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Qualitative Assessment the Effect of Glucose on Hydrogel Used for Nanomedicine Drug Delivery

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ABSTRACT

Oral cancer is the sixth among the most common pathological types of cancer in the world and its prevalence is increasing in many countries. The conventional treatments known for this disease are by combination of surgery, chemotherapy as well as radiotherapy. The emergence of the nanoparticles drug delivery system which applies the use of topical drugs becomes a better option in the oral cancer therapy due to its high efficiency of drug delivery options. Therefore, this study aims to characterize hydrogels to be used for effective localized drug delivery in the oral cancer treatment. The hydrogels studied in this project involve three types of natural polysaccharides which were kappa-carrageenan, iota-carrageenan, and xanthan gum. The carrageenan hydrogels were formulated with two different concentrations where iota-carrageenan (1.0% and 1.5%) and kappa-carrageenan (0.5% and 1.0%). While xanthan gum was formulated with three different concentrations (1.0%,1.5% and 2.0%). All hydrogels were added with different glucose concentrations (5%, 10% and 15%). The effects of sugar on the physical properties of the carrageenan were studied qualitatively. Result concluded ι-carrageenan and xanthan gum would be the suitable candidates to be used in oral nanoparticle drug delivery system.

Keywords: *Oral cancer, drug delivery, nanotechnology, carrageenan, xanthan gum, glucose*

ABSTRAK

Kanser mulut adalah jenis kanser patologi keenam yang paling umum di dunia dan penigkatan kes dapat dilihat di banyak negara. Rawatan konvensional untuk penyakit ini adalah melalui gabungan antara pembedahan, kemoterapi serta radioterapi. Kemunculan sistem penghantaran ubat nanopartikel sebagai ubat topikal menjadi alternatif yang baik dalam terapi kanser mulut kerana penghantaran ubat yang efisien. Oleh itu, kajian ini bertujuan untuk mengenal pasti ciri hidrogel untuk digunakan sebagai ubat yang berkesan dalam rawatan kanser mulut. Hidrogel yang dikaji dalam projek ini melibatkan tiga jenis polisakarida semula jadi iaitu kappa-carrageenan, ι- -carrageenan dan gam xanthan. Hidrogel carrageenan telah dirumuskan dengan dua kepekatan berbeza di mana iota-carrageenan (1.0% dan 1.5%) dan κ-carrageenan (0.5% dan 1.0%). Manakala gam xanthan pula dirumus dengan tiga kepekatan berbeza (1.0%,1.5% dan 2.0%). Semua hidrogel ditambah dengan kepekatan glukosa yang berbeza (5%, 10% dan 15%). Kesan gula terhadap sifat fizikal karagenan dikaji secara kualitatif. Keputusan menyimpulkan ι- karagenan dan gam xanthan akan menjadi material yang sesuai untuk digunakan dalam sistem penghantaran ubat nanozarah oral.

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Kata Kunci: *kanser mulut, penghantaran ubat, nanoteknologi, carrageenan, xanthan gum, glukosa*

Introduction

Oral cancer is a malignant tumour that mostly develops in the squamous cells found in the mouth, tongue, lips and throats. The incidence of oral cancer is increasing in many countries and become one of the most threatening diseases. This life-threatening disease have often been late discovered after the cancer has spread to the lymph nodes of the neck and deeply invade into the local structures. Early detection and treatment of oral cancer is the important for survival of a patient. According to World Health Organization, about 657,000 of oral cancer reported each year with 330,000 deaths. The main factors leading to oral cancer are smoking, chewing tobacco and alcohol consumption (Shimpi et al., 2018). Other risk factors include human Papillomavirus (HPV) infection, weakened immune system and also genetic syndrome. In the early stage of oral cancer, the symptoms are not easily noticed and usually patient feel no pain until the disease spread to develop and affects the oral tissues. The treatments for oral cancer include combination of surgery, chemotherapy, radiotherapy as well as targeted therapy.

The conventional treatment of oral diseases or specifically the oral cancer has some disadvantages such as the chemotherapy can causes side effects on the patients, drug resistance to occur and toxicity towards healthy tissue. As for the oral medicine applications, drug resistance and drug delivery has become the major barriers in the treatment of oral mucosal diseases. Referring to the article written by Sankar et al., (2011), the therapeutics drugs may undergo enzymatic degradation, have poor tissue penetration and accidental swallowing could happen. Furthermore, the topical treatments are inappropriate to be used for oral mucosal treatment due to its drug design which only fits for dermatological conditions. The drugs are not suitable for mouth condition due to the aqueous environment as the drugs can be easily washed off or swallowed. Thus, the need for an improvement in the mechanism of the targeted drug delivery has been suggested by the combination of two biomaterials to become an excellent site targeting vehicle.

The hybrid biomaterials are formed by incorporating the therapeutic nanoparticles into hydrogel network. Hydrogels are polymer with many unique properties such as having tissue-like mechanical properties, able to tune in any shaped they are confined and retain high water content (Prashant et al., 2018). The hydrogel network has the swelling and shrinking properties which can control drug release and its physical structure is amendable to manipulation so that drugs can be directly delivered to specific site as well as retained much longer in mouth (Gao et al., 2016). Furthermore, hydrogels are non-toxic, biocompatible as well as biodegradable making it convenience for drug delivery development study. Hydrogels have become a good candidate for nanoparticles drug encapsulation for oral cancer treatment because it is safe, site-specific and could effectively delivered towards targeted area of disease.

Therefore, the main purpose of this study was to formulate and characterize hydrogels encapsulation of topical drugs to protect as well as to increase drug adhesion on the infected for better drug delivery system. The natural polymer-based hydrogels carrageenan and xanthan gum were used as the potential hydrogels to be used in the nanoparticles drug encapsulation for oral cancer treatments. The respective hydrogels are non-toxic, biodegradable, and safe for human consumption. Moreover, they are extracted from plant and bacteria fermentation making them convenient for Muslims in terms of halal issues. Sugar with different concentrations was added to the hydrogels and the effects on the physical properties of the hydrogels was observed. Also, the characterization of hydrogels on its morphology, rheology and surface charge were reviewed in general. The aim of this study is to characterize hydrogels to become an effective localized drug delivery for oral cancer treatment.

Material and Methods

Methods

Gels Preparation

The carrageenan samples, ι- (1.0 w/v% and 1.5 w/v%), κ-(1.5 w/v% and 2.0 w/v%) and xanthan gum (1.0 w/v%, 1.5 w/v%, 2.0 w/v%) were prepared. Dry hydrogels powder was weighed using an analytical balance and transferred into a beaker. Glucose was added into the same beaker. The concentrations of the sugar were 5%, 10% and 15%. Hydrogels samples were formulated by adding 100 mL total volume of 0.05 M PBS solution into a beaker. The beakers with each sample concentrations were covered using aluminium foil, stirred at 100 rpm using a magnetic stirrer and heated to approximately 70°C then left to dissolve completely. The samples were then poured into 50 mL falcon tubes, covered with parafilm, and chilled at 4 °C. All sets of samples were prepared in triplicate. For each concentration of the hydrogels sample, control samples were prepared with no addition of glucose. The samples' texture were observed and recorded. The gels preparation steps above were repeated for other hydrogels sample, ι-carrageenan and xanthan gum. Figure 1 illustrates the formulation of , ι-carrageenan, κ-carrageenan and xanthan gum prepared in this study.

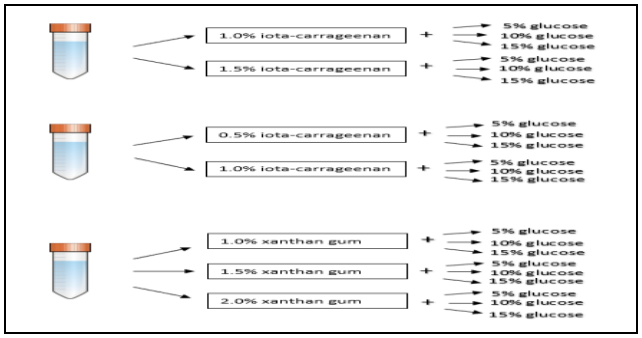


Figure 1: The formulation of hydrogels with glucose.

Result and Discussion

In this study, two types of carrageenan; κ- and ι-carrageenan as well as xanthan gums were being examined. The formulated hydrogels were compared with the control samples containing no glucose. Throughout the experiment, the pH was maintained at pH 7 by using phosphate buffer. The gelling temperature range for hydrogel samples are 40°C-60°C as the preferred heating temperature for them to solubilise (Ali & Ahmed, 2019). The texture of gels were compared and analysed to observe the physical characteristics especially on the viscosity of fluid and their gel structure.

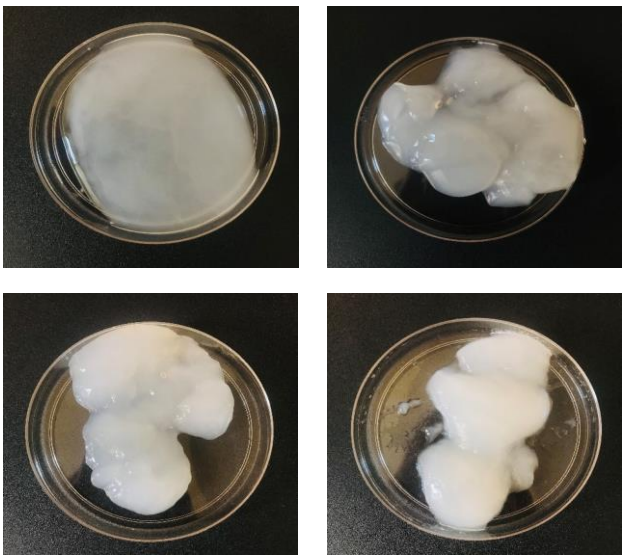
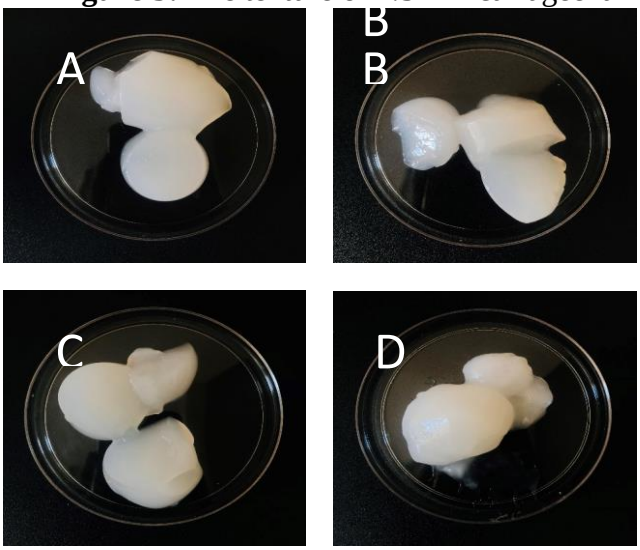


Figure 2: The texture of 1.0% ι -carrageenan with addition of different glucose concentration. (A) Addition of 5% glucose. (B) Addition of 10% glucose. (C) Addition of 15% glucose. (D) Control, with no glucose.

From figure 2 above, 1.0% ι -carrageenan literally formed a creamy-gel structure which is soft, thick and sticky. The texture is elastic

and has good viscosity. The turbidity of the gels exhibits cloudy and whitish colour. As of Figure 3(A), the 1.0% ι -carrageenan with addition of 5% glucose has different form of gels compared to the other three B, C,D). The gel formed is less viscous form with slightly sticky characteristic. The reason of this condition would be an error of the experiment where gel concentration became lower due to loss of sample powder during gel formulation step.

Figure 3: The texture of 1.5% ι -carrageenan



with addition of different glucose concentration. (A) Addition of 5% glucose. (B) Addition of 10% glucose. (C) Addition of 15% glucose. (D) Control, with no glucose.

Based on the **Figure 3**, 1.5% ι -carrageenan literally formed a thick gel which was white in colour. The texture has higher viscosity compared to 1.0% ι -carrageenan gels. The gels formed were elastic, strong and soft. Looking at the 1.5% ι -carrageenan control gel, it has nearly same texture as the 1.0% ι -carrageenan gels. Addition of glucose to 1.5% ι -carrageenan, shows better gel form with good textures were produced. This concentration of 1.5% ι -carrageenan with the addition of glucose would produce gels with good quality as there are no liquid excess from the gel formation.

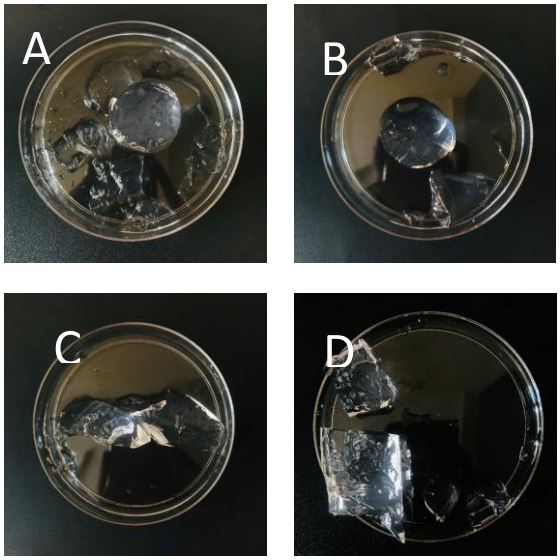


Figure 4: The texture of 0.5% κ-carrageenan with addition of different glucose concentration. (A) Addition of 5% glucose. (B) Addition of 10% glucose. (C) Addition of 15% glucose. (D) Control, with no glucose.

Based on the **Figure 4**, it can be clearly seen that the texture of κ-carrageenan gel is very hard and brittle. The gels are more like agar and when the gels were broken, the agar structure breaks into small pieces. There is not much difference in the texture of the κ-carrageenan even with the addition of different concentration of glucose. Furthermore, there were excess liquid solution from every carrageenan gel sample which indicate that the gel formation process is unusual and distorted.

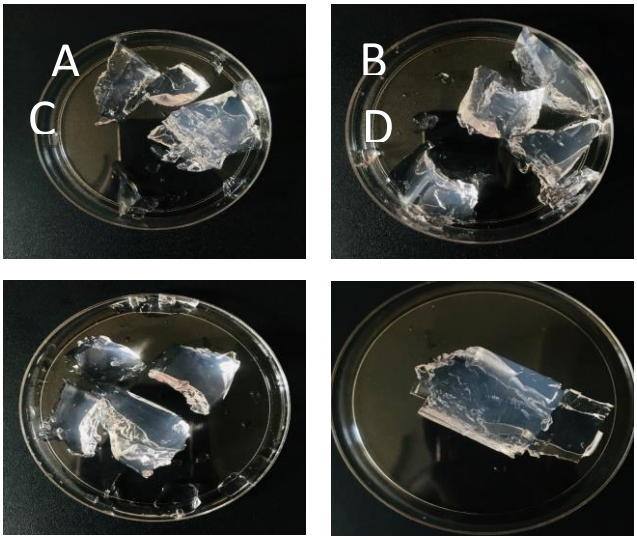


Figure 5: The texture of 1.0% κ-carrageenan with addition of different glucose concentration. (A) Addition of 5% glucose. (B) Addition of 10% glucose. (C) Addition of 15% glucose. (D) Control, with no glucose.

Higher concentration of κ-carrageenan would increase the gel strength. Based on the figures above, 1.0% κ-carrageenan gel is very hard and strong. The solid agar structure is not easily smashed or breaks. The gels of κ-carrageenan at different concentration of glucose shows no different in the physical form and texture. Notably, the excess solution from the carrageenan sample could be the result of poor spreading formulation.

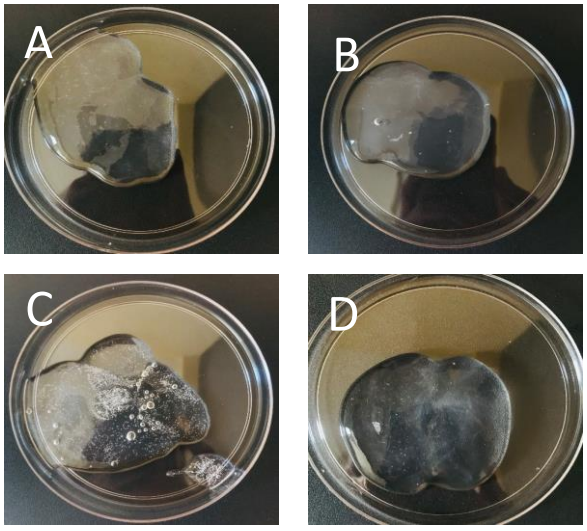


Figure 6: The texture of 1.0% xanthan gum with addition of different glucose concentration. (A) Addition of 5% glucose. (B) Addition of 10% glucose. (C) Addition of 15% glucose. (D) Control, with no glucose.

Based on the **Figure 6**, 1.0% xanthan gum formed a fluid gel which is soft, flowy and slightly sticky. The gel is clear and exhibit no opaque colour. Furthermore, there were no differences in the physical characteristic of the gels formed at each different concentration of glucose added. The viscosity of each gel concentration were difficult to discern as the fluid gel has similar physical texture and characteristics.

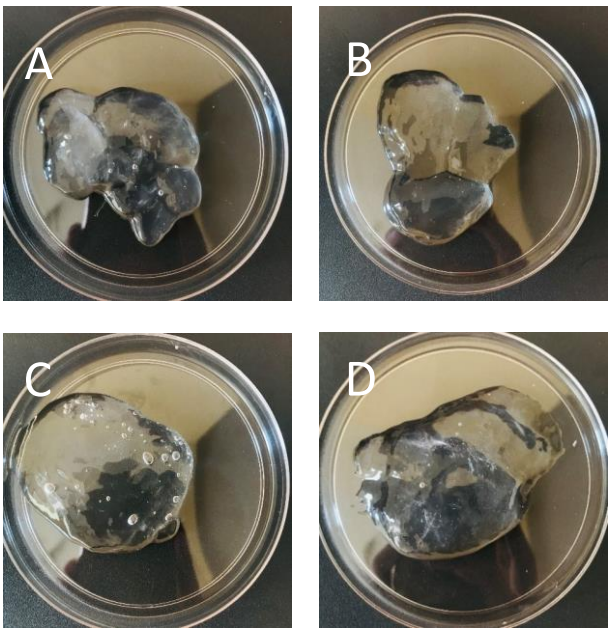


Figure 7: The texture of 1.5% xanthan gum with addition of different glucose concentration. (A) Addition of 5% glucose. (B) Addition of 10% glucose. (C) Addition of 15% glucose. (D) Control, with no glucose.

Based on the **Figure 7,** 1.5% xanthan gum formed a gel that is less viscous, sticky. However, it is slightly thicker than 1.0% xanthan gum gel. Also, the gel is clear and exhibit no turbidity. There were no differences in the physical characteristic of the gels formed at each different concentration of glucose added. Higher concentration of

xanthan gum makes the gel formed more dense and thicker.

From the results, ι- and κ-carrageenan gels show different in the physical properties and texture. ι -carrageenan has soft, sticky and creamy-like texture but κ-carrageenan has hard, strong and brittle with agar structure of gels. By looking at the carrageenan chemical structure, a linear polysaccharide chains with ester-sulphate attached to the sugar unit. The amount and position of the sulphate group attached onto the sugar unit influence the viscoelasticity of the carrageenan (Popescu, Iordan & Boscornea, 2007). Kappa-carrageenan has one sulphate ester group while iota-carrageenan has only one sulphate ester group leaving kappa-carrageenan with more hydrogen bonds in its structure. Polysaccharides tends to be more hydrophobic if they have a greater number of internal hydrogen bonds. This result in softer gel structure and lower gel strength of iota-carrageenan compared to kappa-carrageenan. Also, the sulphate group in iota structure inhibit syneresis of the gel formed due to higher hydrophilicity properties of iota compared to kappa. Furthermore, the addition of glucose would affect the viscosity of the gels where it stabilizes the structure of carrageenan through water binding (OH-groups) interaction between glucose and polysaccharide structure (Kozlowska, Pauter, & Sionkowska, 2018). Higher glucose concentration would make hydrogels become thicker, denser, and more porous. As mentioned by Li & Mooney (2018), toughness of a hydrogel makes them could maintain its structure and resist rupture during bio-adhesion.

Meanwhile, xanthan gum also formed elastic and soft texture of gels. Xanthan gum is biocompatible and has excellent water solubility. Xanthan gum is a long chain polysaccharide with high molecular weight. Xanthan gum is soluble in either cold or hot water but it needs proper and intense agitation when dissolve in a solution to prevent clump formation. Above the pH 4.5, xanthan gum acts as polyanion due to the reduction of 0-acetyl and pyruvyl residues. The pyruvyl content affect the viscosity of the gel formed. Higher pyruvyl content in xanthan gum structure produces high viscosity of gel. Xanthan gum can form gel usually at higher pH and it would di-acylates at pH 9. The factor affecting the gelation of xanthan gum are pH, concentration of xanthan gum in a solution and the concentration of buffer. However, xanthan gum in aqueous solution would exhibit weak gel like properties and could not form true gel at any concentration. The poor mechanical strength of xanthan gels can be improved by manipulating the xanthan structure which is amendable for chemical modification. Therefore, xanthan gum modification would enhance mechanical properties of xanthan gel.

The differences in the texture of each type of hydrogels can be explained through its structure and stability of the polysaccharide. Hydrogels have a fluid-like viscous properties under high shear strain rates. Fluid can be classified into three types according to the flow index of "n" behaviour where n<1 is a non-newtonian pseudoplastic, n=1 is Newtonian and n>1 represents a Non-newtonian dilatant as shown in figure 4.8.

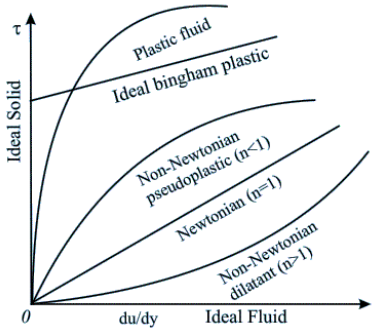


Figure 8: Fluid classification according to the shear rate and shear stress based on thflow behaviour index "n" including non-Newtonian pseudoplastic, Newtonian and Non-Newtonian dilatant. (Jahangiri et al., 2012)

Hydrogels are a non-Newtonian fluid where fluid flow properties are not described by a single constant value of viscosity. The

fluid viscosity depends on applied force to either become more liquid or more solid. The hydrogels pseudo-plastic fluid which has the character of shear thinning effect that would assist in the formation of thin layer adhesion when applied across the mucous membrane and lead to an efficient drug delivery on the target area (Carvalho et al., 2012). The ideal hydrogel formulation for topical drug specifically for oral disease treatment is preferred in a semisolid form or sol-gel formulation. Furthermore, the hydrogels must have the flowable feature that will enhance the efficiency for drug administration. So, kappa-carrageenan with brittle and hard texture is not preferred to be utilized in topical drug formulation due to its non-flowy gel properties.

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Conclusion

In conclusion, the objectives of the study were achieved when the observations show that the different formulation of the carrageenan concentration and salt concentration exhibit different physical properties. The formulation of kappa-carrageenan and NaCl results in a clear, hard, and brittle gel, whereas the iota-carrageenan added with NaCl has a softer texture and more elastic. The formulation of kappa-carrageenan with NaCl was found not suitable to be used as the matrix of nanoparticles for topical administration due to its hard texture. However, the formulation of iota-carrageenan and NaCl has the potential to be used in designing novel topical drug. For 0.5% iota-carrageenan, the addition with 1.5% and 2.0% NaCl has the desired texture for topical drug administration. Whereas for 1.0% iota-carrageenan, the addition of the lowest concentration of salt, which is 0.5% is another suitable candidate for topical drug design. Results revealed that the addition of glucose was seen to affect the physical characteristics for all hydrogels. Increments in the glucose concentration was also seen to compromise the integrity of the gel structure producing a more softer semi solid structure. The occurrence of syneresis seems with the addition of glucose and increase with the concentration of glucose

References

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research*, *6*(2), 105–121. doi: 10.1016/j.jare.2013.07.006

Banerjee, S., & Bhattacharya, S. (2012). Critical Reviews in Food Science and Nutrition Food Gels: Gelling Process and New Applications Food Gels: Gelling Process and New Applications. *Critical Reviews in Food Science and Nutrition*, *52*(July 2013), 334–346.https://doi.org/10.1080/10408398.2010.500234

Calixto, G., Bernegossi, J., Fonseca-Santos, B., & Chorilli, M. (2014). Nanotechnology-based drug delivery systems for treatment of oral cancer: a review. *International journal of nanomedicine*, *9*, 3719–3735. https://doi.org/10.2147/IJN.S61670

Carvalho, F. C., Calixto, G., Hatakeyama, I. N., Luz, G. M., Gremião, M. P. D., & Chorilli, M. (2013). Rheological, mechanical, and bioadhesive behavior of ydrogels to optimize skin delivery systems. *Drug Development and Industrial Pharmacy*, *39*(11), 1750–1757. https://doi.org/10.3109/03639045.2012.734510

Chee, B. S., & Nugent, M. (2019). Electrospun natural polysaccharide for biomedical application. N*atural Polysaccharides in Drug Delivery and Biomedical Applications*, 589–615. doi:10.1016/b978-0-12-817055-7.00026-1

Chen, G., Roy, I., Yang, C., & Prasad, P. N.
David, S., Shani Levi, C., Fahoum, L., Ungar, Y., Meyron-Holtz, E. G., Shpigelman, A., & Lesmes, U. (2018). Revisiting the carrageenan controversy: do we really understand the digestive fate and safety of carrageenan in our foods?. *Food & function*, *9*(3), 1344–1352. https://doi.org/10.1039/c7fo01721a

Gao, W., Zhang, Y., Zhang, Q., & Zhang, L. (2016). Nanoparticle-Hydrogel: A Hybrid Biomaterial System for Localized Drug Delivery. *Annals of Biomedical Engineering*, *44*(6), 2049–2061. https://doi.org/10.1007/s10439-016-1583-9

Gonçalves, C., Pereira, P., & Gama, M. (2010). Self-assembled hydrogel nanoparticles for drug delivery applications. *Materials*, *3*(2), 1420–1460. https://doi.org/10.3390/ma3021420

International Agency for Research on Cancer, & Organization, W. H. (2019). Lip, oral cavity: Globocan 2018. *The Global Cancer Observatory*, 2018–2019. Retrieved from https://gco.iarc.fr/today/data/factsheets/cancers/1-Lip-oral-cavity-fact-sheet.pdf

Kozlowska, J., Pauter, K., & Sionkowska, A. (2018). Carrageenan-based hydrogels:
Effect of sorbitol and glycerin on the stability, swelling and mechanical properties.
Polymer Testing. https://doi.org/10.1016/j.polymertesting.2018.02.016

Li, J., & Mooney, D. J. (2016). Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, *1*(12). doi:10.1038/natrevmats.2016.71

Lopez Hernandez, H., Grosskopf, A. K., Stapleton, L. M., Agmon, G., & Appel, E. A. (2019). Non-Newtonian Polymer–Nanoparticle Hydrogels Enhance Cell Viability during Injection. *Macromolecular Bioscience*, *19*(1), 1–7. https://doi.org/10.1002/mabi.201800275

Malik, N. S., Ahmad, M., Minhas, M. U., Tulain, R., Barkat, K., Khalid, I., & Khalid, Q. (2020). Chitosan/Xanthan Gum Based Hydrogels as Potential Carrier for an Antiviral Drug: Fabrication, Characterization, and Safety Evaluation. *Frontiers in Chemistry*, *8*. doi:10.3389/fchem.2020.00050

Michler, G. H., & Lebek, W. (2016). Electron microscopy of polymers. In *Polymer Morphology: Principles, Characterization, and Processing* (Vol. 66). https://doi.org/10.1002/9781118892756.ch3

Milani, J., & Maleki, G. (2012). Hydrocolloids in Food Industry. Food Industrial Processes - Methods and Equipment. doi:10.5772/32358

Myneedu, L. (2015). Effect of salts on the structure-function relationships of sodium kappa-carrageenan. *Theses and Dissertations Available from ProQuest*, 1–87. Retrieved from http://docs.lib.purdue.edu/dissertations/AAI1598109

Parvez, K. (2019). Characterization Techniques of Two-Dimensional Nanomaterials. Biomedical Applications of Graphene and 2D Nanomaterials, 27–41. doi:10.1016/b9780-12-815889-0.00002-7

Patel, H. B., Patel, H. L., Shah, Z. H., & Modasiya, M. K. (2011). Review on Hydrogel Nanoparticles in Drug Delivery. *American Journal of PharmTech Research*, *1*(3), 20–38.

Popescu, E., Iordan, M., & Boscornea, C. (2007). *Structure and properties of carragenan*.

Pramod, K., Shanavas, S., & Baby, J. N. (2015). Research Article Rheological profiling of a hydrogel drug delivery vehicle. *Journal of Chemical and Pharmaceutical Research, 7*(7): 818-825

Prashant, S., M., et al. (2018). A review on-hydrogel. *Journal of Pharaceutical Technology Resources, 8*(3). Retrieved from http://ajptr.com/assets/upload/publish_article/ AJPTR-83005_3193.pdf

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. *Journal of Food Science and Technology*, *47*(6), 587–597. https://doi.org/10.1007/s13197-010-0162-6

Samimi, S., Maghsoudnia, N., Eftekhari, R. B., & Dorkoosh, F. (2019). Lipid-Based Nanoparticles for Drug Delivery Systems. Characterization and Biology of Nanomaterials for Drug Delivery, 47–76. doi:10.1016/b978-0-12-814031-4.00003-9

Sankar, V., Hearnden, V., Hull, K., Juras, D. V., Greenberg, M., Kerr, A., … Thornhill, M. (2011). Local drug delivery for oral mucosal diseases: Challenges and opportunities. *Oral Diseases*, *17*(SUPPL. 1), 73–84. https://doi.org/10.1111/j.1601-0825.2011.01793.x

Shastry, S., Sanjay, C., Kaul, R., Mahima, V., Doggalli, N., & Patil, K. (2015). Topical drug delivery: An essential aid in the management of oral diseases. *Journal of Advanced Clinical and Research Insights, 2*, 269-275.

Shang, Y., & Xiong, Y. L. (2010). Xanthan enhances water binding and gel formation of transglutaminase-treated porcine myofibrillar proteins. *Journal of food science*, *75*(3), E178–E185. https://doi.org/10.1111/j.1750-3841.2010.01547.x

Singhvi, G., Hans, N., Shiva, N., & Kumar Dubey, S. (2019). Xanthan gum in drug delivery applications. *Natural Polysaccharides in Drug Delivery and Biomedical Applications*, 121–144. doi:10.1016/b978-0-12-817055-7.00005-4

Shimpi, N., Jethwani, M., Bharatkumar, A., Chyou, P. H., Glurich, I., & Acharya, A. (2018). Patient awareness/knowledge towards oral cancer: A cross-sectional survey. *BMC Oral Health, 18*(1), 1–10. https://doi.org/10.1186/s12903-018-0539- x

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