A Review of Mechanical Properties of Scaffold in Tissue Engineering: Aloe Vera Composites

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Abstract: Aloe Vera (AV) is a well-known pharmaceutical herb and traditional biomaterial from thousand years ago. AV is also a great potential material in tissue engineering because of its excellent properties such as biocompatible, biodegradable, antioxidant, anti-inflammatory, anti-diabetic, and antimicrobial. In tissue regeneration, scaffold plays an important role to form template for cell growth or tissue repair. There are some requirements in fabricating scaffold to mimic the native parts of the body such as skin, cardiac, nerve, or bone. Here, mechanical properties of AV aiming to be used as scaffold are reviewed. Tensile strength as the main mechanical property of scaffold made from AV and incorporated with other natural materials (chitosan, alginate, collagen, etc.) and synthetic polymers (PCL, PVA, PLGA, etc.) will be discussed. The methods to measure mechanical properties are also being presented in this brief review article. Finally, some of the perspectives will be given for the future development of AV in tissue engineering.

1. Introduction

To date, tissue engineering has gone through remarkable developments and advancements. It proposes excellent ways to repair or replace lost tissues and damaged organs of the human body, such as skin, cartilage, bone, etc. Generally, the working principles of tissue engineering includes three important elements: (i) living cells/tissue, (ii) biomaterials scaffold, and (iii) growth factors/bioreactor [1]. In tissue engineering, researchers focused on methods and materials that can be used to fabricate porous scaffold that mimics the native extracellular matrix (ECM) of the cell that meet the requirements of tissue regeneration, as well as the suitable technique to produce scaffold for each type of tissue. There are certain criteria that scaffold materials must meet for development and designation in tissue engineering or medicine applications [2].

Among these requirements, mechanical properties are essential factors to consider in fabrication of scaffolds. Mechanical strength is identified by the impact resistance of final products in order to maintain their integrity of scaffold during implantation [3]. Most common mechanical tests to evaluate scaffold include tensile and compressive tests. Each application during tissue remodelling requires a different acceptable range for mechanical properties, because it is desirable for the scaffolds to have mechanical properties close to those of the native tissue or organ, and to be able to avoid “possible side effects” resulting from the stress-shielding mechanism. For example, the native skin has tensile strength values approximately between 5.0 to 30.0 MPa, Young’s modulus in the range of 4.6-20.0 MPa and elongation at break about 35.0-115.0% [4,5]. Therefore, scaffold...
to be used in native skin must exhibit good mechanical strength in order to ensure their stability for angiogenesis, the lymphatic system, nerve bundles, and other structures to generate [4,6,7].

Synthetic polymers and natural materials are two types of materials suitable to fabricate scaffold structure and have been used in last decade. However, every material has limitations to be used in tissue engineering. Synthetic polymers have show several advantages especially in processability and good mechanical properties but exhibit insufficient bioactivity in scaffold products; low potential in cell attachment, hydrophobic, therefore its limit in surface cell recognition sites [8-10]. Natural materials, on the other hand, have excellent biocompatibility, highly hydrophilic, and efficient biological activity when interacting with cells. However, they have low mechanical strength and fast degradation rate [11,12]. To overcome the limitations of each individual type of materials, they have been combined to exploit their favourable properties [13,14].

Aloe Vera (AV) has been known as an ancient therapeutic herb belonging to the Liliaceae family [15]. Its compositions possess numerous bio-actives including antioxidants, anti-inflammators, immunomodulators, hepatoprotective, antitumor, antiulcer and anti-diabetic agents [15-17]. AV is also well known as a great candidate for tissue regeneration because of its ability to promote cell migration, proliferation and growth, especially in wound healing. Moreover, AV has attracted research attention due to its biocompatibility, biodegradability, and low toxicity [18]. AV contains over 75 compounds, including polysaccharides, vitamins, amino acids, enzymes, and other substances [19,20]. Lim Zhe Xi et al. showed the structure of the AV plant and components of the AV leaf is [21]. Most valuable bioactivity comes from the inner gel of Aloe Vera leaf [22].

In the recent years, AV has been used in combination with other materials to form scaffolds for many applications because of its unique bioactivity [23]. When compared to other natural materials used in tissue engineering such as chitosan, alginate or silk, AV showed superior ability in accelerating wound healing. Sheikh Rahman et al reviewed the applications of AV use for tissue engineering, as well as the techniques to fabricate scaffolds from AV, including electrospinning, freeze – drying, phase separation and self – assembly [24]. This review summarizes mechanical properties of scaffolds made from compounds of AV with synthetic polymers and natural materials and discusses testing methods to measure those properties.

2. Experimental study
As mentioned above, when scaffolds is implanted into the human body, it must have adequate mechanical strength to efficiently support the growth of cells. For each application, different requirements of mechanical properties are needed and thus, different tests have been utilized. For example, a scaffold applied in bone requires a compression test [25] while a tensile test is more important for a scaffold to be used in skin or cartilage [26,27]. Furthermore, sample shape and size are also need to be considered when choosing of testing methods. This paper mainly discusses tensile test (which is usually) used in evaluation of composites of AV incorporated with other materials. Generally, the tensile properties are described by the stress-strain curve. Stress and strain are defined by equations (1) and (2):

\[
\text{Stress (}\sigma) = \frac{F}{A} \quad (1)
\]

\[
\text{Strain (}\varepsilon) = \frac{\Delta l}{l} \quad (2)
\]

where F (N) is the force applied to the sample, A (m^2) is the cross-sectional area of the sample, \Delta l (mm) is the extension in length after deformation and L (mm) is the initial length between clamps.

There are several standards for sample preparation and testing method depending on the type of materials and sample sizes. Table I shows list of standards for tensile testing for scaffolds. Most of scaffold samples are prepared as or cut into films or thin sheets for tensile test. Sónia P. Miguel et al. determined the tensile strength of scaffold made from polycaprolactone, aloe vera and chitosan under wet and dry state according to the guidelines of ASTM D3039/A3039M [28]. The test sample was in the form of membrane with thickness in the range of 0.41 to 0.52 mm. Sadegh Khoshgozaran-Abras et al. measured the tensile strength of a film fabricated from chitosan and aloe vera using ASTM D 882 – 02 in thickness ranging from 0.163 to 0.213 mm [29]. Similarly, Ong-Ard Saibutatong et al. also employed ASTM D 882 – 02 to evaluate aloe aera – bacterial cellulose composite films with 0.03 mm thickness [30].
On the other hand, in several situations where it is impossible or difficult to prepare the samples according to the standard, for instance, the length of the sample is too short to grip with the machine, certain have to be made in order to carry out the test. For example, Princeton Carter et al. fixed the film on a paper frame because the sample size was too small and the length of the sample was only 16 mm [31]. The force was zeroed after clamping and the sides of the frame were cut to release the tension of paper.

### Table 1: List of standards for the tensile strength of scaffold.

| Standard               | Name of standard                                      | Thickness        |
|------------------------|-------------------------------------------------------|------------------|
| ASTM D3039/ D3039 M    | Standard Test Method for Tensile Properties of Polymer Matrix Composite Materials | Up to 2.5 mm    |
| ASTM D 638 - 02a       | Standard Test Method for Tensile Properties of Plastics | Up to 14 mm      |
| ASTM D 882 – 02        | Standard Test Method for Tensile Properties of Thin Plastic Sheeting | Less than 1 mm  |

#### 3. Results and discussion

##### 3.1 Aloe Vera/synthetic polymers

Biopolymers are a source of inexpensive materials that possess excellent mechanical properties and are easy to process, making them the first consideration to use for tissue engineering [32]. When combined with natural materials, especially aloe vera, the biological properties of scaffolds fabricated from synthetic polymers are enhanced, which allow the scaffold more to apply in tissue regeneration and greater potential in this field. Table 2 show some aloe vera/ synthetic polymers systems used and their mechanical properties.

### Table 2: Composites of aloe vera/synthetic polymers scaffold in tissue engineering.

| Year | Aloe vera model | Synthetic polymer | Application     | Technique    | Tensile strength (MPa) | Reference |
|------|-----------------|------------------|-----------------|--------------|------------------------|-----------|
| 2017 | Powder          | PLGA             | Wound healing   | Electrospinning | PLGA-AV (1:1) – 4.66 ± 0.90 | [27]      |
| 2016 | Powder          | PCL              | Barrier membrane| Electrospinning | PCL/AV (80/20) – 1.35 | [31]      |
| 2015 | Powder          | PCL              | Wound dressing  | Electrospinning | PCL/AV 5% - 2.00 | [33]      |
| 2014 | Powder          | PCL              | Skin            | Electrospinning | PCL/AV 10% - 6.28 | [16]      |

Until now, several synthetic polymers have been studied to incorporate with aloe vera, such as polyvinyl alcohol (PVA), polycaprolactone (PCL), or polylactic-co-glycolic acid (PLGA) [27,31,34]. Most of the scaffolds from these combinations have been fabricated by electrospinning technique because it is a simple way to achieve the porous structure. Aloe Vera has mostly been used in powder form and small concentrations. Aloe Vera gel contains nearly 55% polysaccharide in dry matter and the rest of components are amino acids, salicylic acid, ascorbic acid, vitamin A and vitamin E [35]. The functional groups – OH, - COOH and – NH in AV pulp have potential to combine with other groups in synthetic polymers [27,36,37]. However, the mechanism of bonding between AV and other materials is still in discussion. It depends on the source of the materials, fabricated techniques, or the solvents used to dissolve components of aloe vera [38,39].

As seen in Table 3, the scaffolds from AV/synthetic polymers have shown good tensile strength that meets the requirements for tissue regeneration. Recently, Ixas Garcia-Orue et al. obtained uniform nanofibrous membranes based on PLGA and AV with fraction 1:1 [27]. This was the highest amount of AV in powder form in the literature. In this case, AV increased the tensile strength of pure PLGA from 3.06 ± 0.35 MPa to 4.66 ± 0.90 MPa, which was adequate mechanical property for use in tissue growth processing. Similarly, S. Suganya et al. synthesized nanofibrous scaffold from PCL and AV at low concentration (5 and 10%) by electrospinning [16]. At 10% AV in the complex scaffold, the tensile strength gained the maximum value of 6.28 MPa compared to PCL (2.43 MPa) and PCL/AV (5%) (2.96 MPa). Furthermore, a PCL/AV blend also displayed better mechanical properties compared to a PCL/collagen blend. S. Agnes Mary and V.R. Giri Dev achieved the same outcome for scaffold with PCL and 5% AV [33]. However, according to Princeton Carter et al., when the concentration of AV exceeds 20%, tensile strength decreases [31]. This is a limitation of AV when incorporated into synthetic polymers. The mechanical strength of
this scaffolds is depended on the bonding of functional groups in the matrix. PLGA and PCL have mainly C=O groups on the chains can be connected with functional groups existed in AV powder (-OH, -COOH). In low concentration, it was assumed that all the number of functional groups in AV got the bonding with functional group of synthetic polymers. However, the high concentration of AV in the composite may have interrupted the bonding of functional groups in the matrix with excess functional groups. This issue can be resolved by using suitable solvent or cross-linker. Kyoung Ran Park, Young Chang Nho also reported biopolymers such as PVA and PVP to be excellent candidates to prepare hydrogel with AV [40]. Compression test results presented increased strength along with increasing concentration of AV gel up to 10%.

3.2 Aloe Vera/natural materials

Natural materials have attracted remarkable attention in tissue engineering. These type of materials exhibit excellent behaviour in tissue regeneration because of their unique biological properties, biodegradability, biocompatibility, low toxicity and suitable structure for live organism [2,41,42]. However, the main disadvantages of natural materials are weak mechanical properties and difficulties in processing. Several biomaterials have been studied to combine with AV in tissue repairing such as chitosan, gelatin, alginate, collagen, bacterial cellulose supplied in Table 3 [26,29,30,43-46].

Table 3: Composites of aloe vera/natural materials scaffolds.

| Year | Aloe Vera model | Natural materials | Technique | Application | Tensile strength MPa | Reference |
|------|----------------|-------------------|-----------|-------------|----------------------|-----------|
| 2017 | Powder         | Chitosan/Collagen | Freeze-drying | Burn skin tissue | At 0.1% AV – 0.17 | [26]     |
| 2016 | Gel            | Gelatin/Chitosan  | Freeze-drying | Skin          | At 0.07% AV – 0.11 | [43]     |
| 2013 | Powder         | Alginate          | Solvent casting | Wound healing | Maximum force at break: 54 N | [45]     |
| 2013 | Powder         | Alginate          | Solvent casting | Wound healing | In dry films in non-crosslinked state: 21.44 to 40.44 At dry films after crosslinking: 42.36 to 50.91 | [46]     |
| 2012 | Gel            | Chitosan          | Solvent casting | Wound healing | At 20% AV: 8.49 ± 0.7 | [29]     |
| 2010 | Gel            | Bacterial cellulose | Solvent casting | Tissue engineering | At 30% AV – 8.67 | [30]     |

Uniform scaffold fabricated from comprising of AV and other natural materials (chitosan and gelatin) using freeze-drying technique have shown excellent porosity and biological properties [26,43]. However, adding AV also decreases the strength of film by using this method. Andini Isfandiar et al. mixed AV powder with chitosan/collagen to form a novel scaffold [26]. It was found that tensile strength decreased when increasing AV concentration from 0.1 % to 0.25 %. The highest tensile strength value was only about 0.17 MPa with 0.1% AV powder. This limited tensile strength can be explained by the presentation of AV which interfered with bonding between collagen and chitosan. Similarly, tensile strength of a gelatin/chitosan blending decreased from 0.11 MPa to 0.04 MPa when AV gel concentration was increased from 0.07 to 0.15%, although other characteristics showed good results [43]. With the in vitro and in vivo assay results, these combinations are worth investigating in tissue engineering.

Another technique, solvent casting, displayed more favourable peculiars in fabrication of scaffolds with strong mechanical properties from a combination of natural materials. By that method, Pereira et al. successfully produced scaffolds from alginate and 5, 15 and 25% AV concentration [45,46]. Although the increasing of AV proportion caused reduction of tensile strength, the change in the values was unremarkable in both dry and wet test conditions. Scaffold gained tensile strength in the range of 21.44 to 40.44 MPa in the dry state without crosslinker and 42.36 to 50.91 MPa after crosslinked by CaCl2. The role of crosslinking agent was clear in this case and is an important factor to consider in scaffold processing. Similarly, chitosan and bacterial cellulose were mixed with AV gel up to 50% amount by solvent casting [29,30]. AV gel improved
the tensile strength of scaffold at low concentration to 8.49 ± 0.7 MPa at 20% incorporate with chitosan and 8.67 MPa at 30% for bacterial cellulose mixing. The higher amount of AV caused a decrease in mechanical properties. However, the resultant product still had adequate properties for tissue regeneration. In short, when AV was incorporated with other individual natural materials, the bonding occurred mostly between functional groups of them and mechanical strength presented good results at low concentration of AV.

3.3 Composite aloe vera/natural/synthetic polymers scaffolds

In composite scaffolds of synthetic polymers and natural materials, these two types of materials are able to balance out each other’s limitations in tissue applications. To enhance the characteristics of this type of composites, AV was added to improve the bioactivity of scaffolds by electrospinning, as shown in table 4 [28,47-49]. All the experiments listed used the electrospinning method to construct scaffolds. This novel group of composites is still in development and has great potential for future work.

### Table 4: Composite aloe vera/natural/synthetic polymers scaffolds.

| Year | Amodel | Incorporate compound | Technique | Application | Tensile strength (MPa) | Reference |
|------|--------|----------------------|-----------|-------------|------------------------|-----------|
| 2017 Gel | PCL/Chitosa n/PEO | Electrospun | Skin | In dry state: 6.39 ± 2.09 In wet state: 6.23 ± 0.33 | [28] |
| 2017 Powder | PCL/ gum tragacanth | Electrospun | Skin | PCL/GT/AV (5%): 0.75 PCL/AV: 0.32 | [47] |
| 2014 Powder | PCL/silk fiber/HA | Electrospun | Bone | PCL/SF/AV: 4.80 PCL/SF/HA/AV: 10.89 | [48] |
| 2014 Powder | PLACL/silk fiber | Electrospun | Cardiac | PLACL/AV: 4.7 PLACL/SF/AV: 3.7 | [49] |

In order to improve biological properties of the scaffold, AV has been considered for use in applications as skin, bone, and cardiac. Miguel et al. established new scaffolds with PCL on the top and chitosan/PEO/AV at the bottom [28]. Mechanical properties of membranes were examined under dry and wet conditions. Although adding 40% AV gel reduced tensile strength from 9.48 MPa to 6.39 MPa (compared to PCL/CS/PEO) in dry state, the achieved result was sufficient to apply in skin repairing [4,5]. Ranjbar-Mohammadi used AV powder mixed with PCL and gum tragacanth (GT) to determine the effect of AV addition [47]. Tensile strength of nanofibers increased from 0.21 to 0.75 MPa by adding 5% AV into PCL/GT. Although there was an increase in tensile strength, the application of new scaffolds in skin regeneration had many limitations. Shanmugavel et al. fabricated scaffolds to apply in bone tissue engineering from a combination of PCL, silk fibre (SF), hydroxyapatite (HA) and AV powder [48]. The role of HA can be seen in increasing tensile strength of PCL/SF/AV from 4.80 to 10.89 MPa. This implied that AV powder was suitable to combine with HA in bone scaffolds, which is a noteworthy point. Furthermore, tensile strength of PCL/SF/AV had higher values compared to PLACL/SF/AV with 3.70 MPa [49]. The AV gel has the variety of functional groups when comparing to powder model. It shows the better benefit in combination with both synthetic polymer and other natural materials.

### 4. Conclusion

Aloe Vera is an excellent natural material and suitable for incorporation with other biomaterials in tissue engineering for improved biological properties; enhanced scaffolds’ biodegradability, porosity, biological properties for the growth of new tissue implanted in the human body. According to certain researches, Aloe Vera showed different mechanical properties behaviours when combined with synthetic polymers or natural materials or both. In most cases, Aloe Vera can enhance mechanical properties at low concentrations as well as improve the bioactivity of scaffolds, especially, in mixture of synthetic polymers (PLGA, PCL, and PVA/PVP) and other natural materials (chitosan, alginate, bacterial cellulose). It depends mainly on the bonding of functional group between AV model and other materials through specific synthetic technique. However, increasing AV concentration over 30% weakens the bonding of scaffold and decreases the mechanical properties. In other cases, combination of AV and compounds of both synthetic polymers and other natural materials exhibited great results. This is a new approach for scaffolds in
future time. Crosslinking agents are another important role in enhancement of mechanical properties of AV scaffolds with other materials. This should be put into consideration when blending different materials.

Acknowledgement

The authors acknowledge financial supported from AUN/SEED-Net scholarship organization (Grant Number: 304/PBAHAN/6050351).

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