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Bio-Ceramic Coating for Knee Implants for better Interface Between Bone Tissues and Titanium Metals

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Abstract—Total Knee arthroplasty or partial knee arthroplasty is conducted when the patient's knee region starts to pain due to osteoarthritis and other bone related diseases.In this surgery a new knee implant made of metal on metal (titanium, cobalt-chromium) or polymer on metal (polyethylene on titanium) is used. A huge disadvantage of this kind of knee implants is that it causes inflammation, infections due to the metal or polymer debris generated on the implant. Infections or inflammation caused by bacterium adherence to an implant surface,a biofilm formation occurs at the implantation site, as well as infections caused by metal debris generated from friction and movement of the knee joint, are referred as implant-associated infections. So, in this project, a bio-ceramic coating on titanium comprising of beta-Tricalcium phosphate, pectin, gelatin and polyvinylpyrrolidone on the titanium screw was developed to increase antibacterial effects and biocompatibility of the implant materials. Bone implant coating will enhance a cell growth around the implant and it give a viable environment for the implanted site. The primary characterization of the coating materials is done by SEM with EDX, FTIR analysis, in-vitro antibacterial testing, anti-inflammatory testing and the in-vitro degradation study is done for the determination of stability of the coating. In the above testing, it is concluded that our coating materials have an increased antibacterial effects and biocompatibility in nature. However, further In-Vivo testing process is needed to confirm the use of bio-ceramic coating for knee implant.

Keywords:TKA**,** Beta Tricalcium Phosphate, PVP, Gelatin, pectin

1. INTRODUCTION

Total knee replacement has been used to relieve the pain caused by arthritis and it has been established as an undefeated treatment for advanced chronic joint pains. The main aim of the total knee replacement for a patient is to recover from the pain. The range of motion of the knee is limited by the anatomy of the bones and ligaments, but allows around 120 degrees of flexion. A special characteristic of the knee is that it allows a small degree of medial and lateral rotation when it is moderately flexed. Instability is common for both primary and revision total knee arthroplasty, accounting for up to 20% in the total knee replacements done. Physical examination should include observations of gait, which might suggest instability in the axial or coronal planes for instance, a vagus or valgus thrust is observed. At least 150 types of knee implant models are currently available worldwide; there are about 500,000 knee replacements annually. The single radius knee is the most common type of knee implant used in surgeries as it offers the greatest level of extension and flexion for the majority of patients, regardless of gender, age and activity level. Either total or partial knee replacement surgery reliably improves pain and function in patients with arthritis.

The foremost importance is that the implanted device not only restores the function of the body but also continues to do that for the lifetime of the patient. To promote the long lifetime for the implant and adverse effects, it should be integrated quickly with the human body by forming the bond with the surrounding bone, which is possible by Biomedical materials, which include alloys, ceramics, synthetic polymers, biopolymers and composites, which are used to repair, restore or replace damaged or diseased tissue in the artificial organs, tissues or in prostheses.

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2. MATERIALS AND METHODOLOGY

A. SYNTHESIS OF THE COATING MATERIAL

In this paper we are using four known biomaterials beta tricalcium phosphate, pectin, PVP and gelatin (purchased from sigma Aldrich) that has successfully been used in orthopedic and dental implants.

The amount of all materials were varied to find out the correct ratio to be used in order to get an exact ratio of materials that would have the appropriate viscosity to get attached on the titanium screw surface and materials to bind together perfectly. The solvent used to dissolve all the materials was Acetic acid in a quantity of 44 mL with 6 mL of distilled water.

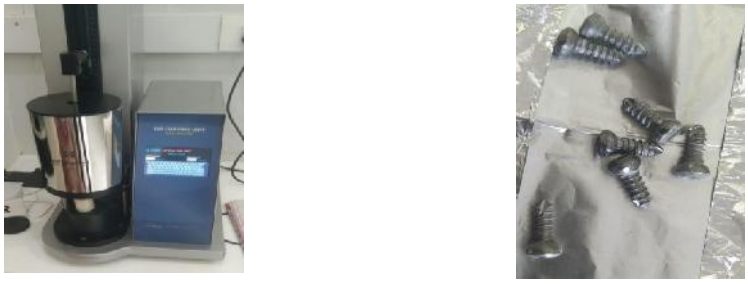
Beta tricalcium Phosphate(g)	Pectin (g)	PVP(g)	Gelatin(g)
1	0.5	0.5	0.5
1.2	0.6	0.5	0.6
1.4	0.7	0.5	0.7
1.6	0.8	0.5	0.8
1.8	0.9	0.5	0.9
2.0	1.0	0.5	1.0

Table 1.shows the total number of material concentrations and ratios tried for getting the coating material ratio.

After doing several trials, the final concentration for the beta tricalcium phosphate, pectin, PVP and gelatin for the coating material mixture was 2:1:0.5:1.

B. Dip coating

The success of these coating mixtures depends on achieving a high crystallinity in the coatings, good adherence between ceramic and metal, control over coating thickness and the ability to coat porous and complex shapes. The electrophoretic deposition process is a good method to coat porous and complex-shaped implants. However, high temperature sintering of such electrophoretically deposited coatings can often lead to cracking at the substrate–coating interface. Dip coating and sol–gel processes are good methods for producing a thin coating on implants, but achieving a thicker coating is often very difficult. In this case, dip coating is selected as it matches the requirement.



(a) (b)

Fig. 1 (a) shows the dip coater apparatus on which the titanium screws were coated with the biomaterials, (b) shows the titanium cortical bone screw after dip coating.

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The coating process was performed electronically using a special dip-coating apparatus (fig 1.2) at a constant dipping and withdrawing rate of 1 mm/s. Dipping and pulling were done at a constant speed without any rapid shaking. The coated substrate was then oven-dried at 130˚C for 1 hour to form a white coating layer, after which it was kept in the oven heating up to 40˚C.

3. Characterization

3.1 SEM

FE-SEM; a super hybrid lens version (SHL/SHLs, two versions with different functions), which enables higher resolution observation and analysis; and the newly-developed semi-in-lens version (i/is, two versions with different functions), which is suited for the observation of semiconductor devices.Furthermore, the JSM-IT800 can also be equipped with a new Scintillator Backscattered Electron Detector (SBED) and a Versatile Backscattered Electron Detector (VBED). The SBED enables the acquisition of images with high responsiveness and produces sharp material contrast even at a low accelerating voltage, while the VBED can help obtain images of 3D, topography and material contrasts. Thus, the JSM-IT800 can help users to obtain information that was not obtainable and to solve problems in measurement.

3.2 FTIR

Fourier Transform Infrared Spectroscopy (FTIR) is a widely used technique for rapid material identification. With the aid of custom-built reference libraries, it can be used to verify or identify unknown materials in a matter of minutes. It is considered to be a non-destructive technique that requires little to no sample preparation in order to perform analysis. The Bruker ALPHA II has a portable benchtop design with interchangeable modules to suit your sampling requirements. The ATR, DRIFT, and External Reflection modules give the flexibility to analyze a wide range of sample types. It can be integrated into routine sampling or be used for more targeted identification and verification. By generating custom calibrations, the ATR module can be used to perform semi-quantitative analysis. This can provide valuable real-time information such as semi-quantitative mineralogy.

3.3 EDX

Energy Dispersive X-ray Spectroscopy (EDS or EDX) is an analytical technique accustomed identify and characterize the fundamental composition of sample material. employing a scanning microscope (SEM) equipped with an x-ray detector, atoms within a sample are excited by a beam employing a scanning microscope (SEM) equipped with an x-ray detector, atoms within a sample are excited by an ray. The cumulative spectrum of the emission energies for a component is exclusive to its element, and hence, it is often accustomed to identify unknown particles in an exceedingly sample or determine sample composition. EDS technology from Particle Technology Labs is beneficial for analyzing the fundamental composition of sample material for elements of mass 12 (Carbon) and up, and might even be wont to quantify the fundamental composition of a sample. Results may be used for foreign material identification, process troubleshooting, reformulations, among other uses.

3.4 Polymer coating stability studies

In order to check the peel-off of polymer composite from the coated screw, polymer coating stability study was performed. Briefly, the polymer coated screw was incubated in two different buffer solutions namely, acetate buffer (pH 5.5) and phosphate buffer (pH 7.4) with two different time points (1st and 3rd day) to estimate the coating stability. After the respective time period of treatment in different buffer solutions, the screw was dried and analyzed in Field-emission scanning electron microscope (FE-SEM).

3.5 ANTIBACTERIAL ACTIVITY OF POLYMER COMPOSITES:

3.5.1 Zone of inhibition (ZOI) measurement

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The antibacterial activities of the polymer composite were studied by agar well-diffusion method using Mueller–Hinton agar (MHA) plate according to NCCLS (1993). The MHA medium was prepared in distilled water (pH 7) and sterilized in an autoclave at 121 ˚C for 15 min. The MHA was poured (around 40 ˚C) in sterile petri plate at aseptic conditions in laminar air flow and solidified. An inoculum containing 106 cfu/mL of each freshly prepared bacterial culture was spread on the MHA plates with a sterile cotton swab moistened with the suspension of gram-positive Streptococcus aureus and gram-negative Escherichia coli. Then, three wells (8 mm in diameter) were punched into the agar medium and filled with different concentrations of polymer composite samples (25 µL, 50 µL, and 100 µL) and allowed to diffuse at room temperature for 2 h, and the culture plates were incubated at 37 °C for 24 h. After incubation, the diameter (mm) of the zone of inhibition was recorded in each plate. The results were expressed as mean value with standard deviation (SD) of a triplicate experiment.

3.5.2 Determination of the minimum inhibitory concentration (MIC)

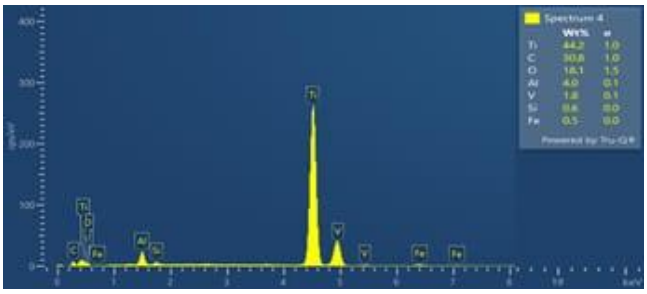
The minimum inhibitory concentration (MIC) was determined by the broth dilution method according to CLSI M07-A8. For example, Streptococcus aureus and Escherichia coli cultures (0.5 McFarland: 1.5 × 108 CFU/mL) were added to the nutrient broth, which contained different dilutions (10-1 to 10-7) of polymer composite. The MIC concentration of the polymer composite is defined as the lowest concentration inhibiting visible growth of Streptococcus aureus and Escherichia coli. After 18 h of incubation, the bacterial growth in the test tubes was observed as turbidity at 600 nm using a UV-visible spectrophotometer. The least concentration which showed more than 75% of bacterial growth inhibition was determined and noted as the MIC value.

3.5.3. Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) is measured by sub-culturing the broths used for MIC determination onto fresh nutrient agar plates. MBC is the lowest concentration of a sample that results in killing 99.9% of the bacteria being tested. All the experiments were performed in triplicate and the results were expressed as mean with standard deviation (SD).

3.6. Anti-inflammatory study (Inhibition of albumin denaturation assay)

A reaction mixture (5 mL) consisting of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of PBS (pH 6.4), and a couple of mL of varying concentrations of test samples (100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL) was incubated at 37 °C in a very biochemical oxygen demand (BOD) incubator for 15 min Then heated at 70 °C for five min. an analogous volume of H2O served as control. NSAID at concentrations of 100 µg/mL, 200 µg/mL, 400 µg/mL, and 800 µg/mL was used as a reference drug and treated similarly for determination of absorbance. the share of inhibition of protein denaturation was calculated by using,
Equation 1: where, Vt is that the absorbance of the test sample and Vc is that the absorbance of control. The drug concentration for 50% inhibition determined from the dose–response curve by plotting percentage inhibition with regard to control against treatment concentration.



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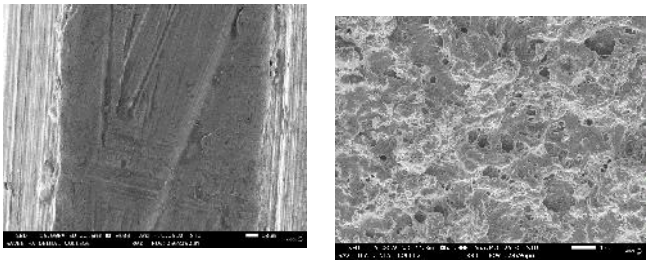
4.RESULT

4.1 MORPHOLOGICAL CHRACTERIZATION

Scanning electron microscope (SEM) reveal the difference of coated and uncoated surface. The diameter of cortical screw was 6mm and length of around 12mm. Fig (a) show the sharp edge and rough surface uncoated surface. Fig(b)SEM micrographs show biomaterial polymer layer on the screw does not see sharp edges and rough surface. The biomaterial polymer coating was well adapted to the metal substrate.



(a) **(b)**

Fig2, (a)shows the SEM analysis of Titanium screw before coating,(b) shows the SEM analysis of coated Titanium screw after coating

EDX analysis of uncoated screws (fig.3(a&b)) confirmed the present highly inorganic group material of 44% titanium, 4% aluminum, 1.8% vanadium, and 0.6% silicon. Since SEM and EDX was done together, The EDX results of coated screws (fig.3 (a&b)) confirmed the uptake of different organic groups of material 16%calcium, 1.6% copper, 43% carbon and 3.9% phosphorus were the main components of the polymer. The polymer mixture is deposit on the screw surfaces.

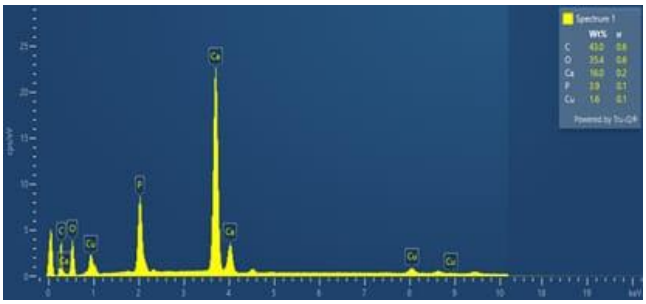


Fig3. shows the EDX analysis of non-coated and coated Titanium screw before coating

4.2 FUNCTIONAL CHARACTERIZATION

The chemical composition of the coating solution was analyzed by attenuated total reflectance–Fourier transform infrared spectroscopy (ATR–FTIR) (Bruker, ALPHA II Compact) in the spectral region of 4000–400 cm^{-1} wavelength with 4 cm^{-1} resolution at total scans of 32.

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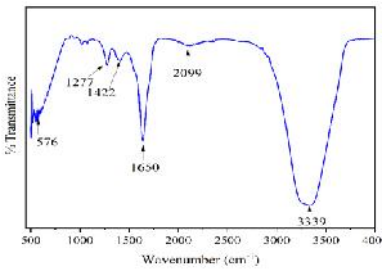

Fig 4. shows the Fourier transform infrared spectroscopy graph for the coating material sample.

The FTIR spectrum of the polymer composite is shown in **Fig 4.**The broad stretching vibration was observed between 3200 - 3400 cm^{-1}, which was attributed to aromatic O-H stretching vibration of polymer composite. Similar peak was observed by Wathoni et al. (2019) for pectin. The characteristics transmittance peaks of the polymer composite were observed at 1277 cm^{-1} (Aromatic C–O stretching), 1422 cm^{-1} (Aromatic C–H stretching), 1650 cm^{-1} (aromatic C=O stretching), and 2099 cm^{-1} (aromatic C-H_2 stretching) (Safo et al., 2019). The spectral band at 576 cm^{-1} was also noted and is attributed to characteristics spectral band of PO_4^{2-} functional group of tricalcium phosphates (Tavares et al. 2013).

4.3. Polymer composite peel-off/degradation studies

Fig.5illustrates the Field-emission scanning electron microscope (FE-SEM) image of uncoated screw and polymer coated screws at two different magnifications. The uncoated screw exhibited sharp blades and edges, after coating on the screw a layer growth over the screw is visible and that was captured through FE-SEM. Followed by the coating the screw was incubated. At different pH (acetate buffer-5.5 and phosphate buffer-7.5) and two different time points (1st and 3rd day) to estimate the coating stability.

The FE-SEM image shows the stability nature of polymer composite coated on the screw (**Fig.6).** The samples which were treated at pH 5.5 showed more dominant peel-off than the other samples that may be owing to the acidic nature of the treatments. Acids usually have the tendency to etch surfaces.

In pH 7.4, soaked screw demonstrates the coating adherence on the internal structures (10 µm) and that adherence was reduced on the third day; similarly, this was noted on the pH 5.5 treated screws. On the third day, lines over the natural screw are visible, which indicates the removal of coated material over the surface. Samples that were treated at pH 5.5 explained the sharp natural structure of the screw compared to other samples that resembled the better coating stability at pH 7.4.

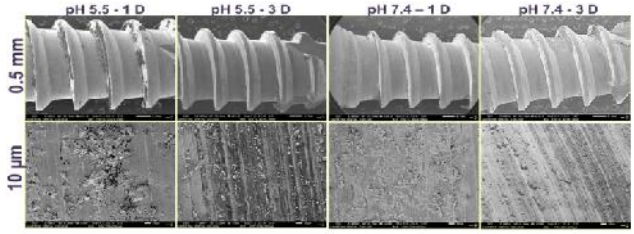

Fig.6. Morphological evaluation of polymers coated screws after exposure with buffer solution with different pH (5.5 and 7.4) at different time points (1 and 3 day), respectively.

4.4 ANTIBACTERIAL ACTIVITY TEST

4.4.1 Zone of inhibition (ZOI) measurement

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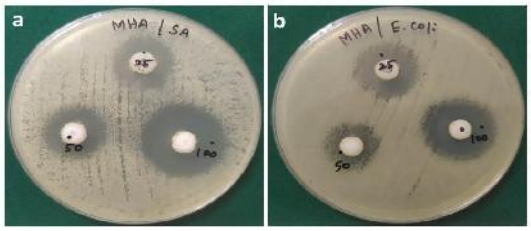
The antibacterial activity of the polymer composite was assessed against Streptococcus aureus and Escherichia coli at three different concentrations (25 µL, 50 µL, and µL).The result shows that the ZOI for composite polymers against both of the tested Gram-positive Streptococcus aureus and Gram-negative Escherichia coli was observed. Fig. 1.5 (a, b) and Fig. 1.6, the zone of inhibition of composite polymers were found as 16±1.5 mm (25 µL), 19±1.0 mm (50 µL), and 23±1.5 (100 µL) for Escherichia coli; whereas, 17±1.0 mm (25 µL), 22±1.5 mm (50 µL), and 30±2.0 (100 µL) for Streptococcus aureus, respectively.



(a) (b)

Fig. 7.(a) & (b) . Zone of inhibition (ZOI) test on MHA plate for composite polymers for (a) Gram positive *Streptococcus aureus* and (b) Gram negative *Escherichia coli*.

It confirms that the prepared polymer composite exhibits excellent antibacterial activity against both Gram positive Streptococcus aureus and Gram-negative Escherichia coli. However, the antibacterial efficacy of the composite polymers is considerably higher against Streptococcus aureus compared to Escherichia coli, which suggests that our material is a promising candidate to control the Gram-positive organisms.

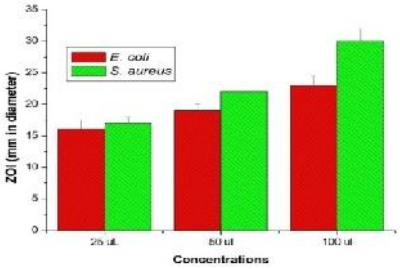


Fig. 8. Zone of inhibition (ZOI) measurement for composite polymers.

4.4.2 Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) result represents that the growth of Streptococcus aureus and Escherichia coli were inhibited (above 75%) by polymer composite at a concentration of 0.75 mg/mL (range of 0.5 mg to 1 mg/mL) and 0.9 mg/mL (range of 0.5 mg to 1 mg/mL), respectively, as illustrated in Fig. 9

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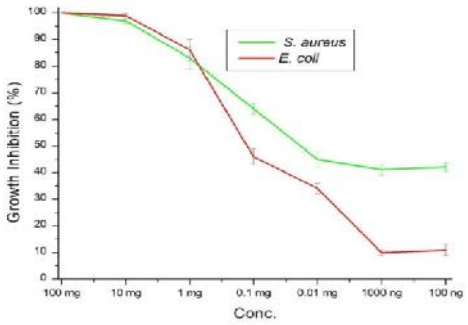
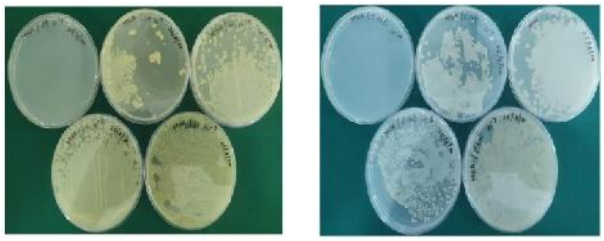


Fig.9. Minimum inhibitory concentration (MIC) test for gram positive S.*aureus* and (b) Gram negative *Escherichia coli*.

4.4.3 Determination of minimum bactericidal concentration (MBC)

After the determination of MIC of polymer composite, aliquots of 50 µL from all the MIC tubes (after 18 h) were seeded on the prepared nutrient agar by spread plate method and incubated in the bacteriological incubator for 24 h at 37 °C. As shown in **Fig.10 (a, b)** the MBC result indicates that the bacterial population (Streptococcus aureus and Escherichia coli) was completely killed at a concentration of 1 mg/mL (10-3) for polymer composite, respectively. The observed results clearly indicate that the obtained value of MBC is almost near to the value of MIC for both of the studied organisms.



(a) **(b)**

Fig. 10.(a) Minimal bactericidal activity (MBC) of composite polymers for *Streptococcus aureus*,(b) Minimal bactericidal activity (MBC) of composite polymers for *Escherichia coli*

4.5 Anti-inflammatory assessment

The in vitro bioassay results of anti-inflammatory potentials of polymer composite materials assessed against denaturation of egg albumin are summarized in Fig.2.7. All tested concentrations (P 0.001) significantly inhibited the denaturation of egg albumin. The maximum inhibition percentage obtained was 26% at a concentration of 0.8 mg/mL. The acetylsalicylic acid used as a standard drug exhibited an inhibition of 65% at a concentration of 0.1 mg/mL. This result indicated that the prepared polymer composite material exhibits reasonable anti-inflammatory effects.

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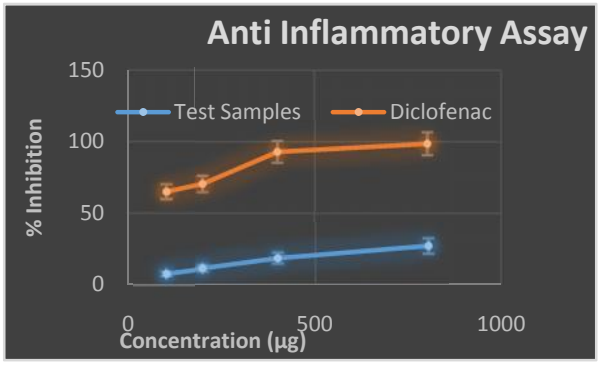

Fig. 11. Anti-inflammatory effect of polymer composite

5. DISCUSSION AND FUTURE ENHANCEMENT

5.1 DISCUSSION

After successful knee replacement surgery, a small fraction of patients had to undergo secondary knee surgery due to infections caused by implant debris and bacterial infection. Biomaterials or biopolymer material are coated on the screw to increase the biocompatibility and antibacterial propertyto overcome the issue. A 12mm cortical bone screw is coated with a mixture of Beta tricalcium phosphate, Pectin, PVP and gelatin in the ratios of 2:1:0.5:1. The coated screw underwent SEM analysis, EDX analysis, FTIR, Anti-bacterial testing,
Anti-inflammatory and Coating stability test to test the antibacterial and biocompatibility properties of the coating. At the end of all the test, results show that the coated screw has increased antibacterial property and biocompatibility.

5.2 FUTURE ENHANCEMENT

The present coating material has passed the basic characteristic testing and in the next step, it has to be tested for tissue – implant interaction for which it has to undergo animal testing and in-vivo and in-vitro testing for bone growth analysis and mechanical testing for finding out its potential as a bone implant substitute.

CONCLUSION

A bio-coating made up of Beta Tri-calcium Phosphate, Pectin, PVP and Gelatin at a ratio of 2:1:0.5:1 is coated on a 12mm cortical bone screw. The cortical bone screw underwent characteristic tests such as SEM, EDX, FTIR, and coating stability tests. The result for the characteristics shows the coating materials are present on the screw surface and the coating is stable in both acidic and basic pH for a week. In the next stage, antibacterial and anti-inflammatory tests were conducted, and the results showed good antibacterial property against E. coli and Streptococcus aureus and no severe inflammatory properties against the coating material. Hence, the polymer material coated on the screw shows greater antibacterial activity and biocompatibility in comparison to existing coated knee implants.

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REFERENCES

[1] N. Wathoni, C. Yuan Shan, W. Yi Shan, T. Rostinawati, R.B. Indradi, R. Pratiwi, M. Muchtaridi, Characterization and antioxidant activity of pectin from Indonesian mangosteen (Garcinia mangostana L.) rind, Heliyon.5(2019)e02299.https://doi.org/10.1016/j.heliyon.2019.e02299

[2] I.A. Safo, M. Werheid, C. Dosche, M. Oezaslan, The role of polyvinylpyrrolidone (PVP) as a capping and structure-directing agent in the formation of Pt nanocubes, Nanoscale Adv. 1 (2019) 3095–3106. https://doi.org/10.1039/C9NA00186G.

[3] Briggs, E & Walpole, AR & Wilshaw, Peter & Karlsson, M & Pålsgård, Eva. (2004). Formation of highly adherent nano-porous alumina on Ti-based substrates: A novel bone implant coating. Journal of materials science. Materials in medicine. 15. 1021-9. 10.1023/B:JMSM.0000042688.33507.12.

[4] Lisheng Zhao,a ‡ Junjie Li,b ‡ Liang Zhang,c ‡ Yu Wang,a Jiexin Wang, d Bin Gu,a Jianfeng Chen,e Tong Hao,b Changyong Wang*b and Ning Wen*a Preparation and Characterization of Calcium Phosphate/Pectin Scaffolds for Bone Tissue Engineering Received 00th January 20xx, Accepted 00th January 20xx DOI: 10.1039/x0xx00000x www.rsc.org/

[5] S. Girod Fullana a,*, H. Ternet a , M. Freche b , J.L. Lacout b , F. Rodriguez a Controlled release properties and final macroporosity of a pectin microspheres–calcium phosphate composite bone cement aUniversité de Toulouse, CIRIMAT, UPS-INP-CNRS, Faculté de Pharmacie, 118 Route de Narbonne, 31062 Toulouse Cedex 4, France bUniversité de Toulouse, CIRIMAT, UPS-INPT-CNRS, ENSIACET, 118 Route de Narbonne, 31077 Toulouse Cedex 4, France

[6] Habraken WJEM, de Jonge LT, Wolke JGC, Yubao L, Mikos AG, Jansen JA. Introduction of gelatin microspheres into an injectable calcium phosphate cement. J Biomed Mater Res A 2008;87A(3):643–55.

[7] Sriamornsak P. Effect of calcium concentration, hardening agent and drying condition on release characteristics of oral proteins from calcium pectinate gel beads. Eur J Pharm Sci 1999;8(3):221–7

[8] Yuan-Tsung Fu a,b , Shi-Yuan Sheu a,c,d , Yueh-Sheng Chen a,e , Kuo-Yu Chen Porous gelatin/tricalcium phosphate/genipin composites containing 3 lumbrokinase for bone repair journal homepage: www.elsevier.com/locate/bone

[9] M. Duskova, E. Leamerova, B. Sosna, and O. Gojis, “Guided tissue regeneration, barrier membranes and reconstruction of the cleft maxillary alveolus,” Journal of Craniofacial Surgery, vol. 17, no. 6, pp. 1153–1160, 2006.

[10] F. P. Strietzel, P. Khongkhunthian, R. Khattiya, P. Patchanee, and P. A. Reichart, “Healing pattern of bone defects covered by different membrane types-a histologic study in the porcine mandible,” Journal of Biomedical Materials Research—part B, vol. 78, no. 1, pp. 35–46, 2006.

[11] A. Sculean, F. Schwarz, G. C. Chiantella et al., “Five-year results of a prospective, randomized, controlled study evaluating treatment of intra-bony defects with a natural bone mineral and GTR,” Journal of Clinical Periodontology, vol. 34, no. 1, pp. 72–77, 2007.J. U. Duncombe, “Infrared navigation—Part I: An assessment of feasibility (Periodical style),” *IEEE Trans. Electron Devices*, vol. ED-11, pp. 34–39, Jan. 1959.

[12] . Parthasarathy, B. Starly, and S. Raman, “A design for the additive manufacture of functionally graded porous structures with tailored mechanical properties for biomedical applications,” Journal of Manufacturing Processes, vol. 13, no. 2, pp. 160–170, 2011.

[13] K. F. Leong, C. M. Cheah, and C. K. Chua, “Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs,” Biomaterials, vol. 24, no. 13, pp. 2363–2378, 2003.

[14] Shi, Jianping; Liang, Huixin; Jiang, Jie; Tang, Wenlai; Yang, Jiquan (2019). Design and Performance Evaluation of Porous Titanium Alloy Structures for Bone Implantation. Mathematical Problems in Engineering, 2019(), 1–9. doi:10.1155/2019/5268280

[15] Zhang, Bill; Myers, Damian; Wallace, Gordon; Brandt, Milan; Choong, Peter (2014). Bioactive Coatings for Orthopedic Implants—Recent Trends in Development of Implant Coatings. International Journal of Molecular Sciences, 15(7), 11878–11921. doi:10.3390/ijms150711878

[16] Hickson, C. J.; Metcalfe, D.; Elgohari, S.; Oswald, T.; Masters, J. P.; Rymaszewska, M.; Reed, M. R.; Sprowson , A. P. (2015). Prophylactic antibiotics in elective hip and knee arthroplasty: an analysis of organisms reported to cause infections and national survey of clinical practice. Bone and Joint Research, 4(11), 181–189. doi:10.1302/2046-3758.411.2000432

[17] Saidin, Syafiqah; Jumat, Mohamad Amin; Mohd Amin, Nur Ain Atiqah; Saleh Al-Hammadi, Abdullah Sharaf (2021). Organic and inorganic antibacterial approaches in combating bacterial infection for biomedical application. Materials Science and Engineering: C, 118(), 111382–. doi:10.1016/j.msec.2020.111382

[18] Madhavi Latha Chinta;Aditya Velidandi;Ninian Prem Prashanth Pabbathi;Swati Dahariya;Sreenivasa Rao Parcha; (2021). Assessment of properties, applications and limitations of scaffolds based on cellulose and its derivatives for cartilage tissue engineering: A review . International Journal of Biological Macromolecules,.doi:10.1016/j.ijbiomac.2021.01.19