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Contents

Biopolymer-based scaffolds for corneal stromal regeneration: A review

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Abstract

The stroma is one of the 5 layers of the cornea that comprises more than 90% of the corneal thickness, and is the most important layer for the transparency of cornea and refractive function critical for vision. Any significant damage to this layer may lead to corneal blindness. Corneal blindness refers to loss of vision or blindness caused by corneal diseases or damage, which is the 4th most common cause of blindness worldwide. Different approaches are used to treat these patients. Severe corneal damage is traditionally treated by transplantation of a donor cornea or implantation of an artificial cornea. Other alternative approaches, such as cell/stem cell therapy, drug/gene delivery and tissue engineering, are currently promising in the regeneration of damaged cornea. The aim of tissue engineering is to functionally repair and regenerate damaged cornea using scaffolds with or without cells and growth factors. Among the different types of scaffolds, polymer-based scaffolds have shown great potential for corneal stromal regeneration. In this paper, the most recent findings of corneal stromal tissue engineering are reviewed.

Key words: biopolymer, tissue engineering, scaffold, corneal stroma

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Introduction

The outer layer of the eye consists of sclera and cornea. The cornea plays an essential role in the ocular light pathway and consists of 5 distinct layers (from outside to inside): the epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. Injuries leading to scarring and diseases such as keratoconus (cornea progressive thinning) and bullous keratopathy (an endothelial dysfunction which causes formations of small vesicles in the cornea) can cause blindness and visual impairment due to corneal damage.[1] Corneal blindness is a widespread problem that is the 4th cause of blindness in the world, with more than 10 million people having bilateral blindness, while only about 185,000 corneal transplants are performed annually worldwide.[2,3] Treatment of corneal blindness imposes considerable economic pressure on the medical system and patients. The management of the pathological conditions seems to be an important issue in reducing the economic pressure and improving patients' quality of life.[4,5]

Corneal transplantation is still the most frequent type of transplant in the world, which can improve the visual function in case of severe corneal damage. Transplanted allograft tissue poses the risk of stimulus-immune responses that may cause transplant rejection; there is also a possibility of transmitting certain diseases from the grafted tissue.[6] In addition, transplantation of an organ or a tissue may be a process with numerous cultural, ethical and legal barriers.[7] To address these issues, many scientists have tried to replace the cornea with a variety of alternative solutions (Fig. 1).

Corneal replacements include 2 categories: keratoprosthesis and tissue-engineered structures. Keratoprosthesis or corneal prosthesis is a surgical alternative to donor transplantation. Various commercially available corneal prostheses, such as Boston KPro and osteo-odonto-keratoprosthesis (OOKP), are used clinically with a different rate of success.[8] Although the material and design of the prostheses vary, poly(methyl methacrylate) (PMMA) is considered as a basic primary material.[9] Retinal detachment, calcification, glaucoma, corneal melting, prosthesis extrusion, and some other complications are reported as a result of using these keratoprostheses.[10]

Researchers have also developed cell-/stem cell-based methods to overcome the limitations of previous approaches. Cell therapy methods are used to regenerate the endothelium and epithelium layers, but rarely for stromal regeneration. Due to the limitations of the current methods, alternative regenerative approaches are required. In corneal tissue engineering, different engineered structures are used to form corneal substitutes. Biomaterials used for corneal regeneration should have several critical features: high transparency, biocompatibility and moisture conservation.[11] Suitable mechanical properties are essential factors of the cornea to protect its structure, morphology and normal functionality.[12]

In this review, we focus on the most recent available corneal stroma replacement approaches. While the corneal endothelium and the ocular surface have been a subject of interest in corneal investigations for several years, stromal regeneration has recently become the subject of equally studied research. This is because this layer is probably the most challenging of all 3 layers to repair, regenerate or replace. The complex structure of the stroma makes it very difficult to be engineered, and therefore a wide range of approaches (including polymer-based scaffolds) are being investigated in order to find an optimal stromal replacement. This paper provides a review of recent polymer-based scaffolds used for corneal stromal tissue engineering.

Anatomy and histology of the cornea

The cornea is a transparent connective tissue with no blood vessels that provides an optical interface. It protects the eye from infections and provides good mechanical support. The human cornea is 12–13 mm in diameter and 0.5 mm in thickness at its center.[13] The 3 distinct cellular layers, including corneal epithelium (external layer), stroma and endothelium (internal layer), are separated by 2 acellular interfaces. Bowman's layer is between the stratified

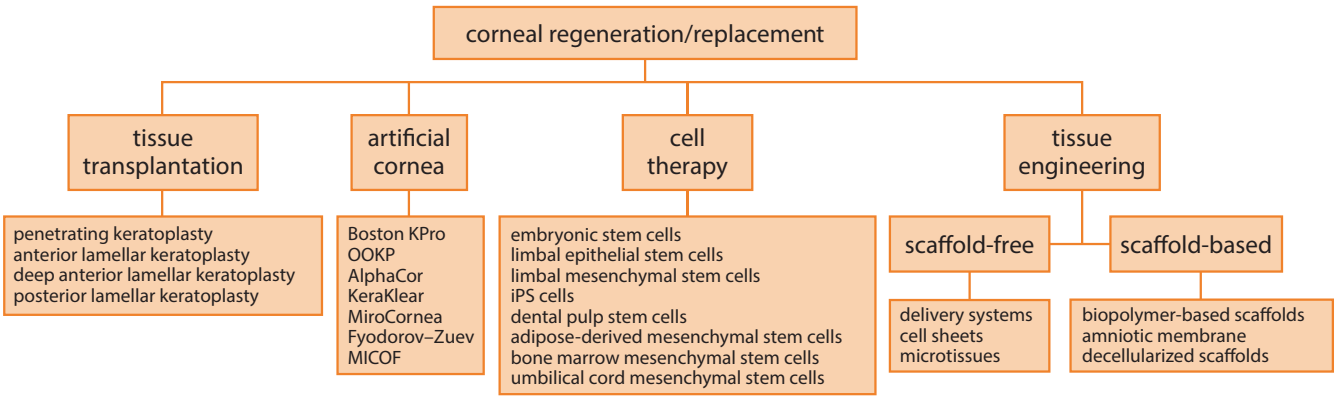

Fig. 1. Therapeutic strategies for corneal replacement/regeneration

epithelium and the stromal layer. Descemet's membrane is the basal lamina of endothelium that separates it from the stromal layer. The cornea tissue is rich in collagen and contains a leucine-rich proteoglycan-like keratan sulfate. The corneal epithelium is a 4–6-layered non-keratinized stratified squamous tissue with 40–50 μm thickness, which is highly innervated.[14,15] Tear film, which covers the outside of the epithelium, provides a smooth surface that can help light refraction. Moreover, it is anti-bacterial and necessary for the proliferation, repair and maintenance of epithelial homeostasis.[16] Bowman's membrane is a condensed layer of collagen 15 μm in thickness located posterior to the epithelium. It is also known as anterior limiting lamina. Bowman's membrane is one of the barriers regulating the transfer of molecules. Approximately 90% of the thickness of the cornea is attributed to the stromal layer, which consists of aligned collagen fibers (lamellae), and there are different collage types, such as collagens type I, V, XIV, XII, and VI.[17] Decorin, lumican and keratocan are small leucine-rich proteoglycans that regulate hydration of the cornea and are also required for its transparency.[13,18] The woven collagen bundles between adjacent lamellae provide mechanical strength needed to withstand shear stress by transferring stress between lamellae. In the embryonic period, keratocytes migrate from neural crest to the corneal stroma and locate between lamellae to produce the matrix components.[19] A thick type VIII collagen-rich basement membrane is located posterior to the stroma named Descemet's membrane. The corneal endothelium layer anchors this membrane. The endothelium removes water from the stroma to maintain corneal transparency.[20,21] The main functions of the cornea are determined as protection, transparency and maintaining optical properties. The stroma is a dense, non-vascularized tissue that contains organized collagen fibrils to protect tissue from tensile strength and shear stress (Fig. 2). Collagen fibers and endothelium function provide the transparency of the cornea, which determines optical properties.[22]

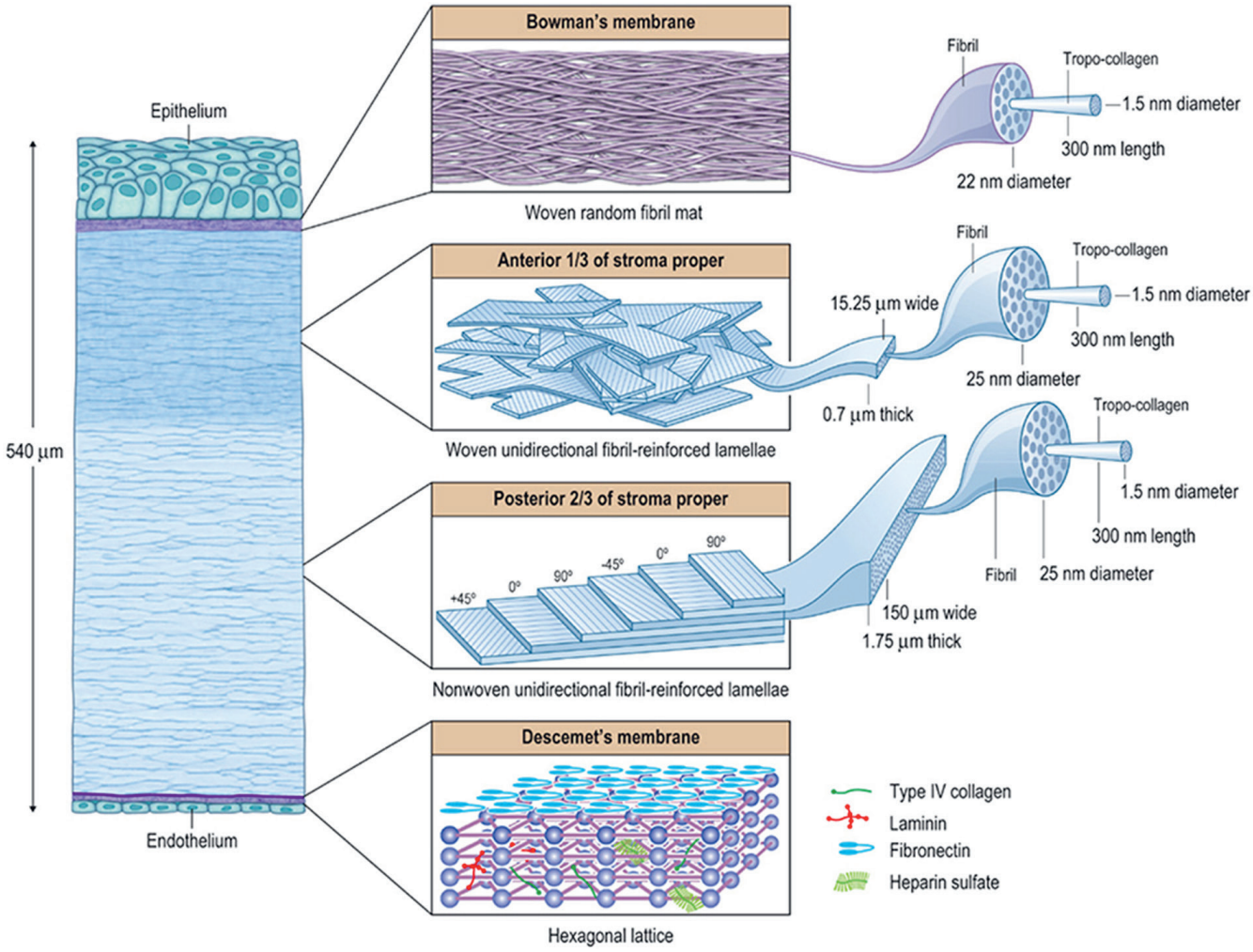

Fig. 2. The structure of cornea indicating that it is composed of 3 cellular layers separated by Descemet's membrane and Bowman's layer. The histology and molecular structures of the cornea are shown to help illustrate different interactions between the corneal tissue components. The anterior third of the corneal stroma is a lamellar interwoven fabric composed of unidirectionally fibril-reinforced lamellae. The posterior two-thirds of this tissue is a non-woven, unidirectionally fibril-reinforced lamellae. This highly specialized structure brings strength and stiffness for corneal tissue. The unidirectional orientation of collagen fibrils in each lamella is critical, because this unique arrangement prevents fibril undulation and also maintains the mechanical properties of the cornea. Reprinted with permission from Elsevier[22]

Tissue transplantation

One of the current clinical approaches is to replace the full-thickness tissue through transplantation of a cornea during penetrating keratoplasty. These corneas are obtained from cadaveric donors. All 5 layers, including the epithelium (as the corneal surface), Bowman's membrane, stroma, Descemet's membrane, and endothelium, would be replaced after penetrating keratoplasty. Some remarkable changes in the functioning and morphology of the corneal surface were observed after this surgery. For example, after penetrating keratoplasty, the metabolism of the epithelial cells decreased in terms of oxygen absorption compared to the healthy eye.[23,24]

Anterior lamellar keratoplasty and deep anterior lamellar keratoplasty are also available transplantation techniques. In these surgeries, the host corneal endothelium and Descemet's membrane are left untouched. In the deep anterior lamellar keratoplasty, the corneal surface and the whole stroma are replaced, but in the anterior lamellar keratoplasty, a part of the patient's stroma is left intact. Both of these techniques are considered partial replacements of corneal tissue. The advantages of these methods compared to penetrating keratoplasty are that they are less invasive and also reduce endothelial damage, which prevents transplant rejection.[25,26]

Posterior lamellar keratoplasty is another technique to replace the corneal endothelium, Descemet's membrane and posterior part of the stroma.[27] Transplantation of the corneal endothelium was first described by Melles et al.[28] They called this technique posterior lamellar keratoplasty. This technique was then improved by Terry and Ousley, who renamed it deep lamellar endothelial keratoplasty. This surgery required manual lamellar dissections within the deep corneal stroma of both the donor and the recipient corneas.[29] The next significant modification of posterior lamellar keratoplasty was Descemet's stripping endothelial keratoplasty. In this procedure, instead of performing a lamellar dissection, the patient's Descemet's membrane is peeled off using specially designed strippers.[30] Compared with deep lamellar endothelial keratoplasty, Descemet's stripping endothelial keratoplasty is easier to perform, and stripping the Descemet's membrane leaves a very smooth recipient interface onto which the donor can be applied. This may lead to better visual results, but has also been implicated as a cause of early postoperative donor dislocations.[27]

Although all of these surgical techniques provide good chances for patients, there is still a significant limitation in the number of donors.

Artificial corneas

As the number of donors decreases, artificial alternatives need to be developed. Pellier de Quengsy used a bio-inert glass as a substitute of cornea for the first time in 1789.[31] In 1953, Stone and Herbert showed that PMMA constructs were well-maintained in the eyes of rabbits for 24 months.[32] Artificial PMMA-based keratoprostheses are now commercially available and used in clinical setting. A keratoprosthesis consists of 2 main parts: a cylindrically shaped optical part (core part) and a surrounding skirt (haptic part) which ensures tight connection to the ocular tissue. The keratoprosthesis should preferably be manufactured through mechanical shaping from one piece of polymer for long-term tight connection between the optic and haptic parts. The polymer should be hydrophobic to avoid interaction with eye medications and dimensional changes. The polymer should also be flexible to allow the skirt to follow the movement of the surrounding tissue and prevent local stress. Moreover, the polymer must be transparent and immunologically safe to be used in human eyes. Therefore, the consortium focuses on the evaluation of various acrylic polymers with a glass transition temperature around 10°C. This allows mechanical shaping at low temperature and flexibility at the temperature of the human eye.[10,33] Different materials are used as optic and haptic parts to produce various keratoprostheses (Table 1).

Boston KPro is likely to be the most well-known keratoprosthesis made of PMMA as the optic part and titanium as the haptic part.[34] Another example of the use of PMMA-based artificial corneas is in OOKP, which uses a piece of tooth as a supporting structure. Many complications such as glaucoma, retinal detachment, prosthesis extrusion, calci-

Table 1. Haptic and optic parts of different keratoprostheses[40–46]

Keratoprosthesis	Type	Haptic part	Optic part	Reference
Boston KPro	Hard KPro	titanium	PMMA	40
OOKP	Hard KPro	a piece of tooth	PMMA	41
Miro®Cornea	Hard KPro	hydrophobic acrylic polymer	hydrophobic acrylic polymer	42
KeraKlear®	Hard KPro	hydrophilic acrylic polymer	hydrophilic acrylic polymer	42
Fyodorov–Zuev KPro	Hard KPro	titanium	PMMA	43
MICOF	Hard KPro	titanium	PMMA	44
AlphaCor	Soft KPro	poly(2-hydroxyethyl methacrylate)	poly(2-hydroxyethyl methacrylate)	45
Legeais BioKPro-III	Soft KPro	polytetrafluoroethylene	polyvinylpyrrolidone-coated polydimethylsiloxane	46

PMMA – poly(methyl methacrylate).

fication, and corneal melting have been reported after employing artificial corneas.[35,36] Many of these complications arise from the hydrophobic nature of the rigid materials used in these constructs. Hydrogel-based skirt prostheses were used in patients with a history of corneal transplant rejection.[37] Fyodorov–Zuev KPro, KeraKlear® and Miro®Cornea are other examples of keratoprostheses which are not widely used in clinics. Fyodorov–Zuev KPro is made of PMMA and titanium, and its properties are similar to the Boston KPro. Fyodorov–Zuev KPro is a common keratoprosthesis for cornea transplantation in Russia and China. KeraKlear® represents a flexible structure developed in the USA. Miro®Cornea has been developed in Germany. One of the main complications of the recent prostheses is the formation of retro-prosthetic membrane after transplantation.[38,39]

Biopolymer-based scaffolds for corneal stromal tissue engineering

The shortage of donors and insufficient application potential of the keratoprostheses have led to numerous research studies investigating the production of tissue-engineered epithelial, stromal and endothelial replacements. Corneal stromal regeneration is a challenge for scientists because it has a complicated structure, and also unique optical transparency and mechanical strength. Corneal stromal regeneration is one of the critical targets for researchers, because creating functional stroma is very important in the treatment of corneal dysfunctions, and obtaining a prosthesis with satisfying mechanical, chemical and morphological properties alike is one of the most challenging issues in corneal stroma tissue engineering.[47] Nowadays, using biopolymer-based scaffolds is a promising approach that has attracted much attention from research teams and is focused on regenerative strategies using different biomaterials in combination with various cell types. Biocompatibility, transparency and strength are considered to be the most important factors for corneal scaffolds. In addition, scaffold-based approaches are focused on the fabrication of constructs that could mimic the microenvironment of the native tissue to support cell adhesion, migration, proliferation, and differentiation.[48]

The researchers have used synthetic polymers as a substrate for engineering corneal stroma because they have adjustable mechanical properties.[49,50] Moreover, some of these scaffolds have the capacity of inducing the differentiation of human stromal stem cells into keratinocyte lineage. For example, poly(ester urethane) has been used as a scaffold in combination with stromal stem cells in order to differentiate these cells to the keratinocyte lineage. Despite the differentiation of the stem cells, there were some weaknesses in the optical properties of the scaffold.[51] Synthetic and natural biopolymers could be blended to improve the biological and optical properties.[48] For example, Ozcelik et al. showed that hydrogel films composed of collagen type I, chitosan, poly(L- and D-lactic acid), and poly(ethylene glycol) present excellent biological, optical and mechanical properties compared with synthetic materials alone.[52] Collagen type I, as a natural biopolymer, has many advantages such as encapsulating living cells, especially in the natural human cornea. This biopolymer is one of the components of stroma, so using this biopolymer as a component of corneal scaffolds could play an important role in corneal stromal regeneration.[53] Collagen type I hydrogels have some weaknesses in mechanical properties that could be partially eliminated by chemical cross-linkers.[54–56] For aligning the arrangement of fibroblasts (similar to the arrangement of the stromal cells), it is possible to produce aligned nanofibers of type I collagen using the electrospinning method, although this reduces transparency.[57]

Silk fibroin has been widely used for a variety of tissue engineering and biomedical applications. Due to the biocompatibility and transparency of silk protein, silk fibroin-based scaffolds are also utilized for corneal stromal tissue engineering.[58] Silk films with well-developed topography, chemical surface modification, degradation rate, and porosity could provide excellent optical, mechanical and biological properties.[59,60] Such optimized silk films seeded with suitable cell types can provide a high potential to be used as a functional corneal tissue equivalent in clinical approaches. Lawrence et al. fabricated silk thin films to replicate corneal stromal tissue architecture.[61] The films were surface-patterned to induce cell alignment. To improve nutrients diffusion and to enhance cell interactions, micropores were introduced into the thin films. Proliferation of corneal fibroblast and expression of corneal extracellular matrix (ECM) on the silk films demonstrated the biocompability of these films. Their optical and mechanical properties were also appropriate to support the corneal stromal functions.

A strategy to improve the biocompatibility of scaffolds is to coat or modify their surfaces.[62,63] In this regard, Ma et al. fabricated PMMA hydrogels surface-modified with amines and then coated with ECM proteins such as collagen I and IV, fibronectin, and laminin. The hydrogels were then surgically implanted into bovine corneas. The results demonstrated that specific surface modifications promote biocompatibility of the hydrogels.[64] In another study, Gil et al. prepared arginine-glycine-aspartate (RGD)-coupled silk lamellar systems and studied the behavior of human corneal fibroblasts (HCF) in the presence of this system.[65] They produced RGD-coupled, porous, patterned, transparent, and mechanically robust silk films. The effect of RGD-coupling on the proliferation, orientation, gene expression of HCF, and ECM organization was assessed. The results indicated that RGD surface modification improved proliferation, cell attachment, alignment, and expression of type I and V collagens, and also increased the expression of biglycan and decorin proteoglycans. They claimed that this system could mimic the structure of corneal stromal tissue and give a useful strategy to achieve an engineered human cornea.

Table 2. Polymer-based scaffolds for corneal stromal regeneration

Polymer-based scaffold	Cross-linker	Cell type	Clinical status	Reference
Gelatin/chondroitin sulfate porous scaffold	carbodiimide	rabbit corneal keratocytes	in vitro	66
Poly(ε-caprolactone)/silk fibroin electrospun scaffold	-	human stromal keratocyte cells	in vitro	67
Poly(ε-caprolactone) electrospun membrane	glutaraldehyde	human corneal stromal cells	in vitro	68
Gelatin/chondroitin sulfate	EDC/NHS	rabbit corneal keratocyte	in vitro	69
Poly(ε-caprolactone)-poly (ethylene glycol)/GelMA hydrogel	–	limbal stromal stem cells	in vitro/in vivo (rat)	70
Silk film	–	human corneal stromal stem cells and dorsal root ganglion neurons	in vitro	71
Porous silk film	–	stromal cells	in vitro/in vivo (multipocket corneal stromal rabbit models)	72
Multilayered silk films	–	human corneal epithelial and stromal stem cells	in vitro	73
Compressed collagen	transglutaminase	corneal stromal cells	in vitro/in vivo (female New Zealand rabbits)	74
Polyglycolic acid (PGA) fibers	–	rabbit corneal stromal cell	in vitro/in vivo (female rabbit)	75
Multi-layered silk film	–	human corneal stromal stem cells	in vitro	76
Poly(ester urethane) urea fibrous substrate	–	human corneal stromal stem cells	in vitro	51
Gelatin/ascorbic acid cryogels	cryogelation technique	rabbit keratocyte	in vitro/in vivo (alkali burn-induced animal model)	77
Aligned poly(ester urethane) urea substrate	–	corneal stromal stem cells and human corneal fibroblasts	in vitro	78
Aligned polycaprolactone nanofibers	–	adult dental pulp cells	in vitro/in vivo (mouse)	79
Keratocyte spheroids fabricated on chitosan coatings	–	rabbit stromal cells	in vitro/in vivo (rabbit corneal stromal defect model)	80
Collagen type I gel	bio-orthogonal strain-promoted azide–alkyne cycloaddition	keratocytes	in vitro	81
Fibrin and fibrin-agarose scaffold	–	–	in vitro	82
Magnetically aligned collagen fibrils	transglutaminase	keratocytes	in vitro	83
Silk fibroin/chitosan scaffold	–	primary rabbit corneal epithelial cells and corneal stromal cells	in vitro/in vivo (New Zealand white rabbits)	84
Collagen/poly (N-isopropylacrylamide) membrane	–	epithelial corneal cells	in vitro/in vivo (rabbit)	85
Methacrylated gelatin	UV	human keratocytes	in vitro	86

EDC – N-ethyl-N'-(3-(dimethylamino)propyl)carbodiimide; NHS – N-hydroxysuccinimide.

The number of studies on corneal stromal regeneration has increased over the last decades. Table 2 contains recent approaches regarding corneal stromal regeneration, applying different biopolymer-based scaffolds.

Conclusions and future perspectives

Various approaches have been developed to replace or regenerate corneas. Each of the described methods has had important contributions to the rapidly evolving field of corneal stromal tissue engineering. The stroma is an important layer in the cornea, and its reconstruction in patients with corneal blindness means a huge improvement in their quality of life and also the possibility to restore their sight. While the ideal stromal replacement has not been established yet, there have been important efforts in the direction of a fully functional and biocompatible stromal transplant. Current corneal blindness treatment options, due to stromal opacities, remain limited to penetrating keratoplasty, anterior lamellar keratoplasty, deep anterior lamellar keratoplasty, and artificial cornea. The shortage of cornea donors and side effects of artificial corneas bring limitations to these methods. Therefore, novel approaches are needed to overcome these limita-

tions. Although some research has confirmed the effectiveness of cell-/stem cell-based strategies to regenerate corneal stroma, their inadequate regenerative potential encourages researchers to use scaffolds as the supporting structures. Significant progress has been made in recent years in corneal tissue engineering regarding regenerating damaged corneal stroma or replacing it using natural and/or synthetic biopolymers. Future studies should focus on combining different methods to achieve transparent and well-maintained stromal replacements that will be able to not only host stromal cells, but also re-establish stromal functionality to restore vision.

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Design and evaluation of the antimicrobial properties of ackee seed extract silver nanoparticle film formulations

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Abstract

Background. Plant-extract-reduced metal nanoparticles provide means of overcoming microbial resistance. Incorporating them into appropriate pharmaceutical formulations will enhance their portability and ease of administration.

Objectives. To synthesize silver nanoparticles using methanol extracts of the seeds of *Blighia sapida* as capping agents and formulating the products in antimicrobial films.

Material and methods. Phytochemical screening of the methanol extract of *Blighia sapida* K.D. Koenig (ackee) seeds was performed and its antioxidant properties were determined using DPPH (1,1-diphenyl--2-picrylhydrazyl) assay. The green synthesis of ackee seed extract silver nanoparticles (ASAgNPs) was accomplished with reacting 1 mM of aqueous silver nitrate ($AgNO_3$) and the methanol extract in a flask; the bioreduction was performed at 37°C for 72 h. The resulting nanoparticles were lyophilized and characterized using UV-visible spectrophotometry, Fourier-transform infrared spectroscopy (FTIR) and photomicrography. The nanoparticles were further formulated into films using starch and carboxymethyl cellulose using the solvent evaporation method. The extract, biosynthesized nanoparticles and film formulations were screened for antimicrobial activity against several pathogens using the agar well diffusion method.

Results. The methanol seed extracts of the ackee fruit contained saponins, tannins, flavonoids, terpenoids, and anthraquinones. The extract exhibited significant antioxidant properties. The nanoparticles and film formulations had a broader range of activity against microbes than the plant extract, exhibiting significant activity against *Escherichia coli* ATCC 700728, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. Activity was also observed with *Candida krusei*, *C. albicans*, and *Penicillium* sp. It is noteworthy that this last organism showed resistance to fluconazole.

Conclusions. Ackee seed extract silver nanoparticles exhibited a synergistic antimicrobial activity against several pathogens. Film formulations of the nanoparticles retained this antimicrobial activity and allowed the product to be presented in a consumer-ready form.

Key words: silver nanoparticles, ackee seeds, antimicrobial films, *Blighia sapida*

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Introduction

The need for new, potent and affordable drugs for the treatment of microbial infections in the developing world is one of the issues facing global health today. However, finding effective drugs for the treatment of these infections is hindered by factors ranging from microbial resistance to safety, compliance and cost. The use of medicinal plants for curative purposes is as old as mankind, but coupled with the latest developments in nanotechnology, they can be used to treat diseases. A synergistic formulation is expected to result from combining the antimicrobial properties of plant extracts with the metal nanoparticles in the form of film formulations for ease of use.

The green synthesis of metal nanoparticles from plant extracts is an attractive alternative to physical and chemical methods. This method is simple, the costs are low, the production time is short, and it is amenable to large-scale production, does not require extreme temperature or pressure, and eliminates the need for toxic reagents.[1–3] The synthesis of metal nanoparticle using plants has the additional advantage of stabilizing the nanoparticles, since plant biomolecules exert a twofold effect of reducing and capping the biosynthesized nanoparticles.[4–6]

Ackee (*Blighia sapida* K.D. Koenig; Family: Sapindaceae) is a herbaceous, biennial plant. It is native to West Africa and is also cultivated in India and the American tropics. It is well-distributed throughout Nigeria and is found in drier forests of the savannah region.[7] Ackee seeds contain bioactive substances such as saponins, flavonoids, tannins, terpenoids, alkaloids, steroids, and anthraquinones.[8–10] These bio-constituents contribute to its antioxidant, anti-inflammatory, anti-diarrheal, and antimicrobial activities. Ackee provides medicinal value for traditional healers in Nigeria and across Africa for the treatment of several ailments.[11] Ackee fruit is rich in essential fatty acids, vitamin A, zinc, and protein.[8,12]

While several studies have reported the antibacterial activity of silver nanoparticles (SNPs) synthesized from plant extracts, no research has been performed on the synthesis of SNPs from *B. sapida* and subsequent formulation into antimicrobial and antioxidant films for ease of application.

Material and methods

Collection of ackee seeds and preparation of plant material

Seeds of *Blighia sapida* were collected during the fruiting season from the Botanical Garden, University of Ibadan, Nigeria. The seeds were thoroughly washed, rinsed and oven-dried at a temperature of 40°C. The oven-dried seeds were then blended and extracted using methanol.

Method of extraction

Two kilograms of the dried seed sample was transferred into a glass container; 7.5 L of pure methanol was added, then stirred every 2 h with a glass rod and allowed to stand for 72 h. The solvent (now containing the extract) was collected using a muslin bag. The filtrate was further filtered using Wattman No. 1 filter paper. This process was repeated twice with another 5.0 L of pure methanol added each time to the chaff. The combined filtrate was then concentrated with the aid of a rotary evaporator (Heidolph Laborota 400; Heidolph Instruments, Kelheim, Germany) set at 40°C, after which the sample was further concentrated using a vacuum oven set at 40°C. The dried extract was weighed and the percentage yield was calculated.

Both qualitative and quantitative phytochemical screening of the plant extract were performed using standard procedures.

Antioxidant activity according to DPPH scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical thanks to the free electron that is delocalized around the molecule, thereby preventing the dimerization that other free radicals undergo. This delocalization gives the molecule its deep violet color which is characterized by an absorption band in ethanol solution at a wavelength of about 517 nm. The DPPH is reduced and the violet color is lost when it is placed in a substrate that can release a hydrogen atom. To determine the antioxidant potential of the test samples, the change in optical density of DPPH radicals was monitored. The sample extract (0.2 mL) was diluted with methanol and 2 mL of a DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm.[13] The percentage of DPPH radical scavenging was calculated using the equation:

$$\% \text{ inhibition of DPPH radical} = ([A_{br} - A_{ar}]/A_{br}) \times 100,$$

where A_{br} is the absorbance before the reaction and A_{ar} is the absorbance after the reaction had taken place.

Total antioxidant capacity according to phosphomolybdenum complex formation

The measurement of total antioxidant capacity employed a spectrophotometric principle based on the reduction of Mo (VI) to a green phosphate Mo (V) complex by the sample analyte at an acidic pH. In a test tube, 0.1 mL of the sample solution (100 µg) was combined with 1 mL of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tube was covered and kept in a boiling water bath for 90 min, then cooled to room temperature. The absor-

bance of the aqueous solution was measured at 695 nm against a blank using a UV spectrophotometer (CE7400 AQUARIUS, Cambridge, UK).[12,13]

Synthesis of the silver nanoparticles using the *Blighia sapida* seed methanol extract

The methanol extract of ackee seeds was used for the biosynthesis of silver nanoparticles. Mixtures of the methanol extract at concentrations of 1:4 and 1:9 were mixed with 1 mM of an aqueous solution of silver nitrate ($AgNO_3$) in a 250-milliliter Erlenmeyer flask containing 100 mL of 1 mM of the aqueous solution of $AgNO_3$. The resulting ackee seed silver nanoparticles (ASAgNSPs) were placed into an incubator for complete bio-reduction at a temperature of 37°C for 24–72 h and were visually observed for changes in color.[6]

Characterization of the plant extract silver nanoparticles

The biosynthesized ASAgNSPs were characterized using UV-visible spectroscopy. The reduction of $AgNO_3$ to Ag^+ by the plant extract was verified using an UV-visible spectrophotometer (CE7400 AQUARIUS, Cambridge, UK). The absorption spectra of the samples were recorded at intervals of 24–72 h.

Fourier-transform infrared analysis

The Fourier-transform infrared analysis (FTIR) analysis of the ASAgNSPs was performed using a potassium bromide (KBr) pellet (Perkin Elmer, Waltham, USA) in transmission mode. Transmission spectra were obtained using 64 scans at a resolution of 8 cm^{-1} in the spectral range of 4000–400 cm^{-1}.

Antimicrobial characteristics of the synthesized ASAgNSPs

The ackee seed methanol extract and biosynthesized ASAgNSPs were screened for antimicrobial activity using the agar well diffusion method to compare their effectiveness against different microorganisms.

Using the cup-plate method, a sterile nutrient agar was prepared and poured into sterile Petri dishes and allowed to solidify. Each plate was inoculated with 25 µL (containing about 10^8 CFU/mL) of either *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Citrobacter freundii* ATCC 8090, *Salmonella typhi* ATCC 14028, *E. coli* ATCC 700728, or *E. coli* ATCC 11775. Four wells with a diameter of 8 mm were bored using a sterile cup borer. The respective wells were filled with the methanol extract of ackee seeds, the synthesized silver nanoparticles of the extract, the $AgNO_3$solution, and 25 µL of streptomycin (1 mg/mL) serving as a positive control. The plates were incubated at 37°C overnight. The antibacterial activity of each component was measured in terms of the mean diameter (in mm) of the zone of inhibition produced by each component at the end of the incubation period.

Antifungal properties of synthesized *Blighia sapida* nanoparticles

A potato dextrose agar medium was prepared and poured into sterile Petri dishes and allowed to solidify. Fungal pathogens (25 µL) were spread onto respective plates labelled *Aspergillus niger*, *Rhizopus* sp., *Candida albicans*, *C. krusei*, and *Penicillium* sp. Four wells 8 mm in diameter were bored using a sterile cup borer. The methanol extract, fluconazole (as a positive control), dimethyl sulfoxide (as a negative control), the $AgNO_3$ solution, and the silver nanoparticles were transferred into the respective wells and the plates were incubated at 28 ±2°C overnight. The antifungal activity of each component was expressed in terms of the mean diameter (in mm) of the zone of inhibition produced by each component against the fungi at the end of the incubation period.

Formulation of antimicrobial film

Three different kinds of film were formulated: 1 film without silver nanoparticles, serving as the blank or control, and 2 other films with different concentrations of the silver nanoparticles (1:4 and 1:9).

Ten grams of carboxymethyl cellulose (CMC) sodium salt was mixed with 1 L of distilled water in a large beaker using a magnetic stirrer. Corn starch (1.2 g) was gelatinized in 50 mL of distilled water at 80°C for 45 min. The gelatinized starch was added to the CMC solution and allowed to mix for 1 h. Then, 0.6 g of aluminum sulfate in 20 mL of distilled water was added to the beaker in order to investigate the optimal cross-linkage; the solution was allowed to mix for another 30 min. The Petri dishes were spread with 30 mL of the gelatinized starch-CMC solution and the silver nanoparticles were loaded onto them and dried at 70°C until a film formed.

Antimicrobial assay of formulated films

An antimicrobial assay was carried out using the films containing biosynthesized ASAgNSPs, and a control film against the clinical isolates on which the biosynthesized nanoparticles were effective, through the standard disk diffusion method. The 5-millimeter disk-shaped films were placed on sterile microbe-swabbed media in Petri dishes and incubated at 37°C (for bacteria) or 28 ±2°C (for fungi) for 24 h and 72 h, respectively. The diameter of the zone of inhibition was measured and recorded in millimeters.

Thickness and folding endurance of film

After being cut into 1 × 1-inch strips, the films – with or without nanoparticles – had their thickness measured with a micrometer screw gauge. The thickness of the film reflects how well the polymer is incorporated into the formulation. The folding endurance of the film describes the number of times the film can be bent over or folded at a particular point until it breaks.

Statistical analysis

Statistical analysis was carried out with one-way analysis of variance (ANOVA) and the t-test, using GraphPad Prism v. 7 software (GraphPad Software Inc., San Diego, USA). At a 95% confidence interval (95% CI), p-values less than or equal to 0.05 were considered significant.

Results and discussion

Phytochemical analysis of the methanol extract of ackee seeds revealed that they contained phytoconstituents such as saponins, tannins, flavonoids, terpenoids, steroids, alkaloids, and anthraquinones. Cardiac glycosides were not found, though saponin was present (Table 1). Saponins primarily modify the composition of the rumen microbial population, which results in a modification of rumen fermentation. According to Delmas et al.,[14] saponins are very toxic to fungi. The antifungal activity of saponins against *Trichoderma viride* was formerly used as a method of identifying them.

Tannins inhibit extracellular microbial enzymes, reduce bioavailable iron, and form hydrogen bonds, specific interactions with proteins such as enzymes or cell envelopes, and complex formulations with polysaccharides. Tannins have been found to have antimicrobial activity against fungi, bacteria and yeast.[15]

Flavonoids exhibit a wide range of activity, ranging from antimicrobial to anti-inflammatory, analgesic, antiallergic, and antioxidant effects. They help reduce the risk of cancer and prevent menopausal symptoms.[16] Their antibacterial effects are thought to come from their ability to form complexes with bacterial cell walls and extracellular and soluble proteins. Quercetin, a known flavonoid found in apples, has been shown to possess antioxidant properties. Both tannins and flavonoids have been found to propagate synergistic effects, which are responsible for high antioxidant activity.

The seeds of *Blighia sapida* have some alkaloidal content, and alkaloids are very useful defense systems for plants. They protect the plant against herbivores and pathogens. Hence, it can be said that *Blighia sapida* seeds have anti-inflammatory, antioxidative, anticarcinogenic, anti-allergic, immunomodulatory, antifungal, antibacterial, and protective functions. In addition, they are useful in the production of soap due to their high saponin content.

The functional groups identified by the FTIR analysis of ackee seeds were primary and aromatic alcohols, amine, amide, carbonyl, carboxylic, and alkyl halide groups (Fig. 1). These molecules have been indicated in the bio-reduction of silver ions.[6]

The DPPH assay is a fast, reliable, and reproducible parameter for analyzing the in vitro antioxidant activity of pure compounds and plant extracts.[17,18] The percentage of scavenging antioxidant activity is dependent on the concentration of extract used. A decrease in the absorbance value of the methanol extract with a corresponding increase in the concentration of the extract signifies a good radical scavenging activity of the extract (Table 2). The percentage of scavenging activity of the extract increases with the concentration of the extract; the highest percentage of scavenging activity in this study (62.1%) was found at a concentration of 1000 µg/mL. The standard ascorbic acid exhibited a higher percentage of scavenging activity than the extract at the same concentration because it contains more phenolic compounds than the extract. The total antioxidant capacity of the methanol extracts of ackee seeds showed an increase in absorbance values with a corresponding increase in the concentration of the extract, indicating that the extract possesses good antioxidant activity.

The method used for formulating the film dosage form was proposed by Suo et al.[19] and Weerawarna.[20] It in-

Table 1. Phytochemical constituents of *Blighia sapida* seeds

Test	*B. sapida*
Saponins	++
Tannins	+
Flavonoids	+
Cardiac glycoside	–
Terpenoids	+
Steroids	+
Alkaloids	+
Anthraquinones	+

Table 2. DPPH scavenging activity of methanol extracts of *Blighia sapida* seeds

Concentration	Ackee seed methanol extract [%]	Standard [%]
50 µg/mL	–	95.1
100 µg/mL	2.4	95.5
200 µg/mL	9.7	95.6
400 µg/mL	26.3	95.7
600 µg/mL	46.5	95.7
800 µg/mL	58.7	95.8
1000 µg/mL	62.1	96.9

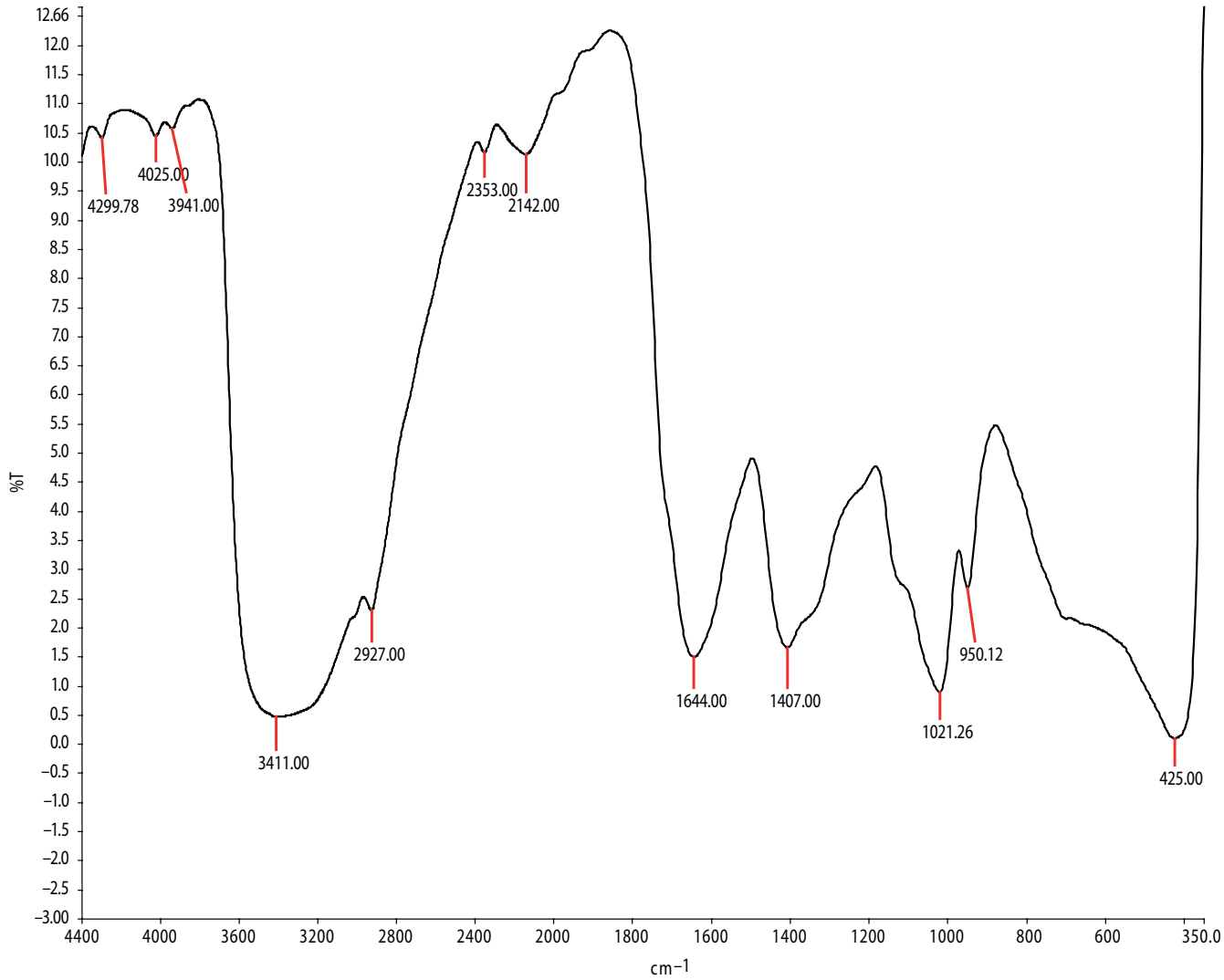


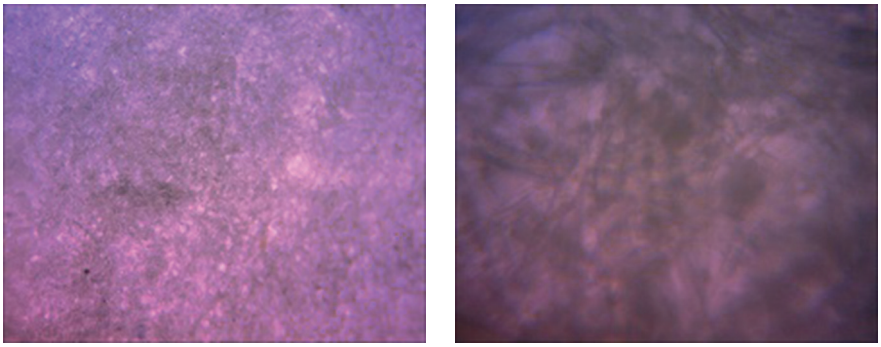
Fig. 1. FTIR spectroscopy of the biosynthesized silver nanoparticle (1:9) from ackee seed extracts

volves using a cross-linker, aluminum sulfate, which helps in holding the polymer chains together to form a film with the desired properties. The use of 2 or more polymers have been found to improve the physical properties of films, such as thickness and mechanical strength; therefore, a blend of sodium carboxymethyl cellulose and corn starch were used in the preparation. A small amount of a plasticizer – 1% glycerol – was also incorporated into the formulation to improve the flexibility of the film. The plasticizer and the polymers used in the preparation were compatible, as this is a crucial criterion that must be met.

The stability of the films was dependent largely on the level of cross-linkage across the polymer chains and the plasticizing effects of the plasticizers used. Using cross-linkers in the preparation improve the physical stability of the film by imparting thickness and mechanical strength and by preventing dissolution. However, ionic functional groups across the chain have been found to encourage water diffusion within the network.[21] It has also been discovered that the concentration of plasticizer in the preparation could result in either brittle or excessively smooth films. Hence, an appropriate amount of plasticizer is required to formulate a film with the desired characteristics (Fig. 2).

In the antimicrobial test carried out on the extract, the extract only demonstrated activity against 1 bacterium, *E. coli* ATCC 25930 (12 mm), and activity against 4 strains of fungi, *C. albicans* (14 mm), *Rhizopus* (22 mm), *C. krusei* (14 mm), and *A. niger* (14 mm). The extract showed the highest level of antifungal activity against *Rhizopus*, with a recorded zone of inhibition of 22 mm in diameter. Therefore, the extract had a more pronounced antifungal effect, encouraging its use in topical or dermatological preparations (Table 3). The biosynthesized nanoparticles (1:4 and 1:9) displayed good antimicrobial activity against the tested pathogenic organisms, as shown in Tables 3, 4.

The 1:9 ASAgNSPs demonstrated activity against 8 bacterial organisms, with most activity against *S. aureus* ATCC 29213 (a 11-mm zone of inhibition). The least activity was observed against *E. coli* ATCC 25930, with zones of inhibi-



×40 magnification ×100 magnification

Fig. 2. Photomicrograph of film strips without silver nanoparticles at different magnifications

Table 3. Antimicrobial activity of ackee seed extract silver nanoparticles (ASAgNPs) against various microorganisms, according to the diameter of the zone of inhibition (in mm)

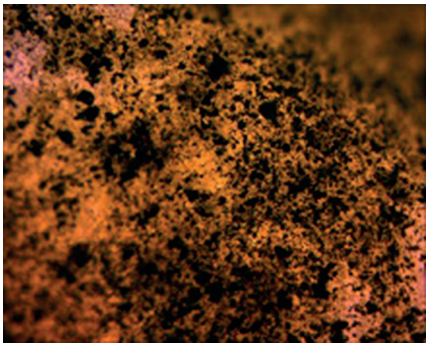
Test organism	*Blighia sapida* methanol extract	ASAgNPs		Streptomycin	Silver nitrate solution
		1:4 concentration	1:9 concentration		
Escherichia coli ATCC 25930	12.0	–	2.0	18.0	6.0
Citrobacter freundii ATCC 8090	–	4.0	9.0	18.0	3.0
Staphylococcus aureus ATCC 29213	–	12.0	11.0	16.0	7.0
Salmonella typhi ATCC 14028	–	9.0	6.0	18.0	12.0
Escherichia coli ATCC 700728	–	6.0	10.0	15.0	3.0
Pseudomonas aeruginosa ATCC 27853	–	12.0	10.0	22.0	9.0
Escherichia coli ATCC 11775	–	9.0	10.0	20.0	–
Escherichia coli ATCC 35218	–	6.0	6.0	20.0	4.0

Table 4. Inhibitory activity of ackee seed extract silver nanoparticles (ASAgNPs) against fungal organisms, according to the diameter of the zone of inhibition (in mm)

Test organism	*Blighia sapida* methanol extract	ASAgNPs		Fluconazole	Silver nitrate solution
		1:4 concentration	1:9 concentration		
Candida krusei	14.0	3.0	5.0	12.0	–
Candida albicans	14.0	2.0	6.0	14.0	2.0
Rhizopus	22.0	6.0	6.0	–	–
Penicillum sp.	–	2.0	4.0	–	2.0
Aspergillus niger	14.0	–	–	–	–

tion 2 mm in diameter. The 1:4 ASAgNSPs showed activity against 7 of the tested bacterial organisms; *E. coli* ATCC 25930 was resistant to it. The highest level of antibacterial activity was against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853, with zones of inhibition 12 mm in diameter. Of the tested fungal pathogens, only *Penicillum* sp. demonstrated resistance to the biosynthesized 1:4 nanoparticle, while the other ASAgNSP (1:9) showed good antifungal activity against 4 fungal pathogens (Fig. 3). The strongest antifungal activity of the biosynthesized 1:4 nanoparticle was against *Rhizopus* (6 mm), while the least antifungal activity was against *C. albicans* (2 mm) and *Penicillum* sp. (2 mm). The biosynthesized 1:9 nanoparticle recorded its highest antifungal activity against *C. albicans* (6 mm) and *Rhizopus* (6 mm), with the least antifungal activity being against *Penicillum* sp. (4 mm). However, *Rhizopus* and *Penicillum* sp. showed resistance to the control (fluconazole) (Fig. 4).

The ASAgNSP films (Tables 5, 6) also demonstrated good antimicrobial activity against both tested bacterial and fungal pathogens (Fig. 5). The highest antibacterial activity for the 1:9 ASAgNSP film was demonstrated against *E. coli* ATCC 700728, *Citrobacter freundii* ATCC 8090 and *P. aeruginosa* ATCC 27853 – each with zones of inhibition 15 mm in diameter. The least antibacterial activity was against *Salmonella typhi* ATCC 14028 (10 mm). However, *Klebsiella pneumoniae* showed resistance to the silver nanoparticle film. The 1:4 ASAgNSP film displayed the most antibacterial activity against *E. coli* ATCC 700728, with a 15-mm-diameter zone of inhibition. The least activity was again against *S. typhi* ATCC 14028 (10 mm), while *E. coli* ATCC 25930 and *K. pneumoniae* showed resistance



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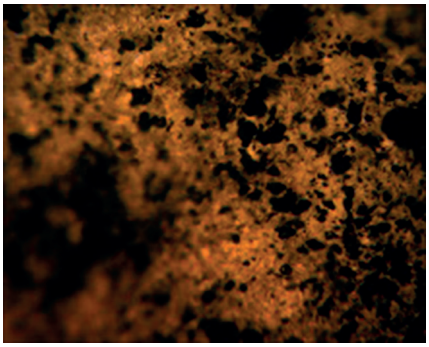

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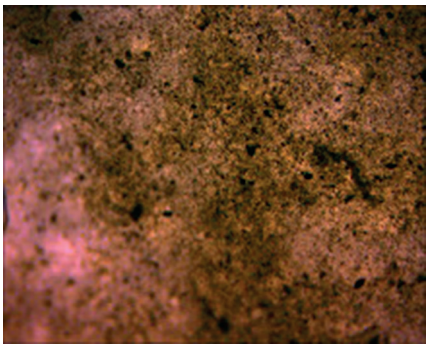
Fig. 3. Photomicrograph of ackee seed extract silver nanoparticles (1:4)



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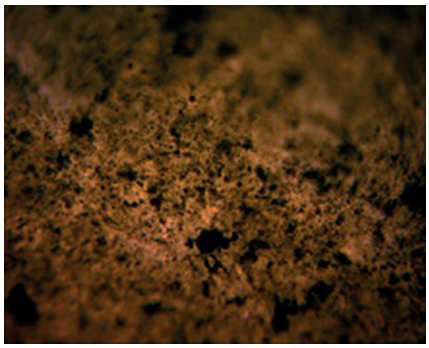

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Fig. 4. Photomicrograph of ackee seed extract silver nanoparticle film strips (1:9)

Table 5. Antimicrobial activity of ackee seed extract silver nanoparticle (ASAgNP) films against various microorganisms, according to the diameter of the zone of inhibition (in mm)

Test organism	Blank film	ASAgNP Film (1:4)	ASAgNP Film (1:9)
Escherichia coli ATCC 25930	–	–	11.0
Citrobacter freundii ATCC 8090	–	12.0	15.0
Staphylococcus aureus ATCC 29213	–	12.0	13.0
Salmonella typhi ATCC 14028	–	10.0	10.0
Escherichia coli ATCC 700728	–	15.0	15.0
Pseudomonas aeruginosa ATCC 27853	–	14.0	15.0
Escherichia coli ATCC 11775	–	12.0	12.0
Escherichia coli ATCC 35218	–	13.0	13.0
Klebsiella pneumoniae	–	–	–
Bacillus cereus	–	11.0	13.0
Proteus sp.	–	11.0	14.0
Aeromonas hydrophila	–	14.0	11.0

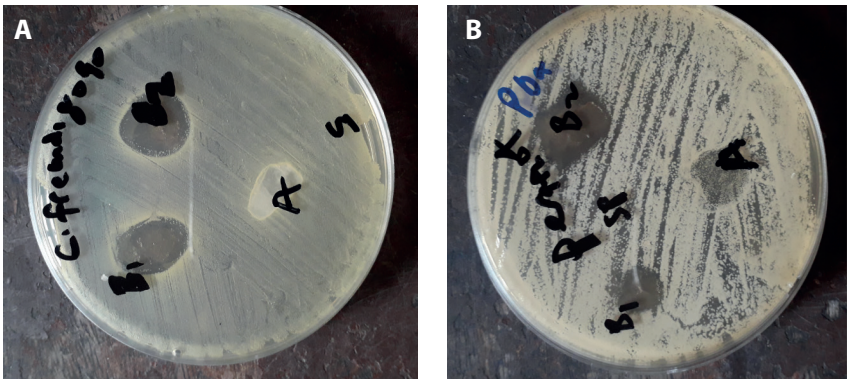

Fig. 5. Antimicrobial activity of ackee seed extract silver nanoparticle film strips against *Citrobacter freundi* (A) and *Penicillium* sp. (B)

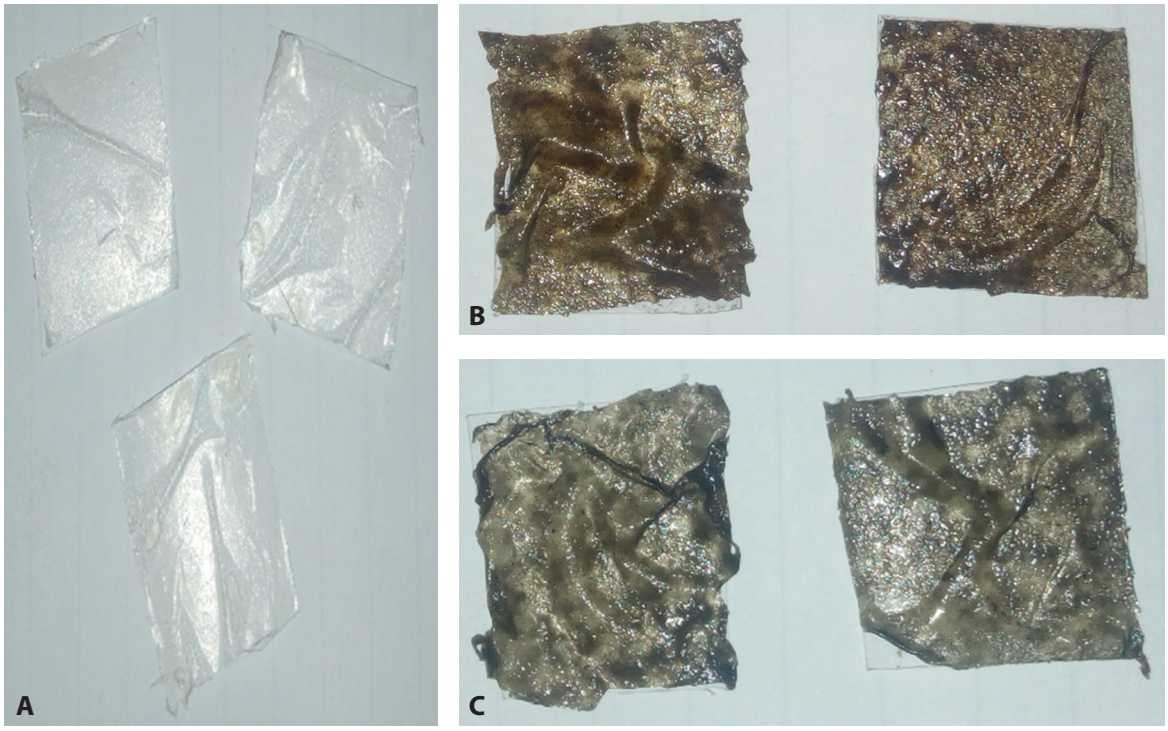

Fig. 6. Blank film strips without silver nanoparticles (A), film strips with ackee seed extract silver nanoparticles at a 1:4 concentration (B) and at a 1:9 concntration (C)

Table 6. Inhibitory activity of ackee seed extract silver nanoparticle (ASAgNP) films against fungal organisms, according to the diameter of the zone of inhibition (in mm)

Test organisms	Blank film	ASAgNP film (1:4)	ASAgNP film (1:9)
Candida krusei	–	7.0	11.0
Candida albicans	–	11.0	15.0
Rhizopus	–	8.0	8.0
Penicillum sp.	–	9.0	13.0
Aspergillus niger	–	–	–

to this silver nanoparticle film. The ASAgNSP film also demonstrated good antifungal activity against 4 out of the 5 tested fungal pathogens. The highest level of antifungal activity for the 1:9 film was seen against *C. albicans* (15 mm), with the least activity being against *Rhizopus* (8 mm); *A. niger* showed resistance to the 1:4 ASAgNSP film (Table 6). The highest level of antifungal activity for this film was also against *C. albicans*, with an 11-mm-diameter zone of inhibition; the least antifungal activity was against *C. krusei* (7 mm). No significant difference was observed in the activity of the 2 formulations. Furthermore, *A. niger* showed resistance to the film. The film without silver nanoparticles (blank) showed no antimicrobial activity. This demonstrates that the other components used in the formulation of the film had no antimicrobial properties themselves.

The biosynthesized silver nanoparticle served as a carrier for the extract, while the extract also served as a capping agent, both showing synergistic effects. The biosynthesized silver nanoparticle contained silver, which boosted the antimicrobial effects of the nanoparticle, as silver is found to have antimicrobial properties. The slightly higher values ($p > 0.05$) of the zones of inhibition of the silver nanoparticle film and the biosynthesized 1:9 nanoparticle in comparison to the 1:4 one suggests that more nanoparticles formed in the former (Table 5).

The thickness of the film is solely dependent on the amount and type of polymers used in the preparation. A film made from 2 or more polymers would yield a film of the desired thickness. The blank film was the thickest (0.21 mm), while the films containing silver nanoparticles were of relatively similar thickness (0.13 mm). The folding endurance is used to evaluate the mechanical properties of the film. It is determined by folding or bending the film at the same point to determine the number of times it can be folded until it breaks or cracks. The more folds, the higher the folding endurance and the higher the mechanical strength of the film. The film strips showed an appreciable level of folding endurance, as the fewest was 6 folds, recorded for the 1:9 ASAgNSP film. The most folds was for the film with no silver nanoparticles (blank), whereas the 1:4 ASAgNSP film was folded 10 times before it finally broke. The presence of pores within the film strips facilitated the diffusion of the materials incorporated into the film into the surrounding inoculated medium, thereby eliciting the antimicrobial activity (Fig. 6).

Conclusions

An environmental-friendly, non-toxic, cost-effective method has been devised for the biosynthesis of silver nanoparticles using *Blighia sapida* methanol extract as a capping agent. This method has been found to be a good alternative compared to the chemical synthesis of silver nanoparticles, and can be used in the commercial production of biosynthesized silver nanoparticles.

The biosynthesized ackee seed silver nanoparticle film displayed good antimicrobial activity against clinical pathogenic organisms, demonstrating a broad spectrum of activity against both gram-negative (*E. coli*), gram-positive (*S. aureus*) and fungal pathogens (*Penicillum* sp.); thus, they could be of great importance as microbial growth inhibitors, making them useful in antimicrobial control systems and medical devices.

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Focus on COVID-19: Antiviral polymers in drugs and vaccines

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Pathogenic viral factors pose a serious epidemiological threat and challenge to the world population, as proven by the scale and rapidity of COVID-19 pandemic outbreak. Polymer macromolecules can be an alternative to the accepted forms of treatment. Polymeric substances can be used as drugs or as adjuvants in vaccines. The most important feature of polymers is their advanced structure and the ability to construct the molecule from scratch, giving it the desired properties. Antiviral properties are influenced by, among other things, electrical charge, form and structure, and composition with other polymers or heavy metals. Depending on the expected properties, molecules can be built from scratch to be capable of transporting drugs or improve the effectiveness of the right drug. They can also be antiviral drugs in themselves. Polymeric compounds allow to reduce the frequency of adverse effects and improve the effect of the drug. They can have a direct antiviral effect by upsetting the lipid membrane of the surrounding viruses. Antiviral action of polymers occurs because of the properties of the polymers alone or in combination with other molecules. Viral epidemics are a motivation for research that can help stop a global pandemic in the future.

Key words: polymers, coronavirus, pandemics, antivirals, antiviral agents

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Introduction

Recurring pandemics threaten public health as known and hitherto unknown pathogens hit the world's population with great force. The danger is multidimensional and requires broad-spectrum counteraction. Researchers look for new solutions or rediscover old ones. At the moment of danger, attention is paid to polymeric compounds. Polymeric materials have a wide range of possible applications and are susceptible to modification. In medicine, they can be used for the transfer of drugs as supportive substances, e.g., as adjuvants in vaccines.[1] Their great advantage is the possibility to adjust the properties of the material according to needs – by enrichment with metal ions or nanoparticles, or by combining these materials with other compounds. Polymeric compounds can act as auxiliary compounds – as transporters of specific substances or vaccine adjuvants. The transport possibilities are determined by the chemical nature of the polymer – they allow for crossing the lipid membrane or for reaching the target cells selectively. Polymers improve the body response to the vaccine and may reduce the number of side effects or reduce the toxicity of the drug. They can be antivirals in themselves. In this work, we have focused on the aspect of the use of polymers as agents in patients. We also discussed the use of polymer compounds in the diagnosis and detection of pathogens. This study focuses on the antiviral effect of polymers, alone or in combination with other molecules, and their usefulness in reducing epidemiological threats. Attention was paid to substances capable of inhibiting coronaviruses and viruses causing respiratory diseases.

Methods

The article discusses the use of polymers in the production of drugs against human coronaviruses (HCoVs), where they act as active or auxiliary substances. In addition, the protective role against the side effects of some drugs and vaccines was emphasized. The systematic search of the literature was performed in October 2020. Research terms comprised a combination of words „polymers", „antiviral" and „coronavirus". We considered SARS epidemics from 2003, MERS-CoV from 2012 and SARS-CoV-2 outbreak from 2019.[2]

Chemical characteristics

The inactivation function of antiviral agents depends on the structure of the polymer chain. The distribution of the electric charge affects the virucidal properties of the substance. Crucial factors are the anionic characteristic of the polymer charge and the hydrophobicity of the backbone. The inhibitory effect of polymers is related to the concentration of glycoproteins in the viral envelope. Non-enveloped viruses are resistant to polymers which are virucidal against enveloped viruses. The interaction of the polymers with the viral envelope makes it difficult for the virion to attach to a cell receptor.[3] Polymers combined with heavy metals affect viral proteins and the genetic material of the virus. Also, salts of toxic ions are more effective in disinfection than nonionic metals. Elevated temperature in environment increases antiviral activity of these polymers. Combining the drug with a polymer reduces toxicity and side effects, but does not reduce drug activity. It has a positive effect on the distribution of the drug in body compartments.[4]

Many faces of virucidal polymers

Polymer compounds show different properties depending on the chemical structure and physical conditions of the environment. The polymers can be virucidal against one type of viruses or have a broad spectrum of activity. Poly(vinylbenzoic acid) (PVBzA) could be a potential antiviral agent with a broad antiviral range (Table 1). It has the ability to inhibit enveloped viruses ZIKV (Zika virus), HIV-1, Flu, Lyssa, Ebola, and SARS. This polycarboxylate showed the broadest spectrum of activity against all viruses. Among the polyphosphates, poly(vinylphosphonic acid) (PVPA) shows a high inhibitory capacity against herpes simplex virus 2 (HSV-2) and SARS; however, due to its very low effectiveness against other viruses, it can only be used to a limited extent. These compounds can be used in drug development.[3] Polymeric compounds can be obtained by deacetylation of naturally occurring chitin. The polymeric compound based on chitosan HTCC (N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride) efficiently inhibitsthe respiratory infection caused by the human coronaviruses HCoVs.[5]

Polymeric compounds are susceptible to modification. In this way, the properties of the initial compound can be modified as required. Ye et al. showed that graphene oxide conjugated with polymer has antiviral properties at different stages of viral infection.[6] They indicated that the charge of the polymer conjugated with the negatively charged graphene oxide (GO) is important. Nonionic PVP (polyvinylpyrrolidone composite) showed greater antiviral potential compared to cationic PDDA (poly(diallyldimethylammonium chloride). The inactivation mechanism is based on the cleavage of the virus by single-layer graphene oxide. Ye et al.[6] suggested the use of conjugated GO as a potential virucidal material. Low concentration of povidone-iodine (PVP-I) showed antiviral activity during 15 s of oral rinsing.[7] Such use of PVP-I is recommended for oral procedures and surgical prophylaxis. The PVP-I shows virucidal activity for use in surface and hand disinfection after contact with infectious SARS-CoV material.[8] Such vaccines contain a backbone made of gold nanoparticles, polymers such as poly(lactic-co-glycolic acid) (PLGA), chitosan, and polyetherimide

Table 1. The chemical characteristics of the polymers and their role in antiviral drugs and vaccines

Author	Compound	Type	Role
Schandock et al.	PVBzA PVPA	polycarboxylates polyphosphates	antiviral agent antiviral agent
Milewska et al.	HTCC	polisaccharides	antiviral agent
Ye et al.	GO-PVP GO-PDDA	composite composite	antiviral agent antiviral agent
Bidra et al. Kariwa et al.	PVP-I	N-vinylpyrrolidone polymer	antiviral agent
Hu et al.	PEG-PLGA	copolymer	drug adjuvant
Honda et al.	delta inulin	polisaccharides	vaccine adjuvant
Garrido et al.	cyclodextrins	oligosaccharydes	drug carrier, antiviral agent, cholesterol trapper, vaccine adjuvants
Wang et al.	metal-organic frameworks	coordination polymer	detector
Lee et al.		DNA-based nanoarchitecture	drug carrier detector

PEG-PLGA – poly(ethylene glycol)-poly(lactide-co-glycolide); PVBzA – poly(vinylbenzoic acid); PVPA – poly(vinylphosphonic acid); HTCC – N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride; PVP – poly(vinylpyrrolidone); PDDA – poly(diallyldimethylammonium) chloride; PVP-I – poly(vinylpyrrolidone)-iodine

(PEI), or protein assemblies. The antiviral agent diphyllin, vacuolar ATPase blocker, is more effective when is encapsulated in poly(ethylene glycol)-block-poly(lactide-co-glycolide) (PEG-PLGA) copolymers than alone.[9]

Polymers as adjuvants for vaccines

As shown above, the polymeric compounds can act directly as an antiviral drug. Polymers are used as auxiliary compounds for other substances, vaccines and drugs. They play the role of adjuvants for vaccines, a transport role, or improve drug distribution in body tissues. Honda-Okubo et al.[10] showed that adjuvants for coronavirus vaccines based on delta inulin can improve the effectiveness of the vaccine by enhancing memory B cells. The addition of an adjuvant speeds up the neutralization of the pathogen. Adjuvant-conjugated vaccines reduce eosinophilic immunopathological side effects in the lungs caused by disproportionate vaccine-induced Th1 response. Inulin delta-based polymers have a positive effect on the efficacy of the vaccine against coronaviruses and reduce the inflammatory response of the body, which causes an adverse immunopathological effect in the form of lung infiltration with eosinophils.

Multirole cyclodextrins

Cyclodextrins (CDs) are oligosaccharides from the dextrin group. A characteristic feature is that CDs form a torus in the solution. Due to the specific distribution of the load, they have a hydrophobic interior. The outer surface can be modified by adding nonionic, anionic or cationic groups. The CDs form inclusion complexes with hydrophobic compounds.[11] Native or modified cyclodextrins can be used as carriers for antiviral drugs. They can enhance drug activity or be used as proper virucidal drugs. The CDs show ability to interact with virus lipid membranes by encapsulating them into cholesterol traps. They can also be used as vaccine adjuvants. Notably, dimethyl-beta-cyclodextrin improves the absorption of low-molecular-weight heparins and can be used as an anticoagulant drug carrier.[12]

Supportive role of polymers

During the pandemic, quick diagnostics is important. It has become crucial to develop low-cost methods of SARS-CoV-2 infection diagnosis, with high sensitivity and specificity. The effective detection from a low number of virions is a great advantage. Coordination polymers can be used as metal–organic framework (MOF) with typical structure porosity. The MOFs in combination with fluorescence technique may be used as virus and antibody detectors in the future. Practical application is hindered by the high detection limit which is practically not available in samples taken for testing.[13] Lee et al.[14] developed modular DNA-based nanoarchitecture that can be used as a secondary carrier or pathogen detector. Their solution allows for building polymers with the desired properties from scratch.

Conclusions

Viral outbreaks are stimulating for research that could help contain future global pandemics. Polymeric compounds reduce the toxicity of the drug and the frequency

of side effects. At the same time, they can improve the effect of the actual therapeutic substance. The possibility of modification of the polymer creates an area for the study of nanoarchitecture, which will allow effective targeted therapy.

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Polymers with antiviral properties: A brief review

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Abstract

Viruses that are pathogenic to humans and livestock pose a serious epidemiological threat and challenge the world's population. The SARS-CoV-2/COVID-19 pandemic has made the world aware of the scale of the threat. The surfaces of various materials can be a source of viruses that remain temporarily contagious in the environment. Few polymers have antiviral effects that reduce infectivity or the presence of a virus in the human environment. Some of the effects are due to certain physical properties, e.g., high hydrophobicity. Other materials owe their antiviral activity to a modified physicochemical structure favoring the action on specific virus receptors or on their biochemistry. Current research areas include: gluten, polyvinylidene fluoride, polyimide, polylactic acid, graphene oxide, and polyurethane bound to copper oxide. The future belongs to multi-component mixtures or very thin multilayer systems. The rational direction of research work is the search for materials with a balanced specificity in relation to the most dangerous viruses and universality in relation to other viruses.

Key words: COVID-19, invasive virions, virucidal properties, antiviral polymers, heavy metal particles

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Introduction

Human epithelial cells of the nose and throat cavity express high abundance of the receptor ACE2 (ACE-R) utilized for high-affinity virus binding and neurolipin-1, which also aids viral invasion. Both create vulnerable environment for the SARS-CoV-2/COVID-19 entry. In addition to its strong binding to ACE2-R, the greater infectivity of SARS-CoV-2 is related to its ability to survive on a variety of surfaces over the long term.[1,2] At a time when the mass of viral infections means that vaccines and effective therapies against human coronaviruses are limited, reducing the density of invasive virions in the environment is gaining great medical and economic importance. The main effort of the medical services is put into the costly fight against the virus that has already infected the patient. A chance to reduce the costs is offered by technologies that will reduce the survival rate of a virus deposited on various objects.[2] Infectious agents can survive from hours to days under favorable conditions. The smooth surface provides a favorable environment for the survival of the virus, but the temperature shortens its half-life. At the same time, the virus is more stable in the indoor environment with low relative humidity.[3] Materials and equipment can be a source of infectious agents because virions remain active outside the host cells. Man-made substances with virucidal properties help to reduce the amount of dangerous viruses and therefore reduce the risk of infection. The SARS-CoV-2 attached on the surfaces can be inactivated through cleaning with solutions containing 0.5% hydrogen peroxide, 70% ethanol, 0.5% povidone iodine, or 0.1 sodium hypochlorite (only temporarily).[1,4] To be effective, the sanitization needs periodical repetition; one should also bear in mind that it cannot be applied to every surface.[1] Materials that lower the survival rate of pathogens are especially useful in medical devices. The SARS-CoV-2 coronavirus pandemic strengthens the demand for microparticles that can be combined with other materials, giving them virus-inactivating properties.[2]

Methods

Natural antiviral active compounds acting on the SARS and MERS-CoV enzymes (nsP13 and 3CL protease helicase), for example: myricetin, scutellarein, flavonoids, tea tree oil, eucalyptus oils and phenolic compounds, are not covered in this article.[1] This paper discusses the use of polymers in reducing human exposure to coronaviruses and some other viruses that cause respiratory disease. The systematic search of the PubMed database was performed in October 2020 and was focused on a part of "man-made" materials classified with indirect mechanisms of antiviral action and chemically modified materials surfaces. Research terms comprised a combination of words "polymer" and "coronavirus". The analysis included data from epidemics: SARS in 2003, MERS-CoV from 2012 and the current SARS-CoV-2.

Bioinspired surfaces

The inactivation function of antiviral agents depends on the structure of the polymer chain. The anionic nature of the polymer charge and the hydrophobicity of the backbone are key.[5] Self-cleaning coatings can be applied to surfaces that are frequently touched and contaminated to avoid adhesion of infectious microdroplets. Some nanostructured, polymer-based coating materials that mimic the natural structures of lotus leaf, gecko bristles, skis, or fly eyes are superhydrophobic.[1]

Physicochemical characteristics of antiviral polymers

Elevated temperature in the environment increases antiviral activity of polymers. Anions of toxic ions are more effective in disinfection than nonionic metals.[3] Sodium acrylonitrile/methallyl sulfonate membranes used in continuous renal replacement therapy have proven to be effective barriers to inhibit SAR-COV-2 penetration.[6] Dres et al.[7] proposed a method of obtaining personal protective equipment in the form of a gluten face mask, which is a biopolymer. Gluten was supposed to be used as a filter material. The electrospinning process creates a mat of carbon nanofibers, which is used to create a filter and a laminate from which the mask is made. The polymers can be easily modified. One of the modifiable features is the electric charge which affects the quality of antivirus protection. Leung et al. improved virus capture efficiency thanks to the use of positively electrifying polyvinylidene fluoride (PVDF) nanofibers.[8] Reusable medical masks (e.g., N95) must be cleaned. The use of a hydrophobic material allows partial self-cleaning. El-Atab et al. developed a method for the preparation of polyimide hydrophobic membranes with nanopores.[9] Personal protective equipment (PPE) in the form of a face mask makes daily communication difficult. Face shields are less effective than face masks because they do not stick to the face. Therefore, it would be a compromise to use a transparent material with appropriate filtering properties. He et al. developed a method of producing masking filters from a nanoporous transparent polylactic acid mat with higher efficiency than standard PPE.[10] Schandock et al.[5] showed that polyvinylbenzoic acid (PVBzA) could be a potential antiviral agent with a wide range of applications. It has the ability to inhibit enveloped viruses ZIKV (Zika virus), HIV-1, influenza, Lyssa, Ebola and SARS. Among the polyphosphates, poly (vinylphosphonic acid) (PVPA) shows a high inhibition capacity for herpes simplex virus 2 (HSV-2) and

Table 1. The chemical characteristics of the polymers and their role in limiting the spread of viruses

Author	Code name	Type	Task
Dres et al.	AMS	sodium acrylonitrile/methallyl sulfonate	CRRT membrane
Schandock et al.	PVBzA	polycarboxylates	antiviral agent
Schandock et al.	PVPA	polyphosphates	antiviral agent
Ye et al.	GO-PVP GO-PDDA	composite	antiviral agent
Ye et al.	GO-PDDA	composite	antiviral agent
Bidra et al. Kariwa et al. Khan et al.	PVP-I	N-vinylpyrrolidone polymer	antiviral agent
Ahmed et al.		metal-combined polymers	antiviral agent
Das et al.	processed gluten		PPE
Leung et al.	positively electrifying PVDF	fluoropolymer	PPE
El-Atab et al.		polyimide	PPE
Behzadinasab et al.	polyurethane with coper oxide (Cu_2O)	composite	antiviral agent
He et al.	nanoporous PLA	polyester	PPE

AMS – acrylonitrile/methallyl sulfonate; CRRT – continuous renal replacement therapy; PEG-PLGA – poly(ethylene glycol)-poly(lactide-co-glycolide); PVBzA – poly(vinylbenzoic acid); PVPA – poly(vinylphosphonic acid); PVP – poly(vinylpyrrolidone); PDDA – poly(diallyldimethylammonium) chloride; PVP-I – poly(vinylpyrrolidone)-iodine; PVDF – poly(vinylidene) fluoride; PLA – poly(lactic acid).

SARS; however, due to its very low effectiveness against other viruses, it can only be used under certain exposure conditions. These compounds can be used to form an antiviral protective layer on external surfaces. Ye et al.[11] has shown that polymer-conjugated graphene oxide has antiviral properties due to the negatively charged graphene oxide (GO)-conjugated polymers. The polymers can be: non-ionic PVP (polyvinylpyrrolidone composite) and cationic PDDA (poly(diallyldimethylammonium) chloride). The inactivation mechanism is based on the cleavage of the virus by a single-layer graphene oxide. The PVP seems to be more effective.

Materials containing heavy metal particles

Some heavy metal nanoparticles have virucidal properties, hence the ideas of weaving them into the structure of various materials, including inorganic ones. Balagna et al.[12] found that materials coated with silver silicate composite nanoparticles inactivate SARS-CoV-2. Also, the copper-covered surface significantly reduces the half-life of SARS-CoV-2 to 4 h compared to the steel-plastic surface where the virus was detectable for 72 h.[13] Disinfection of various items can be difficult or even impossible. The problem can be solved by using covering materials capable of inactivating the virus in production. The addition of copper to components produced on 3D printers may be helpful in increasing the virological safety of parts which, due to the method of manufacture, do not reach 100% sterility.[14] Heavy metal polymers affect viral proteins and the genetic material of the virus.[1,3] The cationic copper nanoparticles coupled with the polymer achieve a higher inhibitory effect than the microparticles or the metal surface. Copper ions lead to DNA denaturation, RNA damage and disturbance of viral protein synthesis. The addition of copper to PPE equipment used by healthcare professionals increases their effectiveness as a virological barrier.[15] Replacing metallic copper in the polymer structure with copper oxide increases the effectiveness of PPE. Behzadinasab et al. proposed to cover some everyday items with polyurethane bound to copper oxide (Cu_2O).[16] Ahmed et al.[17] introduced a novel PPE filter barrier architecture, in which the polymer acts as a cross-linked mechanical barrier and scaffolding. He proposed the use of polylactic acid (PLA) and cellulose acetate (CA) to create an electrospun multilayer barrier scaffold. The nanoparticles of Cu_2O and graphene oxide embedded in it inactivate viral particles that have penetrated into it.

The chemical characteristics of the polymers and their role in limiting the spread of viruses are presented in Table 1.

Conclusions

Accelerated virus inactivation polymers are a promising area of research due to the growing epidemic threat of viral diseases, in particular those caused by coronaviruses. They can make a significant contribution to slowing down the pandemic. Taking into account the different purpose of different materials and the associated physico-chemical characteristics, it seems that the future belongs to multi-component mixtures or very thin multilayer systems. The rational direction of research work is the search for materials with a balanced specificity in relation to the most dangerous viruses and universality in relation to other viruses.

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