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Drug delivery applications of poly-γ-glutamic acid

Olalekan A. Balogun-Agbaje[1], Olubusola A. Odeniyi[2] and Michael A. Odeniyi[1*]

Abstract

Background: Poly-γ-glutamic acid (γ-PGA) is a biopolymer of microbial origin, consisting of repeating units of L-glutamic acid and/or D-glutamic acid. The biopolymer has found use in the fields of agriculture, food, wastewater, and medicine, owing to its non-toxic, biodegradable, and biocompatible properties. Due to its biodegradability, γ-PGA is being tipped to dislodge synthetic plastics in drug delivery application. High cost of production, relative to plastics, is however a clog in the wheel of achieving this.

Main body of abstract: This review looked at the production, nanoparticles fabrication, and drug delivery application of γ-PGA. γ-PGA production optimization by modifying the fermentation medium to tailor towards the production of desirable polymer at reduced cost and techniques for the formulation of γ-PGA nanoparticle as well as its characterization were discussed. This review also evaluated the application of γ-PGA and its nanoparticles in the delivery of drugs to action site. Characterization of γ-PGA and its nanoparticles is a crucial step towards determining the applicability of the biopolymer. γ-PGA has been used in the delivery of active agents to action sites.

Conclusion: This review highlights some of the efforts that have been made in the appraisal of γ-PGA and its nanoparticles for drug delivery. γ-PGA is a candidate for future extensive use in drug delivery.

Keywords: Poly-γ-glutamic acid, Medium optimization, Nanoparticles, Drug delivery

Background

Polyglutamic acid (PGA) is a polymer of repeating units of glutamic acid. The repeating units can be L-glutamic acid and/or D-glutamic acid. There are two isoforms of polyglutamic acid: *α*-PGA and γ-PGA. The classification is based on whether the carboxyl group is attached through *α* or γ-linkages. In poly-γ-glutamic acid (γ-PGA) structure (Fig. 1), amide linkages with the monomers exist between α-amino and γ-carboxyl functional groups [1]. γ-PGA is a non-immunogenic, biodegradable anionic homo-polyamide [1]. Bacterial production of *α*-PGA is tasking and is only achievable with recombinant technology [2], whereas γ-PGA is produced by gram-positive bacteria, chiefly *Bacillus* sp. [3] and some gram-negative bacteria like *Fusobacterium nucleatum* [4]. Compared with γ-PGA produced by bacteria, *α*-PGA synthetically produced has a lower molecular mass (<10kDa) which limits its application. The molecular mass of γ-PGA is higher and commonly varies between ~ 100 and >1000 kDa [5].

The production cost of pure microbial γ-PGA is a major impediment to the widespread industrial use of the biopolymer. Many types of researches, as a result, have focused on optimizing the growth condition of the producing bacteria, with a view to obtaining high yield and desirable molecular mass at a cheaper cost [3]. γ-PGA production and its properties depend largely on the nutrient composition of the fermentation medium as well as the pH of the medium, temperature and period of incubation, and the producing microbe [6].

* Correspondence: deleodeniyi@gmail.com
[1]Department of Pharmaceutics & Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria
Full list of author information is available at the end of the article

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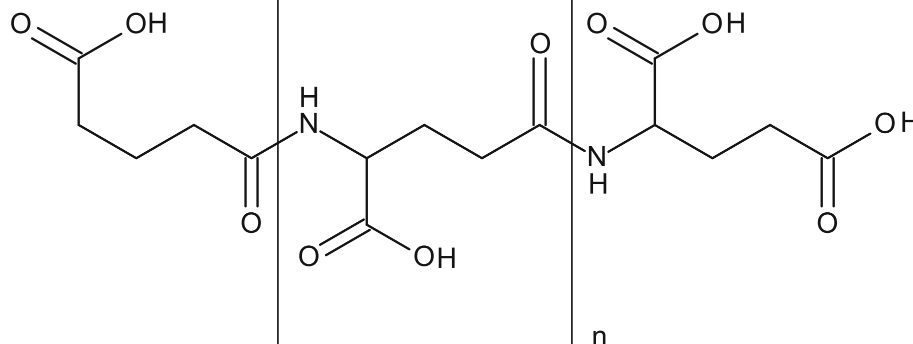

Fig. 1 Chemical structure of γ-PGA

The hydrophilicity, biodegradability, and biocompatibility of γ-PGA make it a suitable candidate for drug delivery [7]. A loose α-carboxyl group, which confers hydrophilicity, is present on every glutamate unit of the biopolymer [8]. The hydrophilicity was exploited to improve the water solubility of paclitaxel in a conjugate of the drug with γ-PGA [9]. The presence of carboxyl groups in the structure of γ-PGA (Fig. 1) also makes the biopolymer amenable to modification, by undergoing ionic interaction with cationic active agents or polymer, to form a stable complex [10]. The negative charge on the anionic γ-PGA has been exploited to form a nanoparticle complex with positively charged chitosan [11] and polymer-drug complex with doxorubicin [12]. γ-PGA can assume a number of conformations, depending on the pH of the solution and polymer concentration [13]. There are strong intramolecular hydrogen bonds between the monomer units of the biopolymer in an unionized state [14]. These hydrogen bonding are shown by Ho et al. [15], using optical rotatory dispersion, to be responsible for the stacked α-helix conformation of free γ-PGA (in aqueous solution) that led to hydrophobicity at pH 2 (unionized). The conformation reportedly changed at pH 4.09 due to a breakup of the bonding, which resulted in 50% of the earlier conformation changing into a random-coil conformation. At pH higher than 6.5 however, γ-PGA existed in a complete linear random-coil conformation [15]. At this conformation, γ-PGA offers multiple binding sites to cationic molecules [15].

Esterification is a desirable method of modifying γ-PGA to allow for the delivery of poorly water-soluble agents [16]. Hydrophobic modification with different esters retains the intramolecular hydrogen bonding stabilized α-helix structure of γ-PGA. This conformation opens up the structure and optimizes electrostatic interaction between γ-PGA and positively charged functional groups, with minimal repulsive effect on like ions [16].

A self-assembled nanoparticulate system of hydrophobically modified γ-PGA with L-phenylalanine ethyl ester was developed by Akagi et al. [17]. The nanoparticle reportedly consists of a L-phenylalanine ethyl ester hydrophobic core and γ-PGA hydrophilic shell. This modification enhanced the loading and stability of the poorly water-soluble antimalarial, quinine [18]. Hydrophobic derivatives such as benzylamidated γ-PGA [16] are capable of assuming a stable helix structure with orderly arranged side chains on the outer edge, resulting in a rigid conformation [16, 19, 20]. The orderly alignment spurs a strong π-π stacking interaction between the benzyl groups of the derivative [21]. The π-π stacking interaction results in higher drug loading efficiency of a drug, gambogic acid, with a conjugated system [22].

In this review, we give an insight into the optimization of fermentation medium for γ-PGA synthesis, formulation and characterization of γ-PGA and nanoparticles, and the potential application of the biopolymer in drug delivery.

Main text

γ-PGA biosynthesis

L-Glutamic acid serves as the precursor for microbial production of γ-PGA. It can be incorporated into the biosynthetic pathway endogenously or exogenously [3]. One of the steps involved in γ-PGA biosynthesis is racemization. This ensures the incorporation of D-glutamic acid (by converting the L-enantiomer supplied exo- or endogenously) into the evolving γ-PGA chain [3]. Ashiuchi et al. [23, 24] reported a homologous pair of glutamate racemase gene (race/glr and yrpC) in *Bacillus subtilis.* The glr is an essential gene for L-glutamate conversion into D-glutamate during γ-PGA biosynthesis in *B. subtilis* [25].

ATP is said to be the catalyst for the polymerization of PGA. The encoded gene, pgsBCA, on polyglutamate synthase (pgs) was also reported to be the lone architect of

γ-PGA polymerization [26]. The two-part system of DegS-DegU, DegQ, and SwrA, as well as the ComP-ComA system, is thought to be responsible for the regulation of γ-PGA synthesis [27, 28] (Fig. 2).

Exo-**γ**-glutamyl peptidase and endo-**γ**-glutamyl peptidase were the enzymes identified as having the potential to degrade γ-PGA in *Bacilli* [29].

γ-PGA production and medium optimization

The major impediment to the widespread industrial application of pure microbial γ-PGA is the production cost. Researchers have, therefore, focused on reducing this cost by utilizing cheap and renewable substrates as well as optimizing the growth condition of the producing bacteria with a view to obtaining high yield and desirable molecular mass [3].

γ-PGA production using cheap and renewable substrate

Odeniyi and Avoseh [30] investigated the production of γ-PGA using agricultural residue as substrate. The best producing bacteria, isolated from decomposing *Coix lacryma-jobi,* was identified as *Bacillus toyonensis* As8. The highest γ-PGA yield of all the agricultural residue studied (sorghum leaves, corn cob, rice bran, *Coix noir* leaves, and cassava peel) was reported to be from cassava peel, used at 20g/l in the fermentation medium.

In a similar work, corn starch (CS) and soybean meal (SBM) were employed as solid substrate in the synthesis of γ-PGA by *Bacillus amyloliquefaciens* JX-6 [31]. The output between sterilized and non-sterilized fermentation medium was compared. Industrial monosodium glutamate (IMSG) was employed in the medium as a γ-PGA precursor. Response surface methodology (RSM) showed the final components of the medium SBM, CS, and IMSG to be in the ratio 5:5:1 [31]. The reported yield for sterilized and non-sterilized solid-state fermentation were 166.99 ± 1.94g/kg and 134.25 ± 4.38g/kg respectively. The authors opined that, although the sterilized fermentation produced a higher γ-PGA yield, non-sterilized fermentation could reasonably reduce the fermentation time and consequently the cost of production.

Mohanraj et al. [32] compared γ-PGA production by *B. subtilis* 2756 using soybean and sago as substrates in both glutamate-dependent and non-dependent fermentation media. The substrates were used at 90 g/l for the glutamate-dependent media and 75 g/l for glutamate independent. RSM was used to optimize the concentration of L-glutamic acid, NaCl, and yeast extract contents of the media. The optimized media resulted in an approximately 7% increase in γ-PGA production [32]. γ-PGA was said to be produced by the organism in both glutamate- and non-glutamate-dependent media. A higher yield was, however, reported in the glutamate containing medium.

Fishmeal wastewater was employed as the source of nitrogen for γ-PGA synthesis by *Bacillus subtilis* A3 [33]. The fermentation medium contained 30 g/l glucose, 15 g/l chemical oxygen demand (COD) of fishmeal wastewater, and 25 g/l L-glutamate. Used alone as fermentation medium, fishmeal wastewater yielded a small amount of γ-PGA which suggested the ability of *B. subtilis* A3 to produce the polymer using fishmeal wastewater [33]. Compared with different sources of nitrogen which included tryptone, peptone, yeast extract, maize flour, fish protein ammonium sulphate, and soy bean, it

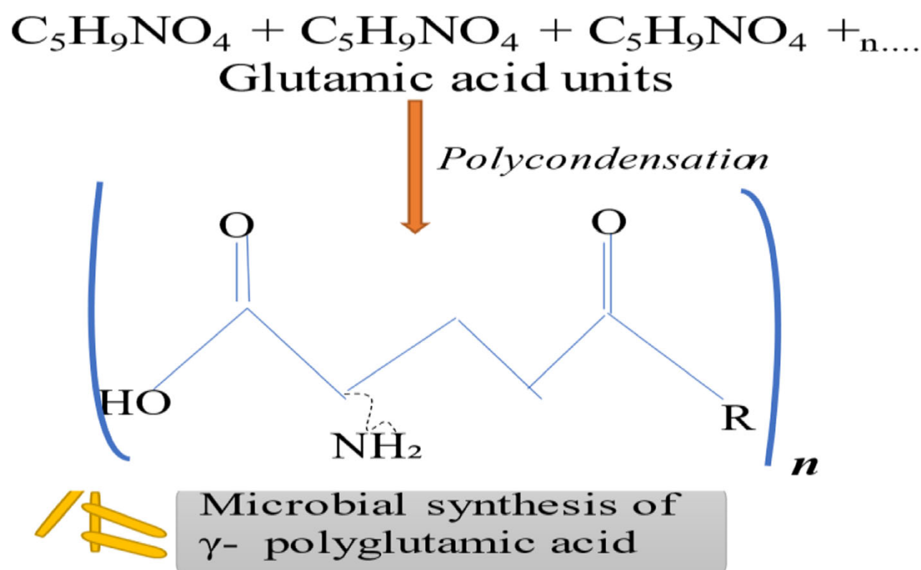

Fig. 2 Schematic representation of microbial production of γ-polyglutamic acid

was reported that only tryptone (29 ± 0.30 g/l) and fish protein (27.05 ± 0.12 g/l) had a greater influence on the yield than fishmeal wastewater (25.01 ± 0.32 g/l).

Optimization of medium composition

Composition of the fermentation medium is one of the most important determinants of both the production rate as well as the properties of γ-PGA produced by microbial fermentation [6]. The effect of medium composition was shown by Birrer et al. [34] when the medium NaCl concentration was varied. The molecular mass of the γ-PGA synthesized by *Bacillus licheniformis* ATCC 9945a changed from 1200 to 2200 kDa when the concentration of NaCl was increased from 0 to 4%.

Bajaj et al. [35] obtained an improved γ-PGA production of 26.12 g/l by *B. licheniformis* NCIM 2324 (from 5.27 g/l for basal medium) from optimized medium. The optimum medium, obtained by RSM, contained 62.4 g/l glycerol, 15.2 g/l citric acid, 8.0 g/l ammonium sulphate, and 20 g/l L-glutamic acid. Further optimization was carried out in which metabolic precursors for γ-PGA production, L-glutamine (0.07 g/l), and -ketoglutaric acid (1.46 g/l) were added to the fermentation medium [36]. The improved γ-PGA yield of 35.75 g/l (compared to the 26.12 g/l) was opined to be due to a better L-glutamic acid utilization caused by the added metabolic precursor.

Culture medium volume and yeast extract proportion are determining factors for γ-PGA production in *B. licheniformis* A13, a glutamate-independent γ-PGA producer [37]. The optimum medium designed by Plackett-Burman produced γ-PGA of 28.2 g/l after 72 h. The medium composed of 50 g/l glucose, 2 g/l yeast extract, 3 g/l NH_4Cl, 0.006 g/l $FeSO_4.4H_2O$, 6.4 g/l K_2HPO_4, 0.8 g/l $MgSO_4.7H_2O$, 0.00084 g/l $CaCl_2.2H_2O$, 0.8 g/l NaCl, and 0.1 ml solution of trace elements. The volume of the culture medium was 25 ml [37].

A higher yield of γ-PGA with optimized medium containing 30 g/l L-glutamate, 40 g/l yeast extract, and 20 g/l glucose with *B. subtilis* ZJU-7 as the producer was reported [38]. The concentration of the glucose in the medium was maintained within the range 3–10 g/l by the fed-batch method. γ-PGA production up to 101.1 g/l, representing up to 3.2-fold increase in comparison to the batch fermentation, was reported.

The effect of amino acids addition to a fermentation medium of *B. subtilis* Z15 has been demonstrated [39]. Increase in γ-PGA yield corresponding to 23.18%, 12.15%, and 31.46% was reported on the respective addition of 3 g/l aspartic acid (at 0 h), 1.5 g/l phenylalanine (at 0 h), and 7 g/l glutamic acid (at 24 h). It was reported that crude extract of glutamic acid after isoelectric crystallization (CEGA) could also be used in place of glutamate for the production of γ-PGA. The optimized amino acid content, arrived at using response surface methodology, contained 9 g/l CEGA, 4g/L aspartic acid, and 1.55 g/L phenylalanine. A yield 63.1% higher than control was reported when the medium was applied in 5-L bioreactor.

Culture condition optimization

Another crucial factor in γ-PGA production is the culture pH [40]. Optimum γ-PGA yield was reported for *B. licheniformis* ATCC 9945A at pH 6.5 [41]. This was however achieved with *B. subtilis* IFO 3335 at a pH of 7 [42].

Wu et al. [43] proposed a process for optimizing glutamate utilization and γ-PGA yield. In the process, the pH of the fermentation medium was sustained at 7 for 24 h to enhance maximal biomass accumulation. The pH was then lowered and maintained at 6.5 for optimal glutamate utilization and consequent γ-PGA yield. This process increased both glutamate utilization and γ-PGA yield from 24.3 to 29.3 g/l and 22.2 to 27.7 g/l respectively [43].

A procedure aimed at reducing the cost of production of γ-PGA by avoiding exogenous addition of L-glutamic acid was developed [44]. This was achieved by co-culturing L-glutamate-dependent *B. subtilis* with *Corynebacterium glutamicum* and utilizing glucose and sucrose as carbon sources. The fermentation time was also reduced by the process. The average molecular mass of the produced γ-PGA was reported to be 1240 kDa.

γ-PGA recovery

The recovery of peptidoglycan bound γ-PGA, as in *Bacillus anthracis*, is difficult to achieve because the polymer is not released into the medium. *B. anthracis* is also known to be toxic, which makes its industrial application untenable [3]. γ-PGA secreted into the medium can be recovered by three methods: precipitation by reducing water solubility, precipitation by complex formation, and filtration [2]. Removal of the microbial biomass is the first step in any of these recovery methods. The biomass is removed by centrifugation or filtration using a 0.45-μm filter [45]. The water solubility of γ-PGA can be reduced by adding ethanol to the supernatant or filtrate from the fermentation medium. This brings about the precipitation of the γ-PGA from solution [45]. This procedure however does not guarantee a pure γ-PGA production due to the fact that proteins and polysaccharides in solution can be co-precipitated with the biopolymer [46]. Precipitation by complex formation involves the use of metals for a complexation reaction with γ-PGA in solution. Commonly used metals include Al^{3+}, Fe^{3+}, Cu^{2+}, and Cr^{3+} [47]. The molecular size of high molecular weight γ-PGA differs from the remaining components of the medium. A series of filtration and

buffer exchange steps capitalize on this difference to effect γ-PGA separation [46].

Characterization of γ-PGA

Characterization of γ-PGA is an important step towards the determination of areas to which the polymer can be applied. Some techniques are available for the analysis and characterization of γ-PGA.

Infrared spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is used for the polymer's identification. The spectra indicate specific functional groups/bonds in the polymer. Ho et al. [15] reported γ-PGA in KBr pellet to indicate the following: a weak C=O absorption at ~1394–1454 cm^{-1}, a characteristic strong amide and C-N groups absorptions at ~1620–1655 cm^{-1} and 1085–1165 cm^{-1} respectively, a strong hydroxyl absorption at around 3400–3450 cm^{1}, characteristic aliphatic N-H stretching between 2900 and 2800 cm^{-1}, and 1600–1660 cm^{-1} and 1390–1450 cm^{-1} for amide and C=O groups respectively.

Nuclear magnetic resonance (NMR)

The degree of esterification and homogeneity of γ-PGA are usually determined using ^{1}H and ^{13}C-NMR [34].

Amino acid analysis

This analysis intends to evaluate the purity of the synthesized γ-PGA. The TLC detection of only glutamic acid is an attestation to the purity of the polymer [1]. The step involves the acid hydrolysis of γ-PGA using 6M HCl at 100 °C for specific hours in a glass vial. The hydrolyzed polymer is dried by evaporating the remnant HCl. The product, after drying, is dissolved in water and the amino acid content TLC-analyzed [48]. Goto and Kunioka [49] analyzed γ-PGA using an amino acid autoanalyzer. The hydrolysis was done using 6N HCl at 150 °C for 3 h in an enclosed glass tube under a nitrogen ambience using a Pico-Tag apparatus.

Molecular mass determination

The properties and eventual fields of application of γ-PGA are determined by the molecular mass of the polymer [50]. Molecular mass of γ-PGA is often measured by gel permeation chromatography (GPC). The molecular mass is determined by calibrating against particular standards [34]. Molecular mass of γ-PGA was determined using GPC [49]. The concentration of the sample used was 1 mg/mL (200 μL volume injected) and 50 mM aqueous solution of NaCl to acetonitrile (4:1) employed as eluant. The rate of flow of the eluant was 0.7 mL/min. Calibration curve to determine molecular mass was made using poly-(styrene sulfonic acid, sodium salt) as standard. There is often the need for molecular mass reduction of γ-PGA intended for drug delivery application. Techniques used for molecular mass reduction include medium composition alteration, ultrasonic degradation, alkaline hydrolysis, and microbial or enzymatic degradation [1].

Formulation of γ-PGA Nanoparticles

Nanoparticles (NPs) have gained tremendous attention in drug delivery system over the years. This is due to their biocompatibility with body cells, low toxicity, and also their ability for controlled release [51]. Nanoparticles, in addition to the ability to sustain drug release at the site of action, enjoy high intracellular uptake and also the capability of improving drug stability [52]. Nanoparticles can be nanocapsules, in which the active agent is contained within an oily reservoir core surrounded by a membrane wall. They can also be nanospheres in which the agent is dispersed in a matrix of the particles [53].

The delivery system also ensures controlled drug release, bioavailability, and retention in site of action [54, 55]. Factors such as solubility of the active agent, molecular weight, biodegradability, surface charge, size requirement, biocompatibility, and type of polymeric system determine the method of choice for nanoparticles preparation [51]. Formulation of γ-PGA as nanoparticles for drug delivery offers a number of merits which include improved drug stability, high drug loading efficiency, and the possibility of drug incorporation without the need for a chemical reaction [56].

Solvent exchange method

γ-PGA nanoparticle formulation using solvent exchange was described by Matsusaki et al. [57]. γ-PGA was the hydrophilic portion while L-phenylalanine ethyl ester or L-leucine methyl ester served as the hydrophobic side. The amphiphilic derivatives of γ-PGA, Phe-γ-PGA and Leu-γ-PGA, were made by dissolving 4.7 unit mmol γ-PGA in an aqueous solution of 0.3N $NaHCO_3$. Carbodiimide, phenylalanine, and leucine were added to the solution in sufficient quantity and then agitated for a period of 30 min at 4 °C. The consequent solution was kept at a room temperature condition for 24 h, thereafter dialyzed, and the derivatives retrieved after 72 h of freeze-drying. Self-assembled nanoparticles were then made by dissolving 10 mg of Leu-γ-PGA and Phe-γ-PGA in a mixture of equal volume of DMSO and distilled water. This was followed by dialysis and lyophilization. Phe-γ-PGA with conversion degrees of 42 and 58 yielded nanospheres of approximately 200 nm (characterized by TEM) [57].

Ionic gelation method

This method involves the interaction between the surface charges of a polymer and another polymer of opposite charge or a polycation or polyanion [58]. A nanoparticle of γ-PGA and chitosan was prepared by Lin et al. [11] using this technique. The nanoparticles were used to deliver oral insulin. In the preparation, 2 mL of a 1mg/ml γ-PGA (pH 7.4) aqueous solution was flushed through a pipette into aqueous chitosan under magnetic stirring. The formed nanoparticles were removed by ultracentrifugation (32,000 rpm for 50 min). The diameters of the NPs were reported to fall between 110 and 150 nm. The zeta potential of the formed NPs depended on the amount of negatively charged γ-PGA and positively charged chitosan used in the preparation [11].

Characterization of γ-PGA nanoparticles

Characterization of nanoparticles is very crucial to understanding the behavior of formulated NPs in order to channel to appropriate application. NP size and size distribution affect important parameters such as drug release, stability, cellular uptake, and tissue penetration [59]. Smaller particles, compared to larger ones, possess a larger surface area but lower drug encapsulation ability. The encapsulated drugs are released faster during degradation because of nearness to the NP surface [60]. Smaller particles however default in stability because of being prone to aggregation [56]. There is thus the need for a balance between what are large and what are small particles.

Particle size and size distribution of γ-PGA NPs are determined by dynamic light scattering (DLS) and photon correlation microscopy, scanning electron microscopy (SEM), atomic force microscopy (AFM), and transmission electron microscopy (TEM) [61, 62].

The surface charge and the surface hydrophobicity are determinants in the interaction of NPs with biological environment. The zeta potential, determined by a zetasizer, is a function of the surface on the NPs. The values can either be negative or positive, depending on the materials making up the particles [63]. Effect of pH on the zeta potential of curcumin-loaded 6-deoxy-6-arginine modified chitosan (DC)/γ-PGA nanoparticles was reported by Su et al. [64]. The charge on DC reportedly increased from +46.6 to +28.8 mV as the pH was raised from 4 to 9. The same pH change resulted in a decrease in the charge on γ-PGA from − 26.2 to − 38.6 mV. The NPs were prepared at an optimum pH of 6, in which interaction between γ-PGA and DC was strongest.

Application of poly-γ-glutamic acid in drug delivery

γ-PGA has been used in several fields of endeavor. The wide application is owed to its unique characteristics. Its properties which include water solubility, non-toxicity, biodegradability, and biocompatibility have endeared the microbial polymer to many fields of application [50]. γ-PGA has been utilized for the delivery of some category of drugs (Fig. 3) [12, 18, 22, 64–69].

Anticancer delivery application

The molecular mass of γ-PGA is crucial to drug delivery as well as the ability to control drug release. Li et al. [9] reported molecular masses between 3×10^4 and 6×10^4 as necessary for conjugation with the anticancer agent, paclitaxel. The conjugate offered a water-soluble derivative of paclitaxel. Apart from this, the conjugate was shown to display a superior therapeutic index, compared to paclitaxel. There was a disappearance of established breast and ovarian cancers within 2 weeks upon injection of the conjugate at a dose equivalent to 40 mg/kg and 160 mg/kg of paclitaxel respectively. The remarkable antitumor activity of the conjugate was due to a greater uptake by the tumor cells, compared to free paclitaxel. Unlike paclitaxel also, the γ-PGA-paclitaxel conjugate exhibited negligible tubulin polymerization activity and did not enhance paclitaxel-dependent CHO mutant cell line growth [9].

Paclitaxel poliglumex, a macromolecular taxane formulated by conjugation between paclitaxel and γ-PGA, was designed by Singer [65]. The conjugate offered a water-soluble alternative to paclitaxel, in addition to an improved pharmacokinetic profile, ensured a longer drug exposure to the tumor cells and minimized systemic exposure. Animal study demonstrated the conjugate to be of higher efficacy and lesser untoward effect, compared to standard paclitaxel [65].

The eligibility of γ-PGA as a vector for the controlled release of anticancer drug, doxorubicin, was evaluated by Manocha and Margaritis [12]. The formulation was made by ionic complexation of the anionic γ-PGA and cationic doxorubicin (DOX). The efficiency of complexation between the drug and the polymer was almost 100%. A pH-dependent in vitro drug release from the complex was reported. Up to 40% burst release of doxorubicin from the complex (in the first 8 h) was observed at pH 2.2. The burst was followed by a slow release over 7 days. The burst was, however, not observed at a pH of more than 2.2. A controlled release of doxorubicin over time is thus achievable with a pH-triggered DOX/γ-PGA complex [65].

Ye et al. [66] formulated a cisplatin-attached γ-PGA. The conjugate produced a water-soluble derivative of cisplatin with molecular weight of conjugation being between 4.5 and 6.0×10^4 Da. An initial burst release of cisplatin from the conjugate (in PBS) within the first 6 h, followed by a subsequent sustained release, was reported. Although less potent than free cisplatin against breast cancer Bcap-37 cell line growth in vitro, γ-PGA-

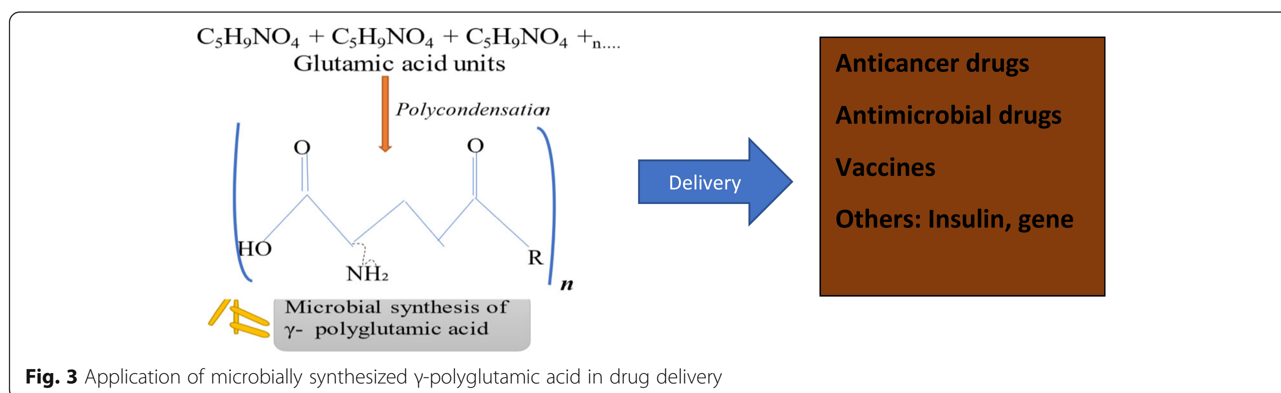

Fig. 3 Application of microbially synthesized γ-polyglutamic acid in drug delivery

cisplatin conjugate injected as 3 doses (with a 2-day interval between doses) reportedly displayed a greater antitumor activity and lesser toxicity in vivo. Bodyweight loss in the mice treated with free cisplatin was not observed with the conjugate [66].

A novel nanoparticle carrier to evaluate curcumin delivery was designed by Su et al. [64]. The nanoparticles were prepared by ionic interaction between 6-deoxy-6-arginine-modified chitosan and γ-PGA. The encapsulation efficiency and drug loading capacity reported were 79.5% and 11.31% respectively. The formulation was also said to be stable at temperatures, 30–60 °C and pH 3–9. Drug release from the nanoparticles was evaluated in simulated gastric and intestinal fluids, and compared with the release of curcumin from ethanol. In the simulated gastric fluid (SG), a burst release (within 30 min) from ethanol and NPs were respectively 38.95 and 12.77%. A total release (in SG) of 69.75% and 31.85% occurred for free curcumin and NPs respectively. Upon incubation in the simulated intestinal fluid (SI), free curcumin was slowly released continuously. A more rapid release was reported for curcumin-loaded NP (from 2 to 4 h), compared with in SG, which resulted in a more significant release at the intestinal pH.

A NP delivery system for the controlled delivery of doxorubicin to tumor cells was designed [67]. The nanoparticle system composed of γ-PGA, graphene oxide (GO), and chitosan oligosaccharide (CO). Graphene oxide was linked covalently with chitosan oligosaccharide and γ-PGA loaded onto the composite to form GO-CO-γ-PGA system. Controlled and sustained release of doxorubicin loaded on the nanocarrier were reported. An in vitro cytotoxicity assay, using MTT, had GO-CO-γ-PGA-DOX display higher efficacy in proliferation inhibition than when γ-PGA was absent in the system [67].

Benzylamidated γ-PGA derivative was used as the drug loading core in a drug delivery system for the natural anticancer, gambogic acid [22]. The outer shell consisted of an amphiphilic hyaluronic acid derivative grafted with all-trans retinoic acid. The drug was incorporated into the benzylamidated γ-PGA via a π-π stacking interaction [22]. An encapsulation efficiency of almost 100% was reported at a drug-polymer ratio of less than 10%. The system also offered a tumor targeting property and an enhanced circulation time. The π-π stacking and hydrophobic interaction with respect to encapsulation efficiency was compared, which depicted a drastic reduction in encapsulation efficiency when benzylamidated γ-PGA was substituted with propylated γ-PGA derivative [22].

Antimicrobial delivery application

γ-PGA was shown to reduce the toxic effect of amphotericin B while retaining the antifungal activity [68]. Zia et al. [68] investigated the ability of γ-PGA NPs to ferry the antifungal, amphotericin B. The in vitro antifungal activity of the γ-PGA-based NP formulation against *Candida albicans* was studied. The formulation was compared with Fungizone®, a marketed brand of amphotericin B. Antifungal activity of the γ-PGA-based NP was comparable to Fungizone® but the NP formulation showed reduced toxicity to erythrocyte and also had an insignificant effect on mammalian cell viability [68] (Table 1).

γ-PGA was hydrophobically modified to enhance the stability and bioavailability of quinine [18]. An amphiphilic polymer was formed with L-phenylalanine ethyl ester as the hydrophobic core. A stable nanodispersion of quinine was formed with the amphiphilic polymer acting as a surfactant. Bioavailability of quinine following this formulation reportedly increased by up to 6.5 folds.

Delivery of oral insulin

The conventional subcutaneous insulin administration causes poor patient compliance and glucose control [70, 71]. Oral insulin administration offers a better ground as the insulin transits the portal circulation and through the liver into the systemic circulation, which mimics physiological insulin [72]. Sonaje et al. [69] formulated a γ-PGA-based nanoparticle system

Table 1 Application of γ-PGA and derivatives in drug delivery

Drug	Formulation	Details	References
Doxorubicin	Ionic complexation with γ-PGA	pH-triggered controlled release	[12]
Quinine	Quinine-loaded hydrophobically modified γ-PGA with L-phenylalanine ethyl ester NP	Improved stability and enhanced bioavailability	[18]
Gambogic acid	π-π stacking interaction with benzylamidated γ-PGA	Improved drug loading	[22]
Curcumin	Curcumin encapsulated modified chitosan/γ-PGA NP	Improved drug release	[64]
Paclitaxel	Complexation with γ-PGA	Water solubility of paclitaxel; reduced systemic effect; longer exposure time to tumor cells	[65]
Cisplatin	Conjugation with γ-PGA	Increased antitumor activity and reduced toxicity	[66]
Doxorubicin	Doxorubicin-loaded NPs of graphene oxide/chitosan oligosaccharide/ γ-PGA	Higher antitumor activity, controlled and sustained release	[67]
Amphotericin B	Amphotericin B-loaded γ-PGA NP	Reduced toxicity to erythrocyte	[68]
Insulin	Enteric-coated Chitosan/γ-PGA NP	Orally bioavailable insulin	[69]

with chitosan as a copolymer in the formulation. The insulin incorporated nanoparticle was pH-dependent and prepared by an ionic gelation method. To prevent insulin degradation in the stomach, the freeze-dried nanoparticle was reportedly protected with an enteric-coated capsule. The result of an in vivo dissolution test indicated the stability of the nanoparticle in the low pH gastric environment but was readily released in the small intestine. Relative bioavailability of insulin was 20% which indicated the ability of the formulation to deliver insulin orally [69].

Conclusions

The application of γ-PGA in drug delivery is on the rise, owing to their tremendous qualities. These qualities include non-toxicity, water solubility, biodegradability, and biocompatibility. The presence of a free α-carboxyl group on the monomer units of γ-PGA presents points of coupling with other polymers or active agent. The ability to form a self-assembled amphiphilic nanoparticle with hydrophobic esters give the biopolymer an added edge for the delivery of poorly water-soluble agents. γ-PGA has been formulated as nanoparticles to exploit the additionally incredible characteristics offered by nanoscale sized system such as high drug encapsulation efficiency, improved stability, solubility and controlled release.

Abbreviations

γ-PGA: Poly-γ-Glutamic acid; α-PGA: Poly-α-Glutamic acid; ATP: Adenosine triphosphate; CS: Corn starch; SBM: Soybean meal; IMSG: Industrial monosodium glutamate; glr: Glutamate racemase; RSM: Response surface methodology; NPs: Nanoparticles; COD: Chemical oxygen demand; CEGA: Crude extract of glutamic acid; FTIR: Fourier-transform infrared; NMR: Nuclear magnetic resonance; TLC: Thin layer chromatography; GPC: Gel permeation chromatography; DMSO: Dimethyl sulfoxide; TEM: Transmission electron microscopy; PBS: Phosphate buffer saline; DLS: Dynamic light scattering; SEM: Scanning electron microscopy; AFM: Atomic force microscopy; CHO: Chinese hamster ovary; DC: 6-Deoxy-6-arginine modified chitosan; DOX: Doxorubicin; SG: Simulated gastric fluid; SI: Simulated intestinal fluid; GO: Graphene oxide; CO: Chitosan oligosaccharide; PEG: Polyethylene glycol

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Declarations

.

Authors' contributions

OAB: methodology, writing—original draft, writing—review and editing; OAO: writing—original draft, writing—review and editing, visualization; MAO: conceptualization, writing—original draft, writing—review and editing. All authors gave their individual critical revision and final approval of the version to be submitted. The authors read and approved the final manuscript.

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Author details
[1]Department of Pharmaceutics & Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria. [2]Department of Microbiology, University of Ibadan, Ibadan, Nigeria.

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