CAMILA CUBAYACHI

Avaliação de formulações a base de fibroína da seda e insulina no tratamento tópico de feridas oculares

Ribeirão Preto
2018

RESUMO

CUBAYACHI, C. **Avaliação de formulações a base de fibroína da seda e insulina no tratamento tópico de feridas oculares.** 2018. 115f. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018.

O processo de cicatrização das feridas na córnea em pacientes com doenças crônicas, como a diabetes *mellitus*, é deficiente, o que pode acarretar em erosões recorrentes, opacidade e até mesmo cegueira, e usualmente não responde aos tratamentos convencionais. Estudos demonstraram o potencial da insulina (INS) na cicatrização de feridas epiteliais da córnea, mas sua administração tópica requer o uso de sistemas carreadores para garantir sua estabilidade e liberação controlada no sítio a ser regenerado. Assim, o objetivo deste trabalho foi desenvolver filmes a base de fibroína da seda (FS) contendo INS e avaliar seu potencial no tratamento tópico de feridas oculares. Os filmes de FS contendo INS na concentração de 100 UI/cm^2 e glicerina como plastificante foram obtidos por evaporação de solvente e apresentaram-se homogêneos, transparentes, permeáveis ao vapor de água, com baixa capacidade de intumescimento e elevada resistência mecânica. Análises de espectroscopia de infravermelho por transformada de Fourier e calorimetria diferencial exploratória sugeriram conformação da INS em folhas-β/Seda II, além de ocorrência de interações não-covalentes entre INS e FS, capazes de estabilizar a estrutura do filme. Um método analítico para quantificar a INS por cromatografia líquida de alta eficiência foi desenvolvido e validado, apresentando-se seletivo, preciso, exato e linear no intervalo de 3 a 100 µg/mL. Estudos de liberação *in vitro* demonstraram que os filmes foram capazes de liberar cerca de 8% da INS nos primeiros 30 minutos, atingindo 22% em 12 horas, totalizando 1 UI (3,5 µg), sem liberação adicional até 24 horas. Entretanto, estudos de degradação enzimática permitem sugerir que as proteinases presentes nas feridas seriam capazes de ocasionar quebras no filme quando aplicado, o que apresentaria um componente adicional na liberação da INS. O espectro de dicroísmo circular de amostras do receptor demonstrou que a insulina liberada dos filmes manteve sua conformação nativa, além de conservar atividade biológica *in vivo*, reduzindo a glicemia sanguínea de ratos Wistar. Estudos *in vitro* em cultura de células epiteliais da córnea humana (HCEC-SV40) demonstraram que o tratamento com os filmes não ocasionou citotoxicidade. Ainda, em modelo inflamatório estabelecido através do estímulo das células com lipopolissacarídeo de E. coli, observou-se que os filmes foram capazes de modular os níveis de mediadores do processo inflamatório, como interleucina 6 e metaloproteinase de matriz 9. Finalmente, estudos de cicatrização *in vivo* utilizando modelo de debridamento do epitélio da córnea de ratos Wistar diabéticos permitiram avaliar a capacidade da INS liberada dos filmes em modular o processo inflamatório e permitir a regeneração do epitélio corneano, favorecendo as características fenotípicas das células epiteliais. Desta forma, os filmes de FS demonstraram ser um sistema de liberação de INS promissor para a administração tópica ocular e tratamento da cicatrização de feridas epiteliais da córnea.

Palavras-chave: Cicatrização da córnea. Fibroína da seda. Insulina. Filme. Sistema de liberação ocular.

1. INTRODUÇÃO

A cicatrização de feridas na córnea é um problema clínico significativo, especialmente devido aos frequentes danos traumáticos à córnea e ao crescente número de cirurgias refrativas. Ainda, alterações não traumáticas na homeostase da córnea frequentemente ocorrem como consequência de doenças sistêmicas, como a diabetes *mellitus* (DM), doenças oculares, como a síndrome do olho seco, ou ainda como efeito adverso da administração de um medicamento (Peterson *et al.*, 2014; Ljubimov e Saghizadeh, 2015).

Estima-se que cerca de 20% da população sofra de algum trauma ocular durante a vida, sendo essa a principal causa de atendimentos nas clínicas oftalmológicas, além de representar a terceira maior causa de cegueira no mundo (Tandon *et al.*, 2010; Ljubimov e Saghizadeh, 2015; Couture *et al.*, 2016). Esse elevado número ilustra a necessidade de um melhor entendimento dos mecanismos de cicatrização da córnea e desenvolvimento de meios efetivos de modular e melhorar esse processo.

O tratamento para cicatrização de feridas na córnea é predominantemente sintomático. Entretanto, o uso de esteróides e antiinflamatórios não-esteroidais suprime a resposta inflamatória e provoca efeitos colaterais significantes, além de favorecer a ocorrência de infecções. Desta forma, terapias específicas que possibilitem o controle da resposta inflamatória a nível celular são desejáveis e vem sendo estudadas (Abdelkader *et al.*, 2011; Ljubimov e Saghizadeh, 2015).

Fatores de crescimento têm um enorme potencial para resolver as deficiências das modalidades atuais de tratamento de feridas na córnea (Goldman, 2004; Behm *et al.*, 2012; Ljubimov e Saghizadeh, 2015). Eles atuam como sinalizadores extracelulares do dinâmico processo de cicatrização, promovendo proliferação e diferenciação celular, quimiotaxia e síntese de matriz extracelular e podem ser incorporados em sistemas de liberação especializados (Caramella *et al.*, 2013). Dentre os diversos fatores de crescimento estudados para promover o reparo de feridas, como o fator de crescimento epidermal (EGF), o fator de crescimento derivado de plaquetas (PDGF) e o fator de crescimento transformante (TGF-β) (Goldman, 2004; Johnson e Wang, 2013), a insulina (INS) é provavelmente o mais prontamente disponível e seu uso em humanos é aprovado pelo FDA (Liu *et al.*, 2009).

A INS é um peptídeo anabólico, presente no fluido lacrimal. Seus receptores foram encontrados na superfície ocular, na córnea, nos tecidos neuronal e vascular da retina e na glândula lacrimal (Rocha *et al.*, 2002a; b; Yu *et al.*, 2010; Abdelkader *et al.*, 2011). A INS é alvo constante de pesquisas envolvendo a promoção da cicatrização da córnea (Bartlett, Slusser*, et al.*, 1994; Bartlett, Turner-Henson*, et al.*, 1994; Zagon *et al.*, 2006; Zagon *et al.*, 2007; Klocek *et al.*, 2009). Estudos *in vitro* demonstraram que ela é necessária para a proliferação das células epiteliais bem como para a manutenção da cultura de células da córnea (Shanley *et al.*, 2004; Berdal *et al.*, 2011). Além disso, a INS preserva as características fenotípicas do epitélio corneano (Guerriero *et al.*, 2007; Alves *et al.*, 2008) e tanto a aplicação por via subcutânea (Zagon *et al.*, 2006) quanto tópica (Zagon *et al.*, 2007) mostrou favorecer a reepitelização da córnea em ratos diabéticos através da promoção de eventos metabólicos e da proliferação celular.

A maioria das formulações de uso tópico disponíveis atualmente para promover a cicatrização de feridas da córnea são na forma de soluções, suspensões e pomadas (Schultz e Morck, 2010). Geralmente são necessárias altas concentrações das substâncias ativas e várias aplicações por dia para manter um nível terapêutico do fármaco no local de ação, o que pode induzir efeitos tóxicos e danos celulares, podendo reduzir a adesão dos pacientes e o sucesso da terapia (Ludwig, 2005).

Desafios relacionados às características físico-químicas e biológicas do fármaco que se pretende administrar topicamente também são muitos. A natureza proteica da INS lhe confere baixa estabilidade conformacional e enzimática, fazendo com que seja rapidamente degradada em meio biológico (Chiou, 1991; Oda *et al.*, 2011; Rawat *et al.*, 2015). Desta forma, o sucesso da terapia depende do aumento de sua biodisponibilidade no local de ação, podendo ser obtido pelo uso de sistemas de liberação (Kim e Peppas, 2003; Li *et al.*, 2015).

No desenvolvimento de um sistema de liberação que seja adequado para a terapia tópica de lesões na córnea, as características do material que o compõem devem ser levadas em consideração, não só para garantir a estabilidade e liberação sustentada do fármaco, mas também para evitar que o mesmo provoque respostas imunológicas e inflamatórias danosas. Desta forma, os polímeros biocompatíveis, ou biopolímeros, vem sendo muito estudados como carreadores de fármacos (Daamen *et al.*, 2007; Nair e Laurencin, 2007; Sionkowska, 2011).

Um biopolímero natural promissor é a fibroína da seda (FS), uma proteína fibrosa presente no casulo do bicho-da-seda (*Bombyx mori) (Altman et al., 2003)*. Este polímero vem ganhando muita atenção em diversos segmentos da pesquisa, principalmente na biotecnologia, devido às suas propriedades únicas como: biocompatibilidade, alta permeabilidade ao oxigênio e ao vapor de água, biodegradabilidade, resistência mecânica, favorável processabilidade quando combinado com outros polímeros e mínima reação inflamatória (Wenk *et al.*, 2011). Verificou-se ainda a interação da FS com fibroblastos (Servoli *et al.*, 2005) (SERVOLI *et al.,* 2005), queratinócitos (Gupta *et al.*, 2007) e células endoteliais (Fuchs *et al.*, 2006). Tais habilidades podem ser muito importantes para sistemas de liberação de fármacos, pois podem direcionar melhor o princípio ativo ao seu alvo específico, melhorando a resposta terapêutica desejada. Trabalhos recentes demonstraram o uso da FS em diversos tipos de formulações usadas para promover a cicatrização de feridas devido às suas propriedades físicas e biológicas (Lawrence *et al.*, 2009; Harkin *et al.*, 2011; Wenk *et al.*, 2011; Harkin e Chirila, 2012).

Desta forma, a prevalência da incidência de feridas crônicas oculares de difícil cicatrização, especialmente as epiteliais corneanas, e a não observância de um tratamento eficaz, aliada a ação da INS no processo de cicatrização fazem crer que a administração controlada deste fármaco a partir de carreadores formados por FS seja promissora para o tratamento de lesões que acometam a região anterior do olho.

7. CONCLUSÃO

A metodologia para obtenção de dispersão aquosas de FS a partir de casulos do bicho-da-seda foi padronizada e permitiu a formação de filmes transparentes e homogêneos, com alto conteúdo em folhas-β, hidratação moderada, permeabilidade ao vapor de água e propriedades mecânicas adequadas para administração tópica ocular, além de possibilitar a incorporação de INS no interior da matriz encapsulante. O método analítico para quantificação da INS por CLAE mostrou-se sensível e seletivo para a INS e foi validado com sucesso. Os filmes de FS foram capazes de estabilizar e liberar a INS de maneira sustentada, conservando sua integridade estrutural e biológica. Estudos *in vitro* demonstraram ausência de toxicidade dos filmes em relação a cultura de células epiteliais da córnea humana, além da capacidade de modulação da expressão de IL-6 e MMP-9 em modelo de inflamação. Finalmente, estudos de cicatrização *in vivo* utilizando modelo de debridamento do epitélio de ratos diabéticos sugerem que a INS liberada dos filmes é capaz de modular o processo inflamatório e permitir a regeneração do epitélio corneano, favorecendo as características fenotípicas das células epiteliais, demonstrando seu potencial no tratamento de feridas na córnea.

9. REFERÊNCIAS[1]

ABDEL-NABY, W. et al. Silk-Derived Protein Enhances Corneal Epithelial Migration, Adhesion, and Proliferation. **Invest Ophthalmol Vis Sci,** v. 58, n. 3, p. 1425-1433, 2017.

ABDELKADER, H. et al. New therapeutic approaches in the treatment of diabetic keratopathy: a review. **Clin Exp Ophthalmol,** v. 39, n. 3, p. 259-70, 2011.

ADELLI, G. R. et al. Diclofenac sodium ion exchange resin complex loaded melt cast films for sustained release ocular delivery. **Drug delivery**, v. 24, n. 1, p. 370-379, 2017.

AHER, N. D.; NAIR, H. A. Bilayered films based on novel polymer derivative for improved ocular therapy of gatifloxacin. **ScientificWorldJournal,** v. 2014, p. 297603, 2014.

ALLARDYCE, B. J. et al. The impact of degumming conditions on the properties of silk films for biomedical applications. **Textile Research Journal,** v. 86, n. 3, p. 275-287, 2016.

ALTMAN, G. H. et al. Silk-based biomaterials. **Biomaterials,** v. 24, n. 3, p. 401-16, 2003.

ALVES, M. E. C. et al. Tear film and ocular surface changes in diabetes mellitus. **Arq Bras Oftalmol,** v. 71, n. 6 Suppl, p. 96-103, 2008.
ANDREANI, T. et al. Preparation and characterization of PEG-coated silica nanoparticles for oral insulin delivery. **Int J Pharm,** v. 473, n. 1-2, p. 627-35, 2014.

ARRANZ-VALSERO, I. et al. IL-6 as a corneal wound healing mediator in an in vitro scratch assay. **Exp Eye Res,** v. 125, p. 183-92, 2014.

BAI, L. et al. Surface modification and properties of Bombyx mori silk fibroin films by antimicrobial peptide. **Applied Surface Science,** v. 254, n. 10, p. 2988-2995, 2008.

BARTH, A. The infrared absorption of amino acid side chains. **Progress in biophysics and molecular biology,** v. 74, n. 3-5, p. 141-173, 2000.

[1] Segundo NBR 6023

BARTLETT, J. D. et al. Toxicity of insulin administered chronically to human eye in vivo. **J Ocul Pharmacol,** v. 10, n. 1, p. 101-7, 1994.

_____. Insulin administration to the eyes of normoglycemic human volunteers. **J Ocul Pharmacol,** v. 10, n. 4, p. 683-90, 1994.

BASTION, M. L.; LING, K. P. Topical insulin for healing of diabetic epithelial defects?: A retrospective review of corneal debridement during vitreoretinal surgery in Malaysian patients. **Med J Malaysia,** v. 68, n. 3, p. 208-16, 2013.

BEHM, B. et al. Cytokines, chemokines and growth factors in wound healing. **J Eur Acad Dermatol Venereol,** v. 26, n. 7, p. 812-20, 2012.

BENOWITZ, L. I.; POPOVICH, P. G. Inflammation and axon regeneration. **Curr Opin Neurol,** v. 24, n. 6, p. 577-83, 2011.

BERDAL, M. et al. Aminated β-1,3-D-glucan has a dose-dependent effect on wound healing in diabetic db/db mice. **Wound Repair Regen,** v. 19, n. 5, p. 579-87, 2011.

BORRA, R. C. et al. A simple method to measure cell viability in proliferation and cytotoxicity assays. **Braz Oral Res,** v. 23, n. 3, p. 255-62, 2009.

BUKOWIECKI, A. et al. Wound-Healing Studies in Cornea and Skin: Parallels, Differences and Opportunities. **Int J Mol Sci,** v. 18, n. 6, 2017.

CAO, Y.; BINDSLEV, D. A.; KJÆRGAARD, S. K. Estimation of the in vitro eye irritating and inflammatory potential of lipopolysaccharide (LPS) and dust by using reconstituted human corneal epithelium tissue cultures. **Toxicol Mech Methods,** v.25, n. 5, p. 402-9, 2015.

CARAMELLA, C. M. et al. New therapeutic platforms for the treatment of epithelial and cutaneous lesions. **Curr Drug Deliv,** v. 10, n. 1, p. 18-31, 2013.

CHEN, W. L. et al. In vivo confocal microscopic findings of corneal wound healing after corneal epithelial debridement in diabetic vitrectomy. **Ophthalmology,** v. 116, n. 6, p. 1038-47, 2009.

CHENG, G. et al. Differences in regenerated silk fibroin prepared with different solvent systems: From structures to conformational changes. **Journal Of Applied Polymer Science,** v. 132, n. 22, 2015.

CHIOU, G. C. Systemic delivery of polypeptide drugs through ocular route. **Annu Rev Pharmacol Toxicol,** v. 31, p. 457-67, 1991.

CHIOU, G. C.; SHEN, Z. F.; ZHENG, Y. Q. Adjustment of blood sugar levels with insulin and glucagon eyedrops in normal and diabetic rabbits. **J Ocul Pharmacol,** v. 6, n. 3, p. 233-41, 1990.

CHO, H. J. et al. Molecular weight distribution and solution properties of silk fibroins with different dissolution conditions. **Int J Biol Macromol,** v. 51, n. 3, p. 336-41, 2012.

CICERONE, M. T.; PIKAL, M. J.; QIAN, K. K. Stabilization of proteins in solid form. **Adv Drug Deliv Rev,** v. 93, p. 14-24, 2015.

COBURN, J. M.; NA, E.; KAPLAN, D. L. Modulation of vincristine and doxorubicin binding and release from silk films. **J Control Release,** v. 220, n. Pt A, p. 229-238, 2015.

COLE, N. et al. Expression of interleukin-6 in the cornea in response to infection with different strains of Pseudomonas aeruginosa. **Infect Immun,** v. 67, n. 5, p. 2497-502, 1999.

COUTURE, C. et al. The tissue-engineered human cornea as a model to study expression of matrix metalloproteinases during corneal wound healing. **Biomaterials,** v. 78, p. 86-101, 2016.

CROSSON, C. E.; KLYCE, S. D.; BEUERMAN, R. W. Epithelial wound closure in the rabbit cornea. A biphasic process. **Invest Ophthalmol Vis Sci,** v. 27, n. 4, p. 464-73, 1986.

CRUZ, E. L. C. M. **Avaliação de formulações de uso tópico a base de insulina no distúrbio das glândulas lacrimais e na regeneração da córnea em ratos diabéticos**. Dissertação de Mestrado. Faculdade de Ciências Farmaucêuticas de Ribeirão Preto, Universidade de São Paulo. Ribeirão Preto, 125p., 2014.

CSÓKA, G. et al. Comparison of the fragility index of different eudragit polymers determined by activation enthalpies. **Journal of thermal analysis and calorimetry,** v. 87, n. 2, p. 469-473, 2006.

CUI, F. et al. Preparation and evaluation of chitosan-ethylenediaminetetraacetic acid hydrogel films for the mucoadhesive transbuccal delivery of insulin. **J Biomed Mater Res A,** v. 89, n. 4, p. 1063-71, 2009.

CUNHA, D. A. et al. Insulin secretion by rat lacrimal glands: effects of systemic and local variables. **Am J Physiol Endocrinol Metab,** v. 289, n. 5, p. E768-75, 2005.

DAAMEN, W. F. et al. Elastin as a biomaterial for tissue engineering. **Biomaterials,** v. 28, n. 30, p. 4378-4398, 2007.

DAVIS, S.; GRANNER, D. Insulina, hipoglicemiantes orais e a farmacologia do pâncreas endócrino. **As bases farmacológicas da terapêutica. 10ª edição, Rio de Janeiro: McGraw Hill**, p. 1263-90, 2003.

DE MORAES, M. A. et al. Silk fibroin and sodium alginate blend: miscibility and physical characteristics. In: (Ed.). **Mater Sci Eng C Mater Biol Appl**. Netherlands: 2014 Elsevier B.V, v.40, p.85-91, 2014.

DE PAIVA, C. S. et al. Corticosteroid and doxycycline suppress MMP-9 and inflammatory cytokine expression, MAPK activation in the corneal epithelium in experimental dry eye. In: (Ed.). **Exp Eye Res**. England, v.83, p.526-35, 2006.

DELMONTE, D. W.; KIM, T. Anatomy and physiology of the cornea. **Journal of Cataract & Refractive Surgery,** v. 37, n. 3, p. 588-598, 2011.

DESHPANDE, P. B. et al. Controlled release polymeric ocular delivery of acyclovir. **Pharmaceutical development and technology**, v. 15, n. 4, p. 369-378, 2010.

DO COUTO, R. O. et al. Combining amino amide salts in mucoadhesive films enhances needle-free buccal anesthesia in adults. **Journal of Controlled Release,** v. 266, p. 205-215, 2017.

DUPPS, W. J.; WILSON, S. E. Biomechanics and wound healing in the cornea. **Exp Eye Res,** v. 83, n. 4, p. 709-20, 2006.

EBIHARA, N. et al. Role of the IL-6 classic- and trans-signaling pathways in corneal sterile inflammation and wound healing. **Invest Ophthalmol Vis Sci,** v. 52, n. 12, p. 8549-57, 2011.

ECKES, B. et al. Fibroblast-matrix interactions in wound healing and fibrosis. In: (Ed.). **Matrix Biol**. Netherlands, v.19, p.325-32, 2000.

EL-SOUSI, S. et al. Hydroxypropylmethylcellulose films for the ophthalmic delivery of diclofenac sodium. **Journal of Pharmacy and Pharmacology,** v. 65, n. 2, p. 193-200, 2013.

ERDINEST, N. et al. Anti-inflammatory effects of alpha linolenic acid on human corneal epithelial cells. **Investigative ophthalmology & visual science,** v. 53, n. 8, p. 4396-4406, 2012.

ESLANI, M. et al. The role of toll-like receptor 4 in corneal epithelial wound healing. **Investigative ophthalmology & visual science,** v. 55, n. 9, p. 6108-6115, 2014.

FAI, S. et al. Randomized Controlled Trial of Topical Insulin for Healing Corneal Epithelial Defects Induced During Vitreoretinal Surgery in Diabetics. **Asia Pac J Ophthalmol (Phila),** v. 6, n. 5, p. 418-424, 2017.

FDA Reviewer Guidance, Validation of Chromatographic Methods. **Center for Drug Evaluation and Research**, US Food and Drug Administration, 1994.

FEENSTRA, R. P. G.; TSENG, S. C. G. Comparison of Fluorescein and Rose Bengal Staining. **Ophthalmology,** v. 99, n. 4, p. 605-617, 1992.

FILBIN, M. T. How inflammation promotes regeneration. **Nature neuroscience,** v. 9, n. 6, p. 715, 2006.

FUCHS, S. et al. Outgrowth endothelial cells isolated and expanded from human peripheral blood progenitor cells as a potential source of autologous cells for endothelialization of silk fibroin biomaterials. **Biomaterials,** v. 27, n. 31, p. 5399-5408, 2006.

GEREMICCA, W.; FONTE, C.; VECCHIO, S. Blood components for topical use in tissue regeneration: evaluation of corneal lesions treated with platelet lysate and considerations on repair mechanisms. **Blood Transfus,** v. 8, n. 2, p. 107-12, 2010.

GOLDMAN, R. Growth factors and chronic wound healing: past, present, and future. **Advances in skin & wound care,** v. 17, n. 1, p. 24-35, 2004.

GRASS, G. M.; ROBINSON, J. R. Mechanisms of corneal drug penetration II: Ultrastructural analysis of potential pathways for drug movement. **Journal of pharmaceutical sciences,** v. 77, n. 1, p. 15-23, 1988.

GUERRIERO, E. et al. Loss of alpha3(IV) collagen expression associated with corneal keratocyte activation. **Invest Ophthalmol Vis Sci,** v. 48, n. 2, p. 627-35, 2007.

GUPTA, M. K. et al. Patterned silk films cast from ionic liquid solubilized fibroin as scaffolds for cell growth. **Langmuir,** v. 23, n. 3, p. 1315-1319, 2007.

HADASSAH, J. et al. Evaluation of succinylated collagen bandage lenses in corneal healing by the expression of matrix metalloproteinases (MMP-2 and MMP-9) in tear fluid. In: (Ed.). **Ophthalmic Res**. Switzerland: 2009 S. Karger AG, Basel., v.42, p.64-72, 2009.

HAFEZI, F. et al. Absence of IL-6 prevents corneal wound healing after deep excimer laser ablation in vivo. **Eye (Lond),** v. 32, n. 1, p. 156-157, 2018.

HARKIN, D. G.; CHIRILA, T. V. Silk fibroin in ocular surface reconstruction: what is its potential as a biomaterial in ophthalmics? **Future Med Chem,** v. 4, n. 17, p. 2145-7, 2012.

HARKIN, D. G. et al. Silk fibroin in ocular tissue reconstruction. **Biomaterials,** v. 32, n. 10, p. 2445-2458, 2011.

HAZRA, S. et al. Non-mulberry Silk Fibroin Biomaterial for Corneal Regeneration. **Sci Rep,** v. 6, p. 21840, 2016.

HERMANS, K. et al. Development and characterization of mucoadhesive chitosan films for ophthalmic delivery of cyclosporine A. **International journal of pharmaceutics,** v. 472, n. 1-2, p. 10-19, 2014.

HERSE, P. R. A review of manifestations of diabetes mellitus in the anterior eye and cornea. **American journal of optometry and physiological optics,** v. 65, n. 3, p. 224-230, 1988.

HIGUCHI, T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. **Journal of pharmaceutical sciences**, v. 52, n. 12, p. 1145-1149, 1963.

HOFER, M.; WINTER, G.; MYSCHIK, J. Recombinant spider silk particles for controlled delivery of protein drugs. **Biomaterials,** v. 33, n. 5, p. 1554-1562, 2012.

HOFMANN, S. et al. Silk fibroin as an organic polymer for controlled drug delivery. **Journal of Controlled Release,** v. 111, n. 1-2, p. 219-227, 2006.

HOGERHEYDE, T. A. et al. Assessment of freestanding membranes prepared from Antheraea pernyi silk fibroin as a potential vehicle for corneal epithelial cell transplantation. **Biomedical Materials,** v. 9, n. 2, p. 025016, 2014.

HRYNYK, M.; NEUFELD, R. J. Insulin and wound healing. **Burns,** v. 40, n. 8, p. 1433-1446, 2014.

HU, X.; KAPLAN, D.; CEBE, P. Determining beta-sheet crystallinity in fibrous proteins by thermal analysis and infrared spectroscopy. **Macromolecules,** v. 39, n. 18, p. 6161-6170, 2006.

HUUS, K. et al. Thermal dissociation and unfolding of insulin. **Biochemistry,** v. 44, n. 33, p. 11171-11177, 2005.

International Conference on Harmonization (ICH). **Guidance for industry Q2B: Validation of Analytical Procedures. Methodology**. 2005.

ISO, B. 10993-5: Biological evaluation of medical devices. **Tests for in vitro cytotoxicity**, 1999.
JAO, D.; MOU, X.; HU, X. Tissue Regeneration: A Silk Road. **J Funct Biomater,** v. 7, n. 3, 2016.

JIA, L.; GHEZZI, C. E.; KAPLAN, D. L. Optimization of silk films as substrate for functional corneal epithelium growth. **Journal of Biomedical Materials Research Part B: Applied Biomaterials,** v. 104, n. 2, p. 431-441, 2016.

JIN, H.-J.; KAPLAN, D. L. Mechanism of silk processing in insects and spiders. **Nature,** v. 424, n. 6952, p. 1057, 2003.

JIN, H. J. et al. Water-stable silk films with reduced β-sheet content. **Advanced Functional Materials,** v. 15, n. 8, p. 1241-1247, 2005.

JOHNSON, N. R.; WANG, Y. Controlled delivery of heparin-binding EGF-like growth factor yields fast and comprehensive wound healing. **Journal of Controlled Release,** v. 166, n. 2, p. 124-129, 2013.

JORGENSEN, L. et al. Adsorption of insulin with varying self-association profiles to a solid Teflon surface—Influence on protein structure, fibrillation tendency and thermal stability. **European journal of pharmaceutical sciences,** v. 42, n. 5, p. 509-516, 2011.

JUNQUEIRA, L.; CARNEIRO, J. **Histologia Básica. 10ª edição. Ed**: Guanabara Koogan S. 2004.

KARAGEORGIOU, V. et al. Porous silk fibroin 3‐D scaffolds for delivery of bone morphogenetic protein‐2 in vitro and in vivo. **Journal of Biomedical Materials Research Part A,** v. 78, n. 2, p. 324-334, 2006.

KARVE, K. A. et al. Effect of β-sheet crystalline content on mass transfer in silk films. **Journal of membrane science,** v. 383, n. 1-2, p. 44-49, 2011.

KAUR, G.; DUFOUR, J. M. **Cell lines: Valuable tools or useless artifacts**: Taylor & Francis 2012.

KAUR, P. et al. Novel nano-insulin formulation modulates cytokine secretion and remodeling to accelerate diabetic wound healing. **Nanomedicine,** v. 15, n. 1, p. 47-57, 2018.

KHUTORYANSKAYA, O. V. et al. Hydrogen‐Bonded Complexes and Blends of Poly (acrylic acid) and Methylcellulose: Nanoparticles and Mucoadhesive Films for Ocular Delivery of Riboflavin. **Macromolecular bioscience,** v. 14, n. 2, p. 225-234, 2014.

KIM, B.; PEPPAS, N. A. In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers. **International journal of pharmaceutics,** v. 266, n. 1-2, p. 29-37, 2003.

KIM, C.-W. et al. Application of SV40 T-transformed human corneal epithelial cells to evaluate potential irritant chemicals for in vitro alternative eye toxicity. **Journal of pharmacological and toxicological methods,** v. 80, p. 82-89, 2016.

KIM, D. K.; SIM, B. R.; KHANG, G. Nature-Derived Aloe Vera Gel Blended Silk Fibroin Film Scaffolds for Cornea Endothelial Cell Regeneration and Transplantation. **ACS Appl Mater Interfaces,** v. 8, n. 24, p. 15160-8, 2016.

KIM, D. W. et al. Effect of silk fibroin peptide derived from silkworm Bombyx mori on the anti-inflammatory effect of Tat-SOD in a mice edema model. **BMB Rep,** v. 44, n. 12, p. 787-92, 2011.

KIM, E. Y. et al. Silk fibroin film as an efficient carrier for corneal endothelial cells regeneration. **Macromolecular Research,** v. 23, n. 2, p. 189-195, 2015.

KIM, J.; SHIN, S.-C. Controlled release of atenolol from the ethylene-vinyl acetate matrix. **International journal of pharmaceutics,** v. 273, n. 1-2, p. 23-27, 2004.

KLOCEK, M. S. et al. Naltrexone and insulin are independently effective but not additive in accelerating corneal epithelial healing in type I diabetic rats. **Exp Eye Res,** v. 89, n. 5, p. 686-92, 2009.

KUNDU, B. et al. Silk fibroin biomaterials for tissue regenerations. **Advanced drug delivery reviews,** v. 65, n. 4, p. 457-470, 2013.

LAWRENCE, B. D. et al. Silk film biomaterials for cornea tissue engineering. **Biomaterials,** v. 30, n. 7, p. 1299-1308, 2009.

LEE, M. C. et al. Fabrication of silk fibroin film using centrifugal casting technique for corneal tissue engineering. **Journal of Biomedical Materials Research Part B: Applied Biomaterials,** v. 104, n. 3, p. 508-514, 2016.

_____. Fabrication of silk fibroin film using centrifugal casting technique for corneal tissue engineering. **J Biomed Mater Res B Appl Biomater,** v. 104, n. 3, p. 508-14, 2016.

LEMOS, C. N. et al. Iontophoresis-stimulated silk fibroin films as a peptide delivery system for wound healing. **Eur J Pharm Biopharm,** v. 128, p. 147-155, 2018.

LI, A. B. et al. Silk-based stabilization of biomacromolecules. **Journal of Controlled Release,** v. 219, p. 416-430, 2015.

LI, X. et al. Functionalized silk fibroin dressing with topical bioactive insulin release for accelerated chronic wound healing. **Mater Sci Eng C Mater Biol Appl,** v. 72, p. 394-404, 2017.

LIU, Y.; PETREACA, M.; MARTINS-GREEN, M. Cell and molecular mechanisms of insulin-induced angiogenesis. **Journal of cellular and molecular medicine,** v. 13, n. 11-12, p. 4492-4504, 2009.

LJUBIMOV, A. V. Diabetic complications in the cornea. **Vision Res,** v. 139, p. 138-152, 2017.

LJUBIMOV, A. V.; SAGHIZADEH, M. Progress in corneal wound healing. **Prog Retin Eye Res,** v. 49, p. 17-45, 2015.

LOPES, M. A. et al. Probing insulin bioactivity in oral nanoparticles produced by ultrasonication-assisted emulsification/internal gelation. **Int J Nanomedicine,** v. 10, p. 5865-80, 2015.

LU, Q. et al. Stabilization and release of enzymes from silk films. **Macromol Biosci,** v. 10, n. 4, p. 359-68, 2010.

LU, S. et al. Insoluble and flexible silk films containing glycerol. **Biomacromolecules,** v. 11, n. 1, p. 143-150, 2009.

LU, X.; RICHARDSON, P. M. Inflammation near the nerve cell body enhances axonal regeneration. **J Neurosci,** v. 11, n. 4, p. 972-8, 1991.

LUDWIG, A. The use of mucoadhesive polymers in ocular drug delivery. **Advanced drug delivery reviews,** v. 57, n. 11, p. 1595-1639, 2005.

MAGOSHI, J. et al. Crystallization of silk fibroin from solution. **Thermochimica acta,** v. 352, p. 165-169, 2000.

MATTEUCCI, E. et al. Insulin administration: present strategies and future directions for a noninvasive (possibly more physiological) delivery. **Drug design, development and therapy,** v. 9, p. 3109, 2015.

MAURI, S. et al. Stabilization of insulin by adsorption on a hydrophobic silane self-assembled monolayer. **Langmuir,** v. 31, n. 32, p. 8892-8900, 2015.

MAYCOCK, N. J.; MARSHALL, J. Genomics of corneal wound healing: a review of the literature. **Acta Ophthalmol,** v. 92, n. 3, p. e170-84, 2014.

MELONI, M. et al. Occludin gene expression as an early in vitro sign for mild eye irritation assessment. **Toxicology in Vitro,** v. 24, n. 1, p. 276-285, 2010.

MICERA, A. et al. Nerve growth factor effect on human primary fibroblastic-keratocytes: possible mechanism during corneal healing. In: (Ed.). **Exp Eye Res**. England, v.83, p.747-57, 2006.

MING, J.; PAN, F.; ZUO, B. Influence factors analysis on the formation of silk I structure. **International journal of biological macromolecules,** v. 75, p. 398-401, 2015.

MORALES, J. O.; MCCONVILLE, J. T. Manufacture and characterization of mucoadhesive buccal films. **European Journal of Pharmaceutics and Biopharmaceutics,** v. 77, n. 2, p. 187-199, 2011.

MOTTA, A. et al. Stabilization of Bombyx mori silk fibroin/sericin films by crosslinking with PEG-DE 600 and genipin. **Journal of Bioactive and Compatible Polymers,** v. 26, n. 2, p. 130-143, 2011.

MOWREY-MCKEE, M.; SILLS, A.; WRIGHT, A. Comparative cytotoxicity potential of soft contact lens care regimens. **Eye & Contact Lens,** v. 28, n. 3, p. 160-164, 2002.

MÓDULO, C. M. et al. Influence of insulin treatment on the lacrimal gland and ocular surface of diabetic rats. **Endocrine,** v. 36, n. 1, p. 161-8, 2009.

NAIR, L. S.; LAURENCIN, C. T. Biodegradable polymers as biomaterials. **Progress in polymer science,** v. 32, n. 8-9, p. 762-798, 2007.

NAKAMURA, M. et al. Promotion of corneal epithelial wound healing in diabetic rats by the combination of a substance P-derived peptide (FGLM-NH2) and insulin-like growth factor-1. **Diabetologia,** v. 46, n. 6, p. 839-842, 2003.

NAKAMURA, M.; NISHIDA, T. Differential effects of epidermal growth factor and interleukin 6 on corneal epithelial cells and vascular endothelial cells. In: (Ed.). **Cornea**. United States, v.18, p.452-8, 1999.

NARUMI, M. et al. Contribution of corneal neovascularization to dendritic cell migration into the central area during human corneal infection. **PloS one,** v. 9, n. 10, p. e109859, 2014.

NOWELL, C. S.; RADTKE, F. Corneal epithelial stem cells and their niche at a glance. **J Cell Sci,** v. 130, n. 6, p. 1021-1025, 2017.

NUNES, K. M. et al. The Monoglyceride Content Affects the Self-Assembly Behavior, Rheological Properties, Syringeability, and Mucoadhesion of In Situ-Gelling Liquid Crystalline Phase. **J Pharm Sci**., v.105, p.2355-64, 2016.

ODA, K. et al. Mechanism underlying insulin uptake in alveolar epithelial cell line RLE-6TN. **European journal of pharmacology,** v. 672, n. 1-3, p. 62-69, 2011.

PAL-GHOSH, S. et al. Removal of the basement membrane enhances corneal wound healing. **Exp Eye Res,** v. 93, n. 6, p. 927-36, 2011.

PANDEY, L. M. et al. Surface chemistry at the nanometer scale influences insulin aggregation. **Colloids and Surfaces B: Biointerfaces,** v. 100, p. 69-76, 2012.

PARK, Y. R. et al. NF-κB signaling is key in the wound healing processes of silk fibroin. **Acta Biomater,** v. 67, p. 183-195, 2018.

PAWAR, H.; TETTEH, J.; BOATENG, J. Preparation, optimisation and characterisation of novel wound healing film dressings loaded with streptomycin and diclofenac. **Colloids and Surfaces B: Biointerfaces,** v. 102, p. 102-110, 2013.

PETERS, E.; ARISMAN, R. Applied Polymer Science—21st Century. **Elsevier: New York**, p. 177-196, 2000.

PETERSON, J. L. et al. The role of endogenous epidermal growth factor receptor ligands in mediating corneal epithelial homeostasis. **Investigative ophthalmology & visual science,** v. 55, n. 5, p. 2870-2880, 2014.

POON, A. C. et al. Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies. **British Journal of Ophthalmology,** v. 85, n. 10, p. 1188-1197, 2001.

RAWAT, S. et al. Molecular mechanism of poly (vinyl alcohol) mediated prevention of aggregation and stabilization of insulin in nanoparticles. **Molecular pharmaceutics,** v. 12, n. 4, p. 1018-1030, 2015.

REIS, C. P. et al. Nanoparticulate delivery system for insulin: design, characterization and in vitro/in vivo bioactivity. **Eur J Pharm Sci,** v. 30, n. 5, p. 392-7, 2007.

REPETTO, G.; DEL PESO, A.; ZURITA, J. L. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. **Nature protocols,** v. 3, n. 7, p. 1125, 2008.

ROCHA, E. M. et al. Identification of insulin in the tear film and insulin receptor and IGF-1 receptor on the human ocular surface. **Invest Ophthalmol Vis Sci,** v. 43, n. 4, p. 963-7, 2002a.

_____. Insulin, insulin receptor and insulin-like growth factor-I receptor on the human ocular surface. **Adv Exp Med Biol,** v. 506, n. Pt A, p. 607-10, 2002b.

ROCKWOOD, D. N. et al. Materials fabrication from Bombyx mori silk fibroin. **Nature protocols,** v. 6, n. 10, p. 1612, 2011.

RODRIGUEZ-NOGALES, A. et al. Effect of aqueous and particulate silk fibroin in a rat model of experimental colitis. **Int J Pharm**, v.511, p.1-9., 2016.

SAGNELLA, A. et al. Effect of different fabrication methods on the chemo-physical properties of silk fibroin films and on their interaction with neural cells. **RSC Advances,** v. 6, n. 11, p. 9304-9314, 2016.

SAINI, J.; KHANDALAVLA, B. Corneal epithelial fragility in diabetes mellitus. **Canadian journal of ophthalmology. Journal canadien d'ophtalmologie,** v. 30, n. 3, p. 142-146, 1995.

SAMBURSKY, R. et al. Sensitivity and specificity of a point-of-care matrix metalloproteinase 9 immunoassay for diagnosing inflammation related to dry eye. **JAMA Ophthalmol,** v. 131, n. 1, p. 24-8, 2013.

SARMENTO, B. et al. Development and validation of a rapid reversed-phase HPLC method for the determination of insulin from nanoparticulate systems. **Biomedical Chromatography,** v. 20, n. 9, p. 898-903, 2006.

SCHULTZ, C. L.; MORCK, D. W. Contact lenses as a drug delivery device for epidermal growth factor in the treatment of ocular wounds. **Clin Exp Optom,** v. 93, n. 2, p. 61-5, 2010.

SERVOLI, E. et al. Surface properties of silk fibroin films and their interaction with fibroblasts. **Macromolecular bioscience,** v. 5, n. 12, p. 1175-1183, 2005.

SHANLEY, L. J. et al. Insulin, not leptin, promotes in vitro cell migration to heal monolayer wounds in human corneal epithelium. **Invest Ophthalmol Vis Sci,** v. 45, n. 4, p. 1088-94, 2004.

SHIH, K. C.; LAM, K. S.; TONG, L. A systematic review on the impact of diabetes mellitus on the ocular surface. **Nutr Diabetes,** v. 7, n. 3, p. e251, 2017.

SHIN, S.-C.; YOON, M.-K. Application of TPX polymer membranes for the controlled release of triprolidine. **International journal of pharmaceutics,** v. 232, n. 1-2, p. 131-137, 2002.

SIEPMANN, J.; SIEPMANN, F. Mathematical modeling of drug delivery. **International journal of pharmaceutics**, v. 364, n. 2, p. 328-343, 2008.

SIONKOWSKA, A. Current research on the blends of natural and synthetic polymers as new biomaterials. **Progress in polymer science,** v. 36, n. 9, p. 1254-1276, 2011.

STEPP, M. A. et al. Wounding the cornea to learn how it heals. **Exp Eye Res,** v. 121, p. 178-93, 2014. ISSN 1096-0007.

SUGAYA, S. et al. Regulation of soluble interleukin-6 (IL-6) receptor release from corneal epithelial cells and its role in the ocular surface. **Jpn J Ophthalmol**., v.55, p.277-282, 2011.

SUZUKI, K. et al. Cell-matrix and cell-cell interactions during corneal epithelial wound healing. **Prog Retin Eye Res,** v. 22, n. 2, p. 113-33, 2003.
TADDEI, P. et al. Silk Fibroin/G elatin Blend Films Crosslinked with Enzymes for Biomedical Applications. **Macromolecular bioscience,** v. 13, n. 11, p. 1492-1510, 2013.

TAKAHASHI, H. et al. Matrix metalloproteinase activity is enhanced during corneal wound repair in high glucose condition. **Curr Eye Res,** v. 21, n. 2, p. 608-15, 2000.

TANDON, A. et al. Role of transforming growth factor Beta in corneal function, biology and pathology. **Current molecular medicine,** v. 10, n. 6, p. 565-578, 2010.

TESTER, J. W. et al. Thermodynamics and its Applications. Prentice Hall PTR, 1997.

The National Eye Institute (NEI). Facts About the Cornea and Corneal Disease. National Institutes of Health (NIH). 2016. Disponível em <https://nei.nih.gov/health/cornealdisease>. Acesso em 01/11/2018.

THOFT, R. A.; FRIEND, J. The X, Y, Z hypothesis of corneal epithelial maintenance. **Invest Ophthalmol Vis Sci,** v. 24, n. 10, p. 1442-3, 1983.

TSENG, C.-L. et al. Synergistic effect of artificial tears containing epigallocatechin gallate and hyaluronic acid for the treatment of rabbits with dry eye syndrome. **PloS one,** v. 11, n. 6, p. e0157982, 2016.

UEBERSAX, L.; MERKLE, H. P.; MEINEL, L. Insulin-like growth factor I releasing silk fibroin scaffolds induce chondrogenic differentiation of human mesenchymal stem cells. **Journal of Controlled Release,** v. 127, n. 1, p. 12-21, 2008.

UETA, M. et al. Triggering of TLR3 by polyI: C in human corneal epithelial cells to induce inflammatory cytokines. **Biochemical and biophysical research communications,** v. 331, n. 1, p. 285-294, 2005.

_____. Intracellularly expressed TLR2s and TLR4s contribution to an immunosilent environment at the ocular mucosal epithelium. **The Journal of Immunology,** v. 173, n. 5, p. 3337-3347, 2004.

USP XXX. **United States Pharmacopeia**, 30 ed., United States Pharmacopeial Convention: Rockville, MD, 2007.

VEPARI, C.; KAPLAN, D. L. Silk as a biomaterial. **Progress in polymer science,** v. 32, n. 8-9, p. 991-1007, 2007.

VOLKOV, V. et al. Phosphorylated silk fibroin matrix for methotrexate release. **Molecular pharmaceutics,** v. 12, n. 1, p. 75-86, 2014.

WANG, H.-Y.; ZHANG, Y.-Q. Processing and characterisation of a novel electropolymerized silk fibroin hydrogel membrane. **Scientific reports,** v. 4, p. 6182, 2014.

WANG, X. et al. Growth factor gradients via microsphere delivery in biopolymer scaffolds for osteochondral tissue engineering. **Journal of Controlled Release,** v. 134, n. 2, p. 81-90, 2009.

WENK, E.; MERKLE, H. P.; MEINEL, L. Silk fibroin as a vehicle for drug delivery applications. **Journal of Controlled Release,** v. 150, n. 2, p. 128-141, 2011.

WESKA, R. F. et al. Effect of freezing methods on the properties of lyophilized porous silk fibroin membranes. **Materials Research,** v. 12, n. 2, p. 233-237, 2009.

WILLOUGHBY, C. E. et al. Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function–a review. **Clinical & Experimental Ophthalmology,** v. 38, p. 2-11, 2010.

WILSON, S. E. et al. The corneal wound healing response: cytokine-mediated interaction of the epithelium, stroma, and inflammatory cells. **Prog Retin Eye Res,** v. 20, n. 5, p. 625-37, 2001.

WIROSTKO, B. et al. Novel Therapy to Treat Corneal Epithelial Defects: A Hypothesis with Growth Hormone. **Ocul Surf,** v. 13, n. 3, p. 204-212 e1, 2015.

YAMAMOTO, T. et al. A Proteomic Approach for Understanding the Mechanisms of Delayed Corneal Wound Healing in Diabetic Keratopathy Using Diabetic Model Rat. In: (Ed.). **Int J Mol Sci**. Switzerland, v.19, 2018.

YAN, C. et al. Targeting Imbalance between IL-1β and IL-1 Receptor Antagonist Ameliorates Delayed Epithelium Wound Healing in Diabetic Mouse Corneas. **Am J Pathol,** v. 186, n. 6, p. 1466-80, 2016.

YANAI, R. et al. Correlation of proliferative and anti-apoptotic effects of HGF, insulin, IGF-1, IGF-2, and EGF in SV40-transformed human corneal epithelial cells. In: (Ed.). **Exp Eye Res**. England, v.83, p.76-83, 2006.

YOON, H. et al. Fabrication of transparent silk fibroin film for the regeneration of corneal endothelial cells; Preliminary study. **Macromolecular Research,** v. 22, n. 3, p. 297-303, 2014.

YOSHIDA, K. et al. Layer-by-layer films composed of poly(allylamine) and insulin for pH-triggered release of insulin. In: (Ed.). **Colloids Surf B Biointerfaces**. Netherlands: 2011 Elsevier B.V, v.91, p.274-9, 2012.

YU, F. S. et al. Growth factors and corneal epithelial wound healing. **Brain Res Bull,** v. 81, n. 2-3, p. 229-35, 2010.

YUCEL, T.; LOVETT, M. L.; KAPLAN, D. L. Silk-based biomaterials for sustained drug delivery. **Journal of Controlled Release,** v. 190, p. 381-397, 2014.

YUN, H. et al. The role of glycerol and water in flexible silk sericin film. **International journal of biological macromolecules,** v. 82, p. 945-951, 2016.

ZAGON, I. S. et al. Use of topical insulin to normalize corneal epithelial healing in diabetes mellitus. **Arch Ophthalmol,** v. 125, n. 8, p. 1082-8, 2007.

ZAGON, I. S.; SASSANI, J. W.; MCLAUGHLIN, P. J. Insulin treatment ameliorates impaired corneal reepithelialization in diabetic rats. **Diabetes,** v. 55, n. 4, p. 1141-7, 2006.

ZHANG, C. et al. Flexibility regeneration of silk fibroin in vitro. **Biomacromolecules,** v. 13, n. 7, p. 2148-2153, 2012.

ZHAO, X. et al. Insulin nanoparticles for transdermal delivery: preparation and physicochemical characterization and in vitro evaluation. **Drug development and industrial pharmacy,** v. 36, n. 10, p. 1177-1185, 2010.

ZHENG, L. L. et al. Comparative in vitro cytotoxicity of artificial tears. **JSM Ophthalmol,** v. 3, n. 1, p. 1026, 2015.

ZHONG, J. et al. Self‐assembly of regenerated silk fibroin from random coil nanostructures to antiparallel β‐sheet nanostructures. **Biopolymers,** v. 101, n. 12, p. 1181-1192, 2014.

ZHOU, J. et al. Facile method to prepare silk fibroin/hyaluronic acid films for vascular endothelial growth factor release. In: (Ed.). **Carbohydr Polym**. England: 2016. Published by Elsevier Ltd., v.143, p.301-9, 2016

_____. Regenerated silk fibroin films with controllable nanostructure size and secondary structure for drug delivery. **ACS applied materials & interfaces,** v. 6, n. 24, p. 21813-21821, 2014.

ZHU, Y.; MEHTA, K. A.; MCGINITY, J. W. Influence of plasticizer level on the drug release from sustained release film coated and hot-melt extruded dosage forms. **Pharmaceutical development and technology,** v. 11, n. 3, p. 285-294, 2006.

ZIAEI, M.; GREENE, C.; GREEN, C. R. Wound healing in the eye: Therapeutic prospects. **Adv Drug Deliv Rev,** v. 126, p. 162-176, 2018.

ZIDAN, G. et al. Medicated ocular bandages and corneal health: potential excipients and active pharmaceutical ingredients. **Pharm Dev Technol,** v. 23, n. 3, p. 255-260, 2018.

ŚLADOWSKI, D. et al. Culture of the primary corneal epithelium as a potential component of test batteries for eye irritancy testing. **Toxicology in vitro,** v. 19, n. 7, p. 875-878, 2005.

ERRATA

CUBAYACHI, C. **Avaliação de formulações a base de fibroína da seda e insulina no tratamento tópico de feridas oculares.** 2018. 115f. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2018.

Folha	**Linha**	**Onde se lê**	**Leia-se**
AGRADECIMENTOS	45	À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) pelo auxílio financeiro	À Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo auxílio financeiro
RESUMO, i	14	INS	FS
RESUMO, i	19	8%	9%
ABSTRACT, ii	17	8%	9%
79	20	os picos	as bandas
79	28	o pico	a banda
80	5	novos picos	novas bandas