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# Engineering a sprayable and elastic hydrogel adhesive with antimicrobial properties for wound healing

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ABSTRACT

Hydrogel-based bioadhesives have emerged as alternatives for sutureless wound closure, since they can mimic the composition and physicochemical properties of the extracellular matrix. However, they are often associated with poor mechanical properties, low adhesion to native tissues, and lack of antimicrobial properties. Herein, a new sprayable, elastic, and biocompatible composite hydrogel, with broad-spectrum antimicrobial activity, for the treatment of chronic wounds is reported. The composite hydrogels were engineered using two ECM-derived biopolymers, gelatin methacryloyl (GelMA) and methacryloyl-substituted recombinant human tropoelastin (MeTro). MeTro/GelMA composite hydrogel adhesives were formed via visible light-induced crosslinking. Additionally, the antimicrobial peptide Tet213 was conjugated to the hydrogels, instilling antimicrobial activity against Gram (+) and (−) bacteria. The physical properties (e.g. porosity, degradability, swellability, mechanical, and adhesive properties) of the engineered hydrogel could be fine-tuned by varying the ratio of MeTro/GelMA and the final polymer concentration. The hydrogels supported *in vitro* mammalian cellular growth in both two-dimensional and three dimensional cultures. The subcutaneous implantation of the hydrogels in rats confirmed their biocompatibility and biodegradation *in vivo*. The engineered MeTro/GelMA-Tet213 hydrogels can be used for sutureless wound closure strategies to prevent infection and promote healing of chronic wounds.



## 1. Introduction

More than 2% of the US population suffers from chronic non-healing wounds, which represent an estimated 20 billion dollars in health care related costs each year [1]. Chronic wounds are characterized by delayed healing and sustained inflammation, as well as impaired extracellular matrix (ECM) function [2]. These wounds can be caused by a number of pathologies including diabetes mellitus, vascular insufficiency, local-pressure effects, compromised nutritional and immunological states, surgeries, and burns [3]. Conventional therapies for chronic wound management, such as skin substitutes or autologous skin grafts often fail to restore tissue homeostasis, and can lead to further health complications [3,4]. In particular, microbial infection at the wound site can severely prolong the healing process, lead to necrosis, sepsis, and even death [5]. Chronic wounds are highly susceptible to colonization by pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermis,* various filamentous fungi and yeasts (i.e. *Candida* spp.) [6–8]. Topical and systemic antibiotic administration is frequently prescribed to patients suffering from chronic wounds. However, the over-

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prescription, abuse, and misapplication of antibiotics have led to an escalating drug resistance in pathogenic microorganisms, which is associated with increased morbidity and mortality [9].

Polymeric hydrogels hold remarkable potential to be used as dressings for the treatment of non-healing wounds [5,6]. Hydrogels are hydrated three-dimensional (3D) networks of natural or synthetic polymers, which can be tailored to mimic the physicochemical properties of human tissues. Natural polymers that are derived from native ECM proteins such as collagen or elastin, are particularly advantageous for tissue-engineered wound dressings because of their inherent biocompatibility and biodegradability both *in vitro* and *in vivo* [10]. Hydrogel-based dressings also absorb wound exudates, which in turn promotes fibroblast proliferation, keratinocyte migration, and the eventual re-epithelialization of the wound [11]. Furthermore, wound healing and infection prevention can be promoted by delivering biomacromolecules, growth factors, and other small molecule agents via polymeric scaffolds [5,6]. In particular, previous works have demonstrated the incorporation of antimicrobial properties to hydrogel-based dressings through integration of different types of biocidal agents, including metal nanoparticles [12,13], cationic polymers [14], and antimicrobial peptides (AMPs) [15].

Despite many biological advantages of hydrogel-based dressings, they often exhibit weak mechanical and adhesive properties on the wound area, when compared to conventional wound closure approaches (i.e. cyanoacrylate-based adhesives) [16]. Cyanoacrylates and aldehyde-based adhesives have been largely associated with tissue inflammation, cell necrosis, and cytotoxicity [17,18]. Hydrogel-based adhesives and biologically derived fibrin glues have been shown to exhibit poor adhesion to wet tissues, and are not able to support tissue regeneration [19]. In addition, poor mechanical properties and prolonged curing times of existing adhesives often lead to impaired performance and tissue bonding [20,21]. An ideal tissue adhesive for wound closure and treatment should be (i) biocompatible and biodegradable, (ii) rapidly crosslinked and easily applicable, (ii) antimicrobial and impervious to antibiotic resistance, (iii) strongly adhesive, (iv) tunable and long lasting, and (v) a promotor of tissue regeneration and wound healing [22,23]. Therefore, new biomaterial-based approaches are needed to address the limitations of currently available alternatives.

Here, we present a new composite class of elastic and antimicrobial hydrogels, for the clinical management of chronic non-healing wounds. The engineered hydrogels are comprised of two biopolymers derived from native ECM proteins, gelatin and tropoelastin. Both gelatin methacryloyl (GelMA) [24,25] and methacryloyl-substituted recombinant human tropoelastin (MeTro) [26,27] have been previously explored to engineer hydrogels through photocrosslinking using ultraviolet (UV) light. Although UV light has been extensively used for photocrosslinking of different biopolymers, it is also associated with DNA and tissue damage [28–31], adverse effects on cell metabolic activity [32], and suppression of the immune system *in vivo* [33]. Here, we describe for the first time the engineering of composite MeTro/GelMA hydrogels through visible light-mediated photocrosslinking. The use of a visible light-activated photoinitiator system eliminates the biosafety concerns associated with UV light, while yielding mechanical properties similar to, or comparatively better than UV-crosslinked hydrogels [34]. The physical, mechanical, and adhesive properties of the engineered MeTro/GelMA hydrogel adhesives were characterized. Additionally, to provide antimicrobial properties to the composite hydrogels, AMP Tet213 (KRWWKWWRRC) [35] was conjugated to the polymeric network. The antimicrobial properties of AMP incorporated MeTro/GelMA (MeTro/-AMP) hydrogels were evaluated against Gram-positive (G+) methicillin resistant *Staphylococcus aureus* (MRSA), and Gram-negative (G-) *E. coli*. Lastly, *in vitro* and *in vivo* cytocompatibility of optimized MeTro/GelMA-AMP hydrogels were investigated. The highly tunable mechanical and adhesive properties of MeTro/GelMA-AMP hydrogels showcase their potential for the engineering of multifunctional, biomaterial-based therapies for the treatment of chronic non-healing wounds.

## 2. Results and discussion

### 2.1. Synthesis and structural characterization of MeTro/GelMA hydrogels

In this study, we present a new composite class of elastic and antimicrobial hydrogels for the treatment of non-healing wounds. The engineered hydrogels were synthesized using MeTro and GelMA biopolymers, which mimic the native composition of the ECM. MeTro is a photocrosslinkable bioelastomer comprised of recombinant human tropoelastin, a highly elastic protein that provides structural integrity and modulates cell function in human tissues (Fig. 1a) [27]. On the other hand, GelMA is a photocrosslinkable biopolymer comprised of a modified form of denatured collagen, providing physiological cell binding motifs and protease-sensitive degradation sites (Fig. 1b) [36]. Due to their biocompatibility and high tunable mechanical properties, UV crosslinkable MeTro and GelMA biopolymers have been explored for various tissue engineering applications [24,26,27]. Here, we incorporated both MeTro and GelMA into a single polymeric network, enabling the modulation of several features of the resulting composite hydrogels such as degradation rate, mechanical properties, porosity, and tissue adhesion. In addition, we used a visible light activated photoinitiator system to minimize the biosafety concerns associated with UV light. Photopolymerizable scaffolds used for tissue engineering applications are generally crosslinked *in situ* using UV light ($250\ nm < \lambda < 400\ nm$). However, the exposure of living cells and tissues to UV radiation can induce DNA damage, leading to cell death and carcinogenesis [37]. To circumvent this limitation, we investigated a method of photocrosslinking of MeTro and GelMA through the use of visible light ($400\ nm < \lambda < 700\ nm$). Visible light is cheaper, safer, and possesses deeper tissue penetration for transdermal implantations, because of its longer wavelength [38,39]. In addition, similar to other photocrosslinking systems, the ability to control radical formation, makes this an ideal method for crosslinking. Visible light-induced crosslinking of MeTro/GelMA composite hydrogels was achieved through the incorporation of the type 2 initiator Eosin Y, with the co-initiator triethanolamine (TEA) and the co-monomer poly(N-vinylcaprolactam) (VC) (Fig. S1) to form an elastic and sprayable hydrogel for wound healing (Fig. 1c–d). This visible light mediated crosslinking scheme has been thoroughly investigated, showing improved cell viability when compared to UV crosslinked hydrogels [40–42]. Briefly, visible light excites dye molecules of Eosin Y into a triplet state, which abstracts hydrogen atoms from TEA. The deprotonated radicals initiate vinyl-bond crosslinking with VC via chain polymerization reactions, which leads to accelerated gelation [43]. The visible light photocrosslinking systems based on this chemistry, such as FocalSeal-L (Genyzme Biosurgery, Inc., Cambridge, MA), was approved by the Food and Drug Administration (FDA) [44].

To verify the degree of crosslinking within the hydrogels, $^{1}H$ NMR (500 MHz) spectra were taken from MeTro (Fig. 1e) and GelMA (Fig. 1f) prepolymers, and partially dissolved MeTro/GelMA-AMP crosslinked hydrogels (Fig. 1g). Results demonstrated that the methacrylated groups in the co-blended MeTro/GelMA-AMP network are involved in the formation of the 3D hydrogel network.