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Advances in skeletal tissue engineering with hydrogels

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Structured Abstract

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Objectives – Tissue engineering has the potential to make a significant impact on improving tissue repair in the craniofacial system. The general strategy for tissue engineering includes seeding cells on a biomaterial scaffold. The number of scaffold and cell choices for tissue engineering systems is continually increasing and will be reviewed.

Design – Multilayered hydrogel systems were developed to coculture different cell types and develop osteochondral tissues for applications including the temporomandibular joint.

Experimental variable – Hydrogels are one form of scaffold that can be applied to cartilage and bone repair using fully differentiated cells, adult and embryonic stem cells.

Outcome measure – Case studies represent an overview of our laboratory's investigations.

Results – Bilayered scaffolds to promote tissue development and the formation of more complex osteochondral tissues were developed and proved to be effective.

Conclusion – Tissue engineering provides a venue to investigate tissue development of mutant or diseased cells and potential therapeutics.

Key words: hydrogels; stem cells; tissue engineering

Introduction

Tissue engineering has demonstrated significant potential for skeletal tissue repair that may be applied to the treatment of craniofacial tissue loss. The general premise of tissue engineering is to provide a functional biological tissue equivalent to replace tissue lost by disease, congenital abnormalities, or traumatic events. The standard approach of tissue engineering is to seed cells on a three-dimensional (3D) biomaterial scaffold (1). The scaffold is designed to create a 3D environment that promotes tissue development of cells that are

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placed on or within the scaffold. Gene vectors, soluble factors, and chemical signals may be incorporated into the scaffold to help promote tissue development during *in vitro* incubation or *in vivo* implantation. Tissue engineering has been applied to many tissues and organs in the body including numerous craniofacial structures including teeth, bone for the cranium, and bone–cartilage structures for the temporomandibular joint (TMJ). The discovery of embryonic stem (ES) cells and the advances in understanding of adult stem cell capabilities have injected excitement into the field of tissue engineering as more powerful cell sources, building blocks of the new tissue, are discovered. This review will discuss a number of the cell and scaffold options available for tissue engineering. A summary of our approach for engineering cartilage and bone using hydrogels will be presented. Case studies for new methods to build more complex tissue structures, improving scaffold function, and understanding craniofacial disease using tissue engineering will be provided.

Clinical need

Current medical and surgical therapies can produce remarkable results for many diseases and pathologies related to cartilage and bone tissues. The treatments for complex fractures, end-stage arthritic joint disease, limb deformities, and craniofacial pathologies have all seen recent dramatic improvements. Patients with disorders of the musculo-skeletal and craniofacial systems have more therapeutic options today than ever before, however, there are still vast improvements in technologies and therapies that need to be realized, especially in the areas of repairing articular cartilage and severe bone defects. Current therapies used to correct articular cartilage defects in joints include penetration of subchondral bone (2–4), mosaiplasty or autograft osteochondral transplant (5, 6), osteochondral allograft placement (7), partial and total joint arthroplasty, and recently, autologous chondrocyte transplantation (ACT) (8, 9) [for an extensive review see (10)]. The need for cartilage in the craniofacial skeleton is also high. Cartilage tissue is often harvested from distant sites and used in nasal and ear reconstructions in Plastic and Reconstructive surgery. The TMJ of the jaw bone is a complex articulation that can be involved in numerous pathologies leading to cartilage wear and failure that requires invasive surgical correction (11).

While providing some benefit, current surgical procedures have important shortcomings such as suboptimal long-term outcome, implantation and long-term presence of alloplastic material, donor site and joint morbidity, invasive surgical approach, risk of infection, and structural failure. Allografting and autografting strategies have other shortcomings such as the possibility of disease transmission, rejection of allograft tissue, insufficient autologous resources, contour irregularities, major histo-incompatibility, graft-vs.-host disease, and potential need for immunosuppression (11–14). The emerging field of tissue engineering has widened the search for better and less invasive treatments for many disease processes.

When designing a tissue engineering system, or improving upon current technologies, one must consider the choice of cell, scaffold, and biological signals or cues that must be provided. A summary of current technology and available 'tools' for assembling a tissue engineering system are described.

Cell Source

There are numerous cell and scaffold choices that are available for engineering cartilage and bone, all with unique advantages and disadvantages. Academic research and clinical therapies have utilized cells alone, cells in combination with a biomaterial scaffold, and biomaterials that induce host cell activities, for skeletal regeneration technologies. Addition of a cellular component to a scaffold may aid in repairing tissue at a faster rate and for repairing larger defects. However, identification of an appropriate cell source remains a significant barrier to the realization of cell-containing materials capable of replacing current bone or cartilage reconstruction techniques.

Cell-based approaches to bone and cartilage tissue engineering generally require a large number of cells for scaffold seeding. Therefore, cells must be able to proliferate extensively while also maintaining the ability to differentiate and retain tissue forming activities, such as, extracellular matrix (ECM) secretion, and mineralization for the case of bone. Both autologous and allogenic cells are being considered as cell sources for tissue engineering, including adult and ES cells.

Fully differentiated cells

Currently, autologous chondrocyte implantation (ACT) is the sole FDA-approved cell-based cartilage repair product available in the United States (9, 15). This procedure requires the harvest of cartilage from a non-weight bearing portion of the knee, isolation and expansion of the chondrocytes from the tissue, and subsequent implantation of the cells into the joint. One limitation is the amount of tissue that can be harvested as well as donor site morbidity (16, 17). As a result, monolayer expansion of the cells is necessary to obtain sufficient cell numbers to construct a clinically useful implant. However, when chondrocytes are removed from their native tissue environment and expanded in monolayer, they lose their chondrogenic phenotype characterized by loss of spherical cell morphology, production of type I collagen instead of type II collagen, and loss of aggrecan gene expression (18). Numerous groups have provided additional evidence for the application of periosteum-derived cell constructs using a variety of scaffold materials in animal studies and clinical cases (19, 20).

Mesenchymal stem cells

A stem cell is capable of dividing to form equal daughter cells (self renewal) and to differentiate into two or more tissue-specific cell lineages when provided the appropriate cues (21–23). These properties are useful for tissue engineers as they are capable of 1) proliferating to achieve the often burdensome cell number requirements to make new tissue and 2) differentiating into multiple cell types to form new repair tissue. Adult cells with stem cell-like properties that can form cartilage have been also isolated from the bone marrow, fat, and muscle, expanding the 'tool box' of cell types that are capable of proliferation and differentiation (24–27).

Adult bone marrow is a major source of hematopoetic stem cells (HSCs) responsible for renewing circulating blood components. The marrow also contains non-HSCs, termed mesenchymal stem cells (MSCs), which contribute to the regeneration of mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, adipose, and stroma (25). MSCs in the body are recruited to repair injured tissues, making them good candidates for cell-based therapies in musculoskeletal tissue engineering (28). MSCs can be easily isolated and expanded in vitro while maintaining their ability to differentiate into chondrogenic, osteogenic, and adipogenic lineages. Cell therapies using MSCs have the flexibility to be applied either autologous, from a patient's bone marrow aspirates, or allogenic, from a cell bank. Recent studies have demonstrated the immuno-privileged status of these cells and the possibility of allogenic application of MSCs. LeBlanc *et al.*, demonstrated that MSCs (both undifferentiated and differentiated) do not elicit alloreactive lymphocyte response. The cells had HLA Class I, but not Class II receptors present on their surface, allowing them to avoid recognition as a foreign cell (Class II receptors were present only intracellular) (29).

Embryonic stem cells

Embryonic stem cells have entered the horizon as a cell source for tissue engineering. Embryonic cells with stem cell properties can be isolated from the inner cell mass of a blastocyst or from the primordial gonadal ridge of the fetus (22, 23). ES cells have generated considerable excitement from tissue engineers as these cells may have the potential to address the need for large numbers of cells that maintain the capability to differentiate and form tissue. ES cells can be potentially expanded indefinitely in an undifferentiated state and differentiate into all tissue found in the body. When ES cells are placed in clusters and allowed to differentiate, they form embryoid bodies (EBs) that contain cell types from all three germ layers randomly distributed (21).

Recently, the ability of chondrocytic cells derived from mouse ESCs to differentiate into an osteogenic cell type has shown that cells from different stages of skeletal developmental processes, such as endochondral ossification, may be isolated from ES cell culture. In addition, mouse and human ES cells have been coerced towards a chondrogenic phenotype (30–33).

There are a number of limitations to the use of ES cells in therapeutic applications. Isolation of homogenous cell populations from ES cells requires selection strategies to ensure isolation of a pure cell-type from spontaneously differentiating cells within EBs. Furthermore, the purity of any isolated population may be questionable because of the plasticity of cells, which are present at various stages of differentiation. ES cells must be applied allogenic, therefore raising the problem of immune responses. However, as we continue

to learn more about ES cell behavior, selection/purification and differentiation strategies will develop that will allow realization of their potential.

Scaffold choices

Naturally-derived and synthetic scaffold materials have been used to exploit the regenerative capacities of host-tissues or transplanted cells (34). The realization of a tissue engineered construct for cartilage and bone repair and replacement poses a number of specific requirements on scaffold materials including biocompatibility, osteoconduction or induction, temporary mechanical support, controlled degradation, and adequate interstitial fluid flow.

Initial attempts at creating alternatives to conventional bone grafts (allografts and xenografts) were to develop synthetic bone replacements. Numerous investigations led to development of a number of bone-void filling materials and graft extending materials, some with current clinical availability. Investigations by multiple research groups led to a long list of biomaterials with osteoconductive properties, including: titanium (35), natural/coralline hydroxyapatite (HA) (36), porous HA and calcium phosphates (37), photopolymerizable polyanhydrides, bioglass (38), and polyester copolymers [poly(lactic-co-glycolic acid; PLGA)] (39). Further, investigation revealed that modifications of scaffold architecture and material properties could improve bone growth. This led to a research thrust aimed at creating scaffold materials with biomimetic properties that would mimic the role of the ECM in many cell functions, including: adhesion, migration and proliferation. For example, median pore size has been found to influence conductive properties of tissue engineering scaffolds (40, 41). Additionally, several biomaterials have been modified with adhesive peptide domains prevalent in ECM proteins (42, 43). These studies showed that osteoblasts seeded on functionalized biomaterials promoted cell adhesion and attachment (42, 43), and expression of ECM proteins (44). A number of other surface properties have been found to effect cell function within polymeric biomaterials including texture, hydrophobicity, charge and chemical composition (39–45). Further engineering of biomaterial surface and bulk properties will allow biospecific interactions between appropriate cell types and scaffold materials.

Scaffolds for cartilage tissue engineering applications have different biological and physical requirements compared with bone scaffolds. Scaffolds composed of synthetic and natural materials in a variety of physical forms (fibers, meshes, gels) have been applied to cartilage tissue engineering (16, 45). Solid scaffolds provide a substrate upon which cells may adhere, while liquid and gel scaffolds function to physically entrap the cells. An example of a solid scaffold is poly(glycolic acid), PGA. PGA meshes have been successfully used to engineer tissues including cartilage and bone both *in vitro* and *in vivo* (45–47). Recently, two solid scaffold systems, polycaprolactone and PGA, have been applied to chondrogenesis of MSCs with some success (48–50). Scaffolds derived from hyaluronic acid have also demonstrated positive tissue forming abilities using chondrocytes and MSCs (51, 52).

Hydrogels are a class of scaffolds that have been studied in tissue engineering and include alginate, Pluronics, chitosan, and fibrin glue as examples. Fibrin glue is a biological gel that has been used to encapsulate chondrocytes, but the resulting gel is weak and there is little control in the network formation (53). Alginate, a polysaccharide, forms an ionic network in the presence of divalent or multivalent ions. Many groups have investigated the activity and biological properties of cells entrapped in alginate *in vitro* (54, 55). Alginate has also been examined *in vivo* for use in craniofacial cartilage replacement and as cartilage plugs to prevent vesicoureteral reflux (56, 57). Researchers have also modified alginate with adhesion peptides in order to encapsulate anchorage dependent and independent cells (58, 59). Alginate and agarose (or ionic and thermoresponsive polymers in general) provide little control over the gelation process, particularly in a clinical setting. Once crosslinking is induced, by the addition of an ionic solution or a temperature change, the process cannot be stopped or accelerated. Thus, the need for a new biomaterial or method for cell encapsulation that provides control over gel formation and shape maintenance led to the development of the photopolymerization system for tissue engineering applications (60). These initial studies investigated photopolymerization and tissue regeneration in a non-degradable system.

Biological cues that can be incorporated into tissue engineering systems range from inorganic minerals to adhesion peptides or growth factors. Anseth and

colleagues have attached the adhesion peptide RGD to an injectable hydrogel to enhance development of tissue by bone marrow-derived cells (61, 62). Mooney incorporated RGD peptides into alginate and demonstrated greater cartilage matrix production when the peptide was present (58, 63). More complex systems that are able to tether growth factors or even protease sensitive peptide sequences can be integrated into the hydrogels (64). Soluble growth factors present another potent regulator of cell function that can be utilized to control cell fate in and around biomaterial implants. Furthermore, increasing evidence shows that several of these soluble factors mediate cell signaling through interactions with ECM components (65–67). Since the identification of bone morphogenic proteins (BMPs) as the osteoinductive component of demineralized bone matrix, researchers have been working to create scaffold materials that exploit growth factor signaling. Several groups have utilized synthetic, biodegradable polymer constructs for local delivery of BMPs and transforming growth factors (TGFs), which may have potential for eliciting tissue growth *in vivo* (68–70). Further applications of localized drug delivery include the incorporation of vascular endothelial growth factor into scaffold materials (71, 72). This known angiogenic growth factor may be essential in promoting blood vessel formation to provide nutrient transport within implanted constructs.

Additional research efforts have been aimed at fabricating biomaterials that allow for minimally invasive surgical procedures and flexible implantation. These would improve current grafting techniques that make custom fit at the implantation site difficult and incur additional bone loss and trauma to surrounding tissues. As previously discussed, photopolymerizing hydrogels have been used to investigate bone and cartilage tissue engineering; these systems can provide a unique minimally invasive system for cell-based tissue engineering application that can also function as localized drug carriers (70, 73). Furthermore, photopolymerizing hydrogel systems have been fabricated with controlled degradation characteristics to improve tissue formation for both bone and cartilage repair.

Hydrogels for skeletal tissue engineering

Our laboratory is investigating the application of hydrogels to skeletal tissue engineering systems. Hydrogels are crosslinked polymeric systems that are capable of absorbing large volumes of aqueous solution. Cells can be encapsulated during the hydrogel formation process and hydrogels may be formed *in situ* within a defect site in the body, facilitating application to tissue engineering strategies. Physical properties such as the porosity of hydrogels can be modulated by altering the crosslinking density which in effect changes the volume water that is absorbed in the hydrogel and the mechanical properties (74, 75). These physical properties are important for tissue engineering applications as the water content influences the viability of cells encapsulated in the hydrogel and the rate of tissue development (76). Furthermore, different mechanical properties may be required for harsh environments such as the joint compared to subcutaneous environments in the cranium where mechanical stiffness may be less important. While it may appear that hydrogel-based materials do not have strong enough mechanical properties for application in the skeletal system, they provide a matrix for accelerated tissue formation which will in turn provide mechanical integrity. As with other biomaterials, the chemical and physical properties of hydrogel scaffolds may be altered to improve tissue development.

The following case studies represent an overview of our laboratory's investigations into the efficacy of different cell sources for tissue engineering cartilage and bone and the development of bilayered scaffolds to promote tissue development and the formation of more complex osteochondral tissues. Finally, the application of tissue engineering to study tissue development by mutant or diseased cells will be addressed.

Cell sources for cartilage repair

Over the years, we have investigated the ability of three major cells types to form cartilage in hydrogels; chondrocytes, marrow-derived stromal cells, and ES cells (Fig. 1). Chondrocytes were initially investigated to screen novel materials and methods for cell encapsulation and cartilage tissue engineering both *in vitro* and *in vivo* (77, 78). The advantage of using these cells for cartilage engineering is that primary chondrocytes do not have to differentiate and are immediately able to secrete large amounts of cartilage-specific ECM components. Primary chondrocytes (isolated from a bovine joint) produce cartilage-like tissue that has significant matrix deposition and appears very similar to native

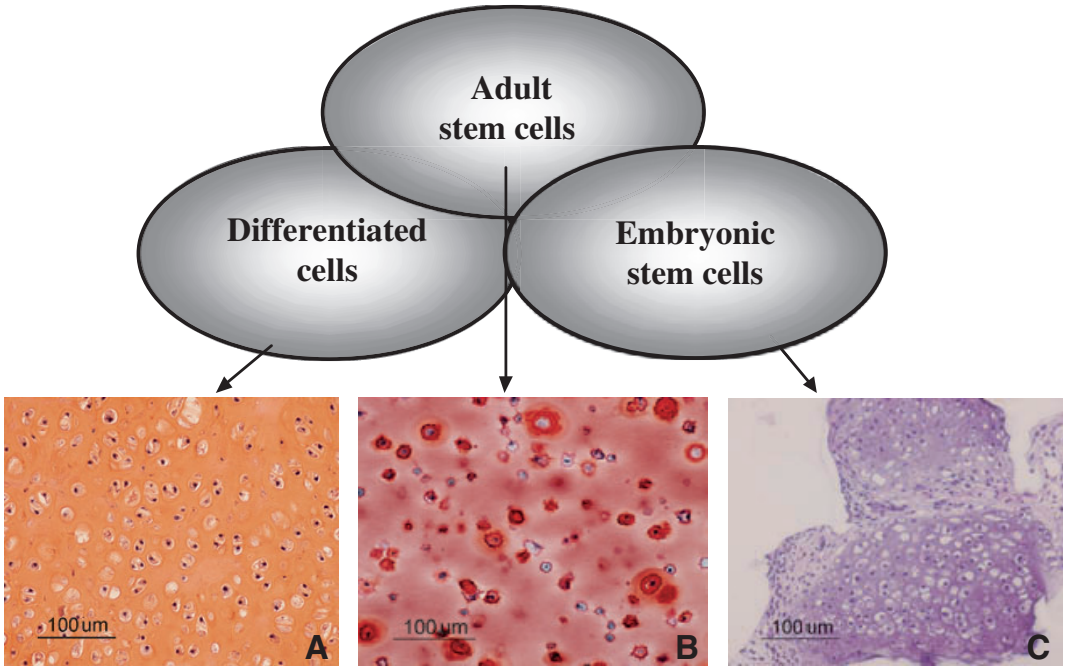

Fig. 1. Chondrocytes, bone marrow-derived stem cells, and embryonic stem cells were encapsulated in hydrogels and incubated in vitro form cartilage-like structures.

tissue in <2 months (Fig. 1A). One limitation of utilizing chondrocytes is the amount of tissue that can be harvested in people as well as donor site morbidity. As a result, monolayer expansion of the cells is necessary to obtain sufficient cell numbers to construct a clinically useful implant. To overcome this issue, marrow-derived cells can be isolated from a patient and expanded while retaining their tissue forming capabilities. Again, cartilage-like tissue may be formed from these cells (Fig. 1B).

Embryonic stem cells can also be encapsulated in hydrogels and have the ability to self replicate and differentiate into cells from all three germ layers, providing a potentially powerful tool for tissue regeneration applications. Unfortunately, researchers still do not understand how to control the differentiation of ES cells and obtain homogenous cell populations. 3D microenvironments with appropriate growth factors and biological cues may be very useful for studying ES cell differentiation. We examined the chondrogenic differentiation capability of ES-derived EBs in photopolymerizing poly(ethylene glycol)-based hydrogels. EBs were formed from mouse ES cells and were cultured for 5 days. The EBs were then mixed with a macromer solution and polymerized to form poly ethylene glycol (PEG) gels. EB-PEG hydrogels (3D) were cultured *in vitro* for 17 days in chondrogenic differentiation medium with TGF-β1. Histological and morphological analysis of 3D culture with TGF-β1 demonstrated basophilic ECM deposition characteristic of neocartilage that was homogenous (Fig. 1C).

Marrow stromal cells for cartilage and bone tissue engineering

The biological signals required for MSC differentiation into mesenchymal tissues such as cartilage, bone, and adipose have been defined and incorporated into *in vitro* and *in vivo* tissue engineering systems (25). Chondrogenesis of bone marrow-derived MSCs with TGFβ has been demonstrated in pellet culture and on poly glycolic acid (PGA) scaffolds (24, 49, 79). We encapsulated MSCs in hydrogels to demonstrate chondrogenesis of MSCs in 3D photopolymerizing hydrogels which may be more suitable for clinical application and for use in our multilayered composite hydrogels (Fig. 2A). Adult goat MSCs were photoencapsulated in hydrogels, cultured, and harvested after 3 weeks. Histological, biochemical, and RNA analyses were performed to evaluate both the differentiation of MSCs into a chondrogenic phenotype as well as the accumulation of ECM products in the hydrogels. Figure 2B visualizes viable cells in the hydrogel by staining with a mitochondrial metabolic marker. Positive staining for proteoglycans is observed after 3 weeks (Fig. 2C) (80).

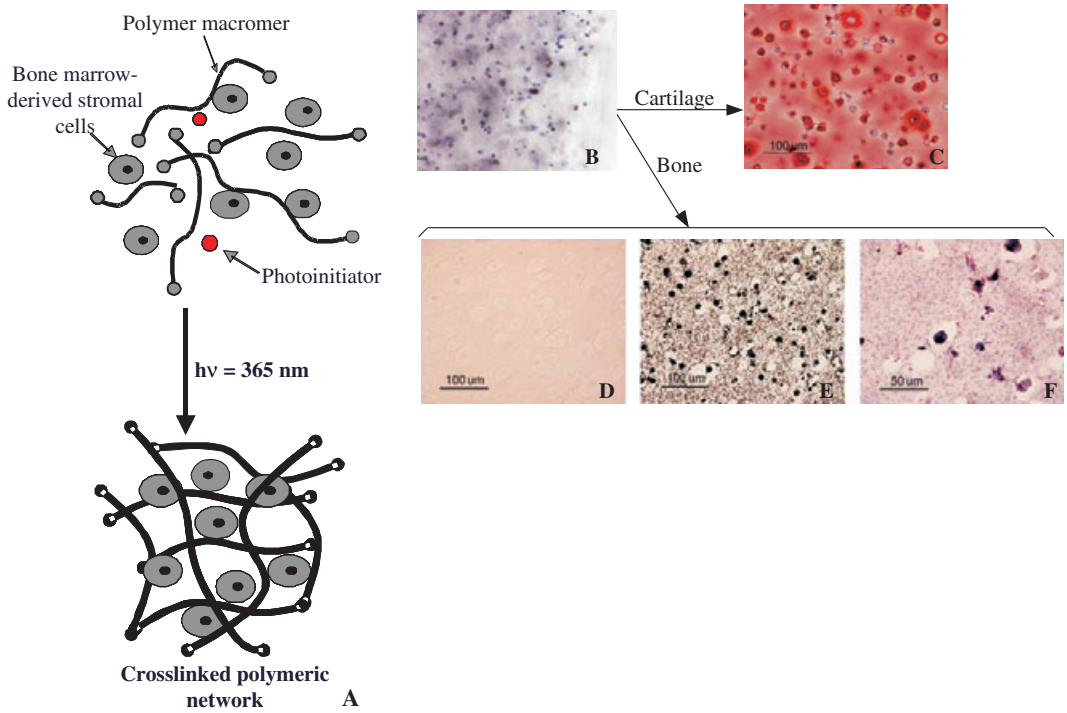

Fig. 2. (A) Photoencapsulation of bone marrow-derived cells by exposure of a liquid polymer solution containing a photoinitiator to light. (B) Mitochondrial metabolic straining of MSCs encapsulated in a hydrogel and resulting neotissue after (C) exposure to TGFbeta1 (safranin O staining), (D) Standard growth medium (von Kossa staining), and osteogenic conditions showing (E) calcium deposition (von Kossa staining), and (F) no cartilage formation by Safranin O staining.

Bone marrow-derived MSCs may also be directed to undergo osteogenesis after encapsulation. Photoencapsulated goat MSCs were stimulated with medium containing dexamethazone and beta-glycerophosphate with or without BMP-2 to promote osteogenesis. Hydrogels became opaque after approximately 1 week and calcium contents significantly increased compared with control hydrogels incubated in MSC growth medium (MSCGM, Clonetics, Fig. 2D). Type II collagen and aggrecan gene expression were negative while Type I collagen was positive, indicating that cells did not undergo chondrogenesis. Histological sections demonstrated positive von Kossa staining for calcium and negative Safranin-O staining for proteoglycans after 3 weeks in culture (Fig. 2E,F, respectively).

New challenges arise when moving *in vitro* systems to the complex *in vivo* environment. One of the main purposes of applying the photopolymerization system to cell encapsulation was the potential for *in situ* polymerization. *In situ* polymerization requires that a macromer solution be injectable and solidify or gel *in vivo*. This allows the macromer to take the form of the irregular shape of the defect. Fig. 3 pictures a defect created on the femoral condyle of a goat. A thin film is placed over the defect and macromer solution is injected underneath the film which serves as a mold. The solution is subsequently polymerized using light, causing a gel to form. The final implant therefore is formed within the defect and is shaped to the surface of the condyle (Fig. 3).

Multilayered hydrogels for osteochondral engineering

Engineering osteochondral tissues that comprise zones of cartilage and bone is desirable for creating structures to replace larger defects and for articular tissue engineering applications such as the TMJ. Furthermore, engineered cartilage is difficult to integrate with host cartilage, while bone can be more easily integrated. Thus, creation of a bone layer adjacent to engineered cartilage may provide an anchor for integration of a cartilage implant. To address tissue engineering of osteochondral tissue, a multilayered hydrogel system was developed (Fig. 4A). The multilayered hydrogel is created by partially polymerizing the first layer to form a semisolid. A second layer of macromer solution is added and both are polymerized. The multilayered hydrogel system allows distinct cell types to be cocultured in 3D systems (81, 82). Encapsulated stem cells (both differentiated and undifferentiated) produce

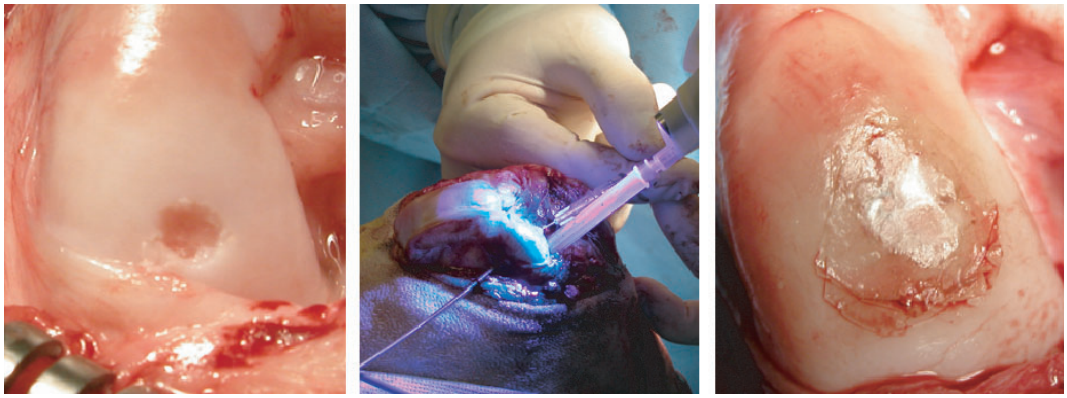

Fig. 3. In situ polymerization of hydrogels in a goat chondral defect. Step 1. Creation of a chondral defect. Step 2. Injection and exposure of polymer to light that forms a contoured surface.

growth factors and ECM molecules that may influence the proliferation and/or differentiation of other cells (84). As the multilayered gel system maintains the cells separate in the layer in which they were encapsulated, the effects of cell coculture on gene expression, matrix production and tissue development can be evaluated.

A bilayered hydrogel was synthesized containing chondrocytes and MSCs in each layer (Fig. 4B). The construct was incubated in osteogenic medium for three weeks. Histological analysis of the bilayered construct reveals that the chondrocytes produced cartilage-like tissue as visualized by positive Safranin-O staining of proteoglycans (Fig. 4C) and did not calcify (no staining by von Kossa). The adjacent MSC layer showed no Safranin-O staining yet contained small calcified structures which stained positive (black) upon von Kossa staining suggesting mineralization (Fig. 4D). Each cell type remained in their respective layer, producing cartilage and bone-related markers.

In vivo osteochondral tissues and formation of a TMJ

Tissue engineering techniques may also be applied to articular joints in the cranium. Numerous attempts have been made to construct a TMJ (83). While the photopolymerizing hydrogel system is amenable to the development of injectable technologies, it may also be applied to *ex vivo* scaffold synthesis to create osteochondral implants in the shape of a TMJ. In collaboration with dental colleagues, Mao *et al.*, a mold was made from a human TMJ (Fig. 5A–C). Subsequently,

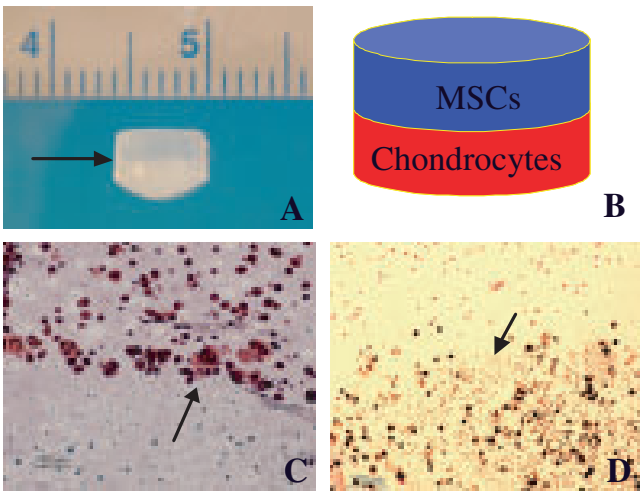

Fig. 4. (A) Gross picutre of a bilayered hydrogel including the interface (arrow), (B) Chondrocytes and MSCs were encapsulated in the bilayered hydrogels, and (C) Safranin O staining for proteoglycans demonstrates the upper layer of chondrocytes while (D) von Kosso staining for calcium delineates the MSC layer.

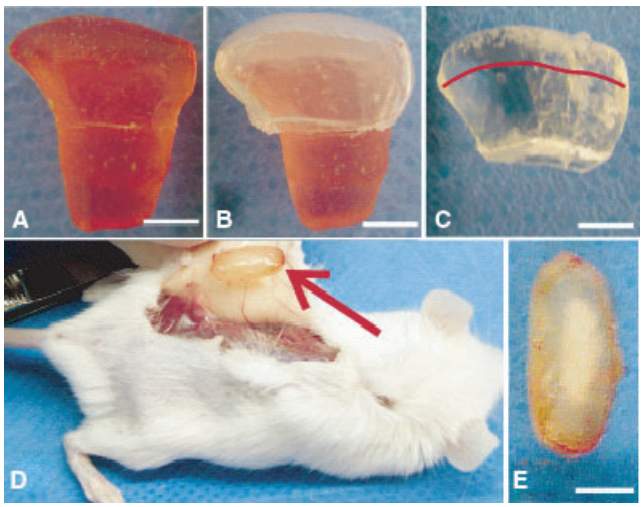

Fig. 5. The bilayered technology can also be extended to the engineering the temporomandibular joint. A mold made from a TMJ (A) was created and a first hydrogel for the cartilage layer is formed (B) and a subsequent layer for bone (C). The bilayered implant is placed in the subcutaneous pocket of a rat (D) and after 12 weeks demonstrates two separate layers of tissue formation (E).

the hydrogel was photopolymerized in the mold in layers to form a cartilage and bone region. Rat MSCs were pre-differentiated down the cartilage and bone lineages before encapsulation in the bilayered gel. The gels were then implanted subcutaneous in a rat for 12 weeks (Fig. 5D). The explanted construct grossly demonstrated an opaque region corresponding to the cartilage layer and vascular ingrowth was observed in the bone layer. Gene expression and histological analysis was performed to further confirm the formation of an osteochondral tissue structure in the shape of a human TMJ (85).

Tissue engineering applied to craniofacial disease

Tissue engineering is generally considered an application oriented technique focused on creating tissue implants. However, tissue engineering may also be applied to *in vitro* analysis of diseased cells and their tissue development. Innumerable genetically modified mice have been created to help understand and mimic human disease. In general, the disease-associated changes in the mice are evaluated *in situ* or *in vivo*. Using the hydrogel system we were able to investigate the tissue development of cells from a mouse model of Apert syndrome, a disease related to fibroblast growth factor receptor function (FGFR) that results in craniosynostosis (86). Cell encapsulation in a 3D hydrogel better mimics the *in vivo* environment of a cell compared with monolayer culture and allows the mutant population of cells to be studied in an isolated and defined condition.

The heterozygous $FGFR2^{+/S252W}$ mouse model of Apert Syndrome was developed by collaborator E. Wang Jabs (86). Osteoprogenitor and mesenchymal cells were isolated from limbs of newborn mice (mutant and normal phenotype siblings). The cells were expanded in monolayer culture and then photoencapsulated

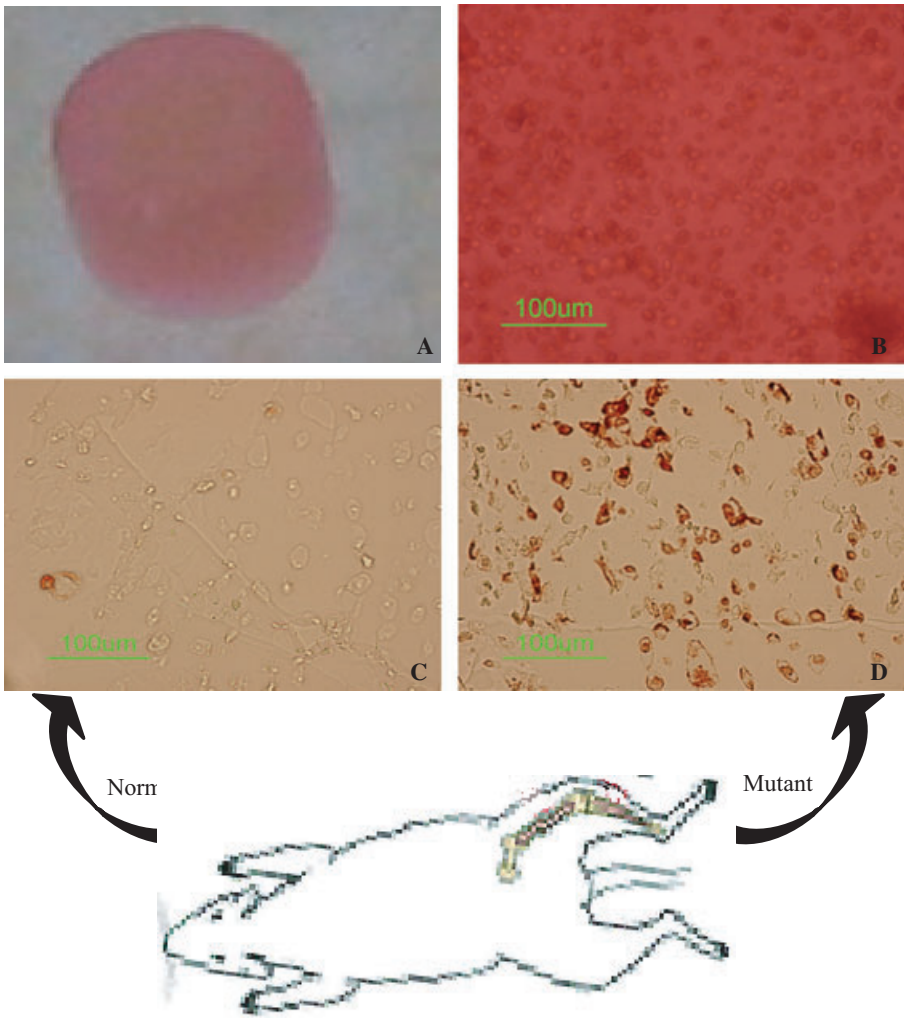

Fig. 6. Encapsulation of cells from a FGFR mutant mouse in a hydrogel grossly (A) and by light microscopy (B). After incubation in osteogenic conditions, the cells isolated from the normal mouse are negative for Type II collagen (C) while the mutant cells in the same condition are positive (D).

(20 M cells/ml) in 10% w/v of poly(ethylene oxide) diacrylate hydrogels (Fig. 6A,B). Both groups were cultured in osteogenic medium for 3 weeks. Biochemical, histological, immunohisto-chemistry analyses were performed. Von Kossa staining demonstrated mineralization in the pericellular regions in both groups with similar quantified calcium accumulation in the mutant (4.25 ± 0.38% dry weight) and control constructs (3.66 ± 0.67% dry weight). Immunohistochemical staining for Col I was also present in all of the cell-hydrogel constructs. Immunohistochemical staining for Col II is negative in constructs containing normal cells while is positive in those containing mutant cells (Fig. 6C,D). The Apert mouse exhibits abnormal cartilage nodules below the cranial sutures, signifying that the expression of the cartilage specific matrix molecule, Type II collagen, in the engineered tissue mimics the mutant mouse disease state. Alkaline phosphatase is a marker of early bone formation and its accumulation in the constructs containing control cells (0.28 ± 0.04 U/L/wet wt) was higher than those with mutant cells (0.04 ± 0.01 U/L/wet wt), with $p < 0.01$. Reduced bone forming activity is in accordance with the function of the FGF/FGFR pathway. When the FGF pathway is activated, cell proliferation increases while bone formation is blocked. The Apert mouse mutation is activating, causing constitutive activation of the FGFR. Thus, the cell behavior in the hydrogel scaffolds reflects that of the disease and may be used to evaluate tissue development and potential therapeutics.

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