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Repopulation of porcine kidney scaffold using porcine primary renal cells

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ABSTRACT

The only definitive treatment for end stage renal disease is renal transplantation, however the current shortage of organ donors has resulted in a long list of patients awaiting transplant. Whole organ engineering based on decellularization/recellularization techniques has provided the possibility of creating engineered kidney constructs as an alternative to donor organ transplantation. Previous studies have demonstrated that small units of engineered kidney are able to maintain function *in vivo*. However, an engineered kidney with sufficient functional capacity to replace normal renal function has not yet been developed. One obstacle in the generation of such an organ is the development of effective cell seeding methods for robust colonization of engineered kidney scaffolds. We have developed cell culture methods that allow primary porcine renal cells to be efficiently expanded while maintaining normal renal phenotype. We have also established an effective cell seeding method for the repopulation of acellular porcine renal scaffolds. Histological and immunohistochemical analyses demonstrate that a majority of the expanded cells are proximal tubular cells, and the seeded cells formed tubule-like structures that express normal renal tubule phenotypic markers. Functional analysis revealed that cells within the kidney construct demonstrated normal renal functions such as re-adsorption of sodium and protein, hydrolase activity, and production of erythropoietin. These structural and functional outcomes suggest that engineered kidney scaffolds may offer an alternative to donor organ transplant.

Statement of Significance

Kidney transplantation is the only definitive treatment for end stage renal disease, however the current shortage of organ donors has limited the treatment. Whole organ engineering based on decellularization/recellularization techniques has provided the possibility of creating engineered kidney constructs as an alternative to donor organ transplantation. While previous studies have shown that small units of engineered kidneys are able to maintain function in animal studies, engineering of kidneys with sufficient functional capacity to replace normal renal function is still challenging due to inefficient cell seeding methods. This study aims to establish an effective cell seeding method using pig kidney cells for the repopulation of acellular porcine kidney scaffolds, suggesting that engineered kidneys may offer an alternative to donor organ transplant.

1. Introduction

Chronic kidney disease (CKD) is defined as a reduced glomerular filtration rate, increased urinary albumin excretion, or both, and is a significant public health issue [1]. Roughly 1 in 10 adult Americans has CKD, and these patients are at risk for decreased quality and length of life [2]. CKD may progress to end stage renal disease (ESRD), and the number of patients with ESRD is increasing in the United States. This is, in part, due to the recent increase in the rate of diabetes [3]. Current therapies for this condition are dialysis and renal transplantation [3,4]. Dialysis is not an effective long-term option, as only 50% of the US dialysis patients are still alive 3 years

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after start of dialysis [5]. Renal transplantation is the only definitive treatment for ESRD [4]. However, a shortage of donor organs and increasing demand for renal transplantation has resulted in a long list of patients awaiting transplant. To address this shortcoming, development of an engineered, transplantable kidney has been proposed.

Recent studies in the field of tissue engineering have demonstrated that whole organ engineering is a viable strategy for the generation of transplantable organ constructs, and that this technology may be used to create engineered kidneys. The whole organ engineering technique is based on the decellularization of native organs and recellularization of the acellular organ scaffold [6–10]. The purpose of "decellularization" of native organs is to remove cellular components which cause an immunogenic responses upon implantation [10]. Current decellularization methods are based on the removal of cells by detergents or enzymes while leaving the extracellular matrix and vascular basement membranes intact. The acellular matrix is then recellularized with organ-specific cell types that repopulate the organ and restore organ function. Recently, the successful implantation of a bioengineered rat kidney showed the feasibility of using the decellulariza tion/recellularization approach for the generation of renal tissue [11–13]. Several cell sources have been tested for the recellularization of acellular kidney matrices, including embryonic stem cells [13] and explanted renal tissue [14]. While these preliminary experiments suggest that functional kidney could be engineered through recellularization of a renal matrix, the field of whole organ tissue engineering is still in its infancy, and numerous challenges have to be addressed before these techniques can be used in the clinic.

One of critical challenges for clinical translation is establishment of effective recellularization of acellular kidney by functional renal cells. The use of embryonic stem cells [12,13], which has pluripotent cell capability, in terms of cell growth as well as multi-differentiation has been tested for recellularization of kidney scaffold. However, teratoma (tumor) formation and ethical issues have limited the potential for clinical application. The use of patient's own cells can address the limitation raised by ESC. To this end, induced pluripotent stem cells (iPSC) can be considered as another promising cell source for the whole kidney construction. Recent progress in the cell culture and differentiation technique resulted in the iPSC-derived renal population such as tubular cells and podocytes [15,16]. However, the safety issue such as teratoma formation still needs to be resolved prior to clinical application. Another issue is the lack of renal structure formation resulting in limited renal function. A recent study has demonstrated the feasibility of implanting engineered rat kidney [11], however the cell seeding technique employed in the study did not produce fully functional renal structures within the renal scaffold. Intravascular and ureter cell injection used in this study resulted in repopulation of the glomerular compartments, but no recellularization of the renal tubules [17]. Therefore, efficient recellularization techniques that restore functional renal structures have yet to be developed.

Towards this end, the current study aims to establish an effective recellularization technique using appropriate cell sources that are capable of creating fully functional renal tubular structures within acellular kidney scaffolds. To achieve this goal, we developed a cell isolation and expansion method that produces a sufficient number of porcine primary renal cells for the repopulation of acellular porcine renal scaffolds. The use of primary renal cells from patients can be considered as a practical cell source that can eliminate safety and ethical issues from the ESC and iPSC. These cells are then applied to the acellular renal scaffold using a novel technique that results in the formation of functional renal tubular structures. The microarchitecture of the recellularized constructs were confirmed by histological and immunohistochemical analysis. Additionally, the functional capacity of the engineered kidneys was determined by electrolyte and protein adsorption, hydrolase activity, and erythropoietin production. The results of this study indicate that functional engineered kidneys generated by the described methods may someday be used to restore renal function in patients with ESRD.

2. Materials and methods

2.1. Culture of primary porcine renal cells

Kidneys harvested from Yorkshire pigs (female, 40–50 kg, 3–6 months) (n = 10 pigs) were used for renal cell isolation. All surgical procedures were performed in accordance with Wake Forest University Animal Care and Use Committee approved protocols. Following kidney harvest and subsequent removal of the renal capsule and renal pelvis, the kidney cortical tissue, separated from the medulla, was minced and digested at 37 °C under mild shaking in an enzyme solution containing 1% Liberase Blendzyme (Roche, Mannheim, Germany) and 1% antibiotic/antimycotic (HyClone, South Logan, UT) in PBS buffer. Following 45 min digestion, the tissue digestion was stopped by mixing the enzyme solution with same volume of growth medium, followed by filtering through a 100 micron cell strainer (BD Biosciences, San Jose, CA) and centrifugation. The supernatant was removed and the centrifuged cells were re-suspended in renal cell media, followed by plating in tissue culture dishes.

The renal cell media is composed of 1:1 mixture of the following media: one part keratinocyte serum free media (Gibco® Grand Island, NY USA) containing 2.5% fetal bovine serum (FBS) (HyClone), 1% Penicillin–Streptomycin (P/S) Solution (HyClone), 0.4% ITS (Insulin, Transferrin, Selenium) liquid media (Sigma–aldrich, St. Louis, MO), and supplements for Keratinocyte serum free media (Epidermal Growth Factor, Bovine Pituitary Extract) (Gibco®), and one part DMEM high glucose media (HyClone) containing 10% FBS and 1% P/S Solution.

For primary culture, the media was changed at day 4 and cells were 90% confluent by day 7. For cell passaging, 0.05% trypsin (HyClone) was used to detach cells from the culture dishes. The cell culture was maintained at 37 °C, supplemented with 20% O_2, 5% CO_2, and 30 mL of growth medium were added to a 15 cm culture dish. During the cell culture, the cell morphology was examined by a microscopic observation.

2.2. Characterization of the primary renal cells

The proliferative capability of the isolated renal cells was determined by counting cells at each passage (every 3–4 days) during 13 days of culture. For each passage, an initial cell density was 10^6 cells in a 15 cm culture dish (140 cm^2) (Corning). To confirm renal specific phenotypes, immunofluorescence was performed on the cultured cells using several renal cell markers including: aquaporin 1 (Aqp1), aquaporin 2 (Aqp2), aquaporin 4 (Aqp4), Ezrin, and podocin, which are expressed by proximal tubular and descending Loop of Henle cells, Loop of Henle and distal tubular cells, distal tubular and collecting duct cells, and proximal tubular cells, podocyte/tubular cell population [18], and podocyte respectively. The cells on monolayer were fixed with 2% paraformaldehyde (Polysciences Inc., Washington, PA) at 4 °C for 10 min. After antigen retrieval using cold methanol and subsequent non-specific antigen blocking, the treated cells were incubated with primary antibodies for 1 h at room temperature, followed by treatment with Alexa 594 conjugated secondary antibodies (Invitrogen). Cells were subsequently imaged with a

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