Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 12 (7): 693-697, 2012
ISSN 1812-5654 / DOI: 10.3923/jas.2012.693.697

Evaluation of *Stryphnodendron* sp. Release Using Natural Rubber Latex Membrane as Carrier

[1]Karoline M. Romeira, [2]Bruno C. Drago, [1]Heitor D. Murbach, [1]Patricia B. Aielo,
[1]Regildo M.G. da Silva, [3]Carlos A. Brunello and [1]Rondinelli D. Herculano
[1]Departamento de Ciências Biológicas, FCLAs-UNESP. Av. Dom Antonio 2100,
CEP: 19806-900, Assis, Brazil
[2]Departamento de Engenharia de Materiais, UFSCar. Rodovia Washington Luís (SP-310),
Km 235, CEP: 13565-905, São Carlos, Brazil
[3]Departamento de Física, FFCLRP-USP. Av. Bandeirantes 3900, CEP: 14040-901, Ribeirão Preto, Brazil

Abstract: Natural rubber latex from *Hevea brasiliensis* has interesting characteristics related to this work such as: it is easy to manipulate, low cost, can stimulate the natural angiogenesis, is a biocompatible material and presents high mechanical resistance. The aim of this study was to develop a novel sustained delivery system for *Stryphnodendron* sp. based on Natural Rubber Latex (NRL) membranes and to study the *Stryphnodendron* sp. delivery system behavior. *Stryphnodendron* sp., commonly known as barbatimão is extensively used in folk medicine for the treatment of diarrhoea, gynaecological problems and for healing wounds. The stem bark of this species is mentioned in the Brazilian Pharmacopeia with a content of at least 20% of tannins. Previous studies showed significant cicatrizant properties, anti-inflammatory activity and gastric anti-ulcerogenic effects for the stem bark crude extract. One possible way to accelerate the tissue repair process, it was incorporated the *Stryphnodendron* sp. extract in NRL membranes. Stryphnodendron sp extract was incorporated into the NRL, by mixing it in solution for in vitro protein delivery experiments. Results show that the NRL membrane can release *Stryphnodendron* sp. for up to 49.89% of its *Stryphnodendron* sp. content for up 400 h. The kinetics of the extract release could be fitted with double exponential function, with two characteristic times of 0.78 and 133.22 h. In this study, we demonstrated that the induced angiogenesis provided by NRL membranes combined with a controlled release of extract is relevant for biomedical applications.

Key words: *Stryphnodendron* sp., biomaterials, natural rubber latex, drug delivery system, barbatimão

INTRODUCTION

The controlled release of drugs/extracts can be efficient if a suitable encapsulation procedure is developed, which requires biocompatible materials to hold and release the drug/extracts. In this study, NRL membrane is used to deliver Stryphnodendron sp extract. Latex membrane from Hevea brasiliensis is an important inductor of wound healing, regeneration of organism. (Ereno *et al*., 2010; Sampaio *et al*., 2010; Herculano *et al*., 2011a; Ciapetti *et al*., 1994; Mendonca and Coutinho-Netto, 2009). In addition, the treatment of diabetic and phlebopathic ulcers with the biomembrane prepared from the natural latex showed the presence of a vascular growth factor in the latex (Balabanian *et al*., 2006; Mendonca and Coutinho-Netto, 2009; Ferreira *et al*., 2009; Frade *et al*., 2001; Mrue *et al*., 2004). *Stryphnodendron* sp., commonly known as barbatimão, is extensively used in folk medicine for the treatment of diarrhoea, gynaecological problems and for healing wounds. The stem bark of this plant contains a considerable amount of tannin (10-37%) and prominent presence of several flavan-3-ols, proanthocyanidins and prorobinetinidins (Lopes *et al*., 2005; Lima *et al*., 1998; Holetz *et al*., 2005; Santos *et al*., 2002). The crude extract, in the form of decoction or infusion, is traditionally used by the local population for the treatment of leucorrhoea and diarrhea, as well as an anti-inflammatory and healing agent (Di Mambro *et al*., 2005; Castro *et al*., 2009; Eurides *et al*., 1995). Some studies of the crude extract from the stem bark of *Stryphnodendron adstringens* have revealed significant anti-inflammatory activity and gastric anti-ulcerogenic effects. It has also been observed that a decoction of the stem bark of *Stryphnodendron* sp. accelerates the healing of wounds and decreases inflammation (Minatel *et al*., 2010). One possible way to accelerate the tissue repair

Corresponding Author: Rondinelli D. Herculano, Departamento de Ciências Biológicas, FCLAs-UNESP. Av. Dom Antonio 2100, CEP: 19806-900, Assis, Brazil

process, it was incorporated the *Stryphnodendron* sp. extract in NRL. *Stryphnodendron* sp. extract was incorporated into the NRL, by mixing it in solution for *in vitro* protein delivery experiments. NRL membranes have already been used to deliver drugs (Herculano *et al*., 2006, 2007, 2009, 2010).

In this study, we propose a novel release system based on the encapsulation of *Stryphnodendron* sp. in NRL membrane for the sustained and controlled delivery of *Stryphnodendron* sp. for future applications in medicine as wound healing. Results show that the NRL membrane can release *Stryphnodendron* sp. for up to 400 h, which is relevant for biomedical applications.

MATERIALS AND METHODS

The NRL used in the present study was commercial high-ammonia natural rubber latex (ESALQ-USP, Piracicaba, Brazil) of about 60% dry rubber content (DRC), 4-5% weight of non-rubber constituents such as protein, lipids, carbohydrates and sugar and 35% of water (Hashim and Subramaniam, 1986; Yip *et al*., 1994; Yip and Sussman, 2000). After extraction, ammonia was used to keep the latex liquid. The deproteinization of natural rubber latex was performed by centrifugation at 8,000 g. The cream fraction after centrifugation was re-dispersed to make 60 wt.% of dry rubber content latex and was washed twice by centrifugation to prepare the deproteinized natural rubber latex and then reduce the cytotoxic protein content on NRL. *Stryphnodendron* sp., commonly known as barbatimão were collected in March 2011 at Paranapanema Valley region, (Sao Paulo State, Brazil). The barks extract (20 g) was extracted by stirring in an Erlenmeyer at room temperature with acetone/water 7:3 (4×100 mL). These extract was evaporated under vacuum to 30% volume and filtered to remove fats and then freeze-dried. Extract of *Stryphnodendron* sp. was incorporated by mixing 2 mL of natural rubber latex with 2 mL of extract solution (10 mg mL^{-1}). These membranes were prepared by pouring the latex+extract solution in a stainless steel plate with 5.00±0.05 cm diameter and 200±5.00 μm thick. Typically the membranes were left for 2 days to fully polymerize before use. For the study of the release of extract, latex membrane was placed in 200 mL of an aqueous solution, from which aliquots were collected during an interval ranging from 10 to 24,000 min. The extract released into the solution was monitored by measuring the UV-VIS spectra with a BEL ENGINEERING SF 200 ADV spectrophotometer, as *Stryphnodendron* sp. extract has a maximum absorption at 297 nm.

RESULTS AND DISCUSSION

The goal of controlled-release delivery systems is to provide desirable delivery patterns so that predictable plasma drug/extract levels can be achieved, which requires the characterization of the basic pharmacodynamic and pharmacokinetic properties of a drug/extract. For *Stryphnodendron* sp. studied here, it has been found that it absorbs at 297 nm. In Fig. 1 shows the absorbance intensity as a function of *Stryphnodendron* sp. concentration in solution.

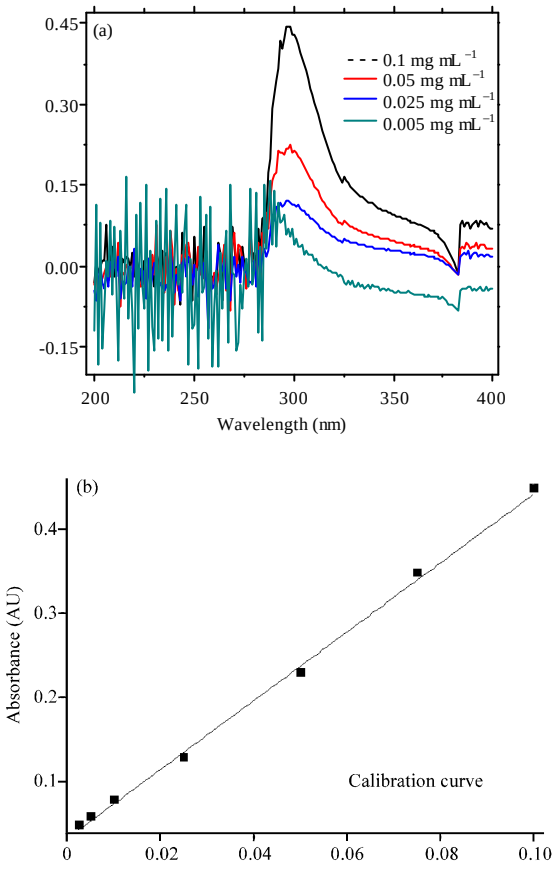

Fig. 1(a-b): Absorbance intensity as a function of *Stryphnodendron* sp. concentration in solution

The sustained delivery system for *Stryphnodendron* sp. has been successfully developed based on NRL. The release profile for *Stryphnodendron* sp. in a NRL matrix in Fig. 2 shows that the first, fast step of burst release corresponded to the *Stryphnodendron* sp. near or on the surface of the NRL membrane (Herculano *et al*., 2011b). The slower release process would be associated with *Stryphnodendron* sp. diffusing slowly through the matrix. Results demonstrated that the NRL membrane can release *Stryphnodendron* sp. for 15 days.

The experimental data in Fig. 2 were fitted using a bi-exponential function $y(t) = y_0+A_1e^{-t/\tau1}+A_2e^{-t/\tau2}$, where y(t) was the amount of *Stryphnodendron* sp. in the NRL at a

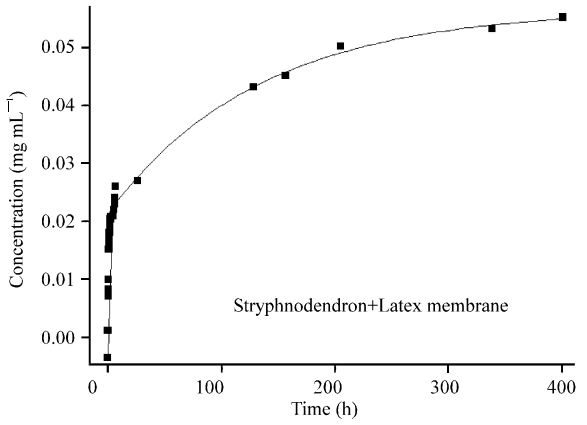

Fig. 2: *Stryphnodendron* sp. release as a function of time for the NRL membrane

given time, t, y_0 is the initial content of *Stryphnodendron* sp., A_1 and A_2 are constants equal to -0.030 and -0.035, respectively, the characteristic times are $\tau_1 = 0.78$ h and $\tau_2 = 133.22$ h. Upon integrating the curve until 400 h, the total amount of *Stryphnodendron* sp. released by the membrane in the 200 mL aqueous solution was 17.95 mg (44,89%).

As mentioned earlier the controlled release of drugs/extracts are of interest for medical applications, since the dose can be adjusted according to the necessity of the patient (Shilpa *et al*., 2003; Kim *et al*., 2008; Sirianni *et al*., 2010; Malcolm *et al*., 2004; Rahimi *et al*., 2010) . In this work, it was used the method proposed by Langer and Folkman (1976), that is, to mix the drug/extract (*Stryphnodendron* sp.) with the polymer (latex) in a colloidal state, in order to create a membrane that works as a delivery system.

Several procedures are used to control the release of substances by polymers. For example Woo *et al*. (2001) used a combination of 3 different biodegradable microspheres of poly (D,L-lactide-co-glycolide (PLGA)), using different molecular weight and terminal endings of the polymeric chain (hydrophilic or hydrophobic) to determine the best delivery system of BMP. They conclude that the best bone healing results were achieved using high dose and slow delivery rate systems.

Already Lobler *et al*. (2000) developed a device based in polyhydroxyalkanoates (PHA) for implantation of a glaucoma drainage system. In this study, polyhydroxyalkanoates (PHA) based on hydroxybutyric acid were tested in terms of their potential suitability to manufacture mechanically stable tube components of drug delivery drainage systems and in terms of biocompatibility.

Wang *et al*. (2005) prepared uniform-sized chitosan microspheres by membrane emulsification technique. Uniform chitosan microspheres were further used as a carrier of protein drug (BSA). They observed that BSA loading efficiency was highest when pH value was 8.09 and it decreased with an increase of the crosslinking degree.

Herculano *et al*. (2011b) proposed a drug release system based on NRL for the sustained and controlled delivery of metronidazole (MET). They concluded that the release time of MET in *in vitro* tests was very promising for the kinetics of release.

Our results indicate that with very simple changes in preparation of NRL membrane, we could control *Stryphnodendron* sp. release up to 15 days, according to Woo *et al*. (2001), Wang *et al*. (2005), Herculano *et al*. (2011b), Malcolm *et al*. (2004) and Langer and Folkman (1976) results, or in other words with a slow release rate.

CONCLUSION

We have prepared natural rubber latex membranes containing *Stryphnodendron* sp. as a model system for tissue regeneration. The method of preparation is reproducible and the natural rubber latex membrane is very stable. Results demonstrated that the NRL membrane can release *Stryphnodendron* sp. for 15 days, thus making them promising materials for protein/extract release in *in vivo* applications. We have observed that 44.89% of the initial *Stryphnodendron* sp. content inside NRL was released in 400 h. Our results indicate that NRL could be used in the future as an active membrane that could accelerate tissue healing.

ACKNOWLEDGMENTS

This work was supported by PROPe/UNESP and FAPESP (Processes: 2011/17411-8, 2011/06856-9).

REFERENCES

Balabanian, C.A.C.A., J. Coutinho-Netto, T.L. Lamano-Carvalho, S.A. Lacerda and L.G. Brentegani, 2006. Biocompatibility of natural latex implanted into dental alveolus of rats. J. Oral Sci., 48: 201-205.

Castro, A.H.F., R. Paiva, A.A. de Alvarenga and S.M.M. Vitor, 2009. Callogenesis and contents of total phenols and tannins in barbatimao [*Stryphnodendron adsrtingens* (Mart.) Coville]. Cienc. Agrotec., 33: 385-390.

Ciapetti, G., S. Stea, A. Pizzoferrato, L. Checchi and G.A. Pelliccioni, 1994. A latex membrane, as an alternative device in the GTR technique: Preliminary report on its biocompatibility. J. Mater. Sci. Mater. Med., 5: 647-650.

Di Mambro, V.M., F.D. Marquele and M.J.V. Fonseca, 2005. Methodology for evaluating *in vitro* of antioxidant action in antiageing topical formulations. Cosmet. Toiletries, 17: 74-78.

Ereno, C., S.A. Guimaraes, S. Pasetto, R.D. Herculano and C.P. Silva *et al*., 2010. Latex use as an occlusive membrane for guided bone regeneration. J. Biomed. Mat. Res., 95: 932-939.

Eurides, D., A. Mazzanti, M.E. Belleti, L.A.F. da Silva and M.C.S. Fioravante *et al*., 1995. Morphological and morphometrical studies on tissue repair of mice cutaneous wounds treated with barbatiman aqueous solution (*Stryphynodendron barbatiman* Martius). Uruguaiana, 2/3: 30-40.

Ferreira, M., R.J. Mendonca, J. Coutinho-Netto and M. Mulato, 2009. Angiogenic properties of natural rubber latex biomembranes and the serum fraction of *Hevea brasiliensis*. Braz. J. Phys., 39: 564-569.

Frade, M.A.C., R.V. Valverde, R.V.C. de Assis, J. Coutinho-Netto and N.T. Foss, 2001. Chronic phlebopathic cutaneous ulcer: A therapeutic proposal. Int. J. Dermatol., 40: 238-240.

Hashim, H. and A. Subramaniam, 1986. Composition of lipids in latex of *Hevea brasiliensis* clone RRIM 501. J. Nat. Rubber Res., 1: 30-40.

Herculano, R.D., C.A. Brunello and C.F.O. Graeff, 2006. Solid state nitric oxide sensor using a latex rubber matrix. Macrolomecular Symp., 245-246: 529-532.

Herculano, R.D., C.A. Brunello and C.F.O. Graeff, 2007. Optimization of a novel nitric oxide sensor using a latex rubber matrix. J. Applied Sci., 7: 3801-3805.

Herculano, R.D., C.P. Silva, C. Ereno, S.A. Catanzaro-Guimaraes, A. Kinoshita and C.F. de Oliveira Graeff, 2009. Natural rubber latex used as drug delivery system in Guided Bone Regeneration (GBR). Mater. Res., 12: 253-256.

Herculano, R.D., S.A.C. Guimaraes, G.C. Belmonte, M.A.H. Duarte, O.N. Oliveira Jr., A. Kinoshita and C.F. de Oliveira Graeff, 2010. Metronidazole release using natural rubber latex as matrix. Mat. Res., 13: 57-61.

Herculano, R.D., A.A.A. de Queiroz, A. Kinoshita, O.N. Oliveira Jr. and C.F.O. Graeff, 2011a. On the release of metronidazole from natural rubber latex membranes. Mat. Sci. Engin., 31: 272-275.

Herculano, R.D., L.C. Tzu, C.P. Silva, C.A. Brunello, A.A.A. de Queiroz, A. Kinoshita and C.F.O. Graeff, 2011b. Nitric oxide release using natural rubber latex as matrix. Mater. Res., 14: 355-359.

Holetz, F.B., T. Ueda-Nakamura, B.P.D. Filho, J.C.P. de Mello, J.A. Morgado-Diaz, C.E.M. de Toledo and C.V. Nakamura, 2005. Biological effects of extracts obtained from *Stryphnodendron adstringens* on *Herpetomonas samuelpessoai*. Memorias Inst. Oswaldo Cruz, 100: 397-401.

Kim, S., S.S. Kim, S.H. Lee, S.E. Ahn and S.J. Gwak*et al*., 2008. *In vivo* bone formation from human embryonic stem cell-derived osteogenic cells in poly(d,l-lactic-co-glycolic acid)/hydroxyapatite composite scaffolds. Biomaterials, 29: 1043-1053.

Langer, R. and J. Folkman, 1976. Polymers for the sustained release of proteins and other macromolecules. Nature, 263: 797-800.

Lima, J.C.S., D.T.O. Martins and P.T. de Souza, 1998. Experimental evaluation of stem bark of *Stryphnodendron adstringens* (Mart.) Coville for antiinflammatory activity. Phytother. Res., 12: 218-220.

Lobler, M., K. Sternberg, O. Stachs, R. Allemann and N. Grabow *et al*., 2000. Polymers and drugs suitable for the development of a drug delivery drainage system in glaucoma surgery. J. Biomed. Mater. Res. Part B, 97B: 388-395.

Lopes, G.C., A.C.C. Sanches, C.V. Nakamura, B.P.D. Filho, L. Hernandes, J.C.P. de Mello, 2005. Influence of extracts of *Stryphnodendron polyphyllum* Mart. and *Stryphnodendron obovatum* Benth. on the cicatrisation of cutaneous wounds in rats. J. Ethnopharmacol., 99: 265-272.

Malcolm, R.K., S.D. McCullagh, A.D. Woolfson, S.P. Gorman, D.S. Jones and J. Cuddy, 2004. Controlled release of a model antibacterial drug from a novel self-lubricating silicone biomaterial. J. Controlled Release, 97: 313-320.

Mendonca, R.J. and J. Coutinho-Netto, 2009. Cellular aspects of wound healing. An. Bras. Dermatol., 84: 257-262.

Minatel, D.G., A.M.S. Pereira, T.M. Chiaratti, L.O. Pasqualin and J.C.N. Couto *et al*., 2010. Clinical study for the validation of the efficacy of ointment containing barbatimao (*Stryphnodendron adstringens* (Mart.) Coville)* on healing of decubitus ulcers. Rev. Bras. Med., 67: 250-256.

Mrue, F., J. Coutinho-Netto, R. Ceneviva, J.J. Lachat, J.A. Thomazini and H. Tambelini, 2004. Evaluation of the biocompatibility of a new biomembrane. Mater. Res., 7: 277-283.

Rahimi, M., A. Wadajkar, K. Subramanian, M. Yousef, W. Cui, J.T. Hsieh and K. Nguyen, 2010. Nguyen. In vitro evaluation of novel polymer-coated magnetic nanoparticles for controlled drug delivery. Nanomed. Nanotechnol. Biol. Med., 6: 672-680.

Sampaio, R.B., R.J. Mendonca, A.R. Simioni, R.A. Costa and R.C. Siqueira, 2010. Rabbit retinal neovascularization induced by latex angiogenic-derived fraction: An experimental model. Curr. Eye Res., 35: 56-62.

Santos, S.C., W.F. Costa, J.P. Ribeiro, D.O. Guimaraes, P.H. Ferri, H.D. Ferreira and J.C. Seraphin, 2002. Tannin composition of barbatimao species. Fitoterapia, 73: 292-299.

Shilpa, A., S.S. Agrawal and A.R. Ray, 2003. Controlled delivery of drugs from alginate matrix, 2003. J. Macromol. Sci., Part C Polym. Rev., 43: 187-221.

Sirianni, R.W., E.H. Jang, K.M. Miller and W.M. Saltzman, 2010. Parameter estimation methodology in amodel of hydrophobic drugrelease from a polymer coating. J. Controlled Release, 142: 474-482.

Wang, L.Y., G.H. Ma and Z.G. Su, 2005. Preparation of uniform sized chitosan microspheres by membrane emulsification technique and application as a carrier of protein drug. J. Controlled Release, 106: 62-75.

Woo, B.H., B.F. Fink, R. Page, J.A. Schrier and J.Y. Woo *et al*., 2001. Enhancement of bone growth by sustained delivery of recombinant human bone morphogenetic protein-2 in a polymeric matrix. Pharm. Res., 18: 1747-1753.

Yip, E. and G. Sussman, 2000. Allergenicity of latex gloves with reference to latex protein sensitive individuals in a Canadian population. J. Rubber Res., 3: 129-141.

Yip, E., K. Turjanmaa, K.P. Ng and K.L. Mok, 1994. Allergic responses and levels of extractable proteins in NR latex gloves and dry rubber products. J. Nat. Rubber Res., 9: 79-86.