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3D printed chitosan / hydroxyapatite scaffolds for potential use in regenerative medicine

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Abstract: *The present study investigates properties of direct 3D printed chitosan/hydroxyapatite scaffolds. Chitosan is biodegradable which is beneficial for certain indications in the field of regenerative medicine. Hydroxyapatite on the other hand improves the mechanical properties and occurs as a major component in the mineral phase of bones. These two materials were combined and processed on an optimized 3D printing method to produce scaffolds with promising mechanical properties.*

Keywords: *Chitosan, Hydroxyapatite, 3D printing, regenerative medicine*

Introduction

Research on biomaterials for bone implantation and replacement has expanded considerably over the last four decades [1]. Nowadays novel biomaterials consist of bioactive and bioresorbable substances which mimic the natural function of bone and activate in vivo mechanisms of tissue regeneration. Such composite materials based on biodegradable polymers and bioactive ceramics, are suitable for regenerative medicine. These composites exhibit tailored physical, biological and mechanical properties as well as adaptable degradation behaviour [1]. The natural biopolymer chitosan is currently a subject of interest in tissue engineering. Chitosan is characterized by excellent biostimulation which comes from its chemical properties [2] [3]. Hydroxyapatite (HA) is one of the major constituents of the inorganic component in human hard tissues (bones and teeth). Therefore a composite of HA and chitosan is expected to be a good biomaterial [4]. 3D printing is a technology where a three dimensional CAD component is created in layers using liquid binder for solidification. Especially customizable scaffolds with complex structures and high cavity can be easily produced using 3D-printing.
In the present paper, we report the preparation and characterization of 3D printed chitosan/HA composites. To analyze mechanical properties and surface structures, measurements of compressive strength (CS), IR spectroscopy and scanning electron microscopy (SEM) were performed.

Materials and Methods

Materials
Chitosan was obtained from Eastar Holding Group (Dongchen, China). Hydroxyapatite Medicoat AG (Mägenwil, Switzerland). Lactic acid (Fluka) and sodium hydroxide (Scharlau) was used during the printing process and post treatment.

Methods
To define the suitable binder solution preliminary tests were performed with 20 wt.%, 30 wt.%, 40 wt.% lactic acid (LA), citric acid (Roth) and acetic acid (Fluka). The potential binder solutions were added to the powder composite (25 wt.% chitosan) and mixed with a spatula. Wettability, viscosity and solidification time were assessed.

An adapted 3D-Printing system (Z-Corp, Z-510) was used to produce hydroxyapatite/chitosan fully dense cylinders of Ø 10 x 10 mm and probes with open porous structures as shown in Fig. 1. The structures were printed with 40 wt.% LA as binder solution. Specimens with different composite ratios (20 wt.%, 25 wt.%, 30 wt.% chitosan) were printed. In the post hardening process the specimens were immersed in 2N NaOH for 3 sec and then in LA (7.5 wt.%, 10 wt.%, 20 wt.%) for 10 sec, drying duration 2 d, RT.

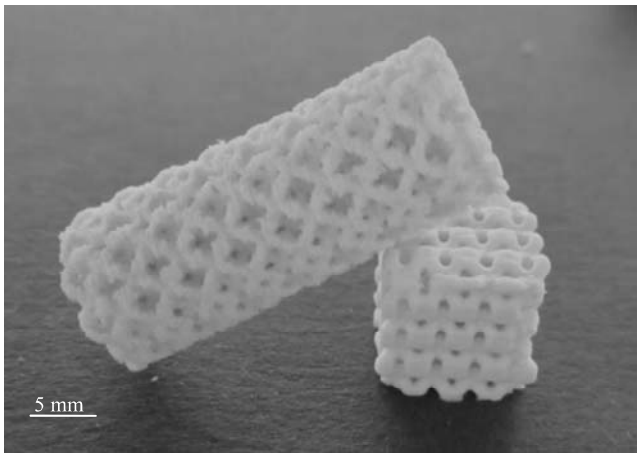

Figure 1: 3D-Printed structures with an open porous geometry composed of 25wt.% chitosan and 75wt.% HA.

Analysis
The samples were characterized by SEM (TM-1000, Hitachi), IR spectroscopy (Varian 670-IR, Agilent Technologies) and compression strength (Hydropulser LFV-5-PA/ECD 120, walter + bai ag).

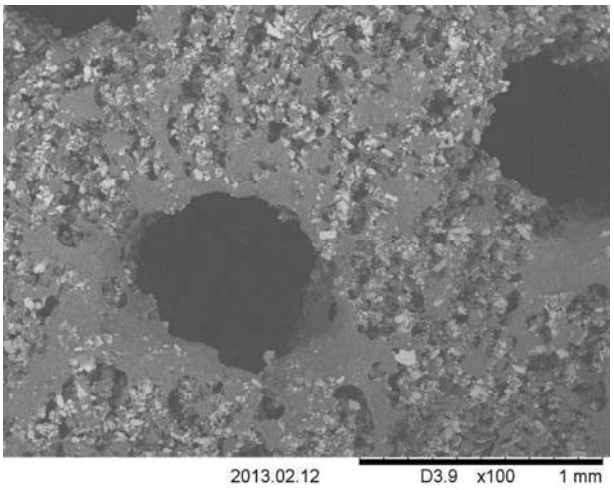

Figure 2: Chitosan/HA mesh structures after immersion into 7.5 wt.% lactic acid. It can be seen that the acidic treatment was not sufficient to evoke a closed and smooth chitosan layer.

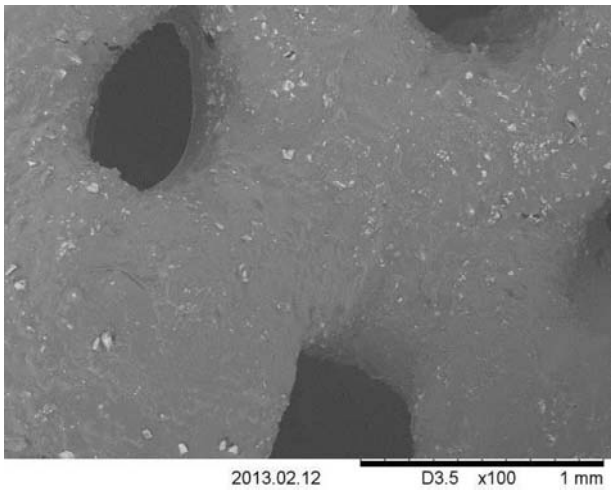

Figure 3: Chitosan/HA mesh structure after immersion into 10 wt.% lactic acid. The acidic treatment resulted in close and smooth chitosan top layer.

Results

40 wt.% LA was considered as best suitable for the printing process due to its medium viscosity, high wettability and short solidification time. The most stable and accurate green parts were printed with a composite ratio of 20 wt.% chitosan and with 40 wt.% LA as binder.
While solid specimens could be post treated with 40 wt.% LA, open porous structures could only be treated with 10 wt.% LA. They collapsed in contact with higher concentrated LA. The overall best mechanical properties were observed with solid specimens printed with 40 wt.% LA, composite ratio 25 wt.% chitosan and post treatment with 40 wt.% LA. Compression strength of these specimens resulted in 16.32 MPa (s = 2.843), E Modulus 4.4 GPa (s=2.113) with a porosity of 37.1%. Along all fabrication steps the fully integrity of all initial components could be demonstrated by means of IR spectroscopy measurements.

Discussion

Although bioactive HA and degradable polymers are promising biomaterials, 3D printing of mechanically stable composite structures is still a challenge. Desired mechanical properties could not be achieved by high temperature sintering methods, because chitosan would decompose above 220°C [5].With SEM pictures a compact layer of chitosan can be observed after immersion in 10 wt.% LA as illustrated in Figure 3. Compared to that, the immersion in 7.5 wt.% LA showed a lower coverage. Literature claims a compressive strength of 3D-printed composites with other bio polymers in a range of 0.5-5 MPa [6] [7]. This emphasizes the potential of the herein investigated material with its 16.3 MPa CS. However the insolubility of the manufactured specimens needs to be further improved to ensure long-lasting mechanical stability in body like environments. Furthermore the applied post hardening regime is only applicable to the outermost shell of approximately 1.2 mm. A reduction of this effect would additionally increase compressive strength.

Acknowledgement

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