Prospects for Polymer Therapeutics in Parkinson's Disease and Other Neurodegenerative Disorders

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Abbreviations:

PD, Parkinson's disease; BBB, blood brain barrier; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; HD, Huntington's disease; MS, multiple sclerosis; TBI, traumatic brain injury; SCI, spinal cord injury; PLGA, poly(lactic-co-glycolic acid); PLL, poly-L-lysine; PEG, polyethylene glycol; PEI, polyethylene imine; DMAEMA, 2-dimethylaminoethyl methacrylate; PAMAM, poly(amido amide); 6-OHDA, 6-hydroydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;

Table of Contents

Abstract

Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons and represents a growing health burden to western societies. Like many neurodegenerative disorders the cause is unknown, however, as the pathogenesis becomes ever more elucidated, it is becoming clear that effective delivery is a key issue for new therapeutics. The versatility of today's polymerization techniques allows the synthesis of a wide range of polymer materials which hold great potential to aid in the delivery of small molecules, proteins, genetic material or cells. In this review, we capture the recent advances in polymer based therapeutics of the central nervous system (CNS). We place the advances in historical context and, furthermore, provide future prospects in line with newly discovered advancements in the understanding of PD and other neurodegenerative disorders. This review provides researchers in the field of polymer chemistry and materials science an up-to-date understanding of the requirements placed upon materials designed for use in the CNS aiding the focus of polymer therapeutic design.

1. Introduction

Parkinson's Disease (PD) is a movement disorder that was described in 1817 by James Parkinson in his famous text "*An Essay on the Shaking Palsy"*. PD is a progressive neurodegenerative disorder which can give rise to a range of symptoms including the well-known triad of bradykinesia, rigidity and tremor. Parkinson's disease (PD) and many neurodegenerative disorders (such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD) and multiple sclerosis (MS)), exhibit complex pathological features. Whilst these pathologies are being ever further elucidated, the causes (with the exception of genetic predispositions such as huntingtin gene mutations for HD) are more ambiguous. Many neurodegenerative disorders (including PD) are age related disorders and represent a growing healthcare burden to ageing populations, and as of yet, lack disease modifying intervention. Medications such as the gold standard levodopa for PD, result in a marked improvement in patient quality of life, but for a limited period [1]. They treat the symptoms of the disease not the underlying progression of the disease which, in the case of PD, is the dying back of the dopaminergic neurons in the midbrain. Disease modifying interventions are being sought based on gene therapies, new drug formulations, stem cells and other techniques, for which polymers of a variety of chemistries and structures are being considered for increasingly important roles.

PD patients can exhibit a variety of phenotypic features, which can differ in severity from patient to patient depending of the underlying pathology, and can be accompanied by dementia and/or depression. Its cause can be divided into sporadic PD or familial PD where a genetic risk factor is known, and the age of onset can vary. In short, the pathology is complex and not consistent between patients, a matter that must be born in mind when discussing the merits of different medications, or during the design of future therapies. However, several inherent features of PD could be considered to offer relatively more straightforward opportunities for intervention than

some other neurological disorders, which for the purpose of this review, makes PD an attractive example to address. The first of these features is the generally slow progressive nature of the disease. Unlike diseases such as ALS, which typically displays a rapid progression with 50% or patients dying within 2.5 years [2], PD usually progresses at a much slower rate [3] giving a longer period for potential intervention. The second feature, which sets PD (and other progressive diseases) apart from acute conditions such as traumatic brain injury (TBI), stroke, or spinal cord injury (SCI), is that at the time of diagnosis there is a modest window of opportunity within which, if the disease progression could be halted, a reasonable quality of life could be maintained. Clinical symptoms present after an approximate 50% loss of nigral neurons and an approximate striatal dopamine loss of 80% [4], providing a rationale for developing neuroprotective therapies to preserve these remaining neurons. Another key feature of PD for therapeutic intervention is the relatively select area of the brain that is affected. Whilst AD affects the hippocampus and cortex, and MS can affect any region of the CNS, PD predominantly affects the nigrostriatal pathway: the substantia nigra (where the cell bodies reside) and the striatum (where the neurons project). In summary, a therapeutic intervention aimed at treating Parkinson's disease optimally could have an effect lasting years, and be targeted to the nigrostriatal pathway, either through direct stereotactic injection, or translocation across the blood brain barrier (BBB). This is clearly a formidable task, and whilst progress is being sought via clinical trials in gene therapies, direct protein infusions and cell therapies, there is an emerging field of polymer therapeutic research, for neurodegenerative diseases as a whole [5]. Growth factors such as glial cell line derived neurotrophic factor (GDNF), Neurturin (NTRN), brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) all show promise as a means of achieving neuronal protection or re-innervation [6-10], and polymer therapeutics are likely to play a significant role in overcoming the problem of effective delivery [11, 12]. Although the field is in its infancy in terms of clinical translation, polymer science research is being developed in many areas (as outlined below) which may ultimately impact upon future PD therapies.

2. Areas for Polymer Therapeutic Intervention

There are several PD research areas which may result in future interventions involving therapeutics based either in part (the majority), or entirely (the minority), on polymer formulations. Figure 1 highlights potential areas for polymeric therapeutics in PD, where it can be observed that the majority of applications involve assisting delivery; either drugs, nucleic acids, cells, or proteins. For assisting the systemic delivery of pharmaceuticals to the brain, polymers may be used to alter solubility or circulation time, form micelles for drug encapsulation, or be used as linkers for targeting moieties. Alternatively, cationic polymers may be used to deliver negatively charged nucleic acids by condensing the nucleic acid, protecting it from degradation and assisting intracellular delivery. Due to the short half-lives of therapeutic proteins such as neuron protecting growth factors GDNF or NGF etc., sustained delivery platforms are being sought that protect the protein, releasing it slowly for a more prolonged effect. More recently, efforts have turned to developing platforms to assist cell therapies, such as injectable adherent microparticles or protective hydrogels.

---FIGURE 1---

3. Polymer Design Considerations

Polymers intended for therapeutic use in the brain or spinal cord must be synthesized holding several key design considerations in mind. Some of the most important considerations are outlined individually below. However, it must also be noted that often parameters may well be closely linked, such as the polymer degradation profile and the polymer toxicity, where one affects the other, so cannot be treated as independent entities.

3.1 Toxicity and Host Response

Perhaps the first and most important aspect of polymer therapeutic design is the consideration of how the polymeric biomaterial will affect the host tissue/organism. Depending on the application, the expected toxicity can vary greatly [13]. For example cationic polymers for gene delivery often suffer an efficiency/toxicity tradeoff [14], whereas scaffolds for regeneration or cell delivery can be expected to exhibit little or no elevation in host response above that of a sterile media control injection [15]. The direct cellular toxicity of polymeric gene vectors is well documented *in vitro*. Indeed, the vast majority of research into new gene vectors comes accompanied by a report on cytotoxic affects. However, the situation in the brain or spinal cord is not only more complex than a 2D cell culture system, but also far less studied. Whilst *in vitro* toxicity analysis can give an indication of the direct toxicity in the brain, there are other host response effects which do not correlate so well, such as microglial activation and astrocyte reactivity. For example one study showed that direct toxicity to the striatum, caused by polymeric gene vectors, was inversely proportional to the density of microglia or astrocyte density surrounding the injection site [16].

It is not clear whether the initiation of a microglial response by an injected biomaterial is necessarily detrimental to the surrounding tissue or not. For example, microglial activity has been correlated with angiogenic microvessel formation after stroke injury in Sprague-Dawley rats [17]. For biomaterial implants containing a large number of transplanted cells, this microglial activity surrounding a graft may or may not be beneficial depending on whether the microglia are assisting microvessel formation thus promoting a new blood supply to the graft, or clearing foreign material. However, the glial scar formed around an injury to the CNS by reactive astrocytes [18, 19], including an injury left by a needle tract [16, 20, 21], is broadly considered to hinder axon growth and thus would hinder the ability of the graft to integrate with the host tissue during neuron replacement therapies for PD. Thus a polymer designed to reduce the reactivity of astrocytes may be beneficial, but it is not clear how this could inherently be achieved. However, polymer based hydrogels have extensively been shown to be capable of protein loading [22-25], thus hydrogels designed to assist cell based therapies to the brain could be loaded with the enzyme chondroitinase ABC, to allow a more permissive integration of the graft with the host. Chondroitinase ABC is an enzyme that digests chondroitin sulfate proteoglycans, the inhibitory component of the glial scar, and whilst much of the focus of its use has been towards SCI therapies [26, 27], use in the brain for assisting transplants should not be overlooked.

Whilst there is ambiguity surrounding the exact effect of the microglial response to direct brain injection [28], the beneficial effect of immunosuppression towards the graft is also variable. For example, studies show immunosuppression to increase xenograft (human to rat) survival qualitatively [29] and quantitatively [30], but allograft transplantation into the striatum was not affected between immune-deficient/immuno-competent mice [31] other than to favor neuron differentiation [32]. The brain is now no longer considered a completely immuno-privileged region there is a degree of immune privilege, and the immune response is a major consideration for cell

therapy [33]. Therefore materials designed to suppress negative effects of the host response could possibly play an important role in graft survival. To this end a hydrogel has been developed that releases an anti-inflammatory cytokine, interleukin 10, which is triggered by matrix-metalloproteinase 9 secreted by activated microglia [34]. Consideration of immunogenic properties of hydrogels for graft protection is of course also critical. There is little point delivering beneficial therapeutics if the material itself invokes a larger immune response than that of the graft alone. To this end synthetic materials and lab synthesized peptide materials offer a means of lowering host immune responses to implanted materials.

For any biomaterial intended for use in the brain certain design features can be inherently included to reduce the toxicity, including: reduction of surface charge, surface modification with inert polymers such as polyethylene glycol (PEG) (which can also reduce charge densities), use biodegradable polymers built from well tolerated monomers as a base material (such as the copolymer poly(lactic-co-glycolic) acid (PLGA)), use of degradable crosslinkers to assist material breakdown or, use synthetic/natural polymer hybrids to try to mimic the surrounding tissue more closely.

Polymers designed for systemic administration have more complications which must be considered. The first and most obvious is the hemocompatibility or how the material interacts with blood. New polymers, designed for systemic applications, can be subjected to a simple battery of hemocompatibility tests *in vitro*, using human blood to assess parameters such as platelet activation, plasma clotting time and hemolysis [35]. Dendrimers, the highly-ordered, three dimensional branched polymers, which can be built up, one generation at a time, have been extensively researched for therapeutic delivery, including to the brain. However, studies have shown that generation 7 cationic poly(amidoamine)(PAMAM) dendrimers aggressively initiate blood clot formation which is likely caused through electrostatic interaction between the dendrimer amine group and the negatively charged fibrinogen [36]. A study from a different research group suggests that surface coating PAMAM with PEG may offer a significant safety improvement for systemic application. PEGylation reduced red blood cell hemolysis *in vitro*, which was most effective when PEG chains over 5KDa were grafted to the PAMAM core [37], suggesting that such strategies could be useful if the PEG layer does not interfere with the intended use of the dendrimer.

---FIGURE 2 ---

The second large toxicity/host response issue associated with systemic delivery applies to all vector systems and is independent of the polymer vehicle itself. This is the problematic effect of off-target delivery. Not only is brain targeted systemic delivery a task difficult unto itself, with a large proportion of off-target delivery to the liver, spleen or kidney [38], but the exact site of delivery within the brain is likely to be crucial for future protein/growth factor therapies. Early clinical efforts, administering the growth factor NGF to the brain via direct injection into the lateral ventricles, resulted in painful side effects that outweighed the therapeutic benefits [39]. The ventricle spans a large region of the brain (see figure 2), meaning that proteins delivered intraventricularly can exert their effect over a large area of the brain, with off-target effects. These effects were identified as Schwann cell hyperplasia and sprouting of sensory and sympathetic neurites in the subpial region of the medulla oblongata, giving rise to symptoms of pain [40]. While the lateral ventricles lie in close proximity to the striatum where the dopaminergic neurons lost in PD terminate (see

figure 2), their proximity to large brain regions such as the cortex render them an unlikely route for polymer therapeutic delivery for PD therapies. Indeed, a more recent trial involving NGF delivered directly to the basal forebrain in AD patients, via polymer encapsulated NGF secreting cells [41, 42], did not result in any of these painful side effects experienced after intraventricular delivery, highlighting the importance of reducing off-target delivery.

3.2 Degradability

Another key design consideration for polymer therapeutics, is whether degradability should be incorporated into the polymer structure, and if so, by what means should it degrade, and which regions of the polymer should be cleavable (i.e. backbone or side-chains). Although at first it might seem likely that the majority of biomaterials for use in the brain or CNS should be biodegradable, this may not be the case, and would instead be application dependent. For instance, incorporating biodegradability into the polymer structure of nanoparticles designed for the delivery of genes or drugs to the striatum or substantia nigra, would eliminate accumulation issues, could assist in the release of cargo, and probably reduce toxicity (if the break-down products are non-toxic themselves). However, the NGF cell secreting implants mentioned earlier, composed of the non-biodegradable materials polyethersulphone and polyeurethane, were well tolerated [41, 43], and in general inert metal probes such as for deep brain stimulation (DBS) are well tolerated although accompanied by a persistent glial scar [44, 45]. One study looked specifically at the host response to degradable (fast or slow) or non-degradable hydrogels, implanted into the striatum/substantia nigra of Sprague-Dawley rats [46]. The number of astrocytes within the vicinity of the implant was significantly lower for the slow or non-degrading hydrogels than the fast degrading hydrogel, suggesting that the continually changing environment created by hydrogel degradation causes more astrocyte activation. Thus, the decision of introducing biodegradability into the polymer structure is not clear-cut and will depend on the application. Non-degradable cell delivery devices essentially composed of poly-l-lysine coated glass beads appear to be well tolerated in the striatum (in terms of microglial activation)[47], whereas non-degradable PAMAM or polyethyleneimine (PEI) gene vectors cause tissue loss accompanied by a strong astrocyte/microglial response [16].

3.2.1 Hydrolysable Polymers

Polymers containing esters, amides, acetals, carbonates, anhydrides, urethanes and phosphates are susceptible to cleavage in the presence of water, and the relative merits of each have been discussed elsewhere in the context of biomedical applications [48]. Incorporating such groups into the polymer backbone can thus facilitate biodegradation *in vivo*, however, rates depend on many factors, such as the number of groups (if copolymers with other non-degradable monomers), molecular weight of the polymer and monomer composition. For example, microspheres prepared from high molecular weight poly($_{D,L}$ –lactic acid)(PLA) showed little degradation compared to the those composed of low molecular weight PLA which degraded much faster [49]. PLA is well known to degrade slower than poly(glycolic acid)(PLG), so the degradation profile can be tuned by varying the blend of the two monomers. This FDA approved copolymer, PLGA, has become one of the most researched polymers for biomedical applications for its tunable degradation rate and highly biocompatible nature (natural breakdown products) [48, 50].Much work has focused on the delivery of drugs or growth factors to the brain via targeted PLGA products which is discussed in more detail in later sections.

3.2.2. Enzymatically Cleavable Polymers

Naturally occurring polymers such as the proteins collagen, fibrin and elastin, or natural polysaccharides such as hyaluronic acid, heparin and chitosan, have been used as base materials for degradable biomaterial preparation [51, 52]. Semi-synthetic biomaterial scaffolds can be produced by combining these natural biopolymers with synthetic polymers such as PEG, with the aim of representing or mimicking host tissue more closely [53, 54], allowing cellular attachment [24], introducing enzyme cleavability [55], or improving the binding of growth factors [56]. These semi-synthetic materials are often designed in such a way that a functionalized PEG molecule (often branched) is used to crosslink the biopolymer either via an intrinsically present amine [57], via EDC/NHS activated carboxyl groups [58], or via a previously added functional group [59].

A variety of hydrogels or scaffolds have been proposed for tissue regeneration in the CNS for neurodegenerative diseases therapies [60]. Many are used without transplanted cells and are designed to act as a supportive network (predominantly for applications in SCI, TBI or stroke). However, for applications in PD, many are used in conjunction with replacement cells, stem cells, or cytokine producing cells. However, regardless of strategy, allowing the host tissue to remodel the injected/implanted biomaterial by proteolytic activity of proteases such as matrix metalloproteinases (MMPs) would be advantageous. MMPs produced by neurons or supporting cells such as microglia, can allow invasion of dorsal route ganglion cells into PEGylated fibrinogen hydrogels in an *in vitro* model system, but the authors show that invasion does not occur in the presence of MMP inhibitors (see figure 3 adapted from [61]).

---FIGURE 3---

Much recent work has focused on the design of peptide sequences that are susceptible to MMP cleavage, which can be incorporated into hydrogel or biomaterial design [62]. A group with much experience in starPEG-heparin biomaterials has recently shown a versatile approach to incorporate two peptides (one MMP cleavable, and one laminin-derived adhesion peptide) into a hydrogel structure [59]. Prior functionalization of maleimide terminated starPEG firstly with a cell adhesion peptide (Seq: SIKVAVGWCG), then a MMP cleavable domain with a protected second cysteine group (Seq: GCGGPQGIWGQGGCG) allows a peptide functionalized molecule that can crosslink maleimide functionalized heparin *in situ* via Michael addition (cysteine to maleimide) (see figure 4) [59].

---FIGURE 4---

In this case dorsal root ganglion penetration of the gel was dependent on the presence of the cell adhesion peptide, and similar starPEG-heparin hydrogels have shown good biocompatibility in the rodent brain [24]. Semi-synthetic materials therefore offer a practical route to degradable materials provided that their design always takes into account correct natural polymer sourcing, reproducibility of production, sterilization, and ease of use during the surgical procedures.

3.2.3. Intracellular Degradation (acid/reducing cleavage)

Whilst large microspheres or macro scale structures such as hydrogels, scaffolds or nerve guidance conduits may rely on hydrolysis or enzymatic action for biodegradation, small (low micro, or nanoscale) delivery vectors may use intracellular cues for biodegradation. There is a growing

research field aimed at developing polymer vectors to deliver drugs, genes or proteins to the central nervous system. Polymers therapeutics designed for systemic use may not benefit from introducing hydrolysable degradation into the structure. Instead, these vectors are designed to target and translocate the blood brain barrier, the highly selective endothelial layer which forms the largest area for exchange between the main blood supply and brain tissue [63]. In such a case it is likely that degradation upon cell entry would be more beneficial, allowing the vector to remain intact during transport in the blood. The two main methods of intracellular degradation use a change in pH to mediate cleavage within the endosome, or the presence of reducing conditions such as intracellular glutathione to break up the vector and potentially release the cargo [64].

---FIGURE 5---

There are many acid cleavable groups which may be incorporated into polymer vectors, such as acetal or ketal linkages, orthoesters or the amine containing oxime or hydrazone groups, reviewed in detail elsewhere [64]. Figure 5 shows a library of acetyl containing acid cleavable polymers created by Fréchet and coworkers [65]. These forms of acid cleavable linkage, are also easy to incorporate into living polymerizations [66, 67], the preparation method of choice for many gene vectors [68, 69]. However, to date, the vast majority of degradable polymers for delivery to the brain use a reduction sensitive moiety (disulfide bond) instead.

The presence of disulfide bonds in the structure of polymeric gene vectors allows a reversal of the well-reported correlation between increasing toxicity with increased transfection performance [14]. Non-degradable polymer vectors often showed higher transfection capabilities with increasing molecular weight, but this trend would typically be accompanied by increasing toxicity [70, 71]. However, by linking small (2kDa) PEI chains via disulfide bonds, Goepferich and coworkers were able to make a vector large enough for efficient gene transfer but degradable in a sufficient time scale to reduce toxicity [14]. More recently, Kim and coworkers produced branched PEIs based on the linkage of thiol terminated 1.2kDa branched PEIs macro monomers (see figure 6) for marker gene (red fluorescent protein) delivery to the rodent brain [72]. *In vitro* toxicity analysis of these vectors delivering microRNA (mRNA) to Neuro2a cells showed that polyplexes (polymer complexed with nucleic acids via electrostatic attraction) formed at the optimum polymer/mRNA ratio of 13.3/1 the degradable vector allowed ~ 70% cell viability compared to ~25% cell viability for the non-degradable PEI control [73].

---FIGURE 6---

Poly(β-amino esters)(PAEs) are much studied for non-viral gene delivery applications as the ester group gives rise to their inherently degradable properties [74-76]. However, a large study comparing a variety of PAEs showed that the addition of disulfide bonds via cysteine produced higher transfection results of small interfering RNA (siRNA)(presumably through quick siRNA release) but interestingly resulted in a decline in DNA transfection compared to non-reducible controls [77]. Another intriguing study shows that a disulfide bond incorporated into a cyclized polymer structure, that consists of single chains attached to themselves via intramolecular cyclizations, allows a high transfection capability and low toxicity even though reduction of the bond does not result in cleavage of the polymer, but instead an “untying” of the polymer chains [78].

---FIGURE 7---

Whilst some studies have shown a reduced performance of reducible gene vectors [79], others have reported an increase in efficiency [14, 80]. Using the reducing conditions within the cell to break down polymeric delivery devices clearly has much potential to improve delivery efficiency and lower toxicity, two factors presently presenting a barrier to the true realization of non-viral gene delivery to the brain.

3.3. Adding Functionality (Targeting, Cell Entry etc.)

Currently, the vast majority of drugs administered for diseases such as PD, are administered orally, requiring them to enter the blood stream and finally cross the blood brain barrier (BBB) to mediate their effect in the brain. This non-invasive approach has high patient acceptance but severely limits the type of drug that can be administered, with proteins and charged molecules being prevented entry to the brain [81]. Routes to bypass the BBB, such as stereotactic surgery, are a possible option for one-off interventions (such as cell replacement therapies), but remain a difficult task for continuous infusion [82-85]. Other routes to CNS administration involve BBB disruption and/or nasal administration [86, 87]. Whilst these areas of research are gaining momentum (reviewed elsewhere [81]), polymer therapeutics are still likely to require specific functionality in their design. ~~Another hurdle hindering the progress of therapeutic delivery to the CNS is the ability to design polymer vectors capable of crossing the blood brain barrier, targeting specific cell types and avoiding off target delivery to organs such as the liver and spleen.~~ Even vectors delivered by stereotactic injection, which is the main injection method analyzed to date for non-viral gene delivery to the brain [88], may still require the addition of specific cell targeting moieties [89, 90], for cell surface receptors [91], cell penetrating peptides or nuclear localization sequences [80] to deliver with high efficiency. There is clearly an argument for polymer vectors with a simple design and much of the polymer vector research by Wang et al., has focused on very simple “one pot” synthesis strategies, such as deactivation enhanced ATRP [66, 92] or RAFT polymerization [67], to allow easy scale up and increased reproducibility. However, nature’s highly effective adenoviral vectors are not such a simple design, and consist of a highly organized structure containing multiple proteins [93-95]. There is therefore perhaps a tradeoff between simplicity/translation to the clinic/viable upscale (supply), and complexity/efficiency/demand. The aim of much of Wang’s research is therefore to make simple and efficient vectors as a starting point which contain free vinyl groups [71, 96, 97] to allow simple post synthesis functionalization via Michael addition for example with antibody fragments [98]. However, more complex delivery systems may include multiple functional moieties such as a polymalic acid vector developed for systemic delivery of antisense oligonucleotides against glioblastoma (see figure 8)[99]. The vector includes two targeting antibodies, pH triggered endosomal escape moiety, carboxylate groups to improve solubility, and a tracking Alexa Fluor.

---FIGURE 8---

Adding targeting moieties into the structure of polymeric gene or drug carriers for improving systemic delivery to the brain has been extensively investigated (reviewed elsewhere) [100, 101], but has also been proposed for assisting access to the CNS via intra-nasal administration [102-105]. The discovery of transferrin receptors specifically on the endothelium of capillaries of the brain [106] has led to much research using either the monoclonal antibody against transferrin or

conjugating the transferrin glycoprotein itself to the polymer vector [107-112]. However, due to high endogenous levels of transferrin nearly saturating the receptors, antibodies against the transferrin receptor, that do not compete with transferrin binding, such as OX26 (rat) and RI7217 (mouse) offer practical alternatives for *in vivo* studies [113, 114]. Research has also turned towards the use of antibody fragments to functionalize polymers [98], due to the improved tissue penetration, reduced immunogenicity, or increased packing density of these small fragments compared to the parent molecule [115]. Immunoliposomes targeted with the OX26 antibody showed greater gene delivery than non-targeted liposomes [113], whilst in a comparative study RI7217 targeted liposomes showed better brain uptake than four other targeting moieties [114].

Many targeting ligands have been used to improve the efficiency of gene protein or drug delivery to the brain such as rabies viral glycoprotein (RVG) [116-118], lactoferrin [38, 119, 120], peptide TGN [121-123], or Tet1 [90] to name a few (more reviewed elsewhere [88]). The improvements that ligands such as these provide, although sometimes modest, give a strong rationale for the inclusion of specific groups into the polymer design, to aid cell/tissue targeting, cell uptake or nuclear localization etc. It is also noteworthy that cell penetrating peptides such as Arg-9, penetratin, and TAT, themselves maybe neuroprotective, thus offering an addition advantage for functionalized vectors, but obscuring from where benefits arise [124]. Whilst very efficient delivery to brain has recently been achieved using anti-glioblastoma RGD modified polymer micelles [125], PD therapeutics may also require specific targeting of the striatum, or the dopaminergic neurons themselves, to overcome the off-target effects mentioned previously [39, 40]. As a study with targeted GDNF fusion proteins has shown, reducing off target growth factor effects are difficult [126], and perhaps specific astrocyte or neuron targeting would be more feasible for gene therapy. The use of a region specific promoter, such as GFAP or tyrosine hydroxylase, could restrict expression to astrocytes [127] or to catecholaminergic neurons in the brain respectively [128, 129].

3.4. Structure

Designing a polymer therapeutic requires consideration of the toxicity, degradability, and functionality, which in turn will affect the choice of monomer composition. However, another important design consideration is the polymer structure, which will not only affect the choice of monomers used, but also how they are to be arranged in the polymeric structure. Depending on whether a macroscale hydrogel is being designed or a nanoscale drug delivery device, the polymer structure may be vastly different. The desired structure will not only determine whether crosslinking monomers (such as divinyls) are used, but also how drugs are loaded [130], nucleic acid interactions [96, 131, 132], and mechanical properties [133] to name but a few.

Progress in polymer synthesis techniques has allowed the formation of a variety of polymer structures. Whilst conventional free radical polymerization can easily be used to form linear polymers, particularly from monomers containing a single vinyl group, control over the molecular weight and the distribution of molecular weight can be poor. The initiation process is slow and continuous, and the termination process is uncontrolled, leading to chain growth starting or stopping at different times, hence the formation of a variety of chain lengths, (or high polydispersity index) [134]. Controlled radical or "living" radical polymerization however, creates a dynamic equilibrium between the active species capable of propagation, and the deactive species (not permanently terminated) halting the chain growth until next activated (see figure 9) [135].

---FIGURE 9---

In this way the reaction procedure can be controlled so that the desired molecular weight can be achieved with a narrower polydispersity. A variety of polymer architectures can be formed depending on the polymerization technique used (e.g. nitrox-oxide polymerization, atom transfer radical polymerization or reversible addition fragment polymerization), the monomers used (e.g. mono functional, multi-functional), and/or strategy employed (e.g. starting with a multifunctional core for star polymer synthesis) [69].

---FIGURE 10---

Figure 10 shows the major polymer architectures that could be used for designing polymer therapeutics for the CNS. A brief overview is given below, because many of these structures will appear in the last section of this review, where polymer therapeutics specifically used for PD applications are discussed. A simple example, where the specific structure of a polymer bears critical importance to its function, can be seen when a dendrimer can alter whether it does or does not inhibit the aggregation of the Parkinson's disease protein alpha-synuclein [136]. The function of the outlined polymer structures is not limited to nanoscale delivery devices; they can be designed as crosslinkers for materials such as microparticles/macroscopic hydrogels, tethering agents for functional moieties, or simply side chains to adjust hydrophilicity.

3.4.1. Linear Polymers

---FIGURE 11---

These can typically be formed via simple ring opening polymerization reactions, to form polyesters such as poly(lactic acid), poly(glycolide), poly(caprolactone) and the copolymer PLGA [137]. Controlled radical polymerization can also be used as another simple example, whereby linear chains are formed by the polymerization of monomers containing a single vinyl group, such as 3-aminopropyl methacrylamide (APMA), 2-dimethylaminoethyl methacrylate (DMAEMA) or N-(2-hydroxypropyl)methacrylamide (HPMA) (see figure 11) [68, 69]. Being simple to produce with good size control, linear polymers are much studied e.g. linear PEG chains for altering drug solubility/circulation times, or linear PEI for transfection agents. However, the functionality of linear polymers is limited to the monomers used, and the two end groups. In contrast, branched polymers contain a greater number of chain ends per single molecule, allowing greater possibility for post functionalization. Another way to introduce functionalization for applications such as gene delivery, is via living copolymerization, either forming diblock copolymers which can self-assemble into micelles etc., or forming statistical or graft copolymers containing different groups or chains attached to a linear backbone [138].

3.4.2. Branched Polymers

As figure 10 shows, branched polymers can be formed as a variety of structures, such as branched or hyperbranched polymers, highly ordered three dimensional dendrimers or chains organized in a star shape via a central core unit, to name some common types. The occurrence of branching gives rise to the possibility of greater functionalization due to the greater number of chain ends. Cationic polymers for applications in gene delivery, such as PEI, are commercially available as a linear structure (typically 22kDa) or branched structure (typically 25kDa) where the linear 22kDa

PEI typically shows a higher transfection capability [139, 140]. However, in contrast, studies of the tertiary amine containing DMAEMA polymer show that branched polymers, either hyperbranched or in a star shape, show a higher transfection capability than a linear DMAEMA control polymer [97, 141]. Dendrimers, branched polymers with a highly ordered structure [142], have been extensively researched for a wide range of biomedical applications [143], some of which could be tailored to PD therapeutics, such as gene/protein/drug delivery or to prevent harmful protein aggregation (see section 4).

---FIGURE 12---

Several groups using controlled living polymerizations as a route to branched structures report the presence or likelihood of both intermolecular cross-links (between different polymer chains) and intramolecular (internal cyclizations) within the same polymer chain [144-146]. Furthermore, Gao and Matyjaszewski used atom transfer radical polymerization (ATRP) [147, 148] to homopolymerize a divinyl monomer under dilute conditions to synthesize nanogel cores as a new route to star polymer architectures (see figure 12) [149]. Recently Wang and co-workers have been able to shift the intermolecular/intramolecular balance to form either utmost hyperbranched polymers, or single chains self-linked by intramolecular crosslinks by *in situ* deactivation enhanced ATRP [150]. By controlling the monomer to initiator ratio, along with the percentage of the deactivating Cu^{II} species (enhances deactivation depicted in figure 9), single cyclized molecules can be formed which can be used for gene vectors for neural cells (figure 13) [78, 96] ~~(add in new reference)~~.

---FIGURE 13---

3.4.3. Cross-linked Networks

Networks of cross-linked polymer chains can occur on a variety of scales, from macroscopic gels that retain water (hydrogels), to finite networks on the microscale or nanoscale (microgels or nanogels). The term “cross-linked network” may refer to either intermolecular or intramolecular crosslinks and may refer to either a covalent bond (chemically cross-linked) or physical interactions such as electrostatic interactions, hydrogen bonding, or hydrophobic interactions (physically cross-linked). Aside from core precursors for star polymers (see figure 12), nanogels may be designed for therapeutic delivery [151, 152]. Macroscopic hydrogels/scaffolds may be designed for neuron guidance applications, cell delivery systems or growth factor/drug depots for sustained release [153-156]. Network formation may be induced via a variety of methods. For applications in Parkinson’s disease, where the material must be injectable, crosslinking can only occur prior to injection if nano/microscopic networks are formed [157], or if the hydrogel has shear thinning properties [158, 159], allowing release through the injection cannula. Therefore, much attention has been given to *in situ* gelling materials, which form a hydrogel upon or soon after the injection procedure [160]. In this case, the precursor materials must not be toxic, ruling out the much used EDC/NHS chemistry, and must form a hydrogel at 37^{o}C in a suitably short time frame, but not too quickly as to clog the injection cannula during the injection procedure (usually greater than two minutes). To this end, one obvious stimuli to trigger gelation is temperature. Temperature sensitive polymers, which are a liquid solution at room temperature, can form hydrogels when the lower critical solution temperature (LCST) is surpassed (typically below 37^{o}C for biomedical applications), due to the change in the hydrophobicity [161, 162]. Pure PEG based polymers or

other hydrophobic hydrogels suffer the drawback that they are unsuitable for cell delivery applications as cells attach poorly. Semi synthetic hydrogels offer an attractive means to providing a substrate for cell attachment, and can be designed to form a gel *in situ* by mixing of crosslinker, such as PEG, immediately prior to injection, to crosslink naturally occurring materials such as collagen [15] or heparin [24]. One final means to forming a cross-linked network, worth mentioning in the context of PD therapies, has been to use a branched PEG polymer containing reducible (disulphide) and non-reducible crosslinkers, activated to gel by the addition of glutathione [157]. The reducing agent glutathione and its precursor N-acetyl cysteine have been studied as a means to reducing reactive oxidative species in the brain [163]. Although efficient glutathione (or precursor) delivery remains an obstacle, greater superoxide activity and decreased levels of reduced glutathione are well documented in PD, indicating oxidative stress is a mechanism of cell loss [164-166]. The PEG based polymer relied on non-reducible ethylene glycol dimethacrylate (EGDMA) as branch points, and on glutathione sensitive links, to be reduced to thiols, which could then spontaneously crosslink with free vinyl groups remaining on the polymer chains to form a crosslinked network (see figure 14)[157]. Although this was very preliminary research, the concept could, in principle, be applied to PD cell transplantation therapies for a combined gel formation and glutathione delivery. However, cell attachment should again be considered, perhaps by adding cell adhesion ligands such as RGD into the hydrogel structure [59].

---FIGURE 14---

4. Applications in Parkinson's disease: Current Progress and Future Perspectives

As figure 1 attempts to depict, there are several possible areas where polymer therapeutics may impact on PD. Nanoscale polymers may assist drug or gene delivery, or inhibit protein aggregation, whilst microscale materials may allow more effective protein delivery, such as growth factors. Macroscopic polymer hydrogels might be able to improve the transplantation process by providing a protective medium to which cells can attach. In addition, hydrogels may also be used to deliver growth factors with the aim of protecting neurons, providing neuron guidance cues or improving cell survival post transplantation. Current progress in the above mentioned fields are highlighted below.

4.1. Polymers as Drug Carriers

Dopamine, the neurotransmitter depleted in Parkinson's disease, acts upon the dopaminergic pathways as shown in figure 2: mesolimbic/cortical pathway, tuberoinfundibular pathway and nigrostriatal pathway. In PD, the death of neurons in the latter pathway results in a deficit of dopamine, in particular in the putamen where only 1% of dopamine was found in patient autopsies compared to healthy controls [167]. The replacement of dopamine, via the precursor levodopa (or L-DOPA) (which can cross the BBB) forms the mainstay treatment for PD [168], however, it is associated with undesirable side-effects such as dyskinesias and a host of non-motor complications such as nausea, hallucinations etc., [168, 169]. The inevitable intermittent nature of oral delivery produces peaks and troughs of L-DOPA levels in the blood and brain, which appears to compound these side-effects [170-172] and better means of delivery are being sought [172]. Indeed, for many existing PD drugs, drug delivery systems are being developed either to sustain more constant pharmacological levels or improve therapeutic efficiency by better targeting/delivery across the BBB [173, 174]. Polymer therapeutics may therefore play a role in

reducing side effects and improving efficiency of current drugs, or may allow the development of new drugs capable of crossing the BBB [175, 176].

The biodegradabe linear polysaccharide, chitosan, contains a primary amine group per repeating unit, allowing interaction with hydrophilic drugs such as dopamine. Dopamine loaded chitosan nanoparticles could be formed with an average diameter of 110nm [177]. Dopamine is too hydrophilic to pass through the BBB as a free drug, however, intraperitoneal administration of dopamine loaded chitosan nanoparticles caused an increase in striatal dopamine output [178]. Chitosan has also been shown to transport nucleic acids such as siRNA to the brain (more on gene delivery to the brain in section 4.2) when functionalized with PEG and the TAT peptide [179], or deliver peptides to inhibit caspase activity [180, 181].

PLGA is another biodegradable material (figure 15) that has been used for drug delivery to the brain for applications in PD.

---FIGURE 15---

Microparticles of PLGA encapsulating a drug can be formed using emulsions, for example oil-in-water/solvent evaporation [182], or water-in-oil-in-water emulsions [183]. In this way, particles containing the free drug can be purified, freeze-dried and stored for later use. As mentioned earlier, the intermittent administration of oral L-DOPA administration can be problematic so a more controlled release of the drug could potentially improve the side effects of L-dopa induced dyskinesias. More recently a prodrug of L-DOPA has been produced for dopaminergic stimulation, termed L-dopa-α-lipoic acid. It is less susceptible to enzymatic conversion (e.g. by catechol-O-methyltransferase and monoamine oxidase), resulting in longer periods of activity compared to standard L-DOPA. D'Aurizio et al., encapsulated L-dopa-α-lipoic acid within PLGA microparticles and showed that striatal dopamine levels could be increased post subcutaneous administration of such a delivery system (see figure 16) [184]. The remarkable feature of this work is the pharmacokinetic properties of the delivery, which gave rise to relatively steady striatal dopamine levels over a period of four days.

---FIGURE 16---

Two further studies showed that the delivery of levodopa methyl ester in conjunction with benserazide (a peripheral dopa-decarboxylase inhibitor) via PLGA particles could reduce L-DOPA induced dyskinesias [183, 185]. A reduction in phosphorylation of the non-dopmainergic glutamatergic receptor α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was observed [185], a receptor previously shown to be involved in L-DOPA induced dyskinesias [186].

To model L-DOPA induced dyskinesias, firstly a commonly reported model of PD was established using the toxin 6-hydroxydopamine (6-OHDA) that (when given at the appropriate dose) selectively destroys dopaminergic neurons, and then L-DOPA is administered over a period of weeks in a manner that mimics the intermittent dosing that results from taking tablets at intervals during the day. The acute toxin models of PD, such as 6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone models, comprise the most common models but are not without criticisms [187] and more pathologically relevant models are being sought [188-192]. However, the chronic toxin exposure models can indeed mimic the human PD nigrostriatal lesion

[193] and so the toxin induced PD models are still used to validate the therapeutic efficiency of new therapies.

---Table 1---

Aside from the dopamine precursor L-DOPA, agonists that act upon the dopamine receptors are widely used, often as a first line treatment [169]. These are not without their side effects (many similar to L-DOPA treatment) and research groups are also investigating the use PLGA based microscale carriers as a means of sustained release of agonists such as apomorphine [194] or delivery of rotigotine [195, 196] to the brain. Wang et al., were able to show that rotigotine loaded PLGA microspheres administered in conjunction with L-DOPA, reduced the abnormal involuntary movements (dyskinesias) associated with L-DOPA therapy compared to the control group (L-DOPA alone) in the 6-OHDA rat model of PD [195]. These preclinical studies, showing the ability of PLGA micro/nanoparticles to deliver therapeutic agents to the animal brain, could mean that a fundamental change in the way PD drugs are administered might not be too far from reality. Combining drugs that are already approved by regulatory bodies, and PLGA that already has FDA approval for a host of devices, could mean an easier route to acceptance for tackling the problem of “on-off” symptoms than more radical approaches such as viral delivery of enzymes involved in the dopamine synthesis pathway [197-200]. PLGA spheres could also be used to deliver other therapeutic agents such as MAO-B inhibitors [201] and attempt to modify the disease process by delivering antioxidants [202], cytoprotective drugs such as urocortin [104, 203], or neuroprotective growth factors (covered in section 4.4).

4.2. Polymeric Gene Vectors

The number of non-viral transfection studies performed in the mammalian brain to date is well over 65 [88]. The majority of these studies are with liposome or polymer vectors, and many use marker genes to assess the effectiveness of the vector. For example, the early studies in the Demeneix group used liposomes and PEI to deliver the marker genes for β-galactosidase or luciferase to the newborn mouse brain [204, 205]. Since then much work has focused on the difficult task of transfecting the adult rodent brain with functional genes for a therapeutic outcome in an experimental model of disease. This section will highlight some of the recent progress made towards accomplishing this goal.

Despite the early success of PEI vectors for the delivery of marker genes to the rodent brain[204], the more recent studies, delivering therapeutic nucleic acids to models of disease, have focused heavily on poly-L-lysine (PLL) based vectors. PEI has been shown to cause significant toxicity in the striatum, thus researchers have sought to reduce the toxicity through delivery via collagen spheres [16], encapsulation within liposomes [206], or using PEG to reduce the charge density [90]. PLL, the natural homopolymer of L-lysine, has been modified by the addition of neurotensin for gene delivery directly to dopaminergic neurons to act on their high affinity neurotensin receptors (the entire work is concisely reviewed elsewhere [207]). The delivery of the gene encoding human GDNF, via the neurotensin-PLL vector, to the rat substantia nigra one week after the 6-OHDA lesion, was able to induce re-innervation of the striatum where the dopaminergic neurons project (see figure 17) [208].

---FIGURE 17---

Another PLL based variant used L-cysteinyl-poly-L-lysine conjugated to 10kDa PEG to form a transfection vector that could compact DNA into polyplexes of 11nm diameter or less. This small size allows the transfection of post-mitotic cells and could mediate GDNF transgene expression in the rat striatum for up to three weeks post intrastriatal injection [209]. A follow up study showed that GDNF transgene expression could be observed for up to six months and showed that GDNF levels were higher in the 6-OHDA model than in the healthy brain [210]. Since the 6-OHDA causes a large upregulation of astrocytes in the rat 6-OHDA model [211] it is likely that this increased GDNF level is due to the transfection of astrocytes, being greater in number in the 6-OHDA model than the healthy brain [210]. A previous study has shown that primary astrocytes extracted from the midbrain of the newborn rat are far less amenable to non-viral transfection than immortalized Neu7 astrocytes [96]. However, a comprehensive study of a library of poly(β-amino esters) showed that one specific composition (1,4-butanediol diacrylate “backbone”, 4-amino-1butanol “side chain”, with the end-capping agent 1-(3-aminopropyl)-4-methylpiperazine) was able to transfect healthy astrocytes where others, including the lipofectamine2000 control, failed to do so [75]. The ability of polymer gene vectors to be efficient at transfecting astrocytes is probably an important design criteria [127], especially for secreted proteins such as GDNF, however it must be borne in mind that astrocyte numbers are not elevated in the human PD condition either in the substantia nigra or the putamen [212]. Another important factor which must be considered when designing gene delivery vectors for stereotactic injection into the brain is the charge of the polymer/nucleic acid complex. Whilst the aforementioned studies show complexes typically cationic in nature, a recent study showed that greater spread away from the injection site could be achieved by anionic complexes, as they have less affinity for cell surface proteoglycans than the cationic counterparts [213].

The above studies, using a PLL based vector, observed the effect of transfection after direct stereotactic injection into the brain, and used the 6-OHDA as a toxin to selectively destroy the dopaminergic neurons. However, the same basic vector has been used mediate GFP transgene expression in several brain regions (including the striatum) after intranasal delivery [85]. In addition, a PLL based vector has also been analyzed for its ability to mediate GDNF transgene activity in the rat brain after systemic injection [214]. To facilitate translocation across the BBB, this dendrigraft PLL was functionalized with PEG and angiopep, a ligand that specifically binds to the low-density lipoprotein receptor-related protein which is overexpressed on the BBB. This study used the pesticide rotenone to model PD, which, when administered systemically, can cause loss of the dopaminergic neurons and α-synucleinopathy, or just dopaminergic neuron loss without the α-synucleinopathy when administered via direct stereotactic injection [215]. The angiopep conjugated PLL vector was able to produce GDNF over-expression in the rat brain in a dose dependent manner, resulting in a greater number of tyrosine hydroxylase (TH) positive (dopaminergic) neurons surviving than a green fluorescent protein control group [214]. The same group functionalized dendrigraft PLL with the rabies virus glycoprotein peptide (RVG29) again via a difunctional PEG, to deliver anti-apoptotic interference RNA to the rat brain [118]. RVG29 is a 29 amino acid peptide capable of binding the nicotinic acetylcholine receptor widely expressed throughout the brain and BBB [216]. Systemic delivery of this PLL loaded with short hairpin RNA to knockdown caspase-3 expression was found to significantly improve motor activity during behavioral tests in the rotenone PD model in comparison to saline control injections or the non-functionalized vectors [118].

Whilst the vast majority of non-viral gene delivery studies, which have focused on a functional (behavioral) outcome in a PD model, have used PLL as the cationic component for nucleic acid condensation, others have used the PAMAM dendrimer. Using a difunctional PEG spacer, a lactoferrin ligand was attached to generation 5 PAMAM [119, 217]. Lactoferrin binds to the lactoferrin receptors present in the brain endothelial capillary cells of the BBB [218, 219], and transport across the BBB is reported to be unidirectional [219], giving lactoferrin the potential to be a highly efficient means of targeting the brain. Delivery of the GDNF encoding gene resulted in a significant behavioral improvement in both the 6-OHDA PD model [119], and the rotenone model [217].

There are two other non-viral vectors, which although do not fit into the classic polymer structures outlined in section 3.4, are worth mentioning due to their high efficacy at delivering nucleic acids across the blood brain barrier. The first, Trojan horse liposomes, are PEGylated liposomes with insulin or transferrin monoclonal antibodies attached (reviewed extensively elsewhere [220]). An early report showed gene expression in the brain following a single intravenous injection [109], and has since been used to deliver therapeutic genes such as GDNF to the brain in PD models [129]. The second, targeted exosomes, are vesicles produced by cells [221], which were functionalized with the rabies virus glycoprotein to deliver siRNA neurons to the mouse brain after systemic injection. The targeted exosomes could mediate successful knockdown of BACE1 – a specific Alzheimer's disease protein, showing great potential for the future gene therapies in neurological disorders (reviewed elsewhere [222]).

Despite much general research [223], polymer vectors based on the tertiary amine containing DMAEMA monomer have received less attention specifically for transfection of neurons or for neuronal applications. Studies have shown that the transfection of primary astrocytes is possible using DMAEMA vectors [96], and that they can mediate transgene expression in the spinal cord when released from collagen conduits in the completely transected rat spinal cord injury model [224]. However, a recent study has assessed the ability of peptide TGN functionalized DMAEMA vectors to mediate gene delivery to the mouse brain [122]. These vectors, formulated with PEG to form micelles, were able to mediate widespread transfection of the brain. Interestingly, non-targeted vectors showed only slight transfection around the lateral ventricle, but the targeted vectors could mediate transfection in the striatum and the substantia nigra, which are both regions of interest for PD therapies.

---Table 2---

Whilst much progress is clearly being made in the field of non-viral gene delivery, clinical trials involving viral delivery of genes such as NGF [225], glutamic acid decarboxylase [226], neurturin [227] (a structural relative of GDNF), and ProSavin (tyrosine hydroxylase, AADC, and cyclohydrolase 1) [228] to the brain are providing mounting evidence for the safety of such therapeutic strategies. By 2012, 28 clinical trials involving gene therapies for neurodegenerative disorders had taken place [229]. Although greater efficacy is being sought, some studies lack significant improvement, which may be in part due to patient selection criteria, which could perhaps be improved by selecting patients in an early stage of the disease process. However, non-viral vectors are not without their advantages, which could materialize in later years once the efficacy has been improved. If viral mediated gene therapy can pave the way to prove the principle that gene therapies can be of benefit to PD patients [230, 231], then perhaps the advantages of

non-viral vectors such as ease of handling/distribution/storage, and ability in most cases for large scale production could emerge allowing widespread therapeutic intervention. In addition, targeted vectors, with the ability to cross the BBB [232], would significantly reduce the cost of intervention. However, the transfection of off target organs such as the liver and kidney must also be overcome as figure 18 serves to highlight [73].

---FIGURE 18---

4.3. Polymeric Inhibitors of Protein Aggregation

The use of polymeric nanoparticles in an attempt to reduce the fibrillation or aggregation of disease causing proteins is an exciting and more recent area of research. Many of the neurodegenerative diseases have pathologies characterized by the presence of protein aggregates, such as intranuclear inclusions in HD and polyglutamine diseases (e.g. huntingtin and atrophin-1 respectively), neuritic plaques and neurofibrillary tangles in AD (e.g. Aβ peptide and hyperphosphorylated tau), or aggregates of the prior protein in Prion's disease, or the α-synuclein protein containing Lewy bodies in PD [233]. Nanomaterials, such as quantum dots, polystyrene spheres, cationic polymers and dendrimers have been used to either reduce the rate, or stop the process of protein aggregation or fibril formation. The fibrillation process starts initially with nucleation of monomeric peptides forming subcritical or critical nuclei of oligomers, followed by elongation to mature fibrils. Early work in this field working with amyloid β protein has shown that the lag phase, before fibril formation, can be increased by using copolymers of N-isopropylacrylamide: N-tert-butylacrylamid, termed NiPAM:BAM [234]. The authors reasoned that this extension of the lag phase is due to the association of monomeric or oligomeric protein to the nanoparticle surface (as shown in figure 19) rather than to each other to elongate the fibril.

---FIGURE 19---

The nature and mechanism of fibril formation is indeed complex with a balance between promoting and preventing fibrillation being shown even for materials of the same composition. For example the aforementioned NiPAM:BAM polymers were previously shown to promote fibril formation of human β2-microglobulin [235]. In addition, increasing the hydrophobicity of the copolymer resulted in a reduction of the lag time of amyloid β protein back to nearly that of the protein incubated in nanoparticle free solution [234]. Another study, which used amino modified polystyrene nanoparticles showed that low concentrations (0.02 or 0.05 mg/mL) caused quicker amyloid β protein fibril formation then the no treatment group [236]. However, in stark contrast, the high concentration (1.1 mg/mL) retarded fibril formation significantly as shown in figure 20.

---FIGURE 20---

In terms of PD, studies have investigated the ability of cationic dendrimers to inhibit fibrillation of α-synuclein, the protein present in Lewy bodies (protein aggregates involved in PD pathology). α-synuclein can exist in a variety of conformation states (e.g. partially folded, oligomeric, or fibrillar or amorphous aggregates) and much evidence links α-synuclein misfolding to both sporadic and familial PD [237].

Reducing the fibrillation/aggregation of α-synuclein may therefore play a role in the affecting the progression of PD. PAMAM dendrimers (generations (G) 4, 5 and 6) used as purchased without

modification were able to reduce α-synuclein fibrillation as measured by the Thioflavin T assay [238]. This occurred for all three generations, and the effect of concentration analyzed for G5 PAMAM showed a concentration dependence on the degree of inhibition. A different study showed that whilst G4 PAMAM showed inhibitory effects on α-synuclein fibrillation, the middle generation G3.5 PAMAM did not [136]. The number of end groups remains the same, however whereas G4 PAMAM has primary amine end groups, the G3.5 PAMAM has carboxyl groups. This would indicate that surface amines effect the protein-polymer interaction (note G3.5 PAMAM still has amine groups within its structure, but not at the surface). Two other studies, both using tertiary amine containing dendrimers, showed inhibitory effects of α-synuclein aggregation [239, 240]. As mentioned before for amyloid β protein, extrapolation of results to fibrillation inhibition mechanisms is difficult. For instance, many other amine containing polymers, including poly-L-lysine and PEI have been shown to increase the rate of α-synuclein aggregation [241], showing that a simple amine-protein interaction is perhaps not all that is required.

Many flavonoids have been shown to inhibit the fibrillation of α-synuclein [242] and a mechanistic study of the flavonoid-protein interaction elucidates that the oxidized form (quinone containing molecule (see figure 21) of the flavonoid plays a major role [243]. To highlight the role that the quinone group plays in inhibiting fibrillation, quercetin, a molecule that self oxidizes to the quinone form, was added to α-synuclein after different incubation times (and therefore different stages of the oxidizing process) [244]. The freshly prepared quercetin exhibited almost no inhibitor effect compared to a large inhibitory effect on fibrillization when the oxidized (left for 48 hours) quercetin was used.

---FIGURE 21---

This data should therefore suggest that other molecules such as dopamine or L-dopa should have intrinsic anti-fibrillation properties in their oxidized form which would contain a quinone group. Indeed, studies have shown that not only does dopamine inhibit fibrillization of α-synuclein [245] but that amyloid fibrils can be disaggregated by dopamine and L-dopa [246]. Future polymer based interventions in protein aggregation may utilize simple chemistries such as the Michael type reaction to link several dopamine molecules together, to form hyperbranched structures similar to the quinone containing polydopamine used as a tissue adhesive [247]. The high branching degree, ability to biodegrade and low toxicity of such materials could be a useful alternative to amine containing dendrimers which typically exert cytotoxic effects. Indeed the toxicity of a material proposed for inhibiting protein fibrillization, is of utmost importance as a recent study using n-acetyl-L-cysteine capped quantum dots to inhibit beta amyloid fibrillation serves to highlight [248]. The quantum dots exhibited a dose dependent toxicity which the authors took into account whilst analyzing the toxicity of beta amyloid aggregates in the presence or absence of the quantum dots in an *in vitro* system. Such studies are important, as they place the inhibition qualities of the material into context by balancing them with the negative effects of the material. To maximize efficiency and minimize off-target effects, the polymers could be designed to incorporate moieties with a specific affinity to the disease protein, such as liposomes containing phosphatidic acid to bind amyloid-β [249]. Care should be also be paid to the use of heparin based hydrogel materials in the brain (see section 4.5) as glycosaminoglycans have also been shown to promote the fibrillation of α-synuclein *in vitro* [250]. Inhibiting the fibrillation of α-synuclein could be of major

importance for future PD intervention, especially considering the recent finding that fibrillation of α-synuclein spreads to anatomically connected areas of the brain [251, 252].

4.4. Growth Factor Delivery Systems

The ability of growth factors such as NGF, GDNF and BDNF to protect neurons in animal models of neurodegenerative diseases is well documented [8, 9]. They act through differing cell signaling pathways (simplified in figure 22) which result in changes in Ca^{2+} release, neurite outgrowth/axon branching, and of particular interest for PD, they exhibit neuroprotection [253, 254].

---FIGURE 22---

In 1993 GDNF was discovered to enhance the survival of midbrain dopaminergic neurons [255], and has since been the focus of extensive study as a potential PD therapy. Just three years after discovery of the protein, GDNF therapy for PD entered initiation stages for clinical trials. One open label phase trial showed positive outcomes [256] not present in the follow up randomized control trial [257]. Meanwhile, patients with moderate to advanced sporadic form of PD had been selected for infusion of GDNF into intracerebroventricular space (ICV) where no improvements were seen and side effects were observed [258]. This was contrary to previous findings in rat and monkey PD models, which showed GDNF administered in a similar fashion, exerted neuroprotection and functional recovery [6, 259-261]. The inconsistency in results obtained by direct administration of the recombinant protein suggests that effective delivery remains the crux of the problem for clinical translation [7].

Research groups have since also focused attention upon biomaterial based strategies to allow sustained release of growth factors from materials such as microspheres and hydrogels [11, 12]. Whilst catheter design has vastly improved since the early trials [262], polymeric implants that sustain the release of growth factors could potentially negate the use of infusion pumps and catheters in the future.

To achieve controlled growth factor release in the brain, one early study embedded a bovine serum albumin/NGF mix into molded poly(ethylene-co-vinyl acetate) [263], whilst another showed that NGF delivered via PLGA microspheres could prevent excitotoxic damage induced by striatal infusion of quinolinic acid [264]. Just as with drug delivery to the CNS (section 4.1), the vast majority of growth factor delivery vehicles are comprised of PLGA, however a microparticle composed of a PLGA outer layer and collagen inner layer was fabricated in an attempt to reduce the burst release typical of PLGA vehicles and most biomaterial delivery systems in general [265]. The GDNF delivered was modified to contain a collagen binding peptide, thus allowing it to have an affinity to the collagen. However, growth factors typically couple to macromolecules with charged domains such as heparin [24, 59, 266] and collagen [267-269], and a study made use of this electrostatic interaction to deliver NGF to neurons *in vitro* via microspheres made of starPEG crosslinked collagen [270].

In terms of microsphere mediated delivery of GDNF to rodent models of Parkinson's disease, early studies showed that impressive behavioral improvements or tyrosine hydroxylase expressing neuron retention could be achieved [271-273]. However, the treatment group in these cases was compared to either empty microspheres or untreated animals, thus not showing the effect of a

single injection of GDNF alone. More recent studies have confirmed these findings [274, 275] with long term functional benefits being observed for up to 30 weeks [275], showing that PLGA mediated delivery of GDNF could be a good future therapy strategy for PD. Interestingly, a PLGA microsphere study that delivered vascular endothelial growth factor (VEGF), previously shown to have neuroprotective effects on dopaminergic neurons *in vitro* and *in vivo* [276], in conjunction with GDNF, showed little or no improvement in a combined effect of both growth factors compared to the GDNF alone group [277] showing the high potency of GDNF itself. It must be noted here that another study, encapsulating both VEGF and GDNF in PLGA nanoparticles did show a synergistic effect on nigral neuron survival in the 6-OHDA rat model [278], so this research area requires more study for clarification.

---Table 3---

It is clear from the numerous studies that using polymers to encapsulate or bind growth factors can lead to functional improvements in animal models of PD. Much progress has been made to sustain the release of bioactive neurotrophic factors, a process necessary to overcome the problem of short protein half-lives *in vivo*. There are a host of other growth factors with neuroprotective properties which could be analyzed in control delivery systems including neurturin [227], insulin like growth factor (IGF-1) [279, 280], conserved dopamine neurotrophic factor [281], fibroblast growth factor [282], or mesencephalic astrocyte-derived neurotrophic factor [283] etc. One problem associated with biomaterial delivery systems is that they often ~~However, the vast majority of the aforementioned studies~~ show a burst release (a rate of release typically much higher at the start than later on) of the growth factor of over couple of days. This release pattern may arguably be useful for a range of applications; however achieving zero-order release kinetics (rate of release is independent of concentration, i.e. steady release) could be really interesting for PD research for better delivery of dopamine or sustaining therapeutic neurotrophin levels for longer. High aspect ratio tubular structures possibly may afford such release, however potential toxicity issues must be considered. Another route could utilize the concept of a “pepper pot” whereby the drug is stored in high quantity behind a release limiting pore size [284], however, scaling such a device down to the microscale for injectability would be no small feat. Lastly, the simple but effective concept of using microfluidic devices to form PLGA with an outer coating of alginate allowed near zero-order release of the model drug rifampicin *in vitro* as shown in figure 23 [285].

---FIGURE 23---

Obviously drugs and growth factors will have vastly different release profiles, and even bovine serum albumin and GDNF have been shown to have differing release kinetics from the same type of microsphere (poly (ε-caprolactone)) [286]. However, designing delivery systems to control the release of therapeutic molecules is of high importance to PD research. Injectable hydrogels present another means of growth factor delivery to the brain either alone [23, 25] or encapsulating loaded microparticles [287]. Studies have focused on hydrogel mediated delivery of growth factors for applications in stroke [25] and HD [23], but to date much of the work using hydrogels for applications in PD has been to assist cell transplantation (covered in the next section).

4.5. Polymers Designed to Assist Cell Transplantation

The progressive death of dopaminergic neurons in PD provides researchers the rationale for cell transplantation. This could be to directly replace the lost neurons with fetal ventral mesencephalon tissue or stem cells engineered to differentiate into genuine ventral mesencephalic-like cells prior to transplantation. This strategy aims to repair the damaged neural circuits by implanting cells that can integrate into the circuitry and take over the function of the cells lost to the disease process. With current technology, the implanted cells have to be placed ectopically into the striatum (their target area) as when they are placed in the substantia nigra they can extend processes locally but not far enough to reach the striatum. Thus, an additional challenge is to find methods of encouraging the processes to grow more extended distances to enable synapses with distant targets. A different cell therapy strategy (although one that could in principle be combined with circuit repair) is to implant cells that are able to protect the remaining neurons by expressing molecules such as neurotrophic factors. Some cells, such as mesenchymal stem cells, may generate such factors naturally, and other may require genetic engineering (see selected reviews on the subject of cell transplantation to the brain [288-291]. One obstacle that hinders the widespread application of such therapies is the tendency of large numbers of cells to die during/soon after the implantation process. To allow for this, large numbers of cells have to be transplanted, which has been a particular problem for primary ventral mesencephalic grafts, meaning that multiple fetal samples are required for each transplant. Furthermore, long-term graft survival can be rather unpredictable, so strategies are needed to improve cell survival post transplantation [292, 293]. Many different biomaterials have been developed for assisting transplantation to the CNS, many of which are for applications in spinal cord injury [294-296], however some research has been directly applicable to PD. This section provides an overview and discussion about how polymer based strategies have been used in an attempt to overcome the second obstacle by trying to improve the survival of cells post transplantation to the brain, with particular focus on PD. The first strategy is closely related to the previous sections of this review, whereby nano/microparticles are co injected with the graft to deliver therapeutic agents such as DNA or neurotrophic factors to assist the survival of the cells post transplantation. The second strategy uses injectable materials (typically a hydrogel) either to provide an adherent substrate (reducing anoikis) or form a soft material barrier between the transplanted cells and their new ectopic environment during the early period post transplantation.

The concept of co-delivering growth factor microparticles with transplanted cells was studied as early as 2001 when Mahoney and Saltzmann pre-cultured cells with NGF releasing PLGA microparticles [297]. This system was used as a means of achieving higher choline acetyltransferase activity (measure of cholinergic cell function) from the transplanted fetal brain cells, and cell survival was not studied. However, cell survival was measured in a study where PLGA microspheres were used to deliver GDNF to the striatum during the grafting of ventral mesencephalic tissue either at the site of the injection or a site directly adjacent to it [298]. Unloaded or loaded microspheres were used, but little beneficial effects were observed, which the authors contributed to the small total amount of GDNF released from the spheres. In addition the *in vitro* release profile showed nearly all of the GDNF being released within the first day. The same group then coated the aforementioned PLGA microspheres with poly-D-lysine (PDL) to allow cell attachment. These pharmacologically active microcarriers were pre-cultivated with ventral mesencephalic cells and the cell/sphere complex was delivered to the 6-OHDA PD rat model [299]. Interestingly, delivering the cells whilst attached to the microcarriers, whether loaded with GDNF or not, vastly improved their survival (as measured by the number of TH positive neurons).

This gives the rationale for other studies to be performed with pre-loaded injectable materials, such as using PLL coated glass spheres to deliver neurons [47], or the further investigation into the use of pharmacologically active microcarriers to deliver MSCs [300]. In the case of MSCs, prior loading of microcarriers with the growth factor NT-3 further promoted MSC survival over the cell transplant alone group [300].

---FIGURE 24---

Injectable materials, which gel in situ upon injection [160], offer another potential means of protecting cells during transplantation to the brain. Many research groups have focused on using biomaterials to deliver cells to the ischemic brain or models of traumatic brain injury [25, 301-306], however, studies have also been performed in the striatum or PD animal model. One such study investigated a series of hydrogels, and determined that the *in vitro* survival of embryonic stem cell derived neural precursor cells was greatest with the growth factor reduced Matrigel. The *in vivo* study showed a larger graft volume could be obtained in the healthy mouse brain when the Matrigel was used to deliver the cells. Interestingly though however, the Matrigel did not reduce the number of cells undergoing apoptosis in the early stages post transplantation (it was in fact increased), but did increase the number of proliferating cells. Matrigel is a hydrogel composed of extracellular matrix proteins from mouse sarcomas, and as such does not have a well-defined structure/composition preferred for clinical translation. Other hydrogel systems, based on collagen, have been used to deliver cells to the rodent brain [15, 307]. The star shaped PEG crosslinker has been used (terminated with succinimidyl glutarate) to crosslink collagen type 1 (via the collagen amine groups) during the injection process, thus forming a hydrogel around the cells post transplantation (see schematic representation in figure 25) [15].

---FIGURE 25---

Despite a reduction in the microglial invasion/astrocyte scar observed around the graft site when the hydrogel was used, no increase in mesenchymal stem cell survival was observed. A study which used a collagen hydrogel, functionalized by the spontaneous binding of two laminin derived peptides, showed an increase in the survival of neural stem cells post transplantation [307]. It should be noted however, that the non-functionalized collagen hydrogel achieved no such increase in cell survival (which is in accordance with the aforementioned starPEG/collagen hydrogel), so the laminin derived peptides must be playing an important role in survival.

---Table 4---

Polymer based hydrogels hold the potential to provide useful assistance for cell transplantation therapies for diseases such as PD. They can reduce the shear stress that cells undergo during injection [312], however, typical flow rates for injection into the brain are very low (1 µl/min) [15, 16]. In addition hydrogels have been shown to reduce the host response at the site of transplantation [15], and they can provide a barrier to the ectopic environment. Whilst the adherent properties of the hydrogel are clearly very important [307], and potentially could be manipulated by the inclusion of dopamine itself [313, 314], there is also a growing rationale for preloading cells to an injectable substrate to mediate improved survival [47, 299, 300]. The development of hydrogel technology and micro contact printing have allowed pre-formed, cell seeded hydrogels of varying sizes to be easily produced [315], which could be adapted for applications in PD.

Perhaps the ideas of injectable hydrogels and pre-adhered cells could be combined in a shear-thinning hydrogel whereby cells at the center of the hydrogel experience very little shear force [159] and remain adhered to the hydrogel throughout the injection process [158]. Then it becomes easy to envisage further modification of the shear thinning hydrogel to incorporate trophic support for neurons [316], or other anti PD drugs in general. Polymer constructs may contribute to improving cell transplantation for PD in other, less foreseeable ways, such as assisting the during the cell growth stage prior to injection. For example the manipulation of stem cells prior to injection [317], or encouraging sphere growth via thermoresponsive patterned culture substrates [318].

5. A Note on Clinical Translation and Clinical Trials

This review has paid particular attention to the potential application of polymer-based therapeutics for PD. To date, research in this field has been largely carried out either *in vitro*, or via the use of *in vivo* models of the disease, with little application in the clinic. Here the authors briefly consider where clinical translation is likely to occur for PD and other neurodegenerative conditions for the aforementioned polymer-based strategies (drug/gene/growth factor/cell delivery).

It is likely that an early translational target will be controlling drug concentrations within the blood stream over extended periods. Currently, coating the active ingredient provides a means of prolonging the delivery of PD drugs ropinirole (clinical trials.gov identifier = NCT00331149, reviewed [319]) and amantadine HCl (clinical trials.gov identifier = NCT02153632). In addition, gel based delivery of drugs may allow sustained delivery, such as interintestinal delivery of DUODOPA® (levoDopa/carbidopa clinical trials.gov identifier = NCT01754129, [320]). To date, gene therapy trials for neurodegenerative diseases have all used a viral vector for the delivery of the genetic information, for example, CERE-120 – Neurturin for PD (clinical trials.gov identifier = NCT00985517, [227] reviewed, [321]), CERE-110 – NGF for AD (clinical trials.gov identifier = NCT00876863, [225] reviewed, [322]) or ProSavin (clinical trials.gov identifier = NCT00627588, [228]). Originally the safety concern associated with the use of viral vectors was a major driving force for non-viral gene vector development; however, as these trials increasingly show safety and tolerability of the vector, it is likely that non-viral vectors will be desired for other reasons. The simplicity and scalability of some methods of polymeric vector manufacture, particularly “one-pot” reactions that keep intrinsic functionality for specific modification [323], could allow a more broad scale translation of the therapy to the vast number of patients with neurodegenerative disorders. Perhaps viral vectors will pave the way, showing proof-of-concept for gene therapy in the human brain and, if efficiency can be improved, lead to a polymer-based therapy on a larger scale. Currently several polymer gene vector clinical trials are underway (reviewed in full elsewhere, [324]) with the majority focusing on anti-cancer activity, with one analyzing the use of a lipid vector for glioma therapy (clinical trials.gov identifier = NCT00734682).

One polymer assisted therapeutic strategy for neurodegenerative disorders which is well underway in clinical investigation is *ex vivo* gene therapy: an overlap between cell therapy and growth factor delivery to the brain. A variety of polymers such as polyethersulfone, poly(acrylonitrile vinyl chloride) and poly-l-lysine to name but a few (see review for full list [325]) have been used to create matrix materials as a hollow fibers/microcapsules that encapsulates cells, usually on a supportive matrix (see Figure 26) [326].

---FIGURE 26---

This encapsulation technology effectively shields transplanted cells from the host immune system, and so can be referred to as immunoisolated cell transplants. Cells that secrete neuroprotective proteins can be encapsulated in these devices so that immune cells cannot enter, but the desired protein can reach the surrounding tissue. With over twenty years since the early studies of encapsulation technology [327], this field of study has entered clinical trials with some promising results and the reader is referred to the following reviews on the subject [42, 328]. This technology has the potential for applications in a range of neurodegenerative disorders such as HD [329], AD [41], and PD (ongoing, clinical trials.gov identifier = NCT01734733). Unlike gene therapy, the protein production can be halted by device retraction. This approach is also limited to *ex vivo* delivery of proteins from the transplanted cells, and of course does not include cell therapies which require the cells to integrate with the host as cell replacement therapies intend. As stated in section 4.5, anoikis may play a significant role in cell death post transplantation [330], and so gelatin microcarriers have also entered the clinic as an injectable cell culture platform for a trial involving the transplantation of retinal epithelial cells for PD [331]. The small quantity, but highly promising data on microcarrier based transplantation, suggests that the development of simple cell adherent platforms have the potential to impact on future cell transplantation strategies.

6. Conclusions

This review has focused on five key areas of neuroscience research where polymer materials may affect future therapies for neurodegenerative diseases such as PD: drug delivery, gene delivery, protein fibrillation, growth factor delivery and cell transplantation. We find ourselves in a situation where delivery is a critical issue to be resolved, especially in the cases where the key drug/cell/molecule is known but present delivery methods render the effects transient, variable, inefficacious or exacerbates side effects. In summary polymer therapeutics may: 1) assist drug delivery to the brain and control release, 2) provide a cheaper/more scalable alternative to viral gene vectors, 3) offer an interesting means of studying protein fibrillation, 4) allow sustained growth factor release, and finally 5) allow better survival of cells that are transplanted to the brain. Presently, the use of polymers in these areas lags behind their non-polymeric counterpart (e.g. oral L-DOPA therapy, adenoviral gene delivery, or fetal cell transplantation), but shows interesting early signs of the improvements that could be made if such technology is embraced and developed further. Although clinical translation is not yet a reality for most of the technologies covered in this review, and some, such as anti-fibrillation polymers for understanding the aggregation and cytotoxicity of aggregated proteins, may remain as powerful research tools, others, such as microcarriers designed to improve cell implantation in the brain, may become a clinical reality in the not too distant future due to the conceptual simplicity and range of regulatory approved cell adherent polymers.

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Delivery System	Drug/ Administration Method	Animal/ Model	Summary	Year/ Reference
Chitosan nanoparticles	Dopamine intraperitoneal	Healthy rat	Acute administration of dopamine loaded nanoparticles induced a dose-dependent rise in striatal DA output	(2011) [178]
PLGA microspheres	Levodopa methyl ester and benserazide subcutaneous	6-OHDA rat	Loaded microspheres improved stepping of the lesioned forepaw and decreased apomorphine-induced turns	(2011) [183]
PLGA microspheres	L-dopa-α-lipoic acid (LD-LA) subcutaneous	Healthy rat	Microsphere delivery provided sustained levels of striatal dopamine for up to 4 days after a single administration	(2011) [184]
PLGA microspheres	Levodopa methyl ester and benserazide subcutaneous	6-OHDA rats with levodopa induced dyskinesias	Levodopa/benserazide delivered by microspheres resulted in lower abnormal involuntary movement (AIM) scores than the administration of the free drug	(2011) [185]
Odorranalectin-conjugated PEG-PLGA nanoparticles	Urocortin peptide intranasal	6-OHDA rat	Odorranalectin modification of the nanoparticles increased brain uptake and therapeutic effect of the Urocortin peptide	(2011) [104]
Lactoferrin conjugated PEG-PLGA nanoparticles	Urocortin peptide intravenous	6-OHDA rat	Urocortin loaded nanoparticles improved behavioral function and striatal innervation	(2011) [194]
PLGA microspheres	Rotigotine intramuscular	6-OHDA rat –subgroup with dyskinesias	The combination of microsphere and pulsatile L-DOPA delivery produced no better therapeutic benefit than mono L-DOPA administration but significantly decreased dyskinesia	(2012) [195]

PLGA microspheres	Rasagiline mesylate (RM) intraperitoneal	Rotenone rat model	RM administered via microspheres resulted in no additional therapeutic than RM in saline, but allowed administration every two weeks	(2012) [196]
PLGA microspheres	Rotigotine intramuscular	Cynomolgus monkey	The safety profile of Rotigotine loaded microspheres was analyzed and deemed safe prior to clinical trials	(2013) [197]

Table 1 - Recent progress in polymer assisted drug delivery for PD

Gene Vector Material	**Functional-ization**	**Gene Delivered/ Administration Method**	**Animal Model**	**Summary**	**Year/ Reference**
PLL	Neurotensin	GDNF intrastriatal	6-OHDA rat	Transfection in the substantia nigra 1 week after 6-OHDA produced biochemical, anatomical, and functional recovery	(2006) [208]
PEG substituted lysine 30-mer peptides	-	GDNF intrastriatal	Healthy rat	Striatal transgene expression lasted up to 8 weeks, at levels at least 100-fold greater than intracerebral injections of naked DNA plasmids	(2009) [209]
PAMAM	Lactoferrin	GDNF intravenous	6-OHDA rat	Multiple injections improved locomotor activity and reduced dopaminergic neuronal loss	(2009) [119]
PAMAM	Lactoferrin	GDNF intravenous	Rotenone rat model	Above mentioned effects also observed in the rotenone PD model	(2010) [217]
PEG substituted lysine 30-mer peptides	-	GDNF intrastriatal	Healthy rat and 6-OHDA rat	GDNF transgene activity observed up to 6 months after a single administration	(2011) [210]
PEG - PLL	Angiopep	GDNF intravenous	Rotenone rat model	Improved locomotor activity and recovery of dopaminergic neurons	(2013) [214]
PEG - PLL	Rabies virus glycoprotein peptide	Anti caspase 3 RNAi intravenous	Rotenone rat model	Weekly administration reduced activated casapse-3 levels and improved locomotor activity and rescued dopaminergic neuronal loss	(2013) [118]

Table 2 - Significant studies using a therapeutic nucleic acid delivered to the rodent brain

Delivery System	**Growth Factor/ Administration Method**	**Model/ Animal**	**Summary**	**Year/ Reference**
PLGA microspheres	GDNF - intrastriatal	6-OHDA rat	Motor behavior restoration and higher fiber density in the GDNF treated striatum	(2009) [274]
PLGA microspheres	GDNF - intrastriatal	6-OHDA rat	Complete behavioral recovery (amphetamine induced rotation test) after 16 weeks of treatment	(2011) [275]
Polybutylcyanoacrylate nanoparticles coated with polysorbate-80	NGF - intraperitoneal	MPTP mouse	Decreased rigidity and increased locomotor activity compared to control mice receiving MPTP alone	(2008) [279]
Polybutylcyanoacrylate nanoparticles coated with polysorbate-80	NGF - intraperitoneal	MPTP mouse	Polysorbate-80 required for brain uptake. Maximum NGF concentration detected 45 mins post administraiton	(2009) [280]
PLGA microspheres	VEGF and GDNF - intrastriatal	6-OHDA rat	GDNF microspheres more successful in bringing functional recovery than VEGF microspheres. Little or no combined effect, suggesting GDNF is dominant.	(2013) [277]
PLGA nanospheres	VEGF and GDNF - intrastriatal	6-OHDA rat	Combined effect of VEGF and GDNF in neuron density in the striatum	(2014) [278]

Table 3 - Recent studies delivering growth factors to the Parkinsonian rodent brain

Cell Type Delivered	Material	Growth Factor	Animal Model	Summary	Year/ References
Ventral mesencephalic tissue/suspension	Polyethylene glycol (PEG)-substituted lysine 30-mers	Gene encoding GDNF	6-OHDA rat	Pretransfected tissue showed a 16-fold increase in surviving neurons, and the pretransfected striatum allowed a seven-fold increase in survival compared to saline treated controls.	(2009) [308]
Embryonic Stem Cell-Derived Neural Precursor Cells	Growth factor-reduced Matrigel (and others)	-	Healthy adult mouse	Matrigel promoted proliferation of grafted cells resulting in a larger graft volume and more dopaminergic neurons in the graft.	(2010) [309]
Multipotent mesenchymal stromal cells	PLL coated PLGA microspheres	NT3	6-OHDA rat	Microsphere cell delivery reduced amphetamine-induced rotational behavior and allowed protection/repair of the nigrostriatal pathway	(2011) [300]
Neural stem cells	Poly ε-caprolactone scaffolds	GDNF	Healthy rat	GDNF loaded scaffolds enhanced transplant survival, proliferation, migration, and neurite growth and suppressed inflammatory reactive astroglia.	(2012) [310]
Neural stem cells	Laminin-derived IKVAV motif functionalized self-assembling peptide $RADA_{16}$	-	Healthy adult rat with cortex biopsy	The hydrogel formed immediately *in situ* enhancing cell survival and reducing the surrounding of glial astrocytes	(2013) [311]
GDNF secreting mesenchymal stem cells	Star PEG crosslinked collagen hydrogel	Secreted GDNF	Healthy adult rat and 6-OHDA rat	The collagen hydrogel reduced the host response to the cells by reducing the recruitment of microglia and astrocytes to the graft site	(2013) [15]

Neural stem cells	Collagen hydrogel functionalized with laminin derived peptide (collagen-binding LG3 (CLG3) and histidine-tagged LP (HLP))	-	Healthy adult rat	The collagen hydrogel improved neural stem cells viability in the early stage after transplantation into the striatum	(2013) [307]

Table 4 - Cell transplantation studies using various biomaterial platforms with the aim of improving cell survival post transplantation to the brain.

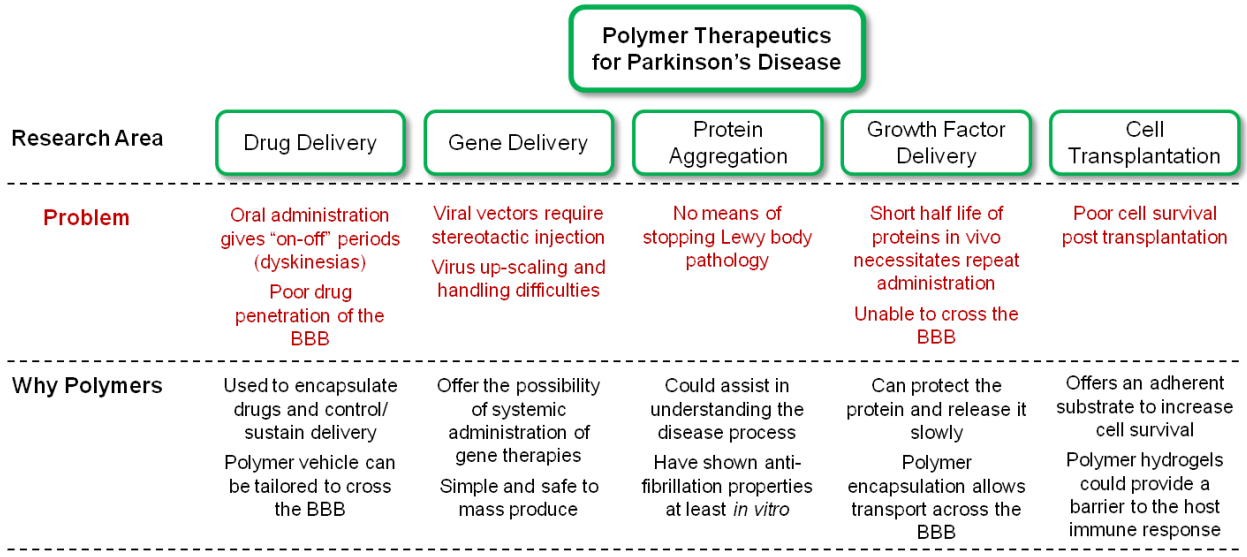

Figure 1 - Representation of how polymers may play a role in Parkinson's disease research

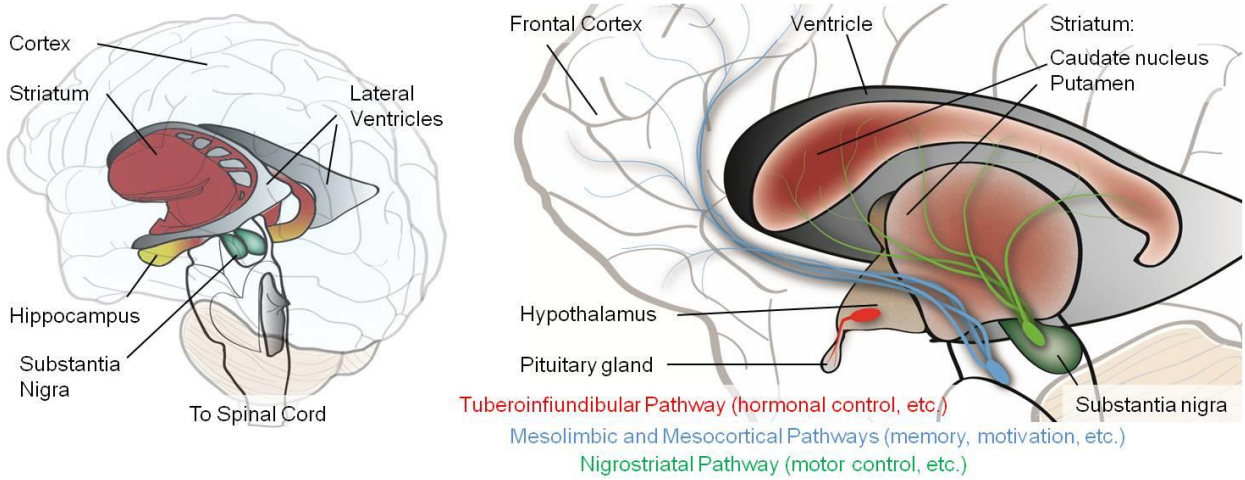

Figure 2 - Schematic representations of the human brain (left) with a zoomed cross-section (right), showing the nigrostriatal pathway (depicted in green) affected in Parkinson's disease. The dopaminergic neuron cell bodies lie in the substantia nigra and project into the striatum.

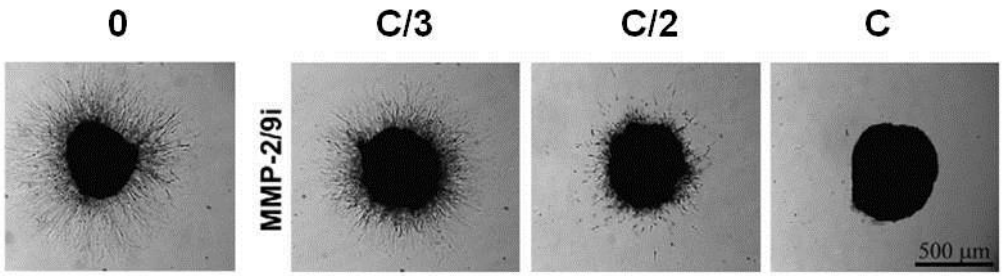

Figure 3 - DRG cell outgrowth within MMP cleavable PEGylated fibrinogen hydrogel. The left hand image shows the control (without MMP) allowing neurite outgrowth through the hydrogel, showing that the cell can manipulate its surrounding. However, MMP-2/9 inhibitor decreased the neuronal but not the nonneuronal outgrowth at the C/3 and C/2 concentrations and obstructed completely any outgrowth at the C concentration (day 2 of experiment); scale bar = 500µm. Image taken with permission from reference [61] and adapted to show one experimental group.

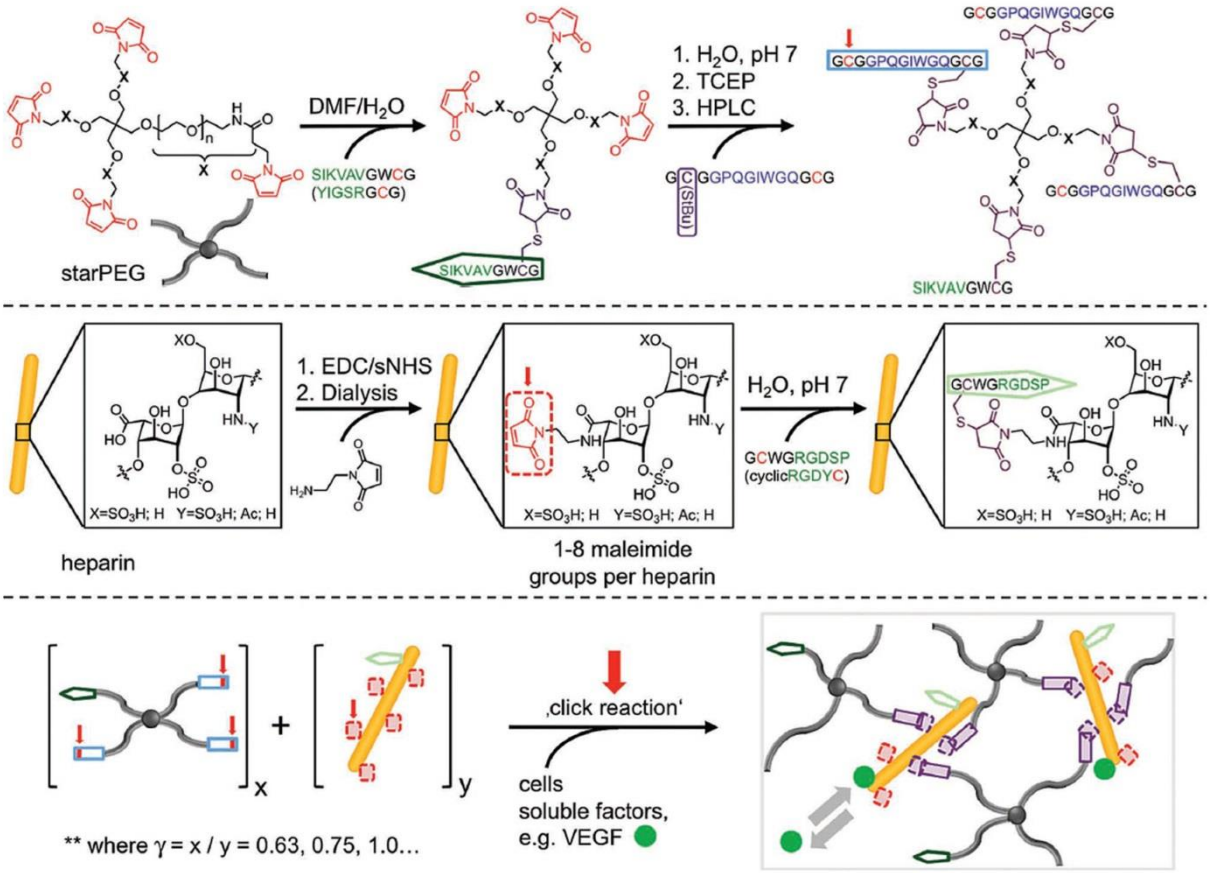

Figure 4 - Schematic diagram showing the route to in situ forming hydrogels formed from starPEG and heparin via Michael type addition. Cell adhesion peptides (green) can be added to mono-substitute the PEG via the maleimide group, while the remaining arms are substituted with a bifunctional MMP cleavable peptide sequence (blue) allowing gelation with maleimide modified heparin. Note that growth factors can also be added during the last step to functionalize the hydrogel. Reprinted with permission from [59].

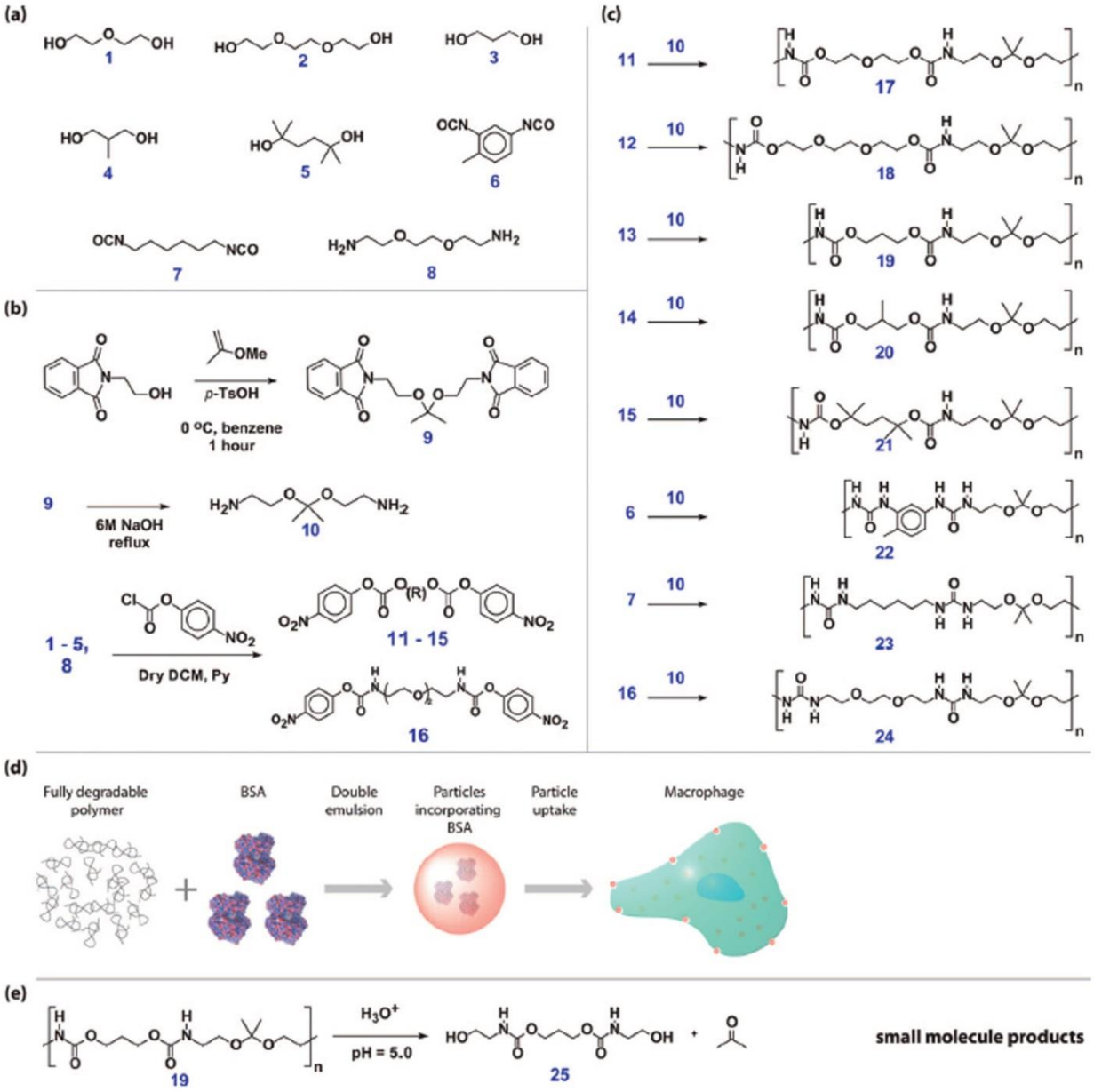

*Figure 5 - A library of monomers (a) can be used to make acid-degradable polymers. Part (b) shows the acid cleavable monomer **10**, and the activation of monomers. Part (c) shows the polymerization of activated monomers with the acid-cleavable monomer, to form fully degradable polymers (via mechanism shown in (e)) capable of protein delivery to cells etc (d). Reproduced with permission from [65]. Copyright (2008) American Chemical Society.*

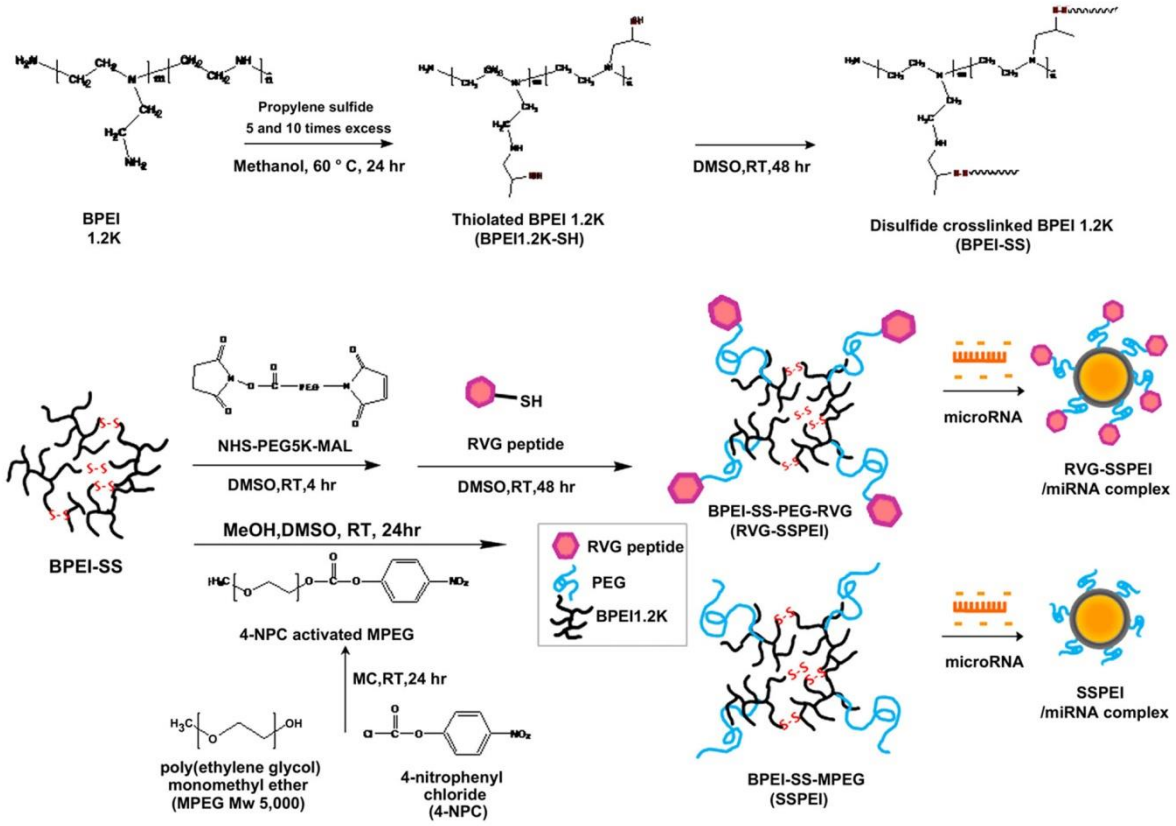

Figure 6 - Synthetic route to biodegradable branched PEI functionalized with the rabies virus glycoprotein for nucleic acid delivery to the brain. Reproduced with permission from [72].

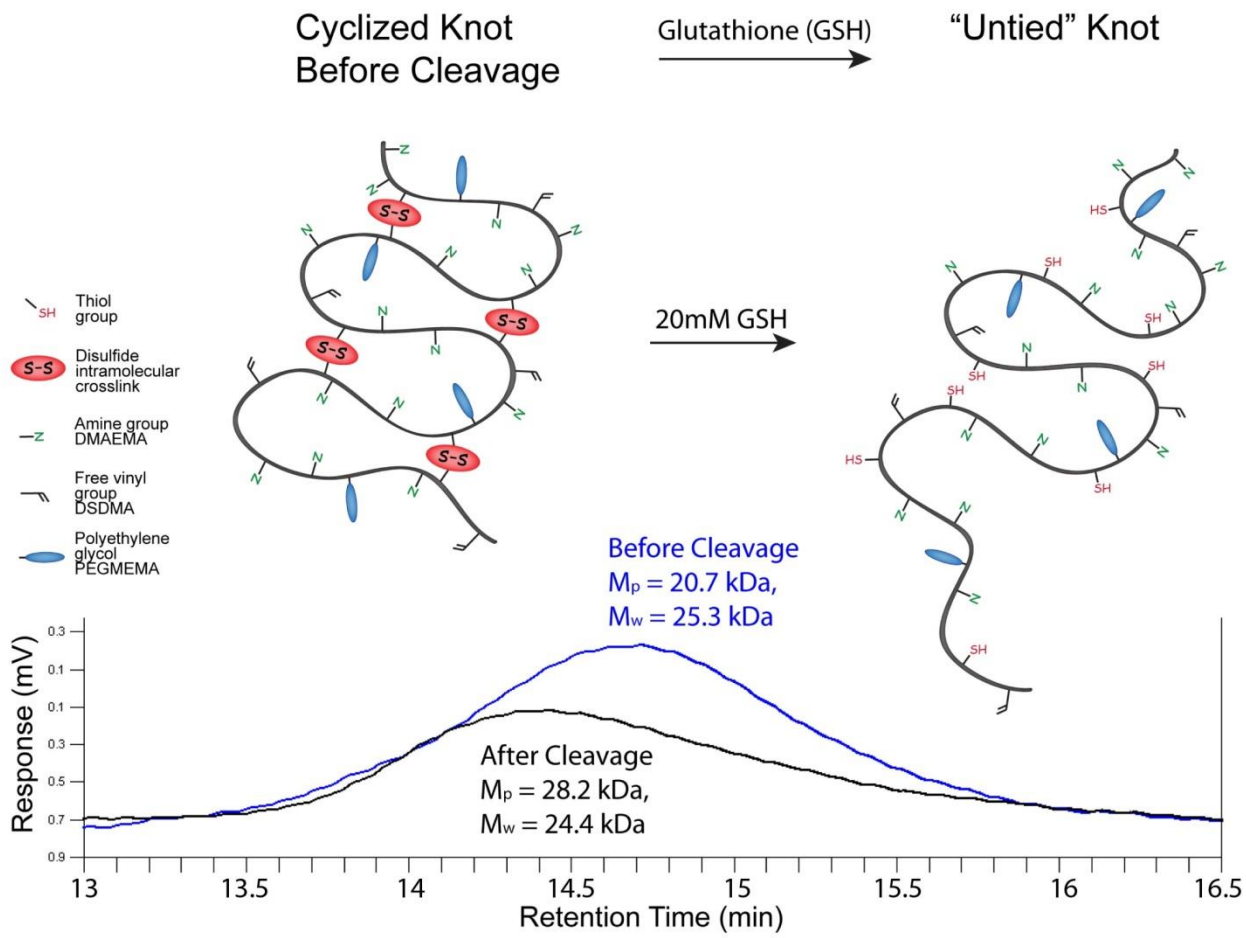

Figure 7 - Schematic depiction and gel permeation chromatography data of a disulfide linked knot structured polymer. Notice how cleavage of the disulfide bond does not affect the carbon-carbon bonded backbone, so no reduction in molecular weight is observed, only an increase due to increased hydrodynamic volume. Reproduced from [78] by permission of The Royal Society of Chemistry.

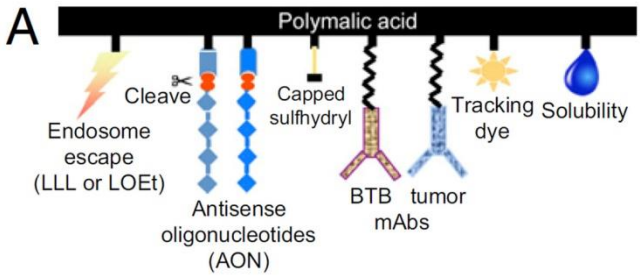

Figure 8 - Schematic representation of the complexities capable for molecule delivery via polymer therapeutics. Reproduced with permission from Proceedings of the National Academy of Sciences of the United States of America (ref. [99]; copyright 2010, National Academy of Sciences, USA).

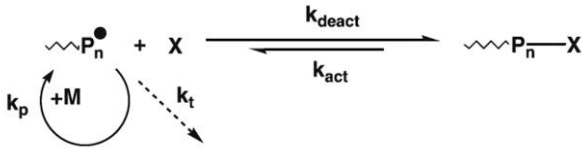

Figure 9 - Mechanism of reversible termination during controlled living radical polymerization, as shown in [135].

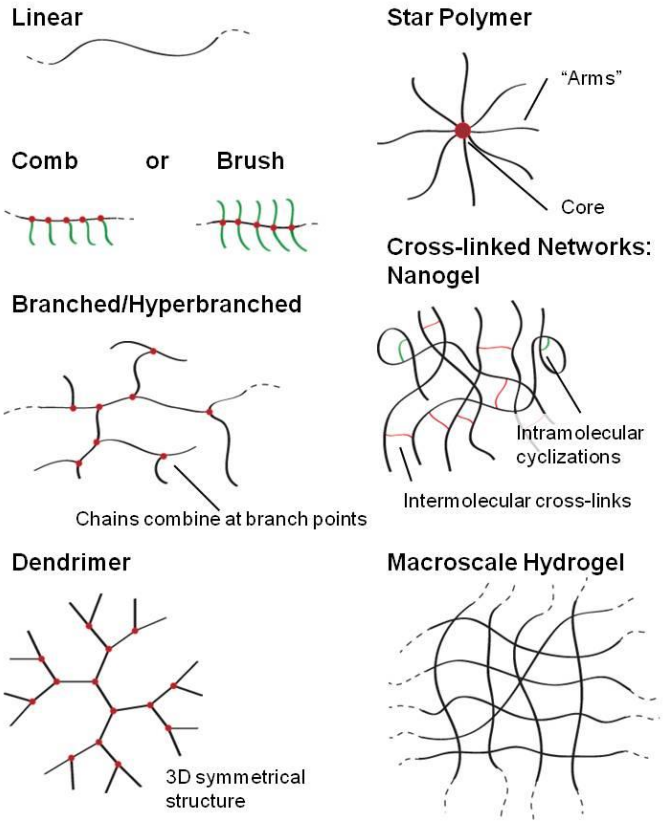

Figure 10 - Schematic depiction of the major polymer structures available for biomaterial synthesis, ranging from small molecules to macroscale crosslinked networks for hydrogel preparation.

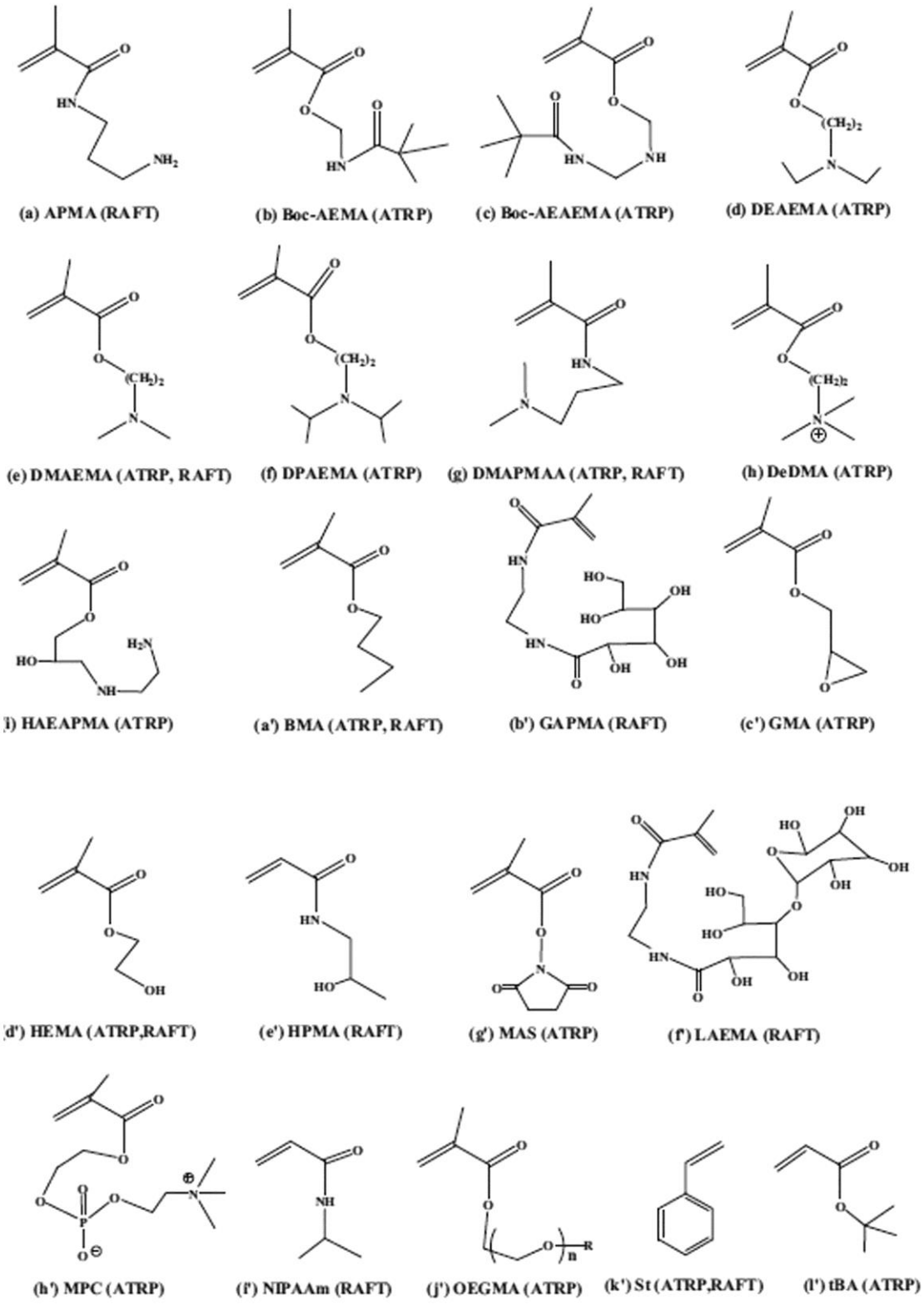

Figure 11 - Examples of vinyl monomers used for radical polymerization (type denoted in brackets), of nucleic acid vectors, taken directly from [68] with permission.

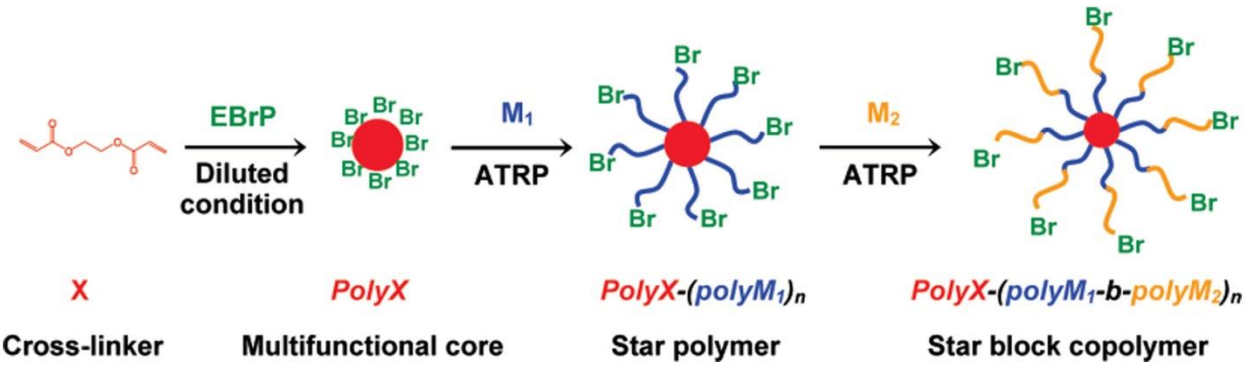

Figure 12 - The preparation of star and star block copolymers by the in situ generated core method, image Reprinted with permission from [149]. Copyright (2008) American Chemical Society.

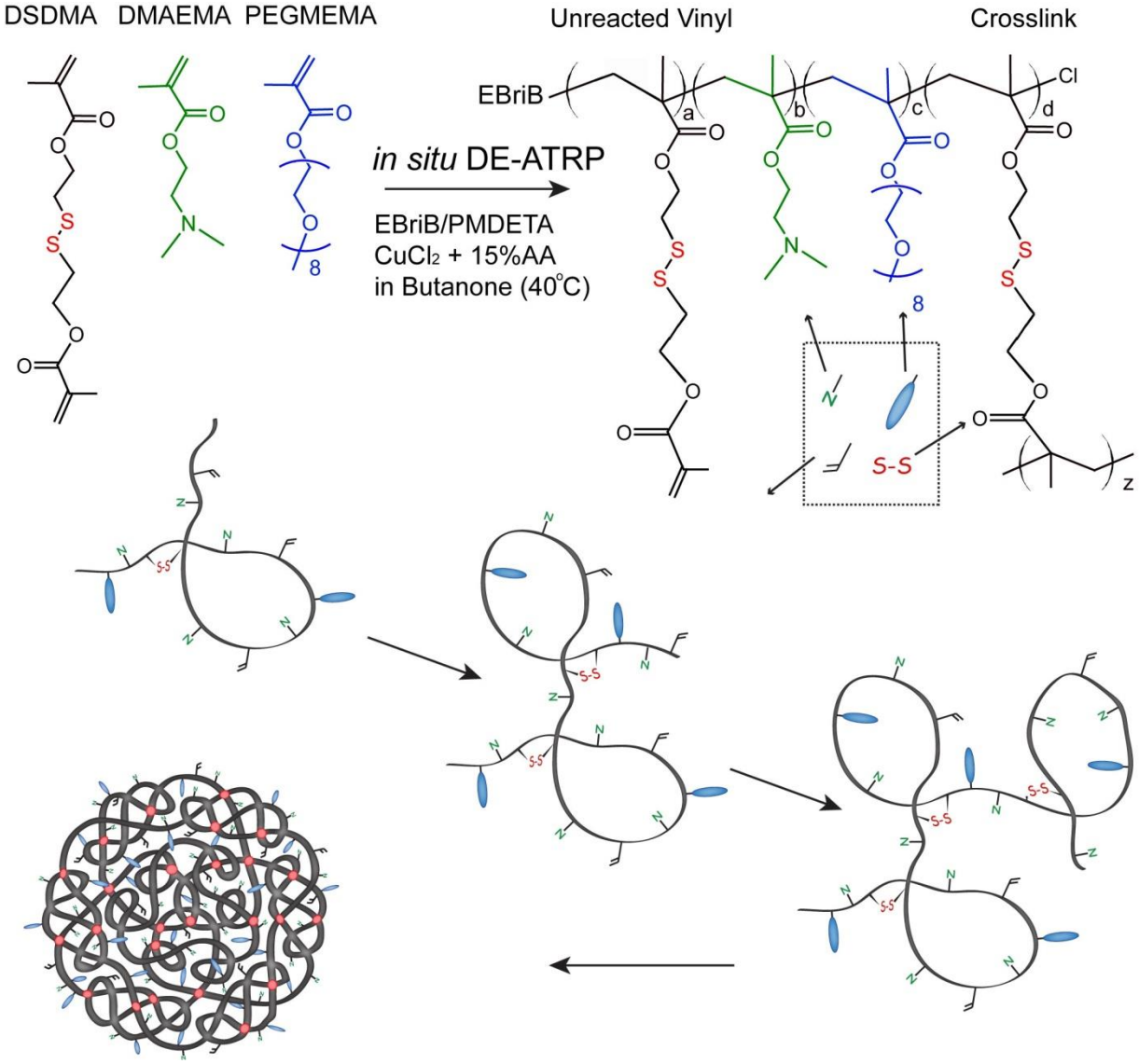

Figure 13 - Atom transfer radical polymerization is a versatile means to allow internal cyclization (intramolecular crosslinks) within the polymer structures developed for gene delivery, reproduced from [78] by permission of The Royal Society of Chemistry.

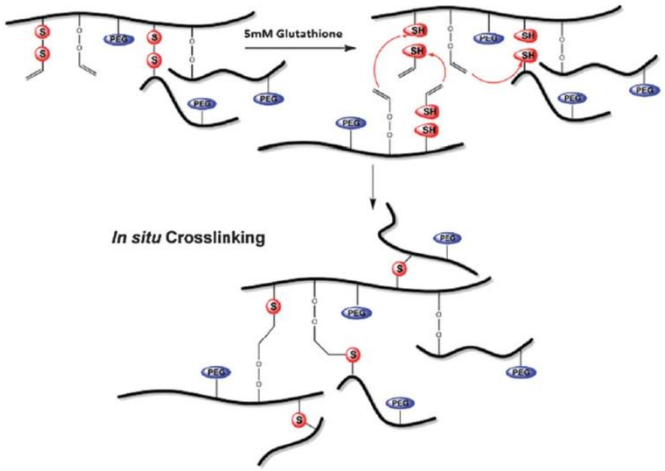

Figure 14 - Schematic representation of a glutathione sensitive polymer that undergoes crosslinking after the addition of cellular concentrations of glutathione. The disulphide bonds are cleaved, forming thiol groups which crosslink the polymer chains via free vinyl groups throughout the polymer structure. Reproduced from [157] by permission of The Royal Society of Chemistry.

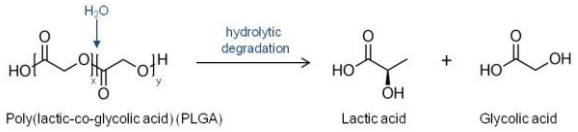

Figure 15 - Chemical structure of PLGA and its subsequent break down products

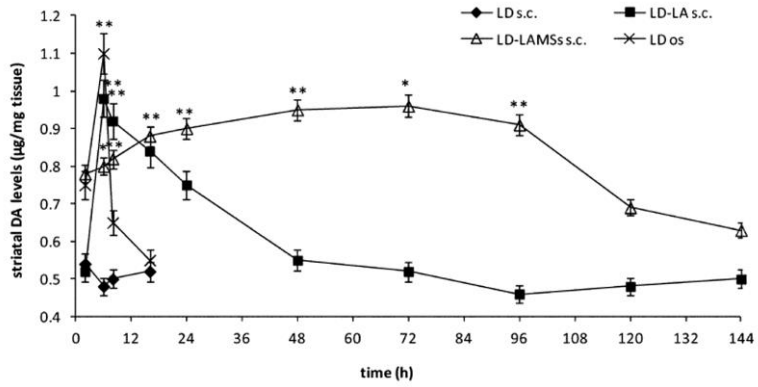

Figure 16 - A graph showing how delivery of L-DOPA via PLGA microspheres allow sustained levels of dopamine in the rat striatum. L-DOPA administered subcutaneously (LD s.c.) or orally (LD os) gave short lived dopamine levels. L-dopa-a-lipoic acid (LD-LA s.c.) gave better duration of dopamine, but when loaded to microspheres (LD-LAMs s.c.) striatal dopamine was elevated for over four days. Graph reproduced with permission from [184]. Copyright (2011) American Chemical Society.

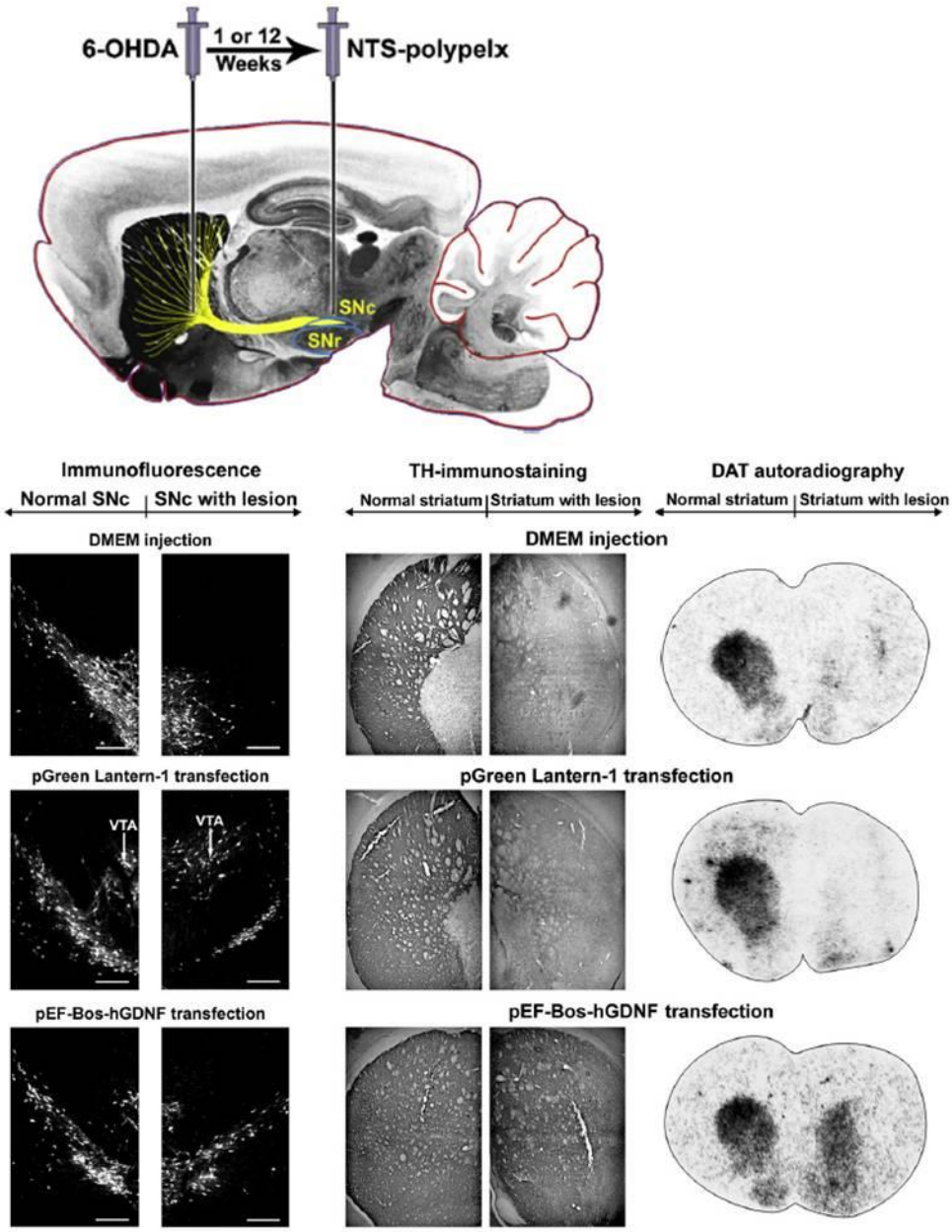

Figure 17 - Neurotensin modified polyplexes for the delivery of the GDNF encoding gene . The upper panel shows the site of the lesion and vector injection. The lower part shows the re-innervation of the substantia nigra (SNc) (left) and striatum (middle/right) following GDNF gene therapy. Figures reproduced with permission from [207].

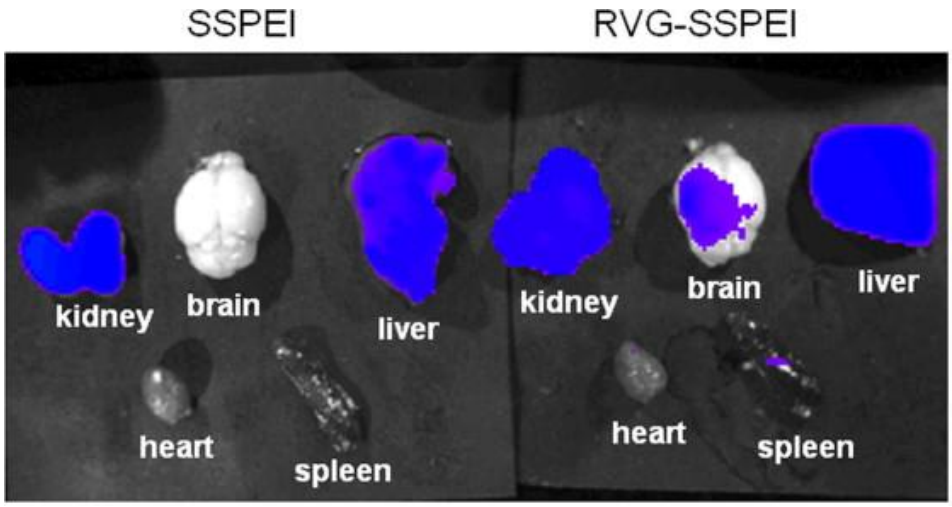

Figure 18 - Image taken with permission from [73], to highlight that although achieving high transfection capability in the brain is an important goal, off-target gene expression is a serious hurdle to overcome. Blue areas represent tissue of high transgene activity, and non-targeted gene administration (left hand side) shows no transgene activity in the brain. RVG targeted delivery shows transgene activity in the brain, but also large off-target delivery, especially in the kidney and liver.

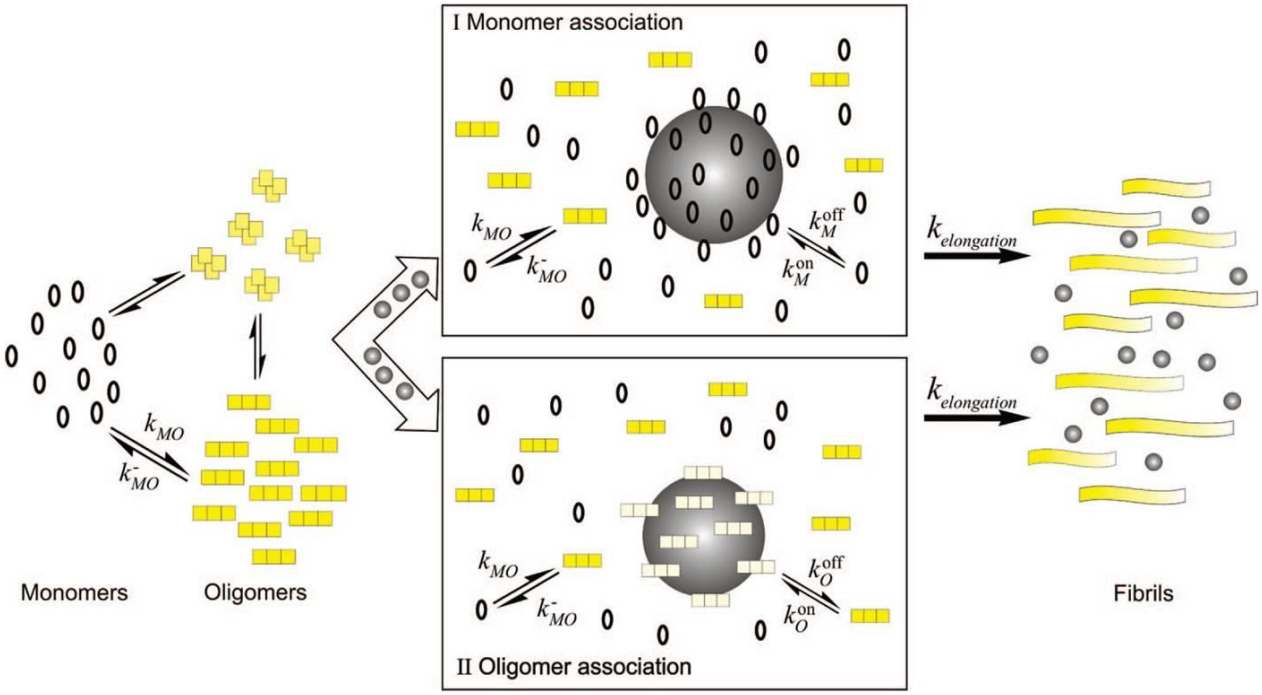

Figure 19 - Showing possible fibrillation mechanisms of amyloid β (monomers shown as open circles, oligomers as yellow squares), and the interaction with polymer nanoparticles (grey). Reprinted with permission from [234]. Copyright (2008) American Chemical Society.

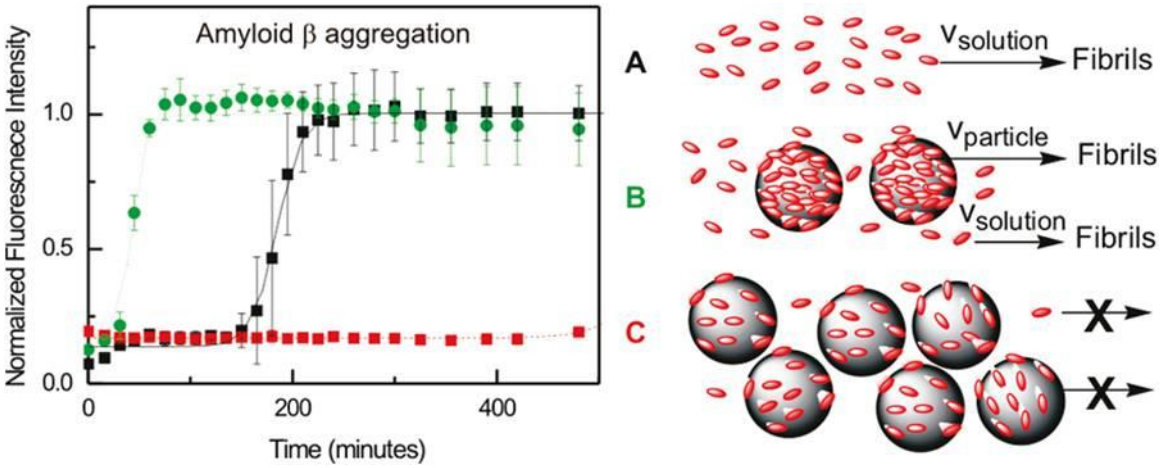

Figure 20 - Inhibition of the fibrillation of amyloid β depends on the concentration of the polmer nanoparticles. Low concentrations (green data) speed up the fibrillation process, whereas high concentrations stopped fibril formation. Reprinted with permission from [236]. Copyright (2010) American Chemical Society.

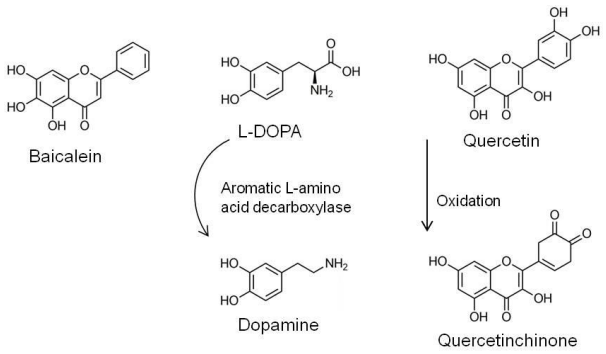

Figure 21 - Chemical structures of molecules used to inhibit α-synuclein aggregation in vitro*, including the flavanoids baicalein, quercetin (and its oxidized form - quercetinchinone).*

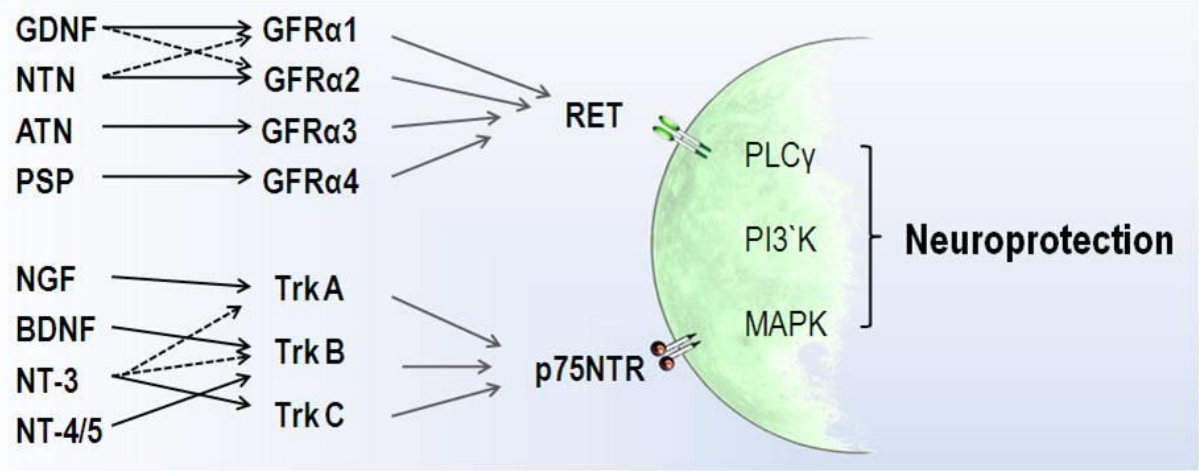

Figure 22 – The action of trophic factors on cell surface receptor complexes. The action of GDNF, neurturin (NTN) and neurotrphin-3 (NT-3) may act on more than one receptor (dashed arrow) though the major interaction is indicated by the solid arrow. The cellular signalling pathways activated cause a variety of outcomes including Ca2+ release, neurite outgrowth and neuron branching, but for PD only the effect of neuroprotection is indicated (inspiration taken from reference [253]). N.B. BDNF = brain derived neurotrophic factor, ATN = artenin, PSP =persephin.

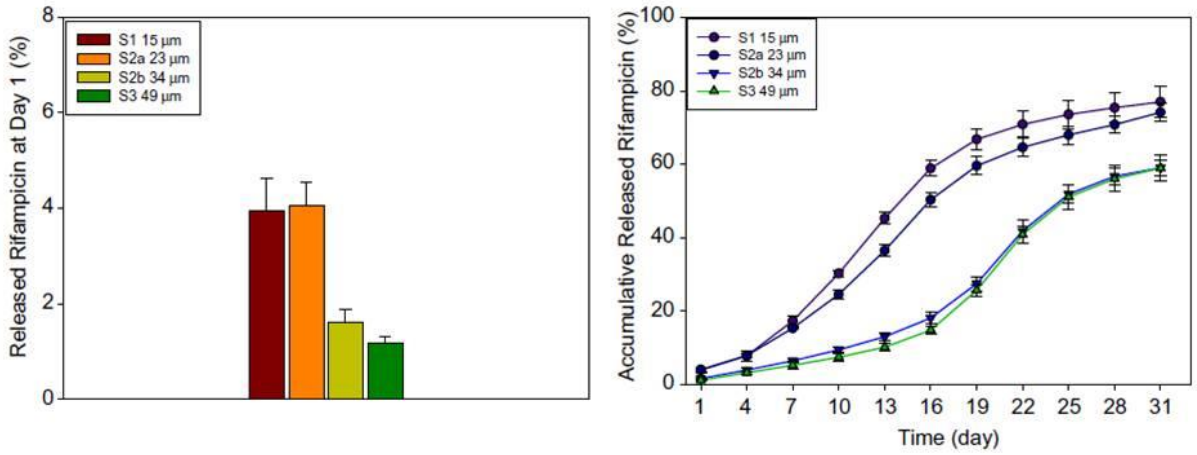

Figure 23 - Drug release (rifampicin) from microspheres of varying diameter, featured here to show the near zero-order release of drug possible from PLGA based microspheres. The left hand panel shows no more than 5% of the drug is released after the first day, and the right panel shows the length of time release is achieved. Selected from [285], with reprint permission.

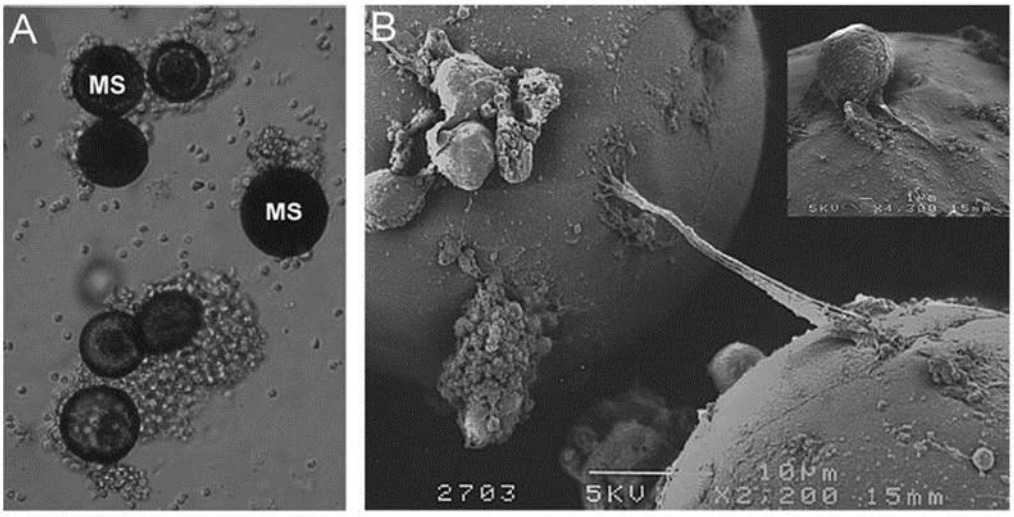

Figure 24 - Light microscope image (A) and scanning electron microscope image (B) showing fetal ventral mesencephalic cells growing on pharmacologically active microcarriers with neurite projections. Images used with permission from [301].

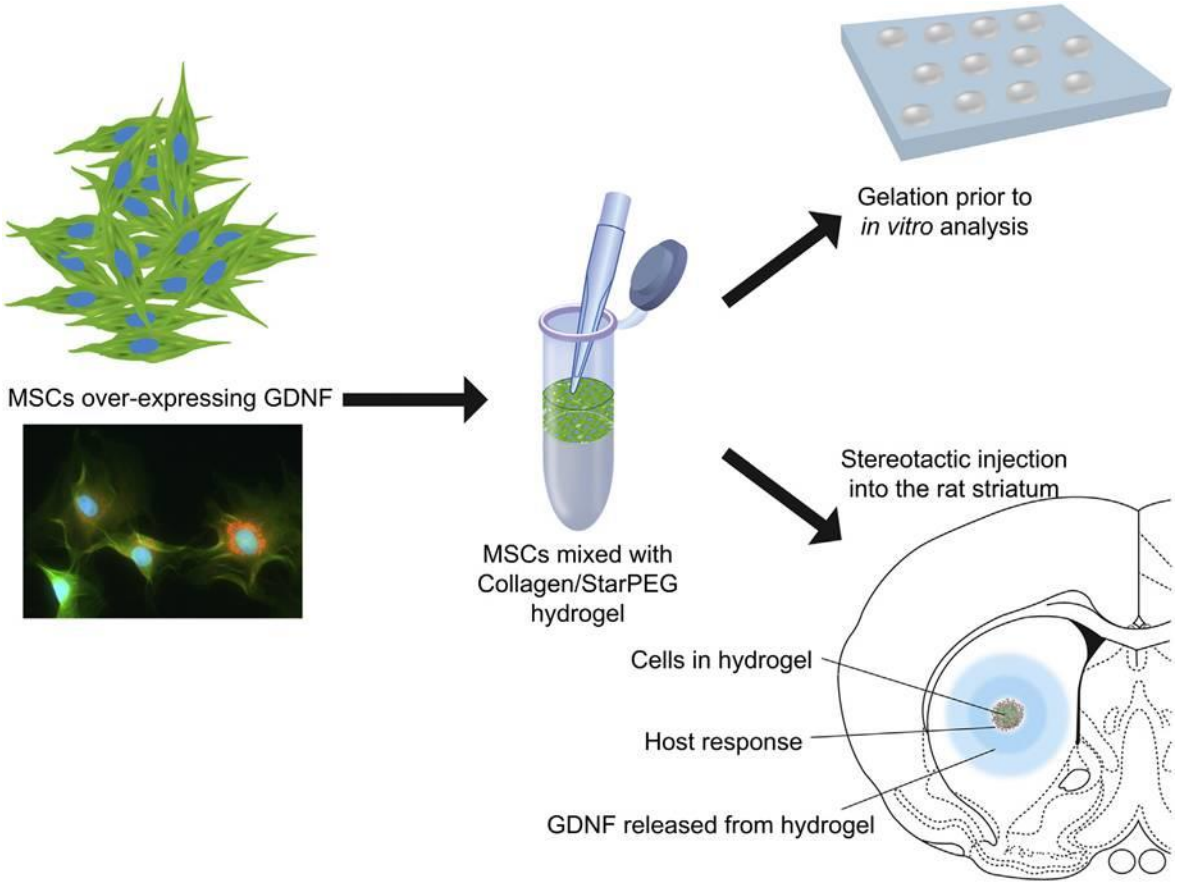

Figure 25 - Schematic presentation of the process of forming cell loaded, in situ forming hydrogels in preparation for in vitro *or* in vivo *analysis. Reproduced with permission from [15].*

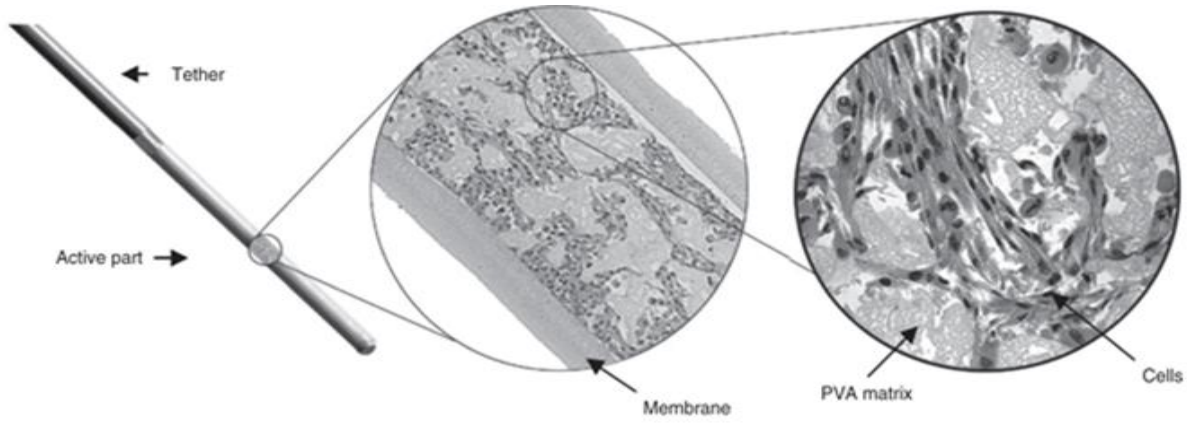

Figure 26 – Representation of a polymer device encapsulating cells for the delivery of therapeutics to the brain. It must be implanted by sterotactic surgery but has the advantage of device removal if required. Reproduced with permission from [326].