formulations as they fine-tuned the release of FD40 in comparison with that of F_{sol}, perhaps because the COL and COL/PEGDM pellets, which cannot permeate the membrane, caused the reservoir solutions to have lower FD40 concentrations, which, in turn, decreased the steepness of the FD40 gradient from the reservoir to the exterior PBS solution. Therefore, the F_{col} and F_{pel} formulations, as sustained drug-release systems, improved the ability to control FD40 release by limiting the rate of FD40 dissolution, with the membrane controlling the diffusion rate via the COL tunnels. Consequently, the release of a drug can be controlled by the COL concentration in the membrane and the drug formulation.

3.3. Release mechanism

To further characterize the FD40 diffusion mechanism, we determined the diffusion coefficients for FD40 through 0.8% (w/v) crosslinked collagen (D_c), PEGDM (D_p), TEGDM (D_t), and water (D_w). D_c, D_p, and D_t were calculated using the FD40 diffusion rates through the gels (Fig. S3), and D_w was calculated using the Stokes–Einstein equation [23]. Because D_c (45.2 $\mu m^2/s$) was 1000 times larger than D_p (0.045 $\mu m^2/s$) and was smaller than D_w (67.9 $\mu m^2/s$), it appears that FD40 diffused through interconnected COLs in the membranes. If the COLs in the membrane are not interconnected, dead-ends are probably present that would inhibit the rate of drug release to the outside. However, once the COL density increases above a permeation threshold (>100 mg COL/ml), which was estimated by SEM as noted above (Fig. 2D–F), the COLs should be sufficiently interconnected that the number of dead-ends is reduced, and permeability is thereby increased. Because D_t was zero, FD40 cannot diffuse through the TEGDM reservoir, which enables unidirectional drug release.

3.4. In vitro BDNF release and bioactivity

To evaluate the release of the neurotrophic factor rhBDNF, capsules were filled with COLs that contained the protein and were tightly sealed with a membrane with a COL concentration of 0, 100, 300, or 500 mg/ml Fig. 4A presents the zero-order kinetic profiles found for rhBDNF release during a 6-week assay period. Apparently, the release kinetics of rhBDNF can be fine-tuned by varying the concentration of the COLs in a membrane in much the same manner as was found for FD40. Additionally, media that had been preincubated with capsules that contained rhBDNF induced the phosphorylation of mitogen-activated protein kinase (MAPK) in RGC5 cells when incubated with those cells as shown by western blotting of the cell extracts (Fig. 4B). BDNF is known to upregulate the expression of phosphorylated MAPK in retinal tissue [24]. In the present study, rhBDNF was found to phosphorylate MAPK in RGC5 cells in a dose-dependent manner by incubating the cells with media spiked with rhBDNF (Fig. 4C), which demonstrated that, when released from the capsule, rhBDNF retained its full activity.

Among the known neurotrophic factors, BDNF is the most potent survival factor for damaged retinal ganglion cells [10,25,26]. However, BDNF is currently administrated to the retina by intravitreal or subretinal injections in PBS [26], adenovirus vectors containing the BDNF gene [26,27], or genetically modified cells that secret BDNF [13,28]. Direct injections, however, result in extreme patient discomfort and complications arise caused by repeated injections or surgical procedures [2]. Because our capsule can contain various drug formulations, the encapsulation of the adenovirus vectors and the genetically modified cells might be possible and, as such, would represent a less invasive path than is currently available.

3.5. Implantation study

Our next challenge was to evaluate the capsule's ability to deliver a protein-type drug to the retina via the sclera. Capsules that had a reservoir (2.6 × 2.6 × 0.6 mm) filled with F_{pel} were sutured to the sclerae of three rabbits' left eyes with 10-0 nylon (Fig. 5A). The capsules abutted the sclerae but did not penetrate the conjunctivae or adjacent areas. Fig. 5B shows a fluorescent image of FD40 within a capsule, and Fig. 5C shows the release of FD40 locally at the sclera but not at the conjunctiva. This unidirectional release should reduce drug elimination by conjunctival lymphatic/blood clearance, thereby resulting in more effective delivery to the retina [29]. One month after implantation, the capsules remained sutured and neither the PEGDM of the membranes (Fig. 5D) nor the reservoirs had eroded (Fig. S4). The COLs in the membranes also survived with little biodegradation (Fig. 5D), most likely because the collagen molecules were stabilized by chemical crosslinking [18]. Although the capsules were loosely covered with connective tissue by the end of the trial, they were easily removed from the implant site. Routine ophthalmological examinations showed no eye-related toxic effects. Intense FD40 fluorescence in the sclerae adjacent to the implantation sites was observed (Fig. 5E). Furthermore, FD40 had migrated to the retinal pigment epithelium (RPE) and adjacent regions (Fig. 5F), which indicated that transscleral delivery of FD40 to the retina had been achieved. To the best of our knowledge, this is the first report that a macromolecule can be delivered to the

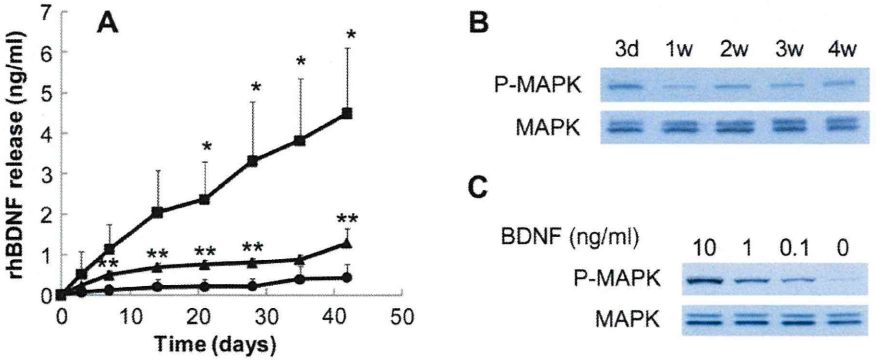

Fig. 4. Release of rhBDNF *in vitro*. (A) rhBDNF-loaded COLs in PBS were added to capsule reservoirs that sealed with a membrane with a COL concentration of 100 mg/ml (circles), 300 mg/ml (triangles), or 500 mg/ml (squares), and the release of rhBDNF was monitored using the reagents of a BDNF-ELISA kit. The release rate of rhBDNF through a PEGDM/COL membrane that contained 100 mg COL/ml was almost the same as one that contained no COLs. Means ± SDs are shown. $^*P < 0.05$ for 300 mg/ml vs. 500 mg/ml $^{**}P < 0.05$ for 100 mg/ml vs. 300 mg/ml. (B) Western blots of RGC5 cells extracts probed with antibody against phosphorylated MAPK (P-MAPK) and total MAPK. (C) The control study showed that rhBDNF could induce MAPK phosphorylation in RGC5 cells in a dose-dependent manner by incubating the cells with media spiked with rhBDNF.

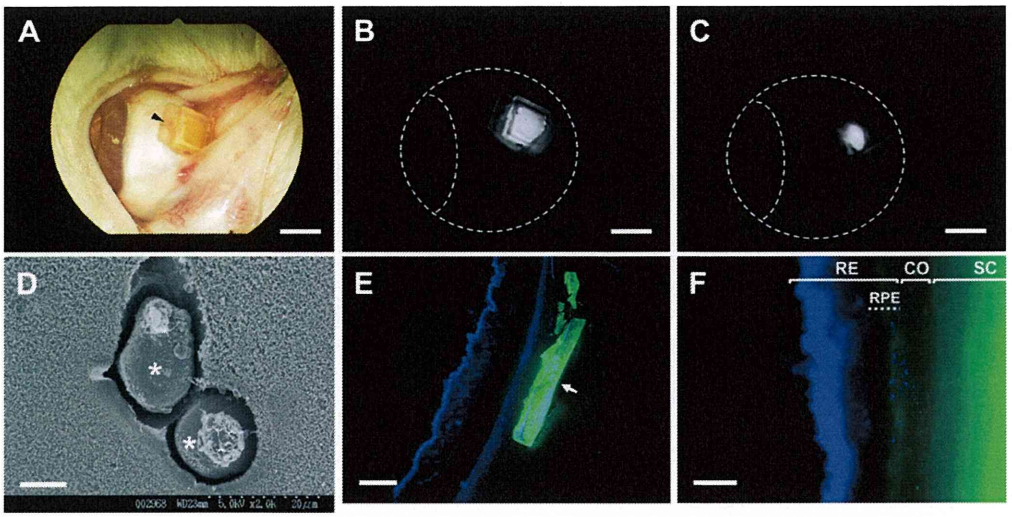

Fig. 5. Episcleral implantation of a capsule. (A) Image of a capsule sutured to the sclera of a rabbit eye 3 days after implantation. An arrowhead indicates the suture site. Fluorescent images around the sclera (B) immediately before and (C) after removal of the capsule 3 days after implantation. Fluorescence is visible as the white areas. (D) SEM image of a COL (asterisks) in the membrane of a used capsule that was removed 1 month after implantation. The COLs were not biodegraded. (E, F) The distribution of FD40 (green) in the retina and sclera around the implantation site 3 days after implantation (arrow: capsule). Cell nuclei were stained with 4,6-diamidino-2-phenylindole (blue). FD40 reached the retinal pigment epithelium. Abbreviations: sclera (SC), retinal pigment epithelium (RPE), choroid (CO), and retina (RE). Bars: 4 mm (A, B, and C), 10 μm (D), 400 μm (E), and 100 μm (F).

retina via a reservoir-based transscleral drug-delivery system, although quantification of the drug distribution still needs to be done. Proteins, as large as 50–75 kDa, penetrate into the choroid/RPE upon periocular injection [30]. Therefore, it may be possible to also deliver BDNF by the transscleral route. Given that the distribution of FD40 was somewhat concentrated at the RPE and adjacent regions, our device may be effective especially for lesions that surround the RPE. The capsule could also be used to deliver anti-angiogenic drugs, e.g., Lucentis and Macugen (for the treatment of age-related macular diseases) [31], to a lesion, e.g., the choroidal neovascular membrane, because delivery by this route will be less invasive and safer than are conventional intravitreal injections. Our non-biodegradable capsule should therefore be suitable for the transscleral delivery of protein-type drugs that require chronic suppressive-maintenance therapy over several weeks or months.

In summary, our capsule design incorporates features, outlined below, that have been absent from intraocular drug-delivery implant systems previously developed. First, the drug release kinetics can be controlled by changing the drug formulation and/or the membrane COL density so that the initial and final bursts are suppressed, which extends the release period. Second, the capsule is a scleral implantable device. To date, two ocular drug-delivery systems, Vitrasert [32] and Retisert [33], which are intravitreal sustained-release implants of ganciclovir and fluocinolone acetonide, respectively, have been marketed. Although these devices release the drugs at relatively constant rates, they must be surgically implanted in and later removed from the vitreous, which may cause complications or patient discomfort. Our capsule can be implanted and removed almost noninvasively by minor surgery. Third, most transscleral drug-delivery systems are designed to deliver low molecular weight drugs; however, ours appears able to deliver drugs of much greater molecular weights, i.e., protein-type drugs. Recent clinical trials and research have shown that many proteins are effective as drugs [9]. However, none of the available devices can deliver protein-type drugs in a controlled-release manner to the retina. Our capsule can be easily modified to accommodate different release rates for protein-type drugs by altering the membrane COL composition and/or drug formulation. Although this report demonstrated the release of only FD40 and BDNF, it should be possible to load and release low molecular weight drugs, protein-type drugs, and even drugs produced by encapsulated cells. The capsule thus has great potential for use in biomedical applications. Our future work will focus on preclinical animal studies to further assess the safety and effectiveness of the capsule.

4. Conclusion

This study reports the design and testing of a transscleral drug-delivery system that is implantable in the episclera and allows for controlled release of BDNF or other protein-type drugs with zero-order kinetics. Our microfabricated capsule consists of a drug reservoir sealed with a controlled-release membrane that contains interconnected COLs, which are the routes for drug permeation. The drug release kinetics can be controlled by changing the drug formulation and/or the membrane COL density so that the size of the bursts is reduced, which extends the release period. The capsule is designed to contain various drug formulations and dosages, allowing for a wide range of biomedical applications. The device thus has great potential as a conduit for continuous, controlled drug release.

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Appendix

Figure with essential color discrimination. Fig. 4 of this article have parts that are difficult to interpret in black and white. The full

color image can be found in the online version, at doi:10.1016/j.biomaterials.2010.11.006.

Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.biomaterials.2010.11.006.

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