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Oxidized alginate hydrogels for bone morphogenetic protein-2 delivery in long bone defects

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ABSTRACT

Autograft treatment of large bone defects and fracture non-unions is complicated by limited tissue availability and donor site morbidity. Polymeric biomaterials such as alginate hydrogels provide an attractive tissue engineering alternative due to their biocompatibility, injectability, and tunable degradation rates. Irradiated RGD-alginate hydrogels have been used to deliver proteins such as bone morphogenetic protein-2 (BMP-2), to promote bone regeneration and restoration of function in a critically sized rat femoral defect model. However, slow degradation of irradiated alginate hydrogels may impede integration and remodeling of the regenerated bone to its native architecture. Oxidation of alginate has been used to promote degradation of alginate matrices. The objective of this study was to evaluate the effects of alginate oxidation on BMP-2 release and bone regeneration. We hypothesized that oxidized-irradiated alginate hydrogels would elicit an accelerated release of BMP-2, but degrade faster in vivo, facilitating the formation of higher quality, more mature bone compared to irradiated alginate. Indeed, oxidation of irradiated alginate did accelerate in vitro BMP-2 release. Notably, the BMP-2 retained within both constructs was bioactive at 26 days, as observed by induction of alkaline phosphatase activity and positive Alizarin Red S staining of MC3T3-E1 cells. From the in vivo study, robust bone regeneration was observed in both groups through 12 weeks by radiography, micro-computed tomography analyses, and biomechanical testing. Bone mineral density was significantly greater for the oxidized-irradiated alginate group at 8 weeks. Histological analyses of bone defects revealed enhanced degradation of oxidized-irradiated alginate and suggested the presence of more mature bone after 12 weeks of healing.

1. Introduction

Musculoskeletal injuries account for two-thirds of all injuries each year in the USA [1,2]. Of the 6.3 million bone fractures that occur annually in the USA, over 500,000 require bone grafts, accounting for ~$2.5 billion in medical expenses [3]. Substantial loss of bone tissue caused by traumatic injury or tumor resection presents a significant clinical challenge for reconstruction. Among these injuries, critically sized bone defects are particularly difficult to repair and often require subsequent surgeries or result in a non-union. Currently, the gold standard of care is autograft harvested from the iliac crest, but the limited graft tissue available and associated donor site pain and morbidity [4] warrant the study of more effective therapeutics.

Tissue engineering and regenerative medicine approaches, based on the delivery of osteoinductive cells, growth factors, and matrix materials, have emerged as a promising alternative platform. One clinically viable tissue engineering strategy is to deliver an osteogenic growth factor within a biomaterial scaffold to the site of injury and thereby stimulate the endogenous bone repair process [5]. A critical factor in the effectiveness of the carrier is the ability to provide the necessary temporal and spatial presentation of the growth factor for sufficient recruitment and differentiation of endogenous stem cells [5]. As members of the transforming growth factor-β (TGF-β) super family of growth factors, bone morphogenetic proteins (BMPs) promote migration of many cells including osteoprogenitors [6] and osteogenic differentiation of mesenchymal stem

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cells [7,8]. Both BMP-2 and BMP-7 are approved by the US Food and Drug Administration for clinical use [9,10], and BMP-2 has been widely studied as an osteoinductive protein for bone regeneration. Although BMP-2 delivered on an absorbable collagen sponge has shown success in long bone healing and spinal fusion [11,12], concerns regarding the use of supraphysiological doses and associated complications including heterotopic mineralization and inflammation [13] necessitate the development of biomaterial carriers that promote greater regenerative efficacy with lower doses of growth factors [14].

Alginate hydrogels have been used as delivery vehicles for a multitude of proteins, including BMP-2 [15–18]. Alginate is a polysaccharide derived from algae that exhibits minimal binding interactions with cells and can be ionically crosslinked into hydrogels using divalent cations such as calcium [19]. Alginate does not degrade enzymatically [18], but alginate hydrogels can degrade slowly due to dissociation of the ionic crosslinks [20]. Scaffold degradation is a crucial regulator of not only growth factor release but also extracellular matrix deposition [21]. Ideally, as the scaffold degrades, space for new bone is created. As such, the rate of scaffold degradation should be similar to the rate of new tissue formation to allow for successful coalescence of the newly formed bone. In the case of alginate, various modification techniques, including gamma-irradiation and partial oxidation, have been utilized to enhance degradation of the scaffold [22–26].

Gamma-irradiation lowers the molecular weight of the alginate polymer chains, allowing the polymers to more readily dissociate from the alginate matrix [22]. Previously, irradiated alginate led to improved cellular infiltration and tissue healing compared to unmodified alginate [18,22]. Irradiated alginate hydrogels have been used to deliver proteins such as BMP-2 and facilitate functional regeneration in our critically sized rat femoral segmental defect model [27–30]. In previous subcutaneous implant studies, irradiation of the alginate led to enhanced tissue infiltration, bone architecture, and bone area fraction [18,22], as well as greater mineral density and extent of alginate degradation [22] compared to unmodified alginate.

Partial oxidation, whereby a small percentage of the uronate residues are oxidized, allows the polymer chains to be more susceptible to hydrolysis and increases the degradation rate in vitro [23–26]. Unlike the degradation of unmodified alginate, oxidized alginate breakdown occurs primarily via hydrolysis, specifically at the oxidized sugar residues [23]. Oxidation of the alginate creates a more open-chain structure while maintaining the ionic cross-linking capacity [24] and biocompatibility of the alginate [23]. In previous work, oxidized alginate hydrogels served as a carrier for chondrocytes [24] and growth factors (BMP-2 and TGF-β3) [25], and in both studies facilitated an increase in cellular infiltration and matrix formation subcutaneously compared to unmodified alginate. Additionally, oxidized alginate hydrogels loaded with vascular endothelial growth factor (VEGF) mitigated tissue loss in a mouse hind limb ischemia model [26]. Oxidized-irradiated alginate hydrogels were used recently for adipose stem cell delivery, promoting the formation of new adipose tissue subcutaneously [31]. In the present study, we utilized this same alginate modified by both irradiation and oxidation – i.e., a lower molecular weight and hydrolytically degradable alginate – as a growth factor delivery system for bone tissue engineering in an orthotopic model.

In our rat segmental defect model, irradiated alginate hydrogel surrounded by a poly(ε-caprolactone) (PCL) nanofiber mesh provided a more sustained release of BMP-2 and augmented bone regeneration compared to the clinically used collagen sponge [28,29]. However, a portion of the alginate material was shown to persist at 30 weeks and may have hindered functional remodeling of the newly formed bone tissue [32]. The interplay between carrier degradation, growth factor availability, and tissue ingrowth remains poorly understood. With these design parameters in mind, this work evaluated the regenerative capacity of an oxidized-irradiated alginate hydrogel as a delivery vehicle for BMP-2 in a well-established rat long bone defect model. The objectives of the study were: (i) to compare BMP-2 release and bioactivity from irradiated alginate and oxidized-irradiated alginate, and (ii) to evaluate bone regeneration, alginate degradation, and quality of regenerated bone in vivo using these two alginate formulations. We hypothesized that the oxidized-irradiated alginate hydrogels would elicit an accelerated release of BMP-2, but degrade faster in vivo, facilitating the formation of higher quality, more mature bone.

2. Materials and methods

2.1. Alginate hydrogel preparation

Sodium alginate rich in guluronic acid blocks (MVG alginate, FMC BioPolymer) was used for all experiments. A low molecular weight alginate formulation was made by treating MVG alginate with gamma-irradiation, reducing its molecular weight from ~250 kDa to ~50 kDa [26]. For preparation of the oxidized-irradiated alginate, irradiated alginate was exposed to sodium periodate, resulting in ~1% oxidation of the uronate residues and creating a hydrolytically labile polymer [24]. The alginates were functionalized with RGD peptide sequences (two sequences per polymer chain) [22] to promote cell adhesion. Alginates were reconstituted in alpha-minimum essential medium (αMEM, Gibco) and mixed with recombinant human BMP-2 (rhBMP-2, R&D Systems) in 0.1% rat serum albumin. Hydrogels (2% (w/v) alginate) were prepared by mixing the alginate+rhBMP-2 solution with calcium sulfate slurry (0.21 g/ml) at a ratio of 25:1 [29]. All hydrogels were incubated at room temperature for 30 min before further manipulation. Hydrogels used for in vivo delivery were stored at 4 °C overnight.

2.2. Nanofiber mesh production

Nanofiber meshes were fabricated as previously described [29]. Briefly, PCL was dissolved in a 90:10 volume ratio of hexafluoro-2-propanol:dimethylformamide (Sigma-Aldrich) to a 12% (w/v) concentration. The solution (5 ml) was electrospun onto a static collector plate for 5–6 h. Using a VLS3.50 laser cutter (Universal Laser Systems) and CorelDRAW software, PCL sheets were cut into 12 × 19 mm rectangles, each with 24 1 mm diameter perforations, and rolled to form tubes 4.5 mm in diameter and 12 mm in length. Meshes were sterilized by ethanol evaporation overnight, rinsed three times in phosphate buffered saline (PBS, Cellgro), and stored in PBS. The meshes used in vivo were transferred to αMEM ~12 h before surgery.

2.3. rhBMP-2 release kinetics

To investigate the release kinetics of BMP-2, 2% (w/v) alginate hydrogels ($n = 8$) containing 500 ng rhBMP-2 per 150 μl were injected into PCL nanofiber meshes and incubated at 37 °C in 1 ml PBS, as previously described [29]. PBS was collected and replaced at 3 and 15 h, and at 1, 2, 3, 5, 8, 14, and 26 days. The BMP-2 remaining in the constructs at 26 days was then eluted by rinsing vigorously with PBS. The BMP-2 not removed by PBS rinsing was eluted using 1 ml 0.1% sodium dodecyl sulfate (SDS) on a rocker plate for 1 h. SDS was removed from solution by an SDS-Out™ Precipitation Kit (Thermo Scientific). The BMP-2 in solution