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AFM Image of Collagen on Induced Aligned Fluoropolymer Substrates

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Abstract: Reconstituted collagen film formed from lyophilized collagen in acetic acid and deposited on glass slide substrate was found to be randomly orientated. In this study PVDF and its copolymer (PVDF-TrFE) were used as a template for depositing collagen, a type of protein abundantly found in human body. The objective of this study was to develop the nucleation and growth of the aligned PVDF and PVDF-TrFE crystals. Collagen films formed, showed strong affinity to these fluoropolymers. The PVDF/PTFE fibres initiated growth of collagen films present in coiled collagen twisted structures around the PVDF and PVDF-TrFE fibres. The thickness of the collagen on the PTFE film obtained from AFM approximately 15 nm. Upon deposition of collagen on PVDF-TrFE/PTFE films, the thickness increased to 40-110 nm. The collagen was observed to orientate in the direction of PVDF-TrFE/PTFE. The collagen film formed on 72-28 PVDF-TrFE/PTFE film template was observed to be relatively less disorder and can be used in biomedical application. The dielectric properties of the collagen may be investigated for the application in electro-mechanical applications.

Key words: Collagen, PTFE, PVDF, PVDF-TrFE, Atomic Force Microscopy (AFM)

INTRODUCTION

Glass substrates are usually replaced by plastics substrates due to its flexibility and toughness. Fluoropolymer plastics are utilized as a substrate for protein deposition due to their inert characteristics. Some study showed that PVDF were used as membranes in immunoblot analysis for detecting high molecular weight protein for patients suffering from tubular proteinuria (Natzir, 2004). Due to its piezoelectric nature, PVDF were also used in designing of spike shoes for sprint ad hurdles athletes in order to achieve great stride frequency and good timing (Si *et al*., 2011).

Collagen, a type of protein in human body has been widely used in biomedical application, mostly in the form membrane and gels (Garno *et al*., 2002; Mao and Kisaalita, 2004; Yann-Astier and Howorka, 2005). Many studies were carried out to investigate structures and surface morphology of collagen adsorbed on various polymer (De Cupere and Rouxhet, 2001; Dufrene *et al*., 1999a; Dupont-Gillain *et al*., 1999). Some of the observations evidently indicate that surface properties of the substrate can influence the nanoscale organisation of adsorbed collagen films and its evolution upon drying. Critical substrate height variation close to the collagen molecule thickness may also affect the mobility of the adsorbed molecules and their tendency to aggregate (Dufrene *et al*., 1999b; Dupont-Gillain and Rouxhet, 2001) The dot-like and random elongated collagen films are easily produced but collagen films with aligned features are most challenging to produce (Dufrene *et al*., 1999b). Reconstituted collagen film produced from native collagen was mostly found to be randomly orientated Therefore, several studies have been conducted in promoting aligned collagen film (Denis *et al*., 2002; Maeda, 1999). The aligned PTFE film produced by frictional transfer method (Bodo and Schott, 1996; Motamedi *et al*., 1994; Wittmann and Smith, 1991) were used to orient PVDF homopolymer and PVDF-TrFE (72, 65, 51 VDF molar% compositions) copolymers, which were used as film templates to promote the alignment of the collagen film. Previous studies show that collagen and PVDF-TrFE composites are biocompatible and inert and thus suitable to be used in bio medical application (Goissis *et al*., 1999; Plepis *et al*., 1999). These oriented collagen structures (Jiang *et al*., 2004; Maeda, 1999) are of utmost importance

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in this study as they are provide relatively high piezoelectricity when compared with other biopolymers such as keratin and fibrin (Fukada, 1995). The objective of this study was to develop the nucleation and growth of the aligned PVDF and PVDF-TrFE crystals.

MATERIALS AND METHODS

Lyophilised collagen powders were dissolved in dilute hydrochloric acid (pH less than 2.5) to form a collagen concentration of 5 mg mL^{-1} and placed in a closed container to avoid dust or contaminants. The collagen solution was stored at 5°C to prevent denaturing of collagen at ~34°C. The collagen films (control) were formed by dipping the collagen solution on glass substrates. The fluoropolymer, which were PTFE, homopolymer of PVDF and copolymers of PVDF-TrFE (72, 65 and 51 VDF molar %composition) were utilized as film templates to aid the orientation of collagen. The first layer the film template, PTFE was produced by friction transferred method which resulted in highly aligned PTFE films (Bodo and Schott, 1996; Wittmann and Smith, 1991). The homopolymer PVDF and copolymer PVDF-TrFE were spin coated on the aligned PTFE films to form a second layer of the film template (PVDF / PTFE, 72-28 PVDF-TrFE/ PTFE, 65-35 PVDF-TrFE / PTFE and 51-49 PVDF-TrFE/ PTFE). All of these films templates were annealed at 140°C for 2 h in an electric oven before they were dipped in collagen solution for 2 min, then dried in a desiccator at ambient temperature for two to three days. Vibration or motions of the slides were avoided at all times in order to obtain a uniform collagen film.

RESULTS

Collagen film on glass substrates: The surface morphology, structures and orientation of collagen formed on glass, PTFE, PVDF, PVDF-TrFE films were observed using AFM, ThermoMicroscopes AFM (Nanoscope IIIa, Digital instruments Inc, Santa Barbara, CA). Figure 1a shows the AFM images of random collagen on glass slide with 5 mg mL^{-1} concentration. The homogeneity and thickness of collagen film formed on glass slides were estimated by the surface roughness observed from the profile of the AFM film (Fig. 1b).

Collagen deposited on aligned PTFE and annealed PVDF / PTFE and PVDF-TrFE / PTFE film: The microstructures of collagen on aligned PTFE film are shown in Fig. 2a and b while the thickness of the collagen layer was

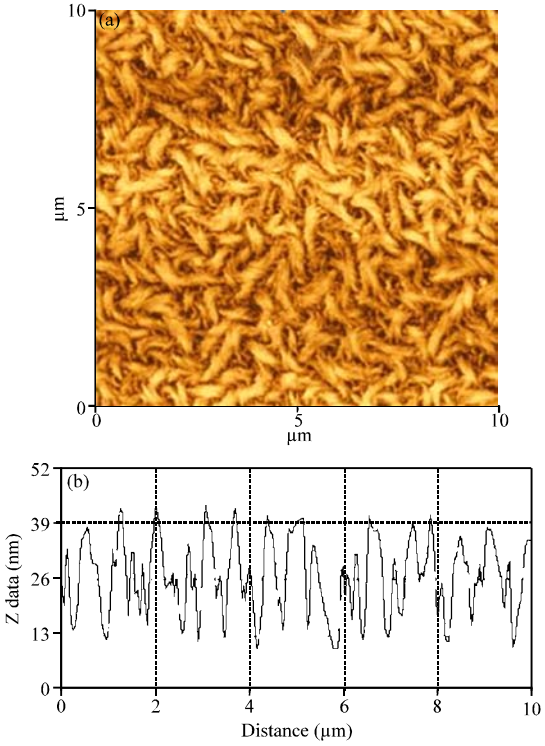

Fig. 1(a-b): (a) AFM image and (b) Height profile of random collagen on glass slide (5 mg mL^{-1} concentration)

determined by the difference between the height of the cross-section of the 'holes' or the uncoated areas of PTFE fibers with the PTFE fibre presented in Fig. 2c. The thickness for collagen film on PTFE was 15 nm.

Figure 3a shows the image of collagen molecules formed on an annealed PVDF / PTFE glass substrate. The growth of collagen observed being aligned by the direction of PTFE film. The thickness of the collagen film deposited on the PVDF was approximately 50 nm (Fig. 3b).

The AFM morphology of collagen deposited on annealed 72-28 PVDF-TrFE / PTFE was shown in Fig. 4a which shows that the growth of collagen growth is influenced by the direction of PVDF-TrFE / PTFE with the thickness approximately of 110 nm (Fig. 4b).

Figure 5a shows the image of collagen molecules formed on annealed 65-35 PVDF/PTFE glass substrate shows a certain degree of alignment on the copolymer surfaces with thickness of the collagen film deposited on the PVDF-TrFE /PTFE was less than 50 nm (Fig. 3b).

Figure 6a shows the surface morphology of collagen deposited on annealed 51-49 PVDF-TrFE/PTFE film with film thickness less than 40 nm thin compared to 72-28 PVDF-TrFE/PTFE and 65-35 PVDF-TrFE/PTFE.

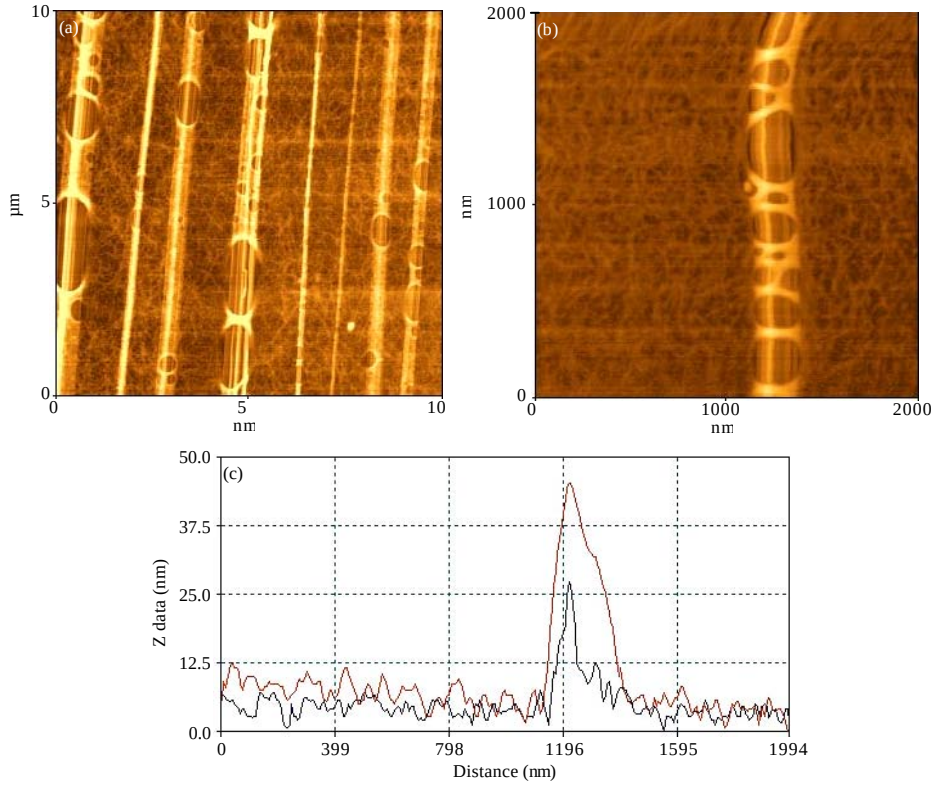

Fig. 2(a-c): (a) Scan area of 10×10 µm (b) Scan area 1×2 µm for AFM images of collagen (5 mg mL^{-1}) on PTFE and (c) Cross section profile, Red and blue lines are the line profile along the collagen and PTFE fibers, respectively

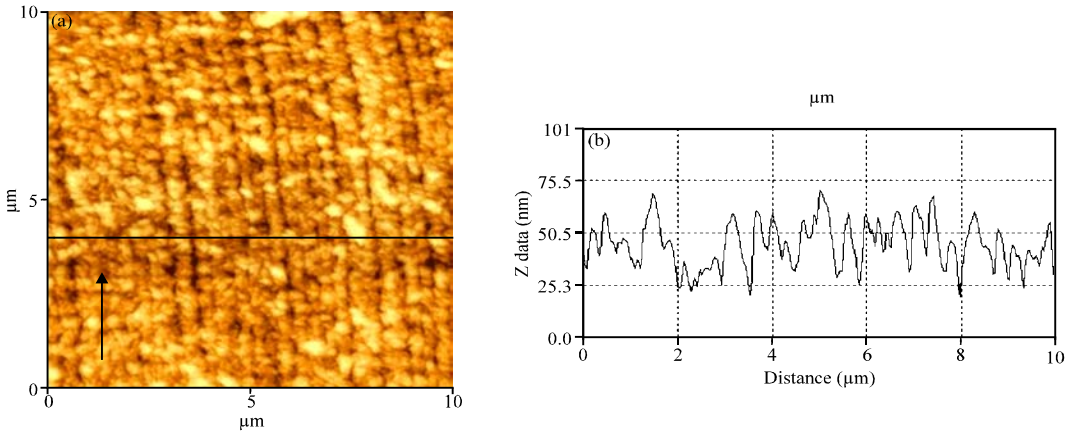

Fig. 3(a-b): (a) AFM images and (b) Surface roughness profile of collagen deposited on annealed PVDF/PTFE, arrow indicates direction of PTFE film

DISCUSSION

The major advantages of Atomic Force Microscopy (AFM) over conventional optical and electron microscopy for imaging include its non-requirement of a special coating and vacuum and its capability to perform imaging in various environments. AFM force-distance measurements have become a fundamental tool in the

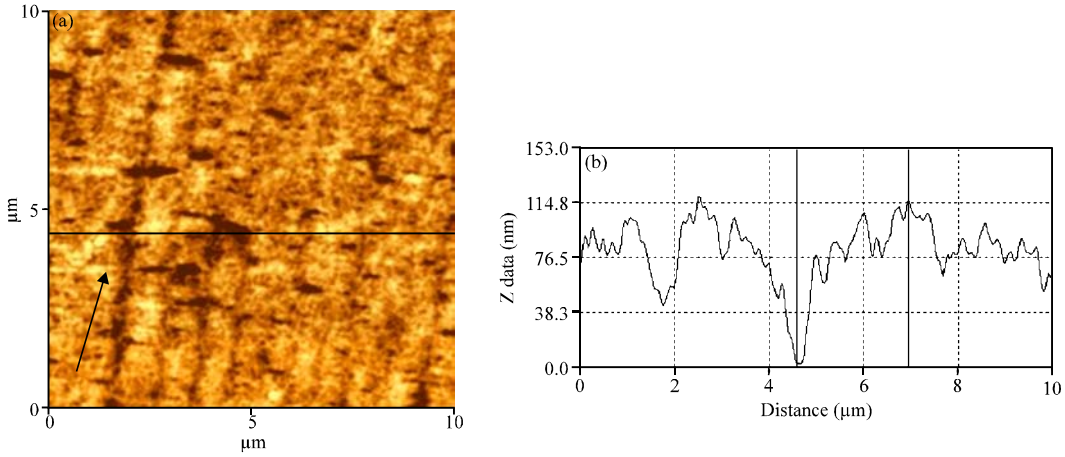

Fig. 4(a-b): (a) AFM images, arrow indicates direction of PTFE film and (b) Surface roughness profile of collagen deposited on annealed 72-28 PVDF-TrFE/PTFE

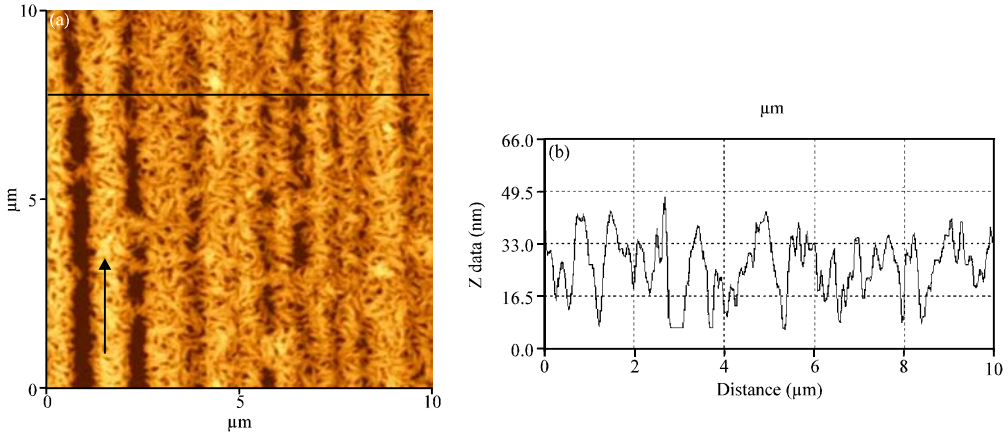

Fig. 5(a-b): AFM images, arrow indicates direction of PTFE film and (b) Surface roughness profile of collagen deposited on annealed 65 -35 PVDF-TrFE/PTFE

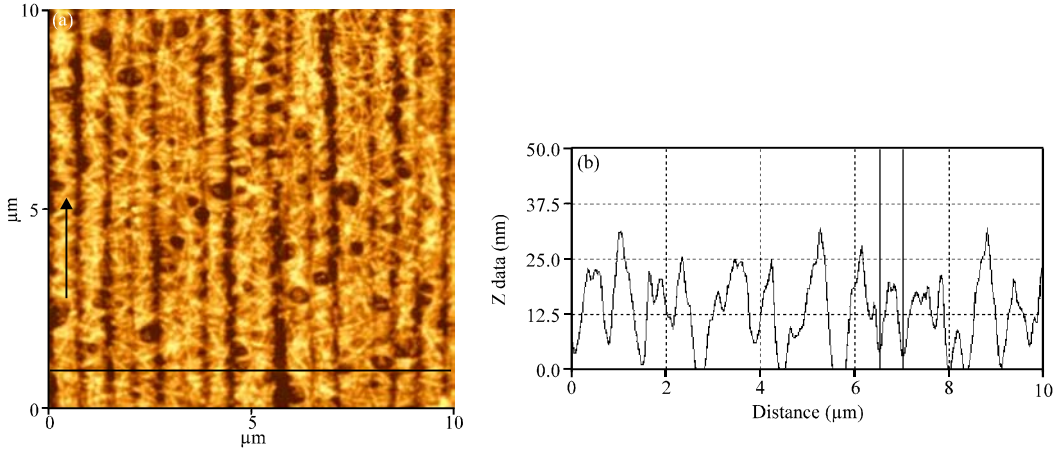

Fig. 6(a-b): AFM images, arrow indicates direction of PTFE film and (b) Surface roughness profile of collagen deposited on annealed 51-49 PVDF-TrFE/PTFE

fields of surface chemistry, biochemistry and materials science (Lee *et al*., 2011). The surface topographies of the films were recorded in a non contact mode at a localized region of 10 um^2 and the surface roughness of the films was visualized from the surface profile analysis, by using a plot of height verses distance. Figure 1a shows assemblies of undulated fibre structure, similar features as that of the natural collagen fibre but seven fold wider in diameter than a native collagen fibre. However, the length of collagen fibre was 150 nm which was half the magnitude of the native collagen (Kastelic *et al*., 1978; Plodinec *et al*., 2010). The collagen features were consistent to those observed by previous authors at the same concentration (Maeda, 1999). The roughness of the dipped coated collagen film was approximately 25 nm (Fig. 1b). The collagen molecules are partially hydrolyzed in the mild acidic medium of the hydrochloric acid and undergo partial coil reversal of the chain transitions. Upon drying, the assembly of acidic collagen solution resulted in the formation of secondary structures. The characteristics of a banding pattern of the native collagen fibres were not observed (Jiang *et al*., 2004; Kadler *et al*., 1996). This was possibly due to the acidic medium used as most of the striation in collagen fibril as a consequences of fibers formed in neutral pH (Goh *et al*., 1997). The deposition of collagen on the PTFE fibers (Fig. 2a, b) was due to the hydrophobic surface of the PTFE fiber that caused the collagen to change their conformation to expose their hydrophobic regions of their molecule. This increased the deposition of the collagen on the PTFE surface and optimized contact between the collagen molecule and PTFE. Hence it is evident that collagen has shown great affinity to the PTFE fibres. Figure 3a shows the PVDF/PTFE fibers have initiated the growth of collagen molecules on the substrate as evident from the coiled collagen twisted around the PVDF fibers. The collagen fibers were found to be orientated in the direction of the PTFE fibers.

When the collagen was deposited on the PVDF-TrFE copolymers films of 72, 65 and 51 VDF molar % composition, known as annealed 72-28 PVDF-TrFE/PTFE, 65-35 PVDF-TrFE/PTFE and 51-59 PVDF-TrFE/PTFE) respectively, the collagen was found to be orientated on the PVDF-TrFE strands (Fig. 4a-6a). This was attributed to the ordered array of the PVDF-TrFE/PTFE crystals which induced the orientation of the collagen on their surfaces. The thickness of the collagen on the PVDF-TrFE/PTFE film obtained by AFM profile was in the range of 40-110 nm. Although, the collagen deposited on annealed 65-45 PVDF-TrFE / PTFE and annealed 51-49 PVDF-TrFE/PTFE film templates show a certain degree of alignment on the copolymer surfaces, the collagen film indicated some disentanglement of the collagen fibrils. This may be due to some disoriented copolymer molecules formed on the film surface which may caused the collagen fibrils to come apart (Fig. 5a, 6a). Due to this, the thicknesses of the collagen films deposited on 65-45 PVDF-TrFE/PTFE and 51-49 PVDF-TrFE/PTFE were far less than the thickness of the collagen film deposited on the annealed 72-28 PVDF-TrFE/PTFE (Fig. 4b-6b). This may suggests an improve binding between the collagen and the 72-28 PVDF-TrFE/PTFE film template. The blackened areas observed in most of the AFM images of collagen on PVDF-TrFE/PTFE may indicate the presence of uncoated areas of PTFE. This again supports the notion that the collagen deposition is very much dependent on the coverage of PTFE.

CONCLUSION

The 72-28 PVDF-TrFE / PTFE can be used as a new substrate for potential biomedical application for promoting aligned collagen film. The collagen deposition on 72-28 PVDF-TrFE / PTFE produced aligned collagen film along to the direction of the PTFE transfer. The collagen too demonstrated strong affinity to the 72-28 PVDF-TrFE / PTFE film in comparison to other copolymer of 65 and 51 VDF molar % compositions, hence making it favorable for used as coating in ferroelectric devices. The alignment of the collagen may be quantified using a high magnification polarized microscope with an attached compensator. The dielectric properties of the collagen may be investigated for further application in electro-mechanical applications.

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