Investigation of Chitosan Film Degradation in Tissue Engineering Applications

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Abstract. In this paper, the biological and physical properties of chitosan, a potential polysaccharide resource, were investigated. The proposed film was synthesised by mixing chitosan (CS) solution and polyvinyl alcohol (PVA) using solvent casting. The modified chitosan/PVA films were then subjected to analysis by Fourier transform infrared spectroscopy (FTIR), in which intermolecular hydrogen bonding between the molecules of CS and PVA was clearly demonstrated. The swelling degree of chitosan along with the contact angle were also examined. After the addition of genipin, which increased chitosan concentration the swelling degree, degradation rate, and contact angle was decreased. The results confirm that the properties of chitosan are such that they can be employed for tissue engineering applications.

Keyword: chitosan, degradability, lysozyme, bone tissue engineering.

1. Introduction
Over recent decades, tissue engineering has been widely studied due to the necessity for developing substitutes for damaged, diseased, or injured parts of the body. Cells with high proliferation and differentiation the required environments are crucial for the tissue regeneration process [1]. One of the most widely focused on materials for this application has been chitosan, a cationic linear copolymer polysaccharide, due to its-remarkable properties, which include biocompatibility, biodegradation, biological activity, porous structure, high affinity towards biological molecules, and low-toxicity [2], in addition to its ability to provide a promising environment for cell growth [3]. However, pure chitosan is not recommended for such uses to its poor mechanical strength and fragile nature. To overcome these drawbacks, CS combined with other materials has been considered for tissue engineering, with mixes such as CS/collagen, CS/PVA and CS/Alginate [4]. Poly vinyl alcohol (PVA) is a synthetic polymer used in many industrial applications due to its compatible structure, hydrophilic properties, high stability, and low manufacturing cost [5], while genipin is a natural crosslinking agent material obtained from the fruit of Gardenia Jasminoides [6]. Genipin can crosslink biopolymers such as chitosan, alginate, and collagen, leading to the formation of covalent bonding, which enhances their mechanical properties [7, 8]. Simona et al. studied different concentrations of genipin in chitosan scaffolds in PBS solutions with lysozymes. The results exhibited effective changes in the degradation of chitosan scaffolds such that, when the concentration of genipin was increased, the degradation of the scaffold was decreased. They thus recommended utilisation of cross-linked chitosan using genipin tissue engineering [9]. Santosh et al., studied the degradation of chitosan- and chitosan -PVA blends. Degradation rates were measured by weight loss when the samples were placed in the PBS media. The weight loss of the CS-PVA blend was lower than that of chitosan due to PVA’s stability in water, which means that it does not degrade unless the temperature ≥ 60 °C [10]. Long, et al., prepared scaffolds from chitosan and collagen with different concentration of genipin to study the degradation behaviour of scaffolds at periods 7 to 21
days after creation. The degradation behaviours of the scaffolds were decreased when the concentrations of genipin increased [11]. Thi-Hiep, et al also investigated the mechanical and physical properties of PVA/GE crosslinking with genipin. Their degradability study showed that PVA/GE crosslinked at various different concentrations appropriate for the production of scaffolds for bone regeneration [12].

In this work, the synthesis and characterisation of chitosan/PVA for tissue regeneration was investigated. Additionally, the effect of genipin cross-linking agent on the properties of the film was evaluated, and the physical properties of synthesised film cross-linked with genipin were observed.

2. Experimental

1-Materials

Chitosan (CS) at 75% deacetylation with a molecular weight of 161 g/mol was obtained from Xianm Shaanxi (China.); acetic acid (99%) was purchased from Chem-lab NV (Belgium.); PVA with molecular weight 1300- to 2300g/mol was obtained from CDH (India.); Genipin was obtained from CN Lab Nutrition, Asian Group (China.); phosphate buffer saline (PBS) of, pH7.2 was purchased from HIMEDIA (India.); and lysozyme (LZ, ≥ 90 % proteins, activity ≥ 40 000 U/mg ) was also obtained from CDH (India).

2-Preparation of Chitosan (CS) –PVA Film

Mixes of 1wt% and 3wt% of chitosan were prepared by dissolution in an aqueous solution of acetic acid; these were then mixed by a magnetic stirrer at 1,400 rpm at 50 °C for 1hr. simultaneously 1wt% of PVA was dissolved in distilled water and stirred at 120 °C. The blend was prepared by mixing the two polymer solutions (CS: PVA) (90:10). A constant concentration of genipin (0.2% wt%) was added to each of the blends and stirred for 1h at 50 °C. Table 1 shows the composition of the films blends. The solutions were placed in a petri dish and dried for 3 days at room temperature.

Table (1): Composition of the films blends.

| samples | CS con. | PVA con. | ratio | Genipin wt% |
|---------|---------|----------|-------|-------------|
| A       | 1%      | 1%       | 90:10 | -           |
| B       | 3%      | 1%       | 90:10 | -           |
| C       | 1%      | 1%       | 90:10 | 0.2         |
| D       | 3%      | 1%       | 90:10 | 0.2         |

2-1 Fourier transform infrared spectroscopy (FTIR)

By using FTIR microscopy [BRUKER, Germany], the vibrations of the functional groups of the films were determined to indicate the interaction between the chitosan and PVA blend.

2-2 Swelling degree

The swelling degree of the films with blended CS:PVA with and without the addition of genipin in dimensions 1cmx1cm as shown in figure 1, was carried out according to ASTM D4546-08(13) by immersing the blend films in phosphate buffer saline solution at pH 7.2 at room temperature for 24 h. The excess water was removed from the surfaces of the film using tissue paper and then the weight was recorded. The swelling degree was determined via the following equation:
Swelling degree (%) = \[\left(\frac{W_2 - W_1}{W_1}\right) \times 100\]  \hspace{1cm} (14)

where, \(W_1, W_2\) are the weights of dried and swollen samples, respectively.

![Sample for swelling test](image1)

Figure (1): Sample for swelling test

2-3 Degradation behaviour study

The degradation studied were performed to ASTM standard F1635-04a (15). The degradation rate of films of dimensions 2cm×2cm, as shown in figure 2, were determined by weighting the dry samples (\(W_0\)). Then, the films were immersed in PBS solution containing lysozyme 0.0001g/L and incubated at 37 °C for up to 5 weeks, with the degradation medium refreshed every third day. After each week of incubation, the films were removed from the degradation media, washed with distilled water and dried at room temperature. The weights of the dried scaffolds were recorded as \(W_d\). The degradation rate of the film was calculated as

\[
\text{Weight loss (\%)} = \left(\frac{W_0 - W_1}{W_0}\right) \times 100\]  \hspace{1cm} (16)

where \(W_0\) is the dry weight before degradation and \(W_1\) the dry weight after degradation.

![Degradation test sample](image2)

Figure (2) Degradation test sample

2-4 Water contact angle test

It was important to know the maximum time required for films to become more hydrophilic in order to enhance cell attachment to the films. This was determined by measuring the contact angle of the droplet (Young-Laplace fitting method) on surface films with dimensions 2cm×2cm, as shown in figure 3. This test was accomplished to ASTM standard D5946-04 (17).

![Contact angle sample](image3)

Figure (3) Contact angle sample
3. RESULT

1-FTIR

Figure 4 demonstrates the FTIR spectra obtained for the samples. The spectrum of chitosan without blending is shown in Fig (4a). The peaks located at 3388 and 2924 cm can be related to N-H and C-H stretching, respectively. The peak observed at 1631 cm is associated with N-H stretching in amide II. PVA spectrum is obviously demonstrated in Figure (4b). The bands located at 3253 and 2927 cm are linked to O-H and C-H bonds, respectively. After mixing chitosan with the PVA, another peak clearly occurs at 1743 cm that indicates interaction between O-H (for PVA) and N-H (for chitosan), as shown in figure (4c) [18]. Figure (4d) shows peaks located at 1253 cm, 1405 cm, and 1629 cm that can be assigned to C-O, CH3, and C=C bonds, respectively. These show the successful interaction between amine groups of chitosan and the carboxymethyl group of genipin [9]. The enhanced properties can be attributed to the interactions between chitosan and PVA in the blend by means of hydrophobic side-chain aggregation and intermolecular and intra-molecular hydrogen bonds, as shown in Figure 5 [19, 20].

2-Swelling degree

The swelling degree of the CS-PVA blended films in PBS solution is shown in Figure 6. From the figure, the cell attaches and penetrates through the blended films. Thus, the inter-reactions between the two polymer CS-PVA blends help to form a flexible or relaxed network that offers blended films the ability to swell. After adding a constant percentage of the cross-link agent, the swelling degree of films is decreased due to cross-link bonds between chitosan and genipin, which increases the bonding forces between the polymer chains, reducing the penetration of the liquid [21].

3- Degradation behaviour study

Weight loss is widely employed to investigate the enzymatic degradation of biodegradable polymers. Figure 7 demonstrates the degradation rate of cross-linked CS-PVA blended films. Generally, throughout the degradation process, the enzyme penetrates the film and reacts with the polymer blend. The results show that the penetration of enzyme through the film is limited by increasing the concentration of the cross-linking agent. In addition, the weight loss of samples significantly improved during the degradation time, probably due to the degradation of chitosan by lysozyme, which hydrolyses the glucosamine–glucosamine linkages [22].

4- Contact angle

The wettability of all films prepared was determined using contact angle analysis. The contact angles of all films are shown in figure 8. Generally, the addition of genipin to the blend of chitosan and polyvinyl alcohol at constant concentration decreased the contact angle. The results showed that the films became more hydrophilic with time, which is favourable for bone tissue engineering [9].

4. Conclusion

This paper, successfully investigated and evaluated the degradation behaviours of chitosan/PVA blends cross-linked with genipin for tissue engineering applications. The effect of adding the cross-link agent on the swelling rate was also examined. The results demonstrated that the proposed films enhanced the degradation process, improving the biological properties after the addition of genipin and offering an increased chitosan concentration. The superior properties of the proposed films make them suitable candidates for tissue regeneration applications.
Figure (4): FTIR of the films
Figure (5): Chitosan and PVA interaction.

Figure (6): Swelling degree of the films

Figure (7): Weight loss of the films
Figure (8): Contact angle testing of the films

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