Preparation of collagen based composite materials with synthetic polymers for potential wound dressing applications

Potansiyel yara örtü uygulamaları için kollajen esaslı kompozit malzemelerin sentetik polimeler ile hazıranması

Research Article

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ABSTRACT

Collagen is very important part of the fibrous proteins in the living organism. Animal tissues, especially tendons main structure contain collagen. In this investigation, natural collagen (coll) modified with different synthetic monomers such as 2-hydroxy ethylmethacrylate (HEMA) and acrylamide (AAm) was prepared as p(Coll-co-HEMA) and p(Coll-co-AAm) composites to enhance collagen natural characteristics. Biocompatibility of the prepared interpenetrating polymeric network (IPN) was tested with MTT Assay and found biocompatible. Model drugs, such as Trimetoprim (TMP) and Naproxene (NP) were used as antibacterial active agents for release studies from the synthesized composite hydrogel-collagen IPN matrices. The drug-loaded IPN films release studies were carried out in bio-mimetic media. It was found that collagen-synthetic polymer matrices can be readily prepared and used for drug delivery system in the release of active agents, providing great potential in wound dressing applications.

Key Words

Collagen, collagen-hydrogel IPN composites, drug delivery devices, biocompatibility.

ÖZET

Kollajen yaşayan organizmada fibril proteinlerin önemli bir kısmıdır. Hayvan dokularında özellikle tendonların ana yapısı kollajen içerir. Bu çalışmada doğal kollajen (coll) 2-hidroksil etilmetakrilat (HEMA) ve akrilamid (AAm) gibi değişik sentetik monomerler ile modifiye edilerek p(Coll-ko-HEMA) ve p(Coll-ko-AAm) şekilde kollajenin doğal karakteristiklerini artırmak için kompozit olarak hazırlanmışlardır. Hazırlanan iç içe geçmiş ağ yapıların (IPN) biyouyumluluk testleri MTT assay ile yapılmış ve biyouyumlu bulunmuştur. Trimetoprim (TMP) and Naproksen (NP) gibi antibakterial aktif ajanlar model ilaç olarak kullanılmış ve sentezlenen kompozit-hidrojel-kollajen IPN matrikslerinden salım çalışmaları yapılmıştır. İlaç yüklenen IPN-filmleri salım çalışmaları biyomimetik ortamda yapılmıştır. Kollejen-sentetik polimer matrikslerinin kolayca hazırlanabildiği ve aktif ajan salımı için ilaç salım sistemi olarak kullanılabilirliği sonucu çıkarılarak yara örtü uygulamalarınnda büyük potansiyele sahip olduğu bulunmuştur.

Anahtar Kelimeler

Kollajen, kolajen-hidrojel IPN kompozit, ilaç salım cihazları, biyouyumluluk.

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INTRODUCTION

Collagen as the most abundant fibrous natural protein is mainly employed in the production of leather [1,2]. Due to its natural characteristic as biopolymer such as hydrophilicity, biocompatibility, non-antigenic and mechanical durability, collagen has been widely investigated for novel material design in biomedical applications as nanofiber, minipelet, fibriler multilayer films [3-12]. As unmodified collagen can not be directly exploited due to some down-sides such as calcium deposition, and high thrombogenicity, it requires chemical modification, structural alteration and introduction of new functionalities to render desired properties while eliminating undesirable possessions [13]. Synthetic polymeric hydrogels are soft and flexible materials and are constructed from hydrophilic functional group containing monomer or polymers [14]. Even though hydrogels are versatile and mostly biocompatible, they sometimes suffer from weak mechanical characteristics. The natural collagen on the other hand, have resourceful characteristic with triple helical structure and contains various functional groups [15]. Therefore, in this investigation, we prepared collagen containing hydrogel composites interpenetrating polymeric network as composite films using various hydrophilic monomers.

The interpenetrating polymer network hydrogels (IPN) were prepared as films in controlled thickness by casting monomer/polymer-collagen mixture prior to chemical crosslinking. To analyze the collagen based composites biocompatibility, MTT test was used [16]. The IPN matrices were loaded with various drugs by placing them into concentrated aqueous corresponding drug solutions for 24 h. The model drugs such as Trimetoprim (TMP) and Naproxen (NP) were used as antibacterial active agents for the release studies from the synthesized composite hydrogel-collagen matrices. The drug loaded IPN films were placed in various bio-mimetic media for release studies. It was demonstrated that these types of collagen based hydrogel IPN films can have potential applications in biomedical field e.g., they can be used for wound dressing materials for open wounds and can be used as patch to treat foot fungi and so on.

Materials and Methods

Collagens from calf skin (water soluble) and from Bovine Achilles tendon (non water soluble) were obtained from Sigma-Aldrich. Acrylamide (AAm) (99% Sigma-Aldrich) and 2-hydroxyl ethyl methacrylate (HEMA) as monomers, N, N methylenebisacrylamide (MBA) (99%, Sigma) and ethylene glycol dimethacrylate (EGDMA) as crosslinkers, ammoniumpersulfate (APS) (98%, Aldrich) as an initiator, and N, N, N′, N′-tetramethylethylenediamine (TEMED) (Across) as an accelerator were used in the hydrogel IPN preparation. HCl, HNO_3 and NaOH were used also products of Sigma-Aldrich. All the reagents were of analytical grade or highest purity available, and used without further purification. The DI water was 18.2 MΩ.cm (Millipore Direct-Q3 UV) and was used throughout the absorption experiments. The active agents, Trimethoprim and Naproxen were obtained from a local vender.

Hydrogel Preparation

The non-soluble collagen was degraded in the mixture of three volume of concentrated HCl and one volume of concentrated HNO_3 (aqua regia). Upon disintegration of the collagen in acidic medium, the solution was neutralized with concentrated NH_3 and their aqueous solution were mixed with the synthetic monomer such as 2-hydroxyl ethyl methacrylate (HEMA), Acrylamide (AAm). The crosslinking agent ethylene MBA was employed also to generate three dimensional hydrogel composite films. Ammonium persulfate (APS) was used as redoks initiator. Composite materials based on collagen with various monomers were prepared in aqueous solution of their appropriate mixture with 0.05-20 mole percent of crosslinkers via redox polymerization techniques. The amount of used collaged was used in this investigations was approximately 100 mg per gram synthetic materials. The precursor solutions were placed

on petri dishes or between two glass slides before polymerization and crosslinking reactions. The prepared composites films in desired thickness were placed in distilled water to remove impurities for 24 h and replacing waste water for every 8 h.

MTT assay of IPN Hydrogel Composite Materials and drug release studies

Lymphocytes isolated from whole blood were used in the study FicollPaque. Each culture (RPMI 1640, serum, penicillin, streptomycin) 2 ml of 5 x 10^5 cell/ml lymphocytes were prepared and used. Release layers left in distilled water and sterilized after 24 hours were added to the culture medium. Two separate cultures of each IPN films were prepared. At the end of this period, designated as 24 hours treatment time, to determine the cytotoxicity MTT assay method was used. At the end of this treatment time, 200 μl cell suspension was added to each well followed by addition of 20 MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide). At 37°C under 5% CO_2 for 4 h in an incubator, 100 μl DMSO/ethanol (v/v) mixture was added and 1 h later absorption measurements were done at 330 nm. By using the absorption values obtained for control and hydrogel film layer treated cell, the cell inhibition percent of hydrogel IPN composites were determined.

The cleaned composite hydrogel films were dried in oven at 50°C to a constant weight and placed in concentrated drug solutions in distilled water, ethanol or methanol. The drug release studies were performed in phosphate buffer saline (pH 7.4). And the release amounts of drug were determined from their corresponding calibration curves constructed at their maximum absorption wavelengths (TMP: 280 nm and NP: 330 nm).

Results

The introduction of collagen into hydrogel matrices were expected to increase the mechanical durability and the biocompatibility. As illustrated in Figure 1, the disintegrated collagen pieces can be entrapped within the 3-D structure of p(HEMA) and p(AAM) matrices.

The potential use of these collagen containing IPN hydrogels films can be wound dressing materials as they have sufficient water content due to the hydrophilic nature of the hydrogel as base materials. Here, trimetetoprim was chosen that is a bacteriostatic antibiotic and mainly used to treat infections with bacteria. Naproxen is a nonsteroidal anti-inflammatory drug commonly used for the reduction of pain, fewer, inflammation and stiffness. These drugs were used for the release studies from the prepared collagen containing p(HEMA) and p(AAm) based hydrogels. Collagen containing (IPN) hydrogel films loaded with various drugs were placed in phosphate buffer solutions (PBS) at pH 7.4 for release studies. The released amounts of drug was determined from a calibration curve constructed with UV-Vis spectrophotometer (TMP

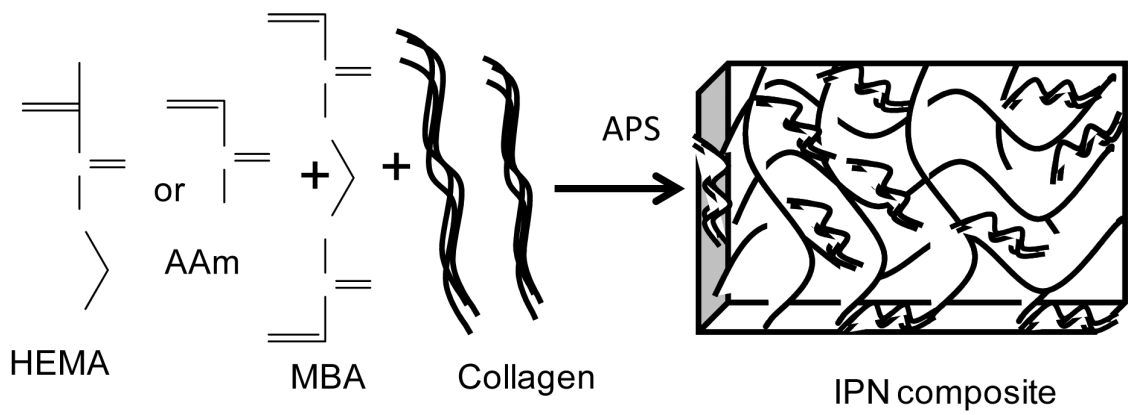

Figure 1. Schematic representation p(Coll-co-HEMA) or p(Coll-co-AAm) composite network formation.

has absorption maxima at 280 nm and NP has absorption maxima at 330 nm). Different amounts of drugs absorbed by the various polymer-collagen matrices were determined. The loading and release efficiencies were varied depending upon the amount of collagen in composite and the nature of the synthetic materials. Figure 2 shows, (a) the chemical structures of model drug, TMP, and it's the release profile from (b) p(Coll-co-AAm) and p(AAm), and (c) p(Coll-co-HEMA) and p(HEMA) hydrogel matrices.

As can be seen from Figure 2, p(Coll-co-AAm) release more drugs than p(AAm) (Figure 2(b), whereas p(p(Coll-co-HEMA) composite and p(HEMA) materials release about the same amounts of TMP with similar release characteristics.

Another model drug with different, chemical structure, Naproxene, NP as shown in Figure 3 (a) can be loaded to collagen-synthetic composites.

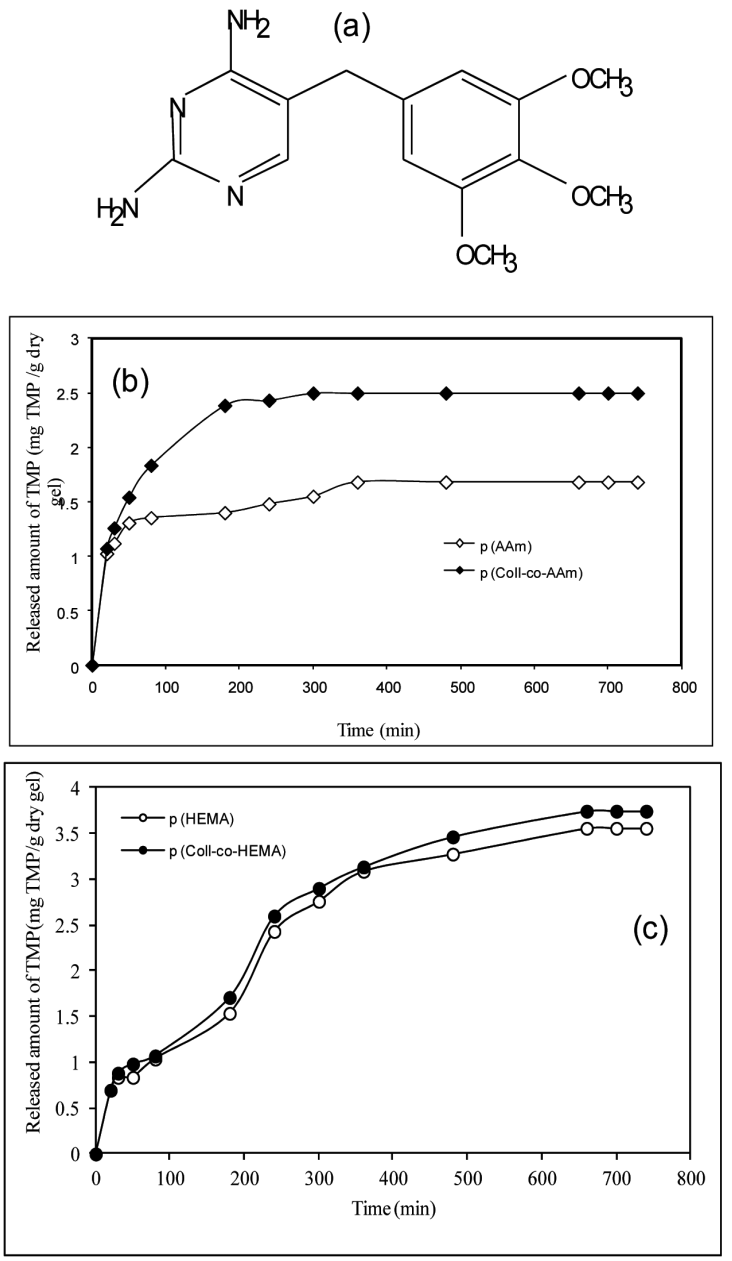

Figure 2. (a) the structure of Trimethoprim (TMP), and (b) the release profiles of TMP from p(Coll-co-AAm) and p(AAm) and (c) from p(Coll-co-HEMA) and p(HEMA).

The release profiles of this drug from p(Coll-co-AAm) and p(AAm) as (b), and from p(Coll-co-HEMA) and p(HEMA) hydrogel matrices as (c) in Figure 3 were illustrated.

As can be seen from the Figure 3 (b), p(Coll-co-AAm) absorbs and release more NP than p(AAm) while p(Coll-co-HEMA) composite and p(HEMA) materials release about the same amounts but less than p(Coll-co-AAm) and p(AAm) even though they both exposed to the same loading procedures. On the other hand, p(Coll-co-HEMA) and p(HEMA) hydrogel matrices release NP in a linear drug release fashion up 330 min. Therefore, by controlling various parameters such the amount of collagen and by choosing the appropriate hydrogel material compositions in the IPN structure, it is possible to control the amount of release drug as well as its kinetics. In general, the amount drug loading was increased

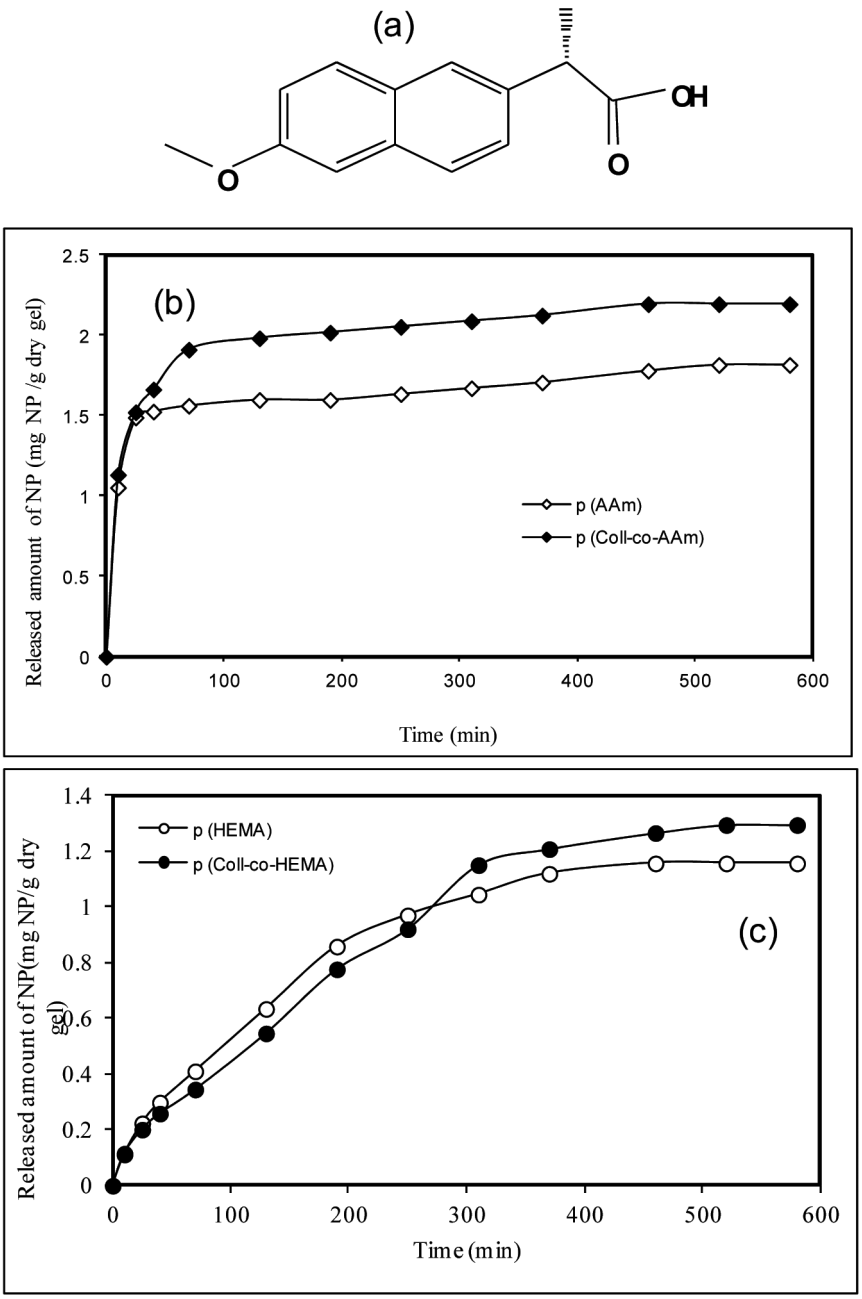

Figure 3. (a) The chemical structure of Naproxen (NP), (b) NP release profile from p(Coll-co-AAm) and p(AAm) , and (c) from p(Coll-co-HEMA) and p(HEMA).

as the collagen content of the hydrogel matrix was increased.

We also tested the prepared hydrogel films biocompatibility by using very well known MTT assay. For any biomaterials to be used in biomedical application required to be biocompatible. Table 1 illustrates the MTT result of prepared hydrogel films.

Table 1. MTT assay of prepared hydrogel IPN films.

	Absorbance	Proliferation inhibition (%)
Control	0.392	0
P(AAm)	0.346	0.12
P(Coll-co-AAm)	0.291	0.26
P(Coll-co-HEMA)	0.336	0.14
P(HEMA)	0.291	0.26

As can be seen from table 1, there is no cell death in control samples and very little for all the prepared hydrogel matrices. Therefore, it can said that p(Coll-co-AAm) and p(Coll-co-HEMA) can be considered as biocompatible materials as well as drug delivery devices.

Conclusion

It was demonstrated with this investigation that polymeric films based on collagen with some synthetic materials can be readily prepared and used as potential materials for drug delivery purpose. These types of materials can be applicable as wound and/or burn dressing materials as healing and prevention from various bacteria while providing comfortable aqueous and soft environments and as a hygiene environment. As described here, the antibacterial drugs such as TMP, NP can be released from the synthesized IPN networks with promising application for the treatment of various skin related discomforts. The collagen based-synthetic matrices can be prepared from different functional group containing monomers and polymers for the drug with different chemical functional groups. Therefore, types of collagen based hydrogel films can have potential applications in various biomedical fields.

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References

1. G. Reich, From Collagen To Leather-The Theoretical Background, Basf, (2007)
2. B. Madhan, P. Thanikaivelan, V. Subramanian, R.J. Raghava, B.U. Nair, T. Ramasami, Molekular mechanics snd dynamics studies on the interaction of gallic acid with collagen-like peptides, Chem. Phys. Letter., 346, (2001) 334.
3. J. Li, A. Mak, Transfer of collagen coating from porogen to scaffold: collagen coating within poly (dl-lactic –co-glycolic acid) scaffold, Composites: Part B, 38 (2007), 317.
4. H. Kim, H. Hobbs, I. Wang M. Rutten C. Wamser, Biocompatible composites of polyaniline nanofibers and collagen, Synthetic Metal., 159 (2009), 1313.
5. M. Maeda, S. Tani, A. Sano, K. Fujioka, Microstructure and release characteristics of the minipellet, a collagen-based drug delivery system for controlled release of protein drugs, J. Cont. Release., 62 (1999), 313
6. C. Chaubaroux, E. Vrana, C.,Debry, P. Schaaf, B., Senger, J. Voegel, Y. Haikel, C. Ringwald, J. Hemmerlé, P. Lavalle, and F. Boulmedais, Collagen-based fibrillar multilayer films crosslinked by a natural agent Biomacromolecules, 13 (2009) 2128.
7. Z. Cheng, S. Teoh, Surface modification of ultra thin poly(e-caprolactone) films using acrylic acid and collagen, Biomaterials, 25 (2004) 1991.
8. Y. Di, R. Heath, Collagen stabilization and modification using a polyepoxide, triglycidylisocyanurate, Polym. Degrad. Stability, 94 (2009) 1684.
9. Y. Li, T.T. Thula, S. Jee, S.L. Perkins, C. Aparicio, E.P. Douglas, L.B., Gower, Biomimetic mineralization of woven bone-like nanocomposites: role of collagen cross-links, Biomacromolecules, 13 (2012) 49.
10. J.T. Lu, C. Lee, S. Bent, H. Fishman, E.E. Sabelman, Thin collagen film scaffolds for retinal epithelial cell culture, Biomaterials, 28 (2007) 1486.
11. W.T Brinkman, K. Nagapudi, B.S. Thomas, E.L. Chaikof, Photo–cross–linking of type I collagen gels in the presence of smooth muscle cells: mechanical properties, cell viability, and function, Biomacromolecules, 4 (2003) 890 .
12. B. Hoyer, A. Bernhardt, S. Heinemann, I. Stachel, M. Meyer, M., Gelinsky, Biomimetically mineralized salmon collagen scaffolds for application in bone tissue engineering, Biomacromolecules, 13 (2009) 1059.
13. K. Nam, T. Kimura, A. Kishida, Physical and biological properties of collagen-phospholipid polymer hybrid gels, Biomaterials, 28 (2007) 3153.

14. N. Sahiner, A facile method for the Preparation of Poly(4-vinylpyridine) nanoparticles and their characterization. Tr J. Chem., 33 (2009) 23.
15. M. Harmancioğlu, Deri Kimyası, Ege Ü. Ziraat Fak. Yayınları, 1998.
16. C.Yang, L. Xu, Y., Zhou, X. Zhang, X. Huang, M. Wang, Y. Han, M. Zhai, S. Wei, and J. Li, A green fabrication approach of gelatin/cm-chitosan hybrid hydrogel for wound healing, Carbohyd. Polym., 82 (2010) 1297.
17. C. Sılan, A. Akcali, M.T. Otkun, N. Ozbey, S. Butun, O. Ozay, N. Sahiner, Novel hydrogel particles and their IPN films as drug delivery systems with antibacterial properties. Colloid Surf. B., 89 (2012) 248.