# Nature nurtures: lens regeneration, a breakthrough in ophthalmology

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Pediatric cataract is a major cause of treatable blindness worldwide (1,2). The prevalence of cataract in children has been estimated between 1–15/10,000 children (3). There are 200,000 children blind from cataract worldwide, and 20,000 to 40,000 children with developmental bilateral cataract are born each year (3). Pediatric cataracts are responsible for more than 1 million childhood blindness in Asia alone (3). Pediatric cataract blindness presents an enormous problem in terms of human morbidity, economic loss, and social burden. Despite the availability of meticulous surgery, cataract is still the leading cause of blindness worldwide in children (4). There is no "ideal" lens to date that can replace the natural lens and at the same time preserving all the properties of the natural lens and devoid of complications. We are in an era where stem cells have revolutionized the treatment of many human diseases to the extent of changing blood groups as well. It is high time that we focus on this major cause of treatable blindness with treatments that would bring about a paradigm shift in the outcome of surgery.

Early recognition, surgical intervention and appropriate follow-up after surgery can result in good visual outcomes in pediatric cases. However, several factors may impact the outcome of pediatric surgery including age at the time of surgery, limitation of intraocular lens power and type, availability of appropriate health services, surgical expertise and follow-up in terms of pharmacotherapy as well as visual rehabilitation (5). It is unsettling to know that despite timely intervention, post-surgical complications like visual axis opacification (VAO), refractive errors, amblyopia and glaucoma leave our treatment inadequate and incomplete (6,7). Pediatric cataract patients and their parents have to

be equipped for a long haul. In the light of the existing literature, it is important to examine these patients frequently and carefully for the development of these sequelae. If this problem continues to grow unchecked, the available techniques and resources will no longer be sufficient to sustain useful vision in children.

Conventional surgical approach to treat cataract in children includes early intervention with anterior and posterior capsulorhexis; anterior vitrectomy and rehabilitation by intraocular lens implantation (IOL)/ contact lens (6,8-10). There are both proponents and opponents of primary IOL implantation in infants (6,8-10). The use of compatible intraocular lens combined with better instrumentation, technique and expertise has led to a decrease in all the above mentioned complications (8). This is just the closest we have reached till the 21st century to replacing the natural lens and the search for a better and natural alternative goes on as evidenced by Lin *et al.* (11). Giving a child an almost brand new "natural lens" may sound like a fantasy, but not after we read the work of Lin and colleagues (11). Their innovative work on lens regeneration using endogenous stem cells has given us what we can call "A Eureka moment". There is no "elixir" at the moment to regenerate the lens but the lens heals itself from the residual epithelial cells after cataract surgery. Their article gives us "food for thought" in exploring the regenerative potential of the lens epithelial cells.

Though research on stem cells in is on since 1825, the trials initially progressed at a snail's pace, many a times influenced by the lack of *in vivo* experiments (12). The actual process by which the lens regenerates is becoming better understood as a result of trials in mammals but lens regeneration in adult

animals is limited to lower vertebrates only. Regeneration of the lens in adult newts was observed first by Collucci in 1891 and independently by Wolff in 1895, and the process is named after him as "Wolffian regeneration" (13). There has been a debate whether the lens needs favorable growth factors from the cornea as well as the retina to regenerate (14,15). But in contrast to lens regeneration in newts, where the lens regeneration is dependent on iris epithelial cells, lens regeneration in mammals is dependent on lens epithelial cells (16-23). Lens epithelial cells have been home to various lens regeneration experiments which explore the potential benefits and barriers of the technique.

The lens is an organ that keeps growing. Throughout life, new lens fibers are added continuously within the lens periphery, forming concentric growth shells. In an adult eye, exterior lens capsule has anterior lens epithelial cells positioned on the interior of the lens capsule. The posterior part of the lens is free from lens epithelial cells (24). Being ectodermal in origin, lens regenerates in response to injury. But the lens regeneration is possible only in the presence of a right "milieu" to the lens. The nourishment provided from the aqueous and vitreous chambers serves as an excellent home for regeneration. The essential ingredients include an intact anterior and posterior capsule, residual epithelial cells at the equatorial capsule, as well as no adherence between anterior and posterior capsules (25,26). The capsule needs to be relatively intact. In the words of textor "the lens regeneration depends on the lesion on the anterior capsule" (27). Polishing of capsule which is believed to disrupt lens epithelial cells; does not remove all residual lens epithelial cells and reformation of the new lens occurs even after polishing (19).

The authors had ample laboratory evidence that the human lens epithelium is capable of proliferation and differentiation. After cataract formation seen both after pediatric and adult cataract surgery is also a proof of the regenerative capacity of lens epithelial cells. Lens epithelial cell proliferation diminishes with age clinically evidenced by the increased rates of VAO in children. The authors used bromodeoxyuridine (BrdU), a synthetic nucleoside that is an analog of thymidine. BrdU is commonly used in the detection of proliferating cells in living tissues. By labeling cells with BrdU, the regenerative capacity of lens epithelial cells was established along with decrease in the same with age and an 11-fold increase after injury.

The molecular and biochemical aspects of the present study along with the *in vitro* experiments are indispensible for future research in humans. The microscopic analysis

of regenerated lenses in mice suggested new growth with significant contribution from Pax6 in adult lens fibers. Lentoid bodies formed after lens epithelial cell proliferation in rabbits and demonstrated crystallins as found in a mature lens. Otx2 has recently been shown to play an important role in regulating Notch induced FoxE3 expression (a forkhead transcription factor related to Xenopus lens1), which is essential for lens formation (28). The roles of crytallins in maintaining the clarity of the lens cannot be ignored (28). Sox proteins and PAX6 also play roles in regulating crystallin expression (29,30). While the study portrays the molecular aspects of lens regeneration, the inductive signals that trigger lens regeneration were also highlighted in the form of BMI-1; loss of which leads to lens opacification. The results are in concordance with studies that believe FGFs and BMPs induce lens differentiation from ectoderm (31).

In the background of the available data, the authors devised a unique way of capsulorhexis to maximize the utility of the residual lens epithelial cells. By reducing the size of the capsulorhexis opening and moving it from center to periphery; the lens epithelial cells were maximally preserved. After experiments in rabbit eye *in vivo* and macaques; similar technique was performed in 24 eyes of children aged less than 2 years. The regenerated lenses reached sizes like a natural lens by 8 months and also developed an accommodative potential of 2.5 D. Also the increase in visual acuity was comparable to the controls operated by aspiration, anterior and posterior capsulorhexis with aphakia.

The success of the treatment in the study by Lin and colleagues raises some issues to be addressed in future. The authors were careful in their patient selection. There were no children with traumatic or hereditary or metabolic cataract. Nearly 50% of the cataracts are not idiopathic (3). Inherited cataracts represent a major contribution to congenital cataracts, especially in developed countries. Secondly the follow-up is relatively short to document an effect on the visual outcome and possible long-term complications like glaucoma. On the contrary a long period of 8 months for the lens to mature can be majorly amblyogenic in a child less than 6 months. It would be interesting to know the changes taking place within this time frame which could affect the visual potential. The process of speeding up transdifferentiation by using cultured cells could be an option to explore. Since we are not addressing the root cause of cataracts, there could be ongoing factors which disrupt lenticular transparency. Although the follow-up of 8 months did not show development of cataractous lens fibres, in the long-term we might see that the cataract is not eliminated but just delayed.

Lin *et al.* have reported a success rate of 100%. None of the regenerated lens/ areas of the lens were optically imperfect. Earlier studies in mammals suggest the regeneration is slow and disorganized (13,19,32). There have been reports of vacuolated masses, opacification, and traction folds in the regenerated lens in animals and these problems disappear only after embryonic implants (12). Since Lin and colleagues did not encounter any such surprises, there are a lot of hidden metabolic regulators and inflammatory agents responsible for lens epithelial cells regenerating into the new lens fibers. Care should be taken to avoid too much inflammatory response.

Lens epithelial cells multiply and sometimes cause visual axis obscuration. Transformation of a residual lens epithelial cell hence may not be that critical a factor. Rather it is the ability of fibers to arrange themselves in the form of natural lens fibers that is surprising. The response might be concentration based. Regeneration is directly related to the thickness of cortical layers left behind, especially those at the equatorial region of the capsule; but regeneration was still possible if no cortical matter remained in experimental studies (27). We need to leave some residual cortical matter to assist growth by physically separating the anterior and posterior capsules. It has been shown that differentiated mammalian cells can also be reprogrammed to become induced pluripotent stem cells that subsequently differentiate to different tissues. Early events of dedifferentiation occur through day 8 of the regeneration process and cell proliferation is initiated by day 4 post-lensectomy in newts. It takes a time for visible proliferation to develop. This lag period before lens epithelial cells proliferate may cause an adhesion between the collapsed anterior and posterior capsule (13). Refilling the capsule with some material to prevent adhesions has also been suggested (12).

Once lens differentiation has started, the index study believes that the process is remarkably similar to lens development. Although the regenerated lens appears normal morphologically; we are yet to prove it histologically in adults. The disorganized regrowth of doughnut-like lens tissues that have been observed after congenital cataract removal in infants have never been functionally tested for biological lens function. The accommodation apparatus namely the zonules and ciliary muscle also have to be studied for any change in function. The pathophysiology of congenital and hereditary cataracts differs in fundamental ways from that of age related cataracts. The lens regeneration will obviously be slower in them. The adherence of the capsules might be a bigger obstacle in adult lens fibers which will take slower to regenerate.

The study advocates that we can restore a "natural lens" in children *de novo*. Identifying key signals and their targets could allow pharmacological stimulation of lens fiber differentiation and further avenues of research extending into adult lens as well. The optimal combination of choosing the right patient, an apt surgical technique along with postoperative care will lead to a breakthrough in regenerative ophthalmology. The surgical procedure of keyhole surgery needs to be studied further and practiced for perfection in the small eyes of infants with compromised space. We have tried this in a few infants and feel it would have a learning phase. Creating a rhexis flap attached at a hinge to the main capsule followed by repositing the flap after lens aspiration could be an alternative surgical technique which we have used in a few odd patients (unpublished). There have been instances when the cataract seems uncomplicated but once aspiration was commenced the cataract seemed hard requiring phacoemulsification or there was a posterior polar opacity of a large size warranting a change of plan (33). Replacing the lens with a new natural and accommodating lens and not any other material will be a dream come true. The study by Lin *et al.* is praiseworthy as they have given an insight into what the future holds for pediatric cataract management and the need for further research which would help in validating this technique and at the same time appreciating the complications and the modifications required.

## Acknowledgements

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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