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A novel approach to enhance the spinnability of collagen fibers by graft polymerization

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ABSTRACT

Collagen is an important natural biopolymer that cannot be electrospun easily due to the lost properties occurs in the associated degrading chains while dissolving and spinning. Grafting polymerization of methyl methacrylate-*co*-Ethyl Acrylate was applied to modify the surface of acid soluble collagen (ASC). The branched copolymer on the surface of collagen significantly influenced the initial viscosity. Since chain entanglement is crucial for fiber formation during electrospinning, the dependency of entanglement concentration on branch densities possessing the approximate same viscosity was investigated; in which the mean fiber diameters of all considered samples remained broadly constant. Increasing the number of branching onto ASC chains significantly decreased the deteriorative impact of the electrospinning conditions. It has also increased the stability of the collagen-based fibers under high humidity conditions. The short chain branched ASC-g-P(MMA-*co*-EA) can effectively influence the thermal stability of electrospun collagen fibers while the long chain branched ASC-g-P(MMA-*co*-EA) can provide a higher chain entanglement density leading to the more fiber uniformity.

1. Introduction

Development of newly engineered materials with desired properties e.g. thermal, mechanical or biological for specific applications is an important field in material sciences [1–3]. In biomaterial and particularly in natural-based polymers, it is essential to modify their properties to desired conditions for specific end uses [4,5].

Collagen and its associated derivations are the leading natural biomaterials with excellent biological and physiochemical properties that have been used in a variety of applications such as in drug delivery [4,6], filtration [7,8], tissue scaffolds [9,10], protective clothing [11], wound dressing [12]. Attempts have been made to modify collagen by graft polymerization to benefit from its natural properties while at the same time, to add value by introducing monomer(s) to its main chain [13,14].

Thus far, the main objective of many research groups has been focused on reducing the highly hydrophilic behavior of collagen chains and benefiting in a controllable degradation by using the graft polymerization method [13,15–18]. This method is well known for producing collagen-based hydrogels [15,19–22].

When it comes to promoting the final product with a high surface area, the electrospinning technique is an attractive method for the processing of polymeric fibers with a wide range of diameters from a few microns down to 100 nm. In general, the electrospun fibers can be achieved by altering the processing and polymer melt/solution parameters [23,24].

However, Zeugolis et al. reported that physiochemical properties of the pure collagen are lost when it is electrospun into fibers [25]. They observed a lowered denaturation temperature in electrospun collagen chains that was also confirmed by some recently published studies [26,27]. In a similar work, Yang et al. revealed that 45% of the collagen mass is denatured during electro-spinning [28]. Furthermore, due to a significant conformational change in collagen chains, it has been reported that the electrospun collagen fibers do not swell in aqueous media and may immediately dissolve in water [9,28–31].

To reduce the negative effect of processing methods on the sensitive structure of collagen chains, surface modification methods on collagen fibers using crosslinking agents have been extensively recommended as post-treatment [23,24,32,33]. For instance, chemical crosslinking agents such as aldehydes have been typically applied to protect the collagen fibers obtained from electrospinning [23,24]. However, due to random crosslinking, the end material is more likely to lose its desired morphology after post-treatment [34]. Also, the rigid and fixed collagen chains are incapable to represent good mechanical properties rendering the material to suffer under non-stable humidity conditions [23].

To the best of our knowledge, the effect of flexible branching chains

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onto the structure of collagen has not been given attention when the grafted polymer is electrospun into fibers. We hypothesize that during the electrospinning process, the branching on the main backbone of collagen can significantly preserve the collagen from an extensive conformation change by the electrostatic repulsion force occurring between its chains in acidic solvents. And branch densities can present different velocities by influencing chain entanglement which is essential in fiber formation.

In this study, Collagen was solubilized by acid treatment. Subsequently, binary different vinyl monomers of Methyl Methacrylate (MMA) and Ethyl Acrylate (EA) in varied feed ratios were grafted onto the acid soluble collagen (ASC) to achieve collagen graft polymers with varied branch densities. To exploit the advantages of the chain flexibility of the structurally branched copolymer, the collagen graft copolymers were then processed through the electrospinning method in low concentrations. The effect of entanglement density on fiber diameters was investigated. Our aim is to benefit from the amphiphilic behavior, the firmness and the plasticizing capacity of the resulted copolymer under unstable humidity conditions. Since electrospinning is believed to be a challenging processing method for the sensitive nature of collagen chains, we performed surface characterizations on the electrospun fibers from the collagen graft copolymers. To investigate the degradation and water absorption capacity of the collagen-based fibers, a series of comparisons were performed between the unprocessed bulky graft collagen copolymers and the fibers. This controlled branching can be used as a predictable method to preserve the morphology of the collagen-based fibers, in contrast with randomly crosslinking methods which pose significant challenges.

2. Materials and methods

2.1. Materials

Collagen from cow skin was provided by Devro Company Inc., UK. Methyl methacrylate (MMA, 99%, Alfa Aesar), Ethyl Acrylate (EA, 99%, Alfa Aesar) were used as monomers and were passed through a column of 5% sodium hydroxide acquis solution to remove inhibitors existing in the monomers. Benzoyl peroxide (BPO, 97%, Alfa Aesar) was used as initiator and recrystallized in Acetone before applying. Acetic acid (AA, 99.7%, Alfa Aesar), Formic Acid (FA, 99%, Alfa Aesar), distilled water and methanol (MeOH, 99.9%, Alfa Aesar) were applied as received.

2.2. Graft polymerization onto acid soluble collagen (ASC)

ASC was prepared using pure collagen from cow skin in 100 mmol AA and distilled water (Table 1) to reach pH of 3 ± 1. The mixture was incubated for 5 h at 45 °C in a 250-ml triple necked round-bottom flask and a stirrer bar was then added. This step was terminated by the suddenly increased temperature of 80 °C, the threshold of achieving ASC in water, seen as a homogenous solution.

Free radical polymerization was used to synthesize the graft copolymers of MMA and EA onto ASC in distilled water. This procedure is given in detail elsewhere [35,36,51]. In this step, the previous 250-ml triple necked round-bottomed flask was served as a reaction vessel. Nitrogen gas was applied through the solution while stirring. Once the desired temperature (80 °C) was achieved, dissolved BPO in 2 ml Acetone as the initiator, was added gradually to the reaction vessel within 10 min. Distilled MMA and EA in the rates mentioned in Table 1, were then introduced to the mixture via a syringe in 30 min. The temperature and reaction time were fixed at 80 °C and 60 min after adding the initiator and the monomers. The stirrer speed was also fixed at 2400 rpm during the reaction. Precipitation of the graft copolymer occurred after 15 min of reaction time yielding a milky white solution. The reaction mixture was then added to excess cool methanol for complete precipitations. The solution was then filtered with a glass sinter filter and dried in a vacuum oven at 25 °C until a constant weight was achieved. Accordingly, 5 samples (S1P2… S5P2) were obtained as listed in Table 1.

As with any conventional free radical copolymerization reaction, the formation of P(MMA-*co*-EA) always arises along with that of the desired copolymer (ASC-g-P(MMA-*co*-EA) due to reactivity ratio effects or the segregation of macromonomers from main and side chains. An extraction step was required to remove ungrafted ASC, unreacted EA and MMA macromonomer and P(MMA-*co*-EA) from the collagen graft copolymers.

A simple isolation method with selective solvent extraction based upon the difference in the solubility was employed. Therefore, the grafted copolymers were extracted by repeated washings with hot water followed by acetone at room temperature to remove the associated ungrafted ASC and P(MMA-*co*-EA). The supernatants were then separated from the graft copolymer using a sintered glass filter under reduced pressure. All samples were dried in a vacuum oven at room temperature until constant weight was achieved. The grafting percentage (GP) and the grafting efficiency (GE) were calculated by the following equations:

$$GP = \frac{W1 - W0}{W0} \quad (1)$$

$$GE = \frac{W1 - W0}{W2} \quad (2)$$

where W_0, W_1, and W_2 are the weights of the initial ASC and ASC-g-P (MMA-*co*-EA) and the used comonomers, respectively [36,37].

To calculate the Yield of the graft collagen, the collagen graft copolymer was hydrolyzed in HCl and the weight of branch copolymer was evidence to measure the Yield of the graft collagen using the given equation:

$$\text{Yield of the graft collagen} = \frac{(W1 - W2) - W0}{W0} \quad (3)$$

where W_0, W_1, and W_2 were the weights of the initial ASC, ASC-g-P (MMA-*co*-EA), and the grown branches, respectively [38]. The molecular weight of the isolated grafted branches was then determined by viscometric measurements in Acetone at 30 °C, based on the relation $(\eta) = 7.70 \times 10^{-3} M_n^{0.70}$ [38].

Table 1
Change of graft performance with water content and initiator concentration based upon the feed ratio composition for reactants used in the synthesis of grafted copolymers (initial amount of ASC was set at 11 g).

Sample	Comonomer cont. in feed (mmol)	EA cont. (%)	Feed ratio ASC:(MMA-*co*-EA) (wt:wt)	Water cont. (mL)	Initiator (mmol)	GP (%)	GE (%)	Yield of grafted collagen (%)	$Mn * 10^{-3}$ of branch copolymer	Wight ratio of ASC: side grafts	Nitrogen cont. %
S1P2	109.83	0.50	1:1	85	4.51	11.42	10.79	6.26	9.54	1:0.96	7.46
S2P2	219.71	5.00	1:2	106	9.10	37.14	16.47	21.85	7.89	1:1.09	4.69
S3P2	329.65	10.00	1:3	120	13.62	49.85	15.68	32.47	7.08	1:1.33	4.59
S4P2	493.47	15.00	1:4	145	18.16	57.42	13.49	34.37	8.06	1:1.68	4.35
S5P2	549.39	20.00	1:5	160	22.70	51.71	10.34	32.13	9.11	1:1.53	5.95