ORIGINAL ARTICLE

Modification of polycaprolactone electrospun nanofibers with hydroxyapatite nanoparticles in improving the properties of bone graft

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ABSTRACT

Introduction: Electrospun nanofiber has become ideal bone scaffold due its nanostructured that mimicking native extracellular matrix (ECM). Polycaprolactone (PCL) nanofiber has been used widely for bone tissue engineering due to its biocompatibility and biodegradation properties, but with lack of bioactivity owing to its hydrophobicity. Eventhough hydroxyapatite (HA) is an important mineral with good hydrophilicity, fabrication of HA nanofiber is very challenging due to its brittleness. Therefore, by reinforcing HA with PCL, the distinct advantages from both biomaterials are synergized in improving the scaffold properties. **Materials and Methods:** This study focused on the fabrication of PCL nanofibers with HA nanoparticles by using an electrospinning technique. HA was incorporated into the PCL matrix at 0 wt.%, 0.25 wt.%, 0.50 wt.%, 0.75 wt.%, 1.00 wt.% and 2.00 wt.%. The electrospun nanofiber scaffolds were characterized by using various scanning electron microscopy (SEM), energy dispersive x-ray (EDX), Fourier-transform infrared spectroscopy (FTIR) and water contact angle (WCA) to evaluate their morphological, chemical, and surface properties. **Results:** The results of EDX and FTIR show the presence of HA in the PCL/HA nanofibers. The addition of HA into PCL reduced the nanofiber's diameter and at the same time improved the hydrophilicity properties of the nanofibers. **Conclusion:** The most suitable formulation was PCL nanofibers with 0.75wt.% HA as it gave the desired nanofibers morphology, element composition and good wettability. Overall, the fabricated PCL/HA electrospun nanofiber scaffolds will hold great potential as biomimetic scaffolds for bone tissue engineering, providing a favorable microenvironment for cell growth and bone regeneration.
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INTRODUCTION

Bone is a complex process of mineralization mostly consisting of cancellous bone and cortical bone [1]. The major constituents of bone matrix are nano apatite crystal which make up around 65% of its total mass with hydroxyapatite (HA) as the main mineral component, while the organic component consists mostly of types I collagen, along with a little amount of lipids, polysaccharides, non-collagen protein, and other things [2]. Meanwhile, bone graft is an attractive choice in orthopedic surgical area for augmenting bone regeneration [3]. There are three approaches to bone graft: autograft, allograft, and xenograft with time-consuming of healing process, especially in larger bone defects [4]. Therefore, undoubtedly, bone tissue engineering (BTE) is become an emerging field that has been growing rapidly in these recent years with the aims to combat the limitation of traditional bone grafts [5].

Much effort has been devoted to investigating novel bone substitutes in creating the ideal bone scaffold through the utilisation of many innovative scaffold construction techniques [5]. In order to construct an ideal bone scaffold, features such as mimicking the natural structure and properties of bone extracellular matrix (ECM), providing temporary mechanical support, controlling degradation, stimulating bone cell migration, promoting osteogenic differentiation, and increasing cellular activity toward scaffold host tissue integration are required [6]. Thus, an appropriate selection of biomaterials and fabrication methods is crucial. Organic and inorganic biomaterials such as proteins, polysaccharides, polymers, metals, and ceramics are used to develop an ideal bone scaffold [7].

The remarkable advancement of nanotechnology in bone tissue engineering offers an alternative to employing nanofiber by electrospinning as it possesses porosity qualities similar to the extracellular matrix (ECM) which appropriate substrate for cell infiltration and growth due to due to large surface area, aspect ratio, permeability, and porosity [8-10]. Electrospun nanofibers have garnered significant interest due to their unique structural properties and controllable fiber diameter, making them an attractive candidate for scaffold fabrication. Nanofibers are light in weight and have a high surface-to-volume ratio, which makes them outstanding compared to the conventional fibrous structure in supporting cellular ingrowth.

Polycaprolactone (PCL) is a food and drug administration (FDA) approved biodegradable polymer widely used as nanofiber for bone tissue engineering due to its excellent processability, biocompatibility, and tunable degradation rate [11,12]. Pure PCL has drawbacks such as hydrophobicity and lack of OH functional groups, this nature of PCL can cause a decrease in cell adhesion [13]. Therefore, various study has been conducted either based on blending or nanocomposites in improving the PCL nanofibers properties for bone applications [12]. However, pure PCL scaffolds also lack the necessary bioactivity and osteoconductivity for efficient bone regeneration due to non-existent of bone mineral component.

On the other hand, hydroxyapatite is one of the popular materials used for bone scaffolding due to its chemical resemblance that is similar to the mineral phase of bone [14]. Hydroxyapatite is an inorganic mineral with 39.68% of calcium and 18% of phosphorus resulting in a Ca/P mole ratio of 1.67 [15]. By elevating the levels of calcium ions in the surrounding area, hydroxyapatite can promote the multiplication of osteoblasts and the specialization of mesenchymal stem cells (MSC) by elevating the levels of calcium ions in the surrounding area [16]. Thus, hydroxyapatite has gained significant usage in bone regeneration due to its notable characteristics, including elevated bone conductivity, biocompatibility, non-immunogenicity, and bioactivity [17].

However, hydroxyapatite particles showed some weaknesses in term of mechanical strength and durability with low fracture toughness due to its brittle nature [17]. Therefore, hydroxyapatite remains a critical challenge in certain bone grafts application especially in the HA nanofiber fabrication due to these properties. Therefore, modification need to be done to incorporate the HA into the nanofibers scaffold.

One of the solutions to overcome this problem is by reinforcing HA with PCL, well-known polymeric material for bone nanofibers. Combination of PCL with hydrophilic HA at the same time able to improve hydrophobic properties of PCL nanofibers. Therefore, in this study, electrospinning technique was used to fabricate PCL and HA nanofibers at different HA concentrations in finding the optimal concentration HA in improving the surface properties of the nanofibers that will lead to better performance for bone tissue regeneration application.

MATERIALS AND METHODS

Materials

Polycaprolactone (average Mn 80.000) was purchased from Sigma Aldrich (UK), Hydroxyapatite nanoparticle (< 200nm particle size) was purchased from Sigma Aldrich (USA), Dichloromethane (M.Wt 94.93 g/mol) was purchased from QReC (Malaysia), and N,N-Dimethylformamide (M.W. 73.09) was purchased from R&M Chemicals (Malaysia).

Preparation of PCL/HA Polymer Solution

PCL solution was prepared at 14wt.% PCL in DCM: DMF binary solution with a ratio of 1:1. Then, the solution was stirred by using a magnetic stirrer for 1 hour until all the PCL pellets dissolved at room temperature. Pure PCL electrospun nanofibers act as the control in this study. Then, PCL/HA polymer solution was prepared at different concentrations which were 0.25 wt.% HA, 0.50 wt.% HA, 0.75 wt.% HA, 1.00 wt.% HA, and 2.00 wt.%. HA. HA was added into the PCL solution and was stirred using the magnetic stirrer until the HA powder dissolved. The solution was sonicated for 5 minutes with an amplitude of 15 and then placed back into the magnetic stirrer for further homogenization for 1 hour.

Electrospinning

The polymer solution was filled into the syringe by avoiding the formation of bubbles in the solution as it will affect the quality of the resulting nanofiber. The extrusion of the polymer solution inside the syringe is controlled by using a syringe pump (model: NE-300 Just Infusion Syringe Pump New Era Pump Systems Inc. (US)). The electrospinning was conducted at 16 kV at 0.2 ml/h of flow rate and the distance between the needle tip and the collector was adjusted to 15 cm. The electrospinning process was carried out at room temperature. The duration of this process lasted for 2 to 3 hours. The sample was cut into a size of square with 1cm x 1cm before undergoing a series of characteristic analyses, which include the scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), energy dispersive x-ray (EDX), and water contact angle measurement.

SEM and EDX Analysis

SEM (Hitachi TM3000 Tabletop) was used to examine the fibers morphology. ImageJ was used to calculate the diameter of the fibers based on an average of 20 nanofibers. The SEM image with the magnification of 3000x for each sample was used to measure the diameter and operating at an acceleration voltage of 15kV. The element composition of the samples was analyzed by using EDX that combined with SEM (Hitachi TM3000 Tabletop). As the electron beam is focused on the sample, there will be an emission of the characteristic X-rays. The spectrum produced will then analyze and discussed afterward. All the samples were sputtered coat with platinum.

FTIR Analysis

The samples were subjected to FTIR analysis to determine the functional groups present. The software, OriginLab was used to find out the peak of the spectrum. The functional group was identified based on the peak value.

Wettability Analysis

Water contact angle (VCA Optima) was conducted to measure the samples wettability. A small drop of water was applied to the sample and the video that lasted for 2 minutes was recorded. The video captured was analyzed and the contact angle was measured. In this study, the water contact angle with 0s, 30, 60s, and 90s was taken for further analysis.

RESULTS

Morphology Analysis

The morphology of the electrospun nanofibers were observed using SEM. Fig. 1 a, b,c,d shows the SEM image of each sample. The SEM image for each sample looks similar. As shown in Fig. 1, the fiber distributions were not uniform for all samples. Besides, the electrospun nanofibers formed were not aligned or random, which in a nonwoven mat form. Other than that, the fibers formed were continuous and beadless. On the other hand, as the concentration of HA increased, the more pronounced the white particle appears within the fibrous structure.

Fiber Diameter Analysis

The mean fibers diameter of each sample was displayed in Table I. The fibers diameter decreased as HA concentration increased. Meanwhile, the pure PCL was having greater fiber diameter compared to the rest of the samples. There are contradictory studies for previous studies where the diameter of nanofiber decreases as the

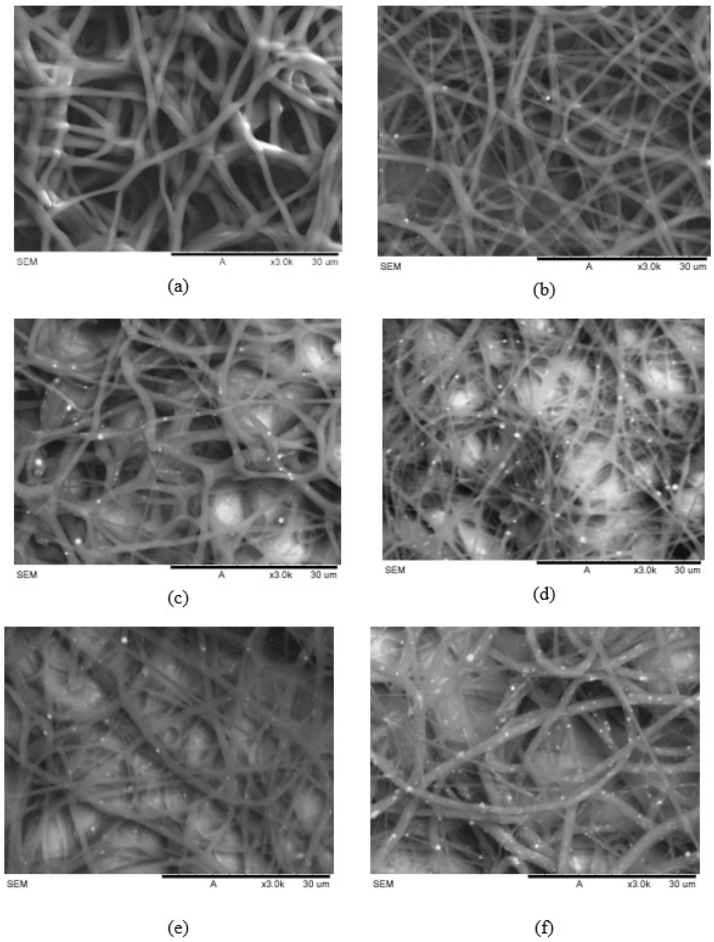

Fig. 1: SEM images of PCL/HA electrospun nanofiber with (a) 0 wt.%, (b) 0.25 wt.%, (c) 0.50 wt.%, (d) 0.75 wt.%, (e) 1.00 wt.%, (f) 2.00 wt.%.

concentration of HA increases [18], while diameter of nanofiber increased as the concentration of HA increases [13]. However, the nanofibrous substrates with high specific surface area, small fiber diameter and porosity are more appropriate for bone tissue regeneration because osteoblasts are anchorage dependent cells [12].
Element Composition Analysis

The element composition from EDX analysis is shown in Table 2. For the electrospun pure PCL nanofibers, the elements found were only carbon and oxygen. Whereas for the electrospun nanofiber of PCL/HA, the composites found were carbon, oxygen, calcium, and phosphorus.

Table I: The diameter of nanofibers of PCL and different concentrations of HA (0.25, 0.50, 0.75, 1.00 and 2.00 wt.%)

Sample	Mean diameter (m)
PCL	1.381
PCL with 0.25 wt.% HA	0.956
PCL with 0.50 wt.% HA	0.930
PCL with 0.75 wt.% HA	0.880
PCL with 1.00 wt.% HA	0.858
PCL with 2.00 wt.% HA	0.866

Table II: The EDX analysis of PCL nanofibers with different concentrations of HA (0.25, 0.50, 0.75, 1.00 and 2.00 wt.%)

	Carbon (%)	Oxygen (%)	Calcium (%)	Phosphorus (%)
PCL	63.609	36.391	0.000	0.000
PCL + 0.25wt.% HA	62.653	35.647	0.963	0.737
PCL + 0.50wt.% HA	62.272	34.590	2.050	1.089
PCL + 0.75wt.% HA	60.562	36.067	2.187	1.184
PCL + 1.00wt.% HA	58.296	37.666	2.834	1.203
PCL + 2.00wt.% HA	55.530	37.572	4.895	2.003

FTIR Analysis
Fig. 2 shows the FTIR spectrum for both PCL electrospun scaffold and PCL/HA electrospun scaffold at 1.00 wt.% HA. According to Fig. 2, a significant peak was observed in both electrospun scaffolds at wavenumbers of 2342 cm-1 and 2939 cm-1, respectively. This indicates the stretching of the C-H bonds in the PCL material. Furthermore, for both nanofibers the notable peaks detected at 1719 cm-1 and 1722 cm-1 are indicative of the carbonyl (C=O) stretching vibration in the PCL ester group [19]. However, the intensities of these peaks are marginally different in the PCL/HA sample due to the influence of HA. The peaks at 1238 cm-1 and 1161 cm-1 correspond to the C-O stretching of PCL [20]. The peaks at lower wavenumbers (1366 cm^{-1}, 1292 cm^{-1}, 1238 cm^{-1}, 1161 cm^{-1}, 1044 cm^{-1}, 961 cm^{-1}, and 730 cm^{-1}) in the pure PCL spectrum exhibit varying intensities

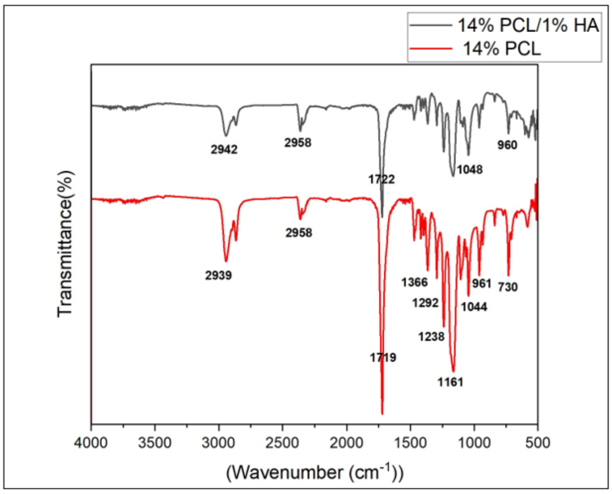

Fig. 2: FTIR result of PCL electrospun scaffold and PCL/HA (1.00 wt.% HA) electrospun scaffold.

in comparison to the corresponding peaks in the PCL/HA spectrum. Some of the peaks may be less intense or shifted as a result of the presence of HA

Wettability Analysis
The wettability of the fabricated electrospun nanofiber scaffold was analyzed through water contact angle measurement. Fig. 3 shows the water contact angle for each sample. There were four angle measurements were taken for each sample, from left to the right, which indicates the angle measurement for the 0s, 30s, 60s, and 90s. The value of the water contact angle can be found in the top right of the image. From Fig. 3, it was clear that the nanofiber for pure PCL is hydrophobic in nature and lack of functional groups as the water contact angle gave the value of 119.90° at the 90s. The addition of 0.25 wt.% and 0.50 wt.% HA into PCL did not show a profound improvement in the hydrophilicity of the scaffold as the water contact angle at the 90s still remains at the angle of greater than 90°, which was 118.60° and 119.20° respectively. At 0s, the water contact angle for PCL with 0.75 wt.% HA gave the water contact angle of 104.80°, which was also considered hydrophobic, however, as time passed, the water contact angle kept decreasing which proved that the increase in HA content can aid in enhancing the hydrophobic property of PCL. The electrospun nanofiber of PCL with 1.00 wt.% HA and 2.00 wt.%HA, gave the contact angle of less than 90° at the 30s that showed the higher hydrophilic property compared to other nanofibers.

DISCUSSION

Previous studies show that low doses of 5% of HA retain particles within the fibrous structure [18], while 10% of HA led to agglomeration of HA nanoparticles on the surface of scaffold (> µm size) [13]. Therefore, concentration under 5% were chosen to minimize the agglomeration and improve the properties. The fibers deposited in a nonwoven mat form because of the

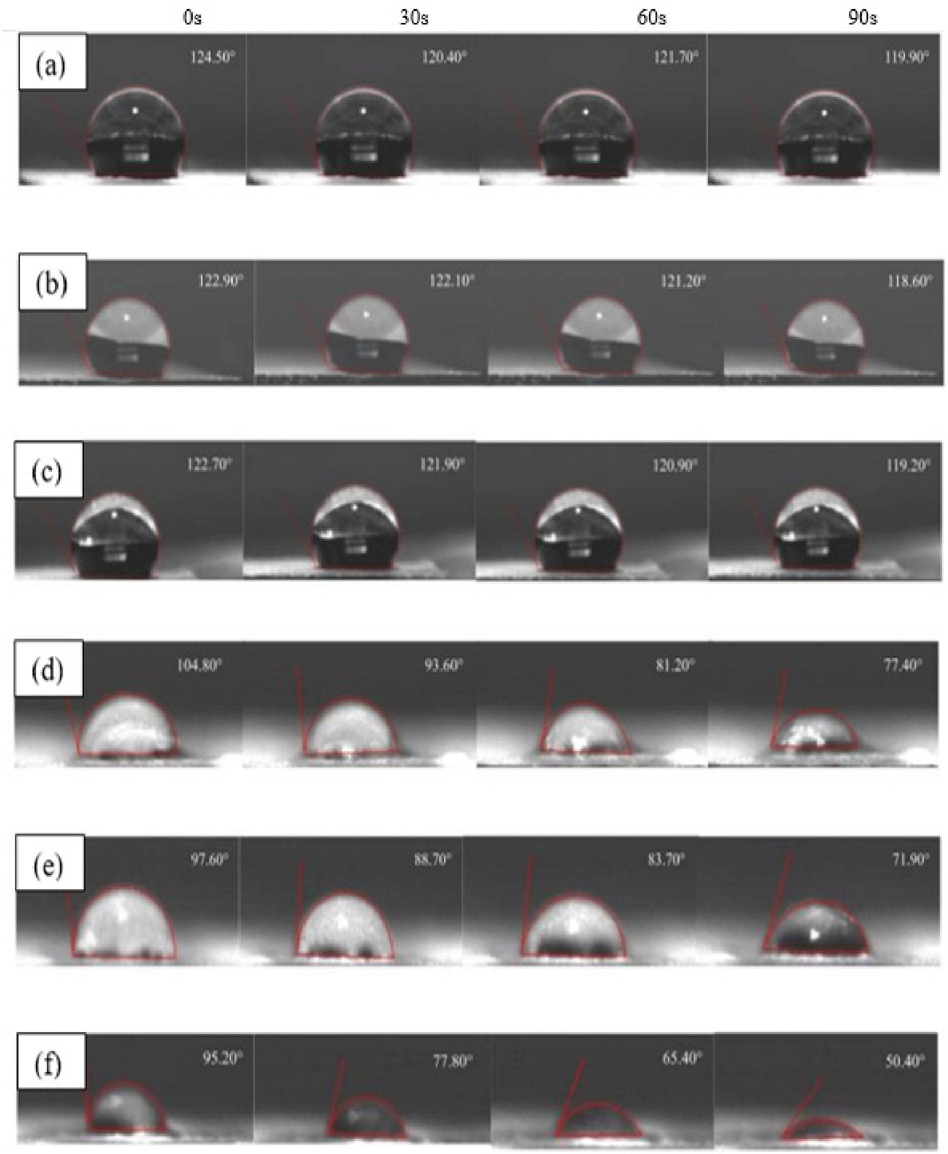

Fig. 3: Water contact angle of of PCL/HA electrospun nanofiber with (a) 0 wt.%, (b) 0.25 wt.%, (c) 0.50 wt.%, (d) 0.75 wt.%, (e) 1.00 wt.%, (f) 2.00 wt.% of HA.

bending instability [21]. The formation of continuous fibers most probably because the solution flow rate used was similar to the critical value of the polymer solution [22]. The formation of beadless fibers is influenced by the solvent's evaporation rate, with DMF in binary solvents reducing DCM volatility, preventing needle tip drying and hindering electrospinning [22]. Our results were inconsistent with previous study [18], the spinning of solutions with hydroxyapatite particles serves to significantly reduce the diameters of the fibers. The reduction of fibers diameter might be due to the addition of HA that had changed the solution conductivity and charge distribution. The introduction of HA influences the stretching and elongation of the electrospinning jet, as confirmed by other experimental work [23]. In addition, it was believed that changes in polymeric solution viscosity have a significant impact on the stretching of the charged jet during electrospinning [24]. Therefore, the addition of hydroxyapatite particles into the polymer solution might affect the viscosity of the solution. The reduction in fibers diameter gave advantageous for bone scaffold applications as it closely mimics the nanoscale architecture of the extracellular matrix, promoting cell attachment, proliferation, and tissue integration [12].

Calcium, phosphate, and oxygen indicate hydroxyapatite [25], which means that HA was successfully introduced into PCL/HA composite scaffold. Based on the results, the percentage of calcium and phosphate increased gradually as the concentration of HA increased which shows that the content of incorporated HA in the nanofibers increased.

In general, the intensities of certain peaks in the 14% PCL spectrum are higher than those in the PCL/HA spectrum, suggesting that the presence of specific functional groups in 14% PCL is more pronounced. The introduction of HA alters the intensity and potentially the position of specific peaks, which is indicative of changes in the chemical structure and interactions within the composite material. Based on the results, the bands of 1048 cm^{-1} and 960 cm^{-1} which correspond to the asymmetric and symmetric stretching of the PO43– ions, that indicated the presence of HA nanoparticles in the nanofiber scaffold. This is consistent with finding from previous studies [26,27]. The range of the O-H band is around 3400-3700 cm^{-1}, however, as observed from Fig. 2, the peaks of the O-H group are not notable where it only showed a small fluctuated waveform. The reason why the O-H band was not prominent may be due to the low concentration of HA in the sample.

As the concentration of HA increased, the hydrophilicity of the scaffold improved. This was because HA consists of the hydroxyl groups which have a strong affinity to the water molecules through hydrogen bonding [28]. Begin with the addition of 0.75 wt.% HA to the PCL already shows the hydrophilic property. For the electrospun nanofiber of PCL with 1.00 wt.% and 2.00 wt.%, gave the contact angle of less than 90° at the 30s, which again affirms that the addition of HA can improve the wettability of the scaffold. Blending the PCL with HA can improve hydrophilicity of a scaffolds [29]. Besides, the addition of HA resulted in the increased of surface roughness, which in turn increased surface hydrophilicity [30]. Increased hydrophilicity is desirable for bone scaffold applications as it is prone to protein adsorption with the highest affinity toward fibronectin binding [31] and promotes cell adhesion, nutrient diffusion, and overall biocompatibility.

CONCLUSION

The nanofiber for the bone scaffold of PCL incorporated with different weight percent of HA was successfully fabricated via the electrospinning technique. After reviewing the properties in terms of the fiber diameter, hydrophilicity, and chemical composition, the most suitable electrospun nanofiber for bone scaffold application was PCL with 0.75 wt.% HA due to its reasonable diameter of the nanofibers, and it showed great hydrophilicity. Therefore, PCL/HA electrospun nanofibers have the potential for bone tissue engineering, which offers improved fiber morphology in enhancing scaffold-cell interactions for bone regeneration.

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