Research

Cytotoxic effect of polyvinyl alcohol-magnetite composite

Karla Baca Ramos[1] · Imelda Olivas Armendáriz[1] · Christian Chapa González[1] · Vera A. Álvarez[2] · Perla E. García-Casillas[3]

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Abstract
Polymeric hydrogel is a promising candidate for drug delivery applications due to its ability to encapsulate and release drugs. Incorporating magnetic particles enables controlled and specific release, providing sustained and targeted delivery. This study aimed to assess the cytotoxicity of the magnetite-polyvinyl alcohol (MPVA) hydrogel, specifically its magnetite content, using 3T3 fibroblast cells. The findings indicate that the MPVA hydrogel with magnetite nanoparticles was compatible with the cells and did not induce cell death. Incorporating magnetite nanoparticles into the PVA hydrogel improved its thermal stability and degradation temperature, disrupting the chain order, decreasing melting behavior, and fractional crystallinity of the hydrogel. The MPVA hydrogel demonstrated a higher gel fraction and crosslink density compared to the PVA hydrogel due to the presence of magnetite nanoparticles. The interaction between PVA and magnetite nanoparticles occurred through non-covalent forces, allowing for reversible interactions and dispersion of the nanoparticles within the PVA matrix. Although the cytotoxicity of the MPVA gel was similar to that of the PVA gel, the viability of fibroblast cells within the MPVA gel varied depending on the concentration. The MPVA hydrogel exhibited stronger attachment and induced irregular changes on the cell surface compared to the PVA hydrogel. Furthermore, the MPVA gel displayed paramagnetic behavior and controllable magnetization, as demonstrated by the hysteresis loop. These magnetic properties make the MPVA gel suitable for potential biomedical applications, including drug delivery, tissue engineering, and magnetic resonance imaging (MRI) contrast agents.

Keywords Cytotoxicity · Hydrogel · Magnetite · Cell interaction · Polyvinyl Alcohol · Composite

1 Introduction

New delivery systems have emerged as promising alternatives to conventional treatments for drug delivery, aiming to enhance the effectiveness of drug delivery [1]. These innovative materials, such as hydrogels, nanoparticles, capsules, and micelles, have unique properties that enable efficient drug release and targeted delivery [2, 3].

Polymers are the most commonly used materials in drug delivery systems because they can remain in the bloodstream for an extended period. The ability of polymers to stay in the bloodstream for an ample time is often attributed to their size, shape, and surface properties, which can influence their circulation kinetics and biodistribution [4]. Polymers used in drug delivery systems can be designed to have a larger molecular weight and size, which can help them evade rapid clearance by the body's immune system and extend their circulation time. The prolonged circulation of polymers in the bloodstream can have several advantages for drug delivery. It can increase the bioavailability of drugs by extending their exposure time in the systemic circulation, allowing for a

✉ Perla E. García-Casillas, perla.garcia@ciqa.edu.mx | [1]Universidad Autónoma de Ciudad Juárez, Av. del Charro No. 450. Col. Partido Romero, 32310 Ciudad Juárez, Chihuahua, México. [2]Universidad del Mar de Plata, Solís 7575, 7600 Mar del Plata, Argentina. [3]Centro de Investigación en Química Aplicada, Blvd. Enrique Reyna Hermosillo No. 140, Saltillo, Coahuila, México.

more sustained therapeutic effect [5, 6]. It can also reduce dosing frequency, improving patient compliance and convenience [7, 8].

Hydrogels are three-dimensional networks of hydrophilic polymers that can hold a large amount of water, making them ideal for drug delivery. They can be designed to have specific characteristics, such as high biocompatibility, biodegradability, and responsiveness to external stimuli. This allows for the controlled release of drugs at the desired site of action [9, 10]. Hydrogels can be formulated into various forms, such as gels, films, or coatings, making them versatile for different administration routes, including oral, topical, and injectable. Using these nanostructures as drug delivery systems have shown promising results in enhancing the effectiveness of drugs. They offer advantages such as improved drug stability, enhanced bioavailability, controlled release, and targeted delivery, improving therapeutic outcomes and reducing side effects [11, 12]. However, additional research and development are necessary to optimize their properties, safety, and efficacy for clinical applications. Nonetheless, these innovative materials hold significant potential for revolutionizing drug delivery and improving patient outcomes in various disease treatments [13–15].

Polyvinyl alcohol (PVA) hydrogel is a type of polymer network with unique properties that make it suitable for drug delivery applications. One of the critical characteristics of PVA hydrogel is its ability to absorb and release a large amount of liquid, resulting in a swollen structure [16]. This high swelling capacity creates a hydrophilic environment, which can be advantageous in avoiding capture by phagocytes, immune cells that can remove foreign particles from the body [17, 18]. The rate of swelling of PVA hydrogels can be controlled by adjusting the resistance of the polymer to changes in volume. This means that the swelling behavior of PVA hydrogels can be manipulated to achieve desired drug release profiles. For example, modifying the PVA polymer's crosslinking density or molecular weight can adjust the swelling and drug release rate to meet specific therapeutic needs [19].

Controlled swelling of PVA hydrogels is optimal for drug delivery, as it allows for targeted drug release in specific tissues or organs. For instance, in localized drug delivery applications, such as drug delivery to tumors or inflamed tissues, PVA hydrogels can be designed to swell in response to specific environmental cues, such as changes in pH, temperature, or enzyme activity [20]. This can result in targeted drug release at the desired site, minimizing off-target effects and improving the therapeutic efficacy of the drug [21].

Moreover, PVA hydrogels can also be used with other drug delivery strategies, such as incorporating nanoparticles that respond to an external stimulus. This can further enhance the drug delivery capabilities of PVA hydrogels by providing controlled drug release and improving drug stability [22, 23]. Magnetite nanoparticles can generate a magnetic response under an external stimulus. One of the promising applications of magnetite nanoparticles is their incorporation into polymer structures to create "intelligent" drug delivery systems. These systems are designed to respond to an external magnetic field, allowing for controlled and targeted drug release in specific body areas [24]. The magnetite nanoparticles act as a trigger or "switch" that can be activated by an external magnetic field, releasing drugs from the polymer matrix. Magnetite has been widely used in drug delivery, demonstrating its ability to release anticancer drugs [25] effectively. Additionally, it has shown potential for the transfer of the neurotrophic factor that promotes the growth and survival of neurons in the brain [26–28].

The incorporation of magnetite nanoparticles into polymer structures can be achieved through various methods, such as physical mixing, chemical conjugation, or encapsulation during the fabrication process of the polymer matrix. The magnetite nanoparticles' size, shape, and surface properties can be carefully controlled to achieve desired drug delivery characteristics, such as release rate, drug loading capacity, and responsiveness to external magnetic fields [29, 30].

When an external magnetic field is applied to the polymer matrix containing magnetite nanoparticles, the magnetic response of the nanoparticles can cause changes in the polymer matrix, such as deformation, disruption, or disruption of the drug-loaded matrix. This can result in the release of drugs from the polymer matrix in a controlled and targeted manner, allowing for precise drug delivery to a specific area of the body [31]. Due to their magnetic properties, magnetite nanoparticles can also serve as contrast agents for evaluating the status of tissues and organs during drug release [32]. Several advantages make this approach promising for improving drug delivery in various biomedical applications. These include controlled drug release, targeted drug delivery, enhanced drug stability, non-toxicity and biocompatibility, versatility, and non-invasiveness. However, further research and development are needed to optimize this technology and ensure its safety and efficacy for clinical translation [33, 34]. One of the most important methods for determining biocompatibility is studying the response of tissue-polymer interaction in vitro using cell culture techniques [35, 36].

In general, PVA is considered biocompatible and has low cytotoxicity. It is widely used in biomedical applications due to its non-toxicity and biocompatibility. However, adding magnetite nanoparticles to PVA hydrogel may affect its cytotoxicity. Magnetite nanoparticles are commonly used in drug delivery systems and have been

studied for their cytotoxicity. While they are generally considered biocompatible, they may induce cytotoxic effects at high concentrations or prolonged exposure.

The cytotoxicity of MPVA depends on several factors. These include the PVA concentration, the magnetite nanoparticles' concentration and size, and the exposure duration. It's important to note that the cytotoxicity may vary depending on the specific formulation, experimental conditions, and cell types used in the study. Comprehensive biocompatibility and cytotoxicity assessments following relevant guidelines and standards are necessary. Careful evaluation of the specific PVA hydrogel and magnetite nanoparticle formulation intended for biomedical application is also essential. This paper describes how magnetic particles change the behavior of PVA hydrogels and their interaction with 3T3 fibroblasts.

2 Results y discussion

The molecular weight of polyvinyl alcohol (PVA) used in the hydrogel synthesis significantly impacts the properties of the resulting hydrogel [37]. When a high molecular weight PVA, such as 155,000 g/mol, is dissolved in water, it forms a highly viscous solution that can make the in-situ synthesis of magnetite nanoparticles difficult. This can result in an inhomogeneous distribution of nanoparticles within the hydrogel. On the other hand, when a lower molecular weight PVA, such as 40,000 g/mol, is used, a higher number of freeze–thaw cycles may be required to achieve proper crosslinking of the hydrogel. The behavior observed in this study aligns with Sonker's findings, where it was demonstrated that PVAs with lower molecular weights tend to have lower viscosity. As a result, this lower viscosity can lead to a decrease in cross-linking density. To compensate for this, additional freeze–thaw cycles may be required to achieve the desired cross-linking level [38]. An average molecular weight PVA is often used to strike a balance between the solution's viscosity and the resulting hydrogel's crosslinking density. It provides an optimal viscosity that allows for a better distribution of nanoparticles during in-situ synthesis while also achieving the appropriate crosslinking density required to reach an equilibrium swelling level in an aqueous solution.

Choosing the appropriate molecular weight of PVA is an essential consideration in hydrogel synthesis, as it can significantly impact the final properties of the hydrogel, including its homogeneity, crosslinking density, and swelling behavior [10].

The properties of magnetic polyvinyl alcohol (MPVA) gel depend on the content of magnetite nanoparticles. Table 1 shows differences in physical properties between PVA gel and MPVA gel. One notable difference is the degradation temperature (Td), which is higher in MPVA gel compared to PVA gel by 13 degrees. This suggests that the presence of magnetite nanoparticles may enhance the thermal stability of the hydrogel, resulting in a higher degradation temperature. However, the increased degradation temperature of MPVA gel is attributed to the reinforcing effect of the nanoparticles on the swelled gel structure. The presence of magnetite nanoparticles can alter the degradation behavior of the hydrogel, potentially leading to slower degradation [30]. The melting temperatures (T_m) of the hydrogels show an inverse relationship, indicating that the addition of magnetite nanoparticles decreases the melting behavior of the hydrogel. The fractional crystallinity (X_{cr}) also decreases with the addition of magnetite, suggesting a lower formation of crystalline regions in MPVA gel than in PVA gel. This may be due to the disruption of chain ordering of PVA caused by the presence of nanoparticles, as noted by Paradossi et al. in their study [39]. It is also mentioned that hydrogels are not homogeneous and can have regions with different degrees of swelling. High crosslink density and low swelling regions in hydrogels can indicate cluster formations, as reported by Drumheller and Hubbell [40].

Table 1 Physical parameters of PVA and MPVA

	T_d (°C)	T_m (°C)	X_{cr} %	GF %
PVA	282.5 ± 0.7	224.7 ± 1.1	40.3 ± 0.9	74.5 ± 2.2
MPVA	295.6 ± 3.5	213.2 ± 0.8	31.7 ± 0.2	84.0 ± 1.1

Figure 1 indicates that PVA's crosslink density is higher than MPVA, resulting in less swelling of PVA hydrogel. Crosslink density refers to the degree of chemical or physical crosslinking within a hydrogel network, which can affect its swelling behavior [41].

In this case, the polymer's gel fraction (GF) is used as an indicator of its crosslinking density. The gel fraction refers to the portion of the polymer that remains insoluble in a solvent after swelling or degradation, and it is often used to estimate the degree of crosslinking in a hydrogel.

Ricciani determined that the swelling is influenced by the degree of crosslinking in the PVA hydrogel. MPVA exhibited a higher gel fraction (GF) than PVA in this case, indicating a higher crosslinking density in the MPVA hydrogel. The increased crosslinking density in the MPVA hydrogel can be attributed to magnetite nanoparticles, which facilitate crosslinking and result in a more densely cross-linked network. This higher crosslinking density in the MPVA hydrogel reduces swelling behavior compared to the PVA hydrogel, as observed in Fig. 1 [41].

In hydrogels, the swelling mechanism can be described as a diffusion process followed by a relaxation process. The initial swelling rate is determined by the diffusion of water

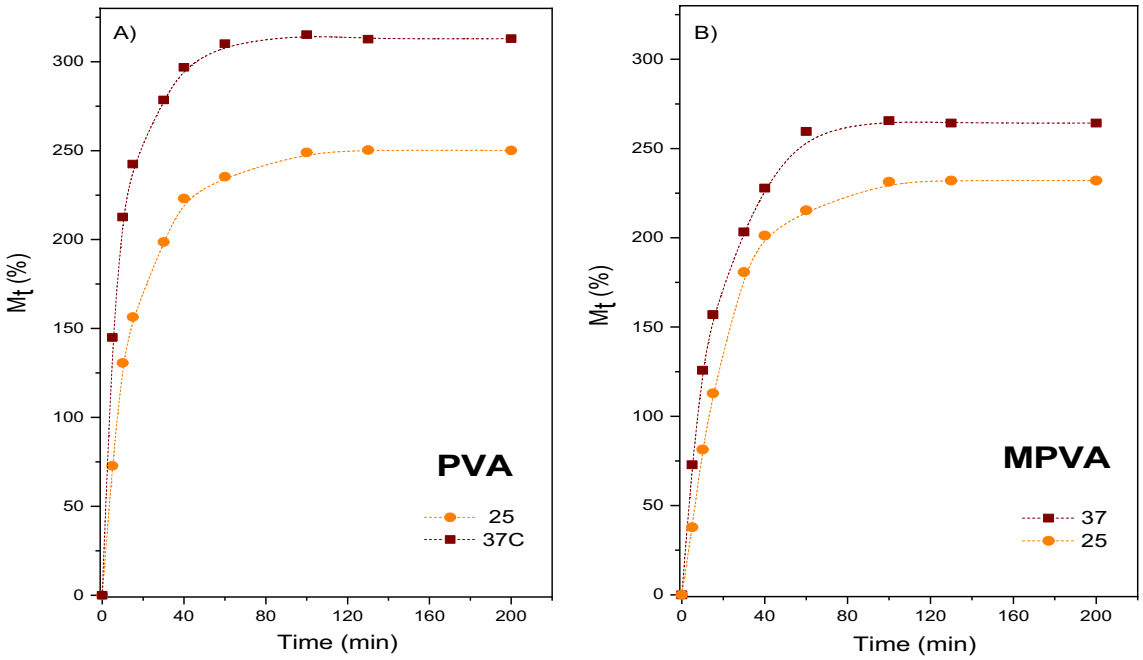

Fig. 1 PVA and MPVA gel swelled in a physiological fluid at 25 y 37 °C

into the hydrogel network, followed by a slower absorption process involving the relaxation of the polymeric chains [42, 43].

According to Sirousazar [44], the strength of hydrophobic interactions increases with temperature, leading to the contraction of hydrogels through polymer chain association. The swelling behavior of hydrogels is temperature-sensitive due to the association/dissociation of hydrogen bonds. This behavior has been observed in PVA and MPVA hydrogels in physiological fluid, as depicted in Fig. 1A and B. Higher temperatures result in greater relaxation of polymeric chains and increased swelling in PVA hydrogel. In contrast, the presence of magnetic nanoparticles in MPVA hydrogel restricts the mobility of PVA chains, limiting the overall swelling behavior, as shown in Fig. 1B.

The X-ray diffraction (XRD) pattern of PVA and MPVA gels is shown in Fig. 2. The characteristic diffraction peaks of semi-crystalline PVA are observed at 19.8° and 22.9°, corresponding to the reflection planes (1 0 1) and (1 0 $\bar{1}$), respectively [45]. These peaks indicate the presence of crystalline regions in the PVA matrix. In the XRD pattern of MPVA, an additional peak at 35.5° appears, which can be attributed to the diffracted plane (3 1 1) of the magnetite nanoparticles, confirming the presence of iron oxides in the PVA matrix. This suggests that the magnetite nanoparticles are dispersed within the PVA matrix and have a crystalline structure. The full width at half maximum (FWHM) of the most intense peak of magnetite can be used to estimate the crystallite size using the Scherrer formula. In

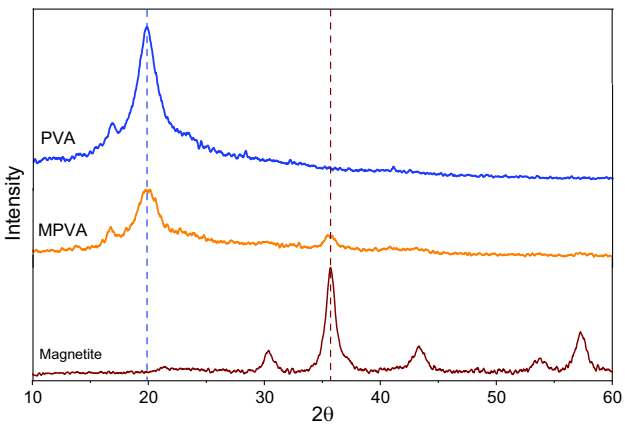

Fig. 2 Diffraction patterns of PVA an MPVA

this case, a crystallite size of 8 nm was obtained, indicating that the magnetite nanoparticles in the MPVA gel have a relatively small crystalline size.

As confirmed by the XRD pattern, the incorporation of magnetite nanoparticles in the MPVA gel indicates their potential impact on the hydrogel's crystalline structure and properties. Similar findings were observed by Mahmood [46], who determined that the inclusion of iron oxide nanoparticles (IONPs) in the PVA-PVP copolymer matrix substantially enhanced the material's mechanical strength. This enhancement could have significant

implications for the hydrogel's overall performance and potential applications.

The composition of magnetite nanoparticles gives them a net charge on their surface, which enables them to interact with PVA polymer chains through electrostatic and van der Waals forces. Panja calculated the van der Waals interaction energy for nanoparticles of different sizes (5 nm and 15 nm). The van der Waals interaction energy is slightly higher than the dipole–dipole interaction energy [47]. Therefore, in this case, the van der Waals interaction contributes to explaining the dispersion behavior of Fe3O4 magnetic nanoparticles in PVA. Notably, the FTIR analysis did not reveal the presence of a new band, indicating the absence of covalent bonding between the nanoparticles and PVA. Instead, the interaction primarily relies on non-covalent forces, facilitating reversible interactions and the potential dispersal of nanoparticles within the PVA matrix.

In the structure of magnetite (Fe_3O_4), the iron ions (Fe_{2+} and Fe_{3+}) represent the positive components, which are particularly attractive for interactions with the hydroxyl groups (-OH) present in PVA. These hydroxyl groups can establish hydrogen bonds with the functional groups on the nanoparticle surface, facilitating the dispersion of nanoparticles within the polymer matrix.

Incorporating nanoparticles and PVA chains induce significant modifications in the properties of the resulting MPVA composite, especially in terms of its mechanical characteristics [46]. The nanoparticles serve as reinforcing agents, providing structural support to the polymer matrix and resulting in improved mechanical strength, increased tensile resistance, and enhanced elasticity.

Furthermore, the addition of nanoparticles can influence the thermal and magnetic properties of the composite [48]. For instance, magnetite nanoparticles can alter the composite's thermal conductivity, thereby impacting its heat transfer capabilities.

The interaction between PVA and magnetite nanoparticles also affects the magnetic response of the MPVA composite. The presence of magnetite nanoparticles allows for manipulating and controlling the material's magnetic properties, offering the opportunity for external regulation by applying a magnetic field [30]. This particular characteristic makes MPVA a promising candidate for various applications, such as controlled and prolonged drug release systems, where the release of drugs can be precisely regulated using an external magnetic field.

Figure 3 shows the magnetic hysteresis loop of MPVA, which provides information about the magnetic properties of the gel. The coercive force (Hc) of MPVA is negligible at 1.5 Oe, indicating a paramagnetic characteristic. This suggests that the MPVA gel is not firmly magnetized and does not retain any significant magnetization after removing the magnetic field, which could be advantageous for biomedical applications where minimal residual magnetization is desired [49].

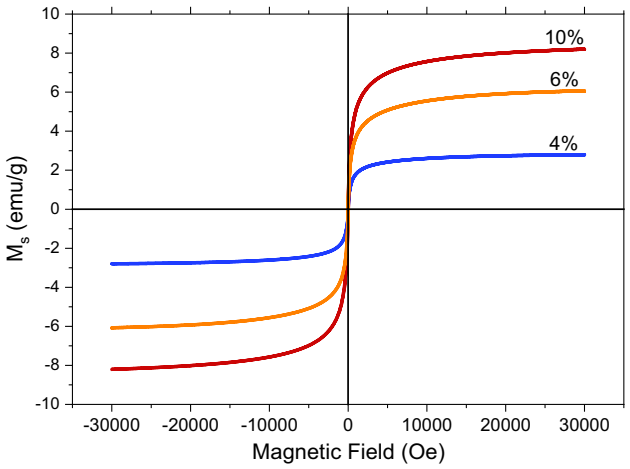

Fig. 3 Magnetic hysteresis loop for MPVA

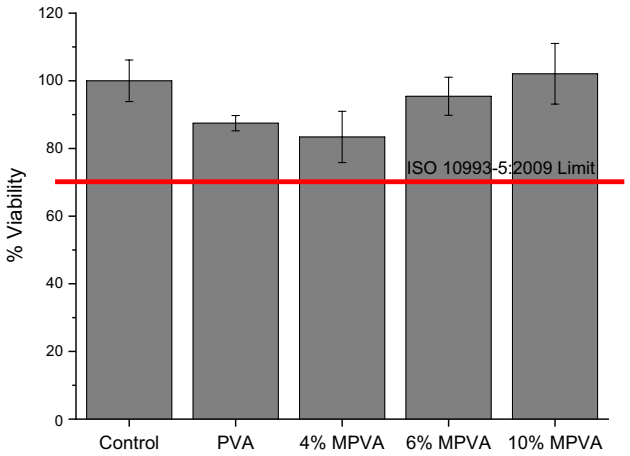

Fig. 4 Viability of PVA and MPVA

The magnetic saturation (M_s) of MPVA, representing the maximum magnetization that can be achieved in the presence of a magnetic field, increases with the magnetite content. For MPVA gels with 4%, 6%, and 10% iron oxide content, the M_s values are 3.2, 5.9, and 8.2 emu/g, respectively. This indicates that the magnetization of the MPVA gel can be controlled by varying the magnetite content, with higher magnetite content resulting in higher magnetic saturation.

Figure 4 shows that PVA gel exhibited lower cell viability than the control group when evaluated with spindle-shaped fibroblast cells (3T3 fibroblast). The PVA gel was sterilized with UV irradiation, which caused dehydration of the gel. Upon rehydration with culture media, the reduced culture media used to hydrate the gel may have inhibited

cell proliferation, resulting in lower cell viability. Hydrating the hydrogel is an inherent characteristic and an essential aspect of its final application. Therefore, evaluating this condition is necessary to assess any potential cell death caused by the nature of the material. According to the ISO 10993–5:2009 standard, a cell viability value above 75% is considered non-cytotoxic. Based on this standard and the variance value obtained, the difference in cell viability between PVA and MPVA gel is negligible, suggesting that incorporating magnetic nanoparticles into the polymeric network does not significantly affect cytotoxicity [50].

The cell viability of MPVA gel with 3T3 fibroblast cells depends on the magnetite concentration. 4% MPVA gel and PVA gel showed lower cell viability than the control group. A study by Chapa Gonzalez et al. (2014) suggested that the slightly basic pH of physiological fluids could cause oxidation/reduction of magnetite, generating iron ions that may induce cell death. However, cell viability increased with higher magnetite content, with 10% MPVA gel showing higher cell viability than the control group [51]. This suggests that the presence of magnetite nanoparticles may have a concentration-dependent effect on cell viability.

During the degradation of polymers, chemical disintegration of the molecule occurs, resulting in lower molecular weight fractions. In the case of PVA, degradation in the presence of -OH and -H groups can lead to the formation of ketones and enols [52]. This process can alter the production of reactive oxygen species (ROS), which are chemical species containing oxygen that are produced intracellularly through multiple mechanisms and can have various effects on cells and tissues [53]. Excessive ROS production has been implicated in mitochondrial DNA mutations, aging, and cell death [54]. Magnetite nanoparticles in MPVA gel may strengthen the gel structure and decrease swelling, resulting in a higher crosslink density and slower degradation, which could lead to less abrupt changes in ROS levels. This behavior may be consistent with an increasing incubation time of the cell culture, as shown in Fig. 5, where cell viability is slightly lower at 48 h but not significantly different according to the Student's t-test ($p = 0.05$) compared to other incubation times and materials. Further studies may be needed to fully understand the impact of magnetite content and degradation behavior of MPVA gel on cell viability and ROS production.

The average size of fibroblasts can vary depending on different factors, such as species, age, and physiological state of the cells. Generally, fibroblasts tend to have a size ranging from 20 to 50 µm (µm) diameter [55]. In Fig. 6A, the fibroblasts attached to the hydrogel surface measure approximately 35 µm and exhibit an elongated, spindle-shaped morphology due to their adaptation to the production and remodeling of the extracellular matrix.

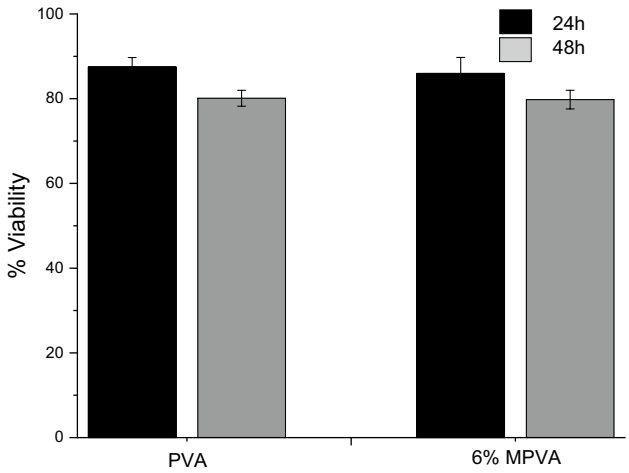

Fig. 5 Viability cell of PVA and MPVA at 24 y 48 h

Figure 6B shows 10 microns of smooth rounded fibroblast after being incubated with PVA hydrogel. In the cell adhesion process, an initial adsorption step occurs; fibroblasts can swell and adopt a more rounded shape due to the influx of fluids into the cell, relaxation of the cytoskeleton, or changes in osmotic pressure.

Subsequently, cell adhesion initiates, and the hydrogel components generate surface charges or functional groups that facilitate interaction with the fibroblast cell membrane. Chen's research revealed that hydrophobic groups facilitate cell adhesion to the polymer. These interactions enable the formation of cell–matrix junctions and promote cell proliferation and differentiation on biomaterial surfaces. These interactions can occur through electrostatic forces or chemical affinity, as described by Chen [56].

Receptors on the fibroblast's cell membrane, such as integrins, can recognize and bind to ligands present on the hydrogel surface. These receptor-ligand interactions facilitate a more robust and stable attachment between the fibroblast and the MPVA hydrogel [57]. After 48 h of interaction, Fig. 6C shows an irregular fibroblast surface with multiple bulges. The properties of the hydrogel and cellular polarity changes can influence this change in shape. The rigidity or surface irregularities of the PVA hydrogel can play a role in determining cell shape. A softer substrate allows cell reorganization and contraction, forming a rounded form. Additionally, the surface ligands present in the hydrogel have the potential to influence cellular polarity [56].

The interaction between actin filaments and microtubules plays a vital role in maintaining proper cell shape; any alterations in the distribution of actin filaments and microtubules can lead to changes in cell shape, causing the components of the cytoskeleton to contract and reorganize, resulting in a more rounded cell shape

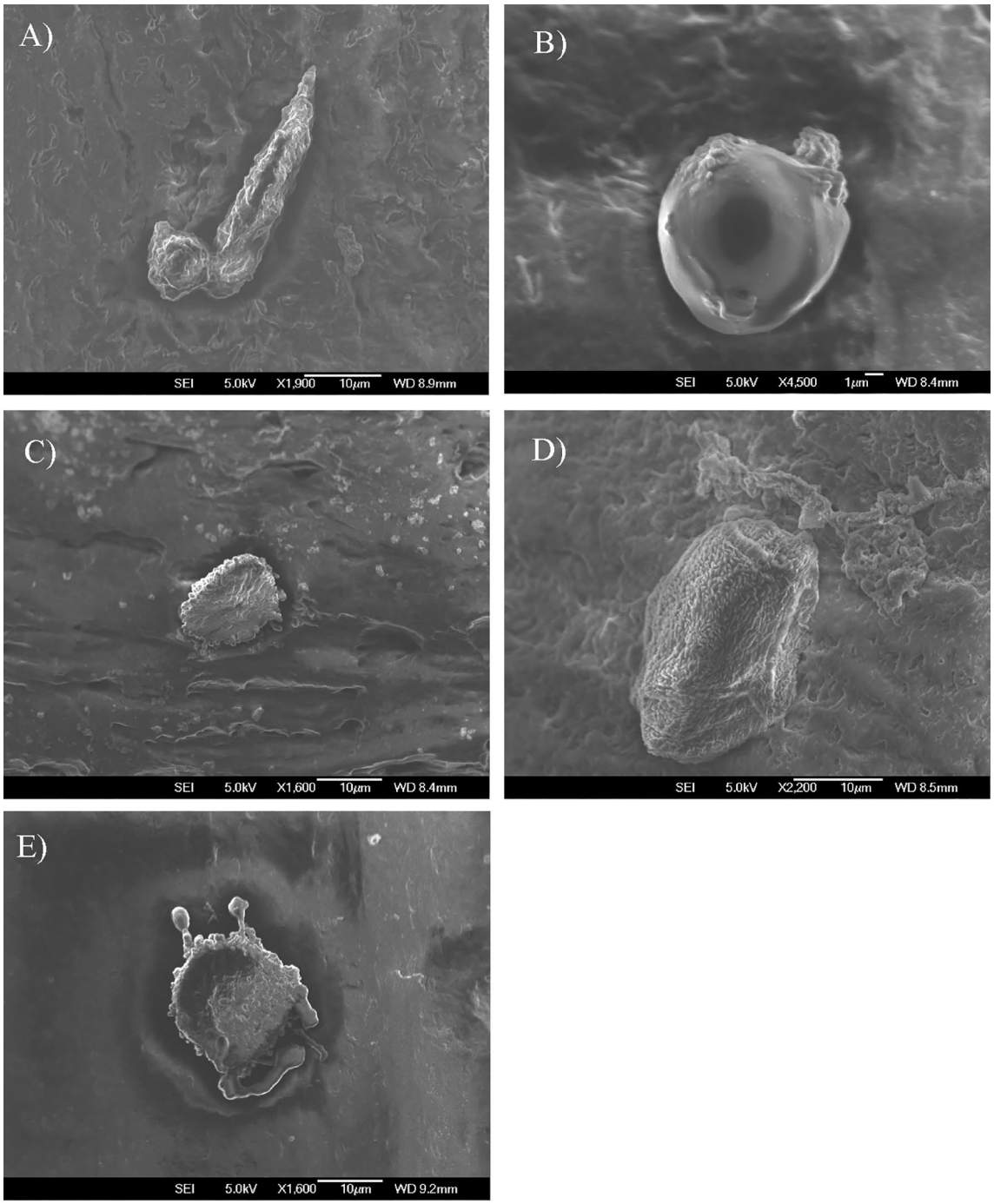

Fig. 6 **A** fibroblast onto PVA hydrogel, Fibroblast after being incubated for **B** 24 and **C** 48 h with a PVA hydrogel, Fibroblasts onto MPVA after **D** 24 and **E** 48 h of incubation

[58]. When the fibroblasts were incubated with MPVA, Fig. 6D shows that the cells exhibited a swollen appearance after 24 h, with a size of approximately 30 microns. This size was double the size of the cells incubated with PVA alone. However, unlike the pure PVA condition, the cells had no rounded shape. After 48 h of incubation, the incubated cells exhibit an irregular shape with protrusions measuring approximately 15 microns in size (Fig. 6E).

The interaction of the MPVA with fibroblasts can trigger a process of oxidative stress and cellular damage. The magnetite present in the composite can generate reactive oxygen species, producing free radicals that damage cellular components such as lipids, proteins, and DNA.

This cumulative cellular damage can provoke an inflammatory response and lead to cell death through necrosis. Necrosis is characterized by cellular collapse, inflammation, and the release of intracellular content into the surrounding environment [59, 60]. Cells undergoing necrosis often exhibit increased size and a swollen appearance, as shown in Fig. 6B and D. As necrosis progresses, the plasma membrane of the cells may rupture and lose its integrity. This can result in the release of cellular contents into the extracellular environment, triggering an inflammatory response.

On the other hand, the interaction of the MPVA can also activate cell death signaling pathways, such as apoptosis. Oxidative stress and cellular damage can trigger an internal signaling cascade that leads to caspase activation and DNA fragmentation. Apoptosis is a programmed and orderly form of cell death in which cells shrink, the nucleus condenses, and apoptotic bodies are formed. These apoptotic bodies can be phagocytosed without eliciting a significant inflammatory response. To precisely determine the mechanism of cell death, further studies, such as applying a flow cytometry assay demonstrated by Albukhaty [61], are required.

3 Conclusion

- The presence of magnetite nanoparticles in the PVA hydrogel enhances its thermal stability and degradation temperature. These nanoparticles also disrupt the chain order and decrease the hydrogel's melting behavior and fractional crystallinity.
- The crosslinking density of the PVA hydrogel is higher than that of the MPVA hydrogel, resulting in less swelling. While higher temperatures promote a more significant bump in the PVA hydrogel, the presence of magnetic nanoparticles restricts the swelling behavior of the MPVA hydrogel.
- The MPVA gel exhibits a higher gel fraction and crosslink density than the PVA gel due to the incorporation of magnetite nanoparticles.
- The interaction between PVA and magnetite nanoparticles occurs through non-covalent forces, allowing for reversible interactions and dispersion of the nanoparticles within the PVA matrix.
- The cytotoxicity of the MPVA gel is similar to that of the PVA gel. However, the cell viability of the MPVA gel with fibroblast cells may vary depending on the concentration.
- Fibroblast cells demonstrate different morphologies and interactions with PVA and MPVA hydrogels, with the MPVA hydrogel promoting stronger attachment and irregular changes on the cell surface.
- The magnetic properties of the MPVA gel, as revealed by the hysteresis loop, indicate its paramagnetic behavior and controllable magnetization. This makes it suitable for potential biomedical applications, such as drug delivery, tissue engineering, or magnetic resonance imaging (MRI) contrast agents.

3.1 Experimental method

PVA gel was prepared by dissolving PVA (CAS 9002-89-5; M_w 89,000-98,000; Sigma Aldrich; Germany) in deionized water with a concentration of 10% w/v and heating the solution at 80 °C for 4 h with constant magnetic stirring. After cooling, the solution was placed in nonstick molds and underwent three freeze–thaw cycles to achieve crosslinking.

MPVA gel was prepared by obtaining magnetite nanoparticles in-situ within the PVA matrix. Iron (III) chloride hexahydrate (CAS 10025-77-1; Sigma Aldrich; Germany) and Iron (II) sulfate heptahydrate (CAS 7782-63-0; Sigma Aldrich; Germany) were added to the PVA solution in a ratio of 1:2 and heated at 80 °C for 4 h. After cooling to 60 °C, Ammonium hydroxide (CAS1336-21-6; Sigma Aldrich; Germany) was added to adjust the pH to 10 and achieve magnetite nanoparticles. The MPVA resulting gel was cooled, placed in nonstick molds, and cross-linked through 3 freeze–thaw cycles.

Cytotoxicity of the MPVA gels on the 3T3 fibroblast cell line was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT; CAS 298-93-1; Sigma Aldrich; Germany). 3×10^4 cells were seeded in a 24-well microplate with a 0.5×0.5 cm polymer composite sample. The cells were incubated for 24 h and 48 h. After incubation, the media was removed, and the samples were washed with phosphate-buffered saline ($10 \times$ PBS; Sigma Aldrich). 50 µl of MTT solution (5 mg/ml) and 450 µl of Dulbecco's Modified Eagle's Medium–high glucose (D-MEM; D5796 Sigma Aldrich; Germany) media supplemented with fetal bovine serum (FBS; F8067 Sigma Aldrich; Germany) and solution Stabilized, with 5,000 unit's penicillin–streptomycin (Sigma Aldrich; Germany) were added to each well. The cells were incubated for 3 h at 37 °C in a humidified atmosphere with 5% CO2 in a D180-P-Co2 incubator. Next, dimethyl sulfoxide (DMSO; CAS67-68-5; Sigma Aldrich; Germany)) was added to the cells to dissolve the formazan product, and the microplate was gently shaken for 20 min. The absorbance of the solution was measured at a wavelength of 570 nm using a Benchmark Plus microplate spectrophotometer. Control was used, and each experiment was repeated three times for reliable results.

Authors contributions Conceptualization, PEGC; methodology, IOA and VAA; formal analysis, KBR and PEGC; investigation, KBR and CCCh writing—review and editing, KBR and PEGC

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Availability of data and materials Data is contained within the article.

Declarations

Conflict of interest There are no economic or personal interests.

References

1. Rosenblum D, Joshi N, Tao W, Karp JM, and Peer D (2018) Progress and challenges towards targeted delivery of cancer therapeutics, In Nature Communications, 9(1) (2018).
2. Tiwari G, Tiwari R, Bannerjee S, Bhati L, Pandey S, Pandey P, Sriwastawa B (2012) Drug delivery systems: an updated review. Int J Pharm Invest 2(1):2
3. Abasian P, Ghanavati S, Rahebi S, Nouri Khorasani S, Khalili S (2020) Polymeric nanocarriers in targeted drug delivery systems: a review. Polym Adv Technol 31(12):2939–2954
4. Nasongkia N, Chen B, Macaraeg N, Fox ME, Fréchet JMJ, Szoka FC (2009) Dependence of pharmacokinetics and biodistribution on polymer architecture: effect of cyclic versus linear polymers. J Am Chem Soc 131(11):3842–3843
5. Vilar G, Tulla-Puche J, Albericio F (2012) Polymers and drug delivery systems. Curr Drug Deliv 9(4):1–28
6. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin HS (2018) Nano based drug delivery systems: recent developments and prospects. J Nanobiotechnol 16:1
7. Ekladious I, Colson YL, Grinstaff MW (2019) Polymer–drug conjugate therapeutics: advances, insights, and prospects. Nat Rev Drug Discov 18(4):273–294
8. Jain D, Raturi R, Jain V, Bansal P, Singh R (2011) Recent technologies in pulsatile drug delivery systems. Biomatter 1(1):57–65
9. Willner I (2017) Stimuli-controlled hydrogels and their applications. Acc Chem Res 50(4):657–658
10. Hoffman AS (2013) Hydrogels for biomedical applications. Adv Drug Deliv Rev 65:10–16
11. Bordbar-Khiabani A, Gasik M (2022) Smart hydrogels for advanced drug delivery systems. J Mol Sci 23(7):23073665–23073716
12. Zeb A, Gul M, Nguyen TTL, Maeng HJ (2022) Controlled release and targeted drug delivery with poly(lactic-co-glycolic acid) nanoparticles: reviewing two decades of research. J Pharm Investig 52(6):683–724
13. Jo MJ, Jin IS, Park CW, Hwang BY, Chung YB, Kim JS, Shin DH (2020) Revolutionizing technologies of nanomicelles for combinatorial anticancer drug delivery. Arch Pharm Res 43(1):100–109
14. Mchenry ME, Laughlin DE (2000) Nano-scale materials development for future magnetic applications. Acta Mater 48(1):223–238
15. Berry CC, Curtis ASG (2003) Functionalisation of magnetic nanoparticles for applications in biomedicine. In *J Phys D: Appl Phys,* 36.
16. Wei L, Cai C, Lin J, Chen T (2009) Dual-drug delivery system based on hydrogel/micelle composites. Biomaterials 30(13):2606–2613
17. Cavalieri F, Chiessi E, Villa R, Viganò L, Zaffaroni N, Telling MF, Paradossi G (2008) Novel PVA-based hydrogel microparticles for doxorubicin delivery. Biomacromol 9(7):1967–1973
18. Howard KA, Dash PR, Read ML, Ward K, Tomkins LM, Nazarova O, Ulbrich K (2000) Infuence of hydrophilicity of cationic polymers on the biophysical properties of polyelectrolyte complexes formed by self-assembly with DNA. Seymour, Biochimica et Biophysica Acta (BBA) 1475(3):245–255
19. Peppas NA, Bures P, Leobandung W, Ichikawa H (2000) Hydrogels in pharmaceutical formulations. Eur J Pharm Biopharm 50(1):27–46
20. Abdullah O, Usman Minhas M, Ahmad M, Ahmad S, Barkat K, Ahmad A (2018) Synthesis, optimization, and evaluation of polyvinyl alcohol-based hydrogels as controlled combinatorial drug delivery system for colon cancer. Adv Polym Technol 37(8):3348–3363
21. Usta A, Asmatulu R (2016) Synthesis and analysis of electrically sensitive hydrogels incorporated with cancer drugs. J Pharm Drug Deliv Res 5:2
22. Akhlaq M, Azad AK, Ullah I, Nawaz A, Safdar M, Bhattacharya T, Uddin ABMH, Abbas SA, Mathews A, Kundu SK, Miret MM, Ananda Murthy HC, Nagaswarupa HP (2021), Methotrexate-loaded gelatin and polyvinyl alcohol (Gel/pva) hydrogel as a ph-sensitive matrix. *Polymers*, *13*(14)
23. Chen T, Hou K, Ren Q, Chen G, Wei P, Zhu M (2018) Nanoparticle-polymer synergies in nanocomposite hydrogels: from design to application. Macromol Rapid Commun 39:21
24. Sun Y, Duan L, Guo Z, Duanmu Y, Ma M, Xu L, Zhang Y, Gu N (2005) An improved way to prepare superparamagnetic magnetite-silica core-shell nanoparticles for possible biological application. J Magn Magn Mater 285(1–2):65–70
25. Bajpai AK, Gupta R (2011) Magnetically mediated release of ciprofloxacin from polyvinyl alcohol based superparamagnetic nanocomposites. J Mater Sci–Mater Med 22(2):357–369
26. Ebadi M, Buskaran K, Bullo S, Hussein MZ, Fakurazi S, Pastorin GA (2021) Drug delivery system based on magnetic iron oxide nanoparticles coated with (*polyvinyl alcohol-zinc/aluminium-layered double hydroxide-sorafenib*). Eng J 60(1):733–747
27. Albukhaty S, Naderi-Manesh H, Tiraihi T, Sakhi Jabir M (2018) Poly-l-lysine-coated superparamagnetic nanoparticles: a novel method for the transfection of pro-BDNF into neural stem cells. Artif Cells, Nanomed Biotechnol 46(3):S125–S132
28. Al-Musawi S, Ibraheem S, Mahdi SA, Albukhaty S, Haider AJ, Kadhim AA, Kadhim KA, Kadhim HA, Al-Karagoly H (2021) Smart nanoformulation based on polymeric magnetic nanoparticles and vincristine drug: a novel therapy for apoptotic gene expression in tumors. Life 11(1):1–12
29. Bertoglio P, Jacobo SE, Daraio ME (2010) Bertoglio. J Appl Polym Sci 115(3):1859–1865
30. Oliveira PN, Bini RD, Dias GS, Alcouffe P, Santos IA, David L, Cótica LF (2018) Magnetite nanoparticles with controlled sizes

via thermal degradation of optimized PVA/Fe(III) complexes. J Magn Magn Mater 460:381–390
31. Liao J, Huang H (2020) Review on magnetic natural polymer constructed hydrogels as vehicles for drug delivery. Biomacromol 21(7):2574–2594
32. Textor SC, Glockner JF, Lerman LO, Misra S, McKusick MA, Riederer SJ, Grande JP, Gomez SI, Carlos JC (2008) The use of magnetic resonance to evaluate tissue oxygenation in renal artery stenosis. J Am Soc Nephrol 19(4):780–788
33. Ghanbari M, Shamspur T, Fathirad F (2017) In situ preparation of magnetic Fe3 O4 nanoparticles in presence of PLGA and PVA as magnetite nanocarrier for targeted drug delivery. J Pharm Drug Deliv Res 06:02
34. Shagholani H, Ghoreishi SM, Mousazadeh M (2015) Improvement of interaction between PVA and chitosan via magnetite nanoparticles for drug delivery application. Int J Biol Macromol 78:130–136
35. Jung K, Corrigan N, Wong EHH, Boyer C (2022) Bioactive synthetic polymers. Adv Mater 34:2
36. Rodríguez-Rodríguez R, García-Carvajal ZY, Jiménez-Palomar I, Jiménez-Avalos JA, Espinosa-Andrews H (2019) Development of gelatin/chitosan/PVA hydrogels: thermal stability, water state, viscoelasticity, and cytotoxicity assays. J Appl Polym Sci 136:10
37. Hdidar M, Chouikhi S, Fattoum A, Arous M (2017) Effect of hydrolysis degree and mass molecular weight on the structure and properties of PVA films. Ionics 23(11):3125–3135
38. Sonker AK, Rathore K, Nagarale RK, Verma V (2018) Crosslinking of polyvinyl alcohol (PVA) and effect of crosslinker shape (aliphatic and aromatic) thereof. J Polym Environ 26(5):1782–1794
39. Paradossi G, Cavalieri F, Chiessi E, Spagnoli C, Cowman MK (2003) Poly(vinyl alcohol) as versatile biomaterial for potential biomedical applications. J Mater Sci Mater Med 14(8):684–691
40. Drumhellere PD, Hubbell JA (1995) Densely crosslinked poly(ethylene glycol) polymer networks in trimethylolpropane triacrylate for cell-adhesion-resistant surfaces. J Biomed Mater Res 29(2):207–215
41. Christie M, Peppas A, Christie N (2000) Biopolymers, Advances in Polymer Science, 153 (2000).
42. Mun G, Suleimenov I, Park K, Omidian H (2010), Superabsorbent Hydrogels. In *Biomedical applications of hydrogels handbook* (pp. 375–391).
43. Chai Q, Jiao Y, Yu X (2017) Hydrogels for biomedical applications: their characteristics and the mechanisms behind them. Gels 3(1):3010006
44. Sirousazar M, Kokabi M, Hassan ZM (2012) Swelling behavior and structural characteristics of polyvinyl alcohol/montmorillonite nanocomposite hydrogels. J Appl Polym Sci 123(1):50–58
45. Ricciardi R, Auriemma F, Gaillet C, de Rosa C, Lauprêtre F (2004) Investigation of the crystallinity of freeze/thaw poly(vinyl alcohol) hydrogels by different techniques. Macromolecules 37(25):9510–9516
46. Mahmood HS, Habubi NF (2022) Structural, mechanical and magnetic properties of PVA-PVP: iron oxide nanocomposite. Appl Phys A Mater Sci Process 128:11
47. Panja S, Maji S, Maiti TK, Chattopadhyay S (2015) A smart magnetically active nanovehicle for on-demand targeted drug delivery: where van der waals force balances the magnetic interaction. ACS Appl Mater Interfaces 7(43):24229–24241
48. Darwish MSA, Al-Harbi LM, Bakry A (2022) Synthesis of magnetite nanoparticles coated with polyvinyl alcohol for hyperthermia application. J Therm Anal Calorim 147(21):11921–11930
49. Thorat ND, Shinde KP, Pawar SH, Barick KC, Betty CA, Ningthoujam RS (2012) Polyvinyl alcohol: an efficient fuel for synthesis of superparamagnetic LSMO nanoparticles for biomedical application. Dalton Trans 41(10):3060–3071
50. Roacho-Perez JA, Gallardo-Blanco HL, Sanchez-Dominguez M, Garcia-Casillas P, Chapa-Gonzalez C, Sanchez-Dominguez CN (2018) Nanoparticles for death-induced gene therapy in cancer (review). Mol Med Rep 17(1):1413–1420
51. Chapa Gonzalez C, Roacho Pérez JA, Martínez Pérez CA, Olivas Armendáriz I, Jimenez Vega F, Castrejon Parga KY, Garcia Casilla PE (2014) Surface modified superparamagnetic nanoparticles: interaction with fibroblasts in primary cell culture. J Alloys Compd 615(1):S655–S659
52. Zhang SJ, Yu HQ (2004) Radiation-induced degradation of polyvinyl alcohol in aqueous solutions. Water Res 38(2):309–316
53. Orrenius S (2007) Reactive oxygen species in mitochondria-mediated cell death. Drug Metab Rev 39(2–3):443–455
54. Cha MY, Kim DK, Mook-Jung I, Cha MY, Kim DK, Mook-Jung I (2015), Experimental Mol Med, 47, 3.
55. Driskell RR, and Watt FM (2015), Understanding fibroblast heterogeneity in the skin. In *Trends in cell biology,* 25, 2, 92–99. Elsevier Ltd.
56. Chen Q, Zhang D, Zhang W, Zhang H, Zou J, Chen M, Li J, Yuan Y, Liu R (2021) Dual mechanism β-amino acid polymers promoting cell adhesion. Nat Commun 12:1
57. Kim SH, Turnbull J, Guimond S (2011) Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. J Endocrinol 209(2):139–151
58. Ojima K, Lin ZX, Rosa de Andrade I, Costa ML, Mermelstein C (2016) Distinctive effects of cytochalasin B in chick primary myoblasts and fibroblasts. PLoS ONE 11:4
59. Lee YH, Cheng FY, Chiu HW, Tsai JC, Fang CY, Chen CW, Wang YJ (2014) Cytotoxicity, oxidative stress, apoptosis and the autophagic effects of silver nanoparticles in mouse embryonic fibroblasts. Biomaterials 35(16):4706–4715
60. Los M, Mozoluk M, Ferrari D, Stepczynska A, Stroh C, Renz A, Herceg Z, Wang ZQ, Schulze-Osthoff K (2002) Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. Mol Biol Cell 13(3):978–988
61. Albukhaty S, Al-Musawi S, Mahdi SA, Sulaiman GM, Alwahibi MS, Dewir YH, Soliman DA, Rizwana H (2020) Investigation of dextran-coated superparamagnetic nanoparticles for targeted vinblastine controlled release, delivery, apoptosis induction, and gene expression in pancreatic cancer cells. Molecules 25(20):25204721