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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EVALUATION OF QUINCE SEED CONTAINING HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

by

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İZMİR

EVALUATION OF QUINCE SEED CONTAINING HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

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M.Sc THESIS EXAMINATION RESULT FORM

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EVALUATION OF QUINCE SEED CONTAINING HYDROGELS FOR TISSUE ENGINEERING APPLICATIONS

ABSTRACT

One of the most important components of tissue engineering is the tissue scaffold, which offers a proper environment for cell development and promotes tissue regeneration. To allow effective mass transfer of nutrients and oxygen, an ideal scaffold should be biocompatible, biodegradable, and porous. Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. Quince seed has phenolic compounds, organic acids and free amino acids. The antioxidant functions of its phenolic compounds are superior to that of chlorogenic acid and ascorbic acid as standard antioxidants. Recent studies indicated that quince seed mucilage accelerated wound healing. The aim of this study is to produce quince seed mucilage containing carboxymethyl chitosan hydrogels for skin tissue regeneration. For this purpose, quince seed mucilage was mixed with aqueous carboxymethyl chitosan solution and they were crosslinked with poly (ethylene glycol) diglycidyl ether at three different concentrations. Then the samples were freeze dried to obtain 3D porous structure. The morphologies, chemical structures, mechanical properties and swelling capacities of the hydrogel scaffolds were characterized by SEM, FTIR, TPA and swelling test, respectively. Also, the viability of MCF-7 breast adenocarcinoma cells on the scaffolds was evaluated by WST-1 colorimetric assay. As a result, the obtained hydrogels could be promising scaffolds for skin tissue engineering applications.

Keywords: Quince Seed, carboxymethyl chitosan, hydrogel, bioactivitiy, tissue scaffold, porous materials

AYVA ÇEKİRDEĞİ İÇEREN HİDROJELLERİN DOKU MÜHENDİSLİĞİ UYGULAMALARINDA DEĞERLENDİRİLMESİ

ÖZ

Doku iskelesi, hücre büyümesi için uygun bir ortam sağlayan ve doku yenilenmesine izin veren doku mühendisliğinin ana bileşenlerinden biridir. İdeal bir iskele biyolojik olarak uyumlu, biyolojik olarak parçalanabilir olmalı ve besinlerin ve oksijenin verimli bir şekilde toplu olarak taşınmasını sağlamak için gözenekli yapıya sahip olmalıdır. Şifalı bitkiler, tedavi edici değeri olan bileşenler içerdiğinden, yüzyıllardır insan hastalıklarına çare olarak kullanılmıştır. Ayva tohumunda fenolik bileşikler, organik asitler ve serbest aminoasitler bulunur. Fenolik bileşiklerinin antioksidan fonksiyonları, standart antioksidanlar olarak klorojenik asit ve askorbik asitinkinden üstündür. Son çalışmalar, ayva tohumu müsilajının yara iyileşmesini hızlandırdığını göstermiştir. Bu çalışmanın amacı, cilt dokusunun yenilenmesi için karboksimetil kitosan hidrojelleri içeren ayva tohumu müsilajı üretmektir. Bu amaçla ayva çekirdeği müsilajı sulu karboksimetil kitosan çözeltisi ile karıştırılarak üç farklı konsantrasyonda poli (etilen glikol) diglisidil eter ile çapraz bağlanmıştır. Daha sonra 3 boyutlu gözenekli yapı elde etmek için numuneler dondurularak kurutulmuştur. Hidrojel doku iskelelerin morfolojileri, kimyasal yapıları, mekanik özellikleri ve şişme kapasiteleri sırasıyla SEM, FTIR, TPA ve şişme testi ile karakterize edildi. Ayrıca, MCF-7 meme adenokarsinom hücrelerinin yapı iskeleleri üzerindeki canlılığı, WST-1 kolorimetrik tahlil ile değerlendirildi. Sonuç olarak, elde edilen hidrojeller cilt dokusu mühendisliği uygulamaları için umut verici yapı iskeleleri olabilir.

Anahtar kelimeler: Ayva çekirdeği, karboksimetil kitosan, hidrojel, biyoaktivite, doku iskelesi, gözenekli malzemeler

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CHAPTER ONE
INTRODUCTION

Tissue engineering is a relatively new field whose goal is to create or regenerate artificial tissues and organs. Over 4000 years ago, the idea of replacing a broken body part with natural materials was advocated and even tried. Living tissues, on the other hand, were not engineered until recently, giving rise to the area of tissue engineering. However, it was not till very recently that living tissues were engineered, giving rise to the field of tissue engineering. Tissue engineering is an emerging field that holds strong promises to provide replacements for damaged tissues by combining three main elements, namely cells, scaffolds, and biomechanical or biochemical signals (Mhanna & Hasan, 2017).

In the study carried out within the scope of the thesis, it is aimed to produce a hydrogel scaffold containg quince seed mucilage for skin tissue engineering applications. Hydrogels were produced by crosslinking carboxymethyl chitosan (CMCht) with poly (ethylene glycol) diglycidyl ether (PEGDE) and quince seed mucilage, which plays a role in wound healing was used as an additive to improve the bioactivity of the hydrogel scaffolds. Scanning electron microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and thermogravimetric differential thermal analysis (TGA/DTA) were used for the morphological, chemical and thermal behaviour analysis of the hydrogel scaffolds respectively. Besides their swelling and mechanical properties were evaluated. Finally, cytotoxicity of the hydrogel scaffold on MCF-7 breast adenocarcinoma cells was also investigated.

CHAPTER TWO
THEORETICAL BACKGROUND

In this part of the study, tissue engineering, tissue engineering scaffold, hydrogel, carboxymethyl chitosan, quince seed and poly (ethylene glycol) diglycidyl ether are discussed.

2.1 Tissue Engineering

Tissue damage and loss arising from disease, injury or trauma are one of the major health care problems in all over the world (Chapekar, 2000). In order to reconstruct the devastated tissues, tissue transplantation and implantation of biomedical devices are the most commonly used treatment strategies (Sahithi, Swetha, Ramasamy, Srinivasan & Selvamurugan, 2010). In the tissue transplantation strategy, autografts (tissue transplantation from one site to another on the body of a patient) are promising and lifesaving solution, but they have limited donor availability and also harvesting autografts is expensive and painful. Allografts (tissue transplantations from one person to another) do not limit by a donor and could be alternatives to autografts. However, they have the potential risk of immune response and carrying the infection or disease from the donor to the patient (O'brien, 2011; Vasita & Katti, 2006). On the other hand, biomedical devices are generally used for temporary solutions because they do not repair all the functions of damaged tissue. Infection and potential rejection of the implant are other important disadvantages of this procedure. The rapid increment in the need for tissue treatment has elicited the development of alternative treatments to overcome the disadvantages of traditional treatment strategies (Chen, Ushida, & Tateishi, 2002; Mikos & Temenoff, 2000).

2.2 Components of Tissue Engineering

Basically, tissue engineering strategies involve three approaches. The first strategy, cell transplantation, involves the direct injection of healthy cells removed from donor tissue into the damaged tissue but the probability of new tissue formation is about 10% in this technique (Ebnesajjad, 2012). The second strategy is related to delivering

biochemical factors to the damaged tissue. These two strategies are more suitable for the treatment of smaller defects. In addition, the need for producing the tissue with practical size scale and predetermined shapes limits the use of these approaches (Ma, 2004). Therefore, the third approach, implanting the scaffold into the damaged area has become more important and more relevant with the concept of tissueengineering. This scaffold-guided regeneration involves using the scaffold into the damaged area to improve tissue regeneration. In addition, cell loaded scaffold implantation are the basis of most current tissue engineering strategy. In this approach, isolated healthy cells from a patient are subjected to an *in-vitro* environment for their expansion first and then, they were seeded onto a scaffold. These cell-scaffold constructs are implanted into the defect area, scaffold gradually degrades over time and meanwhile, new tissue forms (Dhandayuthapani, Yoshida, Maekawa & Kumar,2011; Ebnesajjad, 2012; Martina & Hutmacher, 2007; Shin, Jo, & Mikos,2003).

In the last approach, cell-scaffold constructs, it is aimed to produce an artificial extracellular matrix (ECM) that is a suitable environment for cell growth just like in their native tissue. ECM is the non-cellular and three-dimensional network that surrounds cells and present in all tissues. It gives mechanical support for the cellular constituents and also serves to regulate many cellular behaviors (Frantz, Stewart, & Weaver, 2010; Kim et al., 2011). The scaffold provides a microenvironment for cell attachment, proliferation, and differentiation, and new tissue formation by mimicking extracellular matrix. Also, it helps in carrying the biomolecular signals for cells. Therefore, scaffold plays a vital role in tissue regeneration and repair process (Liu, Xia, & Czernuszka, 2007; Ma, 2008). The schematic illustration of the tissue engineering approaches is seen in Figure 2.1 (El-Sherbiny &Yacoub, (2013).

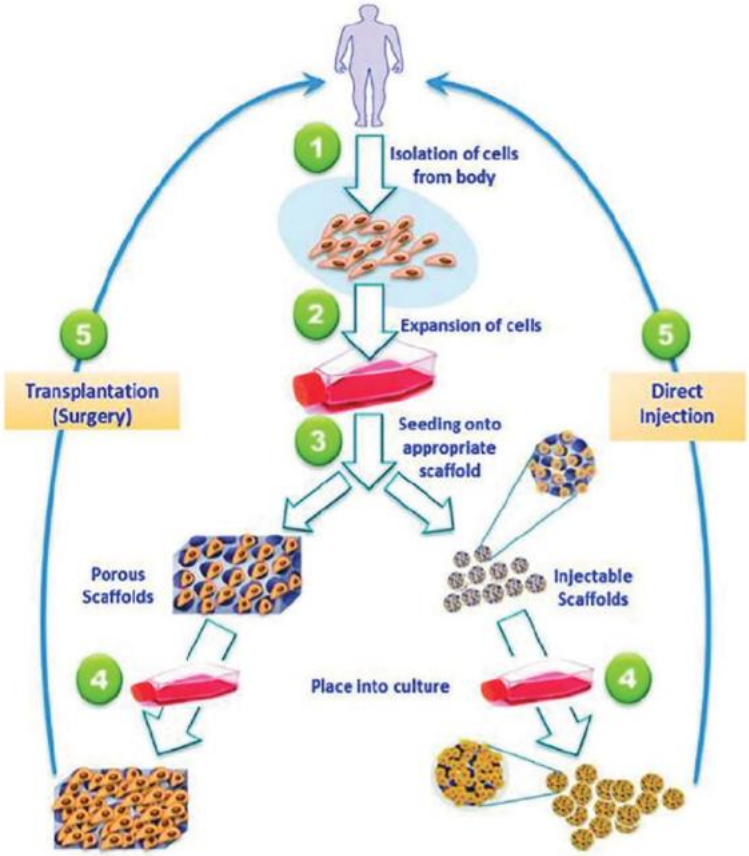


Figure 2.1 Schematic representation of tissue engineering approaches (El-Sherbiny & Yacoub, 2013)

2.3 Properties of the Tissue Engineering Scaffold

Biocompatibility is the first and very important criterion of the scaffold. It is defined as the "ability to be in contact with a living system without producing an adverse effect" according to IUPAC Recommendations (Vert et al., 2012). The scaffold must be biocompatible that cells could attach and migrate through the structure, and proliferate to form new tissue. In addition, scaffold material should not induce any inflammation or adverse response after implantation (O'brien,2011).

Scaffolds are not permanent implants and they act only as temporary ECM until the new tissue formation is completed in the damaged area. Therefore, biodegradability of the scaffolds is a critical requirement. Since in the regeneration process, scaffold degradation allows cells to produce their own extracellular matrix and eventually grown tissue replace the scaffold, it is important that the degradation rate of the

scaffold should match the rate of new tissue formation. In addition, the degradation products of the scaffolds should be non-toxic and can be removed from the body (Ma, 2004; O'brien,2011).

The scaffold must have adequate mechanical properties consistent with the implanted site to ensure support to the tissue growth. For example, scaffold could deform when the compressive strength of the scaffold is too low and do not allow tissue growth. Besides, very high compressive strength can lead to inadequate conditions to support cell growth in vivo. In addition, if the difference of elastic moduli value between the scaffold and natural tissue is too high, delamination of the scaffold from the surrounding tissue can occur. The scaffold must be handled without any deformation during the implantation and it is also very important that it should maintain its mechanical integrity until new tissue formation is occurred (Ebnesajjad, 2012; Ma, 2004; Sachlos & Czernuszka,2003).

Scaffolds are three-dimensional porous solid tissue engineering construct sand in the production of the scaffold, cost-effective manufacturing technology become important. The scaffold should be easily fabricated into the desiredthree-dimensional structure with different shapes and sizes (Dhandayuthapani, Yoshida, Maekawa, & Kumar, 2011; O'brien, 2011; Sachlos & Czernuszka, 2003).

2.4 General Information About Hydrogels

Hydrogels are three-dimensional hydrophilic polymer networks that consist of water-soluble polymers and are provided to be water-insoluble after crosslinking by physical or chemical methods. Hydrogels can be composed of natural polysaccharides, proteins or synthetic polymers. In aqueous phases, they can hold a high amount of water in their structure compared to their own weight.

Since the biological application studies of methacrylate-based hydrogel 60 years ago, hydrogels have been used in drug delivery systems, biological uses of chemically modified implants, devices used for diagnostic purposes and tissue engineering (Peppas, Hilt, Khademhosseini & Langer, 2006). Hydrogels to be used in this field

have a variant to be developed to modify. The most important of these variables are the type of polymer, the form and method of crosslinking, biodegradability, cellular interference, and the need for a three-dimensional hydrogel cell.

2.4.1 Classification of Hydrogels

The hydrogel products can be categorized on different bases (Garg & Garg, 2016).

2.4.1.1 Classification based on source

Hydrogels can be classified into two groups based on their natural or synthetic origins (Zhao et al., 2013). Natural biopolymers include polysaccharides, e.g. chitosan, alginate, cellulose, starch; proteins, e.g. collagen, silk, gelatin and polynucleotides (DNA, RNA) (Dhandayuthapani, Yoshida, Maekawa, & Kumar, 2011). These polymers are from renewable resources, environmentally-friendly polymers and they can be mentioned as the first biodegradable biomaterials used for tissue regeneration (Dhandayuthapani, Yoshida, Maekawa, & Kumar, 2011; Okamoto & John, 2013).

Synthetic biopolymers have attracted great attention in the biomedical field, including tissue engineering due to their advantages over natural biopolymers. Even though, natural biopolymers have the advantage of biological recognition, they have important drawbacks such as possible adverse immune responses and transportation of pathogenic impurities, poor mechanical properties, uncontrollable biodegradability, batch-to-batch non-uniformity and high cost (Armentano, Dottori, Fortunati, Mattioli, & Kenny, 2010; Liu & Ma, 2004).

2.4.1.2 Classification according to polymeric composition

a. Homopolymeric hydrogels: The most basic structural unit of polymeric networks are monomers. Homopolymeric hydrogels are polymeric networks containing a single type of monomer in their structure (Iizawa et al., 2006).

b. Copolymeric hydrogels: These are made up of two or more monomer species,

each containing at least one hydrophilic component, arranged in a random, block, or alternating pattern throughout the polymer network's chain (Yang, Chu & Fix, 2002).

c. Multipolymer: Interpenetrating polymeric hydrogels (IPN) are a type of hydrogel that is made up of two independent cross-linked synthetic and/or natural polymer components that are contained in a network shape. One component of a semi-IPN hydrogel is a cross-linked polymer, while the other is a non-cross-linked polymer. (Maolin et al., 2000).

2.4.1.3 Classification based on configuration

This hydrogel classification is made according to physical structure and chemical composition (Garg & Garg, 2016).

a. Amorphous (not crystalline)
b. Semi-crystalline structure: A complex mixture containing amorphous and crystalline phases.
c. Crystalline

2.4.1.4 Classification based on type of cross-linking

Hydrogels can be divided into two groups on the basis of their chemical or physical behaviour of the cross-link junctions. Chemically cross-linked networks have stable junctions, while physical networks have temporary junctions that results from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds or hydrophobic interactions (Hacker et al., 2011).

2.4.1.5 Classification based on physical appearance

According to the polymerization procedure used in the hydrogel forming process, hydrogels can be produced as matrix, film or microsphere form. In addition, hydrogels can be examined in 3 basic groups as non-ionic (neutral), anionic or cationic hydrogels, depending on the presence of electrical charge in cross-linked chains (Garg & Garg, 2016).

2.4.2 Crosslinking in Hydrogels

Crosslinking cannot be properly classified as a quality of hydrogels because it is more of a cause of the material's other qualities. The degree of crosslinking varies greatly. The network of the hydrogel can be achieved in a variety of methods. Physical crosslinking occurs as a result of hydrophobic contacts between chains, ionic interactions between a polyanion and a polycation (complex coacervation), or ionic interactions between a polyanion and multivalent cations (complex coacervation) (ionotropic hydrogel). Chemically bonded gels fall into the second type. Ultraviolet irradiation, heating or chemical crosslinking via a crosslinker with a large collaborative reaction can all be used to crosslink the molecules (Garg & Garg, 2016).

2.4.3 Swelling Behavior in Hydrogels

Cross-linked polymer networks swelling in a liquid media are known as hydrogels. The absorbed liquid works as a selective filter, allowing some solute molecules to diffuse freely, while the polymer network acts as a matrix, holding the liquid together. The hydrogel connection swells as the polar groups are hydrated, exposing hydrophobic groups, which additionally interact with water molecules, resulting in hydrophobically-bound or secondary bound water. Total bound water is a term that encompasses both primary and secondary bound water (Garg & Garg,2016).

The swelling rate of hydrogels can be measured by calculating the ratio of the weight difference to the dry weight, after measuring the dry weight (w0) and the wet weight after swelling (ws). (Garg & Garg,2016).

2.5 Tissue Scaffold Components General Information

2.5.1 Quince Seed (Cydonia oblonga Miller)

Quince (Cydonia oblonga Miller) is a deciduous tree in the Rosaceae family that is used as a medicinal and nutritious plant in the Middle East, South Africa, and Central Europe (Zargari, 1986). In folk medicine, various portions of the plant have been used to treat a range of ailments. After decoction, the leaves were utilized for their calming, antipyretic, and antidiarrheic effects. The fruits were traditionally used to treat diarrhea (Duke &Ayensu,1985). Soaking or boiling the seeds releases the mucilage from the seed coat, resulting in a jelly-like consistency that can be used for sore throats, bronchitis, and as a bulk laxative. Mucilage is frequently used externally to treat small burns and other minor injuries (Grieve M,1971).



Figure 2.2 Quince fruit (Cydonia oblonga) (Personal archive, 2018)

With the increasing interest in low-cost and natural materials, the trend towards fruit seeds is increasing. When considered chemically, it is desired to be used in hydrogel production since it has phytochemical components. Biochemical identification tests were used to evaluate the presence of plant chemical constituents such as carbohydrates, protein, flavonoids, sterols, alkaloids, tannins, saponins, resin, phenol, and terpenoids (Patel, Pandya, Shah & Mahajan, 2011).

2.5.2 Carboxymethyl chitosan

Carboxymethyl chitosan (CMCht) is the most commonly used chitosan derivative. It is produced by carboxymethlation of chitosan which introduced the carboxymethyl ($COOCH_3$) group in the primary or secondary hydroxyl groups and the amino groups bonded to the glucopyranose unit. Derivatives of CMCht can be amino substituted (N-CMC), hydroxyl substituted (O-CMC) or both amino and hydroxyl substituted (N, O-CMC). Figure 2.3 shows the chemical structure of carboxymethyl chitosan (Huang, Yu, Zhang, Qi & Zhang, 2017). CMCht has desirable water-solubility due to carboxymethyl groups on hydroxyl sites of the glucosamine units. It also maintains the advantages of chitosan such as low toxicity and high biocompatibility in addition to its good water-solubility (Bhattarai, Gunn, & Zhang, 2010; Gonçalves, da Silva, Signini, & Naves, 2017; LogithKumar et al., 2016).

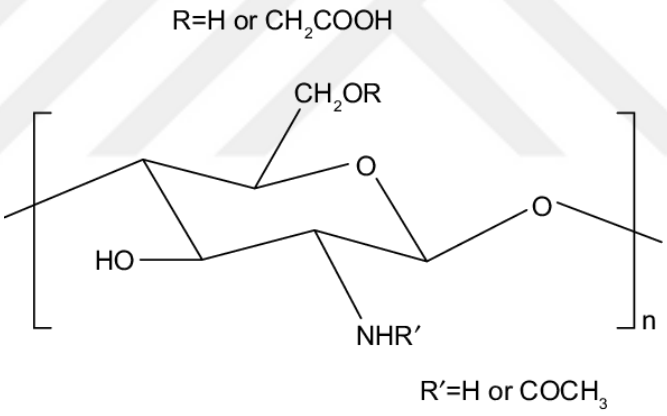


Figure 2.3 Chemical structure of carboxymethyl chitosan (CMCht) (Huang, Yu, Zhang, Qi & Zhang, 2017).

2.5.3 Poly (ethylene glycol) diglycidyl ether (PEGDE)

Poly (ethylene glycol) diglycidyl ether (PEGDE) is widely used as an additive for cross-linking polymers bearing amine, hydroxyl or carboxyl groups (Monticelli, et al., 2019). Figure 2.4 shows the chemical structure of (PEGDE). (Monticelli, et al., 2019).

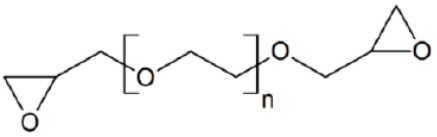


Figure 2.4 Chemical structure of polyethylene glycol diglycidyl ether (PEGDE) (Monticelli, et al., 2019)

Crosslinking by polyethylene glycol diglycidyl ether (PEGDE) has been more recently introduced and showed attractive features in terms of viscoelastic properties and reduced biodegradation (Tezel & Fredrickson, 2008).

It is a water-soluble epoxyresin and has two epoxide functional groups (cyclical ether with a three-membered ring) located at both ends of each molecule. It has been used as a cross-linking agent for polysaccharides and proteins *via* ring opening of the epoxide group by the chemical reaction with the amino, carboxyl and hydroxyl functional groups (Gámiz González, Edlund, Vidaurre, & Gomez Ribelles, 2017; Lu, Xu, Zheng, Zhang, & Su, 2006; Vargas, Acevedo, López, & Romero, 2008).In particular, the crosslinking parameters play a major role in determining the rheological and swelling properties of the hydrogel (Tezel & Fredrickson, 2008).

CHAPTER THREE
LITERATURE STUDY

The effects of quince seed mucilage at concentrations of 50, 100, 200, and 400 g/mL on human skin fibroblast proliferation as an element of wound healing were examined by Ghafourian et al., (2014). At concentrations less than 400 g/mL, cells treated with quince seed mucilage improved their proliferative activity. The concentration of 50 μg/mL was the most effective dose after 72 hours treatment. Test result indicates that quince seed mucilage has the ability to stimulate proliferation of human skin fibroblast. This effect suggests that this compound can act as a wound healing agent.

In the study of Silva et al., the phenolic compounds, organic acids and free aminoacids of quince seeds were determined. Quince seeds were found to have caffeoylquinic acids, especially, C-glycosyl flavones. Caffeoylquinic acids were about 34 to 37% of the determined phenolics, with 5-O-caffeoylquinic acid was the most abundant one (19-24%). C-glycosyl flavones were in the range of 63-66%, with isoschaftoside was the major one (about 18%). The total phenolic content of the seed was around 0.1 g/kg. The six organic acids identified in the seeds were citric, ascorbic, malic, quinic, shikimic and fumaric acids. The total organic acid content was around 0.5 to 0.8 g/kg. The sum of malic acid plus quinic acid (45-61%) dominated the total organic acid content. Citric (20-27%) and ascorbic acids (13-27%) were also present at high percentages. 21 free amino acids were identified in seeds which ranged from 1.3 to 1.7 mg/kg. Glutamic acid, aspartic acid and asparagine were found to be the three major free amino acids and they were about 60 to 75% of the total free amino acids.

In the study of Moghbel & Tayebi 2015, the aim was to achieve a drying method for promotion of quince seeds mucilage stability and also to study differences in physicochemical and mechanical properties when influenced by the drying method. The mucilage was divided into two equal parts, one group dried by cold and the other by the warm method. The condition for the cold method (lyophilization) was 24 hours for both stages of freezing (-30°C) and drying (-50°C and 50 mTorr pressure). The

temperature for drying by an oven was 50°C. Although quince seeds dried mucilage by lyophilization had less yield amounts yet in view of purity and physicochemical and mechanical properties, it was significantly better than mucilage prepared by the oven method.

Tamri, Hemmati & Boroujerdnia 2015, studied the healing efficiency of quince seed mucilage formulated as 5%, 10%, and 20% creams in eucerin base with especial attention on growth factors involving in wound healing. Results showed that there were statistically significant differences in wound contraction between quince seed mucilage 10% and 20% creams treatments groups and control groups in most of the days. Rabbits treated with quince seed mucilage 20% cream had the best results (completed healing in 13 days, higher hydroxyproline content, higher tissue resistance and higher wound fluid levels of evaluated growth factors).

Xie, Yin, Liu, Zhu & Yang 2018, studied a variety of innovative pH-sensitive polymers consisting of Chinese quince seed gum (CQSG) and poly (*N*, *N*-diethylacryl amide-co-methacrylic acid) which were synthesized via free radical polymerization for controlled drug delivery. Results suggested the hydrogel polymers were composed of CQSG chains physically entangled in poly (*N*, *N*-diethylacryl amide-co-methacrylic acid) networks. Swelling properties of these hydrogels were strongly affected by the contents of CQSG and crosslinkers. Attributes imply that the new proposed CQSG/poly (*N*,*N*-diethylacryl amide-comethacrylic acid) hydrogel polymers can be used as a good medium for oral delivery of proteinaceous drugs.

In the study of Ashraf et al., 2016 micromeritic properties, water absorption capacity, stimuli (pH, ethanol and saline) responsive swelling-deswelling (on-off switching) and controlled drug releasefrom the polysaccharide glucuronoxylan isolated from the seeds of quince were investigated. The water retention capacityof Quince hydrogel (QH) was found admirable. The hydrogel also exhibited privileged swelling in waterand basic buffers (pH 6.8 and 7.4) while insignificant swelling in acidic buffer (pH 1.2). The swelling kinetics of QH follows second order. These results signify QH a smart material for sustainedrelease formulations.

Gonzalez, Edlund, Vidaurre & Ribelles, 2017 studied highly swellable hydrogels which were produced by crosslinking of high molecularweight carboxymethylated chitosan (CmCHT) with poly (ethylene glycol) (PEG) oligomers. The crosslinking density was used to modulate the hydrogel swelling capacity, which ranged from 900 to 5600 percent. The hydrogels had good homogeneity in the swelled state, with a high interconnected porosity and nanodomains rich in CmCHT and others rich in PEG diglycidyl ether. The hydrogel with the highest crosslinking density had a storage modulus of 27 kPa, which, together with the fact that it was enzyme degradable with lysozyme after 59 days, suggests that these hydrogels could be used in delivery systems or soft tissue regeneration.

CHAPTER FOUR
EXPERIMENTAL STUDY

In this part of the study, the methods used for quince seed mucilage extraction, production of tissue scaffolds and characterization of their structural and chemical properties were examined.

4.1 Materials of the Hydrogel Scaffolds

Quince fruits were purchased from the local market in İzmir, Turkey. O-carboxymethylated (CMCht) with a deacetylation degree around 91% was obtained from Santa Cruz Biotechnology, USA. Poly (ethylene glycol) diglycidyl ether (PEGDE) (Mn=500) were obtained from Sigma-Aldrich, USA.

4.2 Production Stages of the Hydrogel Scaffolds

4.2.1 Preparation of Quince Seed Mucilage

Quince seed mucilage (QSM) was prepared by putting 7 grams of quince seeds in 50 ml of distilled water for 24 hours. QSM mixture was filtered with the help of a cheesecloth and the seeds were discarded. Ethyl alcohol (70°C) was added to the filtrate for the precipitation of mucilage in a rich phase. After excess ethyl alcohol was discarded, the precipitate was kept in the refrigerator for 1 day. Next, lyophilization was performed at -25°C (TELSTAR – LyoQuest -85) for 48 hours for drying. Finally, QSM was obtained as a white solid in spongy form. Images from QSM preparation stages are given in Figure 4.1.

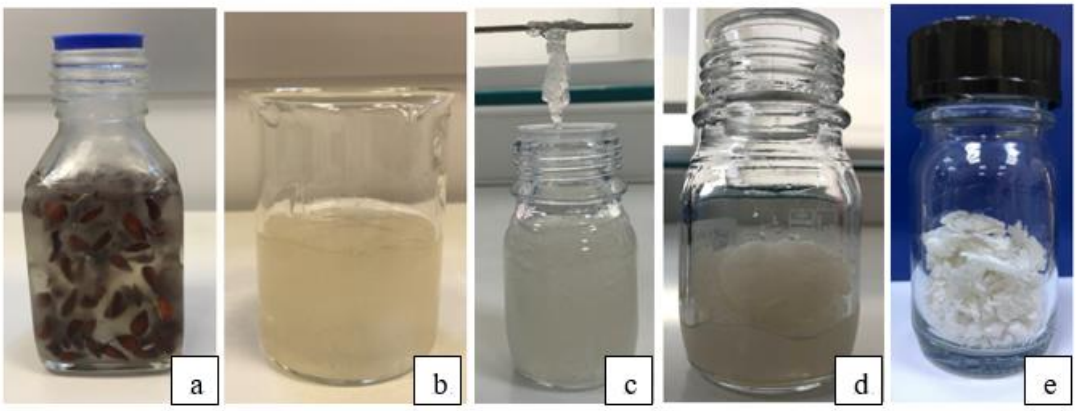


Figure 4.1 Images from quince seed mucilage preparation stages (a) soaking in water for 24 hours, (b) after filtering, (c) precipitation with ethyl alcohol (d) the precipitate before lyophilization (e) white solid spongy structure of the quince seed mucilage after lyophilization

4.2.2 Preparation of CMCht Solution

Dissolving CMCht in distilled water and stirring at room temperature overnight yielded a CMCht solution with a concentration of 5% (w/v).

4.2.3 Production of Quince Seed Containing Hydrogel Scaffolds

Samples were composed of 5% (w/v) CMCht (carboxymethyl chitosan), QSM (quince seed mucilage) solution at a concentration of 10 mg / 10 ml and PEGDE (polyethylene glycol diglycidyl ether) as the cross-linker. Samples of the determined concentrations were prepared as given in Table 4.1. In order to ensure cross-linking, samples were kept in an oven at 40°C for 24 hours. Finally, 3D porous scaffolds were obtained after lyophilization at -25°C (TELSTAR – LyoQuest -85) at for 48 hours.

Table 4.1 Nomenclature of the hydrogel scaffolds based on their PEGDE contents. (CMCht: Carboxymethyl chitosan, PEGDE: polyethylene glycol diglycidyl ether, QCM: quince seed mucilage)

Tissue scaffold nomenclature	Tissue scaffold content	PEGDE concentration (percent by weight (w/v))
A	CMCht-PEGDE	0.01
B	CMCht-PEGDE	0.03
C	CMCht-PEGDE	0.05
D	CMCht-PEGDE-QCM	0.01
E	CMCht-PEGDE-QCM	0.03
F	CMCht-PEGDE-QCM	0.05

4.3 Characterization of the Hydrogel Scaffolds

4.3.1 SEM Analysis

The scaffolds cross-section morphological studies were carried out using a SEM (JEOL JSM-6060) with a 5 kV accelerating voltage. Prior to examination, all of the scaffolds were coated with a thin layer of gold/palladium.

4.3.2 ATR-FTIR Analysis

ATR-FTIR was used to determine the chemical structures of the tissue scaffolds (Perkin Elmer Spectrum BX). Each spectrum was produced by scanning 25 times in the wavenumber range 4000-650 cm-1 at a resolution of 4 cm-1.

4.3.3 Swelling Ratio Test

The tissue scaffolds were tested for swelling in phosphate buffered saline (PBS) solution. The dried scaffolds were first weighed (w0), then submerged in PBS for 24 hours at 37°C. Scaffolds were weighted (ws) again after excess water was removed with filter paper. The following equation was used to compute the swelling ratios of the hydrogel wound dressings (4.1).

$$\text{Swelling ratio } (\%) = \left(\frac{ws - wo}{wo}\right) \text{ x } 100 \quad (4.1)$$

4.3.4 Texture Profile Analysis (TPA)

Scaffolds were submitted to a two-cycle compression test employing texture profile analysis in order to evaluate their mechanical behavior (TA XT Plus Texture Analyzer with a 5 kgf load cell). The scaffolds were squeezed twice using a probe (25 mm diameter) at a test rate of 0.5 mm/sec up to 40 percent deformation, with a diameter of 12 mm and a thickness of 6 mm. All of the trials were done in triplicate at room temperature. The obtained force-time plot was used to determine parameters like as hardness, cohesiveness, springiness, resilience, and adhesiveness.

4.3.5 Mechanical Test

A TA XT Plus Texture Analyzer with a 5 kgf load cell was used to determine the mechanical properties of the produced scaffolds. The cylindrical samples, which had a diameter of 5 mm and a thickness of 10 mm, were crushed at a continuous rate of 0.5 mm/sec until they reached an 80 percent strain. All of the experiments were carried out in triplicate at room temperature. The compressive strength was calculated using stress values at an 80 percent strain. The scaffolds' compressive moduli were calculated by slope fitting the initial linear section of stress-strain curves.

4.3.6 DTA / TGA

The thermal characteristics of the experimental and control group scaffolds were determined by DTA/TGA (Shimadzu DTG-60H). Analyses were carried out in a nitrogen environment with a 10°C/min heating rate and a temperature range of 25-800°C.

4.4 In-Vitro Biocompatibility Studies of the Scaffolds

The in-vitro biocompability tests were performed at the Department of Biomechanics in Dokuz Eylül University.

4.4.1 Cell Line and Culture Conditions

MCF-7 breast adenocarcinoma cells were used to determine cytotoxicity of scaffolds with and without quince extract. The cell line was obtained from Dokuz Eylul University, Department of Molecular Medicine at passage 26. They were cultured in a complete RPMI-1640 medium which was supplemented with 10% (v/v) fetalbovine serum (FBS, Cegrogen Biotech GmbH, Germany), 1% (v/v) L-glutamineand 1% (v/v) penicillin/streptomycin (Thermo Fisher Scientific, USA) in T-75 tissue culture flasks with filter cap (SPL Life Sciences Inc., Korea). Cells were incubated in a humidifed atmosphere at 37^0C in a 5% (v/v) CO_2 in a Heraeusincubator (Heracell, Germany).

4.4.2 Passaging of Cells and Cell Seeding of Scaffolds

Adherent cells were washed with PBS and detached with Trypsin-EDTA solution (0.25%, sterile-filtered solution, Sigma-Aldrich, U.S.A) for 3–5 min incubation at 37ºC and used observed by using an Inverted microscope (Olympus, CKX41SF, Tokyo, Japan). The cell suspension was centrifuged (Hettich, Universal 32, Germany) at 1200 rpm for 5 minutes. The cells were then resuspended in a culture medium. The cells were counted by using hemocytometer and trypan blue solution before seeding on scaffolds for further analysis. The cell suspension sample was taken by a micro pipette and diluted with PBS (1:10) before cell counting. The percentage viability of cells was calculated by a formula from (Crowley L.C, Marfell B.J, Christensen M.E, & Waterhouse N.J. 2016)

$$\% \text{ Viability } = \frac{Number\ of\ colorless\ cells}{Total\ number\ of\ cells} \times 100 \qquad (4.2)$$

The scaffolds were cut by using a special cutter device and sterilized by UV light

on both sides as it was mentioned by (Sharifi et al., 2018). $1x10^4$ cells per scaffold was seeded on each with a culture medium and they were waited to adhere. The medium was changed every other day and incubated at 37°C in a humidifed atmosphere containing 5% CO_2.

4.4.3 Cell Viability Analysis

The scaffolds were cultured for 5 days and the viability was analyzed by using the WST-1 colorimetric assay (Cayman Chemical, USA) on days 1, 3 and 5 in three replicates according to the kit instructions at 450 nm in a Synergy HTX multi-mode reader (Bio-Tek, USA). The WST-1 mixture was prepared by mixing Electron Mediator Solution and WST-1 Developer Reagent Solution equally, immediately before the experiment. The culture medium was changed with the WST-1 mixture supplemented media and waited for 2 hours at 37°C in a CO_2 incubator. The formazan activity was determined by using the colour change in culture media by using a spectrophotometer device at 450 nm.

CHAPTER FIVE
EXPERIMENTAL RESULTS AND DISCUSSIONS

It is very important to check and investigate the physical, chemical, mechanical and biocompatibility properties of materials prepared for use in biological applications. The structure produced as a tissue scaffold must have appropriate three-dimensional properties and be able to mimic the extracellular matrix of the target tissue. A functional tissue scaffold should have sufficient porosity. Pore sizes should be large enough for cells, nutrients, and biosignal molecules to be transported and provide space for tissue growth. The tissue scaffold must be manufactured to be resistant to the loads to be exposed during implantation and in-vivo. In addition, it should have biodegradable properties in accordance with the development process of new tissue.

Within the scope of this thesis, hydrogel scaffolds were produced from quince seed mucilage and O-carboxymethyl chitosan, both of which have been proven to be biocompatible, to be used in the treatment of skin tissue damage. Although both materials have previously taken part in tissue engineering studies, the hydrogel structure consisting of these two materials was produced for the first time and its properties were investigated.

5.1 Preparation of the Hydrogel Scaffolds

Hydrogel scaffolds were prepared by using quince seed mucilage (QSM) and O-carboxy methyl chitosan (CMCht) polymer and hydrogel properties were acquired by cross-linking CMCht by PEGDE as the crosslinker. Three dimensional hydrogel scaffolds were obtained after freeze-drying. The names of the tissue scaffolds based on their contents and PEGDE concentrations are given in Table 4.1. The images of the produced A, B, C (control group) and quince seed mucilage containing D, E, F (experimental group) hydrogel scaffolds are shown in Figure 5.1. It is observed that polymerization shrinkage is more pronounced in the control group and therefore the samples including QSM have more dimensional stability. When the gelation time was examined it was observed that A had the slowest gelation rate as expected due to its lowest PEGDE concentration. As well as it had a gel time longer than D meaning that

QSM had a positive effect on gelation.

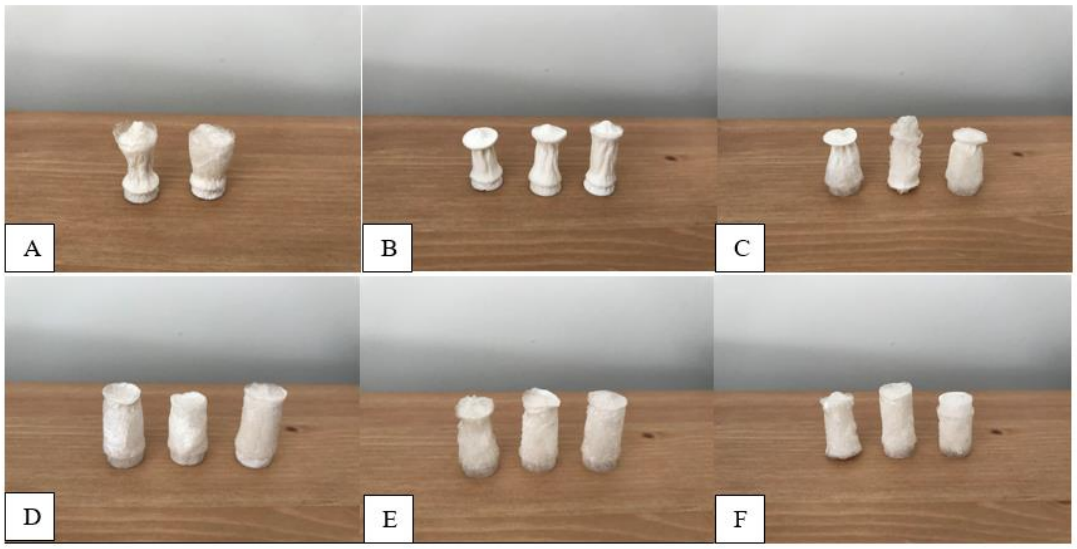


Figure 5.1 Images of the freeze-dried hydrogel scaffolds (Personal archive, 2019)

5.2 SEM Analysis

Surface properties are of great importance for polymeric materials to be used in the biomedical field. The first reactions after implantation of any material used in this area will occur on the surface. Scanning electron microscopy (SEM) was used to determine the surface morphologies of the fabricated scaffolds.

Pore size and structure are important in tissue scaffolds for cell attachment and growth. While the desired pore size may vary depending on the application area and target cell type, the pore structure must be open-pored. The cross-sectional morphologies of the B and QSM containing E hydrogel scaffolds were examined by SEM. The SEM images at 50x and 100x magnification were given in Figure 5.2. It is seen that the hydrogel scaffolds have an open-porous structure.

The most important factor is to ensure the integration of quince seed mucilage, which is used as an additive in the hydrogel scaffolds, with the carboxy methyl chitosan matrix. When the SEM images of the tissue scaffold produced at 0.03 (v: v) PEGDE concentrations were examined, it was observed that the quince seed mucilage

was well integrated in the carboxy methyl chitosan structure.

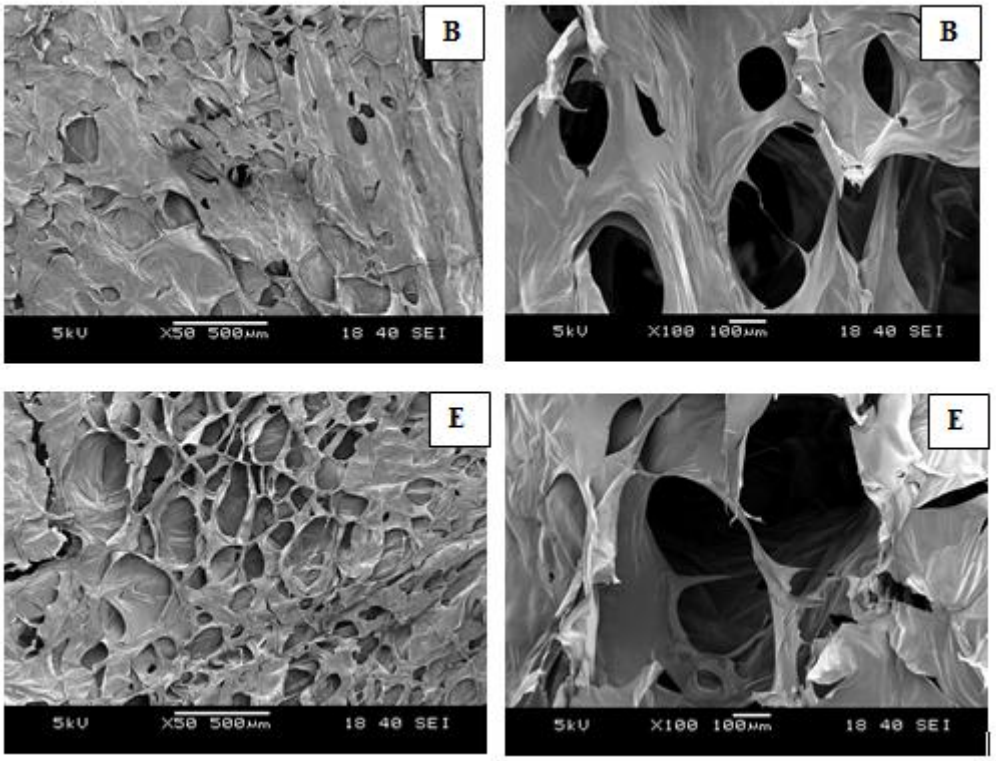


Figure 5.2 Cross-sectional SEM images of the B and E hydrogel scaffolds cross-linked with 0.03 (w/v) of PEGDE

5.3 ATR-FTIR Analysis

The FTIR spectra of CMCht and PEGDE are given in Figure 5.3. In the FTIR spectrum of CMCht, O–H and N–H stretching vibrations appeared at 3400–3200 cm^{-1} and C–H stretching vibration was at around 2900 cm^{-1}. The asymmetric and symmetric stretching vibrations of the carboxy group were at 1587 and 1410 cm^{-1}, respectively. Also, the asymmetric vibration of the carboxy group overlapped with the N-H bending vibration of the amine group. Finally, the strong absorption band of secondary hydroxyl group appeared at 1053 cm^{-1}. In the FTIR spectrum of PEGDE, C-H stretching, C-H bending and C-O stretching bands were at 2868 cm^{-1}, 1456 cm^{-1} and 1096 cm^{-1}, respectively. The bands at 911 cm^{-1} and 855 cm^{-1} were due to the epoxy group vibration (Colpankan Gunes, O. (2019). Production Of Antibacterial, Biodegradable And Biocompatible Materials For Tissue Engineering Applications.

Phd Thesis, Dokuz Eylül University, İzmir).

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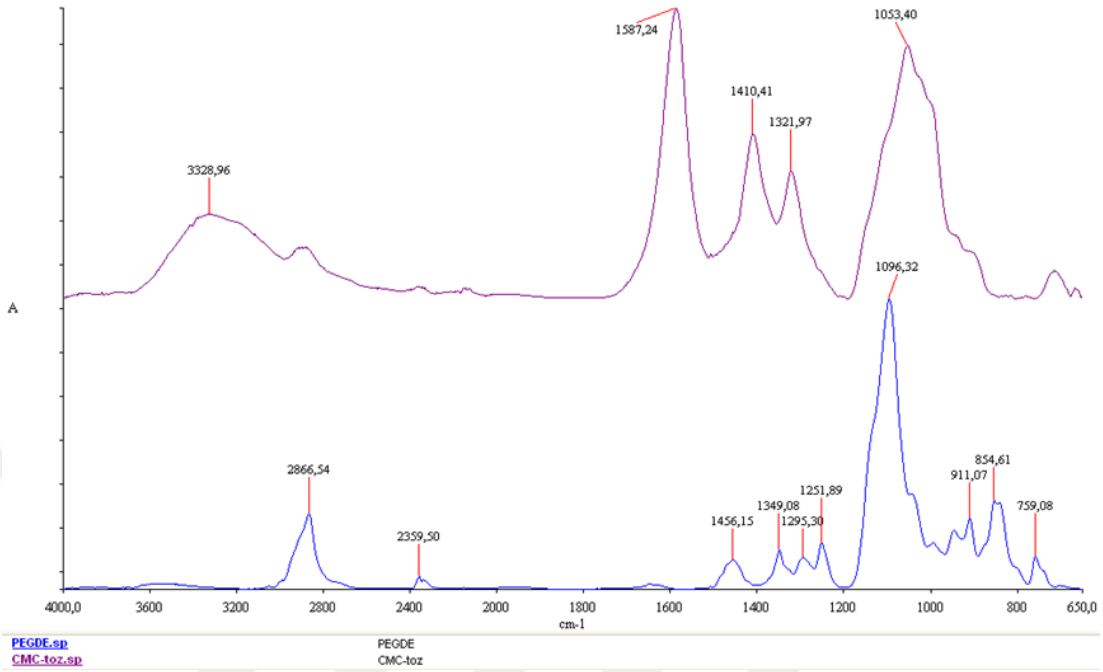


Figure 5.3 FTIR spectra of CMCht and PEGDE

The cross-linking behaviour of the hydrogel scaffolds as well as the presence of QSM in the hydrogel structure were evaluated by comparing the FTIR spectra of QSM, cross-linked B and E scaffolds, and uncross-linked A and D scaffolds. Figure 5.4 shows the FTIR spectra of the uncross-linked A and D samples, as well as QSM. The broad peak at around 3400–3200 cm^{-1} in the FTIR spectra of uncross-linked A and D referred to both O–H and N–H stretching vibrations. The stretching vibration of the C–H group was at 2876 cm^{-1}. The asymmetric and symmetric stretching vibrations of the carboxy group were ascribed to the peaks at 1586 and 1410 cm^{-1}, respectively. Furthermore, the secondary hydroxyl group's absorption peak was found at 1056 cm^{-1}. (Chenetal., 2017; Chenetal., 2015; Kumar Singh Yadav & Shivakumar, 2012). However, any peak that belongs to the quince mucilage cannot be detected due to low concentration of QCM in the hydrogel composition.

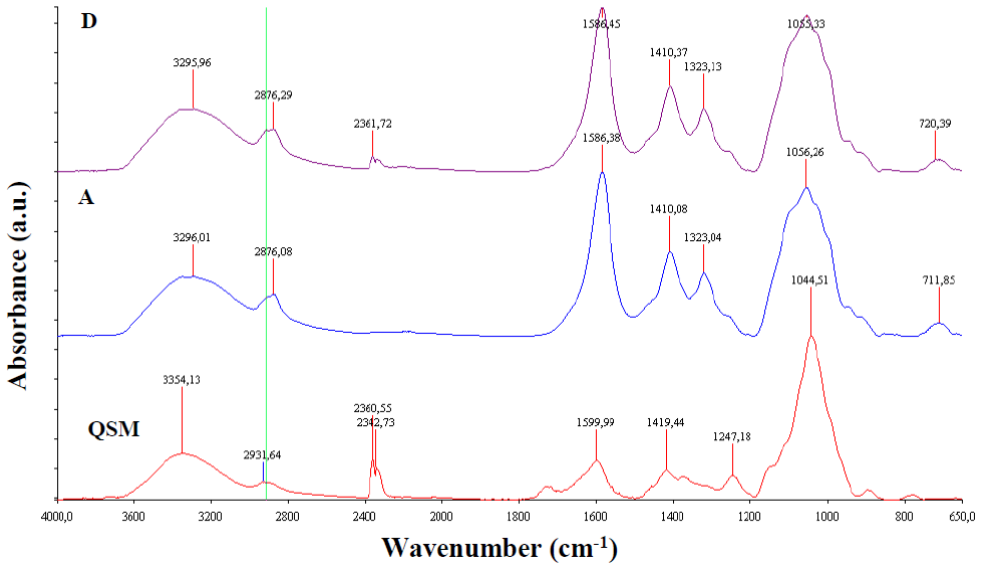


Figure 5.4 FTIR spectra of uncross-linked A, uncross-linked D tissue scaffolds and QSM

FTIR spectrum of cross-linked B and E samples showed the broad absorption band of O-H and N-H groups at around 3350 cm^{-1} and C-O stretching vibrations at about 1090 cm^{-1} (Figure 5.5 and Figure 5.6). Also, C–H stretching and N-H bending vibrations appeared at 2871 cm^{-1} and 1592 cm^{-1}, respectively. The ring-opening interaction of the epoxy groups in PEGDE with the amine and hydroxyl groups in CMCht caused changes in the wavenumbers of the N-H and C-O absorption bands compared to the uncrosslinked samples (Achilias, Karabela, Varkopoulou, & Sideridou, 2012; Badr, Amer, & Shehat, 2010; Fouchal, Knight, & Dickens, 2004; González, Cabanelas, & Baselga, 2012). The crosslinking mechanism between CMCht and PEGDE is given schematically in Figure 5.7. The crosslinking reaction resulted in hydrogel formation containing CMCht and PEG blocks linked together with amine and ether linkages (Gamiz Gonzalez, Edlund, Vidaurre, & Gomez Ribelles, 2017).

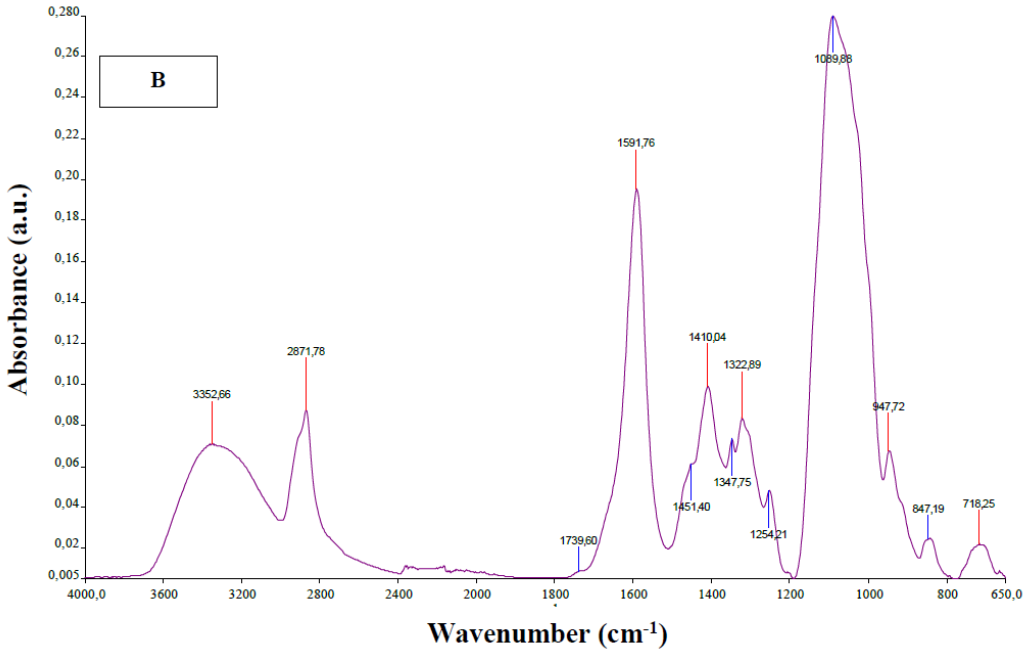


Figure 5.5 FTIR spectra of the hydrogel scaffold B

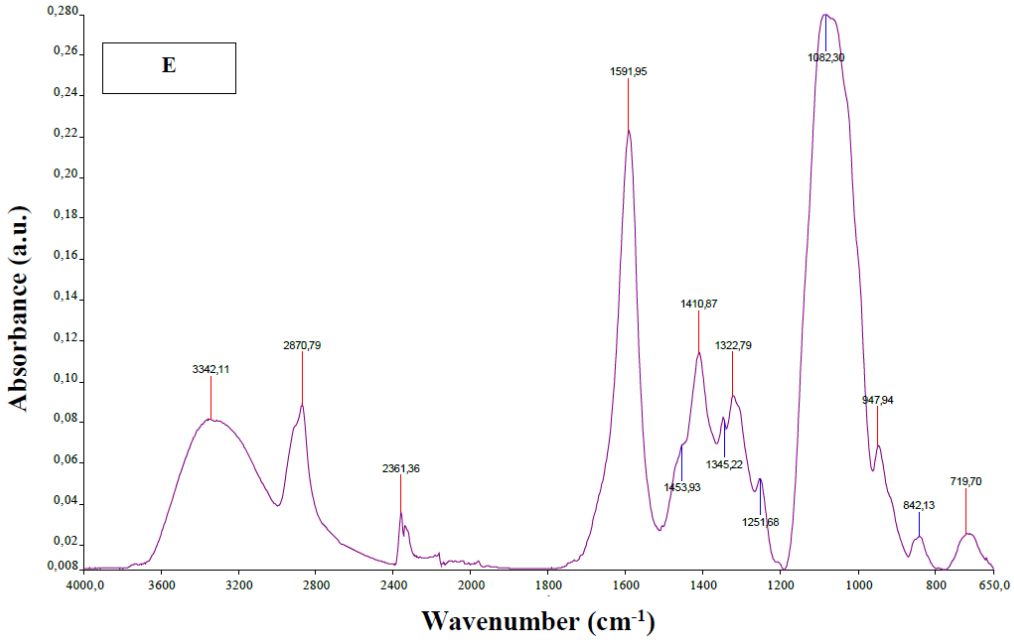


Figure 5.6 FTIR spectra of the hydrogel scaffold E

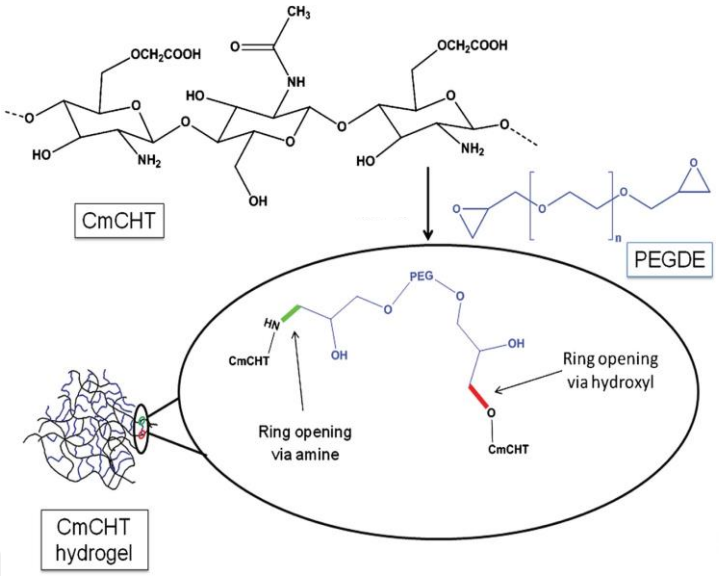


Figure 5.7 Schematic representation of the hydrogel formation from CMCht and PEGDE (Gamiz Gonzalez, Edlund, Vidaurre, & Gomez Ribelles, 2017).

5.4 Swelling Behavior

The scaffolds swelling behavior was studied in a PBS solution at 37°C for 24 hours. After incubation in PBS, lightly-crosslinked hydrophillic CMCht polymer chains, underwent swelling. Meanwhile, dissolution of the polymer chains was restricted by the cross-linking sites. The swelling ratios of the hydrogel scaffolds were calculated according to Equation 4.1., and they are given in Figure 5.6 and Table 5.1. For all the samples, as the concentration of PEGDE used as the crosslinker increased, a decrease in the swelling ratios was observed, as expected. Besides, QSM addition improved the swelling potential of the hydrogel scaffolds compared to the control group.

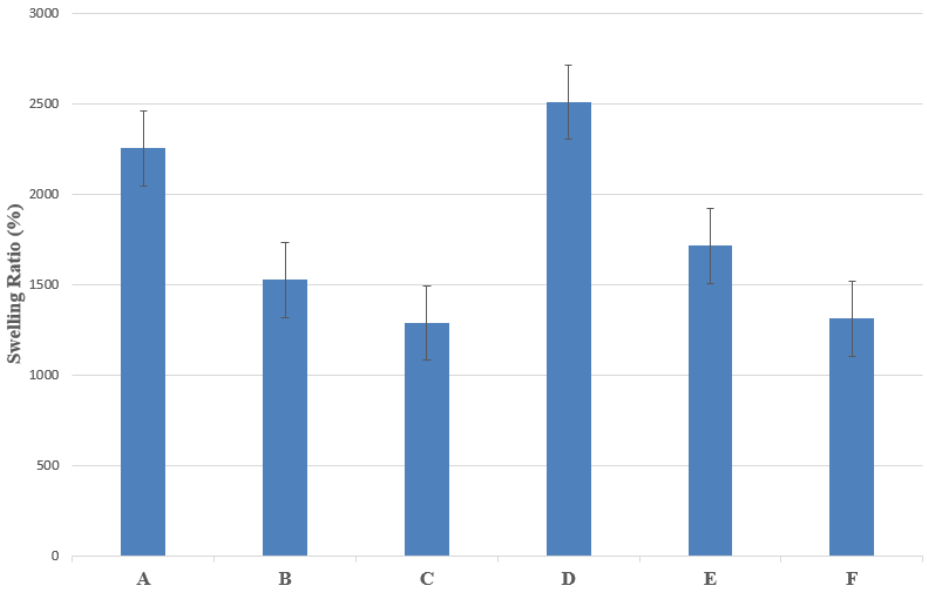


Figure 5.8 Swelling rates of tissue scaffolds

Table 5.1 Swelling ratio values of the tissue scaffolds

Tissue scaffold nomenclature	**Swelling ratio (%)**
A	2256,58 ± 164
B	1527,42 ± 191
C	1288,6 ± 74
D	2510,9 ± 179
E	1715,5 ± 205
F	1312,93 ± 232

5.5 Texture Profile Analysis

Jones, Woolfson, and Djokic (1996) presented texture profile analysis (TPA) as an acceptable tool for characterization of semisolid pharmacological dosage forms. TPA has long been used to characterize solid and semisolid foods, but it has lately gained popularity in the characterisation of pharmaceutical and polymeric systems (Ferreira, Calvinho, Cabrita, Schacht, & Gil, 2006). TPA can be used to determine a variety of mechanical properties including as hardness, cohesiveness, springiness, adhesiveness, and compressibility (Hurler, Engesland, Poorahmary Kermany, & Škalko Basnet,

2012). Force-time graphs of the produced hydrogel scaffolds are shown in Figure 5.7. From these graphs in order to obtain the mechanical parameters some points (numbered from 1 to 6), areas between these points (A1-2, A2-3, A1-3, A4-6), and time intervals (T1-2, T4-5) were determined first and as an example they were illustrated in the force-time graph of the hydrogel scaffold B (Figure 5.8).

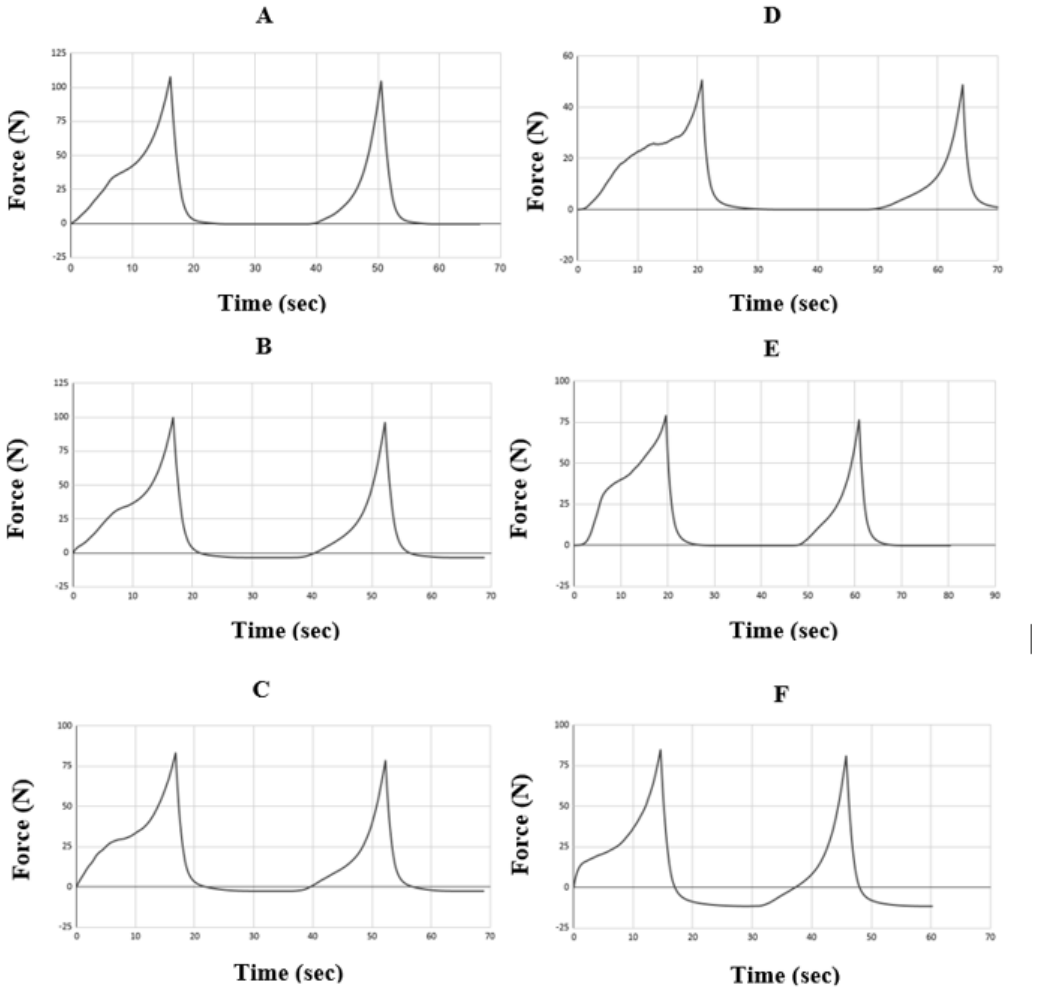


Figure 5.9 Force-time graphs of the hydrogel scaffolds

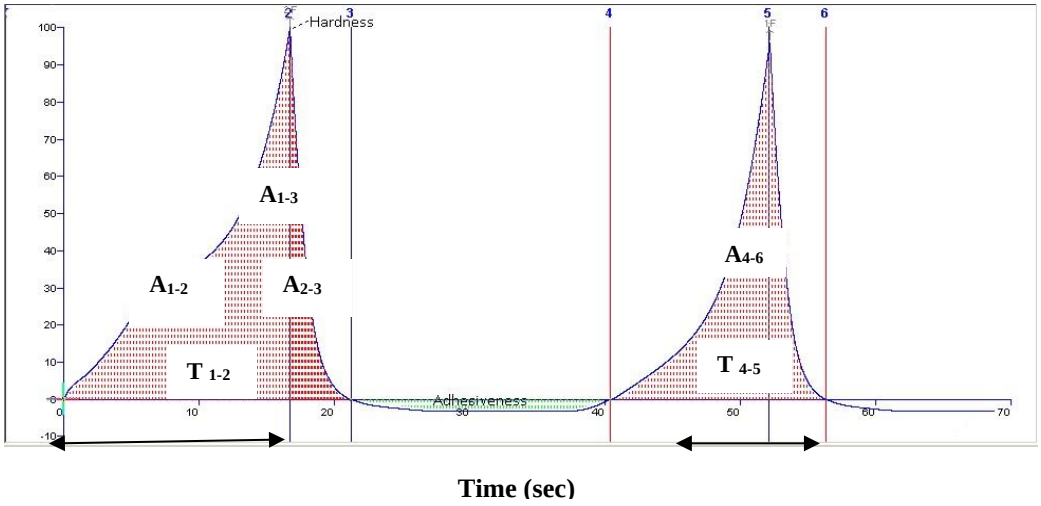


Figure 5.10 Labelled force-time graph of the hydrogel scaffold B

The maximum force of the first compression of the scaffolds was specified as hardness value, which was given as the force required for a pre-determined deformation of 40% (Figure 5.8). Cohesiveness is a measure of how effectively a sample resists a second deformation in comparison to how it responds to the first. It was calculated as (A4-6/A1-3), the ratio of the energies sprawled in the first and second compression. Springiness, also known as elasticity, refers to a sample's ability to physically spring back after being deformed; it was computed as (T4-5/T1-2). Instant springiness, also known as resilience, is a measurement of how well a sample "fights to regain its former height." The ratio of the initial compression's upstroke and downstroke energy (A2-3/A1-2) was used to calculate it (Ferreira, Calvinho, Cabrita, Schacht, & Gil, 2006; Hurler, Engesland, Poorahmary Kermany, & Škalko Basnet, 2012; Król, Malik, Marycz, & Jarmoluk, 2016; Rosenthal, 2010). Adhesiveness is measured as the negative work between the two cycles, In other words, adhesiveness is the work needed to overcome the attractive force between the sample and the probe. (Friedman, Whitney & Szczesniak, 1963).

The mechanical properties of the TPA-produced hydrogel scaffolds are listed in Table 5.2. At lower cross-linker concentrations, the hardness of the samples reduced with QSM addition; however, QSM addition had no effect on hardness at the highest cross-linker concentration. It was also observed that the hardness values of the control

group decreased, whereas experimental group increased with increasing PEGDE concentration. It might be suggested that PEGDE units might disturb the strong secondary interactions among the CMCht chains leading to a decrease in hardness, on the other hand, QSM by interacting with the polar groups of CMCht might also be effective in the decrement of the strength of CMChts intermolecular interactions at lower PEGDE concentrations. At constant QSM concentration as the cross-linker concentration increased, hardness values of the samples increased as expected. On the other hand, all samples had a shoulder in the first cycle meaning that scaffolds had some permanent damages during the first compression which lead to a smaller area for the second cycle. Average cohesiveness values decreased slightly upon addition of QSM. On the other hand, the springiness values were congener to each other except sample F which showed the highest adhesiveness. Finally, there was no significant difference between the resilience values of the samples.

Table 5.2 TPA parameters for tissue scaffolds

Scaffold	**Hardness (N)**	**Cohesiveness**	**Springiness**	**Resilience**	**Adhesiveness**
A	107.52±0.03	0.54±0.02	0.69±0.01	0.20±0.06	-4.61±0.03
B	99.78±0.12	0.57±0.09	0.70±0.03	0.19±0.11	-52.68±0.07
C	83.13±0.06	0.53±0.02	0.76±0.05	0.15±0.09	-34.21±0.02
D	50.57±0.15	0.45±0.07	0.77±0.02	0.12±0.04	-0.10±0.04
E	78.97±0.17	0.48±0.03	0.70±0.02	0.12±0.04	-1.36±0.06
F	83.00±0.09	0.49±0.05	0.58±0.02	0.15±0.04	-175.73±0.09

5.6 Mechanical Properties

Compression testing was used to examine the mechanical properties of the scaffolds, which involved compressing the scaffolds at a continuous deformation rate of 0.5 mm/sec up to an 80 percent strain. The compressive strength and computed compressive modulus values of the scaffolds are shown in Figures 5.9 and 5.10, respectively. The scaffolds containing QSM (D and E) had slightly lower strength values compared to the scaffolds without QSM (A and B), whereas sample C turned out to be too brittle because of the highest cross-link density. Furthermore, increasing the cross-linker concentration in the hydrogels marginally enhanced compressive modulus, as expected, but there was no significant variation in compressive strength values.

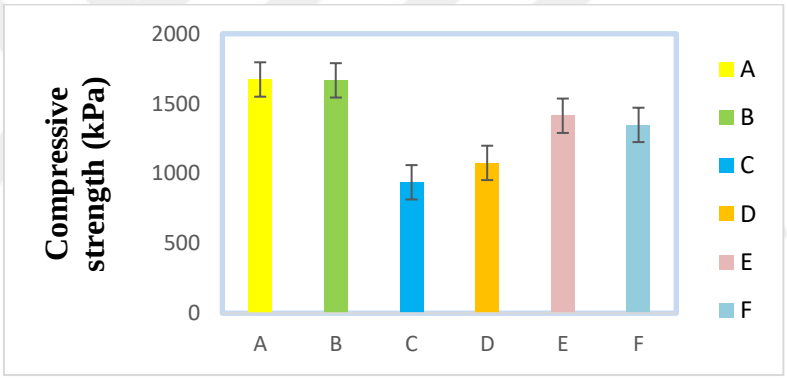


Figure 5.11 Compressive strength values of the prepared hydrogel scaffolds

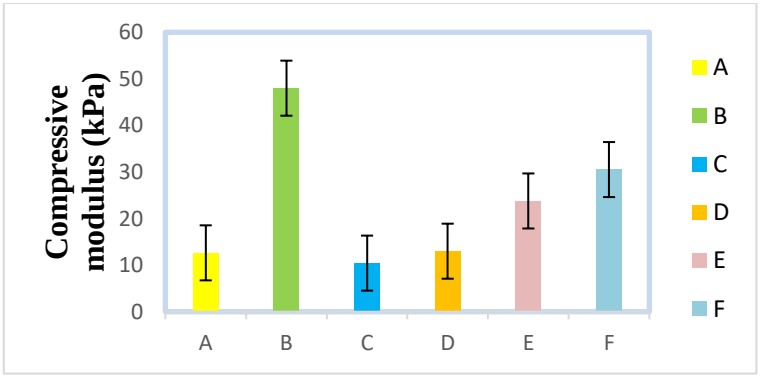


Figure 5.12 Compressive modulus values of the prepared hydrogel scaffolds

5.7 DTA / TGA

TGA-DTA analyses were performed to investigate the thermal behavior of the hydrogel scaffolds. Figures 5.11 and 5.12 show the DTA - TGA curves of the hydrogel scaffolds B and E, respectively.

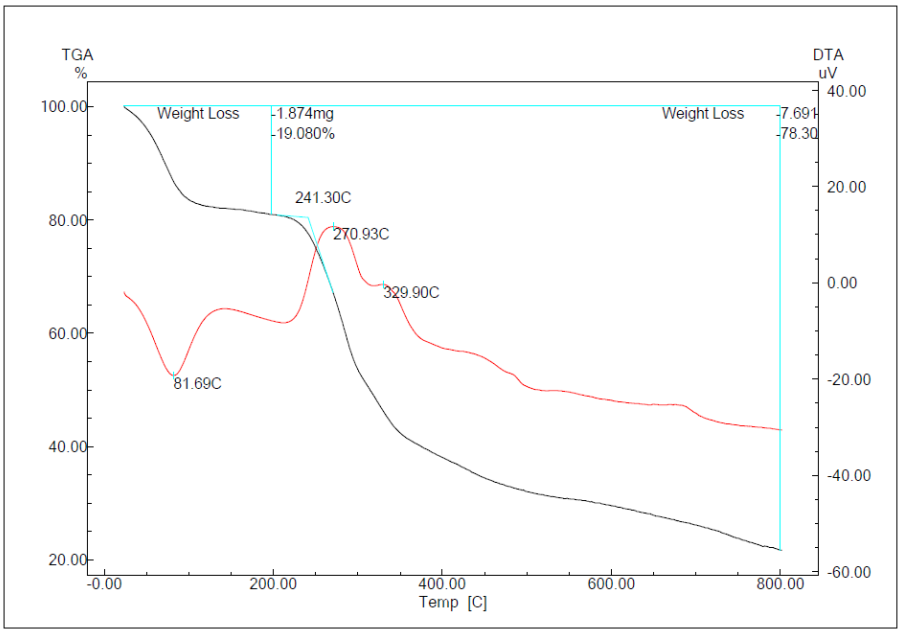


Figure 5.13 DTA - TGA curves of the hydrogel scaffold B

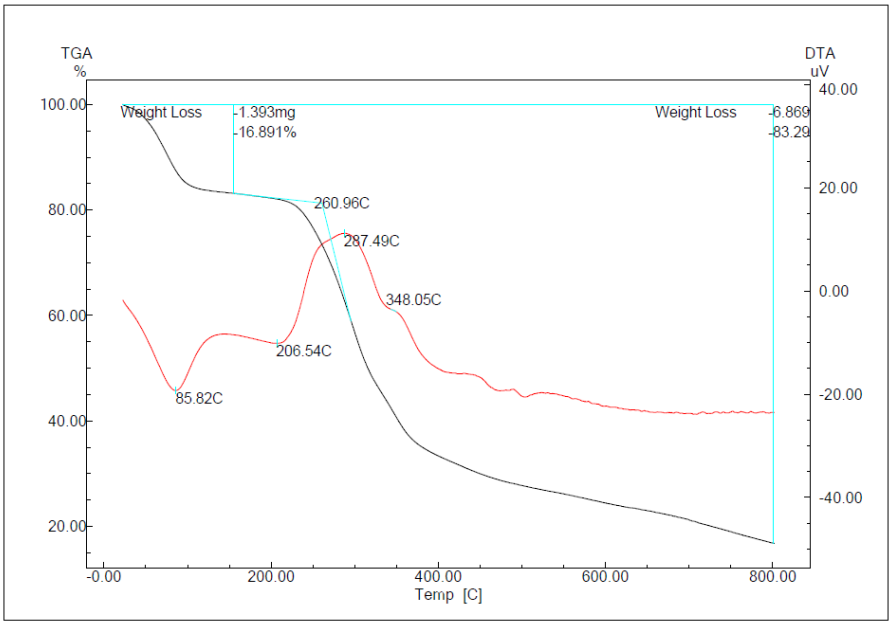


Figure 5.14 DTA - TGA curves of the hydrogel scaffold E

When the TGA curve of the hydrogel scaffold B without QSM was examined, it was first seen that the adsorbed water, corresponding to about 19% weight loss, disappeared from the structure. Onset decomposition temperature of the hydrogel scaffold B was 241ºC (T_{onset}). After this temperature, decomposition of the organic bonds in the hydrogel started with the weight loss of 59 % till 800ºC. From the DTA curve it was determined that the first decomposition stage was endothermic and the second stage consisting of two different steps was exothermic (Figure 5.11). When the TGA curve of the hydrogel scaffold E with QSM was examined, adsorbed water, corresponding to 16% weight loss, disappeared from the structure as the first stage. Next, deterioration of the organic structures with the weight loss of 66 % took place after the onset decomposition temperature of the hydrogel scaffold E which was 260ºC (T_{onset}). DTA curve showed that the first decomposition stage was endothermic and the second stage having two different steps was exothermic (Figure 5.12) The primary degradation steps of CMCht and PEGDE were found to occur at temperatures of 210-310°C and 325-425°C, respectively, in the literature. (Gámiz González, Edlund, Vidaurre, & Gomez Ribelles, 2017). So, the former step of the second stage should be due to the decomposition of CMCht and the latter step should be due to the decomposition of PEGDE. It was also determined that the QSM addition increased the thermal stability of the hydrogel scaffolds by increasing the onset decomposition temperature from 241ºC to 260ºC.

5.8 In-vitro Cellular Activities

The viability of MCF-7 breast adenocarcinoma cells on the scaffolds E and B (with and without quince extract) was evaluated by WST-1 colorimetric assay. Figure 5.13 shows cell viabilities at 1, 3, and 5 days of cultivation. The proliferation rate showed a regular increase in both groups. Moreover, the scaffolds with quince seed extract showed a better proliferation rate as there was a statistically significant difference among days 1-3 ($p=0.0476$) and days 1-5 ($p=0.0015$). However, there was only a significant difference among days 1-5 ($p=0.0021$) for scaffolds without quince seed extract. It can be concluded that, quince seed extract has a positive effect on cell proliferation rate. Also, scaffolds should be tested with other cells like mesenchymal

stem cells, healthy fibroblasts, etc. for a detailed research. However, both scaffolds are non-toxic and biocompatible.

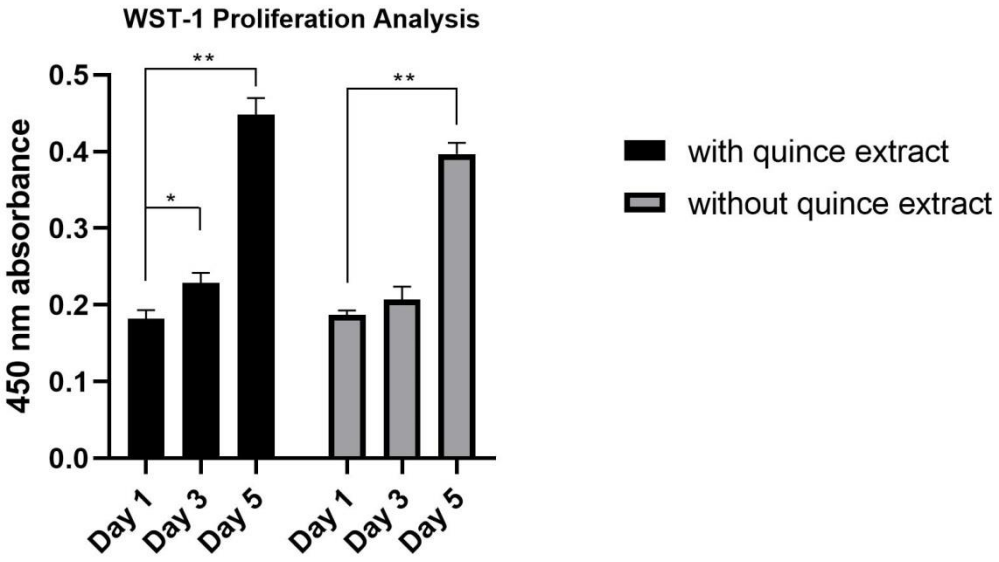


Figure 5.15 WST-1 Proliferation test results of the MCF-7 cells on scaffolds. (*p<0.1, **p<0.01)

CHAPTER SIX
GENERAL EVALUATION AND CONCLUSION

In this study, tissue scaffolds were produced from quince seed mucilage (QSM) and carboxy methyl chitosan (CMCht) polymer, which have characteristic features such as bioactivity and biocompatibility. Although both materials have taken part in tissue engineering studies, the hydrogel scaffolds designed from these two materials were produced for the first time and their properties were examined.

Poly (ethylene glycol) diglycidyl ether (PEGDE) was used as a crosslinker to provide a lightly coss-linked hydrogel structure and it was aimed to benefit from the natural gelation behaviour of quince seed mucilage. The gelation time of the hydrogel scaffolds produced in the study was optimized as 24 hours.

With the freeze-drying process used as the production method, three-dimensional hydrogel scaffolds with an open pore structure were obtained.

According to the swelling test results, QSM addition increased the swelling ratio values of the hydrogel scaffolds compared to the control group. Also, as the concentration of PEGDE increased, a decrease was observed in the swelling ratios of the scaffolds.

Mechanical test results indicated that QSM decreased the compressive strength, hardness and resilience values of the hydrogel scaffolds at lower cross-linker concentrations, but at the highest cross-linker concentration, the hardness, cohesiveness and resilience values remained nearly the same with that of the control group.

It was determined that the degradation of hydrogel scaffolds consisted of two main stages. While the removal of the adsorbed water is observed in the first stage, the degradation of organic groups in the structure is observed in the second stage. When the onset decomposition temperature of the control group and the onset decomposition

temperature of the experimental group were compared, it was observed that the QSM addition improved the thermal stability of the hydrogel scaffolds.

According to the WST-1 cell proliferation test results, MCF-7 cells showed a better proliferation rate on the hydrogel scaffolds with QSM extract compared to the contro group. It can be concluded that, QSM extract has a positive effect on MCF-7 cell proliferation rate.

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