Editorial

Lens, heal thyself: a significant advance in regenerative ophthalmology

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A few animals, notably Cnidarians, including Hydra and some species of jellyfish, and Planaria possess unlimited regenerative capacity and effectively elude both senescence and biological aging (1). However, humans, and most other vertebrates, exhibit limited regenerative capability for a few tissues that declines with age. Given the isolation of human embryonic stem cells (ESCs), the creation of induced pluripotent stem cells (iPSCs), and the discovery of many adult stem cell types, the drive to harness these cells to reverse the ravages of disease and age captivates the imagination of the public and medical scientists alike. The enthusiasm for unlocking human regenerative potential for clinical use currently drives both private and public research initiatives, giving hope to millions of sight impaired and blind people. For example, the Audacious Goals Initiative, sponsored by The National Eye Institute of the United States, specifically seeks to fund cross-disciplinary research leading to the restoration of vision through retina regeneration.

Although most efforts for regenerative medicine in ophthalmology focus on the retina, lens disease resulting in cataracts remains the leading cause of human blindness (2). Fortunately, surgical removal of the cataract with replacement by a synthetic intraocular lens (IOL) provides excellent potential for vision restoration in adults with access to the procedure. However, sight threatening complications of cataract surgery remain a problem, particularly for pediatric patients (3). The recent article by Lin and colleagues provides an alternative strategy for treating pediatric cataracts that relies on the inherent ability of lens epithelial cells to regenerate new lens fiber cells to replace the excised, cataractous fiber cell mass (4).

Some species of frogs and newts are the only terrestrial vertebrates exhibiting lens regeneration following complete lens loss or surgical removal (5). In both types of amphibians, lens regeneration results from the conversion of a previously differentiated tissue into lens tissue through a process known as transdifferentiation. In frogs, lens regeneration occurs through transdifferentiation of corneal epithelial cells and this process only occurs in larval tadpoles prior to metamorphosis. In contrast, adult newts regenerate the lens from transdifferentiation of pigmented epithelial cells of the dorsal iris. Regeneration by transdifferentiation involves widespread genetic and epigenetic reprogramming and requires dedifferentiation, proliferation and subsequent differentiation of the missing cell type.

In contrast to newts and frogs, mammals do not spontaneously regenerate lens by transdifferentiation of other existing tissues. However, as early as 1827, surgeons in France noted the regeneration of rabbit lenses in eyes following surgical removal of the lens fiber cell mass while leaving the lens capsule intact (6). Lens regeneration from epithelial cells remaining adherent to the lens capsule following removal of the fiber cell mass has since been demonstrated in dogs, cats, sheep, guinea pigs, rats, mice and non-human primates (7-9). In these experimental animals, the quality of lens regeneration depended on the state of the lens capsule and freedom from excessive inflammation. In particular, filling the evacuated capsule to prevent wrinkling and adhesion of the capsule surfaces, followed by sealing the capsule wound, resulted in accelerated regeneration and improved lens size (10). Also, the speed and efficiency with which mammals regenerate their lenses declines with age.

The current standard treatment for cataracts involves creating a reasonably large hole in the anterior lens capsule

followed by the removal of the fiber cell mass. After cataract removal, a synthetic IOL, placed into the remaining lens capsular bag, replaces the function of the natural lens. The major complication of cataract surgery results from proliferation and fibrosis of lens epithelial cells that remain following cataract extraction. These cells often migrate onto the posterior lens capsule or onto the IOL causing capsular wrinkling and creating opacities within the visual axis. This requires additional procedures to remove the posterior capsule and/or adherent lens cells to clear the visual axis. The overall frequency of visual axis opacification (VAO) has declined in recent years as the result of both better IOL design and more complete removal of lens epithelial cells during surgery. However, in contrast to the adult population, infant cataracts present unique challenges and these patients continue to experience high rates of complications, including VAO, amblyopia and glaucoma, following the initial cataract extraction.

Lin and colleagues revisited previous evidence demonstrating the potential of mammalian lens regeneration. In so doing, they used BrdU incorporation to reaffirm that the proliferative potential of human lens epithelial cells declines with age. However, human central lens epithelial cells, where cell division is normally rare, ramped up their BrdU incorporation rate 11-fold following removal of the fiber cell contents from the capsular bag. Also, as previously shown, the authors demonstrated that neonatal rabbit lens epithelial cells could be maintained in culture and form lentoid bodies that express elevated levels of αA-, β- and γ-crystallins, proteins characteristic of lens fiber cells.

The continuous postnatal growth of mammalian lenses depends on lens epithelial cell proliferation, mostly in the germinative zone slightly anterior to the lens equator, followed by differentiation of equatorial epithelial cells into secondary fiber cells. Investigators in the Lin *et al*., study sought to investigate the role of *Bmi-1*, a member of the Polycomb Repressor Complex 1 required for self-renewal of stem cells, in lens epithelial cell proliferation. To do this, they deleted the *Bmi-1* gene using the Nestin-Cre deleter strain where Cre is active in the developing lens epithelium (11). *Bmi-1* deficient mouse lenses exhibited normal prenatal development and remained clear through 2 months of age. However, by 7 months of age, *Bmi-1* deficient lenses developed cataracts and most lens epithelial cells lost expression of both *Pax6* and *Sox2*. Progressive reduction of lens epithelial cell proliferation in the *Nestin-Cre/Bmi-1* floxed mice and reduced proliferation in human lens epithelial cells following RNAi-mediated knockdown *Bmi-1* expression supported the notion that *Bmi-1* plays an essential role in long term maintenance of lens epithelial cell self-renewal required for post-natal lens growth. However, the authors failed to note any overall decrease in lens size or reduction in static lens epithelial cell number with age in the *Bmi-1* deficient mouse lenses.

The existence of a distinct stem cell population within the lens epithelium remains a controversial topic. Lens epithelial cells express telomerase, a protein typically restricted to stem cells and cancer (12). Stem cells generally require a specialized location or niche which maintains their stem cell properties. No clear consensus has emerged as to specifically where lens stem cells might reside or the location of their niche. Clearly, both central and equatorial lens epithelial cells retain the ability to both proliferate and differentiate into lens fiber cells. Although a number of investigations suggested the possibility of specific mammalian lens stem cells within different locations of the epithelium (13,14), or even outside the lens (15), a recent paper suggested a mathematical alternative—the penny pusher model—explaining continuous lens growth without the need for a specific lens stem cell population (16). In this model, all lens epithelial cells are equivalent and differential proliferative behaviors depend only on the relative positions of cells within the epithelial layer.

Armed with repeated previous demonstrations of the regenerative capacity of mammalian lens epithelial cells, Lin and colleagues developed a modified cataract extraction (capsulorhexis) method with the goal of maximally preserving the anterior lens capsule with intact lens epithelial cells. In this respect, the authors sought to exploit the regenerative capacity of these cells to recreate an optically clear lens, rather than treat these epithelial cells as complications leading to VAO. The key here involved making a small (1–2 mm) incision peripheral to the visual axis from which phacoemulsification extracted the fiber cell mass. They initially demonstrated the potential of this "minimally invasive" anterior continuous curvilinear capsulorhexis (ACCC) technique in rabbits where they followed the fate of the remaining capsular bag for up to 5 months post-surgery. Despite initial adherence of the posterior and anterior capsules, within 4–5 weeks lens fiber cells began regenerating from the periphery toward the center of the lens, such that a biconvex, clear regenerated lens with an average of 15.6 dioptres had formed by 5 months post-surgery.

Following the success of their surgical procedure

in rabbits, Lin *et al*., tested their technique in juvenile long-tailed macaques as a proxy for human infants 4–12 months old. As seen in the rabbits, the surviving macaque lens epithelial cells fueled the curvilinear pattern of lens regeneration resulting in clear, biconvex lenses within 5 months of surgery. Furthermore, in the six macaques undergoing this procedure, no post-surgical complications common to pediatric cataract surgery in humans occurred. Given this outcome, the authors took the bold step of testing their minimally invasive ACCC technique in human infants with bilateral cataracts.

The human trial consisted of 12 infants who received the modified ACCC and 25 control infants that received standard cataract treatment with ACCC through a large anterior capsule opening. The standard treatment was subsequently followed, in the majority of cases, by laser capsulotomy or posterior continuous curvilinear capsulorhexis and anterior vitrectomy with or without the insertion of an IOL. All infants were followed by slit-lamp microscopy to follow process of lens regeneration *in vivo*. Amazingly, the capsular opening healed and a transparent biconvex lens regenerated in all 24 eyes receiving the minimally invasive ACCC treatment within 3 months of the surgery. These regenerated lenses reached sizes comparable to native lenses by 8 months following surgery and, in contrast to the standard treated eyes, achieved a mean accommodative response of 2.5 dioptres. Furthermore, the mean visual acuity achieved in eyes treated with the modified ACCC was indistinguishable from that of eyes receiving the standard treatment.

The large reduction in post-operative complications using the minimally invasive ACCC technique argues in favor of clinical superiority to standard ACCC treatment. As expected, the majority (92%) of eyes receiving standard treatment developed complications including 84% that developed VAO that required additional surgery. In contrast, only 17% of the modified ACCC treated eyes developed complications and only one eye (4.2%) developed VAO. Although all of the lenses treated with the modified ACCC developed a scar resulting from localized fibrosis at the site of capsular opening, the peripheral scar failed to interfere with transparency of the visual axis. As noted in mice (8), an initial fibrotic response to fiber cell removal by the lens epithelium apparently preceded a more normal fiber cell differentiation response in human infants.

Lin and colleagues provide a compelling case to re-examine current standard treatment for pediatric cataracts. Exploiting the regenerative capacity inherent in the native lens represents a desirable, less invasive and by all measures a seemingly superior strategy that works with rather than against lens biology. However, some important considerations remain. First among these likely rests in the selection of appropriate candidates for the procedure. While this technique likely holds promise for patients with cataracts resulting from traumatic or infectious causes, this route may prove less successful in patients with cataracts resulting from genetic causes. There seems little reason to suggest that abnormalities in lens fiber cell clarity resulting from a genetic deficiency or gain of function mutation would improve upon regeneration without correction of the underlying genetic lesion. As a significant portion of bilateral congenital and pediatric cataracts result from genetic abnormalities (17,18), this remains an important consideration when selecting patients for this procedure.

Since age-related cataract removal remains among the most commonly performed surgical procedures the potential of minimally invasive ACCC to treat elderly patients merits serious consideration. However, as pointed out by Lin and colleagues age related cataracts present a number of different challenges than pediatric cataracts. Among these are the hardness of the senile cataracts, and the reduced proliferative capacity of aged lens epithelial cells relative to those of pediatric lenses. Also, the standard treatment for age-related cataracts enjoys higher rates of success and fewer complications than for pediatric cataracts. Even under the best of circumstances, restoration of sight by the modified ACCC will require several months as the lens undergoes the process of regeneration. Patients and clinicians will need to carefully weigh the benefits and risks of this newer treatment in light of the effectiveness of standard treatments.

Further basic research may also reveal ways to improve the potential of lens regeneration for the treatment of age-related cataract. The differentiation of lens progenitor cells from both human ESCs and iPSCs (19,20) suggests the possibility that "new" lens epithelial cells might be generated from age-related cataract patients for use in seeding the capsular bag with lens cells with regenerative potential more akin to those of infants. These approaches might also result in the correction of genetic anomalies leading to cataracts in iPSCs from pediatric cataract patients before differentiation of lens progenitor cells for the same purpose. Lin and colleagues effectively demonstrate how basic research in animal models can fuel paradigm-shifting advances in clinical practice. These advances provide an example and set the stage for new advances to achieve

the goal of rapidly growing new, natural lenses to replace synthetic lenses for the treatment of both pediatric and age-related cataracts.

The regeneration of retinal neurons in human patients remains an important goal in the treatment of human blindness and sight impairment. However, at present, many challenges remain to achieve clinically significant retina regeneration in human patients. In contrast, limbal stem cell transplantation already shows widespread clinical success in regenerating corneal epithelium in patients with limbal stem cell deficiency (21). Lin and colleagues have now elegantly demonstrated that endogenous lens epithelial cells provide the basis for clinically relevant vision restoration in pediatric cataract patients with fewer complications than current surgical practice.

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Footnote

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References

1. Petralia RS, Mattson MP, Yao PJ. Aging and longevity in the simplest animals and the quest for immortality. Ageing Res Rev 2014;16:66-82.
2. Bourne RR, Stevens GA, White RA, et al. Causes of vision loss worldwide, 1990-2010: a systematic analysis. Lancet Glob Health 2013;1:e339-49.
3. Medsinge A, Nischal KK. Pediatric cataract: challenges and future directions. Clin Ophthalmol 2015;9:77-90.
4. Lin H, Ouyang H, Zhu J, et al. Lens regeneration using endogenous stem cells with gain of visual function. Nature 2016;531:323-8.
5. Henry JJ, Tsonis PA. Molecular and cellular aspects of amphibian lens regeneration. Prog Retin Eye Res 2010;29:543-55.
6. Cocteau MM, D'Etoille L. Expériences Relatives a la Reproduction du Cristallin. J Physiol Exp Path (Paris) 1827;7:30-44.
7. Lois N, Dawson R, McKinnon AD, et al. A new model of posterior capsule opacification in rodents. Invest Ophthalmol Vis Sci 2003;44:3450-7.
8. Call MK, Grogg MW, Del Rio-Tsonis K, et al. Lens regeneration in mice: implications in cataracts. Exp Eye Res 2004;78:297-9.
9. Gwon A. Lens regeneration in mammals: a review. Surv Ophthalmol 2006;51:51-62.
10. Gwon A, Gruber LJ, Mantras C. Restoring lens capsule integrity enhances lens regeneration in New Zealand albino rabbits and cats. J Cataract Refract Surg 1993;19:735-46.
11. Cammas L, Wolfe J, Choi SY, et al. Integrin-linked kinase deletion in the developing lens leads to capsule rupture, impaired fiber migration and non-apoptotic epithelial cell death. Invest Ophthalmol Vis Sci 2012;53:3067-81.
12. Colitz CM, Davidson MG, McGAHAN MC. Telomerase activity in lens epithelial cells of normal and cataractous lenses. Exp Eye Res 1999;69:641-9.
13. Oka M, Toyoda C, Kaneko Y, et al. Characterization and localization of side population cells in the lens. Mol Vis 2010;16:945-53.
14. Zhou M, Leiberman J, Xu J, et al. A hierarchy of proliferative cells exists in mouse lens epithelium: implications for lens maintenance. Invest Ophthalmol Vis Sci 2006;47:2997-3003.
15. Remington SG, Meyer RA. Lens stem cells may reside outside the lens capsule: an hypothesis. Theor Biol Med Model 2007;4:22.
16. Shi Y, De Maria A, Lubura S, et al. The penny pusher: a cellular model of lens growth. Invest Ophthalmol Vis Sci. 2014;56:799-809.
17. Bermejo E, Martínez-Frías ML. Congenital eye malformations: clinical-epidemiological analysis of 1,124,654 consecutive births in Spain. Am J Med Genet 1998;75:497-504.
18. Rahi JS, Dezateux C. National cross sectional study of detection of congenital and infantile cataract in the United Kingdom: role of childhood screening and surveillance. The British Congenital Cataract Interest Group. BMJ 1999;318:362-5.
19. Qiu X, Yang J, Liu T, et al. Efficient generation of lens progenitor cells from cataract patient-specific induced

pluripotent stem cells. PLoS One 2012;7:e32612.
20. Yang C, Yang Y, Brennan L, et al. Efficient generation of lens progenitor cells and lentoid bodies from human embryonic stem cells in chemically defined conditions. FASEB J 2010;24:3274-83.
21. Atallah MR, Palioura S, Perez VL, et al. Limbal stem cell transplantation: current perspectives. Clin Ophthalmol 2016;10:593-602.

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