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Carbon nanotubes for bone tissue osseointegration engineering: A review

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Abstract

In bone tissue engineering, latest advancements have drastically improved the process of surgical healing by carbon nanotubes that have grown as biocompatible products to address bone damage or deficiencies. The latest reconstructive solutions related to the bone tissue engineering industry have several benefits, but still, some inconveniences continue to emerge. The integration of carbon nanotubes as a replacement medium for implantable bone tissue enhances dramatically the mechanical properties of different biomaterials to scaffold the damaged region, which imitates natural bone. Carbon nanotubes and bio-matters have been improved recently for bone tissue applications. This review aims to analyse the utilisation of carbon nanotubes as bio-composite in bone tissue by comparing the biophysical and chemical properties of carbon nanostructures and discussing the functionality in bone tissue regeneration and how it can be utilised in the future in Malaysia.

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1.0 Introduction

1.1 Bone tissues

Bones are tissues that support the body's weight and are the centre of action for muscle attachments. Although bone tissues are naturally self-healing, complex fractures and large bone defects are difficult to heal, which could lead to non-unions. Fracture cure is usually classified as (i) direct healing without callus formation, which occurs during appropriate cure and repairing, and (ii) intermediate healing with callus formation, which allows the bridging of bone fragments. The most popular method of fracture cure is indirect fracture healing (Marsell & Einhorn, 2011). The first two days of recovery right after the wound is inflammatory. Blood deformation is worrisome as a cause in the growth of bone marrow, bone cortex, and periosteum. Tumours grow out of control and can allow cancer to spread. This is caused by the growth of tumours and the immune response. Tumour necrosis factor (TNF) is a molecule that reaches the body and induces inflammation. Inflammation is where the immune system responds and destroys cancer (Baht et al., 2018).

The bone mineral consists of hydroxyapatite (HA) carbonated. The bulk of the chemical composition of calcium phosphate is given by HA–$Ca_5(PO_4)_3(OH)$. HA is the most stable chemical type in the aqueous state of these minerals (Franco et al., 2010). The use of HA as bone regeneration biomaterial is prolific between 0 and 43 percent of its total weight, depending on the bone (Nudelman et al., 2010). Biphasic calcium phosphate, β-tricalcium phosphate (β-TCP), and HA are the most widely used phosphate biomaterials for calcium (BCP) (Yamada et al., 1997).

New bone formation is induced by precipitation of calcium phosphates in the extracellular matrix (ECM) atmosphere by the production of HA crystal mineralisation (Barrère et al., 2006). The gully materials that exogenously supply these minerals will speed up bone formation. Exogenous calcium has shown that they either function chemotactically, encouraging osteogenic cell propellant migration, or act on osteogenesis regulators (Topala et al., 2009). These osteogeny signals were also found to be mediated by alkaline phosphatase activity in reaction to phosphate ions with increasing mineralisation (Lakhkar et al., 2013).

The tissue engineers' interests are the greatest because the porous structure permits the transport and redevelopment of nutrients and minerals, all are essential to tissue growth. The cellulose bone at the nanoscale consists of parallel collagen fibrillar type I, over which the platelets are produced with HA carbohydrate crystals (Chen et al., 2021). They are longitudinally aligned with the osteons and lamellas plane. The collagenic component of this formation is regarded as a long triple helix Tropocollagen molecule rod-like as shown in Fig. 1 (1.50 nm diameter and 300 nm long).

1.2 Tissue engineering

Tissue engineering is a field that has grown in biomaterials production, which involves incorporating scaffolds, cells, and biologically active molecules into tissues. It deals with health conditions caused by tissue and organ injury and loss. It is also an interdisciplinary area, as defined by Langer and Vacanti in 1999, that applies the principles of technology and the life sciences to the production of bio-substitutes that restore, maintain, and improve the role of the tissue. Tissue technology aims to assemble functional structures that repair, retain, or strengthen weakened tissues or entire organs. Popular tissue engineering techniques concentrate on constructing cell life-supporting systems of scaffolds. In comparison to the effort to replace the complex functions and exceptional properties of biological tissues directly, these scaffolds are constructed to promote the stable growth of tissue-built cell populations.

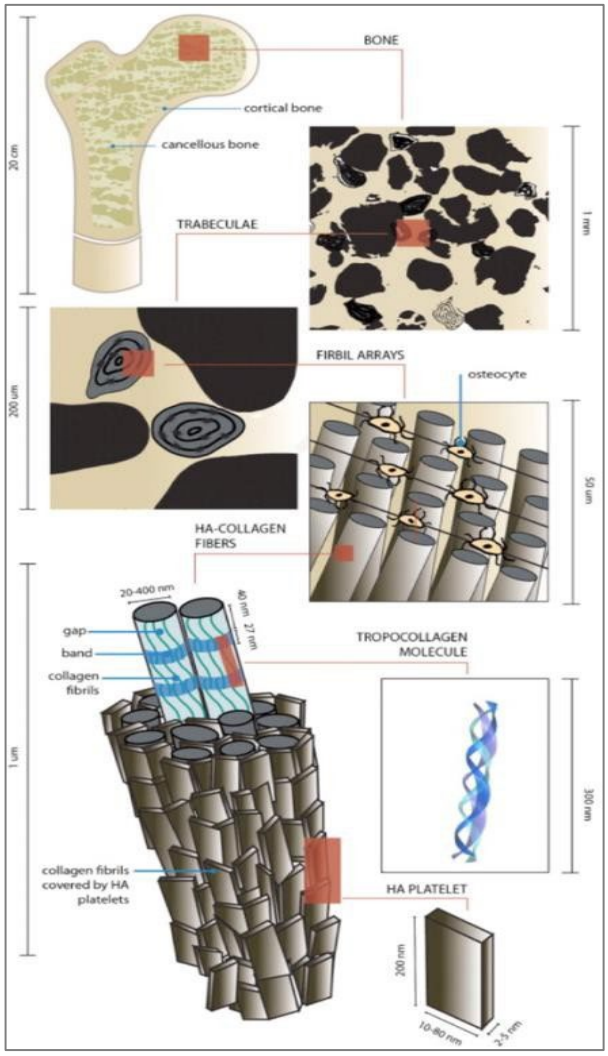

Fig. 1: Demonstrations of the structural hierarchy of multi-length formation in the bone (Tanaka et al., 2020)

Tissue engineers are equipped to create cell-sustaining environments. In the tissues, an ECM is considered to sustain a normal cell environment. The ECM is in a cell-secret biochemical assembly that supports cells' structure and chemical. A centre of some 300 distinct proteins is used to generate the standard ECM of the mammalian. The ECM provides structural and biochemical protection to neighbouring cells, a three-dimensional network of extracellular macromolecules including collagen, enzymes, and glycoproteins. Because of the individual multicellularity of multiple multicellular lines, the composition of ECM varies between multicellular structures; however, cell-to-cell connectivity and differentiation functions are typical functions of the ECM. ECM protein consists of repeating units forming long fibre-like chains of nanoscale insoluble, crosslinked macromolecules. The proteins are roughly defined into three distinct groups: collagen, glycoproteins, and proteoglycans.

There are 28 different kinds of collagen, each with its special structure and molecular shape, made from 43 various collagen subunits (Ricard-Blum & Ballut, 2011). However, the various forms of collagen, the composition of collagen, and the preparation and assembly of the different ECM are varied in all collagen participants.

Cells communicate by cell molecules, e.g., integrins, to the extracellular environment. Extracellular environment or space refers to the section of a multicellular organism outside of the cells, which is typically carried outside and filled by fluid. The term extracellular in cell biology, molecular biology and related fields means "outside the cell". This relationship influences cell attachment and cytoskeleton development. These changes in the substratum have been shown to influence cell adhesion, migration, development, and gene expression. Living cells, whether in the body or an artificial setting, are regulated by a complex integration of biochemical,

mechanical, and architectural elements exchanged by proteins, enzymes, and other molecules in an extracellular sense (Murphy et al., 2014).

In 2006, Berry et al. revealed the elastic features of the cell fate of human mesenchymal stem cells as an extracellular environment (hMSCs) (Berry et al., 2006). They showed soft substrate neurogenesis, while stiffer substrates stimulated osteogenesis, which was consistent with the tissue elasticity of the phenotype induced. These observations reflect the disparity in substrate rigidity, which results in variations in cell-transmitted and sensed forces (Zernicka-Goetz, 2002). Cells translate physical inputs into biochemical signals through certain mechanical transudative processes, regulating cell proliferation and behaviours of differentiation (Kim et al., 2012). It has been determined that regions on a cell that are of concern are limited to produce the same effects. Cells restricted to areas with comparatively limited compactness (ratio of the width of forms, divided by their region as in Fig. 1), such as a ring, lead to less intracellular forces in comparison with areas with high compactness, including slender rectangles, in which the cell has greater extracellular environment forces (McBeath et al., 2004).

1.3 Biomaterials for tissue engineering

Biomaterials can be organic and artificial by their origin in certain medical applications, help bolster, or removes the healing process in or out of an organism. In Ancient Egypt, the use of biomaterials for catheter repair is common, as are liver and lung bandages and both liver and kidney transplants. Besides, bio-printed anatomical structures (to be inserted in patients) was also successfully used in the same period. These early deployments of biomaterials ensured a proper functional substitute of the tissue substituted. Therefore, materials selected for such purposes were targeted in a host that also resulted in extremely inert materials, such as dental gold, to produce a minimally damaging reaction (Singh et al., 2009).

The use of biomaterials-free systems that sustain cell life is one technique for tissue engineering. A bioactive scaffold efficiency must be achieved by a changeable biomaterial, therewith it substitutes the following cells or tissue, and it is to suit its function, and it can be used in the area where the patient lives (Ji et al., 2011). A class of bio ceramics that are promising for applications in bone tissue processing is bioactive porous ceramic scaffolds. There were plenty of experiments performed on ceramics for the following purposes: hydroxyapatite, β-tricalcium phosphate, biphasic calcium phosphate, and bioactive glass. These materials share structural similarity with bones and have a high calcium and phosphate minerals composition (Lakhkar et al., 2013). The regenerative mechanism of cure by release and replacement of these minerals in the bone has been supported by such a composition. The presence of these minerals is also considered to facilitate a beneficial climate for osteogenic cell attachment, spread and differentiation. Also, there are various techniques available to make these ceramics into bone-like architectures, as well as being compositionally like bone (Hutmacher, 2000).

1.4 Nanotechnology for bone tissue engineering

Nanotechnology typically covers the area in applied science and technology, in which cohesive emphasis is the analysis in the molecular-level matter in dimensions down to 1 micrometre, normally 1 to 100 nm, and the production of products within a certain scale spectrum.

A nanometre is one billionth of a meter. Illustration to be portrayed on nano content is 25,400,000 nanometres per inch, a newspaper sheet is about 100,000 nanometres thick and a comparable measure, if marble is a nanometre, then one meter will be the Earth's dimension. A scale restriction to the 1–100 nm scale, the area that is scale-dependent quantum effects weigh heavily, also seems to exclude numerous properties of materials, especially in the pharmaceutical sector, for being focused on a sub-100 nm range.

Due to its low durability and susceptibility to fractures, the use of bioactive ceramics to rejuvenate bone tissue was restricted (Li et al., 2016). The materials resulting from this are poorly stable and potentially disastrous. Attempts have switched to substitute ceramic materials, including calcium silicones, to fix these issues. Generally, defining the advantages of dissolution products that cause HA-mineralisation and contribute to new bone growth, calcium silicates are better suited than calcium phosphates (Venkatraman & Swamiappan, 2020).

1.5 Carbon nanotubes for bone tissue engineering

By referring to Table 1, the strength of the multi-walled carbon nanotubes (MWCNTs) is higher than the steel and epoxy, and its diameter is greater than that of single-walled carbon nanotubes (SWCNTs), between 2 nm and 100 nm. CNTs in composites can be treated as biomimetic nanocomposites of collagen fibres at the

level of the cell hierarchy for bone tissue engineering and regeneration (Ahadian et al., 2016). Not only can they have a beneficial effect on cell adhesion enhancement by a strong cell-binding relationship, but also cell morphology and stem cell differentiation and acceleration because of preferential affinity to cell binding, thereby promoting osteoblast distinguishment and apathy mineralisation (Li et al., 2012).

Table 1: Density, tensile strength, and Young's modulus of carbon nanotubes contrasted with other materials (Osmani et al., 2014)

Material	Young's Modulus (GPa)	Tensile Strength (GPa)	Density (g/cm^3)
Single wall nanotube	1054.000	150.000	-
Multiwall nanotube	1200.000	150.000	2.600
Steel	208.000	0.400	7.800
Epoxy	3.500	0.005	1.250
Wood	16.000	0.008	0.600

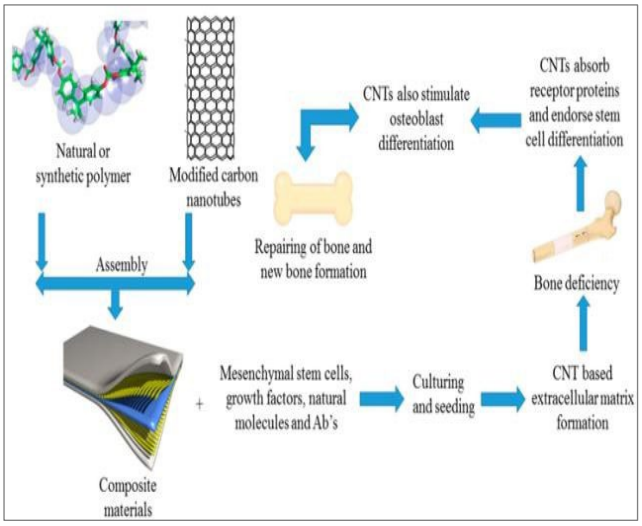

Fig. 2: Carbon nanotubes (CNT) as a nanocomposite-based scaffold (Lekshmi et al., 2020)

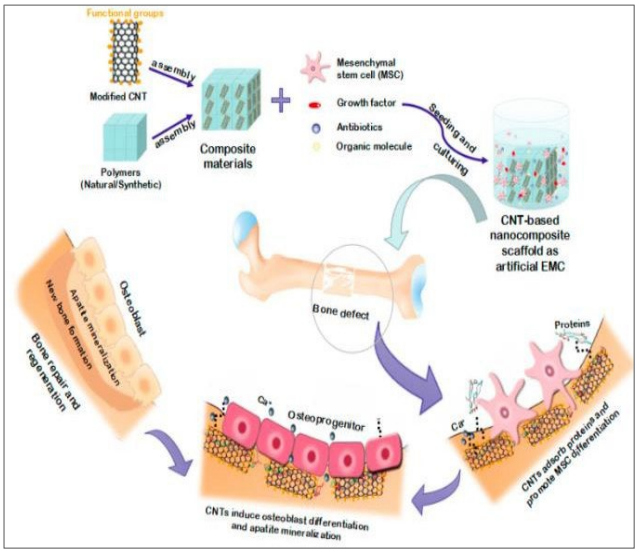

Fig. 3: Diagram explaining the role of carbon nanotubes (CNTs) as scaffold composites (Pei et al., 2019)

It should be concerned, however, that single CNT products cannot be used for regenerative bone tissue cultivation. The pure CNT skinning module has much higher axial strength, durability, and modulus than the bone tissue that does not correlate well to human tissue. CNTs can only make use of their mechanical, electrical, or surface features through the composite application with other materials and enhance overall physical and chemical characteristics and the conductivity of bones of the composite materials. The way how CNTs are being used in bone tissue scaffolds is depicted in Fig. 2 where the CNTs act as a nanocomposite-based scaffold.

Fig. 3 explains how CNTs are being conjoined to be a composite until the tissue regenerates accordingly and the reason why single CNT products cannot be used for regenerative bone tissue cultivation solely.

2.0 Comparison of CNTs-based scaffolds for bone tissue engineering

2.1 Material

For bone tissue engineering osseointegration scaffold, MWCNTs that are being used in this review is the one produced by carbon vapour deposition (CVD). The reason of why CNTs synthesised from CVD is selected because it can be produced in mass easily compared to the arc discharged method and laser vaporisation method (Mikael et al., 2012) and contain less toxicity and have the potential to extend their biological effects (Allaedini et al., 2016). Thus, the MWCNTs will be compared for bone tissue scaffold engineering for the composite of biodegradable and non-biodegradable. The comparison consists of carbon nanotubes-hydroxyapatite (CNTs-HA) and CNTs-natural biopolymer. Fig. 4 shows the scaffolds for bone regeneration include biodegradable and nonbiodegradable scaffolds, as well as composite scaffolds comprised of a combination of each material.

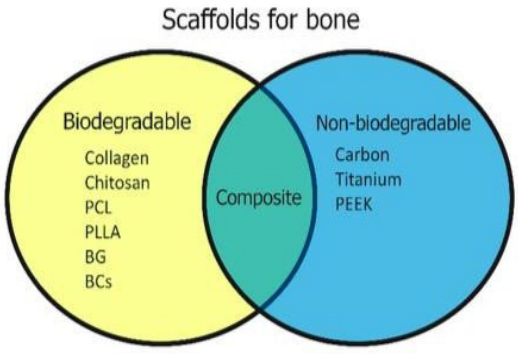

Fig. 4: Scaffolds for bone regeneration (Tanaka et al., 2020)

2.2 *Calcium phosphate material: carbon nanotubes-hydroxyapatite (CNTs-HA)*

Hydroxyapatite is excellent for simulating the natural function of bone, with good biocompatibility, and is thus commonly utilised in artificial bone-like scaffolding. However, because of their exceedingly delicate nature, their tensile strength and toughness to crack are poor due to their tremendous weights, such as bone. The mechanical features of CNTs can be used to optimise the bone engineering bioavailability of HA. Fig. 5 shows the preparation process of bone scaffolds using the composite of carbon nanotubes and hydroxyapatite.

As shown in Fig. 6, the implant of CNTs-HA scaffolds is placed in the rabbit's radius orthopaedic.

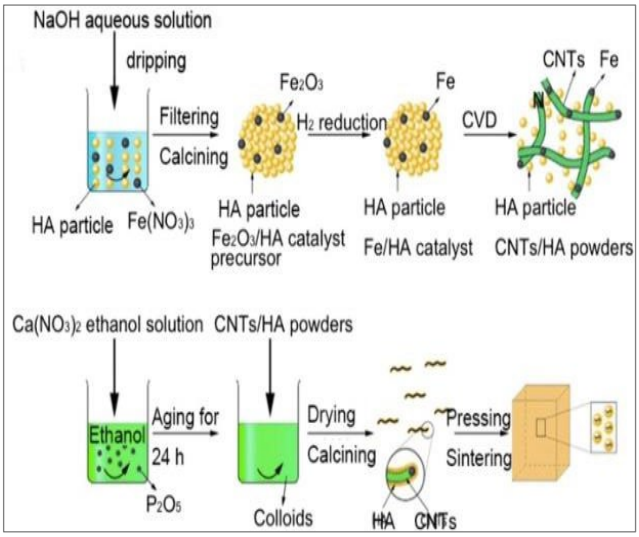

Fig. 5: Schematic diagram of the CNTs-HA preparation process (Huang, 2020)

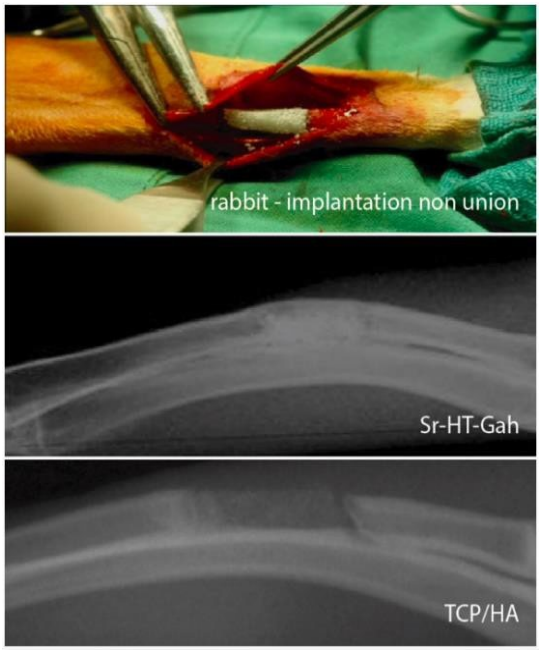

Fig. 6: Implant of CNTs-HA scaffolds (Li et al., 2016)

The scaffolds' dimension is 13mm and after 12 weeks of implants, the X-ray shows the bone formation of the defect site.

By modifying the number of carbon nanotubes and hydroxyapatite that is used when making the composite, the length, shape, and amount of the HA layers that are produced within the structure can all be regulated as shown in Fig. 7. Owing to the homogeneity dispersion and strong interfacial linkages between the nanoparticles and the matrix, the flexural tensile force of CNT-HA composites was increased to 83 MPa, approximately 1.6 times greater than that of pure HA. These composites were also very biocompatible and promote the growth of the cells of the bone and fibroblasts in in-vitro significantly.

Hydroxyapatite, which can absorb chondroitinase, provides the required conditions for the proper operation of bone and marrow, so it is widely used to produce the crafting of synthetic bone-like scaffolds. Although possessing a brittle nature where they break easily, their tensile strength would not be very strong when subjected to heavy loads, such as bones. Carbon nanotubes (CNT) have several different mechanical optical property variations that can be used to build the optimum conditions in a bone interface. Li and co-author made CNT-reinforced poly(acrylonitrile) reinforced glass (PA-HAC) composite effectively via a double in situ synthesis of the chemical vapour deposition process and a mixed sol-gel system, as the fluorochrome labelling images at 120 days after implantation showing new bone (golden yellow) and old bone (deep sea green). The thickness, shape, and composition of the HA layer are based on the ratio of CNT molecules to the average amount of HA molecules that are added during the CNT–HA synthesis.

2.3 *Natural biopolymer: carbon nanotubes chitosan (CNT-CS)*

As a promising biomaterial with an extended application potential, because of its high biocompatibility, biodegradation and antibacterial activity, Chitosan (CS), a linear polysaccharide extracted from Chitina, played a major role. Chitosan is typically easy to turn into different new traits, both organic and inorganic, and features a cationic nature with an Ag^{+} core and a hydrophobic surface which attracts various negative proteoglycans and promotes bone mineralisation after implantation. Fig. 8 displays typical structure of deacetylated chitosan.

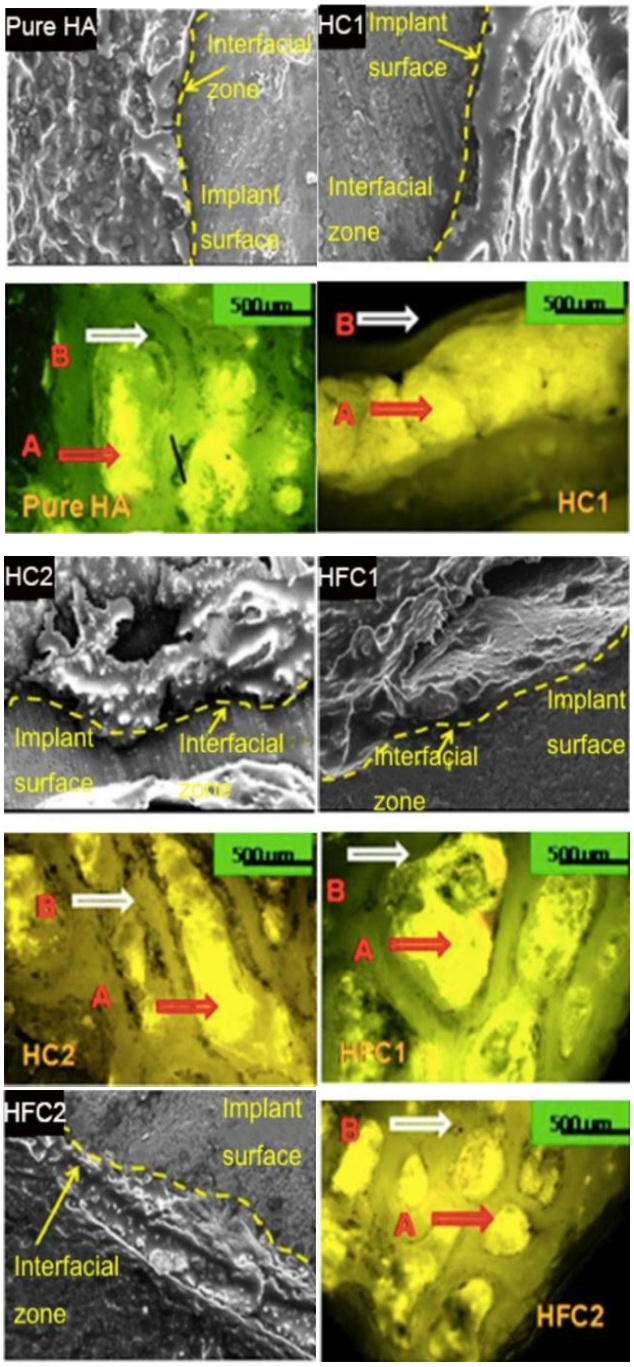

Fig. 7: SEM micrographs (grey) of implantation the host-implant. (HA: hydroxyapatite; HC1: HA + 1% MWCNT; HC2: HAC + 2% MWCNT; HFC1: HA + 1% functionalised-MWCNT, and HFC2: HA + 2% functionalised-MWCNT) (Pei et al., 2019)

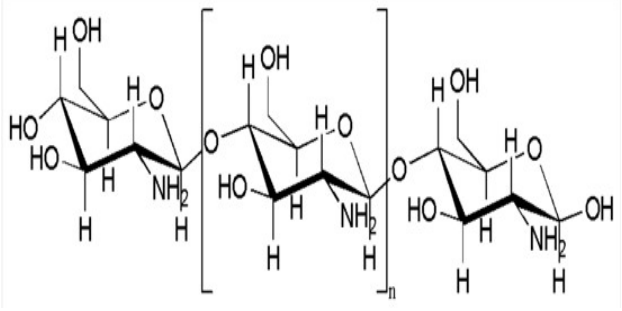

Fig. 8: Structure of fully deacetylated chitosan (Gupta & Ravi Kumar, 2000)

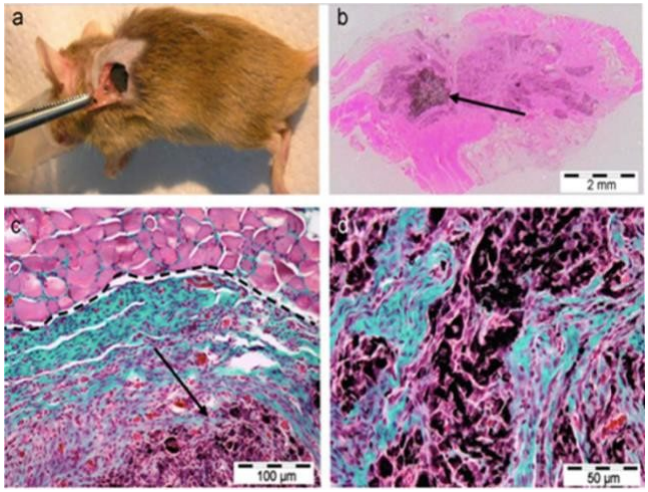

Fig. 9: The surgical implantation of MWCNTs-CS (Pei et al., 2019)

Fig. 9 shows the surgical implantation of rhBMP-2 adsorbed MWCNT–CS scaffolds into the subcutaneous muscular pocket of a mouse (a). While the picture in (b) is the optical microscope micrograph of regenerated bone tissue of the muscular pocket of the same mouse, and (c) and (d) are the optical micrograph in detail of regenerated bone tissue in blue-green, the remaining scaffold is in black colour, and plenty of fibroblasts of the purple colour after major disassembly.

As an orthopaedic material, CS can be quickly modified into different types including films, fibres, beads, and more complicated shapes for orthopaedic therapy. The cationic nature of "mycotoxins" is responsible for the attraction of various negative charged proteoglycans (glycosaminoglycans). Typically associated with osteosynthesis; porous materials have a very high connection to the bone implantation process. Acetic acid can be gotten from a falling solution of it in a mould. This is what helps the membranes change shape such as where the AC (acetic acid) turns solid. There are also various compounds and salts to the CS that have been combined with a variety of other components such as HA, alginate, ammonium chloride, hyaluronic acid, calcium phosphate, poly-(methyl methacrylate), poly-l-lactic acid, and growth factors to be used in orthopaedics.

An antimicrobial silver-zinc, containing composite scaffold was prepared by combining silver sulfadiazine and MWCNTs (Bakhsheshi-Rad et al., 2019). The nanofibers were used as a coating to enhance the biocompatibility and antibacterial properties of the metallic alloy. Cells treated with nanofibers in the presence of silver sulfadiazine demonstrated statistically important ($p < 0.005$; > 80%) decreases in

the numbers of viable cells between 4 and 6 hours in colonies of equine bone marrow mesenchymal stem cells in contrast to the untreated cells. CS–CNT polymeric (or composite) fillers can strengthen the structure of biomimetic tissue in dentistry, facilitate growth of new bone cells, and greatly increase corrosion resistance, even while encouraging bone regrowth. However, more experiments are required to examine the in vivo scientific studies and the mechanical properties of CS–CNTs to adjust the structure and establish a biocompatible internal medium for the human body in its physiological state.

The in vivo and in vitro reinforced chitosan bioactivity can be seen clearly in the graph of the Fig. 10. The optical density of CNTs-CS osteoblast increased significantly in the 6th day in (a) as well as the protein estimation of the composite in (b). The (c) shows a significant alkaline phosphate (ALP) activity of an osteoblast difference between days 1 and 14.

Further systematic research is required, however, to study and improve in vitro and in vivo CS-CNT-based biomaterials with a mechanical property to achieve a biocompatible internal atmosphere in human conditions.

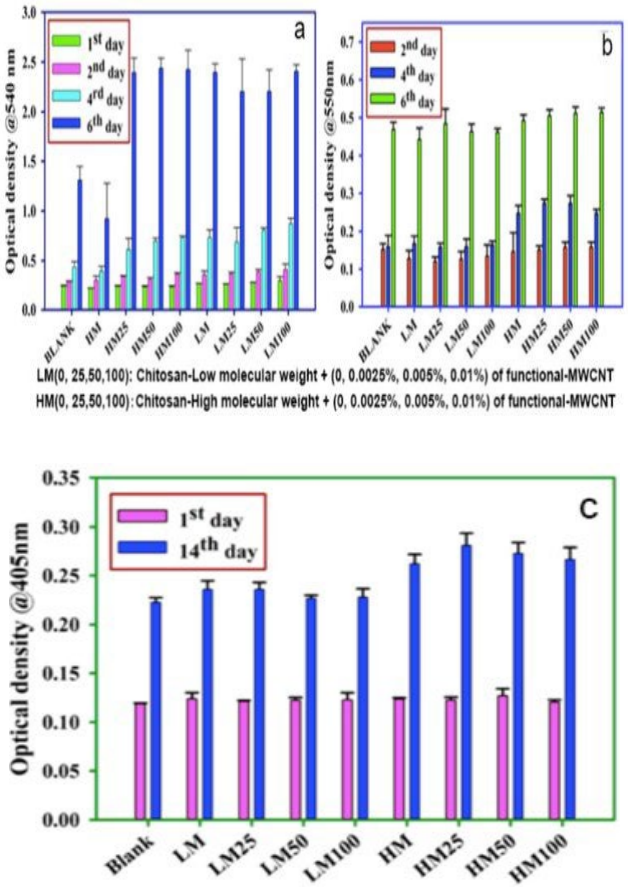

Fig. 10: Carbon nanotube-reinforced chitosan (CNT-CS) biomaterials (Pei et al., 2019)

2.4 Synthetic biopolymers: carbon nanotubes-polycaprolactone (CNTs- PCL)

The semicrystalline polymer, polycaprolactone (PCL), is used in many different products as a tissue-built bone tissue binding materials and for its good biocompatibility, biodegradability, and drug solubility. However, Structural Vinyl Chloride laboratory analysis showed a high level of hydrophobic, poor cell affinity (anti-transplant cells) and insufficient mechanical properties for load-bearing substances. The other materials with which a composite is made, including the use of semiconducting CNTs, are popular to address the shortcomings of the supercapacitor. The PCL–MWCNT scaffold prepared by the screw-assisted extrusion-based additive manufacturing process was as uniformly distributed as possible, with small, normal pores.

The use of mesoporous cubic-nitrogen (MCN)-tetraethers in the pore structure of the PCL scaffold, that is, the strengthening and growth of the inside pore system, contributed to raising the surface roughness by the slight movement in the molecular level and contributed to the greater pore size through the porous system structure and porosity as depicted in Fig. 11. This composite, which stores fat embedded with between 366 and 397 µm deep pores, supported the early-stage attachment and proliferation of human stem cells and helped improve the protein adsorption (Huang et al., 2012).

When rats had a CNTs-PCL inserted into their subcutaneous tissue, there were no noticeable signs of inflammation from where the composite was placed into a scaffold, except for the new blood vessels that were developing in the scaffold pores. The study describes a scaffold system that produces promising new productions to produce angiogenesis, cell forming

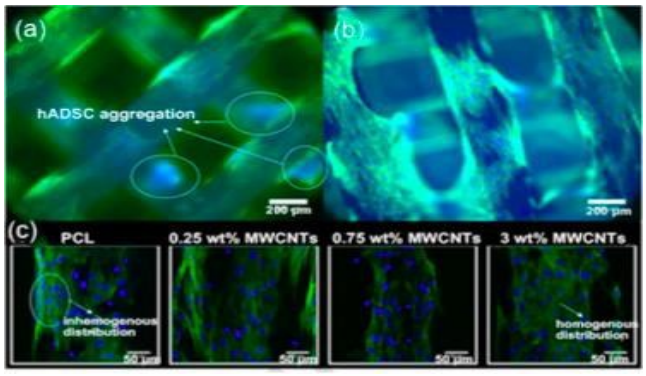

Fig. 11: Fluorescence microscopy images of (a) PCL and (b) PCL-3 wt.% MWCNTs; and (c) conceal images of cell morphology on surface of all MWCNT-PCL scaffolds at 14th day (Prins et al., 2014)

and tissue growth for bone regeneration. The researchers found some promising new candidates for this application.

3.0 Prospect of bone tissue engineering and the utilisation in Malaysia

The nanotechnology of carbon nanotubes is a new field to be discovered in Malaysia, even though it has been two decades since its inception. A lot of things need to be catered for this technology to be utilised, especially in the medical field focusing on bone tissue scaffold engineering. Biocompatibility, controlled degradability, mechanical stability, inductivity for vascularisation, excellent bone control, osteoconductivity and osteoinductivity can be considered as the specification criteria for bone tissue involvement that the researchers here in Malaysia need to table up.

Polymer-bearing biomaterials possess some of the above properties, but a few are still missing (for example, mechanical integrity). To enhance the properties of biomaterials and promote osteoblastic cell growth and differentiation, CNTs have excellent mechanical, thermal and electrical connections, enabling their use as refurbishments or additives in many biomaterials.

While some big steps in bone regenerative medicine have been introduced over the years, there are still several drawbacks to existing therapies such as bone graft. Furthermore, despite substantial advances in materials science in the field of bones substitution, no adequate bone replacement has been developed which still poses a major challenge to orthopaedic and reconstructive surgeons for large bone defects/injuries. Bone tissue engineering has arisen here as a legitimate alternative to existing bone regeneration/substitution therapies. Bone tissue engineering is focused on understanding the development of tissues and regeneration and seeks to stimulate the new usable tissues rather than merely insert new replacement sections, unlike the classic biomaterial approach. The goal of this analysis is to provide a detailed summary of all components available for the effective treatment of bone tissue engineering.

This review only limits to rely on studies on hybrids of CNTs with hydroxyapatite, chitosan, and PCL. For HA, hydroxyapatite, the combination of CNTs-HA with TCP, a combination as biphasic and amorphous calcium phosphates (BCPs and ACPs) are common types of CPCs used in bone tissue engineering. Even though the mechanical strength of ceramics is greater than compared to polymers, it is still inferior to natural bones especially in terms of tensile and torsion strength. Nonetheless, HA has a great compressive (500–1000 MPa) and bending strength (115–200 MPa) in comparison with cortical human bone (100–230 and 50–150 MPa respectively); however, its fracture toughness (1 MPa $m^{0.5}$) is much less (2–12 MPa $m^{0.5}$).

For chitosan, the composite of CNTs-CS scaffolds has minimal immunological rejections, high histocompatibility, and high osteoconductive, osteo-inductive, and osteogenic properties. They are also very low-cost, which makes them an excellent therapy. However, their use is limited, as they are almost impossible to procure, due to the need for extra surgery, morbidity caused by donor sites, and the complexity of acquiring. Allogeneic or xenogeneic scaffolds may have both osteoconductive and osteo-inductive effects. They even have no extra surgery and can be surgically installed. However, these are not necessarily fully donor-site-unsterilised and can mostly pose a risk of disease transmission.

For PCL, the fabrics are made up of eco-friendly components, biodegradable and can be conveniently fabricated into various shapes. They will also provide mechanical assistance for tasks requiring the use of an unlimited number of tools. While in vivo, the CNTs-PCL exhibit high compressive strengths and regulated degradation time; however, they lose their strengths due to rapid degradation in vivo and create local acidic condition, which can render adverse tissue responses.

From these three reviews comparison, it is clear to state that the usage of CNTs with HA is the best, but with addition with TCP will give a better result for the scaffold. These composites will also provide a better biocompatibility and promote the growth of the cells of the bone and fibroblasts in in-vitro significantly. CNTs-HA method needs to be studied further to close any gaps to ensure success in its utilisation in Malaysia. Rooting from here, the bone tissue engineering can be a better studied field in years to come as the bone tissue engineering is quite new in this country to answer the question of what the optimal scaffold is to be used for bone tissue engineering and osseointegration.

Acknowledgement

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