American Journal of Pathology, Vol. 161, No. 3, September 2002

Technical Advance

A New Method for Large Scale Isolation of Kidney Glomeruli from Mice

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Here we report a new isolation method for mouse glomeruli. The method is fast and simple and allows for the isolation of virtually all glomeruli present in the adult mouse kidney with minimal contamination of nonglomerular cells. Mice were perfused through the heart with magnetic 4.5-μm diameter Dynabeads. Kidneys were minced into small pieces, digested by collagenase, filtered, and collected using a magnet. The number of glomeruli retrieved from one adult mouse was 20,131 ± 4699 (mean ± SD, $n = 14$) with a purity of 97.5 ± 1.7%. The isolated glomeruli retained intact morphology, as confirmed by light and electron microscopy, as well as intact mRNA integrity, as confirmed by Northern blot analysis. The method was applicable also to newborn mice, which allows for the isolation of immature developmental stage glomeruli. This method makes feasible transcript profiling and proteomic analysis of the developing, healthy and diseased mouse glomerulus. ***(Am J Pathol 2002, 161:799–805)***

As the genome projects are near completion,[1,2] an important step in the functional analysis of genome data are the determination of transcriptomes corresponding to specific cellular functions and states of differentiation. Such analyses require methods allowing for the isolation of highly homogenous population of cells and/or microorgans from *in vivo* situations. One such microorgan is the kidney glomerulus. Glomeruli constitute ~10% of whole kidney tissues and are unique structures of microvasculature mainly made up of three highly specialized cell types; fenestrated endothelial cells, mesangial cells, and podocytes. These cell types together with the glomerular basement membrane form the permeable barrier across which blood is filtered to produce primary urine. During the past decade several gene products have been shown to play essential roles in glomerulus development,[3–5] function, and pathology.[6] However, our knowledge of the molecular mechanisms governing glomerulus morphogenesis and development of the specialized features of its individual cells is still very limited. An obvious difficulty in addressing these issues stems from the low abundance of the glomerulus cells and the inability of the glomerulus cell types to retain their differentiated features in cell culture. Podocytes, for example, make up less than 2% of kidney tissues. Although endothelial cells and pericytes exist outside the glomerulus, their phenotype within the glomerulus is quite distinct from related cells elsewhere.[7]

We describe a new protocol for the isolation of glomeruli from mice. The protocol is fast and allows for the isolation of virtually all glomeruli present in a mouse kidney at 97% purity. The method thus allows for transcript profiling and proteomic analysis of the glomerulus using standard procedures.

Materials and Methods

Reagents

Collagenase A was purchased from Roche (Roche Diagnostics GmbH, Mannheim, Germany). Deoxyribonuclease I and Hanks' balanced salt solution (HBSS) were from Invitrogen (Invitrogen AB, Lidingö, Sweden). Dynabeads M-450 tosylactivated (ϕ 4.5 μm) and magnetic particle concentrator (MPC) were from Dynal (Dynal A.S., Oslo, Norway). The surface of the Dynabeads was inactivated

Supported by grants from the Swedish Cancer Foundation, Vetenskapsrådet, the Inga-Britt and Arne Lundberg Foundation, the Novo Nordisk Foundation, and an European Molecular Biology Organization (EMBO) postdoctoral fellowship (to H. G.).

Accepted for publication May 28, 2002.

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according to manufacturer's instructions before use. Cell strainers were from Falcon (BD Biosciences, Stockholm, Sweden). The mouse nephrin cDNA was kindly provided by Dr. Heli Putaala of Karolinska Institute, Stockholm, Sweden. The mouse Tie 2 cDNA was kindly provided by Dr. Tom Sato of University of Texas Southwestern Medical Center, Dallas, TX.

Mice

Mice used were C57BL6 and 129/sv or hybrids between the two. Mice were housed at the Department of Experimental Biomedicine at Göteborg University according to Swedish animal research regulations. All experiments were approved by a local committee for ethics in animal research.

Isolation of Glomeruli

Mice were anesthetized by an intraperitoneal injection of Avertin (2,2,2-tribromoethyl and tertiary amyl alcohol; 17 μl/g mice) and perfused with 8×10^7 Dynabeads diluted in 40 ml of phosphate-buffered saline through the heart. The kidneys were removed, minced into 1-mm^3 pieces, and digested in collagenase (1 mg/ml collagenase A, 100 U/ml deoxyribonuclease I in HBSS) at 37°C for 15 minutes (for newborn mice) or 30 minutes (for adult mice) with gentle agitation. The collagenase-digested tissue was gently pressed through a 100-μm cell strainer using a flattened pestle and the cell strainer was then washed with 5 ml of HBSS. The filtered cells were passed through a new cell strainer without pressing and the cell strainer washed with 5 ml of HBSS. The cell suspension was then centrifuged at $200 \times g$ for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in 2 ml of HBSS. Finally, glomeruli containing Dynabeads were gathered by a magnetic particle concentrator and washed for at least three times with HBSS. During the procedure, kidney tissues were kept at 4°C except for the collagenase digestion at 37°C.

Morphological Studies

Dynabead-perfused kidneys were snap-frozen for cryostat sectioning. Sections were stained with hematoxylin and eosin (H&E) and were examined by light microscopy. Isolated glomeruli were examined by both light and electron microscopy (EM). Specimens for EM were fixed with 2% paraformaldehyde and 2.5% glutaraldehyde. Glomeruli intended for transmission EM were subjected to ferrocyanide-reduced OsO_4, dehydration, and plastic infiltration before ultrathin sectioning. For scanning EM glomeruli were osmicated according to the OTOTO protocol[8] and dried using hexamethyldisilazane evaporation.[9]

Analysis of RNA Integrity

Total RNA was isolated using the RNase mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instruction. Northern blot analysis was performed as described previously[10] using ^{32}P-labeled nephrin cDNA, Tie 2 cDNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-actin cDNA probes.

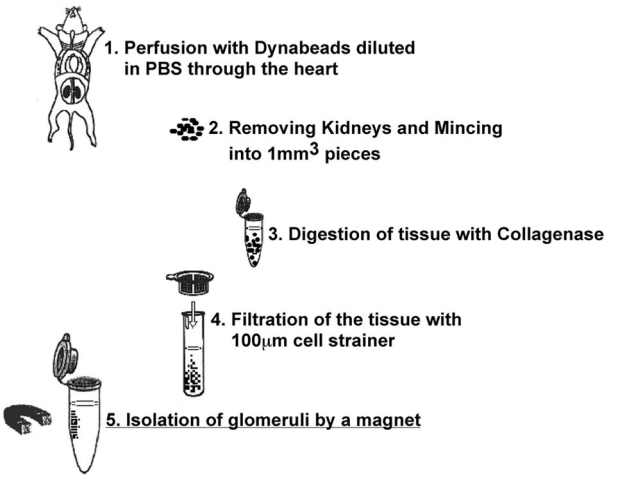

Figure 1. Flow chart of the isolation technique of mice glomeruli.

Results

Isolation of mice glomeruli was performed by perfusion of spherical superparamagnetic beads, termed Dynabeads (Ø 4.5 μm) through the heart (Figure 1). Kidneys were then removed, minced, and digested in collagenase followed by filtration. H&E staining of the kidneys from mice perfused by Dynabeads revealed that the beads were mainly distributed in the glomeruli, and that only a few beads could be detected in the surrounding renal tissues (Figure 2, A and B). Dynabeads targeted all glomeruli, irrespective of their location in the kidney cortex (data not shown). Collagenase digestion of the kidney had little effect on the glomerular structure. Dynabeads accumulated in the glomerular vessels, making the glomeruli easy to isolate using a magnet with a low degree of contaminating tissues (Figure 2; C to E). Almost all glomeruli isolated were lacking the Bowman's capsule and some of them had part of the afferent and/or efferent arterioles still attached. Scanning EM revealed that the glomeruli had retained a roughly spherical shape through the isolation procedure. The capillary loops were kept in close contact and were covered by an intact podocyte coat with seemingly undisturbed topography of the primary and interdigitating secondary foot processes (Figure 3, A and B). Transmission EM on sections confirmed the preservation of the structural elements of the filtration barrier. The podocyte foot processes formed intact filtration slits with bridging diaphragms and adhered closely to the basal lamina. Only occasionally, minor detachment of podocytes could been seen. Also the fenestrated glomerular endothelium adhered to the basal lamina as *in vivo*, but the endothelial cells often showed a highly convoluted surface toward the capillary lumina with numerous cytoplasmic protrusions

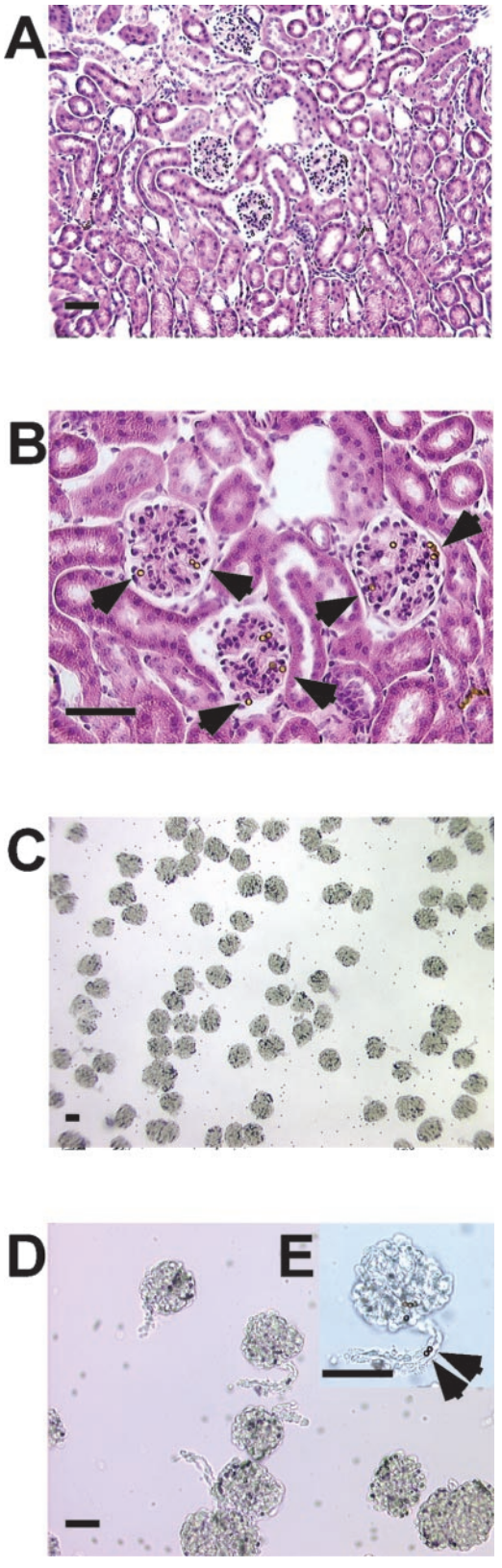

Figure 2. A and **B:** Histological examination of adult mouse kidney after Dynabeads perfusion. A 4-week-old mouse was perfused with magnetic beads through the heart. Kidneys were removed, snap-frozen, and stained with H&E. **C** and **D:** Histological examination of mice glomeruli subjected to magnetic bead perfusion. An adult mouse was perfused with magnetic beads through the heart and glomeruli were isolated. **Arrowheads** indicate Dynabeads. Scale bars, 50 μm.

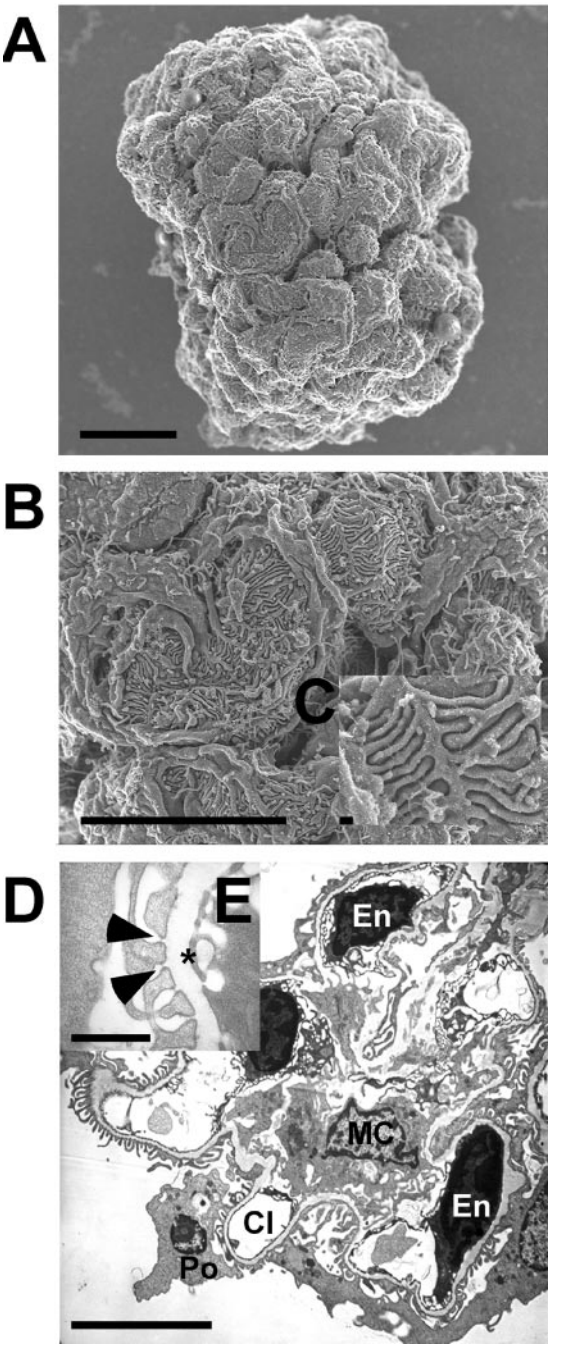

Figure 3. Electron micrographs of isolated adult mice glomeruli. **A:** Scanning EM recording at low magnification demonstrates the closed, spherically bulging, shape of a whole glomerulus. **B** and **C:** At higher magnifications the intricate pattern of the interdigitating pericyte foot processes is shown. Note the constant width of the filtration slit in **C**. **D:** Transmission EM overview of transected glomerulus including capillaries and a mesangial cell (MC). One podocyte (Po) and a capillary lumen (Cl) are indicated, other lumina are partly collapsed. The different tissue elements remain in close contact after isolation. **E:** Detailed view of the filtration barrier including part of fenestrated endothelium, an apparently unaltered basal lamina (**asterisk**), and filtration slits with diaphragms (**arrowheads**). Scale bars: 10 μm (**A**, **B**); 100 nm (**C**, **E**); 2.5 μm (**D**).

(Figure 3, D and E). Frequently, Dynabead particles occupied the capillaries. The subcellular organization of cytoplasm- and organelle-rich cells, the podocytes and the mesangial cells, appeared normal, and the

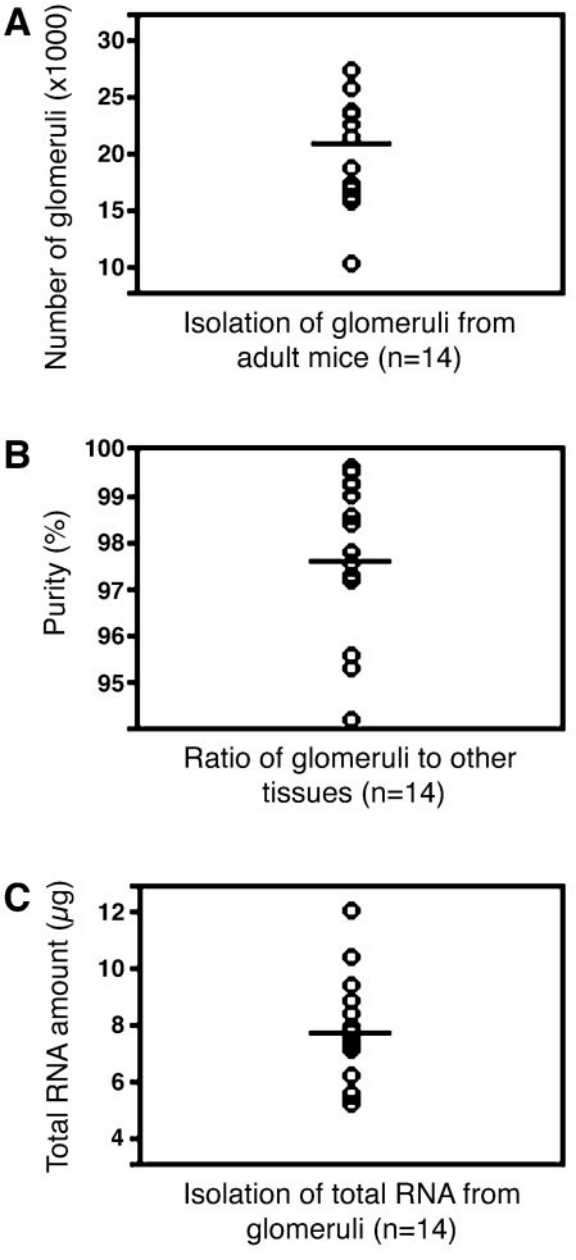

Figure 4. Yield and purity of isolated glomeruli. Fourteen mice, 3 to 24 weeks of age, were perfused with magnetic beads and glomeruli were isolated. The number of glomeruli and the purity were counted at least three times for each mouse and the mean value plotted. **A:** Number of glomeruli isolated by Dynabeads perfusion per adult mouse. **B:** Purity of isolated glomeruli. **C:** Total RNA in isolated glomeruli. RNA was extracted from the glomeruli and the amount of RNA from each mouse plotted. Bars indicate the mean value of 14 mice.

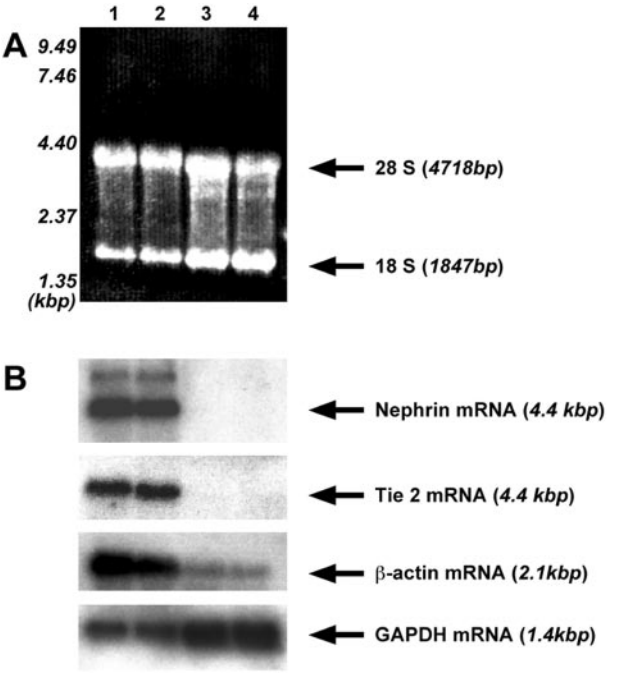

Figure 5. Evaluation of RNA integrity and gene expression. **Lanes 1** and **2** contain 8 μg of total RNA from glomeruli isolated using Dynabeads perfusion. **Lanes 3** and **4** contain 10 μg of total RNA from snap-frozen whole kidney. **A:** Electrophoresis EtBr staining confirm integrity of the isolated RNA. **B:** Northern blot analysis of RNA from glomeruli. Nephrin cDNA probe was used as a podocyte marker and Tie 2 cDNA probe was used as a capillary endothelial marker. β-actin and GAPDH cDNA probes were used to assess expression of housekeeping genes.

interstitial volume of the mesangium did not seem widened as compared to an intravital situation.

By counting the glomeruli under a light microscope, the number of glomeruli collected from one adult mouse was estimated to 20,131 $\pm$ 4699 and the estimated purity was to 97.5 $\pm$ 1.7% (Figure 4, A and B). Because the number of isolated glomeruli correspond approximately to the number of glomeruli known to exist in mouse kidney,[11] we conclude that our method allows for isolation of most glomeruli present in a mouse kidney, and with minimal contamination of nonglomerular tissue.

The amount of total RNA retrieved from isolated glomeruli of one adult mouse was estimated to 7.9 μg on average (Figure 4C). The integrity of the RNA was kept high as there were no signs of RNA degradation during our procedure (Figure 5). Northern blot analysis confirmed RNA integrity as well as enrichment of glomeruli. We used nephrin transcripts as a marker for podocytes and transcripts for the tyrosine kinase receptor tie-2 as a marker for endothelial cells. Complementary DNAs corresponding to both these mRNAs recognized abundant transcripts in glomerulus total RNA but not in whole kidney total RNA (Figure 5; weak signals were seen also in whole kidney RNA on prolonged exposure; data not shown). Ethidium bromide staining and hybridization against GAPDH and β-actin probes verified the equal integrity and loading of the RNA samples.

As the glomeruli continue to form and differentiate for up to 2 weeks postpartum,[12] newborn mice were used to study whether we could collect glomeruli at different stages of maturation using our isolation technique. Perfused Dynabeads were distributed in the S-shaped, cup-shaped, as well as in maturing capillary loop stages, but not in the early vesicle stages (Figure 6; A to D). Developing glomeruli containing beads were isolated with a magnet and the contamination of other renal tissues was low (Figure 6E). Structures reminiscent to the S-shaped, cup-shaped, and maturing stages were readily visible and individually collectable under the microscope. The

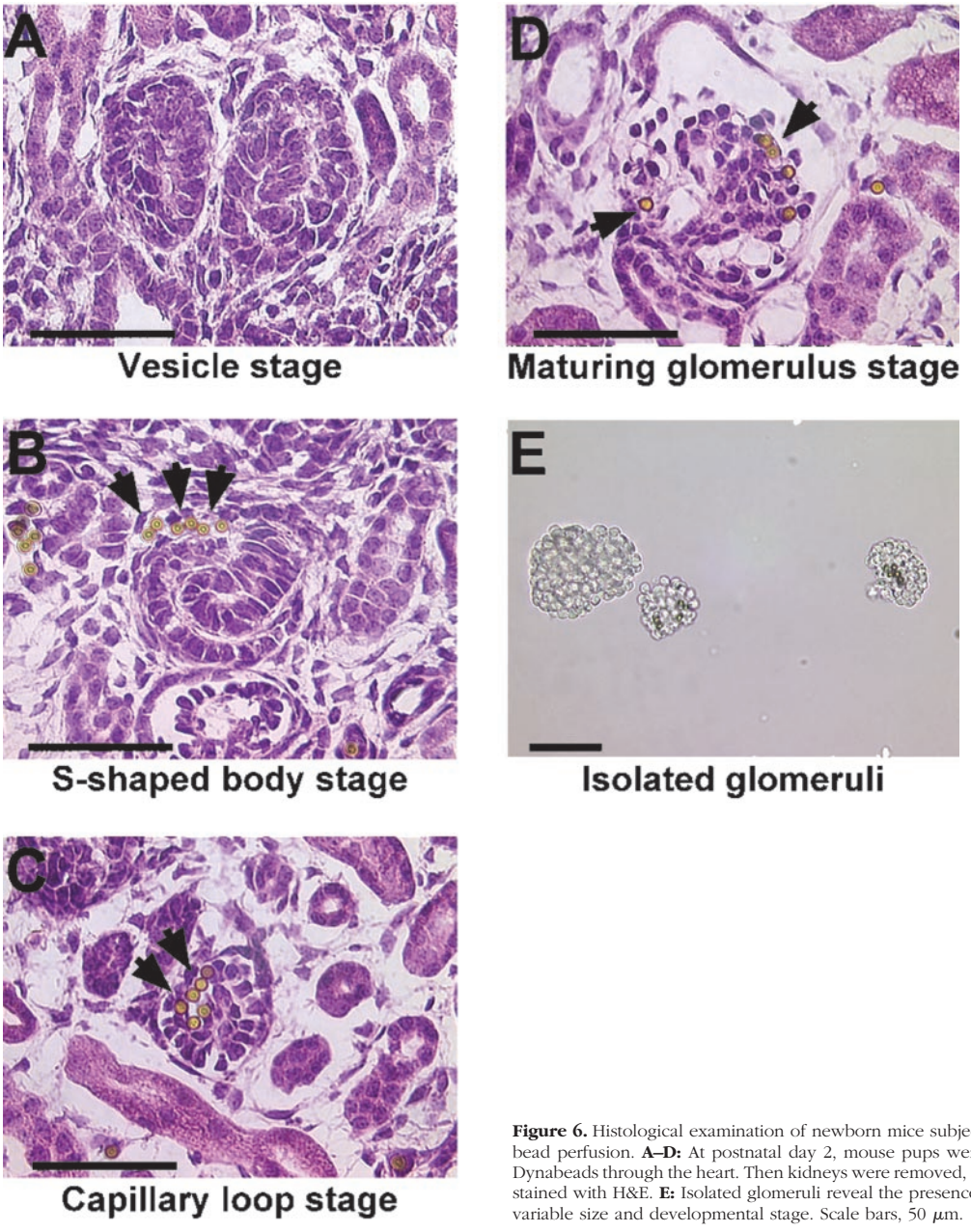

Figure 6. Histological examination of newborn mice subjected to magnetic bead perfusion. **A–D:** At postnatal day 2, mouse pups were perfused with Dynabeads through the heart. Then kidneys were removed, snap-frozen, and stained with H&E. **E:** Isolated glomeruli reveal the presence of glomeruli of variable size and developmental stage. Scale bars, 50 μm.

number of postnatal day 2 mice glomerular structures isolated per mouse was 3560 ± 879 (n = 5). RNA isolation revealed that the newborn pup glomeruli contained 30 to 50 times more RNA per cell than the adult glomerulus cells (data not shown).

Discussion

The kidney glomerulus is a tuft of capillaries composed of many loops surrounded by epithelial cells (podocytes) and held together by a core of mesangial cells and matrix. The main function of the glomerulus is to constitute a permeable, size-selective, barrier across which blood is filtered to produce primary urine. To allow efficient selective filtration, cellular and matrix components of the glomerulus are endowed with specialized features. Recently many types of knockout and transgenic analyses in mice have been instrumental in identifying molecules important for kidney development, especially in the early stages of nephron development.[3–5] However, our knowledge is still limited concerning molecules of importance in late-stage glomerulogenesis[7,13,14] and in the steady-state function of the mature glomerulus. Improved methods for glomerulus isolation would constitute an important step toward further molecular analysis of glomerular development and function.

Methods to isolate glomeruli from rat[15] and rabbit[16] using sieving techniques have already been described. However, it has been difficult to isolate pure glomeruli from mouse by sieving because the diameter of mouse glomeruli is rela-

tively similar to their tubules. As mice are widely used as experimental models of development and disease, it is important to be able to isolate glomeruli from mice. Isolation of mouse glomeruli after Fe_3O_4 perfusion has been reported,[17] however the isolation efficiency was rather limited.

Here we describe a highly efficient method for the isolation of mice glomeruli using spherical Dynabeads containing iron. Dynabeads are made of a monodisperse polymer and exhibit magnetic properties within a magnetic field.[18] Their surface is smooth with a coated polymer shell that reduces the direct damage to the tissues when they are perfused and protect from toxic exposure to iron. For our purpose, the Dynabeads were perfectly fitted not only to specifically embolizing the glomerular capillaries but their use also simplified and shortened the time of the isolation procedure which reasonably reduced cell damage to a minimum. The collagenase digestion and gentle filtration steps detached effectively the morphologically intact glomeruli from surrounding tissues. The method has good advantages not only for morphological studies but also for keeping intact the *in vivo* mRNA and protein profiles. The isolated glomeruli were well covered by podocytes and we could readily observe the fine structures of the podocyte slit diaphragms and fenestrated endothelial cells by electron microscopic analysis. The method can be applied to both developing glomeruli and to models of adult glomerular disease. In the Northern blotting analysis, we used nephrin cDNA and Tie 2 cDNA to verify the enrichment of glomeruli and their integrity in our preparations. Nephrin is a recently identified novel protein expressed only at the glomerular podocyte slit diaphragm. Nephrin has been reported to be mutated in congenital nephrotic syndrome of the Finnish type.[19] Tie-2 is an endothelium-specific receptor tyrosine kinase, which binds to angiopoietin-1 and -2.[20] Its strong expression in the kidney glomerulus compared with whole kidney probably reflects the fact that 50% of the glomerulus consists of endothelial cells, whereas such cells are far less abundant in the rest of the kidney. Thus, it seems clear that mRNAs with expression in a low proportion of cells are hard to detect when analyzing whole organs because of their dilution.

The yield in our isolation method was ~20,000 glomeruli per adult mouse, which is consistent with the amount of glomeruli found in an adult mouse.[11] This was also confirmed by the lack of glomeruli in the rest of the tissues not collected by the magnet (data not shown). Thus, the method described here is an efficient large-scale method for isolation of intact glomeruli from mice. The method enables the application of techniques for systematic analysis of gene and protein expression, such as Expressed Sequence Tag (EST) sequencing,[21] serial analysis of gene expression,[22] DNA microarray hybridization,[23] and proteomics.[24] The speed of the method is important for preserving the *in vivo* transcript and protein profile. The use of mice for these types of analyses opens up the possibility of exploring numerous transgenic models of developmental and pathophysiological disturbance of glomerular function at the molecular level. Because our method is based on perfusion, it will not work in cases in which there is a complete fallout of glomerular capillaries. However, it seems to work in cases of incomplete capillary development, as shown by the efficient isolation of immature stage glomeruli. The method also works well for diabetic mice and transgenic models with increased accumulation of mesangial matrix (our own unpublished observations). Thus, the method should prove useful when analyzing changes in gene and protein expression profiles during the later stages of glomerular development and during early stages of glomerular disease.

Acknowledgments

We thank Mrs. Yvonne Josefsson and Mrs. Gunnel Bokhede at the Electron Microscopy Unit, Institute of Anatomy and Cell Biology, Göteborg University, Sweden, for technical assistance.

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