Comparative Study

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Comparative Study of Natural Polymers and Titanium as a Medical Implant in Terms of Safety

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Abstract.

Titanium has been utilized as an implant material because of its mechanical properties, corrosion resistance, and biocompatibility. However, there are some issues in using titanium for medical implants such as particle release that could be toxic to the native environment. Therefore, it is necessary to find safer substitute materials such as natural polymers, which are found to be great in biocompatibility and less toxic. In this paper, we discussed the safety characteristics such as biocompatibility, biodegradability, and mechanical properties for both materials. The source of information was gathered through online databases, PubMed, using keywords such as titanium, 3D bioprinting, and implant, and further screened with biocompatibility, mechanical characteristics, gelatin, fibrin, cellulose, alginate, agarose, silk, in-vitro and in-vivo**.** Journal publications that did not discuss biocompatibility, biodegradability, mechanical qualities, could not be opened, and were not research articles were excluded. The 9 journals were selected based on the inclusion, title, and abstract. It can be concluded that natural polymers could be a titanium alternative based on its safety characteristics. Further studies are required to do more research about their safety to be used as medical implant material.

Keywords : titanium, natural polymers, implant, biocompatibility, mechanical properties

Abstrak

Titanium telah digunakan sebagai bahan implan karena sifat mekanik, ketahanan korosi, dan biokompatibilitasnya. Namun, ada beberapa masalah dalam menggunakan titanium untuk implan medis seperti pelepasan partikel yang dapat menjadi racun bagi lingkungan sekitar. Oleh karena itu, perlu untuk menemukan bahan pengganti yang lebih aman seperti polimer alam, yang ditemukan memiliki biokompatibilitas yang baik dan kurang beracun. Dalam makalah ini, kami membahas karakteristik keamanan seperti biokompatibilitas, biodegradabilitas, dan sifat mekanik untuk kedua bahan tersebut. Sumber informasi dikumpulkan melalui database online, PubMed, menggunakan kata kunci seperti titanium, bioprinting 3D, dan implan, dan selanjutnya disaring dengan biokompatibilitas, karakteristik mekanik, gelatin, fibrin, selulosa, alginat, agarosa, sutera, in-vitro dan dalam -vivo. Publikasi jurnal yang tidak membahas biokompatibilitas, biodegradabilitas, kualitas mekanik, tidak dapat dibuka, dan bukan merupakan artikel penelitian dikecualikan. Kesembilan jurnal tersebut dipilih berdasarkan inklusi, judul, dan abstrak. Dapat disimpulkan bahwa polimer alam dapat menjadi alternatif titanium berdasarkan karakteristik keamanannya. Penelitian lebih lanjut diperlukan untuk melakukan penelitian lebih lanjut tentang keamanannya untuk digunakan sebagai bahan implan medis.

Kata kunci : titanium, polimer alam, implan, biokompatibilitas, sifat mekanik

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BACKROUND

Medical implant insertion is a medical procedure that usually uses materials which are not originating from the human body to be implanted inside the human body. Titanium (Ti) is one of the materials used to be implanted to substitute the damaged organ that has supporting function. However, titanium was found to have toxicity towards the human body (Friehs et al., 2016; Kim et al., 2019; Pichat, 2010). The titanium particles that are introduced to human tissues are reported to have various tissue reactions due to formation of metal-protein complexes initiated by protein adsorption and desorption mechanisms (Friehs et al., 2016; Kim et al., 2019; Pichat, 2010). Tissue reactions include the inflammation or microbial biofilm formation which could lead to a locally acidified environment (Friehs et al., 2016; Kim et al., 2019; Pichat, 2010). According to meta-analysis research from Atieh et al. (2012), through MOOSE (Meta-Analysis of Observational Studies in Epidemiology) guidelines, prevalence of peri-implantitis was 18.8% and 63.4% for peri-implant mucositis (Atieh et al., 2012). Peri-implant disease has been a part of complication on implant insertion with titanium material and could persist for years (Kim et al., 2019 ; Atieh et al., 2012 ; Wilson et al., 2015). Journals that discussed complications of titanium material for implant insertion, were not the least. Therefore, to overcome this issue, it needs an alternative material which could lead to substituting titanium as an medical implant. In this paper, natural polymers will be suggested to be used as a medical implant alternative material which is produced by a 3D bioprinting process.

3D Bioprinting is a process of placing biomaterials combined with living cells to make a desired pattern layer by layer under the instruction of computer-aided design (CAD), resulting in living tissues or organs that are needed (Bociaga et al., 2019; P. et al., 2018; Seol et al., 2014; Željka et al., 2018; B. Zhang et al., 2018). The bioprinting process usually uses several methods depending on the machine. The combined biomaterials and cells could be called as bioink. Bioink materials could be divided into two types: scaffold-based and scaffold-free. Scaffold-based uses hydrogel as the main materials that will be the media of the cells to be loaded (Dissanayaka & Zhang, 2020; Ozbolat, 2015). While scaffold-free, not using exogenous biomaterials where the main material is the cells itself that will be first formed into neo-tissue and followed by deposited in specific patterns where they will then be fused and matured to become larger-scale function tissue (Dissanayaka & Zhang, 2020; Ozbolat, 2015). The scaffold-based bioink could be derived from natural and synthetic materials. The natural materials or can be called as natural polymers include Gelatin, Fibrin, Cellulose, Agarose, Alginate, and Silk while Pluronic or polyethylene glycol (PEG) is considered synthetic (Dissanayaka & Zhang, 2020; Ozbolat, 2015).

Since bioink natural polymers are a combination between living cells and natural materials, the biocompatibility of the implant would be increased. In other words, the cytotoxicity effect of those materials are expected to be less than titanium. On the other side, natural polymers are expected to fulfill the safety characteristic of being a medical implant. Research has been done evaluated the biocompatibility and cytotoxic properties of titanium and natural polymers in both in-vitro and in-vivo, but until recent studies, the comparison between bioink natural polymer and titanium has not been described in safety characteristic such as biocompatibility, biodegradability, and mechanical properties. With the research gap from above, the aim of this paper is to compare the safety characteristics between titanium and natural polymers which can be a good use for medical implant insertion.

METHODOLOGY

In this literature review (Sugiyono, 2019), the source of information was gathered through online databases from PubMed. The journals were searched using keywords based on research problem formulation steps, such as "titanium", "3D bioprinting", and "implant". The selected journals were targeted within the last ten years (2012-2022). Firstly, the study was screened by title and abstract, using the advanced keywords in PubMed such as "biocompatibility", "mechanical properties", "gelatin", "fibrin", "cellulose", "alginate", "agarose", "silk", "in-vitro", as well as "in-vivo". The inclusion of this study was selected based on the advanced keyword, research article, and include all the safety characteristics. While the exclusion criteria of this study are not primary research articles, the link is unavailable opened, and doesn't have safety characteristics such as biocompatibility, mechanical properties, and biodegradability.

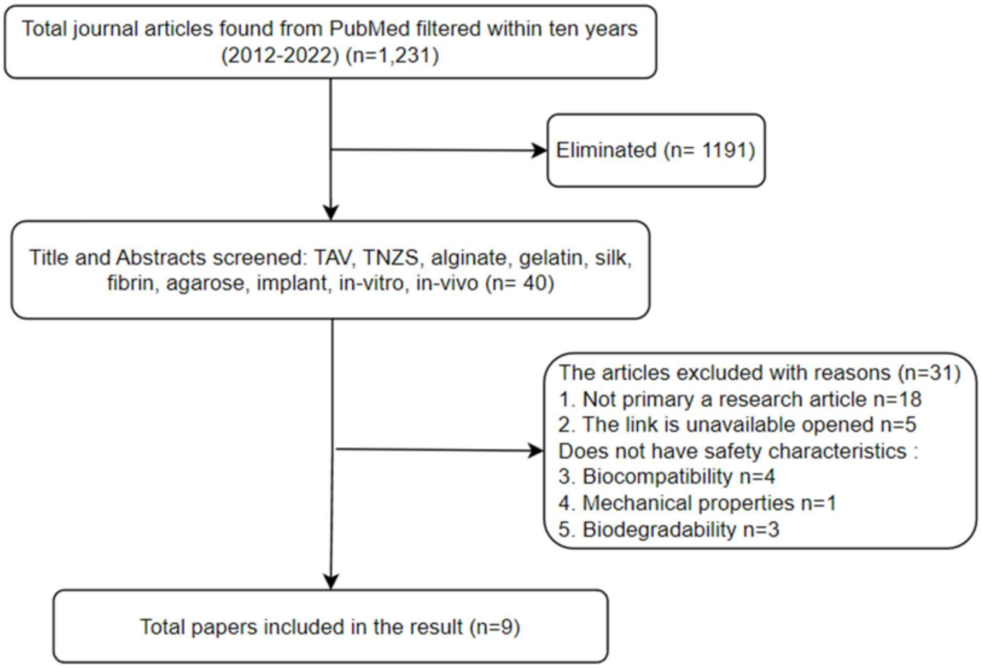

Figure 1. Flowchart of Journal Selection Method

Figure 1. shows that a total of 1,231 studies were located in the databases. The criteria was added into the search keywords in the databases and resulted in 40 journals. After the exclusion criteria was added, the authors selected 9 journals to be discussed in which described titanium and natural polymers biocompatibility in in-vitro and in-vivo study. The analysis of biological results from in-vitro study as the biocompatibility and mechanical properties evaluation of selected journals, can be seen in table 1.

RESULT AND DISCUSSION

According to the result from literature review, as can be seen in table 1, natural polymer and titanium component-based implants have significant differences at their mechanical strength and cytotoxicity. The safety of these materials include characterization and content of the implant, as well as biological responses that are related through the choices material, considered to be discussed.

Table 1. Analysis of Biocompatibility Evaluation in In-vitro Study of Selected Journals

No	Author (year)	Title	Objective	Study Design	Content of an implant	Biological Result	Ref
1	Wang et al. (2018)	Enhanced Osseointegration of Titanium Alloy Implants with Microgrooved Surfaces and Graphene Oxide Coating	Evaluating the biocompatibility of Ti-6Al-4V with laser treatment and Graphene Oxide coating in improving cell adhesion, proliferation and differentiation	In-vitro study	-Enhanced Ti-6Al-4V plate with Texturing and Graphene Oxide - BMSCs cells	The rougher surface of the implant increases the cell proliferation, adhesion and differentiation. (Non-cytotoxic)	87
2	He et al. (2020)	Titanium and zirconium release from titanium and zirconia implants in mini pig maxillae and their toxicity in vitro	Comparison of ZrO2/Ti release into bone tissues between ZrO2 and Ti implants	In-vitro study In-vivo study	- Ti and ZrO2 grade 4 implants - Pig's Maxillae - PDL-hTRET cells	There are particles released, causing toxicity to the cells. The number of Ti particles released was higher than ZrO2, with nanoparticles having higher toxicity compared to nanoparticles . (Have cytotoxic)	91
3	K. Zhang et al. (2017)	3D bioprinting of urethra with PCL/PLCL blend and dual autologous	Evaluation of the urothelial cells (UCs) and smooth muscle cells	In-vitro study	- A dissolved fibrinogen in calcium-free high glucose	The cells were well proliferated	96

		cells in fibrin hydrogel: an in-vitro evaluation of biomimetic mechanical property and cell growth environment	(SMCs) viability in cell-laden fibrin hydrogel for cell growth		DMEM - SMCs and UCs cells	in hydrogel. (Non-cytotoxic)	
4	Cordeiro et al. (2022)	Corn cob Cellulose Scaffolds: A New Sustainable Temporary Implant for Cartilage Replacement	Evaluation of the corncob-derived cellulose in PCL scaffold to replace wood-cellulose to be use as the potential cartilage-replacement	In-vitro study	-Poly-Ɛ-caprolactone (PCL) and corncob cellulose (CC) - L929 mouse fibroblasts	Cell viability and proliferation were increased. (Non-cytotoxic)	97
5	Zhang, Yahui; Yu, Yin; Akkouch, Adil; Dababneh, Amer; Dolati, Farzaneh; Ozbolat (2012)	In vitro study of directly bioprinted perfusable vasculature conduits	Evaluation of dehydration, swelling, degradation characteristic, perfusability, mechanical strength, and permeability capabilities of vasculature conduits	In-vitro study	**-** crosslinked alginate with a 4% CaCl2 solution - HUVSMCs cells	Cell viability decreased right after printing and increased the day after. (Non-cytotoxic)	95
6	Campos et al. (2015)	The stiffness and structure of three-dimensional printed hydrogels direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages	Evaluation of the mesenchymal stroma cell (MSC) viability in agarose hydrogel as a printable scaffold	In-vitro study	- cell-free Agarose hydrogel 3% with dissolving 3 g of agarose in tap water - MSC-osteogenic and MSC-adipogenic differentiation cells	Cell viability increased. (Non-cytotoxic)	98

7	Das et al. (2015)	Bioprintable, cell-laden silk fibroin–gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs	Optimization of the silk-fibroin bioink for cell encapsulation and printing	In-vitro study	-8SF-15G bioink (silk fibroin - gelatin crosslinked) - hTMSCs cells	Cells viable over 1 month in 8SF-15G-T are constructed in a stable 3-D structure. (Non-cytotoxic)	53

1. Titanium-based implant

Skeletal injuries have been found to be one of the common issues in the orthopedic field. Bone disorders such as fractures, osteoarthritis or bone defects caused by resection of the tumor are usually treated through implantation procedure. These days, osteo implant insertion use titanium as the main materials due to their properties, such as bio inertness, excellent biocompatibility, high fatigue and tensile strengths, low allergenicity and light in weight. In addition, titanium (Ti) has the ability to induce the formation of new bone and form tight bonds with newly formed bones. Thus, titanium can be called osteoconductive (Ahn et al., 2018).

Before titanium was found to be the materials for implantation, implants were discovered firstly using metal as the first generation. It is used due to the inertness and strength of the materials component itself, making it compatible to become an osteo implant.[13] However, scientists found that to become an implant, the materials need to cover the biocompatibility and bio inertness. Hence, it is needed to meet the factor of contact with body fluid and cells. In order to overcome it, titanium was used as it matched with the biocompatibility factor in the human body (Ahn et al., 2018; Nair & Elizabeth, 2015). As the second generation, pure Ti was used. The third generation of an implant then can be discovered to fulfill the ability of the materials to combine the factors needed, such as bioactivity and biodegradability. To overcome these factors, various methods have been used to modify Ti alloys to have excellent biocompatibility, including the cellular responses (Ahn et al., 2018; Nair & Elizabeth, 2015).

2. Titanium Types Used for an Implant

a. Ti-6Al-4V (TAV)

One type of Titanium that can be used for implantation to the human body is Ti-6Al-4V (TAV). It is a multi-phase Ti alloy composed of α and β structures, with 6% of aluminum and 4% of vanadium. The combination of these materials, make it possesses high beneficial properties, such as high strength, corrosion resistance and has high capability to create bonding with the bones and tissues (Ashok Raj et al., 2017; Mao et al., 2013; Philip et al., 2019). The corrosion resistance of these materials could be due to the stability of oxide layers presented as well as the low tendency of ion formation when contacting aqueous environment (Ganesh et al., 2012). It also found that it has higher strength than the commercially available titanium (pure Ti) (Ganesh et al., 2012; Hussein et al., 2015). However, the elastic moduli of TAV are reported to be comparatively higher than the normal human bone. Thus, could lead to bone fracture or loosening of an implant (Li et al., 2019).

b. Ti-24Nb-4Zr-7.9Sn (TNZS)

The other titanium alloy is Ti-24Nb-4Zr-7.9Sn (TNZS), which consist of titanium, niobium, zirconium and tin powder materials (Guo et al., 2012). It has microstructure with β-phase monolithic where it is found to be non-toxic. It has low elastic modulus, resistance to corrosion and high strength (Zhan et al., 2020). This alloy has a low Young's modulus of just 42–72 GPa and a maximum recoverable strain of 3.3%, making it an excellent choice for use in medical applications. The difference in elastic moduli between the two phases, the elastic modulus of the α-phase being greater than the β-phase, can be attributed to the alloy's change in elastic modulus. Although the elastic modulus of the alloy is not linearly dependent on the percentages of the phases, as is implied by the mixing rule (perhaps caused by inaccuracies in the measurements of elastic modulus or phase compositions), they are positively correlated (Li et al., 2020).

c. 3D bioprinting and bioink

3D bioprinting, which is denoted as 3D printing and biology, is a technique of layer-by-layer manufacturing of tissues (Hagenbuchner et al., 2021). It also can be defined as a printing process using cells that have been patterned through automated machines (Vanaei et al., 2021). In this technique, the biological materials, living cells, and biochemical materials are positioned precisely together with controlling the functional components placement, in order to fabricate the 3D structures (Hagenbuchner et al., 2021). There are several technologies in processing tissue bioprinting, which are microextrusion, inkjet, and laser-assisted printing. The difference between these technologies relies on the printing mechanism. Inkjet printers are also called drop-on demand machines which will proceed the printing process as a droplet. However, this machine can only be done using liquid form of biological materials (Guillotin et al., 2010). On the other hand, microextrusion has features to control the temperature while handling the materials for both the system of stage and dispensing. This printing technology has the ability to process very high densities of cells. Laser-assisted bioprinting is the printing method using a laser with rapid gelation kinetics requirement in order to achieve high shape fidelity (Jones, 2012; Lee Ventola, 2014).

3D bioprinting has been applied to many medical applications, such as tissue and organ fabrication; manufacturing prostheses, implants, and anatomical models; and pharmaceutical research including drug discovery, administration, and dosage form (Klein et al., 2013; Lee Ventola, 2014). This method also can be used in tissue engineering, which has the purpose of regeneration of the damaged tissue and reconstruction. Therefore, cell-laden structure is a need for tissue engineering. Cell-laden is a desired pattern of cells which has the ability to mimic the native cell's functions and structure. To obtain a successful cell-laden structure, highly versatile, non-toxic, and outstanding bioactive bioinks with great printability, suitable mechanical sustainability, and controllable biodegradability are essential (Ashammakhi et al., 2019).

In order to produce the desired form for medical purposes, bioink is the essential component which will be crosslinked or stabilized during the printing process or immediately after. The bioink selection also depends on the tissue constructs' purposes and application, to support the printing process and also the bioactivity of the cells (Gungor-Ozkerim et al., 2018). A single biomaterial typically cannot fulfill all mechanical and functional requirements required to generate biomimetic tissue-like constructions (Ashammakhi et al., 2019; Daly et al., 2016). Therefore it is usually combined with other materials, or it can be called as multi-component biomaterials.

There are two types of material as bioinks in 3D printing. In the biomedical sciences, the polymers that are produced as a biomaterial from natural resources are referred to as "natural biomaterials". These biomaterials have a more diverse advantage than synthetic material. The abilities are biomimicking of extracellular matrix structure, have the abilities of biodegradation and biocompatibility properties, self-assembling, and non-toxicity (Gopinathan & Noh, 2018). The second one is synthetic material which has several advantages. Nevertheless, the benefits that come from synthetic polymers are not present in natural polymers namely pH and temperature responses, controllable of mechanical stability, even tuning the biodegradation and biological properties to comply with tissue-specific degradation and mechanical property requirement of the target tissues and organs. Yet, it still represents a small number of the systems used in bioprinting because of their limitations such as using hazardous solvents, melting points greater than body temperature, and trouble encapsulating cells, also lack of cellular recognition sites and other biological signals inherent in natural ECM for stimulating cellular proliferation and differentiation (Khoeini et al., 2021).

3. Natural Polymers as Bioink Materials

a. Gelatin

Gelatin is the materials of bioink that are categorized as a natural protein which has amphoteric behavior because of the presence of alkaline and acidic amino acids functional groups (Mobaraki et al., 2020). This material is derived from collagen hydrolysis that usually can be extracted from animal's connective tissues. Gelatin has several advantages as bioink, such as being non-cytotoxic, can promote cell adhesion, it is water-soluble and has low immunogenicity (Hospodiuk et al., 2017). The other properties that allow the gelatin to be used as bioink materials is the biocompatible and biodegradability. With all these properties that gelatin has, it can be used in the 3D bioprinting in the form of gelatin methacryloyl (GelMA) (Bertassoni et al., 2014; B. H. Lee et al., 2016; Nichol et al., 2010). The other advantage is gelatin is a polymer that has temperature-controlled phase transition in physiological conditions. However, gelatin tends to be fast degraded due to its limitation of inferior mechanical strength. Therefore, it limits the exclusive usage (Singh et al., 2019). Because of the properties that gelatin has, it is allowed to be combined with the other materials.

Types of gelatin that are usually used as bioink is known as Gelatin methacryloyl (GelMA) which is made from gelatin that is already being chemically modified with methacrylic anhydride (MAA) (B. H. Lee et al., 2016). GelMA hydrogels have high similarity to natural dermal ECM. It has higher biocompatibility and better mechanical and degradation properties compared to other types of hydrogel, such as collagen (Piao et al., 2021). Gelatin also has lower antigenicity compared to collagen (Yue et al., 2015). Because of all the properties GelMA hydrogel has, it can be mixed with other components in order to make it have appropriate properties for tissue engineering.

b. Fibrin

Fibrin is a biopolymer that originated in the human body which plays a role in healing processing during the blood coagulation which is derived from fibrinogen (de Melo et al., 2020). The viscosity of pure fibrinogen will not increase linearly when the concentration is increased. The commencement of fibrin clot formation (gelation) causes substantial changes in mechanical properties, which may be identified by a change in turbidity and an increase in the elastic or shear modulus in rheological studies (Shpichka et al., 2020). The resulting fibrin gel possesses extraordinary and unique viscoelastic capabilities among polymers, which are connected to its molecular structure with complicated multi-scale hierarchy. The pure fibrinogen has low viscosity, thus making it very suitable for inkjet bioprinting methods (Panwar & Tan, 2016). However, the gel's mechanical properties are relatively poor because of its fast fibrin gelation and irreversibility, making it usually performed in low temperature. It could also be combined with alginate, gelatin, hyaluronic acid, or collagen according to its applications (Zhao et al., 2014).

Fibrin gel is cell supporting and allows for adequate cell development and function. It has also been demonstrated that combining fibrin with gelatin alginate bioinks resulted in comparable cell viability to constructs using solely gelatin alginate (S.V. et al., 2013). On the other hand, fibrin also has tunable properties which can lead the cells and allow it to determine substance release kinetics, hence making it suitable for use in skin treatment (Shpichka et al., 2019). Fibrin-based product has been approved by FDA to be used as sealant, hemostat, and adhesive (Roberts et al., 2020). Sealants are designed to prevent fluid leakage by producing a barrier that may cling to or be mechanically interlocked with tissues. Adhesives (or glues) are meant to stick to structures and bind them together, therefore providing or restoring mechanical integrity (Spotnitz, 2014). Although sealants and adhesives physically halt the flow of blood, they do not actively produce hemostasis. Fibrin is the only clinically authorized substance that can perform all three functions, making it appealing for a number of therapeutic circumstances (Mandell & Gibran, 2014).

Because of its strong bioactivity and mechanical strength after crosslinking, it has been employed as an additive with other biopolymers for extrusion bioprinting. The incorporation of fibrin with gelatin, alginate, and collagen has been shown to offer a bioactive cue as well as mechanical support to bioprinted constructions, resulting in improved form fidelity (Das et al., 2015). By incubating fibrin gels with thrombin at room temperature, fibrinogen is crosslinked. Thrombin protease cleaves fibrinogen at two places, producing symmetrical structures that assemble non-covalently. The primary drawback of utilizing fibrin is that it gels quickly and irreversibly at body temperature, making bioprinting problematic (Desimone et al., 2015). To avoid early crosslinking, thrombin and fibrinogen blends can be printed at moderate temperatures simultaneously, or separate thrombin deposition can be performed over a construct for crosslinking following 3D bioprinting (Zhao et al., 2014;Desimone et al., 2015).

c. Cellulose

Cellulose is a primary structural material in plant cell walls due to its rigid structure. The complex composition of cell walls offer a favorable basis in design and fabrication with materials having superior properties. The form of cellulose such as lignocellulose, bleached pulp, and dissolving pulp are available in the market (Wang et al., 2018). But recently, microsized and nanosized cellulose including microcrystalline cellulose (MCC), cellulose nanofibrils (CNF), cellulose crystalline (CNC) and bacterial cellulose with tailor made properties could be isolated (Isogai & Bergström, 2018). Cellulose materials without chemicals are impracticable in bioprinting methods such as extrusion or sintering based 3D printing (Desimone et al., 2015). Whereas, nanocellulose hydrogels might be considered as extrudable precursors for 3D printing. In order to achieve a good minimum requirement for extrusions, it needs to have good extrudability through sized nozzles and good shape fidelity of the dispensed filament (Wang et al., 2018). Both properties can make nanocellulose hydrogel to be a great potential for biomedical and other applications.

Carboxymethyl cellulose (CMC), a water soluble cellulose, has been used to modify the viscosity with the other polymers (Benwood et al., 2021). A researcher found that when CMC combined with a poly(lactic-co-glycolic acid) bioink it could create the ideal viscosity for deposition, meaning it would not obstruct the syringe tips. Thus, it can create bone tissue (Sawkins et al., 2015). Another researcher has combined CMC with glycol chitosan hydrogels to produce a gel-based ink that has both stability and cell compatibility, and it helps to stabilize and shape fidelity to the final construct (Janarthanan et al., 2020).

Cellulose nanocrystals is another form of cellulose that can be used for its mechanical strength along with shear thinning behavior (Wu et al., 2018). It is incorporated into many different bioinks, improving the elasticity, strength, and porosity of the constructs created, and when blended with other materials can also improve the viscosity of bioinks (Benwood et al., 2021). In addition, this form doesn't authorize the bacteria to grow, it will make a good benefit option for wound dressing instead (Markstedt et al., 2015). In other words, cellulose will promote all the benefits into a bioink and make a good result for the human body.

d. Alginate

Alginate is a biocompatible anionic polymer derived from brown seaweed, and has been used widely for many biomedical applications because of its biocompatibility, low toxicity, relatively low cost, and mild gelation. Alginate hydrogels may be created using a variety of cross-linking techniques, and because of their structural resemblance to extracellular matrices found in live tissues, they have a wide range of uses in the treatment of wounds (K. Y. Lee & Mooney, 2012). Alginate wound dressings keep the wound site's surroundings physiologically wet, reduce bacterial infection, and speed up the healing process. Depending on the cross-linker types and cross-linking procedures used, drug molecules ranging from tiny chemical medicines to macromolecular proteins can be released from alginate gels in a regulated way (K. Y. Lee & Mooney, 2012). Alginate can use hydrogels to deliver cells for tissue formation and tissue engineering.

Alginate has low viscosity and zero shear viscosity so it can retain its shape. There is a disadvantage of alginate-based bioink. Proteins are poorly absorbed due to the very hydrophilic nature of alginate, limiting cell adhesion (Hospodiuk et al., 2017). Alginate is unique among other bioink that it has very low biological activity which can lead to unsupported cell proliferation. To overcome this limitation, it needs to be incorporated with other bioactive materials (or growth factors) (J. Lee et al., 2020). Hoffman mentioned that bioceramics combined with alginate-based-cell-laden structures have been widely used (Wüst et al., 2014).

e. Agarose

If alginate is derived from brown seaweed, then agarose is derived from red seaweed and contains D-galactose and 3,6-anhydro-L-galactopyranose (Benwood et al., 2021). Agarose has been widely used for bioprinting and tissue engineering applications because of their biocompatibility. It can be prepared as a thermal-reversible gel. There are three steps to produce agarose gelation; induction, gelation, and pseudo equilibrium, and finally these steps will result in a formed gel in the helical structure of the agarose molecule. Agarose hydrogel can be formed without the need for toxic crosslinking agents like genipin and making it a biocompatible polymer (Zarrintaj et al., 2018). There are some reports approving agarose-alginate to be a suitable material for 3D bioprinting for cartilage tissue engineering and promote an excellent cell viability for 21 days in culture (López-Marcial et al., 2018).

f. Silk

Silk is a natural biopolymer produced from silkworms, spiders, and insects such as flea, ants, cricket, *and Bombyx mori (B. mori)* (Can & Ateş, 2016). It has many functions for biomedical application based on its diversity. There are some reports that exhibit the silk natural biopolymer has been used in a variety of applications by using its bioactive molecules, growth factors and signaling cues, proliferation, and differentiation for cell or tissue reconstruction (Chimene et al., 2016). The natural form of silk consists of a filament core protein, silk fibroin and sericin proteins. Fibroin and sericin is a primary protein, each of the protein contains serine amino acids, glycine, and alanine (Can & Ateş, 2016). The amino acid sequences of silk proteins are various between species, therefore it will have a wide range of mechanical properties.

Silk fibroin from mulberry and non-mulberry silk have been well-explored and used as the most common biomaterial. Non-mulberry silk such as *Philosamia ricini* called Eri, has superior mechanical properties in comparison to mulberry silk. The presence of poly-alanine sequences in Eri enhances its mechanical properties. But either mulberry and non-mulberry silk are suitable polymers for bioink constructions (Singh et al., 2019). Silk fibroin (SF) is a part of primary protein that has been used in biomedical application because of their controllable degradability, biocompatibility, effectively support cell functionally, and well-defined mechanical properties (Singh et al., 2019).

The components of silk allowed it to be combined with other materials, such as gelatin. The components between silk and gelatin were used as crosslinker-free hydrogel. They come to demonstrate a biocompatibility either in vitro and in vivo, providing enhancement in soft tissue regeneration (Singh et al., 2019). Using cross linkers such as glutaraldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, and genipin resulted in a downgrade in printing resolution. They have negative effects such as toxicity to cells, structural alteration to polymers, and cost escalation (Irvine & Venkatraman, 2016; Roseti et al., 2018). The usage of crosslinkers is linear with the cytotoxic effects. The higher and longer term of using a crosslinker, it will increase its cytotoxicity.

However, the drawback was found in using this biomaterial since SF has low viscosity and frequent clogging during printing. But to overcome this issue, it needs to mix with another polymer such as gelatin and optimize for its rheology matters (Mobaraki et al., 2020).

5. The Safety Characteristics

a. Titanium Implants

To find out a more suitable implant, the materials should have structural and surface compatibility as biocompatibility. The structural compatibility depends on its dimensions, geometry, as well as its strength and elastic modulus (Mukherjee et al., 2018; Zheng et al., 2022). In this case, titanium could fulfill the structural compatibility standard. On the other hand, surface compatibility depends on its surface morphology, tribology, corrosion properties, wettability of implants, etc. To fulfill the surface compatibility requirements, it is usually used titanium compounds that could coat the surface of the titanium implant (Mukherjee et al., 2018; Zheng et al., 2022). The other factor that is important as the biocompatibility for titanium is the surface wettability. When titanium is introduced into the human body, the first contact that will occur is between the material and human body fluid (Ashok Raj et al., 2017; Philip et al., 2019). Therefore, it is necessary to ensure that the titanium is eligible for contact with fluid for long periods of time.

Following the requirements of titanium implants, some mechanical properties must be considered, such as modulus of elasticity, tensile strength, yield strength, ductility, and hardness/toughness. Modulus of elasticity is a property that is concrete with elasticity. It has the important role of responding to mechanical stress. In order to balance the impact of the externally applied forces of occlusion or muscular activation, an implant must be loaded to produce forces (F) and stresses within the bone. A state of static equilibrium may be created by these forces (Amarnath et al., 2011). The function of elasticity property is to balance mechanical strain within the bone and the implant. It must be sure that the implant will make a uniform distribution of stress without changing the integrity of metallurgic and clinical (Saini, 2015).

When a force is applied to a biomaterial or bone, one of the additional qualities that results in a change in dimension (strain) that is proportional to the elastic modulus is tensile strength. In other words, it should have the result to improve functional stability and prevent any fractures (Amarnath et al., 2011). Yield strength has a function to prevent brittle fracture under cyclic stress, an implant material should have a high yield strength and fatigue strength.[75] Ductility also plays a role. If an implant possesses ductility and won't alter or compromise its integrity, it can shift into the required shape. One of the qualities that stop the implant from fracturing is hardness and toughness (Amarnath et al., 2011).

Another important requirement for an implant is the surface properties. The tension and energy of the surface will determine the implant's wettability. Wettability is described as a liquid's ability to sustain contact with a solid surface, which can be determined by the intermolecular forces at the interacting phases' interface (Chi et al., 2021). Where it is important to have good wettability in order to allow the materials interacting with body fluids. Osteoblast is found to have improved adhesion on implant surfaces when it has higher wettability (Saini, 2015).

b. Natural Polymers as Bioink in 3D Bioprinting

To reach a desired physicochemical, bioink needs to fulfill an important requirement before being used for 3D printing. The ideal desires of physicochemical properties include proper properties, chemical, rheological, and biological characteristics. These ideological properties are needed for making adequate mechanical properties and robustness, stabilization and convertible gelation to reach high shape of fidelity, biodegradability mimicking the natural microenvironment of the tissues, has a capacity to be chemically modified to meet tissue-specific requirements, and the ability to produce on a big scale with minimal batch-to-batch variance (Gungor-Ozkerim et al., 2018). As spoke in introduction, there are two approaches in 3D bioprinting. The first one is scaffold based bioink, which are 3D tissue structures that are developed by printed biomaterial and live cells. The scaffold biomaterial dissolves in this environment, and the encapsulated living cells develop and occupy the space to produce the pre-designed tissue structure (Gungor-Ozkerim et al., 2018;Hospodiuk et al., 2017). The second approach is scaffold-free based bioink, where living cells are directly printed in a process similar to embryonic development. The neo tissues are formed by a selected set of living cells, which are later deposited in a precise configuration to produce merged big functional tissue structures throughout time (Gungor-Ozkerim et al., 2018;Hospodiuk et al., 2017).

f. Issues, Similiarities, Differences, and Research Gaps

Titanium is a widely used implant material that has been known to have good quality, such as high strength, good mechanical properties, and corrosion resistance (Dias Corpa Tardelli et al., 2020). The titanium compound has been used as an implant material for a long time. However, the long-term use of titanium-based implants was found to have approximately 70% of failures due to the patient's immune reaction (Albrektsson et al., 2012; Derks, n.d.; Messous et al., 2021; Moraschini et al., 2015). It could induce inflammation, as a result, causing diseases such as mucositis, peri-implantitis, or periodontitis (Albrektsson et al., 2012;Cecchinato et al., 2013; Mehrotra N, 2022). On the other hand, natural polymers materials were derived from nature and combined with living cells (native environment), creating a new model of implant insertion. Based on Table 2, the combination of natural polymers will be discussed in one.

Furthermore, there is another issue found by Shi L, et al. (2020) that titanium alloys, which is Ti-6Al-4V (TAV), had to have a higher elastic modulus than the human bone.[9-] The significance of mismatch between the elastic modulus of implants and human bone may become the causes of stress, shielding, bone resorption and implant loosening. The moduli mismatch is more severe in osteoporosis than in normal bone tissue, which is expected to result in increased rates of implant failure and loosening. As a result, a new implant with a low elastic modulus and great strength is required (Shi et al., 2013). They found the novel Ti-24Nb-4Zr-7.9Sn (TNZS) acts as an implant, which is a more suitable environment for osteogenic differentiation than TAV because of its low elastic modulus and high ALP (alkaline phosphatase) activity. But still, both implants had particles released to the bone which can cause toxicity to native environment. It was found that the number of particles released of Ti content was higher compared to Zr content. Moreover, in comparison within sizes of particles released, nanoparticles were found to cause more toxicity compared to microparticles (He et al., 2020).

On the contrary, the bioink natural polymers are known to have less tendency to be toxic material as they are mostly derived from non-synthetic materials and there is no risk of particles being released to cause any toxicity. Natural polymers resemble the extracellular matrix's native structure and composition. Its stimulating activities enable the addition of growth factors and other proteins able to improve cellular processes. However, it is found to quickly degrade, due to their low mechanical strength (De la Puente & Ludeña, 2014). To overcome these issues, natural polymers were not solely used alone but combined with the others (synthetic material or other natural polymers), allow it to have higher mechanical strength and less tendency to degrade easily. Hence, natural polymers have unique characteristics of their own, allowing them to be combined and made into any desired organs, unlike titanium. The other issues that are found in the natural polymers as a medical implant takes a lot of cost and is complex for regulatory approval. The cost is much higher than the synthetic polymer that can limit the uses to be medical implant material. The regulatory approval will take a lot of time due to their unclear current guidance.

In addition, all the types of bioink natural polymers are great for implant insertion because of their low toxicity. In the in-vitro studies, these kinds of natural polymers have their own potential to print a specific organ with a combination of native living cells, without giving toxicity effects to the cells. For instance, alginate is also known to be one of the natural biomaterials that are not only compatible but also provide the substrate needed for the cells to attach and proliferate (H. Zhang et al., 2021). Practically, it has low cost, low toxicity, and excellent biocompatibility (Duan et al., 2013). Even though alginate has been widely used in the biomedical field, research still needs to be done to improve its biocompatibility and mechanical properties, as well as providing good oxygen requirements for the cells that have been microencapsulated in the scaffold (H. Zhang et al., 2021). A study by Zhang Y, et al., (2015), demonstrated the biocompatibility of alginate to be used as perfusable vasculature conduits (Zhang, Yahui; Yu, Yin; Akkouch, Adil; Dababneh, Amer; Dolati, Farzaneh; Ozbolat, 2012). Based on the result, 4% alginate was generally the better concentration to be used, since the higher concentration of the alginate will provide higher tensile strength, but too high will lower cell survival (Zhang, Yahui; Yu, Yin; Akkouch, Adil; Dababneh, Amer; Dolati, Farzaneh; Ozbolat, 2012).

CONCLUSION

The safety of an implant for both titanium and natural polymers depends on the mechanical properties, biodegradability, and its biocompatibility. The terms of safety can be referred to whether it causes toxic to the cells or not, controllable biodegradability for the long-term performance, and its mechanical properties provide a good environment for the native cells. Study showed that improving the pores structure of titanium implants could increase cell proliferation and differentiation, and also improve their biological activity. Even though titanium serves the good mechanical properties and corrosion resistance to be an implant material, study showed that the toxicity of using titanium as an implant material is still quite high. The evidence showed that the possibility of the release of titanium particles could cause toxicity to humans and a high elastic modulus in titanium, such as TAV, which could be a problem for long term uses. On the other side, studies for all natural polymers discussed in this study showed no toxicity. In-vitro study described that the natural polymers allowed the cells to proliferate. Meanwhile, the degradation rate for each biomaterials is different and it affects the long-term uses. This challenge will be studied in the future.

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