Brazilian Dental Science
Ciência Odontológica Brasileira
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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE
doi: 10.14295/bds.2017.v20i4.1495

Scaffold architecture for dental biomaterials: influence of process parameters on the structural morphology of chitosan electrospun fibers

Arquitetura de arcabouços para biomateriais: influência dos parâmetros do processo na morfologia estrutural de fibras de quitosana eletrofiadas

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ABSTRACT

Objectives: The electrospinning is a widely adopted technique used to produce the polymeric fibers. The process depends on process parameters (voltage, flow rate and distance from capillary to the collector). The present study aimed to evaluate the influence of these parameters on chitosan fibers, a biopolymer used as scaffolds in dental and oral tissue engineering. **Material and Methods:** A solution of 7%(m/w) was prepared dissolving 0.7g of chitosan in 7 mL of trifluoroacetic acid (TFA) and 0.3mL of dichloromethane (DCM) (TFA/DCM - 70:30). After 12 hours, polymer solution (5 mL) was drawn into the syringe and pumped through needles of 0.4 mm internal diameter, at a rate of 0.8 mLh^{-1}, different needle-tip-to-collector distances and voltages, for 10 min. Electrospun fibers micrographies were obtained using the Scanning Electron Microscope for morphological analysis. **Results:**All voltages showed significant difference ($p < 0.0001$) between them. At 15kV fibers showed higher concentration of beads. At 10 and 12 cm of distance there was no statistical significance ($p > 0.0001$) but at 15 cm beads formation increased significantly ($p < 0.0001$). At 12 cm fibers showed lowest fibers diameter in comparison to 10 and 15 cm ($p < 0.0001$). There was no difference between 10 and 12 cm ($p > 0.0001$) but in comparison to 15 cm both distances presented significantly difference ($p < 0.0001$). **Conclusion:** Thus, it can be concluded that morphology, in chitosan electrospun fibers, is influenced by the voltage and distance and this could describe the mohphological control of these structures.

RESUMO

Objetivo: A eletrofiação é uma técnica amplamente adotada para produzir fibras poliméricas. O processo depende dos parâmetros do processo (tensão elétrica, razão de fluxo e distância do capilar ao coletor). O presente estudo teve como objetivo avaliar a influência desses parâmetros sobre fibras de quitosana, um biopolímero utilizado como arcabouçosna engenharia de tecidos dentários and orais. **Material e Métodos:** Uma solução de 7% (m / w) foi preparada dissolvendo 0,7 g de quitosana em 7 mL de ácido trifluoroacético (TFA) e 0,3 mL de diclorometano (DCM) (TFA / DCM - 70:30). Após 12 horas, a solução (5 mL) foi colocada em uma seringa e bombeada através de agulhas de 0,4 mm de diâmetro, sob uma taxa de 0,8 mLh^{-1}, diferentes distâncias e tensões, por 10 min. Micrografias de fibras eletrofiadas foram obtidas usando o Microscópio Eletrônico de Varredura para análise morfológica. **Resultados:** Todas as tensões apresentaram diferença significativa ($p <0{,}0001$) entre elas. Sob 15kV fibras apresentaram maior concentração de grânulos. Sob 10 e 12 cm de distância não houve significância estatística ($p>0{,}0001$), mas sob 15 cm os grânulos aumentaram significativamente ($p <0{,}0001$). Com 12 cm as fibras de quitosana apresentarammenor diâmetro em comparação às de 10 e 15 cm ($p <0{,}0001$). Não houve diferença entre 10 e 12 cm ($p> 0{,}0001$), mas em comparação com 15 cm, ambas as distâncias apresentaram diferença significativa ($p <0{,}0001$). **Conclusão:** Assim, pode-se concluir que a morfologia das fibras eletrofiadas de quitosana é influenciada pela tensão elétrica e distância, descrevendo o controle morfológico dessas estruturas.

KEYWORDS

Chitosan; Fibers; Electrochemical.

PALAVRAS-CHAVE

Quitosana; Fibras; Eletroquímica.

INTRODUCTION

The electrospinning is a widely adopted technique used to produce polymeric nanofibers [1].These materials have different mechanical, electrical and thermal propertiesfunctionality [2], and demonstrate potential in many areas, with important structural characteristics for applications in biological systems [3,4].

These applications are facing the challenges of regenerative strategies that use scaffolds to provide a surface on which cells may adhere, grow, and form an organized arrangement according to tissue complexity, such as dental and oral structures [5,6].

In this sense, chitosan scaffolds appear to be suitable for a variety applications. Chitosan, is a biopolymer used in the medical and pharmaceutical area, obtained from the deacetylation of chitin[7], that is widely studied because of its characteristics, such as antimicrobial activity [8], analgesic effect [9] and biodegradability [7,10,11].

A currently available technique for nanofiber synthesis is the electrospinning [12] that, essentially, consists of a capillary containing polymer solution, a high voltage source and a conductive collector.

The high voltage source is responsible for creating an electric field that will act on the surface of a polymer solution producing liquid jet ejection, which generates solid fibers during the solvent-evaporation process [13,14].

The process depends on parameters of solution (viscosity; superficial tension and conductivity and type of polymer], of the process (voltage, flow rate and distance from capillary to the collector)and environmental (humidity and temperature)[15,16], as can be seen in Figure 1.

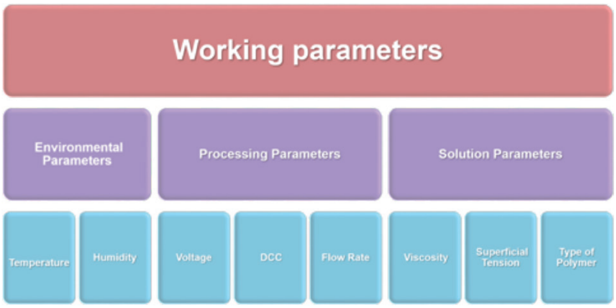

Figure 1 - Working parameters of electrospun nanofibers synthesis

From this, electrospun fibers commonly reported discontinuities, called beads [17], related in some studies to the instabilities of the polymer solution jet [18,19].

Often considered as a defect in fiber morphology, the formation of granules is strongly influenced by several parameters, some already mentioned, such as process and solution parameters [20,21].

Thus, the aim of the present study was to evaluate the influence of electrospinning parameters on chitosanfibers to characterizethe scaffolds architecture for guided tissue regeneration.

MATERIAL AND METHODS

Table 1 - Materials used in the study according to manufacturer's information

Material	Manufacturer	Composition	Lot #
Chitosan	Sigma-Aldrich, Saint Louis, USA	$C_6H_{11}O_4N$	SLBH2747V
Trifluoroacetic Acid	Sigma-Aldrich, Saint Louis, USA	$C_2HF_3O_2$	SHBF6609V
Dichlorome-thane	Sigma-Aldrich, Saint Louis, USA	CH_2Cl_2	STBF0286V

Solution Preparation

A solution of 7%(m/w) was prepared dissolving 0.7g of medium molecular weight chitosan (Sigma-Aldrich) in 7 mL of trifluoroacetic acid (TFA) (Sigma-Aldrich) and 0.3mL of dichloromethane (DCM) (Sigma-Aldrich, Saint Louis, USA) (TFA/DCM - 70:30).

Initially, the chitosan was dissolved gradually as TFA by placing the assembly in a shaker (IKA RH Basic, Staufen, Germany),at room temperature. Then DCM was added gradually.

Electrospinning

After 12 hours, polymer solution (5 mL) was placed in the syringe and pumped through needles of 0.4 mm internal diameter, ata rate of 0.8mLh^{-1}, for 10 min anddifferent needle-tip-to-collector distances and voltages, as shown in Table 2.

Table 2 - Parameters used for electrospinning

Samples		Voltage		
		10 kV	12 kV	15 kV
Distance	10 cm	A	D	G
	12 cm	B	E	H
	15 cm	C	F	I

Analysis Scanning Electron Microscope (SEM)

Electrospun nanofibers micrographies were obtained using the Scanning Electron Microscope with high-vacuum equipment (Inspect S 50, FEI Company, Brno, Czech Republic) operating at 20-25 kV, 5.0 spot and magnifications of 1000, 2000 and 5000x.

Mean diameter and beads formation

Mean diameter and beads formation of nanofibers were analyzed using the ImageJ image analysis software (Version 1.44o, National Public of Health) with micrographies obtained from the SEM, that were divided into 12 frames (Figure 2).

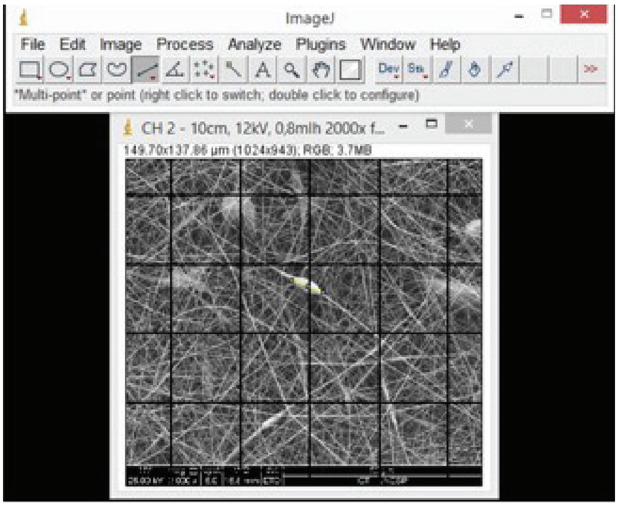

Figure 2 - Image analysis for accounting beads

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to determine working parameters effects on mean diameter and beads formation.

RESULTS

SEM analysis is presented in Figure 3.

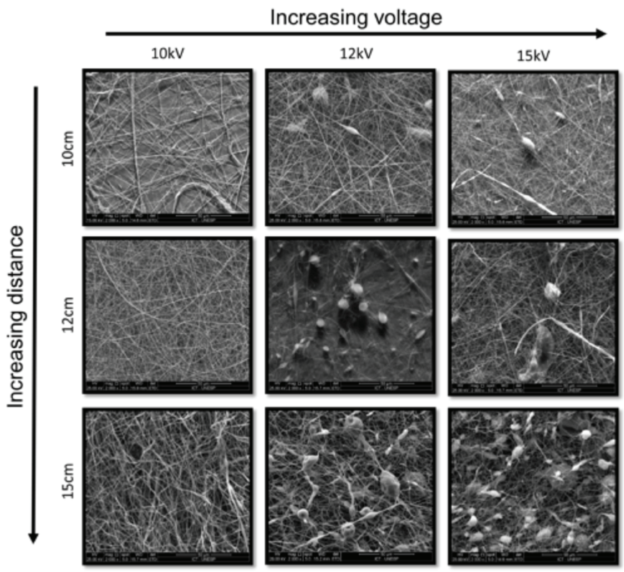

Figure 3 - SEM images of the fibers under different experimental conditions

From this figure, it is clear that there is a tendency to bead formation at higher voltages.

These beads were statistically analyzed as shown on Figure 4.

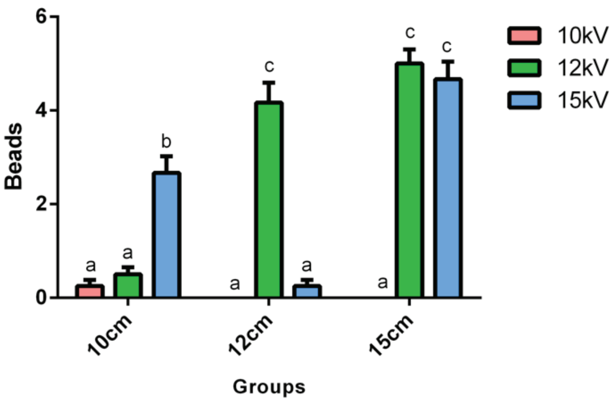

Figure 4 - Bar graph profile showing beads formulations

Voltage presented influence on beads formation. All voltages showed significant difference ($p < 0.0001$) between them. At 15kV fibers showed higher concentration of beads.

At 10 and 12 cm of distance between electrospinning tip and collector there is no statistical significance ($p > 0.0001$) but at 15 cm beads formation increased significantly ($p < 0.0001$).

Fiber diameters measured by image analysis software (mean and standard deviations) and subsequent statistical analysis are presented in Figure 5.

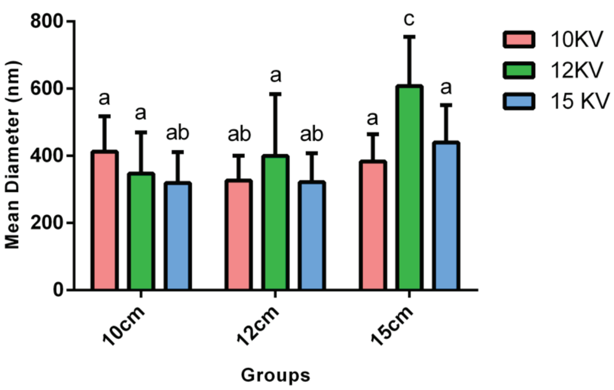

Figure 5 - Mean diameter (with SD) of nanofibers

At 12 cm fibers showed lowest fibers diameter in comparison to 10 and 15 cm ($p < 0.0001$).

Distance influenced the fiber diameter. There is no difference between 10 and 12 cm ($p > 0.0001$) but in comparison to 15 cm both distances presented significantly difference ($p < 0.0001$).

DISCUSSION

Scaffolds for tissue engineering has been employed in intraoral applications such as periodontal and peri-implant regenerative medicine, oral and maxillofacial reconstructive surgery [22-24].

Thus, it is important to characterize the morphology structure of these materials. In this paper, it is shown the influence of electrospinning process parameters on chitosan scaffolds.

There is no consensus regarding theapplied voltages influenceon diameter of electrospun fiber. Some studies showed that electric field has no influence on the diameter fibers[25]. However, several groups suggested that higher voltages can increase the electrostatic repulsive force on the charged jet, favoring the narrowing of fiber diameter[26].

In this study, higher voltages (12 and 15 kV), at 10cm, promoted significant diameter decrease. And, under 12 and 15 cm, respectively, the 12kV presented the lowest diameters.

In this context, the needle-tip-to-collector distance can also affect the fiber diameter [27]. According to some studies, short distances do not promote enough time to fibers solidify before reaching the collector, whereas at long distances, beads formation can be presented[26,28].

In this sense, this study presented results that corroborate with previous studies and showed lowest diameters, for chitosan fibers,at 10 cm and higher beads formation at 15 cm.

The beads formation was observed in the electrospinning process [29]considered as focal defects of electrospun fibers [30]attributed to different causes such as lower surface tension[30], higher voltage [31,32], viscosity solution [33] and polymer concentration [21, 34].Thus, the hypothesis was raised that with the voltage increase there is a form change of the solution surface from which the electrospinning jet originates, resulting alterations of the nanofibers morphology [12].

Also it was observed that the intensity of the electric voltage applied to the electrospinning can change the shape of the Taylor cone at the tip of the syringe, increasing and modifying beads formation[19]. This result agrees with the results of the present study, which found that higher voltages (12 and 15kV] showed a significant increase of beads formation.

This can also related to the flow rate and voltage together. Some study showed that If one of these two parameters is changed alone beads can be formed[35].

Thus, these results suggest that electrospinning parameters influence the scaffold morphology.

CONCLUSION

It can be concluded that electrospinning parameters influenced the structural morphology of chitosan electrospun fibers and this is important for optimization research of oral mucosal regenerative biomaterials. The further biological characterization of chitosan nanofibers will support the development of this promising biomaterial into a successful tissue-engineering scaffold.

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Date submitted: 2017 Oct 23

Accept submission: 2017 Dec 06