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REVIEW

Cell encapsulation using biopolymer gels for regenerative medicine

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Abstract There has been a consistent increase in the mean life expectancy of the population of the developed world over the past century. Healthy life expectancy, however, has not increased concurrently. As a result we are living a larger proportion of our lives in poor health and there is a growing demand for the replacement of diseased and damaged tissues. While traditionally tissue grafts have functioned well for this purpose, the demand for tissue grafts now exceeds the supply. For this reason, research in regenerative medicine is rapidly expanding to cope with this new demand. There is now a trend towards supplying cells with a material in order to expedite the tissue healing process. Hydrogel encapsulation provides cells with a three dimensional environment similar to that experienced in vivo and therefore may allow the maintenance of normal cellular function in order to produce tissues similar to those found in the body. In this review we discuss biopolymeric gels that have been used for the encapsulation of mammalian cells for tissue engineering applications as well as a brief overview of cell encapsulation for therapeutic protein production. This review focuses on agarose, alginate, collagen, fibrin, hyaluronic acid and gelatin since they are widely used for cell encapsulation. The literature on the regeneration of cartilage, bone, ligament, tendon, skin, blood vessels and neural tissues using these materials has been summarised.

Keywords Tissue engineering · Regenerative medicine · Hydrogels · Cell encapsulation · Biomaterials

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Introduction

As the mean life expectancy of the developed world has increased, there has been an ever increasing demand for the development of new strategies for the repair of diseased and damaged tissues (Crimmins and Saito 2001). Ceramic, metallic and polymeric materials have been investigated widely for the direct replacement of tissues with good success (Zhang and Webster 2009). While the majority of licensed treatments consist of a synthetic material alone, there is a current trend towards the delivery of cells within the material matrix in order to expedite healing (Kretlow et al. 2009). Much of this work has involved the use of sponge-like scaffold materials exhibiting interconnected porosity (Rosa et al. 2008). Although within such structures, the cells are arranged spatially in three dimensions with respect to one another, they still attach to a two dimensional surface and as such do not exhibit a phenotype that would be expected in native tissue. Since cell

Table 1 Biopolymer gels used for cell encapsulation and tissue regeneration and their key features

Biopolymer	Protein/polysaccharide	Source	Gelation method	Mechanism of degradation	Cell adhesivity
Agarose	Polysaccharide	Seaweed	Thermal	Non-degradable	Low
Alginate			Ionotropic cross-linking	Ion exchange	
Collagen	Protein	Animal/human	Neutralisation	Enzymatic cleavage	High
Fibrin			Proteolytic cleavage		
Gelatin			Thermal		
HA	Polysaccharide		Chemical cross-linking		

HA hyaluronic acid

phenotype is critical to correct healing of the damaged tissue, a number of workers have attempted to deliver cells within a matrix that is more akin to the extracellular matrix (ECM) than sponge-like polymers or ceramics. The structure of a hydrogel is morphologically similar to that of the ECM and when used as an encapsulation medium (Bokharia et al. 2005), it enables the cell population to exhibit phenotypes more similar to those in vivo than when the cells are grown in monolayer culture (Abbott 2003). A vast range of different hydrogel based materials are available with contrasting chemistries which may or may not allow cell attachment (Lee and Mooney 2001). In this review, we discuss approaches that have been taken to repair diseased or damaged tissues using biopolymeric hydrogels for cell encapsulation. We give a basic overview of the most frequently used biopolymer gel materials (Table 1) and also discuss their application in the repair of a number of tissue types.

Biopolymer gels

Alginate

Alginate is a polysaccharide isolated from brown algae which has been used with great success as a wound dressing (Augst et al. 2006) and as a food additive. On dissolution in an aqueous medium, alginate forms a hydrocolloid, which gels ionotropically following the addition of multivalent cations. Alginate is formed from polysaccharides derived from a range of seaweeds found world-wide and consists of a mixture of β-D-mannuronic acid (M) and α-L-guluronic acid residues (Lee and Mooney 2001). The ratio of M to G blocks can vary significantly depending upon the source of the raw materials used in alginate manufacture. Both cell adhesion and hydrogel stiffness can be influenced by M to G ratio (Wang et al. 2003). Since hydrogel formation occurs following electrostatic interaction between the carboxylic moieties on the G blocks of alginate and multivalent cations, the higher the ratio of G:M, the stiffer the resulting gel. Cell adhesion to alginate gels can be increased by covalently modifying the polymer with molecules such as RGD (Rowley and Mooney 2002).

Fibrin

Fibrin gels occur widely in the human body and are important in haemostasis following injury (Janmey et al. 2009). Fibrin gels form following the cleavage of fibrinogen by thrombin to expose regions on the fibrin molecules that interact allowing self assembly of protofibrils which aggregate and lengthen. At certain points in the fibrin network, branching occurs, which increases the volume of the gel. The presence of RGD motifs within the fibrin network allow cell adhesion and binding of a range of important growth factors. In the presence of mammalian cells, however, fibrin can degrade rapidly due to the localised secretion of proteolytic enzymes (Ye et al. 2000). To overcome this problem fibrin degradation inhibitors and fibrin stabilisers, such as aprotinin, factor XIII and ε-amino-*n*-caproic acid, have been added to the gels to maintain the structure over longer time periods (Mol et al. 2005; Park et al. 2005; Ye et al. 2000). Furthermore, the importance of sufficient thrombin and calcium to prevent excessive degradation has been identified (Eyrich et al. 2007).

Collagen

Collagen is the most abundant form of protein in humans, constituting 30% of all protein found in the body. There are 29 different forms of collagen in the body, the most ubiquitous which is type I collagen, which comprises triple α-helices, which in the correct environmental conditions self assemble to form a fibrillar structure (Pachence 1996). The self-assembling tendencies of type I collagen have led to it being used as a hydrogel for use in tissue engineering. Rat tail collagen, for example, may be dispersed in acid medium and when neutralised in culture forms gels which have been widely used since they allow cell adhesion. The two major limitations of collagen-based scaffolds are their weak nature and their extensive contraction by encapsulated cells. One group has sought to address both of these drawbacks by plastic compression of the collagen gel immediately after its formation (Brown et al. 2005).

Gelatin

Gelatin is formed from the hydrolysation of collagen. Two different forms of gelatin with different isoelectric points can be formed depending on the hydrolysation protocol. Gelatin dissolves in water at 60°C then gels as the solution cools to room temperature (Young et al. 2005). Gelatin is widely used in pharmaceutical and medical applications due to its biodegradable nature (Ikada and Tabata 1996; Miyoshi et al. 2005).

Hyaluronic acid

Hyaluronan is found throughout the body in a number of tissues, including skin and cartilage. Colloids of hyaluronic acid (HA) can be gelled by prior chemical modification of hyaluronan with thiols, methacrylates or tyramines, or can be cross-linked in situ using formaldehyde or divinyl sulphone. One of the key advantages of using HA gels for tissue engineering is that their degradation can be mediated by hyaluronidase, an enzyme secreted by a multitude of mammalian cell types (Peppas et al. 2006).

Agarose

Agarose can be formed into a gel that once heated to 90°C forms a polymer solution. When the temperature of this solution is lowered to room temperature gelation will occur. Agarose is used widely in molecular biology and since it is well accepted following implantation it has been evaluated for immunoisolation purposes (Lahooti and Sefton 2000a, b, c). The non-degradable nature of the gel, however, means that it has not been widely used in tissue engineering since scaffold materials used in tissue engineering applications should degrade over time to allow space for accumulation of new tissue.

The application of biopolymer gel encapsulation in regenerative medicine

Cell encapsulation in biopolymer hydrogels was initially investigated for immunoisolation of cells producing therapeutic proteins for treatment of diseases. Some of these studies are summarised in Table 2. More recently, encapsulation of mammalian cells has been used in the regeneration of an array of different tissues. Table 3 summarises a broad cross section of the literature covering the majority of the body tissues. The remainder of the review, however, will focus on the use of biopolymer gels for the encapsulation of cells for use in musculoskeletal, neural, skin, hepatic and cardiovascular tissue engineering.

Musculoskeletal tissue engineering

During embryogenesis musculoskeletal tissue develops from the mesoderm. Mesenchymal stem cells (MSCs) differentiate to form the individual musculoskeletal tissues, which include muscle, cartilage, bone, tendons and ligaments. The musculoskeletal system enables locomotion and with the ageing population, novel approaches to regenerate parts of the musculoskeletal system and therefore restore patient mobility are gaining increasing attention.

Muscle

In musculogenesis MSCs differentiate to myoblasts, the muscle cell precursors, which further differentiate

Table 2 Biopolymer gel cell encapsulation for treatment of diseases with non-autologous cells

Biopolymer gel	Encapsulated cell type	Secreted therapeutic protein	Disease treatment	References
Agarose	Islet cells	Insulin	Diabetes	Gazda et al. (2007)
	Kidney cells	Hepatic lipase	Hyperlipidemia	Lahooti and Sefton (2000a)
	Fibroblasts	Alkaline phosphatase	Osteoporosis	Lahooti and Sefton (2000b)
Alginate	Fibroblasts	VEGF	Cardiovascular disease	Keshaw et al. (2005)
	Kidneys cells	Endostatin	Brain tumour	Joki et al. (2001), Read et al. (2001a, b)
	Myoblasts	Human factor IX	Haemophilia	Hortelano et al. (1996)
	Fibroblasts	Human factor IX	Haemophilia	Liu et al. (1993)
	Fibroblasts	Human growth hormone	Dwarfism	Chang et al. (1994)
	Islet cells	Insulin	Diabetes	Calafiore et al. (2006), Soon-Shiong (1999), Zimmermann et al. (2007)
Collagen	Fibroblasts	Alkaline phosphatase	Osteoporosis	Lahooti and Sefton (2000c)

and fuse to form multinucleated myotubes. These myotubes mature into myofibres which bundle together to form the skeletal muscle (Berendse et al. 2003). Encapsulated skeletal myoblasts have been shown to remain viable and differentiate to form myotubules in collagen gel when mechanical conditioning is used after 7 day's culture in vitro. Cell alignment was shown to occur uniaxially in the direction of applied tension (Cheema et al. 2003).

Cartilage

Cartilage is found in joints and prevents bone to bone contact. The tissue consists of mainly chondrocytes and an ECM containing collagens type I and II and aggregan (Sterodimas et al. 2009). A large amount of research has focussed on tissue engineering cartilage by the encapsulation of chondrocytes in a variety of hydrogels including fibrin, collagen, chitosan and alginate. Chondrocytes encapsulated in fibrin gels have been maintained for 5 weeks in vitro (Park et al. 2005). Park et al. (2005) also showed that addition of HA gel to the fibrin gel may be beneficial since after 4 weeks in vivo culture the degree of contraction was reduced and ECM production was higher in fibrin/HA gels when compared with pure fibrin gels. Chondrocytes encapsulated in pure HA gels have been evaluated both in vivo up to 12 weeks and in vitro up to 2 weeks post-encapsulation. The constructs were shown to maintain or increase in size and encapsulated chondrocytes were shown to deposit ECM both in vivo and in vitro. The amount of ECM produced was also shown to be enhanced under mechanical loading (Chung et al. 2008).

Chondrocytes encapsulated in collagen and gelatin gels were shown to produce cartilage specific matrix over 21 days in vitro culture. The collagen gel was shown to contract after 1 day and an increase in cell number was observed throughout the first 7 days. The viable cell number however, fell gradually over the subsequent 14 days culture. In contrast, the viability of chondrocytes encapsulated in gelatin hydrogels was maintained, with no significant change in cell number (Hoshikawa et al. 2006). Maintaining the viability of encapsulated chondrocytes can be problematic due to insufficient perfusion of scaffolds with a large volume. The addition of microchannels to polymeric gels has been proven to maintain the viability and function of alginate encapsulated chondrocytes (Choi et al. 2007), and this may prove effective for other polymeric gels.

Ligament and tendon

Tendons and fibroblasts are rich in fibroblasts which secrete ECM containing collagen type I/III, elastin and proteoglycans (Carvalho et al. 2000; Chan and Leong 2008; Cheema et al. 2007; Cleary et al. 1967). Collagen and fibrin gel encapsulation of fibroblasts have been shown to have promise for tissue engineering of tendons and ligaments. Marenzana et al. (2006) showed that tendon fibroblasts encapsulated in

Table 3 A summary of key references from the current literature describing cell encapsulation for the regeneration of a number of tissues

Biopolymer gel	Encapsulated cell type	Engineered tissue	References
Agarose	MSCs	Cartilage	Pelaez et al. (2009)
Alginate	Dorsal root ganglia	Neural	Bellamkonda et al. (1995)
	Fibroblasts	Dermis	Hunt et al. (2009a)
	Chondrocyctes	Cartilage	Choi et al. (2006), Hong et al. (2007)
	MSCs	Bone	Smith et al. (2007), Wang et al. (2003)
	Osteoblasts	Bone	Kong et al. (2003)
	Hepatocytes	Liver	Khattak et al. (2007)
	Neural progenitor cells	Neural	Zielinski and Aebischer (1994)
Chitosan	Chondrocyctes	Cartilage	Hong et al. (2007)
	Fibroblasts	Neural	Zielinski and Aebischer (1994)
Collagen	Myofibroblasts	Skeletal muscle	Cheema et al. (2003)
	Fibroblasts	Dermis	Brown et al. (2005), Nazhat et al. (2006), Marenzana et al. (2006)
	Fibroblasts	Ligament	Murray et al. (2006)
	Fibroblasts	Tendon	Marenzana et al. (2006)
	Chondrocyctes	Cartilage	Hoshikawa et al. (2006)
	Osteosarcoma cells	Bone	Bitar et al. (2007)
Fibrin	Myofibroblasts	Cardiac muscle	Ye et al. (2000), Birla et al. (2005)
	Myofibroblasts	Blood vessel	Mol et al. (2005)
	Smooth muscle cells	Blood vessel	Grassl et al. (2003)
	Fibroblasts	Dermis	Tuan et al. (1996), Brown et al. (1993), Meana et al. (1998), Cox et al. (2004)
	Fibroblasts	Ligament	Chun et al. (2003)
	Keratinocytes	Epidermis	Bannasch et al. (2000, 2008)
	Chondrocytes	Cartilage	Park et al. (2005), Eyrich et al. (2007), Mesa et al. (2006)
	MSCs	Bone	Catelas et al. (2006), Hou et al. (2008)
	Hepatocytes	Liver	Bruns et al. (2005)
	Dorsal root ganglia	Neural	Herbert et al. (1998)
	Urethral cells	Urethra	Bach et al. (2001)
	Keratocytes	Corneal stroma	Alaminos et al. (2006)
Gelatin	Chondrocyctes	Cartilage	Hoshikawa et al. (2006)
	Hepatocytes	Liver	Wang et al. (2006)
Hylauronic acid	Chondrocytes	Cartilage	Chung et al. (2008)
	Dorsal root ganglia	Neural	Horn et al. (2007)

collagen gels attached, spread and were orientated in the parallel to the long axis of the collagen fibrils. The fibroblasts produced tensile forces and remodelled the matrix (Marenzana et al. 2006). Ligament fibroblasts encapsulated in collagen gels proliferated and secreted further collagen up to day 14, then maintained the collagen level and cell number for a further 7 days culture. Gel contraction was seen to occur over the 21 days culture (Murray et al. 2006). Similarly, ligament fibroblasts encapsulated in fibrin gels have been shown to proliferate, contract the gel and secrete ECM (Chun et al. 2003).

Bone

Bone tissue consists mainly of osteoblast secreted mineralised ECM containing collagen type 1, collagen type IV, fibronectin and heparan sulphate (Narayanan

et al. 2009; Williams et al. 1989). Fibrin gel encapsulation has been investigated as an approach for bone tissue engineering. Where MSCs have been used, their osteogenic differentiation is measured by expression of ALP, osteopontin, bone sialoprotein (BSP), and osteocalcin. MSCs encapsulated in fibrin gel have been shown to proliferate, but at a slower rate than in monolayer culture. Encapsulation was seen to enhance osteogenic differentiation and ECM production, compared with monolayer culture. Mineralised tissue was seen to accumulate in the pores that formed in the fibrin gel, which were surrounded by numerous cells (Hou et al. 2008). Catelas et al. (2006) also showed that MSCs encapsulated in fibrin gels proliferate, show osteogenic differentiation, and secrete mineralised tissue. They, however, observed that the MSCs did not fully differentiate to mature osteoblasts within the 28 days of in vitro culture.

Skin

Skin is composed of two anatomically distinct layers known as the epidermis and the dermis. It comprises loose connective tissue rich in collagen type I that contains blood capillaries, smooth muscle fibres, sweat glands and sebaceous glands and their ducts, hair follicles and sensory nerve endings. The epidermis consists mainly of tightly packed stratified keratinocytes upon a basement membrane (Fuchs 2008; MacNeil 2007; Williams et al. 1989). For tissue engineering skin fibrin has been used to encapsulate both fibroblasts, to develop a dermal analogue (Cox et al. 2004; Meana et al. 1998) and keratinocytes to create an epidermal analogue (Bannasch et al. 2000, 2008). Encapsulated fibroblasts were shown to proliferate within the fibrin matrix, but also allow for the stratification of the co-cultured keratinocytes on the surface of the gel both in vivo and in vitro (Meana et al. 1998). Fibrin-encapsulated keratinocytes applied to the surface of Alloderm produce a continuous epithelium with a cornified layer and basement membrane after 4 weeks in vivo (Bannasch et al. 2008).

Brown et al. (2005) and co-workers have shown that plastic compressed collagen gel can be used to encapsulate fibroblasts for tissue engineering the dermis. The compression was only seen to reduce cell viability of encapsulated human dermal fibroblasts by 10% as long as the gel did not become desiccated. A concern when increasing the collagen content of the hydrogel is that diffusion of nutrients and waste products through the scaffold would not be sufficient to maintain cell viability over extended periods of time. To address this problem Nazhat et al. (2007) incorporated micro-channels into the gels using soluble phosphate glass fibres and after 24 h the cell viability of encapsulated fibroblasts was seen to be greater than 80%.

We have shown that fibroblasts encapsulated in alginate hydrogel serve as a dermal analogue allowing for a stratified epithelium to form on the surface of the alginate from the surface seeded keratinocyte co-culture (Hunt et al. 2009a). Furthermore, we have shown that these cells remain mitotically inhibited up to 33 days encapsulation (Hunt et al. 2009b) and that the cells remain viable for at least 150 days encapsulation. The hydrogel was shown to degrade by acellular mechanisms in vitro to release the encapsulated fibroblasts, which were seen to subsequently secrete ECM (Hunt et al. 2009a). We have also observed that the alginate is not contracted by the encapsulated fibroblasts, and this may result in reduced detrimental scar contraction which is often seen in large skin wounds (Harrison and MacNeil 2008).

Neural tissue

Chronic neurological diseases and physical injuries can result in loss of neuronal cell bodies, axons and associated glia support. Since the central nervous system has limited or no capacity to replace the lost neurons, there is a significant interest in the engineering of neural tissue (Nisbet et al. 2008). One of the major challenges facing researchers in this area is stimulation of guided axonal extension (Norman et al. 2009). To this end, Horn et al. (2007) encapsulated chick dorsal ganglia in HA and fibrin gels which were evaluated both in vitro. Both gels were shown to support neutrites within the first 60 h, but after 192 h a 50% increase in neutrite length was seen in HA gels when compared with the fibrin gels. Despite this, when the neurons encapsulated in the HA gels were implanted, there was no restoration of spinal cord function. Herbert et al. (1998) also investigated the use of fibrin gels for neural tissue engineering. Encapsulated chick dorsal root ganglia were successfully

maintained in the hydrogels and displayed neutrite outgrowth. Bellamkonda et al. (1995) showed that PC12 neural progenitor cells and chick dorsal ganglia encapsulated in agarose also produced neutrites of 900 μm in length after 4 days in culture.

Another approach to neural regeneration is the localised and sustained release of nerve growth factor (NGF) to stimulate neural differentiation of precursor cells. For example, Zielinski and Aebischer (1994) have shown that a genetically modified fibroblast cell line provided sustained release of nerve growth factor (NGF) during encapsulation in chitosan hydrogels. The amount of NGF secreted was sufficient to induce the differentiation of co-cultured neural progenitor PC12 cells. The limitation of this approach, however, was that fibroblasts were seen to aggregate and after 2 weeks which resulted in necrosis in the centre of the aggregates.

Liver

Hepatic tissue is formed of hepatocytes which are organized into a polarized epithelium with distinct apical and basal domains (Dunn et al. 1989). Hepatocytes have a high metabolic activity and produce many liver specific molecules such as urea, amino acids, glycogen and bile. The successful tissue engineering of liver is often assessed by measuring the secretion of these liver specific products.

Hepatocytes encapsulated in fibrin showed maintained viability over the first 3 days culture in vivo, but thereafter showed significant loss despite the maintenance of an even distribution of cells. Neotissue formation, however, was observed with good integration into the host tissue after 7 days in vivo as well as maintained liver specific function and phenotype in viable cells (Bruns et al. 2005).In order to facilitate the diffusion of nutrients within hydrogels encapsulating hepatocytes to maintain cell viability and function, various approaches have been taken. For example, the incorporation of micro-channels into gelatin hydrogels was shown to maintain 90% viability and metabolic activity of the encapsulated hepatocytes after 45 days in vitro culture (Wang et al. 2006). Similarly, Khattak et al. (2007) have shown that perfluorocarbon incorporation can improve metabolic activity and viability of encapsulated hepatocytes for 2 weeks in vitro culture.

Cardiovascular tissue

The cardiovascular network is essential to maintaining the viability and functions of all living tissues. The network can become obstructed of damaged due to disease or injury and thus efforts towards the tissue engineering of blood vessels and cardiac tissue are being made (Stegemann et al. 2007). In order to engineer vascular tissue, myofibroblasts have been encapsulated in fibrin gels (Mol et al. 2005; Ye et al. 2000) and viability maintained for up to 6 weeks in vitro. The cells were also seen to proliferate and secrete collagen. Smooth muscle cells have also been encapsulated fibrin gels in order to produce an arterial media equivalent. After 3 weeks in vitro culture the arterial equivalent was seen to have a 95% reduction in volume and was shown to accumulate collagen, which was aligned in the circumferential direction of the tubular structure. The accumulation of collagen and the gel contraction was associated with an increase in ultimate tensile strength of the construct (Grassl et al. 2003).

Cardiac tissue has successfully been engineered in vivo by encapsulation of neonatal cardiac myocytes in fibrin gel within a silicon chamber implanted near an artery to maintain cell viability. After 3 weeks the implant was found to be viable with large amounts of muscle tissue which contracted in response to electrical stimulation and had neovascularisation throughout (Birla et al. 2005).

Conclusion

Biopolymer gel encapsulation of mammalian cells is finding increasing application in engineering a variety of different tissues. This review has summarised the most widely used biopolymeric hydrogels in tissue regeneration as well as the response of a range of cell types to encapsulation. Researchers have studied a variety of factors including cell viability, growth, matrix production and differentiation. Due the variation in culture conditions and material formulation, it is often difficult to make conclusions on the effectiveness of a particular polymeric gel for tissue regeneration applications and there is a need for an increase in systematic research in this field. What is clear is that alginate, collagen, fibrin, hyaluronic acid, and gelatin all show promise as materials for the

encapsulation of cells for tissue engineering of cartilage, bone, ligament, tendon, skin, blood vessels and neural tissues and there is no doubt that polymeric cell encapsulation has great potential in the future of regenerative medicine. The potential importance, however, of tailoring degradation, ensuring sufficient diffusion through the biopolymer scaffolds and mechanical conditioning to ensure success of tissue engineering by encapsulation of cells in biopolymer gels have been highlighted in this review.

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