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RESEARCH ARTICLE

NANO ENCAPSULATION OF BERBERINE AND COLLAGEN 1

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ABSTRACT

Berberine has long been used as a traditional medicine because of its potential activity against Bacterial, fungal, viral diseases. Berberine have a complex and diverse chemical structure provide a base for different biological targets. The present study deals with the novel encapsulation of biopolymer called Collagen1 with berberine by Electrospinning method in different compositions, parameters, and method for preparation. By electrospinning not only we get desired mechanical and biological properties of encapsulation studies, but also used to combine these two different materials with wide range of drug delivery science and tissue engineering properties to produce electrospun nanofibres at different compositions. The physical and thermal properties of electro spun nanofibres interaction and were examines under FE-SEM. resultant studies may be used in variety of applications including Chemical, Genetical, Immunological and industrial purposes.

Key words: Nano encapsulation, Berberine, Collagen

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INTRODUCTION

Encapsulation studies were mainly useful for tissue engineering (Dechwisissakul, 2000) and meeting a wide range of applications in Biotechnology, Environmental, and Medicine fields (Ganesan, 2015). Mainly these studies made possible in nano particles engineering, which enhances the site specific drug delivery system and improves the pharmaco kinetics properties to drug (Namba, 1985). There are many methods to perform encapsulation studies, in this study we discuss abut electrospinning method. Electro spinning is a method which uses electrical energy as a source and fabricate fibres in different diameter ranges from microns to nano meters. Berberine is a plant alkaloid with many biological activities (Hattori, 1995). Preclinical *in vitro* and *in vivo* studies carry diverse pharmacological actions of berberine that could be potentially helpful in the management of infectious, anti-bacterial anti-inflamatory and metabolic diseases (Ray, 1976). To study more about we conducted the study in nanotechnology by infusing berberine in collagen (Rungsimakan, 2001) for drug delivery experiments for that we will get to know about encapsulation nature of drug. The purpose of this study is to identify the potential of drug by using electrospinning (Chang, 1964) at different compositions of berberine infused collagen.

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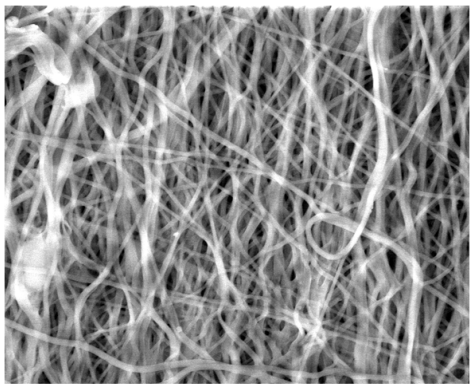
MATERIALS AND METHODS

Collagen cross linking

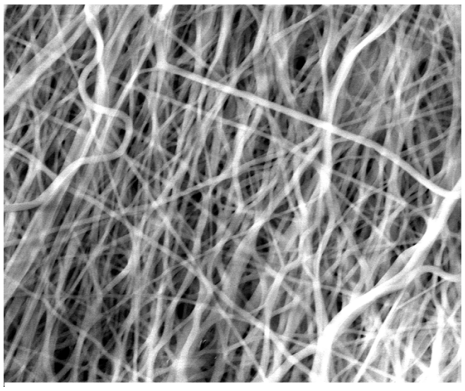
The collagen 1 was cross-linked (Lim, 1980) by dealing with glutaraldehyde vapor, soaked with 20% glutaraldehyde solution at room temperature for different time period (Löhr,, 1999), followed by treatment with 0.2M glycine aqueous solution to lump un reacted Amino acids (Löhr, 2001).

Preparation of solutions: Extracted (Lohr, 2003) collagen type I and the proteins were dissolved in HCL for 8 hours at room temperature. Whereas the berberine solution (Murua, 2008) was prepared by dissolving 50 mg of berberine in 2ml of methanol and then the emulsify (Sakai, 2005) it with 1% Pva solution and run in cyclo mixture for 2 hours to homogenise the solution. The mixture of berberine and collagen solutions were prepared (Cellesi, 2004) in the weight ratio of 1:2, 1:1, 2:1 for encapsulation.

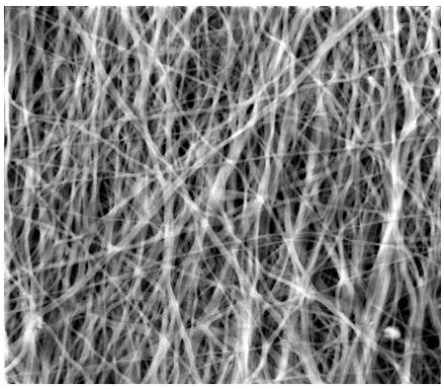
Parametres: The electrospinnig instrument can be used for various solutions (Govan, 1981), but every solution has its own properties. To prepare the encapsulation (Otterlei, 1991) mainly we need ,Drum rotation speed, Syringe translation speed, Solution flow rate, Voltage required the following table has listed the parameters we used for encapsulation (Espevik, 1993). Preliminary experiments revealed that, independent of the conditions used, continuous fibres could not be spun from acidic aqueous solutions of pure collagen (Soon-Shiong, 1991).



SEM Image of 2 parts of Berbenine and 1 part of collagen



SEM Image of 1 part collagen and 1 part berberine



SEM image of 2 part of collagen and 1part of berberine

It is known that by addition of sodium chloride to the solution, formation of fibres by electrospinning becomes possible due to the increase in solution conductivity . Moreover, the presence of NaCl can induce hydrophobic interactions (Clayton, 1991) in or between the protein molecules and thus contribute to the production of continuous fibres The higher net charge increases the force exerted on the jet and at a concentration of 42.5mM NaCl and spinning voltages between 10 and 25 kV, fibre formation was observed. However, continuous jets could not be produced and only short beaded fibres were obtained. This latter phenomenon is known to be favoured by the presence of a high electric field, which leads to capillary breakup of the electrospinning jet (Orive, 2006). Increasing the viscosity of the spinning solution is a way to overcome the formation of beads and a suitable polymer for this purpose we use PVA (De Vos, 1997). Addition of this polymer to the protein solution, which also contained 42.5mM NaCl, allowed a much better control over fibre formation. The voltages necessary to obtain a continuous fibre (De Vos, 1999) were dependent on the weight ratio of collagen to Berberine. Collagen and berberine solutions (Dusseault, 2006) (2% w/v) containing 42.5mM sodium chloride, having a collagen and berberine weight ratio of 1:2 and spun at 21 kV, afforded the formation of a continuous jet (Tam, 2006). However, the collected fibres were not completely dry and resulted in meshes of highly fused fibres. Increasing the weight ratio between collagen and Berberine (King, 2003) to 1:1 or 2:1 required higher potentials of 22 and 23.5 kV, respectively. However, at a collagen and berberine weight ratio of 1:2, beaded and highly fused fibres were obtained. Obviously, under these conditions (Strand, 2003) the water evaporation from the fibres was not complete. Dry fibres entirely devoid of beads and with a narrow diameter distribution (Boontheekul, 2008) (average fibre diameter ¼ 0.4070.05 mm) were produced at a weight ratio of collagen and berberine equal to 1:1.

Electrospinning: The electrospinning method consists (Orive, 2003) a syringe needle, electrode, stainless sheet paper on drum and electric supply. 5ml syringe filled with polymer solution which is linked to syringe pump. Solutions (Strand, 2001) will be hard pressed through tube on a rotating drum which is covered by stainless steel paper. The needle (distance between needle and rotate drum should be 6cm to collect the fibres) was associated (Calafiore, 1999) to high voltage supply (Maximum volts 40 kv and the experiment has to be carried at room temperature). The solution (Wang, 1997) will get a positive voltage which is 30 kv and the fibres were collected (Haque, 2005) on steel paper with the speed of 0.5 ml/hour

SEM

The resultant Microscopic images of different composition (Green, 2005) of polymer encapsulation (Chen, 2007) has viewed under FESEM-EXT-501 microscope.

RESULTS

Different compositions of Berberien and collagen mixtures (2:2, 1:1, 1:2) were performed to produce fibres from soluble berberine. As with collagen solutions, addition of NaCl at a concentration of 42.5mM and PVA at a concentration of 1% w/v were necessary to obtain a continuous fibres., it was possible to spin fibres at a voltage of 10 kV and a flow rate of 50 ml/ min. These fibres had an average thickness of 0.5 mm, The fibres have a rough surface and appear to be composed of 5–10nm wide filaments, oriented parallel to their longitudinal axis similarly to native elastin fibres As with collagen, the Berberine appear to preserve the ability to self organize into the native structure during fibre formation in the electrospinning process. The fibres were easily produced, but difficult to collect because of substantial splaying. Splaying occurs when the radial forces derived from the electrical charges carried by the jet, overcome the cohesive forces in the jet itself. The single jet divides into many charged jets before reaching the collecting plate. In the arterial wall, collagen and elastin are both present and constitute together with the extracellular matrix and the cells, a fibre-reinforced composite structure of which the mechanical properties are mainly determined by the fibrous network.

The presence of both proteins is necessary to confer the vessel its strength but also its elasticity. In the arterial wall, collagen and Berberine are both present and constitute together with the extracellular matrix and the cells, a fibre-reinforced composite structure of which the mechanical properties are mainly determined by the fibrous network. The presence of Both mixture concentrations is necessary to confer the vessel its strength but also its elasticity Aqueous solutions comprising collagen, Berberine (weight ratios are, 2:1, 1:1, 1:2) and The degree of cross linking was estimated by determining both the denaturation temperature and the residual amount of free amine groups of (non-)cross linked samples. Formation of crosslinks in the collagen/ Berberine spun matrices increased the denaturation temperature of the samples. As a consequence of crosslinking, the amount of free amino groups present in the samples decreased Independent of the weight ratio of collagen to Berberine, the relative percentage of free amino groups left after cross linking of the fibres was decreased to approximately

30% of the original value. Moreover, by means of SEM, it was verified that no NaCl crystals were present at the surface of the fibres after EDC/NHS crosslinking.. Crosslinked collagen/berberine scaffolds with different weight ratios (2:1, 1:1, 1:2,) of the two solutions were formed. In all cases, the formation of a confluent multi-layer of SMC, growing on top of each other was observed by means of histology The possibility to electrospin collagen and berberine solutions into fibres composed of a homogeneous blend of the two solutions can lead to the production of scaffolds with extraordinary properties, completely different from those observed in analogous mixtures of Solutions, for which the separate contributions can always be well distinguished. Evaluation of the specific interactions occurring in or between soluble collagen and soluble berberine and theoretically resulting in formation of collagenous fibrillar structures and aggregation of collagen and berberine might result in a better understanding of the potential of the application of collagen/berberine electrospun scaffolds in different fields, like tissue engineering. Further investigations are actually being performed in this direction.

Conclusion

Electrospinning was used as a successful technique to produce non-woven meshes from aqueous solutions of collagen type I and Berberine. In all cases, the addition of NaCl was necessary to spin homogeneous and continuous fibres. Composition of the solution, net charge density and applied electric field were parameters influencing the morphology of the obtained fibres. Spinning collagen/elastin solutions yielded meshes composed of fibres with diameters ranging from 220 to 600nm. Stable scaffolds were prepared by cross linking with EDC/NHS. After cross linking, scaffolds completely devoid of NaCl were obtained. SMCs were successfully cultured on cross linked scaffolds and a confluent layer of cells was observed after 14 days on the surface of the different meshes. One of the advantages of electrospinning aqueous solution of collagen and Berberine is the formation of scaffolds with high porosity and surface area, two essential requisites for tissue engineering. Electrospinning solutions of the two solutions separately from each other can also give the possibility to produce multilayered scaffolds with controlled morphology and/or mechanical properties. Moreover, in this study, it has been shown that fibres, in which the two solutions cannot be distinguished, can be electrospun from a mixture of collagen and Berberine. This may result in fibres with extraordinary mechanical properties, different from those observed in analogous mixtures of the insoluble solutions. Further investigations are currently being done in this direction.

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