A step towards clinical application of acellular matrix: A clue from macrophage polarization

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Abstract

The outcome of tissue engineered organ transplants depends on the capacity of the biomaterial to promote a pro-healing response once implanted in vivo. Multiple studies, including ours, have demonstrated the possibility of using the extracellular matrix (ECM) of animal organs as platform for tissue engineering and more recently, discarded human organs have also been proposed as scaffold source. In contrast to artificial biomaterials, natural ECM has the advantage of undergoing continuous remodeling which allows adaptation to diverse conditions. It is known that natural matrices present diverse immune properties when compared to artificial biomaterials. However, how these properties compare between diseased and healthy ECM and artificial scaffolds has not yet been defined.

To answer this question, we used decellularized renal ECM derived from WT mice and from mice affected by Alport Syndrome at different time-points of disease progression as a model of renal failure with extensive fibrosis. We characterized the morphology and composition of these ECMs and compared their in vitro effects on macrophage activation with that of synthetic scaffolds commonly used in the clinic (collagen type I and poly-L-(lactic) acid, PLLA).

We showed that ECM derived from Alport kidneys differed in fibrous protein deposition and cytokine content when compared to ECM derived from WT kidneys. Yet, both WT and Alport renal ECM induced macrophage differentiation mainly towards a reparative (M2) phenotype, while artificial biomaterials towards an inflammatory (M1) phenotype. Anti-inflammatory properties of natural ECMs were lost when homogenized, hence three-dimensional structure of ECM seems crucial for generating an anti-inflammatory response. Together, these data support the notion that natural ECM, even if derived from diseased kidneys promote a M2 protolerogenic macrophage polarization, thus providing novel insights on the applicability of ECM obtained from discarded organs as ideal scaffold for tissue engineering.

Introduction

Chronic kidney disease (CKD) has reached pandemic levels in the last decade [1]. Unfortunately, etiology and pathophysiology are unclear and treatment is often inadequate. Ideally, patients with CKD should receive therapies aimed at counteracting disease causes, as well as at enhancing endogenous repair mechanisms. However once patients reach end stage renal disease (ESRD) kidney transplantation is

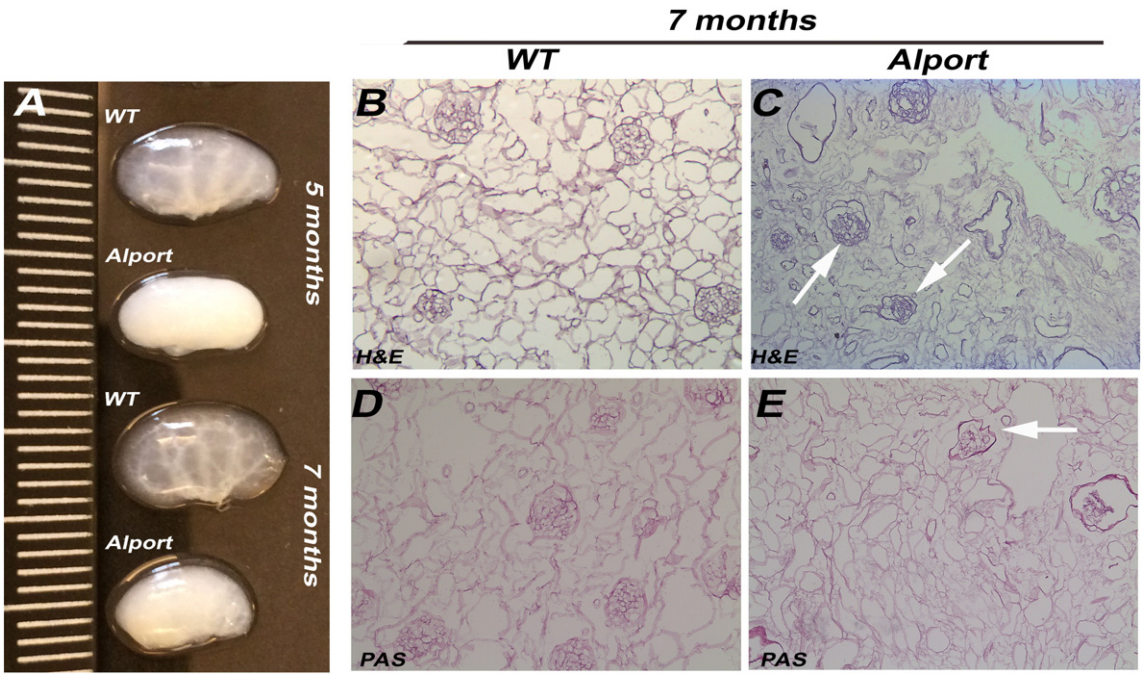

Fig. 1. Histological characterization of mrECM from WT and Alport mice. A. Representative images of WT and Alport kidneys at 5 and 7 months of age. Alport kidneys appear smaller in size and opaque while WT kidneys appear translucent (measurement in cm). B–E. Representative bight field images of H&E (B–D) and PAS staining (C–E) of WT mrECM at 7 months (B, D) and Alport mrECM at 7 months (C, E) showing differences in matrix deposition between later stage disease and WT matrices. Alport matrix showed disorganization of the glomerular structure (arrows) and vessels. Magnification 20×.

the optimal treatment option, but this opportunity is limited by an inadequate supply of transplantable grafts and by chronic toxicity of lifelong immunosuppression [2]. Therefore, many patients remain on dialysis instead of benefiting from a renal transplant, while the mortality rate of patients on the waiting list is progressively increasing [3].

Ex vivo organ/tissue bio-fabrication has been proposed as a strategy to increase the transplantable organ pool. So far, translation of tissue engineering technologies to clinical nephrology has been hampered – among other factors – by the lack of scaffolds capable of mimicking morphological and physiological characteristics of the kidney extracellular matrix (ECM) and securing a pro-healing response after transplantation [4].

In the past decade, acellular ECM scaffolds obtained from animal or human kidneys by decellularization have offered a valuable platform for kidney tissue engineering. The rationale for using natural scaffolds lies on the evidence that the ECM defines the physical and chemical interactions that control cellular physiology and fate and provides mechanical and structural support to cells and tissues [5]. While initial experiments focused on the rodent [4,6–8], porcine [9,10]; and non-human primate ECMs [11–12], we proposed the use of human discarded kidneys as a source of acellular ECM scaffolds [4,13].

The ECM of human kidneys seems to be an extremely useful biomaterial for tissue engineering specifically because they maintain the framework of the innate vasculature intact, which is critical for implantation in vivo. Preliminary investigations have shown that such ECM can be successfully and consistently produced from discarded kidneys [4,13] that are devoid of immunogenic cell membrane proteins but retain their complex architecture, gross molecular composition and numerous growth factors (GFs) [14], which in turn provide a bioactive environment for stem cells cellular activity [15].

Shortly after implantation, scaffolds are infiltrated by immune cells that can modulate the inflammatory response via paracrine and autocrine signaling.

Among them, macrophages are shown to be actively involved in the initial immune response [16–18]. In particular, the delicate balance between their pro-inflammatory (M1) and reparative (M2) phenotype is key for the fate of the graft and functional regeneration in vivo [16–18].

In this work, we first studied structure and composition of kidney matrices derived from WT mice and from mice affected by Alport Syndrome, where a mutation in the collagen IVα5 gene causes an altered matrix deposition within the glomerulus with consequent renal fibrosis, chronic inflammation and kidney failure [19]. We investigated macrophage