Fabrication of biocompatible hydrogels from pine resin

by VIDHURA MAHENDRA

Centre for Rapid and Sustainable Product Development
Polytechnic Institute of Leiria
Corresponding author email: vidhura.mahendra@ipleiria.pt

Abstract This short review aims to look at some of the applications based on potential pine associated resin (rosin) composites for cell culture studies via hydrogel fabrication. Although hitherto there are only a few links to pine resin based hydrogel formation in the public domain, literature work based rosin incorporated drug delivery studies and its associated uses can be useful for extensive works on the cell interaction and viability. Rosin in such case may be optimised to afford similar characteristics hence applications.

1 Introduction

Pine resins are exudates ranging from the volatile terpenes to non-volatile material known as rosin (Figure 1). They are isolated by tapping the tree, approximately contains 70% rosin, 15% turpentine 15% debris and water [1]. At room temperature it is brittle and softens at higher temperatures. It is used in paper sizing, printing inks, surface coatings, adhesives and rubber additives together with some more advanced applications in biomedical and construction industry [2].

Figure 1: Blocks of rosin

2 Hydrogel fabrication from nature

The usual composition of hydrogels could be up to 99% water and as a result are similar to human tissues [3]. By tuning their shape, physical properties, chemical composition and infusing them with cells, biomedical engineers have successfully used hydrogels as three– dimensional molecular scaffolds that can be filled with cells, molecules for bodily injection or application in order to release drugs and stimulate tissue regeneration. Alginate hydrogels [4] have been studied as it is a biocompatible polysaccharide obtained from natural brown seaweed and its degradation kinetics can be tuned to suit drug molecules encapsulated in the gel hence delivery.

One of the most useful and naturally biocompatible polymers is cellulose and the research works related to them are encouraging. Peng et al [5] have reported on developing novel cellulose based hydrogels to overcome weak mechanical strength, poor biocompatibility and lack of antimicrobial activity which may induce skin allergy of the body in commercial diapers with a simple chemical cross-linking of quaternized cellulose (QC) and native cellulose in sodium hydroxide and urea aqueous solution. The prepared hydrogel was shown superabsorbent property, high mechanical strength, good biocompatibility and excellent antimicrobial efficacy against *Saccharomyces cerevisiae* (Figure 2). The resulting data encouraged the use of these hydrogels for hygienic application such as disposable diapers.

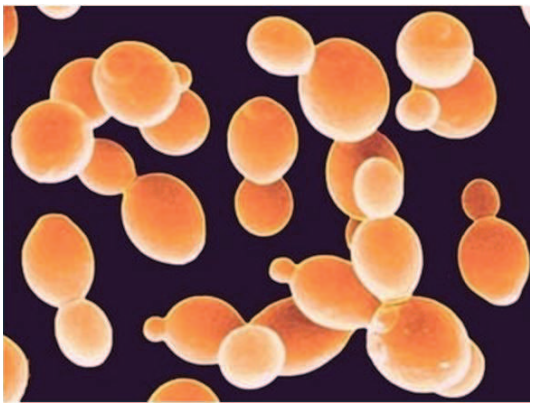


Figure 2: Saccromyces cerevisiae (unicellular fungi) Image credit: http://www.scientistlive.com/content/19429.

Kobayashi [6] describes the use of cellulose originat-

ing from bagasse wastes (Figure 3) from the food industry to fabricate hydrogel films with flexible and bioactive properties for tissue engineering.

Figure 3: Pile of baggase waste
Image credit:
http://www.endswasteandbioenergy.com/article/1290438/cuba-unveils-plan- 765mw-biomass-power

A natural plant polymer was regenerated from *Agave tequilana Weber* bagasse from Corralejo Penjamo, Guanajuato, Mexico. It was subsequently converted to lignocellulose. A phase inversion process with a new preparation technique was followed for cellulose hydrogel films. The hydrogel film preparation and characteristics were demonstrated from perspectives of bioactive applications with cytotoxicity of fibroblast cell cultivation on a scaffold film. Experimental evidence was established showing the resultant hydrogel films have exclusive properties displaying good mechanical and viscoelastic films even in their water-swollen condition. Hydrogel behaviours in cellulose structure and characteristics were clarified using several analytical methods for cell growth on the scaffold which was prepared to show different cellulose morphologies. Different effects of cellulose fibre nanostructures of the hydrogel films were described for their cytotoxicity for tissue engineering applications.

A new series of *in situ* forming antibacterial conductive degradable hydrogels using quaternized chitosan (QCS) grafted polyaniline (Figure 4) with oxidized dextran as cross- linker has been reported [7].

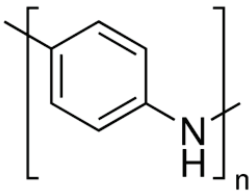
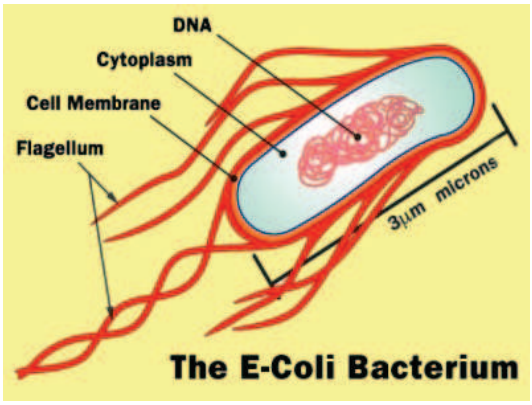
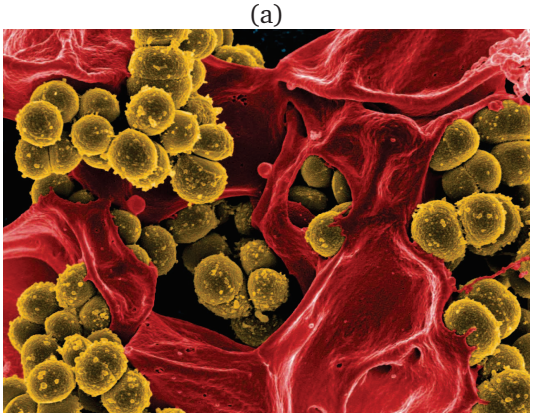


Figure 4: Chemical structure of polyaniline

The chemical structures, morphologies, electrochemical property, conductivity, swelling ratio, rheological property, *in vitro* biodegradation and gelation time of hydrogels were characterized. Injecting ability was verified by in vivo subcutaneous injection on a Sprague Dawley [8] rat. The antibacterial activity of the hydrogels was initially evaluated employing antibacterial assay using *Escherichia coli* and *Staphylococcus aureus in vitro* (Figure 5). The hydrogels containing polyaniline showed enhanced antibacterial activity compared to QCS hydrogel, especially for hydrogels with 3 wt% polyaniline showing 95 kill% and 90 kill% for *E. coli* and *S. aureus*, respectively. Compared with QCS hydrogel, the hydrogels with 3 wt% polyaniline still showed enhanced antibacterial activity for *E.coli in vivo*. The adipose-derived mesenchymal stem cells (ADMSCs) have been used to evaluate the cytotoxicity of the hydrogels, and hydrogels with polyaniline showed better cell compatibility than QCS hydrogel. The electroactive hydrogels could significantly enhance the proliferation of C2C12 myoblasts compared to QCS hydrogel. This work opens the way to fabricate in situ forming antibacterial and electroactive degradable hydrogels as a new class of bioactive scaffolds for tissue regeneration applications.



(a)



(b)

Figure 5: (a) Escherichia coli *and (b)* Staphylococcus aureus
Image credit (a):
http://www.nature-education.org/water-testing.html
Image credit (b):
This scanning electron micrograph shows the methicillin-resistant Staphylococcus aureus.
http://www.sci-news.com/medicine/science- antibiotics-methicillin-resistant-staphylococcus-aureus-01548.html

Franco et al [9] have described the anomalous

swelling behaviour of a scleroglucan (a water soluble nature-derived polysaccharide produced by fermentation of the filamentous fungus *Sclerotium rolfsii*) in borax hydrogel by different physico-chemical approaches and means of molecular dynamics simulations. The role of polymer combinations forming interpenetrated structures was explained in terms of specific properties which significantly differ from those of the constituent polymers thus allowing appropriate tailoring of the delivery rates. Finally the wide possibilities of applications of nano-gel structures which allow combination therapies for cancer treatment and the suitability for intracellular targeting have also been reported. The studies on polysaccharide hydrogels are still in progress and emphasize future researches to be more stimulated.

It is also noteworthy mentioning that natural biomaterials such as gelatine (derived either from animals or plants e.g. pectin or pectic polysaccharides) are directly involved in cell culture studies due to their biocompatibility. The chemical functionalities present in gelatin [10] (e.g. carboxylic acid, thiol, hydroxyl) allow for potential covalent modification of the gelatine methacrylated (GelMA) with growth factors or cytokines to further promote cell viability and function. Therefore, GelMA could potentially be tailored to different cell or tissue types or growth factor and drug delivery applications based on the specific type of gelatin precursor.

3 Rosin in biological applications

Along with the other naturally occurring materials previously discussed rosin has been studied as an application for drug delivery [11] obtained from *Pinus palustris* [12] (Figure 6), the long leaf pine. It has potential as pharmaceutical excipients [13], that is natural or synthetic substance formulated alongside the active ingredient of a medication in terms of biodegradability, ease of availability, matrix forming coating, microencapsulating and binding. The studies further reveal it had been found as an anti-inflammatory and antitumor activity. A semisolid preparation such as skin cream shows good homogeneity and spreading ability. Moreover consists of prominent properties for the sustained release drug system with most of the drug and dosage form.

Further study on rosin has been used to prepare spherical microcapsules by a method based on phase separation via solvent evaporation [14]. Rosin based polymer has been used as film coating materials; coated pellets were prepared using diclofenac sodium [15] as a model drug and sustained release of the drug was probed [16]. Rosin polymer has been used as the transdermal drug delivery system. Its combination with polyvinyl pyrrolidone and dibutyl phthalate (30% w/w) produces smooth film with improved elongation and tensile strength.

Figure 6: Longleaf pine (Pinus palustris) forest

Rosin has appropriate hydrophobic properties that can be utilized as matrix forming agent of water soluble drug such as dilitiazem hydrochloride [17] to prolong the release. The drug release followed first order kinetics and the Higuchi model, thus indicates that there was no erosion of the matrix and the tablet maintained its shape and surface area [18].

Moreover, rosin esters are reported to have good film forming properties and can be used for enteric coating and delayed release of drugs. Rosin and rosin-based polymers have drug delivery applications achieving sustained/controlled release profiles [19-20]. This further exemplifies the role of rosin as a barrier to migrate molecules in the medium.

Derivatives of rosin polymers (RD-1 and RD-2) had been synthesized in the laboratory and evaluated for physicochemical properties [21], polydispersity (Mw/Mn), molecular weight (Mw), and glass transition temperature (Tg). The derivatives of rosin have further been evaluated for pharmaceutical film coating by characterizing the release of a model drug (diclofenac sodium) from pellets coated with the derivatives. The studies have revealed that pellet film coating could be achieved without agglomeration of the pellets within a reasonable operation time and drug release was sustained up to 10 hours with the two rosin derivatives. These results have suggested the application of rosin derivatives (RD-1 and RD-2) for film coating.

In vitro tests related to rosin have been studied [22] to determine its biochemical and physical compatibilities. Free films of rosin (2 cm $\times$ 1 cm $\times$ 0.4 mm, 120 mg) were subjected to *in vitro* degradation by placing them in 10.0

mL of 0.2M phosphate buffered saline (PBS) (pH 7.4, 37 ^{o}C) and maintained on a rotating container [23]. The PBS was changed every 8 hours for the first day, every day for the first week and weekly thereafter to keep the pH relatively constant. 15 Films were withdrawn at intervals of 30, 60, and 90 days, washed with distilled water, dried and subjected to analysis. The films have been subcutaneously implanted on the backs of male Wistar [24] rats (200-300g) to monitor the *in vivo* degradation. Anaesthesia was induced by intraperitoneal injection of a mixture of ketamine HCl (85 mg/kg bodyweight) and xylazine (12 mg/kg body weight). Tetracycline, 10 mg/kg dose, was given at the time of surgery. An incision (2.5 cm) was inflicted laterally about the mid-portion of the back. Subcutaneous pockets were formed around each incision, free film was inserted, and the wounds have been sealed by intermittent nylon stitches at 0.5 cm apart. Films were explanted at 30, 60, and 90 days for analysis [25-26]. In these studies the authors have broadly justify the biocompatibility of rosin as a material for exclusive use in living organism.

Satturwar et al [27] have studied rosin, for its degradability and compatibility in and with the physiological environment with the aforementioned methodologies and revealed rosin has shown faster degradation *in vivo* as compared with *in vitro* studies. Subsequent placement in PBS, the rosin films showed MW loss of 14.7%, with the films being recovered at the end of 90 days. After *in vivo* implantation in rats, the free films showed MW loss of 60% at around day 75 and complete loss at the end of 90 days. Bulk degradation is evident both *in vitro* and *in vivo*. Although rosin degrades over a period of 2 to 3 months, it provides good compatibility compared with Poly (DL-lactic-co-glycolic acid) (PLGA) to the extent investigated in the paper. This finding presumably will lead to new applications of rosin in the field of drug delivery.

Nande et al have discussed the derivatives of rosin synthesized by a reaction with polyethylene glycol 200 and maleic anhydride proved suitable for sustaining drug release from matrix tablets and pellets [28]. Furthermore polymerised rosin films containing hydrophobic plasticisers showed excellent potential as coating materials for the preparation of sustained release dosage forms have been reported [29].

In a slightly different approach to what we have discussed so far, Kaith et al [30] have recently reported on reducing the gum rosin [31] from its rosin acid to alcohol form via a typical reducing agent, sodium borohydride and cross-linked subsequently with the addition of an acrylamide to afford its co-polymer as gum rosin-acrylamide (GrA-cl-poly(AAm) hydrogel, an application for removal of malachite green dye from waste water. This reaction resembles to the reaction of gelatine with acrylic anhydride followed by the cross-linking to afford gelatine methacrylamide (GelMA), a hydrogel which is widely used in cell culture studies as mentioned before. The paper has further explained the versatility of rosin in terms of modifying the structure to adopt a useful route in creating a hydrogel. Most importantly it also suggests the rosin acids collectively have no influence on reduction preferences to its alcohol form enabling to fabricate hydrogels as the final product.

4 Conclusion

In this short review we have looked into the ways of incorporating rosin as a useful precursor for potential biological studies similar to many other natural materials and its advancement. Number of researches carried out on rosin as a precursor for drug transport in biomedical applications and has been shown as a useful insulator for prolong released drugs *in vivo* with no influence in the overall interaction *per se*. Furthermore, it has also been studied for structural modifications especially in the hydrogel fabrications, where the rosin had undergone typical reduction reaction followed by polymerisation to afford the co-polymer. These studies have also proved that rosin withstands to structural modifications with its functional groups acting as the site of interest without the distortion of its overall fused ring system. These developments have encouraged for potential hydrogel fabrication for cell studies *in vitro* and *in vivo*.

Acknowledgement

We would like to acknowledge the Portuguese Foundation for Science and Technology (FCT — UID/ Multi/04044/2013), for the funding of the research work.

References

[1] Zhang J, Rosin-based chemicals and polymers, Smithers Rapra, 2012

[2] H F Enos, Current and potential use of Rosin, presented at Paper and Textile Chemistry Division Meeting, March 23, 1977, p.75.5 B. E. Avla and S. Pekkala, Journal of the American Chemical Society

[3] New method for synthesizing a biocompatible hydrogel could speed up research and development of several promising applications in tissue engineering http://tinyurl.com/zvj5xg2

[4] Alexander D. Augst, Hyun Joon Kong, David J. Mooney, Alginate Hydrogels as Biomaterials, Volume 6, Issue 8, August 7, 2006 , Pages 623–633, DOI: 10.1002/mabi.200600069

[5] Na Peng, Yanfeng Wang, Qif Ye, Lei Liang, Yuxing An, Qiwei Li, Chunyu Chang Biocompatible cellulose-based superabsorbent hydrogels with antimicrobial activity, Carbohydr. Polym, 2016 Feb 10; 137:59-64

[6] Takaomi Kobayashi , Chapter 1 – Fabrication of Cellulose Hydrogels and Characterization of Their Bio-

compatible Films, Studies in Natural Products Chemistry 45:1-5, Jan 2015

[7] Xin Zhao, Peng Li, Baolin Guo, Peter X. Ma, Antibacterial and conductive injectable hydrogels based on quaternized chitosan-graft-polyaniline/oxidized dextran for tissue engineering, Acta Biomater. 2015 Oct; 26:236-248

[8]Info from http://www.criver.com/products-services/basic-research/find-a-model/sprague-dawley-rat

[9] Franco Alhaique, Maria Antonietta Casadei, Claudia Cencetti, Tommasina Coviello, Chiara Di Meo, Pietro Matricardi, Elita Montanari, Settimio Pacelli, Patrizia Paolicelli, From macro to nano polysaccharide hydrogels: An opportunity for the delivery of drugs, J, Drug Delivery Science and Technology, 32, 88-99, 1 Apr 2016

[10] Jason W. Nichol, Sandeep T. Koshy, Hojae Bae, Chang M. Hwang, Seda Yamanlar, Ali Khademhosseini, Cell-laden microengineered gelatin methacrylate hydrogels, Biomaterials 31 (2010) 5536-5544

[11] Om Prakash Pal, Rishabha Malviya, Vipin Bansal, Pramod Kumar Sharma, ROSIN AN IMPORTANT POLYMER FOR DRUG DELIVERY: A SHORT REVIEW, International Journal of Pharmaceutical Sciences Review and Research, Volume 3, Issue 1, July – August 2010; Article 007 ISSN 0976 – 044X, pp 3-37

[12] Boyer, W. D. and J. B. White, Natural Regeneration of Longleaf pine, pp 99-113 in Proc. of the Symposium on the management of Longleaf Pine, Farrar RM (ed.) General Technical report 50–75

[13] Bhattacharyya, Lokesh; Schuber, Stefan; Sheehan, Catherine; William, Roger (2006). "Excipients: Background/Introduction". In Katdare, Ashok; Chaubal, Mahesh. Excipient Development for Pharmaceutical, Biotechnology, and Drug Delivery Systems. CRC Press.

[14] Bohme K, Buprenorphine in a transdermal therapeutic system–a new option, Clin Rheumatol, 21, 2002, Suppl 1: S13-16.

[15] Small RE, Diclofenac sodium, Clin. Pharm. 1989 Aug;8(8):545-58

[16] Pathak YV, Dorle AK, Rosin and rosin derivatives as hydrophobic matrix materials for controlled release of drugs, Drug Des Delivery, 6, 1990, 223-227.

[17] Info from http://www.rxlist.com/cardizem-cd-drug.htm

[18] Prashant MS, Suniket VF, Avinash KD, Evaluation of polymerized rosin for the formulation and development of transdermal drug delivery system: A Technical Note, AAPS PharmSciTech, 6(4), 2005, E649- E654.

[19] Pathak YV, Shinghatgiri M, Dorle AK, *in vivo* performance of pentastergum coated aspirin microcapsules, J Microencapsul, 4, 1987, 107Y110.

[20] Sheorey DS, Dorle AK, Release kinetics & drugs from rosin-glycerol ester microcapsules prepared by solvent evaporation technique, J Microencapsul, 8, 1991, 243Y246.

[21] Lakshmana PS, Shirwaikar AA, Shirwaikar A, Kumar A, Formulation and evaluation of sustained release microspheres of rosin containing aceclofenac, 50(2), 2009, 51-62.

[22] Lu L, Garcia CA, Mikos AG. *in vitro* degradation of thin poly (DL-lactic-co-glycolic acid) films. J Biomed Mater Res. 1999; 46: 236-244.

[23] Suggs LJ, Krishnan RS, Garcia CA, Peter SJ, Anderson JM, Mikos AG. *in vitro* and *in vivo* degradation of poly (propylene fumarate-co-ethylene glycol) hydrogels. J Biomed Mater Res. 1998; 42: 312-320.

[24] Info from http://www.criver.com/products-services/basic-research/find-a-model/wistar-rat

[25] Schakenraad JM, Nieuwenhuis P, Molenaar I, Helder J, Dijkstra PJ, Feijen J. *in vivo* and *in vitro* degradation of glycine/DL-lactic acid copolymers. J Biomed Mater Res. 1989; 23: 1271-1288.

[26] Gogolewski S, Jovanovic M, Perren SM, Dillon JG, Hughes MK. Tissue response and *in vivo* degradation of selected polyhydroxyacids: polylactides (PLA), poly (3-hydroxybutyrate) (PHB) and poly(3- hydroxybutyrate-cp-3-hydroxyvalerate) (PHB/VA). J Biomed Mater Res. 1993; 27: 1135-1148.

[27] Prashant M. Satturwar, 1 Suniket V. Fulzele, 1 and Avinash K. Dorle 1, Biodegradation and *in vivo* Biocompatibility of Rosin: a Natural Film-Forming Polymer, AAPS PharmSciTech 2003; 4 (4) Article 55 (http://www.aapspharmscitech.org), pp 1-6

[28] Nande, V.S.; Barabde, U.V.; Morkhade, D.M.; Patil, A.T.; Joshi, S.B. Synthesis and characterization of PEGylated derivatives of rosin for sustained drug delivery. Reactive Funct. Polym. 2006, 66, 1373-1383.

[29] Fulzele, S.V.; Satturwar, P.M.; Dorle, A.K. Polymerized rosin: novel film forming polymer for drug delivery. Int. J. Pharm. 2002, 249, 175-184.

[30] B. S. Kaith, Rajeev Jindal and Rachna Sharma, Synthesis of a Gum rosin alcohol-poly(acrylamide) based adsorbent and its application in removal of malachite green dye from waste water, RSC Adv., 2015, 5, 43092–43104, DOI: 10.1039/c5ra04256a

[31] Y. Zheng, K. Yao, J. Lee, D. Chandler, J. Wang, C. Wang, F. Chu and C. Tang, Macromolecules, 2010, 43, 5922–5924.