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The Engineering of Craniofacial Tissues in the Laboratory: A Review of Biomaterials for Scaffolds and Implant Coatings

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A majority of craniomaxillofacial reconstructive procedures are performed to replace or construct missing or damaged skeletal structures. These operations require the harvesting of bone or soft tissue from distant donor sites. The donor site operation often results in greater morbidity than the primary reconstructive procedure and there may not be adequate quantities of bone available for harvesting in children. Furthermore, there is unpredictable loss of bone graft volume during the remodeling process.

Langer and Vacanti define tissue engineering as "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain or improve tissue function" [1]. One tissue engineering strategy is based on harvesting

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progenitor or stem cells, expanding and then, differentiating them into cells that have the potential to form new tissue (eg, bone) or organ (eg, tooth). The harvested cells are seeded on scaffolds. These scaffolds are fabricated in the laboratory to resemble the structure of the desired tissue or organ to be replaced. Much of the current tissue engineering research is directed toward the areas of cell manipulation (isolation, expansion, and differentiation) and scaffold design (biomaterials, design, and fabrication). This article reviews biomaterials available for use in craniomaxillofacial tissue (bone) engineering, coatings applied to scaffolds, and scaffold fabrication techniques.

Biomaterials for bone tissue engineering

The role of the scaffold in tissue engineering is to provide a matrix of a specific geometric configuration on which seeded cells may grow to produce the desired tissue or organ. The physical and chemical characteristics of a scaffold play a significant role in cell proliferation and tissue in-growth.

Biomaterials used as scaffolds for bone tissue engineering are classified into two broad categories: naturally derived and synthetic. Advantages of naturally derived scaffolds include the ability to support cellular invasion and proliferation. Synthetic materials offer ease of processing and mechanical strength [2].

Biomaterials used in tissue engineering also may be divided into ceramics and polymers [3]. These biomaterials may be produced in solid blocks, sheets, porous sponges or foams, or hydrogels. Historically, many of these substances have been used as bone substitutes, sutures, meshes, fixation devices, and dressings.

Bone is composed of an organic (polymer) component, primarily collagen, and a mineral (ceramic) component, primarily hydroxyapatite (HA) [4]. Currently, these individual components are being studied for use as scaffolds in tissue engineering. Novel biodegradable materials with improved mechanical properties, cell-interaction properties, and process ability also are being developed [5].

Scaffolds for use in bone tissue engineering must allow for: (1) easy cell penetration, distribution, and proliferation [6]; (2) permeability of the culture medium [7]; (3) in vivo vascularization (once implanted) [8,9]; (4) maintenance of osteoblastic cell phenotype; (5) adequate mechanical stiffness [10]; (6) proper biodegradation (rate and inflammatory response) and eventual total replacement by bone [11]; and (7) ease of fabrication (including 3-D printing). To date, the ideal scaffold that meets all these criteria has not been developed.

Biomaterials used as scaffolds: ceramics

Natural or synthetic HA and beta-tricalcium phosphate (β-TCP) are ceramics used in bone tissue engineering. Ceramic biomaterials structurally are similar to the inorganic component of bone. They are biocompatible,

osteoconductive, and may bind directly to bone. They are protein-free and, thus, stimulate no immunologic reaction [12]. Furthermore, ceramic materials have long degradation times (many years) in vivo [3].

HA is a well-known biomaterial used for many decades as a bone substitute for small defects of the jaws. It may be derived from bovine bone (deproteinized) or coralline or made as a pure synthetic. It was one of the first biomaterials used as a scaffold, seeded with osteoprogenitor cells from periosteum or bone marrow, for bone and cartilage engineering [3,13]. Currently, investigators (primarily in Japan and Europe) continue to study HA for use as a scaffold in bone tissue engineering [14,15]. Major disadvantages of HA are that it is brittle, it has little mechanical strength, it does not resorb, and the pore size cannot be controlled easily by conventional processing methods [16].

Harris and Cooper assessed the osteogenic potential of bone marrow–derived human mesenchymal stem cells (hMSC) seeded on HA scaffolds [17]. The constructs (hMSC + HA scaffolds) were implanted into a dorsal pouch in the skin of mice for 5 weeks. Regardless of the type of HA scaffold used (coralline HA, bovine bone-derived HA, or synthetic HA/TCP), histomorphometric analysis revealed minimal bone formation. The most bone formation (only 13.8% of total surface area) was documented in the synthetic HA/TCP scaffolds [17]. In contrast to this study, Boo and colleagues show "active bone formation" when using a HA scaffold [18]. Others find a higher cell density on HA scaffolds when combined with TCP and fibrin [19].

TCP is a naturally occurring material comprising calcium and phosphorous and is used as a ceramic bone substitute in craniomaxillofacial and orthopedic surgery. This material has the advantage that it can be made into specifically shaped scaffolds by 3-D printing technology. Olsen and coworkers use β-TCP to fabricate 3-D printed scaffolds for in vitro bone engineering using porcine bone marrow progenitor cells. TCP scaffolds are shown to maintain their shape and allow for good cell penetration and bone formation in this in vitro model. The comparison of β-TCP scaffolds with PLGA (D,L-lactic-co-glycolic acid) scaffolds shows that similar cell penetration and bone formation occur with both materials [20].

Boo and colleagues compared β-TCP and HA as scaffolds for bone engineering. The scaffolds were seeded with MSC and implanted in subcutaneous sites. Histologic examination after 8 weeks revealed active bone formation in HA and TCP scaffolds [18].

TCP degrades either through osteoclastic resorption (phagocytosis) or by chemical dissolution by the interstitial fluid [21]. β-TCP is expected to degrade 3 times faster than HA; however, degradation in vivo remains controversial [22,23]. In vivo experiments using rabbits demonstrate that TCP may resorb and be replaced by newly formed bone within 3 months [24]. Handschel and coworkers, however, show that no TCP degradation occurred, even after 6 months, under nonloading conditions in a rat model. Generally, the predictability of ceramic degradation is poor [25]. Furthermore, the

extent of degradation depends on many factors, such as crystallinity, porosity, density, form, size, the host, and implantation site [26]. Furthermore, HA and TCP are not strong enough scaffolds to provide mechanical strength when replacing load bearing skeletal structures.

Biomaterials used as scaffolds: polymers

The common polymers studied for craniomaxillofacial bone tissue engineering include synthetic polyesters, such as polyglycolic acid (PGA), polylactic acid (PLA) [27], and polycaplactone (PCL) [28]. Natural polymers, such as collagen and hyaluronic acid, alginate, and agarose, also are studied as scaffolds. Recently, copolymers of polyethylene oxide and polypropylene oxide, known as pluronics, have been developed in the form of injectable hydrogels [29].

Polymers seeded with chondrocytes were used to engineer a human ear, temporomandibular joint disc, and meniscal-shaped constructs [30–32]. Advantages of synthetic polymers include the ease and control of synthesis, their unlimited supply, and non–cell-mediated degradation. Biodegradable synthetic polymers can be formulated to possess desirable pore features and shape [33–35]. Disadvantages include lack of mechanical strength, difficulty in 3-D fabrication (specifically, 3-D printing), uncontrollable shrinkage, questionable cell-polymer interactions, and possible local toxicity resulting from acidic degradation products [35].

PGA has been used for many years as a resorbable suture (for example, Dexon [American Cyanamid, Pearl River, New York]). It is the first polymeric scaffold used to tissue engineer cartilage [3]. PGA is insoluble in water, and glycolic acid is the final product of degradation resulting local acidosis and potential tissue damage [35].

PLA is the polymer of lactic acid and is used as a scaffold. PLA is more hydrophobic than PGA and more resistant to hydrolysis. It is degradated into lactic acid, which also can be locally toxic to tissues [35].

PLGA is a copolymer of PGA and PLA. The suture material, Vicryl (Ethicon, Somerville, New Jersey), is composed of PLGA. Abukawa and coworkers used PLGA to make a 3-D scaffold in the shape of a porcine mandibular condyle (Fig. 1). The scaffold was seeded with porcine MSC in this autologous model. Bone formation occurred, however, only at the surface of the construct after 6 weeks of in vitro culture [36]. Abukawa and colleagues designed and fabricated a novel scaffold composed of PLGA with heterogenous pore sizes (small, 20-m to 200-m diameter, and large, 1-mm to 2-mm diameter), called the fused interconnected scaffold. MSC harvested from the ilium of a minipig were combined with these scaffolds. After only 10 days in a bioreactor, cultured constructs were implanted into mandibular defects of the same minipig and allowed to heal for 6 weeks. Histologic examination showed bone to bridge the defects (Fig. 2) [37]. The degradation rate or resultant local tissue effects were not studied, however. Furthermore, these scaffolds lacked strength and are not amenable to easy 3-D printing technology.

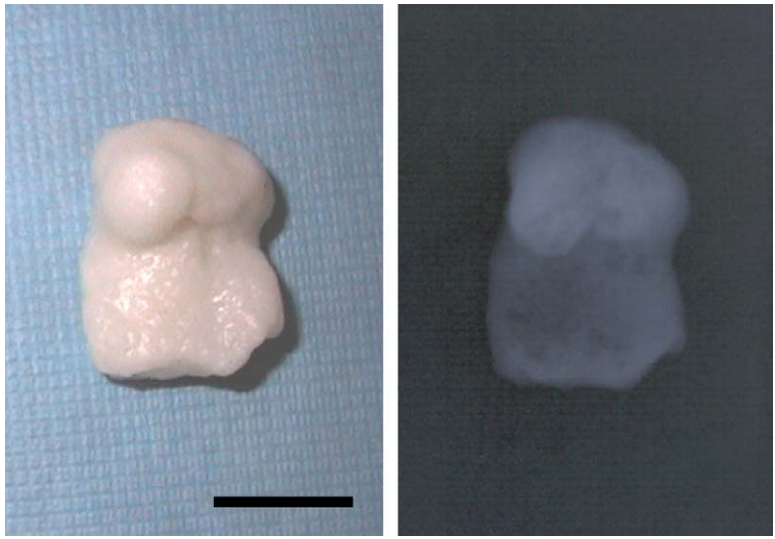

Fig. 1. Formation of a mandibular condyle in vitro by tissue engineering. Engineered construct consisting of bone and scafffold. Bar = 15 mm. (*From* Abukawa H, Terai H, Hannouche D, et al. Formation of a mandibular condyle in vitro by tissue engineering. J Oral Maxillofac Surg 2003;61:98; with permission.)

Currently available scaffold materials are less than ideal because of inadequate bone formation, lack of sufficient penetration of cells and bone throughout the scaffold, inadequate degradation properties, or inadequate mechanical stiffness.

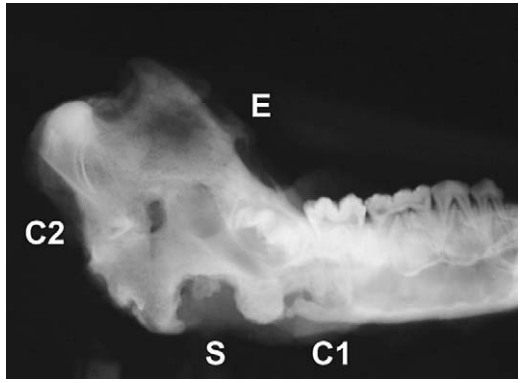

Fig. 2. Reconstruction of mandibular defects with autologous tissue-engineered bone. Reconstructed mandible with empty control (E), experimental contructs (C1 and C2), and control scaffold only (S). (*From* Abukawa H, Shin M, Williams WB, et al. Reconstruction of mandibular defects with autologous tissue-engineered bone. J Oral Maxillofac Surg 2004;62:604; with permission.)

New technologies for scaffold fabrication

New scaffold fabrication techniques are being developed, such as solid freeform fabrication (SFF). Products are designed on a computer screen as 3-D models with information from CT or MRI scans. Ideally, after implantation, a construct is organized into normal healthy tissue as the scaffold degrades. The goal of this technology is to fabricate a scaffold with accurate patient specific macrostructure (3-D shape) and microstructure (porosity and interconnected channels) for ideal nutrient flow and tissue and vascular in-growth.

This technology is relatively new and SFF machines for medical applications are available only at a few institutions, such as University of Michigan and Massachusetts Institute of Technology. Hollister's group uses this technology to tissue engineer bone with HA [38], PLA [39], and PCL [10]. Hollister's group finds this technology successful in producing bone in an immunocompromised mouse model [38]. Lin and coworkers also demonstrate that this method could produce highly porous structures that match human trabecular bone by introducing the homogenization-based topology optimization alogorithm [40]. Scheck and colleagues [40a] use genetically modified primary human gingival fibroblasts and HA scaffolds to produce bone. Williams and coworkers use PCL scaffolds and bone morphogenetic protein-7 (BMP-7)–induced human gingival fibroblasts cells to produce bone [10].

One SFF technique, the 3-D printing technology, is a manufacturing process that creates parts directly from a computer model used in the production of a complex 3-D scaffold. The parts are built by spreading a layer of powder repetitively and selectively joining the powder in the layer through the inkjet printing of a binder material [41]. Moreover, using multiple feeds, it becomes possible to manufacture scaffolds with various architectural qualities that can maintain multiple cell types on each layer, thus closely mimicking the anatomic features of a tissue or organ. Tissue engineering bone using this technique demonstrates ability of bone formation in vitro using porous PLGA/TCP composite scaffolds [42].

Lessons learned: implant coatings

The application of coatings to dental and orthopedic implants began approximately 20 years ago and has become a common practice in implant production [43]. The aim of coating implants was to increase biocompatibility and improve bone formation at the implant-bone interface.

HA is used more than any other coating to enhance osseointegration of titanium dental implants [44]. Several recent studies to measure the effect HA coatings have on titanium implants. One study followed 120 patients who received a total of 634 implants to assess the effect of implant coating (HA) on osseoointegration. Osseointegration was measured as a function of

probing depth and micromobility. One year after implantation, results revealed a significantly smaller degree of micromobility in the HA-coated implants compared with noncoated ones [45]. This difference between the two groups declined steadily, and 3 years after implantation, the groups had no significant difference in micromobility. It was concluded that HA accelerated the initial rate of osseointegration.

Schwartz-Arad and colleagues compared marginal bone loss and 12-year survival rates of HA-coated implants to those of pure titanium implants. The average marginal bone loss was significantly higher ($P < .001$) among the HA-coated implants compared with the pure titanium implants, but the 12-year survival rates for the HA-coated implants were significantly higher than for those with the pure titanium implants [46].

Issues concerning the degradation of implant coatings have been raised, as it is believed that HA coatings tend to "peel" away and, because this product is not biodegradable, may cause implant failure [47].

One of the potential benefits of using implant coatings is that the materials can be used as a drug delivery system. This would be most useful in tissue engineering scaffolds. These may include growth factors and osteogenic supplements. In a recent experiment, recombinant human BMP-2 (rhBMP-2) was incorporated into the structure of calcium phosphate coatings used to coat titanium implants. The objective of this experiment was to combine these osteoinductive properties of rhBMP-2 with the osteoconductive properties of calcium phosphate coatings. It was found that the bioactive properties of rhBMP-2 were not affected by the process of being integrated with the HA [48].

In a similar experiment, hepatocyte growth factor (HGF) was incorporated into discs made of HA. HGF is a growth factor known to promote angiogenesis. This is a desirable property for implant coatings, because vascularization is an essential part of the bone formation. In this experiment, the effect of the HGF on osteoblast differentiation was observed in vitro. The results show that the HGF coatings induced alkaline phosphatase activity to a much greater extent than the plain HA coatings [49].

Recently, Wang and coworkers studied the prospect of using a mother-of-pearl coating on dental implants [47]. Previous studies found that nacre (mother of pearl) could stimulate bone cell differentiation and induce bone formation [50]. An advantage to nacre coatings is that the material is biodegradable, so it should not remain trapped at the implant/bone interface and interfere with long-term implant integration.

Smart scaffolds: the future

One of the basic roles of a scaffold in bone tissue engineering is to act as a carrier for cells and to maintain the space and create an environment in which the cells can proliferate and produce the desired bone matrix. Transplanted cells often lose the desired function upon transfer from the in vitro

culture system to the in vivo recipient site [51,52]. To address these problems, scaffolds with the ability to deliver biochemical factors at a predetermined rate for a definitive time period are being developed [53]. These smart scaffolds have the advantage of being able to: (1) promote early capillary invasion [54], (2) maintain cell activity and desired phenotype [55], and (3) induce osteoblastic differentiation of existing progenitor cells in the recipient tissue [56,57].

Early reconstitution of the capillary system (ie, vascularization) is critical for tissue-engineered bone survival and function. Smith and colleagues report that sustained delivery of vascular endothelial growth factor enhances vascularization at the location of transplanted cells, which contributes to their survival [9]. The transplanted cells, therefore, subsequently proliferate and produce bone matrix at the reconstruction site.

Adult stem cells can be differentiated into osteoblasts when triggered by osteogenic supplements (100 nM dexamethasone, 50 μg/mL ascorbic acid, and 10 mM beta-glycerophosphate) [58,59]. Based on this data, Kim and colleagues designed a biodegradable poly (PLGA) scaffold that releases osteogenic media (containing dexamethasone and ascorbic acid) in vitro and in vivo [55,60]. Similarly, Zhang and coworkers used an ascorbic acid-containing polymer scaffold (lysine-di-isocyanate [LDI]-glycerol-polyethylene glycol [PEG]-ascorbic acid [AA]) that supports osteoblast proliferation and bone formation [61].

BMP are shown to initiate osteogenic differentiation in stem cells [62,63]. Furthermore, BMP have the unique ability to induce de novo bone and cartilage formation when implanted at ectopic sites [56,57]. A PLGA scaffold system capable of sustained BMP-4 is combined with bone marrow cells and reported to promote bone formation [54,64].

Bone tissue development is a highly coordinated process that involves various biologic factors. The ability to deliver multiple growth factors to a recipient site also may be a promising strategy to enhance bone formation, and the combination BMP-4 and vascular endothelial growth factor released from PLGA scaffolds is reported to enhance bone formation [54,64].

Release kinetics in drug delivery systems are predictable in vitro. In vivo, however, the environment is more complex, making it more difficult to predict the material degradation process. Therefore, maintaining drug release within the therapeutic range is one of the keys of an effective drug delivery system for tissue engineering bone in vivo. In fact, degradation products of polymers create an acidic environment in vivo [65,66]. An acidic environment associated with biodegradation increases the release of rhBMP-2 from the PLGA/calcium-phosphate cement composite in vitro compared with PLGA [67]. Zhang and colleagues demonstrate that the degradation products of LDI-glycerol-AA polymer do not affect the pH [68]. For effective controlled release, further experiments using biodegradable materials should be performed to optimize drug delivery system, for bone tissue engineering.

These "smart" materials may revolutionize tissue-engineering research, because controlled release of biochemical and growth factors from a scaffold may enhance cell penetration, proliferation, differentiation, and bone matrix production and improve vascularization of grafts.

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