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Biomedical applications of chitin hydrogel membranes and scaffolds

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ABSTRACT

Chitin is a non-toxic, biodegradable and biocompatible natural polymer. It is used in several biomedical applications. Chitin is insoluble in most of the organic solvents due to its rigid crystalline structure. However, it can be dissolved in calcium chloride dehydrate methanol (Ca solvent) solvent system. The α- and β-chitin hydrogels can easily be developed using the Ca solvent system. Using these hydrogels, it is able to develop scaffolds and membranes for the variety of biomedical applications such as tissue engineering and wound dressing. In this paper, we present the preparation and biomedical applications of chitin hydrogel membranes and scaffolds.

1. Introduction

Chitin is known to be a biodegradable polymer in nature and in the body (Hirano et al., 1990; Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1990) and to be of low toxicity when administered. For these reasons, chitin is useful for several biomedical applications (Jayakumar, Prabaharan, Nair, & Tamura, 2010; Jayakumar, Prabaharan, Nair, Tokura et al., 2010; Nishimura et al., 1985; Okamoto et al., 1993). However, chitin is insoluble in general organic solvents due to its rigid crystalline structure, which is based on the hydrogen bond between the acetamide group, hydroxyl group, and carbonyl group (Austin, 1975; Delacruz et al., 1992; Gardner & Blackwell, 1975; Kaifu, Nishi, & Tokura, 1981; Minke & Blackwell, 1978; Tamura, Nagahama, & Tokura, 2006). Chitin has different patterns of crystalline structure due to its origin. The outer skeletal of crab and shrimp consists of α-chitin, and squid pen consists of β-chitin. α-Chitin has been proposed to have a more rigid crystalline structure than β-chitin (Scheme 1). Several studies have been reported about the solubility of chitin (Tokura, Nishi, & Noguchi, 1979). In recent years, the calcium solvent system was found to be a good solvent system to dissolve the chitin under mild conditions (Tamura, Nagahama et al., 2006; Tokura, Nishimura, Sakairi, & Nishi, 1996). It has also been found that chitin hydrogels can be prepared using this calcium solvent system (Jayakumar & Tamura, 2008; Nagahama, Nwe et al., 2008; Tamura, Nagahama et al., 2006; Tamura, Sawada, Nagagama, Higuchi, & Tokura, 2006).

Chitin finds a lot of applications in various fields like cosmetics, water purification and separation material and as a food additive. Attempts have been made to use chitin in biomedical fields such as wound dressings and scaffolds due to their wound healing, antibacterial and anti-inflammatory properties (Jayakumar, Nwe, Tokura, & Tamura, 2007; Jayakumar, Selvamurugan, Nair, Tokura, & Tamura, 2008; Madhumathi, Binulal et al., 2009; Maeda, Jayakumar, Nagahama, Furuike, & Tamura, 2008). However, due to the insoluble nature of chitin, its applications are limited. Recently, researchers modified chitin into a chitin hydrogel for biomedical applications using the calcium solvent. (Jayakumar & Tamura, 2008; Nagahama, Higuchi, Jayakumar, Furuike, & Tamura, 2008; Nagahama, Kashiki et al., 2008; Nagahama, Nwe et al., 2008; Peter et al., 2009; Tamura, Nagahama et al., 2006; Tamura, Sawada et al., 2006). Using the chitin gel, it is possible to prepare membranes and scaffolds easily. In this review, we review the preparation and biomedical applications of chitin hydrogel membranes and scaffolds.

2. Preparation of chitin hydrogel membranes

α-Chitin hydrogel can be prepared by suspending α-chitin in water (approximately 0.1% w/v) and filtered through a saran-meshed filter to remove the water. The resulting chitin thread is pressed between filter papers at room temperature for 20 h.

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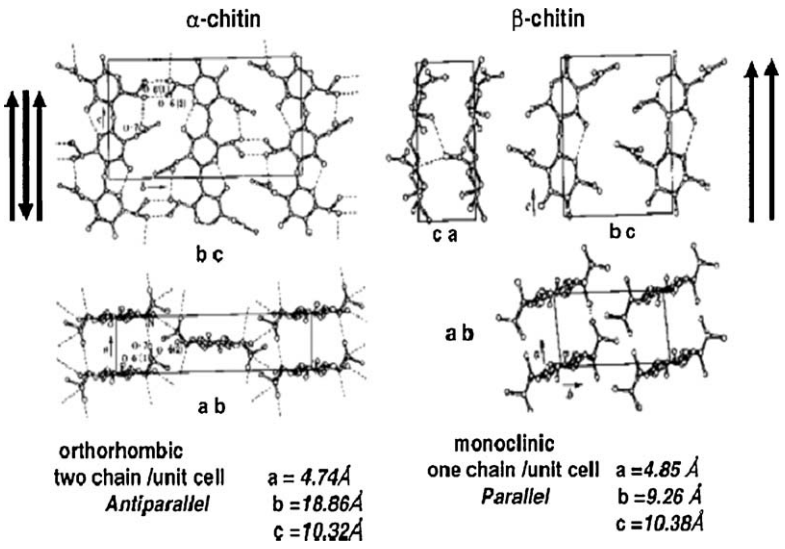

Scheme 1. Crystalline structures of chitin (Minke & Blackwell, 1978).

Finally α-chitin membranes are obtained (Nagahama, Kashiki et al., 2008; Nagahama, Nwe et al., 2008; Tamura, Nagahama et al., 2006). Similarly β-chitin hydrogel membranes can also be prepared using the β-chitin hydrogel (Madhumathi, Binulal et al. (2009); Nagahama, Kashiki et al., 2008; Nagahama, Nwe et al., 2008; Tamura, Nagahama et al., 2006). Fig. 1 shows the SEM images of α- and β-chitin hydrogel membranes (Nagahama, Nwe et al., 2008).

3. Preparation of chitin hydrogel scaffolds

α-Chitin hydrogel scaffolds can be prepared by the following method. The α-chitin hydrogel is transferred into a 24 well culture plate and frozen at −20 °C for 12 h to freeze the water in the hydrogel. The frozen hydrogel is lyophilized at −80 °C for 48 h to obtain α-chitin scaffolds (Peter et al., 2009). The β-chitin hydrogel scaffolds can also be prepared by lyophilization (Maeda et al., 2008). Fig. 2 shows the SEM images of β-chitin scaffolds (Maeda et al., 2008).

4. Applications of chitin hydrogel membranes and scaffolds

4.1. Tissue engineering

Tissue engineering is a multidisciplinary science, encompassing diverse fields like materials engineering and molecular biology in efforts to develop biological substitutes for failing tissues and organs. Tissue engineering thus seeks to replace diseased and damaged tissues of the body. A number of biodegradable polymers have been explored for tissue engineering purposes. These include synthetic polymers like poly(caprolactone), poly(lactic-co-glycolic acid), poly(ethylene glycol), poly(vinyl alcohol) and natural polymers like alginate, collagen, gelatin, chitin and chitosan etc (Khor & Lim, 2003; Kim et al., 2008). Of these, chitin and its derivatives had shown tremendous promise as tissue supporting materials.

The α- and β-chitin membranes were prepared using chitin hydrogel with and without *N*-acetyl-D-glucosamine (GlcNAc) for tissue engineering applications (Nagahama, Kashiki et al., 2008; Nagahama, Nwe et al., 2008). The mechanical, swelling, enzymatic degradation, thermal, and growth of NIH/3T3 fibroblast cell studies of the membranes were reported. Fibroblast cells were totally well separated and proliferated on the surface of each membrane with polygonal morphology. So, these chitin membranes are promising biomaterials that can be useful for tissue engineering applications (Nagahama, Kashiki et al., 2008; Nagahama, Nwe et al., 2008).

Jayakumar et al. (2009) developed α- and β-chitin membranes using α- and β-chitin hydrogel for tissue engineering applications. The bioactivity and cell adhesion studies of these membranes were also studied using MG63 osteoblast-like cells. The cells were adhered and spread over the membrane after 24 h of incubation. These results indicated that the chitin membranes could be used for tissue engineering applications (Jayakumar et al., 2009).

The α-chitin/gelatin composite membranes were prepared by mixing α-chitin hydrogel with gelatin (Nagahama et al., 2009). In addition, mechanical, swelling, enzymatic degradation, thermal and bioactivity studies were also studied. Biocompatibility of the α-chitin/gelatin membrane was performed in human MG63 osteoblast-like cells. After 48 h, the results indicated that the cells

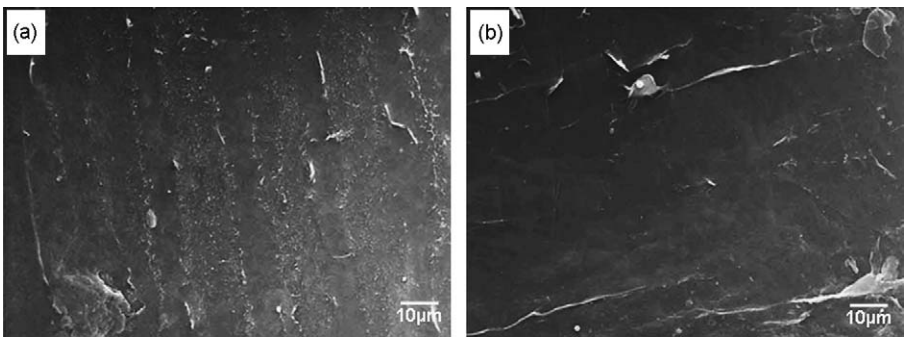

Fig. 1. SEM images of (a) α-chitin and (b) β-chitin membranes (Nagahama, Nwe et al., 2008).

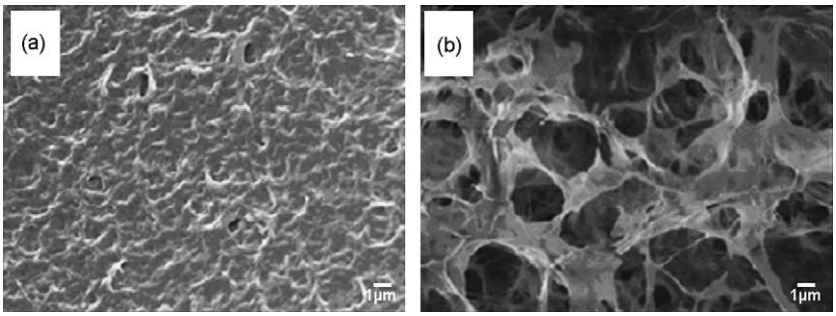

Fig. 2. SEM images of (a) surface and (b) cross section morphology of β-chitin scaffold (Maeda et al., 2008).

were attached to the surface and completely spreaded on the membrane surface. These results indicated that α-chitin/gelatin membranes are bioactive and are suitable for cell adhesion suggesting that these membranes can be used for tissue engineering applications (Nagahama et al., 2009). In the same way, the biological properties of the β-chitin membranes were also reported (Tamura, Nagahama et al., 2006).

Chitin has poor mechanical properties. It can be used as a bone substitute for bone repair and reconstruction if its mechanical properties can be improved with the addition of hydroxyapatite (HAp) (Huang, Dong, Chu, & Lin, 2008). HAp has been used in orthopedics and dentistry due to its osteoconductivity and osteophilicity (Aoki, 1994; LeGeros, 1991). HAp is a natural inorganic component of bone and teeth. Addition of HAp enhances the mechanical properties and cell attachment of chitin when used for orthopedic and dental purposes. HAp-coated chitin can be useful for bone reconstruction. Biopolymer-HAp composites have been synthesized by many methods like blending (Mi et al., 2003), biomimetic process using simulated body fluid (SBF) (Zhang et al., 2004), in situ precipitation (Chen, Wang, & Lin, 2002), electrochemical deposition (Huang et al., 2008) etc. These processes are either complex or time consuming.

Madhumathi, Binulal et al. (2009) reported a simple method for the preparation of β-chitin–HAp composite membranes. The preparation of these membranes was based on the wet synthesis of HAp using alternate soaking method in $CaCl_2$ (pH 7.4) and Na_2HPO_4 as described earlier (Tagushi, Kishida, & Akashi, 1998) and this method does not need high processing temperature or special equipment. The apatite forming ability of β-chitin membranes was also studied at different time intervals using various characterization tools. The results showed that the presence of apatite layer was higher on surface of β-chitin membranes, and the amounts of size and deposition of apatite layers were increased with increasing number of immersion cycles. Human mesenchymal stem cells (hMSCs) were used for evaluation of the biocompatibility of pristine as well as composite membranes for tissue engineering applications. The presence of apatite layers on the surface of β-chitin membranes enhancing the cell attachment and spreading (Fig. 3) suggested that β-chitin–HAp composite membranes could be used for tissue engineering applications.

Suzuki et al. (2008), developed β-chitin sponges with chondrocyte culture. The absorption efficiencies of chondrocytes in β-chitin sponges were found to be around 98%. Results from the histochemical and immunohistochemistry suggest that the cartilage like layer in the chondrocytes-sponge composites of β-chitin sponges was similar to hyaline cartilage. However, only immunohistochemistry staining of type II collagen in the β-chitin sponge was closer to normal rabbit cartilage than other types of sponges. This β-Chitin sponge was superior to other sponges concerning the content of extracellular matrices of collagen. β-chitin hydrogel scaffolds were also developed by lyophilization technique (Maeda et al., 2008). The bioactivity studies of β-chitin scaffolds were studied using SBF solution. The bioactivity studies showed that there is a calcium phosphate layer on the surface as well as in the cross section of β-chitin scaffolds. It seems that the β-chitin scaffolds can also be used for tissue engineering applications.

Bioactive glass ceramics are silicate-based materials used for bone repair. Bioactive glass was developed by Hench as a biomaterial to repair bone defects (Hench, 1991) and is widely used in orthopaedic and dentistry. Bioactive glass ceramic coatings on the surface of titanium are superior to HAp in their ability for osteointegration (Wheeler, Montfort, & McLoughlin, 2000). Moreover bioactive glass ceramic can also bond to soft and hard tissue (Verrier, Blaker, Maquet, Hench, & Boccaccinia, 2004). The bonding ability of these materials is attributed to the formation of carbonated apatite layer on the surface of the coated materials (Kokubo, 1991). Bioactive glass ceramics have been reported to influence osteoblast and bone marrow stromal cell proliferation and differentiation (Bosetti & Cannas, 2005; Foppiano, Marshall, Marshall, Saiz, & Tomsia, 2007). It has also been reported that bioactive glass could directly influence cells at the genetic level (Hench, 2009). Many groups have reported that bioactive glass ceramics influence osteoblastic cell differentiation with an increase in the level of differentiation markers like ALP, osteocalcin and osteopontin (Valerio, Pereira, Goes, & Leite, 2004; Xynos, Edgar, Buttery, Hench, & Polak, 2000).

Bioactive glass ceramic nanoparticles (nBGC) were prepared using sol–gel technique (Peter et al., 2009). The α-chitin/nBGC composite scaffolds were prepared using α-chitin hydrogel with nBGC by lyophilization technique (Peter et al., 2009). The composite scaffolds showed adequate porosity, swelling and degradation

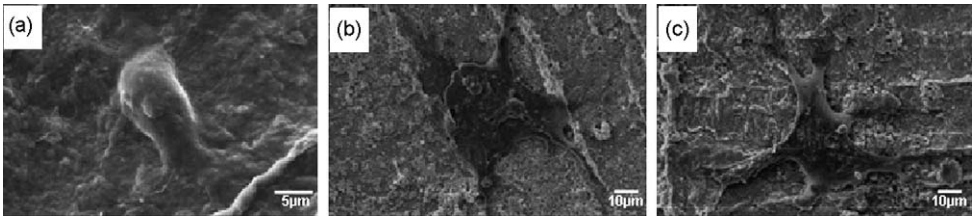

Fig. 3. Cell adhesion and spreading morphology of β-chitin membranes (a) 0 (control), (b) 3 and (c) 5 cycles soaking in $CaCl_2$ (pH 7.4)/Na_2HPO_4 solutions (Madhumathi, Binulal et al., 2009).

properties along with their ability of bioactivity. The biocompatibility of the composite scaffolds was studied in MG63 using MTT assay and cell attachment. Results indicated no sign of toxicity and the MG63 cells were attached on the scaffolds. These results suggested that the developed composite scaffold have suitable applications in the tissue engineering field (Peter et al., 2009).

Addition of silica nanoparticles enhances the bioactivity and biocompatibility of chitin. Madhumathi, Sudheesh Kumar et al. (2009) developed α-chitin composite scaffolds containing nanosilica using α-chitin hydrogel. Bioactivity, swelling ability and cytotoxicity of α-chitin composite scaffolds were analyzed *in vitro*. These scaffolds were found to be bioactive in SBF and biocompatible when tested with MG63 cell line. The α-chitin/nanosilica composite scaffolds showed higher biocompatibility. These results suggest that α-chitin/nanosilica composite scaffolds can be useful for bone tissue engineering applications.

4.2. Wound dressing

Recently, Madhumathi et al. (2010) developed α-chitin/nanosilver composite scaffolds for wound dressing applications using α-chitin hydrogel with silver nanoparticles. The antibacterial activity, blood clotting and cytotoxicity of the prepared composite scaffolds were studied. These α-chitin/nanosilver composite scaffolds were found to be bactericidal against *Staphylococcus aureus* and *Escherichia coli* with good blood clotting ability. These *in vitro* results suggested that α-chitin/nanosilver composite scaffolds could be used for wound dressing applications. Similarly, Sudheesh Kumar et al. (2010) also prepared β-chitin/nanosilver composite scaffolds for wound healing applications using β-chitin hydrogel with silver nanoparticles. The antibacterial, blood clotting, swelling, cell attachment and cytotoxicity studies of the prepared composite scaffolds were evaluated. The prepared β-chitin/nanosilver composite scaffolds were bactericidal against *E. coli* and *S. aureus* and it showed good blood clotting ability as well. Cell attachment studies using Vero (epithelial cells) showed that the cells were well attached on the scaffolds. These results suggested that β-chitin/nanosilver composite scaffolds could be a promising candidate for wound dressing applications.

5. Future perspectives

In this review, we presented the preparation and biomedical applications of novel chitin membranes and scaffolds prepared from chitin hydrogel. Since chitin has showed enhanced antibacterial activity and blood clotting ability, it can be used as wound dressing material. This review also summarizes that α- and β-chitin hydrogel membranes and scaffolds can be used for tissue engineering applications. The eventual production and clinical use of such types of implants awaits the take-up of these materials on a more commercial basis that would see the introduction of chitin based implantable devices.

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