Bio-Based Materials Step into the Operating Room

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A new class of implantable medical materials — constructed from naturally derived and renewably sourced polymers — is poised to transform clinical medicine.

The search for novel materials that have optimum compatibility with the human body and the emergence of a new sustainable bioeconomy have begun to intersect. Naturally sourced biopolymers may be ideal for the design of new biomedical devices, as such polymers can effectively interface with human cells and tissues. Moreover, the chemical, physical, and mechanical properties of bio-based materials can be easily tuned to match the native properties of a variety of target tissues.

This article discusses the evolving field of bio-based materials for biomedical implants, and provides examples of successful applications of these materials in wound closure, tissue repair, and tissue regeneration.

What are bio-based materials?

Bio-based materials, also referred to as biopolymers or bio-derived materials, are engineered materials made from substances derived, in whole or in part, from living matter. These materials are classified into three main categories based on their origin and production *(1)*:

• *biomass derived*. These bio-based materials are directly extracted or removed from biomass. Examples include polysaccharides (carbohydrates) such as starch, cellulose, alginates, carrageenan, pectin, dextran, chitin, and chitosan, and proteins such as casein, glutein, whey, silk proteins, soy proteins, and corn proteins.

• *biomonomer derived*. These materials are produced via classical chemical synthesis using monomers obtained from renewable agricultural resources. An important example is polylactic acid (PLA) — a polyester made from renewably derived lactic acid monomers derived from renewable sources. The monomers themselves can be obtained through fermentation of agricultural carbohydrate feedstocks, such as corn starch.

• *microorganism derived*. The polyhydroxyalkanoate (PHA) family of polymers is the most well-known material produced by microorganisms. Other examples include xanthan and bacterial cellulose.

Biomedical material specs

A biomedical material is a nonviable material used in a medical device, intended to interact with biological systems *(2)*. An essential characteristic of biomedical materials is biocompatibility — the ability to function appropriately in the human body to produce the desired clinical outcome without causing adverse effects.

Biomedical materials must meet stringent performance requirements. They must have sufficient physical, biological, and mechanical similarity to the natural physiological environment. In addition, the biomedical material construct and any degradation products must be nontoxic and non-inflammatory. The implanted material must not interfere with wound healing nor induce an immune response.

New biomedical materials must be assessed throughout the development process to ensure their suitability for medical applications. Characterization must include mechanical

properties, physical and chemical properties, biological properties, shelf stability, and usability. The surgical target will determine the precise technical specifications for a given biomaterial. Clinician input is indispensable to the design process, with surgeon needs and patient needs guiding the material design.

As the prevalence of chronic conditions such as cardiovascular disease, diabetes, arthritis, and neurodegenerative disease rises in the global community, the need for innovative biomaterials that interact optimally with the human body will continue to increase. Bio-based polymers are increasingly being recognized as biocompatible materials that can mimic the body's natural, functional, bioactive structures. For instance, bio-based polymers have demonstrated success in wound closure, tissue repair, and tissue engineering.

Success story: Carbohydrates for wound closure

Despite advancements in suturing and stapling techniques, physicians continue to struggle with the problem of leakage from internal wounds. The demand for tissue adhesives to augment or replace sutures and staples for internal wound repair is, therefore, significant. Polysaccharide (carbohydrate)-based tissue glues are a promising alternative.

Although tissue glues made from synthetic chemicals such as cyanoacrylates or glutaraldehydes have been developed and commercialized, such adhesives have limited clinical usage, due to biocompatibility and performance problems. A family of hydrogel tissue adhesives based on the natural polysaccharide dextran overcomes these limitations of existing tissue glues.

Synthesized from sucrose, dextran is a high-molecular-weight polysaccharide composed of chains of D-glucose units. The polysaccharide is manufactured by some of the same bacteria that produce lactic acid, including *Leuconostoc mesenteroides*, *Streptococcus mutans*, and *Lactobacillus brevis*, as well as by *Aerobacter capsulatum*. Dextran already has a long history of clinical use as a plasma volume expander for the treatment of circulatory shock.

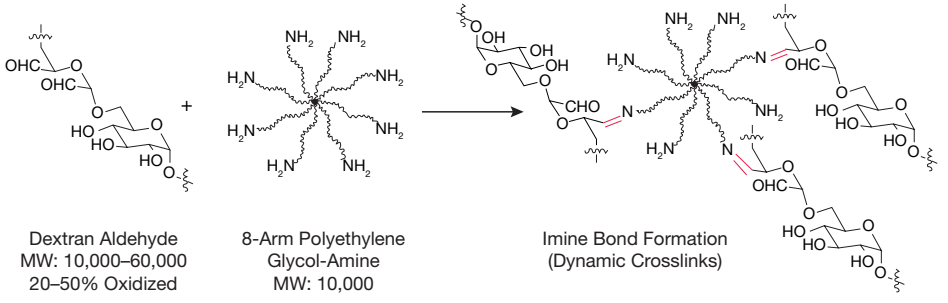
Dextran-based tissue glues have been produced by reacting dextran aldehyde with multi-arm polyethylene glycol-amines (Figure 1) to form a crosslinked hydrogel *(3)*. This crosslinking reaction occurs on both moist and dry tissues. In addition, the polysaccharide-based tissue adhesive is free of blood products, so there is no potential for transmission of infection. This property distinguishes the polysaccharide-based tissue adhesives from commercial fibrin glues, which contain the blood proteins fibrinogen and thrombin.

The results of *in vitro* testing of dextran-based tissue glues with clinically relevant cell lines reveal that these adhesives are noncytotoxic to connective tissue fibroblasts, and they do not elicit the release of inflammatory mediators; in contrast, commercial tissue adhesives based on cyanoacrylate are highly cytotoxic to connective tissue fibroblasts. The biocompatibility, biodegradability, adhesion properties, and convenience of polysaccharide-based tissue glues make these adhesives an effective system for treating a wide variety of wounds. Their chemistry enables fine-tuning of sealant properties, including cure rate, degradation rate, and swelling, to meet the needs of specific clinical targets.

In preclinical studies, dextran-based tissue adhesives have been successfully applied to a variety of difficult-to-close surgical incisions and wounds, including intestinal incisions during colorectal surgery, vascular incisions during vascular graft implantation, and traumatic wounds to internal organs *(4)*. The tunable properties of the dextran-based sealant enable the adhesive to close a wide range of incisions and wounds; the sealant will thus be useful for both elective and emergency surgeries. The dextran-based sealant is well-tolerated in short-term and long-term studies; it remains on the target site without injuring adjacent tissues.

A particularly exciting finding is that the polysaccharide-based tissue adhesive is capable of sealing corneal incisions, and is nontoxic to corneal cells *(5)*. A small amount of tissue adhesive (1–2 mL) was able to strongly and robustly seal a clear corneal incision through the first five days of healing *(6)*. This is an important finding, as it suggests that the adhesive can be utilized to close and prevent leakage from clear corneal incisions made during cataract surgeries.

Tissue glues based on naturally derived polysaccharides therefore represent a promising platform for sealing and



▲ **Figure 1.** Polysaccharide-based tissue glues are produced by combining oxidized dextran polysaccharide with polyethylene glycol-amine. These two polymers react to form a crosslinked hydrogel network. Source: *(4)*.

healing soft tissues. These materials will find clinical utility in general surgery, cardiothoracic surgery, vascular surgery, emergency medicine, trauma surgery, and ophthalmology, to name just a few of the potential medical applications.

Success story: Soy for bone repair

Bio-based materials have demonstrated potential not only for wound closure in soft tissues, but also for repair of bony defects. Damages and defects in bone can result from traumatic events or surgical procedures; when the defect reaches a critical size, the bone is unable to spontaneously regenerate, and bone fillers are required to support the formation of new bone.

Bone reconstruction requires materials that are easy to handle, biodegradable, noncytotoxic, nonimmunogenic, and capable of inducing bone regeneration. Currently there are no commercial bone fillers that meet all of these technical requirements. Soybeans (Figure 2) are the source of an attractive alternative material for bone repair because they contain bioactive phytoestrogens that can induce differentiation of osteoblasts (bone-forming cells).

▲ **Figure 2.** Soybeans can be a rich source of materials for biomedical implants, including bone fillers and bone-regeneration scaffolds. Image courtesy of the U.S. Dept. of Agriculture.

Soybean-based biomaterials are synthesized by simple thermosetting of defatted soybean flour; the resulting material can be processed into films, membranes, porous scaffolds, and granules for various surgical applications *(7)*. Alternatively, soybean-based formulations can be obtained by extracting a fraction of the soybean that is enriched in the main soy components to produce a soft hydrogel.

These soy-based fillers are ductile and therefore easily adapt to the shape of the implantation site. They absorb water, with the swollen material assuming a rubbery consistency; this property contributes to biocompatibility, as it minimizes irritation to surrounding cells and tissues. The soy-based fillers also degrade in a controlled fashion, so that their lifetime in the body is predictable.

Studies have shown that soybean-based granules are bioactive *in vitro* — they reduce the activity of inflammatory cells and bone-removing cells, and increase the activity of bone-forming cells. These results suggest that upon implantation, the soybean-based bone filler may be able to reduce chronic inflammation while simultaneously promoting bone regeneration by stimulating bone cells.

Importantly, the production of soybean-based bone fillers is cost-competitive with commercial bone fillers *(8)*. In addition, unlike existing bone fillers, which are loaded with expensive growth factors, soybean-based bone fillers do not require the addition of exogenous growth factors for bioactivity.

In preclinical *in vivo* tests in rabbits, soybean-based bone fillers have shown efficacy in inducing bone formation during the eight weeks following implantation *(9)*. Treatment with soybean-based granules stimulated bone repair and healing, with progressively maturing structural features of bone, as well as cellular features superior to those in a nontreated bony defect that healed naturally. Soybean-based bone fillers may be suitable for orthopedic, maxillofacial, and periodontal surgeries.

Soybean-based biomaterials have been combined with gelatin and hydroxyapatite composites to create injectable foamed bone cements *(10)*. After the soy/gelatin/hydroxyapatite foam is injected into the bone defect, it forms interconnected pores; this porosity allows the bone-forming cells to infiltrate the soy scaffold. Because soy-based bone cements are injectable, they could be used for bone regeneration in a minimally invasive fashion. Clinical applications for these novel foamed cements include the treatment of vertebral fractures and the fixation of implants.

Success stories: Silk for scaffolding tissues

Just as glues made from polysaccharides may transform soft-tissue closure and fillers made of soy may advance bone repair, silk-based biomaterials have the potential to enhance tissue engineering *(11)*. Silk protein fibers are produced by

both silkworms and spiders (Figure 3), and are characterized by a unique combination of high strength and extensibility *(12)*. The toughness of silk fibers is superior to that of any commercially available synthetic high-performance fiber. Silk fibers composed of the silk fibroin protein have been in clinical use as sutures for centuries; they are biocompatible and degrade slowly over several weeks *in vivo*. Because the fibers can slowly and predictably transfer a load-bearing burden to nascent biological tissues *(13)*, silk is an ideal platform for tissue engineering.

Silk hydrogels have been prepared from aqueous solutions of silk protein, derived from *Bombyx mori* silkworms, via sonication-induced gelation *(14)*. One particular silk hydrogel has been formulated to yield mechanical properties similar to those of cartilage. These scaffolds can support the proliferation of chondrocytes (cartilage cells), and may be utilized for cartilage tissue engineering *(15)*.

Silk nanofibers can be manufactured by aqueous-based electrospinning of silk and blends of silk with polyethylene oxide *(16)*. Electrospun silk protein scaffolds have been evaluated for vascular tissue engineering, and can support the growth of human aortic endothelial cells and human coronary artery smooth-muscle cells. Moreover, electrospun silk scaffolds stimulate the formation of interconnecting networks of capillary tubes *(17)*. Electrospun silk nanofibers can be shaped into tubular materials with sufficient mechanical strength to withstand human blood pressures, and may find utility as tissue-engineered vascular grafts.

Silk scaffolds have also demonstrated potential for bone tissue engineering and ligament tissue engineering.

In bone tissue engineering, silk scaffolds have been chemically modified with covalently bound arginine-glycine-aspartate (RGD) peptide sequences; such RGD sequences are naturally found in cell adhesion molecules,

▲ **Figure 3.** Spider silk fibers can serve as substrates for tissue engineering and stem cell adhesion, proliferation, and differentiation.

Even more types of naturally derived materials are on the horizon for clinical medicine.

and RGD sequences can support cellular adhesion to silk scaffolds. These scaffolds promote the attachment of mesenchymal stem cells derived from human bone marrow, which can differentiate into bone, cartilage, or muscle. When utilized for bone tissue engineering, silk scaffolds in combination with mesenchymal stem cells support the formation of organized bonelike structures *(18)*. This indicates that silk scaffolds can be useful for bone repair.

In another clinical application, silk-fiber matrices have been designed to match the mechanical requirements of a native human knee ligament, including fatigue performance, suggesting their use for ligament replacement *(19)*. Silk-based biomaterials have even demonstrated the ability to support neuronal outgrowth *(20)*, so silk-based conduits may enable neural regeneration following traumatic spinal cord injuries.

Given the outstanding mechanical properties and aqueous processability of silk fibers, as well as the ability of silk scaffolds to support numerous cellular populations including stem cells, silk-based biomaterials may eventually find use in tissue engineering in every organ system of the body.

Future directions

The success stories of polysaccharide-based tissue glues for wound closure, soybean-based biomaterials for bone reconstruction, and silk-based scaffolds for tissue engineering all illustrate the versatility and capability of bio-based materials as biological implants. Even more types of naturally derived materials are on the horizon for clinical medicine.

Synthesizing new polymers using monomers obtained from agricultural resources is one avenue for future innovation. For instance, films and plastics composed of corn-derived 1,3-propanediol have been shown to be noncytotoxic and noninflammatory to clinically relevant cell lines *(21)*. Such materials may be readily adapted for biomedical implants. Agricultural resources such as soy, kenaf, flax, and cellulose may also provide useful starting materials for implantable medical devices.

Moreover, additional polymers derived from microbial production are under exploration. Polyhydroxyalkanoates, for example, are naturally occurring polyesters that are synthesized by many bacteria, and these materials are being investigated for tissue engineering *(22)* and targeted drug delivery *(23)*.

Continued work in both biomedical engineering and biochemical engineering will be required to realize the

potential of bio-based materials for medicine and surgery. Chemical engineers in particular will be crucial for introducing naturally derived materials into clinical practice. Specific challenges include:

• process development to enable reliable, cost-effective, scaled-up production of bio-derived polymers with desired physical, mechanical, chemical, and biological properties

• detailed physiological models to facilitate understanding of cellular proliferation and tissue repair during states of disease and health

• mechanistic studies to allow insight into interactions between natural biopolymers with cells, tissues, and organs.

Chemical engineering advances in these areas will soon provide physicians and surgeons with novel bio-derived materials for clinical applications. Chemical engineers can then consider themselves not only part of the research and development team, but also as part of the patient-care team. CEP

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Literature Cited

1. **Weber, C. J.,** "Biobased Packaging Materials for the Food Industry: Status and Perspectives," European Union Directorate 12, Royal Veterinary and Agricultural Univ., Frederiksberg, Denmark (2000).
2. **Ratner, B. D., *et al.*,** "Biomaterials Science: An Introduction to Materials in Medicine," Academic Press, New York, NY (1996).
3. **Bhatia, S. K., *et al.*,** "Interactions of Polysaccharide-Based Tissue Adhesives with Clinically Relevant Macrophage and Fibroblast Cell Lines," *Biotechnology Letters*, **29** (11), pp. 1645–1649 (Nov. 2007).
4. **Bhatia, S. K., *et al.*,** "Polysaccharide-Based Tissue Adhesives for Closure of Surgical Wounds," Proceedings of the AIChE Annual Meeting, Philadelphia, PA (Nov. 16–20, 2008).
5. **Bhatia, S. K., *et al.*,** "Polysaccharide-Based Tissue Adhesives for Sealing Corneal Incisions," *Current Eye Research*, **32** (12), pp. 1045–1050 (Dec. 2007).
6. **Chenault, H. K., *et al.*,** "Sealing and Healing of Clear Corneal Incisions with an Improved Dextran Aldehyde-PEG Amine Tissue Adhesive," *Current Eye Research*, **36** (11), pp. 997–1004 (Nov. 2011).
7. **Santin, M., *et al.*,** "A New Class of Bioactive and Biodegradable Soybean-Based Bone Fillers," *Biomacromolecules*, **8** (9), pp. 2706–2711 (Sep. 2007).
8. **Santin, M., and L. Ambrosio,** "Soybean-Based Biomaterials: Preparation, Properties and Tissue Regeneration Potential," *Expert Reviews in Medical Devices*, **5** (3), pp. 349–358 (May 2008).
9. **Merolli, A., *et al.*,** "A Degradable Soybean-Based Biomaterial Used Effectively as a Bone Filler *In Vivo* in a Rabbit," *Biomedical Materials*, **5** (1), Article no. 015008 (Feb. 2010).
10. **Perut, F., *et al.*,** "Novel Soybean/Gelatine-Based Bioactive and Injectable Hydroxyapatite Foam: Material Properties and Cell Response," *Acta Biomaterialia*, **7** (4), pp. 1780–1787 (Apr. 2011).
11. **Slotta, U., *et al.*,** "Synthetic Spider Silk Proteins and Threads," *Chem. Eng. Progress*, **108** (5), pp. 43–49 (May 2012).
12. **Omenetto, F. G., and D. L. Kaplan,** "New Opportunities for an Ancient Material," *Science*, **329** (5991), pp. 528–531 (Jul. 30, 2010).
13. **Horan, R. L., *et al.*,** "*In Vitro* Degradation of Silk Fibroin," *Biomaterials*, **26** (17), pp. 3385–3393 (Jun. 2005).
14. **Wang, X., *et al.*,** "Sonication-Induced Gelation of Silk Fibroin for Cell Encapsulation," *Biomaterials*, **29** (8), pp. 1054–1064 (Mar. 2008).
15. **Chao, P.-H. G., *et al.*,** "Silk Hydrogel for Cartilage Tissue Engineering," *Journal of Biomedical Materials Research B: Applied Biomaterials*, **95B** (1), pp. 84–90 (Oct. 2010).
16. **Jin, H.-J., *et al.*,** "Electrospinning *Bombyx Mori* Silk with Poly(Ethylene Oxide)," *Biomacromolecules*, **3** (8), pp. 1233–1239 (Aug. 2002).
17. **Zhang, X., *et al.*,** "*In Vitro* Evaluation of Electrospun Silk Fibroin Scaffolds for Vascular Cell Growth," *Biomaterials*, **29** (14), pp. 2217–2227 (May 2008).
18. **Meinel, L., *et al.*,** "Engineering Bone-Like Tissue *In Vitro* Using Human Bone Marrow Stem Cells and Silk Scaffolds," *Journal of Biomedical Materials Research A*, **71A** (1), pp. 25–34 (Oct. 2004).
19. **Altman, G. H., *et al.*,** "Silk Matrix for Tissue Engineered Anterior Cruciate Ligaments," *Biomaterials*, **23** (20), pp. 4131–4141 (Oct. 2002).
20. **Huang, W., *et al.*,** "Regenerative Potential of Silk Conduits in Repair of Peripheral Nerve Injury in Adult Rats," *Biomaterials*, **33** (1), pp. 59–71 (Jan. 2012).
21. **Bhatia, S. K., and J. V. Kurian,** "Biological Characterization of Sorona Polymer from Corn-Derived 1,3-Propanediol," *Biotechnology Letters*, **30** (4), pp. 619–623 (Apr. 2008).
22. **Hoefer, P.,** "Activation of Polyhydroxyalkanoates: Functionalization and Modification," *Frontiers in Bioscience*, **15** (1), pp. 93–121 (Jan. 2010).
23. **Grage, K., *et al.*,** "Bacterial Polyhydroxyalkanoate Granules: Biogenesis, Structure, and Potential Use as Nano-/Micro-Beads in Biotechnological and Biomedical Applications," *Biomacromolecules*, **10** (4), pp. 660–669 (Apr. 2009).