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Composite Alginate-Hyaluronan Sponges for the Delivery of Tranexamic Acid in Postextractive Alveolar Wounds

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ABSTRACT

The management of wounds in patients on anticoagulant therapy who require oral surgical procedures is problematic and often results in a nonsatisfactory healing process. Here, we report a method to prepare an advanced dressing able to avoid uncontrolled bleeding by occluding the postextractive alveolar wounds, and simultaneously, capable of a fast release of tranexamic acid (TA). Composite alginate/hyaluronan (ALG/HA) sponge dressings loaded with TA were prepared by a straightforward internal gelation method followed by a freeze-drying step. Both blank and drug-loaded sponges were soft, flexible, and elegant in appearance and nonbrittle in nature. Scanning electron microscopy analysis confirmed the porous nature of these dressings. The integration of HA influenced the microstructure, reducing the porosity, modifying the water uptake kinetic, and increasing the resistance to compression. TA release from ALG/HA sponges showed a controlled release up to 3 h, and it was faster in the presence of HA. Finally, an *in vitro* clotting test performed on human whole blood confirmed that the TA-loaded sponges significantly reduce the blood clotting index by 30% compared with ALG/HA_{20} sponges. These results suggest that, if placed in a socket cavity, these dressings could give a relevant help to the blood hemostasis after dental extractions, especially in patients with coagulation disorders.

Introduction

Tooth extractions, even if considered as minor oral surgery, are one of the most routinely performed treatments among dental surgical techniques, and like all surgical procedures often cause large socket wounds, particularly after large tooth extraction. Under normal conditions, the management of oral surgical wounds is simple, but for some category of patients, such as those on anticoagulant therapy, it is problematic and still controversial. Generally, when tooth extraction is required in such patients, the pharmacological therapy is reduced or stopped for several days before the surgery, increasing the risks of uncontrolled bleeding and, most of all, thromboembolism, which is considered a major complication.[1-3] However, a different approach based on the use of local hemostatic agents would make it possible to operate without any interruption or diminution of the anticoagulant treatment, avoiding risks caused by clogged blood flow due to suspending drug regimen.[4-6]

The term "socket healing" generally refers to a series of local alterations that arise in both hard and soft tissues to close the socket wound after tooth extraction and to restore tissue homeostasis.[7] The most common complication following tooth extractions is the alveolar osteitis (dry socket) which may develop when an inflammation of the alveolar bone occurs, resulting in intense pain and delayed wound healing.[8,9] Dry socket is often a consequence of the removal or dissolution of the blood clot at the site of the tooth extraction before the wound has healed.[10] During tooth extraction, the formation of a blood clot is essential because it serves as a protective layer over the underlying bone and nerve endings in the empty tooth socket. The clot also provides the foundation for the growth of new bone and for the development of soft tissue.

For this purpose, an advanced wound dressing able to control wound bleeding and enhance clot formation could be very useful for the prevention of alveolar osteitis and pain following tooth extraction. The wound dressing can act both as socket plug limiting

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the bleeding, but also as a local release platform for different drugs, including antifibrinolytics. In particular, the association of tranexamic acid (TA) with postoperative compression showed good results in preventing postoperative bleeding.[6,11] TA was already widely used as mouthwash[12] or as socket irrigation immediately after extraction[13] to prevent postextraction bleeding in patients on warfarin. However, this approach exhibits several limitations such as poor handiness and control over the delivered dose, as well as poor efficiency due to bleeding, which tends to quickly wash out the drug away from the administration site.

Macroporous alginate (ALG) sponges are considered a very interesting platform system for local drug release, and for this purpose, they have been extensively developed for a wide range of applications, such as bone tissue engineering, wound dressing, and drug delivery.[14-16] Through the years, ALG has gained a leading role among the wound dressing materials due to peculiar characteristics including the high absorbency and the promotion of healing and epidermal regeneration.[15] Its natural origin and simple extraction process from marine brown algae biomass, associated with their characteristics in terms of biocompatibility and biodegradability under physiological conditions, make this polysaccharide ideal for use as socket-dressing materials.[17] Furthermore, it has been demonstrated that calcium alginate materials activate platelet and blood coagulation and for this reason, they have also been used as hemostatic dressings.[18]

Common techniques for producing macroporous ALG dressings from a hydrogel or a polymer solution include air drying,[19] solvent evaporation,[20] or freeze drying.[21] However, because of their hydrophilic polymeric backbones, ALG dressings easily dissolve in water unless radical, chemical, or physical crosslinks are present. To overcome this limitation, internal gelation of ALG through $CaCO_3$-GDL (d-glucono-δ-lactone) system has been recently proposed by our group as a versatile and straightforward strategy to obtain homogenous cross-linked composite ALG hydrogels.[22,23] In addition, we found that the integration of hyaluronan (HA), an extracellular glycosoaminoglycan extensively involved in all phases of wound healing[24] in these ionically cross-linked ALG matrix has proved to be a versatile strategy to promote the wound healing process. Beside its function as the main component of extracellular matrix and cartilage, HA is also an important component of both soft periodontal tissues such as gingiva and periodontal ligament and of the hard tissue, such as alveolar bone and cementum. For these reasons, as recently reported by Casale et al.,[25] the topical application of HA could have a positive action on the healing of mineralized and nonmineralized tissues of the periodontium. Moreover, a recent pilot study in dogs demonstrated how HA may enhance bone formation and accelerate wound healing in infected sockets.[26]

In this work, we present an alginate/hyaluronan (ALG/HA)–based composite sponge dressing loaded with TA useful for reducing bleeding after tooth extraction and, at the same time, reducing the risk of alveolar osteitis. Moldable, biocompatible, and bioresorbable dressing were prepared by internal gelation followed by a freeze drying to obtain solid macroporous sponges loaded with TA. The gradual release of calcium ions directly from the inside of an ALG solution results in a homogeneous crosslink, with consequent improved mechanical properties, and allow an easy integration and a uniform distribution of drugs that are soluble in hydrogels or aqueous solutions. We examined in depth the behavior of the sponges when they came in contact with simulated biological fluids, evaluating the swelling rates, the degradation behaviour, and the drug release in relation to the composition and to the microstructure of the sponges. The mechanical properties and the adhesion profile on a simulated wound surface were also evaluated. Finally, the *in vitro* toxicity of the platform was tested in normal adult human primary epidermal keratinocyte cell lines and hemostatic efficacy evaluated through an *in vitro* dynamic whole blood clotting test.

Materials and Methods

Materials

Sodium alginate (from *Macrocystis Pyrifera*, medium viscosity, 360 cps at 25°C) was purchased from Farmalabor (Italy). HA sodium salt from *Streptococcus equi* (1.5-1.8 × 10^6 Da), tranexamic acid (TA), GDL, calcium chloride dihydrate, potassium chloride, sodium chloride, sodium phosphate dibasic, calcium carbonate, and dimethylsulfoxide were obtained from Sigma–Aldrich (St. Louis, MO). Ethanol (laboratory grade) was purchased from Carlo Erba (Italy). Media, sera, and antibiotics for cell cultures were from ATCC (American Type Culture Collection). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Fisher Scientific (Leicestershire, UK). Deionized ultrafiltered water was used throughout this study.

ALG/HA Sponge Preparation

A 2% w/v ALG solution containing 2 different HA amount (10% or 20% of ALG weight to give ALG/HA_{10} and ALG/HA_{20}, respectively) was prepared and gelled with a freshly prepared GDL solution as previously described.[22,27] To obtain TA-loaded ALG/HA sponges, TA was dissolved directly in the initial ALG/HA solution to give a final drug concentration of 2% (w/v). Solutions were cast in 24-well culture plate (1 mL) to form circular disks 5 mm in thickness and 15 mm in diameter. The well plates were capped, sealed with Parafilm®, and gelled on a horizontal surface at room temperature for 24 h. After gelation, the ALG disks were washed with deionized water, frozen overnight at −20°C, and then lyophilized at 0.01 atm and −60°C in a Modulyo apparatus (Edwards, Crawley, UK). The freeze-dried sponges were stored at room temperature under vacuum.

Physical Characterization

The bulk morphology of the ALG/HA sponge was analyzed through scanning electron microscopy (SEM). Samples were mounted on a metal stub by means of carbon adhesive tape and coated with a 20-nm thick gold/palladium layer with a modular high-vacuum coating system Emitech K575X. Images at different magnification were acquired using Quanta 200 FEG (FEI, Hillsboro, OR) microscope.

The average porosity of the ALG/HA sponge was determined by a fluid displacement method. Ethanol was chosen as the displacement liquid because it penetrates easily into the pores and did not induce shrinkage or swelling. The geometrical volume (V_s) of the sponge samples was calculated by measuring diameter and height, and the pore volume (V_p) was measured by ethanol displacement method. The dry sponges ($n = 3$) were weighed (W_0) and immersed in absolute ethanol at room temperature, and then placed in a degasser for 5 min to remove air bubbles from the sponge pores. After wiping gently with a filter paper to remove surface ethanol, samples were weighed immediately (W_e) to reduce ethanol evaporation. The porosity of the sponge was calculated according to Equation 1:

$$\text{Porosity} = \frac{V_p}{V_s} \times 100 = \frac{W_e - W_0}{\rho_e V_s} \times 100 \quad (1)$$

where ρ_e represents the density of ethanol (0.789 g/cm^3). An average value of 5 replicates for each sample was taken.