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Review of Materials for the Fabrication of Microparticles in the Context of Bone Tissue Engineering

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ABSTRACT

Bone tissue engineering (BTE) is a rapidly advancing field that seeks to repair or regenerate damaged or diseased bone tissue. Microparticles play an increasingly significant role in BTE by serving as drug delivery systems, cellular carriers, and scaffold components. This review aims to provide a comprehensive understanding of the variety of materials used in the fabrication of microparticles for bone tissue engineering applications. Natural polymers discussed include chitosan, collagen, gelatin, hydroxyapatite (HA), and silk fibroin, each offering unique biocompatibility and biochemical properties. Synthetic polymers such as ceramics, poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), and polycaprolactone (PCL) offer advantages in terms of mechanical stability and controlled degradation. The review also explores composite materials that combine the strengths of natural and synthetic polymers for enhanced biocompatibility, mechanical strength, and bioactivity. The functionalization and surface modification of these microparticles to meet specific requirements in bone tissue engineering are additionally covered. The objective is to guide researchers in selecting the most appropriate materials for specific applications within the realm of bone tissue engineering, considering factors such as biocompatibility, mechanical properties, and bioactivity.

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INTRODUCTION

Microparticles, spherical carrier scaffolds, have recently received extensive interest for their potential therapeutic applications in a diverse range of clinical and regenerative medical settings. Not only can these versatile subunits be used as cell culture scaffolds, their innate structure reduces the degradation of encapsulated biologically active molecules and also allows their exploitation as a localised injectable delivery system. Above all, the biodegradability of the scaffold is what differentiates it from permanent implants (Dvir et al., 2011). The complete biodegradability of the scaffolds prevents the need for additional surgical procedures to remove or replace the scaffold. The scaffold can be implanted directly into the injured site to initiate tissue regeneration in-vivo. Therefore, a variety of materials and diverse modification protocols and technologies are utilised in order to enhance the mechanical properties and biocompatibility of scaffolds fabricated from microparticles, with the aim of improving regenerative medicine.

Microparticles are made from synthetic or natural biomaterials to provide sustained and controlled delivery of therapeutic drug (Kulchar et al., 2021). Utilising biomaterials has several advantages for the proper growth and development of cells. In addition to serving as a physical attachment for cells, biomaterials also provide biochemical cues and activate the molecular signalling mechanisms. The choice of biomaterial for microparticle synthesis depends on the intended use, length of therapy, desired nature of the polymer (for example, hydrophobic or hydrophilic, neutral or charged), bioactive agents to be delivered (for drug delivery applications), and the chemistry needed for further functionalization and modification. Most recent microparticle research has concentrated on controlled drug release, site-specific drug delivery, and reducing drug toxicity and degradation (Sree Giri Prasad, 2014). Microparticles can be made from either natural or synthetic materials. Biopolymer-based biodegradable biomaterials can be divided into naturally occurring and synthetic options (Reddy et al., 2021). The synthetic polymer microparticles often have a positive surface charge in order to facilitate attachment of negatively charged cells, although this can make it more difficult to harvest the cells from the microparticles.

NATURAL POLYMERS

Chitosan, is a popular choice in the field of regenerative medicine as it is a biodegradable, naturally occurring polymer, has drawn considerable attention in recent years as a scaffolding material in tissue engineering and regenerative medicine. Chitosan is a cationic polysaccharide, which is constructed of β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit) (Bharathi et al., 2022). It has a similar structure to hyaluronic acid (Valachová & Šoltés, 2021). Hyaluronic acid, is one of a critical development, which is involved in the regulation of tissue responses during injury, repair, and regeneration (Dovedytis et al., 2020).

Chitosan is obtained from chitin by the deacetylation process (Antonino et al., 2017). The free amino groups in chitosan allow it to bind with negatively charged molecules. Chitosan's amine groups structure can be chemically modified to increase its solubility and biological activity (Zhang et al., 2010; Chen et al., 2022). The biocompatibility increases with degree deacetylation increase. The degradation rate of chitosan is primarily determined by its degree of deacetylation, but it is also affected by the distribution of N-acetyl D-glucosamine residues and its molecular mass (Cheung et al., 2015). Chitosan is particularly appealing as a bone scaffold material because it promotes osteoblast cell attachment and proliferation (Chua et al., 2008; Hesaraki & Nezafati, 2014; Oh et al., 2021; Sukul et al., 2021), as well as the formation of mineralized bone matrix (Hu et al., 2017). Positive chitosan charges can also bind to negative chitosan charges on red blood cells (RBC), allowing chitosan to be used as a haemostatic agent (Lestari et al., 2020). Chitosan can be transformed into a variety of physical forms, including fibres

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(Ranganathan et al., 2019; Salim et al., 2022), powders (Sukpaita et al., 2021), pastes (Maharjan et al., 2021), and even films (Prakash et al., 2020). Chitosan in combination with various biocomposite materials was extensively studies and has been shown to promote the differentiation and mineralization of osteoblasts by the up-regulation of genes that are responsible for controlling these processes, hence making it as a realistic materials for use in bone tissue engineering applications. Chitosan is especially attractive as a bone scaffold material because it supports the attachment and proliferation of osteoblast cells as well as formation of mineralized bone matrix (Levengood & Zhang, 2014). Its surface is hydrophilic, which promotes the adhesion, differentiation and proliferation of cells (Brun et al., 2021). It has been shown to promote the differentiation and mineralization of osteoblasts by the up-regulation of genes that are responsible for controlling these processes and so has promising applications in bone tissue engineering.

Hydrogels have been used widely for local delivery of drug in bone applications (Bae et al., 2011; Park et al., 2013). It has three dimensional, hydrophilic, cross-linked polymeric networks are a heterogeneous group of biomaterials and can be either naturally derived or synthetic. They are able to swell without disintegrating and can absorb up to several times their dry weight in water (Ahmed, 2015). Due to their high water content, hydrogel biomaterials can emulate the physical properties of soft tissues, making them highly suitable as scaffolds for tissue engineering (Lee & Mooney, 2001; Bryant et al., 2004). Examples of hydrogels include the combination of collagen, gelatin, and poly (ethylene glycol) which have the distinct advantage of being able to swell with water, making them very easy to load with drugs (Ahmad et al., 2022). In addition, modifications of hydrogels can be performed in order to impart desired characteristics, such as degradation or retention of bioactive factors. A novel fluvastatin-releasing hydrogel with fluvastatin covalently tethered via lactic acid linkages into a poly (ethylene glycol) hydrogel and released by degradation of lactic acid into the surrounding area (Shah et al., 2015). However, several disadvantages of hydrogel include non-biocompatible properties of hydrogel, batch to batch variation which causes differences in final formulation and generally suffer from weak mechanical properties, lack of a sustained supply of nutrients or simulants to maintain the cell functions, which severely limits their further practical applications (Zhu & Marchant, 2011; Wang et al., 2018). While Zorlutuna et al., (2018) suggests that microfluidic technologies can be used to produce shape-controlled hydrogels that may be relevant to tissue engineering (Zorlutuna et al., 2018).

Collagen is a natural protein that is found in the extracellular matrix of many tissues and provides structural and mechanical support to tissues (Parenteau-Bareil et al., 2010). It is biocompatible, has low antigenicity and is able to bind to cells (Li et al., 2021). Multiple variants of collagen, which is the predominant extracellular matrix (ECM) protein, contribute to significant tensile strength and structural integrity to connective tissues, tendons, and the skin (Kjær, 2004). When collagen is used as a biomaterial in vitro, it not only provides structural integrity but also regulates cell proliferation and differentiation. It has been shown to promote cell and tissue attachment as well as growth, and has also been combined with other materials such as the bioactive ceramic, b-tricalcium phosphate and an osteoinductive growth factor, bone morphogenetic protein (BMP-2) to enhance bone regeneration (Lee et al., 2021). Collagen powder could improve cell therapy (Rico-Llanos et al., 2021). In a rabbit alveolar cleft model, collagen powder scaffolded human umbilical cord-derived mesenchymal stem cells (huMSCs). The authors reported significantly better bone repair than when implanting collagen without cells and, when focusing on certain parameters, even when implanting collagen with BMP-2. In a separate literature, mesenchymal stem cells (MSCs) showed increased osteogenic potential when cultured in collagen-infused polymer scaffolds (Kundu & Putnam, 2006).

Gelatin is a molecule derived from collagen by irreversible denaturation of collagen proteins. Gelatin and collagen share a very similar molecular structure and function, and are therefore frequently substituted for collagen in cell and tissue culture for biomaterial purposes (Rico-Llanos et al., 2021). It is a naturally occurring polymer, exhibits structural similarities to its precursor collagen, which is a highly

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prevalent constituent of the extracellular matrix. Gelatin has good biocompatibility, biodegradability and low toxicity, and has been incorporated into some scaffolding systems. Unlike hydrogels which is a cross-linked polymers that can absorb significant amounts of water and permit solutes within their swollen matrices, and provide sustained delivery of the solutes they have absorbed, gelatin has hydration properties such as swelling and solubility; gelling behaviour such as gel formation, texturizing, thickening, and water-binding capacity; and surface behaviour like emulsion and foam formation, stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity. However, gelatin-based hydrogels have been shown to be useful for different applications with important and innovative characteristics especially in bone tissue reconstruction and drug delivery (Andreazza et al., 2023).

Hyaluronic acid (HA) may be synthetic or natural. Natural HA is derived from specific species of coral or bovine bone and may contain trace amounts of Mg, Na, CO3, and F. Synthetic HA is produced by sintered granules or blocks that are dense or microporous (Fiume et al., 2021). In vitro and in vivo evaluations of a composite bi-layer type-1 collagen-HA/Mg scaffold for osteochondral regeneration were conducted by Calabrese et al., 2017. In vitro and in vivo osteochondro differentiation was induced by the combination of this scaffold with mesenchymal stem cells (MSC) derived from adipose tissue (hADSCs) under specific differentiation conditions (Calabrese et al., 2017). Hayati et al., (2011) further discussed the incorporation of HA which significantly increased the physicochemical properties, osteoblasts bioactivity and osteogenesis ability of polymer scaffolds. For example, after the reinforcement of HAN with 10%, poly (3-hydroxybutyrate) (PHB) scaffolds at a porosity of 77% showed a 2 times improvement of compressive strength and modulus, when compared with pure polymer (Nemati Hayati et al., 2011). However, HA suffer some disadvantages. Firstly, the ionic crosslinking method can circumvent harsh chemical conditions and some cytotoxic cross-linkers. Furthermore, it has obvious limitations such as low stability and low mechanical properties (Park & Lee, 2011; Yan et al., 2014).

Silk fibroin (SF) is a naturally occurring biocompatible protein polymer that is derived from the cocoons of the silkworm Bombyx mori, is an FDA approved biomaterial and has been widely recognized for use in tissue engineering applications (Sun et al., 2021). SF exhibits versatility in its ability to undergo various processing techniques, resulting in a diverse range of forms and structures suitable for application as scaffolds in the context of bone regeneration (Sun et al., 2021; Youn et al., 2021). SF is widely reported to be biocompatible and biodegradable, and to have high cell adhesion moieties, low antigenicity, superior mechanical properties, and ease of handling. SF is frequently solubilized in an aqueous medium and can be readily reconstituted into diverse material structures, such as films, mats, hydrogels, and sponges, utilising a range of fabrication methods including spin coating, electrospinning, freeze drying, physical, and chemical crosslinking techniques (Sun et al., 2021). The outstanding mechanical properties of SF fibres include a high break strain (4–26%), ultimate strength (300–740 MPa), and toughness (70–78 MJ m2) (Koh et al., 2015). The utilisation of three-dimensional (3D) SF scaffolds has demonstrated potential in the field of bone-void-filling applications (Deshpande et al., 2022). Wang et al., (2021) have developed an injectable, functional, silk-based hydrogel to stimulate angiogenesis while Ajiteru et al. have conjugated graphene oxide (GO) to silk in order to create an electroconductive bio-ink (Wang et al., 2021). A study developed by Singh, et al., (2016) on hydrogels of mulberry and non-mulberry silk fibroin blended with agarose for cartilaginous tissue formation suggested that the blended hydrogels showed enhanced cellular proliferation and ECM production in comparison of pure agarose hydrogels in-vitro (Singh et al., 2016). Results from this study suggested the potential alternatives for cartilage repair using the silk fibroin blended with agarose. Study by Ghalei et al., (2022) reported the fabrication of nitric oxide (NO)-releasing inter penetrating network (IPN) hydrogels based on gelatin methacryloyl (GelMA) and silk fibroin (SF) scaffold using a two-step cross-linking/swelling technique which mainly aim to inhibit bacterial colonization (Ghalei et al., 2022). In this study, the incorporation of SF with a more hydrophobic and crystalline structure reinforces the GelMA scaffolds, rendering them more stable for potential clinical applications. It was also suggested that strong antibacterial responses

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were induced by a high initial NO release from SNAP-swollen GelMA-SF hydrogels, followed by a more sustained NO release over 24 hours.

SYNTHETIC POLYMERS

The best biomaterials derived from natural polymers however, their poor mechanical properties and rapid biodegradability limit their application. The utilisation of composites composed of hybrid blends of synthetic and natural polymers has been widely explored in the field of medical applications. This is primarily owing to the advantageous of the mechanical and biological properties of each component (Chau et al., 2008). They are biocompatible and bioactive, encouraging adhesion and growth of cells on their surface. (Jin et al., 2021). Synthetic polymers can further divided into two main groups particularly the non-biodegradable and biodegradable. However this review is focusing only the biodegradable materials which aim for the fabrication of microparticles and scaffold specifically for tissue engineering.

Ceramics and synthetic polymers are also being investigated as carriers for statins which demonstrate good mechanical properties and osteoconductiveness. Ceramic bone cements are readily available in FDA-approved to provide a framework upon which bone can grow. In some cases, it is resorbable, allowing native bone to slowly replace it, and has been well characterized as a delivery vehicle for other small molecule drugs such as antibiotics. Nevertheless, the scaffolds fabricated from ceramics usually demonstrate poor integration with surrounding bone tissues (Eugênio Villaboim de Castro Lima Jimmy Cavalcanti Calixto Ana Lia Anbinder et al., 2011). The four most common types of ceramic biomaterials used for tissue regeneration are calcium phosphate (CaP), which includes hydroxyapatite (HA) (Ca10[PO4]6[OH]2), beta-tricalcium phosphate (BTF) (Ca3[PO4]2), and biphasic calcium phosphate (mixture of hydroxyapatite and beta-tricalcium phosphate); bioglass and alumina (ZrO2).

Poly (lactic acid) (PLA) is a biodegradable synthetic polymer that has attracted massive interest as an effective substitute material in regeneration medicine due to its good biodegradability and biocompatibility (Abdal-Hay et al., 2016). Specifically, poly (lactic acid) (PLA) is one of the most widely used synthetic polymers in this field due to the non-toxicity of lactic acid, which is naturally present in the human body, and is FDA-approved (Chen et al., 2010; Wang et al., 2010). It is a poly (α-hydroxyacid) and can exist in 3 different forms, PLA, PLA and a racemic mixture, PDLLA (Puppi et al., 2010). It is degraded by hydrolysis of the ester linkage to form lactic acid, a normal metabolic breakdown product, however the increase in acidity than can ensue following the degradation of PLA can lead to inflammation and swelling (Silva et al., 2018). There are some major limitations such as the hydrophobic nature and poor ductility of PLA which hinder its practical use as substitute materials in tissue regeneration. It is known that the surface wettability reflects the adhesion, growth of cells, and protein absorption on the surface of the material (Abdal-Hay et al., 2013). Some researchers noticed that the porous scaffolds fabricated from PLA floats on cell culture medium (Sarapirom et al., 2014). Thus, the hydrophobic nature of PLA is a serious problem in a predominantly hydrophilic bioenvironment where the cells fail to have initial attachment to the implanted scaffolds. This means PLA suffers from high hydrophobicity due to the lack of hydrophilic groups on its surface, which lends to its poor affinity for cell (Farah et al., 2016). PLA is often modified with other compounds or combined with other bioactive materials in order to deal with some of the issues above. Composite materials have also been produced, such as the electrospinning of hydroxyapatite (HA) nanoparticles, one of the major inorganic components of bone (Lopresti et al., 2020). HA is often used due to its osteoconductivity and bioactivity (Jaiswal et al., 2013). The composite membrane showed enhanced osteoblast cell growth in comparison to a membrane composed of PLA alone, a higher elastic modulus and lower strain at failure, in addition to slowing down the degradation rate of the membrane (Nazir & Iqbal, 2021). Jaiswal et al. (2018) blended PLA with gelatin to form nanofibrous scaffolds and then mineralized the scaffolds with HA (Jaiswal et al., 2013). They found that the mineralized scaffolds exhibited higher ALP activity leading them to conclude that HA increases the osteogenic capacity of the scaffold. The inclusion of HA also leads to

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increased surface roughness, which is important for protein absorption, cell attachment and proliferation. With regards to the degradation rate the addition of the gelatin appeared to increase the degradation rate of the scaffolds, and the HA switched the mechanism from bulk to gradual degradation.

PLGA is an FDA approved biosynthetic polymer, belonging to the poly (α-hydroxyacids) which is one of the most commonly used synthetic materials for preparing fibrous scaffolds in tissue engineering (Venkatesan et al., 2019; Tran et al., 2020; Hua et al., 2021; Su et al., 2021). The polymers are commercially available with different molecular weights and copolymer compositions. The degradation time can vary from several months to several years, depending on the molecular weight and copolymer ratio (Makadia & Siegel, 2011). The forms of PLGA are usually identified by the monomers ratio used. For example, PLGA 50:50 identifies a copolymer whose composition is 50% lactic acid and 50% glycolic acid. Poly (lactic acid) (PLA) has also been used to a lesser extent than PLGA due to the lower degradation rate (Gadad et al., 2012).

PLGA has been used to create microparticles, which are generally considered to fall within the size range of 1-100 μm (Su et al., 2021b) for use in tissue engineering applications, including for the delivery of growth factors (Kirby et al., 2011) and also plasmid deoxyribonucleic acid (DNA) (Soderquist et al., 2010). PLGA loaded with insulin growth factor 1 (IGF-1) have also been incorporated into PVA hydrogel structures in order to facilitate cartilage regeneration (Wei et al., 2020). Poly (lactic-co-glycolic acid) (PLGA) is one of the most successfully used biodegradable polymers because its hydrolysis leads to metabolite monomers, lactic acid and glycolic acid. Because these two monomers are endogenous and easily metabolized by the body via the Krebs cycle, minimal systemic toxicity is associated with the use of PLGA for drug delivery or biomaterial applications (Masloub et al., 2016). The rate at which this occurs should be compatible with the rate at which the tissue regenerates and the products of the degradation must be acceptable within the body. These products are involved in the normal metabolism of cells and hence are non-toxic and water -soluble. The four important processes involved in the biodegradation of PLGA; hydration, initial degradation, further degradation and solubilization (Elmowafy et al., 2019). The aqueous medium first penetrates the polymer and relaxes the structure, reducing its glass transition temperature (Tg) . It is in these hydrated regions that the initial degradation begins, with the hydrolysis of the ester bonds within the polymer backbone structure (Hua et al., 2021). As the backbone is degraded the molecular weight of the polymer is reduced and the PLGA maintains its integrity but loses mechanical strength. As it moves onto the next degradation step, the molecular weight is reduced to such an extent that the polymer loses its integrity. Finally, the smaller fragments are broken down into the monomer molecules leading to solubilization of the PLGA (Hirenkumar & Steven, 2012). The ratio of lactic acid to glycolic acid in the PLGA has an impact on the rate of polymer degradation. Factors which influence the degradation rate of the microparticles are the porosity, molecular weight, geometry and the nature of the aqueous environment. A desirable feature of these systems is the retention of porosity whilst at the same time being able to withstand mechanical stresses. With the adequate selection of polymers an additional factor that needs to be considered is the type of scaffolds used.

Polyether polyethylene glycol (PEG) also known as poly (ethylene oxide) and poly(oxyethylene) is synthetic, water soluble, and biodegradable is also another synthetic polymer approved by FDA (López-Cano et al., 2021). PEG is a unique polymer that is soluble in both water (water-soluble polyether) and common organic solvents. The water-solubility of PEG has been exploited to produce a non-fouling polymer-grafted surfaces (Kikuchi, 2017). Since the 1970s, PEG has been utilised to modify therapeutic proteins and peptides in order to increase their solubility, decrease their toxicity, and extend their circulation half-life. PEG has been investigated as cell scaffolds and drug delivery systems (Zustiak & Leach, 2010). It exhibits considerable potential in the realm of spinal cord tissue engineering due to its favourable biological and material characteristics. PEG is usually combined with gelatine and hydrogels in biodegradable synthetic scaffold (Kong et al., 2017). In a study by Shubin, A. D. et al., PEG hydrogels was observed to support the survival and proliferation of primary submandibular gland cells as

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multicellular spheres in-vitro (Shubin et al., 2017). Using surface embedding technology, Sun et al. (2023) successfully created a composite hydrogel material of PEG nanogold particles (AuNPs). Experiments in vitro demonstrated that the substance induced the differentiation of bone mesenchymal stem cells (BMSCs) into osteoblasts and increased the mineralization activity. Gold nanoparticles (AuNPs) have been reported to promote osteogenic differentiation of mesenchymal stem cells and osteoblasts (Zhang et al., 2017). Incorporating a PEG-based hydrogel and nano-HA also showed significant impact in promoting bone regeneration. As described by Cao Z. et al. (2018), the addition of nano-HA with a large specific surface area to the PEG-based hydrogel network supplied the necessary nucleation sites for the initiation of mineralization (Cao et al., 2018).

POLY(E-CAPROLACTONE)

Polycaprolactone (PCL) a hydrophobic polyesters are widely employed hydrolytically degradable polymer produced by ring-opening polymerization of -caprolactone. PCL is widely used in long-term implants, bone tissue engineering, and slow-releasing drug delivery applications due to the polymer's hydrophobic and semicrystalline properties (Arakawa & DeForest, 2017). The best thing about PCL, is that it can be used in many different ways due to the fact that it has a good biocompatibility, a slow degradation rate, the potential for loadbearing applications, and produces less acidic breakdown products than other polyesters do (Yang et al., 2021). Its low melting point makes it easy to shape and good for 3D printing. In general, 3D printing is a layer-by-layer fabrication technique using powder, liquid, or solid material substrates. Each newly formed layer is triggered to adhere to the previous layer and gradually increasing the size of the structure. Due to the thermoplasticity of PCL, fused deposition modelling (FDM) is the most common 3D bioprinting technique. Driven into a heated printhead, PCL melts and deposits thin layers precisely and sequentially (Turnbull et al., 2018). The printing environment cools the molten PCL, which quickly fuses to form a scaffold. However, the increased temperatures impose constraints on the incorporation of biomolecules and hydrogels (Augustine, 2018). Besides its recent usage in 3D bioprinting, PCL microparticles have been thoroughly investigated previously specifically in the bone regeneration. Study conducted by Zhou et al (2016) and Guarino et al (2012) demonstrated fabrication of spherical PCL particles via an electrospraying process (Guarino et al., 2012; Zhou et al., 2016).

COMPOSITE MATERIAL

Tissue engineering is an interdisciplinary field that aims to repair tissues and organs and the primary challenge in the field of tissue engineering is to mimic the naturally occurring extracellular matrix. Due to the limitations of a single material in terms of biological, physical, and chemical properties, composite biomaterials offer improved biological properties and multiple performances for bone regeneration. Combining two or more biodegradable polymers into a single biomaterial composite compensate for the deficiencies of each one used alone (Hajebi et al., 2021). The rate of resorption of composite materials in the body should correspond to the rate of new tissue formation. Additionally, the composite should have greater mechanical properties compared to either polymer used alone, and also greater structural integrity and flexibility than brittle ceramics. In the context of dentistry and engineering, composite materials typically consist of a matrix (polymer resin) and reinforcement materials (such as glass fibers, carbon fibers, or ceramic particles). The matrix holds the reinforcement materials together, resulting in a material that is stronger, more durable, and possesses desirable properties. Numerous composites also show excellent resistance to abrasion, corrosion, and exposure to high temperatures (Zweben, 2015). There is a growing interest in utilising composite materials composed of biodegradable polymers and bioactive ceramics, such as tricalcium phosphate, hydroxylapatite, and bioactive glasses, as scaffolds in tissue engineering (Boccaccini A.R et al., 2002). These materials are particularly promising for the regeneration of bone and cartilage tissue.

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Certain bioactive ceramics such as tricalcium phosphate and hydroxyapatite as well as bioactive glasses, such as 45S5 Bioglass®, react with physiologic fluids to form tenacious bonds with hard (and in some cases soft) tissue (Boccaccini, 2003). However, these bioactive materials are relatively stiff, brittle and difficult to form into complex shapes. Conversely, synthetic bioresorbable polymers are easily fabricated into complex structures, yet they are too weak to meet the demands of surgery and the in vivo physiologic environment. Composites of tailored physical, biologic and mechanical properties as well as predictable degradation behaviour can be produced combining bioresorbable polymers and bioactive inorganic phases (Khanna C. et al., 2021). Another study by Jang et al., (2020) have proposed PCL/HA hybrid microspheres that induce osteogenic differentiation of human periosteum-derived cells and significantly promote bone formation in vivo (Jang et al., 2020). Composite materials composed of PCL and hyaluronic acid (HA) widely distributed in the ECM, were shown to increase the differentiation potential. (Hwang, H. S., et al.,2023) reported fabrication of GelMA/PCL-TCP composite fibrous membrane promoted osteogenic differentiation of aBMSCs in vitro and pronounced bone formation in vivo. In their study, the composite has a strong potential as a promising membrane for guided bone regeneration. Besides, a uniform porous networkof β-TCP particles were successfully integrated within the fibers. In comparison with pure PCL and GelMA/PCL, GelMA/PCL-TCP membranes led to increased cell attachment, proliferation, mineralization, and osteogenic gene expression in alveolar bone-derived mesenchymal stem cells.

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