2.3.2 Wound dressings

The treatment of acute and chronic wounds is a pressing need in many facets of medicine, and alginate-based wound dressings offer many advantageous features. Traditional wound dressings (e.g., gauze) have provided mainly a barrier function – keeping the wound dry by allowing evaporation of wound exudates while preventing entry of pathogen into the wound [61]. In contrast, modern dressings (e.g., alginate dressings) provide a moist wound environment and facilitate wound healing [62]. Alginate dressings are typically produced by ionic cross-linking of an alginate solution with calcium ions to form a gel, followed by processing to form freeze-dried porous sheets (i.e., foam), and fibrous non-woven dressings. Alginate dressings in the dry form absorb wound fluid to re-gel, and the gels then can supply water to a dry wound, maintaining a physiologically moist microenvironment and minimizing bacterial infection at the wound site. These functions can also promote granulation tissue formation, rapid epithelialization, and healing. Various alginate dressings including AlgicellTM (Derma Sciences) AlgiSite MTM (Smith & Nephew), Comfeel PlusTM (Coloplast), KaltostatTM (ConvaTec), SorbsanTM (UDL Laboratories), and TegagenTM (3M Healthcare) are commercially available. A variety of more functional and bioactive alginate based wound dressings have also been studied to date. The sustained release of dibutyryl cyclic adenosine monophosphate, a regulator of human keratinocyte proliferation, from partially oxidized alginate gels accelerated wound healing, leading to complete re-epithelialization of full thickness wounds within 10 days in a rat model [63]. Alginate gels releasing stromal cell-derived factor-1 were also effective in accelerating wound closure rates and reducing scar formation in pigs with acute surgical wounds [64]. Incorporation of silver into alginate dressings increased antimicrobial activity and improved the binding affinity for elastase, matrix metalloproteases-2 (MMP-2), and pro inflammatory cytokines (e.g., TNF-_, IL-8). The addition of silver into alginate dressings also enhanced the antioxidant capacity[65]. Alginate fibers cross-linked with zinc ions have also been proposed for wound dressings, as zinc ions may generate immunomodulatory and anti-microbial effects, as well as enhanced keratinocyte migration and increased levels of endogenous growth factors[66]. Blends of alginate, chitin/chitosan, and fucoidan gels have been reported to provide a moist healing environment in rats, with an ease of application and removal[67].

2.3.3 Cell delivery

Alginate gels are increasingly being utilized as a model system for mammalian cell culture in biomedical studies. These gels can be readily adapted to serve as either 2-D or more physiologically relevant 3-D culture systems. The lack of mammalian cell receptors for alginate, combined with the low protein adsorption to alginate gels allows these materials to serve in many ways as an ideal blank slate, upon which highly specific and quantitative modes for cell adhesion can be incorporated (e.g., coupling of synthetic peptides specific for cellular adhesion receptors). Further, basic findings uncovered with in vitro studies can be readily translated in vivo, due to the biocompatibility and easy introduction of alginate into the body. RGD-modified alginate gels have been most frequently used as in vitro cell culture substrates to date. The presence of RGD peptides in alginate gels allows one to control the phenotype of interacting myoblasts , chondrocytes [68], osteoblasts[69], ovarian follicle[70], as well as bone marrow stromal cells (BMSCs)[71]. For example, the adhesion and proliferation of myoblasts cultured on alginate gels were dramatically enhanced by chemical conjugation of RGD peptides to the alginate backbone, compared with non-modified alginate gels (Fig.3) [72]. Further, the number of cells adherent to the gels, as well as the growth rate, were strongly dependent on the bulk RGD density in the gels. The length of the spacer arm between the RGD peptide and the alginate chain is a key parameter in regulation of cellular responses. The adhesion and growth of primary human fibroblasts cultured on alginate gels modified with a peptide with the sequence of (glycine)n–arginine–glycine–aspartic acid–serine–proline (GnRGDSP) was dramatically influenced by the spacer arm length, irrespective of the same total concentration of the peptides in the gels (Fig.4). At least four glycine units as a spacer arm allowed proper binding to the cellular receptors, but using more than 12 glycine units led to no further improvement in cell adhesion and growth [73]. The number of RGD peptides per alginate chain, and the spacing between clusters of RGD peptides, even independently of the overall density of RGD ligands, dramatically impact the response of cells to RGD-modified alginate gels[74], likely due to the ability of these variables to affect the clustering of integrin receptors[75]. While the presence of the RGD ligands typically enhances cell adhesion and differentiation, chondrogenic gene expression and matrix accumulation of BMSCs encapsulated in RGD-alginate gels (3-D) was inhibited with an increase of the RGD density in vitro[76]. Interestingly, alginate gels have recently been formed in a microfluidic device through light-triggered release of caged calcium using DM-nitrophenTM compounds, and used as a 3-D cell culture substrate. Preosteoblasts (MC3T3-E1) and human umbilical vein endothelial cells were co-cultured in the microfluidic device using photo-patterning of alginate hydrogels, and this system may provide a useful means for integrating 3-D culture microenvironments into microfluidic systems .

Recent studies utilizing alginate gels as 3-D cell culture substrates have revealed key insights regarding stem cell and cancer biology. The fate of mesenchymal stem cells was demonstrated to be controlled by the elastic modulus of the RGD-alginate gels in which they were encapsulated, as differentiation down fat and bone pathways was promoted at different values of gel stiffness. Strikingly, and in contrast to 2-D culture systems used in previous mechanotransduction studies, the control over stem cell fate was related to the number of adhesive bonds formed between the gel and the cells, as well as alterations in the receptors cells utilized to adhere to the RGD peptides in 3-D versus 2-D culture. The cells actively reorganized on the nanoscale the adhesion ligands presented from the gels [77]. Alginate gels have also been used to examine how a 3-D

culture microenvironment influences cancer cell signaling and tumor vascularization. Integrin engagement within a 3-D tumor microenvironment (i.e., encapsulation in RGD-alginate gels) dramatically altered how cancer cells signal to recruit blood vessels, and this finding may lead to the development of new anti-angiogenic cancer therapies[78].

A crucial limitation of most 3-D cell culture systems is the difficulty in analyzing and quantifying cell-matrix interactions, particularly in a non-invasive, real time manner. However the development of several FRET techniques has recently enabled a previously unprecedented ability to quantitatively probe the relation between cell adhesion and decision-making. In one FRET technique, cell membranes are pre-stained with florescent molecules (i.e., acceptor), and a different fluorophore (i.e., donor) can be coupled to the cell adhesion peptides conjugated to the polymer chains. This FRET technique allows one to quantify cell receptor-ligand binding, and a similar FRET technique provides information on cell mediated rearrangements, at the nanometer size scale, of the adhesion ligands attached to gels [79]. The relationship between cell behavior and the number of receptor–ligand bonds was investigated by encapsulating either preosteoblasts (MC3T3-E1) or myoblasts (C2C12) in alginate gels presenting RGD peptides using a FRET technique. The adhesive interactions can be directly visualized, as the green emission of fluorescein in the cell membrane was greatly decreased and the red emission of rhodamine at the interface between cells and gels was increased when the cells were encapsulated in rhodamine- G4RGDASSKY-alginate gels, due to FRET (Fig.5). The proliferation and differentiation of both cell types were significantly dependent on the number of receptor–ligand bonds calculated using the FRET signal. This type of analysis may allow one to predict cell behavior, particularly in 3-D culture, and to design proper 3-D cell culture substrates for many applications.

2.3.4. Bone

Despite recent progress, treatment of bone injuries is still often limited due to poor healing, and alginate gels have found potential in bone regeneration by delivery of osteoinductive factors, bone-forming cells, or combination of both. Alginate gels have advantages for bone and cartilage regeneration, as compared to other materials, due to their ability to be introduced into the body in a minimally invasive manner, their ability to fill irregularly shaped defects, and the ease of chemical modification with adhesion ligands (e.g., RGD) and controlled release of tissue induction factors (e.g., BMP, TGF). However, alginate gels do not have sufficient mechanical properties to allow load bearing in the initial stages of regeneration without fixation. They are also not inherently degradable in physiological conditions, as reviewed earlier, highlighting the need to control their degradation in order that residual gels do not interfere with regeneration. Alginate gels have proven useful in animal models for the delivery of growth factors that can effectively drive bone regeneration (e.g., bone morphogenetic proteins). The use of RGD-alginate gels allows complete regeneration of critical-sized femoral defects in rodents with a low dose of BMP [80]. Alginate gels that deliver DNA encoding bone morphogenetic proteins (BMPs) have also demonstrated significant bone tissue can be regenerated [81]. The delivery of multiple factors, either in combination or sequence, is also being explored, in a similar manner as described for angiogenesis. Sequential delivery of BMP-2 and BMP-7 using alginate gels enhanced osteogenic differentiation of bone marrow derived stem cells in vitro[82], and co-delivery of BMP-2 and VEGF releasing from alginate gels enhanced the repair and regeneration of critical sized bone defects [83].

The transplantation of stem cells using alginate hydrogels has been widely explored in bone tissue engineering. The thickness of calcium cross-linked alginate gels was demonstrated to alter the behavior of rat bone marrow cells; however, different geometries did not influence cell differentiation . Bone marrow stromal cells, after being induced down the osteoblast pathway in vitro and mixed with calcium cross-linked alginate gels, repaired horizontal alveolar bone defects in dogs. Alginate/ chitosan gels entrapping mesenchymal stem cells and bone morphogenetic protein-2 also showed potential for trabecular bone formation in mice [84].

Alginate has also been combined with inorganic materials to enhance bone tissue formation. Alginate/ hydroxyapatite (HAP) composite scaffolds with interconnected porous structures were prepared by a phase separation method, which enhanced the adhesion of osteosarcoma cells [85]. Cell-encapsulating alginate gel beads were introduced into calcium phosphatecement, and demonstrated potential for bone tissue engineering under moderate stress-bearing conditions [86]. In addition, alginate gels containing collagen type I and -tricalcium phosphate enhanced adhesion and proliferation of human bone marrow stromal cells that do not readily attach or proliferate on pure alginate gels[87].

2.3.5 Cartilage

Repair of damaged or degraded cartilage is still one of the major challenges facing the orthopedics field, but tissue engineering approaches have recently shown potential in cartilage regeneration. Alginate gels have proved to be useful for transplanting chondrogenic cells to restore damaged cartilage in animal models. Early studies utilized a suspension of chondrocytes in an alginate solution mixed with calcium sulfate, and injected into molds of facial implants in order to produce pre-shaped cartilage. These constructs formed cartilage with three-dimensional shape retention after 30 weeks of subcutaneous implantation into mice and sheep, and the contents of proteoglycan and collagen as well as the elastic modulus of the engineered cartilage reached about 80% of those found in native cartilage [88]. Shape-memory alginate gels were subsequently developed to engineer cartilage with desired shape and size in vivo following minimally-invasive delivery. In brief, macroporous alginate gels with predefined geometries were compressed into a significantly smaller form (dry state) and introduced into mice through a small catheter. The gels were then rehydrated in situ with a suspension of primary bovine articular chondrocytes, and recovered their original

shape and size within 1 h, which allowed cartilage formation in mice with the desired geometry [89].

The use of stem cells in cartilage regeneration is very attractive, due to the invasive and destructive processes required to obtain primary chondrocytes from tissues. Encapsulation in alginate can regulate differentiation of stem cells, and in particular chondrogenesis may be enhanced. It has been demonstrated that chondrogenic lineage of adult stem cells could be regulated via the introduction of soluble factors and biophysical cues in 3D cell culture systems [90]. In addition, it has been hypothesized that chondrogenesis of stem cells relates to the morphology of the encapsulated cells (i.e., rounded cell shape) [91], and alginate gels promote a rounded morphogology that may promote the cellular differentiation process [92].

Human mesenchymal stem cells (MSCs) encapsulated in alginate gel beads have been cultured in serum-free medium with the addition of transforming growth factor (TGF)-1, dexamethasone, and ascorbate 2-phosphate for more than one week, and demonstrated to form cartilage in large osteochondral defects [93]. Rabbit bone marrow stromal cells cultured in alginate gels have also been injected into osteochondral defects in rabbit knees without the use of a periosteal patch, which significantly enhanced the cellular proliferation and chondrogenic differentiation of BMSCs. This resulted in histologically and mechanically improved tissues in osteochondral defects[94]. The chondrogenic potential of human adipose derived stem cells (hASCs) suggests these cells as a possible cell source for cartilage regeneration, and chondrogenic differentiation of hASCs seeded in alginate gels was greatly improved in the presence of TGF-_1 [95]. Pre-differentiated hASCs induced by transduction with an adenovirus carrying a TGF-_2 plasmid maintained the chondrogenic phenotype in vivo and led to new cartilage formation when the cells were encapsulated into alginate gel beads and subcutaneously transplanted into mice[96].

2.3.6 Miscellaneous tissues and organ (Muscle, nerve, pancreas, and liver)

The potentiality of alginate gels are also being actively investigated for their ability to mediate the regeneration and engineering of a variety of other tissues and organs, including skeletal muscle, nerve, pancreas, and liver. Recent strategies for skeletal muscle regeneration include cell transplantation, growth factor delivery, or a combination of both approaches [97], and alginate gels have found potential in these strategies. A combined delivery of VEGF and insulin-like growth factor-1 (IGF-1) from alginate gels was used to modulate both angiogenesis and myogenesis. The localized and sustained delivery of both growth factors led to significant muscle regeneration and functional muscle formation, due to satellite cell activation and proliferation, and cellular protection from apoptosis by the released factors [98]. Long-term survival and outward migration of primary myoblasts into damaged muscle tissue in vivo from RGD-alginate gels were dramatically enhanced by the sustained delivery of hepatocyte growth factor (HGF) and fibroblast growth factor 2 (FGF 2) from the gels [99]. This led to extensive repopulation of host muscle tissues and increased the regeneration of muscle fibers at the wound site [99]. Alginate gels have also been investigated for the repair of the central and peripheral nerve systems. Alginate-based highly anisotropic capillary gels, introduced into acute cervical spinal cord lesions in adult rats, were integrated into the spinal cord parenchyma without major inflammatory responses and directed axonal regrowth[100]. Alginate gels, covalently cross-linked with ethylenediamine, were useful to restore a 50-mm gap in cat sciatic nerves [101], and promoted the outgrowth of regenerating axons and astrocyte reactions at the stump of transected spinal cords in young rats[102]. Alginate gels were also used as glue for repair of peripheral nerve gaps that could not be sutured[103]. Alginate gels may be useful for cell-based neural therapies, as mouse-derived neural stem cells cultured in calcium alginate beads maintained their capacity for multi lineage differentiation into neurons and glial cells. Alginate gels modified with a peptide containing the YIGSR (Tyr-Ile-Gly-Ser-Arg) sequence promoted adhesion of NB2a neuroblastoma cells and neurite outgrowth from the cells, depending on the peptide density in the gels [104].

The alginate gel has a potential scope to provide hepatic tissues for replacement of a failing liver, and alginate gels encapsulating hepatocytes may offer a suitable platform for developing a bio artificial liver as they are easily manipulated and can be cryopreserved [105]. The hydrophilic nature of alginate gels processed to exhibit an interconnected porous structure allows efficient seeding of hepatocytes into the gels, while maintaining high hepatocellular functions [106]. Primary rat hepatocytes maintained viability in alginate gels and appeared to synthesize fibronectin, which was deposited on the spheroids and promoted their functional expression[107]. Hepatocyte engraftment was improved when hepatocytes were transplanted into the liver lobe of Lewis rats using VEGF releasing porous alginate gels[108].

One of the first applications of alginate gels in tissue engineering involved the transplantation of encapsulated pancreatic islet allografts and xenografts in an effort to cure Type I diabetes. In this approach, the gel was used to provide protection from the host immune system, in order to avoid the use of immunosuppressive drugs that would otherwise be required to prevent graft rejection. This approach has been successfully used to treat animal models of Type I diabetes without the use of immunosuppressive drugs [109]. These alginate beads encapsulating islets are generally coated with poly (amino acids), such as poly-L-lysine, to decrease the outer pore size, while keeping a liquid core structure [110]. The transplant volume of microencapsulated islet cells can be reduced by choosing an appropriate alginate composition and purity [111]. However, the mechanical and chemical instability of alginate beads is believed to be a limiting factor for long term survival of the transplanted islets in vivo, prompting continued investigations into the use of different poly (amino acids) as a coating material and the use of various micro fabrications methods [112].

CONCLUSION

Alginate has proved itself as an important pharmaceutical and biomedical drug delivery tool due to its versatile favorable characteristics. Especially in the field of controlled released delivery, wound healing and tissue engineering. The most important features of alginate for these applications include non toxicity, biocompatibility, mild gelation and easy modification technique. Alginate has a very good track record of clinical use both in biomedical and pharmaceutical field. Due to extensive research of alginate properties such as chemical modification, cross linking strategy, establish the structure activity relationship, improving the physical properties of alginate for particular use has increased the potentiality of alginate use in many purposes. The engineering of new classes alginates with precisely designed physical and chemical properties designed for the particular application could revolutionize the use of these materials and so far this is the demand of time.

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