Chapter 3
Drug Release Behavior of Chitosan-Silver-Gelatin Nanohybrids & Scaffolds

3.1 Introduction
Metronidazole (MTZ, 1-[2-hydroxyethyl]-2-methyl-5-nitroimidazole) is an antibacterial and antiprotozoal drug that has been in use for over 35 years. Now-a-days, it is one of the most essential drugs mostly used during pregnancy. Metronidazole has also been used in women to prevent preterm birth associated with bacterial vaginosis, amongst other risk factors including the presence of cervicovaginal fetal fibronectin (fFN). At present, drug delivery seems to be the topic of interest with a better understanding of the basics in chitin and chitosan chemistry, such as chemical modifications, association of chitosan with inorganic compounds, mucoadhesion, biodegradation, effects on various tissues, distribution to various body organs and advanced technological transformations.

Low molecular weight chitosan (LMWC) (average MW < 10 kDa) could reversibly open the tight junctions between intestinal epithelial cells in a Caco-2 cell model and thus subsequent recovery of initial levels of trans-epithelial resistance was much faster than that of high molecular weight chitosan (HMWC). This ability of LMWC to quickly and reversibly open tight junctions could be a useful characteristic for a carrier of drug molecules, especially for the oral delivery. Chitosan can be degraded by ubiquitous enzymes in the human body and oligomers can activate macrophages and stimulate synthesis of hyaluronan. Moreover, chitosan nanoparticles provide building blocks for the reconstruction of extracellular matrix components.

Gelatin is a protein produced by partial hydrolysis of collagen extracted from bones, connective tissue organs and some intestines of animals such as domesticated cattle, pigs and horses. It has traditionally been used in three major areas: food, pharmaceutical and photographic industries. In the pharmaceutical and health industry, gelatin is used to make shells of hard and soft capsules for medicines, dietary/health supplements, syrups, etc. It is commonly used as a biological substrate also to culture adherent cells. Enzymatically cross-linked gelatin gels have been shown to be biocompatible to both human and bacterial cells and both have been crosslinked into three dimensional matrices.
of gelatin with good cell survival $7$-$9$. Gelatin/chitosan biopolymer systems have a distinct advantage because of their superior biocompatibility and low costs. Thus, we have chosen gelatin and chitosan as a material for the present investigation.

On the other hand, silver (Ag) nanoparticles have attracted considerable interest in biological studies because of their ease of preparation, good biocompatibility and relatively large surface $10$. It is also powerful killer of many viruses and a potential antibacterial agent $11$-$14$. Consequently, ‘Nano Silver’ has two properties which render it to have superior to kill pathogenic organisms. The current interest of chitosan-Ag nanoparticles in biomedical applications is based on the fact that a versatile system must have antibacterial activity towards germs upon contact, without the release of toxic biocides. Polymer-silver nanoparticle systems have opened a window to a new range of applications in the biomedical field, which are highly effective and safe. The increase in flexibility of the scaffolds could improve the contact between the scaffold material and the tissue, hence, promoting penetration of the polymeric chains into the tissue to form strong adhesion $15$-$17$.

In this work, therefore, we prepared chitosan-silver-gelatin nanohybrids or scaffolds and investigated their drug delivery behavior using MTZ as a model drug. We investigated the effect of Ag nanoparticles on physical properties, microstructures and cell growth, along with drug-release behavior of the prepared nanohybrid films or scaffolds.
3.2 Experimental

3.2.1 Materials: Chitosan (CS) \( (M_v \ 1.5 \times 10^5) \), degree of deacetylation: 85\% and silver nanopowder (>100 nm) were obtained from Aldrich. \( l \)-Lactic acid (purity 90\%) was purchased from M/s. Purac Biochem, U.S.A. Metronidazole and gelatin (bovine skin) type B powder were purchased from Aldrich.

3.2.2 Preparation of Nanohybrids and drug loaded Scaffolds: Nanohybrid films were prepared by aqueous dispersion of Ag nanoparticles (NP) and suspension of CS in \( l \)-lactic acid, followed by dehydration and drying. Silver nanoparticles were sonicated in distilled water for 20 minutes and 20\% gelatin was dissolved in distilled water and then suspension of CS was added into the aqueous dispersion of Ag nanoparticles and gelatin, followed by heating up to 70 °C with continuous dehydration for 40–45 minutes. This solution was cast and dried for 12 h at 60 °C and subsequently in vacuum oven for 6 h.

To obtain porous scaffolds, the chitosan-Ag-gelatin solution was freeze-dried at -56 °C using 9 well tissue culture plates for several times. The dimension of obtained cylindrical scaffolds was 20 x 20 mm. After dehydration and cooling, the 24 hr stirred MTZ (10 \%)-loaded solution in water was casted to obtain films and freeze dried to obtain porous scaffolds, respectively. The prepared chitosan-gelatin-Ag nanohybrids with various compositions with and without MTZ are compiled in Table 3.1.
Table 3.1: Various compositions of silver particles, drug and gelatin in chitosan

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Chitosan (gm)</th>
<th>Ag (NPs)</th>
<th>Gelatin (%)</th>
<th>Drug (%)</th>
<th>Drying Process</th>
<th>Sample#</th>
<th>Remark</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>Vacuum</td>
<td>CM-1</td>
<td>Nanohybrid</td>
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<tr>
<td>2</td>
<td>1</td>
<td>x</td>
<td>60</td>
<td>x</td>
<td>Vacuum</td>
<td>CM-2</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>x</td>
<td>x</td>
<td>10</td>
<td>Vacuum</td>
<td>CM-3</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>x</td>
<td>20</td>
<td>10</td>
<td>Vacuum</td>
<td>CM-4</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0.05</td>
<td>20</td>
<td>10</td>
<td>Vacuum</td>
<td>CM-5</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.20</td>
<td>20</td>
<td>10</td>
<td>Vacuum</td>
<td>CM-6</td>
<td>&quot;</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>x</td>
<td>x</td>
<td>10</td>
<td>Freeze</td>
<td>CM-7</td>
<td>Scaffold</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>x</td>
<td>20</td>
<td>10</td>
<td>Freeze</td>
<td>CM-8</td>
<td>&quot;</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>0.05</td>
<td>20</td>
<td>10</td>
<td>Freeze</td>
<td>CM-9</td>
<td>&quot;</td>
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<tr>
<td>10</td>
<td>1</td>
<td>0.20</td>
<td>20</td>
<td>10</td>
<td>Freeze</td>
<td>CM-10</td>
<td>&quot;</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>x</td>
<td>20</td>
<td>10</td>
<td>Freeze</td>
<td>CM-11</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

#- Chitosan has been dissolved in 1% L- Lactic acid.
3.3 Characterization

3.3.1 Surface topography: Scanning Electron Microscopy (SEM) model JEOL Stereoscan 440, Cambridge was used for measuring surface morphology and pore size of scaffolds.

3.3.2 Tensile properties: For tensile measurements, the scaffold specimens were prepared with the dimension of $20 \times 10 \times 1$ mm whereas the nanocomposite films were cut in the size of $1 \times 6$ cm specimens. Both ends of tensile specimens were clipped with a special gripper. The tensile strength and percentage elongation at break of the scaffolds were measured by a Universal Testing Machine (Instron 4204) with a load cell of 1kN and the crosshead speed was $1 \text{ mm min}^{-1}$ at room temperature. Average value out of six measurements is reported for each sample.

3.3.3 In-Vitro drug–release: Drug-loaded films and scaffolds were immersed in 40 ml aliquots of 0.1 M sodium phosphate buffer (pH 6.4) and incubated at a constant temperature shaking bed at 37 °C with continuous stirring. After specific intervals, 3 ml aliquot of sample was withdrawn and immediately replaced with the same amount of fresh medium. UV-visible spectroscopy (Perkin-Elmer Lambda 650/850/950) was used to determine the amount of ‘MTZ’ released at 320 nm via the use of predetermined standard concentration-intensity calibration curve.

3.3.4 Cell Culture: Cells of the murine fibroblast cell line L929 were cultured in Eagle’s minimal essential medium supplemented with 10% (v/v) fetal calf serum, 2mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. Culture was performed in 75 cm$^2$ cell culture flasks in a humidified atmosphere of 95% air and 5% CO$_2$ at 37°C. Subculturing was achieved by trypsin treatment [0.25% (v/v) trypsin]. For all measurements, cells were seeded in a density of $2 \times 10^4$ cells/cm$^2$ and cultured for 48h.

3.3.5 Cytotoxicity Assay: MTT assay is generally used as a standard tool for investigating cell toxicity $^{18, 19}$. In this work, however, we have done trypsinization of cells to know the cell viability and growth. After being cultured our adherent cells under a certain condition, we checked cell viability by trypan blue staining as follows $^{20,21}$; 200 μg of polymer beads and uncoated glass slides as negative controls were incubated with L929 cells [$2 \times 10^4$ cells] for 48 hours in 24 well plates. Specimens of each sample were sterilized in 70% ethanol and then washed in culture medium before the cell culture
procedure was performed. After incubation, cells were tripsinized and counted to see the cell viability and growth. Cells grown under similar conditions were fixed in acidic acid, methanol fixative and photographed using a phase contrast microscope.

3.4 Results

3.4.1 Tensile Properties of Nanohybrid Films: Tensile properties of film specimen of nanohybrids were measured and their results are presented in Figure 3.1 which shows the results of tensile strength and percentage elongation at break for drug loaded chitosan and chitosan-Ag-gelatin nanohybrid films. During experiments, thickness of the nanocomposite films was always fixed at 223 ± 1.2 µm. No significant difference in thickness was observed from that of the chitosan film, indicating that the thickness was not affected by compositing with the Ag nanoparticles. Tensile strength of the chitosan-Ag-gelatin nanohybrids is greater than those of neat chitosan and increases with increasing the silver content in the matrix. In this way, CM-6 (Ag = 0.20 g) has higher tensile strength than that of CM-5 (Ag = 0.05 g) and CM-1 (Ag = 0, 00 g) nanohybrid films. Such a reinforcing effect on chitosan films through compositing with nanoparticles could be mainly attributed to a possible strain-induced alignment of the nanoparticles into the polymer matrix 22 that might depend directly upon the average length of the dispersed particles and, hence, the aspect ratio. The use of gelatin also increased tensile strength of chitosan due to the hydrogen bonding between amine and alcohol groups of chitosan and amide linkage of gelatin in the nanohybrid film (Please compare CM-1 with CM-2 and CM-3 with CM-4, respectively.). The elongation at break of the nanoybrid films were, however, generally decreased with the increasing contents of Ag nanoparticles, which is directly related to the flexibility and toughness of the materials. The elongation at break was not noticeably dependent on the contents of gelatin.
Figure 3.1: Tensile strength (a) and percentage elongation at break (b) of chitosan-gelatin-Ag nanocomposites films
3.4.2 In Vitro Drug Release Behavior of Nanohybrid Films: The release of the drug MTZ was monitored by measuring the UV-visible absorbance of MTZ at 320 nm as a function of incubation time. Drug release from CS-based particulate systems depends upon the extent of cross–linking, morphology, size and density of the particulate system, and physicochemical properties of the drug as well as the presence of adjuvant. In vitro release also depends upon pH, polarity and presence of enzymes in the dissolution media. The release of drug from CS particulate systems involve three different mechanisms: (a) release from the surface of films, (b) diffusion through the swollen rubbery matrix and (c) release due to polymer erosion. So that, in case of drug release from all nanohybrid films surface, adsorbed drug is instantaneously dissolved into the media when it contacts with the same media. Nearly 50-60% of the incorporated drug was released within short period i.e. 40 minutes. Drug release by diffusion involves the conversion of glassy polymer into rubbery matrix then to swollen rubbery matrix.

Figure 3.2: Cumulative release of MTZ by chitosan-gelatin-Ag films. (■) CM 3, (●) CM 4, (▲) CM 5, and (▼) CM 6.
Hence, initially the release rate of drug is faster, but later it becomes somewhat slower and nearly 60-80% drug was released in the next 40 minutes. Remaining drug might be released due to polymer erosion. Figure 3.2 shows the release profiles of prepared nanohybrid films where CM-3 and CM-4 have shown slightly higher drug release rate than those of CM-5 and CM-5. But the differences are not so high. CM-6 exhibited slower (not that impressive) release rate than other drug loaded nanohybrid films. It could be, as mentioned above, due to the fact that the release of guest molecules also depends upon the density of the particulate system. Silver nanoparticles are highly dense in case of CM-6 nanohybrid film than others and thus the CM-6 exhibits slower release. But we must accept that overall impression of all nanohybrids with respect to their drug release property is more or less same. All have shown similar MTZ release behavior with same mechanistic stage (three, mention above).

3.4.3 Cytotoxicity Assay of Nanohybrid Films: Cytotoxicity was evaluated by plating L929 cells onto various chitosan nanocomposite films. The incubation time of 3 days ensures close contact of the sample films with the cells over several cell cycles in their exponential growth phase and ensures that residual solvents, monomers, and/or degradation products of the films are released or leached into the medium. Proliferation of L929 grown in the medium containing various compositions of chitosan-silver-gelatin films are shown in Figure 3.3 at 24 hr interval for three days. The number of cells attached to each sample increased with time. The hydrophobic/hydrophilic balance, electrostatic interaction, chemical functionality and biological cues of a biomaterial surface are known to affect the adhesion of cells thereon. In Figure 3.3, the CM-2 containing chitosan and gelatin (without a drug) exhibited the highest numbers of cells attached to the sample up to 72 hrs, whereas the CM-1 containing only a drug as well as the control sample showed comparable numbers of cells attached to each sample. The results are expected from the biodegradability of chitosan, the antibacterial and antiprotozoal effect of the drug, MTZ, and the gelatin as a cell adhesive protein in cell proliferation.
Figure 3.3: Number of cells attached on the surface of film samples including control. Blue, Red, Green, Purple, Indigo, and Orange are the building block captions of Control, CM-1, CM-3, CM-5, CM-6, and CM-2, respectively.

The number of cells attached to the surface of CM-2 (with 60% gelatin) exceeded by 70% than that of the neat chitosan and chitosan-Ag nanohybrids at 72 hr of incubation time proliferation. Gelatin is a kind of cell adhesive protein and thus is located on the cell surface involved with the binding with other cells or with the extracellular matrix i.e. chitosan-Ag nanocomposites. Moreover, high hydrophilicity (surface energy) of gelatin can also induce biological responses such as cell adhesion and proliferation. The low cytotoxicity of CM-3 in comparison to the CM-1 as well as the control sample is certainly due to the presence of the drug, though the cytotoxicity of the CM-3 is not better than that of the CM-2. The effect of Ag on the cytotoxicity is slightly complicated. Though the cytotoxicity of the nanohybrids is decreased with increasing Ag contents, the Ag containing nanohybrids (CM-5 and CM-6) exhibited higher cytotoxicity than that of CM-3 without Ag. However, the initial adsorption of a thin layer of negatively charged
extracellular matrix proteins from the cell culture medium onto the positively charged Ag surface may promote initial cell attachment, potentially explaining the increased attachment of cells on the chitosan-Ag-gelatin nanohybrid films such as CM-6\textsuperscript{20}.

3.4.4 Cell attachment and growth: In general, cells proliferated over the 3-day period for most of the composites, exhibited the classic growth cycle of slow initial growth at 24 h, followed by exponential expansion to the 72 h time point.

![Figure 3.4: Optical photomicrographs of L929 cells attachment and growth on the surfaces of neat chitosan film (a) and chitosan-gelatin (60\%) (b) Film after 72hrs](image)

However, a slight decrease in cell proliferation was observed for the neat chitosan film (CM-1) and chitosan-silver nanocomposites than gelatin exceeded nanocomposite film (CM-2) after 72 h. Figure 3.4a and 3.4b present optical photomicrographs of L929 cells attachment and growth on the surfaces of neat chitosan film(CM-1) and chitosan-gelatin(60\%, CM-2) film after 72hrs, respectively. Moreover, the comparative results indicated that the adhesion and spreading of cells were better on the surface of CM-2 than on the surface of neat chitosan film (CM-1).

3.4.5 Morphology of Scaffolds: Figure 3.5 shows the typical scanning electron morphology of the top, middle and bottom portions of the drug-loaded scaffolds (sample
CM-10). The interconnected porous morphology resulted from phase separation between polymer-poor and polymer-rich phases. The polymer-poor phase is of mostly solvent i.e. water. The water molecules were removed by sublimation of ice crystals, which leads to the formation of pores. On the other hand, the polymer-rich phase i.e. chitosan-Ag, nanocomposite with drug, consists mostly of the polymer solution and forms the cell walls around the pores. The dimensions of cylindrical well in tissue culture plates used are about ~25 mm thickness and 25 mm diameter. When a polymeric solution is exposed in such dimension, for instance, during quenching, the bottom layer is exposed to the interface of solution and freezer where the cooling rate is relatively fast. As a result, the surface of bottom portion of the scaffold has small and interconnected pores.

![Figure 3.5](image)

**Figure 3.5:** Scanning electron micrographs of (a, b) top, (c, d) middle and (e, f) bottom portions of freeze dried porous scaffolds (sample CM-10)

The top surface of solution, on the other hand, is exposed to the interface of the solution and vacuum where the rate of quenching is comparatively slow because of the temperature gradient. Under such a slow rate, *ice-crystal nuclei* may have enough time to
grow resulting in large and well-interconnected pores. However, the quenching rate at the middle part of the solution will be different from that of both top and bottom portion. Thus, the *ice-crystal nuclei* can have relatively enough time to grow and the crystal growth would be directed by the temperature gradient. This is confirmed by elongated pore morphology in mid-section of the obtained scaffolds. In addition, the ratio between CS matrix and gelatin kept constant with varied nanoparticle content and drug. The mean pore diameter were controlled within the range of 1-10 μm by varying the freezing temperature and hence the ice crystal size.

**3.4.6 Tensile Properties of Porous Scaffolds:** The mechanical properties of chitosan scaffolds formed by the lyophilization technique are mainly dependent on the pore size and orientations; it implies that tensile strength of scaffold with small pore size will be higher than that of big pore size scaffold. The scaffolds / implants based on polymeric materials should be resistant towards the stress exerted by different parts of the body. Figure 3.6 shows the tensile strength and percentage elongation at break of chitosan scaffold. Although the tensile strength of CM-8 (Gelatin=20%) was lowest and CM-10(Ag=0.20gm) and CM-9(Ag=0.05gm) were significantly higher than that of CM-7 and CM-8 (Ag=0.00 gm) but CM-11(CS=200%) exhibited the highest tensile strength. The region behind the higher tensile strength of CM-11 is the higher viscosity of chitosan molecule inside the scaffold, showing high hydrogen bonding with each other. The viscosity of pre-freezing solution has relations with its concentration. The viscosity of the solution is high, which is unfavorable to the water and other molecular chains moving in the solution. Therefore, the number of ice nuclei in the solution of CM-11 with high concentration is smaller, and it is more difficult to grow to larger pore than that of the low concentration one (CM-7). So, the pore size is inversely proportional to the concentration of the scaffolds. It means that the scaffolds with higher concentration (CM-11) are much finer and closer in structure than that with lower concentration (CM-7), leading to much stronger scaffold within the tensile strength test. This is evident that no clear relation exists between the gelatin and silver nanoparticles weight percentages and the tensile strength of the scaffold.
Figure 3.6: Tensile strength (a) and percentage elongation at break (b) of chitosan-gelatin-Ag nanocomposites scaffolds

The percentage elongation at break as a function of Ag or gelatin contents showed almost reverse trends to the tensile strength, as expected; that is, the percentage elongation at
break of CM-10 and CM-9 were significantly lower than that of CM-7 and CM-7. Meanwhile, CM-10 showed higher tensile strength but lower elongation at break than other scaffolds, as already explained for nanohybrids. Among all, the CM-11 (CS=200%) exceptionally exhibited the highest elongation at break, showing that more concentration of chitosan (CM-11) has better mechanical properties than others, though the reason is not so clear at moment.

3.4.7 In Vitro Drug Release behavior of Porous Scaffolds: Figure 3.7 shows the release profiles of the prepared porous scaffolds. No significant effect was found on the release rate of MTZ. When the chitosan concentration is high for the scaffolds, however, the cumulative release rate was low (see CM-11). It may be due to the higher concentration of chitosan causes higher viscosity of solution and greater ionization of amine groups. These results showed that the CS-Ag-gelatin scaffold could be suitable for sustained drug release in tissue engineering applications.

![Figure 3.7: Cumulative release of MTZ by chitosan-gelatin-Ag scaffolds. (-----) CM 7, (-) CM 8, (-----) CM 9, (-----) CM 10, and (-----) CM 11](image)
3.4.8 Cytotoxicity Assay of Porous scaffolds: Proliferation of L929 grown in medium containing various compositions of chitosan-silver-gelatin scaffolds is shown in Figure 3.8 at 24 hr interval for three days. There was much variation in cell proliferation for the first two days of culture and the growth of cell has been increased with incorporated gelatin into the chitosan-Ag composites, similarly as with increasing silver nanoparticles. Though similar trend was observed up to day 3, the extent of proliferation was decreased compared to day 2 for all set of scaffolds.

![Graph showing cell proliferation](image)

**Figure 3.8:** Number of cells attached on the scaffolds including control. Gray, Purple, Green, Red, Dark blue, and Orange are the building block captions of Control, CM-7, CM-8, CM-9, CM-10, and CM-11, respectively

Porous scaffolds have three well dimensional pores with flexibility; which could improve the contact between the scaffold material and the tissue. Penetration of the polymeric chains into the tissue forms strong adhesion. It is expected that the presence of pores may play a critical role in early phase of cell adhesion because that phase of cell adhesion is primarily governed by simple electrostatic interaction between cells and substrates. Thus, in the initial time of proliferation, pores feeling were high up to day 2 and it was decreased with time i.e. day 3. Moreover, gelatin acted as adhesive protein in case of
CM-8 to CM-11 and thus the extents of the proliferation of cells were higher than that of the neat chitosan and chitosan-Ag nanocomposites.

3.5 Discussion
Herein, two main techniques have been discussed for sustained drug release behavior and cell line study of chitosan-Ag-gelatin porous scaffolds and nanohybrids, where both are useful in bio-medical applications. In case of porous scaffold morphology with different phases i.e. top, middle and bottom, water molecule played a crucial role as an ice crystal during being sublimated, to make different pores with different phases. This exceptional behavior of water molecules to make scaffold evoked the thought that the presence of hydrophobic silver particles in the natural hydrophilic polymer (chitosan) solution can alter the size and distribution of ice crystals with different phases formed at the time of freezing and thus can change the whole pores of scaffold made by freeze drying. Moreover, the presence of hydrophobic silver nanoparticles in the chitosan solution may give enhanced strength after embedded itself into the wall scaffold structure. Indeed, in our study we observed that porous scaffolds exhibited higher tensile strength than that of nanohybrids of chitosan-Ag-gelatin (please see Figures 3.1 and 3.6). In both cases, silver nanoparticles played significant roles i.e. with increasing the silver nanoparticles corresponding tensile strength was generally increased but the elongation at break was decreased. In case of porous scaffolds, in particular, silver nanoparticles were dispersed throughout the wall framework of scaffolds and consequently the strength of wall and overall the scaffold was enhanced. On the other hand, with increasing the viscosity of hydrophilic polymer (chitosan), pores of the scaffold would be blocked or shrunk, resulting in the enhanced mechanical properties of porous scaffolds. While increasing the amount of chitosan, however, pore diameter of scaffolds was reduced due to its concentration effect. Then, the numbers of walls of scaffolds were increased, leading to further high mechanical strengths due to the flexibility (viz. non-brittleness) effect of scaffolds as well as its concentration effect. But in case of nanohybrid films, aggregation of silver nanoparticles corresponding to the less flexibility gave lower elongation at break. In this way, mechanical properties of scaffolds are in general higher than those of
nanohybrid films, which may be a benefit for future production of bio-materials in biomedical fields.

Sustained drug release of Metronidazole was investigated with same techniques, i.e. with drug loaded scaffolds and nanohybrids of chitosan-Ag-gelatin. As we have mentioned above, the drug release behavior depends basically on three different mechanisms. Sustained release behavior of drugs from nanohybrid films was in general better than porous scaffolds. The release of drugs from freeze-dried scaffolds can be observed to be faster and higher than that from the smooth nanohybrid films. The higher release rate of porous scaffolds (CM-7 to CM-10) can be attributed to the fact that the inclusion of solvent and diffusion of drug molecules may be rapid in porous scaffolds than in film samples. This result is consistent with their swelling behaviors. In the swelling controlled release system, drugs are dispersed within a glassy polymer. Upon contact with biological fluids, the polymer is swollen but no drug diffusion occurs through the polymer phase. As penetrate enters the glassy polymer, glass transition temperature of the polymer is lowered due to the relaxation of the polymer chains and drugs could diffuse out of the swollen rubbery polymer. This type of system is characterized by two moving boundaries: the front separating the swollen rubbery portion and the glassy region, which moves with a front velocity and the polymer fluid interface. The rate of drug release is controlled by the velocity and position of the front dividing the glassy and rubbery portions of the polymer. Here, in both cases the release behavior was similar at glassy region, i.e., exhibiting linear drug release behavior.

The exceptional behavior of nanohybrid films showing better release of drug is based on its three different mechanisms from surface release to polymer erosion but in case of scaffolds only one kind of mechanism is effective, giving continuously drug release within a short period, i.e. in the swollen rubbery region. It is expected that the presence of pores of scaffolds were instantly contacted with the swollen rubbery region, and thus with the release media, leading to fast release. Apparently, pores of scaffold played a crucial role for releasing behavior. Nevertheless; the viscosity of hydrophilic polymer (chitosan) played also more essential role in the control release than the case of less viscous scaffolds having less chitosan concentration. It was already explained above that with
increasing the viscosity of polymer, pore diameter of the scaffolds would be reduced, affecting the control release behavior.

Cell adhesion study was aiming to characterize the influence of presence of silver nanoparticles in the nanohybrids and scaffolds of chitosan-Ag-gelatin nanocomposites but it was expected that the presence of silver nanoparticles inside the scaffold and nanohybrid may not play any crucial role. In fact, cell growth was increased with increasing the concentration of silver particles. Gelatin has been reported for its excellent cell adhesive property. This can lead to potential cell growth against nanohybrids and scaffolds of chitosan-Ag-gelatin nanocomposites. However, a similar phenomenon was not found in case of hybrids and porous scaffolds without gelatin. It is worthy to note that the cell count in scaffolds was higher than that of nanohybrids. Analysis of growth kinetics of L929 cells on chitosan-Ag-gelatin scaffolds revealed that pores of scaffolds directly influenced on the cell proliferation at early phase of culture (up to day 2) but with increasing time the cell count would be reduced. This may be due to the filling of the pores with early phase of time and having less favorable to the cultured cells. Moreover, porous scaffold is a better cell culture material than that of nanohybrid of chitosan-Ag-gelatin. Additionally, we may expect that the porous scaffold has better bio-medical application than nanohybrid of chitosan-Ag-gelatin. Furthermore, it should be noted that the drug release behavior can be controlled with respect to the crosslink agent like glutaraldehyde.
3.6 Conclusion

The present study was to examine the potential uses of nanocomposites based on chitosan-gelatin and silver nanoparticles as biomaterials. The nanohybrid films and scaffolds, which are successfully prepared by vacuum drying and freeze-drying, respectively, exhibited different morphologies such as smooth films and porous scaffolds, respectively. Ag nanoparticles were found to increase the tensile strength with decreasing the elongation at break. Significant difference in drug release from the smooth films and porous scaffolds was observed. Porous scaffolds have shown higher and faster drug release than smooth films. Designed scaffolds could improve both mechanical properties through toughening mechanisms and tissue regeneration through improved control of cell adhesion. This study examined how gelatin affects on the biological activity and antibacterial efficiency of chitosan-silver nanocomposites. We summarize that a combination of gelatin with biodegradable polymeric chains and silver nanoparticles reinforcement can be applied to achieve desired combination of properties (mechanical and biological activity) of materials used for biomedical applications.
3.7 References