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Preparation and Optimization of Cellulose Nanofibers for Controlled Drug Delivery in Regenerative Endodontics

Meshram Rishikesh K[1*], Hegde Vibha R[1], Daryapurkar A[2], Sathawane N[2]

[1]Department of Conservative Dentistry Endodontics, Swargiya Dadasaheb Kalmegh Smruti Dental College & Hospital, Hingna Nagpur, Maharashtra, India
[2]Department of Basic Sciences, Indian Institute of Information Technology, Butibori, Nagpur Maharashtra, India

ABSTRACT

Nowadays, search for different drug delivery strategies is going on in endodontics to preserve the vitality of stem cells for regeneration. Cellulose, a linear polymer composed of glucose units, is the most abundant of all naturally occurring organic materials. Electrospun Cellulose Nanofibers (ECN) is being used in medical field for targeted drug delivery in cancer patients. ECN is a promising option and can be a great source of constant drug delivery for disinfection and dental stem cell survival in regenerative endodontics. Hence the purpose of this study was to optimize the different electrospinning parameters to prepare cellulose nanofibers for using it as a drug delivery agent in regenerative endodontics. In this study, Cellulose Acetate (CA) nanofibers were electros punned using Tri Fluoroacetic Acid (TFA) by varying the concentrations, voltage and flow rate while keeping the Tip to Collector Distance (TCD) constant. Three different concentrations 9wt%, 11wt% and 13wt% polymer solutions were prepared and tested for NF formation. Field emission scanning electron microscopy (FESEM) was done to find out the percentage of NF. The results of the present study showed that 11% concentration at 26.5 KV and at 0.1ml/hr produces maximum amount of cellulose NF intermingling with each other as compared to 9wt% and 13wt%.

Keywords: Electrospinning, Cellulose, Nanofibers, Drug delivery

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Corresponding author: Meshram Rishikesh K

e-mail✉: dr.rishikm@gmail.com

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INTRODUCTION

Regenerative Endodontic Procedures (REP) can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp dentin complex [1,2]. Disinfection of root canal is an important step in REP [3]. Traditionally, this has been achieved with the use of calcium hydroxide and other different antibiotic formulations as intracranial medicaments. Triple antibiotic paste and Double antibiotic paste have also been recommended for use as medicaments in REP. However, various studies have revealed that the use of these drugs might kill any remaining pulpal cells, including stem or progenitor cells known to be present in dental pulp tissue [4-7]. These drugs even when used at clinically recommended doses can harm the stem cells of apical papillae. Hence, in order to obtain optimum effect of these drugs, a constant rate of delivery to the targeted site is important. This can be made possible by using a drug carrier in Nano form.

Lot of research in medical field on anticancer therapy is focused on target cell therapy which constitutes the use of Nano Fibers (NF). Due to their high surface to volume proportion, drug incorporation and mass transport properties are improved. These nanofibers can be obtained through various techniques which includes phase separation, nanofiber seeding, template synthesis, self-assembly and electrospinning [8-13]. Electrospinning is most resourceful and cost effective technique that can be used to synthesize nanofibers [14]. In this technique, viscoelastic polymer solution is stretched using electrostatic power and nanofibers are obtained [15,16]. This polymer solution can be either natural or synthetic. There are an extensive number of studies available on the use of synthetic polymer sources which includes Polyglycolide (PGA), Poly (L-lactide) (PLA), Poly (Lactic-co-Glycolic Acid) (PLGA)

and Poly (caprolactone) (PCL) [17]. However, some of these materials show increase biodegradable time and are associated with toxic effect. Nowadays, natural polymers also known as biopolymers have gained much attention as a better alternative to overcome some of the limitations posed by synthetic materials. Cellulose, being most abundant in nature, is among the most renewable materials to be targeted for nanomaterials [18,19]. Also, it possesses excellent biodegradability and biocompatibility in biological environments which mimics natural ECMs in design that allow cells to accomplish important tasks like signal transmission, proliferation, differentiation etc20. Hence the purpose of our study was to optimize the different parameters like the concentration of polymer solution, voltage and flow rate in order to obtain good cellulose NF.

MATERIAL AND METHODS

Experimental Material

Cellulose acetate powder 1.95 grams, (Sigma Aldrich Company, Germany), Trifluoroacetic acid 33ml, (Sigma Aldrich Company Germany), distilled water (Recombigen laboratories pvt Ltd, Delhi, India) butter paper, electromagnetic stirrer (MH Enterprises, Hyderabad, India), glass syringe with 0.5mm internal diameter (KF Technology, Life science tools, Italy) 25 gauge needle (Hindustan syringes and medical devices, Ltd, Faridabad, India) aluminum foil, 40 ml glass beakers, glass measuring cylinder (Borosil, Mumbai, India) were taken.

Preparation of Polymer Solution

Three different concentrations of polymer solutions were prepared group I -9wt%, group II- 11wt% and group III-13wt% by dissolving 0.9 grams Cellulose Acetate (CA) powder in 10ml TFA liquid, 0.55 grams cellulose acetate powder in 5ml TFA liquid and 0.65 grams in 5ml TFA liquid respectively. When 0.9 grams CA powder was gradually added to 10 ml TFA and kept on electromagnetic stirrer for first 6 hours, the solution was found to be more viscous (non-flow able) due to very rapid rate of evaporation of acidic liquid. Only 5ml solution was left which was too viscous. Hence, we modified the polymer solution preparation procedure.

Modified CA Polymer Solution Preparation Procedure

A new CA polymer solution was prepared by taking 20ml TFA liquid (5ml evaporation/6 hours) whereas the polymer powder was kept constant in the first group. In group II, to prepare an 11wt% solution, 0.55 grams CA powder was gradually added to 5ml TFA liquid under continuous magnetic stirring for 12 hours. The beaker was tightly sealed with aluminum foil at all times except during adding the powder. 13 wt% solutions was prepared by adding 0.65 grams in 15 ml of TFA liquid (additional 10 ml TFA liquid was taken since no sealing of beaker with aluminum foil). Each of the solution was kept for magnetic stirring at 250 rpm for 12 hours. The CA powder was added in very small increments so that it dissolves completely before adding the next increment.

After 12 hours, the beakers were removed from the magnetic stirrer and the polymer solutions were transferred to the respective glass syringes with internal diameter of 0.7mm labeled as group I, II and III. The final solution remained in the beakers after loss of liquid was 10ml in group I and 5ml and in group II and III. Each glass syringe was then mounted on the stand and locked inside the electrospinning machine (E-spin Nanotech Pvt Ltd, Kanpur, India).

Electrospinning the CA Polymer Solutions

Each of the polymer solutions were subjected to Electrospinning Machine (EM) after mounting the aluminum foil on the collector drum and adjusting the voltage power, flow rate and needle tip to collector distance. The tip of the needle to collector distance was kept constant at 11 cm whereas voltage was varied between 24.5 KV, 25.5 KV and 26.5 KV and flow rate between 0.1ml/hr, 0.2ml/hr and 0.3ml/hr. The speed of the collector drum was adjusted to 1000-1050 rpm and humidity at 29%. Each of the three CA polymer solutions i.e. 9%, 11% and 13% were electrospunned by varying the above parameters and their effect on formation of NF was studied. After deposition of fibers, each of the aluminum foil (9%, 11% and 13%) was removed from the collector drum and kept in electric oven at 50 degree C for 24 hrs. for drying. After air drying, a representative area from each sample group was cutted and the specimen was gold sputtered and examined under field emission scanning electron microscope (FESEM) at 1000X, 10000X 50000X and 100000X magnifications for presence of NF.

RESULTS

Scores were given to each image on the basis of percentage of NF formation as shown in Tables 1 and 2 as well as Figures 1 to 4.

Table 1: Scoring Criteria for NF Quantification.

Range of NF percentage	Score allotted
75-100	F1
50-75	F2
25-50	F3
0-25	F4

Table 2: Comparative Scoring of NF with Respect to Different Parameters.

Flow rate	CA wt%	24.5 KV	25.5 KV	26.5 KV
		Fiber score		
0.1ml/hr	9	F3	F4	F4
	11	F4	F3	F1
	13	F4	F4	F4
0.2ml/hr	9	F3	F4	F4
	11	F2	F4	F4
	13	F4	F4	F4
0.3ml/hr	9	F4	F4	F4
	11	F4	F4	F4
	13	F4	F4	F4

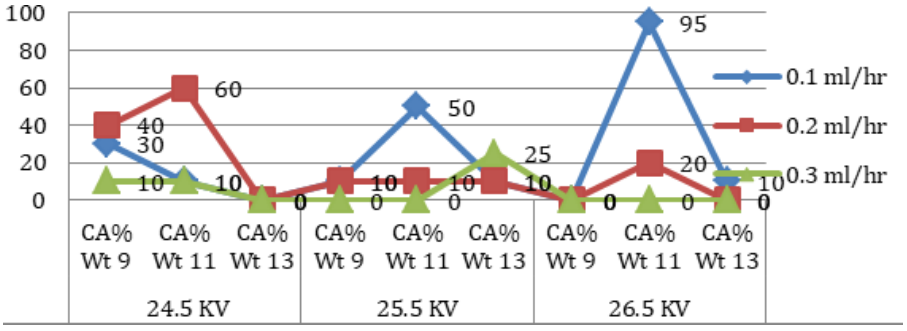

Figure 1: Percentage of NF with respect to different concentrations of polymer solution, voltage and flow rate

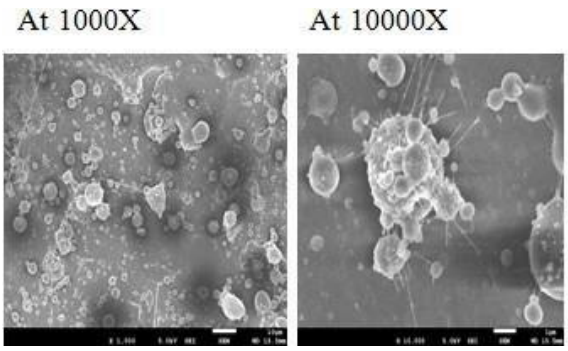

Figure 2: 9wt% CA polymer solution.

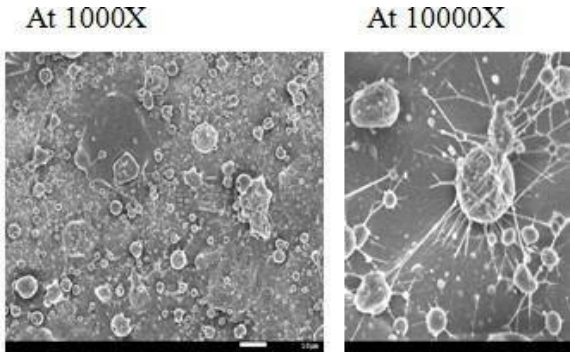

Figure 2: 11 wt% CA polymer solution.

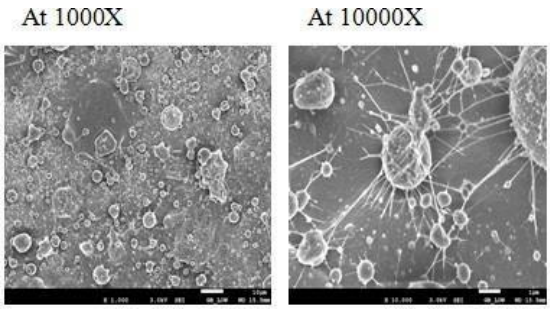

Figure 3: 13 wt% CA polymer solution.

DISCUSSION

Disinfection of the root canal is an important step in regenerative endodontics. To accomplish this, antibiotic mixture was introduced in 2001 in which the intracanal antibiotic paste (i.e., TAP or DAP) has been the most used inter-appointment medicament18. But these drugs, even when used at therapeutic doses have found to have negative effect on endodontic regeneration. To overcome this problem, various studies have been found in which the authors tried to design different drug delivery strategies. These drug delivery systems basically focused on controlling the drug dosage and its rate of release. One of the most effective strategies for this is targeted drug delivery approach which nowadays is more popular in cancer therapy. NF prepared from natural cellulose polymer has also been tried for medical applications as a source of constant drug delivery. But researches on use of cellulose NF as drug delivery agents in endodontics for dental tissue regeneration are lacking. So, in the present study, we tried to optimize the different parameters for preparation of cellulose NF and quantify the amount of NF with respect to the different concentrations of polymer solution.

Previous studies [20] have shown that electrospinning of CA in a combined solvent system produces ultrafine CA fibers as compared to when used alone. Acetone can be used in combination with water, dimethyl sulfoxide, dimethylacetamide, methanol, chloroform and formic acid to produce electrospinned NF. However, in our study since the NF obtained were aimed to be used for carrying drug against stem cells, hence it might be possible that these mixture of solutions can exert toxic effect on stem cells and compromise their survival. In a study conducted by Hashemi-Beni, et al. [21] NF prepared from TFA liquid were evaluated for their effect on stem cells and no cytotoxic effect was noted. Omollo, et al. [22], evaluated TFA for electrospinning of CA at concentrations of 10 wt%, 13wt% and 15wt% and concluded that uniform cellulose NF can be obtained at 13 and 15 wt% of polymer solution and at flow rate of 0.4 ml/hr and voltages of 15-25KV and flow rate of 0.2ml/hr. In our study, very few fibers were obtained at 10, 13 and 15wt% of polymer solution, and voltage of 15-25 KV. This might be attributed to variation in viscosities of the polymer solution due to differences in solution preparation procedure.

In the present study, three different concentrations of CA polymer solutions were prepared, 9 wt%, 11wt% and 13wt%. After going through literature review, it was found that 8 wt% CA polymer solutions produce good fibers. Hence we started first with 8wt% CA solution, but it was more fluid in nature. Then 9wt% sol was planned

and gradually it was increased to 11wt% and 13wt%. 9 wt % means 9 grams of CA powder in 100 ml of TFA liquid. Likewise, to prepare 10ml, 0.9 grams and for 5ml, 4.5 grams CA powder needs to be dissolved. The same protocol was followed to prepare 11% and 13% polymer solution. Initially, when 0.9 gms in 10ml solution was kept for magnetic stirring without sealing the container, very rapid evaporation (5ml) was observed after 6 hrs. Hence, the procedure was modified by taking excess liquid to compensate for the lost amount (5ml/6hrs).

The originality of solution concentration was maintained at 9wt%, 11wt% and 13wt%. In Group I – 10ml liquid had taken whereas in Group II and Group III, 5ml TFA liquid was taken. This was done so in order to study the evaporation effect on the quantity of solution. Since, we want to investigate the effect of taking 10ml liquid as compared to 5ml on the polymer concentration and we found that when 10 ml liquid was taken, thin non intact NF obtained at 26.5 KV and at 0.2 ml/hr flow rate. Out of all the three groups, Group I was less viscous as compared to Group II & Group III. Group I showed good NF (F3:25-50%) at 0.2 ml /hr and at 24.5 KV whereas Group II showed F2 score (50-75%) at the same parameters. Group II had good viscosity and flow as a result of which it showed numerous NF intermingling with each other along with tiny globules. Maximum amount of NF (F1: 75-100%) were obtained with this concentration of polymer solution. Group III i.e. 13% had higher viscosity and could not be able to produce fibers at 0.3 ml/hr flow rate and at all voltages. Only scattered tiny globules were found. The results of the present study also confirms the findings of the study where TFA was used as a solvent and NF were obtained at 15-25KVP. In our study also, predominant NF were obtained but at 26.5 KV and flow rate was 0.1 ml/hr.

CONCLUSION

Within the limitations of the present study, it can be concluded that, CA polymer can be used with TFA liquid for electrospinning at a optimum concentration of 11 wt % with flow rate of 0.1ml/hr and voltage of 26.5 for producing NF. Also, further studies need to be done with respect to addition of suitable drugs to the polymer solution and evaluate its drug release profile for endodontic regeneration.

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