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MANUFACTURING AND ALIGNING THE PCL NANOFIBERS ALONG REQUIRED DIRECTIONS BY SWITCHING METHOD OF ELECTROSPINNING VOLTAGE FIELD FOR DESIGNING SCAFFOLDS IN TISSUE ENGINEERING

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ABSTRACT

Nowadays optimization of nanofibers web scaffold has been experienced in various scientific applications like biomedical prosthesis and scaffolds in which the nanofibers orientation and morphology in those of webs are important. Many attempts have been made to arrange nanofibers in horizontal or vertical directions for cell growing of special tissues such as tendon, ligaments, nerves, etc. In this research a novel method has been presented to control and arrangement pattern and orientation of nanofibers in required directions. For this reason a special collector set up was manufactured for electrospinning of nanofibers included control circuit and collector surface of parallel wires. The switching of parallel wires could be controlled by computer and controller circuit and could change arrangement of nanofibers collecting between collector wires. Also simulation software was written to model movement direction of nanofibers according to the field equations of electrospinning and switching pattern of collector wires. The prepared software could predict and model movement direction of nanofiber from nozzle to collector wires for various parameters of electrospinning. It was possible to change electrospinning parameters and find the optimum condition for any desired nanofibers orientation or pattern on collector. In the experimental part the nanofibers was modeled and produced with various switching patterns and the results of nanofibers orientation modeling and SEM images were compared to consider ability of switching set up of collector and reliability of simulation software. It was observed that results of modeling and experiments confirmed together. The presented method can simulate nanofibers web before production to save time and cost of scaffold preparation from electrospinning technique.

KEYWORDS: Orientation, Nanofibers, Electrospinning, Modeling, Simulation, Electric Field

INTRODUCTION

Nanofiber production is one of the important subjects of nanotechnology in a wide range of bio-scaffolds and filtration applications [1, 2]. The most applications of nanofibers webs can be summarized in micro-layer scaffolds for cell growing as artificial tissues and micro–nano porous filters for special membranes [3, 4]. Layers of nanofibers provides some magic features such as high special surface, high porosity, nano-micro canals, light weight and high permeability [5,6]. Electrospinning is the most commonly used and applicable methods for nanofibers production. Although many researchers have been tried to control nanofibers orientation in a unique or spiral directions but electrospinning process is not sufficiently controllable, because the electric field between nozzle and collector is not controllable. The only method for increasing insurances of nanofibers orientation is controlling the electric field through electrospinning spaces. Some of researches have been focused on using interface electrodes between a rotating or parallel collector and nozzle. These methods provided parallel controlled nanofibers web but the exact control of nanofibers distribution was even impossible [7, 8]. For example Ishiet al. used two collector surfaces in parallel location and switching method. They were succeeded to make some drawn patterns of nanofiber but their attempts were failed to make other controlled distributions [9]. The other researchers suggested some rotating discs, rings, and electrodes to achieve different patterns of nanofibers distribution, approximately. Their methods were valuable but they could not present any simulated or controlled models of nanofibers distribution where trial error methods were very time consuming and expensive [10-11]. In this research a novel collector set up with parallel wires has been prepared and the orientation of nanofibers has been adjusted by using switching of wires for electric field control. Also, movement direction of nanofibers has been calculated from electric field equations and modeled by writing special simulation software.

MATERIAL AND EXPERIMENTAL PROCEDURE

In this research, poly-caprolacton (PCL)$\overline{M_n}$ =8000 was used for approving the applicability of presented method for nanofibers distribution control. Whereas PCL is the most commonly applicable polymer in nanofiber scaffolds a huge number of pervious researchers have been used this polymer for scaffolds and successful reports for its electrospinning have been presented in solution of di methylformamide (DMF): di chloromethane (DCM) with Vt% 20:80 and 12 wt% as optimum concentration [12]. In this research this polymer solution was used too. Feed rate of solution was adjusted 0.2 ml/hour in voltage 9 kV. The solution was homogenized by a magnet for 18 to 20 hours. Solution transferred to electrospinning syringe and feeding pump adjusted on 0.2 ml/hour. In this investigation collector was parallel wires which were connected to a controller circuit.

According to Fig. 1the wires collector could be selected by controller circuit from computer and each selected wire was connected to negative terminal of high voltage. The nozzle of electrospinning syringe was connected to positive terminal of high voltage. The direction of electric field could be adjusted by switching pattern of collector wires which was controlled by computer and controller circuit. Several samples of nanofibers web were produced in different switching patterns and times and analyzed by image processing technique of those SEM images and compared to model of movement direction of nanofibers.

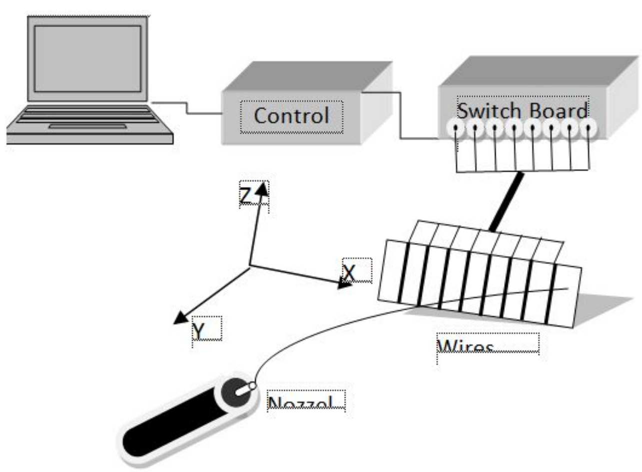

Figure 1: Control Set Up

MODELING OF NANOFIBER MOVEMENT DIRECTION

In each period of switching time, the nanofiber drown and spun from head of nozzle to the wire collector which is connected to negative terminal as a simple dipolar electric field. By this assumption, the movement direction of nanofiber are analyzed from switching time (the time of connecting the wire in negative terminal), throw put mass of solution in period time of switching, impedance and electric on throw put solution, distance between both terminals (nozzle to wire) and distance between wires. In a general acting force equation, $\vec{F}$is the acting force vector to each particle of nanofiber, q is positive charge, $\vec{E}$ is electric filed, $m$the mass of nanofiber particle solution throw put in switching time and$\vec{a}$is acceleration vector of particle. Equation (1) to 6 dives 3D force vector in Cartesian system.

$$\vec{F} = m\vec{a}= m\left[\frac{d^2x}{dt^2}\hat{x} + \frac{d^2y}{dt^2}\hat{y} + \frac{d^2z}{dt^2}\hat{z}\right] \quad (1)$$

$$\vec{F} = \vec{E}.q \quad (2)$$

$$\vec{E}(x,y,z) = E_x(x,y,z)\hat{x} + E_y(x,y,z)\hat{y} + E_z(x,y,z)\hat{z} \quad (3)$$

$$m\frac{d^2x}{dt^2} = qE_x(x,y,z) = F_x \quad (4)$$

$$m\frac{d^2y}{dt^2} = qE_y(x,y,z) = F_y \quad (5)$$

$$m\frac{d^2z}{dt^2} = qE_z(x,y,z) = F_z = 0 \Rightarrow z(t) = V_{0z}t + Z_o \Rightarrow Z = 0 \quad (6)$$

$$F = \frac{-1}{4\pi\varepsilon_0}\frac{q^2}{r^2}\hat{z} \quad (7)$$

Where in equation (7) is:

$$\varepsilon_0 = 8.854187817 \times 10^{-12}\ (\frac{F}{m}) \quad (8)$$

$$q = I.t \quad (9)$$

From electricly relation of electric field on nanofiber particle equations (7) to (9) is presented where $\varepsilon_0$vacuum permittivity is constant. In equation (9) Iis the current of high voltage circuit which is calculated from electric consuming of electrospinning set up or high voltage power supply and t is the delay time of each switching period or the time of connection of selection wire to negative terminal. The mass of particle in switching time (kg/s) is calculated from equation (10), when m' is throwput of syring and m'' is the mass of 1 ml polymeric solution.

$$\begin{cases} m'\left(\frac{ml}{h}\right) \times \frac{1}{3600\,s} = \frac{m'}{3600}\ (\frac{ml}{s}) \\ m''\left(\frac{g}{ml}\right) \times \frac{1}{1000} = \frac{m''}{1000}\ (\frac{kg}{ml}) \end{cases}$$

$$\Rightarrow m = \frac{m'}{3600}\left(\frac{ml}{s}\right) \times \frac{m''}{1000}\left(\frac{kg}{ml}\right) = \frac{m' \times m''}{3600000}\ (\frac{kg}{s}) \quad (10)$$

The acting force of electric field on each nanofiber particle is calculated from equation (11) between head of nozzle to selected wire of collector (for example the first wire) when l and d are horizontal and vertical distance of wire from nozzle respectively as figure 1. As an approximation with assuming constant force, the equation (11) is formed to equation (12) and (13) by combination of equal to equation (4) and equation (5).

$$\overrightarrow{F_1} = \frac{-q^2}{4\pi\varepsilon_0}\frac{(0-l)\hat{x}+(d-0)\hat{y}}{\left(\sqrt{d^2+l^2}\right)^3} \quad (11)$$

$$\overrightarrow{F_1} = \overrightarrow{F_{1x}} + \overrightarrow{F_{1y}} \quad (12)$$

$$\overrightarrow{F_{1x}} = m \times \frac{d^2x}{dt^2} = \frac{-q^2}{4\pi\varepsilon_0}\frac{l}{\left(\sqrt{d^2+l^2}\right)^3} \quad (13)$$

Where "$\overrightarrow{a_x} = \frac{d^2x}{dt^2}$"isassumed as an constant value which is presented as $\propto$.

$$\frac{d^2x}{dt^2} = \propto = \frac{-q^2}{4\pi\varepsilon_0}\frac{l}{\left(\sqrt{d^2+l^2}\right)^3} \times \frac{1}{m} \quad (14)$$

By integration of equation (14), equation (15) is achieved when c_1 is the primary velocity of nanofiber particle.

$$\frac{dx}{dt} = \propto t + c_1 \quad (15)$$

Integration of equation (15) leads to equation (16) which is relation of horizontal movement direction of nanofibers particle.

$$x(t) = \propto \frac{t^2}{2} + c_1 t + c_2 \quad (16)$$

In equation (16) the primary velocity "c_1"is zero and "$c_2 = l$ "is primary horizontal distance or distance of nozzle to wire.

$$x(t) = \propto \frac{t^2}{2} + l \quad (17)$$

These relations are repeated for vertical direction as equation (18) to equation (21).

$$\overrightarrow{F_{1y}} = m \times \frac{d^2y}{dt^2} = \frac{-q^2}{4\pi\varepsilon_0}\frac{d}{\left(\sqrt{d^2+l^2}\right)^3} \quad (18)$$

$$\frac{d^2y}{dt^2} = \propto' = \frac{-q^2}{4\pi\varepsilon_0}\frac{d}{\left(\sqrt{d^2+l^2}\right)^3} \times \frac{1}{m} \quad (19)$$

$$\frac{dy}{dt} = \propto' t + c'_1 \quad (20)$$

$$y(t) = \propto' \frac{t^2}{2} + c'_1 t + c'_2 \quad (21)$$

While"$c'_1 = 0$" is primary velocity and c'_2 is vertical distance of nozzle to wire. For example this distance for the wire located exact in the front of nozzle is zero, and equation (22) calculates time of switching.

$$y(t) = \propto' \frac{t^2}{2} \Rightarrow t^2 = \frac{2y}{\propto'} \Rightarrow t = \sqrt{\frac{2y}{\propto'}} \quad (22)$$

By combined equation (22) with equation (17) the relation of x, y is calculated which is referred to nanofiber particle direction in surface of collector as equation (23).

$$x = \propto \frac{t^2}{2} + l \xrightarrow{t=\sqrt{\frac{2y}{\propto'}}} x = \propto \frac{2y}{2\propto'} + l \Rightarrow x = \left(\frac{\propto}{\propto'}\right) y + l \quad (23)$$

RESULTS AND DISCUSSIONS

In this research the collector was constructed by parallel wires. If all of wires had connected to the ground or negative terminal of high voltage power supply scan random semi-oriented fiber might be spun. The switching of wires in controlled delay time and pattern of switching was considered as simulation model of nanofiber movement direction web in electric fields. To consider and approve the reality of model some samples were prepared by electrospinning in different switching time of 0.2 to 0.5 seconds in hieratical switching pattern. Images of electro spun nanofiber were captured by SEM. After that, SEM images were analyzed by Fourier angular power spectrum in Matlab R2009A software. The orientation distributions of nanofibers in SEM image were sketched from Fourier angular power spectrum as orientation diagrams. From presented diagram the orientation of nanofibers could be traced. All of analyzed SEM image were introduced to the software in similar cubic dimension to ensure FFT analysis.

Figure 2 (a) and (b) show thediagram of angular power spectrum andgraph the movement curvatures of nanofibers between nozzle and collector related to SEM image of PCL nanofiber in figure 2(c)in field switching 0.2 seconds. According to images in figure 2, the switching in 0.2 seconds seems to produce semi-oriented even random nanofibers, obviously. There are several sharp peaks of power spectraum in 48, 126 and 234 degree in Figure 2(a) that show different direction in nanofiber orientation. From equation (23), movement curvatures of nanofibers between nozzle and collector can be modeled according to Figure 2(b). Figure 2(b) presents 2D movement direction of fibers (show of main movement 3D curvature on the collector surface). These curvatures give the morphology of nanofibers on the collector surface, where the curvature is flat in horizontal direction (P) the nanofibers are oriented in horizontal direction. If the nanofibers are oriented in wire direction or vertical form, the curvature is uniform in sharp movement form along vertical direction (M). The first point of the first wire is the point (0, 0) in graphs. Each point of curvature in switching time has been shown by circles. The nozzle was 3 cm from the first wire and five wires had been location in parallel form by 0.5 cm distance together.As a result, In field switching 0.2 second, it is concluded fast switching leads to random orientation of fibers and maybe this situation is suitable for some special tissues like skin scaffolds.

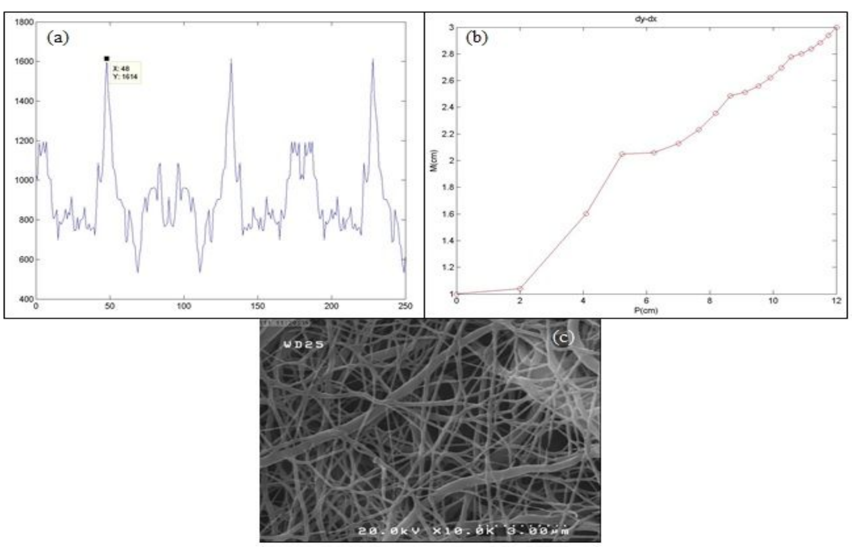

Figure 2: a) Diagram of Angular Power Spectrum,
b) Graph the Movement Curvatures of Nanofibers between Nozzle and Collector and
c) SEM Image of PCL Nanofiber in Field Switching 0.2 second

In Figure 3 the diagram of angular power spectrum, graph the movement curvatures of nanofibers between nozzle and collector and SEM image of PCL nanofiber in field switching 0.3have been shown. In this sample, SEM image presents more oriented fiber but in semi-entangled forms. The orientation is clearly more than field switching 0.2 second in previous sample. Also, diagram of power spectrum presents two peaks in 62 to 118 degree. This observation guides us to have more regularity in parallel nanofibers by increasing switching time which is more suitable in nerve and tendon scaffold applications.

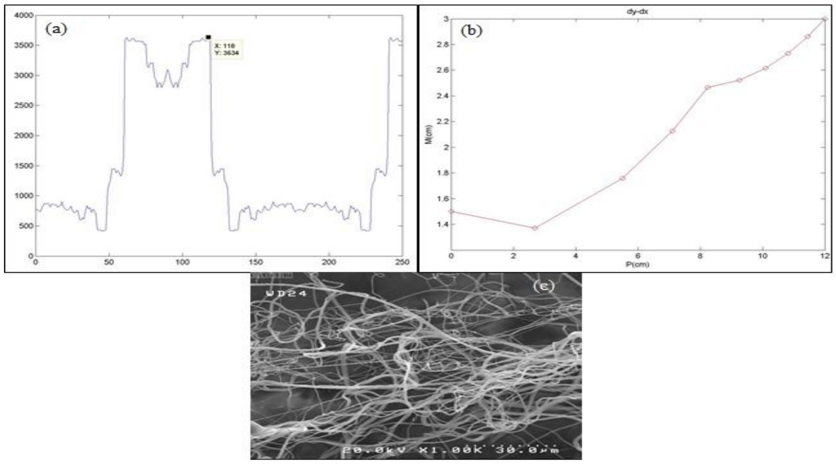

Figure 3: a) Diagram of angular Power Spectrum,
b) Graph the Movement Curvatures of Nanofibers between Nozzle and Collector and
c) SEM Image of PCL Nanofiber in Field Switching 0.3 Second

The diagram of angular power spectrum, graph the movement curvatures of nanofibers between nozzle and collector and SEM image of PCL nanofiber in field switching 0.4 have been shown in Figure 4. Similar to field switching time 0.3 second the orientation increases by increasing switching time. Power spectrum diagram for this sample displays high orientation in parallel fibers and regularity between 0 and 180 degree. This situation is very suitable in production of parallel nanofibers in cross direction of collector wires.

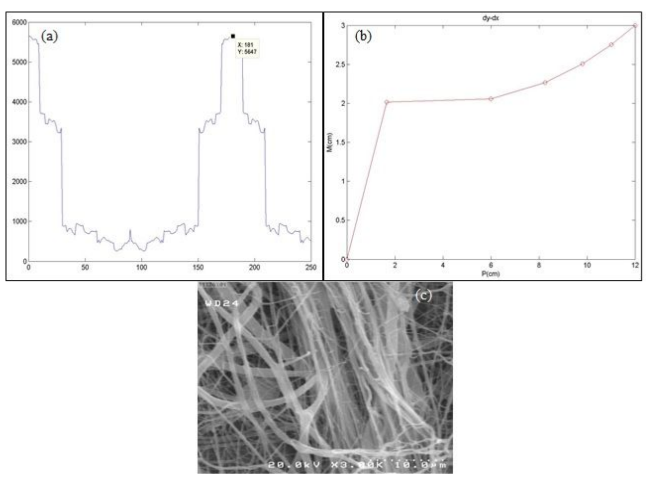

Figure 4: a) Diagram of Angular Power Spectrum
b) Graph the Movement Curvatures of Nanofibers between Nozzle and Collector and
c) SEM Image of PCL Nanofiber in Field Switching 0.4 Second

In Figure 5 the diagram of angular power spectrum, graph the movement curvatures of nanofibers between nozzle and collector and SEM image of PCL nanofiber in field switching 0.5have been shown. Figures show a very distributed structure for nanofiber web. Power spectrum of Figure 5(a) presents peaks among 82 to 98 degrees which is referring to wire directions. It means that increasing switching time more than 0.4 seconds leads to collect some of nanofibers straightly in wire direction because the time of switching may be more than movement times from nozzle to collector and some of nanofibers are collected randomly between wires spaces.

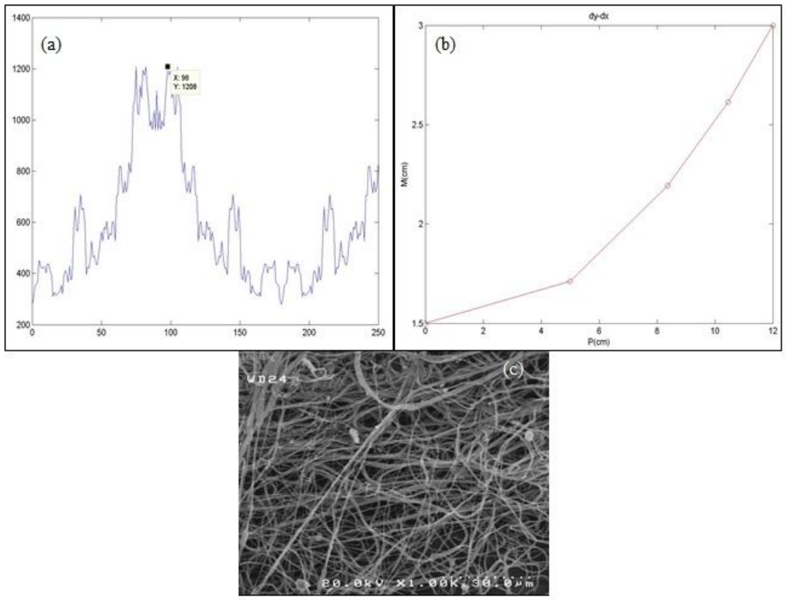

Figure 5: a) Diagram of Angular Power Spectrum
b) Graph the Movement Curvatures of Nanofibers between Nozzle and Collector and
c) SEM Image of PCL Nanofiber in field Switching 0.5 Second

According to mentioned result of different field switching from 0.2 to 0.5 second, it can be concluded that switching time is effective on nanofibers distribution and optimum delay of switching relatives to movement time, acceleration and velocity of nanofibers during electrospinning from nozzle to collector.

Table 1 shows some characteristics of angular power spectrum like major angular of spectrum and the degree of movement curvature in initial and final point of graph the movement curvatures of nanofibers between nozzle and collector. According to Table, samples with 0.3 and 0.4 field switching time have more major angular in angular power spectrum which is related to samples with high orientation in parallel fibers with 172 and 168 values, respectively. These results are similar to degree of movement curvature in initial points which have more values in comparison by field switching time 0.2 and 0.5. Also, the degree of movement curvature in final point of movement curvature of nanofibers between nozzle and collector has no specific trend. It can be justified that the degree of movement curvature in final point is may related to randomized shape of fibers after initial movement in the electrical field. Mentioned results approve the proper compatibility between experimental results of SEM images and the model of movement curvature nanofibers.

Table 1: Some Characteristics of Angular Power Spectrum and Movement Curvature of Nanofibers between Nozzle and Collector

Field Switching Time	Major Angular in Angular Power Spectrum	Degree of Movement Curvature in Initial Point	Degree of Movement Curvature in Final Point
0.2	128	5	53
0.3	172	35	55
0.4	168	75	37
0.5	146	20	60

From considering the curvature form of movement direction for different switching time, it is clear that the nanofibers distribution moves toward straightly curveted morphology of fibers in horizontal direction up to 0.4 seconds delay time or switching time. This matter is confirmed by results of power spectrum and visual observation of SEM images of samples. The switching function leads to stretching the nanofiber toward the next wire and it is a good justification for arrangement of nanofibers toward horizontal form. From the modeled graphs of power spectrums and SEM images, it is concluded that short delay in switching time leads to return movement phenomena and fibers return to the first wire before collecting into the collector. Also, it is concluded that extra delay can't support stretching process and nanofibers movement is a randomly movement similar to collector without switching. The result of power spectrum of SEM images and movement curvatures confirm together. The presented software of simulation model can be easily applied in various situation by change in variables of nozzle distance, wire distance, number of wires, high voltage of electrospinning, throw put value, percentage of solution, etc. the processing time of presented software is very short and this software can be applied for any type of material and in time and cost by prediction of nanofibers movement direction before sample production.

CONCLUSIONS

In this research, electric field switching of collector in shape of parallel wires was applied to control orientation of nanofibers in web scaffold in various morphologies. The modeling and experiments confirmed together and they presented that it is possible to control nanofiber orientation by different switching patterns in which the switching operation stretches nanofiber from nozzle in calculated dynamic from the first wire to the next ones according to the acting force of electric field between nozzle and the final collector wire. The presented method software was capable to model, predict and design the morphology and orientation of nanofibers in the scaffold web by control the switching function using computer simulation. The result confirmed capability of the method and software. This method can be used to reduce costs and time for nanofibers scaffold design according to target applications.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human or animal subjects. Also, there are no conflicts of interest at this article.

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