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Phosphorous-Containing Polymers for Regenerative Medicine

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Abstract

Disease and injury have resulted in a large, unmet need for functional tissue replacements. Polymeric scaffolds can be used to deliver cells and bioactive signals to address this need for regenerating damaged tissue. Phosphorous-containing polymers have been implemented to improve and accelerate the formation of native tissue both by mimicking the native role of phosphorous groups in the body and by attachment of other bioactive molecules. This manuscript reviews the synthesis, properties, and performance of phosphorous-containing polymers that can be useful in regenerative medicine applications.

Keywords

phosphorous polymer; polyphosphoester; polyphosphazene; phosphorylcholine; regenerative medicine; tissue engineering

I. Introduction

Many tissues in the body are susceptible to damage from injury or disease and are not capable of regeneration, resulting in high demand for functional replacement tissues. The fields of regenerative medicine and tissue engineering hold promise in meeting these demands, by employing scaffolds in various combinations with cells and bioactive signals to return damaged tissue to its original form and function.[1] Ideally, these scaffolds should degrade at a rate similar to the growth rate of the regenerating tissues. While great strides have been made in developing biomaterials to help address clinical needs, much work still remains.[2,3]

Polymers are amongst the most popular materials used in regenerative medicine. Biological polymers are naturally-occurring molecules that can be harvested from plants and animals. These polymers are often used because of their favorable biological characteristics that can improve cellular interaction, but their inherent properties such as degradation and mechanical strength cover a limited range.[4] Synthetic polymers are chemically-synthesized molecules that can be easily manufactured with properties that fit a wide range of desired regenerative medicine applications but often require further modification to include biologically active domains to improve cellular interaction.[4]

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Phosphorous-containing groups have been incorporated into both biological and synthetic polymers in an attempt to mimic the role of phosphorous groups in native tissues and encourage tissue formation. For instance, a variety of phosphorylated proteins produced by cells are used to facilitate calcium binding and mineralization[5] while phosphocholine groups form a major component of cell membranes. Additionally, many phosphorous bonds are hydrolytically degradable, and phosphoester bonds can be catalytically broken by a number of proteins in the body, thereby making them promising candidates for use in regenerative scaffolds. Moreover, phosphorous groups can be easily modified to incorporate moieties that fit desired applications. This article initially examines the methods of synthesis of phosphorous-containing polymers and the inherent properties these polymers possess that could be useful for tissue regeneration. The focus then shifts to the evaluation of phosphorous-containing polymers for *in vitro* and *in vivo* regenerative medicine applications. Finally, a brief summary discusses the future implementation of phosphorous-containing polymers to regenerate native tissues.

II. Synthesis and Composition

a. Polymers with Pendant Phosphorous Groups

Phosphorous can be incorporated into polymers via pendant groups attached to the polymer backbone, or incorporated into the polymer backbone itself. Pendant phosphorous groups can be incorporated through a variety of methods. For example, phosphorous-containing monomers (Fig. 1) such as mono-methacryloxyethyl phosphate (mMEP),[6] mono-acryloxyethyl phosphate (mAEP),[6,7] 2-methacryloyloxyethyl phosphorylcholine (MPC),[8–10] and vinyl phosphonic acid (VPA)[11,12] can be homo- or co-polymerized with other vinyl monomers to form co-polymers with varying degrees of phosphorous incorporation. Additionally, the bis- form of two of the previously mentioned monomers, bis-methacryloxyethyl phosphate (bMEP) and bis-acryloxyethyl phosphate, have been used as cross-linking agents in hydrogels.[13] Furthermore, pendant phosphorylcholine has been attached to poly(lactic acid) (PLA) backbones by adding L-alpha-glycerophosphorylcholine to the ring opening polycondensation of lactide.[14]

Pendant phosphorous groups can also be incorporated by post-polymerization modification of existing pendant groups such as alcohols or acids via condensation reactions,[15,16] amines via Mannich-type reactions with phosphorous acid,[17] or oxidation of alcohols with phosphorous pentoxide or metaphosphate.[18–20] The bioactivity of polymers with a diverse array of mechanical and chemical properties has the potential to be improved by attaching pendant phosphorous groups.

b. Polymers with Backbone Phosphorous Groups

Due to the ease of phosphorous group modification, bioactive molecules can be readily attached to polymers with phosphorous groups for regenerative medicine applications. Mechanical and chemical properties, such as stiffness and degradation rate, can also be easily tuned by altering the non-phosphorous groups. Polyphosphoesters (PPEs) are biodegradable polymers with a backbone comprising alternating phosphates and R groups as depicted in Fig. 2A. PPEs can be synthesized via polycondensation reactions with a variety

of alcohols[21–24] or ring opening polymerizations of cyclic phosphoesters.[25,26] PPE co-polymers can be synthesized with lactide in condensation reactions with dichlorophosphates.[27] Phosphate groups that are diols can be used to form PPE urethanes.[28] The properties of PPEs are highly dependent on the R groups between the phosphates and the pendant groups of the polymer backbone, giving two separate ways to tune PPE properties to fit the desired application. Functional pendant groups such as acrylates,[26,29] alkynes,[30] and amines[31] can be present during polymerization and later chemically modified to allow for chemical cross-linking of the polymer or attachment of groups to enhance polymer performance. To improve the biological function of some PPEs, cell-adhesive peptides, such as the GRGDS peptide, can be attached to the polymer backbone following polymerization via reaction with free P-OH groups.[23] Thermoresponsive polymers can be useful in regenerative medicine applications because they have the potential to deliver cells and growth factors to form scaffolds *in situ*.[32] Thermoresponsive PPEs have been synthesized by ring opening co-polymerization of cyclic phosphoester monomers with differing pendant groups. The transition temperature can be tuned by varying the relative monomer feeds.[25,33]

Polyphosphazenes (PPZs) are biodegradable polymers with an inorganic backbone of alternating nitrogen and phosphorous atoms, linked by alternating single and double bonds, and two pendant groups attached to each phosphorous atom. PPZ synthesis is usually performed by first synthesizing poly(dichlorophosphazene) via ring opening polymerization of hexachlorocyclotriphosphazene, followed by attaching pendant groups via condensation reactions, giving the final product illustrated in Fig. 2B.[34]A variety of functional groups can be added in this manner, such as amino acid esters,[35,36] adhesive peptides,[37] choline groups,[38] or groups that impart thermoresponsiveness.[37] Furthermore, pendant thiol groups have been attached to PPZs, yielding injectable liquids that cross-link to form gels at physiologic temperature.[39] As with PPEs, the properties of PPZs are highly dependent on the pendant groups attached to the polymer backbone, and groups can be selected to fit the desired application.

III. Physical Properties

a. Mechanical Properties

Scaffolds used for regenerative medicine applications should provide mechanical support until enough native extracellular matrix is deposited to sustain the generated tissue. The mechanical support necessary is heavily dependent on the load placed on the tissue. Scaffolds made from phosphorous-containing polymers exhibit a wide range of mechanical properties that are highly dependent on the type of phosphorous groups within the polymer as well as the groups attached to or incorporated into the polymer. Compressive moduli have been reported to range from 20 KPa in hydrogels[13] to over 700 MPa in cross-linked PPEs.[40] This broad range of mechanical properties allows phosphorous-containing polymers to be used in both hard and soft tissue applications.

b. Degradation

It is important for polymers used in regenerative medicine applications to undergo biodegradation so that they can be replaced with native tissue. Many polymers with backbone phosphorous groups are biodegradable, making them a promising platform for attaching molecules that fit the desired purpose. PPEs can readily undergo hydrolytic degradation that can be further accelerated via catalytic degradation by alkaline phosphatase (ALP).[28,29,41,42] These degradation mechanisms also apply to polymer chains cross-linked via phosphoester bonds if the individual polymer chains are soluble under physiologic conditions. Many polymers with pendant phosphate groups can readily have these phosphate groups cleaved by the above-mentioned mechanisms, leaving behind the un- phosphorylated polymer. However, the degradation of remaining polymer is dependent upon its composition. PPZ backbones can undergo hydrolytic degradation, but the rate is strongly influenced by the pendant groups, accelerating with increasing pendant hydrophilicity.[36,43,44] Consequently, PPZ degradation rate can be tuned to match the rate of tissue regeneration.

c. Calcium Binding

Binding of calcium ions helps to catalyze the deposition of hydroxyapatite, one of the primary extracellular components of bone. Consequently, phosphorous-containing molecules are often used in bone tissue engineering to facilitate binding of calcium ions and improve bone formation. (Meth)acryloxyethyl phosphate has been shown to promote *in vitro* calcium binding in both its mono-[6,45] and bis-[13] forms. Similarly, improved *in vitro* calcium binding via attachment of pendant phosphate groups has been documented in natural[19,20,46] and other synthetic[16] polymers. Furthermore, vinyl phosphonic acid has displayed improved calcium binding when incorporated into both natural[47] and synthetic polymers.[12]

Phosphorous incorporated into polymer backbones has demonstrated improved mineralization as well. Poly(ethylene glycol) (PEG)-based[42] and aminohexyl- propylene-based[31] PPE hydrogels have been shown to facilitate mineralization, and coating scaffolds with PPEs has increased calcium deposition as well.[48] The PPZs poly(ethyl oxybenzoate) phosphazene and poly(propyl oxybenzoate) phosphazene have been reported to facilitate calcium deposition *in vitro*.[49] Likewise, incorporation of PPZs into blends with other polymers to form scaffolds improves calcification.[50,51] The ability of phosphorous to bind calcium ions when attached to a variety of R groups makes phosphorous-containing polymers promising materials for bone regeneration.

IV. *In Vitro* Studies

a. Protein Adsorption

In vitro studies can be used to elucidate biological properties of scaffolds such as protein adsorption and cellular interactions to help determine which materials are suitable for *in vivo* evaluation. Depending on the target tissue for regeneration, adsorption of bioactive proteins on polymeric scaffolds can have a variety of effects, ranging from favorable to destructive. Protein adsorption is highly dependent on the groups attached to the phosphorous molecules,

allowing for tuning to fit the desired application. Many phosphorous-containing polymers have been reported to adsorb proteins, which can improve their biological function.[7,11,15,52–55] However, in vascular applications, protein adsorption can lead to thrombus formation that can prevent blood flow. MPC incorporation in a variety of co- polymers has been reported to result in decreased protein adsorption and blood cell adherence.[14,56] Furthermore, the phosphorylcholine group of MPC is similar to the surface of phospholipid cellular membranes and has been reported to increase phospholipid adsorption in co-polymers.[57] Analogous results have been observed when phosphorylcholine groups were grafted onto surfaces of polymeric scaffolds after fabrication.[58] Consequently, phosphorylcholine-based polymers have been used to coat many clinically-used device surfaces to improve performance.[10] Particularly, the hemocompatibility of phosphorylcholine-based polymers make these materials promising candidates for use in vascular regeneration applications.

b. Cellular Surface Interaction

The ability of a polymer to stimulate native tissue production by positively interacting with cells is vital for regenerative medicine. Ideally, scaffolds used for tissue regeneration should induce differentiation of stem cells down the desired lineage and support the growth, function, and infiltration of the cells of the regenerated tissue. Specifically, *in vitro* studies can be used to evaluate cellular viability and phenotype as well as quantify deposition of extracellular matrix components that are necessary for tissue function.

Both pendant phosphorous groups as well as pendant R groups of phosphorous- containing polymers have been shown to improve function of a variety of cell types. For example, many polymers with pendant phosphate groups have been shown to improve function of osteoblast-like cells. Oligo(poly(ethylene glycol) fumarate)/bMEP gels have demonstrated improved attachment, proliferation, and ALP production of human fetal osteoblasts.[13] Phosphorylation of poly(vinyl alcohol) has been reported to increase adherence, proliferation, and osteoblastic differentiation of human osteoblast-like cells, as well as increase mineralization of the scaffold.[15] Similarly, the presence of VPA has exhibited improved osteoblast- like cell adhesion and proliferation both as a homo-polymer[11] and in co-polymers with acrylamide.[54,59] Likewise, phosphorylation of chitosan nanofibrous scaffolds has been shown to improve proliferation and osteogenic differentiation of human osteoblast-like cells.[52] Though pendant phosphate groups have primarily been used in scaffolds for bone regeneration, interactions of these groups with other cell types have also been investigated. For instance, grafting of mMEP onto poly(hydroxyethyl methacrylate) hydrogels has resulted in improved corneal limbal epithelial cell attachment and proliferation,[60] demonstrating an additional bioactive role that unmodified phosphate groups can play within the body.

Polymers with phosphorous in their backbone can be modified to fit a wide variety of applications, but bone regeneration has been most extensively studied. For instance, PPE/PLA block co-polymers have displayed increased osteoblast attachment, proliferation, osteoblastic expression, and mineral deposition with PPE incorporation.[48] PPZs have been functionalized with groups composed of bioactive amino acids (AAs) to form biodegradable

amino acid-containing PPZ (AA-PPZ) scaffolds that have been evaluated for bone regeneration applications.[61] Blending of AA-PPZs with poly(lactic-co-glycolic acid) (PLGA) has been reported to result in scaffolds with elastic moduli similar to that of trabecular bone that support rat osteoblast adhesion and proliferation, as well as enhance osteoblastic expression.[51,62] The polyester chains of these blends have been shown to degrade more quickly, which can impart scaffold porosity into the AA-PPZ scaffolds *in situ*, allowing for cellular infiltration.[63] Similarly, porous scaffolds composed of AA-PPZ/PLA blends have exhibited improved osteoblast growth and adhesion compared to PLA scaffolds.[64] Furthermore, AA-PPZs substituted with bulky AA groups have been used in combination with nano-hydroxyapatite to form porous sintered microparticle composite scaffolds with mechanical properties similar to trabecular bone. Osteoblasts seeded on these composites have displayed adhesion, proliferation, and osteoblastic expression similar to that of PLGA/hydroxyapatite composites.[65] In addition to the aforementioned prefabricated scaffolds for load- bearing bone regeneration, PPZs can be substituted with groups that facilitate *in situ* formation. AA-PPZs and nano-hydroxyapatite mixed with 0.5% phosphoric acid have been used to form injectable pastes that can harden *in situ* to form scaffolds with mechanical properties similar to those of trabecular bone. Moreover, partial substitution of phenylphenoxy groups on these AA-PPZs demonstrated improved osteoblastic expression of murine osteoblast-like cells.[66]

Further demonstrating their versatility, AA-PPZs have also been functionalized for use in soft tissue regeneration.[67] These PPZs were used to modify poly(ε-caprolactone) scaffolds, resulting in improved human mesenchymal stem cell (MSC) adhesion, infiltration and tendon differentiation.[68] Electrospun AA-PPZ scaffolds have also been investigated for vascular applications. Additionally, AA-PPZs functionalized with the ethyl ester of phenyl alanine and have been shown to promote growth of rat endothelial cell monolayers on tubular scaffolds.[69] Moreover, PPZs have been applied as a platform for attaching a range of bioactive molecules that can be used to target interactions with specific cell types, such as neuroblastoma cells.[70]

Finally, phosphorylcholine-containing polymers have frequently been used in vascular regeneration applications due to their hemocompatibility. For instance, drug-releasing phosphorylcholine-grafted polyurethane vascular stents have been investigated *in vitro* for the inhibition of rat smooth muscle proliferation and demonstrated impaired platelet deposition with phosphorylcholine incorporation.[71] The hemocompatibility of MPC has been further utilized in attempting to recreate the luminal side of complex bioartificial organs exposed to blood. For instance, a polysulfone/MPC blend has been used to make asymmetrical membrane surfaces, with one side used to promote renal tubule epithelial cells and the other used to promote hemocompatibility to facilitate surface renal tube generation.[72] Furthermore, phosphorylcholine incorporation has also shown promise for use in non-vascular applications. MPC co-polymers have been used as coatings for permeable hollow fiber membrane bioreactors and displayed improved hepatocyte adhesion, urea synthesis, and albumin synthesis when compared to similar co-polymers without phosphorylcholine.[73] The above examples illustrate the broad range of cell types phosphorous-containing polymers can stimulate, further highlighting their potential use in tissue regeneration.

c. Cellular Encapsulation

Cellular encapsulation within tissue engineering scaffolds can accelerate tissue regeneration, as tissue production can begin throughout the scaffold immediately upon implantation, rather than relying solely upon the migration of host cells into the scaffold from the periphery. A variety of phosphorous-containing polymers are capable of forming hydrogel scaffolds under the mild conditions necessary for encapsulated cell survival. For instance, in an effort to regenerate bone tissue, mMEP co-polymerized with PEG-diacrylate has been shown to improve encapsulated human MSC viability and mineral deposition.[7] Furthermore, PPEs functionalized with a PEG-acrylate were shown to support encapsulated goat MSCs for up to three weeks and facilitate scaffold mineralization.[31] Likewise, PEG-based PPEs have improved cell viability of both encapsulated murine osteoblasts[26] and human MSCs[21] when compared to cross-linked PEG gels without phosphorous, demonstrating their potential for use in bone regeneration. Analogous gels have also demonstrated increased osteogenic expression and mineralization of encapsulated goat MSCs in osteogenic media,[42] as well as improved chondrogenesis of encapsulated rabbit MSCs receiving chondrogenic growth factors.[74] Summarily, phosphorous-containing hydrogel scaffolds have the potential to be functionalized to deliver cells and to support and accelerate orthopedic tissue regeneration in non-load-bearing applications.

V. *In Vivo* Studies

a. Bone Regeneration

In vivo studies present opportunities to evaluate how the biological and physical properties of regenerative medicine scaffolds come together to help facilitate generation of native tissue within living organisms. Successful materials provide the necessary mechanical support, facilitate cellular infiltration and differentiation into the desired phenotype, and degrade as they stimulate the deposition of extracellular matrix to generate native tissue in their place. Furthermore, these scaffolds should not elicit a strong immune response, which could lead to unfavorable tissue formation.

Given the general success of calcium phosphate-based bone cements in regenerative medicine applications,[75] it is not surprising that many different phosphorous-containing polymers have also been investigated for similar uses. As mentioned in previous sections, many phosphorous-containing polymers have been found to enhance osteoblastic differentiation and proliferation, as well as to facilitate mineralization, making them promising candidates for use in bone regeneration. Moreover, regeneration of bone tissue often has the added difficulty of requiring that the scaffold maintain mechanical support under large loads while facilitating the growth of native tissue. A variety of phosphorous-containing polymer scaffolds have mechanical properties similar to trabecular bone, supporting their use in load-bearing applications.

By blending AA-PPZs with PLGA, scaffolds with similar mechanical properties to trabecular bone can be fabricated. These polymer blend scaffolds have been reported to improve osteoblast growth rates *in vitro* and biocompatibility *in vivo*, as indicated by decreased inflammation and fibrous capsule thickness in a rat subcutaneous implantation

model.[62] Similarly, other AA-PPZ scaffolds have demonstrated fibrous capsule thickness and vascular density around the implants similar to that of poly(ε-caprolactone)-based reference materials following 12 week of subcutaneous rat implantation according to International Organization for Standardization (ISO) protocols.[76] Furthermore, bioactive AA-PPZs have been blended with PLGA and investigated for use in scaffolds. The rapid degradation of the PLGA led to an interconnected porous AA-PPZ scaffold that facilitated cellular infiltration and collagen deposition with minimal immune response in rat subcutaneous implants.[77] AA-PPZ scaffolds have been further evaluated in a variety of long bone defects. For instance, AA-PPZ scaffolds implanted in rabbit metaphyseal distal femur defects demonstrated similar bone growth and tissue response to an FDA-approved PLGA fixation device for fractures.[78]

Moreover, non-load-bearing PPZ scaffolds have been used in bone regeneration. For example, AA-PPZs membranes that have been modified to have a sustained release of both antibiotic and anti-inflammatory agents promoted healing of sub- critical rabbit tibial and mandibular defects, with minimal inflammatory response.[79,80] Additionally, thermoresponsive PPZ polymers with adhesive peptides grafted onto the backbone have been utilized to encapsulate rabbit MSCs. In murine subcutaneous implantation models, these cell-laden hydrogels demonstrated increased mineralization and osteogenic markers as well as ectopic bone formation at four weeks.[37]

A variety of phosphorylated natural products have also been implemented for bone regeneration. These natural products are often selected for regenerative medicine applications because they are biocompatible and biodegradable. N- methylene phosphonic chitosan scaffolds improved *in vitro* expression of ALP and collagen type I, elicited minimal inflammatory response, and increased bone formation in a rat tibial defect.[81] Phosphorylated chitosan and phosphorylated chitin have been used to reinforce calcium phosphate cements and slow degradation rates in order to improve regeneration of rabbit radial and tibial defects.[82,83] Finally, phosphatidylserine incorporation with collagen and bioactive glass composites has displayed improved osteoblastic attachment and differentiation *in vitro* as well as improved *in vivo* bone formation when used in combination with MSCs in rat femoral defects.[84]

However, not all polymers evaluated for bone regeneration have had promising results. For instance, N-vinylpyrrolidone/mMEP co-polymers have displayed large amounts of hydroxyapatite deposition in rat cranial defects. However, there was minimal trabecular bone formation within the scaffold.[85] This study highlighted that calcification alone is not sufficient for bone regeneration, as without scaffold degradation, native tissue cannot replace synthetic polymer. Similarly, surface-phosphorylated hydroxyethyl methacrylate/ methyl methacrylate co-polymer scaffolds facilitated osteointegration and new bone formation at rabbit femoral diaphysis defect borders, but could not be replaced with native tissue as the scaffolds did not degrade.[18] Though it was not clear why, mAEP/ hydroxyethyl methacrylate co-polymers scaffolds showed no calcification in rat subcutaneous implants despite demonstrating *in vitro* calcification within the same time period.[86] This study highlights the importance of *in vivo* evaluation, as factors not present

during *in vitro* studies can lead to changes in physical and biological properties of scaffolds after implantation.

b. Vascular Regeneration

As in other regenerative applications, the promotion of native cell growth and inhibition of undesired cell growth is important for scaffolds used in vascular regeneration. Particularly, scaffolds are commonly designed to promote endothelial cell coverage and inhibit smooth muscle cell proliferation to facilitate native vessel formation. However, the most vital requirement of materials used for vascular regeneration is the prevention of extensive clot formation, which can occlude blood flow through the vessel. Consequently, phosphorylcholine's excellent hemocompatibility has led to its use in vascular regeneration platforms. For instance, polyurethane-MPC blends have been evaluated for small vessel vascular prostheses in rabbit carotid arteries and showed no thrombus or pseudointima formation eight weeks after implantation.[87] Furthermore, MPC/methacrylic acid co-polymers have been used to coat the lumen of small diameter rat aortic grafts of poly(ester urethane)urea leading to decreased platelet adhesion *in vitro* and *in vivo* along with improved growth of endothelial cells on the lumen of the graft.[88] Additionally, MPC/methacyloxyethyl butylurethane co-polymers blended with poly(ester urethane)urea demonstrated decreased smooth muscle cell proliferation and decreased platelet adhesion *in vitro* as well as improved patency with endothelial coverage and tissue integration when implanted in rat aortas.[89] The above examples illustrate how phosphorylcholine can be incorporated into a variety of polymer scaffolds to improve hemocompatibility and promote native cell growth in vascular regenerative medicine applications.

c. Other Regenerative Techniques

While phosphorous-containing polymers have been most commonly used in bone and vascular tissue regeneration, they have also been evaluated for use in a number of other tissues. In particular, MPC incorporation into biologic polymers has had promising results for corneal tissue regeneration, which requires promotion of nerves and endogenous epithelial cells while also inhibiting the growth of any cells that could introduce opacities to the cornea. MPC/recombinant human collagen co-polymers have been used to replace damaged rabbit corneas and were found to promote epithelial cell and nerve repopulation as well as resistance to neovascularization.[90] Similar corneal implants composed of MPC-cross-linked collagen were used in a guinea pig model and exhibited comparable regenerative results.[91] Likewise, MPC-based polymer coatings have been shown to inhibit fibroblast adhesion, decreasing unwanted fibrous tissue formation around subcutaneous silastic elastomer plates implanted in rats according to ISO protocols.[92] These studies help illustrate how MPC incorporation into polymers can improve biocompatibility of scaffolds for regenerative medicine.

Phosphorous-containing polymers have also been investigated for nerve regeneration applications such as nerve guide conduits, which can be used to provide an environment that can direct axonal growth to the appropriate location. High molecular weight PPE nerve guide conduits have been evaluated for use in a critical-size rat sciatic nerve defect model. These conduits demonstrated healing over 90% of the defects at three months.[93]

Additionally, PPE conduits that have been modified to release nerve growth factor further improved nerve regeneration in the same rat model.[94] The above examples show that like PPZs, PPEs can be tuned to fit a desired regenerative medicine application.

Another technique for tissue regeneration is polymer attachment directly to individual cells to direct cellular function. Recently, phosphatidylethanolamine-containing molecules attached to polymer backbones have been evaluated for interaction with hepatocytes. It was found that 1,2-dimyristoil-sn-glycerol-3-phosphatidylethanolamine could be used to anchor polymers to murine hepatocytes without affecting cellular function *in vivo* and *in vitro*.[95] The ability of the phosphorous-containing polymers to anchor onto hepatocytes has the potential to be utilized for cellular-based therapies in the future.

VI. Concluding Remarks

The ease with which phosphorous groups can be modified presents a platform for a broad range of bioactive molecules to be attached to phosphorous–containing polymers. Furthermore, phosphorous groups themselves can be attached to mimic their role in native tissues, such as phosphate groups facilitating mineralization and phosphatidylcholine-containing moieties functioning as inert cell membranes. Phosphorous incorporation into polymer scaffolds has been shown to improve interaction with a wide variety of cell types as well as to influence their attachment and function both *in vitro* and *in vivo*. In particular, the ability of phosphorous groups to bind calcium and proteins, their positive influence on osteoblastic cells, and their wide range of mechanical properties make phosphorous-containing polymers promising materials for bone regeneration. While extensive work has been done characterizing the cytotoxicity both *in vitro* and localized effects in small animal models of many phosphorous-containing polymers, a major challenge moving forward will be the implementation and evaluation of these materials in large animal models. Furthermore, additional evaluation of the short and long term cytotoxicity of both the materials and their degradation products will be necessary to move many of these regenerative medicine applications into the clinic. Additionally, the excellent hemocompatibility of phosphorylcholine groups lends itself for use in vascular regeneration as well as regeneration of complex tissues that have direct blood contact. Moreover, the success of phosphorylcholine-based polymer coatings on medical devices is promising, and translating this success towards the implementation of phosphorylcholine-based regenerative medicine applications in the clinic is a challenge moving forward. Furthermore, as a greater understanding of biological interactions with biomaterials is accumulated, more expansive investigation of easily modifiable phosphorous-containing polymers in other regenerative medicine applications is an important direction of future research. Further work in these areas has the potential to lead to clinically useful materials capable of restoring tissue function.

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List of Abbreviations

ALP	alkaline phosphatase
AA	amino acid
AA-PPZ	amino-acid-containing polyphosphazene
bMEP	bis-methacryloxyethyl phosphate
ISO	International Organization for Standardization
MSC	mesenchymal stem cell
MPC	2-methacryloyloxyethyl phosphorylcholine
mAEP	mono-acryloxyethyl phosphate
mMEP	mono-methacryloxyethyl phosphate
PEG	poly(ethylene glycol)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PPZ	polyphosphazene
PPE	polyphosphoester
VPA	vinyl phosphonic acid

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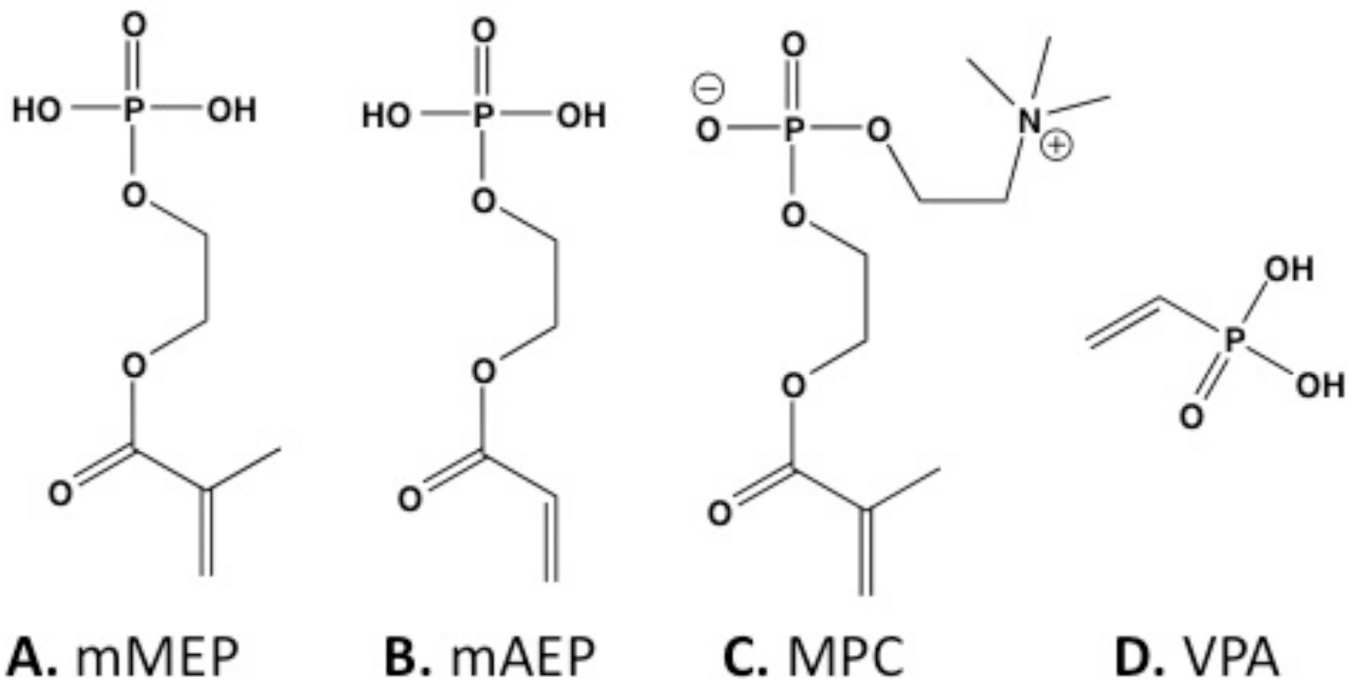

Figure 1.

Phosphorous-containing monomers commonly used to incorporate pendant phosphorous groups into polymer. **A**. mono-methacryloxyethyl phosphate (mMEP). **B**. mono-acryloxyethyl phosphate (mAEP). **C**. 2-methacryloyloxyethyl phosphorylcholine (MPC). **D**. vinyl phosphonic acid (VPA).

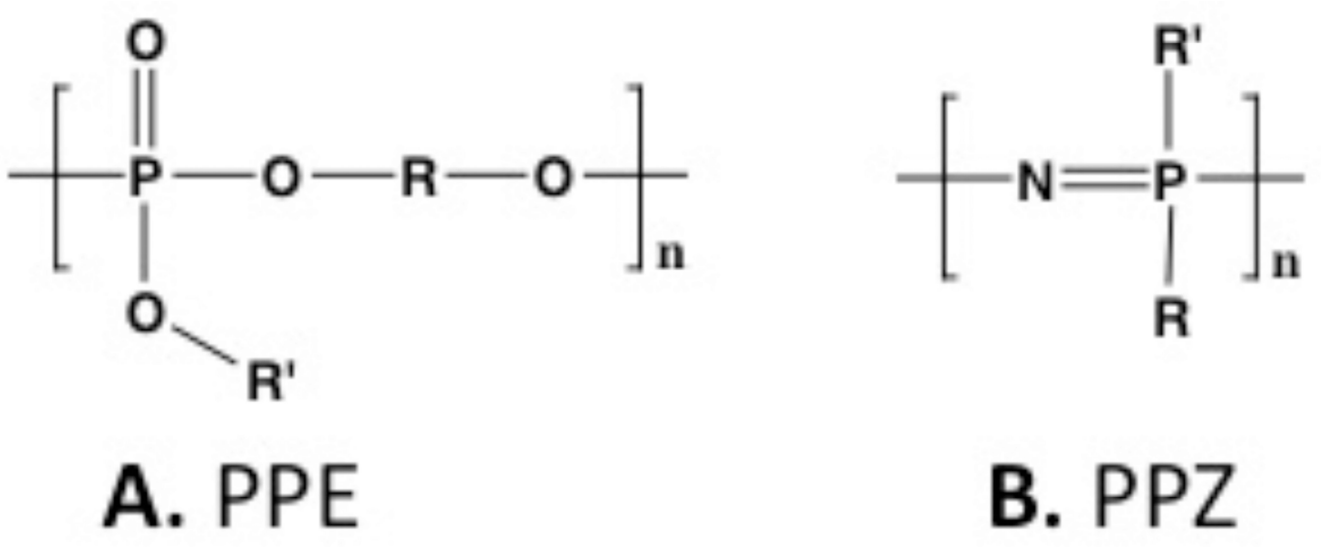

Figure 2.
General structure of **A**. polyphosphoester (PPE) and **B**. polyphosphazene (PPZ).