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MECHANICAL, TRIBOLOGICAL AND CYTOCOMPATIBILITY PROPERTIES OF CELLULOSE NANOFIBER-REINFORCED ULTRA-HIGH MOLECULAR WEIGHT POLYETHYLENE COMPOSITES

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By

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Chair : Hidayah binti Ariffin, PhD
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Ultra-high molecular weight polyethylene (UHMWPE) tibial insert in knee prosthesis possesses limited longevity due to the continuous sliding and rolling movement of the highly stiff metal femoral counterpart onto its surface which leads to the abrasion and wear. This generates wear debris that subsequently contributes to the inflammation. The use of the appropriate nanofiller in the UHMWPE may improve the polymer stiffness and wear resistance. Hydrophilic cellulose nanofiber (CNF) was selected as the nanofiller in this research. The effects of blending processing conditions and residual lignin in CNF on the characteristics of the UHMWPE/CNF bionanocomposites were evaluated. Optimization of the melt blending processing conditions was conducted by varying the temperature, rotational speed, and mixing time using a central composite design (CCD) of response surface methodology (RSM). The mechanical properties of the UHMWPE/CNF bionanocomposites were greatly influenced by the temperature and mixing time, while the rotational speed moderately affected the Young's modulus. At the optimum processing conditions (150°C, 60 rpm, and 45 minutes) a homogeneously distributed CNF contributed to the highest values of Young's Modulus, yield strength, tensile strength, and elongation at 366 MPa, 22.8 MPa, 28.0 MPa, and 462%, accordingly. These values surpassed the standard requirement of fabricated UHMWPE for a joint application. A non-melt blending process through ethanol mixing was then evaluated to elucidate the effect of the blending process on the mechanical properties of the bionanocomposites. The field emission scanning electron microscopy (FE-SEM) analysis revealed a better mechanical interlocking between UHMWPE and CNF from the bionanocomposites sample produced through the melt blending process which resulted in higher yield strength, elongation at break, Young's modulus, toughness, and crystallinity by 28%, 61%, 47%, 45%, and 11%, respectively, compared to those produced through ethanol

mixing (non-melt blending processing). Increasing the CNF content via melt blending processing did not improve the tensile properties of the bionanocomposites due to the weakened hydrophobic interfacial interaction between UHMWPE molecules in the presence of high CNF content. Residual lignin in the CNF was postulated to improve the interaction between UHMWPE and CNF, and hence ligno-cellulose nanofiber (LCNF) with 22.5% lignin was incorporated in to the UHMWPE. It was interesting to note that the tensile strength, toughness, and flexural strength were increased by 21%, 9%, and 51%, respectively, when 0.5 wt.% LCNF was used in the UHMWPE, as compared to 0.5 wt.% CNF. Hydrophobic lignin in LCNF is expected to form a better interaction between UHMWPE and LCNF. The efficiency of CNF and LCNF as fillers for improving the wear resistance was also confirmed through a wear testing analysis using a pin-on-disk tribometer. The enhancement of wear resistance properties by 33% and 42% was achieved for UHMWPE/0.5%CNF and UHMWPE/0.5%LCNF, respectively. This is supported by the reduction in abrasive wear as evidenced through the FE-SEM analysis. Cytocompatibility of UHMWPE/0.5%CNF and UHMWPE/0.5%LCNF on osteoblast-like cells MG-63 was evaluated to further characterize the materials' suitability for knee prosthesis application. The spindle-like appearance of cells on both bionanocomposites with nearly zero shape factors (0.2 to 0.4) indicated a good cell adherence and attachment. Cell viability and ALP activity of MG-63 were higher and similar to the UHMWPE/0% filler, respectively, indicating the non-toxic effect of CNF and LCNF inclusion in the UHMWPE bionanocomposites. Overall, the incorporation of CNF and LCNF in UHMWPE through melt blending contributed to a better wear resistance, higher Young's modulus and excellent cytocompatibility against osteoblastic MG-63 cells. These findings indicate the suitability and potential of CNF and LCNF as nanofiller in UHMWPE for the tibial insert application, with the advantages of being renewable, organic and non-toxic.

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SIFAT-SIFAT MEKANIKAL, TRIBOLOGI DAN SITOKOMPATIBILITI KOMPOSIT POLIETILENA BERAT MOLEKUL ULTRA-TINGGI DIPERKUKUH SELULOSA GENTIAN NANO

Oleh

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Sisipan tibial polietilena berat molekul ultra tinggi (UHMWPE) dalam prostesis lutut mempunyai jangka hayat yang terhad disebabkan oleh pergerakan gelongsor dan berguling yang berterusan oleh komponen femoral logam yang sangat kaku, lalu membawa kepada lelasan dan haus. Hal ini menjana serpihan haus yang seterusnya menyumbang kepada keradangan. Penggunaan pengisi nano yang sesuai dalam UHMWPE boleh meningkatkan kekukuhan polimer dan rintangan haus. Selulosa gentian nano hidrofilik (CNF) telah dipilih sebagai pengisi nano dalam penyelidikan ini. Kesan keadaan pemprosesan campuran dan sisa lignin dalam CNF ke atas ciri-ciri bionanokomposit UHMWPE/CNF telah dinilai. Pengoptimuman keadaan pemprosesan pengadunan leburan telah dijalankan dengan mengubah suhu, kelajuan putaran, dan masa pencampuran menggunakan reka bentuk komposit pusat (CCD) metodologi permukaan tindak balas (RSM). Sifat mekanikal bionanokomposit UHMWPE/CNF sangat dipengaruhi oleh suhu dan masa pencampuran, manakala kelajuan putaran mempengaruhi modulus Young secara sederhana. Pada keadaan pemprosesan optimum (150°C, 60 rpm, dan 45 minit), serakan CNF yang sekata menyumbang kepada nilai modulus Young, kekuatan alah, kekuatan tegangan, dan pemanjangan ketika putus yang paling tinggi, iaitu masing-masing pada 366 MPa, 22.8 MPa, 28.0 MPa, dan 462%. Nilai ini melepasi keperluan standard UHMWPE fabrikasi untuk aplikasi sendi. Seterusnya, penambahan maleik anhidrida dicantum polietilena (MAPE) sebagai penyerasi adalah tidak bererti untuk menambah baik sifat mekanikal bionokomposit. Proses pengadunan tidak cair melalui pencampuran etanol telah dinilai untuk menjelaskan kesan proses pengadunan ke atas sifat mekanikal bionokomposit. Analisis mikroskop elektron pengimbas pelepasan medan (FE-SEM) menunjukkan jalinan mekanikal yang lebih baik antara UHMWPE dan CNF dalam sampel yang dihasilkan melalui pemprosesan campuran lebur dengan kekuatan alah, pemanjangan ketika putus, modulus Young, keliatan dan kehabluran yang lebih tinggi,yang mana masing-masing meningkat sebanyak 28%, 61%, 47%, 45%, dan 11%,

berbanding yang dihasilkan melalui pencampuran etanol (pengadunan tidak cair). Peningkatan kandungan CNF melalui pengadunan cair tidak meningkatkan sifat tegangan bionokomposit disebabkan oleh interaksi antara muka hidrofobik yang lemah antara molekul UHMWPE dengan kehadiran kandungan CNF yang tinggi. Sisa lignin dalam CNF dipostulat dapat meningkatkan interaksi antara UHMWPE dan CNF, dan oleh itu ligno-selulosa nanofiber (LCNF) dengan 22.5% lignin telah dimasukkan ke dalam UHMWPE. Adalah menarik untuk diperhatikan bahawa kekuatan tegangan, keliatan dan kekuatan lentur masing-masing meningkat sebanyak 21%, 9%, dan 51%, apabila LCNF 0.5 wt.% digunakan dalam UHMWPE, berbanding dengan 0.5 wt.% CNF. Lignin hidrofobik dalam LCNF dijangka membentuk interaksi yang lebih baik antara UHMWPE dan LCNF. Kecekapan CNF dan LCNF sebagai pengisi untuk meningkatkan rintangan haus juga telah disahkan melalui analisis ujian haus menggunakan tribometer pin pada cakera. Peningkatan sifat rintangan haus masing-masing sebanyak 33% dan 42% telah dicapai untuk UHMWPE/0.5%CNF dan UHMWPE/0.5%LCNF. Ini disokong oleh pengurangan lelasan haus seperti yang dibuktikan melalui analisis FE-SEM. Sifat keserasian UHMWPE/0.5%CNF dan UHMWPE/0.5%LCNF pada sel seperti osteoblas MG-63 telah dinilai untuk mencirikan lagi kesesuaian bahan untuk aplikasi prostesis lutut. Penampilan sel seperti gelendong pada kedua-dua bionokomposit dengan faktor bentuk hampir sifar (0.2 hingga 0.4) menunjukkan pegangan dan perlekatan sel yang baik. Peratusan kebolehhidupan sel dan aktiviti ALP MG-63 masing-masing adalah lebih tinggi dan serupa dengan UHMWPE/0%, menunjukkan kesan bukan toksik kemasukan CNF dan LCNF dalam bionanokomposit UHMWPE. Secara keseluruhannya, penggabungan CNF dan LCNF dalam UHMWPE melalui pengadunan lebur menyumbang kepada rintangan haus yang lebih baik, modulus Young yang lebih tinggi dan keserasian sel yang sangat baik terhadap sel MG-63 osteoblastik. Penemuan ini menunjukkan kesesuaian dan potensi CNF dan LCNF sebagai pengisi nano dalam UHMWPE untuk aplikasi sisipan tibial, dengan kelebihan boleh diperbaharui, organik dan tidak toksik.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ANOVA	Analysis of variance
ASTM	American Standard Testing Method
BNC	Bacterial nanocellulose
Ca.	circa/approximately
CCD	Central Composite Design
CF	Carbon nanofiber
CNC	Cellulose nanocrystals
CNF	Cellulose nanofiber
CNT	Carbon nanotube
CO_2	Carbon dioxide
COF	Coefficient of friction
CSA	Canadian Standards Association
DMEM	Dulbecco modification of Eagle's basal medium
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
EDT-Na	Ethylenediaminetetraacetic acid disodium salt
FBS	Fetal bovine serum
FESEM	Field emission scanning electron microscopy
FESEM	Field-emission scanning electron microscopy
GO	Graphene oxide
Gr	Graphene
HA	Hydroxyapatite
HDPE	High density polyethylene

HNT	Halloysite Nanotube
HXPE	highly crosslinked polyethylene
IL	Interleukin
ISO	International Organization for Standardization
LCNF	Lignin-containing cellulose nanofiber / lignocellulose nanofiber
LDPE	Low density polyethylene
LLDPE	Linear low-density polyethylene
MA	Maleic anhydride
MAPE	Maleic anhydride grafted polyethylene
MCP	Monocyte chemoattractant protein
MEM	Minimum essential medium
MIP	Macrophage inflammatory proteins
MTT	Methyl thiazolyl tetrazolium
Mw	Molecular weight
n	number of replicates
NaOH	Sodium hydroxide
ND	Nano-diamond
Ø	Diameter
OPEFB	Oil palm empty fruit bunch
PBS	Phosphate buffered saline
PDGF	Platelet-derived growth factor
PE	Polyethylene
PEEK	Polyether ether ketone
PLA	Polylactic acid
pMDI	Polymeric diphenyl-methane diisocyanate
PMMA	Poly (methyl methacrylate)

pNPP	p-nitrophenyl phosphate
POM	Polyoxymethylene
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PVA	Polyvinyl alcohol
RSM	Response surface methodology
SEM	Scanning electron microscope/scanning electron microscopy
SEM-EDS	Scanning electron microscope-energy dispersive spectroscopy
TAPPI	Technical Association of the Pulp and Paper Industry
TG	Thermogravimetric
TGF	Transforming growth factor
TKA	Total knee arthroplasty
TKR	Total knee replacement
TNF	Tumor necrosis factor
UHMWPE	Ultra-high molecular weight polyethylene
VE	Vitamin E
XRD	X-ray diffractometer

CHAPTER 1

INTRODUCTION

1.1 Background

Polymeric tibial insert is one of the components for prosthetic or artificial knee joint. The general component of the artificial joint consists of the metal femoral component, polymeric tibial inserts, and metal tibial tray. To date, ultra-high molecular weight polyethylene (UHMWPE) has been used as material for tibial insert for over 50 years due to its excellent properties of chemical inertness, lubricity, impact resistance, and abrasion resistance (Bracco et al., 2017; Kurtz, 2016f). However, this polymeric component has limited longevity of approximately 10 to 15 years upon implantation. By years, the sliding and rolling movement of the high stiffness metal femoral component on the UHMWPE tibial inserts surface with the exertion of external force can lead to abrasion and fatigue. This eventually causes subsequent generation of wear, component failure, inflammation to adverse tissue, and bone osteolysis (Baena et al., 2015). The wear effect can be intensified by the presence of grain boundary during consolidation, instability of the material in biological condition (*i.e.,* lipid, cholesterol), and adhesion-friction phenomenon during knee movement (Baena et al., 2015; Bhushan, 2003; James et al., 2016).

Therefore, the improvement of UHMWPE mechanical properties and wear resistance is imperatively needed, not limited to structure modification by crosslinking, but also through filler embodiment as UHMWPE composite. Filler incorporation could improve the strength of the current UHMWPE components in total joint arthroplasty without sacrificing their other excellent attributes such as biocompatibility, chemical stability, and wear resistance (Gupta et al., 2013; Puértolas & Kurtz, 2016). Embedded nano-sized filler generally contributed to Young's modulus and tensile strength enhancement of most polymers. According to Zhou et al. (2017), the presence of filler may contribute to some load-bearing capacity, such as increased stiffness and hardness, thus reducing the stress exerted on the polyethylene matrix. For example, the use of 1 wt.% carbon nanofiber as filler successfully improved the strength and modulus of UHMWPE, contributing to the decrement of wear rate up to 42%, 64%, and 83% in air, artificial, and natural lubricant accordingly (Yousef et al., 2017). Yet, concerns over the biocompatibility of these fillers and their particulate debris which are detrimental have hindered the clinical use of UHMWPE as a prosthetic joint component (Wang et al., 2016).

In comparison to other well-studied fillers such as carbon nanofibers, carbon nanotube, and graphene, the use of nanocellulose including cellulose nanofiber (CNF) in the UHMWPE matrix is much less and yet to be widely studied. This could be related to the incompatibility between cellulosic materials with UHMWPE. Nevertheless, nanocellulose is an interesting material to be studied because it is known to be biocompatible and organic. It was reported that the use of cellulose nanocrystal, which is a type of nanocellulose, contributed to the reduced generation of wear debris (Li et al., 2017b; Wang et al., 2016). Not only that, the recorded tensile strength surpassed the minimum requirement of fabricated UHMWPE for joint, which is 27 MPa (Wang et al., 2016). Reduced generation of wear debris indicates a lower wear rate hence reduced risk of failure. CNF, a high aspect ratio nanocellulose, is expected to contribute to the same effect with the advantage of being more effective in improving Young's modulus of UHMWPE. A higher aspect ratio filler can generally cause a material to be stiffer (Kumar et al., 2015; Qiao et al., 2017).

The main challenge in mixing UHMWPE with nanocellulose would be the extremely high melt viscosity of UHMWPE attributed to its high molecular weight ranging from 3 to 6 million g/mol (Bracco et al., 2017; Kurtz, 2016f). Wang et al. (2016) and Li et al. (2017b) prepared UHMWPE/CNC bionanocomposites by dispersing the CNC and UHMWPE in ethanol to produce a homogenous mixture. Despite the success in making UHMWPE/CNC bionanocomposites through this method, this may be difficult to be applied in large-scale processing. It is hence important to optimize the melt blending processing method for producing UHMWPE/nanocellulose bionanocomposites by considering parameters such as temperature, rotational speed, and duration.

Nevertheless, nanocellulose with numerous free hydroxyl groups is highly hydrophilic and incompatible with hydrophobic thermoplastics, thus may affect the composites mechanical performance (Kargarzadeh et al., 2018b; Maia et al., 2019; Nair et al., 2017). Improvement in filler matrix interaction is hence essential by introducing uses of compatibilizer or coupling agent that will interact with both filler and matrix or enhance the filler's hydrophobicity (Kalia et al., 2014; Kargarzadeh et al., 2018b; Yang et al., 2019). This includes the utilization of lignin-containing CNF (LCNF) due to its possible enhancement of compatibility through the presence of hydrophobic lignin (Chen et al., 2019; Espinosa et al., 2019; Ferrer et al., 2016).

In terms of cytocompatibility, utilization of cellulose nanocrystal (CNC) as UHMWPE filler was proven to be non-toxic, indicated by significantly higher MC3T3-E1 preosteoblast cells viability and lower inflammatory response by macrophage RAW 264.7 cells (Wang et al., 2016), despite a possible inflammatory reaction by internalization of rod-like CNC by macrophage phagocytic function (Ventura et al., 2020). However, having a higher aspect ratio and length than CNC, CNF utilization as UHMWPE filler may elicit different toxicological effects, even though it is generally not phagocytized and does not cause inflammatory response (Ventura et al., 2020). Therefore, cytocompatibility evaluation of the UHMWPE/CNF and UHMWPE/LCNF bionanocomposites is imperative and essential. A study by Wang et al. (2016) showed significantly higher viability of MC3T3-E1 preosteoblast cells and lower inflammatory response of macrophage RAW 264.7 cells when grown on UHMWPE sample containing cellulose nanocrystals (CNC), in comparison to the UHMWPE itself.

1.2 Problem statement

The polymeric UHMWPE tibial inserts has limited longevity due to its relatively lower stiffness than the metal femoral component and subsequent wear. Inclusion of natural and non-toxic filler for UHMWPE properties improvements such as CNF and LCNF may enhance its longevity. Relatively lower wear volume was generated by incorporating CNC (Li et al., 2017b; Wang et al., 2016), indicating the nanocellulose potential as UHMWPE filler for improving wear resistance. Additionally, the use of CNF and LCNF in UHMWPE as filler has not been reported elsewhere. Therefore, it is hypothesized that the inclusion of CNF as filler in UHMWPE matrix may enhance it stiffness while at the same time contributing to an improved wear resistance property. The optimization of UHMWPE/CNF melt blending processing parameter, evaluation of blending process, and the investigation on lignin residue in CNF effect could lead to the production of UHMWPE/CNF with good filler dispersion and matrix-filler interaction. It is also expected that the CNF inclusion with or without the presence of lignin would not affect the biocompatibility of UHMWPE against osteoblastic cells MG63.

1.3 Objectives

The general objective of this study was to evaluate the effect of blending process and residual lignin in CNF on the mechanical, wear resistance and cytocompatibility properties of the UHMWPE/CNF bionanocomposites, aimed for tibial insert application. The specific objectives of this research were:

1. To optimize the UHMWPE/CNF bionanocomposite melt blending processing parameters by using response surface methodology (RSM).

2. To compare the characteristics of UHMWPE/CNF bionanocomposites prepared via melt blending and ethanol mixing.

3. To evaluate the effect of lignin residue on UHMWPE/CNF bionanocomposite mechanical and thermal stability properties.

4. To determine the tribological properties of UHMWPE/CNF and UHMWPE/LCNF bionanocomposites

5. To evaluate the cytocompatibility of UHMWPE/CNF and UHMWPE/LCNF bionanocomposites against osteoblastic MG63 cells.

1.4 Scope of study

This study began with optimizing the bionanocomposites processing through melt blending in terms of mixing temperature, rotational speed, and mixing time (Objective 1). Preliminary experiments were conducted for the range determination of those three factors, and optimization was performed using the response surface methodology (RSM) approach. The UHMWPE/CNF bionanocomposites produced through the optimized process were then analyzed for their mechanical properties and filler dispersion. Afterward, the influence of compatibilizer, maleic anhydride-*grafted*-polyethylene (MAPE) was also evaluated. Next, the effect of different blending processes was investigated (Objective 2). A comparison between UHMWPE/CNF produced through melt blending, and ethanol-mixing processing was carried out in terms of mechanical properties, morphological characteristics, and crystallinity indices. Herein the effect of filler content was also investigated. The best blending procedure was then selected to produce bionanocomposites with different filler types, CNF and lignin-containing CNF (LCNF) (Objective 3).

The evaluation of wear resistance properties of the UHMWPE/CNF and UHMWPE/LCNF was carried out in Objective 4, whereby the coefficient of friction and wear rate during pin-on-disc wear testing were determined. In addition, the worn surfaces were analyzed through atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM) for furrow depth scar and width identification, respectively. Lastly, in Objective 5, cell cytocompatibility evaluation against MG63 osteoblastic cells was conducted. The cells morphology, viability, and bioactivity (total protein and alkaline phosphatase activity) were determined. The overall experimental overview is as shown in Figure 1.1.

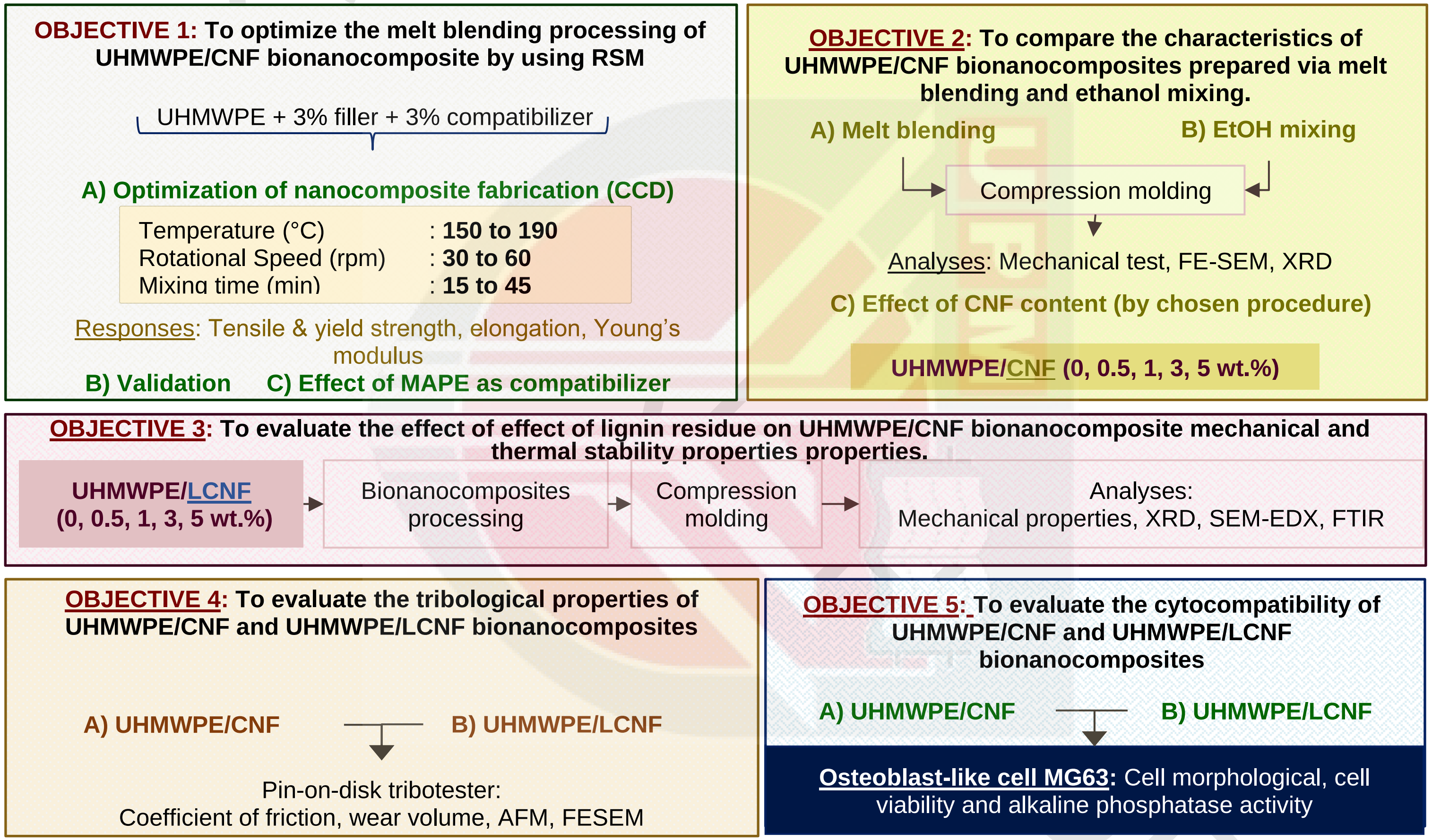

Figure 1.1: The overall experimental overview of this research.

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