

Nutraceutical Aloe Vera Gel Scaffolds for Supporting a Healthy Muscle Movement

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ABSTRACT

Nutraceutical *Aloe vera* gel scaffolds, such as acemannan, promote soft tissue organization in biomedical applications and polymeric matrix. *Aloe vera* hydrogels can induce a much-controlled release of active ingredients and be used as bio-functional scaffolds in a biodegradable form. The before or after questionnaire response for locomotive syndrome was administered: Nutraceutical supplement composed of enzyme-hydrolyzed natural eggshell membrane and curcumin, with *Aloe vera* juice (AVJ) scaffolds, showed the possible

improvement of joint comfort and flexibility. The efficacy for pain and stiffness associated with arthralgia of the knee and hip was evaluated based on locomotive syndrome risk test. Furthermore, ω -3polyunsaturated fatty acids (EPA and DHA) and L-Arginine with AVJ revealed a possible dietary efficacy for Parkinson's disease (PD) patients at stage 3. Prophylactic possible efficacies of AVJ as bio-functional scaffolds are fully expected for increasing collagen synthesis in knee and/or hip joint healing and for mitigation of risk factors of PD patients with diabetes.

Key words: Nutraceutical; *Aloe vera* gel scaffolds; Healthy muscles support; Questionnaire response

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INTRODUCTION

Biomaterials are regarded as natural or synthetic materials used to replace part of a living system or to function in intimate contact with living tissue. They can serve as a scaffold designated to replace, repair, and sustain organ structures. Nanotechnology being one of the most promising technologies shows huge potential in the field of tissue engineering to mimic the porous topography of natural extracellular matrix. Natural scaffolds are made up of protein or carbohydrates with particular biochemical properties and low toxicity, and are found to be biodegradable and biocompatible. The use of *Aloe vera* gel as one of tissue engineering scaffolds was discussed with a special focus on electron spun nanofibers. *Aloe vera* based scaffolds can be fabricated using a variety of techniques. Electrospinning and freeze drying are two techniques which have been investigated for production of *Aloe vera* based scaffolds. Molecular self-assembly, and phase separation induced by non-solvent are two potential techniques that could be used to fabricate *Aloe vera* based scaffolds. The use of *Aloe vera* as tissue engineering scaffolds, gels and films is discussed, with a special

focus on electrospun nanofibers by Rahman *et al.*^[11]. The usage of *Aloe vera* in the form of hydrogels, nanoparticles, nanocomposites, nanofibers and bio-inspired sponges has been extended its well established application and the growing interest in consuming and synthesizing materials based on green or ecofriendly methods also highly encourages the use of numerous plant-based natural products including *Aloe vera*.

Present review discusses the effect of acemannan (*Aloe vera* gel polysaccharide with partial acetyl groups) promoting soft tissue organization in biomedical applications. Furthermore, the promising *Aloe vera* gel scaffold blended collagen, chitosan, alginate, silk fibroin, curcumin, and enzyme hydrolyzed eggshell membrane (EHM), is highlights. Finally, it was suggested that the before or after questionnaire: response of *Aloe vera* juice (AVJ) with EHM and curcumin showed possible supports for a healthy range of motion in muscle movement and joints, and EPA/ DHA and arginine supplement with AVJ have prophylactic benefits in the prevention of PD patients with diabetes.

ACEMANNAN AS A BIOACTIVE SCAFFOLD FOR PERIODONTAL TISSUE REGENERATION

Acemannan (ACM), primary freeze dried polysaccharide of *Aloe vera* inner gel has been shown to stimulate pulpal fibroblasts, gingival fibroblasts, and cement-blasts. Periodontal disease is a common infectious disease causing the destruction of the periodontium. The periodontium is a complex structure composed of both soft and hard tissues, thus an agent applied to generate the periodontium must be able to stimulate periodontal ligament, cementum and alveolar bone regeneration. ACM stimulates both soft and hard tissue healing. Chantarawatit *et al.*^[2] investigated effect of ACM as a bioactive molecule and scaffold for periodontal tissue regeneration, and suggested that ACM could be a candidate biomolecule for periodontal tissue regeneration. Songsiropraduboon *et al.*^[3] investigated the action of ACM as a direct pulp capping material in human primary teeth with deep caries. The histopathological results indicated that the ACM-treated group had significantly better histopathological responses compared with the calcium hydroxide-treated group ($p < 0.05$). These data suggest ACM offers a valuable alternative biomaterial for vital pulp therapy in primary teeth.

Gonna *et al.*^[4] compared the histopathological effects of ACM to that of formocresol as pulp-dressing agents in primary teeth underwent pulpotomy. The authors suggest that ACM sponge may be a suitable agent for pulpotomy in primary teeth, and the inflammatory response was less severed and no necrosis was noted after pulpotomy with ACM compared to formocresol. ACM could function as a bio-active molecule inducing bone-formation by stimulating bone marrow stroma cells proliferation, differentiation into osteoblasts. ACM may be a potential biomaterial for bone regeneration. Boonyagul *et al.*^[5] synthesized the biocompatible ACM scaffold for biochemical application by chemical-crosslinked method. Adipic acid and carbodiimide were employed as a cross-linker and a coupling agent, respectively. The three dimensional porous structure was constructed by the salt leaching method and then with freeze-dried process. The result shows that the amount of adipic acid affected on the swelling rate and *in vitro* degradation. Biochemical assay demonstrated that ACM scaffold exerted on biocompatibility by incubated with ST2 cells. These data suggest that adipic acid could be used as a cross-linker which affected on physical properties of ACM scaffold.

ALOE VERA SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

Aloe vera gel is rich in several compounds, nutrients and polysaccharides. Gels are colloidal systems constituted of a liquid phase dispersed in spatially extended networks, usually assembled by synthetic or natural polymers jointed by scattered connections. Such junction points could be formed by either reversible physical associations or chemical bonds. When the liquid phase is removed without generalized collapse of the gel solid structure a highly porous material (aerogel) could be produced. Aerogel are materials with the highest specific area available, mesoporous structures, and relatively unique features. Combining the functional properties of the structures of gels and aerogels will be result in innovative materials with potential of use in several fields as biomedicine, pharmaceutical, tissue engineering, etc. It is possible to obtain two types of ACM gels and aerogels with different structural and chemical features. One type of gel was produced after extensive ACM deacetylation. ACM gelation was achieved by molecular self-assembly induced with a non-solvent (acetone) vapor diffusion. With supplement drying technology, ACM aerogels with high surface porosity can be obtained after gelation. The gels have the potential to be employed in several fields since they are high-porous materials with high specific surface area making them suitable for diverse adsorption/adsorption of substances such as drugs, cell components, etc.

Silva *et al.*^[6] reported *Aloe vera* gel as membrane and sponge-like scaffolds and the study using *Aloe vera* gel complex structure and chemical composition, associated with freeze-drying, to produce *Aloe vera* sponges. To increase the structures stability in aqueous media, a thin coating of gellan gum (GG), was applied onto *Aloe vera* gel. *Aloe vera*-based sponges showed a heterogeneous porous formation, interconnected pores and good porosity (72-77%). The coating with GG layer onto *Aloe vera* influenced the stability, swelling behavior and mechanical properties of the resulting sponges. Swelling behavior of the sponges was dependent on the pH. Sponges provided the sustained release of BSA-FTIC, used as a model protein, over 3 weeks. Developed *Aloe vera*-based sponges have potential use in biomedical applications. Silva *et al.*^[7] examined that the chitosan/*Aloe vera*-based membranes that were developed displayed satisfactory degradation, roughness, wettability and mechanical properties. A high antibacterial potency was displayed by the blended membranes. Moreover, *in vitro* assays demonstrated that these blended membranes have good cell compatibility with primary human dermal fibroblasts. The chitosan/*Aloe vera*-based membranes might be promising wound dressing materials.

Chokboribal *et al.*^[8] investigated the role of ACM's acetyl-groups on its physical and biological properties. Deacetylated ACM (DeAcAM) was prepared and characterized. The physical properties and microscopic structure of DeAcAM were evaluated using water solubility, contact angle, X-ray diffraction, and scanning-electron microscopy. Increased ACM deacetylation reduced its water solubility and hydrophilicity. Complete deacetylation altered ACM's conformation to a partial crystal structure. The bioactivity of ACM was reduced corresponding to its deacetylation. ACM induced cell proliferation, and VEGF and collagen I expression; however, 100% DeAcAM did not. The stimulated structures of the ACM diad and the completely deacetylated diad were different. The authors concluded acetyl-groups affect ACM's structure and physical/biological properties. Jansisanont *et al.*^[9] investigated the effect of ACM on tooth socket healing, 3 months after surgical removal of mandibular partial bony impacted third molars. ACM is a safe biomaterial for

post-extraction alveolar bone ridge preservation. The radiographic data indicated that ACM induced bone density in the tooth socket 3-months post-operation. Thus, ACM sponge effectively held the blood clot in the socket. This sponge can also function as a scaffold to provide structural support for cell migration, attachment, and growth, and act as a reservoir for growth factors. Minjares-Fuentes *et al*^[10] investigated the main effects of different drying procedures: spray-, industrial freeze-, refractance window- and radiant zone-drying, on ACM. All the drying procedures caused a considerable decrease in the ACM yield (~40%). Degradation affected not only the backbone, as indicated by the important losses of (1-4)-linked mannose units, but also the side-chains formed by galactose. In addition, methylation analysis suggested the deacetylation of mannose units (> 60%), which was confirmed by ¹H-NMR analysis. Interestingly, all these changes were reflected in the functional properties which were severely affected. Thus, water retention capacity values from processed samples decreased ~50%, and a reduction greater than 80% was determined in swelling and fat adsorption capacity values. Therefore, these important modifications should be taken into consideration, since not only the functionality but also the physiological effects attributed to many *Aloe vera*-based products could also be affected. Andriani *et al*^[11] evaluated the efficacy of a new bio-adhesive patch, *Aloe vera* hydrogel (Alovex patch) for the treatment of aphthous stomatitis. An open, not controlled study was performed in 31 pediatric out-patients, aged 6-14 years, affected by mouth ulcers were enrolled consecutively in the 3 Gps Depts+ of San Marino Republic. The results of this study underline the good efficacy and compliance of the path for the treatment of the aphthous stomatitis; also the limit of topical available therapies, linked to the contact time, to develop their therapeutic action, seems not to be evinced on the basis of this study, so the application of this patch seems to be more easy and beneficial.

IMPROVED WOUND HEALING BY ALOE VERA SPONGE AS A BIOLOGICAL VEHICLE

Aloe vera increases the penetration of skin by water hydration and increase of compound solubility. *Aloe vera* mucilage gel acts to keep moisture in wound. Wound healing is a dynamic and complex sequence of events of which the major one is the synthesis of extracellular matrix components. In wound healing, collagen is a tough protein that connects the incisional wound tissues together. *Aloe vera* stimulates fibroblasts to increase wound tensile strength. The mechanical properties of skin and the measurement of wound tensile strength have been studied and the importance of water, proteoglycans, vitamin C, zinc and whole plant *Aloe vera* and *A. vera* mucilage (1:1ratio), were discussed by Davis^[12]. The stimulating influence of *Aloe vera* verifies the modulation and vehicle effect in wound healing. The influence of *Aloe vera* on the glycosaminoglycan (GAG) components of the matrix in a healing wound was studied by Chithra *et al*^[13]. The authors studied the influence of *Aloe vera* on the content of GAG, its types in the granulation tissue of healing wounds and the levels of a few enzymes involved in matrix metabolism. The amount of ground substance synthesized was found to be higher in the treated wounds, and in particular, hyaluronic acid and dermatan sulfate levels were increased. The levels of the reported glycohydrolases were elevated on treatment with *Aloe vera*, indicating increased turnover of the matrix. Both topical and oral treatments with *Aloe vera* were found to have a positive influence on the synthesis of GAGs and thereby beneficially modulate wound healing. Collagen is the major protein of the extracellular matrix, and

is the component which ultimately contributes to wound strength. It plays the major role in establishing wound tensile strength. The influence of *Aloe vera* on the collagen content and its characteristics in a healing wound was investigated by Chithra *et al*^[14]. *Aloe vera* influences the wound healing process by enhancing collagen turnover in the wound tissue. It was observed that *Aloe vera* increased the collagen content of the granulation tissue as well as its degree of crosslinking as seen by increased aldehyde content and decreased acid solubility. The type I/type III collagen ratio of treated groups were lower than that of the untreated controls, indicating enhanced levels of type III collagen. Wounds were treated either by topical application or oral administration of *Aloe vera* to rats and both treatments were found to result in similar effects. The cross-sectional randomized interventional study to evaluate the effectiveness of *Aloe vera* in the healing of post extraction sockets was performed by Nimma *et al*^[15]. *Aloe vera* has been proved a unique property that is implicated in better healing than other group without any side effects. *Aloe vera* is economical, effective, powerful nutritional supplement and antioxidant that protects wound healing.

ALOE VERA GEL SCAFFOLDS WITH SEMI-SYNTHETIC OR NATURAL (CURCUMIN AND EGGHELL MEMBRANE) BIOMATERIALS

Aloe vera gel scaffolds with semi-synthetic biomaterials

A methodology to produce hydrogel-film composed of alginate and *Aloe vera*, for wound healing and drug delivery applications was described by Pereira *et al*^[16]. Alginate films with different *Aloe vera* contents (5, 15 and 25%) were prepared and its properties evaluated in terms of thickness, transparency, swelling behavior and *in vitro* degradation. Results show a positive influence of *Aloe vera* on the transparency of the films, in both dry and wet state. Films were immersed in acetate buffer at pH 5.5 simulating the value of the skin, to evaluate its water absorption capacity. It was found that water absorption increases as the *Aloe vera* content increases, suggesting that *Aloe vera* enhances the hydrophilic properties of the films.

The *in vitro* degradation tests were performed through the incubation of the films for 10 weeks, in a simulated body fluid at 37°C. After this period, films kept its structure integrity exhibiting a weight loss in the range of 14.1-18.6%. Hydrogel films composed of alginate and *Aloe vera* gel were prepared by Pereira *et al*^[17] and characterized for application in the treatment of several types of wound (e.g. exuding, painful and dry). The films were prepared through a two-step procedure encompassing the film formation by a solvent-casting method and an additional crosslinking step to improve their properties. The physical, morphological and water absorption properties were characterized by introducing several variations in the *Aloe vera* content. The *in vitro* degradation behavior of the films was investigated in the presence of the enzyme alginate-lyase for 2 weeks. Results showed that *Aloe vera* improves the water absorption and the *in vivo* degradation rate of the films. In conclusion, this study indicates that the alginate/*Aloe vera* hydrogel films can be potentially explored as wound dressing for the treatment of different wound types.

Collagen-Chitosan (COL-CS) scaffolds supplemented with different concentrations (0.1-0.5%) of *Aloe vera* (AV) were prepared and tested *in vitro* for their possible application in tissue engineering by Jithendra *et al*^[18]. After studying the microstructure and mechanical properties of all composite preparations, a 0.2% AV blended COL-CS scaffold was chosen for further studies. The incubation of AV to COL-CS increased the thermal stability as well

as hydrophilicity of the scaffolds. Cell culture studies on scaffold showed enhanced growth and proliferation of fibroblast (2T3L1) without exhibiting any toxicity. Also, normal cell morphology and proliferation were observed by fluorescence microscopy and scanning electron microscope. The rate of cell growth in the presence/absence of AV in the scaffolds was in the order: COL-CS-AV>COL-CS>TCP (tissue culture polystyrene plate). These results suggested that the AV gel-blended COL-CS scaffolds could be a promising candidate for tissue engineering applications. Suganya *et al.*^[19] presented the works to combine the biological properties of AV and the advantages of electro-spun meshes to prepare a potent transdermal biomaterial. The polycaprolactone (PCL) containing 5 and 10 weight % of lyophilized powder of AV was studied for electrospinning into nanoscale fiber mats and compared with PCL/Collagen blend for dermal substitutes. Scanning electron microscopy revealed the average diameters of PCL, PCL-AV 5%, PCL-AV 10% and PCL-Collagen nanofiber scaffolds in the range of 519 ± 28 , 264 ± 46 , 215 ± 63 and 249 ± 52 nm, respectively. PCL 10% nanofiber scaffolds showed finer fiber morphology with improved hydrophilic properties and higher tensile strength of 6.28 MPa with a Young's modulus of 16.11 MPa desirable for skin tissue engineering. The nanofibers were then used to investigate differences in biological responses in terms of proliferation and cell morphology of mice dermal fibroblasts. It was found that PCL-AV 10% nano-fibrous matrix favored cell proliferation compared to other scaffolds which almost increased linearly by 17.79% and 21.28% compared to PCL on six and ninth day. CMFDA dye expression, secretion of collagen and F-actin expression were significantly increased in PCL-AV 10% scaffolds compared to other nano-fibrous scaffolds. The obtained results proved that the PCL-AV 10% nano-fibrous scaffold is a potential biomaterial for skin tissue regeneration. The usage of AV in the form of hydrogels, nanoparticles, nanocomposites, nanofibers and bio-inspired sponges has extended its well established application spectrum in the fields of wound healing, tissue engineering and drug delivery. Suganya *et al.*^[20] focused on incorporation of bioactive agents namely AV and silk fibroin (SF) with 4% hydroxyapatite (HA) in the poly (lactic acid-co-caprolactone) (PLACL) to fabricate PLACL-AV-SF-HA (4%) nano-fibrous scaffolds by electrospinning which can afford close biomimicry to natural bone constitution. Morphology, composition, hydrophilicity and mechanical properties of the electro-spun PLACL-AV-SF-HA (4%) nano-fibrous scaffold along with other controls namely PLACL, PLACL-AV-SF, PLACL-AV-SF-HA (2%) nono-fibrous scaffolds were examined by field emission scanning electron microscopy. Human mesenchymal stem cells cultured on PLACL-AV-SF-HA (4%) nano-fibrous scaffolds showed significant increments in cell proliferation, osteogenic differentiation, osteocalcin expression and mineral deposition in comparison with different controls. Obtained results highlight the synergistic effect of osteo-inductive property of AV along with osteo-conductive hydroxyapatite in enhancing the differentiation and biological performance of human mesenchymal stem cells to osteoblasts with suitable mechanical support provided by skin fibroin, proving PLACL-AV-SF-HA (4%) biocomposite to be a highly ideal nano-fibrous scaffold for osteo-regeneration related therapeutics. Balaji *et al.*^[21] discussed the works related to recent advancements made in the use of AV especially in the form of biomaterial-based nanostructures.

Selvakumar *et al.*^[22] investigated the works to fabricate guided bone regeneration (GBR) with an anti-infective electro-span scaffold by ornamenting segmented polyurethane (SPU) with two-dimensional AV wrapped mesoporous hydroxyapatite (Al-mHA)

nano-rods. Al-mHA ornamented scaffolds exhibit tremendous improvement (175%) in the mechanical properties with promising antimicrobial activity against various human pathogens. After confirmation of high osteo-conductivity, improved biodegradation, and excellent biocompatibility against osteoblast-like MG63 cells (*in vitro*), the scaffolds were implanted in rabbits as an animal model by subcutaneous and intraosseous (tibial) sites. Improved *in vivo* biocompatibilities, biodegradation, osteo-conductivity, and the ability to provide an adequate biomimetic environment for biomineralization for GBR of the scaffolds (SPU and ornamented SPUs) have been found from the various histological sections. Early cartilage formation, endochondral ossification, and rapid bone healing at 4 weeks were found in the defects filled with Al-mHA ornamented scaffold compared to pristine SPU scaffold based on combinatorial soft segments of poly (ϵ -caprolactone), poly (ethylene carbonate), and poly (dimethylsiloxane) by a biomimetic approach and the advantages of an AV wrapped mHA frame in promoting osteoblast phenotype progression with microbial protection for potential GBR applications. Kim *et al.*^[23] investigated to fabricate transparent ultrathin film scaffolds with nature-derived AV gel and silk fibroin (SF) for corneal endothelial cells (CECs). Field emission scanning electron microscopy (FESEM) images revealed that the critical morphology of CECs was formed on the AV gel in the blend with SF rather than in the scaffold with SF alone. The cell proliferation, phenotype, and specific gene marker expressions for CECs were determined by MTT assays, immunofluorescence, and reverse transcriptions polymerase chain reactions. Incorporation of a small amount of AV gel increased the cell viability and maintained its functions well. The scaffolds were easily handled for transplantation into rabbit eyes with small incisions and examine by their transparency after transplantation and histological staining. The scaffolds attached to the surface of the corneal stroma and integrated with surrounding corneal tissue without a significant inflammatory reaction. These results indicate that AV blended SF film scaffolds might be a suitable substitute for alternative corneal grafts for transplantation.

Torres-Giner *et al.*^[24] reported the use of electro-hydrodynamic processing (EHDP) to encapsulate AV using both synthetic polymers, i.e. polyvinylpyrrolidone (PVP) and poly (vinyl alcohol) (PVOH), and naturally occurring polymer, i.e., barley starch (BS), whey protein concentrate (WPC), and maltodextrin. The AV leaf juice was used as the water-based solvent for EHDP, and the resultant biopolymer solution properties were evaluated to determine their effect on the process. Fourier transform infrared spectroscopy confirmed the successful encapsulation of AV in the biopolymer matrices, presenting both encapsulants a high chemical integration with the bioactive components. Ultraviolet-visible spectroscopy showed that, while PVP nanofibers offered a poor effect on the AV degradation during UV light exposure (~10% of stability after 5 h), WPC nano-beads delivered excellent protection (stability >95% after 6 h). This was ascribed to positive interactions between WPC and the hydrophilic components of AV and the inherent UV-blocking and oxygen barrier properties provided by the protein. Therefore, electro-spraying of food hydrocolloids interestingly appears as a novel potential nanotechnology tool toward the formulation of more stable functional foods and nutraceuticals.

Commercial guided tissue regeneration (GTR) membranes are typically composed of synthetic polymers that have mild clinical success mostly because of their lack of proper bioactivity and appropriate degradation profile. Carter *et al.*^[25] investigated a natural polymer AV was blended with polycaprolactone (PCL) to create nano-fibrous GTR membranes by electrospinning. AV has proven

anti-inflammatory properties and enhances the regeneration of periodontium tissues. PCL, a synthetic polymer, is well known to produce miscible polyblends nano-fibers with natural polymers. Nano-fibrous membranes with varying composition of PCL to AV were fabricated, and several physicochemical and biological properties, such as fiber morphology, wettability, chemical structure, mechanical strength, and cellular compatibility of the membranes were analyzed. PCL/AV membranes with ratios from 100/00 to 70/30 showed good uniformity in fiber morphology and suitable mechanical properties, and retained the integrity of their fibrous structure in aqueous solutions. Experimental results, using cell viability assay and cell attachment observation, showed that the nano-fibrous membranes support 3T3 cell viability and could be a potential candidate for GTR therapy. Isfandiary *et al.*^[26] sought the procedures to synthesize composite scaffolds for burns utilizing collagen-chitosan-AV to find the best concentration of AV as scaffolds application. The synthesis of scaffolds was performed with collagen constituent composition as follow: collagen-chitosan (1:1) was dissolved in 0.05M acetic acid, then subsequent variations of AV, namely 0% AV, 0.1% AV, 0.15% AV, 0.2% AV, and 0.25% AV were added into collagen-chitosan solution, and freeze dry method was applied. Sample characterization was done by FTIR, tensile strength test, SEM test, cytotoxicity assay and degeneration test. In conclusion, sample with 0.2% AV was potential as scaffolds for burns skin tissue.

Aloe vera gel scaffolds with natural (curcumin and eggshell membrane) biomaterials

In spite of multiple ranges of reported pharmacological effects, including anti-oxidant, anti-inflammatory and anti-tumor activity, curcumin was limited in its clinical applicability by its low bioavailability during oral administration. As Osler W. stated; a man is as old as his arteries, stiffness of the central arteries increases with aging and is an independent risk factor for cardiovascular diseases. Efficacy of exercise and potential effects of curcumin on arterial stiffness was reviewed by Shimatsu *et al.*^[27]. Jimenez-Osorio *et al.*^[28] reviewed that curcumin supplementation has been proposed to improve insulin resistance (IR) through the activation of the insulin receptor and its downstream pathway in several experimental models, pointing out that its clinical use may be a good and innocuous strategy to improve IR-related diseases. Research at the laboratory, translational and clinical levels that supports the use of curcumin for various musculo-skeletal disorders, such as osteoarthritis, osteoporosis, musculo-cartilaginous disorders, and sarcoma was comprehensively summarized by Peddada *et al.*^[29]. Curcumin, GRAS-drug approved by FDA, was targeted as one of the most extensively applied drugs to age-associated disorders.

Tummalapalli *et al.*^[30] reported that AV and curcumin loaded oxidized pectin-gelatin (OP-Gel) matrices were used as antimicrobial finishes on nonwoven cotton fabrics to produce composite wound care devices. The drug release characteristics of the bio-composite dressings indicated that curcumin is released through a biphasic mechanism-erosion of the polymeric matrix, followed by diffusion, while AV is released upon leaching of the polymeric matrix. A 50/50 composition of AV/curcumin was used to fabricate OP-Gel-Aloe-Curcumin dressings. However, OP-Gel-Aloe-Curcumin dressings exhibited lesser antimicrobial activity compared to OP-Gel Aloe and OP-Gel-Curcumin dressings. The cyto-compatibility of the fabricated dressings was evaluated using NIH3T3 mouse fibroblast cells. OP-Gel-Aloe treated fibroblasts had the highest viability, with the matrices providing a substrate for good cell attachment and proliferation. On the other hand, OP-Gel-Curcumin and OP-Gel-

Aloe- Curcumin seemed to have induced apoptosis in NIH3T3 cells. *In vivo* wound healing analysis was carried out using an excisional splint wound model on C57BL/6J mice. OP-Gel-Aloe treated wounds exhibited very rapid healing with 80% of wound healing in just 8 days. Furthermore, AV exerted a strong anti-inflammatory effect and prominent scar prevention. Histological examination revealed that an ordered collagen formation and neovascularization could be observed along with migration of nuclei. Therefore, OP-Gel-Aloe bio-composite dressings are proposed as variable materials for effective wound management. The composite wound dressing was found to exhibit very rapid wound healing, and AV showed a strong anti-inflammatory effect and promised scar prevention. The biopolymers; AV, silk fibroin and curcumin incorporated into polycaprolactone (PCL) as suitable substrates for tissue engineering were investigated by Karuppuswamy *et al.*^[31].

Different combinations of PCL with natural polymers-PCL/AV, PCL/silk fibroin, PCL/AV/silk fibroin, PCL/AV/silk fibroin/curcumin were electrospun into nano-fibrous scaffolds. The fabricated two dimensional nano-fibrous scaffolds showed high surface area, appropriate mechanical properties, hydrophilicity and porosity, required for regeneration of diseased tissues. Anjum *et al.*^[32] reported the development of a composite material for wound dressing containing nanosilver nanohydrogels (nSnH) along with *Aloe vera* and curcumin that promote antimicrobial nature, wound healing and infection control. Polyvinyl alcohol/polyethylene oxide/carboxymethyl cellulose matrix was used as gel system to blend with nSnH. The histological studies showed AV based dressings to be the most optimum one. These results suggest that nSnH along with AV based dressing material could be promising candidates for wound dressings.

Danesch *et al.*^[33] demonstrated the results of the six centers, open label clinical studies which were conducted to evaluate the efficacy and safety of natural eggshell membrane (NEM) as a treatment for pain and inflexibility associated with osteoarthritis of the knee and/or hip in European population. Supplementation with NEM significantly reduced pain, both rapidly (10 days) and continuously (60 days) demonstrating that it is a safe and effective therapeutic opinion for the treatment of pain associated with osteoarthritis of the knee and/or hip. A single-center, open-label clinical study was conducted by Brunello *et al.*^[34] to evaluate the efficacy and safety of NEM as natural treatment for pain and stiffness associated with osteoarthritis of knee in an Italian population. Twenty-five subjects received oral NEM, 500mg once daily for four weeks. There were no adverse events or serious adverse events reported during the study and the treatment was reported to be well tolerated by study participants. Supplementation with NEM significantly reduces both pain and stiffness rapidly (10 day) and this effect continued to improve through 30 days. Sah *et al.*^[35] delineated the preparation and physico-chemical characterization of soluble eggshell protein (SEP) modified silk fibroin (SF)-polyvinyl alcohol (PVA) scaffolds and its application in bone tissue engineering. The scaffolds were found with suitable swelling behavior and biodegradability to support cell proliferation till replaces native osseous tissue. *In vitro* cyto-compatibility and differentiation study showed that SEP (SF-PVA) supports viability, proliferation and differentiation of cord blood derived human mesenchymal stem cell. Further, *in vivo* study in mice model showed that the scaffolds are non-immunogenic and support tissue growth. In conclusion, SEP modified SF-PVA scaffold could be a better option for tissue engineering. An asymmetric electro-spun membrane was produced to mimic both layers of skin. It comprises a top dense layer (manufactured with polycaprolactone) that was

designed to provide mechanical support to the wound and a bottom porous layer (composed of chitosan and AV) aimed to improve the bacterial activity of the membrane and ultimately the healing process. Miguel *et al.*^[36] obtained the results that the produced asymmetric membranes displayed porosity, wettability, as well as mechanical properties similar to those presented by the native skin.

Yagi^[37] reported that some of the phytochemicals; such as emodin, aloe-emodin in latex and ACM, aloe lectin (verectin) in inner gel of AV, provide relief to rheumatoid arthritis patients through promoting wound healing as well as reducing inflammation and relieving pain, which are common symptoms of rheumatoid patients. Our dental study^[4] on pulpotomy in primary teeth showed that *Aloe vera* high molecular materials (AHM) could serve as a bioactive molecule that has the capability of bone formation as it stimulates bone marrow stromal cells proliferation, differentiation into osteoblasts. ACM and aloe lectin (verectin) containing in small amount in AV gel, as the controlled releasing active ingredients, may be a potential biomaterial for bone regeneration^[4]. Prophylactic aloe components; aloe-emodin, emodin and fermented butyrate, on autoimmune diseases, such as rheumatoid arthritis, were presented in a previous paper^[38].

The protective effects of fermented butyrate on bone mass are associated with inhibition of osteoclast differentiation and bone resorption *in vitro* and *in vivo*. Lucas *et al.*^[39] showed that short chain fatty acids (SCFAs) are regulators of osteoclast metabolism and bone mass *in vivo*, and identified that butyrate and propionate induce metabolic reprogramming of osteoclasts resulting in enhanced glycolysis at the expense of oxidative phosphorylation, thereby downregulating essential osteoclast genes such as TRAF6 and NFATc1. The data identified SCFAs as potent regulators of osteoclast metabolism and bone homeostasis. Ohlsson *et al.*^[40] demonstrated that the gut microbiota is a crucial regulator of bone metabolism and that modulation of the gut microbiota composition by probiotic interventions can prevent castration-induced bone loss, and introduced a new term “osteomicrobiology” for the rapidly emerging research field of the role of the microbiota in bone health. This research was aimed to bridge the gaps between bone physiology, gastroenterology, immunology, and microbiology.

Al-Hijazi *et al.*^[41] studied the effect of topical application of AV on bone healing by histological examination and immune-histochemical evaluation of BMP7 (a protein that in humans is encoded by the BMP7 gene), and observed that AV treated group exhibited increased proliferation of osteogenic cells and enhanced the expression of BMP7 compared to untreated group. Wang H *et al.*^[42] explored bone tissue engineering by use of ceramic reinforced polymer-matrix composites, and found the novel hydroxyapatite-reinforced AV-coated metallocene polyethylene with a better mechanical and anti-thrombogenic nature. These reports identified AV as potent regulators of osteoclast metabolism and bone homeostasis. Yagi *et al.*^[43] reported that verectin composed of carbohydrate and protein in a ratio of 10.7 and 82.0%, respectively, with molecular weight of 29 KD, in AV gel inhibited Cox-2 and thromboxane A₂ synthase, suggesting the participation to human immune system. Oral AV gel treatment for osteoarthritis could have utility as a prophylactic against the gastrointestinal irritant effects of non-steroidal anti-inflammatory drugs. Extraction, purification and gel preparation of AV pectin and evaluation of the biocompatibility of the pectin gels were studied by Gentilini *et al.*^[44]. Extracted AV pectin possesses interesting properties to be exploited for the production of mechanically stable gels by inotropic gelation and high rhamnose content matrices for application in regenerative medicine. Nimma *et al.*^[15] evaluated the effectiveness of AV gel in the healing of post extraction sockets by cross-sectional

randomized interventional study. AV soaked gel foams group showed a significant decrease in pain after two hours on the day of extraction followed by second, third and seven day which was statistically significant and better than the control group.

Guided bone regeneration (GBR) scaffolds are unsuccessful in many clinical applications due to a high incidence of postoperative infection. Selvakumar *et al.*^[45] investigated to fabricate GBR with an anti-infective electrospun scaffold by ornamenting segmented polyurethane with two-dimensional AV wrapped mesoporous hydroxyapatite (Al-mHA) nanorods. The advantages of Al-mHA frame in promoting osteoblast phenotype progression with microbial protection for potential GBR application were established. Our earlier pre-clinical trials showed that aloe high molecular weight fraction (AHM) exhibited the efficacy as immune-modulators for viral infection-induced hepatic periportal fibrosis and type 2 diabetic patients, and as wound and ulcer-healing ointment to patients suffering from bed sore and lichen planus. Therefore, AHM were considered to be a novel low-cost and safe drug of natural origin, indicating a possible therapeutic efficacy in prevention of age-related diseases by slowing aging process^[46]. Composite scaffolds, which are a combination of natural or synthetic biomaterials, are highly biocompatible with improved tensile strength for effective skin tissue regeneration. Appropriate knowledge of the properties of various biomaterials and scaffolds will accelerate the production of suitable scaffolds for skin tissue regeneration applications.

Natural eggshell membrane (NEM) is a novel dietary supplement ingredient that contains naturally occurring glucosaminoglycans and proteins essential for maintaining healthy joints. Ruff *et al.*^[47] demonstrated the randomized, multicenter, double-blind, placebo-controlled osteoarthritis pain treatment incorporating hydrolyzed NEM (hydrolyzed NEM contain glucosamine up to 1% dry weight, chondroitin sulfate up to 2%, collagen I up to 25%, hyaluronic acid 0.5-2%) clinical study to evaluate the efficacy and safety of hydrolyzed NEM as a treatment for pain and stiffness associated with osteoarthritis of the knee. Rapid responses were seen for mean pain sub-scores (15.9% reduction, $p = 0.036$) and mean stiffness sub-scores (12.8% reduction, $p = 0.024$) occurring after only 10 days of supplementation. There were no serious adverse events reported during the study and the treatment was reported to be well tolerated by study participants. Supplementation with hydrolyzed NEM, 500mg taken once daily, significantly reduced both pain and stiffness compared to placebo at 10, 30, and 60 days. The clinical trial registration number for this study is NCT00750477. The harvested NEM was subjected to moist heat (autoclaving) and was characterized using suitable techniques. Pillai *et al.*^[48] demonstrated that autoclaved eggshell membrane can be a potential and interesting natural scaffold matrix for meniscal tissue engineering.

RESPONSE OF ALOE VERA JUICE WITH THE SUPPLEMENT (CURCUMIN AND NATURAL EGGSHHELL MEMBRANE) FOR SUPPORTING HEALTHY MUSCLE AND JOINTS: THE STUDY BEFORE OR AFTER QUESTIONNAIRE

The efficacy and tolerability of *Aloe vera* juice (AVJ) with or without the supplement for the relief of the pain and discomfort associated with osteoarthritis of knee and/or hip muscle and joints, were evaluated by questionnaire assessment. Questionnaire evaluation shows the personal experiences on administration of 100ml to 500ml of International Aloe Science Council (IASC)-certificated AVJ with

or without the supplements, and was based on the following scheme in Table 1 and 2. Table 1 and 2 were conducted under the agreement of the principles of the Helsinki declaration.

All subjects who were seeking relief of mild to moderate pain due to osteoarthritis of around knee, hip are considered for enrollment in the study. Subjects are excluded if they were currently receiving remission-inducing drugs. Other exclusion subjects are a known allergy to egg or egg products and are pregnant or breastfeeding women.

The study evaluates the effectiveness of AVJ without or with the supplementation during Jan.1 to Mar. 31, 2017. Questionnaire assessment was composed of volunteer: 13 male (34-68 years) and 20 female (22-84 years), and the recovery ratio was 92%. Subjects take three soft gels daily. One soft gel; 0.75 g is composed of enzyme hydrolyzed eggshell membrane (EHEM), curcumin and additives (supplement: Forever Move).

*In the recovery % in total, pains in a stiff shoulder and muscle, and a scruff of the neck etc., with AVJ ingestion are included. Three main locomotive pains and the other pains in total are listed in Table 1.

The study was assessed based on the locomotive syndrome risk test (25-question risk assessment)^[49]. All subjects are composed of 14 male (25-70 years) and 137 female (30-79 years), and the recovery ratio of the response was 2%. The subjects take one soft gel composed of EHEM, curcumin and additives daily with AVJ from Aug. 22 to Oct. 22, 2017.

CASE REPORTS OF ALOE VERA JUICE WITH ω -3 POLYUNSATURATED FATTY ACIDS (PUFA) FOR SUPPORTING HEALTHY MUSCLE IN PARKINSON'S DISEASE PATIENTS

The importance of DHA and EPA has been reported in patients with major depressive and bipolar disorders, Alzheimer's disease, Parkinson's disease (PD), and amyotrophic lateral sclerosis by Zarate *et al*^[50]. Hohen and Yahr staging and the Unified Parkinson's Disease Rating Scale are commonly used for clinical evaluation of PD^[51]. Present studies were carried out using AVJ with ω -3PUFA (DHA and

Table 1 Response of the supplement (EHEM and curcumin) with or without AVJ supporting healthy muscles and joints

Responses by subjects (n = 33)	Without AVJ (%)	With AVJ (%)	Recovery (%) in total*
Low back pain	60	83.3	73.3
Hip arthralgia pain	75	100	87.5
Knee joint pain	71.4	80	76.5

Table 3 Response of ω -3 PUFA supplements (DHA and EPA), L-Arginine with AVJ to Parkinson's disease: case reports. The study was assessed based on PD stages. Patients with diabetes daily took in ω -3PUFA, L-Arginine, and EHEM/curcumin supplements with AVJ from Apr. 2016 (case 2), May 2017 (case 3), and Nov. 2017 (case 1).

	Prescription	Remission
Case 1		
Patient: Female, 72 y; PD stage 3, diabetic. Start on Nov. 2017	PD-drug; AVJ; 300ml, DHA/EPA, each 0.3g; Arginine; 5g/3times/day, Move* ¹ , 0.75g/capsule, 3 capsules/day	Jan. 2018: PD stage 2 Apr. 2018: PD stage 2. Jan.2018: HbAc1 6.5; (normal: \leq 6.2) HOMA-R 2.3; (normal: \leq 1.6)
Case 2		
Patient: Male, 79 y; PD stage 3, diabetic. Start on Apr. 2016	PD-drug; AVJ; 600ml; DHA/EPA, each 0.6g; Arginine; 5g/3times/ day; Move* ¹ , 0.75g/capsule, 3 capsules/day	Apr. 2/2018; PD stage 2, Diabetic assessment: recovered.
Case 3		
Patient: Female, 65y; PD stage 3; Start on May, 2017	PD-drug; AVJ* ¹ ; 200ml, Arginine 5g/2times/day	July 2017; PD stage 0

*1. AVJ supplement (Forever Move) composed of egg shell protein, curcumin and additives.

EPA) and L-Arginine to PD patients with diabetes from April 2016.

RESULTS

Aloe vera juice (AVJ) with the supplements (curcumin, enzyme-hydrolyzed NEM (EHEM) and L-Arginine) may supports the subjects in daily pain troubles of motion, enhances joint comfort and flexibility, promotes healthy cartilage, decrease of stiffness in joints, and promotes fast recovery from exercise in healthy subjects. The decreased pain troubles were shown on Table1. The subjects having pain troubles more than 32 points in locomotive syndrome risk test decreased after ingestion of the supplement (curcumin, EHEM, and arginine) with AVJ in a ratio of 13.9%, and the subjects having in daily pain troubles less than 14 point increased to 70.2% on Table 2. No side effect from consuming the supplement was confirmed.

The PD patients with diabetes in case 1 and 2 were in remission PD-stage 2 by treatment of PD-drug and ω -3PUFA/L-Arginine supplements with AVJ after the treatment of several months (Table 3). The PD patient in case 3 is in remission PD-stage 0 by the treatment of PD-drug and L-Arginine supplement with AVJ after two months (Table 3). Preclinical case studies showed that treatments of PD-drug with ω -3PUFA, L-Arginine with AVJ may improve cognition and muscle stretching. No side effect from consuming the supplement was confirmed.

SUMMARY

In present review the efficacy of *Aloe vera* and acemannan as a biomedical scaffold, was discussed to be useful for tissue engineering as a wound dressing material and drug-eluting implant.

Response to questionnaire for locomotive syndrome was administered. The study before or after questionnaire pointed out that AVJ with curcumin and EHEM supplement support healthy cartilages and muscles, demonstrating for the treatment of pain and stiffness associated with osteoarthritis of the knee and/or hip. Furthermore, locomotive syndrome risk (LSR) test suggested that

Table 2 Assessment of efficacy and tolerability with the supplementation of AVJ and the supplement (EHEM and curcumin) before or after ingestion.

Subjects with AVJ and the supplement: EHEM and curcumin (n = 151)	Before ingestion (%)	After ingestion (%)
Subjects in daily pain troubles, locomotive risk: less than 14 point	72; 47.7	106; 70.2
Subjects in locomotive risk 1: 14 to 32 point	32; 21.2	24; 15.9
Subjects in locomotive risk 2: more than 32 point	47; 31.1	21; 13.9

subjects suffering from daily pain troubles risk 1 and 2 in LSR test may be improved in daily pain troubles by EHEM and curcumin supplementation with AVJ.

Case reports on ω -3PUFA, L-Arginine and AVJ supplement to PD patient with/without diabetes showed that the PD patients at the stage 3 showed possible remission in movements by treatment of PD-drug, 3- ω PUFA and L-Arginine supplement with AVJ. The benefits of AVJ with L-Arginine to chronic fatigue languid syndrome, constipation and muscle pains on adult subjects were reported in previous paper^[52].

Prophylactic effects of nutraceutical AVJ as a bio-functional scaffold with EHEM, ω -3PUFA, curcumin, and L-Arginine are fully expected for increasing collagen synthesis in knee and/or hip joint healing and for mitigation of risk factors in PD patients with diabetes.

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