Fathi *et al*., ***BioImpacts,*** 2015, 5(4), 159-164
doi: 10.15171/bi.2015.31
http://bi.tbzmed.ac.ir/

Hydrogels for ocular drug delivery and tissue engineering

Marzieh Fathi, Jaleh Barar, Ayuob Aghanejad, Yadollah Omidi*

Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

Article Info

Article Type:
Editorial

Article History:
Received: 18 Dec. 2015
Accepted: 22 Dec. 2015
ePublished: 28 Dec. 2015

Keywords:
Cell therapy
Drug delivery
Eye
Hydrogels
Ocular diseases
Tissue engineering

Summary

Hydrogels, as crosslinked polymeric three dimensional networks, possess unique structure and behavior in response to the internal and/or external stimuli. As a result, they offer great prospective applications in drug delivery, cell therapy and human tissue engineering. Here, we highlight the potential of hydrogels in prolonged intraocular drug delivery and ocular surface therapy using stem cells incorporated hydrogels.

Authors Biosketch

Dr. M. Fathi (PhD) is a postdoctoral fellow at RCPN, working on development of advanced biopolymers used for drug delivery and targeting and tissue engineering.

Dr. J. Barar (PharmD, PhD) is professor of biopharmaceutics, working on biological impacts of advanced nanobiosystems used for drug delivery and targeting and cell therapy.

Dr. A. Aghanejad (PhD) is an assistant professor at RCPN, working on pharmaceutical chemistry and drug discovery.

Dr. Y. Omidi (Pharmd, PhD) is a professor of pharmaceutical sciences, working on advanced multi-functional nanobiosystems used for drug delivery and targeting and tissue engineering.

Introduction

Technically, hydrogels are crosslinked polymeric networks whose complexation occurs mainly via hydrophilic ionic interactions between polymeric long chain entities and water molecules. Architecturally, such three-dimensional (3D) complex system within the liquid form some kind of porous structures giving some kind of gelling behavior to the hydrogels which are mostly liquid by weight. One may consider a hydrogel as an open system with semipermeable properties that allow movement of water and solute molecules, but not ionized entities. The 3D porous architecture of hydrogels make them capable of restraining large quantities of water molecules and undergoing macroscopic changes in dimensions which grant some degrees of flexibility alike to natural tissues.

These advanced soft matters can be engineered using natural biopolymers and semi-synthetic/synthetic polymers though chemical (covalent bonds) and/or physical (ionic bonds, entanglements, hydrogen bonding, van der Waals or hydrophobic interactions) crosslinking methods.[1-3] Remarkable hydrophilicity, flexibility and elasticity properties of hydrogels make them suitable for widespread uses in drug delivery and targeting (for small and large molecules), tissue engineering, regenerative medicine, macromolecule separation and encapsulated cell technology. Various physical and/or chemical gelation phenomena are involved in the formation of hydrogels. Mechanistically, in situ physical gelation may happen via (a) hydrophobic interactions, (b) charge interactions via the oppositely-charged polymer/small molecule crosslinkers, (c) hydrogen bond interactions between geometrically-compatible biopolymers (e.g., methylcellulose and hyaluronic acid), (d) stereo-complexation (e.g., L- and D- lactide polymer chains polymerization), (e) Supramolecular complexation (e.g., polyethylene oxide and cyclodextrin molecules polymerization). In situ covalently cross-linked hydrogels is considered as another approach. Perhaps, one of the best paradigms for these hydrogels is the controllable gelation of dextran-tyramine and hyaluronic acid-tyramine complexation and formation as hydrogels using horseradish peroxidase and hydrogen peroxide cross-linkers (with gelation time ranging from 5 sec to 9 min depending on the reactant concentrations used). This approach can further be

***Corresponding author:** Yadollah Omidi, Email: yomidi@tbzmed.ac.ir

compatibilized using human-based biopolymers such as serum albumin molecules cross-linked with tartaric acid, which were reported to result in formation of extremely tissue adhesive hydrogel with the capability of controlling the liberation of anticancer agents such as doxorubicin.[4] Furthermore, hydrogels can be engineered through polymer-polymer cross-linking as reported for hyaluronic acid cross-linked by hydrazine bonds for prolonging the release of drugs such as tissue plasminogen activator and budesonide.[5,6] Fig. 1 shows the structures of selected natural and synthetic polymers commonly used for the production of hydrogels.

By virtue of its prospective, an ideal hydrogel should possess high biocompatibility with cells/tissue within any biological milieu. Depending on the purpose of the hydrogels applications, they can be engineered as bioadhesive systems to integrate with the contacting biological entities, or with hydrophilic surface that pose low interfacial free energy in contact with body fluids to decrease the adherence tendency to proteins and cells. Moreover, the soft and rubbery nature of the hydrogels are in favor with their in vivo uses, in large part because of the no/minimized irritation of the surrounding tissue by these systems.[3,7-9]

It should be noted that the swelling rate and water adsorption capacity of hydrogels appear to be the most key parameters which control the release kinetics of water molecules, solutes and drugs from these polymeric networks.[10] As a general rule, it can be noted that the higher the hydrophilicity of the impregnated drugs within the hydrogel, the faster the liberation of the drug molecules from the hydrogel. However, the interaction of drug molecules with polymeric backbone of hydrogel can significantly affect the liberation of hydrophilic drugs from the hydrogel-based drug delivery systems (DDSs). For example, the charged functional groups (both anionic and cationic entities) of the carbohydrate-based polymers can impose substantial interactions with the drug molecules with opposite charge, resulting in significant prolongation of drug release. For a hydrogel to become smart and behave as a sensor and actuator, its degree of crosslinking should be low enough to enable the polymeric network to go through significant complex conformational alterations in response to a designated stimuli, while such parameter should be high enough to warrant the mechanical stability of hydrogel in order retain its functionality after several cycles of use.[11] Here, we provide concise information on environmentally sensitive of stimuli-responsive hydrogels.

Environmentally sensitive hydrogels

Environmentally sensitive hydrogels (the so-called intelligent/smart or stimuli-responsive hydrogels) possess unique characteristics in terms of morphology alteration by swelling/de-swelling (as volume collapse or phase transition) in response to the designated physical or chemical stimuli (Fig. 2). These hydrogels offer a wide and diverse range of applications in engineering of artificial muscles, biomimetic biosensor/bio-actuator, immobilization of enzymes and cells, bio-separation and preparation of self-regulated DDSs. It should be highlighted that the in situ hydrogel formation makes it more applicable for the delivery of small molecules and macromolecules, tissue engineering, and as a simple and safe system for tissue coverage and other in vivo uses.[12,13] Further, multiple stimuli-responsive hydrogels have attracted substantial interests because dual use of pH- and temperature-sensitive functions can provide an improved system for biological uses by mimicking the responsive biomacromolecules in biological settings.[14,15]

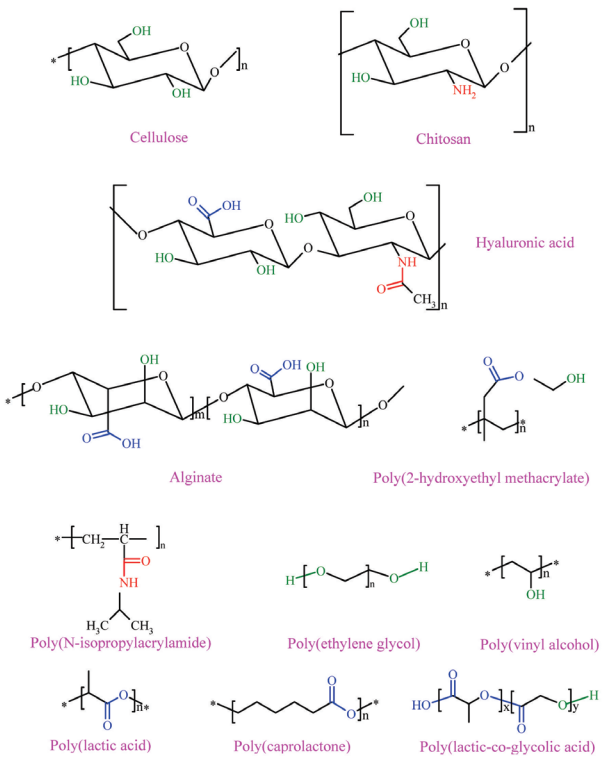

Fig. 1. Chemical structures of natural and synthetic polymers used as hydrogels.

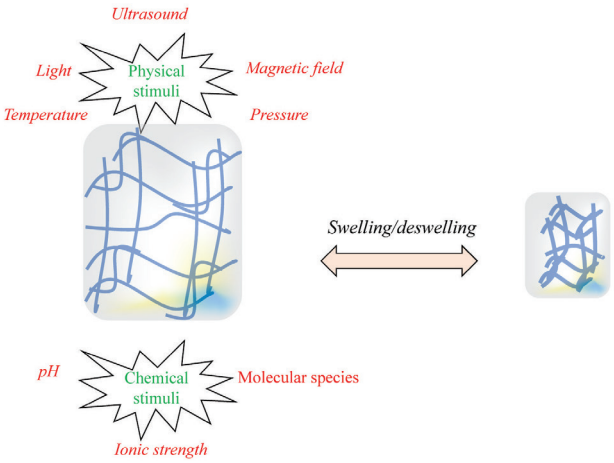

Fig. 2. Hydrogel collapsing in response to physical and chemical stimuli.

Temperature, light and pH sensitive hydrogels

The stimuli responsive hydrogels are remarkably unique tools for engineering intelligent systems (e.g., for drug delivery, tissue engineering) with good enough responsiveness to the external and/or internal stimuli such as light, temperature, pH and different biomolecules. Recently, Kharkar et al. devised a multimodal degradable tunable patient-specific hydrogel as an injectable delivery system responsive to the externally applied light and physiologically reducing microenvironments stimuli. These researchers capitalized on thiol and maleimide ended polyethylene glycol (PEG) macromers and used click chemistry for complexation (i.e., reacting maleimide and thiol end functionalized four-arm PEG macromolecules through a Michael-type addition reaction). The engineered hydrogel encompassed (a) maleimide end functionalized PEG representing the photodegradable (by UV, visible, or two-photon IR light) macromere with an o-nitrobenzyl ether (o-NB) and (b) thiol end functionalized four-arm PEG macromere representing the reducing-environment sensitive linkage. They showed that the hydrogels can be degraded via bulk degradation and/or by surface erosion, in which the liberation of entrapped cargo entities (i.e., fluorescent nanobeads with diameter ~100 nm) appeared to be modulated by both external (light) and internal (reducing physiologic condition) stimuli.[16]

On the basis that the pH gradients can be found in the gastrointestinal tract, tumor tissues and inside the cells (cytosol, endosome and lysosome), the pH-responsive hydrogel can be of great interest. The pH-responsive polymers characteristically contain weak acidic (e.g., carboxylic and sulfonic acids) or basic (e.g., ammonia) functional groups, which are commonly used for engineering hydrogels with capability of drug liberation in response to alterations in pH of the biological environment. Under certain pH conditions the functional groups presented along the backbone and side chains of the polymer undergo ionization that leads to a conformational change(s) in the polymer resulting in its swelling or dissolution charge status. Some paradigms for the pH-sensitive polymers are poly(acrylic acid), poly(L-lysine), poly(ethyleneimine), and poly(N,N-dimethylaminoethyl methacrylate).[7,10]

Temperature-sensitive polymers are another interesting class of advanced materials. The hydrophilicity and hydrophobicity of polymers are in close relation with solubility boundaries of (a) the upper critical solution temperature (UCST) and (b) the lower critical solution temperature (LCST) depending upon the molar mass and the pressure, therefore it should be emphasized that hydrogels formed using these polymers would be fully miscible at temperatures below LCST. When the temperature is above LCST, the polymer becomes hydrophobic and its conformation alters from a linear expanded soluble status to an entangled globular insoluble status. Poly(N-alkyl substituted acrylamides) and poly(N-vinylalkylamides) with transition temperatures of ~32–40°C are the main classes of thermo-responsive polymers that have widely been studied. It should be stated that there exist some other polymers such as poly(N-vinyl piperidine) with transition temperatures at a range of 4–5°C. Among the polymers used for preparation of stimuli-responsive hydrogels, the poly(*N*-isopropylacrylamide) (PNIPAAm) based hydrogels are the most popular temperature-responsive systems because of exhibiting thermally reversible shrinkage or collapse above the LCST (~32°C) in water.[10,17] PNIPAAm exhibits a swollen and hydrophilic state when the temperature is below the LCST, however it shows abrupt volume shrinkage and becomes hydrophobic when the temperature is above the LCST. It should be noted that incorporation of more hydrophilic moieties (e.g., itaconic acid) with PNIPAAm hydrogel can literally bestow pH sensitivity and enhance the LCST of PNIPAAm hydrogel.[18]

Injectable hydrogels

Hydrogels that undergo a "sol-gel" transition provide great opportunity for the development of injectable DDSs and systems usable in tissue engineering and as implants. They offer the advantages of good alignment with the irregular structures in cells/tissue and allow easy cell incorporation. Moreover, from the clinical point of view, implantation surgery can be avoided and replaced by a simple injection procedure. Injectable hydrogels can be implanted into the body in the form of liquid at about 23-25°C. Once injected, they show in situ gelation at temperature above 32°C. Importantly, prior to the gelation of hydrogels, the designated cells and macromolecules (e.g., growth factors) can be incorporated and suspended into the hydrogel in the "sol" state, which can provide a relatively uniform cell seeding and easy implantation.[19] Of a number of polymers, diblock copolymers of PEG and poly(lactic acid), and their triblock copolymers have been introduced as biodegradable and injectable DDSs under physiological conditions. Further, gelatin, agarose, amylase, amylopectin, cellulose derivatives and gellan, exhibit thermoreversible gelation behaviors. For examples, a chitosan-based, injectable thermogels has been engineered through grafting an appropriate amount of PEG onto the chitosan backbone and used for prolonged drug release in vitro.[20]

Hydrogels applications in ocular diseases

The eye is a complex organ that is divided into two main compartments including anterior and posterior segments. Within these intraocular segments, the traverse of exogenous and endogenous substances are restrictedly controlled by the ocular physiologic and biologic barriers.[21] As shown in Fig. 3, to enter into the ocular compartments, the locally or systematically administered drugs must cross several physiologic and biologic barriers including (a) the tear film and lacrimation with fast turn over time, (b) the corneal epithelial barrier that include 5-6 cell layers displaying highly tight junctional interactions among the nonkeratinized flattened superficial cells surrounded by glycocalyx, (c) the endothelial barrier of iris capillary,

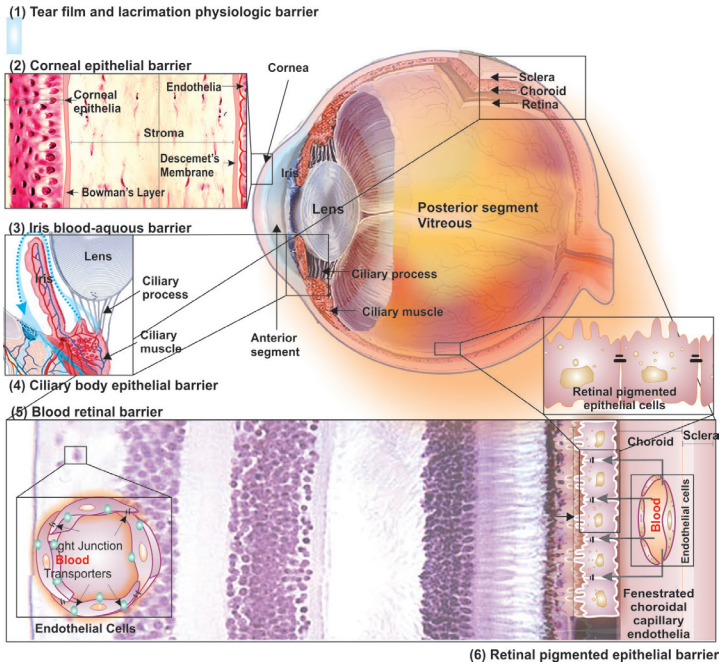

Fig. 3. The eye and its physiologic and biologic barriers.

(d) the epithelial barrier of ciliary body, (e) the retinal capillary endothelial barrier (the inner retinal barrier), and (f) the retinal pigmented epithelial barrier (the outer retinal barrier).[22]

A number of ocular DDSs and devices have been developed to prolong the therapeutic concentrations of ophthalmic drugs within the anterior and/or posterior segments. Of these, ocular nanomedicines and hydrogels seem to provide outstanding therapeutic outcomes.[23-26] In fact, there exist a number of ocular diseases that need implementation of advanced DDSs (e.g., multifunctional nanomedicines and stimuli-responsive hydrogels). Some paradigms for these ocular diseases include inflammation-based diseases (e.g., scleritis, keratitis, uveitis, iritis, conjunctivitis, chorioretinitis, choroiditis, retinitis, retinochoroiditis), ocular hypertension and neuropathy, age-related macular degeneration and mucopolysaccharidosis due to accumulation of glycosaminoglycans. It should be noted that both anterior and posterior segments of the eye along with the stroma of the cornea encompass naturally occurring hydrogel (a water-swollen polymer network), which may to some extend follow the basic physicochemical principles of the hydrogels.[27]

Hydroxypropyl cellulose, hydroxypropyl methylcellulose, chitosan, dextran, poly(acrylic acid) derivatives such as carbomer 934 and polycarbophil have been reported as the most appropriate bioadhesive polymers for the ocular drug delivery. In addition, the high viscosity of the carbomer hydrogels ensures the prolonged retention, improving the ocular bioavailability of the incorporated drugs within the hydrogel complex network. For example, pranoprofen-loaded poly(lactic-co-glycolic acid) nanoparticles dispersed into carbomer hydrogel (i.e., formulated as a semi-solid system) was used for the treatment of edema on the ocular surface.[28,29]

Given that the hydrogels are biocompatible systems for drug delivery and targeting, they have successfully been used for the delivery of hydrophilic drugs into the anterior and posterior segments of the eye. Nevertheless, the hydrophobic drug delivery through hydrogel may encounter with some issues including (a) non-homogeneous loading of drug moolecules, (b) problematic drug release pattern within an aqueous microenvironment such as ocular chambers. Such issues could be resolved through copolymerization and grafting of more soluble side chains; readers are referred to an excellent review of Hoare and Kohane.[4]

It should be also pointed out that the hydrogels have widely been used for the ocular tissue engineering. For example, as one of common issues in ocular diseases, the ocular surface (OS) disorders occur by the limbal stem cell deficiency (LSCD), leading to an impaired vision and blindness. Transplantation of amniotic membrane (or amnion) is the main treatment modality of LSCD, while it can often associate with some risks and limitations including disease transmission, imperfect transparency, variable and unstable quality and low mechanical strength.[30,31] On the basis that hydrogels are biocompatible uniform viscoelastic structures yet amenable for modification(s) to become alike body tissue, they can be used an alternative treatment modality that can be equipped with desired cells as simply applied on the OS. Further, unlike amniotic membrane transplantation modality, the application of hydrogels for tissue engineering demand no requirement for an extensive serological screening for histo-compaitibility.[31] So far, various polymers have been used to devise different types of hydrogel applied for the tissue engineering of OS, including bilayer composite hydrogel composed of corneal stroma crosslinked to poly(ethylene oxide),[32] alginate and agarose based systems,[33] modified calcium alginate,[34] gelatin based constructs,[35] PEG-stabilized collagen-

chitosan crosslinked systems,[36] polyvinyl alcohol modified poly (epsilon-caprolactone) nanofibrous system,[37] genipin crosslinked chitosan,[38] chitosan-PEG systems,[39] collagen and chondroitin sulfate based hydrogel,[40,41] fibrin,[42,43] silk fibroin,[31] keratin, polymethacrylate, and cellulose hydrogel.[31,44] Taken all, it appears that a biocompatible hydrogel can be used as an artificial cornea in the case of LSCD, in which reconstruction of the OS with autologous stem cells embedded onto the hydrogel may provide great clinical impacts.

Further, the main components of hydrogel lens materials are relatively hydrophilic polymers such as poly (2–hydroxyethyl methacrylate) and other monomers that are added to alter the ionicity and water content to improve the wettability, the flexibility, the oxygen permeability and the fluid transport of hydrogel lenses. The oxygen permeability of these hydrogels is dependent on water content; and, therefore, is limited by the solubility of oxygen in water.

Silicone hydrogels combine the positive attributes of a soft lens with the excellent solubility of oxygen in silicone.[45] Because of the moderate hydrophobic nature of the silicone that can be inherently incompatible with the OS, the hydrophilicity feature of currently available silicone hydrogel lenses can be enhanced by surface treatment or by the incorporation of soluble polymers (as the internal wetting agents) within the bulk materials to form an interface between the lens and the tear film.[45]

Final remarks and outlooks

Conclusively, it should be articulated that despite significant advancements upon engineering and modification of hydrogels used for the ocular drug delivery and tissue engineering, some imperative issues must be addressed to ensure about the safety of log-term use, the maximal usefulness and the clinical outcomes of the ocular hydrogels. Of these concerns, the homogenous incorporation of hydrophobic drugs and macromolecules with desired release kinetics as well as optimized sol-gel properties of the hydrogels without clogging during injection can significantly improve the applicability of the hydrogels as the ocular DDSs. It should be inferred that the clinical outcomes of the transplantation of amniotic membrane modality may be limited due to some serious concerns (e.g., transparency issues, inevitable risk of disease transmission from donor to recipient, and undesired inflammation and immunologic responses). Therefore, cytocompatible hydrogels can alternatively provide a much safer modality for the treatment of ocular surface disorders. Such approach can be further implemented for safe engineering of the other ocular cells/tissues within the posterior segments (e.g., treatment of retinal diseases), for which it is necessary to examine the biocompatibility of hydrogels in both ocular in vitro cell-based models[22] and in vivo animal models. Taken all, we believe that hydrogels can offer great potential in ocular drug delivery and tissue engineering if we consider the biological mimicry of hydrogels with target tissues and encapsulated cells.

Acknowledgments

Authors would like to acknowledge the Iranian National Science Foundation (INSF) for the financial support (Grant No: 93030668).

Ethical issues

There is none to be declared.

Competing interests

No competing interests to be disclosed.

References

1. Hamidi M, Azadi A, Rafiei P. Hydrogel nanoparticles in drug delivery. *Adv Drug Deliv Rev* **2008**; 60: 1638-49.
2. Ahmed EM. Hydrogel: preparation, characterization, and applications. *J Adv Res* **2015**; 6:105-21.
3. Hennink W, Van Nostrum CF. Novel crosslinking methods to design hydrogels. *Adv Drug Deliv Rev* **2012**; 64: 223-36.
4. Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. *Polymer* **2008**; 49: 1993–2007. doi: 10.1016/j.polymer.2008.01.027
5. Yeo Y, Bellas E, Highley CB, Langer R, Kohane DS. Peritoneal adhesion prevention with an in situ cross-linkable hyaluronan gel containing tissue-type plasminogen activator in a rabbit repeated-injury model. *Biomaterials* **2007**; 28: 3704-13. doi: 10.1016/j.biomaterials.2007.04.033
6. Yeo Y, Adil M, Bellas E, Astashkina A, Chaudhary N, Kohane DS. Prevention of peritoneal adhesions with an in situ cross-linkable hyaluronan hydrogel delivering budesonide. *J Control Release* **2007**; 120: 178-85. doi: 10.1016/j.jconrel.2007.04.016
7. Joglekar M, Trewyn BG. Polymer-based stimuli-responsive nanosystems for biomedical applications. *Biotechnol J* **2013**; 8: 931-45.
8. Deligkaris K, Tadele TS, Olthuis W, van den Berg A. Hydrogel-based devices for biomedical applications. *Sensors and Actuators B: Chemical* **2010**; 147: 765-74.
9. Radhakrishnan J, Krishnan UM, Sethuraman S. Hydrogel based injectable scaffolds for cardiac tissue regeneration. *Biotechnol Adv* **2014**; 32: 449-61.
10. Chan A, Orme RP, Fricker RA, Roach P. Remote and local control of stimuli responsive materials for therapeutic applications. *Adv Drug Deliv Rev* **2013**; 65: 497-514.
11. Alvarez-Lorenzo C, Concheiro A. Intelligent drug delivery systems: polymeric micelles and hydrogels. *Mini Reviews in Medicinal Chemistry* **2008**; 8: 1065-74.
12. Jeong B, Kim SW, Bae YH. Thermosensitive sol–gel reversible hydrogels. *Adv Drug Deliv Rev* **2002**; 54: 37-51.
13. Qiu Y, Park K. Environment-sensitive hydrogels for drug delivery. *Adv Drug Deliv Rev* **2012**; 64: 49-60.
14. Casolaro M, Casolaro I, Lamponi S. Stimuli-responsive hydrogels for controlled pilocarpine ocular delivery. *Eur J Pharm Biopharm* **2012**; 80: 553-61.
15. Fathi M, Entezami AA, Arami S, Rashidi MR. Preparation of N-Isopropylacrylamide/Itaconic Acid Magnetic Nanohydrogels by Modified Starch as a Crosslinker for Anticancer Drug Carriers. *International Journal of Polymeric Materials and Polymeric Biomaterials* **2015**; 64: 541-9.
16. Kharkar PM, Kiick KL, Kloxin AM. Design of Thiol- and Light-sensitive Degradable Hydrogels using Michael-type Addition Reactions. *Polym Chem* **2015**; 6: 5565-74. doi: 10.1039/C5PY00750J
17. Fathi M, Entezami AA, Pashaei-Asl R. Swelling/deswelling, thermal, and rheological behavior of PVA-g-NIPAAm nanohydrogels prepared by a facile free-radical polymerization method. *Journal of Polymer Research* **2013**; 20: 1-11.
18. Saleh-Ghadimi L, Fathi M, Entezami AA. Heteroarm star-

shaped poly (N-isopropylacryamide-co-itaconic acid) copolymer prepared by glucose core as ATRP initiator. *International Journal of Polymeric Materials and Polymeric Biomaterials* **2014**; 63: 246-55.
19. Park H, Temenoff JS, Tabata Y, Caplan AI, Mikos AG. Injectable biodegradable hydrogel composites for rabbit marrow mesenchymal stem cell and growth factor delivery for cartilage tissue engineering. *Biomaterials* **2007**; 28: 3217-27.
20. Bhattarai N, Ramay HR, Gunn J, Matsen FA, Zhang M. PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release. *J Control Release* **2005**; 103: 609-24.
21. Willoughby CE, Ponzin D, Ferrari S, Lobo A, Landau K, Omidi Y. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function–a review. *Clin Experiment Ophthalmol* **2010**; 38: 2-11.
22. Barar J, Asadi M, Mortazavi-Tabatabaei SA, Omidi Y. Ocular drug delivery: impact of in vitro cell culture models. *J Ophth Vis Res* **2009**; 4: 238-52.
23. Adibkia K, Omidi Y, Siahi MR, Javadzadeh AR, Barzegar-Jalali M, Barar J, et al. Inhibition of endotoxin-induced uveitis by methylprednisolone acetate nanosuspension in rabbits. *J Ocul Pharmacol Ther* **2007**; 23: 421-32. doi:10.1089/jop.2007.0039
24. Adibkia K, Siahi Shadbad MR, Nokhodchi A, Javadzedeh A, Barzegar-Jalali M, Barar J, et al. Piroxicam nanoparticles for ocular delivery: physicochemical characterization and implementation in endotoxin-induced uveitis. *J Drug Target* **2007**; 15: 407-16. doi: 10.1080/10611860701453125
25. Omidi Y, Barar J, Hamzeiy H. Nanomedicines Impacts in Ocular Delivery and Targeting. *Nanotechnology in Health Care* **2012**; 43.
26. Barar J, Omidi Y. Nanoparticles for Ocular Drug Delivery. *Nanomedicine in Drug Delivery* **2013**; 287.
27. Yasuda H. Biocompatibility of Nanofilm-Encapsulated Silicone and Silicone-Hydrogel Contact Lenses. *Macromol Biosci* **2006**; 6: 121-38.
28. Abrego G, Alvarado H, Souto EB, Guevara B, Bellowa LH, Parra A, *et al.* Biopharmaceutical profile of pranoprofen-loaded PLGA nanoparticles containing hydrogels for ocular administration. *Eur J Pharm Biopharm* **2015**; 95(Pt B): 261-70. doi: 10.1016/j.ejpb.2015.01.026.
29. Kaur IP, Smitha R. Penetration enhancers and ocular bioadhesives: two new avenues for ophthalmic drug delivery. *Drug Dev Ind Pharm* **2002**; 28: 353-69.
30. Wright B, Mi S, Connon CJ. Towards the use of hydrogels in the treatment of limbal stem cell deficiency. *Drug Discov Today* **2013**; 18: 79-86. doi: 10.1016/j.drudis.2012.07.012
31. Feng Y, Borrelli M, Reichl S, Schrader S, Geerling G. Review of alternative carrier materials for ocular surface reconstruction. *Curr Eye Res* **2014**; 39: 541-52. doi: 10.3109/02713683.2013.853803
32. Perez EP, Merrill EW, Miller D, Cima LG. Corneal epithelial wound healing on bilayer composite hydrogels. *Tissue Eng* **1995**; 1: 263-77. doi: 10.1089/ten.1995.1.263
33. Ahearne M, Yang Y, El Haj AJ, Then KY, Liu KK. Characterizing the viscoelastic properties of thin hydrogel-based constructs for tissue engineering applications. *J R Soc Interface* **2005**; 2: 455-63. doi: 10.1098/rsif.2005.0065
34. Wright B, Cave RA, Cook JP, Khutoryanskiy VV, Mi S, Chen B, et al. Enhanced viability of corneal epithelial cells for efficient transport/storage using a structurally modified calcium alginate hydrogel. *Regen Med* **2012**; 7: 295-307. doi: 10.2217/rme.12.7
35. Mimura T, Amano S, Yokoo S, Uchida S, Yamagami S, Usui T, et al. Tissue engineering of corneal stroma with rabbit fibroblast precursors and gelatin hydrogels. *Mol Vis* **2008**; 14: 1819-28.
36. Rafat M, Li F, Fagerholm P, Lagali NS, Watsky MA, Munger R, et al. PEG-stabilized carbodiimide crosslinked collagen-chitosan hydrogels for corneal tissue engineering. *Biomaterials* **2008**; 29: 3960-72. doi: 10.1016/j.biomaterials.2008.06.017
37. Bakhshandeh H, Soleimani M, Hosseini SS, Hashemi H, Shabani I, Shafiee A, et al. Poly (epsilon-caprolactone) nanofibrous ring surrounding a polyvinyl alcohol hydrogel for the development of a biocompatible two-part artificial cornea. *Int J Nanomedicine* **2011**; 6: 1509-15. doi:10.2147/IJN.S19011
38. Grolik M, Szczubialka K, Wowra B, Dobrowolski D, Orzechowska-Wylegala B, Wylegala E, *et al.* Hydrogel membranes based on genipin-cross-linked chitosan blends for corneal epithelium tissue engineering. *J Mater Sci Mater Med* **2012**; 23: 1991-2000. doi: 10.1007/s10856-012-4666-7
39. Ozcelik B, Brown KD, Blencowe A, Daniell M, Stevens GW, Qiao GG. Ultrathin chitosan-poly(ethylene glycol) hydrogel films for corneal tissue engineering. *Acta Biomater* **2013**; 9: 6594-605. doi: 10.1016/j.actbio.2013.01.020
40. Chae JJ, Mulreany DG, Guo Q, Lu Q, Choi JS, Strehin I, et al. Application of a collagen-based membrane and chondroitin sulfate-based hydrogel adhesive for the potential repair of severe ocular surface injuries. *Mil Med* **2014**; 179: 686-94. doi: 10.7205/MILMED-D-13-00360
41. Xiao X, Pan S, Liu X, Zhu X, Connon CJ, Wu J, et al. In vivo study of the biocompatibility of a novel compressed collagen hydrogel scaffold for artificial corneas. *J Biomed Mater Res A* **2014**; 102: 1782-7. doi: 10.1002/jbm.a.34848
42. Wittmann K, Dietl S, Ludwig N, Berberich O, Hoefner C, Storck K, *et al.* Engineering vascularized adipose tissue using the stromal-vascular fraction and fibrin hydrogels. *Tissue Eng Part A* **2015**; 21: 1343-53. doi: 10.1089/ten.TEA.2014.0299
43. Ahmed TA, Dare EV, Hincke M. Fibrin: a versatile scaffold for tissue engineering applications. *Tissue Eng Part B Rev* **2008**; 14: 199-215. doi: 10.1089/ten.teb.2007.0435
44. Patchan MW, Chae JJ, Lee JD, Calderon-Colon X, Maranchi JP, McCally RL, et al. Evaluation of the biocompatibility of regenerated cellulose hydrogels with high strength and transparency for ocular applications. *J Biomater Appl* **2015**. doi: 10.1177/0885328215616273
45. Stapleton F, Stretton S, Papas E, Skotnitsky C, Sweeney DF. Silicone hydrogel contact lenses and the ocular surface. *Ocul Surf* **2006**; 4: 24-43.