

# ALTERNATIVES TO HUMAN CORNEAL DONOR TISSUE



Using discarded lenticules as scaffolding for engineered corneal tissue.

BY LISA MARTÉN, MD, MPH

## DECELLULARIZATION AND RECELLULARIZATION OF CORNEA: PROGRESS TOWARDS A DONOR ALTERNATIVE

Fernández-Pérez J, Ahearne M<sup>1</sup>

*Industry support: No*

### ABSTRACT SUMMARY

This review article explores corneal bioengineering as a method of generating transplantable tissue. Fernández-Pérez and Ahearne examine various techniques of decellularization, analytical techniques to confirm decellularization efficiency, different cell sources for recellularization, and recellularization methods. These alternative sources of tissue are being developed in response to a global shortage of human corneal donor tissue. The issue of immune rejection is also being addressed through the use of other transplantable tissues.<sup>2</sup>

### DISCUSSION

Collagen hydrogel and extracellular matrix (ECM) are the main components of transplantable tissue. Biologic tissue alternatives use collagen-based materials, scaffolding with hydrogel matrices, and, most recently, 3D printing.<sup>3,4</sup>

ECM can be created in vitro, but the process is expensive and takes longer than desired.<sup>5</sup> ECM derived from decellularized corneas is an attractive alternative. In decellularization, mechanical agitation and detergents, chemicals, biologic agents, or organic acids are used to break down the cells.<sup>6,7</sup> Decellularization is validated in three main ways: (1) the absence of cell nuclei is demonstrated, (2) anti-double-stranded DNA is quantified, and (3) the length of DNA remnants below a certain length is maintained.<sup>8</sup> The structure, transparency, and composition of the corneal tissue

must be maintained for it to be usable. Thus far, the results with different techniques have not been reproducible.

Tissue from other species has been used to study decellularization, but it can be highly immunogenic to humans if transplanted.<sup>9</sup> For research purposes, human tissue has recently become available in the form of corneal stromal tissue from refractive surgery procedures that would normally be discarded.<sup>10</sup>

Recellularizing decellularized corneas with human cells is a process that can be used to create more viable tissue for possible transplantation.<sup>7</sup> Because keratocytes are derived from neural crest mesenchyme, there are several sources for corneal recellularization. Autologous cells from the contralateral eye, for example, are a safe source of cells. The process of recellularization is difficult, however, and requires seeding the cells directly into the stroma.<sup>11</sup>

Must corneal tissue be recellularized before transplantation? This is currently unknown. Acellular transplants have been successful in animal models.<sup>12</sup> The few human studies conducted involved patients who needed anterior lamellar corneal transplants; researchers reported no benefit from using recellularized tissue versus acellular matrix tissue.<sup>13</sup>

Additional studies are required to determine proper protocols for and to standardize methods of decellularizing and recellularizing corneal tissue to be used for human transplantation.

## STUDY IN BRIEF

- A worldwide scarcity of corneal tissue is spurring research on alternative tissue options. This review article examines multiple sources of tissue, including animals, human hydrogel, and decellularized and recellularized extracellular matrix tissue.

### WHY IT MATTERS

Patients who are blind from bilateral corneal disease can benefit from corneal transplantation. More viable options for corneal tissue would assist a greater number of these patients.

## ENGINEERING A CORNEAL STROMAL EQUIVALENT USING A NOVEL MULTILAYERED FABRICATION ASSEMBLY TECHNIQUE

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*Industry support: No*

### ABSTRACT SUMMARY

In an attempt to fabricate corneal substitutes for transplantation simply and quickly, Fernández-Pérez et al

sought to construct mature tissue from decellularized porcine corneal sheets layered with collagen hydrogel. They evaluated three key parameters to determine utility: (1) transparency, (2) cellular viability, and (3) phenotype.

The investigators reported success with decellularization and recellularization of the lenticules used—high cell viability, keratocyte-like phenotype, and integration into the host cornea with acceptable optical properties. Tissue could be surgically manipulated and sutured without tearing.

## DISCUSSION

Globally, one human donor cornea is available for every 70 needed.<sup>15</sup> Fernández-Pérez et al interlaced layers of decellularized porcine cornea with hydrogels that were laden with human corneal cells to produce a new corneal substitute. Tissue was created by decellularizing porcine corneal tissue. DNA content and degradation were tested, and human cell cultures were isolated. Four gels and five sheets of matrix were combined and allowed to grow for 3 weeks.

The cells were prepared and examined with laser scanning confocal microscopy, transparency and transmittance were tested, and a polymerase chain reaction was performed to measure the amount

# STUDY IN BRIEF

► This study evaluated decellularized porcine corneal sheets layered with human hydrogel tissue as a possible alternative to corneal tissue. Investigators measured key parameters of corneal viability, including transparency, anti-double-stranded DNA content, histology, suturability, and tissue strength.

## WHY IT MATTERS

New sources of human cells for recellularization of the corneal extracellular matrix may increase the availability of viable corneal transplant tissue.

of cell RNA remaining. Histologic and immunohistochemical analyses were done on the tissue as well. Additionally, tissue was sutured to assess its strength and clinical viability.

Results showed highly transparent cells with no cell nuclei remaining and demonstrated a mild effect on corneal thickness from swelling during decellularization. Based on the parameters measured, this corneal stromal equivalent appears to be a viable alternative to corneal tissue. ■

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