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Characterization of chitosan films and effects on fibroblast cell attachment and proliferation

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Abstract Chitosan has been researched for implant and wound healing applications. However, there are inconsistencies in reports on the tissue and fibroblast responses to chitosan materials. These inconsistencies may be due to variations in chitosan material characteristics. The aim of this study was to correlate fibroblast responses with known chitosan material characteristics. To achieve this aim, chitosan was characterized for degree of deacetylation (DDA), molecular weight (MW), residual protein and ash contents, and then solution cast into films and characterized for hydrophilicity by water contact angle. The films were seeded with normal human dermal fibroblasts and the number of attached cells was evaluated for after 30 min. Cell proliferation was evaluated over 5 days. This study found no relationship between DDA, contact angle, cell attachment, and or proliferation. General trends were observed for increasing proliferation with increasing residual ash content and decreasing residual protein. These data indicate that chitosan characteristics other than DDA may be important to their biological performance.

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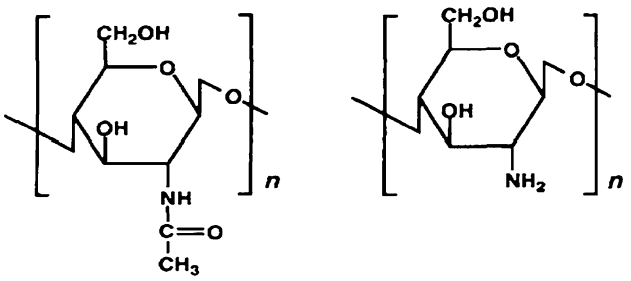
Introduction

The use of chitin and chitosan as a wound treatment has a long history from ancient Japanese fishermen placing powdered crab shells on lacerations [1] to the United States Army haemostatic agent to treat battlefield injuries employing chitosan as a key component [2, 3]. As a wound dressing, chitosan has shown improved and scarless healing associated with: higher numbers of mitotic cells in the wound bed, greater macrophage infiltration into the site, faster re-epithelialization of the wound, increased angiogensesis, and greater collagen deposition resulting in enhanced healing rates and wound strengths [4–9]. When evaluated as a biomaterial, chitosan has shown increased cell attachment and cytokines and growth factors production [10–13]. Chitosan is structurally similar to hyaluronic acid, a polymer component of the extracellular matrix [14]. In addition to healing, chitosan may also provide analgesic effects for serious burns [15] as well as antibacterial properties [16].

Chitosan is a co-polymer of *N*-acetyl-glucosamine and *N*-glucosamine units (Fig. 1). Either an acetamido group ($-NH-COCH_3$) or an amino group ($-NH_2$) is attached to the C-2 carbon of the glucopyran ring. When more than 50% of the C-2 attachment is an amino group, the material is termed chitosan. The degree of deacetylation (DDA) represents the percentage of amino groups. Ideally, chitin is a linear polysaccharide of β-(1–4)-2 acetamido-2-deoxy-D-glucopyranose where all residues are comprised entirely of the acetamido group $-NH-COCH_3$. This is termed fully acetylated or 0% DDA.

In general, data on the use of chitosan for biomedical applications are promising, but there are conflicting and contradictory reports. For example, chitosans of approximately 82–100% DDA implanted in connective tissues have shown increased angiogenesis, promotion of stromal cell



N-acetyl_glucosamine unit N-glucosamine unit

Fig. 1 The chemical structure of chitin/chitosan monomeric units. When more than 50% of the monomeric units of the polysaccharide molecule are *N*-glucosamines, the molecule is referred to as chitosan

migration and differentiation, and reorganization of collagen extracellular matrix [7–9, 17]. Yet, when chitosans of varying DDAs were examined in bone, chitosans of 94% and 100% DDA, were associated with fibrosis while lower % DDA chitosans supported bone formation [18]. *In vitro*, fibroblast proliferation was either not affected or was increased with exposure to chitosans of 80, 89 and 91% DDA [19–21] while in another study, proliferation was inhibited on chitosan films ranging from 52.5 to 97.5% DDA [22]. No difference in one day growth of osteoblasts or fibroblasts was noted between an 80% DDA, 1400kD chitosan or a 70% DDA, 270kD chitosan, though initial attachment on chitosans was greater for the osteoblasts [23]. On the otherhand, an increase in fibroblast apoptosis was observed with an increase in DDA of chitosan materials [24]. The increase in apoptosis was attributed to the increase in binding of fibroblasts cells by the increase in cationic charge of chitosans with increasing DDA. While differences in these studies may be attributed in part to the source of chitosan (fungal vs. arthropod), implant site (bone vs skin) and or types of cells (primary vs. transformed), chitosan material characteristics such as molecular weight, residual ash content, manufacturing processes or other characteristics are rarely provided. This information is important since molecular weight is important to crystallinity and degradability of chitosans, which along with preparation and source (e.g. crab, shrimp, fungus etc) can influence materials properties and biological performance [19, 25–31]. Therefore the aim of this study was to evaluate fibroblast attachment and proliferation to a series of chitosan materials characterized with respect to DDA, molecular weight, MW, surface properties, and residual protein and ash content.

Materials and methods

Materials

Chitosan

Chitosan powders of crab origin were obtained from Vanson HaloSource (Redman, WA) through generous donation and purchase. Films were made by casting solutions of 1% chitosan dissolved in 0.2 M acetic acid (Sigma-Aldrich St. Louis, MO) in 96 well culture plates. The plates were dried in a laminar flow culture hood for the first and last 24 hours of a week period. In between drying periods, the plates were stored at a constant temperature of 21 °C. The films were then rinsed in copious amounts of phosphate buffered solution (PBS) and ethylene oxide gas sterilized for cell culture tests.

Cells

Normal Adult Human Dermal Fibroblasts (NAHDF) (Clonetics/Cambrex, Baltimore, MD) were maintained at 37 °C in a 5% CO_2 atmosphere under sterile conditions. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum and 1% antibiotic–antimycotic (Gibco/Invitrogen Carlsbad, CA). Cells were subcultured with 1% trypsin-EDTA (Gibco/Invitrogen Carlsbad, CA). Only cells between the third and sixth passage were used.

Material characterization

Degree of deacetylation determination

The powders were tested for degree of deacetylation, DDA, by titration [32]. Chitosan (0.5 g) was dissolved in 20 ml of 0.3 N Hydrochloric Acid (Sigma-Aldrich St. Louis, MO). After adding 400 ml of distilled water, this solution was titrated with 1 N NaOH solution (Sigma-Aldrich St. Louis, MO). A titration curve of pH vs. NaOH titration volume was generated. The curve's inflection points were found for each indicated transition. The volume of NaOH at the each inflection point was applied to the equation:

$$\%NH_2 = 16.1 * (y - x)/M \quad (1)$$

where M is the weight of chitosan used (0.5 grams), x is the first inflection point on the graph of measured pH vs. titration volume, y is the second inflection point [32].

Molecular weight measurement

Molecular weight, MW, was determined by dilute solution viscometry [33]. Briefly, solutions of various chitosan were dissolved in 0.25 M acetic acid and 0.25 M sodium acetate (Sigma-Aldrich St. Louis, MO) solvent to concentrations of 0.0025 to 0.03 g/dl, depending on the sample. The samples were analyzed at 25 °C using an Ubbelohde viscometer (Cannon, State College, PA). The intrinsic viscosity was calculated. The molecular weight was found by using the

Mark-Houwink equation:

$$[\eta] = K'M^{\alpha} \quad (2)$$

where $[\eta]$ is the intrinsic viscosity and M is molecular weight. The constants, K' and α, are 1.14×10^{-4} dL/g and 0.83, respectively [33].

Contact angle measurement

Sessile drop air/water contact angle measurements were performed on non-hydrated films using a contact angle goniometer (Rame'-Hart model 100; Mountain Lake, NJ). The contact angle was determined from five samples for each DDA chitosan.

Ash content determination

Chitosan ash content was determined using a constant weight crucible. The crucible weight, W_0, was stabilized to a tolerance of ± 0.5 mg by repeatedly placing it into an oven at 550 °C ± 20 °C for 30 min and allowing it to cool for 30 min until the weight was constant. Chitosan (2–5 g) was combusted in the constant weight crucible and placed in an oven at 550 °C ± 20 °C for 3 hours. The sample was removed, cooled in a desiccator for 30 min, and re-weighed (W_1). This heating and cooling process was repeated every 1.5 hours until a constant weight was established (W_2). The ash percentage was calculated by the equation:

$$Ash\% = \frac{W_2 - W_0}{W_1 - W_0} \times 100 \quad (3)$$

where W_0 is the constant weight of crucible, W_1 is the weight of sample and crucible, W_2 is the weight of ash and crucible [34]. The ash content was determined from two samples for each DDA chitosan.

Protein

Film protein concentrations were determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL). The films were tested with the BCA reagents according to the manufactures instructions and read at 560 nm (μQuant Universal Microplate Spectrophotometer; Bio-Tek Instruments, Inc., Winooski, VT. The protein was determined from five samples for each DDA chitosan.

Cell attachment assay

Normal adult human dermal fibroblast cells were exposed to sterilized chitosan films for 30 min in a serum-free media. Then the media and non-adherent cells were removed. The films were rinsed twice with PBS. Media was added to the chitosan films. After 1 hour at 37 °C, Promega CellTiter reagent (Promega, Madison, WI) was added. The cell number was then assessed by reading the plate at 490 nm on a μQuant Universal Microplate Spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT). Tissue culture plastic (TCP) was used as the control substrate.

Cell proliferation assay

Growth on sterilized chitosan films was determined by cell counting at 3 and 5 days after seeding 5000 or 1000 cells/cm^2, respectively. Cell proliferation was determined by the addition of Promega's CellTiter MTS Cell Proliferation Assay Kit (Promega, Madison, WI) which was read after one hour by the microplate spectrophotometer at 490 nm. Absorbance values were converted to cell number using a standard curve of known cell number vs. absorbance.

Statistical analysis

Material characterization test utilized 2 to 5 samples. Triplicate samples of each film were used in cell culture studies. Cell culture studies were repeated three times. Post-hoc multi-comparison tests using F-protected LSD were used to determine where statistical differences exist. Statistical differences were declared at $p < 0.05$.

Results

Material characteristic

The DDA, MW, contact angle, and residual ash and protein content values for the test chitosans are shown in Table 1. The DDAs obtained by titration were similar to those reported by Vanson HaloSource (Redman, WA). The MW ranged form 5.13×10^5 D for the 80.6% DDA chitosan to 1.24×10^5 D for the 91.9% DDA chitosan. The molecular weight, found by dilute solution viscometry, is an estimated MW that is between the weight average MW and the number average MW. The same K' (1.40×10^{-4} dL/g) and α (0.83) values for the Mark-Houwink equation were used for all DDA. These values have been previously reported to be valid for the range between 71 and 95% DDA [33]. There was no relationship noted between DDA and molecular weight of the chitosans.

The water contact angles measured ranged from 87.7° for 80.6% DDA to 62.1° for 95.6% DDA (Table 1). There were three statistical groups detected in the contact angle data. All groups had more than one sample in the group and overlapped with the next group. There was a weak trend ($R^2 = 0.3$) for

Table 1 Chitosan material characteristics

Vanson lot number	Vanson DDA[1] (%)	Titration DDA[2] (%)	Molecular weight[3] (*10^5Daltons)	Ash content[4] (%)	Contact angle[4] (degree)	Protein[4] (Micrograms/cm^2)
VNS-389	76.1	76.21 ± 1.86	2.00	0.932 ± 0.026^{d}	85.6 ± 0.7^{b,c}	159.4 ± 10.4^{c}
03-ASDQ-122	78.7	78.85 ± 4.32	2.35	3.574 ± 0.006^{f}	72.5 ± 1.9^{a,b}	287.1 ± 44.8^{d}
02-CISC-0920	80.6	82.66 ± 1.87	5.13	0.239 ± 0.002^{a}	73.7 ± 2.3^{a,b}	101.0 ± 14.0^{b,c}
03-ASSQ-0212	87.4	85.85 ± 3.68	4.66	2.456 ± 0.019^{e}	89.7 ± 1.6^{c}	67.6 ± 17.5^{b}
00-CESC-0915	91.9	91.92 ± 2.67	1.24	0.766 ± 0.044^{c}	73.9 ± 0.7^{a,b}	142.1 ± 23.4^{c}
01-CESQ-1415	92.3	92.31 ± 3.72	4.69	0.524 ± 0.009^{b}	63.9 ± 0.5^{a}	5.3 ± 4.8^{c}
98-AECQ-0136	95.6	96.50 ± 3.23	1.52	0.408 ± 0.001^{b}	62.1 ± 1.1^{a}	110.5 ± 56.2^{b,c}

[1]As reported by manufacturer
[2]Determined by titration [32]
[3]Determined by viscosity [33]
[4]Superscripts indicate statistical groups ($P < 0.05$)

the contact angle to decrease, or wettability to increase, as the DDA of the chitosan increased (Fig. 2).

Residual ash and protein content of the specimen varied greatly (Table 1). The 78.7% DDA sample had the highest ash content, 3.57%, while the 80.6% DDA chitosan had the lowest, 0.24%. Residual protein content varied from less than 6 micrograms/cm^2 on the 92.3% DDA films to 287.1 micrograms/cm^2 for 78.7% DDA. Most test materials exhibited residual protein contents from 101 to 159 micrograms/cm^2. There was no correlation between residual ash or protein content, and DDA, MW or contact angle measurements.

It is noted that there is no universal standard for ash or protein content of chitosan materials that is known to the authors. Instead, each manufacture sets their standards. For example, Dalwoo (Dalwoo, Seoul, Korea) has set ash content for their medical/pharmaceutical grade chitosan to $<0.2\%$ while LipoSan Ultra (Primex, Siglufjordur, Iceland) has set their limit to $<2\%$. The ash content for the chitosan samples in this study was above allowable levels for medical/pharmaceutical grade set by Dalwoo. However, all chitosan materials, with the exception of the 78.7 and 87.4% DDA samples were within acceptable range set by LipoSan Ultra.).

Cell attachment assay

Attachment of cells on all chitosan films was statistically greater than on the control TCP substrate (Fig. 3). Chitosan films of 76.1, 80.6, 87.4, and 92.3% DDA had the lowest number of attached cells. Yet, these films attracted 2.5 times more cells than the control. The 76.1 and 78.7% DDA films showed the next highest number of attached cells which was three times the control. The 91.9 and 95.6% DDA had the highest number of cells attached to the surface, averaging more than four times the cell number attached to TCP controls. There was no correlation between cell attachment and DDA or residual ash or protein contents of the films. However there was a weak correlation ($R^2 = 0.79$) between cell attachment and MW of the test chitosan films (Fig. 4).

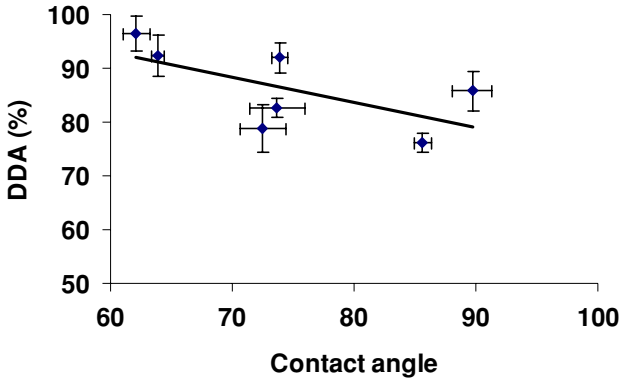


Fig. 2 DDA of chitosan films vs. water contact angle. There was a weak trend ($R^2 = 0.3$) for the contact angle to decrease as the DDA of the chitosan films increased. (DDA values determined by titration [32], $n = 3$; contact angle measurements, $n = 5$)

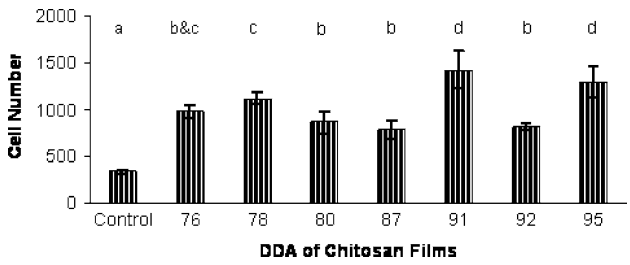


Fig. 3 Attachment of human dermal fibroblast cells (mean ± standard deviation, $n = 9$) on chitosan films after 30 minutes in serum-free medium. Cell attachment was statistically greater on all chitosan films as compared to tissue culture plastic control ($p < 0.05$). There was no correlation between the number of attached cells and the DDA of the chitosan films. Columns with different superscripts are significantly different ($p < 0.05$). ($n = 9$)

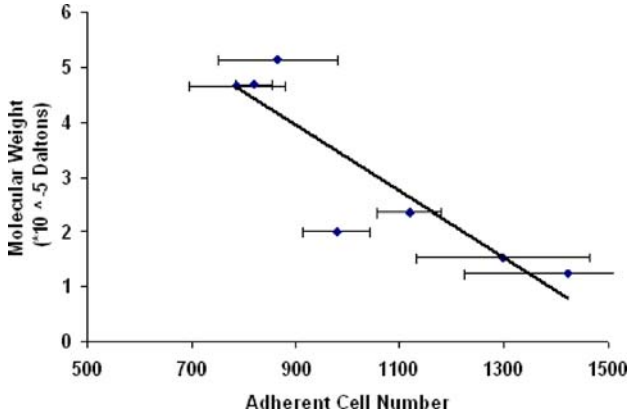


Fig. 4 Molecular Weight of the chitosans vs. Cell Attachment. An inverse relationship between molecular weight and the number of attached human dermal fibroblast was observed. As molecular weight increased, number of attached cells decreased ($R^2 = 0.794$)

Cell proliferation assay

There was no correlation between proliferation of the NAHDF cells and DDA of the test chitosan films in either three- or five-day cultures (Fig. 5). In the three-day tests (Fig. 5a), only films of 78.7, 87.4 and 92.3% DDA supported fibroblast growth at comparable or greater levels than controls. Interestingly, 78.7% DDA film which supported the greatest three-day growth had the highest residual ash and protein contents. In the five-day tests (Fig. 5b), only chitosan films of 92.3 and 95.6% DDA supported cell proliferation levels comparable to control. All other chitosans films exhibited reduced proliferation compared to the TCP control.

While there was no correlation between fibroblast proliferation and DDA, correlations were detected between three-day cell growth and residual ash and protein contents (Fig. 6). As shown in Fig. 6A, three day cell growth increased with increasing residual ash content ($R^2 = 0.77$). In Fig. 6B, with the 78.7% DDA chitosan material excluded, three-day cell growth increased with decreasing residual protein content ($R^2 = 0.69$). After five days, correlations between ash and protein content and fibroblast proliferation were not evident, though there was a weak trend ($R^2 = 0.54$) for increased profileration with decreasing protein content when the 78.7% DDA material is excluded (Fig. 6C).

Discussion

In this study, test chitosan materials exhibited a wide range of physiochemical characteristics. However, there was no correlation between DDA, MW, residual ash or protein content of the test materials. This was interesting in that it is generally reported that with increased processing to increase DDA, MW is also reduced [35]. It may also be reasonably assumed that residual ash and especially protein contents might be reduced with DDA due to increased processing, since DDA is usually increased using alkali [35]. It should be noted that the conditions and or processes used to manufacture the test chitosans is not known to the authors. Nevertheless, the expected relationship among the chitosan characteristics, may be more typical of chitosan materials prepared from the same initial lots, whereas the chitosans in this study were prepared from different lots and at different times. Hence, the lack of correlation may be attributable to variability in the raw staring materials and or changes in processing conditions used to produce the different lots of chitosan. These data suggest that each lot of chitosan should be characterized prior to use in order to begin to understand biological responses.

Characterization of the test chitosans did indicate that all of the materials exhibited MW values in the 10^5 Dalton range, which is typical for chitosans used in biomedical applications [9–14, 19, 21–26, 28, 36]. There was a weak correlation ($R^2 = 0.79$) between MW and cell attachment (Fig. 4) but not with cell proliferation. As MW increased, adhesion decreased. A relationship between MW and adhesion may be present in other studies comparing fibroblast and keratinocytes response to chitosans with different MW and DDA's [22]. It is not clear from these studies if this relationship is an artifact. However, if this correlation is true, it may help explain the differences seen among chitosans of different DDA where MW was not examined as a factor. A potential mechanism by which MW may influence cell attachment may have to do with how MW influences the development of microstructural crystalline domains within the film. Changes in crystallinity are known to affect polymer degradation which in turn may influence biological behavior [18, 27, 29, 30]. Additional characterization of crystalline organization and *in vitro* testing of chitosan films composed of different DDA but with the same MW may provide additional insight into this phenomenon.

In this study, it was interesting that there was a positive correlation between residual ash content and three-day cell proliferation, though the correlation was lost in the five-day growth studies. It is not clear from these results why cell proliferation would increase with increasing residual ash content, though it may be related to the composition of the ash. While the composition of the residual ash was not analyzed, it is most likely to be a calcium-based material since the main mineral component of arthropod cuticle, the source of the chitosan, is calcium carbonate [37]. Calcium-based materials (e.g. Ca-P and Ca-Sulfate) are widely used in biomedical devices and applications and have been shown to support and bone cell growth [38, 39]. It may be that after several days in culture, the ash is either dissolved out of the films or masked by other cell matrix and culture medium components, such that it no longer affects the cells. Additional research is needed to clarify the role of residual ash on the bioactivity of

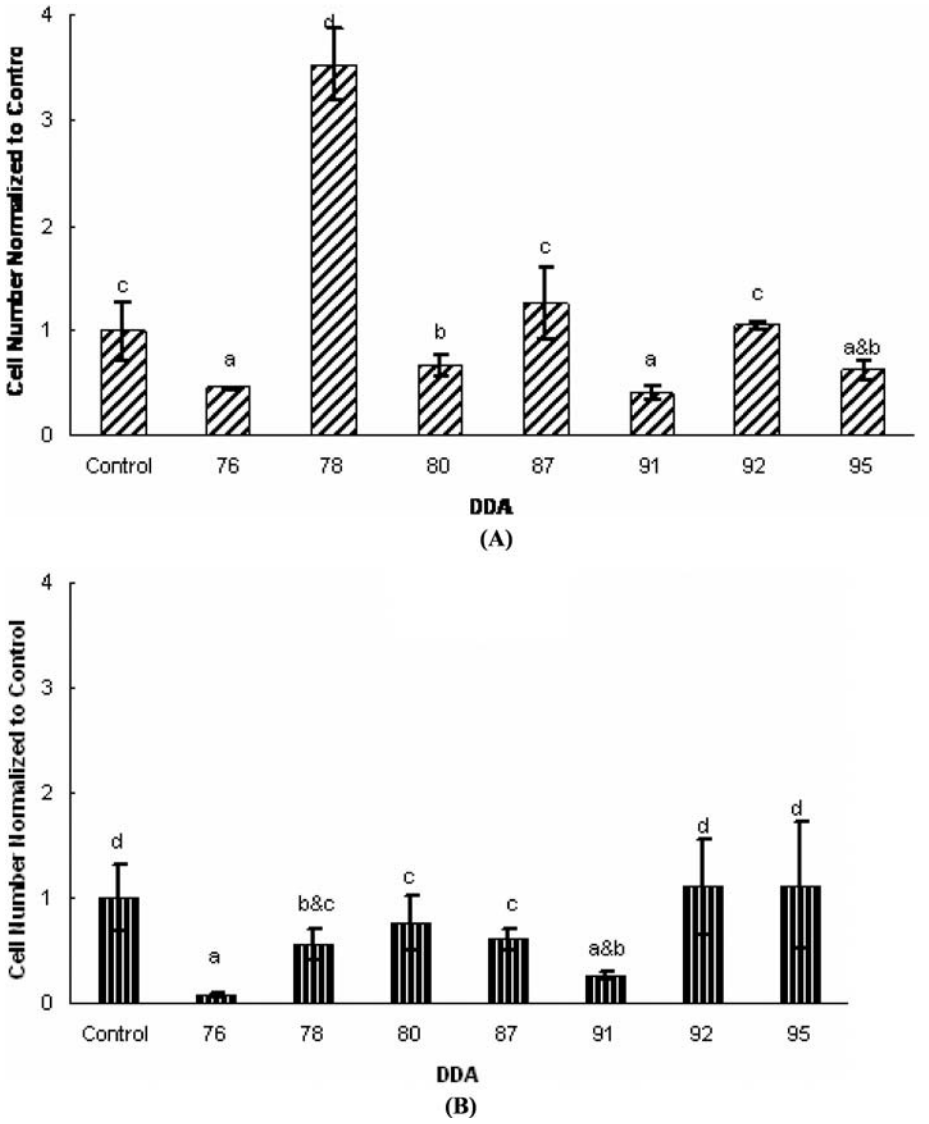


Fig. 5 Proliferation of NAHDF on chitosan films (mean ± standard deviation, $n = 9$) in three-day cultures (**A**) and in five-day cultures (**B**). The cell number was assessed via colorimetric assay (CellTitre® , Promega, Madison, WI). There was no correlation between either the three-day or five-day cell growth and DDA of the chitosan films. Most chitosan films inhibited cell growth as compared to tissue culture plastic controls. The 78.7% DDA film, which supported more than three times the cell growth of controls in the three-day cultures, contained the highest levels of residual ash and protein content of the test materials. Columns with different superscripts are significantly different. $P < 0.05$

chitosan. Nevertheless, these data indicate that ash content may be an important factor in the biological performance of chitosan materials.

Data also indicated that there was a correlation between residual protein content and cell growth. When the 78.7% DDA material is excluded, there was a general trend for cells growth to increase with decreasing residual protein contents. When the 78% DDA material is included, the trend is lost, and this may be due to its high ash and protein content. Percot *et al*. [40] reported that residual proteins from shrimp shells were largely comprised of acidic amino acids such as aspartic and glutamic acids, with alkaline amino acids such as lysine and histidine in lesser amounts. It was also noted by Percot *et al*. [40] that proteins involved in mineralization and bonding of the polysaccarhide are largely composed of these amino acids. It may be that these residual proteins play a role in the generally accepted osteogenic properties of chitosan materials [4, 9, 10, 12, 14, 17, 18, 23, 35]. Nevertheless, residual proteins may have a large influence on biocompatibility of chitosans since they may influence binding/adsorption of other proteins and have potential to elicit immunological reactions. These results, similar to residual ash contents, indicate that characteristics other than DDA influence and module the bioactivity of the chitosan materials.

Contact angle measurements of the test chitosan films were similar to those previously reported [41–44] and there was a trend for the contact angle to decrease or wettability to increase as the DDA of the chitosan increased. The increase in wettability may be related to an increase in the number of free amine groups with increasing DDA. These amines may become protonated under neutral pH resulting in a high positive surface charge that promotes wettability as well as cell and protein adsorption [26–28, 44]. The ability to wet

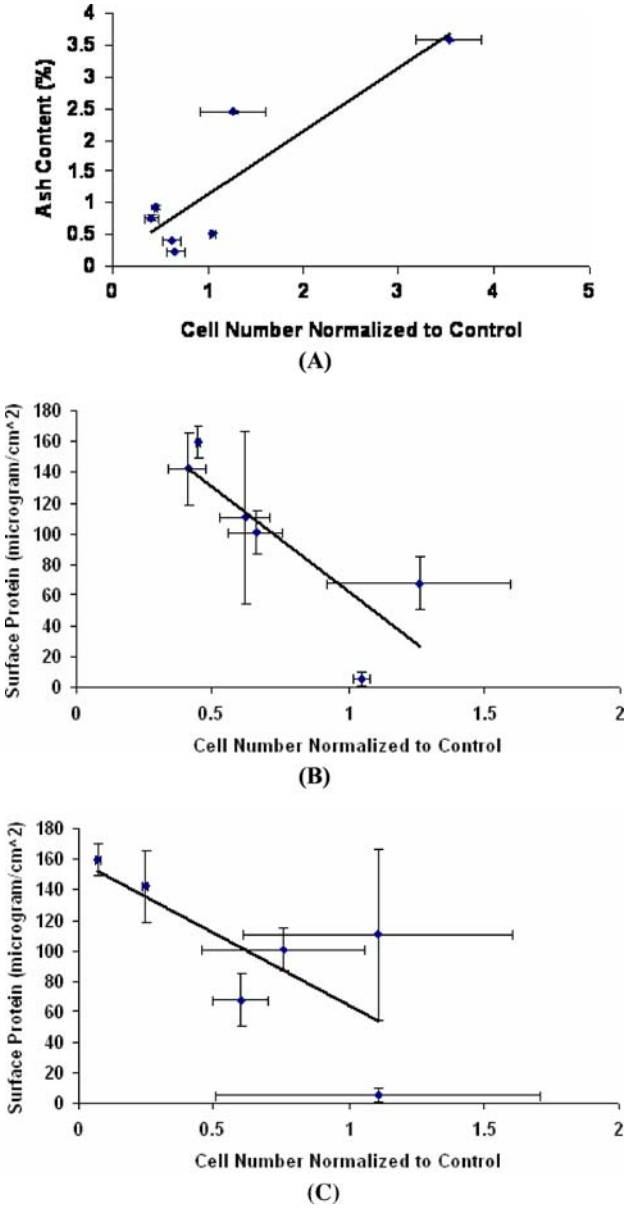


Fig. 6 Residual ash and protein contents vs. proliferation of human dermal fibroblast. **(A)**: three-day cell growth increased with increasing residual ash content ($R^2 = 0.77$). [Note for several of the points the ash content error bars are too small to be seen.] **(B)**: with the 78.7% DDA chitosan material excluded, three-day cell growth increased with decreasing residual protein content ($R^2 = 0.69$). **(C)**: after five days, there was a weak trend ($R^2 = 0.54$) for increased proliferation with decreasing protein content when 78.7% DDA material is exclude. Points are mean values with standard deviations indicated. For ash content, the standard deviations are too small to be seen

and absorb large amounts of protein and cells may be important in chitosans ability to bind and retain clotting and wound repair growth factors and cells which are responsible for the enhanced wound healing rates observed *in vivo* [6, 37, 44–49]. Indeed, even though there were differences in the attachment of cells among the chitosans tested, cell attachment was greater on all test chitosans than on TCP controls.

Contact angle values however, were not predictive of cell attachment or proliferation as has been reported [22, 24, 26]. Serum-free medium was used in this study in an effort to determine if there were differences in the attraction and binding of cells to different chitosan films. However, even if there was a difference in the initial electrostatic binding of the cells to the different films, these differences may have been lost due to the washing steps of the procedure. Serum in the media provides binding proteins such as fibronectin and vitronectin which are first absorbed to the substrate and allow for initial cell attachment. Hence interpretation of the effects of any physiochemical characteristic of the films on cell attachment is limited. It is noted though that large amounts of fibronectin and albumin were absorbed on to a 92.3% DDA chitosan coatings as compared to uncoated titanium [44]. Future studies are planned to evaluate the protein absorption phenomena as well as adhesion of cells in serum containing medium.

In general, the low fibroblast proliferation on the chitosan films is in agreement with related studies [12, 19, 20, 22–24, 42]. This low growth is generally attributed to a high binding of the fibroblast cells by the highly cationic chitosan. However, there was no correlation between cell growth and DDA of the chitosan films as has been reported [19, 22, 26, 42]. Mao *et al*. [24] also did not observe any relationship between cell growth and DDA, and suggested that DDA was not important to cell growth since cells may begin to degrade the chitosan materials [24]. It may be that once cells have attached to chitosan materials, other factors, as has been suggested in this study, begin to affect the cell-material interactions. Additional investigations will be needed to clarify this issue.

The physiochemical characteristics of the chitosan materials evaluated were determined in the non-sterilized condition. This was because numerous studies have shown ethylene oxide, an appropriate sterilization procedure, to have little affect on polymer molecular weight or other mechanical and physical properties [10, 50, 51]. For example, Marreco [51] demonstrated that ethylene oxide gas sterilization had no effect on chitosan-membrane morphology, tensile strength, percentage of strain at break, or *in vitro* cytotoxicity as compared to non-sterilized materials. Roa and Sharma [50] did note an increase in water contact angle with ethylene gas sterilization, and a decrease in hemolysis of rabbit red blood cells with the sterilized materials [50]. It has been postulated that since ethylene oxide is an alkylator, it may generate cross-links via the amine groups which would reduce the net positive charge on the films [44]. In our preliminary measurements, water contact angles were the same for sterile and non-sterile films (data not shown) and no distinction was made for subsequent measurements and data

analyses. This was unfortunate in retrospect and investigations will be undertaken regarding the effects of cross-linking due to ethylene oxide sterilization not only on physiochemical properties of the films, but also on biological behavior of protein and cell adsorption, cell growth and biological degradation.

Conclusion

This study found no clear relationship between chitosan material characteristics such as DDA, wettability and MW, and cell attachment or proliferation. Residual ash and protein contents appear be important to cellular-material interactions since there was a general trend of increasing proliferation with increasing ash and decreasing protein contents. Additional studies are needed to determine the role of these and other factors (e.g. crystallinity, degradation) on fibroblast response to chitosan and to help improve the understanding of the relationships between chitosan's physical and chemical properties to cellular/tissue responses.

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