SYNTHESIS AND CHARACTERIZATION OF INJECTABLE BONE CEMENT PREPARED FROM BIPHASIC CALCIUM PHOSPHATE EXTRACTED FROM LAMB AND BOVINE BONES

USMAN TARIQ

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DEDICATION

Dedicated to my family and friends for their continuous moral support during whole of my academic career

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ABSTRACT

Dicalcium phosphate (DCP) cements such as dicalcium phosphate dihydrate (DCPD) also known as brushite and dicalcium phosphate anhydrous (DCPA) also known as monetite have received considerable attention of researchers due to their potential applications in dental, maxillofacial and orthopaedic surgery. Quick setting and poor injectability due to liquid–solid phase separation limit the clinical use of brushite and monetite cements. The presence of certain ions (Mg, Zn, Na, Sr, Co, Ag etc.) in the cement during the setting process can influence setting time and the properties of the cement. In this study we report preparation of injectable dicalcium phosphate (DCP) bone cement using biphasic calcium phosphate (BCP) extracted from lamb and bovine femur bones. BCP was extracted by calcinating the defatted lamb and bovine bones at 1450 °C. BCP was extracted from three batches each for lamb and bovine bones. EDX analysis showed the presence of Mg and Na ions as trace elements in extracted BCPs. X-ray diffraction pattern of the prepared cement showed the formation of brushite along with monetite as minor phase along with a small quantity of hydroxyapatite. Monetite phase diminished gradually with the decrease in powder to liquid ratio (PLR). The values of initial and final setting times were observed to be well within the preferable range 3-8 minutes, for initial and less than 15 minutes for final setting time, as recommended for orthopedic applications. Exceptional injectability (>90 %) was achieved for almost all the PLR formulations used for preparation of DCP cement. A decrease in the compressive strength was observed with increasing liquid phase in the cement, which was attributed to the resulting higher degree of porosity in the set cement. Moreover, for the DCP cement prepared from three different batches of BCP extracted from bovine and lamb bones, there were no noticeable variations in the setting time, injectability or compressive strength. Apatite layer formation on the cement surface was studied by immersing cement samples in simulated body fluid (SBF) for up to 7 days. A formation of apatite layer and an increase in the compressive strength from 2.71 ± 0.22 to 9.68 ± 0.36 MPa were observed. These results indicate that bone cement prepared from BCP extracted from lamb and bovine femur bones can be considered for orthopaedic applications as a potential bone substitute for regeneration and repairing of bone defects.

ABSTRAK

Simen dikalsium fosfat (DCP) seperti dikalsium fosfat dihidrat (DCPD) yang juga dikenali sebagai brusyit dan dikalsium fosfat anhidrat (DCPA) juga dikenali sebagai monetit telah mendapat perhatian para penyelidik kerana aplikasi potensinya dalam pembedahan pergigian, maksilofasial dan ortopedik. Penetapan pantas dan kebolehsuntikan lemah yang disebabkan oleh pemisahan fasa pepejal-cecair membataskan penggunaan klinikal untuk simen brusyit dan monetit. Kehadiran ion tertentu (Mg, Zn, Na, Sr, Co, Ag dll.) dalam simen semasa proses penyuntikan boleh mempengaruhi masa penetapan dan sifat simen tersebut. Dalam kajian ini, kami melaporkan penyediaan suntikan simen tulang dikalsium fosfat (DCP) menggunakan kalsium fosfat dwifasa (BCP) yang diekstrak daripada tulang femur kambing dan lembu. BCP telah diekstrak dengan mengkalsinkan tulang kambing dan lembu nyahlemak pada suhu 1450 °C. Analisis EDX menunjukkan kehadiran ion Mg dan Na sebagai unsur surih dalam BCP yang telah diestrak. Corak pembelauan sinar-X pada simen yang disediakan menunjukkan pembentukan brusyit dan monetit sebagai fasa minor bersama hidroksiapatit dalam kuantiti yang kecil. Fasa monetit berkurang secara beransur dengan pengurangan nisbah serbuk terhadap cecair (PLR). Nilai masa tetapan awal dan akhir didapati terletak dalam julat yang dikehendaki iaitu 3-8 minit, untuk masa tetapan awal dan kurang daripada 15 minit untuk masa tetapan akhir, seperti yang dicadangkan untuk aplikasi ortopedik. Kebolehsuntikan luar biasa (>90 %) dicapai untuk hampir kesemua formulasi PLR yang digunakan untuk penyediaan simen DCP. Penurunan kekuatan mampatan dengan peningkatan fasa cecair dalam simen, telah dicerap, dan ini disebabkan oleh darjah keliangan lebih tinggi dalam simen yang disediakan. Tambahan pula, bagi simen DCP yang disediakan daripada tiga kelompok berbeza yang diesktrak daripada tulang lembu dan kambing, tidak terdapat variasi ketara dalam masa penetapan, kebolehsuntikan atau kekuatan mampatan. Pembentukan lapisan apatit pada permukaan simen telah dikaji dengan merendamkan sampel simen ke dalam bendalir badan tersimulasi (SBF) sehingga 7 hari. Pembentukan lapisan apatit dan peningkatan kekuatan mampatan daripada 2.71 ± 0.22 kepada 9.68 ± 0.36 MPa telah dicerap. Keputusan ini menunjukkan bahawa simen tulang yang disediakan daripada ekstrak BCP daripada tulang femur kambing dan lembu boleh dipertimbangkan untuk aplikasi ortopedik sebagai tulang gantian yang berupaya untuk pemulihan dan pembaikan kecacatan tulang.

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LIST OF ABBREVIATIONS

HA	-	Hydroxyapatite
TCP	-	Tricalcium Phosphate
CaP	-	Calcium Phosphate
CPC	-	Calcium Phosphate Cement
CS	-	Compressive Strength
CDHA	-	Calcium Deficient Hydroxyapatite
βTCP	-	β- Tricalcium Phosphate
SBF	-	Simulated body fluid
BG	-	Bioactive Glasses
BCP	-	Biphasic Calcium Phosphate
MCPM	-	Monocalcium phosphate monohydrate
MCPA	-	Monocalcium phosphate anhydrous
DCPD	-	Dicalcium phosphate dihydrate (Brushite)
DCPA	-	Dicalcium phosphate anhydrous (Monetite)
OCP	-	Octacalcium Phosphate
αTCP	-	α-Tricalcium Phosphate
TTCP	-	Tetra calcium Phosphate
ACP	-	Amorphous calcium phosphate
DDW	-	Doubled distilled water
FTIR	-	Fourier Transform Infrared Spectroscopy
XRD	-	X-Ray diffraction
EDX	-	Energy-dispersive X-ray spectroscopy
FESEM	-	Field Emission Scanning Electron Microscopy
ICP-OES	-	Inductively coupled plasma optical emission spectroscopy
SD	-	Standard deviation
BMR	-	Brushite to monetite ratio
PLR	-	Powder to liquid ratio

LIST OF SYMBOLS

ρ_o	-	Density of water
φ	-	Porosity
m_1	-	Mass of dry sample in air
m_2	-	Mass sample submerged in water
m_3	-	Mass of wet sample in air
F	-	Force
W_F	-	Mass of syringe filled with bone cement before injection
W_A	-	Mass of syringe filled with CPC after injection
W_B	-	Mass of empty syringe before injection
A	-	Area of cross section
2θ	-	Brags' diffraction angle

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bone is an essential part of the human body as it provides strength and framework to the body and helps in carrying out metabolic, synthetic and mechanical functions. In recent past, for the damaged or deceased hard tissues, surgeries are performed to remove the damaged part to provide relief to the sufferer. But it is not a preferred strategy now-a-days, as the researchers are focusing on developing such techniques which involve least surgical procedures. In this regard, injectable biomaterials like calcium phosphate cements (CPCs) have become extremely important research field.

Since the discovery of first calcium phosphate cements (CPCs) in 1980s, lot of efforts have been devoted to improve the performance of such orthopedic products. Generally these CPCs are based on a powder-liquid concept, which are mixed prior to use [1]. Mixing of solid and liquid material results in a paste which subsequently sets to give a hard mass [2]. Usually, one or several calcium phosphate compounds serve as a solid phase for CPC, whereas, liquid phase comprises of water or a solution containing calcium or phosphate [3]. Despite the different forms and compositions of CPCs, they are categorized into apatite and dicalcium phosphate (DCP) cements depending upon the final product formulation reactions [4]. Apatite cement can be hydroxyapatite (HA) or calcium-deficient hydroxyapatite (CDHA). While, the DCP cement family has two members; dicalcium phosphate dihydrate (DCPD) often termed as brushite and the dicalcium phosphate anhydrous (DCPA) also referred to as monetite [5].

Dicalcium phosphate (DCP) (one of the members of calcium phosphate family) has become materials of great interest due to their orthopedic and dental surgery applications [6]. DCP cements are effectively prepared using reagents monocalcium phosphate monohydrate, phosphoric acid, sulfuric acid, citric acid, and pyrophosphoric acid. [7–12]. However, monocalcium phosphate monohydrate (MCPM) is the commonly used reagent for brushite formation [7,13–16]. The vicinity of a water particle in monocalcium phosphate monohydrate (MCPM) encourages the setting reaction of the cement by donating one of the two water molecules required by DCP precipitates to form dicalcium phosphate dihydrate (DCPD). Therefore, MCPM is preferred over monocalcium phosphate anhydrous (MCPA) in the preparation of brushite cement. The preparation of brushite involves dissolution-precipitation process [13]. Brushite is preferred over hydroxyapatite among calcium phosphate based cements due to its good biocompatibility and higher resorption under physiological conditions [17].

Since the clinical needs for synthetic bone graft materials are growing so, it has encouraged the researchers to develop injectable self-setting calcium phosphates. Injectability and optimal in situ setting time of DCP cement makes it possible to avoid painful surgeries by decreasing the invasiveness during surgeries. These DCP cement formulations can also be advantageous for patients and the medical system as it aids in reducing the recovery time.

Major issue with the injectability of DCPD (brushite) bone cement is solid-liquid phase separation during injection process. One of the solution to address this issue is to incorporate ions in the cement matrix [13,14]. The incorporation of ions improve the setting time, and paste homogeneity, which reduces the phase separation thereby, improving the injectability [13,18]. The pure brushite has poor mechanical strength (≈ 1MPa) and requires improvement in the injectability. The compressive strength of the cement is directly related to porosity of the specimen [19]. While mechanical properties of the injectable bone cement can be enhanced by adding substances like pyrophosphates, carboxylic acids, sulfates and ions (Mg^{2+}, Sr^{2+}, Zn^{2+} and Si^{2+}) [20]. However, incorporation of ions in the brushite cement formulation require extra effort during material preparation.

Beta tricalcium phosphate (β-TCP) is one of the reagents commonly, used in the brushite cement preparation. In common practice, the β-TCP is prepared synthetically but it can also be extracted from biological resources such as mammalian bones. Mostly calcination of mammalian bone at 1200 °C or more results in a biphasic calcium phosphate (BCP) which contains both β-TCP and HA [21,22]. BCP derived from mammalian bone also naturally contain ions like Na, Mg, Zn, Sr, K [22–24] which might be helpful in enhancing the injectability and mechanical properties of the cement without using extra additives during cement preparation.

1.2 Problem Statement

The most desirable way to repair damaged bone is to regrow natural, undamaged bone in its place. Unfortunately, if large volume of tissue is removed, the body cannot regrow an entire new piece of bone. In these cases, the need for an artificial substitute is unavoidable [25]. The ideal bone substitute would be a material that forms a secure bond with the tissues by allowing and encouraging new cells to invade. One way to achieve this is to use injectable bone cement. Injectable bone cements involves minimum invasive surgery procedures which is a highly preferred treatment technique of bone defects [26,27]. Injectable calcium phosphate cements are very effective bone replacement materials.

Available bone cements prepared from synthetic routes with no additives, are difficult to inject due to very short setting time (the time in which cement gains enough strength so that it can maintain its shape) and solid-liquid separation during injection. The desirable range of setting time for surgeons is 3 to 8 minutes in order to perform injection of cement comfortably. But the setting time of pure DCP cement is approximately 30 seconds which makes it impossible to be used as an injectable cement to practice minimal invasive surgical procedures. So, the setting time of synthetically prepared bone cement need to be improved by manually addition of ions and polymers during its synthesis making the process more laborious. In addition, their preparation involves the use of toxic chemical like ammonium hydroxide that can never be preferable if a chemical free alternative is available.

Therefore, the present work focuses on the preparation of injectable bone cement using BCP extracted from mammalian (lamb and bovine) bones with potentially adequate setting time and mechanical properties along with good injectability. Bone cements prepared from β-TCP are already being used for bone healing. Since, BCP extracted from natural biological resources also contains β-TCP which helps cement formation and naturally existence of ions in BCP can be helpful in improving the setting time and injectability of the bone cement. Thus, bone cement with potentially better injectability can be prepared using BCP extracted from natural bones. Moreover, the use of toxic chemical like NH_4OH can also be avoided as its higher dose can cause bronchitis, severe lung irritation, pulmonary edema etc.

1.3 Objectives

The main objective is to synthesize and characterize injectable dicalcium phosphate bone cement using BCP extracted from lamb and bovine bones.

i. To extract biphasic calcium phosphate (BCP) from lamb and bovine bones and characterize for chemical properties.
ii. To synthesize dicalcium phosphate (DCP) bone cement using BCP extracted from lamb and bovine bones and characterize for chemical properties.
iii. To determine setting time, injectability and mechanical properties of the synthesized dicalcium phosphate bone cement.
iv. To study the in vitro resorption of selected dicalcium phosphate bone cement samples in Simulated Body Fluid (SBF).

1.4 Scope of Research

This study involves the extraction and physiochemical characterization of BCP derived from two types of bones (bovine and lamb bone) and dicalcium phosphate cement preparation for potential orthopedic application.

The biphasic calcium phosphate (BCP) is extracted from the lamb and bovine bones by calcination process. The injectable dicalcium phosphate bone cement is prepared by mixing together BCP (extracted from bovine and lamb bone) with MCPM in optimized ratio 1:0.8 and then mixing with liquid phase in various powder to liquid ratios (PLR) such as 2.6, 2.8, 3.0, 3.2 and 3.4 g mL^{-1}. The repeatability of the procedure is determined by synthesizing the dicalcium phosphate cement using BCP extracted from 3 batches each of bovine and lamb bones acquired from random animals.

BCP extracted from all batches of bovine and lamb bones and corresponding bone cement specimens are characterized using XRD and FTIR for phase analysis and functional groups identification. While only extracted BCP samples are subjected to EDX for elemental identification.

The setting time, injectability, porosity and compressive strength of the prepared bone cement samples all different batches are determined. In vitro ion release of the specific bone cement samples (selected based upon experimental results) is measured after immersion in SBF solution for 1, 3, and 7 days. In addition to ion release, the porosity and compressive strength of the samples is also determined after immersion in SBF over the course of 7 days. Changes in morphology of the specific bone cement samples are also studied using FESEM after 1, 3 and 7 days of immersion in SBF to observe the bone-like apatite formation on the surface of cement samples to confirm the bioactivity of the bone cement.

1.5 Significance of the Study

The proposed method for cement preparation opens new doors in the field of injectable bone cement preparation. This research contributes to explore new combinations for producing injectable bone cements with good injectability. This method of bone cement preparation allows the utilization of bone waste. Extraction procedure of BCP from bones is fairly simple as compared to synthetic preparation procedure of β-TCP. The proposed method allows to avoid the use of toxic chemical like ammonium hydroxide and reduces the cost for surgical procedure.

1.6 Thesis Outline

A novel way of bone cement preparation has been reported in this thesis. This document describes the synthesis and characterization of injectable bone cement prepared from β-TCP derived from bovine and lamb bones.

Chapter 1 briefly discusses the background of the research theme, problem statement of the research and objectives of this project. An overview of the structure of bone, bone healing process, various biomaterials, bone cements and factors affecting their properties are elaborated in chapter 2. Extraction methodology of βTCP from lamb and bovine bones as well as characterizations techniques are described in chapter 3. Chapter 4 consists of six sections related to extraction of BCP and preparation of cement. Section 4.1 deals with the general overview of the chapter while extraction and characterization of BCP from bovine and lamb bone are illustrated in sections 4.2 and 4.3 respectively followed by characterization of prepared cement presented in sections 4.4 and 4.5 respectively. Whereas, in vitro biocompatibility studies of prepared bone cement specimens are presented in section 4.6 and summary of results and discussion chapter is presented in section 4.7. At the end, Chapter 5 includes the conclusion and recommendations for the future work.

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