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# Tunning Surface Hydrophilicity of PVA Nanofiber Bone Scaffolds Via Amino Acid and Nanohydroxyapatite Incorporation

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## ABSTRACT

Polyvinyl alcohol (PVA) is a biodegradable and biocompatible polymer with potential use in bone tissue engineering. However, its excessively high hydrophilicity led to poor cell adhesion, limiting its suitability as a bone scaffold. This study investigates the modification of PVA nanofibers through the incorporation of negatively charged amino acids (aspartic acid, glutamic acid) and nano-hydroxyapatite (nHA) to tailor surface hydrophilicity. Electrospun nanofiber composites of Asp/PVA/nHA and Glu/PVA/nHA were fabricated with varying nHA concentrations. Higher nHA content was found to decrease hydrophilicity, whereby Asp/PVA/nHA and Glu/PVA/nHA with nHA concentration of 3.5% were within the ideal range for optimal cell adhesion and proliferation characterized by contact angle between 40°-70°. Samples containing Asp exhibited a lower hydrophilicity compared to their respective Glu-containing counterparts, which may correlated to the difference side chain of Asp and Glu structure. Statistically significant differences ($p \leq 0.05$) suggest that nHA and amino acids effectively modulate surface characteristics, supporting the potential of fine-tuning PVA hydrophilicity via nHA and amino incorporation for improved performance in bone tissue engineering applications.

**Keywords**: amino-acid, bone scaffold, contact angle, electrospinning. PVA, nano-hydroxyapatite, hydrophilicity modulation

## INTRODUCTION

Biomaterial surface hydrophilicity plays a significant and important role in the adhesion, proliferation, and differentiation of different cell types, including the osteoblasts responsible for bone production [1-2]. Ideal scaffolds need to have optimal hydrophilicity, as extreme values can negatively affect cell attachment and function. Super hydrophobic surfaces repel water and proteins thus restrict cell adhesion and proliferation [3]. On the other hand, super hydrophilic surfaces can result in poor cell adhesion, which influences the initial stage of cell adhesion [4]. Previous work has established that moderate hydrophilicity, defined by water contact angles of 40°-70°, is conducive to optimal cell adhesion and proliferation for a majority of cell types, including mesenchymal stem cells [2-5]. This Moderate hydrophilicity is crucial to ensuring favorable interactions between scaffolds and cells.

Polyvinyl alcohol (PVA) is a biodegradable and biocompatible polymer with potential use in tissue engineering [6-8]. In real application PVA can be easily fabricated into nanofibers that closely mimic wthe structural morphology of the extracellular matrix, thus providing an ideal microenvironment for cell growth and attachment [9]. However, its high hydrophilicity can, nonetheless, discourage initial cell adhesion. This can consequently undermine its utility in scaffold-related applications. To overcome these limitations, changes like the addition of nano-hydroxyapatite (nHA) and amino acids such as aspartic acid (Asp) and glutamic acid (Glu) are investigated [10]. Previous study has shown incorporation of nHA to a polymers, enhance mechanical strength, bioactivity, and cell adhesion in PVA scaffolds [11]. Other study shows blending PVA with amino acids incorporates cell-adhesive hydrophilic domains, altering its wettability behavior by introducing surface charge or functional group changes [10]. All these modifications act together to upgrade PVA's surface properties to facilitate better cell-material interactions and overcome its intrinsic limitations for use in tissue engineering.

While the properties of PVA/nHA composites have been widely studied, Effect of incorporating of structurally similar negatively charged amino acid into the PVA/nHA composites to its surface properties remains largely unexplored. Comparison Asp and Glu is interesting since between Asp and Glu only differ by a single carbon atom in their aliphatic side chain. This allows us to investigate how subtle difference influences the exposure of carboxyl groups on the nanofiber surface. This may provide a novel mechanism to control macroscopic hydrophilicity.

This study investigates the wettability of nanofiber composites composed of Asp/PVA/nHA and Glu/PVA/nHA at different nHA concentrations (1%, 2.5%, and 3.5%), comparing them to pure PVA nanofiber as a control. The nanofiber composites were fabricated using an electrospinning method. Statistical analysis ANOVA was used to analyze the significance of each parameter observed.

## METHODS

### Preparation and Fabrication of AA/PVA/nHA Nanofiber

Amino Acid/PVA/nHA composite solution for electrospinning were prepared by dissolving a mixture of PVA and n-HA powders in a 1 mM solution of negatively charged amino acids (glutamic acid and aspartic acid). The ratio between the mixture of PVA/n-HA and the amino acid solution was maintained at 10 wt%. The solution was heated to a temperature of 85°C and stirred continuously at a speed of 300 rpm until all the dry ingredients dissolved. In this study, the presence of amino acids, the types of amino acids, including aspartic acid and glutamic acid and the ratio of n-HA/PVA (1.0%, 2.5%, and 3.5%) were varied. Variation of concentration for each sample was summarized in TABLE 1.

**TABLE 1.** Sample Variation

| Sample | PVA/nHA concentration ratio (wt/wt %) | Amino acid |
|---|---|---|
| Asp/PVA/nHA 1% | 1.00% | Aspartic acid |
| Asp/PVA/nHA 2.5% | 2.50% | Aspartic acid |
| Asp/PVA/nHA 3.5% | 3.50% | Aspartic acid |
| Glu/PVA/nHA 1.0% | 1.00% | Glutamic Acid |
| Glu/PVA/nHA 2.5% | 2.50% | Glutamic Acid |
| Glu/PVA/nHA 3.5% | 3.50% | Glutamic Acid |

The electrospinning process of the AA/PVA/nHA solution is described in FIGURE 1. The electrospinning process of AA/PVA/nHA solution was carried out with a 12 kV DC voltage at a distance between the needle and the collector of 10 cm. The solution feeding is controlled by a syringe pump with a feeding rate of 0.6 µL/h. The collector used in this study is a stainless-steel wire mesh collector. Obtained nanofiber composite scaffold morphology was characterized using Scanning electron microscope (SEM) to confirm the presence of nanofiber structure.

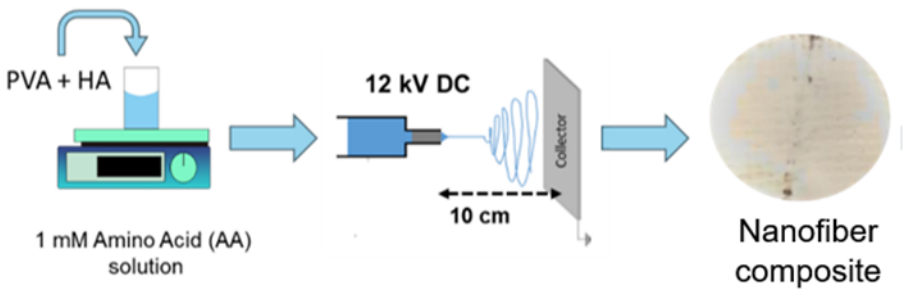



**FIGURE 1.** Electrospinning process of AA/PVA/nHA solution.

### Surface Hydrophilicity Measurement

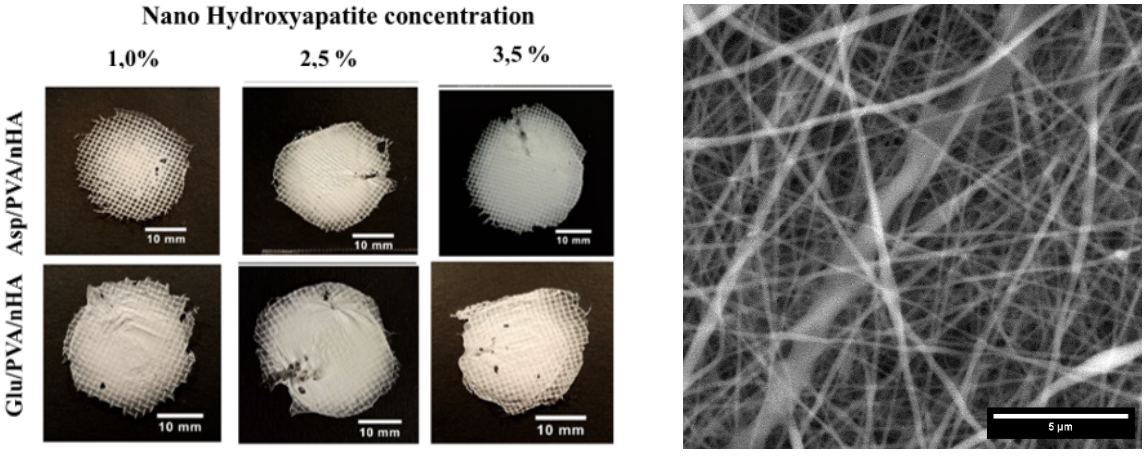
Surface hydrophilicity of amino-acid, PVA, and nano-hydroxyapatite composite were measured using static contact angle method by modified Zadeh et.al procedure [12]. Briefly Deionized water droplet (5 µL) dropped on 3 different parts of sample and a camera captured the droplet image after 10s droplet falls. The Captured droplet image was analyze using ImageJ image analysis software equipped with Dropsnake plugin to measure the contact angle of each droplet [13]. Measurements were repeated three times (n = 3) for each composite sample to ensure reproducibility.

### Statistical Analysis

The contact angle data was analyzed using one-way ANOVA to determine the significance of differences in contact angles between groups. A p-value of less than 0.05 is considered statistically significant. The ANOVA test was conducted using OriginLab software via originlab ANOVA function. Throughout the results, data are presented as the mean ± standard deviation, with error bars reflecting the variance across the sample.

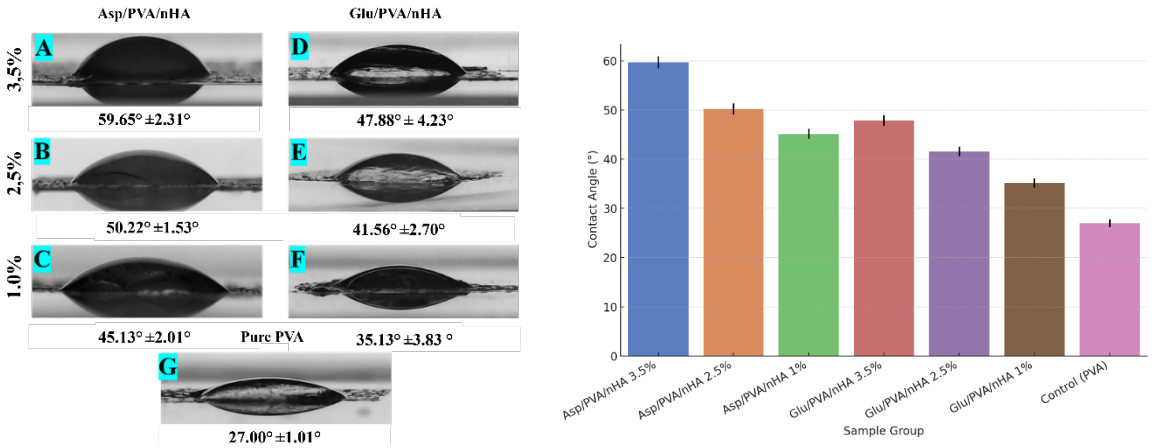
## RESULT AND DISCUSSION

FIGURE 2 shows the morphology of AA/PVA/HA nanofiber scaffolds with varying HA concentrations and negatively charged amino acids. The scaffolds exhibited a relatively uniform shape with an average diameter of 3 cm. From the SEM image the AA /PVA/nHA the nanofiber formation was confirmed. The formed nanofiber observed in all fabricated samples exhibit a dense, nonwoven fiber with random orientation. The fiber diameters were measured with average diameters of 145±23 nm which consistent with previously reported electrospun PVA based nanofiber [14-15].




(a) (b)

**FIGURE 2.** (a) Fabricated nanofiber composite AA/PVA/nHA in various concentrations; (b) SEM image of fabricated nanofiber.




(a) (b)

**FIGURE 3.** (a) Contact angle measurement of water droplet on top of nanofiber composite Asp/PVA/nHA 3,5%, (A), Asp/PVA/nHA 2,5%, (B), Asp/PVA/nHA 1%, (C). Glu/PVA/nHA 3,5% (D), Glu /PVA/nHA 2,5% (E), Glu /PVA/nHA 1% (F) and PVA as control (G); (b) contact angle histogram. All sample containing amino acids exhibits the optimal contact angle for biomaterials which on the range of 40°-70° degree.

FIGURE 3 presents the contact angles of deionized water on the surfaces of pure PVA nanofibers (G), Asp/PVA/nHA (A, B, C), and Glu/PVA/nHA (D, E, F). The contact angle measurements show noticeable differences in hydrophilicity among the nanofiber composites. Pure PVA exhibited the highest hydrophilicity, with a contact angle of 27.00°, confirming PVA's inherent high hydrophilicity as also observed in other PVA-based composite studies [12-13]. However, For Asp/PVA/nHA composites, the contact angle decreased with decreasing nHA content. Asp/PVA/nHA 3.5% showed the highest contact angle (59.65°), followed by Asp/PVA/nHA 2.5% (50.22°) and Asp/PVA/nHA 1% (45.13°). the more hydrophobic than pva nature of hydroxyapatite may play mayor role increase overall surface hydrophobicity [18]. The same trend was observed in Glu/PVA/nHA composites.

It is noteworthy that glutamic containing samples exhibited greater hydrophilicity compared to their aspartic containing sample counterparts, as indicated by the contact angle values. This difference may be due to structural variations between glutamic and aspartic amino acid and their interaction with the PVA matrix. Glutamic acid have longer side chain than aspartic acid which may allow its terminal carboxyl groups to be more exposed at the composites surface [12]. This more exposed carboxyl group may enhance hydrogen bonding interaction with water. The observed contact angles for Asp/PVA/nHA 3.5% (59.65°) and Glu/PVA/nHA 3.5% (47.88°) are within this optimal range for cell adhesion and spreading (40°-70°) [2, 5].

The observed water contact angle for the amino acid incorporates PVA/nHA nanofiber scaffolds demonstrate significant physical improvement over pure PVA. As established by previous studies, cellular adhesion is thermodynamically favored on moderate hydrophilic surface (contact angle between 40° to 70°) [21-23]. While pure PVA's extreme hydrophilicity tightly binds a hydration layer that inhibits the stable anchoring of extracellular matrix proteins. the incorporation Asp and Glu successfully shifts the surface energy into the biologically optimal window. These findings align with the recent research around amino acid

functionalize polymer nanofiber-based scaffolds, confirming that amino acid functionalization provides a predictable method to tailor biocompatibility of scaffolds and in this case by modulation surface wettability [24, 25].

One-way ANOVA was conducted to verify these differences statistically. The analysis yielded an F-statistic of 866.31 (df = X, Y) with a p-value of $3.62\times10^{-17}$, indicating a statistically significant difference between the groups ($p < 0.05$). These observations confirm that variations in nHA concentration and the addition of amino acid additives have a significant effect on the hydrophilicity of the nanofiber composites.

## CONCLUSION

Incorporation of negatively charged amino acid and nano-hydroxyapatite (nHA) has been found to decrease the excessive hydrophilicity of PVA nanofibers. The surface hydrophilicity was found to be decreased proportionally to the increase of nHA concentration. Sample containing aspartic acid (Asp) exhibited a greater decrease of hydrophilicity (higher contact angles) compared to their respective Glutamic Acid (Glu) counterparts. Nevertheless, the contact angles for both Asp/PVA/nHA and Glu/PVA/nHA nanofiber composites remained within the optimal range for cell adhesion and spreading (40°-70°), demonstrating superior surface properties for bone tissue engineering compared to PVA nanofiber alone. These findings strongly suggest nHA and amino acids effectively modulate surface characteristics, supporting the potential of fine-tuning PVA hydrophilicity via nHA and amino incorporation which may lead to improvement performance in bone tissue engineering applications.

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