induce foreign body and inflammatory reactions. The consecutive fibrous tissue overgrowth diminishes the diffusion properties for nutrients and oxygen as well as hormones and waste products. Additionally, stimulated macrophages secrete nitric oxide (NO), which penetrates these barriers and destroys the encapsulated islets, although not recognized as foreign themselves.

Purpose

We propose the encapsulation of islets of Langerhans with a layer of autologous chondrocytes and their matrix to prevent immunorecognition and destruction of transplanted allogeneic or xenogeneic islets (Figure 2).

Using autologous cells as encapsulation material would prevent the foreign body reaction and consecutive consequences of fibrous tissue overgrowth and macrophage activation.

In these studies we use bovine, porcine, and rat chondrocytes and rat islets as an in vitro model to demonstrate the long-term function of the glucose-insulin feedback of islets of Langerhans within a tissue engineered capsule of chondrocytes, which may serve as an immunoisolation barrier utilizing the immunoprivileged properties of the chondrocyte matrix. This approach of xenograft, allograft, and autoimmune immunoisolation might offer a therapeutic strategy for patients with diabetes mellitus, using recipient own chondrocytes from a cartilage biopsy as encapsulation material.

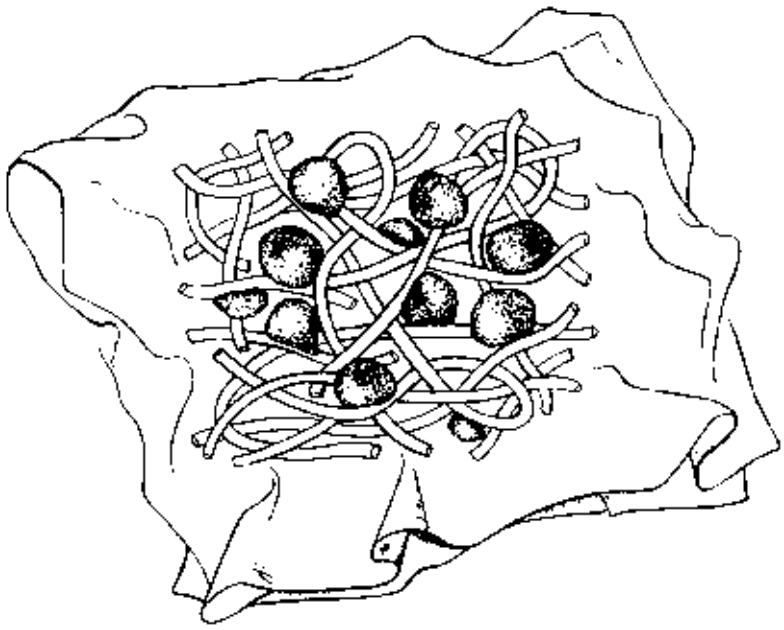

Figure 2: *Method of encapsulation of islets of Langerhans seeded on biodegradable PGA polymer with a confluent layer of chondrocytes. The islets of Langerhans are attached to the biodegradable PGA polymer fibers to prevent macroaggregation. The membrane of chondrocytes is wrapped tightly around the islets on PGA.*

Material and methods

Chondrocyte isolation

Chondrocytes were isolated from bovine, porcine and rat articular cartilage using a collagenase digestion method.

Chondrocyte culture

5 x 10^5 chondrocytes were seeded onto polystyrene culture dishes of 10 cm diameter and grown in culture until a confluent monolayer had formed using a M-199 medium, supplemented with 25 µg ascorbic acid, 10% fetal bovine serum, and 1% glutamine/penicillin/streptomycin. The maturity of the chondrocyte layer was estimated using phase contrast microscopy.

Polymer

Biodegradable highly porous poly-glycolic acid polymer (PGA) meshes were custom fabricated to the density of 40 to 52 mg/cc and thickness of 0.6 mm.

Islet isolation

Islets of Langerhans were isolated from adult, male Lewis-rats with an average weight of 150 to 300 g using a collagenase digestion method. The animals were kept until the day of operation in the Animal Research Facility of Children's Hospital, Boston, MA, in accordance with NIH guidelines for the care of laboratory animals or of the University Hospital Hamburg-Eppendorf, in accordance with German national guidelines for the care of laboratory animals (Deutsches Tierschutzgesetz).

Islet seeding and encapsulation

Chondrocytes formed a confluent layer in the culture dish. This membrane of confluent chondrocytes was detached mechanically from the floor of the culture dish using a cell scraper. A 1.0 x 1.0 x 0.06 cm PGA-polymer scaffold, previously seeded with islets of Langerhans, was placed on the membrane. Using two fine forceps the membrane of chondrocytes was grasped and flipped over the polymer with the attached islets (Figure 2). The constructs underwent microscopic examination for integrity.

Culture conditions and glucose challenge

The encapsulated constructs and the non-encapsulated controls were kept under standard culture conditions (37° C, 5% CO_2, saturated humidity). The culture medium was exchanged every 24 hours and medium samples were stored at –80°C until further analysis. The encapsulated islet constructs and the non-encapsulated controls were exposed to an intermittent glucose challenge, adding culture medium with a glucose concentration of 400 mg/dl. To test the response to the glucose challenge, the insulin concentration of the medium samples was measured.

Insulin measurement

The insulin content of all medium samples of all groups was measured using a radio-immuno-assay (RIA) for insulin.

Histology

Hematoxylin and Eosin (H&E) staining was performed for histological evaluation, assessing the viability and morphological integrity of the islets of Langerhans as well as the structure of the formed cartilage.

Heidenhain staining was performed to differentiate the different cell populations (α-, β-, and δ-cells) within the islets of Langerhans. The α-cells can be depicted by their shiny red granules. The β-cells stain yellow-orange with gray-orange granules. The δ-cells display a typical blue color.

Toluidin blue specifically stains components of the cartilage matrix (i.e. glycosaminoglycans) and was used to evaluate the maturity and structure of the chondrocyte capsule.

Immunohistochemical staining for insulin was carried out to demonstrate the maintained insulin production within the β-cells of the islets of Langerhans, using a guinea pig polyclonal anti-insulin antibody and counter staining with Gill's Hematoxylin.

Results

1. Viable primary chondrocytes could be isolated from bovine, porcine, and rat articular cartilage and cultured to form a confluent and scrapable membrane.

2. Between 300 and 1100 islets of Langerhans were isolated per rat pancreas. The islets, seeded on PGA-polymer matrix attached to the polymer fibers.

3. A method of islet encapsulation with a chondrocyte membrane was successfully developed. The quality of encapsulation could be confirmed using phasecontrast microscopy. All capsules were intact without damage or wholes.

Bovine chondrocyte encapsulation

4.1. From one calf joint an average of 18 ± 3 g of cartilage pieces was harvested. After digestion and filtration 5.5 ± 1.5 x 10^6 chondrocytes with a viability of 98 ± 1 % could be retained. After seeding 5.0 x 10^5 viable bovine chondrocytes per culture dish, it took between 3 to 4 weeks in culture to form a confluent scrapable monolayer of bovine chondrocytes and their matrix.

4.2. During the initial observation period of 6 days insulin levels could be measured in all of the encapsulated and non-encapsulated constructs. An increase in insulin concentration in the culture medium after glucose challenge on days 3 and 5 could be demonstrated.

4.3. During the entire second observation period of 30 days insulin levels could be measured in all of the encapsulated and non-encapsulated constructs.

4.4. H&E histology demonstrated viability and intact morphology of the chondrocyte membrane encapsulated islets of Langerhans, completely covered with several layers of bovine chondrocytes of up to 6 weeks in culture. It appears as if the islets integrated the PGA polymer fibers, with strong affinity to the biodegradable material. There were no signs of cell necrosis or damage. The chondrocyte membrane displayed the typical features of cartilage morphology.

4.5. Immunohistochemistry showed positive staining for insulin within the β-cells of the islets after 6 weeks in culture.

4.6. Two weeks after implantation into the subcutaneous space of nude mice the encapsulated constructs could easily be recovered. H&E histology demonstrated viability of the islets of Langerhans completely covered with several layers of chondrocytes. Cartilage specific matrix was deposited. Immunohistochemistry showed positive staining for insulin within β-cells of the encapsulated islets.

Porcine chondrocyte encapsulation

5.1. From one porcine elbow joint an average of 24 g of cartilage pieces was harvested with an average yield of 9.3×10^6 chondrocytes per joint with a viability of 98 %. Seeding 5.0×10^5 viable chondrocytes per cell culture dish, it took between 3 to 10 weeks of culture to form a confluent scrapable monolayer of porcine chondrocytes and their matrix.

5.2. Insulin levels could be measured in all of the encapsulated and non-encapsulated constructs during the entire observation period of 35 days. There was an obvious increase of the insulin concentration in the culture medium after the glucose challenges.

5.3. H&E histology demonstrated viability of the islets of Langerhans after 35 days in culture for both the encapsulated and non-encapsulated groups. The islets were attached to the PGA-polymer fibers. In the encapsulated constructs an intact layer of neo-cartilage could be seen.

5.4. Heidenhain staining showed an intact micro-morphology of the islets.

5.5. Toluidin blue staining proved the presence of viable chondrocytes surrounded by the positively stained specific components of the chondrocyte matrix.

5.6. Immunohistochemistry for insulin confirmed insulin production within the β-cells of the islets after the 35 days of culture in both the encapsulated and non-encapsulated groups.

Rat chondrocyte encapsulation

6.1. From the joints of one 14 days old Lewis rat an average yield of $1.8 \pm 0.3 \times 10^6$ chondrocytes with a viability of 95 % could be retained. Seeding 5.0×10^5 viable chondrocytes per culture dish, it took between 4 to 7 weeks of culture to form a confluent scrapable monolayer of rat chondrocytes and their matrix.

6.2. Insulin levels could be measured in all of the encapsulated and non-encapsulated constructs during the entire observation period of 30 days. There was an obvious increase of insulin secretion after the glucose challenges.

6.3. H&E histology showed viable, completely encapsulated islets after 30 days in culture. The islets were individually attached to the PGA fibers.

6.4. Immunohistochemistry for insulin showed positive staining for insulin within the β-cells.

6.5. The intact micromorphological structure of the islets (α-, β- and δ–cells) could also be demonstrated by Heidenhain staining.

6.6. Toluidin blue staining revealed viable cartilage surrounding the islets.

Conclusions

1. We were able to isolate chondrocytes from three different species
   - bovine
   - porcine and
   - rat,

   and could grow them in culture to form a confluent and intact membrane.

2. A new immunoisolation method of encapsulation of islets of Langerhans using this chondrocyte membrane was developed and reliably reproduced. In contrast to other current experimental immunoisolation strategies, which use artificial materials and thereby induce an unspecific foreign body reaction leading to a destruction of the transplanted cells inside the capsule, we used cartilage, as encapsulation material. Cartilage is an avascular tissue and the chondrocyte matrix provides an immunoprivileged environment.

3. Applying our encapsulation method, chondrocyte membrane encapsulated islets of Langerhans continued to secrete insulin for at least 30 days in culture, preserving their functional activity.

4. The glucose-insulin feedback mechanism of the encapsulated islets of Langerhans remained intact for at least 3 weeks in culture, indicating intact specific function.

5. Morphological analysis, such as
   - H&E histology,
   - Immunohistochemistry,
   - Toluidin blue staining,
   - Heidenhain staining

   demonstrated viability and intact micromorphology of the encapsulated islets, and the surrounding chondrocyte capsule during the entire culture period, as evidence of preserved tissue specific function.

6. Bovine chondrocyte membrane encapsulated islets of Langerhans preserved viability and function after implantation into nude mice.

7. We can conclude, that encapsulation of cells within autologous avascular tissue, providing a semipermeable barrier is a promising immunoisolation strategy avoiding immunosuppression and the unspecific immune reaction against the capsule material.

8. We propose, that for clinical application the chondrocytes could be isolated from a cartilage biopsy from the later recipient of the islet graft.

9. In our experiment we were able to successfully encapsulate up to 1000 islets per cm^2. We estimate that up to 8000 islets per cm^2 could be encapsulated. This means that for clinical application with a necessity to transplant approximately 10000 islets per kg body weight to cure diabetes mellitus, capsules of 1.25 to 10 cm^2 per kg body weight would be necessary.

10. We consider our autologous-cell-encapsulation immunoisolation technique for islet transplantation a promising and applicable method for the treatment of patients with diabetes mellitus, which is the most frequent metabolic disorder in the developed countries.

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Publications

Sano K, Cusick RA, Lee HM, Pollok JM, Kaufmann PM, Uyama S, Mooney D, Langer R, Vacanti JP: Regenerative signals for heterotopic hepatocyte transplantation. Transplant Proc 28(3):1857-1858, 1996.

Pollok JM, Ibarra C, Vacanti JP: A new method of xenotransplantation using autologous cartilage as an immunoisolation barrier for the transplantation of xenogeneic Islets of Langerhans. Transplant Proc 29(1-2):909-911, 1997.

Pollok JM, Ibarra C, Vacanti JP: Immunoisolation of xenogeneic Islets using a living tissue engineered cartilage barrier. Transplant Proc 29(4):2131-2133, 1997.

Cusick RA, Lee H, Sano K, Pollok JM, Utsunomiya H, Ma PX, Langer R, Vacanti JP: The effect of donor and recipient age on engraftment of tissue engineered liver. J Pediatr Surg 32(2):357-360, 1997.

Pollok JM, Ibarra C, Broelsch CE, Vacanti JP: Immunisolation xenogener Langerhansscher Inseln in einer mittels Tissue Engineering geformten autologen Knorpel-Kapsel. Zentralbl Chir 123:830-833, 1998.

Pollok JM, Kluth D, Cusick RA, Lee H, Utsunomiya H, Ma PX, Langer R, Broelsch CE, Vacanti JP: Formation of spheroidal aggregates of hepatocytes on biodegradable polymers under continuous flow bioreactor conditions. Eur J Ped Surg 8:195-199, 1998.

Kaufmann PM, Kneser U, Fiegel HC, Pollok JM, Kluth D, Izbicki JR, Herbst H, Rogiers X: Is there an optimal concentration of cotransplanted islets of Langerhans for stimulation of hepatocytes in three dimensional matrices? Transplantation 68(2):272-279, 1999.

Pollok JM, Begemann JF, Kaufmann PM, Kluth D, Broelsch CE, Izbicki JR, Rogiers X: Long-term insulin secretory function of Islets of Langerhans encapsulated with a layer of confluent chondrocytes for immunoisolation. Ped Surg Int 15(3-4):164-167, 1999.

Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Kluth D, Herbst H, Rogiers X: Heterotopic hepatocyte transplantation utilizing pancreatic islet co-transplantation for hepatotrophic stimulation: Morphologic and morphometric evaluation. Ped Surg Int 15(3-4):168-174, 1999.

Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Rogiers X, Kluth D, Herbst H: Interaction of hepatocytes and pancreatic islets cotransplanted in polymeric matrices. Virchows Archiv 435:125-132, 1999.

Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Kluth D, Izbicki JR, Herbst H, Rogiers X: Long term differentiated function of heterotopically transplanted hepatocytes on three-dimensional polymer matrices. J Biomed Mater Res; 47(4):494-503, 1999.

Begemann JF, Pollok JM, Kaufmann PM, Kluth D, Broelsch CE, Rogiers X: In vitro demonstration of survival and secretory function of islets of Langerhans encapsulated with a membrane of tissue engineered chondrocytes for immunoisolation. Transplant Proc 31(8):3250-3252, 1999.

Kaufmann PM, Fiegel HC, Kneser U, Pollok JM, Kluth D, Rogiers X: Influence of pancreatic islets on growth and differentiation of hepatocytes in co-culture. Tissue Eng 5(6):583-596, 1999.

Török É, Pollok JM, Ma PX, Kaufmann PM, Dandri M, Petersen J, Burda M, Kluth D, Perner F, Rogiers X: Optimization of hepatocyte spheroid formation for hepatic tissue engineering on 3-dimensional biodegradable polymer within a flow bioreactor prior to implantation. Cells Tissues Organs 169:34-41, 2001.

Pollok JM, Kölln PA, Lorenzen M, Török É, Kaufmann PM, Kluth D, Bohuslavizki KH, Gundlach M, Rogiers X: Islets of Langerhans encapsulated with a tissue engineered membrane of rat-chondrocytes maintain insulin-secretion and glucose-insulin feedback for at least 30 days in culture. Transplant Proc 33(1-2):1713-1714, 2001.

Dandri M, Burda MR, Török É, Pollok JM, Iwanska A, Sommer G, Rogiers X, Rogler CE, Gupta S, Will H, Greten H, Petersen J: Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology 33:981-988, 2001.

Török É, Pollok JM, Ma PX, Vogel C, Dandri M, Pertersen J, Burda M, Kaufmann PM, Kluth D, Rogiers X: Hepatic tissue engineering on 3-dimensional biodegradable polymers within a pulsatile flow bioreactor. Digestive Surgery (in press) 2001.

Pollok JM, Lorenzen M, Kölln PA, Török É, Kaufmann PM, Kluth D, Bohuslavizki KH, Gundlach M, Rogiers X: In-vitro function of islets of Langerhans, encapsulated with a membrane of porcine chondrocytes for immunoisolation. Digestive Surgery (in press) 2001.