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Tissue Engineering: Current State and Perspectives

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Abstract

Tissue engineering is an interdisciplinary field that involves cell biology, materials science, reactor engineering, and clinical research with the goal of creating new tissues and organs. Significant advances in tissue engineering have been made through improving singular aspects of the approach: materials design, reactor design, or cell source. Increasingly, however, advances are being made by combining several areas to create environments which promote the development of new tissues with properties which more closely match their native counterparts. This approach does not seek to reproduce all the complexities involved in development, but rather, seeks to promote an environment which permits the native capacity of cells to integrate, differentiate, and develop new tissues. Progenitors and stem cells will play a critical role in understanding and developing new engineered tissues as part of this approach.

Introduction

The number of organs available for transplantation is far exceeded by the number of patients needing such procedures. In the U.S. in 2000 alone, approximately 72,000 people were on the waiting list for an organ transplant due to end stage organ failure, but only 23,000 transplants were performed (Port 2002). This shortfall motivated the development

of tissue engineering. The thought was that cells might be able to organize into tissues and, ultimately, organ replacements if they were cultured in three dimensions under the appropriate reactor conditions (Langer and Vacanti 1993). Refined considerably, this approach remains the foundation of the field. The scaffolds to create the three dimensional environment have been designed with particular surface chemistries, architectures, and degradation rates to foster and direct cellular attachment, migration, proliferation, and differentiation (Nguyen and West 2002, Chen, Ushida and Tateishi 2002, Yang, Leong, Du and Chua 2001). Reactor design has been shown to be critical for the development of certain tissues (Bancroft, Sikavitsast, van den Dolder, Sheffield, Ambrose, Jansen and Mikos 2002, Sodian, Lemke, Fritsche, Hoerstrup, Fu, Potapov, Hausmann and Hetzer 2002, Dar, Shachar, Leor and Cohen 2002), along with the appropriate chemical conditions including the presence of growth factors (Kaigler, Krebsbach, Polverini and Mooney 2003, Babensee, McIntire and Mikos 2000, Elisseeff, McIntosh, Fu, Blunk and Langer 2001).

In addition, cell sources for tissue engineering have expanded tremendously: fully differentiated cells, progenitors and stem cells have shown great promise for the creation of the highly complex, functional tissues needed to address the tissue and organ shortfalls in medicine (Solter and Gearhart 1999, Shamblott, Axelman, Wang, Bugg, Littlefield, Donovan, Blumenthal, Huggins and Gearhart 1998, Gage 2000, Morrison, White, Zock and Anderson 1999, Jackson, Majka, Wulf and Goodell 2002, Boheler, Czyz, Tweedie, Yang, Anisimov and Wobus 2002). However, there are significant issues which must be understood and addressed to realize this potential. In particular, while stem cells and progenitors of various origins have been shown to be able to differentiate into a variety of cell types and, in some cases, form functional tissues (Levenberg, Golub, Amit, Itskovitz-Eldor and Langer 2002), it remains challenging to differentiate the progenitors and stem cells in a controlled, efficient, and reproducible manner which results in terminally differentiated tissue structures. Tissue engineering may provide a means to gain critical insight into the behavior of stem cells by facilitating the control of the stem cell environment both chemically and physically in three dimensions. This, in turn, may lead to the development of new tissue substitutes and replacements.

Scaffold Design: Materials, Architecture, and Surface Modification

The basic premise of tissue engineering is to combine the appropriate cells with a material under conditions that lead to tissue formation. The nature of the material, its physical and chemical properties, is critical to creating the desired conditions for tissue formation. A host of different materials have been used in tissue engineering. Ceramics and metals have been used primarily in orthopedic applications (Hench 1998, Puleo and Nanci 1999, Ducheyne and Qiu 1999, Rosen, Hobbs and Spector 2002). The mechanical properties of metals and ceramics along with the bioactivity of certain ceramics, including hydroxyapatite and bioglass have made them very successful in orthopedic applications, but are less suitable for other tissues. Polymer materials more closely match the chemical and mechanical properties of most biological tissues (Seal, Otero and Panitch 2001).

Both natural and synthetic polymers have been studied for tissue engineering. Natural materials including collagen (Freyman, Yannas, Yokoo and Gibson 2001), Matrigel (Guest, Rao, Olson, Bunge and Bunge 1997), and alginates (Marijnissen, van Osch, Aigner, van der Veen, Hollander, Verwoerd-Verhoef and Verhaar 2002) have been used as scaffolds. Since these materials compose the naturally occurring extracellular matrix (ECM) it seems reasonable to use them as the basis for engineered tissues. In particular, collagen has been used successfully as a scaffold for skin repair (Pomahac, Svensjo, Yao, Brown and Eriksson 1998) and multiple scaffolds based on it are currently available for clinical use. Collagen has also been used for nerve repair (Liu, Peulve, Jin, Boisset, Tiollier, Said and Tadie 1997), and bladder engineering (Atala 2000). Matrigel, a gel composed of basement membrane proteins, is commercially available and has been used to culture a wide variety of cell types as well as in spinal cord repair (Xu, Zhang, Li, Aebischer and Bunge 1999), and vascular networks (Sieminski, Padera, Blunk and Gooch 2002). Alginates have been used for a variety of tissue engineering studies including cartilage (Marijnissen, van Osch, Aigner, van der Veen, Hollander, Verwoerd-Verhoef and Verhaar 2002) and cardiac tissues (Dar, Shachar, Leor and Cohen 2002).

While natural polymers have shown promise there are certain limitations and concerns regarding their use. First, it is challenging to control their mechanical properties and degradation rates over a wide range (Lee, Singla and Lee 2001). Second, there is a

possibility that the naturally derived materials may provoke a serious immune response or may harbor microbes or viruses (Schmidt and Baier 2000). Proper characterization and screening of the natural materials alleviates many of the concerns, but overcoming the materials limitations is more challenging.

Synthetic polymers can be tailored to have a much wider range of mechanical and chemical properties than their natural counterparts. They also avoid the concern regarding immunogenicity, but biocompatibility becomes an issue (Seal, Otero and Panitch 2001). For the purposes of biomaterials, synthetic polymers may be classified as non-degradable and degradable polymers. Non-degradable polymers that are biocompatible and have been used extensively include poly(tetrafluoroethylene) (Teflon) for applications such as vascular grafts (Sayers, Raptis, Berce and Miller 1998), and high density polyethylene for use in hip implants (Garellick, Malchau and Herberts 1999) among other applications. While the non-degradable polymers can be fabricated with an extremely wide range of well controlled properties, their permanence does raise concern regarding their long-term effects, especially with regards to scarring and inflammatory response (Fournier, Passirani, Montero-Menei and Benoit 2003). Thus, there has been a great deal of research into the development of degradable synthetic polymers which would, in theory, have all the properties of their non-degradable counterparts, but also avoid the long term issues by degrading to metabolizable components (Vert, Schwach, Engel and Coudane 1998).

Poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers poly(lactic-co-glycolic acid) (PLGA) have been employed in a number of devices approved for use by the FDA (Lewis 1990, Mooney, Organ, Vacanti and Langer 1994). They degrade by acid hydrolysis to lactic and glycolic acids which are excreted. The degradation rate is controlled by the molecular weight of the polymer, the crystallinity, and the ratio of glycolic acid to lactic acid subunits. Since lactic acid is more hydrophobic than glycolic acid due to the methyl group, hydrolysis is slower. PLA and PLGA in particular are highly processable making them very attractive for the fabrication of complex structures and the degradation can be tailored from a few weeks to years. They have also been studied extensively for drug delivery purposes. However, the PLGA family of polymers are rather brittle, lack functionalities other than end groups for chemical modification, and exhibit bulk rather than surface degradation which can

produce a non-uniform release profile which is less than ideal for certain drugs. The lack of functionalities in PLA, PGA, and PLGA has been addressed by the incorporation of free amines into the structures (Barrera, Zylstra, Lansbury and Langer 1995, Hrkach, Ou, Lotan and Langer 1995). The amines have been used to tether peptides and modulate the attachment and behavior of cells (Cook 1997).

PLA, PGA, and PLGA, while extremely useful for drug delivery, do exhibit limitations including a propensity for a non-uniform release profile with certain drugs. In response to these limitations regarding drug delivery with the lactic and glycolic acid family of polymers, the polyanhydrides were created. Unlike their polyester counterparts, the polyanhydrides exhibit surface degradation over bulk degradation (Dang, Daviau and Brem 1996), and have been approved by the FDA for the delivery of carmustine (BCNU) for chemotherapy. It is the first new synthetic degradable material approved by the FDA in 20 years. Polyanhydrides are now being studied for the delivery of a wide variety of drugs and proteins (Thomas, Padmaja and Kulkarni 1997, Kipper, Shen, Determan and Narasimhan 2002). The surface degradation can produce more constant release profiles for certain drugs which is particularly critical in the case of very potent drugs (Dang, Daviau, Ying, Zhao, Nowotnik, Clow, Tyler and Brem 1996). Drug delivery is critical for the next stage of tissue engineering and is discussed further in a following section.

The mechanical properties of the PLGA family are also limited. Polycaprolactone (PCL), another degradable ester with a longer carbon backbone exhibits more elasticity than its glycolic and lactic acid counterparts, but it does not exhibit the significant elastomeric properties of certain tissues such as tendon. Poly-4-hydroxybutyrate (P4HB) and Polyhydroxyalkanoate (PHA) have excellent elastomeric properties and have been used in a number of applications of tissue engineering (Martin and Williams 2003) including heart valves (Sodian, Sperling, Martin, Egozy, Stock, Mayer and Vacanti 2000). While scaffolds based on P4HB and PHA have highly elastic behavior (Zhao, Deng, Chen and Chen 2003), they can be challenging to chemically modify, although surface modification is possible (Chen, Zhang, Zhu, Zhang and Hu 2003), and, currently, the range of degradation times is narrower than that for the traditional polyesters. Chemists have sought to make synthetic versions of these highly elastomeric materials based on polyurethanes (Fromstein and Woodhouse 2002) and sebacic acid (Wang,

Ameer, Sheppard and Langer 2002, Wang, Kim and Langer 2003) to extend the repertoire of the degradable elastomeric materials.

Hydrogels are another class of biomaterials which had drawn a great deal of interest in the field due to their general high biocompatibility, mechanical properties which parallel the properties of soft tissues, and their ability to be injected as a liquid which gels in situ (Anseth, Metters, Bryant, Martens, Elisseeff and Bowman 2002). Hydrogels are either chemically or physically cross-linked water soluble polymers, and they may be either degradable or non-degradable depending on their chemistry. Chemically cross-linked hydrogels may be polymerized in situ using a chemical initiator (Stammen, Williams, Ku and Guldborg 2001), or in some cases a photoinitiator (Nguyen and West 2002). The photoinitiated hydrogels use initiators that do not become active until they are exposed to a light of a particular wavelength. The advantage of this over standard initiation schemes is that the hydrogel gelation may be tightly controlled; the un-gelled solution may be injected and then gelled in an actively controlled manner. Photoinitiated hydrogels have been shown to be able to be injected with cells with high cell survival (Mann, Gobin, Tsai, Schmedlen and West 2001, Elisseeff, Anseth, McIntosh, Randolph and Langer 1999) as well as used to deliver growth factors (Elisseeff, McIntosh, Fu, Blunk and Langer 2001). Physically cross linked hydrogels rely on phase separation of the blocks of block copolymers to gel. The phase separation is generally temperature dependent and reversible. Examples include poly(ethylene)-b-poly(propylene)-b-poly(ethylene) (Pluronics) which has been used in a number of tissue engineering studies (Liu, Chen, Liu, Cui, Shang, Xia, Wang, Cui, Yang, Liu, Wu, Xu, Buonocore and Cao 2002, Arevalo-Silva, Eavey, Cao, Vacanti, Weng and Vacanti 2000) and degradable block copolymer systems including ones based on poly(ethylene oxide) and poly(lactic acid) (Kwon, Park, Bae, Kim and Char 2002, Peppas, Keys, Torres-Lugo and Lowman 1999). The challenge with hydrogels has been to obtain a broad range of mechanical properties and to be able to create controlled pore architectures. Photopolymerization combined with photolithography is being investigated as a means to obtain distinct architectures (Liu and Bhatia 2002).

Once a particular material has been chosen for a scaffold supporting structure, the material must be processed into a structure with the appropriate architecture to support

cell growth and tissue formation. Early on in the development of tissue engineering, a highly porous scaffold was identified as being critical for the necessary nutrient and waste transport to support the growth of large pieces of tissue replacements (Langer and Vacanti 1993). Mesh networks were successfully employed and have continued to be successfully employed for the culturing of a number of cell types for the replacement and repair of tissue defects including cartilage, bladder, and artery tissue (Freed, Vunjak-Novakovic, Biron, Eagles, Lesnoy, Barlow and Langer 1994). This mesh was made with PGA fibers fabricated via a melt-spinning process. The mesh has been extended in use by combining it with hydrogels (Vacanti, Leonard, Dore, Bonassar, Cao, Stacheleck, Vacanti, O'Connell, Yu, Farwell and Vacanti 2001) as well as combining it with other degradable highly elastic polyesters (Shum-Tim, Stock, Hrkach, Shinoka, Lien, Moses, Stamp, Taylor, Moran, Landis, Langer, Vacanti and Mayer 1999).

A number of other techniques have been borrowed, adapted, and developed to create porous scaffolds with highly controlled porosity with respect to the pore size, structure, and volume within the material. Salt-leaching or more broadly, particulate leaching, has been widely used (Levenberg, Golub, Amit, Itskovitz-Eldor and Langer 2002, Mikos, Lyman, Freed and Langer 1994, Hile, Amirpour, Akgerman and Pishiko 2000, Mikos, Sarakinos, Leite, Vacanti and Langer 1993, Sikavitsas, Bancroft and Mikos 2002). It is a very simple technique which can be used with a wide variety of polymers to quickly make a large number of scaffolds with a controlled pore size and volume based on the size and amount of initial particulate added to the polymer solution (Mikos 1994). However, the thickness of the scaffolds that can be fabricated is limited by the technique motivating the development of other techniques including supercritical CO₂ (Shum-Tim, Stock, Hrkach, Shinoka, Lien, Moses, Stamp, Taylor, Moran, Landis, Langer, Vacanti and Mayer 1999, Nof and Shea 2002, Watson, Whitaker, Howdle and Shakesheff 2002) and hydrocarbon techniques to fabricate porous scaffolds (Shastri, Martin and Langer 2000).

All of the above techniques are easily tailored and lead to scaffolds with a high degree of well-controlled porosity. However, the porosity is generally isotropic in nature. For certain applications, especially those involving cellular orientation, anisotropic pore structures are desirable. Solid-liquid phase separation techniques have been adapted to

create structures with long, oriented pores (Schurgens, Maquet, Grandfils, Jerome and Teyssie 1996). Phase inversion techniques have also been employed to create oriented pore structures (Hunkeler 1997). These techniques have been employed with both the natural and synthetic structures. Hydrogel pore structures are generally defined by their cross-link densities and locations. As discussed above, the combination of photolithography and photopolymerizable hydrogels has been pursued to create patterned, controlled pore architectures (Liu and Bhatia 2002). Architectural control has also been achieved by creating composites of hydrogels with other polymers (Vacanti, Leonard, Dore, Bonassar, Cao, Stacheleck, Vacanti, O'Connell, Yu, Farwell and Vacanti 2001).

The properties of a particular material for tissue engineering are generally divided into two groups, namely bulk properties including the mechanical properties and degradation behavior and surface properties. A great deal of research in the last few years has focused on tailoring the surface properties of materials to control the interaction between the materials and cells. Functionalized versions of the degradable esters (Cook 1997, Lavik, Hrkach, Lotan, Nazarov and Langer 2001, Hrkach 1996) as well as some of the hydrogels (Mann, Gobin, Tsai, Schmedlen and West 2001, Plant, Woerly and Harvey 1997) have been developed to provide a means to tether peptides to the surfaces of these materials. One of the best studied peptides in this context has been the RGD peptide, a ubiquitous cell adhesion peptide found in fibronectin and laminin (Hynes 1987). By tethering this peptide to a hydrogel based on poly(methyl methacrylate), osteoblast spreading and integration was improved (Burdick and Anseth 2002). Other tethered peptides have also been studied with polymers and gels for neurite extension and guidance (Tong and Shoichet 2001, Shaw and Shoichet 2003, Bellamkonda, Ranieri and Aebischer 1995), cell migration (Mann and West 2002, Sakiyama-Elbert and Hubbell 2001). There is also a strong interest in developing surface modifications to resist particular kinds of protein and cellular attachment or to select for specific cellular attachment (Ruiz, Fine, Voros, Makohliso, Leonard, Johnston, Textor and Mathieu 1999, Nicolau, Taguchi, Taniguchi, Tanigawa and Yoshikawa 1999, Morra and Cassinelli 1999, Banerjee, Irvine, Mayes and Griffith 2000).

Reactor Design

In order to achieve large scale tissue structures, there must be appropriate transport of nutrients to and waste from the cells as they begin to form a tissue or organ. Several culture systems have been developed to provide the necessary transport conditions for cell growth in three dimensions including perfusion reactors (Bancroft, Sikavitsast, van den Dolder, Sheffield, Ambrose, Jansen and Mikos 2002), rotating wall vessel reactors (Carrier, Papadaki, Rupnick, Schoen, Bursac, Langer, Freed and Vunjak-Novakovic 1999), and spinning flask reactors (Sikavitsas, Bancroft and Mikos 2002). These reactors have unique flow patterns and a degree of controllable mechanical stimuli derived from the flow, but the primary benefit of these is generally in their transport properties.

While transport is critical to the developing tissue, there are other stimuli including mechanical stimuli which can greatly influence the development of neo-tissue (Ingber 2002). By developing reactors which provide such stimuli, new tissue-engineering structures with properties previously unseen have been created including tissue-engineered arteries which have burst strengths of 2150 ± 706 mm Hg, high enough to make them suitable for arterial grafting (Niklason, Gao, Abbott, Hirshi, Houser, Marini and Langer 1999). Such work demonstrates the key role bioreactors can play in the development of tissue engineered structures.

Drug Delivery: Further creating the appropriate environment for tissue formation and repair

There are a number good reviews of drug delivery for tissue engineering (Babensee, McIntire and Mikos 2000, Langer 1998, Benoit, Faisant, Venier-Julienne and Menei 2000, Saltzman and Olbricht 2002, Whitaker, Quirk, Howdle and Shakesheff 2001). Drug delivery may allow the creation of the appropriate chemical environment via soluble factors to direct cells. Controlled release allows for the possibility of presenting drugs and growth factors over long time periods within scaffolds to promote tissue development and formation. Growth factors have been successfully incorporated into hydrogels to augment the development of tissues including bone (Burdick, Mason, Hinman, Thorne and Anseth 2002) cartilage (Elisseeff, McIntosh, Fu, Blunk and Langer 2001). Growth factors have also been incorporated into PLGA scaffolds successfully and

have led to the formation of more viable tissue structures (Richardson, Peters, Ennett and Mooney 2001, Murphy, Peters, Kohn and Mooney 2000).

One of the most common manners in which growth factors have been incorporated into polymers is through the formation of microspheres (Benoit, Faisant, Venier-Julienne and Menei 2000, Nam and Park 1999, Fu, Harrell, Zinski, Um, Jaklenec, Frazier, Lotan, Burke, Klibanov and Langer 2003). Such microspheres releasing growth factors have been used as scaffolds, providing another means to generate tissue engineered structures with the necessary chemical environment to foster repair (Mahoney and Saltzman 2001). By combining microspheres containing different drugs with unique release profiles, one can start to emulate the environment seen during development. This has profound implications for stem cell research. Through tissue engineering and drug delivery one may recapitulate developmental cues in a manner which is more physiologically relevant than simple addition of growth factors to two dimensional cultures. In so doing this, one may be able to gain greater control and insight into the capacity of stem cells and use this to realize the replacement and repair of complex tissues.

Cell Sources

The essential principle of tissue engineering is to combine a scaffold with cells for tissue replacement or repair. In some cases, ingrowth of the host cells into a scaffold following implantation has been successful at generating new tissue (Ellis and Yannas 1996), but in most cases, a cell source, either host- or donor- derived is necessary. The cell type or types chosen along with their source is critical to the success of a tissue engineered structure. With the identification of a number of stem cell possibilities as well as the incorporation of genetic engineering in cellular biology, there are a whole host of new possibilities for cell sources and types which are being investigated.

Cartilage was one of the first tissues widely and successfully pursued in the field. Autologous cartilage cells were obtained from cartilage biopsies including behind the ear and were successfully expanded and cultured on PGA meshes and PLA scaffolds and implanted to treat defects (Saim, Cao, Weng, Chang, Vacanti, Vacanti and Eavey 2000, Rodriguez, Cao, Ibarra, Pap, Vacanti, Eavey and Vacanti 1999). Autologous cells avoid

rejection concerns, but are not always a viable option. Cells may not be available, viable, or capable of the necessary proliferation to create a new tissue. Modulating the immune response through the host or donor makes allogenic cells an alternative in some cases, but only for cell types which are capable of the necessary proliferation to form a new tissue. In cases where the cells are only needed for chemical support, it may be possible to use encapsulated xenogenic cells, such as for the replacement of pancreatic islets (O'Connell 2002).

However, for many tissue types, primary cells are not accessible or in numbers and with the proliferative capacity to be viable for tissue engineering. Multipotent stem cells and progenitors hold great promise for addressing the need for viable cell sources in tissue engineering, and stem cell research for regenerative medicine is one of the critical stages for the growth of the field. Hematopoietic stem cells (HSCs) which are capable of replacing all the cells in the hematopoietic system were the first to be identified and remain the best characterized stem cells in the field (Alison, Poulsom and Wright 2002, Weissman 1997). Part of the challenge in the field is that the definition of a stem cell is plastic for most cell types outside of the HSCs. For each type of stem cell, there are often several methods for identifying, isolating, and expanding the cells which can lead to confusion; not all stem cells of a particular type (neural, mesenchymal, retinal) necessarily behave in the same manner under identical conditions. Neural stem cells isolated from different tissue or using different techniques may have dissimilar capacities for proliferation, integration, and differentiation.

Nonetheless, stem cells hold great promise, and stem cell biology has made tremendous advances in the identification, isolation, expansion, and controlled differentiation of stem cells from both adult and fetal tissue (Jackson, Majka, Wulf and Goodell 2002). Stem cells have been reported to exist in bone marrow (hematopoietic and mesenchymal), the brain and spinal cord (neural), the heart, pancreas, retina, liver, and lung among others (Jackson, Majka, Wulf and Goodell 2002). Initially, identification and isolation of stem cells was done most often using cloning techniques (Weissman 1997, Lu, Kwan, Kurimoto, Shatos, Lund and Young 2002, Stemple and Anderson 1992); fluorescence activated cell sorting (FACS) has been employed by a number of groups to either sort stem cells based on specific surface markers (Morrison, White, Zock and

Anderson 1999) or based on the efflux of Hoechst dye (Goodell, Brose, Paradis, Conner and Mulligan 1996, Murayama, Matsuzaki, Kawaguchi, Shimazaki and Okano 2002). Several tissue replacements have been studied comprised of polymer scaffolds seeded with stem cells including cartilage (Weber, Steinert, Jork, Dimmler, Thurmer, Schutze, Hendrich and Zimmermann 2002, Ponticiello, Schinagl, Kadiyala and Barry 2000), small intestine (Hori, Nakamura, Kimura, Kaino, Kurokawa, Satomi and Shimizu 2002), and bone (Gao, Dennis, Solchaga, Awadallah, Goldberg and Caplan 2001).

The ability to specifically direct the proliferation and differentiation of multipotent stem cells for tissue engineering has been a challenge. For example, neural stem cells (NSCs) have been shown to form all the cell types of the CNS and to replace cells in injury models (Gage 2000). However, in vitro, only a percentage of NSCs have been directed to differentiate to a specific cell type (O'Connor, Stenger, Shaffer, Maric, Barker and Ma 2000). Likewise, in vivo in larger injury models, the NSC engraftment and differentiation has occurred, but not always completely (Teng, Lavik, Qu, Park, Ourednik, Zurakowski, Langer and Snyder 2002).

Transdifferentiation of multipotent stem cells has drawn significant interest as a possible means for obtaining stem cells which are either not available or not easily obtained in the adult. It has been suggested that it may be possible to induce one type of stem cell into another type such as transdifferentiating bone marrow stromal stem cells to neural stem cells (Mezy, Chandross, Harta, Maki and McKercher 2000, Sanchez-Ramos, Song, Cardozo-Pelaez, Hazzi, Stedeford, Willing, Freeman, Saporta, Janssen, Patel, Cooper and Sanberg 2000) or transdifferentiating neural stem cells to hematopoietic (Bjornson, Rietze, Reynolds, Magli and Vescovi 1999). Most recent research has suggested that rather than transdifferentiation, the cells may be fusing with other cells to give rise to the apparently transdifferentiated cells (Ying, Nichols, Evans and Smith 2002). The existence of transdifferentiation remains a topic of great debate in the stem cell community.

In 1998, human embryonic stem cells (hES cells) were first reported (Shamblott, Axelman, Wang, Bugg, Littlefield, Donovan, Blumenthal, Huggins and Gearhart 1998, Thomson, Itskovitz-Eldor, Shapiro, Waknitz, Swiergiel, Marshall and Jones 1998). Murine embryonic stem cells have been studied for over twenty years and have been

shown to be capable of differentiating into a host of cell-types (Smith 2001). Like multipotent stem cells, ES cells respond to growth factor but not in a complete manner: only a portion of the ES cells differentiate to a particular cell type in response to a tissue (Boheler, Czyz, Tweedie, Yang, Anisimov and Wobus 2002, Kehat, Kenyagin-Karsenti, Snir, Segev, Amit, Gepstein, Livne, Binah, Itskovitz-Eldor and Gepstein 2001). However, when grown on three dimensional scaffolds, hES cells are capable of differentiating appropriately to form neo-tissues including blood vessels, and, upon implantation, successfully integrate with the host resulting in host blood cells flowing through donor vessels (Levenberg, Golub, Amit, Itskovitz-Eldor and Langer 2002).

Building Complex Tissues: Future Directions and Challenges

Tissue engineered skin grafts were one of the first therapies to be approved and commercially available. Cartilage is also being used and is in clinical trials. Researchers are pursuing tissue engineered structures for a host of tissues including bone, liver, artery, nerve, pancreas, skin, kidney, and bladder (Vacanti and Langer 1999).

One of the greatest challenges encountered in the field has been the need to have a functional vascular network for complex tissues and organs. Vascularization remains a critical issue. Growth factors have been added to promote vascularization by the host (Richardson, Peters, Ennett and Mooney 2001), human ES cells have been differentiated into a donor vascular network (Levenberg, Golub, Amit, Itskovitz-Eldor and Langer 2002), cocultures have been used, and preliminary vascular networks have been engineered to act as a scaffold and drug delivery device for complex tissue formation (Mikos, Sarakinos, Lyman, Ingber, Vacanti and Langer 1993). Vascularization is critical whether it be liver tissue or skin; it is not yet clear which approach will be most appropriate for each application.

The greatest impact in the field is being made by combining the materials, drugs, reactors in a manner which emulates enough of the environment present during development to promote the development of new tissues and organs with the necessary functionality. This approach need not replicate all or even many of the complexities of development. For examples the incorporation of two growth factors into a scaffold to provide appropriate delivery to stimulate both bone growth and vascularization had a

major impact on the developing tissue and its functionality (Richardson, Peters, Ennett and Mooney 2001).

The potential of progenitors and stem cells makes this approach possible. Insights gained from the research in tissue engineering have the potential to provide greater understanding of development and the potential of progenitors and stem cells.

Ultimately, though, the motivation for tissue engineering is the development of neo-tissues for transplantation. We are witnessing the realization of this goal with the clinical implantation of tissue engineered vascular grafts in pediatric patients (Matsumura, Hibino, Ikada, Kurosawa and Shin'oka 2003). Perhaps the most exciting aspect of this work is that the tissue engineered grafts appear to be growing with the patients. Similar work is also being conducted by other research groups (Simon, Kasimir, Seebacher, Weigel, Ullrich, Salzer-Muhar, Rieder and Wolner 2003), and while there are clear challenges associated with this work, the promise is there.

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