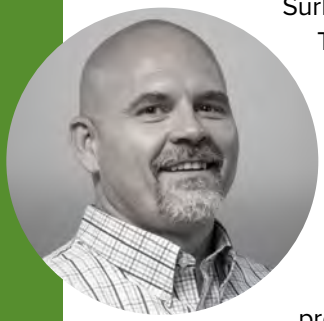
SurFACTS in Biomaterials

SPRING 2022
VOLUME 27, ISSUE 2

INSIDE THIS ISSUE

From President Rob Diller



The spring edition of SurFACTS has arrived. The planning for **BioInterface 2022** is in full swing. We have a comprehensive and innovative technical program planned. A dedicated group of volunteers are planning the workshops and sessions that you have been asking for—including workshops encompassing biodegradable and biostable polymers. As is our tradition, this conference benefits from strong industry participation which nicely complements innovative academic inputs.

We are currently accepting abstracts for Biointerface sessions. Consider submitting an abstract and presenting your work at this years' meeting; the deadline is fast approaching. Biointerface also features a dedicated student session. We are asking for student pitches, no more than five minutes, pre-recorded, and the top three will be selected to perform podium presentations. We look forward to seeing what the students are going to present. We will send out a formal call for student presentations in the months to come.

As we transition into spring, The Surfaces in Biomaterial Foundation is looking forward to our open house. Planning is still underway for this online event at the end of May. Be on the lookout for the specific dates and times of this exciting event. We are currently accepting nominations for this Years' Excellence in Surface Science Award. Think about leaders in our fields and make sure to get your nominations submitted.

I am excited for the direction of the Surfaces in Biomaterials Foundation this year. The planning committee has started scheduling top notch speakers for Biointerface 2022. Please mark your calendars for Nov. 2–4 to be with us in Portland, Oregon. Don't forget to visit the website and our sponsors' and join **our LinkedIn group** as well.

BioInterface 2022

We look forward to seeing you at BioInterface 2022 Workshop and Symposium in Portland, Oregon, Nov. 2–4. Check out the links below for all your planning needs!

Call for Abstracts is open until April 30.

2022 Excellence in Biomaterials Science Award and keynote speaker nominations are now open.

Sign up to sponsor or exhibit at BioInterface.

Register to attend BioInterface!

Reserve your hotel room for BioInterface.

Stay tuned for updates at our website and the Surfaces in Biomaterials Foundation app.

Tissue Decellularization and the Impact of Supercritical Carbon Dioxide

Nina Bionda, Ph.D., and Greg Mouchka, iFyber LLC

Although organ transplantation is a major advancement in modern medicine, the need for tissue vastly outstrips supply. In the US alone, more than 106,000 adults and children are currently on the national transplant waiting list and 17 people die daily while waiting for an available organ [1]. Due to the enormous need for viable organ transplant options, significant developments have been happening in the tissue engineering field. One of the most notable in recent news was the first heart xenotransplant, where a heart from a genetically modified pig was successfully transplanted into a human patient and kept the recipient alive for two months. Non-organ allograft transplants, such as bone, tendons, and ligaments, are also of importance and improve the quality of life for hundreds of thousands of people a year in the US. When the grafts are obtained from donor tissue, one of the main concerns is the patient's potential immune response and organ or tissue rejection. For this reason, significant development efforts are focused on the tissue and organ decellularization process. Decellularization is the removal of cellular components from a tissue or an organ, leaving an extracellular matrix (or ECM) scaffold. The main goal of tissue or organ decellularization is to prepare products containing minimal immunogenic cellular material, thereby minimizing the recipients' immune response upon transplantation, while preserving critical components of the ECM that are required for successful integration in the recipient.

Due to the novelty of this field, there is a lack of regulatory guidance on what objective metrics constitute acceptable decellularization. In a review paper by Crapo, et al. [2], the authors laid forth a set of metrics that are starting to be adopted in the field. They propose the following minimal criteria:

- <50 ng double-stranded DNA per mg ECM dry weight
- <200 base pair DNA fragment length
- Lack of visible nuclear material in tissue sections

Evaluation of nucleic material is the focus of the criteria, not because it is the source of the negative immune response, but because it provides a good correlation to the immunogenic cellular materials and is quantifiable using well-established methods. In addition to minimizing nucleic materials, effectively evaluating the resulting decellularized ECM's properties and fitness for transplantation is of equal importance. For example, tissue that is completely devoid of cellular material, but has been badly damaged by the process itself has poor potential for successful recellularization. Though the specifics of these requirements are tissue or organ dependent, broadly, a successful decellularization process will:

- Retain the morphology and ultrastructure of the original tissue or organ
- Preserve fibrous proteins (i.e., collagen, elastin, laminin, fibronectin) and glycosaminoglycans (GAGs, i.e., keratan and dermatan sulfate, hyaluronic acid)
- Preserve growth factors, cytokines, and other relevant macromolecules

Tissue staining and microscopy methods can be used to subjectively quantify the morphology and ultrastructure of the ECM, and molecular methods such as enzyme-linked immunosorbent assays (ELISAs) can be used to quantify various proteins, GAGs, and growth factors of interest.

Thus far, decellularized ECMs have been successfully obtained from various tissues and organs including nerve tissue, heart valves, small intestinal submucosa (SIS), dermis, lungs, liver, bladder, kidney, and even whole hearts [3]. With a number of ECM products already on the market, the most notable current applications are in soft tissue repair, wound care management, and cardiac and orthopedic applications.

These products are decellularized using processes involving physical (temperature, pressure), chemical (pH, detergents, ionic solutions, and alcohols), and biologic agents (enzymes and chelating agents). Because of the differences in tissue types (e.g., thickness, density, presence of fatty tissue) and the final product application, decellularization processes vary widely; however, there are some generalizations and similarities. For example, a cell lysis step (using osmotic gradients, freeze thaw cycles, or protease inhibitors) is performed to allow access of later treatments to the internal cell contents. Cell separation and removal is frequently done by applying combinations of detergents and solvents and/or enzymes. A final wash with distilled water or buffered saline is performed to remove both cellular debris and chemical residues from the ECM.

Continued on page 4

Tissue Decellularization and the Impact of Supercritical Carbon Dioxide ...

continued from page 3

These "standard" decellularization methods do have some drawbacks. For instance, the use of detergents, which are widely utilized because of their ability to dissolve non-polar components after entering tissues, has been shown to have detrimental effects on the resulting ECM, as well as causing immune response in vivo. The complexity and duration of a protocol is also problematic: thicker, denser tissues can take days or weeks to process [3].

Identifying a decellularization process that meets the criteria for cellular matter, retains the ECM's structure and critical biomolecules, and is performed using a relatively short and straightforward protocol would result in large improvements in the current ECM manufacturing and ultimately expedite the tissue transplantation process. In a review article by Duarte, et al. [4], it was argued that supercritical carbon dioxide (scCO2)-assisted decellularization could be that process. A supercritical fluid is a state of matter when a substance is held at a temperature and pressure above its critical point. These fluids exhibit the diffusivity, viscosity, and surface tension of a gas, but the density of a liquid, resulting in a much more efficient solvent as compared with the gas or liquid states. The relatively low critical point of carbon dioxide (31°C and 73.9 bar) and its chemical inertness makes carbon dioxide a viable option for processing biomaterials and tissues. Carbon dioxide is also inexpensive, readily available, and leaves no toxic residues post-processing.

The scCO2 technology is currently in use in a number of industries, including biomedical applications. However, its use in tissue decellularization has only recently gained more interest. Nevertheless, based on the current body of literature, the scCO2 technology has the potential to significantly advance the field of regenerative medicine and has already been used for evaluating and improving decellularization of several tissue types, including skin, pericardium, myocardium, aorta, optical nerves, tendons, adipose tissues, cartilage, and bone. While in most cases scCO2 processing alone was not sufficient to accomplish decellularization of tissue, the presence of co-solvents, additives, or use of pretreatment steps vastly improved the efficacy of the process. Furthermore, implementation of the scCO2 technology has allowed for significant decreases in processing times and protocol complexity, while demonstrating promising results with respect to maintaining tissue properties and preserving relevant biomolecules.

A recent publication highlighted the processing of tendons using the scCO2 technology and NovaKillTM (NovaSterilis' proprietary additive) and either 0.1% SDS (detergent) or 0.1% EDTA (chelating agent). Although the authors did not achieve target levels of decellularization (Figure 1), this 2-in-1 sterilization and decellularization approach resulted in tendons which were biomechanically superior to those prepared using the standard approach (immersion in 0.1% SDS) [5].

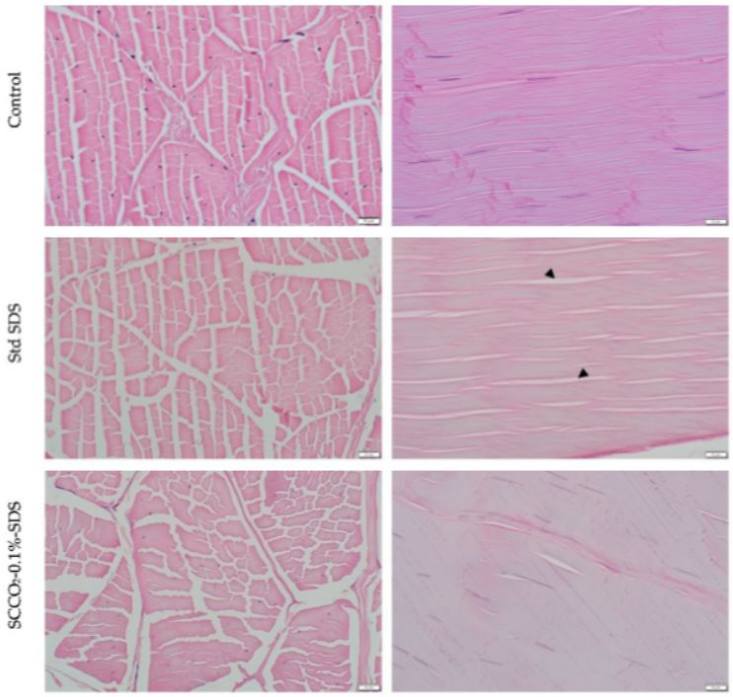


Figure 1. Adapted from reference (5). Histological images of transverse (left panels) and longitudinal (right panels) sections stained with haematoxylin and eosin (H&E). Note the presence of blue nuclei in scCO2-0.1% SDS treated samples and the lack thereof in SDS-only samples. Separation of collagen bundles in the SDS-only sample are indicated with black arrowheads.

The NovaSterilis team has independently evaluated the effects of the scCO2-NovaKillTM technology on the structure of amino acids, which are the building blocks of all proteins, as well as human epidermal growth factor (EGF) as a model protein [6]. They found that with careful selection of processing parameters results is minimal effects on the integrity of the amino acids, with some level of oxidation of methionine and tryptophan residues. Furthermore, processing of lyophilized EGF (vs solubilized) retained its activity (Figure 2) and resulted in minimal structural modifications. Cumulatively, these studies illustrate the use of scCO2 as a relevant tool in the decellularization of various types of tissues. This, coupled with the sterilization properties of the scCO2-NovaKill process, could potentially be a one-stop solution for a number of applications in the regenerative medicine field.

Continued on page 5

Tissue Decellularization and the Impact of Supercritical Carbon Dioxide ...

continued from page 4

Though there is more work to be done, sufficient data exists from a wide variety of researchers that shows scCO2 is a useful tool for obtaining functional biological scaffolds beyond its use as a sterilization modality. NovaSterilis has been a leading provider of scCO2 equipment for over 20 years—initially with a focus on the sterilization of tissue products. Together with iFyber, their laboratory and validation services partner, they can help with feasibility evaluations for new and next generation decellularized products.

References

https://www.organdonor.gov/learn/organ-donation-statistics

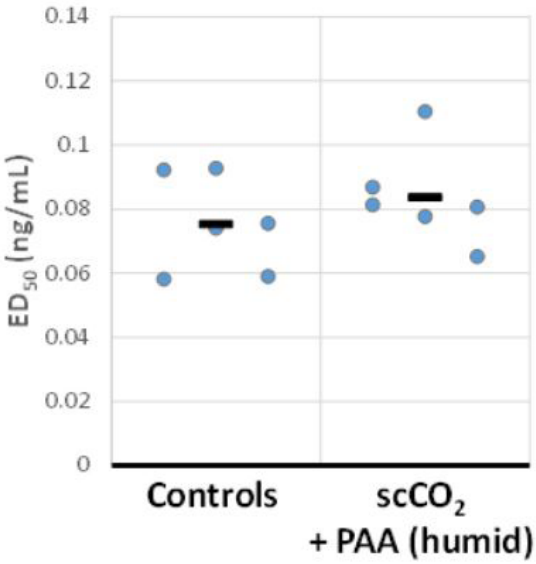
Crapo PM, Gilbert TW, Badylak SF. Biomaterials. 2011, 32, 3233–3243.

Gilbert TW, Sellaro TL, Badylak SF. Biomaterials. 2006, 27(19): 3675-83.

Duarte MM, Silva IV, Eisenhut AR, Bionda N, Duarte ARC, Oliveira AL. Mater Horiz. 2022, 9(3): 864-891.

Sun Y, Lovric V, Wang T, Oliver RA, Walsh WR. Int. J. Mol. Sci. 2020, 21, 1565.

Bednarski DM, Lantz EE, Bobst CE, Eisenhut AR, Eyles SJ, Fey JP. Biochim Biophys Acta Proteins Proteom. 2020, 1868(2): 140334.



	Controls	$scCO_2$ + PAA (humid)
Av. ED_{50} (ng/mL)	0.076	0.084
Std. Deviation	0.015	0.015

Figure 2. Adapted from reference (6). Comparison of activity between control (non-treated) EGF and lyophilized EGF that was subjected to the scCO2 process.

Mentorship Program

Surfaces in Biomaterials is hosting a **mentorship program**. The goal is to build a professional relationship between mentor and mentees. Once matched, mentors and mentees can create a timeline to meet virtually. The goal is to meet twice in the first month and then at least once a month for a total of six months.

Please sign up below to start your mentorship!

Mentors

Surfaces in Biomaterials Foundation is recruiting those interested in serving as a mentor and mentoring incoming industry professionals (graduate students and young professionals).

Click here for the mentor application

Mentees

Surfaces in Biomaterials Foundation is looking to recruit mentees (graduate students and young professionals) that would like to grow within the industry and are seeking to build a relationship with mentors within the Foundation.

Click here for the mentee application

Tissue Engineering: Bringing Progression Toward Health Care Applications

Pritisha S. Khillar, Amit Kumar Jaiswal, Centre for Biomaterials, Cellular and Molecular Theranostics, Vellore Institute of Technology, Vellore, 632014, Tamil Nadu, India

Tissue engineering is an interdisciplinary field that involves the application of life sciences and engineering toward the development of biological substitutes [1]. Tissue engineering can overcome the shortage of specific tissues and organs by repairing or regenerating the damaged or lost tissue and can restore or improve their functions. It involves the cells, growth factors and biologically active materials also called biomaterials to create a functional construct that can mimic the nature of the specific tissue and organ by providing a niche for cell growth and differentiation along with structural support for tissue regeneration [2,3]. Tissue Engineering has a wider application including skin, bone, cartilage, cardiac, and corneal tissues engineering. Besides, it shows involvement in the clinical and health care industry, drug delivery, and 3D bioprinting technology (Fig 1).

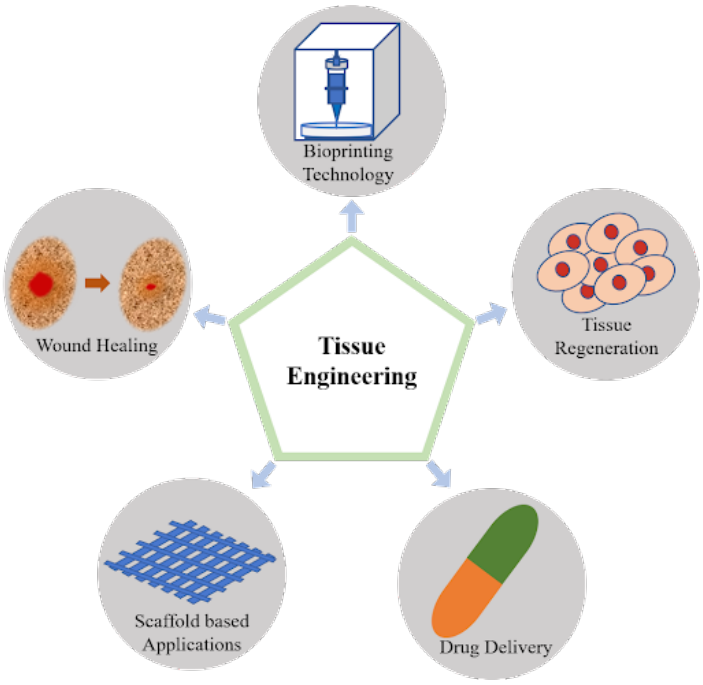


Fig.1. Applications of tissue engineering

Biomaterials act as important factors in Tissue Engineering. They help in stimulating the extracellular matrix mimicking process by maintaining the framework, morphology, and chemistry of the scaffold [4]. They also provide structural support for cell growth and attachment and serve as an integral component for subsequent tissue development [5]. Biomaterials can either be synthetic or natural biopolymers including polysaccharides, proteins, and peptides. Some of the natural biomaterials are alginate, gelatin, collagen, keratin, chitosan, silk, cellulose, hyaluronic acid, and chondroitin sulfate, etc. [6]. Synthetic biomaterials are categorized into metals, ceramics, and polymers. They aimed to mimic the characteristics of natural extracellular matrices (ECMs) [7]. The ceramics include hydroxyapatite, alumina, zirconia, etc. The common metals are stainless steel, cobalt-chromium alloy, titanium alloy, amalgam, etc. While the polymers include polyurethanes, poly (methyl Methacrylate), polycyanoacrylates, polyvinyl Alcohol, polyvinyl pyrrolidone, polyphosphates, etc [8]. However, due to several advantages such as immunomodulating, anti-toxic, and biomimetic properties, natural biomaterials are being frequently chosen over synthetic ones [9].

With the increasing population, orthopedic injuries, including sports-related injuries, musculoskeletal defects have become one of the many serious health problems. In recent times, bone tissue engineering strategies are given greater attention by providing more efficient approaches to treat orthopedic defects that are difficult to heal [10]. The musculoskeletal system provides the structure and support to our body. It is hard and strong, and it has load-bearing capacities. The scaffolds required for Bone Tissue Engineering must retain strong mechanical strength that matches the bone of the defect site. Our lab performs several bone tissue engineering strategies, including implantable bone scaffolds, bone glue/ bone adhesives, bone injectables and 3D printed bone constructs, etc. Currently, our research group is working on the development of scaffolds with improved mechanical properties to meet the above-mentioned requirements.

First, we developed a scaffold using a mixture of polyvinyl alcohol, polyethylene glycol, and hydroxyapatite by following a physical crosslinking method. The crosslinking took place following several freeze-thaw cycles. The fabricated scaffold came out with outstanding mechanical properties and it was found to be durable over a long period. The stress vs strain graph of both wet and dry depict a similar trend showing satisfying mechanical property. It

Continued on page 7

continued from page 6

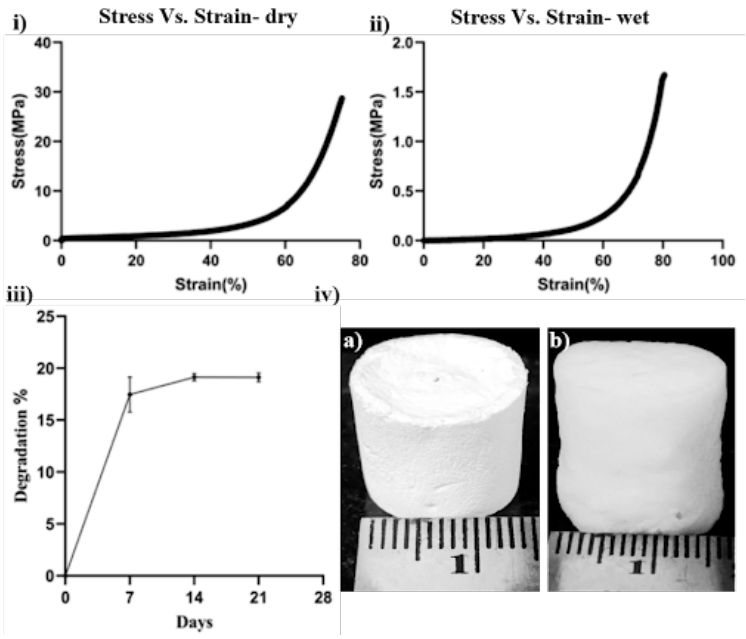


Fig 2. i) The stress Vs. strain graph of dry scaffolds ii) The stress Vs. strain graph of wet scaffolds iii) Quantitative degradation analysis of the scaffold, iv) (a) dry scaffold and b) scaffold after incubation in phosphate buffer for 5 days

showed a slow dissolution where the scaffold was observed to lose only 20% of its whole structure when kept in phosphate buffer saline for 22 days (Fig 2). The endurance of mechanical strength claimed this composition of scaffold to be considered as a promising composite scaffold for bone tissue engineering application.

Second, we developed a hydrogel composed of gelatin, chitosan and oxidized guar gum crosslinked with a mixture of sodium β-glycerophosphate and sodium bicarbonate. This hydrogel has notable properties to be used as a beneficial agent in tissue engineering applications. The hydrogel was prepared following a novel physical and self-crosslinking mechanism. The hypothesis was that the interaction between chitosan and gelatin resulted by the formation of a C–O bond, β-glycerophosphate carried the chitosan units together by ionic bond, gelatin interacted with guar gum and oxidized guar gum by forming a C–N bond and amide bond respectively [11]. FT-IR analysis displayed the presence of particular functional groups and supported the validation of the proposed hydrogel chemistry. The hydrogels showed a favorable gelling time that was confirmed by the inverted vial test. The hydrogels showed sufficient crosslinking efficiency, rheological and mechanical properties. The morphology of the hydrogels showed unevenly distributed porous microstructure throughout the hydrogel construct. As hydrogels are supposed to possess water retention capacity, the designed hydrogels were shown to have an adequate swelling degree and with degradation property like an ideal hydrogel should possess. The hydrogels were observed to possess a very exciting property. When the hydrogels are taken for compression tests, it was observed that they regained about 50% of their original size after a certain time. This confirmed the spongy nature of the hydrogel. All the relevant findings related to the study are given in figure 3. Hence the hydrogels can be surmised to be a powerful candidate for various tissue engineering applications [12].

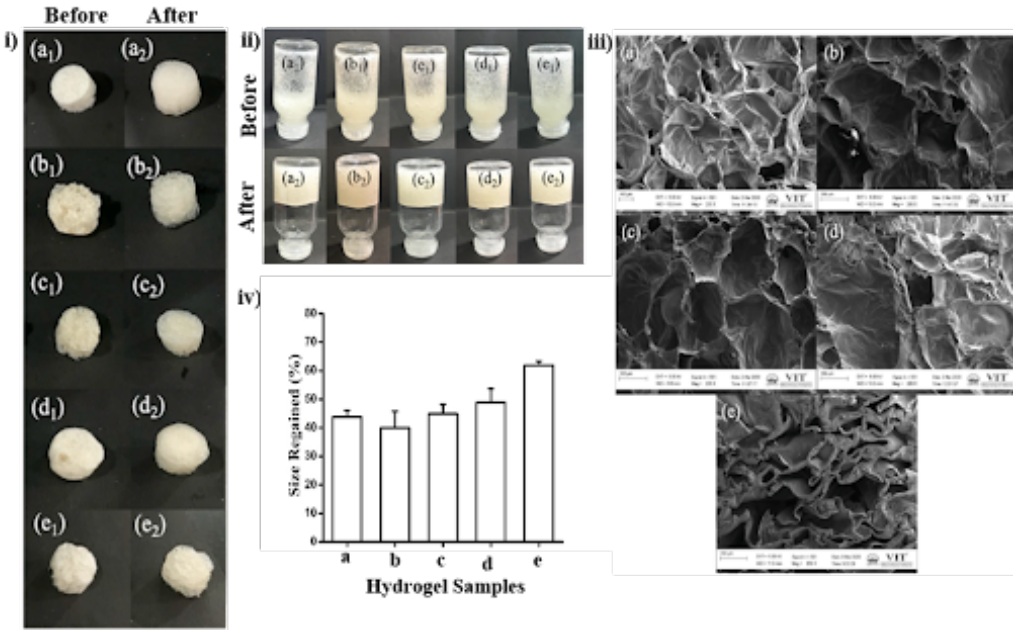


Fig 3. i) Illustration of swelling studies, ii) Illustration of gelling property through inverted tube test iii) Distribution of porous microstructure throughout the constructs for the different hydrogels iv) Size re-gained after being compressed. Here, a, b,c,d and e are the 5 different hydrogels. "Reprinted from polymer testing, 97, Sayoni Maiti, Pritisha S. Khillar, Debasish Mishra, N. Arunai Nambiraj, Amit K. Jaiswal, Physical and self–crosslinking mechanism and characterization of chitosan-gelatin-oxidized guar gum hydrogel, 1-14, Copyright (2021) with permission from Elsevier".

Continued on page 8

Tissue Engineering: Bringing Progression Toward Health Care Applications ...

continued from page 7

In conclusion, tissue engineering includes the use of a vast number of biomaterials for several applications. The modification and development of materials and novel biocomposites play an important role in the field of tissue engineering to prepare several functional scaffolds with hydrogels and bioinks in advanced 3-dimensional bioprinting with improved and tunable properties for soft and hard tissue regeneration along with additional health care applications.

Reference

1. *Howard D, Buttery LD, Shakesheff KM, Roberts SJ (2008). Tissue engineering: strategies, stem cells and scaffolds, Journal of anatomy, 213(1), 66–72. https://doi.org/10.1111/j.1469-7580.2008.00878.x*

2. *Lee EJ, Kasper FK, Mikos AG (2014). Biomaterials for tissue engineering. Ann Biomed Eng, 42(2):323–337. doi:10.1007/s10439-013-0859-6*

3. *Dzobo K, Thomford NE, Senthebane DA, Shipanga H, Rowe A, Dandara C, Pillay M, MotaungK KSCM (2018). Advances in Regenerative Medicine and Tissue Engineering: Innovation and Transformation of Medicine. Stem Cells Int. doi:10.1155/2018/2495848*

4. *Khillar PS, Jaiswal AK (2022). Gums for Tissue Engineering Application. In: Oliveira JM, Radhouani H, Reis RL (eds) Polysaccharides of Microbial Origin. Springer, Cham. http://doi.org/10.1007/978-3-030-42215-8_53*

5. *Chan BP, Leong KW (2008). Scaffolding in tissue engineering: general approaches and tissue-specific considerations. European spine journal : official publication of the European Spine Society. the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society, 17(4), 467–479. https://doi.org/10.1007/s00586-008-0745-3*

6. *Tang X, Thankappan SK, Lee P, Fard SE, Matthew DH, Tran K, Yu X (2014). Polymeric Biomaterials in Tissue Engineering and Regenerative Medicine. Natural and synthetic biomed polym. doi:10.1016/b978-0-12-396983-5.00022-3*

7. *Lutolf MP, Hubbell JA (2005). Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol, 23(1), 47–55. doi:10.1038/nbt1055*

8. *Wang X, Ao Q, Tian X, Fan J, Tong H, Hou W, Bai S (2017). Gelatin-Based Hydrogels for Organ 3D Bioprinting. Polymers, 9(12), 401. doi:10.3390/polym9090401*

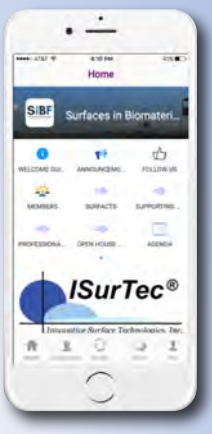
9. *Barua E, Deoghare AB, Deb P, Lala SD (2018). Naturally derived biomaterials for development of composite bone scaffold: A review. IOP Conference Series: Materials Science and Engineering. doi:10.1088/1757-899X/377/1/012013*

10. *Laurencin CT, Ambrosio AMA, Borden MD, Cooper JA (1999). Tissue Engineering: Orthopedic Applications. Annual Review of Biomedical Engineering. doi:10.1146/annurev.bioeng.1.1.19*

11. *Zheng XF, Lian Q, Yang H (2014). Synthesis of chitosan–gelatin molecularly imprinted membranes for extraction of L-tyrosine, RSC Adv, 42478–42485. https:// doi.org/10.1039/C4RA05740F*

12. *Maiti S, Khillar PS, Mishra D, Nambiraj A, Jaiswal AK (2021). Physical and self–crosslinking mechanism and characterization of chitosan-gelatin-oxidized guar gum hydrogel, Polymer Testing. https://doi.org/10.1016/j.polymertesting.2021.107155*

Surfaces in Biomaterials Foundation App is LIVE!



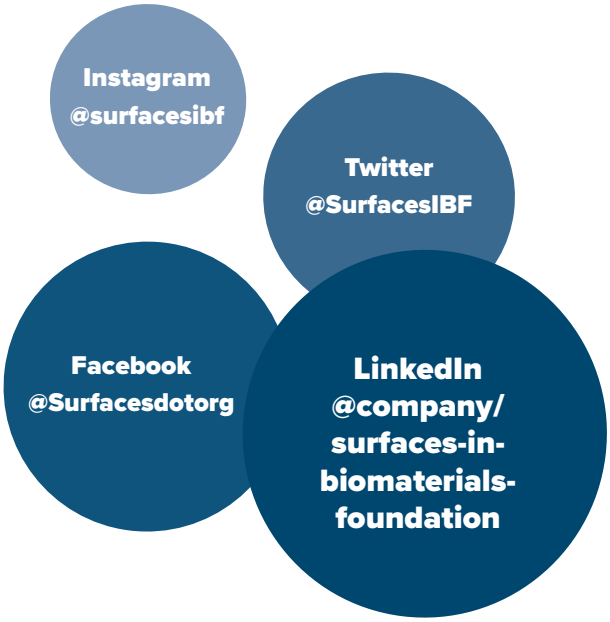
Engage with members, build a professional network, and get updates for BioInterface right to your phone! Stay connected with your colleagues and industry news whenever and wherever you may be—24 hours a day, seven days a week.

Available for download on Google Play and the Apple App Store, with a web app also available for easy access from your computer. Membership to SIBF is required to access all components of the app and to interact with other members.

Access the Web App here!

Click here to download the mobile app.

Log in with your email address. To access all components, login with your email used for membership. Contact info@surfaces.org if you are not sure of your membership status. Employees of Supporting Members need to request access at info@surfaces.org.



SurFACTS in Biomaterials is the official publication of the Foundation and is dedicated to serving industrial engineers, research scientists, and academicians working on the field of biomaterials, biomedical devices, or diagnostic research.

SIBF

Surfaces in Biomaterials Foundation

Thank You to
OUR MEMBERS

APS AMERICAN PRECLINICAL SERVICES NOW PART OF NAMSA®

AST PRODUCTS, INC.

BAUSCH + LOMB

BIOCOAT® INCORPORATED

EVONIK Leading Beyond Chemistry

Elkem

SCHOOL OF BIOMEDICAL ENGINEERING
COLORADO STATE UNIVERSITY

GORE

ISurTec®
Innovative Surface Technologies, Inc.

Medtronic

Learn more about membership at www.surfaces.org/Member-Benefits