Video Article

# Transplantation into the Anterior Chamber of the Eye for Longitudinal, Non-invasive *In vivo* Imaging with Single-cell Resolution in Real-time

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## Abstract

Intravital imaging has emerged as an indispensable tool in biological research. In the process, many imaging techniques have been developed to study different biological processes in animals non-invasively. However, a major technical limitation in existing intravital imaging modalities is the inability to combine non-invasive, longitudinal imaging with single-cell resolution capabilities. We show here how transplantation into the anterior chamber of the eye circumvents such significant limitation offering a versatile experimental platform that enables non-invasive, longitudinal imaging with cellular resolution *in vivo*. We demonstrate the transplantation procedure in the mouse and provide representative results using a model with clinical relevance, namely pancreatic islet transplantation. In addition to enabling direct visualization in a variety of tissues transplanted into the anterior chamber of the eye, this approach provides a platform to screen drugs by performing long-term follow up and monitoring in target tissues. Because of its versatility, tissue/cell transplantation into the anterior chamber of the eye not only benefits transplantation therapies, it extends to other *in vivo* applications to study physiological and pathophysiological processes such as signal transduction and cancer or autoimmune disease development.

## Video Link

The video component of this article can be found at https://www.jove.com/video/50466/

## Introduction

Advances in intravital microscopy have revealed physiological phenomena not predicted by *in vitro* studies[1]. This highlights the challenge in translating findings obtained by conventional *in vitro* methods into the living animal. In the last decade, visualization of tissues in living animals was considerably improved by technological advances in imaging modalities[2, 3, 4, 5, 6]. This has spurred a need for *in vivo* imaging approaches with feasible application in experimental animal models to enable longitudinal visualization of target tissues non-invasively.

Imaging techniques such as magnetic resonance imaging and positron emission tomography or bioluminescence have enabled non-invasive imaging of organs/tissues deep within the body[7-8, 9]. But these techniques cannot achieve single cell-resolution due to high background signals and low spatial resolution, despite the use of high contrast materials or tissue-specific luminescence[4]. This was addressed with the advent of two-photon fluorescence confocal microscopy[10]. Two-photon microscopy enabled intravital imaging studies to visualize and quantify cellular events with unprecedented details[11, 12]. This has led to the characterization of key biological processes in health and disease[13, 14, 15, 16]. While pioneering intravital imaging studies have primarily "mimicked" *in vivo* conditions in excised tissue (*e.g.* lymph nodes), other studies have used invasive approaches to image exposed target tissues *in situ*[17, 18, 19, 20, 21]. Other studies have also used "window chamber models" to circumvent limitations associated with invasive approaches and limited imaging resolution *in vivo*[22, 23, 24, 25]. In the window chamber model, a chamber with a transparent window is surgically implanted into the skin at different locations (dorsal or ear skin, mammary fat pad, liver, etc) on the animal (*e.g.* mouse, rat, rabbit). While this approach clearly enables high-resolution *in vivo* imaging, it requires an invasive surgery to implant the chamber and may not be able to accommodate longitudinal imaging studies over several weeks or months[22].

It was recently demonstrated that combining high-resolution confocal microscopy with a minimally invasive procedure, namely transplantation into the anterior chamber of the eye (ACE) provides a "natural body window" as a powerful and versatile *in vivo* imaging platform[26, 27]. Transplantation into the ACE has been used in the last several decades to study biological aspects of a variety of tissues [28, 29, 30]; and its recent combination with high-resolution imaging enabled studying the physiology of pancreatic islets with single cell-resolution non-invasively and longitudinally[26, 27]. This approach was used to study autoimmune responses during development of type 1 diabetes in animal models

 

(unpublished data). It was also used to study pancreatic development, as well as, in studies of kidney function by transplanting into the ACE pancreatic buds or individual renal glomeruli, respectively (unpublished data). A recent report using this approach further demonstrated its application to study immune responses after pancreatic islet transplantation[31]. Importantly, this study showed that transplantation into the anterior chamber of the eye provides a natural body window to perform: (1) longitudinal, non-invasive imaging of transplanted tissues *in vivo*; (2) *in vivo* cytolabeling to assess cellular phenotype and viability *in situ*; (3) real-time tracking of infiltrating immune cells in the target tissue; and (4) local intervention by topical application or intraocular injection.

Here, we demonstrate how to perform transplantation into the anterior chamber of the eye using pancreatic islets.

## Protocol

The following procedure is performed under the stereoscope in 2 steps, the first step involves loading the islets into the cannula and the second step is the actual transplantation into the ACE. All procedures performed on animals were approved by the institutional animal care and use committee (IACUC) of the University of Miami.

### 1. Loading Islets in Cannula for Transplantation

1. Center the islets in culture dish by spinning the dish in narrowing circles.
2. Disconnect the cannula from the "reservoir" and place the cannula and connecting tubing on a clean surface. The reservoir can be made out of a 300 µl disposable plastic pipette tip without filter (**Figure 1a**).
3. Flush air bubbles out of the reservoir to ensure continuous stream of islets when aspirating into reservoir. Flushing the reservoir is done by driving forward the hands-free motorized syringe-driver using the foot pedal (**Figure 1b, c**). This will also make space in the syringe to allow aspiration of the islets into the reservoir (pre-loaded with sterile solution such as saline, PBS or culture media).
4. Gently aspirate desired amount of islets into the reservoir. Islets will tend to swirl as they enter the reservoir and will remain together towards the bottom. Aspiration is done by driving backward the motorized syringe-driver using the foot pedal.
5. Reconnect the cannula to the reservoir via the connecting tubing.
6. Place the cannula tip back in the culture dish and flush the islets out of the reservoir into the tubing then into the cannula. Ensure that islets remain together as you back-fill the tubing/cannula by gently "flicking" (tapping) the tubing (**Figure 1d**). Stop either before or after all air bubbles ahead of the islets are flushed out the cannula. If not sure, stop as islets enter the back of the cannula as remaining air bubbles ahead of islets can help prevent reflux (backflow) of islets out of the ACE. Will dissipate overnight.
7. At this stage, you are ready to inject the islets into the ACE (please see next steps).

### 2. Islet Transplantation into the Anterior Chamber of the Eye

1. Position the anesthetized mouse on a warm pad under stereoscope.
2. Place the snout of the mouse into anesthesia "mask" connected to oxygen/isoflurane anesthesia machine. The mask is made out of a 1 ml disposable plastic pipette tip (without filter) and connected to anesthesia tubing through the narrow end (**Figure 2a,b**).
3. Gently retract the eye lids of the eye to be transplanted using the index finger and thumb of your free hand and "pop" the eye out for better exposure and easy access (**Figure 2c**). This will require some practice to perfect without impeding breathing of the mouse by excessive pressure on the neck or blocking blood flow to the head.
4. Using a disposable insulin syringe (29 - 31G) as scalpel, carefully penetrate only the tip in the cornea and make a single lateral incision. Make the incision at midpoint between the apex of the cornea and limbus to minimize reflux of the islets during injection out of the ACE (**Figure 2d**).
5. Carefully insert the cannula (preloaded with islets) through the incision.
6. Slowly eject islets out of the cannula and deposit them on top of the iris. To avoid islet reflux due to excessive pressure buildup in the ACE, eject the islets in brief thrusts in as little volume(s) as possible in the quadrant opposite to the incision. This can be further ensured by compacting islets in the tubing while loading the cannula (please see step 1.6).
7. Slowly retract the cannula out of the ACE. This is a critical step, especially, if a large volume of islets was injected as islet reflux due to pressure build up inside the ACE may be inevitable. To eliminate/minimize islet reflux, gently rotate the cannula while inside the ACE to release excess pressure through the incision around the cannula. Check for signs of reflux as you attempt to retract the cannula and, if needed, wait until pressure subsides before completely retracting the cannula out of the ACE.
8. Rinse the transplanted eye with sterile PBS or saline.
9. Inject buprenorphine for post-operative analgesia (0.05-0.1 mg/kg, subcutaneously) for the first 48 hr.
10. Apply erythromycin ophthalmic antibiotic ointment to the transplanted eye immediately after transplantation.
11. Place the animal back in a warmed cage to allow recovery from anesthesia.

## Representative Results

There are a few parameters that define a "good" transplantation. A good transplantation is one that proceeds without bleeding when making the incision as can be seen in the video. Bleeding is prevented/minimized by penetrating only the tip of the scalpel (needle) into the ACE (**Figure 3a**). This will also help prevent contact and puncture of the iris. It will also ensure a small incision which will heal very well without causing cloudiness of the cornea over time (**Figure 3c, d**). Another important aspect to a successful transplantation is to be able to transplant the total desired amount of islets without loss due to reflux out of the ACE. As mentioned in the protocol step 1.6, this can be minimized by ejecting the islets in the least possible volume and, when applicable, by using air bubbles to help seal the incision upon final retraction of the cannula out of the ACE (**Figure 3b**). Moreover, delivering the islets on top of the iris between the edge of the pupil and the limbus positions the islets in a location very amenable for *in vivo* imaging (**Figure 3d**). From practical perspective, having the islets at this intermediate position of the iris reduces the thickness of the imaging z-stacks required to span whole islets (**Figure 4**). This is particularly important during fluorescence confocal/two-photon *in vivo* imaging where a smaller z-stack allows better recovery of specific fluorescence signals in deeper sections of the

imaged tissue with better xy and z resolutions due to less light scatter by the tissue. Moreover, thicker z-stacks require longer acquisition time which increases the likelihood of instrumental or animal drift, especially during *in vivo* imaging.

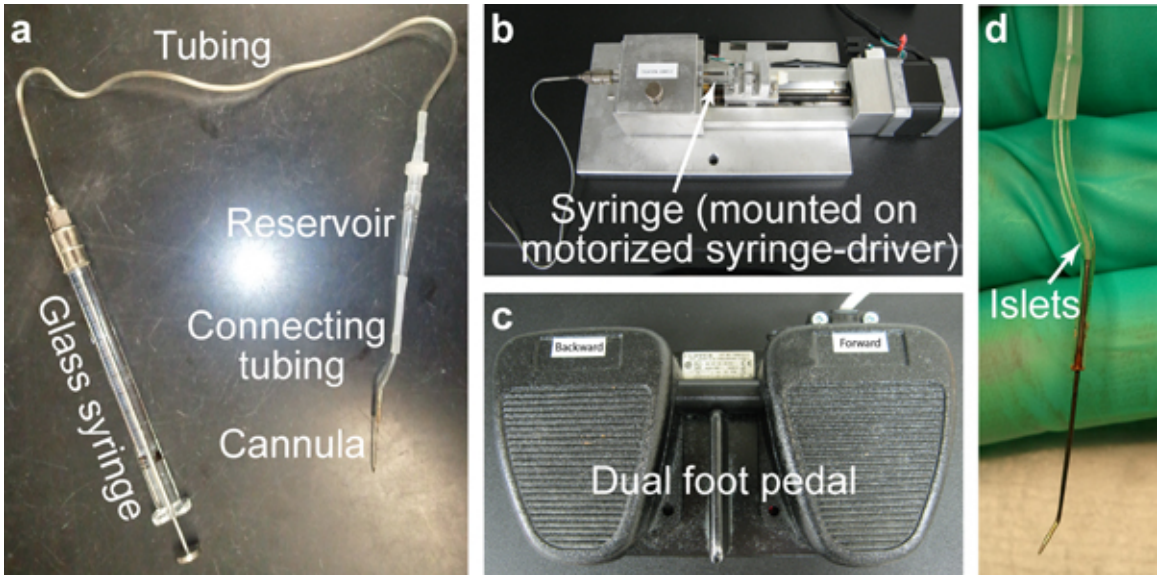


**Figure 1. Photographs of our transplantation apparatus including all parts. (a)** Assembled glass syringe with tubing, reservoir, and cannula. **(b)** Motorized syringe-driver with syringe mounted. **(c)** Dual foot pedal to operate the motorized syringe driver. Pressing either pedal drives the syringe plunger backward (aspiration) or forward (ejection). **(d)** Close-up of the cannula and connecting tubing showing the islets packed at the back of the cannula. This configuration allows delivery of the islets into the anterior chamber of the eye in a minimal volume to reduce reflux and loss of islets.

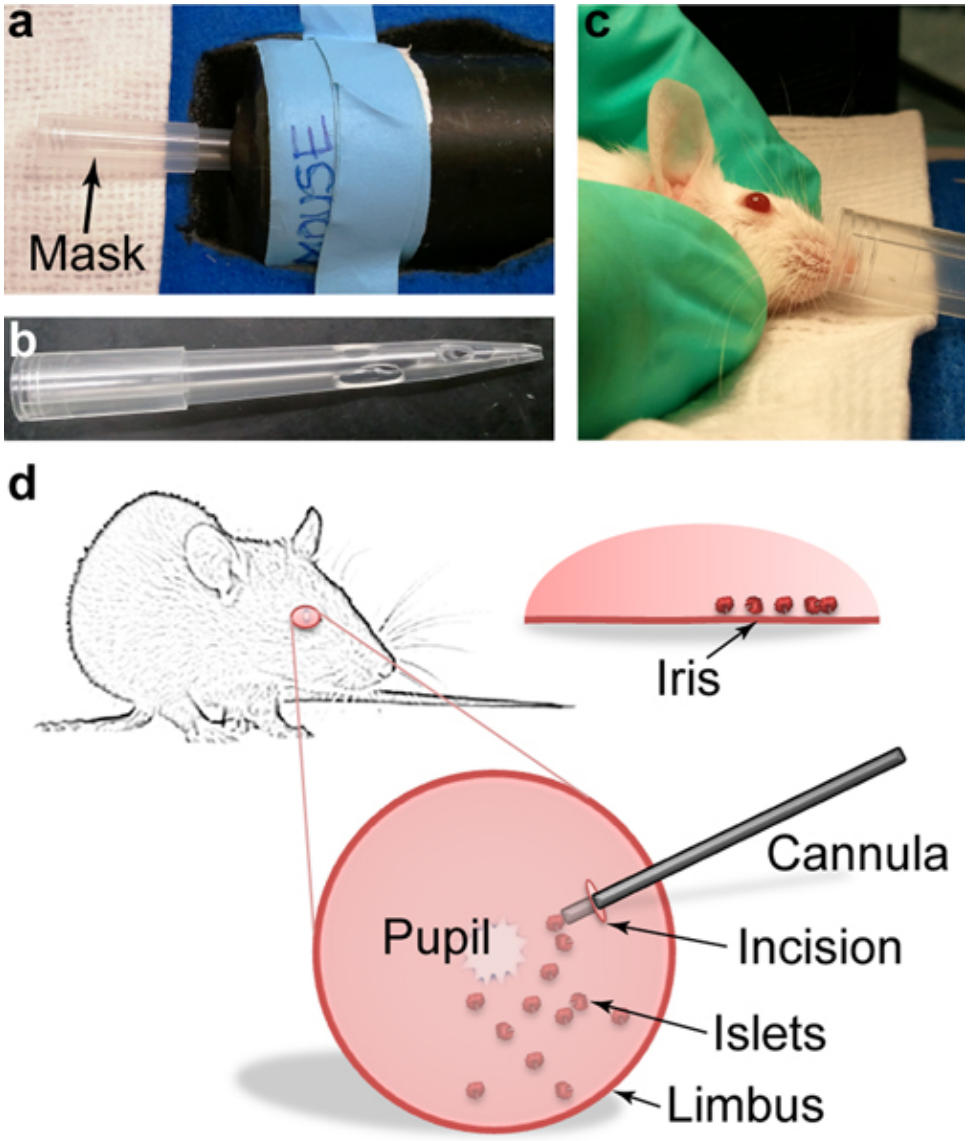


**Figure 2. Depiction of the transplantation procedure into the anterior chamber of the eye (ACE). (a)** Photograph of the mouse anesthesia mask. **(b)** Close-up view of the anesthesia mask made of a 1 ml disposable plastic pipette tip without filter. Several holes were made in the tip to allow mixing of oxygen with isoflurane before reaching the mouse. **(c)** Close-up view showing the eye to be transplanted exposed for better access. The eye is exposed out by stretching the skin of the head using the thumb and index finger. **(d)** Schematic depiction of the transplantation procedure highlighting the location of the incision at midpoint between the apex of the cornea and the limbus. The cannula is inserted through the incision to deliver the islets into the ACE. Islets are deposited on top of the iris where they engraft.

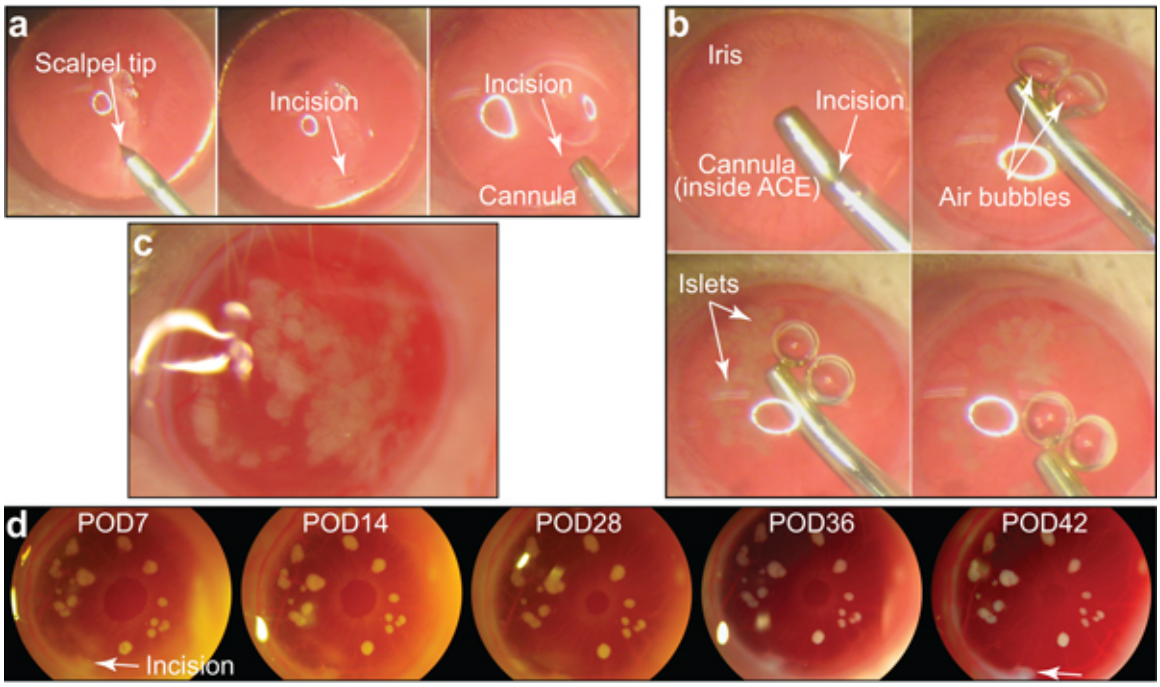


**Figure 3. Representative images of "good" transplantation highlighting critical steps in ensuring successful outcomes. (a)** Series of images showing how far the tip of the scalpel (needle) is pushed into the cornea while making the incision. A small incision is made without bleeding. The incision is slightly larger than the cannula. **(b)** Series of images showing islets being ejected out of the cannula on top of the iris while using air bubbles to prevent reflux. Notice how "bent" the cannula appears due to light refraction once inside the ACE. **(c)** Representative image of a transplanted eye highlighting the clarity of the ACE immediately after transplantation. **(d)** Series of images of the same eye acquired on the specified post-operative days (POD) highlighting the preferred location of islets for *in vivo* imaging and how well-healed and localized the incision is and the clarity of the cornea at 6 weeks after transplantation.

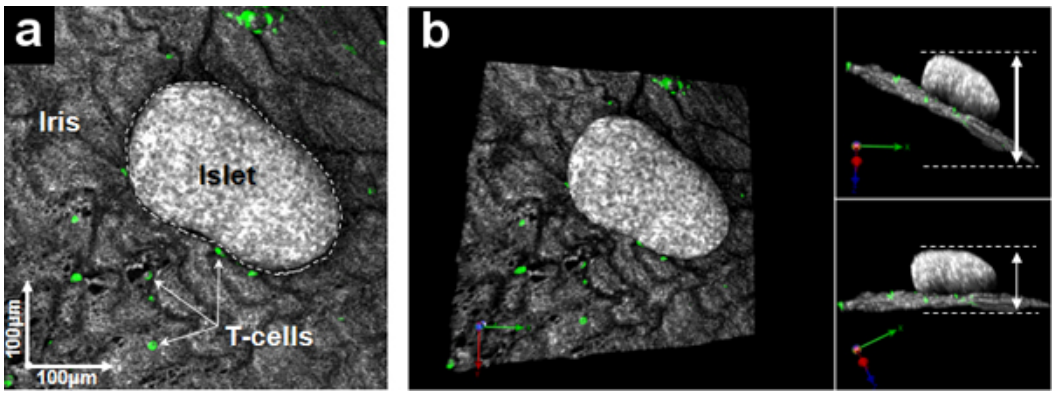


**Figure 4. Representative fluorescence confocal image of a pancreatic islet transplanted in the anterior chamber of the mouse eye (ACE) highlighting the benefits of the islet position on the iris and the ability to resolve individual cells during *in vivo* imaging. (a)** Maximum projection (2-D view) of a z-stack of an islet (outlined with dotted line) on top of the iris of a C57BL/6 transgenic mouse that expresses green fluorescent protein (GFP) in activated and memory T-cells [32]. The image was acquired 5 days after transplantation where a few infiltrating T-cells (green) were detected in the iris surrounding the islet. The islet and iris were visualized by laser backscatter or reflection (grey). **(b)** Three-dimensional (3-D) views of the same islet highlighting the benefits of viewing/imaging angle to reduce the z-stack thickness to acquire the whole islet volume and surrounding structure and immune cells. Notice the xyz axes for rotation of the image.

## Discussion

Murine pancreatic islets were isolated using collagenase digestion followed by purification on density gradients, as described previously [33]. Isolated islets were cultured overnight before transplantation. While this may not be required, it is recommended to allow the islets to recover from the isolation procedure. This is critical when transplantation is performed in diabetic recipients as it will ensure transplantation of surviving/robust islets.

Transplantation is performed under generalized anesthesia with oxygen/isoflurane mixture (1.5-3%) inhalation to effect. Alternative inhalation or injection anesthetics (*e.g.* ketamine) can be used. If injection anesthesia is used, skip step 2.2 in the protocol. Provide the anesthetized animal with a source of heat to prevent hypothermia during the procedure. In some mice, it is possible to break blood vessels when making the incision in the typically avascular cornea. For example, the cornea of nude mice tends to be vascularized; avoid vascularized areas when possible. Use

a new syringe per incision. Avoid puncturing the iris with the needle when making the incision. Preventing contact with the iris can be further ensured by facing the beveled side of the needle tip toward the iris. Do not dry/aspirate aqueous humor after making the incision. It is easier to penetrate the cannula through the incision in a "wet" cornea; add a few drops of sterile PBS or culture media to the cornea if needed.

Post-operative analgesia can be obtained by injecting subcutaneously buprenorphine (0.05-0.1 mg/kg) or preferred analgesic(s) for the first 48 hr. In step 2.9, we administer analgesia immediately after the procedure as the animal is already deeply under general anesthesia. If desired, however, step 2.9 can be performed after step 2.2 in the protocol, with or without a topical anesthetic to the eye (consult with your local IACUC or veterinarian). Alternative ophthalmic antibiotics can also be used.

Here, we used a custom-built microinjection apparatus operated via a foot pedal to drive the 100 µl precision glass syringe to aspirate (load) and eject the islets out of the cannula into the ACE (**Figure 1**). This can be substituted with any 100 µl gas-tight precision glass syringe with a screw-driven plunger that can be operated manually to aspirate/eject the islets; this however will likely require the assistance of another person to operate. In either case, although not required we recommend pre-loading the assembled syringe, tubing, and reservoir with a sterile solution (saline, PBS or culture media) to ensure smooth aspiration and ejection of the islets. This is particularly important if/when the packed islets clog the cannula.

We typically perform our transplantation procedures under clean conditions inside a biosafety cabinet without risk of infections. All used solutions, syringes, cannula, tubing, and gauze are autoclaved or gas-sterilized. While, we cannot ascertain full sterility because of hand contact with the mouse during the procedure, we have not had any issues with islet contamination following the above recommended steps.

We demonstrated here how to transplant pancreatic islets into the ACE for imaging purposes where fewer islets are needed to transplant. In the case where diabetes reversal is desired in the recipient animal, a larger amount of islets needs to be transplanted [26, 27]. While the transplantation procedure is identical to what we showed here, particular attention should be paid to steps 2.6 and 2.7 in the protocol to avoid loss of transplanted islets due to reflux.

Once mastered, this transplantation procedure can be performed in ~ 5 min per mouse. This technique can be used to transplant a variety of tissues into the anterior chamber of the eye. As mentioned above, we have transplanted renal glomeruli as well as embryonic tissue (pancreatic buds) to study pancreatic development in the anterior chamber of the eye *in vivo*.

## Disclosures

P-O.B. is one of the founders of the biotech company Biocrine, which is going to use the anterior chamber of the eye as a commercial servicing platform. A.C. is on the patent protecting this technology.

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