Journal of Medical Devices Technology
jmeditec.utm.my

Review Article

A Comprehensive Review of Biomaterials and Its Characteristic for Bone Tissue Engineering Scaffold

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ARTICLE INFO

Article History:
Received 24 May 2023
Accepted 27 May 2023
Available online 30 May 2023

Keywords:
Bone Scaffold,
Tissue Engineering,
Biomaterials

ABSTRACT

The engineering of bone tissue has emerged as a promising method for the restoration and regeneration of damaged or diseased bone tissue. Bone tissue engineering relies heavily on scaffold materials, which provide a three-dimensional structure that directs cell behaviour and promotes tissue growth. However, the success of bone scaffold applications is contingent on the selection of suitable biomaterials and testing methods to assure their safety and efficacy. This review provides an exhaustive summary of biomaterials and testing methodologies for bone tissue engineering scaffold applications. The review examines the characteristics of metal-based, polymer-based, ceramic-based, composite, as well as nanomaterials that are utilised in bone scaffold applications. In addition, it discusses the properties of the scaffold materials in term of mechanical and biocompatibility properties. The review also includes an examination of the biodegradability properties of scaffolds. This exhaustive review will provide researchers, engineers, and clinicians with valuable insights into the selection and testing of biomaterials for bone scaffold applications, thereby facilitating the development of safe and effective bone tissue engineering therapies.

INTRODUCTION

Bones are impacted by trauma, ageing, and cancer. Bone restoration is difficult and expensive. Conventionally, bone grafting, which may be autograft, allograft, or xenograft, has been used to treat bone injury (Chiarello et al., 2013). Numerous studies have shown that allografts and xenografts have inferior efficacy and higher rejection rates because they are foreign and cause severe tissue inflammation and death (Sohn and Oh, 2019). Moreover, the transmission of diseases is heightened (Hammonds, 2013). Consequently, autograft is the only authorised bone treatment, but the scarcity of harvesting sites and surgical risks limit its practical application (Chen et al., 2020).

Due to the limitations of existing methods, bone tissue engineering can create bone replacements to restore, maintain, and enhance functionalities. In order to simulate bone tissue, tissue engineering employs biodegradable scaffolds, osteogenic cells, and bone-inducing factor. Multiple molecular, cellular, biochemical, and mechanical signals are required for bone defect regeneration. Neovascularization or angiogenesis is essential for the regeneration of bone tissue because it provides oxygen and nutrients to promote growth, differentiation, and tissue function (Wang et al., 2020). Consequently, it is necessary to investigate a scaffold that stimulates angiogenesis, osteogenesis, and mechanical stability during bone regeneration. In designing a bone scaffold, the scaffold materials are the primary concern, as they determine the scaffold's biocompatibility, biodegradability, mechanical behaviour, and other properties (Ngadiman et al., 2017). The biomaterials of the first iteration of bone scaffolds are non-biodegradable metal-based materials. While the second iteration of biomaterials is biodegradable, some metal, polymer, and ceramic-based materials are included.

BIOMATERIALS

Bone tissue engineering scaffold has emerged as a promising technique for repairing and regenerating pathological or damaged bone tissue. Utilising biomaterials to construct a three-dimensional scaffold that fosters the growth of new bone tissue (Fallahiarezoudar et al., 2017). As new bone tissue forms, the scaffold progressively degrades and is ultimately replaced by new bone tissue. The success of scaffold applications for bone

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tissue engineering is dependent on the selection of biomaterials that can mimic the natural extracellular matrix of bone tissue, promote cell attachment, proliferation, and differentiation, and provide the necessary mechanical support for tissue growth. Therefore, the development and utilisation of biomaterials for bone tissue engineering scaffold applications have been the subject of extensive research and have demonstrated enormous clinical application potential. This has resulted in the development of a diverse array of biomaterials, including natural, synthetic, and composite materials, each with its own advantages and disadvantages. The evaluation of a biomaterial's biocompatibility, biodegradability, mechanical properties, and other essential characteristics is necessary for its selection and testing (Oliveira et al., 2020).

METAL-BASED BIOMATERIALS

In the early studies of bone tissue engineering scaffold, non-biodegradable materials such as stainless steel, titanium, and their alloys were investigated. It has been demonstrated that these materials have high mechanical properties, effective resistance to wear, fatigue, and deformation, and that some of these materials also have substantial toughness. (Wu et al., 2017). These materials, however, are not biodegradable. In addition, the risk of metal ions spreading to the adjacent tissues is elevated due to the physiological environment-induced corrosion. This condition may result in toxicity and an adverse effect (Chen et al., 2017). In terms of mechanical properties, the enormous difference between the bone and scaffold Young's Modulus can result in tension shielding. This circumstance inhibits the subsequent transfer of physiological stress to bone tissue (Noyama, et al, 2013). In addition, the non-biodegradability of metal materials led to secondary surgery complications, which has increased interest in biodegradable metals like magnesium, zinc, iron, and their alloys (Wang, et al. 2017). Despite the fact that these three metals are essential for maintaining the normal function of the human body, numerous studies have confirmed that they are biocompatible with human cells and tissues.

In a study conducted by Chen et al. (2017) a biomimetic titanium scaffold was manufactured using a powder metallurgy technique, with magnesium powder serving as the spacer. The scaffold's porosity varies between 30, 40, and 50 %, which indicates that the magnesium particles increased as the porosity increased. The maximum elastic moduli recorded was 44,2 GPa, which exceeded the expected range of 4 to 30 Gpa. Moreover, scaffolds with 30% porosity are the most biocompatible. However, the cell viability of the other two scaffolds was lower than that of the control sample. This is because magnesium residues remaining after the debonding procedure inhibit the ability of cells to adhere to an inert titanium scaffold.

POLYMER-BASED BIOMATERIALS

Polymers are macromolecules composed of repeated constituents linked by covalent bonds. Researchers have favoured biodegradable polymers due to their degradability, which is necessary for bone defect treatment and regeneration. According to their origins, polymers can be categorised as either natural or synthetic. Due to their biodegradability, bioactivity, and biocompatibility, natural polymers like chitosan, alginate, silk fibroin, fibrinogen, collagen, and hyaluronic acid have been extensively researched as bone defect materials. Nonetheless, they have limitations such as unstable sources, poor mechanical properties, high solubility in water, the possibility of denaturation during processing, and the potential for immunogenicity (Qu et al., 2013; Manoukian et al., 2019).

In terms of synthetic degradable polymers, aliphatic polyesters such as PCL, PLA, PGA, and PLGA copolymer are the most extensively researched. PEG and PVA are additional types of polymers (Ngadiman et al., 2015). The majority of these materials are non-toxic to host tissues and all of these materials are biocompatible and degrade at a controlled rate. Nonetheless, when certain synthetic polymers degrade in vivo, their degradation products are acidic, altering the local pH value, accelerating implant degradation, and eliciting inflammatory responses. Moreover, it is possible to accomplish the desired mechanical properties of polymers by controlling the design and synthesis parameters (Shi et al., 2016). Synthetic polymers are more modifiable than natural polymers (Manoukian et al., 2019).

CERAMIC-BASED BIOMATERIALS

Due to their excellent performance, which includes biocompatibility, mechanical compatibility, and precise chemical composition, ceramics have been extensively used in biomedical applications such as tissue engineering over the past few decades (Stansbury and Idacavage, 2016). In bone scaffolds, biodegradable ceramics are the primary focus. These materials are utilised to repair bone fractures and bone defects. Currently, the most commonly used biodegradable ceramics are hydroxyapatite (HA), tricalcium phosphate (TCP), and dicalcium phosphate (DCP). After implantation in the body, they are progressively degraded by solution-driven and cell-mediated processes and eventually replaced with new lamellar bone tissue. Obviously, biodegradable materials have disadvantages, such as weak fracture toughness, brittleness, and extremely high rigidity, and their strength is considerably lower than that of non-absorbable ceramic materials. (Wei et al., 2020).

COMPOSITE BIOMATERIALS

Composite materials based on bioactive ceramics typically refer to materials that combine the benefits of biodegradable polymers and biodegradable ceramics. These composites have exceptional biocompatibility, osteoconductivity, mechanical strength, and osteogenic properties. In addition, with the aid of new fabrication techniques that have emerged in recent years, these composite materials have become the most promising materials for bone defect repair.

Recent research has demonstrated that collagen/HA composite scaffolds can induce osteogenic differentiation of human BMSCs, upregulate osteogenic gene expression, and increase collagen deposition (D'Agostino et al., 2016). Similarly, other investigations of collagen/HA hybrid scaffold have yielded positive results (Mazzoni et al., 2017). Feng et al. (2021) have created a composite cortical scaffold composed of HA, Graphene Oxide (GO), and Chitosan (CTS) (HA/GO/CTS). The resulting compressive strength of the scaffold was close to that of cortical bone (100 – 230 MPa). In vivo bone regeneration, however, the scaffold demonstrated excellent bone formation and vascularization properties.

According to a recent study, PLA/PEG/CaP nanocrystals induce osteogenic differentiation of human mesenchymal stem cells (hMSCs) in contrast to synthetic polymers and ceramic composites. In addition, CaP-rich cortical scaffolds completely

degraded in twelve weeks. However, the scaffold did not meet the expected compressive modulus of natural cortical bone (5 GPa), with a maximum value of 1.76 GPa (Barati et al., 2019). Panseri et al. (2021) have created a PMA/PEG containing two forms of ceramics: HA and -TCP. It was found that the degradation rate of scaffolds containing -TCP is 10 to 20 times that of scaffolds containing HA. As a result, PMA/PEG/HA was identified as a functional structure. The compressive strength of the scaffold is less than 20 MPa, which is lower than the range of cortical bone, and demonstrates brittle failure. Lai et al. (2019) have proposed a new porous PLGA/TCP scaffold incorporating Magnesium (Mg) powder (PTM). In vivo experimental results demonstrate that the PTM scaffold has the dual effects of osteogenesis and angiogenesis, as well as a synergistic effect in fostering the formation of new bone and enhancing the quality of new bone in SAON. Recent research has demonstrated that PCL/silicon-substituted hydroxyapatite (Si-HA) membranes can induce cell growth and differentiation, as well as enhance osteoblast attachment and proliferation; therefore, this material is anticipated to play a significant role in bone defect repair (Lei et al., 2020).

In conclusion, biodegradable materials guarantee an excellent performance scaffold. First, biodegradation is crucial because one of the goals of tissue engineering is to prevent injury and a second operation that imposes a financial burden. In addition, for bone scaffold materials to be clinically qualified, the rate of degradation must match the rate of bone regeneration. The scaffold must be capable of supporting the structure with sufficient mechanical strength for a minimum of 12 weeks. To avoid stress shielding, secondly, scaffold biomaterials must have mechanical properties comparable to those of natural bone. Thirdly, biomaterials must be biocompatible, with degradation products that are non-toxic to host cells. Additionally, it is anticipated that angiogenesis and osteogenesis are induced in vivo.

NANOMATERIALS

Nanomaterials are characterised by their nanoscale size, which enables them to develop critical physical and chemical properties that improve their efficacy and thus make them useful for a wide variety of applications (Fathi-Achachelouei et al., 2019). Nanomaterials are used in tissue engineering applications to adjust mechanical strength, modulate release of multiple bioactive agents such as growth factors, embed novel biomaterials with greater spatiotemporal control within scaffold, utilise simultaneous therapeutic and imaging systems, and minimise toxicity while increasing biocompatibility through specific delivery, as depicted in Figure 1 (Fathi-Achachelouei et al., 2019).

Similar to biomaterials, nanoparticles can be manufactured from a variety of materials, including metals, ceramics, and polymers. In the biomedical industry, metal nanomaterials such as gold, silver, iron oxide, and others have been investigated for extensive tissue engineering applications for many years. Gold nanoparticles (GNPs) and titanium dioxide (TiO2) have been utilised to increase cell proliferation rates for regeneration of bone and cardiac tissue, respectively. GNPs have been demonstrated to be excellent candidates for bone regeneration and are ideal candidates to replace bone morphogenetic proteins (Hasan et al., 2018). Yet, the high cost and propensity of these nanomaterials to cause local inflammation have prompted researchers to seek for alternative bone growth materials.

In addition, nanocomposites polymers containing nanoparticles in the form of hydrogel and electrospun fibres have superior mechanical properties compared to scaffolds lacking reinforcement. By forming interactions between HA and silk fibroin fibres, n-HA, a ceramic-based nanomaterial, improved the mechanical properties of an electrospun silk fibroin scaffold. Furthermore, it has been demonstrated that magnetic nanomaterials (MNPs) (Ansari et al., 2019; Zhang et al., 2017: Ngadiman et al., 2017) and silver nanoparticles (AgNPs) (Chen et al., 2018) enhance the mechanical properties of scaffolds. Moreover, according to Jeevanandam et al. (2018) the toxicity of nanomaterials depends on a variety of factors, such as particle size and shape, crystallinity, surface area, dose and exposure duration, as well as the nanoparticles' aggregation and concentration. As a result, scientists have investigated nanomaterials derived from natural polymers, such as nanocellose (NC), graphene oxide (GO) (Wang et al., 2020) and carbon nanotubes (CNTs) (Kausar, 2020), as they are biocompatible with natural cells and low in toxicity.

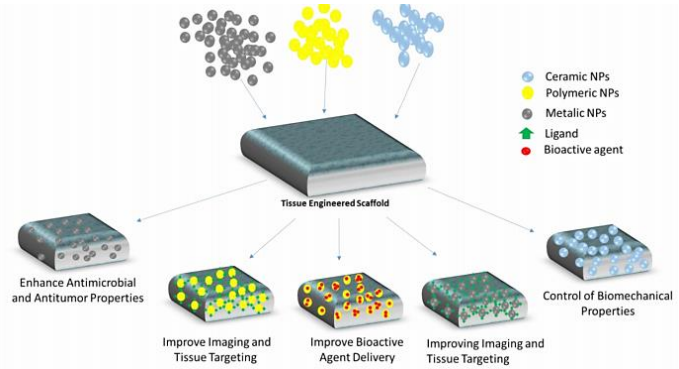

Fig 1 Different types of nanoparticles with various applications in tissue engineering (Fathi-Achachelouei et al., 2019)

MECHANICAL PROPERTIES

After being transplanted into an animal or human body, scaffolds encounter various types of loads in vivo. Compression, tension, shear, torsion, bending, and biomechanical/physiological loads are some of the various types of loads encountered by scaffolding. In scaffold development, it is essential to consider mechanical properties. The scaffold must have sufficient mechanical strength based on the anatomical site at which it will be implanted (Tran et al., 2018). Moreover, it must provide and maintain adequate mechanical support during cell proliferation and tissue regeneration without deforming the new tissue (Joshi et al., 2015). Cortical bone, which is the hard bone, and trabecular bone, also known as cancellous bone, which consists of spongy tissue, are the two natural forms of bone. Both varieties of bone possess distinct mechanical properties. Young's modulus and compressive modulus of cortical bone range from 15 to 20 GPa and 100 to 200 MPa, respectively, whereas trabecular bone ranges from 0.1 to 2 GPa and 2 to 20 MPa (Balagangadharan et al., 2017). In addition, other factors such as pore size, pore interconnectivity, porosity, biomaterials composite, and material density can impact the mechanical integrity of scaffolds (Kumar et al., 2014).

To tailor the mechanical properties of scaffold to a particular application, researchers have incorporated organic nanomaterials such as MCC/NCC as reinforcing agents and biopolymer infill as a replacement for carbon tube. The addition

of MCC/NCC is also anticipated to improve the scaffold's mechanical properties. This was consistent with the findings of (Lee et al., 2009) who created a nanocomposite film from microcrystalline cellulose (MCC) and polyvinyl alcohol (PVA). It was determined that the tensile strength increased as MCC loading increased. Cataldi et al. (2018) extended the study by developing a composite scaffold composed of PVA and nanocrystalline cellulose (NCC) in varying quantities. The author of the study evaluated the tensile properties, which is tension at the break, which increased by 73% when 5 wt% of NCC was incorporated into the PVA biomaterials. Due to the aggregation of filler within the matrix, however, the excessive quantity of NCC has decreased the tensile stress. Polylactic acid (PLA) is a further advantageous biopolymer. It has been reported that PLA by itself lacks mechanical integrity, which is inferior to that of native tissue. In order to address this issue, PLA has been grafted with maleic anhydride and given the designation MPLA. However, the mechanical strength is still insufficient, so NCC was added to MPLA scaffolds (Zhou et al., 2013). At the optimal concentration (5 wt%), these NCC inclusions have been shown to increase tensile strength by 85%. (Zhang et al., 2015) obtained a similar result of mechanical enhancement by the incorporation of NCC into the biomaterial, but in this investigation, NCC was grafted with polyethylene glycol (PEG).

In the in vivo condition, bone scaffold implantation frequently involves compression (Zhang et al., 2019; Charles-Harris et al., 2007) whereas for other applications, such as epidermis or cartilage, the tensile test is more significant (Tran et al., 2018). As a result, researchers have taken an interest in the compressive strength of scaffolds due to their practical application in the human body; scaffolds are more susceptible to compression effects than tensile effects (Charles-Harris et al., 2007). Therefore, Eftekhari et al. (2014) devised a nanocomposite scaffold containing poly-L-lactic acid (PLLA), hydroxyapatite (HA), and MCC. According to the results of the compression test, the addition of MCC and HA nanoparticles to the scaffold enhances its compressive strength and modulus. This significance can be attributed to the improvement of interfacial bonding between the reinforcement and the polymer through the enhancement of hydrogen bonds between the reinforcing agents and the PLLA matrix. Similar results were also obtained by Aleman-Dominguez et al. (2019) whose material designs included MCC-filled PCL and reported a compression modulus in the range of values for spongy bone, and by Chen et al., (2016) whose NCC aerogel-based scaffold exhibited similar compression modulus values.

Other than this, Kumar et al. (2014) have used PVA biomaterials to develop scaffolds by incorporating n-HA and NCCs. This nanocomposite scaffold enhances compression strength from 0.40 to 2.09 MPa and compressive modulus from 0.32 to 16.01 MPa. Additional ovalbumin (OVA) has been added to this analysis (Kumar et al., 2016). The effect of NCC on enhancing mechanical properties at optimal content remains, but the magnitude of compression strength and compression modulus was reported to be lower than in the previous study. Various parameters, such as material processing, porosity, and pore interconnectivity, contribute to the mechanical profile's irregular behaviour in enhancing the mechanical properties. Also, the inclusions of OVA and n-HA have altered the hydrophilic behaviour, reducing the compressive stress–strain behaviour by 0.19–0.37 MPa, depending on the concentrations of NCC and n-HA.

Next, Luo et al. (2019) have devised an in situ nanocomposite porous scaffold composed of PLA/NCC. According to the study, the compressive modulus of scaffolds containing 0.8% NCCs has increased by 368% compared to scaffolds containing PLA alone. Based on these reviews, it has been established that NCCs have the ability to improve the mechanical properties, tensile and compression, of bone scaffolds when the optimal quantity of NCC-filled base biomaterials is utilised. The quantity of NCCs included and tested ranges between 0.5% and 20% by weight. However, the excessive quantity of MCCs or NCCs has rendered the scaffold's biocomposite brittle. No study has yet been conducted to optimise the volume or concentration of NCC in biomaterials for bone tissue engineering scaffolds. Recent studies on the contribution of NCCs to the mechanical properties of bone scaffold materials are presented in Table 2.3.

BIOCOMPATIBILITY PROPERTIES

Biomaterials are considered biocompatible if they are non-toxic to living tissues and can stimulate the host response in the human biological system in an appropriate manner. Biocompatibility is one of the material design requirements for a bone tissue engineering scaffold to perform satisfactorily. The ability of living cells to adhere, proliferate, and integrate with host tissues determines the cytotoxicity of biomaterials. The studies consist of an in vivo study involving live biological entities within the organism and an in vitro study utilising living cells derived from humans or animals.

Shaheen et al. (2019) conducted a cell culture MTT assay with MG63 Osteoblast cells for scaffolds made of chitosan and alginate containing NCCs. Early in the culture, cells have begun to proliferate within the scaffold's apertures. After 72 hours, the filopodium and lamellipodium of cells within the apertures of a three-dimensional structure are observed to be firmly attached. The dense cells grew as colonies with a highly interconnected 3D network structure inside and outside of pores. Consequently, this result indicated that the scaffold exhibited a proliferative propensity, was non-toxic, and conducive to the attachment and growth of MG63 Osteoblast.

In contrast, Luo et al. (2019) examined the viability of M058K cells seeded on PLA and PLA containing NCC. The Alamar blue assay is utilised, and the activity of cells on days 3, 6, and 12 is recorded. As a consequence, the fluorescence intensity of NCC-filled PLA is greater than that of PLA. Green and red stains were applied, accordingly, to living and nonliving cells. Due to its minimal cytotoxicity and high cytocompatibility, the incorporation of NCC into the scaffold is conducive to cell attachment and proliferation. Zhang et al. (2015) have employed the same concept of cell culture. This study differs from the previous study in that hMSCs were cultured for 14 days on PLA and NCC-filled PLA (PLA/NCC) made from the same material. Compared to PLA, the PLA/NCC nanofibrous scaffold contained a greater number of viable cells (green-stained) than PLA alone. However, a small number of dead cells (stained crimson) were also observed. With the addition of NCC, the biocompatibility of PLA was preserved by 5%, according to the results of cell viability and proliferation tests.

In addition, in a study conducted by Zhou et al. (2013) human adipose stem cells (hASCs) were cultured for 7 days on a scaffold composed of PLA/NCC and MPLA/NCC with a constant 5 wt% NCC content. Figure 2 (a-d) depicts the

outcomes of the cell culture experiments. It was reported that the MPLA/NCC-5 contained a greater number of living cells (coloured green) than the PLA/NCC. On nanofibrous MPLA/NCC-5 scaffolds, very few necrotic cells were detected. This result suggested that the cytotoxicity of composites was diminished during hASC cultivation. Alamar Blue proliferation viability testing was also conducted in this study. As shown in Figure 2 (e), the incorporation of NCCs into the scaffold had no effect on the cytotoxicity of human adipose stem cells (hASCs) after seven days. The authors have argued that the low quantity of NCC included was responsible for this circumstance. In future research, a broader range of NCC concentrations must be investigated. Nevertheless, the developed scaffold was able to sustain cell proliferation and exhibited excellent cytocompatibility. Numerous researchers execute the same test using different cell types, including MC3T3 (Chen et al., 2016), mouse fibroblast (Niamsap et al., 2019) and human osteoblast (Herdocia-Lluberes et al., 2015) cells.

From these studies, it can be concluded that the incorporation of NCCs as nanoparticles into bone scaffolds improved the biocompatibility of the scaffolds based on the amount of NCCs included. This is because NCCs are nanomaterials derived from natural sources that were originally biocompatible with natural bone. Notwithstanding, the incorporation of NCCs influences the cellular mechanism during cell culture and in vitro cell viability. Determining the quantity of NCCs that can be incorporated into biomaterials to contribute to bone regeneration is the challenge that arises here.

BIODEGRADABILITY

Biodegradability of scaffold refers to the capability of the scaffold biomaterials to be broken down into simpler substances. Enzymatic degradation, hydrolytic degradation, and biodegradation are the few mechanisms that can result in degradation. When biomaterials are broken down by the action of microorganisms' enzymes, enzymatic degradation occurs. In contrast, hydrolytic degradation involves the hydrolysis of biomaterials by water present in the host's tissue and organ (in vivo). In addition, biodegradation is caused by cell activity, and the degradation of biomaterials is a result of specific biological activity. In addition, the degradation rate is a metric used to assess the biodegradability of a given biomaterial. In tissue engineering, the degradation properties of the scaffold are of utmost importance, as it functions as a transitory template that facilitates tissue regeneration and must degrade over time. This is done so as to avoid protracted allogeneic and xenogeneic reactivity in the hostage, which could lead to other undesirable risks. The rate of tissue degeneration and degradation should be equivalent. Otherwise, the healing process may not be complete.

The rate of degradation of scaffold biomaterials is determined by in vitro and/or in vivo degradation testing. The rate of degradation is calculated by determining the quantity of weight loss over time (Fallahiarezoudar et al., 2017). The scaffold is typically weighed and deposited in a sealed container of simulated body fluid (SBF), which is then allowed to degrade in an incubator at 37 °C (optimum body temperature). Every two weeks, a sample is removed, rinsed with distilled water, dried, and weighed in order to determine the mass loss.

Nanocrystalline cellulose (NCC) is a nanomaterial that has been widely utilised in scaffold biomaterials and has been demonstrated in numerous studies to possess biodegradability properties. However, the high crystallinity and lack of an enzyme that could disrupt the glycosidic linkage of NCC in the human body has resulted in a slow or non-degradable NCC in vivo and in vitro (Kamboj et al., 2020; Lam et al., 2012) . This condition has been studied by Martson et al. (1999) who found that a cellulose-based scaffold underwent a long-term degradation study that resulted in gradual degradation in rat subcutaneous tissue after sixty days. In addition, according to Lam et al. (2012) it has been established that cellulose is degradable in in vivo cases.

Regarding the contribution of NCC incorporation in polymer-based scaffolds, Luo et al. (2019) have devised an in situ NCC-filled PLA nanocomposite scaffold. According to the results of in vitro degradation tests, the weight loss of scaffolding increases as the NCC content rises. This is because the presence of NCC has increased the hydrophilicity of scaffolds, making it more likely for water molecules to diffuse to ester or other hydrophilic groups, resulting in enhanced hydrolysis of ester groups and the breakdown of the PLA molecular chain. In addition, the incorporation of NCC increases the stability during in vitro degradation. In vitro degradation of scaffolds in PBS medium was assessed by measuring mass loss over 30 days. The addition of NCC decreased the in vitro degradation rate of MPLA/NCC scaffolds in PBS by increasing the crystallinity of MPLA and inhibiting the diffusion of water in the polymer matrix, despite increasing the total surface area of the scaffolds. Incorporating NCCs into nanocomposite scaffolds to increase their resistance to degradation (Zhou et al., 2013). In contrast, the incorporation of NCC into PVA/n-HA scaffold decreased the degradation rate as the NCC concentration increased. This is as a result of the decrease in salvation and depolymerization (Kumar et al., 2014). Theoretically, the hydrophilicity of the biocomposite scaffold increased as the amount of NCC increased. The choice of biomaterials, such as polymers, and their modification had varying effects on the degradation rate of the scaffold.

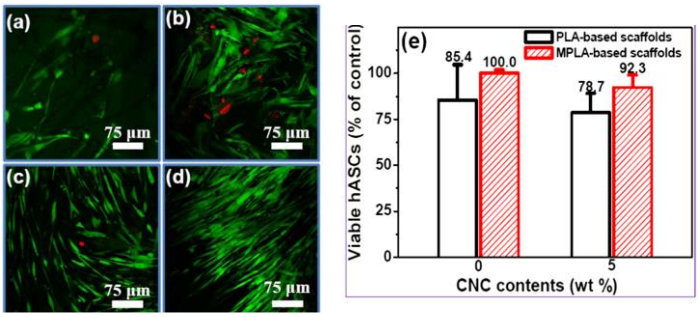

Fig Error! No text of specified style in document. Result of hASCs cultivation on polylactic acid (PLA)/ nanocrystalline cellulose (NCC) and polylactic acid grafted with maleic anhydride (MPLA)/NCC after 7 days. Fluorescence micrograph of (a) PLA, (b) PLA/NCC-5, (c) MPLA, (d) MPLA/NCC-5 and (e) proliferation viability of cells (reproduced from Zhou et al. 2013 with permission. Copyright © 2013, American Chemical Society

CONCLUSION

In conclusion, three major aspects of scaffold biomaterials have been discussed: mechanical properties, biocompatibility, and biodegradability. In applications involving the lower limb or cortical bone, the selection of biomaterials and its fabrication method are crucial. This is due to the fact that biomaterials determine the scaffold's biological and mechanical properties,

while fabrication methods play a crucial role in ensuring that the scaffold has a suitable outer and inner architecture topology. To create a scaffold for cortical bone, it is not sufficient to use a single polymer, as it has lower mechanical properties than natural human cortical bone. Therefore, researchers develop composite materials that combine the same/different categories of materials. In addition, nanomaterials are required in order to improve the efficacy of the scaffold. The issue is that composite biomaterials satisfy the requirements for cortical bone scaffold. First, the scaffold's Young's Modulus must lie within the range of 3 to 30 GPa, but the highest reported value was less than 2 GPa. Regarding biocompatibility, the majority of studies have demonstrated that the utilised biomaterials are biocompatible. However, scaffolds containing metals such as titanium are reported to be toxic due to their degradation. In addition, it is difficult to assess the biodegradation of the cortical bone scaffold due to the paucity of published studies.

ACKNOWLEDGMENT

The authors would like to acknowledge the financial support from Universiti Teknologi Malaysia for the funding under UTMShine (Q.J130000.2451.09G94).

REFERENCES

Aleman-Dominguez M. E., Giusto E., Ortega Z., Tamaddon M., Benitez A. N., and Liu C. 2019. Three-dimensional printed polycaprolactone-microcrystalline cellulose scaffolds. J. Biomed. Mater. Res. Part B Appl. Biomater., 107(3), 521–528, 2019.

Ansari S. A. M. K. et al. 2019. Magnetic iron oxide nanoparticles: synthesis, characterization and functionalization for biomedical applications in the central nervous system. Materials (Basel), 12(3), 465.

Balagangadharan K., Dhivya S., Selvamurugan N. 2017. Chitosan based nanofibers in bone tissue engineering. Int. J. Biol. Macromol., 104, 372–1382.

Barati D., Karaman O., Moeinzadeh S., Kader S., Jabbari E. 2019. Material and regenerative properties of an osteon-mimetic cortical bone-like scaffold. Regen. Biomater., 6(2), 89–98.

Cataldi A., Rigotti D., Nguyen V. D. H., Pegoretti A. 2018. Polyvinyl alcohol reinforced with crystalline nanocellulose for 3D printing application. Mater. Today Commun., 15, 236–244.

Charles-Harris M., Valle S. del, Hentges E., Bleuet P., Lacroix D., Planell J. A. 2017. Mechanical and structural characterisation of completely degradable polylactic acid/calcium phosphate glass scaffolds. Biomaterials, 28(30), 4429–4438.

Chen C.-Y., Chen C.-C., Wang C.-Y., Lee A. K.-X., Yeh C.-L., Lin C.-P. 2020. Assessment of the release of vascular endothelial growth factor from 3D-printed poly-varepsilon-caprolactone/hydroxyapatite/calcium sulfate scaffold with enhanced osteogenic capacity. Polymers (Basel), 12(7), 1455.

Chen S., Yang J., Jia Y.-G., Lu B., Ren L. 2018. A study of 3D-printable reinforced composite resin: PMMA modified with silver nanoparticles loaded cellulose nanocrystal. Materials (Basel), 11(12), 2444.

Chen X., Zhou R., Chen B., Chen J. 2016. Nanohydroxyapatite/cellulose nanocrystals/silk fibroin ternary scaffolds for rat calvarial defect regeneration. RSC Adv., 6(42), 35684–35691.

Chen Y. et al. 2017. Mechanical properties and biocompatibility of porous titanium scaffolds for bone tissue engineering. J. Mech. Behav. Biomed. Mater., 75, 169–174.

Chiarello E. et al. 2013. Autograft, allograft and bone substitutes in reconstructive orthopedic surgery. Aging Clin. Exp. Res., 25, 101–103.

D'Agostino A. et al. 2016. Hydroxyapatite/collagen composite is a reliable material for malar augmentation. J. Oral Maxillofac. Surg., 74(6), 1238.

Eftekhari S., El Sawi I., Bagheri Z. S., Turcotte G., Bougherara H. 2014. Fabrication and characterization of novel biomimetic PLLA/cellulose/hydroxyapatite nanocomposite for bone repair applications. Mater. Sci. C Eng., 39, 120–125.

Fallahiarezoudar E., Ahmadipourroudposht M., Mohd Yusof N., Idris A., Ngadiman N. H. A. 2017. 3D biofabrication of thermoplastic polyurethane (TPU)/poly-L-lactic acid (PLLA) electrospun nanofibers containing maghemite (γ-Fe2O3) for tissue engineering aortic heart valve. Polymers (Basel), 9(11), 584.

Fathi-Achachelouei M. et al. 2019. Use of nanoparticles in tissue engineering and regenerative medicine. Front. Bioeng. Biotechnol., 7, 113.

Feng C. et al. 2021. Co-inspired hydroxyapatite-based scaffolds for vascularized bone regeneration. Acta Biomater., 119, 419–431.

Hammonds, R. L. 2013. The Advancement of Bacterial Cellulose As A Bone and Vascular Scaffolds.

Hasan A., Morshed M., Memic A., Hassan S., Webster T. J., and Marei H. E.-S. 2018. Nanoparticles in tissue engineering: Applications, challenges and prospects. Int. J. Nanomedicine, 13, 5637.

Herdocia-Llubere C. S., Laboy-López S., Morales S., Gonzalez-Robles T. J., González-Feliciano J. A., and Nicolau E. 2015. Evaluation of synthesized nanohydroxyapatite-nanocellulose composites as biocompatible scaffolds for applications in bone tissue engineering. J. Nanomater., 2015, 6.

Jeevanandam J., Barhoum A., Chan Y. S., Dufresne A., Danquah M. K. 2018. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J. Nanotechnol., 9(1),1050–1074.

Joshi M. K. et al. 2015. In situ generation of cellulose nanocrystals in polycaprolactone nanofibers: effects on crystallinity, mechanical strength, biocompatibility, and biomimetic mineralization. ACS Appl. Mater. & Interfaces, 7(35), 9672–19683.

Kamboj N., Kazantseva J., Rahmani R., Rodriguez M. A., Hussainova I. 2020. Selective laser sintered bio-inspired silicon-wollastonite scaffolds for bone tissue engineering. Mater. Sci. Eng. C, 116, 111223.

Kausar A. 2020. Emerging trends in poly (methyl methacrylate) containing carbonaceous reinforcements Carbon nanotube, carbon black, and carbon fiber. J. Plast. Film Sheeting, 36(4), 409–429.

Kumar A., Negi Y. S., Choudhary V., Bhardwaj N. K. 2016. Fabrication of poly (vinyl alcohol)/ovalbumin/cellulose nanocrystals/nanohydroxyapatite based biocomposite scaffolds. Int. J. Polym. Mater. Polym. Biomater., 65(4), 191–201.

Kumar A., Negi Y. S., Choudhary V., and Bhardwaj N. K. 2014. Microstructural and mechanical properties of porous biocomposite

scaffolds based on polyvinyl alcohol, nano-hydroxyapatite and cellulose nanocrystals. Cellulose, 21, 3409–3426.

Lai Y. et al. 2019. Osteogenic magnesium incorporated into PLGA/TCP porous scaffold by 3D printing for repairing challenging bone defect. Biomaterials, 197, 207–219.

Lam E., Male K. B., Chong J. H., Leung A. C. W., Luong J. H. T. 2012. Applications of functionalized and nanoparticle-modified nanocrystalline cellulose. Trends Biotechnol., 30(5), 283–290.

Lee S.-Y., Mohan D. J., Kang I.-A., Doh G.-H., Lee S., Han S. O. 2009. Nanocellulose reinforced PVA composite films: effects of acid treatment and filler loading. Fibers Polym.,10, 77–82.

Lei T. et al. 2020. Silicon-incorporated nanohydroxyapatite-reinforced poly (varepsilon-caprolactone) film to enhance osteogenesis for bone tissue engineering applications. Colloids Surfaces B Biointerfaces,187,110714.

Luo W. et al. 2019. Preparation, characterization and evaluation of cellulose nanocrystal/poly (lactic acid) in situ nanocomposite scaffolds for tissue engineering. Int. J. Biol. Macromol., 134, 469–479.

Martson M., Viljanto J., Hurme T., Laippala P., Saukko P. 1999. Is cellulose sponge degradable or stable as implantation material? An in vivo subcutaneous study in the rat. Biomaterials, 20(21), 1989–1995.

Manoukian O. S. et al. 2019. Biomaterials for tissue engineering and regenerative medicine.

Mazzoni E. et al. 2017. Human adipose stem cells induced to osteogenic differentiation by an innovative collagen/hydroxylapatite hybrid scaffold. FASEB J., 31(10), 4555–4565.

Ngadiman N. H. A., Idris A., Irfan M., Kurniawan D., Yusof N. M., and Nasiri R. 2015. γ-Fe 2 O 3 nanoparticles filled polyvinyl alcohol as potential biomaterial for tissue engineering scaffold. J. Mech. Behav. Biomed. Mater., 49, 90–104.

Ngadiman, N. H. A., Mohd Yusof, N., Idris A., Kurniawan D., Fallahiarezoudar, E. 2017. Fabricating high mechanical strength γ-Fe 2 O 3 nanoparticles filled poly(vinyl alcohol) nanofiber using electrospinning process potentially for tissue engineering scaffold," J. Bioact. Compat. Polym., 32(4), 411–428.

Ngadiman N. H. A., Yusof N. M., Idris A., Misran E., and Kurniawan D. 2017. Development of highly porous biodegradable γ-Fe2O3/polyvinyl alcohol nanofiber mats using electrospinning process for biomedical application. Mater. Sci. Eng. C, 70, 520–534.

Niamsap T., Lam N. T., Sukyai P., 2019. Production of hydroxyapatite-bacterial nanocellulose scaffold with assist of cellulose nanocrystals. Carbohydr. Polym., 205, 159–166.

Noyama Y., Nakano T., Ishimoto T., Sakai T., Yoshikawa H. 2013. Design and optimization of the oriented groove on the hip implant surface to promote bone microstructure integrity. Bone, 52 (2), 659–667.

Oliveira T. C., Gomes M. S., Gomes A. C. 2020. The crossroads between infection and bone loss. Microorganisms, 8(11),1765.

Panseri S. et al. 2021. Bone-like ceramic scaffolds designed with bioinspired porosity induce a different stem cell response. J. Mater. Sci. Mater. Med., 32,1–12.

Qu H., Fu H., Han Z., Sun Y. 2019. Biomaterials for bone tissue engineering scaffolds: A review. RSC Adv., 9(45), 26252–26262.

Shaheen T. I., Montaser A. S., Li S. 2019. Effect of cellulose nanocrystals on scaffolds comprising chitosan, alginate and hydroxyapatite for bone tissue engineering. Int. J. Biol. Macromol., 121, 814–821.

Shi C., Yuan Z., Han F., Zhu C., Li B., 2016. Polymeric biomaterials for bone regeneration. Ann. Jt,1, 27.

Sohn H.-S., Oh J.-K. 2019. Review of bone graft and bone substitutes with an emphasis on fracture surgeries. Biomater. Res., 23, 1,1–7.

Stansbury J. W., Idacavage M. J. 2016. 3D printing with polymers: Challenges among expanding options and opportunities. Dent. Mater., 32(1), 54–64.

Tran T. T., Hamid Z. A., Cheong K. Y. 2018. A review of mechanical properties of scaffold in tissue engineering: Aloe vera composites. Journal of Physics: Conference Series, 1082, 12080.

Wang H., Zheng Y., Liu J., Jiang C., Li Y. 2017. In vitro corrosion properties and cytocompatibility of Fe-Ga alloys as potential biodegradable metallic materials. Mater. Sci. Eng. C, 71, 60–66.

Wang L. et al. 2020. Application of injectable silk fibroin/graphene oxide hydrogel combined with bone marrow mesenchymal stem cells in bone tissue engineering. Colloids Surfaces A Physicochem. Eng. Asp., 604, 125318.

Wang Q. et al. 2020. Osteogenic growth peptide-loaded 3D-printed PCL scaffolds for the promotion of osteogenesis through the ERK pathway. Mater. & Des., 193, 108811.

Wei S., Ma J.-X., Xu L., Gu X.-S., Ma X.-L., 2020. Biodegradable materials for bone defect repair. Mil. Med. Res., 7,1–25.

Wu T., Yu S., Chen D., Wang Y. 2017. Bionic design, materials and performance of bone tissue scaffolds. Materials (Basel)., 10(10) 1187.

Zhang C., Salick M. R., Cordie T. M., Ellingham T., Dan Y., Turng L.-S. 2015. Incorporation of poly (ethylene glycol) grafted cellulose nanocrystals in poly (lactic acid) electrospun nanocomposite fibers as potential scaffolds for bone tissue engineering. Mater. Sci. Eng. C, 49, 463–471.

Zhang H. et al., 2017. Magnetic nanoparticle-loaded electrospun polymeric nanofibers for tissue engineering. Mater. Sci. Eng. C, 73, 537–543.

Zhang S., Vijayavenkataraman S., Lu W. F., Fuh J. Y. H. 2019. A review on the use of computational methods to characterize, design, and optimize tissue engineering scaffolds, with a potential in 3D printing fabrication. J. Biomed. Mater. Res. Part B Appl. Biomater., 107(5), 1329–1351.

Zhou C. et al. 2013. Electrospun bio-nanocomposite scaffolds for bone tissue engineering by cellulose nanocrystals reinforcing maleic anhydride grafted PLA. ACS Appl. Mater. & Interfaces, 5(9), 3847–3854.