Polyvinylidene fluoride doped with hydroxyapatite: cell growth and design of magnetoelectric stimulation

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***Abstract*— To promote tissue regeneration, functional polymer scaffolds mimicking the human extracellular matrix are in high demand. Piezoelectricity and hydrophilicity are key properties that enhance their performance. Polyvinylidene fluoride (PVDF), known for generating surface charges under mechanical stress, is a promising material for such scaffolds. However, hydrophilicity is crucial for proper cell adhesion and growth. This study produced PVDF nanofibers via electrospinning, seeded them with cells, and analyzed their surface using scanning electron microscopy (SEM). Furthermore, the scaffolds underwent cytocompatibility testing to confirm their suitability for supporting cell growth.**

Keywords*— *Scaffold, PVDF, HA, fiber, piezoelectricity

I. Introduction

Piezoelectric polymers, like polyvinylidene fluoride (PVDF), generate electrical voltage when mechanically stressed. PVDF is widely used due to its strong mechanical properties and high piezoelectric response, which can be enhanced by adding dopants like Fe_2O_3 for magnetic properties or hydroxyapatite (HA) for biocompatibility. These composites are promising for advanced tissue scaffolds that support cell growth and regeneration [1].

PVDF is particularly valued for its structural and electromechanical properties, including high melting point, mechanical strength, chemical resistance, and non-toxicity. However, its hydrophobic nature limits cell adhesion, which can be improved through surface treatments like oxygen plasma [2].

Electrospinning is a common method for producing PVDF fibers, allowing control over fiber diameter and crystalline phases. Doping with materials like $BiFeO_3$, TiO_2, or carbon nanotubes (CNTs) enhances piezoelectric and pyroelectric properties. Blending PVDF with CNTs can also improve electrical conductivity and piezoresistive responses under mechanical strain. Single-walled or multi-walled carbon nanotubes are used depending on the desired mechanical and electrical properties [2].

Electrical and magnetic fields influence biological processes. Both AC and DC fields can stimulate cells and promote tissue repair, with new electrode technologies showing positive effects on nerve cell growth. Magnetic fields also affect cell behavior. Weak fields can enhance cell membrane permeability, while stronger fields (>500 μT) promote cell growth. [4].

II. Materials and methods

A. Materials and their Parameters

Electrospun polyvinylidene fluoride (PVDF) fibers containing hydroxyapatite (HA) were fabricated using the electrospinning technique. The process employed a single-needle syringe emitter filled with a polymer solution and a cylindrical collector wrapped in aluminum foil. The polymer solution was delivered through the needle at a flow rate of 25μl/min. Simultaneously, the collector rotated at 2000 revolutions per minute, enabling the polymer jet to be drawn onto its surface, forming a thin layer of nanofibers. A voltage of 50 kV was applied between the emitter and the collector.

PVDF was electrospun in its β-phase. The polymer solution consisted of a 20 % PVDF concentration with the addition of either 5 % or 10 % HA particles. The final mixture was stirred and heated at 80 °C for 24 hours at a stirring speed of 200 rpm.

To obtain high-quality scanning electron microscopy images, the PVDF fiber surface was evenly coated due to its dielectric properties. A 16 nm carbon coating layer was applied to prevent surface charging. Without the coating, it would be difficult to focus on the nanofibers and scan them at high magnification.

B. Cultivation of the Cells

For cytotoxicity testing, HT 1080 (human fibrosarcoma) and K2 (rat sarcoma) cell lines were used. HT 1080 is a human fibrosarcoma cell line established in 1964 from a 35-years old male with a fibrosarcoma, a type of malignant soft tissue tumor. The K2 cell line originates from rat sarcoma and is derived from Rattus norvegicus. Cells were passaged twice a week at 80 %

confluency to maintain consistent growth and avoid contamination. They were cultured at 37 °C in a humidified incubator with 5 % CO_2.

After culturing, cells were separated into test tubes. Sterilized PVDF samples (1.5 cm × 1.5 cm) with 5 % and 10 % HA were prepared. In 60 mm Petri dishes, 30,000 cells were seeded with 4 ml of culture medium (MEM with 10 % FBS and antibiotics). The samples were submerged in the medium and incubated at 37 °C. Cell morphology was observed at 24, 48, and 72 hours using a phase-contrast microscope.

C. *Methods Used for Assessing Properties of the Samples*

1) Scanning Electron Microscopy (SEM): Surface imaging of PVDF fibers was performed using scanning electron microscopy (SEM). A secondary electron detector was selected for the analysis. The working distance between the electron source and the specimen was set to 20 cm, with a beam intensity of 3 keV. The electron beam's emission current was 223.38 µA, and the accelerating voltage was 5 kV. The microscope operated in resolution scan mode.

2) Raman Spectroscopy: Raman spectroscopy was used to identify the phases of PVDF. Analysis of the nanocomposite material, consisting of PVDF nanofibers with 5 % and 10 % HA particles, was conducted using a Nikon CFI S Plan Fluor ELWD objective with 40x magnification and a 380 nm excitation laser. The laser power was 4.9 mW, depending on the sample. The signal was obtained by averaging 50 accumulations, each with an integration time of 10 seconds.

III. Result

A. *Phase composition*

Raman spectroscopy analyzes how light interacts with chemical bonds in a material. By examining peak positions and relative peak intensities, it can reveal the material's chemical structure, crystalline phases, internal stress, contamination, and impurities.

Both samples with 5 % and 10 % HA particles, shown in Figure 1a and 1b and analyzed in the 100–2500 cm^{-1} range, exhibit the presence of the β-phase. Characteristic peaks include 510 cm^{-1} (CF_2, β-phase), 610 cm^{-1} (CF_2, α-phase), 795 cm^{-1} (CH_2 rocking, α-phase), 840 cm^{-1} (CH_2 rocking, β-phase), and 880 cm^{-1} (common to both phases). A peak at 1431 cm^{-1} corresponds to CH_2 bending vibrations [3].

Hydroxyapatite (HA) bonding is identified by phosphate (PO_4^{3-}) vibrations: the bending mode (O-P-O) at 430 cm^{-1}, the symmetric stretching mode (P-O) at 960 cm^{-1}, and the asymmetric stretching mode (P-O) at 1075 cm^{-1}. The (C=C) band appears at 1644 cm^{-1}, indicating structural modifications [5].

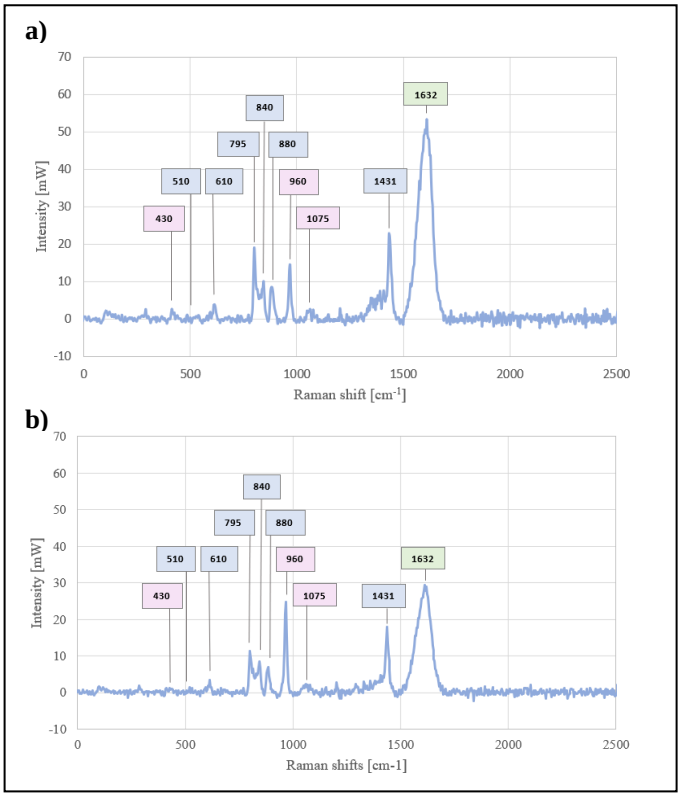

Fig. 1. Raman spectra of PVDF fiber with a) 5 % HA; b) 10 % HA particles

B. *Sample morphology*

SEM observations provided valuable insight into the complex relationship between the surface morphology of PVDF scaffolds, their physical properties, and the mechanical support they offer to growing osteoblastic cells.

Figure 2 presents SEM images of PVDF samples with 5 % and 10 % HA particles after electrospinning. The fibers appear continuous with few beads. In Figure 2a, a micrograph of fibers with 5 % HA particles is shown, using a backscattered electron detector to highlight the contrast between HA and PVDF fibers. Figure 2b show fibers with 10 % HA particles, demonstrating successful incorporation and good dispersion of HA in the membranes.

The average fiber diameter was measured to be approximately 500 nm. A slight increase in fiber thickness was observed with higher HA content, likely due to increased solution viscosity and the presence of solid particles, which can affect jet elongation during electrospinning.

C. *Cytotoxicity test*

Cytotoxicity testing of both samples returned a negative result, indicating that the materials were not cytotoxic under the experimental conditions. In all test groups, the cells demonstrated the ability to recover from initial stress (likely caused by handling or sample introduction) and proceeded to proliferate. This proliferation was observed both randomly across the culture dish and around the samples, suggesting that the materials did not hinder cell growth or attachment in any localized area.

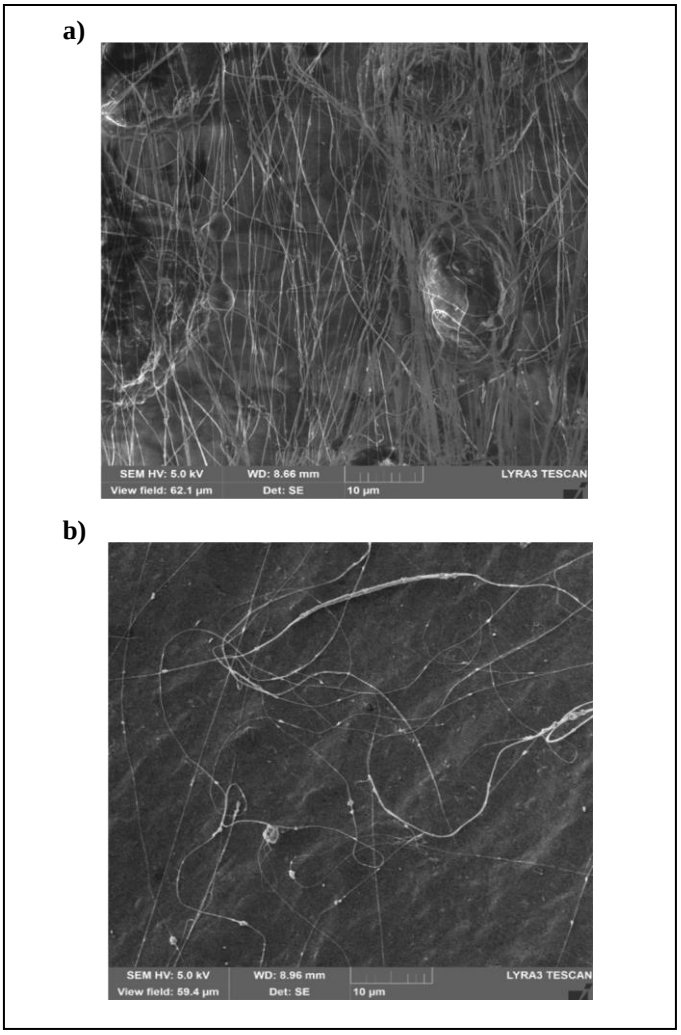

Fig. 2. Representative SEM images of the PVDF composites: a) with HA 5 % particles; b) with HA 10 % particles

Figure 3 presents the third microscopic evaluation after 72 hours, showing 100x magnification images of PVDF samples with 5 % and 10 % HA particles during cell growth.

The visual estimation of cell viability based on the images shows the following results: In Figure 3b, for PVDF + 5 % HA, K2 cells, the cells are well spread, clearly defined, and show no noticeable signs of apoptosis, with a minimal number of dead cells. The estimated viability is approximately 95–98 %. In Figure 3b for PVDF + 5 % HA, HT cells, the cells exhibit a high density and a generally healthy appearance, with slightly more variation in morphology than the K2 cells, but no significant cytotoxicity, resulting in an estimated viability of 90–95 %.

For Figure 3c, for PVDF + 10 % HA, K2 cells, the cells show a small increase in cell growth and proliferation, with larger isolated colonies scattered throughout the dish. The estimated viability is around 88–92 %. Lastly, Figure 3d for PVDF + 10 % HA, HT cells show full recovery, with at least a twofold increase in cell number and normal morphology observed, even near the sample. The estimated viability is 85–90 %.

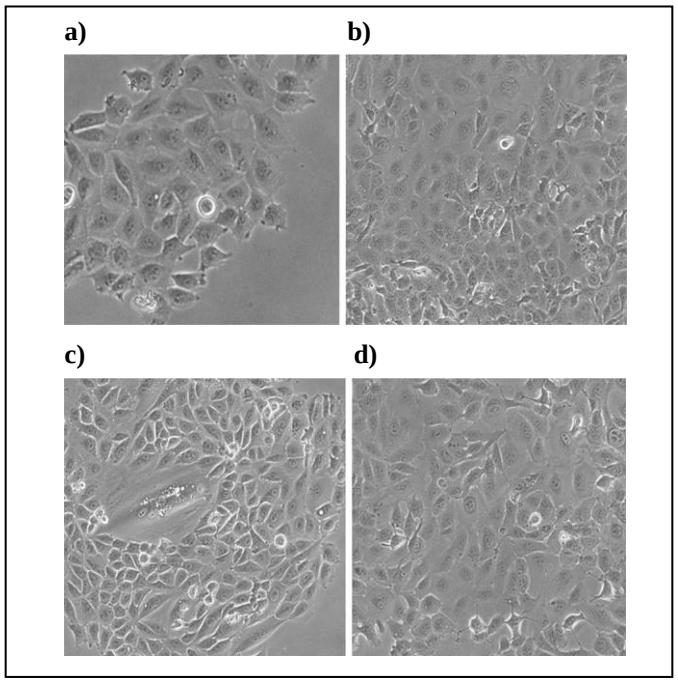

Fig. 3. Third microscopic evaluation: PVDF with HA 5 % particles a) with K2 cells, b) with HT cells; PVDF with HA 10 % particles c) with K2 cells, d) with HT cells.

IV. CONCLUSION

Polymeric electrospun microfiber mats were developed for tissue engineering, regenerative medicine, and drug delivery. PVDF nanofiber scaffolds with 5 % and 10 % HA particles were successfully produced using a single-fluid electrospinning method.

Physical and morphological measurements of the PVDF material were completed, with SEM images requiring a carbon coating to prevent surface charging. HA incorporation was satisfactory, with good particle dispersion throughout the membranes. The average diameter of the fibers was measured to be around 500 nm.

Raman spectroscopy, which analyzes light interactions with chemical bonds, revealed that both the 5 % and 10 % HA particle samples exhibit the β-phase. Characteristic Raman peaks for the α-phase include 610 cm^{-1} and 795 cm^{-1}, while the β-phase is confirmed by peaks at 510 cm^{-1} and 840 cm^{-1}. A common peak for both phases is observed at 880 cm^{-1}.

Cytocompatibility testing with HT 1080 and K2 cells demonstrated high cell viability and healthy morphology, highlighting their suitability for tissue engineering and regenerative medicine.

Future research could examine the interaction between the PVDF scaffold with a magnetic dopant, growing cell tissue, and exposure to an electromagnetic field.

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