UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias e Ingenierías

Systematic Literature Review about Polyvinyl Alcohol as a Biomaterial, in Combination with Natural Polysaccharides in Tissue Engineering Applications

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Ingeniería Química

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RESUMEN

La presente revisión sistemática de literatura tiene como objetivo elaborar un análisis comparativo de estudios relacionados con las aplicaciones del alcohol polivinílico (PVA), en combinación con polisacáridos, en el campo de la ingeniería de tejidos, a través de diferentes parámetros de interés. Se realizó una investigación utilizando PubMed y Scopus como bases de datos para encontrar publicaciones entre 2010 y 2020. Teniendo en cuenta ciertos criterios de inclusión, por ejemplo, los parámetros que afectan las características del PVA como su grado de hidrólisis y su concentración inicial, se obtuvo un total de setenta y nueve publicaciones que incluyen revisiones y artículos de investigación originales de revistas indexadas. Los parámetros tomados para el análisis comparativo incluyeron el grado de hidrólisis de PVA, la concentración inicial de PVA, el tipo de polisacárido, el tipo de tejido diseñado y las pruebas biológicas realizadas. Se encontró que se prefieren grados de hidrólisis más altos, así como concentraciones bajas de PVA. Además, el polisacárido más utilizado es el quitosano, mientras que los tejidos relacionados con la piel son el foco principal en los tejidos manipulados y las pruebas *in vitro* se realizan con mayor frecuencia. Sin embargo, todavía hay aspectos que podrían mejorarse en algunos estudios, como incluir información importante, como el grado de hidrólisis del PVA, que en ocasiones no se menciona. Los materiales de PVA-polisacáridos tienen un gran potencial para aplicaciones de ingeniería de tejidos, pero se necesitan más estudios in vivo para asegurar una comercialización viable.

Palabras clave: Alcohol polivinílico (PVA), ingeniería de tejidos, polisacáridos, pruebas *in vitro* e *in vivo*

ABSTRACT

The present systematic literature review aims to elaborate a comparative analysis of studies related to applications of polyvinyl alcohol (PVA), in combination with polysaccharides, in the field of tissue engineering, through different parameters of interest. A research was done using PubMed and Scopus as databases to find publications between 2010 and 2020. Taking certain inclusion criteria, such as parameters that affect PVA characteristics like PVA hydrolysis degree and initial concentration, into account led to a total of seventy-nine publications including reviews and original research articles from indexed journals. The parameters taken into for the comparative analysis included PVA hydrolysis degree, PVA initial concentration, type of polysaccharide, type of tissue engineered, and biological tests done. It was found that higher hydrolysis degrees are preferred as well as low PVA concentrations. Also, the most used polysaccharide is chitosan, while skin related tissues are the main focus in tissue engineered, and *in vitro* most widely carried out. However, there are still aspects that could be improved in some studies, like including important information, such as PVA hydrolysis degree, which is sometimes not mentioned. PVA-polysaccharide materials have great potential for tissue engineering applications, but greater *in vivo* studies are needed to assure feasible commercialization.

Key words: Polyvinyl alcohol (PVA), tissue engineering, polysaccharides, *in vitro* and *in vivo* tests.

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1. INTRODUCTION

Tissue engineering consists in the development of constructs based on biomaterial scaffolds, cells and molecules in order to restore, maintain or improve tissue functions in a damaged area of the body [1]. Some examples of tissues that could be recovered with this technology include bone, cartilages, and skin [2]. The principle of this field consists in the construction of scaffolds from different biomaterials, such as proteins and biopolymers. Cells are then seeded into the scaffolds to be implanted, and develop viable tissues *in vivo* [3].

As mentioned previously, scaffolds represent one of the key components in tissue engineering constructs. Therefore, most research has been carried out on different biopolymeric systems for this purpose. Some examples of biopolymers that could be use are polyesters, polyanhydrides, polyphosphazenes, polyurethane, poly (glycerol sebacate) [4] and polyvinyl alcohol (PVA), which will be analyzed in this study. PVA, a synthetic material, is one of the most widely used biopolymers due to its good biocompatibility, biodegradability and hydrophilicity features that make it suitable for different biomedical applications. This biopolymer has been particularly useful in the creation of hydrogels [5].

PVA has different properties that affect its possible uses, such as the presence of cross-linking hydrogen bonds, which promotes the gelation and hydrogel network formation. Furthermore, PVA hydrogels have enhanced pH sensitivity, swelling activity and water vapor transmission, important characteristics for materials in wounds healing [6]. However, hydrogels based on PVA only present some important limitations. High concentrations of the polymer are needed to achieve stable structures with appropriate mechanical properties, making their mass application technically and economically unfeasible, since solutions at these concentrations are extremely viscous, and pharmaceutical grade PVA is expensive [7]. That is why other materials are combined with PVA to improve hydrogels properties, providing, at the

same time, the possibility to create other structures of importance, such as nanofibers, nanoparticles, and sponges, among others [8]. To achieve this goal, a great deal of research has been focused on polysaccharides.

Natural polysaccharides are one of the most relevant macromolecules in nature, with important functional diversity that make them promising materials for different biomedical applications, including tissue engineering and drug delivery [9]. Particularly, cellulose, chitosan, hyaluronic acid and alginate are found to be extensively studied for applications in biomedicine [10]. However, they also have some drawbacks, such as poor mechanical properties, high moisture absorption, low stability in aqueous and physiological environments, among others [9]. This could be partially overcome with crosslinking agents, but these are often cytotoxic [11]. Consequently, PVA and polysaccharides can be combined to potentiate their characteristics of interest and mitigate some of their limitations.

As there is a large body or research reporting the development and application of PVA/polysaccharide structures, there is a need to compile relevant findings. A systemic literature review would not only gather and systematize all the information but would also help in identifying research niches that need to be addressed. Consequently, the present work aims to make a comparative analysis of the scientific literature on the combination of PVA and polysaccharides, according to different parameters, such as PVA hydrolysis degree, polysaccharides used, tissues engineered, and biological tests.

2. METHODOLOGY

Indexed literature was searched in PubMed and Scopus as online databases, using a year range from 2010 to 2020. The search terms used were “polysaccharide”, “tissue engineering” and “polyvinyl alcohol” as visualized in Figure 1, where different Boolean terms were used for joining these general terminologies and reach a proper amount of publications to work. Consequently, inclusion criteria refer to parameters that affect the global search terms. Some examples are PVA hydrolysis degree that could alter PVA characteristics, type of polysaccharide used, and biological tests done to determine the efficiency of the scaffolds developed, among others.

On the other hand, studies that include polyvinyl alcohol and polysaccharides in other type of fields like drug delivery, and ones that do not have enough information to carry out a comparative analysis were not considered. Subsequently, an initial recognition of publications was done where titles and abstracts were evaluated to qualify their entire content. The types of documents that were considered include articles from indexed research journals as well as literature reviews of the topics covered. The articles found were used for doing statistics about the different aspects mentioned in the inclusion criteria taken into account.

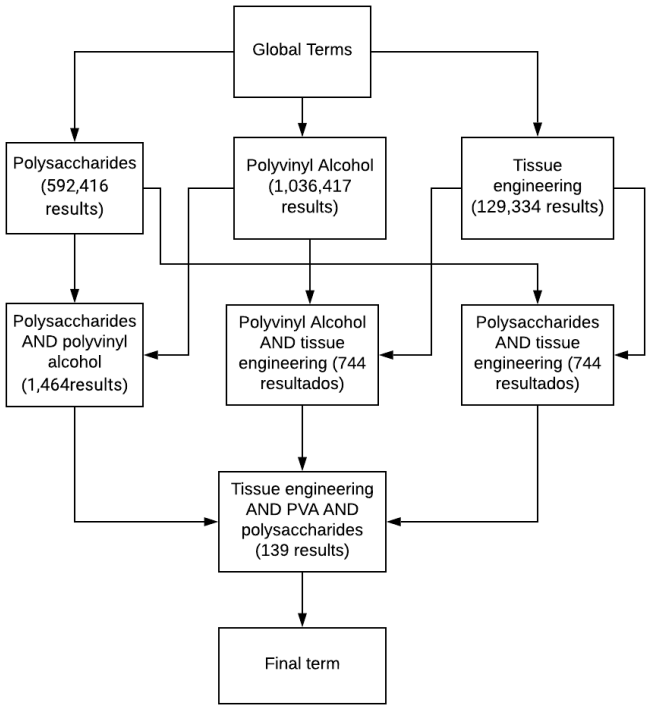

Figure 1. Searching terms use for the digital research from the global terms to a more specific one with the amount of results obtained in each case

3. RESULTS AND DISCUSSIONS

As mentioned previously, this work seeks to elaborate a comparative analysis of different parameters that affect the studies around PVA as a biomaterial, in combination with naturally derived polysaccharides, for tissue engineering applications. For that matter, a total of seventy-nine publications were found, including eight reviews and seventy-one articles from indexed research journals. The aspects that were analyzed include PVA hydrolysis degree, PVA initial concentration, type of structure generated, the polysaccharides combined with PVA, different encapsulated agents, bioactive additives, tissues engineered, and biological tests done. A comprehensive summary of these aspects is provided in Annex 1, at the end of this document. The number of articles that were published in the time laps considered is visualized in Figure 2, showing that in recent years more studies have been done reaching a peak in 2019.

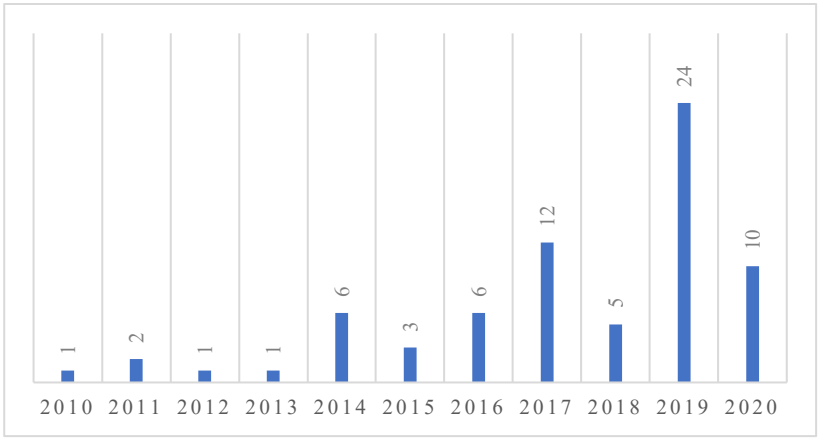

Figure 2. Amount of articles published per year from 2010 to 2020

3.1. PVA in tissue engineering

3.1.1. PVA hydrolysis degree.

PVA is prepared by the hydrolysis of poly (vinyl acetate); therefore, its characteristics depend on the degree of polymerization and on the degree of hydrolysis, which defines the fraction of hydroxyl groups that are present on the backbone [12]. PVA hydrolysis degree (HD)

can affect the nature of the interactions between polymer chains, as well as between the polymer and small additive molecules, such as plasticizers. There are various parameters that could be affected by PVA HD; two of them are the glass transition temperature (Tg), and the cavity radio [12]. Moreover, changes in these parameters allow to detect variations in the fabrication, sterilization and storage of scaffolds in tissue engineering [13].

Furthermore, in most cases high degrees of hydrolysis are used in multiple studies, in this case a 41.25%[14–52] of publications analyzed use a DH form 96% to 100%, and, on the other hand, a 1.25% of the cases use lower hydrolysis degrees from 80% to 83% [53]. However, there is a large number of publications that do not include the hydrolysis degree of the PVA use, which in this case represents 31.25% of the cases [5,15,20,24,25,31,44,54–70].

3.1.2. PVA concentration.

The concentration of PVA directly influences in the construction of scaffolds and their properties. In the study of Yung-Chuan et al, 2005, the fabrication of biocompatible nanocomposites that replicate the properties of cardiovascular tissue was analyzed. In this study, various percentages of PVA were used, from 7.5 to 15%, in combination with bacterial cellulose. In this case, a parameter that could be affected by the variation on concentration is the elastic modulus, which was dependent on the PVA amount in the composite. Furthermore, as the percentage of PVA was higher, the increase of the modulus was faster [71]. In the literature, concentration of PVA is presented in different units like weight percentage [12,14,45,48,49,53,57,60,66–69,17,72,18,20,23,32,34,35,38] and volume percentage [22,24,46,50,54,70,73–76,26,27,30,31,36,40–42], but in general it is mostly used in low concentrations.

3.2. Structures and fabrication methods

3.2.1. Hydrogels.

As previously mentioned, PVA is one of the most widely used biopolymers for scaffold fabrication; however, the main type of scaffolding structure synthetized from it are hydrogels,

water-swollen crosslinked polymer networks that present characteristics such as tissue-like elasticity and mechanical strength [5]. PVA hydrogels can be synthetized using the freeze–thaw method, suitable for biomedical and pharmaceutical applications, where the main parameters include temperature of freezing, time and number of freezing cycles. This method, compared to others, has the advantage that the use of a crosslinking agent is avoided, which could reduce inflammatory responses [77].

On the other hand, in order to obtain a greater variety of hydrogel structures, PVA can be crosslinked with polysaccharides. Through this combination nanoparticles, nanofibers, sponges, nanotubes, microspheres and also hydrogels can be generated. Each structure has its own characteristics; for instance, hydrogels are used to direct cell behavior such as migration, adhesion, differentiation and proliferation [69]. In addition, in the case of these hydrogels the most used synthesis methods are freeze–thaw, and dissolvable-network-based sacrificial molding [78]. Hydrogels represent the structures used to greatest extent, with over 36.9% of the publications taken into account [5,15,21,23,24,26,28,29,31,33,48,50–52,55–58,61,68–70,76,79–83].

Once fabricated, they are tested in different mechanical parameters, such as tensile strength, elongation at break and compressive strength [69]. In addition, they have characteristics of high-water content and porous structure, which can simulate the extracellular matrix of human tissues and promote the exchange of nutrients and metabolic waste [81]. Also, natural polymer-based hydrogels such as collagen, cellulose, chitin, and chitosan show good cell signal transduction and cell-induction characteristics, promising for tissue regeneration. In addition, these natural polymers, once crosslinked with PVA, present appropriate mechanical properties and biocompatibility [69].

3.2.2. Nanoparticles.

In the case of nanoparticles, they are used as encapsulating agents in the fabrication of larger scaffolds, representing 14.3% of the published studies [31,32,36,40,44,45,51,53,54,68,73,76,84]. The

nanoparticles used could be of different sources such as curcumin [54], zinc oxide [31], silver [51], carbonated hydroxyapatite [40], *Cissus quadrangularis* extract [44], and lignin [45], among others. The types of scaffolds that could be developed using nanoparticles are mainly hydrogels, nanofibers and other nanocomposites [31,32,40,51,68,76]. Moreover, there are different methods used for nanoparticles synthesis, such as co precipitation method [40], and double emulsion-solvent evaporation technique [73], among others. Furthermore, nanoparticles have multiple properties like increased permeability, larger surface for protein binding and enhanced scaffold bioactivity [44], which make them good candidates in the field of tissue engineering.

3.2.3. Nanofibers.

Other structures highly used, with approximately 36% of the studies taken into account are nanofibers because of their great potential to mimic natural extracellular matrix in terms of structure, porosity and chemical composition [12,14,30,32,34–36,38–40,42,46,16,49,53,60,62–64,70,72,74,79,17,81,85–88,18–20,22,25,27]. Nanofibrous matrices could show proper elongation and high porosity, while maintaining mechanical stiffness and gradual degradation simultaneously, which are specifications for muscle cell culture [81]. These scaffolds, in addition to mimicking the natural extracellular matrix, can stimulate cell adhesion, proliferation, migration, and differentiation better than particulate structures [74]. The most common method for the preparation of nanofibrous is the electrospinning technique, which consists in obtaining fibers by spinning solutions of polymer though a high electric field to overcome its surface tension forces, and then, fine fibers get expelled from the capillary of the equipment [89]. This technique is highly used for its flexibility in the elaboration of micro and nanosized fibers with unique characteristics [86].

3.2.4. Other structures.

There are other types of structures that are less frequently used, such as cryogels and nanotubes. Cryogels are porous scaffolds usually prepared using chemical crosslinking

methods with agents, such as glutaraldehyde, and its properties depend on the type of PVA used and its initial concentration [47]. Another less conventional structure are nanotubes, and, in this case, the fabrication method is mainly focused on improvement of nanotube dispersion in a PVA matrix and on the enhancement of interfacial interactions [90]. Both of these structures need to be studied more extensively in order to assess their real potential for these applications.

3.3. Polysaccharides used in combination with PVA

3.3.1. Chitosan.

The most common polysaccharide used with PVA is chitosan, with a 64.4% of the cases analyzed [12,15,35–41,43–45,16,48–56,62,17,63,64,66,67,69,70,73,74,78,79,18,80–83,88–90,19,20,27,30,34]. Chitosan is a biocompatible, biodegradable and non-toxic polysaccharide that can be used safely inside or outside the body [54]. It has been used in many biomedical applications such as tissue engineering, wound dressings, and drug delivery systems [79]. Thus, it is widely used in burn wound management. Soft membranes of low molecular weight chitosan with PVA have been studied for antibacterial and wound healing properties, showing significant antibacterial activity towards different pathogens [16].

Furthermore, chitosan´s most promising feature is its ability to be processed into porous structures in cell transplantation and tissue regeneration [56]. This polysaccharide, along with PVA could be used in different scenarios; for example, their scaffolds present enhanced viability and proliferation of nerve cells, which increases the biocompatibility [35]. In addition, chitosan has structural similarities to some extracellular matrix components; therefore, it helps in improving its efficacy in skin tissue engineering [62]. Moreover, chitosan and PVA polymeric blend´s synergic effects have shown important contributions on their physico-chemical properties. Thus, the polymer concentration and miscibility are two of the most important parameters to obtain satisfactory synergistic effects [41].

Different agents have been encapsulated into the PVA-polysaccharide structures to enhance their bioactivity. An example of this is *C. quadrangularis* extract, which has several bioactive compounds that enhance collagen production and have a positive effect on bone fracture healing [44]. Curcumin, on the other hand, is used for being anticarcinogenic, anti-inflammatory, antioxidant, anti-coagulant, antimutagenic, and anti-infective. In addition it has good wound healing potential and enhances the granulation tissue formation, collagen deposition, remodeling of tissues and contraction of wounds [70].

3.3.2. Alginate.

Sodium alginate (SA) consists in a linear polysaccharide with high hydrophilicity, biodegradability, biocompatibility, protein adsorption ability and a relatively economical use [80]. It is one of the most popular polysaccharides in different applications, with over 12.3% of the ones reported in this review [21–24,46,57,76,80,82] . For its combination with PVA in hydrogels, alginates provide physical and biological properties for modeling wound dressing [80]. In addition, sodium alginate raises the water-vapor transmission rate, springiness and permeability, but decreases the gel fraction and flexibility of wound bandages [21]. This polysaccharide could also be used as nanofibers, but its capacity to be electrospun is low due to expansion of the alginate chains in water. Nonetheless, this could be achieved if the electrospinning takes place in organic solvents and in aqueous solutions with synthetic water-soluble polymers such as PVA [86].

An encapsulated agent incorporated in PVA-alginate matrices is honey, a natural wound-healing agent that is used in modern clinical wound care as it has antibacterial, anti-inflammatory, and antioxidant properties. Nanofibrous membranes with honey showed enhanced antioxidant activity, which could provide the ability to control the overproduction of reactive oxygen species (ROS) [22]. On the other hand, a bioactive additive used is hydroxyapatite which incorporated with PVA is able to increase the biocompatibility

and osteoconductivity of the scaffolds [24] . With these agents it could be improve the scaffolds use with this type of polysaccharide and increase the amount of properties.

3.3.3. Starch.

Starch has various advantages, such as being biocompatible, biodegradable, non-toxic and highly abundant, characteristics that make it an appropriate candidate for biomedical applications like wound dressing [30]. In addition, starch is affordable and feasible for the fabrication of synthetic polymer-based composite materials and bio composites because of its easy production from sustainable natural biological resources such as corn, potato, wheat and rice [28]. Nevertheless, native starch does not have adequate mechanical strength to serve as a wound dressing material and can be thereby modified and combined with PVA to mitigate these limitations. Thus, PVA-starch blended films for wound dressing applications possess good degradation, strength, flexibility and water resistance [29]. This polysaccharide is used in 10.96% of the cases taken into account for the statistics [25,26,28,29,31,32,47,88].

In this case an encapsulated agent that has been used is vitamin E, which has shown effective skin care and regeneration functions due to its strong antioxidant activity, anti-inflammatory response, scar prevention properties and availability. Vitamin E was used as nanoparticles, and together with starch and PVA has a good potential for treating skin wounds [32]. Other additives include glycerol with citric acid; they provide better molecular interactions and synergy, which promote the flexibility, plasticity, physicochemical and mechanical properties of PVA-starch films. In addition, citric acid, in appropriate proportions, does not prevent cell growth, and also enhances the antibacterial properties of the wound dressing film, promoting, at the same time, the development of new tissues at the wound site [28].

3.3.4. Cellulose.

This polysaccharide is used in different forms such as bacterial cellulose, nanocellulose, and microcrystalline structure, with important characteristics for biomedical engineering, such

as polyfunctionality, hydrophilicity, and biocompatibility [71]. For instance, nanocellulose used in combination with PVA exhibits collagen-like mechanical behavior, which is typical of soft tissues. Thus, as a hydrogel, PVA-cellulose blends are good contenders for contact lens and other ocular applications [68]. In the case of this polysaccharide, 4.1% of the publications analyzed used it in their studies [58,60,68] .

A crosslinking agent that could be used with cellulose is borax. PVA with borax networks provide a better interaction with the surrounding polymer phase forming hydrogen bonds obtained from the extensive hydroxyl groups. In addition, PVA with borax gels presents well-defined and loose porous networks [58].

3.3.5. Other polysaccharides.

There are other types of polysaccharides that could be used in combination with PVA in tissue engineering but have been less explored. One example is carrageenan, which, in combination with PVA, achieved good hemocompatibility and did not generate adverse inflammatory response [61]. Another example is lignin, which crosslinked with PVA contributed to remarkable improvements in tensile strength and modulus [45]. Other polysaccharide less frequently used is chitin that together with chitosan and PVA are appropriate for the design of biomaterials, such as biodegradable films [87].

3.4. Tissues engineered with PVA-based structures

3.4.1. Skin.

In the case of skin, different types of scaffolds could be used, such as hydrogels [21,26,28,29,31,41,50,51,55,69,70,76,79,80], nanofibers [18,22,25,30,32,38,46,62–64,70,74,79] and nanoparticles [31,32,51,54,76,84]. These structures are applied in the area of wound healing, which remains the most successful case of tissue engineering, as studied in 43.9% of the cases analyzed [16,18,32,38,41,46,50,51,54,55,62,63,21,64,69,70,74,75,77,80,81,83,22,25,26,28–31]. For instance, chitosan and PVA nanofibers are used for skin regeneration, although they present low mechanical properties and

loose of integrity in aqueous media that limit their application. Thus, physical and chemical crosslinking methods are used to improve that inconvenience [79].

Open wounds increase the exposure to oxygen that will generate more reactive oxygen species (ROS), which leads to produce more oxidative stress, causing inhibition of optimum wound healing [70]. Therefore, in the cases that present more damage due to ROS, it is useful to apply PVA structures/scaffolds that have a great affinity for skin and extracellular matrix materials [91]. There have been progresses in this field, with the combination of proper polysaccharides, such as chitosan, that is useful because of its structural similarity to glycosaminoglycans, providing high density matrix and absence of inflamed cells [54].

3.4.2. Bone.

Another highly studied tissue, with 27.3% of the publications taken into account is bone [24,37,69,71,78,82–84,88,89,39,40,43,44,48,60,66,67]. In this case, chitosan is combined with PVA to generate scaffolds because of their cytocompatibility and enhancement of osteoblastic cell proliferation [72]. Moreover, PVA is also used with hydroxyethyl cellulose for the elaboration of nanofibers coated with bone like apatite, which result in a suitable biomaterial for bone engineering [60]. Furthermore, cellulose nanofibers together with hydroxyapatite nanoparticles can be incorporated into starch/PVA matrix crosslinked with citric acid in order to develop a scaffold that is biocompatible, bioactive and that could properly mimic bone extracellular matrix [88].

3.4.3. Cartilage.

Cartilage is another type of tissue that could be engineered; 10.6% of the studies focused on different types of cartilage, such as craniofacial [82] or articular [36,40,49,56,61,76,83]. PVA can be used as hydrogels which show high mechanical properties and biological safety that is why they are used as articular cartilage scaffolds, but cell adhesion on this material is poor [61]. On the other hand, natural polysaccharides, such as chitosan [36,49,56], alginate [82] and carrageenan [61], are able to provide appropriate micro-environments that will modulate the cell attachment

and proliferation, which make their combination a good candidate for a development of artificial grafts [61].

3.4.4. Other tissues.

There are other tissues that can be engineered with the use of PVA-polysaccharide biomaterials, but not enough studies of them have been carried out. Ocular tissue could be engineered using hydrogels, with the unique properties of PVA in combination with nanocellulose [68]. Other type with 4.5% of studies is neural tissue, in which porous nanofiber composites have shown great potential in mimicking nerve extracellular matrix in terms of structure, porosity, and chemical composition [14,35,53]. In addition, these nanofibers in combination with PVA, carbon nanotubes and chitosan can provide the needed structural reinforcement for neural tissue scaffolding due to their high aspect ratio, porosity, high structural and chemical stability [53]. For 1.5% of the publications, cardiovascular tissue is engineered, which used conductive scaffolds because of their similarity to the extracellular matrix of this type of tissue. In this case, electrospun nanofiber scaffolds based on PVA, chitosan, and carbon nanotube were used [19].

3.5. Biological tests

3.5.1. *In vitro* tests.

In order to prove the viability of PVA structures for the regeneration of tissues of interest, *in vitro* and *in vivo* tests in different animal models are performed. Most of the studies, 54.9%, only do *in vitro* tests that are less expensive and a better first approach [12,17,30,32,34–37,39–42,18,43,44,46–49,53,55,57,60,19,61,63,66,67,74,77,79,84,88,20,22,25,26,28,29]. *In vitro* tests could evaluate different parameters such as cell proliferation, biomineralization, biodegradability, cytocompatibility, cytotoxicity, among others [48]. For instance, cytocompatibility is confirmed by the culturing of the desired type of cell, such as mesenchymal stem cells [19], dermal fibroblastic cells [38] and nasoseptal cells [82], in the correspondent PVA-polysaccharide scaffold [75]. Moreover, the

cytotoxicity of the scaffolds could be tested using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on the cells cultured [22].

3.5.2. *In vivo* tests.

For a smaller amount, 3.9%, of the studies, only *in vivo* tests are done, where mainly rabbits and rats, such as Wistar and Sprague-Dawley strains, were used to assay the different types of PVA-polysaccharide scaffolds [21,23,50,52,64,89]. In these scenarios, scaffold biocompatibility was evaluated using the artificial grafts inside the animal; in this way, it could also be evaluated their viability, adhesion, growth and spread [81]. Additionally, wound healing effects and histopathology of the studied structures could be compared to commercial products [21].

3.5.3. Both type of tests.

In some studies, which represent a 17.9% of the publications taken into account, it could apply both types of tests, *in vitro* and *in vivo*, in order to obtain more relevant results [15,31,75,76,80,81,38,51,54,56,62,69,70,73]. For instance, the study of Bi et al, 2019, included an *in vitro* test to determine the biocompatibility of the PVA-Chitosan hydrogels synthetized and an *in vivo* test was used in order to reveal that the surface mineralized double network hydrogel accelerated simultaneous regeneration of bone defects using a rabbit bone model [69]. Another example could be found in the work of Prabhjot et al, 2019, where the *in vitro* test was used to determine the self-adherent, antibacterial and biocompatible of the PVA – Sodium Alginate membrane developed and in the *in vivo* test was found a significant bacterial reduction, wound contraction and reduced inflammation in membrane treated groups in comparison to control group [80].

4. CONCLUSIONS

There is plenty of literature available about the use of PVA in combination with polysaccharides in tissue engineering applications but not enough studies that recompile this kind of information. Thus, this systematic literature review is useful for this matter and could also promote a deeper study in aspects that are not correctly attended. However, for this field of study to advance, there are certain aspects that should be tackled. In some publications certain parameters are not reported, such as PVA hydrolysis degree, which is useful to understand PVA behavior and comprehend better the results. Therefore, this kind of information should be included in the all studies performed. Also, there are important opportunities for further research that are revealed from the present analysis, particularly in the applications on tissues different from skin, bone and cartilage.

Moreover, in several studies, *in vitro* tests are used as results of scaffold biocompatibility, when it should be limited to cytocompatibility or hemocompatibility. To assure biocompatibility, an *in vivo* assessment is required, and it is important to clarify this misunderstanding to establish important consensual conclusions about PVA-polysaccharide biomaterials performance. *In vivo* studies are expensive and complex, but for these biomaterials to be translated from bench to the clinic, they are crucial, and more of them are needed if PVA-polysaccharide structures are to succeed commercially.

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ANNEX A: COMPARATIVE TABLE BETWEEN DIFFERENT PARAMETERS USE IN THE PUBLICATIONS TAKEN INTO ACCOUNT

Reference	Tissue	PVA		Structure	Polisaccharide	Additive	Crosslinking	Type of cell	Biological test	
		HD	Conc.						*In vitro*	*In vivo*
Fong, R. et al, 2018 [12]	N/A	87-98%	10-20 wt%	Films	N/A	Glycerol and propylene glycol	N/A	N/A	N/A	N/A
Jiang, S et al, 2011[5]	Soft tissue, liver	N/A	N/A	Hydrogels	N/A	Dimethyl-sulfoxide	N/A	Hepatocytes	N/A	N/A
Tummala, G et al, 2017 [68]	Soft tissue, ocular	N/A	10 wt%	Nanoparticles, hydrogels	Cellulose	N/A	Cryotropic gelation	Corneal epithelial cells	N/A	N/A

Bi, S. et al, 2019 [69]	Bone	N/A	5 wt%	Hydrogels	Chitosan	Hydroxyapatite	Physical	Rat bone marrow mesenchymal stem cells (rBMSCs)	Normal cell proliferation	Rabbit bone defect
Bano, I. et al, 2019 [16]	Skin (epidermis)	98.0-98.8 %	N/A	Membranes	Chitosan	Glycerol	Tetraethyl orthosilicate	Erythrocytes	N/A	N/A
Shokrgozar, M. et al, 2011 [53]	Neural	80%	12 wt%	Nanofibers	Chitosan	N/A	N/A	Brain-derived cells and U373 cell lines (Astrocytes)	Cytocompatibility	N/A
Pandit, J. et al, 2017 [73]	Ocular	95%	0.50 to 1.0 w/v%	Nanoparticles	Chitosan	Bevacizumab, poly (lactide-co-glycolic acid)	N/A	Epithelial cells	Cytocompatibility	N/A

Kheradmandi, M. et al, 2016 [81]	Skeletal muscle tissue	85-87%	N/A	Hydrogels, nanofibers	Chitosan	N/A	N/A	Mesenchymal stem cells	Cytocompatibility and proliferation.	Rabbit. Biocompatible
Sharma et, P. al, 2016 [27]	N/A	99.45%	5 w/v%	Nanofibers	Chitosan	N/A	N/A	Vero cell	N/A	N/A
Abbas, M. et al, 2019 [70]	Skin, connective tissue	N/A	10 w/v%	Hydrogels, nanofibers	Chitosan	Curcumin	N/A	Red blood cells, fibrocytes, neutrophils	Antioxidant potential	Rabbit, wound healing
Niranjana, R. et al, 2019 [54]	Skin (epidermis)	N/A	10 w/v%	Nanoparticles, patch	Chitosan	Curcumin	Glutaraldehyde	Red blood cells	High antibacterial activity	Albino Wistar rats, wound healing

Zahid, A. et al, 2019 [55]	Skin (fibrous conccetive tissue)	N/A	N/A	Hydrogels	Chitosan	Nitric oxide (NO)	N/A	3T3 fibroblast cells	Enhanced cell migration	N/A
Chen, Q. et al, 2019 [38]	Skin	98%	10 wt%	Nanofibers	Chitosan	Bioglass	N/A	Dermal fibroblastic cells	Enhanced biological properties	Diabetic Mice, cutaneous wound healing and chronic wound healing.
Pon-On, W. et al, 2014 [48]	Bone	98%	7 wt%	Hydrogels	Chitosan	Bioglass, collagen	Freeze/ thawing	Osteoblast-like UMR-106 cell	Cell attachment and growth	N/A
Tamayo, J. et al, 2019 [74]	Skin (Subcutaneous)	87–89%	8 w/v%	Nanofibers	Chitosan	Graphene Oxide	N/A	N/A	Stability in SBF.	Wistar rats, good biocompatibility, antimicrobial activity.

Ruiz, S. et al, 2019 [84]	Skin (Subcutaneous)	87–89%	70-85 wt%	Nanoparticles	Chitosan	Graphene Oxide	N/A	N/A	Degradation assay in SFB	Wistar rats, antibacterial properties.
Cao, L. et al, 2017 [49]	Cartilage	99%	20 wt%	Nanofibers	Chitosan	Graphene Oxide	Glyoxal	Mouse chondrogenic cell (ATDC5)	Cell growth	N/A
Amin, M. et al, 2014 [50]	Skin	98%	10 w/v%	Hydrogels	Chitosan	Honeybee venom	Freeze/ thawing	Fibroblast cells	N/A	Diabetic rats, no bacterial growth.
Hajji, S. et al, 2019 [51]	Skin	99%	2 w/v%	Nanoparticles, hydrogels	Chitosan	Silver	N/A	Red blood cells Control: CHO-K1 cell lines	MTT Cytotoxicity assay. Cytocompatibility	Male healthy young Wistar. Skin wound healing.
Boukari, Y. et al, 2017 [75]	Bone	87–89%	0.3 w/v%	Microspheres	Chitosan	Poly (DL-lactic-co-glycolic acid)	Sodium tripolyphosphate	Human mesenchymal stem cells	Cytocompatibility and cell growth	N/A

Yar, M. et al, 2017 [52]	N/A	98%	N/A	Hydrogels	Chitosan	Heparin	N/A	VERO cell line	N/A	Embryonic chicken, good angiogenic potential.
Pangon, A. et al, 2016 [72]	Bone	87–89%	10 wt%	Nanofibers	Chitosan	Hydroxyapatite	Glutaraldehyde	Osteoblast	N/A	N/A
Hamidabadi, H. et al, 2017 [14]	Neural	96%	5 wt%	Nanofibers	Chitosan	Montmorillonite	N/A	Human Dental Pulp Stem Cells	No cytotoxicity	N/A
Koosha, M. et al, 2019 [79]	Skin	88%	9% w/v%	Hydrogels, nanofibers	Chitosan	Halloysite nanotubes	Glyoxal	Fibroblast cells	No cytotoxicity	N/A

Koosha, M. et al, 2015 [17]	N/A	99%	7 wt%	Nanofibers	Chitosan	N/A	Thermal treatment/ Glutaraldehyde	L-929 Fibroblast cells	Cell attachment and proliferation, without any cytotoxic effect.	N/A
Zou, F. et al, 2017 [18]	Skin	99%	1 wt%	Nanofibers	Chitosan	Collagen	N/A	Porcine iliac artery endothelial cells	Cell adhesion and proliferation.	N/A
Mombini, S. et al, 2019 [19]	Cardiovascular	N/A	8 v/v%	Nanofibers	Chitosan	Carbon nanotube	Glutaraldehyde, citric acid	Mesenchymal stem cells (MSCs)	Exposure of cells to high concentration of carbon nanotubes a promotes oxidative stress and the generation of reactive oxygen species.	N/A

Pandele, A. et al, 2014 [20]	N/A	N/A	1 wt%	Films	Chitosan	Graphene oxide	N/A	Mouse osteoblastic cell line MC3T3-E1	CS–PVA/GO 6 wt.% displayed the lowest cytotoxic potential	N/A
Peng, L. et al, 2019 [56]	Cartilage	N/A	10% wt%	Hydrogels	Chitosan	N/A	N/A	Bone marrow mesenchymal stem cells	Cytocompatibility and cell proliferation.	Rabbit osteochondral defect. Cartilage healing
Jankovic, B. et al, 2013 [85]	Bone, skin, cartilage	88%	N/A	Nanofibers	Chitosan	Polyethylene oxide, hyaluronic acid	Glutaraldehyde	N/A	N/A	N/A
Kaur, P et al, 2019 [80]	Skin	85–89%	10% w/v%	Hydrogels	Alginate	N/A	Boric acid and calcium ions	SK-1 skin epithelial cells	Good self-adherent, antibacterial and biocompatible membrane	Infected murine burn wound. Significant bacterial reduction,

										wound contraction and reduced inflammation.
Choi, Y. et al, 2014 [21]	Skin	99%	6.7% wt%	Hydrogels	Alginate	N/A	N/A	Epithelial	N/A	Male Sprague-Dawley rats. Wound healing
Bichara, D. et al, 2010 [82]	Cartilage	88%	N/A	Hydrogels	Alginate	N/A	N/A	Human Nasoseptal Cells	Higher levels of DNA, glycosaminoglycans, and hydroxyproline in a bioreactor system.	Nude Mice. Compressive modulus increased.
Tang, Y. et al, 2019 [22]	Skin	98–99%	7.2 w/v%	Nanofibers	Alginate	Honey	Glutaraldehyde	NIH/3T3	Non-cytotoxicity and biocompatibility	N/A

Xu, N. et al, 2019 [23]	Lacrimal	99%	3 wt%	Hydrogels	Alginate	Konjac glucomannan	Calcium hydroxide.	Lacrimal canaliculus	N/A	Rabbit. Maintained normal tear meniscus height and increased low tear meniscus area.
George, L. et al, 2017 [76]	Skin and soft tissue	95%	10 w/v%	Hydrogels, nanoparticles	Alginate	Polyallylamine hydrochloride with vitamin C	Cristallization	L929 cells fibroblast	Sustained drug release.	N/A
Jaikumar et, D. al, 2015 [57]	Adipose	N/A	10 wt%	Hydrogels	Alginate	O-carboxymethyl chitosan	Calcium chloride	Adipose Derived Stem Cells	Hydrogel degradation in PBS	N/A

Bendtsen, S. et al, 2017 [24]	Bone	N/A	1 w/v%	Hydrogels (3D)	Alginate	Hydroxyapatite	Calcium chloride	OsteoblasticMC3T3	N/A	N/A
Azarian, M. et al, 2019 [25]	Skin	N/A	N/A	Nanofibers	Starch	Chloroacetated natural rubber	N/A	Human dermal fibroblast cell	Small grade of cytotoxicity and cell viability	N/A
Costa, N. et al, 2020 [26]	Skin	99%	5 w/v	Hydrogels	Starch	Pomegranate peel extract	N/A	Murine fibroblast NCTC clone 929	Nonhemolytic and biocompatible.	N/A
Das, A. et al, 2020 [28]	Skin	98%	2.5, 5, 7.5, 10, 12.5 w/w%	Hydrogels	Starch	Glycerol	Citric acid	Baby Hamster Kidney (BHK-21)	Acceptable degradation, and positive antibacterial effect.	N/A
Das, A. et al, 2019 [29]	Skin	98%	N/A	Hydrogels	Starch	Glycerol	Citric acid	N/A	Good degradation and antibacterial activity.	N/A

Adeli, H. et al, 2019 [30]	Skin	99%	9 w/v%	Nanofibers	Chitosan	Starch	Glutaraldehyde	Mouse fibroblast cell (L929)	Appropriate cytocompatibility and cell viability	N/A
Baghaie, S. et al, 2017 [31]	Skin	N/A	15 w/v%	Hydrogels, nanoparticles	Starch	Chitosan, Zinc oxide	Crystallization	Fibroblast cells L929	Non-cytotoxic.	Male rat
Kheradvar, S. et al, 2018 [32]	Skin	99%	10 wt%	Nanoparticles, nanofibers	Starch	Aloe Vera, Vitamin E	Glutaraldehyde	Fibroblast cells L929	Vitamin E release controlled by Fickian diffusion	N/A
Shao, C. et al, 2019 [58]	Bone	N/A	N/A	Hydrogels	Cellulose	N/A	Borate−diol bonds and hydrogen bonds	N/A	N/A	N/A
Chalal, S. et al, 2014 [60]	Bone	N/A	11 wt%	Nanofibers	Cellulose	N/A	N/A	N/A	N/A	N/A

Islam, T. et al, 2016 [33]	N/A	N/A	9% w/v%	Hydrogels	Carrageenan	N/A	N/A	N/A	N/A	N/A
Zhang, Y. et al, 2015 [61]	Cartilage	N/A	N/A	Hydrogels	Carrageenan	N/A	Physical	ATDC5 cells	Cell attachment and proliferation	N/A
Das, P. et al, 2018 [34]	N/A	99%	7wt%	Nanofibers	Chitosan	N/A	Ar and O2 plasma treatment.	L929 mouse fibroblast cells	Hemocompatibility and cytocompatibility.	N/A
Naghavi, S. et al, 2012 [35]	Neural	98%	10 wt%	Nanofibers	Chitosan	Acetic acid	Glutaraldehyde	PC12 nerve cells	Good cell proliferation	N/A
Garnica, P. et al, 2018 [36]	Cartilage	99%	7,8 w/v%	Hydrogels	Chitosan	N/A	Epichlorohydrin	Cells of auricular cartilage (chondrocytes)	Cytocompatibility and cell adhesion and proliferation.	N/A

Sapru, S. et al, 2018 [62]	Skin	N/A	2 w/v%	Nanofibers	Chitosan	Nonmulberry silk protein	Glutaraldehyde	Human keratinocytes, human monocyte lymphoma cells (U937)	Improved cytocompatibility and hemocompatibility.	Wistar rats. Accelerated wound healing with minimal signs of inflammation.
Golchin, A. et al, 2019 [63]	Skin	N/A	N/A	Nanofibers	Chitosan	Curcumin	N/A	Mesenchymal stem cells (BFP-MSCs)	Low concentrations of Curcumin stimulated cell proliferation, whereas high concentrations had cytotoxic effect.	N/A
Gholipour, A. et al, 2014 [64]	Skin	N/A	10% w/v%	Nanofibers	Chitosan	Poly(caprolactone)	N/A	Mesenchymal stem cells	N/A	Sprague Dawley rats. Cell seeded scaffolds showed smaller scabs than the

										acellular scaffolds.
Zhang, H. et al, 2020 [83]	Bone	N/A	N/A	Hydrogels (3D)	Chitosan	N/A	Chitosan Methacrylate (CHMA)	Bone-marrow-derived mesenchymal stem cells	N/A	N/A
Ghorbani, F. et al, 2020 [37]	Bone	98%	N/A	Porous scaffold	Chitosan	Acetic acid	3-Glycidoxypropyl trimethoxysilane (GPTMS)	MG-63 osteosarcoma cells.	Nontoxic	N/A
Mallakpour, S. et al, 2019 [39]	Bone	99%	N/A	Nanofibers, nanotubes	Chitosan	Ascorbic acid and Bioactive glass	Glutaraldehyde	N/A	Good hydroxyapatite-forming ability in SBF solution.	N/A

Bi, S. et al, 2019 [69]	Skin	N/A	5 wt%	Hydrogels	Chitosan	KOH/urea	Freezing heating	Rat bone marrow mesenchymal stem cells (rBMSCs) and Mouse fibroblast cell line (L929)	Nontoxic	5-week-old rats. Wound healing and reduction in scar tissue formation.
Januariyasa, K. et al, 2019 [40]	Bone (cartilage)	100 %	10 w/v%	Nanoparticles and nanofibers	Chitosan	Carbonated hydroxyapatite	N/A	Mouse osteoblast cells	Better bioactivity after 7 days.	N/A
Tovar, C. et al, 2020 [92]	Bone	87–89%	N/A	Nano-onions	Chitosan	Graphene	N/A	Inflammatory cells	N/A	Wistar rats. Normal material resorption with lower inflammation.
Ergul, N. et al, 2019 [66]	Bone	N/A	1 wt%	Hydrogels (3D printing)	Chitosan	Hydroxyapatite	Neutralization of chitosan´s amino groups.	Human mesenchymal stem cells (MSCs)	Increased cell proliferation	N/A

Garnica, P. et al, 2020 [41]	Skin	99%	10 w/v	Hydrogels	Chitosan	Acetic acid	Genipin	Human dermal fibroblast cells	Noncytotoxic	N/A
Ibrahim et, S. al, 2016 [42]	N/A	99%	20 w/v	Nanofibers	Chitosan	Genisteina	Heating	Human fibroblast cells (W138)	No toxicity	N/A
Kadhim et, I. al, 2020 [67]	Bone	N/A	1 wt%	Films	Chitosan	N/A	Genipin	N/A	Films enhanced the degradation process, improving the biological properties.	N/A
Nie, L. et al, 2020 [43]	Bone	99%	8 wt%	Hydrogels	Chitosan	Calcium Phosphate	Freeze−thaw	Bone marrow-derived mesenchymal stem cells	Improved cytocompatibility	N/A

Thongtham, N. et al, 2020 [44]	Bone	N/A	N/A	Nanoparticles	Chitosan	Collagen, hydroxyapatite	Glutaraldehyde	MC3T3-E1 osteoblast cells	Nontoxic	N/A
Yang, W. et al, 2016 [45]	N/A	99%	1.5 wt%	Films, Nanoparticles	Chitosan	Lignin	N/A	N/A	N/A	N/A
Najafiaslet, M. al, 2020 [46]	Skin	99%	2 and 10 w/v%	Nanofibers	Alginate	Dexpanthenol	Glutaraldehyde	Fibroblast cells L929	Nontoxic	N/A
Ceylan, S. et al, 2017 [47]	N/A	99%	N/A	Cryogel	Starch	N/A	Glutaraldehyde	Mouse Embryonic Fibroblast (MEF) cell line	Cell proliferation	N/A
Milkoreit et al, 2017 [88]	Bone	99%	N/A	Nanofibers	Starch	Hydroxyapatite	Citric acid	Human osteoblast cell line	Good cytocompatibility	N/A