misdirection; an important distinction since each requires a different therapeutic approach.[22-24]

Caution is needed before undertaking HFU examination. A degree of experience in conventional B-scanning is useful to familiarise the operator with the acquisition of images and interpretation of results. Also, HFU will not replace conventional ultrasound, as large lesions in the anterior segment and those in the posterior segment are too deep to be scanned. The sound velocities in ocular tissues, although known with accuracy at the conventional range, are not yet established at higher frequencies. Biometry results therefore should be interpreted with caution using HFU.

Some practical aspects should also be considered. The transducers of HFU are not covered by a membrane,[2] hence the need to conduct examination through a waterbath to prevent corneal abrasion. The safety of the technique has also to be considered. The latest guidelines suggest that spatial peak temporal average power levels (SPTA) must not exceed 100 mW/cm^2 and UBM produces SPTA intensity of 22 mW/cm^2 which is considerable.[2]

There is little doubt, however, that HFU will become an essential addition to conventional ultrasound scanning, and as the technique is further refined, newer applications will be discovered. The article by Aslanides *et al* is an example of such applications and the images illustrated are a yardstick for future studies.

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Stem cells of the ocular surface: scientific principles and clinical applications

The corneal epithelium exists in a state of dynamic equilibrium, with the superficial cells being constantly shed into the tear pool and renewed by proliferation and migration of the remaining cells. Migration occurs centripetally and circumferentially from the limbus and vertically from the basal layer forwards. Any organ that maintains its cell mass by a process of cell loss, renewal, and regeneration must possess a reservoir of 'stem cells'. Stem cells by definition have a high capacity for self renewal throughout the life of the organ(ism).[1] Stem cells are long lived, have a long cell cycle time with a short S-phase duration, have an increased potential to error-free proliferation with poor differentiation, and demonstrate a capability to divide in an asymmetric manner.[2-4] When a stem cell divides, one of the daughter cells remains as its parent and serves to replenish the stem cell pool, whereas the other daughter cell is destined to divide and differentiate with the acquisition of features that characterise the specific tissue. Such a cell is called a transient amplifying cell (TAC) and is less primitive than its parent stem cell. TACs differentiate into post mitotic cells (PMCs) and finally to terminally differentiated cells (TDCs). Both PMCs and TDCs are incapable of cell division.[2] Such a hierarchy of cells exists at the corneoscleral limbus. The more primitive cells demonstrate certain intrinsic features such as the preferential expression of certain enzymes – for example, α enolase, show an abundance of epidermal growth factor receptors, co-express keratin 19 and vimentin, but do not express the differentiation marker, keratin K3/K12. Such cells are believed to represent corneal stem cells.[3 5]

What maintains the 'stemness' of a stem cell is not well understood. In addition to intrinsic factors mentioned above, extrinsic influences from the microenvironment also probably play a role.[3] The microenvironment of the limbus differs from that of the central cornea by the presence and close proximity of blood vessels to the basal epithelial cells.[6] The basement membrane of the limbus is modified to enhance epithelial cell adhesion and also possesses an abundance of type IV collagen which is absent from the central cornea. Conversely, a protein identified by

an antibody AE27, and associated with cells expressing keratin 3, is present in the basement membrane of the central cornea only. This evidence has led to the hypothesis that basement membrane can influence stem cell differentiation in areas containing high levels of type IV collagen and low levels of AE27 binding antigen.[3]

Primary stem cell aplasia occurs in aniridia and in the even rarer condition of congenital erythrokeratodermia. Secondary depletion of stem cells is, however, relatively more common. Thermal and chemical injuries, ultraviolet and ionising radiation, contact lens wear, toxicity of preservatives, Stevens-Johnson syndrome, limbal surgery, and aging are all associated with loss of stem cells. A persistent corneal epithelial defect following keratoplasty is often indicative of stem cell depletion. As our understanding of the role of stem cells in the maintenance of the corneal surface improves, more and more conditions where this process breaks down are being identified. Coster, Aggarwal and Williams, in this issue of the *BJO* (p 977), have reported stem cell depletion related to limbal dermoid, ocular surface malignancy, and chronic trachoma. When the barrier effect afforded by limbal stem cells is lost, cells of the conjunctival phenotype invade and populate the corneal surface.[7] Such 'conjunctivalisation' of the cornea is associated with an irregular unstable epithelium and tear film, erosions and filaments, superficial vascularisation and chronic superficial punctate keratitis and, eventually, calcification and scarring. These changes are associated with symptoms of pain, photophobia (blepharospasm), lacrimation, and blurring/loss of vision. A corneal graft might seem to be an obvious procedure in such situations but is destined to fail. In the absence of a reservoir of stem cells the ocular surface invariably breaks down with recurrence of symptoms.

It is interesting that evidence to support the transformation of conjunctival epithelium into corneal phenotype, a process referred to as 'conjunctival transdifferentiation' (CT), has been presented with some vigour. In fact it has even been mentioned that normal corneal epithelium is maintained and replenished by the surrounding conjunctival epithelium by the process of CT.[8] This concept can now be refuted on several counts: (1) The earlier animal experiments demonstrating CT had failed to completely remove corneal limbal epithelial cells and, as such, the new epithelium was not entirely conjunctival. (2) Transdifferentiated conjunctival epithelium, on ultrastructural examination, demonstrates mucin and goblet cells indicating persistence of the conjunctival phenotype. (3) The protein and keratin composition and the metabolism of 'transdifferentiated' epithelium differs from that of true corneal epithelium. (4) Long term observations of human corneas where partial conjunctivalisation had occurred, failed to show transdifferentiation to corneal phenotype over several months of follow up.[7] On the contrary, tiny 'buds' of corneal epithelium could be seen along the line of contact of corneal and conjunctival phenotypes suggestive of replacement, rather than transdifferentiation, of one phenotype by the other.[7 9] (5) Finally, a repository of conjunctival stem cells was postulated,[9] and later demonstrated to be located at the fornices.[10] The presence of two distinct locations of stem cells, fornicial and limbal, on the ocular surface would suggest that they serve as progenitors of two different phenotypes of epithelial cells. Conjunctival epithelial wounds heal in a centripetal manner from the fornix. Centrifugal migration of cells from the limbus to cover conjunctival defects does not occur beyond a few millimetres[7] indicating that limbal stem cells are programmed primarily to generate cells for the corneal surface.

The successful management of ocular surface disorders secondary to stem cell deficiency became possible with the advent of conjunctival and limbal transplantation. The desired goals of these procedures are the healing of persistent epithelial defects, reduction in the extent of corneal vascularisation, improvement of vision, patient comfort and cosmesis, and finally as a prelude to penetrating keratoplasty. Conjunctival transplantation was introduced by the authors of the hypothesis that corneal epithelium originated from surrounding conjunctival epithelium. This procedure does, at times, achieve the above goals but not in an entirely satisfactory manner. The concept of limbal transplantation was introduced by Tseng and coworkers and has proved to be considerably superior to its forerunner. Tsai *et al*,[11] working on animal models, have shown limbal transplantation to be much more effective than conjunctival transplantation in preventing corneal vascularisation. Keratoepithelioplasty, a procedure wherein lamellar discs of limbal tissue are transplanted to the periphery of the host cornea, is now increasingly being used in the management of ocular surface problems associated with chemical injury, Stevens-Johnson syndrome, and aniridia. Coster *et al*, in their paper, have extended the above principles and successfully transplanted an annulus of peripheral superficial cornea, limbus, and a fringe of conjunctiva in a patient with acid burn. In another patient with chronic trachoma they were able to transplant allogenic tarsal plate together with palpebral, fornicial, and bulbar conjunctiva. Tissue for both conjunctival and limbal grafts can be autologous (from the opposite unaffected eye), from a close family member (improving HLA match), or from a heterologous donor. The latter source is the most common and necessitates the use of systemic immunosuppression. Coster *et al* have reported considerable success with the combined use of systemic azathioprine and cyclosporin A in patients with ocular surface disorders treated with conjunctival and stem cell allografts.

These procedures often require superficial keratectomy which can become complicated by corneal perforation, particularly in thin and irregular corneas. In the foreseeable future, limbal transplants may be combined with large aperture excimer laser photoablation. Systemic immunosuppression may be supplemented or superseded by topical agents such as cyclosporin. In vitro propagation of 'stem cells' on biodegradable membranes may serve as an alternative and effective source of 'donor tissue' with the cells coming increasingly from the recipient patient.

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