New method could offer more precise treatment for corneal disease

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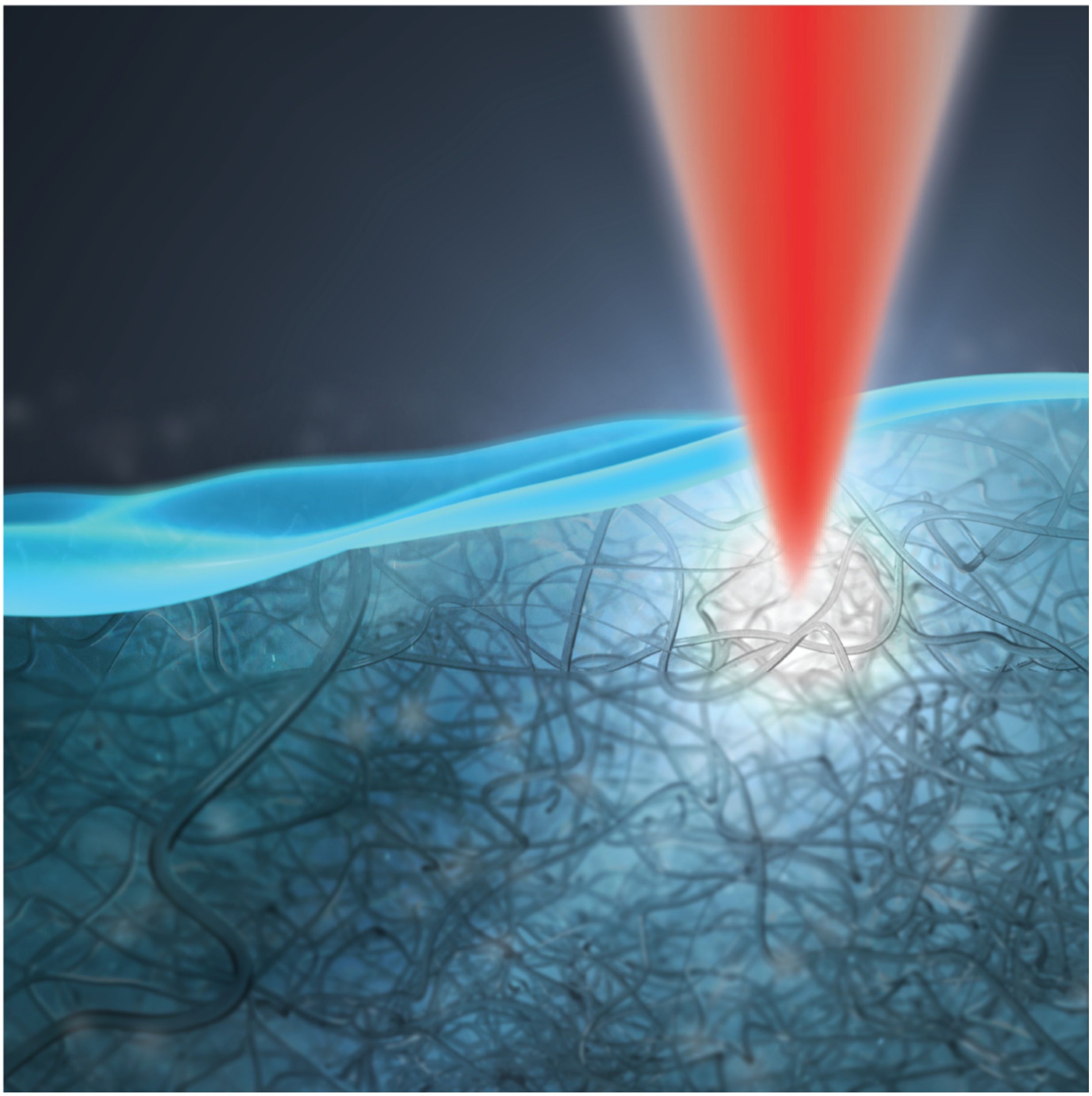

Two-photon collagen crosslinking can selectively stiffen parts of the corneal tissue and might one day offer improved treatment for diseases that weaken corneal tissue. Credit: Seok-Hyun Yun, Massachusetts General Hospital Wellman Center for Photomedicine

Researchers have developed a new light-based technique that selectively stiffens tissue in the cornea and might one day offer improved treatment for eye problems caused by weakened corneal tissue.

In The Optical Society's journal for high impact research, *Optica*, the researchers detail their new technique for strengthening the cornea by precisely crosslinking the collagen fibers that make up corneal tissue. They also demonstrate a specialized microscopy approach that measures tissue stiffness without disturbing untreated areas.

"Because light has the ability to probe deep inside tissue, we're using that property to both go inside and change mechanical properties of tissue and to measure those changes so that we can understand how the tissue changed and visualize it noninvasively," said Sheldon J.J. Kwok, Massachusetts General Hospital Wellman Center for Photomedicine, first author of the paper.

Making crosslinking more precise

Disease can cause the cornea, the clear dome-shaped layer that covers the front of the eye, to gradually weaken until pressure in the eye causes it to bulge and leads to vision problems. A corneal transplant may eventually be necessary as the condition, known as keratoconus, progresses. However, a new treatment called corneal crosslinking is showing promise for slowing or stopping the progression of keratoconus. The procedure is used in Europe, Canada and Japan, and is undergoing clinical trials in the United States.

"Right now, ultraviolet light is used to perform crosslinking across the entire cornea," said Seok-Hyun Yun, Massachusetts General Hospital Wellman Center for Photomedicine, leader of the research team. "However, this comes with a risk of damaging the innermost layer of the cornea, a complication that changes the corneal function and can cause it to become very hazy. There is also no way to predict whether the procedure will actually improve vision in a given patient."

For this reason, the researchers wanted to see if two-photon absorption could be used to precisely control the area of corneal crosslinking. Two-photon absorption uses a near-infrared femtosecond laser to achieve very high spatial resolution confined to a small volume. This approach has been used to harden liquid resins to create microscopic optical components and other 3D structures. Until this research, the technique had not been applied to stiffen tissue, which is initially solid, in a specific area.

Are two photons better than one?

To see if two-photon absorption could indeed induce crosslinking, the researchers used a home-built two-photon microscopy setup that delivers femtosecond Ti:sapphire laser light delivered through the objective lens. They placed a cornea sample under the objective lens, applied a light-sensitive dye to the tissue, and then turned on the laser light and focused it to a specific layer.

After some trial and error, they found that exposing the tissue to 200-milliwatt laser light for ten minutes induced collagen crosslinking without damaging tissue. By scanning the laser through an area of tissue, the researchers found they could induce crosslinking in a specific 3D area.

"If you can crosslink a specific part of the cornea, it might be possible to optimize the visual outcome," Yun said. "With two-photon absorption we can selectively choose and limit the depth of crosslinking to avoid cell damage both on the top and bottom layers of the cornea."

One of the challenges, however, is verifying that crosslinking has taken place. The collagen crosslinking induced by two-photon absorption is not easily visualized with standard imaging methods. Thus, the researchers turned to a specialized technique called Brillouin microscopy, which Yun's lab previously developed and has gradually improved. Brillouin microscopy offers a non-contact way to measure 3D biomechanical tissue properties with high spatial resolution.

"We used Brillouin microscopy to validate that we can induce crosslinking in tissue and showed that the amount of crosslinking produced with two-photon adsorption is almost the same as what can be induced with the single-photon approach used today," Yun said.

Because the two-photon approach induces crosslinking point by point, it is a lengthy process to perform, making it most useful for crosslinking a small region or a thin slice of tissue.

Although more studies are needed to understand how various patterns of crosslinking affect corneal shape and vision, the researchers hope that the new technique could allow crosslinking in a 3D pattern specific to a patient's corneal bulging to maximize the benefits of the treatment.

Two-photon crosslinking might also be useful for tissue engineering applications, where it could be used to selectively modulate the stiffness of 3D cell cultures to more closely match that of tissue in the body, for example.

More information: Sheldon J. J. Kwok et al, Selective two-photon

collagen crosslinking in situ measured by Brillouin microscopy, *Optica* (2016). DOI: 10.1364/optica.3.000469

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